

| SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO          |                                            |
|---------------------------------------------------|--------------------------------------------|
| RAD: 13-240105- -5                                | FECHA: 2014-09-18 18:40:34                 |
| DEP: 2020 DIRECCION DE NUEVAS CREACIONES          | EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I |
| TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II | FOLIOS: 10                                 |
| ACT: 519 PRESENTACION                             |                                            |

DIRECCIÓN DE NUEVAS CREACIONES

2020  
Bogotá, D.C.,  
  
Abogado(a)/Señor(a)  
SILVANA MARIA LOPEZ DIAZ

Asunto:      Radicación:      13-240105- -5  
                 Trámite:            11  
                 Evento:            378  
                 Actuación:           519  
                 Oficio:                    11963  
                 Folios:                    10

|                                     |                   |            |   |                               |  |
|-------------------------------------|-------------------|------------|---|-------------------------------|--|
| Patente de Invención                | X                 |            |   | Patente de Modelo de Utilidad |  |
| Vía Nacional                        |                   |            |   |                               |  |
| Vía PCT (Fase Nacional)             | X                 | Capítulo I | X | Capítulo II                   |  |
| No. Solicitud Internacional         | PCT/US2012/028569 |            |   |                               |  |
| Fecha de Presentación Internacional | 09/03/2012        |            |   |                               |  |

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

|                   |                                  |                 |
|-------------------|----------------------------------|-----------------|
| Descripción:      | Radicado N° 13-240105-00000-0000 | 09/Octubre/2013 |
| Reivindicaciones: | Radicado N° 13-240105-00000-0000 | 09/Octubre/2013 |
| Prioridad:        | US 61 452 017                    | 11/Marzo/2011   |

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

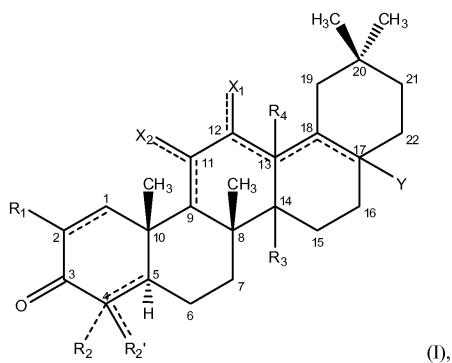
De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por la solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las

cubiertas por la tasa pagada. En el presente caso se estudiarán las reivindicaciones 1 a 84 del capítulo reivindicatorio (Radicado N°. 13-240105-00000-0000).

Título de la solicitud: “Derivados de C4 monometil triterpenoides y sus métodos de uso”

El problema planteado en esta solicitud consiste en la necesidad de proporcionar compuestos derivados de triterpenoides para el tratamiento de trastornos asociados a inflamación.

Para resolver este problema técnico la solicitud en estudio proporciona un compuesto de fórmula (I), composiciones que los contienen y métodos para su elaboración.



El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07J 63/00

#### 4. Excepción a la Patentabilidad (Art 20 D 486 literal (d))

El objeto de las reivindicación 84 no es patentable de acuerdo con el artículo citado ya que corresponde a un método para tratar o prevenir una enfermedad.

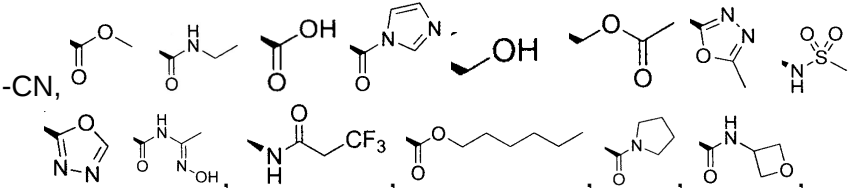
#### 5. De la solicitud de patente

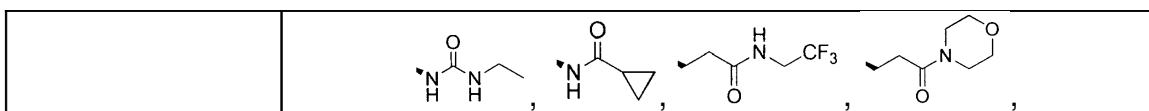
##### Titulo

El título de la solicitud hace referencia al uso de los compuestos solicitados, el cual no es patentable de acuerdo con el artículo 14 D 486, por lo cual se sugiere al solicitante realizar la modificación correspondiente.

##### Reivindicaciones (Art 30 D 486, Art 22 PCT)

Las reivindicaciones 1 y 2 no son concisas porque las definiciones de R1, R2, R3, R4, X e Y son géneros funcionales, se sugiere restringir la solicitud a una razonable generalización del tipo de compuestos que ha sido sintetizado ó probado, es decir, que se encuentran debidamente sustentados.

| Sustituciones  | Soporte de los ejemplos presentados                                                                                                                                                                                                                                               |
|----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| R <sup>1</sup> | -CN, -I, -H,  ,  ,  , |
| R <sup>2</sup> | -CH <sub>3</sub> ,                                                                                                                                                                                                                                                                |
| R <sup>3</sup> | -CH <sub>3</sub> ,                                                                                                                                                                                                                                                                |
| R <sup>4</sup> | -H, -OH,                                                                                                                                                                                                                                                                          |
| X              | =O, -OH, -F.                                                                                                                                                                                                                                                                      |
| Y              |                                                                                                                                                                                               |



La reivindicación 82 comprende compuestos que no cumplen con la formula general reclamada en la reivindicación 1, en la cual el sustituyente del carbono 3 es siempre un grupo oxo. Se le requiere a la solicitante hacer las modificaciones correspondientes.

### Descripción (Art 28 D 486)

En la descripción se mencionan características de los compuestos referidas a que estos presentan a una toxicidad disminuida, una biodisponibilidad aumentada, un efecto terapéutico de mayor duración o una mejoría en su perfil farmacocinético al ser comparados con los compuestos de las anterioridades, sin embargo no se incluyen datos que permitan apreciar estas ventajas, por lo que no se considera que no se encuentran adecuadamente soportadas en la descripción.

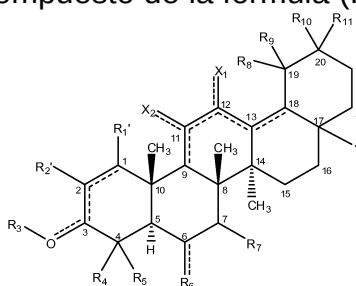
Se requiere a la solicitante hacer las aclaraciones necesarias.

### 6. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: marzo 11 de 2011 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

| Ítem | Documento                                        | Título                                                                                                                                                         | Fecha de Publicación |
|------|--------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| D1   | WO 2009 129546                                   | "Antioxidant inflammation modulators: oleanolic acid derivatives with amino and other modifications at c-17"                                                   | 22/Octubre/2009      |
| D2   | Sun et al, Botanical Studies (2006) 47: 339-368  | "Structure-activity relationships of oleanane and ursanetype Triterpenoids"                                                                                    | Octubre 2006         |
| D3   | Tadashi et al. J. Org. Chem. 1998, 63, 4846-4849 | "Design and Synthesis of 23, 24-dinoroleanolic acid derivative, novel triterpenoid-steroid hybrid molecules"                                                   | 18/Junio/1998        |
| D4   | Tadashi et al. J. Med. Chem. 2000, 43, 1866-1877 | "Novel Synthetic Oleanane and Ursane Triterpenoids with Various Enone Functionalities in Ring A as Inhibitors of Nitric Oxide Production in Mouse Macrophages" | 14/Abril/2000        |

El documento D1<sup>1</sup> divulga un compuesto de la fórmula (I) (Pág.3, líneas 1– 5).



El documento D2<sup>2</sup> relaciona las características estructurales de compuestos triterpenoides derivados de oleanano y ursano y su función como agentes antitumorales, antivirales, antiinflamatorios, hepatoprotectivos, gastroprotectivos, antimicrobianos, antidiabéticos, hemolíticos entre otros.

<sup>1</sup>[http://worldwide.espacenet.com/publicationDetails/originalDocument?](http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=WO&NR=2009129546A1&KC=A1&FT=D&ND=&date=20091022&DB=&&locale=en_EP)

[CC=WO&NR=2009129546A1&KC=A1&FT=D&ND=&date=20091022&DB=&&locale=en\\_EP](http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=WO&NR=2009129546A1&KC=A1&FT=D&ND=&date=20091022&DB=&&locale=en_EP)

<sup>2</sup><http://ejournal.sinica.edu.tw/bbas/content/2006/4/Bot474-01.pdf>

El documento D3<sup>3</sup> divulga compuestos triterpenoides derivados del ácido oleanólico así como métodos de preparación de los mismos.

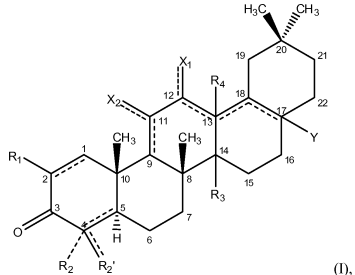
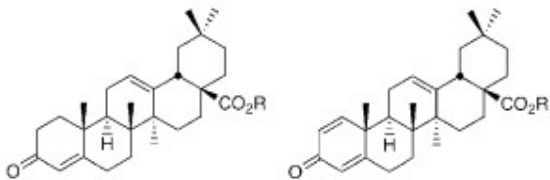
El documento D4<sup>4</sup> divulga compuestos triterpenoides derivados del oleanano y el ursano con modificaciones en el anillo A y su actividad como inhibidores de la producción de óxido nítrico en macrófagos de ratones.

### 7. Examen de Patentabilidad (Art 14 D486)

#### Novedad (Art 16 D486)

La reivindicación 1 consiste en: *“Un compuesto caracterizado porque es de la fórmula.”* (Radicado N°. 13- 240105-00000-00000).

Comparación de las características técnicas esenciales, con las del estado de la técnica:

| Características esenciales                                                                                                                                                                                                                                                                                                             | D3                                                                                                                                                                             |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Un compuesto caracterizado porque es de la fórmula (I) <div>  <p>(I),</p> </div> Donde:                                                                                                                                                              | Compuestos híbridos esteroide-terpenoide: <div>  <p>(Pág. 4846, compuestos 3-6 )</p> </div> |
| <b>X<sub>1</sub> y X<sub>2</sub>:</b> Hidrógeno, halo, hidroxilo amino ó oxo.<br>Con la condición de que X1 no es oxo cuando los átomos de carbono 12 y 13 están conectados entre sí con una unión doble además con la condición de que X2 no es oxo cuando los átomos de carbono 9 y 11 están conectados entre sí con una unión doble | Hidrógeno                                                                                                                                                                      |
| <b>R<sub>1</sub>:</b> Es –H, -CN, halo, -CF3, ó –C(O)R <sub>a</sub> . Donde R <sub>a</sub> es –OH, alcoxi <sub>(C1-4)</sub> , -NH2, alquilamino <sub>(C1-4)</sub> , ó –NH-S(O) <sub>2</sub> -alquilo <sub>(C1-4)</sub> .                                                                                                               | Hidrógeno                                                                                                                                                                      |
| <b>R<sub>2</sub>:</b> Es –H ó se encuentra ausente cuando el átomo al que está unido forma parte de una unión doble.                                                                                                                                                                                                                   | Ausente                                                                                                                                                                        |
| <b>R<sub>2</sub>'</b> : Es –H, =CH <sub>2</sub> , alquilo <sub>(C&lt;8)</sub> , ó alquilo <sub>(C&lt;8)</sub> sustituido sustituido.                                                                                                                                                                                                   | Hidrógeno                                                                                                                                                                      |
| <b>R<sub>3</sub>:</b> hidrogeno, hidroxí, metilo.                                                                                                                                                                                                                                                                                      | Metilo                                                                                                                                                                         |
| <b>R<sub>4</sub>:</b> hidrogeno, hidroxí, metilo                                                                                                                                                                                                                                                                                       | Hidrógeno                                                                                                                                                                      |
| <b>Y:</b> -(CH <sub>2</sub> ) <sub>m</sub> C(O)R <sub>c</sub> Donde m es 0-6 y R <sub>c</sub> es hidrógeno, hidroxí, halo, amino. –NHOH, alquilo <sub>(C&lt;8)</sub> .                                                                                                                                                                 | CO <sub>2</sub> H ó CO <sub>2</sub> CH <sub>3</sub>                                                                                                                            |

Compuestos con las características esenciales del compuesto de la reivindicación 1 habían sido divulgados previamente en el documento D3, el cual da conocer una serie de derivados sintéticos de oleano y ursano que presentan actividad inhibitoria en la producción de óxido nítrico, el documento también presenta métodos de síntesis asociados a estos compuestos.

En consecuencia, la reivindicación 1 no es nueva o carece de novedad, según lo divulgado en el documento D3.

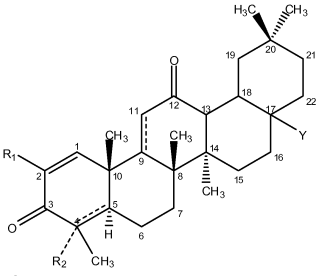
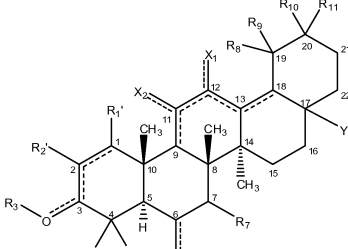
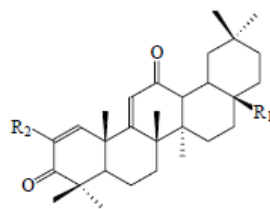
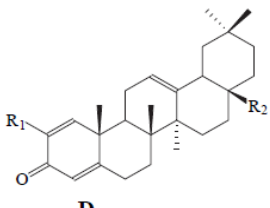
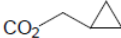
Las reivindicaciones dependientes 5, 6, 8, 10, 12, 15, 22, 25, 26, 29, 31, 32, 35-38 y 51 no mencionan características adicionales que hagan novedoso al compuesto de la reivindicación 1, por lo cual se considera que no son nuevas.

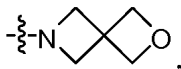
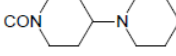
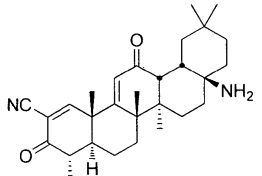
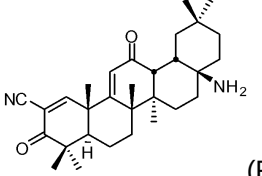
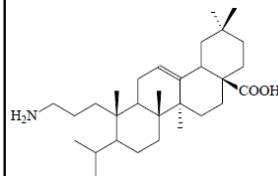
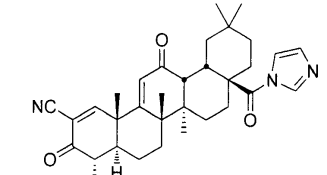
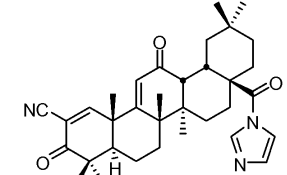
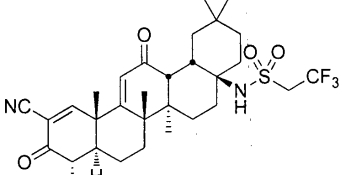
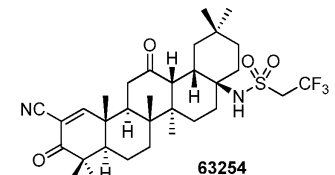
#### Nivel inventivo (Art 18 D486)

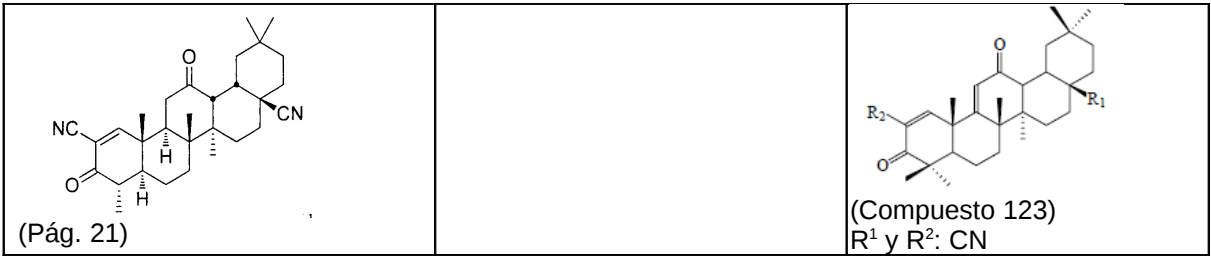
3 <http://pubs.acs.org/doi/abs/10.1021/jo980525y?journalCode=joceah>

4 <http://pubs.acs.org/doi/abs/10.1021/jm000008j>

Comparación de las características técnicas esenciales, con las del estado de la técnica:

| Características esenciales                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | D1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | D2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>Un compuesto caracterizado porque la fórmula (II)</p>  <p>Donde:</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | <p>Un compuesto caracterizado porque es de la fórmula (I)</p>  <p>(I)</p> <p>(Pág. 3, líneas 1-5)</p> <p>Donde:</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  <p>(Pág. 349, compuestos 123-148)</p>  <p>D</p>                                                                                                                                                                                                                                                                                                                                                                                                      |
| <p><b>R<sub>1</sub>:</b> Es -H, -CN, halo, -CF<sub>3</sub>, ó -C(O)R<sub>a</sub>. Donde R<sub>a</sub> es -OH, alcoxi<sub>(C&lt;8)</sub>, -NH<sub>2</sub>, alquilamino<sub>(C1-4)</sub>, ó -NH-S(O)<sub>2</sub>-alquilo<sub>(C1-4)</sub>.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | <p><b>R<sup>2'</sup>:</b> -CN, halo, -OH, ó fluoroalquil<sub>(C&lt;8)</sub>, alquenil<sub>(C&lt;8)</sub>, alquinil<sub>(C&lt;8)</sub>, aril<sub>(C&lt;8)</sub>, heteroaril<sub>(C&lt;8)</sub>, acil<sub>(C&lt;8)</sub>, alcoxi<sub>(C&lt;8)</sub>, ariloxi<sub>(C&lt;8)</sub>, aciloxi<sub>(C&lt;8)</sub>, alquilamino<sub>(C&lt;8)</sub>, arilamino<sub>(C&lt;8)</sub>, amido<sub>(C&lt;8)</sub>, ó una versión sustituida de cualquiera de estos grupos</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | <p><b>R<sup>1</sup> o R<sup>2</sup>:</b> -CN, CO<sub>2</sub>H,</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <p><b>R<sub>2</sub>:</b> Es -H ó R<sub>2</sub> se encuentra ausente cuando el átomo al que está unido forma parte de una unión doble.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | <p><b>R<sup>4</sup> y R<sup>5</sup>:</b> Son independientemente alquilo, o alquilo sustituido.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | <p>-H, -CH<sub>3</sub>,</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <p><b>Y:</b> -H, -OH, -SH, -CN, -F, CF<sub>3</sub>, -NH<sub>2</sub> ó -NCO;<br/> Alquilo<sub>(C&lt;8)</sub>, alquenilo<sub>(C&lt;8)</sub>, alquinilo<sub>(C&lt;8)</sub>, arilo<sub>(C&lt;12)</sub>, aralquilo<sub>(C&lt;12)</sub>, heteroarilo<sub>(C&lt;8)</sub>, heterocicloalquilo<sub>(C&lt;12)</sub>, alcoxi<sub>(C&lt;8)</sub>, ariloxi<sub>(C&lt;12)</sub>, aciloxi<sub>(C&lt;8)</sub>, alquilamino<sub>(C&lt;8)</sub>, dialquilamino<sub>(C&lt;8)</sub>, alquenilamino<sub>(C&lt;8)</sub>, arilamino<sub>(C&lt;8)</sub>, aralquilamino<sub>(C&lt;8)</sub>, alquiltio<sub>(C&lt;8)</sub>, aciltio<sub>(C&lt;8)</sub>, alquilsulfonamino<sub>(C&lt;8)</sub>, o versiones sustituidas de cualquiera de estos grupos;<br/> -alcanodiil<sub>(C&lt;8)</sub> -R<sub>b</sub>, -alquenodiil<sub>(C&lt;8)</sub>-R<sub>b</sub> o una versión sustituida de cualquiera de estos grupos, donde R<sub>b</sub> es:<br/> -hidrógeno, hidroxil, halo, amino o tio, ó.<br/> heteroarilo<sub>(C&lt;8)</sub>, alcoxi<sub>(C&lt;8)</sub>, alqueniloxi<sub>(C&lt;8)</sub>, ariloxi<sub>(C&lt;8)</sub>, aralcoxi<sub>(C&lt;8)</sub> heteroariloxi<sub>(C&lt;8)</sub>, aciloxi<sub>(C&lt;8)</sub>, alquilamino<sub>(C&lt;8)</sub>, dialquilamino<sub>(C&lt;8)</sub>, alquenilamino<sub>(C&lt;8)</sub>, arilamino<sub>(C&lt;8)</sub>, aralquilamino<sub>(C&lt;8)</sub>, heteroarilamino<sub>(C&lt;8)</sub>, alquilsulfonamino<sub>(C&lt;8)</sub>, amido<sub>(C&lt;8)</sub>, -OC(O)NH-alquilo<sub>(C&lt;8)</sub>, -OC(O)CH<sub>2</sub>NHC(O)O-t-butilo, -OCH<sub>2</sub>-alquiltio<sub>(C&lt;8)</sub>, o una</p> | <p><b>Y:</b> Es hidroxil, alcoxi<sub>(C&lt;8)</sub>, ariloxi<sub>(C&lt;8)</sub>, aciloxi<sub>(C&lt;8)</sub>, alcoxi<sub>(C&lt;8)</sub> sustituido, alcoxi<sub>(C&lt;8)</sub> sustituido, ariloxi<sub>(C&lt;8)</sub> sustituido, aciloxi<sub>(C&lt;8)</sub> sustituido, o NR<sub>1</sub>R<sub>2</sub>, donde R<sub>1</sub> y R<sub>2</sub> son independientemente: hidrógeno ó hidroxil; o alquilo<sub>(C&lt;12)</sub>, alquenilo<sub>(C&lt;12)</sub>, alquinilo<sub>(C&lt;12)</sub>, arilo<sub>(C&lt;12)</sub>, aralquilo<sub>(C&lt;12)</sub>, heteroarilo<sub>(C&lt;12)</sub>, heteroaralquilo<sub>(C&lt;12)</sub>, acilo<sub>(C&lt;12)</sub>, alcoxi<sub>(C&lt;12)</sub>, alqueniloxi<sub>(C&lt;12)</sub>, alquiniloxi<sub>(C&lt;12)</sub>, ariloxi<sub>(C&lt;12)</sub>, aralcoxi<sub>(C&lt;12)</sub>, heteroariloxi<sub>(C&lt;12)</sub>, heteroaralcoxi<sub>(C&lt;12)</sub>, tioacilo<sub>(C&lt;12)</sub>, alquilsulfonil<sub>(C&lt;12)</sub>, alquenilosulfonil<sub>(C&lt;12)</sub>, alquinilosulfonil<sub>(C&lt;12)</sub>, arilosulfonil<sub>(C&lt;12)</sub>, aralquilosulfonil<sub>(C&lt;12)</sub>, heteroarilosulfonil<sub>(C&lt;12)</sub>, heteroaralquilosulfonil<sub>(C&lt;12)</sub>, o una versión sustituida de cualquiera de estos grupos; R<sub>1</sub>' es: hidrógeno, ciano, hidroxil, halo, ó amino; ó alquilo<sub>(C&lt;8)</sub>, alquenilo<sub>(C&lt;8)</sub>, alquinilo<sub>(C&lt;8)</sub>, arilo<sub>(C&lt;8)</sub>, aralquilo<sub>(C&lt;8)</sub>, heteroarilo<sub>(C&lt;8)</sub>, heteroaralquilo<sub>(C&lt;8)</sub>, acilo<sub>(C&lt;8)</sub>, alcoxi<sub>(C&lt;8)</sub>, ariloxi<sub>(C&lt;8)</sub>, aciloxi<sub>(C&lt;8)</sub>,</p> | <p><b>R<sup>1</sup> o R<sup>2</sup>:</b> -CN, CO<sub>2</sub>Et,<br/> CO<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub><br/> CO<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>CH<sub>3</sub><br/> CO<sub>2</sub> <br/> CO<sub>2</sub>CH<sub>2</sub>Ph<br/> CO<sub>2</sub>(CH<sub>2</sub>)<sub>7</sub>CH<sub>3</sub><br/> CO-D-Glc(OAc)<sub>4</sub><br/> CO-D-Glc<br/> CONH<sub>2</sub><br/> CONHNH<sub>2</sub><br/> CONHMe<br/> CONH(CH<sub>2</sub>)<sub>2</sub>CH<sub>3</sub><br/> CONH(CH<sub>2</sub>)<sub>5</sub>CH<sub>3</sub><br/> CONHPh</p> |

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>             versión sustituida de cualquiera de estos grupos;<br/> <math>-(CH_2)_mC(O)R_c</math>, donde m es 0-6 y <math>R_c</math> es<br/>             -hidrogeno, hidroxi, halo, amino,<br/> ,<br/>             -NHOH,<br/>             o tio; ó<br/>             alquilo<sub>(c&lt;8)</sub>, alquenilo<sub>(c&lt;8)</sub>,<br/>             alquinilo<sub>(c&lt;8)</sub>, arilo<sub>(c&lt;8)</sub>,<br/>             aralquilo<sub>(c&lt;8)</sub>, heteroarilo<sub>(c&lt;8)</sub>,<br/>             heterocicloalquilo<sub>(c&lt;8)</sub>, alcoxi<sub>(c&lt;8)</sub>,<br/>             alqueniloxi<sub>(c&lt;8)</sub>, ariloxi<sub>(c&lt;8)</sub>,<br/>             aralcoxi<sub>(c&lt;8)</sub>, heteroariloxi<sub>(c&lt;8)</sub>,<br/>             aciloxi<sub>(c&lt;8)</sub>, alquilamino<sub>(c&lt;8)</sub>,<br/>             dialquilamino<sub>(c&lt;8)</sub>, arilamino<sub>(c&lt;8)</sub>,<br/>             alquilsulfonilamino<sub>(c&lt;8)</sub>, amido<sub>(c&lt;8)</sub>,<br/>             -NH-alcoxi<sub>(c&lt;8)</sub>,<br/>             -NH-heterocicloalquilo<sub>(c&lt;8)</sub>,<br/>             -NHC(NOH)-alquilo<sub>(c&lt;8)</sub>, -NH-<br/>             amido<sub>(c&lt;8)</sub>, o una versión<br/>             sustituida de cualquiera de<br/>             estos grupos;<br/>             -NHC(O)<math>R_e</math>, donde <math>R_e</math> es:<br/>             hidrogeno, hidroxi, amino; ó<br/>             alquilo<sub>(c&lt;8)</sub>, alquenilo<sub>(c&lt;8)</sub>,<br/>             alquinilo<sub>(c&lt;8)</sub>, arilo<sub>(c&lt;8)</sub>, araquilo<sub>(c&lt;8)</sub>,<br/>             heteroarilo<sub>(c&lt;8)</sub>,<br/>             heterocicloalquilo<sub>(c&lt;8)</sub>, alcoxi<sub>(c&lt;8)</sub>,<br/>             ariloxi<sub>(c&lt;8)</sub>, aralcoxi<sub>(c&lt;8)</sub>,<br/>             heteroariloxi<sub>(c&lt;8)</sub>, aciloxi<sub>(c&lt;8)</sub>,<br/>             alquilamino<sub>(c&lt;8)</sub>, dialquilamino<sub>(c&lt;8)</sub>,<br/>             arilamino<sub>(c&lt;8)</sub>, o una versión<br/>             sustituida de cualquiera de<br/>             estos grupos;<br/>             ó una sal farmacéuticamente<br/>             aceptable o tautómero del mismo           </p> | <p>             alquiloamino<sub>(c≤8)</sub>, ariloamino<sub>(c≤8)</sub>,<br/>             amido<sub>(c≤8)</sub>, o una versión<br/>             sustituida de cualquiera de<br/>             estos grupos.           </p> | <p> <math>CONHCH_2Ph</math><br/> <math>CONMe_2</math><br/> <math>CON(n-Pr)_2</math><br/> <br/> <br/> <br/> <br/> <br/>  </p> |
| <b>Comparación de ejemplos específicos</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <br>(Pág. 62, compuesto TX63620)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | <br>(Pág. 36,<br>compuesto 63169)                                                                                                       | <br>(Pág. 364, Compuesto 385)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <br>(Pág. 16)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | <br>. 102, compuesto 403)           (Pág                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <br>(Pág. 21)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | <br>63254<br>(Pág. 113, compuesto 63254)                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |



El documento D1 se considera el estado de la técnica cercano a la invención definida en la reivindicación 2 porque divulga compuestos útiles para el tratamiento y la prevención de enfermedades asociadas al estrés oxidativo e inflamación.

La diferencia entre los compuestos definidos en la reivindicación 2 y aquellos anticipados en el documento D1 consiste en que en la posición 4 los compuestos de la solicitud, presentan un grupo metilo mientras que los compuestos anterioridad presentan dos.

El efecto técnico logrado por la eliminación del grupo metilo es el aumento en la potencia de los compuestos reclamados de acuerdo a los valores de IC<sub>50</sub> mostrados en la tabla 1 (Págs. 54-109).

El problema técnico objetivo se considera como la necesidad de modificar los compuestos divulgados en D1 con el fin de aumentar su potencia.

Sin embargo, la modificación del anillo A de compuestos derivados de oleanano y ursano, ya se encuentra divulgada en el documento D2, el cual relaciona las características estructurales de una gran variedad de compuestos triterpenoides derivados de oleanano y ursano y su función como agentes antitumorales, antivirales, antiinflamatorios entre otras.

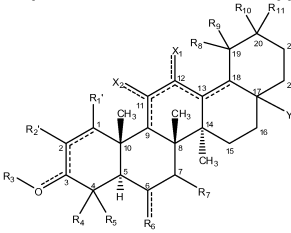
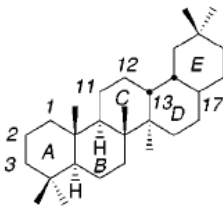
En consecuencia, la persona del oficio normalmente versada en la materia, consideraría la modificación de la estructura de los compuestos del documento D1 de acuerdo a lo planteado en D2 para llegar así al objeto de la reivindicación 2 de la solicitud. Por lo cual se considera obvia.

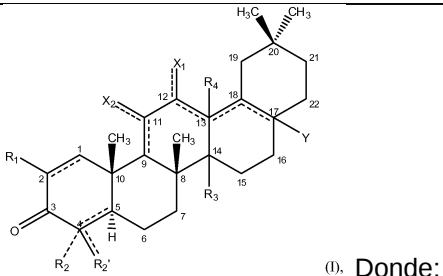
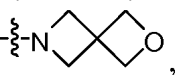
Puesto que las reivindicaciones 3, 4, 7, 9, 11, 13, 14, 16, 17-21, 23, 24, 27- 30, 39-50, 52-79, no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

Conclusión:

Las reivindicaciones 2, 3, 4, 7, 9, 11, 13, 14, 16, 17-21, 23, 24, 27- 30, 39-50, 52-79 cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

La reivindicación 34 consiste en: *“El compuesto de acuerdo con cualquiera de las reivindicaciones 1 y 5-31 caracterizado porque R<sub>4</sub> es hidroxí”*.

| Características esenciales                       | D1                                                                                                                                                                                             | D4                                                                                                                                                                                       |
|--------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Un compuesto caracterizado porque la fórmula (I) | Un compuesto caracterizado porque es de la fórmula (I) <div>  <p>(I)</p> </div> (Pág. 3, líneas 1-5) Donde: | Triterpenoides sintéticos derivados del Ursano y Oleanano <div>  <p>(Pág. 1867, tabla 1)</p> </div> |

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                       |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  <p>(I). Donde:</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                       |
| <p><b>R<sub>1</sub>:</b> Es -H, -CN, halo, -CF<sub>3</sub>, ó -C(O)R<sub>a</sub>. Donde R<sub>a</sub> es -OH, alcoxi<sub>(C&lt;8)</sub>, -NH<sub>2</sub>, alquilamino<sub>(C1-4)</sub>, ó -NH-S(O)<sub>2</sub>-alquilo<sub>(C1-4)</sub>.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | <p><b>R<sup>2'</sup>:</b> -CN, halo, -OH, ó fluoroalquil<sub>(C&lt;8)</sub>, alquenil<sub>(C&lt;8)</sub>, alquinil<sub>(C&lt;8)</sub>, aril<sub>(C&lt;8)</sub>, heteroaril<sub>(C&lt;8)</sub>, acil<sub>(C&lt;8)</sub>, alcoxi<sub>(C&lt;8)</sub>, ariloxi<sub>(C&lt;8)</sub>, aciloxi<sub>(C&lt;8)</sub>, alquilamino<sub>(C&lt;8)</sub>, arilamino<sub>(C&lt;8)</sub>, amido<sub>(C&lt;8)</sub>, ó una versión sustituida de cualquiera de estos grupos</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | <p>-H</p>                                                                                                                                                                             |
| <p><b>R<sub>2</sub>:</b> Es -H ó R<sub>2</sub> se encuentra ausente cuando el átomo al que está unido forma parte de una unión doble.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | <p><b>R<sup>4</sup> y R<sup>5</sup>:</b> Son independientemente alquilo, o alquilo sustituido.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | <p>-CH<sub>3</sub>,</p>                                                                                                                                                               |
| <p><b>R<sub>2</sub>'</b>: Es -H, =CH<sub>2</sub>, alquilo<sub>(C&lt;8)</sub>, ó alquilo<sub>(C&lt;8)</sub> sustituido sustituido.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | <p>-CH<sub>3</sub>,</p>                                                                                                                                                               |
| <p><b>R<sub>3</sub></b> : hidrógeno, hidroxí, metilo</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | <p>-CH<sub>3</sub></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | <p>-CH<sub>3</sub>,</p>                                                                                                                                                               |
| <p><b>R<sub>4</sub></b>: -OH</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | <p>-H</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | <p>-H, -OH, -O-.</p>                                                                                                                                                                  |
| <p><b>Y:</b> -H, -OH, -SH, -CN, -F, CF<sub>3</sub>, -NH<sub>2</sub> ó -NCO;<br/>         Alquilo<sub>(C&lt;8)</sub>, alquenilo<sub>(C&lt;8)</sub>, alquinilo<sub>(C&lt;8)</sub>, arilo<sub>(C&lt;12)</sub>, aralquilo<sub>(C&lt;12)</sub>, heteroarilo<sub>(C&lt;8)</sub>, heterocicloalquilo<sub>(C&lt;12)</sub>, alcoxi<sub>(C&lt;8)</sub>, ariloxi<sub>(C&lt;12)</sub>, aciloxi<sub>(C&lt;8)</sub>, alquilamino<sub>(C&lt;8)</sub>, dialquilamino<sub>(C&lt;8)</sub>, alquenilamino<sub>(C&lt;8)</sub>, arilamino<sub>(C&lt;8)</sub>, aralquilamino<sub>(C&lt;8)</sub>, alquiltio<sub>(C&lt;8)</sub>, aciltio<sub>(C&lt;8)</sub>, alquilsulfonamino<sub>(C&lt;8)</sub>, o versiones sustituidas de cualquiera de estos grupos;<br/>         -alcanodiil<sub>(C&lt;8)</sub> -R<sub>b</sub>, -alquenodiil<sub>(C&lt;8)</sub> -R<sub>b</sub> o una versión sustituida de cualquiera de estos grupos, donde R<sub>b</sub> es:<br/>         -hidrógeno, hidroxí, halo, amino o tio, ó.<br/>         heteroarilo<sub>(C&lt;8)</sub>, alcoxi<sub>(C&lt;8)</sub>, alqueniloxi<sub>(C&lt;8)</sub>, ariloxi<sub>(C&lt;8)</sub>, aralcoxi<sub>(C&lt;8)</sub> heteroariloxi<sub>(C&lt;8)</sub>, aciloxi<sub>(C&lt;8)</sub>, alquilamino<sub>(C&lt;8)</sub>, dialquilamino<sub>(C&lt;8)</sub>, alquenilamino<sub>(C&lt;8)</sub>, arilamino<sub>(C&lt;8)</sub>, aralquilamino<sub>(C&lt;8)</sub>, heteroarilamino<sub>(C&lt;8)</sub>, alquilsulfonamino<sub>(C&lt;8)</sub>, amido<sub>(C&lt;8)</sub>, -OC(O)NH-alquilo<sub>(C&lt;8)</sub>, -OC(O)CH<sub>2</sub>NHC(O)O-t-butilo, -OCH<sub>2</sub>-alquiltio<sub>(C&lt;8)</sub>, o una versión sustituida de cualquiera de estos grupos;<br/>         -(CH<sub>2</sub>)<sub>m</sub>C(O)R<sub>c</sub>, donde m es 0-6 y R<sub>c</sub> es<br/>         -hidrogeno, hidroxí, halo, amino,<br/> <br/>         -NHOH,<br/>         o tio; ó<br/>         alquilo<sub>(C&lt;8)</sub>, alquenilo<sub>(C&lt;8)</sub>, alquinilo<sub>(C&lt;8)</sub>, arilo<sub>(C&lt;8)</sub>, aralquilo<sub>(C&lt;8)</sub>, heteroarilo<sub>(C&lt;8)</sub>, heterocicloalquilo<sub>(C&lt;8)</sub>, alcoxi<sub>(C&lt;8)</sub>, alqueniloxi<sub>(C&lt;8)</sub>, ariloxi<sub>(C&lt;8)</sub>, aralcoxi<sub>(C&lt;8)</sub>, heteroariloxi<sub>(C&lt;8)</sub>,</p> | <p><b>Y:</b> Es hidroxí, alcoxi<sub>(C&lt;8)</sub>, ariloxi<sub>(C&lt;8)</sub>, aciloxi<sub>(C&lt;8)</sub>, alcoxi<sub>(C&lt;8)</sub> sustituido, alcoxi<sub>(C&lt;8)</sub> sustituido, ariloxi<sub>(C&lt;8)</sub> sustituido, aciloxi<sub>(C&lt;8)</sub> sustituido, o NR<sub>1</sub>R<sub>2</sub>, donde R<sub>1</sub> y R<sub>2</sub> son independientemente: hidrógeno ó hidroxí; o alquilo<sub>(C&lt;12)</sub>, alquenilo<sub>(C&lt;12)</sub>, alquinilo<sub>(C&lt;12)</sub>, arilo<sub>(C&lt;12)</sub>, aralquilo<sub>(C&lt;12)</sub>, heteroarilo<sub>(C&lt;12)</sub>, heteroaralquilo<sub>(C&lt;12)</sub>, acilo<sub>(C&lt;12)</sub>, alcoxi<sub>(C&lt;12)</sub>, alqueniloxi<sub>(C&lt;12)</sub>, alquiniloxi<sub>(C&lt;12)</sub>, ariloxi<sub>(C&lt;12)</sub>, aralcoxi<sub>(C&lt;12)</sub>, heteroariloxi<sub>(C&lt;12)</sub>, heteroaralcoxi<sub>(C&lt;12)</sub>, tioacilo<sub>(C&lt;12)</sub>, alquilsulfonil<sub>(C&lt;12)</sub>, alquenilosulfonil<sub>(C&lt;12)</sub>, alquinosulfonil<sub>(C&lt;12)</sub>, arilosulfonil<sub>(C&lt;12)</sub>, aralquilosulfonil<sub>(C&lt;12)</sub>, heteroarilosulfonil<sub>(C&lt;12)</sub>, o heteroaralquilosulfonil<sub>(C&lt;12)</sub>, o una versión sustituida de cualquiera de estos grupos;<br/>         R<sub>i</sub>' es: hidrógeno, ciano, hidroxí, halo, ó amino; ó alquilo<sub>(C&lt;8)</sub>, alquenilo<sub>(C&lt;8)</sub>, alquinilo<sub>(C&lt;8)</sub>, arilo<sub>(C&lt;8)</sub>, aralquilo<sub>(C&lt;8)</sub>, heteroarilo<sub>(C&lt;8)</sub>, heteroaralquilo<sub>(C&lt;8)</sub>, acilo<sub>(C&lt;8)</sub>, alcoxi<sub>(C&lt;8)</sub>, ariloxi<sub>(C&lt;8)</sub>, aciloxi<sub>(C&lt;8)</sub>, alquiloamino<sub>(C&lt;8)</sub>, ariloamino<sub>(C&lt;8)</sub>, amido<sub>(C&lt;8)</sub>, o una versión sustituida de cualquiera de estos grupos.</p> | <p>-CO<sub>2</sub>H,<br/>         -CO<sub>2</sub>Me,<br/>         -CH<sub>2</sub>OH,<br/>         -CH<sub>2</sub>OAc,<br/>         -CH(OH)-,<br/>         -CO-<br/>         -CHO,</p> |

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |  |  |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|--|
| aciloxi <sub>(c&lt;8)</sub> ,<br>dialquilamino <sub>(c&lt;8)</sub> ,<br>alquilsulfonilamino <sub>(c&lt;8)</sub> ,<br>-NH-alcoxi <sub>(c&lt;8)</sub> ,<br>-NH-heterocicloalquilo <sub>(c&lt;8)</sub> ,<br>-NHC(NO <sub>2</sub> )-alquilo <sub>(c&lt;8)</sub> ,<br>-NH-amido <sub>(c&lt;8)</sub> , o una versión sustituida de cualquiera de estos grupos;<br>-NHC(O)R <sub>e</sub> , donde R <sub>e</sub> es:<br>hidrogeno, hidroxilo, amino; ó<br>alquilo <sub>(c&lt;8)</sub> , alquenilo <sub>(c&lt;8)</sub> ,<br>alquinilo <sub>(c&lt;8)</sub> , arilo <sub>(c&lt;8)</sub> , araquilo <sub>(c&lt;8)</sub> ,<br>heteroarilo <sub>(c&lt;8)</sub> ,<br>heterocicloalquilo <sub>(c&lt;8)</sub> , alcoxi <sub>(c&lt;8)</sub> ,<br>ariloxi <sub>(c&lt;8)</sub> , aralcoxi <sub>(c&lt;8)</sub> ,<br>heteroariloxi <sub>(c&lt;8)</sub> , aciloxi <sub>(c&lt;8)</sub> ,<br>alquilamino <sub>(c&lt;8)</sub> , dialquilamino <sub>(c&lt;8)</sub> ,<br>arilamino <sub>(c&lt;8)</sub> , o una versión sustituida de cualquiera de estos grupos;<br>ó una sal farmacéuticamente aceptable o tautómero del mismo |  |  |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|--|

El documento D1 se considera el estado de la técnica cercano a la invención definida en la reivindicación 34 porque divulga compuestos útiles para el tratamiento y la prevención de enfermedades asociadas al estrés oxidativo e inflamación.

La diferencia entre los compuestos definidos en la reivindicación 34 y aquellos anticipados en el documento D1 consiste en que en la posición 13 los compuestos solicitados presentan una sustitución con un grupo –OH, mientras que aquellos encontrados en la anterioridad no presentan sustituciones.

No hay evidencia de un efecto técnico asociado a incluir un sustituyente en la posición 13 de la estructura.

El problema técnico objetivo se considera como la necesidad de obtener compuestos alternativos a los divulgados en D1

Sin embargo, la sustitución en la posición 13 de compuestos triterpenoides derivados de de oleanano y ursano, ya se encuentra divulgada en el documento D4, el cual da a conocer la estructura de derivados sintéticos de estos ácidos y su actividad como inhibidores de la producción de óxido nítrico.

En consecuencia, la persona del oficio normalmente versada en la materia, consideraría la modificación de la estructura de los compuestos del documento D1 de acuerdo a lo planteado en D4 para llegar así al objeto de la reivindicación 34 de la solicitud. Por lo cual se considera obvia.

Puesto que las reivindicaciones 33, 80 - 83, no mencionan características inventivas adicionales tampoco se pueden considerar inventivas

Conclusión:

Las reivindicaciones 33, 80 - 83 cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

8. Conclusión

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

| Íte<br>m | Documento                                           | Reivindicaciones afectadas                                                   | Requisito que afecta |
|----------|-----------------------------------------------------|------------------------------------------------------------------------------|----------------------|
| D1       | WO 2009 129546                                      | 2-4, 7, 9, 11, 13, 14, 16, 17-21,<br>23, 24, 27- 30, 33, 34, 39-50,<br>52-83 | Nivel inventivo      |
| D2       | Sun et al, Botanical Studies<br>(2006) 47: 339-368  | 2-4, 7, 9, 11, 13, 14, 16, 17-21,<br>23, 24, 27- 30, 39-50, 52-79,           | Nivel inventivo      |
| D3       | Tadashi et al. J. Org. Chem.<br>1998, 63, 4846-4849 | 1, 5, 6, 8, 10, 12, 15, 22, 25, 26,<br>29, 31, 32, 35-38, 51                 | Novedad              |
| D4       | Tadashi et al. J. Med. Chem.<br>2000, 43, 1866-1877 | 33, 34, 80- 83                                                               | Nivel inventivo      |

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



**JOSÉ LUIS SALAZAR LÓPEZ**  
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2014-09-23

Elaboró: Ruth Andrea Rodriguez  
Revisó: Sandra Carolina Crespo

# Structure-activity relationships of oleanane- and ursane-type triterpenoids

Hua SUN<sup>1,2,3</sup>, Wei-Shuo FANG<sup>1,3,\*</sup>, Wen-Zhao WANG<sup>1,3</sup>, and Chun HU<sup>2</sup>

<sup>1</sup>*Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, P.R. China*

<sup>2</sup>*Shenyang Pharmaceutical University, Shenyang, Liaoning province, 110016, P.R. China*

<sup>3</sup>*Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine (Peking Union Medical College), Ministry of Education, P.R. China*

(Received September 21, 2005; Accepted March 2, 2006)

**ABSTRACT.** The chemistry of oleanane- and ursane-type triterpenoids has been actively explored in recent years, and their biological and pharmacological activities of these compounds have been found to span a variety of properties. These include antitumor, anti-viral, anti-inflammatory, hepatoprotective, gastroprotective, antimicrobial, antidiabetic, and hemolytic properties as well as many others. This review summarizes the isolation and structure modifications of these triterpenoids as well as the biological and pharmacological activities discovered in the past ten years, with an emphasis on their structure-activity relationships.

**Keywords:** Antidiabets; Anti-inflammatory; Antimicrobial; Antitumor; Antiviral; Gastroprotective; Hepatoprotective; Oleanane; Structure-activity relationship; Ursane.

**Abbreviations:** **GI<sub>50</sub>**, concentration required to inhibit tumor cell growth by 50%; **ED<sub>50</sub>**, concentration caused 50% inhibition of cell proliferation *in vitro*; **CC<sub>50</sub>**, 50% cytotoxic concentration; **LC<sub>50</sub>**, 50% lethal concentration; **TI**, *in vitro* therapeutic index; **MIC**, minimum inhibitory concentration; **PKC**, protein kinase C; **HLE**, human leukocyte elastase; **UIISO-SQC-1**, squamous cervix carcinoma; **OVCAR-5**, human ovarian cancer; **fMLP**, N-formyl-methionyl-leucyl-phenylalanine; **PMA**, phorbol-12-myristate-13-acetate; **AA**, arachidonic acid; **ALT**, alanine aminotransferase; **ID<sub>50</sub>**, doses inhibiting the oedematous response by 50%.

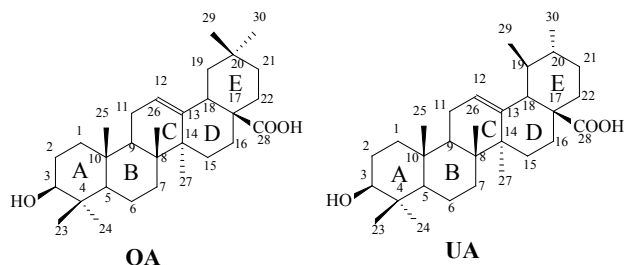
## CONTENTS

|                                                                                                  |     |
|--------------------------------------------------------------------------------------------------|-----|
| INTRODUCTION .....                                                                               | 340 |
| ANTITUMOR ACTIVITIES .....                                                                       | 340 |
| ANTI-VIRAL ACTIVITIES .....                                                                      | 347 |
| ANTI-INFLAMMATORY ACTIVITIES .....                                                               | 350 |
| <i>In vivo</i> studies .....                                                                     | 350 |
| Inhibition of cyclooxygenase (COX) activity .....                                                | 350 |
| Inhibition of complement activity .....                                                          | 353 |
| Inhibition of elastase .....                                                                     | 354 |
| Inhibition of intercellular adhesion molecule (ICAM-1) expression induced by TNF- $\alpha$ ..... | 354 |
| HEPATOPROTECTIVE ACTIVITIES .....                                                                | 355 |
| GASTROPROTECTIVE ACTIVITIES .....                                                                | 356 |
| ANTIMICROBIAL ACTIVITIES .....                                                                   | 356 |
| ANTI-DIABETES ACTIVITIES .....                                                                   | 358 |
| HEMOLYTIC ACTIVITIES .....                                                                       | 360 |
| MISCELLANEOUS .....                                                                              | 362 |
| Spasmolytic activity .....                                                                       | 362 |
| Antipruritic activity .....                                                                      | 362 |
| Anti-thrombotic activity .....                                                                   | 362 |
| Inhibitory activity of ethanol absorption .....                                                  | 363 |
| Effects on nonmalignant prostate cell proliferation .....                                        | 363 |
| LITERATURE CITED .....                                                                           | 364 |

\*Corresponding author: E-mail: wfang@imm.ac.cn; Tel: +86-10-63036794; Fax: +86-10-63017757.

## INTRODUCTION

Oleanolic acid (**OA**) (3 $\beta$ -hydroxy-olean-12-en-28-oic acid) and its isomer, ursolic acid (**UA**) (3 $\beta$ -hydroxy-urs-12-en-28-oic acid) are triterpenoid compounds which exist widely in nature in free acid form or as aglycones for oleanane- and ursane-type triterpenoid saponins (Liu, 1995). Oleanane and ursane are also called  $\beta$ -amyrane and  $\alpha$ -amyrane, respectively. Saponins glycosylated at either C-3 or C-28 are termed monodesmosides, and those glycosylated at both C-3 and C-28 are termed bisdesmosides. These types of triterpene saponins exhibit diverse activities, which may be attributable to the different substructures in the A-, C-, E-rings or other positions. Many comprehensive reviews of two type triterpenoids have been published covering different areas of interest, such as isolation and structure determination (Joseph, 1999; 2000; 2001a; 2001b; 2002; 2003; 2005a; 2005b), and pharmacological activities (Liu, 1995; Safayhi et al., 1997; Setzer and Setzer, 2003; Rios et al., 2000; Baglin et al., 2003), but reviews of structure-activity relationships are scarce. In this review, our discussion will focus mainly on the chemistry and pharmacology of oleanane- and ursane-type triterpenoids discovered in the past ten years, with an emphasis on the relationships between their structures and activities. These triterpenoids are often mentioned simultaneously because they share similar structural features and pharmacological activities. In addition, other pentacyclic triterpenoids such as those of the lupane type are often cited in order to compare their structures and activities with those of oleanane and ursane type triterpenoids.



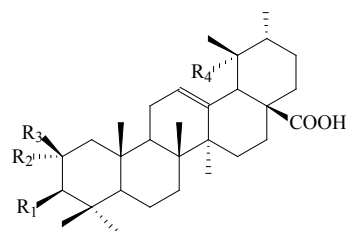
## ANTITUMOR ACTIVITIES

**UA** has been reported to be effective at different stages for tumor prevention and inhibition, e.g. inhibiting tumorigenesis (Huang et al., 1994), differentiation (Lee et al., 1994), and promotion (Tokuda et al., 1986). Moreover, **UA** and **OA** have exhibited potent activity against human leukemia and lymphoma cells. For example, **UA** was effective against P3HR1 cells ( $IC_{50}$ =2.5  $\mu$ g/mL) and chronic myelogenous leukemia cells K562 ( $IC_{50}$ =17.8  $\mu$ g/mL), while **OA** inhibited the growth of P3HR1 cells ( $IC_{50}$ =26.74  $\mu$ g/mL) (Chiang et al., 2003). They also showed anti-angiogenic activities, with **UA** ( $IC_{50}$ =5  $\mu$ M) found more active than **OA** ( $IC_{50}$ =20  $\mu$ M) (Sohn et al., 1995). Bioassay-guided fractionation of *Polylepis racemosa* led to the isolation of four cytostatic

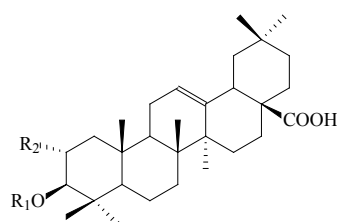
triterpenoids **UA** and **1-3** ( $GI_{50}$ =6.9~>250  $\mu$ g/mL), in which the 19-OH substituted compound **1** was the most active ( $GI_{50}$ =6.9~25  $\mu$ g/mL) (Neto et al., 2000).

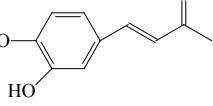
Six triterpenes **UA**, **OA** and **4-7** were isolated from stem bark of *Physocarpus intermedius*, and their  $ED_{50}$  values against five different tumor cells are shown in Table 1 (Kim et al., 2000). While introduction of 2 $\alpha$ -OH substituents showed almost no influence on their activity, 9 $\alpha$ -OH in the **UA** series was detrimental to activity. Coffemoylation of 3 $\beta$ -OH enhanced antitumor activity by several times.

Compound **6**, Corosolic acid, has also been isolated from the fruit of *Crataegus pinnatifida* var. *psilosa*. It displayed both cytotoxicity and PKC inhibition. The  $ED_{50}$  values of **6** and **UA** are shown in Table 2. Both compounds were selectively more potent against solid cancer cells HeLa S<sub>3</sub> and SNU C<sub>4</sub>. Besides, it incompletely inhibited rat brain PKC activity *in vitro* by concentrations higher than 20  $\mu$ g/mL (Ahn et al., 1998).

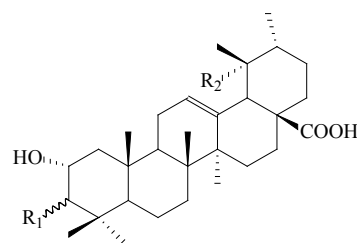


- 1** R<sub>1</sub>=OH R<sub>2</sub>=H R<sub>3</sub>=H R<sub>4</sub>=OH  
**2** R<sub>1</sub>=OAc R<sub>2</sub>=H R<sub>3</sub>=H R<sub>4</sub>=OH  
**3** R<sub>1</sub>=OH R<sub>2</sub>=R<sub>3</sub>=O R<sub>4</sub>=OH



- 4** R<sub>1</sub>= HO- R<sub>2</sub>=H

- 7** R<sub>1</sub>=H R<sub>2</sub>=OH



- 5** R<sub>1</sub>= $\beta$ -OH R<sub>2</sub>=OH  
**6** R<sub>1</sub>= $\alpha$ -OH R<sub>2</sub>=H

**Table 1.** Inhibition ED<sub>50</sub> (μg/mL) of tumor cell proliferation with UA, OA and 4-7.

|           | A549 | SK-OV-3 | SK-MEL-3 | XF498 | HCT15 |
|-----------|------|---------|----------|-------|-------|
| UA        | 4.2  | 3.6     | 4.6      | 4.5   | 4.4   |
| OA        | 16.4 | 12.4    | 18.5     | >30   | 12.1  |
| 4         | 1.6  | 1.6     | 1.7      | 19.8  | 1.7   |
| 5         | >30  | 18.4    | >30      | 30    | >30   |
| 6         | 4.4  | 3.9     | 5.1      | 5.5   | 4.7   |
| 7         | 19.4 | 18.4    | 19.8     | >30   | 15.3  |
| Cisplatin | 1.4  | 0.9     | 0.8      | 0.9   | 2.2   |

A549: non small cell lung; SK-OV-3: ovary; SK-MEL-3: melanoma; XF498: central nerve system; HCT15: colon; Cisplatin was positive control.

**Table 3.** Cytotoxic activity was determined by the MTT.

|        | CC <sub>50</sub> (μg/mL) |     |      |
|--------|--------------------------|-----|------|
|        | HSC-2                    | HSG | HGF  |
| UA     | 29                       | 48  | 25   |
| OA     | 130                      | 230 | >500 |
| 6      | 10                       | 12  | 12   |
| 7      | 21                       | 26  | 24   |
| 13     | 21                       | 25  | 24   |
| 14     | 102                      | 148 | 184  |
| 15, 16 | 22                       | 30  | 50   |

HSC-2: human oral squamous cell carcinoma; HSG: human salivary gland tumor; HGF: human normal gingival fibroblasts.

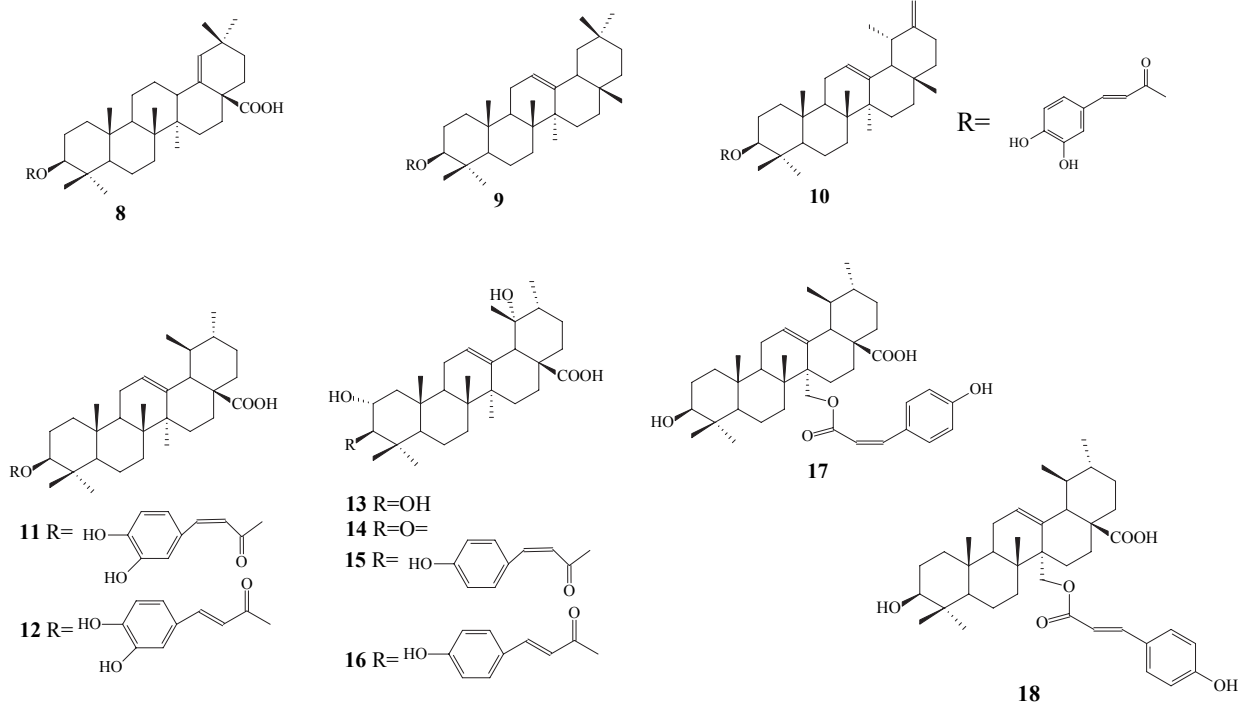
**Table 2.** ED<sub>50</sub> (μg/mL) of corosolic acid and UA against tumor cells.

|    | Hep G <sub>2</sub> | SNU-C <sub>4</sub> | HeLa S <sub>3</sub> | K-562 |
|----|--------------------|--------------------|---------------------|-------|
| 6  | 4.8                | 0.4                | 1.0                 | 4.3   |
| UA | 3.0                | 1.4                | 1.5                 | 12.5  |

Hep G<sub>2</sub>: human hepatocellular carcinoma; SNU-C<sub>4</sub>: human colorectal cancer; HeLa S<sub>3</sub>: human cervix carcinoma.

It is interesting to note that the cinnamoyl substituent can be found in oleanane and ursane type triterpenoids that exhibit antitumor activity. Compounds **4**, **8**, **9**, and **10** were reported to inhibit both free radical and cyclooxygenase I activity (IC<sub>50</sub>=0.9~4.6 μg/mL) (Hamburger et al., 2003). Cis-3-O-*p*-hydroxycinnamoyl ursolic acid (**11**) (GI<sub>50</sub>=18.8~46.4 μM) was slightly more effective than its trans-isomer (**12**) (GI<sub>50</sub>=40~25-100 μM) in inhibiting tumor growth against nine different tumor cell lines (Murphy et al., 2003).

However, the presence of the cinnamoyl segment might not always increase antitumor activity. Callus cultures induced from an axenic leaf of *Eriobotrya japonica* (Rosaceae) produced nine triterpenes **OA**, **UA**, **6**, **7**, **13**, **14**, **15** and **16**. All of these triterpenes exhibited significant activity in terms of CC<sub>50</sub> against HSC-2, HSG and HGF (Table 3), but the activity of the mixture of cinnamoyl esters **15** and **16** was weaker than either non esterified **6** or **13** (Taniguchi et al., 2002). Moreover, when **UA**, 27-*p*-Z-coumaroyloxy UA (**17**) and 27-*p*-E-coumaroyloxy UA (**18**) were isolated through a bioactivity-guided fraction from aerial parts of *Viburnum juncundum* Morton, only **UA** exhibited cytotoxic activity with ED<sub>50</sub> values of ca. 3 μg/mL against HCT-15, UIISO-SQC-1 and OVCAR-5 (Rios et al., 2001).



Due to its ability to repair damaged DNA (Narayan and Wilso, 1996), DNA polymerase is a potential target for adjuvant antitumor therapy, i.e., selective inhibition of this enzyme by other noncytotoxic agents could possibly potentiate chemotherapeutic effects of other DNA-damaging agents. **UA** at 100  $\mu\text{M}$  was tested for inhibitions of calf DNA polymerase  $\alpha$ , rat DNA polymerase  $\beta$ , plant DNA polymerase I ( $\alpha$ -like), and DNA polymerase II ( $\beta$ -like). The percent inhibitions were 92%, 86%, 0%, and 9%, respectively (Mizushina et al., 2000). Four triterpenoids (**OA**, **UA**, **19** and **20**) were isolated from *Baeckea gunniana*, and all showed inhibition of DNA polymerase  $\beta$  ( $\text{IC}_{50}$ =2.5~4.8  $\mu\text{M}$ ). Because **19** and **20** displayed slightly more potent inhibitory activity than that of **UA** and **OA**, it is proposed that the exocyclic double bond on their E-ring might contribute to this improvement (Deng et al., 1999).

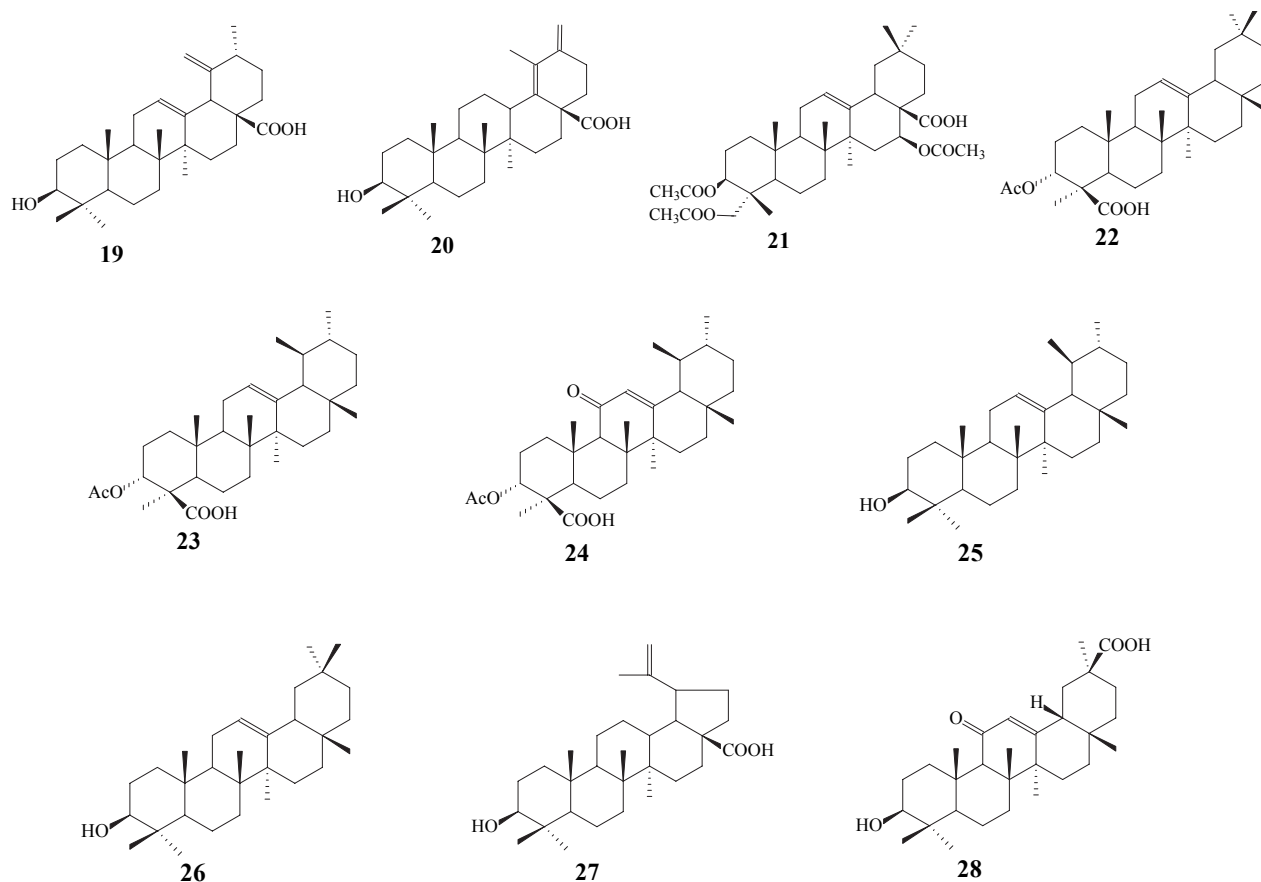
A new polyacylated oleanane triterpene **21** and **OA** were isolated from *Couepia polyandra*. They inhibited the lyase activity of DNA polymerase  $\beta$ , with  $\text{IC}_{50}$  values of 13.0 and 8.8  $\mu\text{M}$ , respectively (Chaturvedula et al., 2003a).

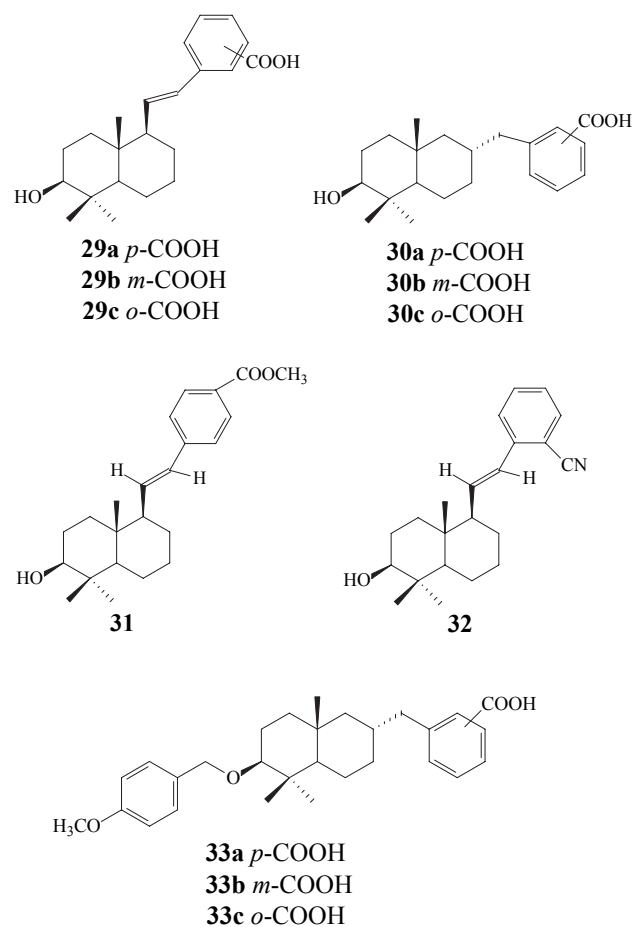
Three acetyl-boswellic acid analogues **22**, **23** and **24** were investigated for their topoisomerase inhibitory activity, and the relative activity **22**>**23**>**24** was observed. The  $\text{IC}_{50}$  values for the inhibition of topoisomerase I and II $\alpha$  catalytic activities by **22** were 3  $\mu\text{M}$  and 1  $\mu\text{M}$ , respectively. Other structurally related pentacyclic

triterpenes, —including **OA**, **UA**,  $\alpha$ -amyrin (**25**),  $\beta$ -amyrin (**26**), betulinic acid (**27**), and 18- $\beta$ -glycyrrhetic acid (**28**), —were tested for both types of topoisomerase inhibition. However, neither the amyrin isoforms nor **28** had significant effects at the concentration range observed for **22**. Of all the compounds, **27** was the most effective with  $\text{IC}_{50}$  values of 43  $\mu\text{M}$  and 5  $\mu\text{M}$  for topoisomerase I and II $\alpha$ , respectively. An analysis of their structural features concluded that the shared pentacyclic ring architecture was important but not sufficient for the inhibition of topoisomerases. Moreover, the combination of carboxyl group at C-4 and two methyl groups at C-20 were important for enhancing the inhibitory activity of the molecule toward both topoisomerases (Syrovets et al., 2000).

A/B-ring partial analogues (**29**–**33**) of **OA** were enantioselectively synthesized. These compounds showed cytotoxicity against human malignant melanoma cell SK-MEL ( $\text{IC}_{50}$ =112~484  $\mu\text{M}$ ), except for **31**, which was not active (Assefa et al., 2001). Some partial analogous e.g. **32** and **33** had activity almost comparable to **OA**.

Triterpene saponins have been extensively explored as antitumor agents. Pentacyclic triterpene glycosides have been reported to be active against various tumors. Considering the diverse structures of both aglycones and attached sugars among various species, one can expect that they will play important roles in anticancer drug discovery.



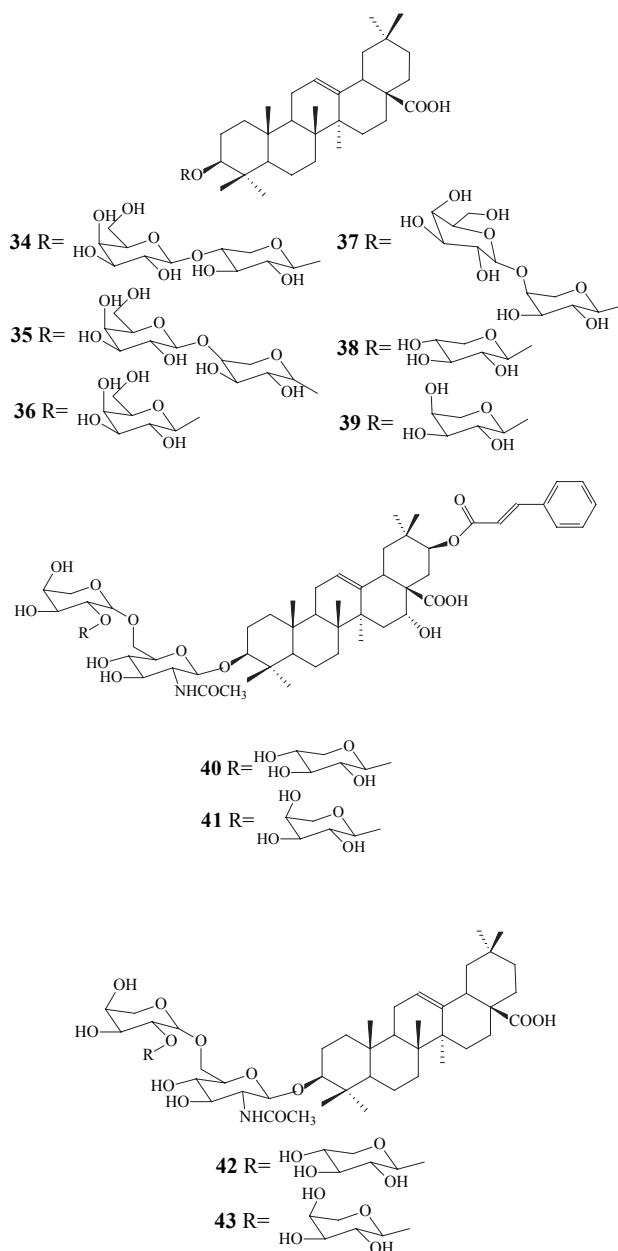


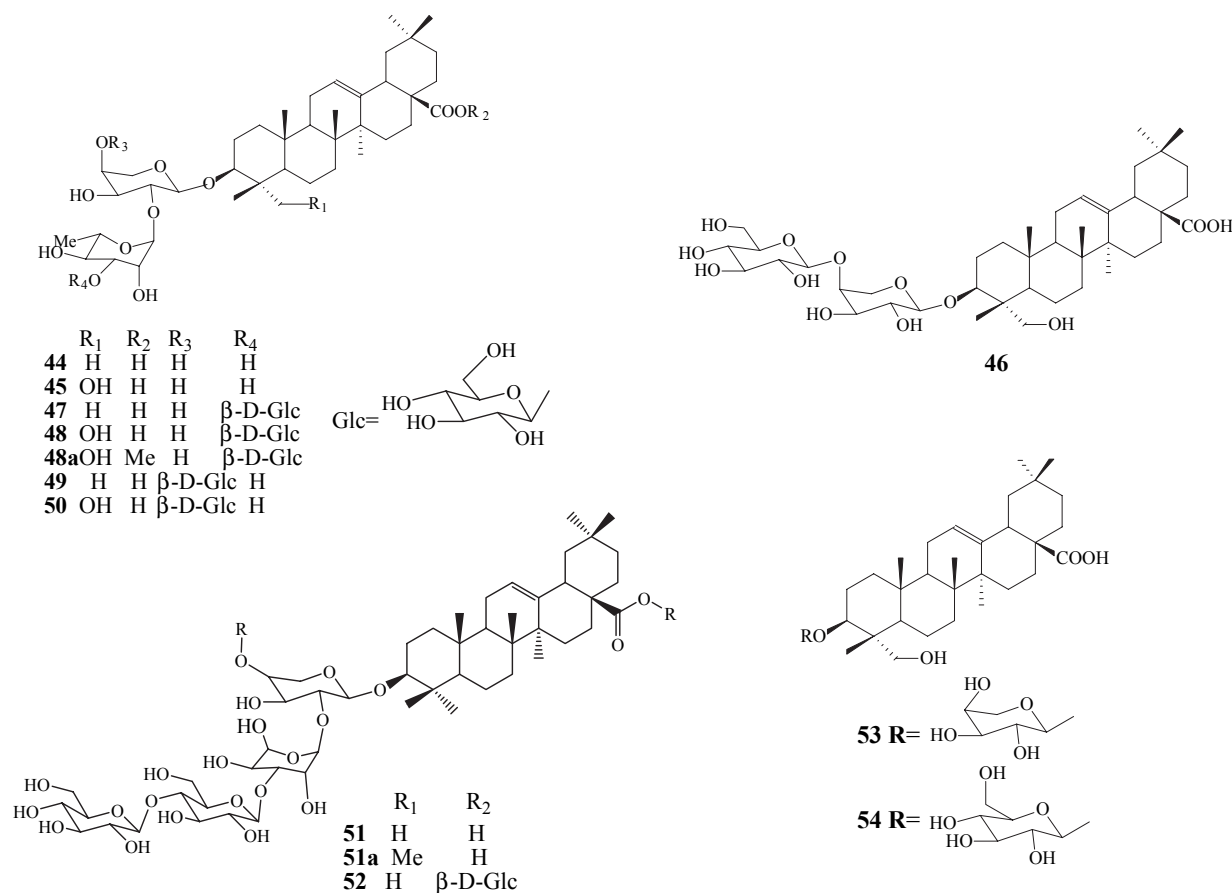
Triterpene saponins **34–37** from *Polyscias amplifolia*, together with two monoglycosides **38** and **39** formed by the partial hydrolysis of **34** and **35**, exhibited cytotoxicity against A2780 ovarian cancer cells (Table 4) (Chaturvedula et al., 2003b). The isolated cinnamoylated compounds **40** ( $IC_{50}$ =13.5  $\mu$ g/mL) and **41** ( $IC_{50}$ =13.0  $\mu$ g/mL) from *Acacia tenuifolia* also showed weak activity against A2780 ovarian cancer. Moreover, saponins **42** and **43**, which possess unique aminosugar moieties, were isolated along with **40** and **41**. These exhibited significant activity against the M109 lung cancer cell line, with  $IC_{50}$  values of 1  $\mu$ M (Seo et al., 2002). Compound **43** was synthesized and tested for cytotoxic activity against the A2780 and M109 lung cancer cell lines with the  $IC_{50}$  0.8 and 1.0  $\mu$ g/mL, respectively (Sun et al., 2003). The above results clearly indicated the importance of 3-O-aminosugar, and the activity was compromised by the presence of C-16 and C-21 substituents in **40** and **41**, as compared to **43**.

Nine triterpenes (**44–52**) containing OA or hederagenin aglycones were isolated from the roots of *Pulsatilla chinensis* and tested for their cytotoxic activity against HL-60 human leukemia cells ( $IC_{50}$ =2.3~>10  $\mu$ g/mL). Compound **46** was the only one that differed in C-3 sugar substituents in these saponins and no activity was detected. The results suggested that the glycoside

**Table 4.** Cytotoxicity against A2780 of compounds **35–43**.

|           | $IC_{50}$ ( $\mu$ g/mL) |
|-----------|-------------------------|
| <b>OA</b> | 20.4                    |
| <b>35</b> | 6.7                     |
| <b>36</b> | 9.2                     |
| <b>37</b> | 10.8                    |
| <b>38</b> | 9.6                     |
| <b>39</b> | 8.9                     |
| <b>40</b> | 8.6                     |
| <b>41</b> | 13.5                    |
| <b>42</b> | 13.0                    |
| <b>43</b> | 0.8                     |





moiety attached to C-3 of the aglycone was essential for cytotoxicity (Mimaki et al., 1999). In addition, there was no significant difference in activity between the OA-based and hederagenin-based saponins. The presence of a C-28 methyl ester imposed no influence on the activity.

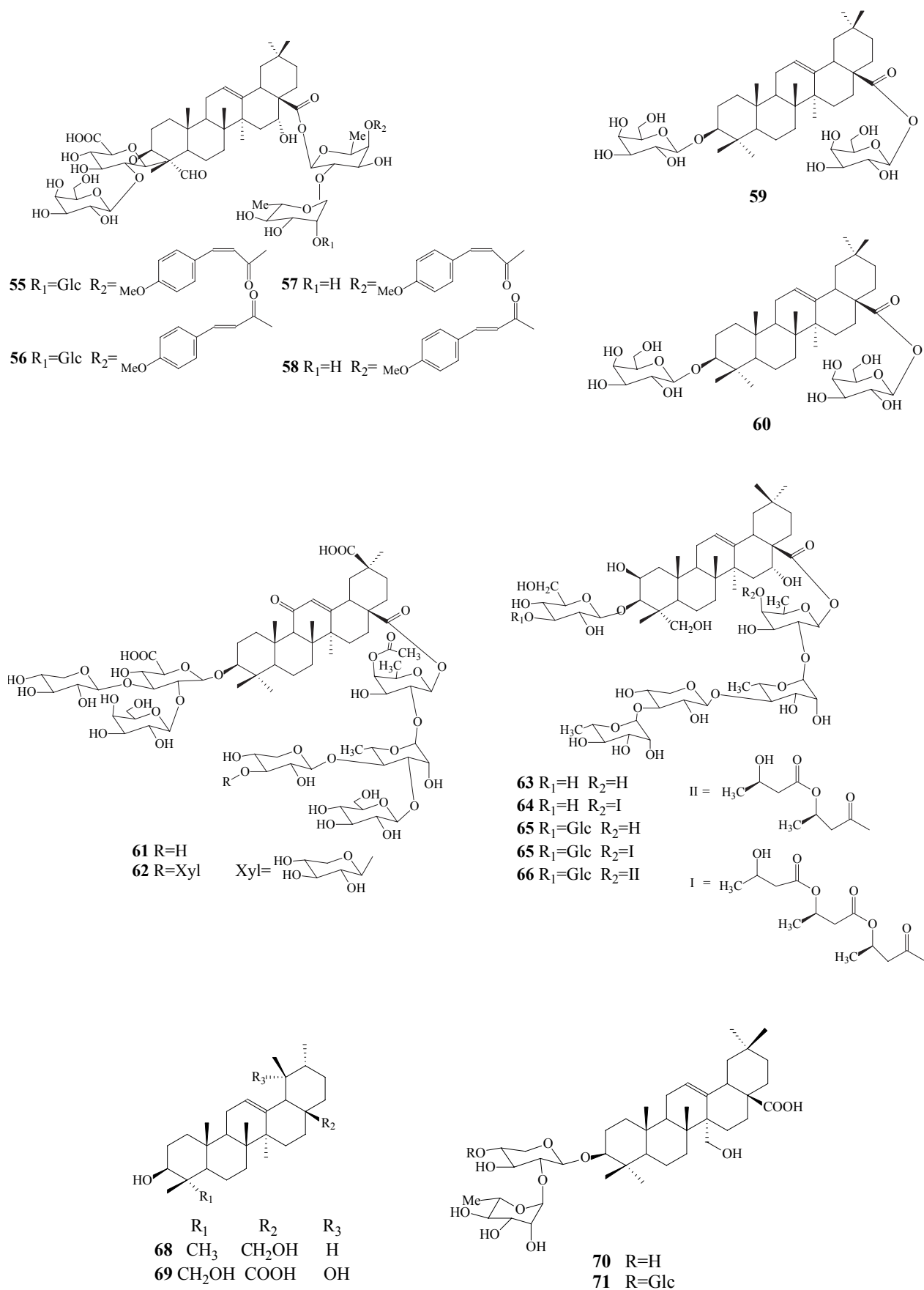
Compound **49** (Hederacolchisid A<sub>1</sub>) was also isolated from *Hedera colchica* K. Koch, and exhibited moderate *in vitro* antiproliferative activities against six human cell lines as well as normal human fibroblasts. Comparison of the cytotoxicity of **49** (IC<sub>50</sub> 4.5~12 μM) with **44** (IC<sub>50</sub> 9~15 μM), **45** (IC<sub>50</sub> 24~36 μM), **50** (IC<sub>50</sub> 24~32 μM), **53** (IC<sub>50</sub> 18~41 μM), and **54** (IC<sub>50</sub> 26~47 μM) from the same plant offers some new information about structure-activity relationships. The sugar sequence OA-3-O-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranoside at C-3 appears essential for antitumor activity of OA monodesmosides. Unlike the previous results (Mimaki et al., 1999), monodesmosides with **OA** as aglycone were more active in this study than those with hederagenin (Barthomeuf et al., 2002).

A study of the synergistic effect of saponins on an additional anticancer drug cisplatin showed that the triterpene saponins jennisenosides A, B, C, and D (**55-58**) increased the accumulation and cytotoxicity of the anticancer agent cisplatin in human colon tumor cells. In contrast, the saponin **59** without the acyl moiety (trans- or cis-*p*-methoxycinnamoyl acid) attached to sugar did

not exert such an effect (Gaidi et al., 2002). This agrees with recent findings that acylating groups might be crucial substructures due to their assumed pore-forming capacity. Saponins **60-67** were tested for any observed difference in the cytotoxic activity between acylated and non-acylated states. It was concluded that the number and structure of sugar chains did not play an important role in their cytotoxic properties, and that acylated saponins were more toxic than non-acylated ones (Barbato et al., 1997).

Superoxide generation can cause DNA damage and thereby initiate tumor-genesis (Gerhäuser et al., 2003). Five compounds (**UA**, **1**, **25**, **68**, **69**) isolated from *Diospyros kaki* were tested for stimulus-induced superoxide generation. Compound **1** inhibited fMLP, PMA and AA-induced superoxide generation more effectively than its 24-hydroxy derivative **69**. The hydroxyl group at R<sub>3</sub> and carboxyl group at R<sub>2</sub> appear to decrease superoxide generating activity by the three stimuli (Chen et al., 2002).

Moreover, the effects of six compounds (**OA**, OA 3-acetate, **44**, **49**, **70**, **71**) isolated from *Anemone raddeana* on fMLP, PMA, and AA-induced superoxide generations were investigated. A methyl group at C-14 caused stronger suppression of the fMLP-induced superoxide generation than a hydroxymethyl at this position. Diglycoside **70** more strongly suppressed PMA and AA-induced superoxide generation than triglycoside **71** (Lu et al., 2001).



Inhibitors of nitric oxide (NO) production in macrophages are potential cancer chemopreventive and anti-inflammatory drugs (Ohshima and Bartsch, 1994). Gribble et al. synthesized a series of **OA** and **UA** derivatives as inhibitors of NO production induced by interferon- $\gamma$  in mouse macrophages (Honda et al., 1997; 1999; 2000a, b and 2002). Some structures and activities were summarized as shown below in Figure 1 and Tables 5-7.

Generally, (1) oleanane and ursane triterpenoids with various enone functionalities in A-ring showed stronger activity. More specifically, a 1-en-3-one functionality in A-ring was important for significant activity (Table 5). (2) Carboxyl, methoxycarbonyl, and nitrile groups at

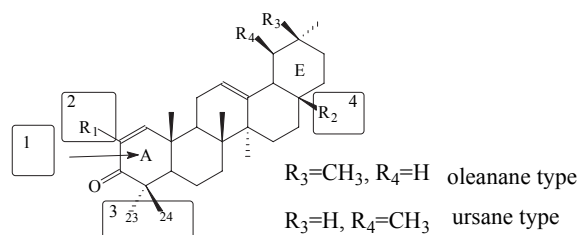
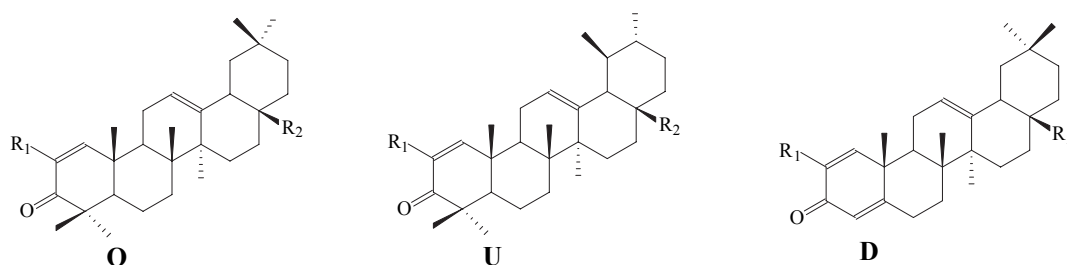


**Figure 1.** Structure-activity relationships of 1-en-3-one derivatives of oleanane triterpenoids.

**Table 5.** NO inhibitory activities of olean- and urs-12-ene triterpenoids with various 1-en-3-one functionalities.



|    | Type | R <sub>1</sub>     | R <sub>2</sub>     | IC <sub>50</sub> (μM) |
|----|------|--------------------|--------------------|-----------------------|
| 72 | O    | H                  | CO <sub>2</sub> Me | 31                    |
| 73 | O    | H                  | CO <sub>2</sub> H  | 5.6                   |
| 74 | D    | H                  | CO <sub>2</sub> Me | >40                   |
| 75 | D    | H                  | CO <sub>2</sub> H  | 13                    |
| 76 | U    | H                  | CO <sub>2</sub> H  | 13                    |
| 77 | O    | OH                 | CO <sub>2</sub> H  | 27                    |
| 78 | O    | CONH <sub>2</sub>  | CO <sub>2</sub> Me | 14                    |
| 79 | O    | OMe                | CO <sub>2</sub> H  | 30                    |
| 80 | O    | CO <sub>2</sub> Me | CO <sub>2</sub> Me | 0.9                   |
| 81 | O    | CO <sub>2</sub> Me | CO <sub>2</sub> H  | 2.2                   |
| 82 | O    | CO <sub>2</sub> H  | CO <sub>2</sub> Me | 0.8                   |
| 83 | O    | CO <sub>2</sub> H  | CO <sub>2</sub> H  | 0.07                  |
| 84 | O    | CHO                | CO <sub>2</sub> Me | Toxic                 |
| 85 | O    | CHO                | CO <sub>2</sub> H  | Toxic                 |
| 86 | O    | Br                 | CO <sub>2</sub> Me | >40                   |
| 87 | O    | Br                 | CO <sub>2</sub> H  | 7.3                   |
| 88 | O    | Cl                 | CO <sub>2</sub> Me | >40                   |
| 89 | O    | Cl                 | CO <sub>2</sub> H  | >40                   |
| 90 | O    | CN                 | CO <sub>2</sub> Me | 0.7                   |
| 91 | O    | CN                 | CO <sub>2</sub> H  | 0.6                   |
| 92 | U    | CN                 | CO <sub>2</sub> Me | 5.1                   |
| 93 | U    | CN                 | CO <sub>2</sub> H  | 6.2                   |
| OA |      |                    |                    | >40                   |
| UA |      |                    |                    | Toxic                 |

C-2 enhanced activity, while hydroxyl, aminocarbonyl, methoxy, chloride, and bromide groups decreased it. A formyl group did not confer activity but only toxicity. (3) 23, 24-Dimethyl groups were also important for significant activity, as **73** was more potent than 23, 24-dinorolean-1-en-3-one derivative **75**. (4) The effect of a free acid or ester form at C-28 was ambiguous. For some analogues, triterpenoids bearing C-28 the carboxyl group were more potent than C-28 methyl; esters, but for others similar activity or even less potent activities were observed when C-28 was carboxylic acid. (5) The oleanane skeleton was usually more potent than the ursane skeleton.

The SARs of modified ring-C oleanane and ursane triterpenoids with  $\Delta^{12}$  functionality (Table 6) were also summarized as follows:

(1) A 9(11)-en-12-one functionality in ring C could enhance activity about 10-100 times; (2) 12-en-11-one, 13(18)-en-11-one, and 12-one functionalities also enhanced potency, and a 9(11)-ene functionality showed similar potency to the original 12-ene; (3) The saturated ring C, 11, 13(18)-diene, and 9, 11-epoxide were less potent than the original 12-ene; as indicated in compounds **110-112**. (4) The combination of a 9(11)-en-12-one functionality with nitrile and carboxyl groups at C-2 enhanced the potency in a synergistic way; Triterpenoids such as **115**, **116** and **119** ( $IC_{50}=0.1$  nM level) were about 10,000 times more potent than **73**. (5) The combination of a 9(11)-en-12-one functionality with amide and formyl groups at C-2 did not enhance potency as strongly as a nitrile or carboxyl group; (6) The combination of a 12-en-11-one and 13(18)-en-11-one functionalities with nitrile group at C-2 also strongly enhanced the potency as 9(11)-en-12-one series by two orders of magnitude.

CDDO (**116**) showed almost the strongest NO inhibitory activity (Table 6) among those synthetic derivatives of **OA**. Moreover, it could also inhibit proliferation of many tumor lines at sub M level and induced cell differentiation of human myeloid leukemia (Suh et al., 2000).

Based on structures of highly active triterpenoids **CDDO**, **115** and **119** (Table 6), a series of oleanane triterpenoids (**123-148**) with various substituents at the C-28 were synthesized (Table 7). Some important SARs regarding substituents at C-17 were also provided:

(1) Nitrile group enhanced potency and ester moieties decreased potency. The less polar the ester, the less was its potency. (2) The carbonyl pyrazole was much less potent than that of 28-COOH, while carbonyl imidazole had higher potency, which might have arisen from high reactivity of imidazole with nucleophiles.

Moreover, many tricyclic compounds (**149-157**), as simplified **CDDO** with same A, B, and C ring architecture, were synthesized and tested for NOS inhibitory activity, the  $IC_{50}$  values were between 0.002 and 1.6  $\mu$ M, in which ( $\pm$ )-**80** showed the most potential ( $IC_{50}=0.002$   $\mu$ M). It was found that the most active compound **157** was only about one-fourth as potent as **CDDO**, so a nitrile group at this position enhanced potency among the typical electron-withdrawing groups such as in compounds **154** and **156** at C-13. Since **151** and **152** were more potent than **149** and **150**, the bis-enone structure for high potency in relatively small molecules is important (Favaloro et al., 2002).

## ANTI-VIRAL ACTIVITIES

Saponins **158** and **159** were isolated from *Gymnoladus chinensis* (Nakashima et al., 1989) and *Gleditsia japonica*, respectively. Both compounds exhibited potent anti-HIV effects against HIV replication in H-9 cells. Their derivatives (**160-173**) were prepared and evaluated for anti-HIV activity (Table 8). The monoterpenyl moieties in **158** and **159** were found to play an important role in modulating the anti-HIV activity of these compounds. In addition, methylation of 28-COOH increased activity while introduction of *n*-butyryl or valeryl groups to the C-3 and/or C-16 hydroxy group decreased it compared to that of **167** (Konoshima et al., 1995).

A series of natural and semi-synthetic **OA**, **UA** and betulinic acid (**27**) derivatives have been isolated (Ma et al., 2002; Kashiwada et al., 1998) or synthesized. Some relationships of anti-HIV activities and structures were summarized in Table 9.

(1) Esters especially dicarboxylic acid hemiesters of 3-OH in **OA**, tended to increase inhibitory activity (Ma et al., 1999). For the 3-acyl chains within five carbons, the HIV-1 PR inhibitory activity of the compounds increased as the lengths of the 3-acyl chains increased (Ma et al., 2000). (2) Methylation of the 28-COOH or the carboxyl

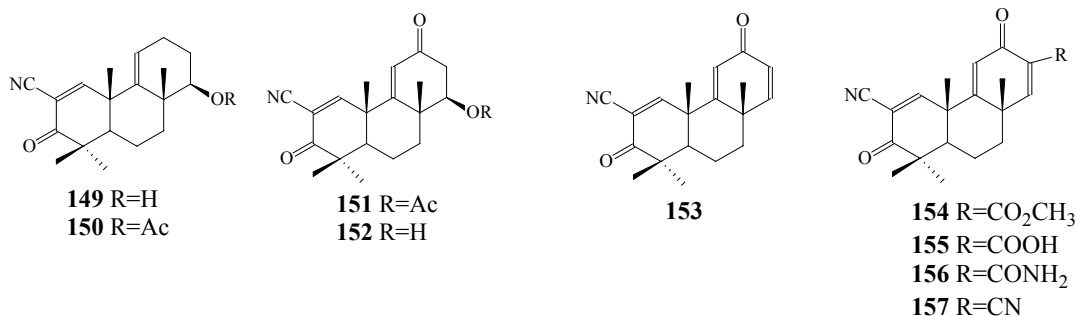


**Table 6.** NO inhibitory activity of olean-1-en-3-one and urs-1-en-3-one triterpenoids with various C-ring functionalities.

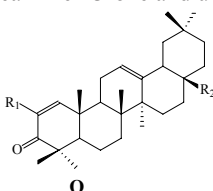
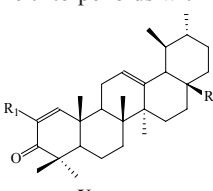
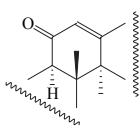
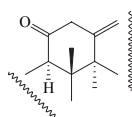
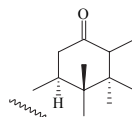
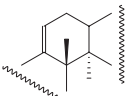
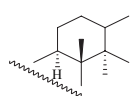
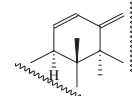
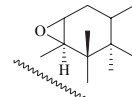
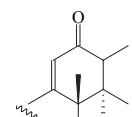
| <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;">  <p><b>O</b></p> </div> <div style="text-align: center;">  <p><b>U</b></p> </div> </div> |      |                                                                                     |                       |                        |                       |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|-------------------------------------------------------------------------------------|-----------------------|------------------------|-----------------------|
|                                                                                                                                                                                                                                                                                                                                                                                | Type | Structure of ring C                                                                 | R <sub>1</sub> at C-2 | R <sub>2</sub> at C-17 | IC <sub>50</sub> (μM) |
| 94                                                                                                                                                                                                                                                                                                                                                                             | O    |    | H                     | CO <sub>2</sub> Me     | 2.8                   |
| 95                                                                                                                                                                                                                                                                                                                                                                             | O    |                                                                                     | H                     | CO <sub>2</sub> H      | 1.1                   |
| 96                                                                                                                                                                                                                                                                                                                                                                             | U    |                                                                                     | H                     | CO <sub>2</sub> Me     | 8.9                   |
| 97                                                                                                                                                                                                                                                                                                                                                                             | U    |                                                                                     | H                     | CO <sub>2</sub> H      | 5.1                   |
| 98                                                                                                                                                                                                                                                                                                                                                                             | O    |                                                                                     | H                     | CH <sub>2</sub> OAc    | >40                   |
| 99                                                                                                                                                                                                                                                                                                                                                                             | O    |                                                                                     | H                     | CH <sub>2</sub> OH     | 3.0                   |
| 100                                                                                                                                                                                                                                                                                                                                                                            | O    |                                                                                     | H                     | CHO                    | 3.8                   |
| 101                                                                                                                                                                                                                                                                                                                                                                            | O    |                                                                                     | CN                    | CO <sub>2</sub> Me     | 0.02                  |
| 102                                                                                                                                                                                                                                                                                                                                                                            | O    |                                                                                     | CN                    | CO <sub>2</sub> H      | 0.04                  |
| 103                                                                                                                                                                                                                                                                                                                                                                            | U    |                                                                                     | CN                    | CO <sub>2</sub> Me     | 0.1                   |
| 104                                                                                                                                                                                                                                                                                                                                                                            | U    |                                                                                     | CN                    | CO <sub>2</sub> H      | 0.8                   |
| 105                                                                                                                                                                                                                                                                                                                                                                            | O    |    | H                     | CO <sub>2</sub> H      | 2.6                   |
| 106                                                                                                                                                                                                                                                                                                                                                                            | O    |                                                                                     | CN                    | CO <sub>2</sub> H      | 0.07                  |
| 107                                                                                                                                                                                                                                                                                                                                                                            | O    |  | H                     | CO <sub>2</sub> Me     | 14                    |
| 108                                                                                                                                                                                                                                                                                                                                                                            | O    |                                                                                     | H                     | CO <sub>2</sub> H      | 3.3                   |
| 109                                                                                                                                                                                                                                                                                                                                                                            | O    |  | H                     | CO <sub>2</sub> H      | 5.2                   |
| 110                                                                                                                                                                                                                                                                                                                                                                            | O    |  | H                     | CO <sub>2</sub> H      | 8.5                   |
| 111                                                                                                                                                                                                                                                                                                                                                                            | O    |  | H                     | CO <sub>2</sub> H      | 9.7                   |
| 112                                                                                                                                                                                                                                                                                                                                                                            | O    |  | H                     | CO <sub>2</sub> H      | 36                    |
| 113                                                                                                                                                                                                                                                                                                                                                                            | O    |  | H                     | CO <sub>2</sub> Me     | 0.7                   |
| 114                                                                                                                                                                                                                                                                                                                                                                            | O    |                                                                                     | H                     | CO <sub>2</sub> H      | 0.2                   |
| 115                                                                                                                                                                                                                                                                                                                                                                            | O    |                                                                                     | CN                    | CO <sub>2</sub> Me     | 0.0001                |
| 116                                                                                                                                                                                                                                                                                                                                                                            | O    |                                                                                     | CN                    | CO <sub>2</sub> H      | 0.0002                |
| 117                                                                                                                                                                                                                                                                                                                                                                            | O    |                                                                                     | CO <sub>2</sub> Me    | CO <sub>2</sub> Me     | Toxic                 |
| 118                                                                                                                                                                                                                                                                                                                                                                            | O    |                                                                                     | CO <sub>2</sub> Me    | CO <sub>2</sub> H      | 0.1                   |
| 119                                                                                                                                                                                                                                                                                                                                                                            | O    |                                                                                     | CO <sub>2</sub> H     | CO <sub>2</sub> Me     | 0.0008                |
| 120                                                                                                                                                                                                                                                                                                                                                                            | O    |                                                                                     | CO <sub>2</sub> H     | CO <sub>2</sub> H      | 0.2                   |
| 121                                                                                                                                                                                                                                                                                                                                                                            | O    |                                                                                     | CONH <sub>2</sub>     | CO <sub>2</sub> Me     | 0.1                   |
| 122                                                                                                                                                                                                                                                                                                                                                                            | O    |                                                                                     | CHO                   | CO <sub>2</sub> Me     | 0.1                   |

**Table 7.** NO inhibitory activities of compounds **123-148**.

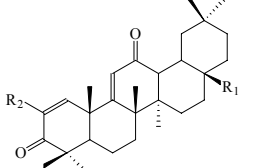
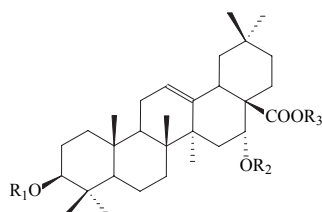
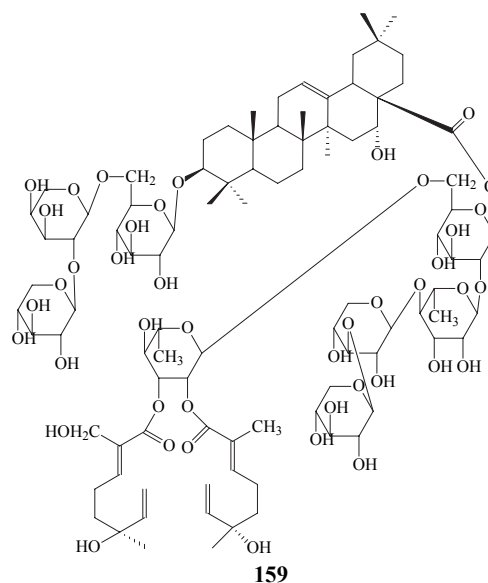
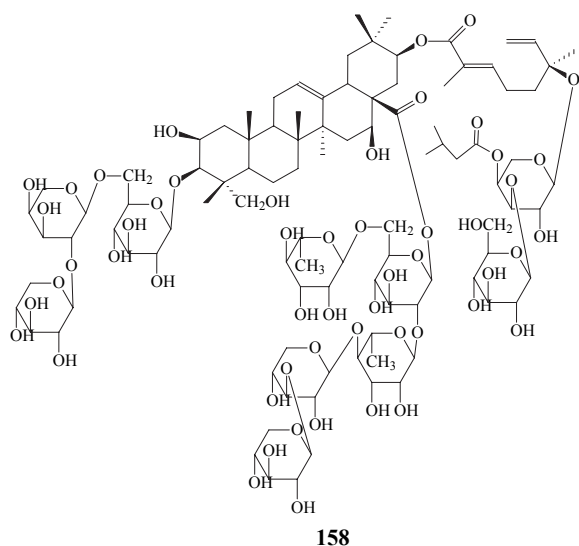
|  |                                                                               |                   |                       |
|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------|-------------------|-----------------------|
|                                                                                   | R <sub>1</sub>                                                                | R <sub>2</sub>    | IC <sub>50</sub> (μM) |
| <b>123</b>                                                                        | CN                                                                            | CN                | 0.0035                |
| <b>124</b>                                                                        | CN                                                                            | CO <sub>2</sub> H | 1.68                  |
| <b>125</b>                                                                        | CO <sub>2</sub> Et                                                            | CN                | 0.80                  |
| <b>126</b>                                                                        | CO <sub>2</sub> Et                                                            | CO <sub>2</sub> H | 7.93                  |
| <b>127</b>                                                                        | CO <sub>2</sub> CH <sub>2</sub> CH=CH <sub>2</sub>                            | CN                | 1.33                  |
| <b>128</b>                                                                        | CO <sub>2</sub> (CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>               | CN                | 6.65                  |
| <b>129</b>                                                                        | CO <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> C <sub>3</sub> H <sub>5</sub> | CN                | 4.45                  |
| <b>130</b>                                                                        | CO <sub>2</sub> CH <sub>2</sub> Ph                                            | CN                | 4.35                  |
| <b>131</b>                                                                        | CO <sub>2</sub> (CH <sub>2</sub> ) <sub>7</sub> CH <sub>3</sub>               | CN                | 60.4                  |
| <b>132</b>                                                                        | CO-D-Glc(OAc) <sub>4</sub>                                                    | CN                | 0.07                  |
| <b>133</b>                                                                        | CO-D-Glc                                                                      | CN                | 10.1                  |
| <b>134</b>                                                                        | CONH <sub>2</sub>                                                             | CN                | 0.098                 |
| <b>135</b>                                                                        | CONHNH <sub>2</sub>                                                           | CN                | 0.26                  |
| <b>136</b>                                                                        | CONHMe                                                                        | CN                | 0.58                  |
| <b>137</b>                                                                        | CONH(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>                           | CN                | 1.50                  |
| <b>138</b>                                                                        | CONH(CH <sub>2</sub> ) <sub>5</sub> CH <sub>3</sub>                           | CN                | 14.9                  |
| <b>139</b>                                                                        | CONHPh                                                                        | CN                | 28.6                  |
| <b>140</b>                                                                        | CONHCH <sub>2</sub> Ph                                                        | CN                | 9.2                   |
| <b>141</b>                                                                        | CONMe <sub>2</sub>                                                            | CN                | 1.55                  |
| <b>142</b>                                                                        | CON(n-Pr) <sub>2</sub>                                                        | CN                | 32.9                  |
| <b>143</b>                                                                        | CON-C <sub>6</sub> H <sub>11</sub>                                            | CN                | 0.80                  |
| <b>144</b>                                                                        | CON-C <sub>7</sub> H <sub>13</sub>                                            | CN                | 0.95                  |
| <b>145</b>                                                                        | CON-C <sub>6</sub> H <sub>11</sub> -N-C <sub>6</sub> H <sub>11</sub>          | CN                | 1.00                  |
| <b>146</b>                                                                        | CON-C <sub>6</sub> H <sub>10</sub> O                                          | CN                | 2.4                   |
| <b>147</b>                                                                        | CON-C <sub>4</sub> H <sub>3</sub> N                                           | CN                | 0.014                 |
| <b>148</b>                                                                        | CON-C <sub>5</sub> H <sub>4</sub> N                                           | CN                | 12.0                  |

**Table 8.** HIV inhibitory effects for 158-173.

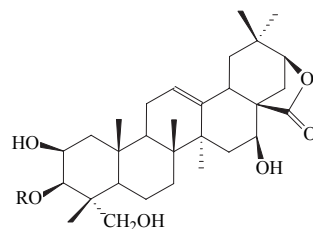
| Compd       | ED <sub>50</sub> (μM) | IC <sub>50</sub> (μM) |
|-------------|-----------------------|-----------------------|
| <b>158</b>  | 1.1                   | 9.8                   |
| <b>159</b>  | 2.7                   | 14                    |
| <b>160</b>  | >100                  | 43                    |
| <b>161</b>  | >100                  | 37                    |
| <b>161a</b> | 9.9                   | 22                    |
| <b>162</b>  | 13                    | 50                    |
| <b>163</b>  | 95                    | 63                    |
| <b>164</b>  | 100                   | >100                  |
| <b>165</b>  | 42                    | 74                    |
| <b>166</b>  | 8                     | 40                    |
| <b>167</b>  | 3.1                   | 4.9                   |
| <b>168</b>  | 5.1                   | 7.6                   |
| <b>169</b>  | 2.3                   | 13                    |
| <b>170</b>  | 27                    | 27                    |
| <b>171</b>  | 15                    | >160                  |
| <b>172</b>  | 30                    | 180                   |
| <b>173</b>  | 31                    | 54                    |

group in the C-3 hemiester chain decreased the inhibitory activity against HIV-1 PR significantly (Kashiwada et al., 1998). Methylation at both sites led to complete loss of activity, e.g. **210** (Ma et al., 1999). (3) Replacement of the 28-COOH with a methyl group resulted in a significant loss of activity (**25** vs **UA**; **26** vs **OA**) (Kashiwada et al., 1998). (4) The structure of E-ring might play an important role in anti-HIV potency. Derivatives of betulinic acid, **27**, were more potent than those of **UA** and **OA** (Kashiwada et al., 2000). (5) Saturation of the C<sub>12</sub>-C<sub>13</sub> double bond could be a major cause of anti-HIV activity enhancement (**238-240**), while a C-3 acyl side chain was essential for optimal activity. In addition, changing the C<sub>28</sub>-carboxyl to aminomethyl could significantly enhance anti-HIV activity (**241**, **242**) (Zhu et al., 2001). (6) Replacement of C-3 hemiester in the **OA** series with hemi-amide retained activity (**219-223** vs **204-205**) (Ma et al., 1999).

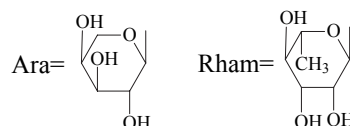
Oleanane-type triterpenoid saponins (**243-258**) were examined for the anti-herpes virus (anti-HSV-1) activity (Table 10). It has been found that (1) the trisaccharide glycosides were more potent than the disaccharide glycosides. The order of activity was **247>250>>253**. (2) The saponins having a glucosyl unit in the central sugar moiety seemed to show greater action. Among the trisaccharide group, the order of activity was **245>244>246>>243**. (3) The carbonyl group at C-22 would be more effective than the hydroxyl group in anti-HSV-1 activity, while the hydroxyl group at C-24 could reduce the activity. Comparing the activities of a group having the same trisaccharide, the order of potency was **254>247>243** (Kinjo et al., 2001).



|             |                                         |         |                        |
|-------------|-----------------------------------------|---------|------------------------|
| <b>160</b>  | Glc <sup>6</sup> -Ara <sup>2</sup> -Xyl | H       | Glc <sup>2</sup> -Rham |
| <b>161</b>  | Glc <sup>6</sup> -Ara <sup>2</sup> -Xyl | H       | H                      |
| <b>161a</b> | Glc <sup>6</sup> -Ara <sup>2</sup> -Xyl | H       | Me                     |
| <b>162</b>  | Glc <sup>6</sup> -Gra                   | H       | H                      |
| <b>163</b>  | Glc                                     | H       | H                      |
| <b>165</b>  | H                                       | H       | H                      |
| <b>167</b>  | H                                       | H       | Me                     |
| <b>168</b>  | Ac                                      | H       | Me                     |
| <b>169</b>  | Ac                                      | Ac      | H                      |
| <b>170</b>  | Butyryl                                 | H       | Me                     |
| <b>171</b>  | Butyryl                                 | Butyryl | Me                     |
| <b>172</b>  | Valeryl                                 | H       | Me                     |
| <b>173</b>  | Valeryl                                 | Valeryl | Me                     |



**164** R=Glc<sup>6</sup>-Ara<sup>2</sup>-Xyl  
**165** R=H



## ANTI-INFLAMMATORY ACTIVITIES

### In vivo studies

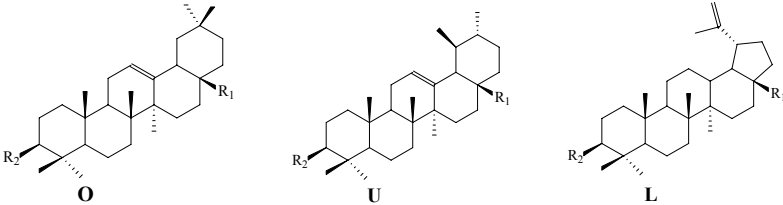
**OA** and **UA** inhibited the Croton oil-induced ear oedema in mice in a dose-dependent manner, and **UA** ( $ID_{50}=0.14 \mu\text{Moles/cm}^2$ ) was twofold more potent than **OA** ( $ID_{50}=0.36 \mu\text{Moles/cm}^2$ ) and indomethacin ( $ID_{50}=0.26 \mu\text{Moles/cm}^2$ ), which was used as a reference non-steroidal anti-inflammatory drug (NSAID) (Ismaili et al., 2001 and 2002; Baricevic et al., 2001).

The anti-inflammatory activities of ten triterpenoids [**25**, **26**, **68**, **259**, **260**, **OA**, Hederagenin (**261**), **28**,  $\alpha$ -glycyrrhetic acid (**262**), and lupeol (**263**)] were evaluated. It was found that the basic carbon skeletons had no influence on the activity; the presence of a C-28 or C-30 carboxylic group and an alcoholic group at C-28 increased the activity in carrageenan and TPA-induced edemas in mice (Recio et al., 1995).

The following mechanistic aspects of anti-inflammatory activities of **OA** and **UA** derivatives have been investigated.

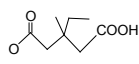
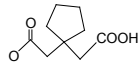
### Inhibition of cyclooxygenase (COX) activity

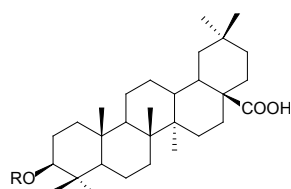
**UA** was a selective inhibitor of COX-2 catalyzed prostaglandin biosynthesis, with  $IC_{50}$  value of  $130 \mu\text{M}$  and a COX-2/COX-1 selectivity ratio of 0.6. **OA** ( $IC_{50}$   $295 \mu\text{M}$ ) was found to be less active than **UA**, but showed a similar selectivity ratio (0.8). Furthermore, no significant inhibition on COX-2 or COX-1 was observed by **28** (Ringbom et al., 1998).  $1 \mu\text{M}$  or less of **CDDO** (**116**) blocked expression of both iNOS and COX-2 protein (Syrovets et al., 2000). Compounds **247**, **260** and 3-epi-**UA** (**264**) (assayed at  $10 \mu\text{M}$ ) blocked the inductive effect of lipopolysaccharide on the production of  $\text{PGE}_2$ , while **OA** and **UA** did not substantially suppress the production of  $\text{PGE}_2$  (Suh et al., 1998). Compounds **5** and **9** strongly

**Table 9.** HIV inhibitory effects for derivatives of **OA**, **UA** and **28**.


|            | Type | R <sub>1</sub>                  | R <sub>2</sub>                                                           | IC <sub>50</sub> (μM) |
|------------|------|---------------------------------|--------------------------------------------------------------------------|-----------------------|
| <b>25</b>  | U    | CH <sub>3</sub>                 | OH                                                                       | 80                    |
| <b>UA</b>  | U    | COOH                            | OH                                                                       | 8                     |
| <b>174</b> | U    | COOCH <sub>3</sub>              | OH                                                                       | 14                    |
| <b>175</b> | U    | COOH                            | OCOCH <sub>3</sub>                                                       | 13                    |
| <b>176</b> | U    | COOH                            | OCOCOOH                                                                  | 7                     |
| <b>177</b> | U    | COOH                            | OCOCH <sub>2</sub> COOH                                                  | 6                     |
| <b>178</b> | U    | COOH                            | OCO(CH <sub>2</sub> ) <sub>2</sub> COOH                                  | 6                     |
| <b>179</b> | U    | COOH                            | OCO(CH <sub>2</sub> ) <sub>3</sub> COOH                                  | 4                     |
| <b>180</b> | U    | COOCH <sub>3</sub>              | OCO(CH <sub>2</sub> ) <sub>3</sub> COOCH <sub>3</sub>                    | >50                   |
| <b>181</b> | U    | COOH                            | OCOC(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub> COOH                 | 30.7                  |
| <b>182</b> | U    | COOH                            | OCOCH <sub>2</sub> C(CH <sub>3</sub> ) <sub>2</sub> COOH                 | 49.5                  |
| <b>183</b> | U    | COOH                            | OCOCH <sub>2</sub> C(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub> COOH | 7                     |
| <b>184</b> | U    | COOH                            | OCOCH <sub>2</sub> OCH <sub>2</sub> COOH                                 | 48.2                  |
| <b>185</b> | U    | COOH                            | OCOCH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>                     | >18.5                 |
| <b>186</b> | U    | COOH                            | OCOCH <sub>2</sub> C(CH <sub>3</sub> ) <sub>3</sub>                      | >18                   |
| <b>187</b> | U    | COO <sup>-</sup> K <sup>+</sup> | OH                                                                       | 1.8                   |
| <b>27</b>  | L    | COOH                            | OH                                                                       | 9                     |
| <b>188</b> | L    | COOCH <sub>3</sub>              | OH                                                                       | >25                   |
| <b>189</b> | L    | COOH                            | COCOOH                                                                   | 7                     |
| <b>190</b> | L    | COOH                            | OCOCH <sub>2</sub> COOH                                                  | 6                     |
| <b>191</b> | L    | COOH                            | OCO(CH <sub>2</sub> ) <sub>2</sub> COOH                                  | 6                     |
| <b>192</b> | L    | COOH                            | OCO(CH <sub>2</sub> ) <sub>3</sub> COOH                                  | 4                     |
| <b>193</b> | L    | COOH                            | OCOCH <sub>2</sub> C(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub> COOH | 4                     |
| <b>194</b> | L    | COOCH <sub>3</sub>              | OCO(CH <sub>2</sub> ) <sub>2</sub> COOCH <sub>3</sub>                    | 40                    |
| <b>195</b> | L    | COOH                            | OCOCH <sub>2</sub> C(CH <sub>3</sub> ) <sub>2</sub> COOH                 | 7.0                   |
| <b>26</b>  | O    | CH <sub>3</sub>                 | OH                                                                       | >100                  |
| <b>OA</b>  | O    | COOH                            | OH                                                                       | 8                     |
| <b>196</b> | O    | COOCH <sub>3</sub>              | OH                                                                       | 20                    |
| <b>197</b> | O    | COOH                            | OCOCH <sub>3</sub>                                                       | 9                     |
| <b>198</b> | O    | COOH                            | OCOCOOH                                                                  | 20                    |
| <b>199</b> | O    | COOH                            | OCOCH <sub>2</sub> COOH                                                  | 8                     |
| <b>200</b> | O    | COOH                            | OCO(CH <sub>2</sub> ) <sub>2</sub> COOH                                  | 4                     |
| <b>201</b> | O    | COOH                            | OCO(CH <sub>2</sub> ) <sub>3</sub> COOH                                  | 4                     |
| <b>202</b> | O    | COOCH <sub>3</sub>              | OCO(CH <sub>2</sub> ) <sub>3</sub> COOCH <sub>3</sub>                    | >50                   |
| <b>203</b> | O    | COOH                            | OCOCH <sub>2</sub> C(CH <sub>3</sub> ) <sub>2</sub> COOH                 | 19.2                  |
| <b>204</b> | O    | COOH                            | OCO(CH <sub>2</sub> ) <sub>4</sub> COOH                                  | 3.0                   |
| <b>205</b> | O    | COOCH <sub>3</sub>              | OCO(CH <sub>2</sub> ) <sub>4</sub> COOH                                  | 7.5                   |
| <b>206</b> | O    | COOH                            | OCO(CH <sub>2</sub> ) <sub>6</sub> COOH                                  | 3.0                   |

Table 9. (Continued)

|     | Type | R <sub>1</sub>                           | R <sub>2</sub>                                                                               | IC <sub>50</sub> (μM)    |
|-----|------|------------------------------------------|----------------------------------------------------------------------------------------------|--------------------------|
| 207 | O    | COOH                                     | OCO(CH <sub>2</sub> ) <sub>8</sub> COOH                                                      | 4.0                      |
| 208 | O    | COOH                                     | OCOCH <sub>2</sub> C(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub> COOH                     | 3.8                      |
| 209 | O    | COOH                                     | OCO(CH <sub>2</sub> ) <sub>4</sub> COOCH <sub>3</sub>                                        | 5.6                      |
| 210 | O    | COOCH <sub>3</sub>                       | OCO(CH <sub>2</sub> ) <sub>4</sub> COOCH <sub>3</sub>                                        | >20                      |
| 211 | O    | COOH                                     | OCO(CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub>                                           | >20                      |
| 212 | O    | COOH                                     | =O                                                                                           | 5.5                      |
| 213 | O    | COOCH <sub>3</sub>                       | =O                                                                                           | 20                       |
| 214 | O    | COOH                                     | =NOH                                                                                         | 5.5                      |
| 215 | O    | COOCH <sub>3</sub>                       | =NOH                                                                                         | 9.5                      |
| 216 | O    | COOCH <sub>3</sub>                       | α-NH                                                                                         | >20                      |
| 217 | O    | COOCH <sub>3</sub>                       | β-NH                                                                                         | >20                      |
| 218 | O    | COOH                                     | =NOCO(CH <sub>2</sub> ) <sub>4</sub> COOH                                                    | 5.5                      |
| 219 | O    | COOCH <sub>3</sub>                       | =NOCO(CH <sub>2</sub> ) <sub>4</sub> COOH                                                    | 4.0                      |
| 220 | O    | COOH                                     | β-NHCO(CH <sub>2</sub> ) <sub>4</sub> COOH                                                   | 3.0                      |
| 221 | O    | COOCH <sub>3</sub>                       | β-NHCO(CH <sub>2</sub> ) <sub>4</sub> COOH                                                   | 3.0                      |
| 222 | O    | COOH                                     | α-NHCO(CH <sub>2</sub> ) <sub>4</sub> COOH                                                   | 2.1                      |
| 223 | O    | COOCH <sub>3</sub>                       | α-NHCO(CH <sub>2</sub> ) <sub>4</sub> COOH                                                   | 3.5                      |
| 224 | O    | COOCH <sub>3</sub>                       | β-NHCO(CH <sub>2</sub> ) <sub>4</sub> CONH(CH <sub>2</sub> ) <sub>3</sub> COOH               | 6.0                      |
| 225 | O    | COOCH <sub>3</sub>                       | β-NHCO(CH <sub>2</sub> ) <sub>4</sub> CONH(CH <sub>2</sub> ) <sub>3</sub> COOCH <sub>3</sub> | >20                      |
| 226 | O    | COOH                                     | β-NHCO(CH <sub>2</sub> ) <sub>4</sub> CONH-β-OA-28-OH                                        | 3.3                      |
| 227 | O    | COOCH <sub>3</sub>                       | β-NHCO(CH <sub>2</sub> ) <sub>4</sub> CONH-β-OA-28-OCH <sub>3</sub>                          | >20                      |
| 228 | O    | CONH(CH <sub>2</sub> ) <sub>5</sub> COOH | COOH                                                                                         | 1.7                      |
| 229 | O    | CONH(CH <sub>2</sub> ) <sub>5</sub> COOH | OCO(CH <sub>2</sub> ) <sub>4</sub> COOH                                                      | 1.7                      |
|     | Type | R <sub>1</sub>                           | R <sub>2</sub>                                                                               | IC <sub>50</sub> (μg/mL) |
| 230 | O    | COOH                                     | OCOC(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub> COOH                                     | 9.7                      |
| 231 | O    | COOH                                     | OCOCH <sub>2</sub> OCH <sub>2</sub> COOH                                                     | 22.1                     |
| 232 | O    | COOH                                     | OCOCH <sub>2</sub> CH(CH <sub>3</sub> )CH <sub>2</sub> COOH                                  | 27.5                     |
| 233 | O    | COOH                                     |           | 4.4                      |
| 234 | O    | COOH                                     |           | 4.7                      |
| 235 | O    | COO <sup>-</sup> K <sup>+</sup>          | OH                                                                                           | 34.3                     |



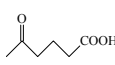
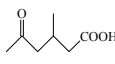
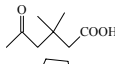
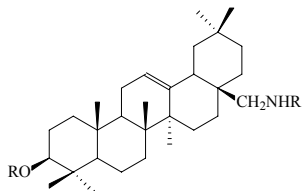
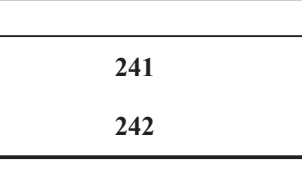
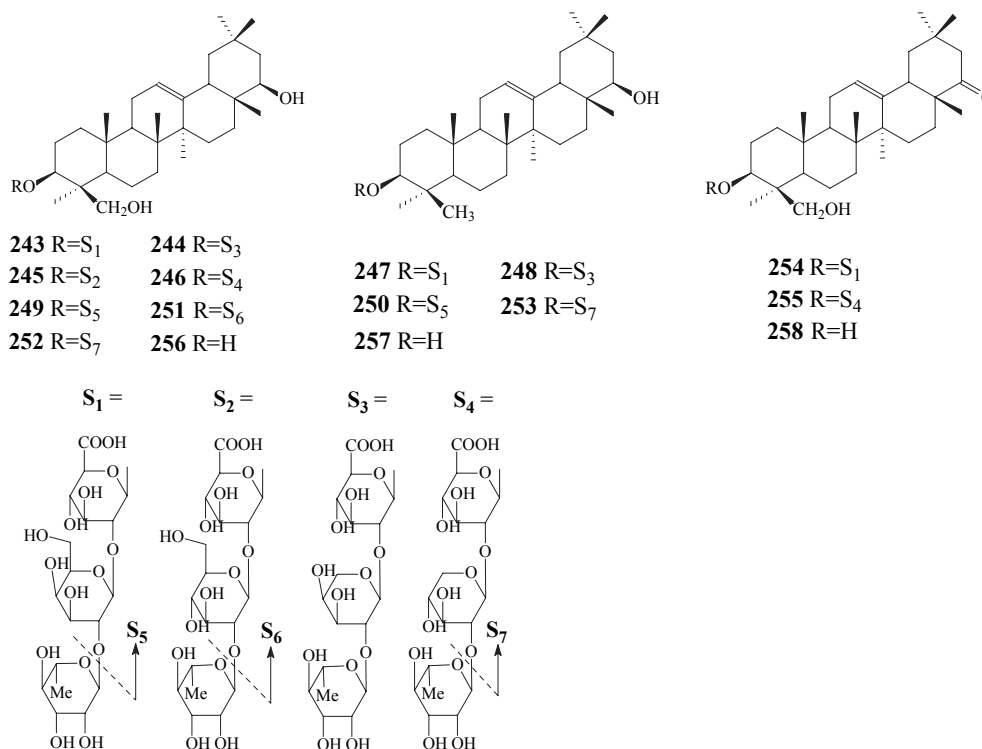
|     | R                                                                                   | EC <sub>50</sub> (μg/mL) |     | EC <sub>50</sub> (μg/mL) |
|-----|-------------------------------------------------------------------------------------|--------------------------|-----|--------------------------|
| 236 | H                                                                                   | 0.5                      | OA  | 1.7                      |
| 237 |  | 2.6                      | 201 | 7.1                      |
| 238 |  | 0.1                      | 232 | 8.3                      |
| 239 |  | 0.1                      | 208 | 1.5                      |
| 240 |  | 0.1                      | 234 | 1.2                      |

Table 9. (Continued)

|     |                                                                                   | R                                                                                 | EC <sub>50</sub> (μg/mL) |
|-----|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|--------------------------|
| 241 |  |  | 0.1                      |
| 242 |  |  | 0.1                      |



inhibited the formation of 6-keto-PGF<sub>1α</sub> with an IC<sub>50</sub> value of approximately 0.12 μM, and synthesis of TXB<sub>2</sub> catalyzed by COX-2 with an IC<sub>50</sub> value in the range of 0.4~2.5 μM (Hamburger et al., 2002). Platycodin D (**265**), isolated from the root of *Platycodon grandiflorum* A. DC. (Campanulaceae), suppressed PGE<sub>2</sub> production at 10 μM in rat peritoneal macrophages stimulated by TPA (Kim et al., 2001).

### Inhibition of complement activity

Complement plays a role in various functions of the adaptive immune response. Yet overactivation of complement is implicated in various inflammatory diseases. A/B-ring partial analogues (**29a-c**, **30a-c** and **33b**) of **OA** also showed complement inhibitory activity (IC<sub>50</sub>=72.3~633 μM). The lack of complement

inhibitory activity with compounds **31** and **32** showed the importance of the free carboxylic group for complement inhibition. The lack of complement inhibitory activity with compounds **33a** and **33c** while the compound **33b** retained the activity may indicate that the *meta* position of the carboxylic group was more favorable for complement inhibitory activity (Assefa et al., 2001).

Some semisynthetic analogues of **OA** (Table 11) were evaluated for their complement inhibitory and cytotoxic activities. The amide derivatives (**268** and **269**), OA-11-oxo **273**, and the 3-acyl derivatives (**203**, **208** and **231**) have retained the complement inhibitory activity. Among these, compounds **269** and **231** showed potency superior to **OA**. Both showed a moderate improvement *in vitro* TI in comparison with **OA** (Assefa et al., 1999).

**Table 10.** Anti-HSV-1 activity, cytotoxicity and selectivity index of **243–258**.

|            | Anti-HSV-1 activity (IC <sub>50</sub> , $\mu$ M) | Cytotoxicity (CC <sub>50</sub> , $\mu$ M) | Selectivity index (CC <sub>50</sub> /IC <sub>50</sub> ) |
|------------|--------------------------------------------------|-------------------------------------------|---------------------------------------------------------|
| <b>243</b> | >75.0                                            | —                                         | —                                                       |
| <b>244</b> | 27.4                                             | 115                                       | 4.2                                                     |
| <b>245</b> | 21.0                                             | >332                                      | >15.8                                                   |
| <b>246</b> | 43.0                                             | >343                                      | >13.7                                                   |
| <b>247</b> | 43.2                                             | 119                                       | 2.8                                                     |
| <b>248</b> | 22.3                                             | 698                                       | 31.3                                                    |
| <b>249</b> | >75.0                                            | —                                         | —                                                       |
| <b>250</b> | 64.1                                             | 641                                       | 10.0                                                    |
| <b>251</b> | 54.0                                             | >393                                      | >7.3                                                    |
| <b>252</b> | >75.0                                            | —                                         | —                                                       |
| <b>253</b> | >75.0                                            | —                                         | —                                                       |
| <b>254</b> | 19.1                                             | >332                                      | >17.4                                                   |
| <b>255</b> | 25.1                                             | >343                                      | >13.7                                                   |
| <b>256</b> | 5.6                                              | 116                                       | 20.7                                                    |
| <b>257</b> | 37.8                                             | >141                                      | >3.7                                                    |
| <b>258</b> | 61.4                                             | 504                                       | 8.2                                                     |

**Table 11.** Classical complement inhibition and cytotoxicity of **OA, 196, 197, 203, 208, 212, 231, and 266–273**.

|            | Complement inhibition<br>IC <sub>50</sub> ( $\mu$ M) | Cytotoxicity<br>IC <sub>50</sub> ( $\mu$ M) | TI   |
|------------|------------------------------------------------------|---------------------------------------------|------|
| <b>OA</b>  | 72.3                                                 | 112                                         | 1.55 |
| <b>197</b> | NA                                                   | NA                                          | 0.06 |
| <b>266</b> | NA                                                   | 70                                          |      |
| <b>267</b> | NA                                                   | NA                                          |      |
| <b>268</b> | 146.6                                                | 115                                         | 0.78 |
| <b>269</b> | 31.8                                                 | 127                                         | 3.99 |
| <b>270</b> | NA                                                   | NA                                          |      |
| <b>271</b> | NA                                                   | 105                                         |      |
| <b>196</b> | NA                                                   | 103                                         |      |
| <b>272</b> | NA                                                   | NA                                          |      |
| <b>273</b> | 233.7                                                | 131                                         | 0.56 |
| <b>203</b> | 108.3                                                | 77                                          | 0.71 |
| <b>231</b> | 31.4                                                 | 93                                          | 2.96 |
| <b>208</b> | 103.0                                                | 86                                          | 0.83 |
| <b>212</b> | NA                                                   | 31                                          |      |

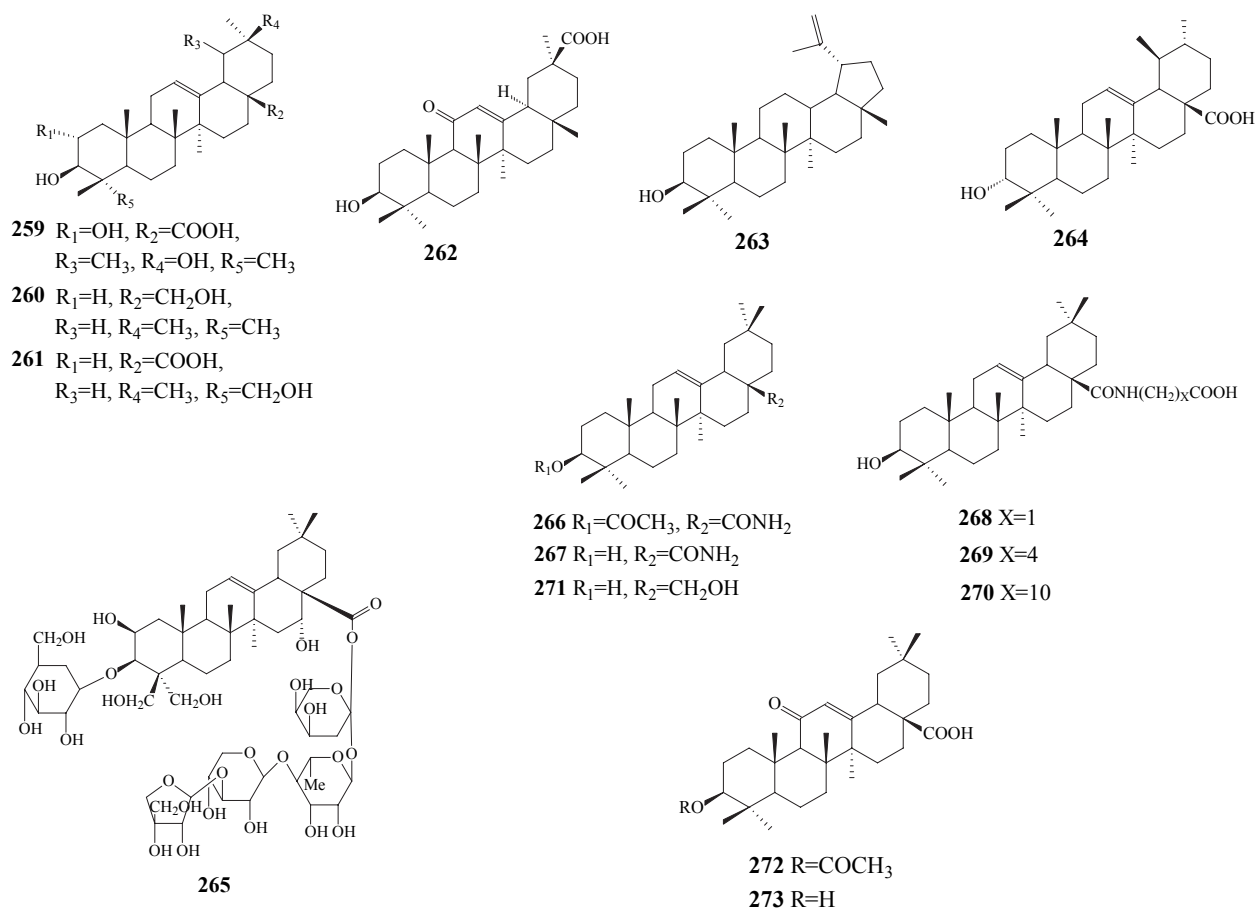
### Inhibition of elastase

The hydrolysis of blood vessel elastin by HLE promotes the transendothelial migration of stimulated proinflammatory cells. The IC<sub>50</sub> values for elastase inhibition by **UA** and **OA** were 4.4 and 6.4  $\mu$ M, respectively (Ying et al., 1991). The  $\alpha$ -boswellic acid (**274**), acetyl-11-keto- $\beta$ -boswellic acid (AKBA) (**275**), **25**, **26**, **UA**, and **28** were tested for the inhibitory activity of HLE. Dual inhibition of 5-lipoxygenase and HLE was unique to boswellic acid series. **UA** and amyrins, which possessed no 5-LO inhibitory properties, blocked the activity of HLE, but **28** had no inhibitory effects at concentrations up to 20  $\mu$ M (Safayhi and Sailer, 1997). The presence of an 11-keto group and hydrophilicity on the A-ring of the pentacyclic ring system was crucial for AKBA's potent 5-LO inhibitory activity (Sailer et al., 1996).

In another report, **UA**, **27**, **263**, and **276** were evaluated as potential inhibitors of HLE. Among these triterpenes, **UA** and **263** showed marked HLE inhibitory activity with IC<sub>50</sub> values of 4.4 and 1.9  $\mu$ M, respectively. The appearance of HLE inhibition may depend on the presence and the orientation of two reactive groups in the tested molecules (Mitaine-Offer et al., 2002).

### Inhibition of intercellular adhesion molecule (ICAM-1) expression induced by TNF- $\alpha$

ICAM-1 is a member of the immunoglobulin superfamily of adhesion molecules, and appears to lead to acute and chronic inflammation. The inhibitory effects of oleanane-type triterpenoids from fabaceous plants on the TNF- $\alpha$ -induced expression of ICAM-1 on THP-1 human monocytic leukemia cells were reported. The activity



of oleanane saponins and sapogenins against ICAM-1 expression appears to be dependent upon the position of the hydroxyl group, in particular upon the status of the C-21 and C-24 positions, and of the glycosyl group at C-3 position (Ahn et al., 2002).

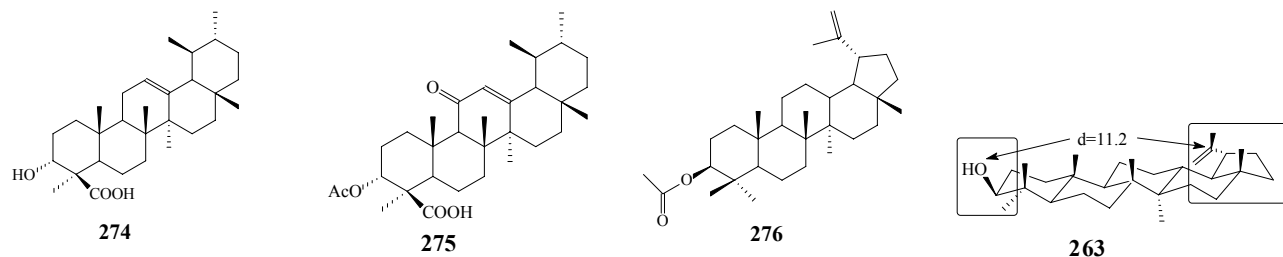
## HEPATOPROTECTIVE ACTIVITIES

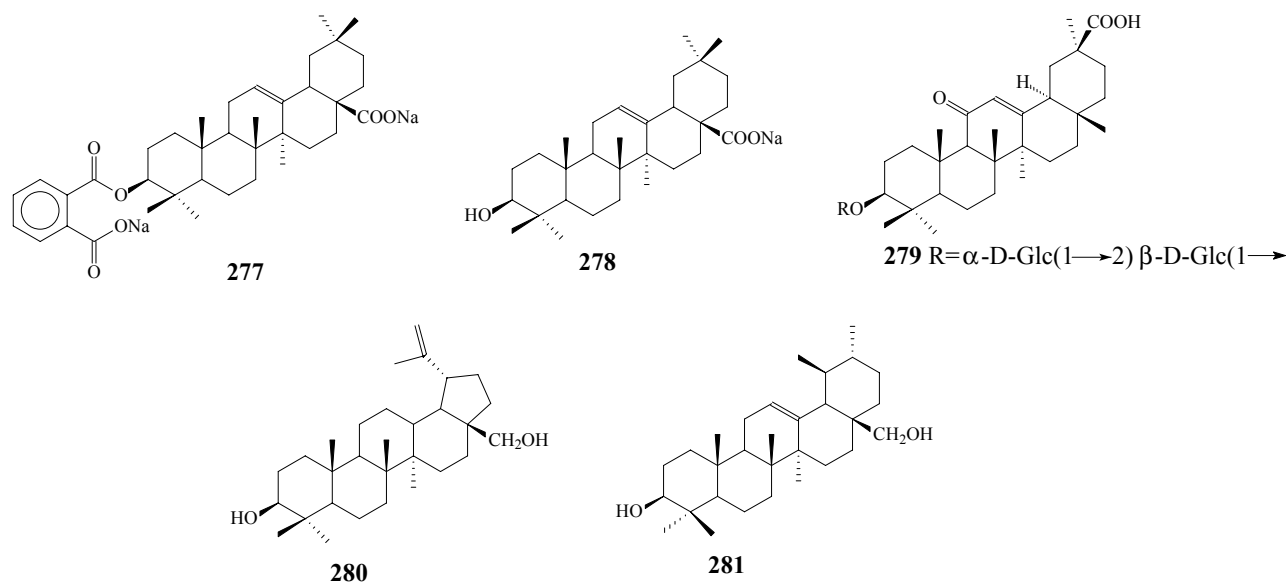
**OA** has been successfully used as an oral drug to treat human acute and chronic liver diseases in China (Qu, 1981). **UA** has been observed to be more potent than **OA** in decreasing chemically induced liver injury in mice (Liu et al., 1994).

**OA** 3 $\beta$ -phthalic monoester disodium salt (**277**) was synthesized in order to increase the solubility of **OA** in water. Compound **277** inhibited the rising of serum ALT

caused by D-galactosamine and  $CCl_4$ , and dramatically decreased liver fat storage, in addition to alleviating the condition of the degeneration of hepatic cells and necrosis (Wan et al., 1998).

**OA** sodium salt (**278**) obviously inhibited the rising of ALT and serum phosphatase caused by  $CCl_4$  when hypodermically injected (100, 50, and 30 mg/kg) (Zhang et al., 2000). When cadmium is administered as  $CdCl_2$  to animals, most of it accumulates initially in the liver. Accordingly, the acute toxic effects of cadmium are observed mainly in the liver. Several triterpenes were investigated for the effect on cadmium toxicity in Hep G2 cells. Among them, 18 $\alpha$ -glycyrrhethinic acid (**262**) and 18 $\beta$ -glycyrrhethinic acid (**28**) had no protective effects, **OA**, **UA**, and glycyrrhizin (**279**) exhibited weak effects, and betulin (**280**) and uvaol (**281**) were more effective in





reducing the toxicity of CdCl<sub>2</sub>. Betulin (**280**), in particular, almost completely abolished the cytotoxicity of CdCl<sub>2</sub> at concentrations as low as 0.1 μg/mL (Miura et al., 1999).

## GASTROPROTECTIVE ACTIVITIES

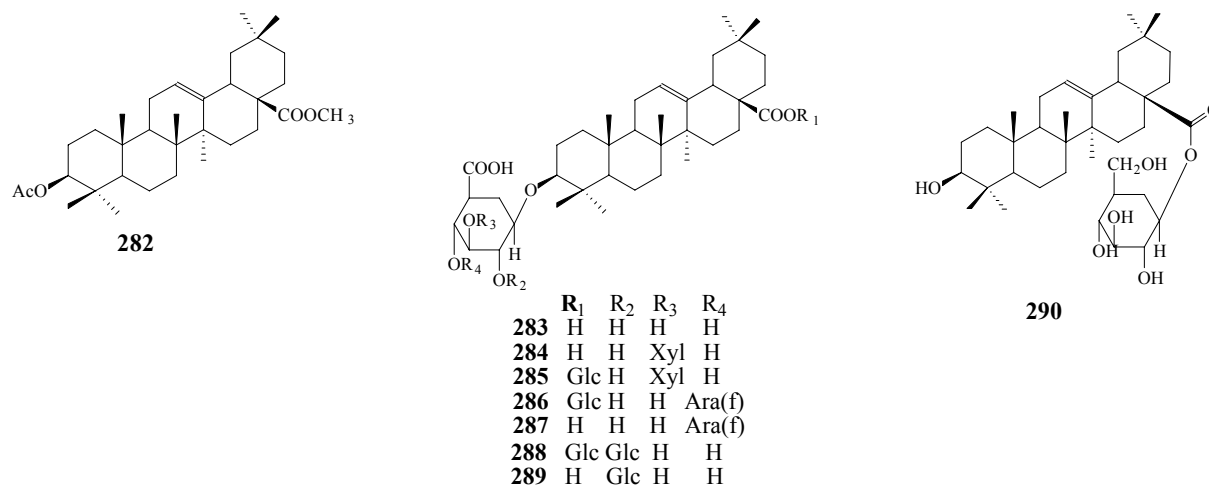
OA displayed gastroprotective effects in three different experimentally induced gastric ulcer models in rats (Astudillo et al., 2001). Five semi-synthetic derivatives of OA (**OA-28-methyl ester**, **282**, **OA-3-acetyl**, **OA-3-oxo**, and **OA-3-oxo-28-methyl ester**) were assessed for gastroprotective effects in the HCl/ethanol ulcer model in mice. The lesion index was 15.7~39.3 mm. Oxidation of the OH at C-3 reduced the activity of OA and its derivatives, while methylation of the 28-COOH with or without acetylation at C-3 did not affect the gastroprotective activities of the compounds (Astudillo et al., 2001).

Eight glycosides (**283-290**) of OA were examined for the effects on ethanol- or indomethacin-induced gastric

mucosal lesions in rats. Saponins **283**, **284** and **287** were effective on ethanol- or indomethacin-induced lesions, but **285**, **286**, **288**, **290** and their aglycone OA did not show such effects. Diglycoside **289** did not inhibit the indomethacin-induced lesions, but **288** (50 mg/kg) did have gastroprotective effect. The results demonstrated that the 3-O-glycoside moiety was essential for the activity, and the 28-ester glucoside reduced the activity (Matsuda et al., 1998b).

## ANTIMICROBIAL ACTIVITIES

OA was reported to have antimicrobial activity against *B. subtilis* (MIC=8 μg/mL), methicillin-sensitive (MIC=8 μg/mL) and resistant (MIC=64 μg/mL) *S. aureus*. In this study, 3-O-(E)-hydroxycinnamoyl OA (**5**) did not show antimicrobial activity (Woldemichael et al., 2003b). In a separate study OA-28-methyl ester showed weak antibacterial activity against *M. luteus* and *E. coli* (Weimann et al., 2002).



**OA**, **UA**, **25**,  $\alpha$ -amyrin acetate (**291**), **UA-3-acetyl**, and **292-295** were isolated from *Alyxia insularis* Kanehira & Sasaki, and examined for antimicrobial activity. Only two compounds, both of which contained an 11-carbonyl group (**292**, **293**), showed inhibitory activity on the growth of *S. epidermidis*, *M. luteus*, *S. aureus*, *B. subtilis*, and *S. faecium* (at 100 and 50  $\mu\text{g/mL}$ ) (Wang et al., 1993). Compounds **296-299** were isolated from *Dillenia papuana* and showed antibacterial activity (Table 12). It was presumed that the carboxylic group and  $\Delta^{12,13}$  double bond played an important role in antibacterial activity (Nick et al., 1994). The MICs of **OA**, **OA-3-oxo**, 3-epi-**OA** (**300**), **28**, **301**, and **302** against *M. tuberculosis* were 50, 16, 16, 128, 64 and 64, respectively. It was concluded that the low polarity pentacyclic triterpenes with a hydroxyl or keto group in the A or B ring and an acid group in the E ring possess moderate antitubercular activity (Caldwell et al., 2000).

3-epi-**UA** (**264**) and **300** showed antimycobacterial activity with MIC values of 8 and 16  $\mu\text{g/mL}$ , respectively (Woldemichael et al., 2003a). Six triterpene saponins **54**, and **303-307**, were isolated from *Hedera helix* L., and tested against *C. albicans*, *B. cereus*, *S. epidermidis* and

*E. coli*. Only compound **303**, a monodesmosidic saponin with **OA** as aglycone, showed inhibitory activity. The MIC values of **303** were 64 ppm against *C. albicans*, and 128 ppm against *S. epidermidis* and *B. cereus*. The MIC value of reference chloramphenicol was 16 ppm for *S. epidermidis* and *B. cereus*. The other saponins (**54**, **304-307**) showed no inhibitory activity. Thus, it was presumed the antibacterial activity was related to the presence of a monodesmosidic saponin with **OA** as aglycone (Bedir et al., 2000).

**OA** and **UA** also showed trypanocidal activity. **UA** stopped the movement of all *T. cruzi* epimastigotes at the minimum concentration ( $\text{MC}_{100}$ ) of 88  $\mu\text{M}$  after 48 h of incubation. **OA** was less active ( $\text{MC}_{100}$ =550  $\mu\text{M}$ ), and **27** was inactive (Abe et al., 2002). In contrast, the methyl esters (**UA-** and **OA-28-methyl ester**) and acetates (**UA-** and **OA-3-acetyl**) of **UA** and **OA** were not active, but **OA-3-oxo** ( $\text{IC}_{50}$ =294.9  $\mu\text{M}$ ), **308** ( $\text{IC}_{50}$ =402.3  $\mu\text{M}$ ) and **309** ( $\text{IC}_{50}$ =56.6  $\mu\text{M}$ ) retained the activity. These results suggested the importance of the polar groups to trypanocidal activity (Cunha et al., 2003).

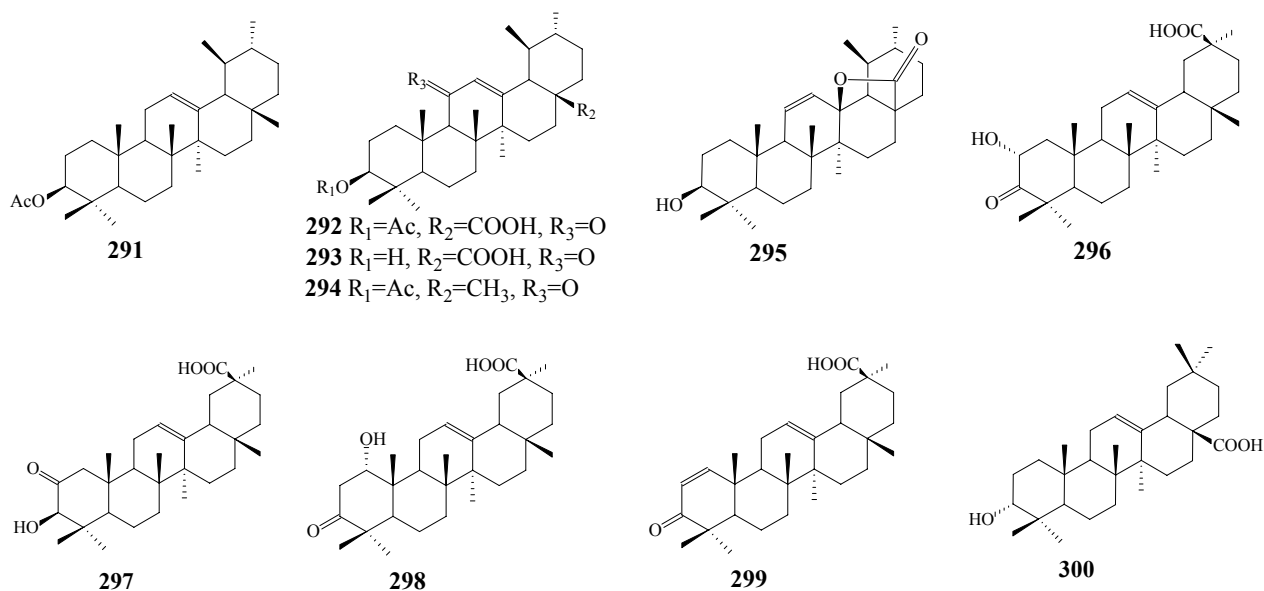
Two **OA** saponins, **290** and **310**, from *Viguiera decurrens* showed insecticidal activity, and their  $\text{LC}_{50}$  values were 1380 and 80  $\mu\text{g/mL}$  against *Epilachna varivestis* larvae, respectively (Marquina et al., 2001). 3-epi-**OA** (**300**) possessed antiprotozoal activity ( $\text{IC}_{50}$ =18.8  $\mu\text{M}$  against *L. donovani* promastigotes; and 28.3  $\mu\text{M}$  against *P. falciparum*) (Camacho et al., 2000).

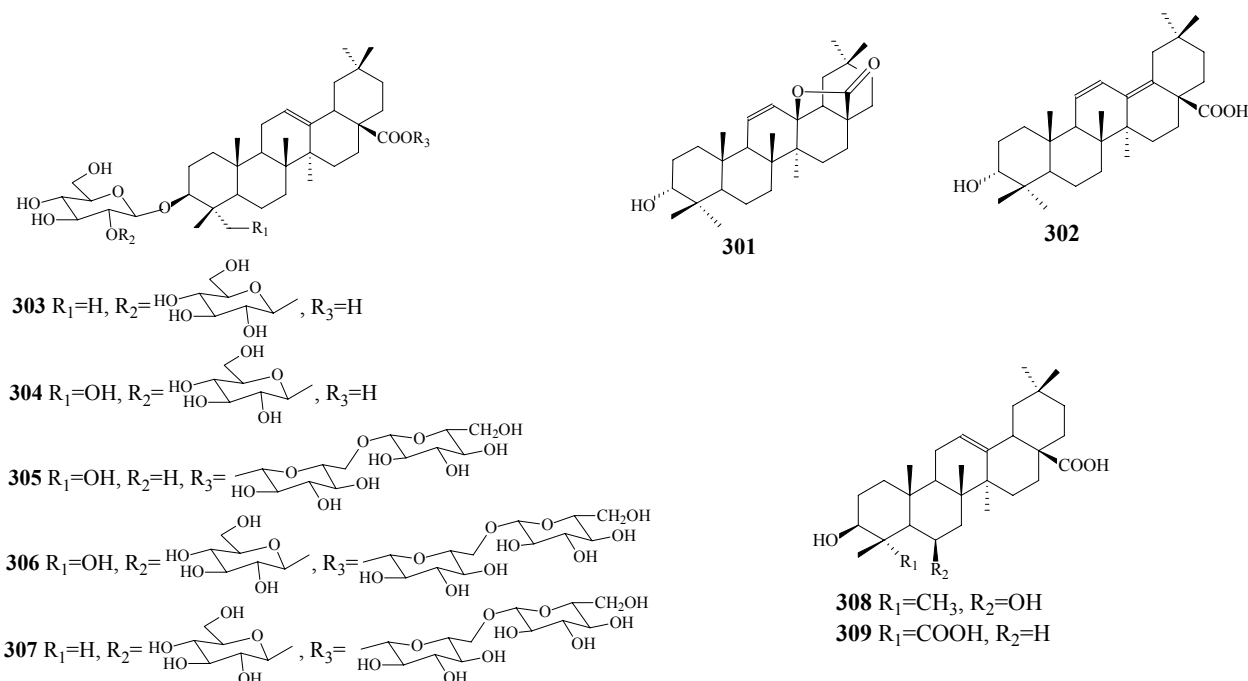
Four saponins (**49**, **50**, **311** and **312**) were isolated from *Serjania salzmänniana* and were molluscicidal, causing 70-100% mortality at 10 ppm against *Biomphalaria alexandrina*. The saponins also showed antifungal activity against *Cryptococcus neoformans* and *Candida albicans* with MIC at inhibitory concentrations of 8 and 16  $\mu\text{g/mL}$ , respectively (Ekabo and Farnsworth, 1996).

**Table 12.** Antibacterial activity of **296-299**.

|            | Minimum growth inhibition ( $\mu\text{g}$ ) |                |                  |
|------------|---------------------------------------------|----------------|------------------|
|            | <i>B. subtilis</i>                          | <i>E. coli</i> | <i>M. luteus</i> |
| <b>296</b> | 2.4                                         | 2.4            | 1.2              |
| <b>297</b> | 2.0                                         | 1.0            | 1.0              |
| <b>298</b> | 2.0                                         | 5.0            | 2.0              |
| <b>299</b> | 1.0                                         | 1.0            | 1.0              |

Positive control: Chloramphenicol MGI=0.1, 0.04, and 0.04  $\mu\text{g}$ , respectively.





Various dosages (1.9 to 3.0 g/kg) of **OA** could affect the survival, growth, and development of the larvae of *heliothis zea*. This might be one of the plant's defense mechanisms against phytophagous insects (Argandona and Faini, 1993). **OA**, **UA**, and their synthesized 3-O-fatty acid ester analogues (**313-318**) were examined for antifeedant activity against the agricultural pest tobacco caterpillar *S. litura* larvae. At the dosages of 150, 100, 50  $\mu\text{g/mL}$ , all of the compounds showed antifeedant activity. At 150  $\mu\text{g/mL}$  dose, all the tested compounds showed more than 50% activity, except compounds **OA** and **318**. At 100  $\mu\text{g/mL}$  dose, the compounds showed 40~78% activity, while compound **318** again showed the lowest activity. Even at 50  $\mu\text{g/mL}$ , compounds **314-317** exhibited more than 50% activity. Compounds **316** (74%) and **317** (71%) were found to exhibit exceptionally potent activity at 50  $\mu\text{g/mL}$  (Mallavadhani et al., 2003).

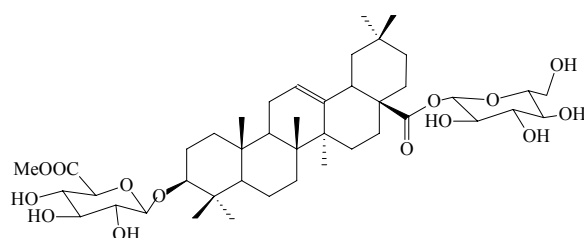
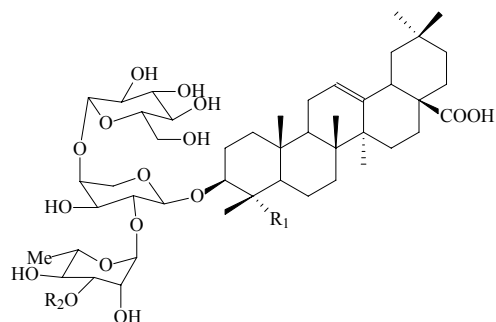
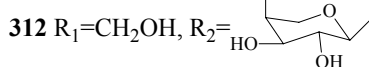
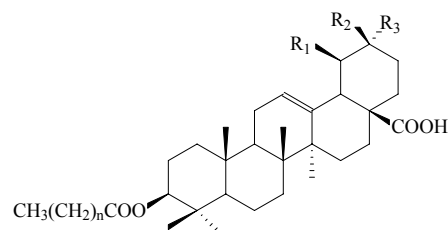
## ANTI-DIABETES ACTIVITIES

Many late complications of diabetes are associated with hypoglycemia. **OA** glycosides (**283**, **286**, **287**, and **319-324**) from the root of *Aralia elata*, which has been used for treating diabetes, were examined for hypoglycemic activity in the oral sucrose tolerance in rats (Table 13). It was concluded the 3-O-glycoside moiety was essential to the activity, while the 28-ester glucosidal moiety significantly reduced the activity. In the 3-O-oligoglucoside structure, the 3'-O-glucopyranosyl moiety tended to decrease the activity, while the 4'-O-arabinofuranosyl moiety increase it (Yoshikawa et al., 1994; 1996a, b; Fujimura et al., 1996). The 6'-methyl ester of the glucuronic acid (**325**) moiety strongly reduced

**Table 13.** Inhibitory effects of **283**, **286**, **287**, **319-324**, **326-329** (100 mg/kg, p.o.) on the rise in plasma glucose level by oral sucrose tolerance test.

|            | Plasma glucose concentration (mg/dl) |      |      |
|------------|--------------------------------------|------|------|
|            | 0.5 h                                | 1 h  | 2 h  |
| <b>319</b> | 18.2                                 | 18.7 | 15.5 |
| <b>320</b> | 76.6                                 | 44.9 | 18.9 |
| <b>321</b> | 27.7                                 | 33.4 | 36.4 |
| <b>323</b> | 71.4                                 | 45.4 | 32.4 |
| <b>322</b> | 31.9                                 | 28.1 | 29.5 |
| <b>287</b> | 18.0                                 | 23.0 | 16.6 |
| <b>283</b> | 26.9                                 | 24.4 | 28.6 |
| <b>324</b> | 55.2                                 | 37.4 | 20.0 |
| <b>286</b> | 36.0                                 | 17.7 | 20.6 |
| <b>OA</b>  | 82.8                                 | 58.1 | 30.8 |
| <b>326</b> | 81.1                                 | 48.9 | 23.4 |
| <b>327</b> | 36.1                                 | 41.9 | 31.2 |
| <b>328</b> | 66.9                                 | 39.3 | 12.4 |
| <b>329</b> | 39.1                                 | 30.0 | 23.4 |

the activity (Matsuda et al., 1998c). The above summaries for SARs were substantiated further by bioassay results of compounds (**326-329**) from *Beta vulgaris* L., among which **327** and **329** showed hypoglycemic activity, **328** showed weaker activity, and **326** was inactive (Yoshikawa et al., 1996c). **OA** sodium salt (**278**) (s.c. 20 mg/kg·d) obviously reduced blood sugar (Zhang et al., 2000).

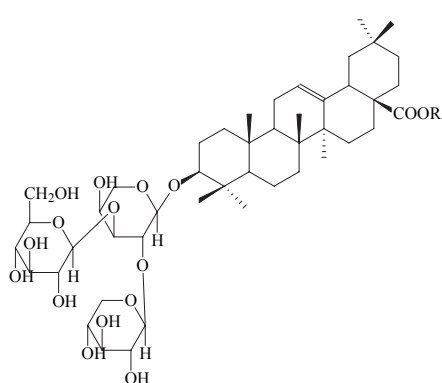
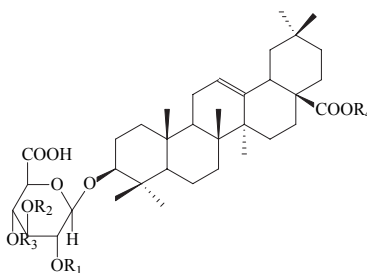
**310****311** R<sub>1</sub>=CHO, R<sub>2</sub>=H**312** R<sub>1</sub>=CH<sub>2</sub>OH, R<sub>2</sub>=**313** R<sub>1</sub>=CH<sub>3</sub>, R<sub>2</sub>=H, R<sub>3</sub>=CH<sub>3</sub>, n=10**314** R<sub>1</sub>=CH<sub>3</sub>, R<sub>2</sub>=H, R<sub>3</sub>=CH<sub>3</sub>, n=12**315** R<sub>1</sub>=CH<sub>3</sub>, R<sub>2</sub>=H, R<sub>3</sub>=CH<sub>3</sub>, n=14**316** R<sub>1</sub>=CH<sub>3</sub>, R<sub>2</sub>=H, R<sub>3</sub>=CH<sub>3</sub>, n=16**317** R<sub>1</sub>=H, R<sub>2</sub>=R<sub>3</sub>=CH<sub>3</sub>, n=14**318** R<sub>1</sub>=H, R<sub>2</sub>=R<sub>3</sub>=CH<sub>3</sub>, n=16

Inhibition of  $\alpha$ -glucosidase can prevent late complications of diabetes by decreasing the postprandial rise in blood glucose (Bischoff, 1994). **OA** and its five synthetic derivatives (**OA-28-methyl ester**, **OA-3-acetyl**, **330**, **331** and **332**) were found to inhibit  $\alpha$ -glucosidase, with IC<sub>50</sub> values of 11.16, 55.097, 19.012, 7.97, 89.71 and 21.63  $\mu$ M, respectively. Compound **330** was the most potent among them (Ali et al., 2002).

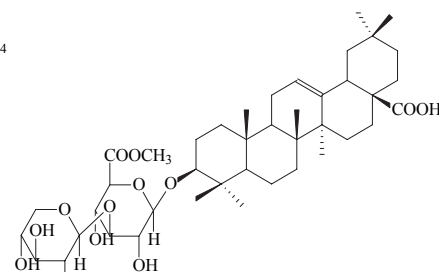
**OA** and **OA-28-aldehyde** were reported to stimulate insulin production in INS-1 cells. At 12.5  $\mu$ g/mL, **OA** increased insulin production by 87.97 ng/mg of protein in INS-1 cells. At 25 and 50 mg/ml of **OA**, the secretion of insulin was reduced considerably in a dose-dependent

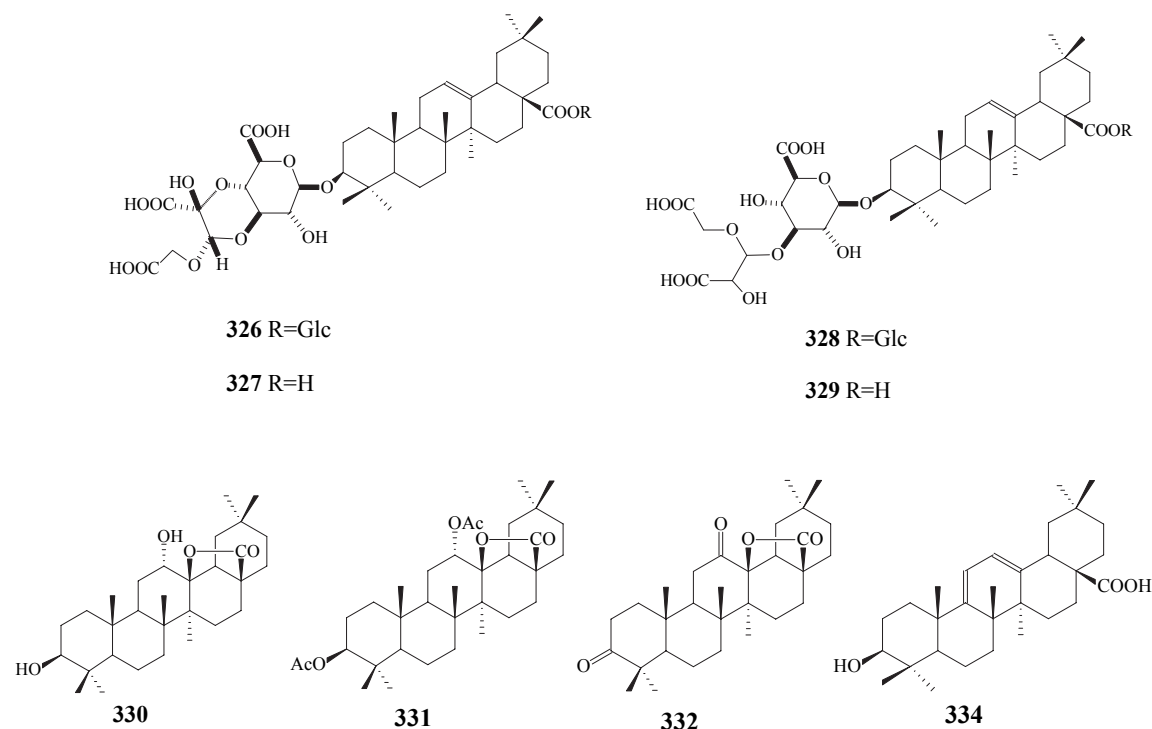
manner, similar to the dose-dependent insulin production by glucose in INS-1 cells. **OA-28-aldehyde** also enhanced insulin secretion by about 4ng/mg in those concentrations (Zhang et al., 2004).

Slowing gastric emptying will prolong the postprandial absorption of food, with a resultant improvement in blood glucose control. **OA** and its oligoglycosides (**283-290**, **333**) were examined for their effects on gastric emptying in mice, and the 3-O-glycosides moiety was found to be essential. The 2'-O- $\beta$ -D-glucopyranosyl moiety of the glucuronic acid reduced the activity, and the 28-ester glucose moiety markedly reduced it (Matsuda et al., 1999).

**319** R=H**320** R=Glc

|            | R <sub>1</sub> | R <sub>2</sub> | R <sub>3</sub> | R <sub>4</sub> |
|------------|----------------|----------------|----------------|----------------|
| <b>321</b> | Xyl            | Gal            | H              | H              |
| <b>322</b> | H              | Gal            | H              | H              |
| <b>323</b> | Xyl            | Gal            | H              | Glc            |
| <b>324</b> | H              | Gal            | Ara            | Glc            |
| <b>333</b> | H              | Ara(p)         | H              | H              |

**325**



## HEMOLYTIC ACTIVITIES

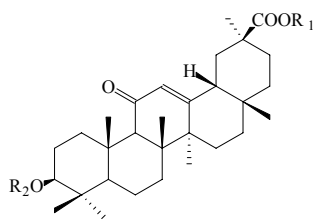
Hemolytic activity is one of the well known characteristics of triterpene saponins. Monodesmosidic OA disaccharides and trisaccharides (**335-355**) were prepared and their hemolytic activity compared (Table 14). The authors concluded that for lactosides of glycyrrhetic acid, its 11-deoxo and 18 $\alpha$ -derivatives, and their methyl esters (**335-341**), it was found that 28-COOH might be a basic requirement for strong hemolytic activity of lactosides. Compound **357**, which possessed no detectable hemolytic properties, differed from the highly active **341** only by interchanged positions of a methyl. Esterification increased the hemolytic activity, but esterification of the most OA lactoside **341** led to the almost non-hemolytic compound **340**. The linkage between rings D and E had remarkable influence on the activity of esters of glycyrrhetic acid lactosides (Ullah et al., 2002). A separate study showed that the connectivity between the sugar units strongly influenced the hemolytic activity of OA disaccharides (**341-346**). The 1 $\rightarrow$ 4 linked saponin **341** showed the highest activity.  $\beta$ -configuration of the outer anomeric position showed higher potency in the 1 $\rightarrow$ 6 linked saponins, while 1 $\rightarrow$ 2 linked glycosides exhibited very low hemolytic properties. Linkage positions 3 or 4 were structural requirements for higher hemolytic activity. The 1 $\rightarrow$ 3 linked saponin was more active than the 1 $\rightarrow$ 4 linked one, but the hemolytic activities of 1 $\rightarrow$ 6 and 1 $\rightarrow$ 2 linked analogues were less active (Seebacher et al., 2000). It was also found that OA trisaccharides (**347-350**) usually proved to be less potent than the corresponding

disaccharides. A  $\alpha$ -configuration of the terminal sugar residue was able to enhance hemolytic activity (Seebacher et al., 1999).

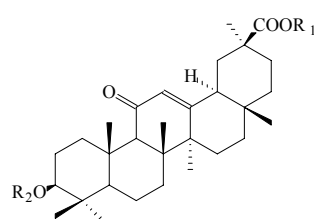
Glycyrrhetic acid disaccharides (**351-355**) were not found to possess detectable hemolytic properties, so the influence of the structure of the aglycon on the hemolytic activity is crucial (Ullah et al., 2000).

**Table 14.** Hemolytic index (HI) of compounds **335-350**.

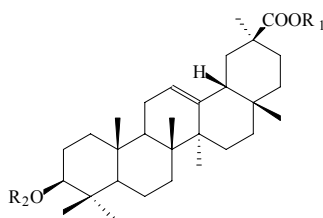
| Saponin    | Class         | HI     |
|------------|---------------|--------|
| <b>335</b> | Monosacchride | <1000  |
| <b>336</b> | Monosacchride | <1000  |
| <b>337</b> | Monosacchride | <1000  |
| <b>338</b> | Monosacchride | 135000 |
| <b>339</b> | Monosacchride | 19500  |
| <b>340</b> | Monosacchride | 10000  |
| <b>341</b> | Monosacchride | 150700 |
| <b>342</b> | Disacchride   | 100100 |
| <b>343</b> | Disacchride   | 3400   |
| <b>344</b> | Disacchride   | 3400   |
| <b>345</b> | Disacchride   | 22000  |
| <b>346</b> | Disacchride   | 35000  |
| <b>347</b> | Trisacchride  | 22000  |
| <b>348</b> | Trisacchride  | 11000  |
| <b>349</b> | Trisacchride  | 19500  |
| <b>350</b> | Trisacchride  | <2000  |



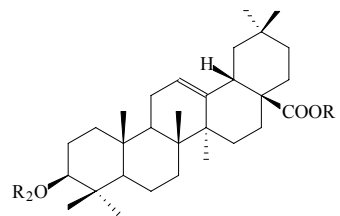
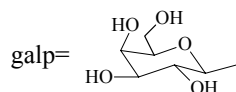
- 335  $R_1=H$ ,  $R_2=\beta$ -D-galp-(1 $\rightarrow$ 4)- $\beta$ -D-glc-(1 $\rightarrow$   
 338  $R_1=CH_3$ ,  $R_2=\beta$ -D-galp-(1 $\rightarrow$ 4)- $\beta$ -D-glc-(1 $\rightarrow$   
 351  $R_1=H$ ,  $R_2=\beta$ -D-glc-(1 $\rightarrow$ 2)- $\beta$ -D-glc-(1 $\rightarrow$   
 352  $R_1=H$ ,  $R_2=\beta$ -D-glc-(1 $\rightarrow$ 3)- $\beta$ -D-glc-(1 $\rightarrow$   
 353  $R_1=H$ ,  $R_2=\alpha$ -D-glc-(1 $\rightarrow$ 4)- $\beta$ -D-glc-(1 $\rightarrow$   
 354  $R_1=H$ ,  $R_2=\beta$ -D-glc-(1 $\rightarrow$ 4)- $\beta$ -D-glc-(1 $\rightarrow$   
 355  $R_1=H$ ,  $R_2=\beta$ -D-glc-(1 $\rightarrow$ 6)- $\beta$ -D-glc-(1 $\rightarrow$



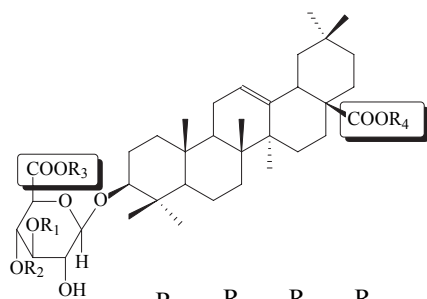
- 336  $R_1=H$ ,  $R_2=\beta$ -D-galp-(1 $\rightarrow$ 4)- $\beta$ -D-glc-(1 $\rightarrow$   
 339  $R_1=CH_3$ ,  $R_2=\beta$ -D-galp-(1 $\rightarrow$ 4)- $\beta$ -D-glc-(1 $\rightarrow$



- 337  $R_1=H$ ,  $R_2=\beta$ -D-galp-(1 $\rightarrow$ 4)- $\beta$ -D-glc-(1 $\rightarrow$



- 340  $R_1=CH_3$ ,  $R_2=\beta$ -D-galp-(1 $\rightarrow$ 4)- $\beta$ -D-glc-(1 $\rightarrow$   
 341  $R_1=H$ ,  $R_2=\beta$ -D-galp-(1 $\rightarrow$ 4)- $\beta$ -D-glc-(1 $\rightarrow$   
 342  $R_1=H$ ,  $R_2=\beta$ -D-galp-(1 $\rightarrow$ 3)- $\beta$ -D-glc-(1 $\rightarrow$   
 343  $R_1=H$ ,  $R_2=\alpha$ -D-galp-(1 $\rightarrow$ 2)- $\alpha$ -D-glc-(1 $\rightarrow$   
 344  $R_1=H$ ,  $R_2=\beta$ -D-galp-(1 $\rightarrow$ 2)- $\beta$ -D-glc-(1 $\rightarrow$   
 345  $R_1=H$ ,  $R_2=\alpha$ -D-galp-(1 $\rightarrow$ 6)- $\beta$ -D-glc-(1 $\rightarrow$   
 346  $R_1=H$ ,  $R_2=\beta$ -D-galp-(1 $\rightarrow$ 6)- $\beta$ -D-glc-(1 $\rightarrow$   
 347  $R_1=H$ ,  $R_2=\beta$ -D-glc-(1 $\rightarrow$ 2)- $\beta$ -D-glc-(1 $\rightarrow$ 6)- $\beta$ -D-glc-(1 $\rightarrow$   
 348  $R_1=H$ ,  $R_2=\beta$ -D-glc-(1 $\rightarrow$ 3)- $\beta$ -D-glc-(1 $\rightarrow$ 6)- $\beta$ -D-glc-(1 $\rightarrow$   
 349  $R_1=H$ ,  $R_2=\alpha$ -D-glc-(1 $\rightarrow$ 4)- $\beta$ -D-glc-(1 $\rightarrow$ 6)- $\beta$ -D-glc-(1 $\rightarrow$   
 350  $R_1=H$ ,  $R_2=\beta$ -D-glc-(1 $\rightarrow$ 2)- $\beta$ -D-glc-(1 $\rightarrow$ 6)- $\beta$ -D-glc-(1 $\rightarrow$



|     | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|-----|-------|-------|-------|-------|
| 356 | H     | H     | Bu    | Glu   |
| 357 | H     | H     | Me    | Glu   |
| 358 | H     | Ara   | Me    | Glu   |
| 359 | Gal   | H     | Me    | Glu   |
| 360 | H     | H     | Me    | H     |
| 361 | H     | Ara   | Me    | H     |
| 362 | Gal   | H     | Bu    | H     |
| 363 | Gal   | H     | Me    | H     |

Membrane-disrupting activity may cause hemolysis and other various biological activities. Fifteen triterpenoid saponins were classified into four types based on their chemistries and membrane-disrupting activities (Hu et al., 1996).

**I: 356-359.** Are glycosylated at two sites (C-3 and C-28), and the carboxylic group of the glucuronic acid residue connecting to C-3 was esterified by an alkyl group. Their actions are so strong that they can cause catastrophic rupture when sufficiently accumulated.

**II: 360-363.** Are glycosylated at a single site (C-3). They bind to the liposomal membrane more strongly than type I, but not enough to cause membrane disruption.

**III: 290, 286 and 324.** Are glycosylated at two sites (C-3 and C-28), and the carboxylic group of the glucuronic acid was free. Although these saponins bind to the membrane as efficiently as type I, their disruptive ability was much weaker.

**IV: 283, 333 and 322.** Are glycosylated at a single site (C-3) with two carboxylic groups. Their membrane disrupting activity could only be shown in the presence of cholesterol.

## MISCELLANEOUS

Oleanane- and ursane-type triterpenoids have many other activities besides the above nine activities, but they have been researched and reported less. The following activities, which have structure-activity relationships, are introduced briefly all together.

### Spasmolytic activity

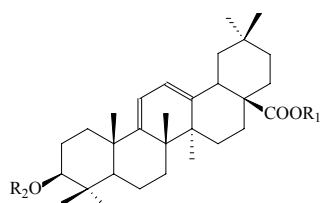
Eucalyptanoic acid (**364**) and its acetyl (**365**) and acetylmethyl (**366**) derivatives, as well as **OA** and **OA** acetyl (**197**) and acetylmethyl (**282**) derivatives, were tested for spasmolytic activity (Table 15). Among them, the presence of the 28-COOMe and C-9(11) double bond enhanced the activity, while the acetoxy group at C-3 decreased it (Begum et al., 2002).

### Antipruritic activity

**OA** 3-O-monodesmosides (**283-285**, **288**, **289**, and **367-370**) were examined for the antipruritic effects using a compound 48/80-induced pruritic model in mice (Table 16). **OA** 3-O-monodesmosides showed antipruritic effects, while **OA** and its 3, 28-O-bisdesmosides did not. Moreover, the 3-O-glucuronides showed more potent activity than the corresponding 3-O-glucosides (Kubo et al., 1997; Matsuda et al., 1998a).

### Anti-thrombotic activity

**OA** showed inhibitory effects on blood platelet aggregation. It not only inhibited blood platelet aggregation induced by adenosine 5'-diphosphate



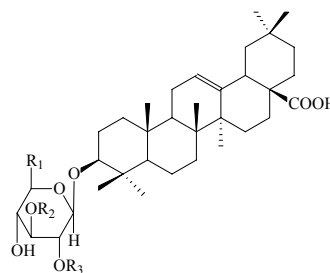
**364**  $R_1=R_2=H$

**365**  $R_1=H$ ,  $R_2=CH_3CO$

**366**  $R_1=CH_3$ ,  $R_2=CH_3CO$

**Table 15.** Effect on Spontaneous conreactions and  $K^+$ -induced contractions.

|            | Effect (% inhibition)    |                             |
|------------|--------------------------|-----------------------------|
|            | Spontaneous conreactions | $K^+$ -induced contractions |
| <b>364</b> | 58.2                     | 31.1                        |
| <b>365</b> | No effect                | No effect                   |
| <b>366</b> | 95.3                     | 85.9                        |
| <b>OA</b>  | No effect                | No effect                   |
| <b>197</b> | No effect                | No effect                   |
| <b>282</b> | 67.7                     | 35.5                        |



**367**  $R_1=CH_2OH$ ,  $R_2=R_3=H$

**368**  $R_1=CH_2OH$ ,  $R_2=Xyl$ ,  $R_3=H$

**369**  $R_1=COOH$ ,  $R_2=Ara$ ,  $R_3=H$

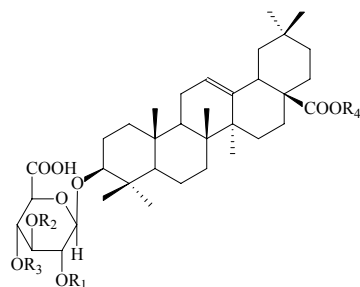
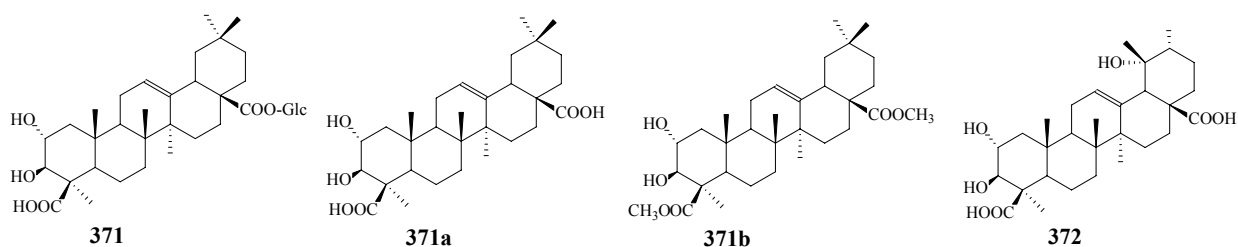
**370**  $R_1=COOH$ ,  $R_2=Glc$ ,  $R_3=Glc$

**Table 16.** Effects on compound 48/80-induced scratch behavior in mice.

|            | Dose (mmol/kg) | Inhibition (%) |
|------------|----------------|----------------|
| <b>OA</b>  | 0.05           | 13.7           |
|            | 0.2            | 9.9            |
| <b>283</b> | 0.05           | -0.9           |
|            | 0.2            | 52.4           |
| <b>284</b> | 0.05           | 2.2            |
|            | 0.2            | 65.8           |
| <b>288</b> | 0.05           | 16.0           |
|            | 0.2            | 2.0            |
| <b>367</b> | 0.05           | -4.5           |
|            | 0.2            | 17.0           |
| <b>368</b> | 0.05           | 28.5           |
|            | 0.2            | 48.9           |
| <b>289</b> | 0.05           | -16.8          |
|            | 0.2            | 64.0           |
| <b>369</b> | 0.065          | 4.2            |
|            | 0.13           | 58.9           |
| <b>370</b> | 0.05           | 43.6           |
|            | 0.10           | 43.6           |
| <b>285</b> | 0.05           | -1.1           |
|            | 0.11           | 9.4            |

Positive control was diphenhydramine hydrochloride, which inhibitions were 26.4 and 84.7 at dosage 0.05 and 0.2 mmol/kg, respectively.

(ADP) and collagen in old mice, but also increased electrophoretic mobility at therapeutical dosage (75-300 mg/kg) (Liu and Wang, 1993). Compounds **371**, **372**, **UA** and **OA** were isolated from *Chaenomeles sinensis*, and tested for the inhibitory activity of tissue factor (TF), which could accelerate the blood clotting. Among these compounds, only **371** and its aglycone **371a** inhibited TF activity, and  $IC_{50}$  values were 0.036 and 0.028 mM/unit, respectively. However, **372**, **UA**, **OA** and the dimethyl ester derivative **371b** of **371a** showed no inhibitory activity. These results indicated that the presence of two free carboxyl groups of **371a** played an important role in exerting the inhibitory activity on TF (Lee and Han, 2003).



|            | R <sub>1</sub> | R <sub>2</sub> | R <sub>3</sub> | R <sub>4</sub> |
|------------|----------------|----------------|----------------|----------------|
| <b>373</b> | Gal            | Gal            | H              | H              |
| <b>374</b> | Gal            | Gal            | H              | Glc            |
| <b>375</b> | H              | Glc            | H              | H              |
| <b>376</b> | H              | Glc            | Ara(f)         | H              |
| <b>377</b> | H              | Glc            | H              | Glc            |
| <b>378</b> | H              | Glc            | Ara(f)         | Glc            |

**Table 17.** Inhibitory activity of **321**, **323**, **373-378** on ethanol absorption.

|            | Dose<br>(mg/kg p.o.) | Ethanol absorption in blood (mg/ml) |      |      |
|------------|----------------------|-------------------------------------|------|------|
|            |                      | 1 h                                 | 2 h  | 3 h  |
| <b>321</b> | 25                   | 0.11                                | 0.13 | 0.02 |
|            | 50                   | 0.01                                | 0.04 | 0.01 |
|            | 100                  | 0                                   | 0    | 0    |
| <b>373</b> | 25                   | 0.56                                | 0.19 | 0.01 |
|            | 50                   | 0.50                                | 0.19 | 0.02 |
|            | 100                  | 0.25                                | 0.18 | 0.02 |
| <b>341</b> | 100                  | 0.57                                | 0.24 | 0.04 |
| <b>374</b> | 100                  | 0.57                                | 0.23 | 0.04 |
| <b>375</b> | 25                   | 0.26                                | 0.20 | 0.03 |
|            | 50                   | 0.03                                | 0.04 | 0.02 |
|            | 100                  | 0.03                                | 0.02 | 0.01 |
| <b>376</b> | 25                   | 0.42                                | 0.21 | 0.03 |
|            | 50                   | 0.34                                | 0.18 | 0.01 |
|            | 100                  | 0.08                                | 0.09 | 0    |
| <b>377</b> | 100                  | 0.58                                | 0.21 | 0    |
| <b>378</b> | 100                  | 0.56                                | 0.23 | 0.04 |

### Inhibitory activity of ethanol absorption

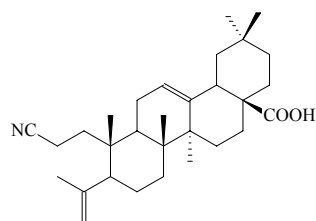
Excessive consumption of ethanol is known to profoundly affect nearly every organ in the body. OA 3-O-monodesmosides (**321**, **373**, **375** and **376**) were found to show potent inhibitory activity on ethanol absorption, while 3, 28-O-bidesmosides (**323**, **374**, **377** and **378**) lacked the inhibitory activity (Table 17). Some OA 3-O-monodesmosides were found to show potent inhibitory activity on ethanol absorption, while 3, 28-O-bidesmosides lacked the inhibitory activity (Yoshikawa et al., 1993 and 1996c).

### Effects on nonmalignant prostate cell proliferation

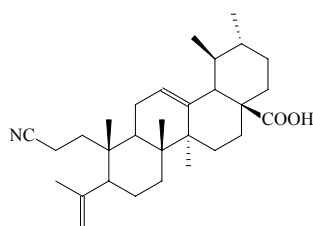
Eight synthesized A-ring cleaved oleanane and ursane analogues (**379-386**) were assessed for their ability to inhibit cell proliferation in NRP.152 (nonmalignant) prostate cells (Table 18). It was found that each pair of ursane and oleanane derivatives exhibited similar activities, and A-ring cleaved compounds were more active. In A ring cleaved series, both conversions of nitriles corresponding aldehydes and reduction of the nitriles to the amines resulted in increased activity (Finlay et al., 1997).

**Table 18.** Inhibitory activity on NRP 152 prostate cell proliferation.

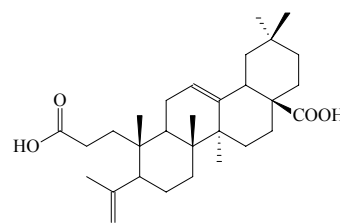
| Compound   | IC <sub>50</sub> (μM) | Compound        | IC <sub>50</sub> (μM) |
|------------|-----------------------|-----------------|-----------------------|
| <b>386</b> | 0.3                   | <b>OA</b>       | >5.0                  |
| <b>385</b> | 0.7                   | <b>UA</b>       | >5.0                  |
| <b>383</b> | 1.5                   | <b>OA-3-oxo</b> | >5.0                  |
| <b>384</b> | 2.4                   | <b>UA-3-oxo</b> | >5.0                  |
| <b>379</b> | 3.8                   | <b>381</b>      | >5.0                  |
| <b>380</b> | >5.0                  | <b>382</b>      | >5.0                  |



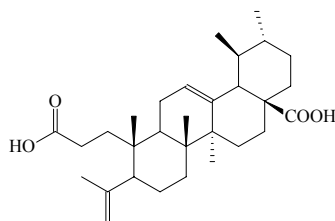
379



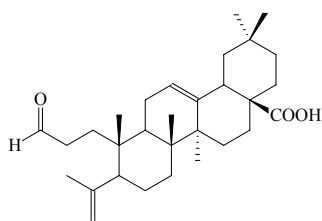
380



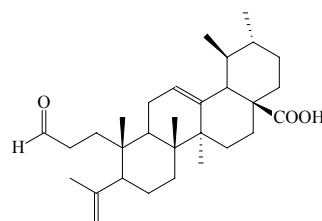
381



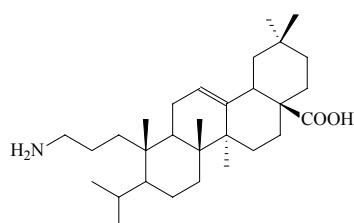
382



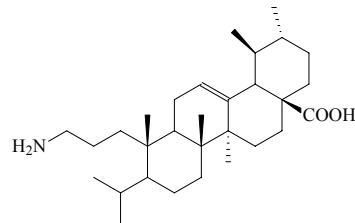
383



384



385



386

## LITERATURE CITED

- Abe, F., T. Yamauchi, T. Nagao, J. Kinjo, H. Okabe, H. Higo, and H. Akahane. 2002. Ursolic acid as a trypanocidal constituent in rosemary. *Biol. Pharm. Bull.* **25**(11): 1485-1487.
- Ahn, K.S., J.H. Kim, S.R. Oh, B.S. Min, J. Kinjo, and H.K. Lee. 2002. Effects of oleanane-type triterpenoids from fabaceous plants on the expression of ICAM-1. *Biol. Pharm. Bull.* **25**(8): 1105-1107.
- Ahn, K.S., M.S. Hahm, E.J. Park, H.K. Lee, and I.K. Kim. 1998. Corosolic acid isolated from the fruit of *Crataegus pinnatifida* var. *psilosa* is a protein kinase C inhibitor as well as a cytotoxic agent. *Plant. Med.* **64**: 468-470.
- Ali, M.S., M. Jahangir, S.S. Hussan, and M.I. Choudhary. 2002. Inhibition of  $\alpha$ -glucosidase by oleanolic acid and its synthetic derivatives. *Phytochemistry* **60**: 295-299.
- Argandona, V.H. and F.A. Faini. 1993. Oleanolic acid content in *Baccharis linearis* and its effects on *Heliothis zea* larvae. *Phytochemistry* **33**(6): 1377-1379.
- Assefa, H., A. Nimrod, L. Walker, and R. Sindelar. 1999. Synthesis and evaluation of potential complement inhibitory semisynthetic analogs of oleanolic acid. *Bioorg. Med. Chem. Lett.* **9**: 1889-1894.
- Assefa, H., A. Nimrod, L. Walker, and R. Sindelar. 2001. Enantioselective synthesis and complement inhibitory assay of A/B-ring partial analogues of oleanolic acid. *Bioorg. Med. Chem. Lett.* **11**: 1619-1623.
- Astudillo, L., J.A. Rodriguez, and G. Schmeda-Hirschmann. 2001. Gastroprotective activity of oleanolic acid derivatives on experimentally induced gastric lesions in rats and mice. *J. Pharm. Pharmacol.* **54**: 583-588.
- Baglin, I., A-C. Mitaine-Offer, M. Nour, K. Tan, C. Cave, and M-A. Lacaille-Dubois. 2003. A review of natural and modified betulinic, ursolic and echinocystic acid derivatives as potential antitumor and anti-HIV agents. *Mini Rev. Med. Chem.* **3**(6): 525-539.
- Barbato, F., M.L. La Rotonda, and F. Quaglia. 1997. Chromatographic indexes on immobilized artificial membranes for local anesthetics: relationships with activity data on closed sodium channels. *Pharm. Res.* **14**: 1699-1705.
- Baricevic, D., S. Sosa, R. Della Loggia, A. Tubaro, B. Simonovska, A. Krasna, and A. Zupancic. 2001. Topical anti-inflammatory activity of *Salvia officinalis* L. leaves: the relevance of ursolic acid. *J. Ethnopharmacol.* **75**: 125-132.
- Barthomeuf, C., E. Debiton, V. Mshvildadze, E. Kemertelidze, and G. Balansard. 2002. *In vitro* activity of hederacolchisid A1 compared with other saponins from *Hedera colchica* against proliferation of human carcinoma and melanoma

- cells. *Plant. Med.* **68**: 672-675.
- Bedir, E., H. Kirmizipekmez, O. Sticher, and I. Calis. 2000. Triterpene saponins from the fruits of *hedera helix*. *Phytochemistry* **53**: 905-909.
- Begum, S., I. Sultana, B.S. Siddiqui, F. Shaheen, and A.H. Gilani. 2002. Structure and spasmolytic activity of eucalyptanoic acid from *Eucalyptus camaldulensis* var. *obtusata* and synthesis of its active derivative from oleanolic acid. *J. Nat. Prod.* **65**: 1939-1941.
- Bischoff, H. 1994. Pharmacology of alpha-glucosidase inhibition. *Eur. J. Clin. Invest.* **24**: 3-10.
- Caldwell, C.G., S.G. Franzblau, E. Suarez, and B.N. Timmermann. 2000. Oleanane triterpenes from *junellia tridens*. *J. Nat. Prod.* **63**: 1611-1614.
- Camacho, M.R., R. Mata, P. Castaneda, G.C. Kirby, D.C. Warhurst, S.L. Croft, and J.D. Phillipson. 2000. Bioactive compounds from *Celaenodendron mexicanum*. *Plant. Med.* **66**: 463-468.
- Chaturvedula, V.S.P., Z.J. Gao, S.M. Hecht, S.H. Jones, and D.G.I. Kingston. 2003a. A new acylated oleanane triterpenoid from *couepia polyandra* that inhibits the lyase activity of DNA polymerase  $\beta$ . *J. Nat. Prod.* **66**: 1463-1465.
- Chaturvedula, V.S.P., J.K. Schilling, J.S. Miller, R. Andriantsiferana, V.E. Rasamison, and D.G.I. Kingston. 2003b. New cytotoxic oleanane saponins from the infructescences of *polyscias amplifolia* from the madagascar rainforest. *Plant. Med.* **69**: 440-444.
- Chen, G., H.W. Lu, C.L. Wang, K. Yamashita, M. Manabe, S.X. Xu, and H. Kodama. 2002. Effect of five triterpenoid compounds isolated from leaves of *diospyros kaki* on stimulus-induced superoxide generation and tyrosyl phosphorylation in human polymorphonuclear leukocytes. *Clin. Chim. Acta* **320**: 11-16.
- Chiang, L.C., W. Chiang, M.Y. Chang, L.T. Ng, and C.C. Lin. 2003. Antileukemic activity of selected natural products in Taiwan. *Am. J. Chin. Med.* **31**(1): 37-46.
- Cunha, W.R., C. Martins, D.S. Ferreira, A.E.M. Crotti, N.P. Lopes, and S. Albuquerque. 2003. *In vitro* trypanocidal activity of triterpenes from *miconia* species. *Plant. Med.* **69**: 470-472.
- Deng, J.Z., S.R. Starck, and S.M. Hecht. 1999. DNA polymerase  $\beta$  inhibitors from *baeckea gunniana*. *J. Nat. Prod.* **62**: 1624-1626.
- Ekabo, O.A. and N.R. Farnsworth. 1996. Antifungal and molluscicidal saponins from *serjania salzmänniana*. *J. Nat. Prod.* **59**(4): 431-435.
- Favaloro, F.G., Jr., T. Honda, Y. Honda, G.W. Gribble, N. Suh, R. Risingsong, and M.B. Sporn. 2002. Design and synthesis of tricyclic compounds with enone functionalities in rings A and C: A novel class of highly active inhibitors of nitric oxide production in mouse macrophages. *J. Med. Chem.* **45**(22): 4801-4805.
- Finlay, H.J., T. Honda, G.W. Gribble, D. David, E.B. Nicole, N. Suh, C. Williams, and M.B. Sporn. 1997. Novel A-ring cleaved analogs of oleanolic acids and ursolic acids which affect growth regulation in NRP.152 prostate cells. *Bioorg. Med. Chem. Lett.* **7**(13): 1769-1772.
- Fujimura, H., J. Yamahara, M. Yoshikawa, and T. Yabuchi. 1996. Saccharide Sbsorption Inhibitor. JP 08040912 A2 13 Feb Heisei, Jpn. Kokai Tokkyo Koho., 5 pp.
- Gaidi, G., M. Correia, B. Chauffert, J.L. Beltramo, H. Wagner, and M.A. Lacaille-Dubois. 2002. Saponins-mediated potentiation of cisplatin accumulation and cytotoxicity in human colon cancer cells. *Plant. Med.* **68**: 70-72.
- Gerhäuser, C., K. Klimo, E. Heiss, I. Neumann, A. Gamal-Eldeen, J. Knauff, G.Y. Liu, S. Sitthimonchai, and N. Frank. 2003. Mechanism-based *in vitro* screening of potential cancer chemopreventive agents. *Mutat. Res.* **523-524**: 163-172.
- Hamburger, M., U. Riese, H. Graf, M.F. Melzig, S. Ciesielski, D. Baumann, K. Dittmann, and C. Wegner. 2002. Constituents in evening primrose oil with radical scavenging, cyclooxygenase, and neutrophil elastase inhibitory activities. *J. Agric. Food Chem.* **50**: 5533-5538.
- Hamburger, Matthias. Ger. Offen. 2003. Antiinflammatory or Pre-neoplastic Lesion Inhibiting Medicaments Containing Caffeic Acid Triterpene or Sterol Esters Having Radical Scavenging Action, Also Useful In Cosmetic Or Nutraceutical Compositions. DE 10215055 A1 30 Oct., 20 pp.
- Honda, T., H.J. Finlay, G.W. Gribble, N. Suh, and M.B. Sporn. 1997. New enone derivatives of oleanolic acid and ursolic acid as inhibitors of nitric oxide production in mouse macrophages. *Bioorg. Med. Chem. Lett.* **7**: 1623-1628.
- Honda, T., B.V. Rounds, L. Bore, F.G. Jr. Favaloro, G.W. Gribble, N. Suh, Y.P. Wang, and M.B. Sporn. 1999. Novel synthetic oleanane triterpenoids: A series of highly active inhibitors of nitric oxide production in mouse macrophages. *Bioorg. Med. Chem. Lett.* **9**: 3429-3434.
- Honda, T., G.W. Gribble, N. Suh, H.J. Finlay, B.V. Rounds, L. Bore, F.G. Jr. Favaloro, Y.P. Wang, and M.B. Sporn. 2000a. Novel synthetic oleanane and ursane triterpenoids with various enone functionalities in ring A as inhibitors of nitric oxide production in mouse macrophages. *J. Med. Chem.* **43**: 1866-1877.
- Honda, T., B.V. Rounds, L. Bore, H.J. Finlay, F.G. Jr. Favaloro, N. Suh, Y.P. Wang, M.B. Sporn, and G.W. Gribble. 2000b. Synthetic oleanane and ursane triterpenoids with modified rings A and C: A series of highly active inhibitors of nitric oxide production in mouse macrophages. *J. Med. Chem.* **43**: 4233-4246.
- Honda, T., Y. Honda, F.G. Jr. Favaloro, G.W. Gribble, N. Suh, A.E. Place, M.H. Rendi, and M.B. Sporn. 2002. A novel dicyanotriterpenoid, 2-cyano-3, 12-dioxooleana-1, 9(11)-dien-28-onitrile, active at picomolar concentrations for inhibition of nitric oxide production. *Bioorg. Med. Chem. Lett.* **12**: 1027-1030.
- Hu, M., K. Konoki, and K. Tachibana. 1996. Cholesterol-independent membrane disruption caused by triterpenoid

- saponins. *Biochim. Biophys. Acta.* **1299**: 252-258.
- Huang, M.T., C.T. Ho, Z.Y. Wang, T. Ferraro, Y.R. Lou, K. Stauber, W. Ma, C. Georgiadis, J.D. Laskin, and A.H. Conney. 1994. Inhibition of skin tumorigenesis by rosemary and its constituents carnosol and ursolic acid. *Cancer Res.* **54**: 701-708.
- Ismaili, H., S. Sosa, D. Brkic, S. Fkih-Tetouani, A. Ilidrissi, D. Touati, R.P. Aquino, and A. Tubaro. 2002. Topical anti-inflammatory activity of extracts and compounds from *Thymus broussonetii*. *J. Pharm. Pharmacol.* **54**: 1137-1140.
- Ismaili, H., S. Tortora, S. Fkih-Tetouami, A. Ilidrissi, R. Della Loggia, A. Tubaro, and R. Aquino. 2001. Topical anti-inflammatory activity of thymus willdenowii. *J. Pharm. Pharmacol.* **53**: 1645-1652.
- Joseph, D.C. and A.H. Robert. 1999. Triterpenoids. *Nat. Prod. Rep.* **16**: 221-240.
- Joseph, D.C. and A.H. Robert. 2000. Triterpenoids. *Nat. Prod. Rep.* **17**: 463-482.
- Joseph, D.C. and A.H. Robert. 2001a. Triterpenoids. *Nat. Prod. Rep.* **18**: 131-147.
- Joseph, D.C. and A.H. Robert. 2001b. Triterpenoids. *Nat. Prod. Rep.* **18**: 560-578.
- Joseph, D.C. and A.H. Robert. 2002. Triterpenoids. *Nat. Prod. Rep.* **19**: 494-513.
- Joseph, D.C. and A.H. Robert. 2003. Triterpenoids. *Nat. Prod. Rep.* **20**: 640-659.
- Joseph, D.C. and A.H. Robert. 2005a. Triterpenoids. *Nat. Prod. Rep.* **22**: 230-248.
- Joseph, D.C. and A.H. Robert. 2005b. Triterpenoids. *Nat. Prod. Rep.* **22**: 487-503.
- Kashiwada, Y., H.K. Wang, T. Nagao, S. Kitanaka, I. Yasuda, T. Fujioka, T. Yamagishi, L.M. Cosentino, M. Kozuka, H. Okabe, Y. Ikeshiro, C.Q. Hu, E. Yeh, and K.H. Lee. 1998. Anti-AIDS agents. 30. anti-HIV activity of oleanolic acid, pomolic acid, and structurally related triterpenoids. *J. Nat. Prod.* **61**: 1090-1095.
- Kashiwada, Y., T. Nagao, A. Hashimoto, Y. Ikeshiro, H. Okabe, L.M. Cosentino, and K.H. Lee. 2000. Anti-AIDS agents 38. anti-HIV activity of 3-*o*-acyl ursolic acid derivatives. *J. Nat. Prod.* **63**: 1619-1622.
- Kim, Y.K., S.K. Yoon, and S.Y. Ryu. 2000. Cytotoxic triterpenes from stem bark of *Physocarpus intermedius*. *Plant. Med.* **66**: 485-486.
- Kim, Y.P., E.B. Lee, S.Y. Kim, D. Li, H.S. Ban, S.S. Lim, K.H. Shin, and K. Ohuchi. 2001. Inhibition of prostaglandin E2 production by platycodin D isolated from the root of *Platycodon grandiflorum*. *Plant. Med.* **67**: 362-364.
- Kinjo, J., K. Yokomizo, T. Hirakawa, Y. Shii, T. Nohara, and M. Uyeda. 2000. Anti-herpes virus activity of fabaceous triterpenoidal saponins. *Biol. Pharm. Bull.* **23**(7): 887-889.
- Konoshima, T., I. Yasuda, Y. Kashiwada, L.M. Cosentino, and K.H. Lee. 1995. Anti-aids agents, 21. triterpenoid saponins as anti-HIV principles from fruits of *Gleditsia japonica* and *Gymnocladus chinensis*, and a structure-activity correlation. *J. Nat. Prod.* **58**(9): 1372-1377.
- Kubo, M., H. Matsuda, Y. Dai, Y. Ido, and M. Yoshikawa. 1997. Studies on *Kochia fructus*. I. antipruritogenic effect of 70% ethanol extract from *Kochia fructus* and its active component. *Yakugaku Zasshi.* **117**(4): 193-201.
- Lee, H.Y., H.Y. Chung, K.H. Kim, J.J. Lee, and K.W. Kim. 1994. Induction of differentiation in the cultured F9 teratocarcinoma stem cells by triterpene acids. *J. Cancer Res. Clin. Oncol.* **120**: 513-518.
- Lee, M.H. and Y.N. Han. 2003. A new in vitro tissue factor inhibitory triterpene from the fruits of *Chaenomeles sinensis*. *Plant. Med.* **69**: 327-331.
- Liu, J. 1995. Pharmacology of oleanolic acid and ursolic acid. *J. Ethnopharmacol.* **49**: 57-68.
- Liu, J., Y.P. Liu, Q. Mao, and C.D. Klaassen. 1994. The effects of 10 triterpenoid compounds on experimental liver injury in mice. *Fund. Appl. Toxicol.* **22**: 34-40.
- Liu, Y.L. and H.S. Wang. 1993. The effects of oleanolic acid on some functions of blood platelet aggregation in old mice. *J. Shenyang College Pharm.* **10**(4): 275-278.
- Lu, J.C., Q.S. Sun, K. Sugahara, Y. Sagara, and H. Kodama. 2001. Effect of six compounds isolated from rhizome of *Anemone raddeana* on the superoxide generation in human neutrophil. *Biochem. Biophys. Res. Commun.* **280**: 918-922.
- Ma, C.M., N. Nakamura, and M. Hattori. 2000. Chemical modification of oleanene type triterpenes and their inhibitory activity against HIV-1 protease dimerization. *Chem. Pharm. Bull.* **48**(11): 1681-1688.
- Ma, C.M., N. Nakamura, H. Miyashiro, M. Hattori, and K. Shimotohno. 1999. Inhibitory effects of constituents from *Cynomorium songaricum* and related triterpene derivatives on HIV-1 protease. *Chem. Pharm. Bull.* **47**(2): 141-145.
- Ma, C.M., N. Nakamura, M. Hattori, and S.Q. Cai. 2002. Isolation of malonyl oleanolic acid hemiester as anti-HIV protease substance from the stems of *Cynomorium songaricum*. *Chin. Pharm. J.* **37**(5): 336-338.
- Mallavadhani, U.V., A. Mahapatra, S.S. Raja, and C. Manjula. 2003. Antifeedant activity of some pentacyclic triterpene acids and their fatty acid ester analogues. *J. Agric. Food Chem.* **51**: 1952-1955.
- Marquina, S., N. Maldonado, M.L. Garduno-Ramirez, E. Aranda, M.L. Villarreal, V. Navarro, R. Bye, G. Delgado, and L. Alvarez. 2001. Bioactive oleanolic acid saponins and other constituents from the roots of *Viguiera decurrens*. *Phytochemistry* **56**: 93-97.
- Matsuda, H., Y. Dai, Y. Ido, T. Murakami, H. Matsuda, M. Yoshikawa, and M. Kubo. 1998a. Studies on *Kochia fructus*. V. Antipruritic effects of oleanolic acid glycosides and the structure-requirement. *Biol. Pharm. Bull.* **21**(11): 1231-1233.
- Matsuda, H., Y.H. Li, T. Murakami, J. Yamahara, and M. Yoshikawa. 1998b. Protective effects of oleanolic acid oligoglycosides on ethanol- or indomethacin-induced

- gastric mucosal lesions in rats. *Life Sci.* **63(17)**: PL245-250.
- Matsuda, H., Y.H. Li, T. Murakami, N. Murakami, J. Yamahara, and M. Yoshikawa. 1998c. Antidiabetic principles of natural medicines. III. Structure-related inhibitory activity and action mode of oleanolic acid glycosides on hypoglycemic activity. *Chem. Pharm. Bull.* **46(9)**: 1399-1403.
- Matsuda, H., Y.H. Li, T. Murakami, J. Yamahara, and M. Yoshikawa. 1999. Structure-related inhibitory activity of oleanolic acid glycosides on gastric emptying in mice. *Bioorg. Med. Chem.* **7**: 323-327.
- Mimaki, Y., M. Kuroda, T. Asano, and Y. Sashida. 1999. Triterpene saponins and lignans from the roots of *pulsatilla chinensis* and their cytotoxic activity against HL-60 cells. *J. Nat. Prod.* **62**: 1279-1283.
- Mitaine-Offier, A.C., W. Hornebeck, M. Sauvain, and M. Zèches-Hanrot. 2002. Triterpenes and phytosterols as human leukocyte elastase inhibitors. *Plant. Med.* **68**: 930-932.
- Miura, N., Y. Matsumoto, S. Miyairi, S. Nishiyama, and A. Naganuma. 1999. Protective effects of triterpene compounds against the cytotoxicity of cadmium in HepG2 cells. *Mol. Pharmacol.* **56**: 1324-1328.
- Mizushina, Y., A. Iida, K. Ohta, F. Sugawara, and K. Sakaguchi. 2000. Novel triterpenoids inhibit both DNA polymerase and DNA topoisomerase. *Biochem. J.* **350**: 757-763.
- Murphy, B.T., S.L. Mackinnon, X.J. Yan, G.B. Hammond, A.J. Vaisberg, and C.C. Neto. 2003. Identification of triterpene hydroxycinnamates with *in vitro* antitumor activity from whole cranberry fruit (*Vaccinium macrocarpon*). *J. Agric. Food Chem.* **51**: 3541-3545.
- Nakashima, H., K. Ohubo, Y. Honda, T. Tamura, S. Matsuda, and N. Yamamoto. 1989. Inhibitory effect of glycosides like saponin from soybean on infectivity of HIV *in vitro*. *AIDS* **3**: 655-658.
- Narayan, S., F. He, and S.H. Wilso. 1996. Activation of the human DNA polymerase beta promoter by a DNA-alkylating agent through induced phosphorylation of cAMP response element-binding protein-1. *J. Biol. Chem.* **271**: 18508-18513.
- Neto, C.C., A.J. Vaisberg, B.N. Zhou, D.G. Kingston, and G.B. Hammond. 2000. Cytotoxic triterpene acids from the peruvian medicinal plant *polylepis racemosa*. *Plant. Med.* **66**: 483-484.
- Nick, A., A.D. Wright, and O. Sticher. 1994. Antibacterial triterpenoid acids from *dillenia papuana*. *J. Nat. Prod.* **57(9)**: 1245-1250.
- Ohshima, H. and H. Bartsch. 1994. Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. *Mutat. Res.* **305**: 253-264.
- Qu, R.Y. 1981. Oleanolic acid in treating virus hepatitis. *Guangzhou Med. J.* **3**: 41-43.
- Recio, M.C., R.M. Giner, S. Máñez, and J.L. Ríos. 1995. Structural requirements for the anti-inflammatory activity of natural triterpenoids. *Plant. Med.* **61**: 182-185.
- Ringbom, T., L. Segura, Y. Noreen, P. Perera, and L. Bohlin. 1998. Ursolic acid from *Plantago major*, a selective inhibitor of cyclooxygenase-2 catalyzed prostaglandin biosynthesis. *J. Nat. Prod.* **61**: 1212-1215.
- Rios, J.L., M.C. Recio, S. Manez, and R.M. Giner. 2000. Bioactive natural products, Part C. Elsevier. *Stud. Nat. Prod. Chem.* **22**: 93-143.
- Rios, M.Y., A. González-Morales, and M.L. Villarreal. 2001. Sterols, triterpenes and biflavonoids of *Viburnum juncundum* and cytotoxic activity of ursolic acid. *Plant. Med.* **67**: 683-684.
- Safayhi, H. and E.R. Sailer. 1997. Anti-inflammatory actions of pentacyclic triterpenes. *Plant. Med.* **63**: 487-493.
- Safayhi, H., B. Rall, E.R. Sailer, and H.P.T. Ammon. 1997. Inhibition by boswellic acids of human leukocyte elastase. *J. Pharmacol. Exp. Ther.* **281**: 460-463.
- Sailer, E.R., L.R. Subramanian, B. Rall, R.F. Hoernlein, H.P.T. Ammon, and H. Safayhi. 1996. Acetyl-11-keto-beta-boswellic acid (AKBA): structure requirements for binding and 5-lipoxygenase inhibitory activity. *Br. J. Pharmacol.* **117**: 615-618.
- Seebacher, W., R. Weis, J. Jurenitsch, K. Rauchensteiner, and E. Haslinger. 2000. Synthesis and hemolytic properties of arvensoside B isomers. *Monatshefte für Chemie.* **131**: 985-996.
- Seebacher, W., R. Weis, J. Jurenitsch, K. Rauchensteiner, and E. Haslinger. 1999. Synthesis and hemolytic activity of oleanolic acid trisaccharides. *Monatshefte für Chemie.* **130**: 1383-1391.
- Seo, Y., J. Hoch, M. Abdel-Kader, S. Malone, I. Derveld, H. Adams, M.C.M. Werkhoven, J.H. Wisse, S.W. Mamber, J.M. Dalton, and D.G.I. Kingston. 2002. Bioactive saponins from *scacia tenuifolia* from the suriname rainforest. *J. Nat. Prod.* **65**: 170-174.
- Setzer, W.N. and M.C. Setzer. 2003. Plant-derived triterpenoids as potential antineoplastic agents. *Mini Rev. Med. Chem.* **3(6)**: 540-556.
- Sohn, K.H., H.Y. Lee, H.Y. Chung, H.S. Young, S.Y. Yi, and K.W. Kim. 1995. Anti-angiogenic activity of triterpene acids. *Cancer Lett.* **94(2)**: 213-218.
- Suh, N., T. Honda, H.J. Finlay, A. Barchowsky, C. Williams, N.E. Benoit, J.W. Xie, C. Nathan, G.W. Gribble, and M.B. Sporn. 1998. Novel triterpenoids suppress inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase (COX-2) in mouse macrophages. *Cancer Res.* **58**: 717-723.
- Suh, N., Y.P. Wang, T. Honda, G.W. Gribble, E. Dmitrovsky, W.F. Hickey, R.A. Maue, A.E. Place, D.M. Porter, M.J. Spinella, C.R. Williams, G.F. Wu, A.J. Dannenberg, K.C. Flanders, J.J. Letterio, D.J. Mangelsdorf, C.F. Nathan, L. Nguyen, W.W. Porter, R.F. Ren, A.B. Roberts, N.S. Roche, K. Subbaramaiah, and M.B. Sporn. 1999. A novel synthetic oleanane triterpenoid, 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid, with potent differentiating, antiproliferative, and anti-inflammatory activity. *Cancer Res.* **59**: 336-341.
- Sun, J.S., X.W. Han, and B. Yu. 2003. Synthesis of a typical N-acetylglucosamine-containing saponin, oleanolic acid

- 3-yl  $\alpha$ -l-arabinopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -l-arabinopyranosyl-(1 $\rightarrow$ 6)-2-acetamido-2-deoxy- $\beta$ -d-glucopyranoside. Carbohydr. Res. **338**: 827-833.
- Syrovets, T., B. Büchele, E. Gedig, J.R. Slupsky, and T. Simmet. 2000. Acetyl-boswellic acids are novel catalytic inhibitors of human topoisomerases I and II $\alpha$ . Mol. Pharmacol. **58**: 71-81.
- Taniguchi, S., Y. Imayoshi, E. Kabayashi, Y. Takamatsu, H. Ito, T. Hatano, H. Sakagami, H. Tokuda, H. Nishino, D. Sugita, S. Shimura, and T. Yoshida. 2002. Production of bioactive triterpenes by *Eriobotrya japonica* calli. Phytochemistry **59**: 315-323.
- Tokuda, H., H. Ohigashi, K. Koshimizu, and I. Yohei. 1986. Inhibitory effects of ursolic and oleanolic acid on skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate. Cancer Lett. **33**: 279-285.
- Ullah, N., W. Seebacher, R. Weis, J. Jurenitsch, K. Rauchensteiner, and E. Haslinger. 2000. Synthesis and hemolytic properties of glycyrrhetic acid glycosides. Monatshefte für Chemie. **131**: 787-794.
- Ullah, N., W. Seebacher, E. Haslinger, J. Jurenitsch, K. Rauchensteiner, and R. Weis. 2002. Synthesis and hemolytic properties of lactosides of glycyrrhetic acid derivatives. Monatshefte für Chemie. **133**: 139-150.
- Wan, L., X.F. Chen, and Z.H. Jiang. 1998. The protective effects of OLA-PENa on experimental liver injury in rats. Chin. Pharm. J. **33**(2): 80-83.
- Wang, S.P., J.S. Lai, and K.F. Huang. 1993. Studies on the constituents of the leaves of alyxia insularis kanehira & sasaki. Chin. Pharm. J. **45**(4): 329-336.
- Weimann, C., U. Göransson, U. Pongprayoon-Claeson, P. Claeson, L. Bohlin, H. Rimpler, and M. Heinrich. 2002. Spasmolytic effects of baccharis conferta and some of its constituents. J. Pharm. Pharmacol. **54**: 99-104.
- Woldemichael, G.M., S.G. Franzblau, F.Q. Zhang, Y.H. Wang, and B.N. Timmermann. 2003a. Inhibitory effect of sterols from ruprechtia triflora and diterpenes from calceolaria pinnifolia on the growth of mycobacterium tuberculosis. Plant. Med. **69**: 628-631.
- Woldemichael, G.W., M.P. Singh, W.M. Maiese, and B.N. Timmermann. 2003b. Constituents of antibacterial extract of *Caesalpinia paraguariensis* Burk. Z. Naturforsch. **58c**: 70-75.
- Ying, Q.L., A.R. Rinchart, S.R. Simon, and J.C. Cheronis. 1991. Inhibition of human leucocyte elastase by ursolic acid. Evidence for a binding site for pentacyclic triterpenes. Biochem. J. **277**: 521-526.
- Yoshikawa, M., E. Harada, H. Matsuda, T. Murakami, J. Yamahara, and N. Murakami. 1993. Elatosides A and B, potent inhibitors of ethanol absorption in rats from the bark of *Aralia elata* Seem.: the structure-activity relationships of oleanolic acid oligoglycosides. Chem. Pharm. Bull. **41**(11): 2069-2071.
- Yoshikawa, M., H. Matsuda, E. Harada, T. Murakami, N. Wariishi, J. Yamahara, and N. Murakami. 1994. Elatoside E, a new hypoglycemic principle from the root cortex of *Aralia elata* Seem.: structure-related hypoglycemic activity of oleanolic acid glycosides. Chem. Pharm. Bull. **42**(6): 1354-1356.
- Yoshikawa, M., T. Murakami, E. Harada, N. Murakami, J. Yamahara, and H. Matsuda. 1996a. Bioactive saponins and glycosides. VII. On the hypoglycemic principles from the root cortex of *Aralia elata* Seem.: structure related hypoglycemic activity of oleanolic acid oligoglycoside. Chem. Pharm. Bull. **44**(10): 1923-1927.
- Yoshikawa, M., T. Murakami, E. Harada, N. Murakami, J. Yamahara, and H. Matsuda. 1996b. Bioactive saponins and glycosides. VI. Elatosides A and B, potent inhibitors of ethanol absorption, from the bark of *Aralia elata* Seem. (Araliaceae): the structure-requirement in oleanolic acid glucuronide-saponins for the inhibitory activity. Chem. Pharm. Bull. **44**(10): 1915-1922.
- Yoshikawa, M., T. Murakami, M. Kadoya, H. Matsuda, O. Muraoka, J. Yamahara, and N. Murakami. 1996c. Medicinal foodstuff. III. Sugar beet. (1): Hypoglycemic oleanolic acid oligoglycosides, betavulgarosides I, II, III, and IV, from the root of *Beta vulgaris* L. (Chenopodiaceae). Chem. Pharm. Bull. **44**(6): 1212-1217.
- Zhang, W.D., W.Q. Wang, Z.Q. Qin, B.L. Wang, and J.Q. Zhou. 2000. Caryophyllin acid sodium and application in pharmacy thereof. Faming Zhuanli Shenqing Gongkai Shuomingshu. CN 1248583 A 29 Mar, 6 pp.
- Zhang, Y.J., B. Jayaprakasam, N.P. Seeram, L.K. Olson, D. Dewitt, and M.G. Nair. 2004. Insulin secretion and cyclooxygenase enzyme inhibition by cabernet sauvignon grape skin compounds. J. Agric. Food Chem. **52**: 228-233.
- Zhu, Y.M., J.K. Shen, H.K. Wang, L.M. Cosentino, and K.H. Lee. 2001. Synthesis and anti-HIV activity of oleanolic acid derivatives. Bioorg. Med. Chem. Lett. **11**: 3115-3118.

## Novel Synthetic Oleanane and Ursane Triterpenoids with Various Enone Functionalities in Ring A as Inhibitors of Nitric Oxide Production in Mouse Macrophages<sup>†</sup>

Tadashi Honda,<sup>‡</sup> Gordon W. Gribble,<sup>\*,‡</sup> Nanjoo Suh,<sup>§</sup> Heather J. Finlay,<sup>‡,||</sup> BarbieAnn V. Rounds,<sup>‡</sup> Lothar Bore,<sup>‡</sup> Frank G. Favaloro, Jr.,<sup>‡</sup> Yongping Wang,<sup>§</sup> and Michael B. Sporn<sup>\*,§</sup>

Department of Chemistry, Dartmouth College, and Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, New Hampshire 03755

Received January 7, 2000

We initially randomly synthesized about 60 oleanane and ursane triterpenoids as potential anti-inflammatory and cancer chemopreventive agents. Preliminary screening of these derivatives for inhibition of production of nitric oxide induced by interferon- $\gamma$  in mouse macrophages revealed that 3-oxooleana-1,12-dien-28-oic acid (**B-15**) showed significant activity ( $IC_{50} = 5.6 \mu M$ ). On the basis of the structure of **B-15**, 19 novel olean- and urs-12-ene triterpenoids with a 1-en-3-one functionality having a substituent at C-2 in ring A have been designed and synthesized. Among them, 3-oxooleana-1,12-diene derivatives with carboxyl, methoxycarbonyl, and nitrile groups at C-2 showed higher activity than the lead compound **B-15**. In particular, 2-carboxy-3-oxooleana-1,12-dien-28-oic acid (**3**) had the highest activity ( $IC_{50} = 0.07 \mu M$ ) in this group of triterpenoids. The potency of **3** was similar to that of hydrocortisone ( $IC_{50} = 0.01 \mu M$ ), although **3** does not act through the glucocorticoid receptor. Interesting structure–activity relationships of these novel synthetic triterpenoids are also discussed.

### Introduction

Oleanane and ursane triterpenoids are pentacyclic compounds with 30 carbon atoms, which are derived biosynthetically by the cyclization of squalene.<sup>1</sup> The group includes a very large number of naturally occurring members that cover an impressive variety of functional groups.<sup>2</sup> Many compounds of this group are reported to have interesting biological, pharmacological, or medicinal activities similar to those of retinoids and steroids, such as anti-inflammatory activity, suppression of tumor promotion, suppression of immunoglobulin synthesis, protection of the liver against toxic injury, induction of collagen synthesis, and induction of differentiation in leukemia or teratocarcinoma cells.<sup>3</sup> However, the potency of these triterpenoids is relatively weak. There are no systematic studies of structure–activity relationships based on chemical modification of oleanane and ursane triterpenoids.<sup>4</sup> We have therefore considered that bioassay-directed systematic drug design and synthesis of derivatives of oleanolic acid (**1**) and ursolic acid (**2**), which are commercially available, could be of great value in discovering novel structures with high biological potency.

The high output of nitric oxide (NO) produced by inducible nitric oxide synthase (iNOS), which is expressed in activated macrophages, plays an important role in host defense. However, excessive production of NO also can destroy functional normal tissues during acute and chronic inflammation.<sup>5</sup> This phenomenon is also closely related mechanistically to carcinogenesis.<sup>6</sup> Thus, inhibitors of NO production in macrophages are potential anti-inflammatory and cancer chemopreventive drugs. Because oleanolic and ursolic acids are already known to have weak anti-inflammatory and anticarcinogenic activity,<sup>3a,3b,3e,3f</sup> we focused our attention on therapeutic agents of these diseases. For this purpose, we have adopted an assay system that measures inhibition of NO production induced by interferon- $\gamma$  (IFN- $\gamma$ ) in mouse macrophages<sup>7</sup> as a preliminary screening assay system. We synthesized various oleanolic and ursolic acid derivatives and tested them as inhibitors of NO production. As a result, we have identified a series of novel olean-12-ene triterpenoids with a 1-en-3-one functionality having carboxyl, methoxycarbonyl, and nitrile groups at C-2 in ring A that show significant inhibitory activity ( $IC_{50} = 0.01$ – $0.1 \mu M$  level) against production of NO induced by IFN- $\gamma$  in mouse macrophages. In particular, 2-carboxy-3-oxooleana-1,12-dien-28-oic acid (**3**) had the highest activity ( $IC_{50} = 0.07 \mu M$ ) in this group of compounds. The potency of **3** was similar to that of hydrocortisone ( $IC_{50} = 0.01 \mu M$ ), although **3** does not act through the glucocorticoid receptor. We report here the synthesis, inhibitory activity, and structure–activity relationships of these novel triterpenoids in detail.

### Chemistry

**Discovery of Lead Compound.** When we started this project, we had no information about a lead

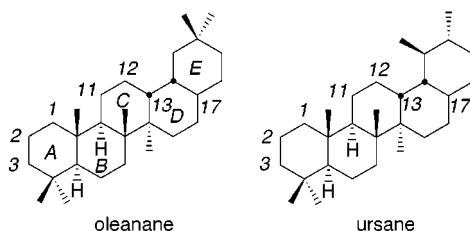
<sup>†</sup> Part of this work has been reported in preliminary form: (a) Honda, T.; Finlay, H. J.; Gribble, G. W.; Suh, N.; Sporn, M. B. New enone derivatives of oleanolic acid and ursolic acid as inhibitors of nitric oxide production in mouse macrophages. *Bioorg. Med. Chem. Lett.* **1997**, 7, 1623–1628. (b) Honda, T.; Rounds, B. V.; Bore, L.; Favaloro, F. G., Jr.; Gribble, G. W.; Suh, N.; Wang, Y.; Sporn, M. B. Novel synthetic oleanane triterpenoids: a series of highly active inhibitors of nitric oxide production in mouse macrophages. *Bioorg. Med. Chem. Lett.* **1999**, 9, 3429–3434.

\* Address correspondence to either author. For G.W.G.: phone, 603-646-3118; fax, 603-646-3946; e-mail, Grib@dartmouth.edu. For M.B.S.: phone, 603-650-6557; fax, 603-650-1129; e-mail, Michael.Sporn@dartmouth.edu.

<sup>‡</sup> Department of Chemistry.

<sup>§</sup> Department of Pharmacology and Toxicology

<sup>||</sup> Present address: The Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 5400, Princeton, NJ 08543-5400.

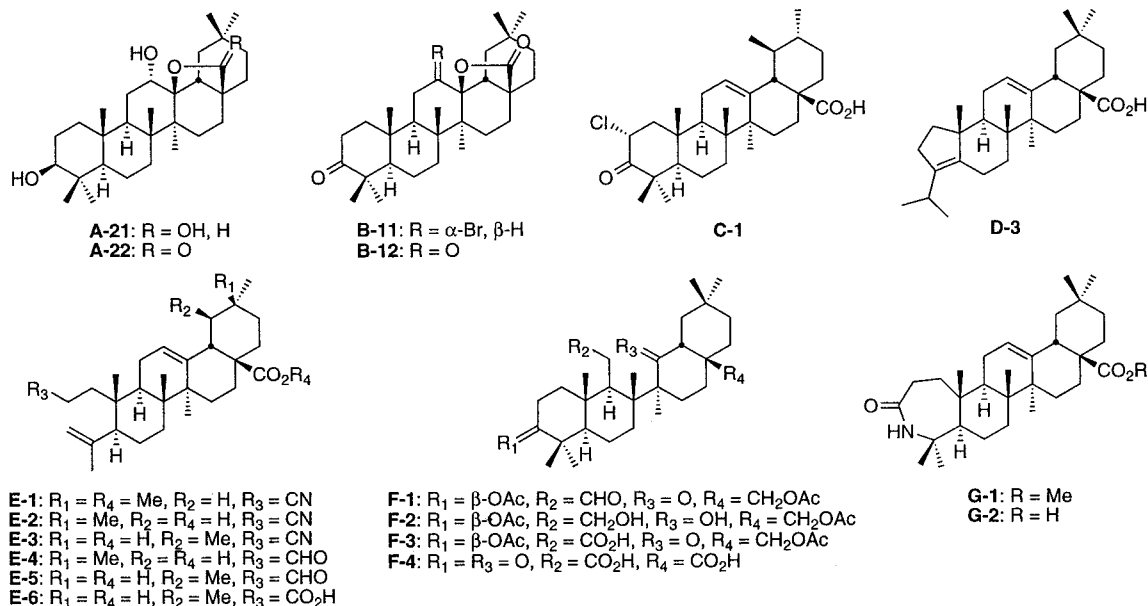
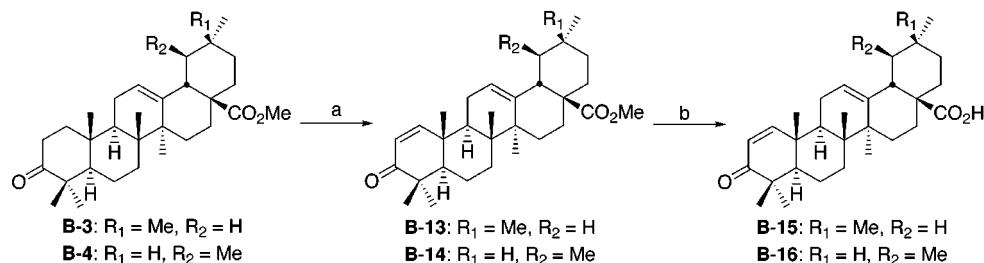
**Table 1.** Preliminary Screening Results of Synthetic Oleanane and Ursane Triterpenoids

| compd                   | skeleton                    | C-3          | C-12                             | C-13        | C-17                | inhibition (%)<br>at 10 $\mu$ M <sup>b</sup> | ref |
|-------------------------|-----------------------------|--------------|----------------------------------|-------------|---------------------|----------------------------------------------|-----|
| <b>1</b>                | olean-12-ene                | $\beta$ -OH  | H                                |             | CO <sub>2</sub> H   | 38                                           | 10  |
| <b>2</b>                | urs-12-ene                  | $\beta$ -OH  | H                                |             | CO <sub>2</sub> H   | 0                                            | 15  |
| <b>A-1</b>              | olean-12-ene                | $\beta$ -OH  | H                                |             | CO <sub>2</sub> Me  | 0                                            | 10  |
| <b>A-2</b>              | urs-12-ene                  | $\beta$ -OH  | H                                |             | CO <sub>2</sub> Me  | 0                                            | 15  |
| <b>A-3</b>              | olean-12-ene                | $\beta$ -OAc | H                                |             | CO <sub>2</sub> Me  | 10                                           | 10  |
| <b>A-4</b>              | urs-12-ene                  | $\beta$ -OAc | H                                |             | CO <sub>2</sub> Me  | 15                                           | 15  |
| <b>A-5</b>              | olean-12-ene                | $\beta$ -OAc | H                                |             | CO <sub>2</sub> H   | 0                                            | 10  |
| <b>A-6</b>              | urs-12-ene                  | $\beta$ -OAc | H                                |             | CO <sub>2</sub> H   | 0                                            | 15  |
| <b>A-7</b>              | olean-12-ene                | $\beta$ -OH  | H                                |             | CH <sub>2</sub> OH  | 0                                            | 28  |
| <b>A-8</b>              | urs-12-ene                  | $\beta$ -OH  | H                                |             | CH <sub>2</sub> OH  | 8                                            | 29  |
| <b>A-9</b>              | olean-12-ene                | $\beta$ -OAc | H                                |             | CH <sub>2</sub> OAc | 4                                            | 28  |
| <b>A-10</b>             | urs-12-ene                  | $\beta$ -OAc | H                                |             | CH <sub>2</sub> OAc | 0                                            | 29  |
| <b>A-11</b>             | oleanane                    | $\beta$ -OAc | $\alpha$ -OH                     | $\beta$ -H  | CO <sub>2</sub> Me  | 0                                            | c   |
| <b>A-12</b>             | oleanane                    | $\beta$ -OAc | $\beta$ -OH                      | $\beta$ -H  | CO <sub>2</sub> Me  | 0                                            | c   |
| <b>A-13</b>             | oleanane                    | $\beta$ -OAc | $\beta$ -OAc                     | $\beta$ -H  | CH <sub>2</sub> OAc | 0                                            | c   |
| <b>A-14</b>             | oleanane                    | $\beta$ -OAc | $\alpha$ -OH                     | $\beta$ -H  | CH <sub>2</sub> OAc | 0                                            | c   |
| <b>A-15</b>             | oleanane                    | $\beta$ -OH  | $\alpha$ -OH                     | $\beta$ -H  | CH <sub>2</sub> OH  | 48                                           | c   |
| <b>A-16</b>             | oleanane                    | $\beta$ -OH  | $\beta$ -OH                      | $\beta$ -H  | CH <sub>2</sub> OH  | 20                                           | c   |
| <b>A-17</b>             | oleanane                    | $\beta$ -OH  | =O                               | $\beta$ -H  | CO <sub>2</sub> Me  | 0                                            | 10  |
| <b>A-18</b>             | oleanane                    | $\beta$ -OAc | =O                               | $\beta$ -H  | CO <sub>2</sub> Me  | 0                                            | 10  |
| <b>A-19</b>             | olean-12-ene                | $\alpha$ -OH | H                                |             | CO <sub>2</sub> H   | 18                                           | 30  |
| <b>A-20</b>             | urs-12-ene                  | $\alpha$ -OH | H                                |             | CO <sub>2</sub> H   | 48                                           | 31  |
| <b>A-21<sup>a</sup></b> | oleanane                    | $\beta$ -OH  | $\alpha$ -OH                     | —O—         | —CH(OH)—            | 21                                           | 32  |
| <b>A-22<sup>a</sup></b> | oleanane                    | $\beta$ -OH  | $\alpha$ -OH                     | —O—         | —CO—                | 13                                           | 32  |
| <b>A-23</b>             | oleanane                    | $\beta$ -OAc | 12 $\alpha$ ,13 $\alpha$ -epoxy- |             | CO <sub>2</sub> Me  | 0                                            | 10  |
| <b>A-24</b>             | oleanane                    | $\beta$ -OH  | $\alpha$ -OH                     | $\beta$ -OH | CH <sub>2</sub> OH  | 22                                           | 32  |
| <b>B-1</b>              | olean-12-ene                | =O           | H                                |             | CO <sub>2</sub> H   | 16                                           | 10  |
| <b>B-2</b>              | urs-12-ene                  | =O           | H                                |             | CO <sub>2</sub> H   | 22                                           | 33  |
| <b>B-3</b>              | olean-12-ene                | =O           | H                                |             | CO <sub>2</sub> Me  | 24                                           | 10  |
| <b>B-4</b>              | urs-12-ene                  | =O           | H                                |             | CO <sub>2</sub> Me  | 16                                           | 15  |
| <b>B-5</b>              | olean-12-ene                | =O           | H                                |             | CHO                 | 11                                           | 34  |
| <b>B-6</b>              | urs-12-ene                  | =O           | H                                |             | CHO                 | 21                                           | 34  |
| <b>B-7</b>              | oleana-11,13(18)-diene      | =O           | H                                |             | CO <sub>2</sub> H   | 47                                           | c   |
| <b>B-8</b>              | oleanane                    | =O           | =O                               | $\beta$ -H  | CO <sub>2</sub> Me  | 3                                            | 10  |
| <b>B-9</b>              | oleanane                    | =O           | =O                               | $\beta$ -H  | CO <sub>2</sub> H   | 37                                           | c   |
| <b>B-10</b>             | oleanane                    | =O           | =O                               | $\beta$ -H  | CHO                 | 38                                           | c   |
| <b>B-11<sup>a</sup></b> | oleanane                    | =O           | $\alpha$ -Br                     | —O—         | —CO—                | 4                                            | 10  |
| <b>B-12<sup>a</sup></b> | oleanane                    | =O           | =O                               | —O—         | —CO—                | 0                                            | 10  |
| <b>B-13</b>             | oleana-1,12-diene           | =O           | H                                |             | CO <sub>2</sub> Me  | 19                                           | 9   |
| <b>B-14</b>             | ursa-1,12-diene             | =O           | H                                |             | CO <sub>2</sub> Me  | 0                                            | d   |
| <b>B-15</b>             | oleana-1,12-diene           | =O           | H                                |             | CO <sub>2</sub> H   | 85                                           | d   |
| <b>B-16</b>             | ursa-1,12-diene             | =O           | H                                |             | CO <sub>2</sub> H   | 41                                           | 14  |
| <b>C-1<sup>a</sup></b>  | urs-12-ene                  | =O           | H                                |             | CO <sub>2</sub> H   | 55                                           | c   |
| <b>C-2</b>              | olean-12-ene                | $\alpha$ -Cl | H                                |             | CO <sub>2</sub> Me  | 2                                            | c   |
| <b>C-3</b>              | olean-12-ene                | $\alpha$ -Cl | H                                |             | CO <sub>2</sub> H   | 0                                            | c   |
| <b>D-1</b>              | oleana-2,12-diene           | H            | H                                |             | CO <sub>2</sub> Me  | 3                                            | 35  |
| <b>D-2</b>              | oleana-2,12-diene           | H            | H                                |             | CO <sub>2</sub> H   | 0                                            | c   |
| <b>D-3<sup>a</sup></b>  | olean-12-ene                |              | H                                |             | CO <sub>2</sub> H   | 0                                            | c   |
| <b>E-1<sup>a</sup></b>  | A-ring cleaved olean-12-ene |              | H                                |             | CO <sub>2</sub> Me  | 21                                           | 36  |
| <b>E-2<sup>a</sup></b>  | A-ring cleaved olean-12-ene |              | H                                |             | CO <sub>2</sub> H   | 33                                           | 37  |
| <b>E-3<sup>a</sup></b>  | A-ring cleaved urs-12-ene   |              | H                                |             | CO <sub>2</sub> H   | 39                                           | 37  |
| <b>E-4<sup>a</sup></b>  | A-ring cleaved olean-12-ene |              | H                                |             | CO <sub>2</sub> H   | 22                                           | 37  |
| <b>E-5<sup>a</sup></b>  | A-ring cleaved urs-12-ene   |              | H                                |             | CO <sub>2</sub> H   | 55                                           | 37  |
| <b>E-6<sup>a</sup></b>  | A-ring cleaved urs-12-ene   |              | H                                |             | CO <sub>2</sub> H   | 10                                           | 37  |
| <b>F-1<sup>a</sup></b>  | C-ring cleaved oleanane     | $\beta$ -OAc |                                  |             | CH <sub>2</sub> OAc | 52                                           | c   |
| <b>F-2<sup>a</sup></b>  | C-ring cleaved oleanane     | $\beta$ -OAc |                                  |             | CH <sub>2</sub> OAc | 12                                           | c   |
| <b>F-3<sup>a</sup></b>  | C-ring cleaved oleanane     | $\beta$ -OAc |                                  |             | CH <sub>2</sub> OAc | 52                                           | c   |
| <b>F-4<sup>a</sup></b>  | C-ring cleaved oleanane     | =O           |                                  |             | CO <sub>2</sub> H   | 28                                           | c   |

Table 1 (Continued)

| compd                   | skeleton     | C-3 | C-12 | C-13 | C-17               | inhibition (%)<br>at 10 $\mu$ M <sup>b</sup> | ref |
|-------------------------|--------------|-----|------|------|--------------------|----------------------------------------------|-----|
| <b>G-1</b> <sup>a</sup> | olean-12-ene |     | H    |      | CO <sub>2</sub> Me | 0                                            | 36  |
| <b>G-2</b> <sup>a</sup> | olean-12-ene |     | H    |      | CO <sub>2</sub> H  | 51                                           | 37  |
| hydrocortisone          |              |     |      |      |                    | 80                                           |     |

<sup>a</sup> Structure shown below this table. <sup>b</sup> Details of the evaluation method are described in the Experimental Section. <sup>c</sup> Unknown compound (synthesis and spectral data will be published elsewhere). <sup>d</sup> Unknown compound (synthesis and spectral data are shown in this paper).

Scheme 1<sup>a</sup>

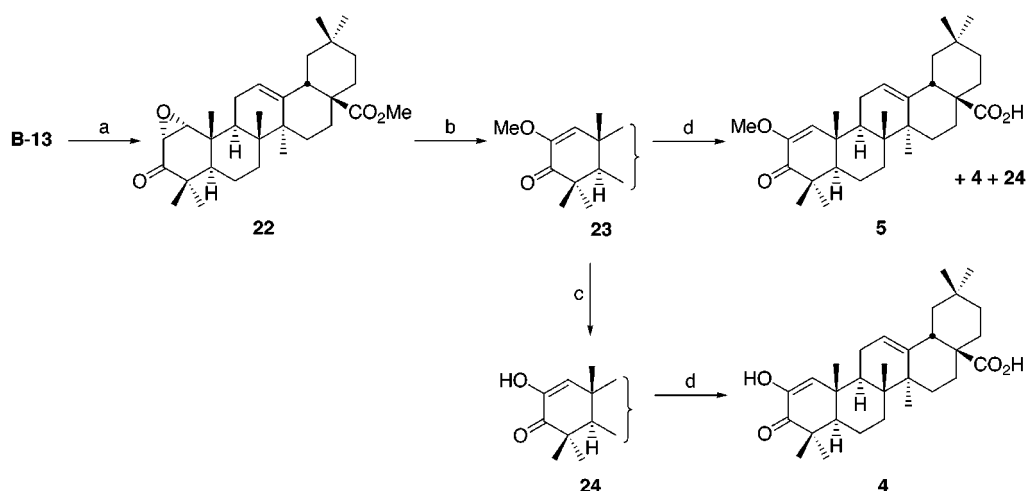
<sup>a</sup> Reagents: (a) PhSeCl, EtOAc; *m*CPBA, pyr, EtOAc; (b) LiI, DMF.

compound. Therefore, about 60 oleanolic and ursolic acid derivatives were initially randomly synthesized. They are divided into seven categories: 3-hydroxy derivatives, **A**; 3-oxo derivatives, **B**; chloro derivatives, **C**; dehydroxy-oleanane derivatives, **D**; A-ring cleaved derivatives, **E**; C-ring cleaved oleanane derivatives, **F**; and lactams, **G** (see Table 1). In the preliminary screen of these derivatives for inhibition of production of NO induced by IFN- $\gamma$  in mouse macrophages, 3-oxooleana-1,12-dien-28-oic acid (**B-15**) was found to show significant activity (inhibition: 85% at 10  $\mu$ M, IC<sub>50</sub> = 5.6  $\mu$ M). (See Tables 1 and 2.)

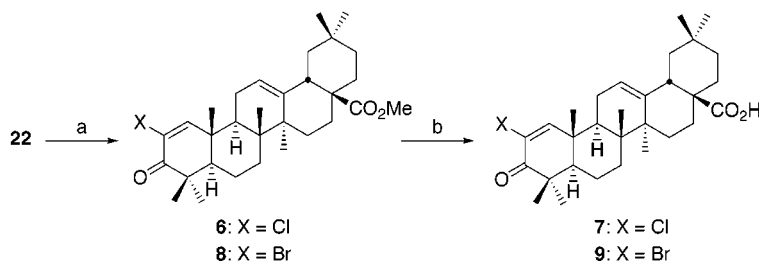
**Design and Synthesis of New Derivatives.** When **B-15** is compared with the other derivatives, it has the following features: first, it is an oleanane; second, it has a 1-en-3-one functionality in ring A; third, it has a carboxyl group at C-17. We focused our attention on the 1-en-3-one functionality in ring A among these features. We therefore designed novel olean- and urs-12-ene triterpenoids with a 1-en-3-one functionality having a substituent at C-2 in ring A, **3-19**, and novel triter-

penoid-steroid hybrid compounds, **20** and **21**<sup>8</sup> (see Table 2). The syntheses of these newly designed derivatives and compounds **B-13-B-16** are illustrated in Schemes 1-6.

Ester **B-13**<sup>9</sup> was synthesized in 62% yield by introduction of a double bond at C-1 of methyl oleanonate (**B-3**)<sup>10</sup> with phenylselenenyl chloride (PhSeCl) in ethyl acetate and sequential addition of pyridine and *m*-chloroperbenzoic acid.<sup>11,12</sup> Acid **B-15** was synthesized in 85% yield by halogenolysis of **B-13** with lithium iodide in *N,N*-dimethylformamide (DMF).<sup>13</sup> Similarly, acid **B-16**<sup>14</sup> was synthesized in 58% yield via ester **B-14** from methyl ursonate (**B-4**).<sup>15</sup> Epoxide **22**<sup>9</sup> was prepared in 99% yield by epoxidation of **B-13** with alkaline hydrogen peroxide. Treatment of **22** with sodium methoxide<sup>16</sup> gave enone **23** (yield, 87%; 98% based on recovered **22**). Diosphenol **24** was synthesized by demethylation of the methyl enol ether at C-2 of **23** with hydrochloric acid in acetic acid (yield, 81%). Halogenolysis of **24** gave acid **4** (yield, 18%). Halogenolysis of **23** gave a desired partial demethylated product **5** in 28% (41% based on recovered

Scheme 2<sup>a</sup>

<sup>a</sup> Reagents: (a) 30% H<sub>2</sub>O<sub>2</sub>, NaOH(aq), THF; (b) NaOMe, MeOH; (c) HCl, AcOH; (d) LiI, DMF.

Scheme 3<sup>a</sup>

<sup>a</sup> Reagents: (a) HX, AcOH, CHCl<sub>3</sub>; (b) LiI, DMF.

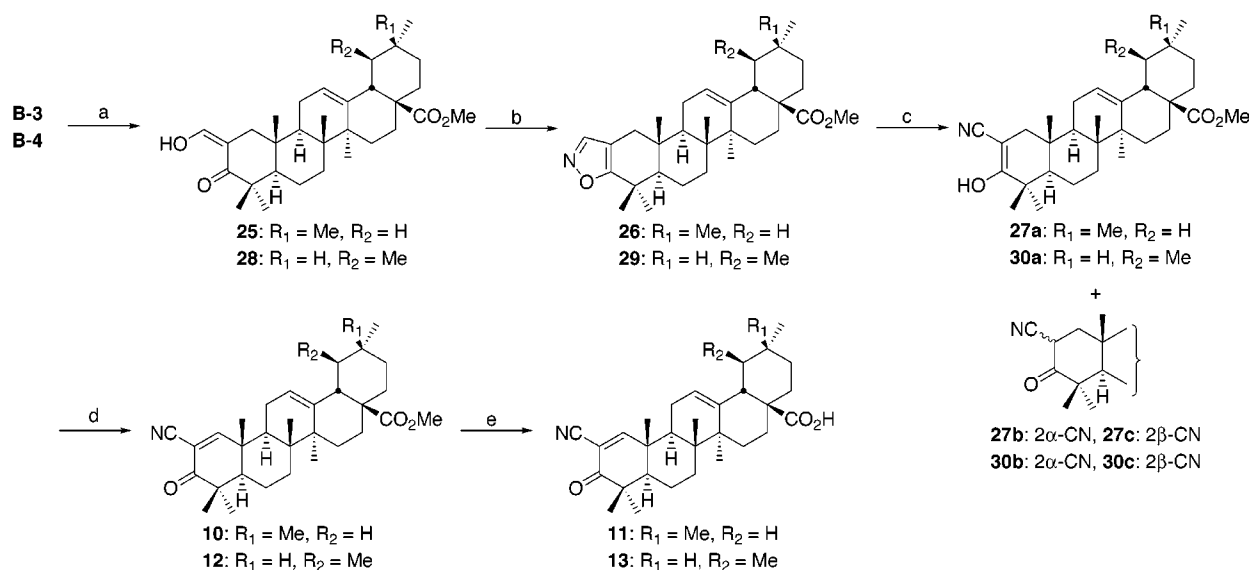
**23**) yield.<sup>17</sup> Chloride **6** was synthesized in 81% yield from **22** with hydrogen chloride in acetic acid and chloroform.<sup>18</sup> Halogenolysis of **6** gave chloride **7** in 77% yield. Similarly, bromides **8** and **9** were prepared from **22** and **8** (yield, 96% and 76%), respectively. Hydroxymethylene **25**<sup>19,20</sup> was prepared in 95% yield by formylation of **B-3** with ethyl formate in the presence of sodium methoxide in benzene.<sup>21</sup> Isoxazole **26** was prepared in 86% yield by condensation of **25** with hydroxylamine.<sup>22</sup> Cleavage of the isoxazole moiety of **26** with sodium methoxide gave nitrile **27** in 99% yield.<sup>22</sup> <sup>1</sup>H NMR showed that **27** is a mixture of three tautomers [**27a**, **27b** (2 $\alpha$ -cyano), and **27c** (2 $\beta$ -cyano)] and that **27a** is the major one in CDCl<sub>3</sub>. Enone **10** was prepared in 88% yield by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) oxidation of **27** in benzene, although the same method as for **B-13** gave **10** in only 35% yield. Halogenolysis of **10** gave acid **11** in 71% (91% based on recovered **10**) yield. Similarly, ursane derivative **12** was synthesized in 52% yield via **28**,<sup>20,23</sup> **29**, and **30** from **B-4**. Acid **13** was prepared in 74% yield by halogenolysis of **12**. Enal **14** was prepared from **25** by PhSeCl–pyridine in methylene chloride and sequential addition of 30% hydrogen peroxide<sup>24</sup> (yield, 71%; 79% based on recovered **25**). Halogenolysis of **14** did not give acid **15** but a complex mixture. Therefore, the synthesis of acid **15** from oleanonic acid (**B-1**)<sup>10</sup> was attempted. Formylation of **B-1** with ethyl formate in the presence of sodium methoxide in tetrahydrofuran gave **32**<sup>20</sup> (yield, 45%; 66% based on recovered **B-1**). Acid **15** was prepared from **32** according to the same method as for **14** (yield, 71%; 84% based on recovered **32**). Jones oxidation of **14** gave acid **16** in 30% (39% based on recovered **14**)

yield. Because this yield was not enough to synthesize derivatives **3** and **17–19** from **16**, an alternative route was adopted. Ester **31** was prepared in 74% (89% based on recovered **B-3**) yield from **B-3** by Stiles' reagent (methoxymagnesium methyl carbonate) in DMF,<sup>25</sup> followed by methylation with diazomethane. <sup>1</sup>H NMR showed that **31** is the single tautomer in CDCl<sub>3</sub> as depicted in Scheme 5. Enone **17** was prepared from **31** according to the same method as for **14** (yield, 83%; 90% based on recovered **31**). Hydrolysis of **17** with potassium hydroxide in aqueous methanol gave acid **16** selectively in 97% yield because the methoxycarbonyl group at C-17 of **17** is sterically hindered. Halogenolysis of **16** gave dicarboxylic acid **3** in 58% yield. Methylation of **3** with methanol under acidic conditions gave ester **18** selectively in 78% yield because of the steric hindrance of the carboxylic acid at C-17 of **3**. Amide **19** was prepared selectively in 96% yield from **17** with saturated ammonia–methanol.

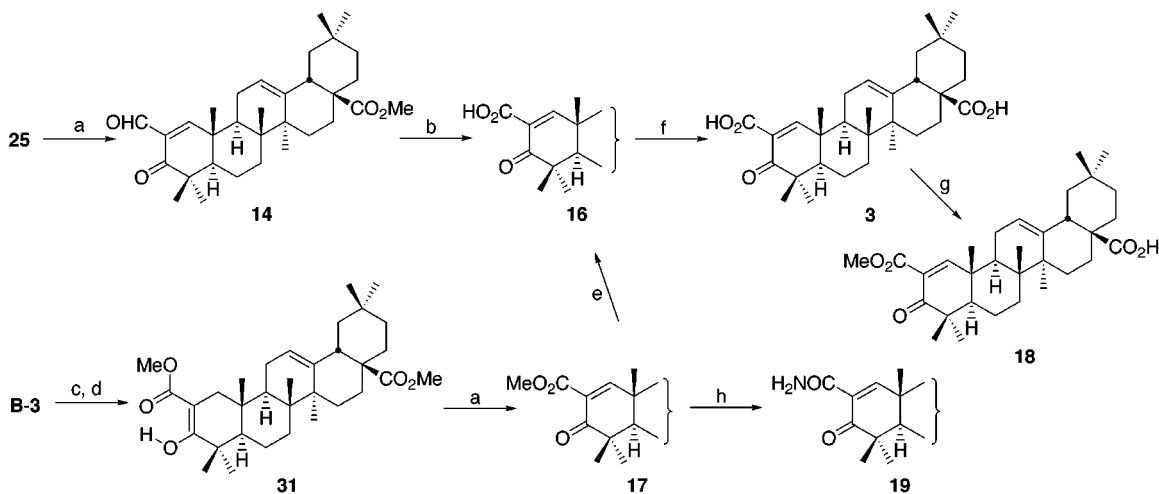
## Biological Results and Discussion

The inhibitory activities [IC<sub>50</sub> ( $\mu$ M) value] of compounds **B-1**, **B-13**, **B-15**, **B-16**, **1–21**, and hydrocortisone (a positive control) on NO production induced by IFN- $\gamma$  in mouse macrophages are shown in Table 2. These derivatives are arranged according to the strength of Taft's  $\sigma^*$  values<sup>26</sup> of substituents at C-2. These results provide the following interesting structure–activity relationships:

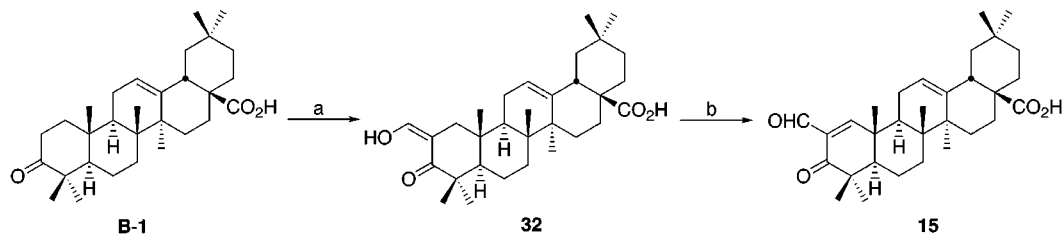
(1) In the A ring, a 1-en-3-one functionality is important for significant activity. The lead compound **B-15** is much more potent than the C-3 ketone **B-1** and the

Scheme 4<sup>a</sup>

<sup>a</sup> Reagents: (a) HCO<sub>2</sub>Et, NaOMe, PhH; (b) NH<sub>2</sub>OH·HCl, aq EtOH; (c) NaOMe, Et<sub>2</sub>O, MeOH; (d) DDQ, PhH; (e) LiI, DMF.

Scheme 5<sup>a</sup>

<sup>a</sup> Reagents: (a) PhSeCl, pyr, CH<sub>2</sub>Cl<sub>2</sub>; 30% H<sub>2</sub>O<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>; (b) Jones; (c) Stiles' reagent, DMF; (d) CH<sub>2</sub>N<sub>2</sub>, Et<sub>2</sub>O, THF; (e) KOH, aq MeOH; (f) LiI, DMF; (g) H<sub>2</sub>SO<sub>4</sub>, MeOH; (h) NH<sub>3</sub>, MeOH.

Scheme 6<sup>a</sup>

<sup>a</sup> Reagents: (a) HCO<sub>2</sub>Et, NaOMe, THF; (b) PhSeCl, pyr, CH<sub>2</sub>Cl<sub>2</sub>; 30% H<sub>2</sub>O<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>.

C-3 alcohol **1** (oleanolic acid). Also, the ursane derivative **B-16** is more potent than the C-3 alcohol **2** (ursolic acid).

(2) A correlation between Taft's  $\sigma^*$  values of substituents at C-2 and biological activity is not observed. This result shows that the activity does not depend on the strength of electron-withdrawing effect of a substituent at C-2.

(3) Carboxyl, methoxycarbonyl, and nitrile groups at C-2 enhance activity. Compounds **3**, **10**, **11**, **16**, and **17** are about 10–100 times more potent than **B-15**. In

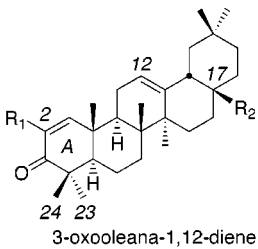
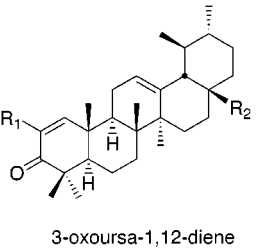
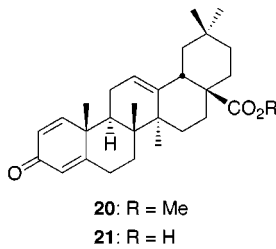
particular, **3** showed the highest activity ( $IC_{50} = 0.07 \mu M$ ) in this series of compounds. The potency of **3** was similar to that of hydrocortisone ( $IC_{50} = 0.01 \mu M$ ).

(4) Hydroxyl, aminocarbonyl, methoxy, chloride, and bromide groups decrease activity. Compounds **4–9** and **19** are much less potent than **B-15**.

(5) A formyl group does not confer activity but only toxicity.

(6) 23,24-Dimethyl groups are important for signifi-

**Table 2.** Activity of Olean- and Urs-12-ene Triterpenoids with Various 1-En-3-one Functionalities

| <div style="display: flex; justify-content: space-around; align-items: flex-end;"> <div style="text-align: center;">  <p>3-oxooleana-1,12-diene</p> </div> <div style="text-align: center;">  <p>3-oxoursa-1,12-diene</p> </div> <div style="text-align: center;">  <p>20: R = Me<br/>21: R = H</p> </div> </div> |                       |                       |                        |                                              |                                                                       |                       |                                                |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|-----------------------|------------------------|----------------------------------------------|-----------------------------------------------------------------------|-----------------------|------------------------------------------------|
| compd                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | skeleton <sup>a</sup> | R <sub>1</sub> at C-2 | R <sub>2</sub> at C-17 | Taft's $\sigma^*$<br>value of R <sub>1</sub> | formula                                                               | analyses <sup>b</sup> | activity <sup>c</sup><br>IC <sub>50</sub> (μM) |
| <b>B-13</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | O                     | H                     | CO <sub>2</sub> Me     |                                              | C <sub>31</sub> H <sub>46</sub> O <sub>3</sub>                        | ref 9                 | 31                                             |
| <b>B-15</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | O                     | H                     | CO <sub>2</sub> H      |                                              | C <sub>30</sub> H <sub>44</sub> O <sub>3</sub> ·3/4H <sub>2</sub> O   | C, H                  | 5.6                                            |
| <b>20</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | D                     | H                     | CO <sub>2</sub> Me     |                                              | C <sub>29</sub> H <sub>40</sub> O <sub>3</sub> ·1/4H <sub>2</sub> O   | C, H                  | > 40                                           |
| <b>21</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | D                     | H                     | CO <sub>2</sub> H      |                                              | C <sub>28</sub> H <sub>38</sub> O <sub>3</sub> ·1/3H <sub>2</sub> O   | C, H                  | 13                                             |
| <b>B-16</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | U                     | H                     | CO <sub>2</sub> H      |                                              | C <sub>30</sub> H <sub>44</sub> O <sub>3</sub>                        | ref 14                | 13                                             |
| <b>5</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | O                     | OH                    | CO <sub>2</sub> H      | 1.34                                         | C <sub>30</sub> H <sub>44</sub> O <sub>4</sub> ·1/2H <sub>2</sub> O   | C, H                  | 27                                             |
| <b>19</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | O                     | CONH <sub>2</sub>     | CO <sub>2</sub> Me     | 1.68                                         | C <sub>32</sub> H <sub>47</sub> O <sub>4</sub> N·3/4H <sub>2</sub> O  | C, H, N               | 14                                             |
| <b>4</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | O                     | OMe                   | CO <sub>2</sub> H      | 1.81                                         | C <sub>31</sub> H <sub>46</sub> O <sub>4</sub> ·1/2H <sub>2</sub> O   | C, H                  | 30                                             |
| <b>17</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | O                     | CO <sub>2</sub> Me    | CO <sub>2</sub> Me     | 2.00                                         | C <sub>33</sub> H <sub>48</sub> O <sub>5</sub>                        | C, H                  | 0.9                                            |
| <b>18</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | O                     | CO <sub>2</sub> Me    | CO <sub>2</sub> H      | 2.00                                         | C <sub>32</sub> H <sub>46</sub> O <sub>5</sub>                        | C, H                  | 2.2                                            |
| <b>16</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | O                     | CO <sub>2</sub> H     | CO <sub>2</sub> Me     | 2.08                                         | C <sub>32</sub> H <sub>46</sub> O <sub>5</sub> ·1/2H <sub>2</sub> O   | C, H                  | 0.8                                            |
| <b>3</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | O                     | CO <sub>2</sub> H     | CO <sub>2</sub> H      | 2.08                                         | C <sub>31</sub> H <sub>44</sub> O <sub>5</sub>                        | C, H                  | 0.07                                           |
| <b>14</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | O                     | CHO                   | CO <sub>2</sub> Me     | 2.15                                         | C <sub>32</sub> H <sub>46</sub> O <sub>4</sub>                        | C, H                  | toxic <sup>d</sup>                             |
| <b>15</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | O                     | CHO                   | CO <sub>2</sub> H      | 2.15                                         | C <sub>31</sub> H <sub>44</sub> O <sub>4</sub> ·1/2H <sub>2</sub> O   | C, H                  | toxic <sup>d</sup>                             |
| <b>8</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | O                     | Br                    | CO <sub>2</sub> Me     | 2.84                                         | C <sub>31</sub> H <sub>45</sub> O <sub>3</sub> Br                     | C, H                  | > 40                                           |
| <b>9</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | O                     | Br                    | CO <sub>2</sub> H      | 2.84                                         | C <sub>30</sub> H <sub>43</sub> O <sub>3</sub> Br·H <sub>2</sub> O    | C, H                  | 7.3                                            |
| <b>6</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | O                     | Cl                    | CO <sub>2</sub> Me     | 2.96                                         | C <sub>31</sub> H <sub>45</sub> O <sub>3</sub> Cl                     | C, H                  | > 40                                           |
| <b>7</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | O                     | Cl                    | CO <sub>2</sub> H      | 2.96                                         | C <sub>30</sub> H <sub>43</sub> O <sub>3</sub> Cl·1/4H <sub>2</sub> O | C, H                  | > 40                                           |
| <b>10</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | O                     | CN                    | CO <sub>2</sub> Me     | 3.30                                         | C <sub>32</sub> H <sub>45</sub> O <sub>3</sub> N·1/4H <sub>2</sub> O  | C, H, N               | 0.7                                            |
| <b>11</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | O                     | CN                    | CO <sub>2</sub> H      | 3.30                                         | C <sub>31</sub> H <sub>43</sub> O <sub>3</sub> N·1/2H <sub>2</sub> O  | C, H, N               | 0.6                                            |
| <b>12</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | U                     | CN                    | CO <sub>2</sub> Me     | 3.30                                         | C <sub>32</sub> H <sub>45</sub> O <sub>3</sub> N·3/4H <sub>2</sub> O  | C, H, N               | 5.1                                            |
| <b>13</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | U                     | CN                    | CO <sub>2</sub> H      | 3.30                                         | C <sub>31</sub> H <sub>43</sub> O <sub>3</sub> N·H <sub>2</sub> O     | C, H, N               | 6.2                                            |
| <b>B-1</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                       | oleanonic acid        |                        |                                              | C <sub>30</sub> H <sub>46</sub> O <sub>3</sub>                        | ref 10                | 37                                             |
| <b>1</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                       | oleanolic acid        |                        |                                              | C <sub>30</sub> H <sub>48</sub> O <sub>3</sub>                        | ref 10                | > 40                                           |
| <b>2</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                       | ursolic acid          |                        |                                              | C <sub>30</sub> H <sub>48</sub> O <sub>3</sub>                        | ref 15                | toxic <sup>e</sup>                             |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                       | hydrocortisone        |                        |                                              |                                                                       |                       | 0.01                                           |

<sup>a</sup> O, 3-oxooleana-1,12-diene; D, 23,24-dinor-3-oxooleana-1,4,12-triene; U, 3-oxoursa-1,12-diene. <sup>b</sup> C, H, and N analyses were within  $\pm 0.4\%$  of the theoretical values. <sup>c</sup> Details of the evaluation method are described in the Experimental Section. IC<sub>50</sub> values of **3** and hydrocortisone were determined in the range of 0.1 pM–1 μM (10-fold dilutions). The other compounds were assayed in the range of 0.01–40 μM (4-fold dilutions). Values are an average of two separate experiments. <sup>d</sup> Compounds **14** and **15** were toxic to cells above 1 μM and were not active below 1 μM. <sup>e</sup> Ursolic acid (**2**) was toxic to cells above 10 μM and was not active below 10 μM.

cant activity. **B-15** is more potent than 23,24-dinor-olean-1-en-3-one derivative **21**.

(7) The oleanane skeleton is more potent than the ursane skeleton. **B-15**, **10**, and **11** are more potent than **B-16**, **12**, and **13**, respectively.

(8) The role of methoxycarbonyl and carboxyl groups at C-17 is ambiguous. In some analogues, the carboxyl group is more potent than the methoxycarbonyl group: acids **B-15**, **3**, **9**, and **21** are more potent than esters **B-13**, **16**, **8**, and **20**, respectively. For other analogues, the carboxyl and methoxycarbonyl groups show similar activity: acids **11** and **13** show similar activity to esters **10** and **12**, respectively. Lastly, acid **18** is less potent than ester **17**.

The inhibitory activity of new triterpenoids **3** and **11** was not blocked by the glucocorticoid antagonist, RU-486,<sup>27</sup> which reverses the action of hydrocortisone (see Figure 1). These data strongly suggest that the actions of these triterpenoids on the iNOS system are not mediated by their interaction with the glucocorticoid receptor.

On the basis of these structure–activity relationships, further lead optimization is in progress. Further biological evaluation and studies on the mechanism of action of **3** are also in progress.

## Experimental Section

**General Experimental Procedures.** Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Optical rotations were measured with a Jasco DIP-181 digital polarimeter. UV and IR spectra were recorded on a Hewlett-Packard 8451A UV/VIS spectrophotometer and a Perkin-Elmer 600 series FTIR spectrophotometer, respectively. <sup>1</sup>H (300 MHz) and <sup>13</sup>C (75 MHz) NMR spectra were recorded on a Varian XL-300 Fourier transform spectrometer. The chemical shifts are reported in  $\delta$  (ppm) using the  $\delta$  7.27 signal of CHCl<sub>3</sub> (<sup>1</sup>H NMR) and the  $\delta$  77.23 signal of CDCl<sub>3</sub> (<sup>13</sup>C NMR) as internal standards. Low-resolution mass spectra and high-resolution MS data were obtained on a Micromass 70-VSE unless otherwise stated. Elemental microanalysis was performed by Atlantic Microlab Inc. TLC and preparative TLC (prep-TLC) were performed with Merck precoated TLC plates silica gel 60 F<sub>254</sub>. Flash column chromatography was done with Select Scientific silica gel (230–400 mesh). The standard work up method was as follows: an organic extract was washed with saturated aqueous NaHCO<sub>3</sub> solution (three times) followed by saturated aqueous NaCl solution (three times), then dried over anhydrous MgSO<sub>4</sub>, and filtered. The filtrate was evaporated in vacuo.

**Methyl 3-Oxooleana-1,12-dien-28-oate (B-13).**<sup>9</sup> A solution of methyl oleanonate (**B-3**)<sup>10</sup> (2.00 g, 4.27 mmol) and phenylselenenyl chloride (98%) (1.00 g, 5.12 mmol) in EtOAc (85 mL) was stirred at room temperature for 3 h. To the stirred

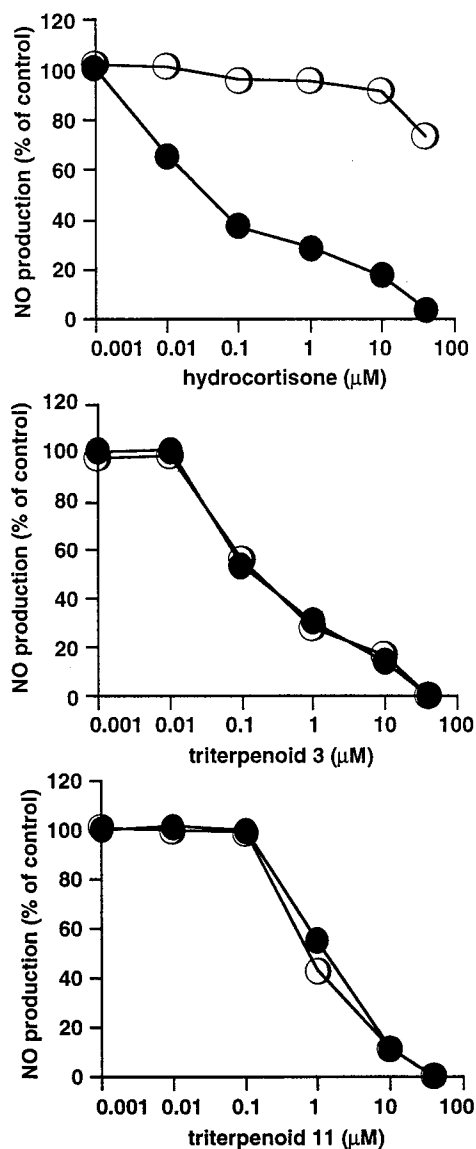


**Figure 1.** Blockage by glucocorticoid antagonist RU486 of hydrocortisone-inhibited NO production but not of triterpenoid (**3** and **11**) inhibited NO production in primary mouse macrophages. Macrophage cells were incubated with IFN- $\gamma$  (20 ng/mL) together with hydrocortisone or triterpenoids without RU486 (●); in some cases RU486 (1  $\mu$ M) was added simultaneously to both hydrocortisone- and triterpenoid-treated cell wells (○). RU486 itself does not interfere with NO production at the concentration tested.

mixture, saturated aqueous  $\text{NaHCO}_3$  solution was added. After most of the aqueous layer was removed, pyridine (844 mg, 10.7 mmol) and *m*-chloroperbenzoic acid (50–60%) (3.68 g, 10.7 mmol) were added to the organic layer. The mixture was stirred at room temperature for 1 h. The mixture was washed with 5% aqueous NaOH solution (three times), saturated aqueous  $\text{NH}_4\text{Cl}$  solution (three times), and saturated aqueous NaCl solution (three times); dried over anhydrous  $\text{MgSO}_4$ ; and filtered. The filtrate was evaporated in vacuo to give a solid. The solid was subjected to flash column chromatography [hexanes–EtOAc (5:1)] to give **B-13** as a crystalline solid (1.23 g, 62%): mp 159–161  $^\circ\text{C}$ ;  $[\alpha]_D^{25} +103^\circ$  (c 0.64,  $\text{CHCl}_3$ ). UV (EtOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 230 (3.92) nm. IR (KBr): 2946, 2867, 1728, 1660  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.04 (1H, d,  $J = 10.1$  Hz), 5.81 (1H, d,  $J = 10.1$  Hz), 5.36 (1H, t,  $J = 3.7$  Hz), 3.64 (3H, s), 2.90 (1H, dd,  $J = 4.6, 13.9$  Hz), 1.17 (3H, s), 1.16 (6H, s), 1.10, 0.94, 0.91, 0.83 (each 3H, s).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  205.5, 178.4, 159.3, 144.5, 125.2, 121.9, 53.6, 51.8, 47.0, 45.9, 44.7, 42.2, 42.0, 41.7, 40.3, 39.7, 34.1, 33.3, 32.7, 32.5, 30.9, 28.0, 27.9, 26.0,

23.8, 23.5, 23.2, 21.8, 19.1, 18.8, 17.5. EIMS (70 eV)  $m/z$ : 466  $[\text{M}]^+$  (73), 451 (11), 407 (31), 262 (57), 203 (100). HREIMS: Calcd for  $\text{C}_{31}\text{H}_{46}\text{O}_3$ : 466.3447. Found: 466.3446.

**Methyl 3-Oxoursa-1,12-dien-28-oate (B-14).** **B-14** was prepared from methyl ursonate (**B-4**)<sup>15</sup> according to the same method as for **B-13** to give an amorphous solid (66%):  $[\alpha]_D^{26} +93^\circ$  (c 0.77,  $\text{CHCl}_3$ ). UV (EtOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 232 (3.95) nm. IR (KBr): 2974, 2935, 2871, 1725, 1669  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.06 (1H, d,  $J = 10.1$  Hz), 5.81 (1H, d,  $J = 10.1$  Hz), 5.33 (1H, t,  $J = 3.8$  Hz), 3.63 (3H, s), 2.28 (1H, d,  $J = 11.5$  Hz), 1.17, 1.15 (each 3H, s), 1.10 (6H, s), 0.95 (3H, d,  $J = 5.4$  Hz), 0.87 (3H, d,  $J = 6.3$  Hz), 0.85 (3H, s).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  205.5, 178.2, 159.5, 139.0, 125.2, 125.0, 53.7, 53.3, 51.7, 48.4, 44.7, 42.6, 41.9, 40.5, 39.5, 39.2, 39.1, 36.8, 33.0, 30.8, 28.2, 28.1, 24.4, 23.7, 23.5, 21.8, 21.4, 19.1, 19.0, 17.7, 17.2. EIMS (70 eV)  $m/z$ : 466  $[\text{M}]^+$  (14), 406 (12), 262 (74), 203 (100). HREIMS: Calcd for  $\text{C}_{31}\text{H}_{46}\text{O}_3$ : 466.3447. Found: 466.3442.

**3-Oxooleana-1,12-dien-28-oic Acid (B-15).** A mixture of **B-13** (100 mg, 0.21 mmol) and LiI (500 mg) in dry DMF (2 mL) was heated under reflux for 6 h. The mixture was acidified with 5% aqueous HCl solution and then extracted with a mixture of  $\text{CH}_2\text{Cl}_2$  and  $\text{Et}_2\text{O}$  (1:2) three times. The extract was worked up according to the standard method to give a solid (110 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (5:1) followed by hexanes–EtOAc (2:1)] to give **B-15** as an amorphous solid (82 mg, 85%):  $[\alpha]_D^{26} +103^\circ$  (c 0.45,  $\text{CHCl}_3$ ). UV (EtOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 230 (3.75) nm. IR (KBr): 2941, 2866, 1732, 1695, 1671  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.04 (1H, d,  $J = 10.2$  Hz), 5.81 (1H, d,  $J = 10.2$  Hz), 5.35 (1H, t,  $J = 3.3$  Hz), 2.86 (1H, dd,  $J = 4.2, 13.4$  Hz), 1.16, 1.152, 1.147, 1.07, 0.94, 0.91, 0.84 (each 3H, s).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  205.5, 184.5, 159.2, 144.2, 125.3, 122.1, 53.5, 46.8, 45.8, 44.7, 42.1, 41.9, 41.3, 40.2, 39.7, 34.0, 33.3, 32.6, 32.5, 30.9, 28.0, 27.8, 26.0, 23.7, 23.5, 23.0, 21.8, 19.0, 18.9, 17.7. EIMS (70 eV)  $m/z$ : 452  $[\text{M}]^+$  (8.8), 437 (3.8), 406 (6.8), 248 (80), 233 (14), 203 (100). HREIMS: Calcd for  $\text{C}_{30}\text{H}_{44}\text{O}_3$ : 452.3290. Found: 452.3289. Anal. (Table 2).

**3-Oxoursa-1,12-dien-28-oic Acid (B-16).** **B-16** was prepared from **B-14** according to the same method as for **B-15** to give an amorphous solid (88%):  $[\alpha]_D^{26} +91^\circ$  (c 0.84,  $\text{CHCl}_3$ ). UV (EtOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 230 (3.99) nm. IR (KBr): 3306, 2973, 2930, 2870, 1729, 1695, 1669  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.07 (1H, d,  $J = 10.1$  Hz), 5.82 (1H, d,  $J = 10.1$  Hz), 5.33 (1H, t,  $J = 3.7$  Hz), 2.24 (1H, d,  $J = 11.2$  Hz), 1.18, 1.16, 1.11, 1.09 (each 3H, s), 0.96 (3H, d,  $J = 6.1$  Hz), 0.88 (3H, d,  $J = 6.4$  Hz), 0.88 (3H, s).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  205.5, 183.9, 159.4, 138.8, 125.3, 53.6, 52.9, 48.3, 44.7, 42.5, 41.9, 40.5, 39.6, 39.2, 39.0, 36.8, 32.9, 30.8, 28.2, 28.1, 24.2, 23.7, 23.4, 21.8, 21.3, 19.0, 17.8, 17.2. FABMS (NBA)  $m/z$ : 453  $[\text{M} + \text{H}]^+$  (100) (by a Micromass ZAB-SE). HRFABMS: Calcd for  $\text{C}_{30}\text{H}_{44}\text{O}_3 + \text{H}$ : 453.3369. Found: 453.3335 (by a Micromass 70-SE-4F).

**2-Carboxy-3-oxooleana-1,12-dien-28-oic Acid (3).** A mixture of **16** (109 mg, 0.21 mmol) and LiI (520 mg) in dry DMF (1.5 mL) was heated under reflux for 1 h. After 5% aqueous HCl solution was added, the acidic mixture was extracted with EtOAc three times. The extract was washed with water (three times) and saturated aqueous NaCl solution (three times), dried over anhydrous  $\text{MgSO}_4$ , and filtered. The filtrate was evaporated in vacuo to give a residue (108 mg). The residue was subjected to flash column chromatography [ $\text{CH}_2\text{Cl}_2$ –MeOH (15:1) followed by  $\text{CH}_2\text{Cl}_2$ –MeOH (10:1)] to afford **3** as a crystalline solid (61 mg, 58%): mp  $>250^\circ\text{C}$  dec;  $[\alpha]_D^{26} +81^\circ$  (c 0.53,  $\text{CHCl}_3$ ). UV (EtOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 234 (3.88) nm. IR (KBr): 3389, 2943, 2872, 1752, 1696, 1637  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  8.43 (1H, s), 5.37 (1H, t,  $J = 3.5$  Hz), 2.87 (1H, dd,  $J = 3.8, 13.9$  Hz), 1.25, 1.22, 1.18, 1.15, 0.95, 0.93, 0.88 (each 3H, s).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  209.0, 183.9, 173.2, 165.2, 144.2, 123.4, 121.7, 52.4, 46.8, 45.7, 45.5, 42.3, 41.4, 41.1, 40.6, 40.4, 34.0, 33.2, 32.5, 32.3, 30.9, 28.4, 27.8, 26.0, 23.7, 23.5, 23.0, 22.0, 19.0, 18.4, 17.8. EIMS (70 eV)  $m/z$ : 496  $[\text{M}]^+$  (3.0), 478 (3.4), 452 (7.6), 248 (56), 231 (35), 203 (100). HREIMS: Calcd for  $\text{C}_{31}\text{H}_{44}\text{O}_5$ : 496.3189. Found: 496.3196. Anal. (Table 2).

**2-Hydroxy-3-oxooleana-1,12-dien-28-oic Acid (4).** **4** was prepared from **24** according to the same method as for **B-15**

except that the reaction time was 2 h. The reaction mixture was subjected to flash column chromatography [hexanes–EtOAc (5:1) followed by hexanes–EtOAc (4:1)] to give **4** as an amorphous solid (18%):  $[\alpha]_D^{25} + 99^\circ$  (c 0.46, CHCl<sub>3</sub>). UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 272 (3.71) nm. IR (KBr): 3434, 2938, 1698, 1667, 1649 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  6.35 (1H, s), 5.96 (1H, brs), 5.34 (1H, t,  $J = 3.5$  Hz), 2.86 (1H, dd,  $J = 3.8, 13.9$  Hz), 1.23, 1.22, 1.14, 1.11, 0.94, 0.92, 0.83 (each 3H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  201.2, 184.0, 144.1, 143.9, 128.4, 122.3, 54.0, 46.8, 45.8, 44.1, 43.3, 42.2, 41.3, 40.2, 38.7, 34.0, 33.3, 32.6, 30.9, 27.8, 27.4, 26.1, 23.8, 23.6, 23.0, 22.0, 19.9, 18.9, 17.7. EIMS (70 eV)  $m/z$ : 468 [M]<sup>+</sup> (3.2), 248 (13), 203 (23), 149 (42), 84 (100). HREIMS: Calcd for C<sub>30</sub>H<sub>44</sub>O<sub>4</sub>: 468.3240. Found: 468.3222. Anal. (Table 2).

**2-Methoxy-3-oxooleana-1,12-dien-28-oic Acid (5).** A mixture of **23** (230 mg, 0.46 mmol) and LiI (1045 mg) in dry DMF (3.5 mL) was heated under reflux for 4 h. The reaction mixture was worked up according to the same method as for **B-15** to give a solid (230 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (3:1) followed by hexanes–EtOAc (2:1), then hexanes–EtOAc (1:1)] to give **24** (35 mg; 16%, 23% based on recovered **23**), **23** (74 mg), **4** (27 mg; 12%, 18% based on recovered **23**), and **5** as an amorphous solid (63 mg; 28%, 41% based on recovered **23**):  $[\alpha]_D^{26} + 96^\circ$  (c 0.29, CHCl<sub>3</sub>). UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 266 (3.84) nm. IR (KBr): 3307, 2947, 2862, 1732, 1693, 1622 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  5.96 (1H, s), 5.36 (1H, t,  $J = 3.3$  Hz), 3.56 (3H, s), 2.87 (1H, dd,  $J = 4.2, 13.9$  Hz), 1.17 (9H, s), 1.11, 0.94, 0.91, 0.84 (each 3H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  200.0, 184.4, 149.1, 144.4, 126.1, 122.1, 55.0, 53.2, 46.8, 45.9, 45.4, 43.3, 42.2, 41.3, 40.2, 38.5, 34.0, 33.3, 32.5, 30.9, 28.6, 27.8, 26.1, 23.8, 23.0, 22.0, 20.4, 19.2, 17.6. EIMS (70 eV)  $m/z$ : 482 [M]<sup>+</sup> (11), 415 (6.5), 245 (18), 203 (33), 157 (100). HREIMS: Calcd for C<sub>31</sub>H<sub>46</sub>O<sub>4</sub>: 482.3396. Found: 482.3375. Anal. (Table 2).

**Methyl 2-Chloro-3-oxooleana-1,12-dien-28-oate (6).** A solution of **22** (99 mg, 0.21 mmol) in AcOH including 1 M HCl (2.5 mL) and CHCl<sub>3</sub> (2.5 mL) was stirred at room temperature overnight. The mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub>. After it was washed with water three times, it was worked up according to the standard method to give a solid (96 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (6:1)] to afford **6** as an amorphous solid (84 mg, 81%):  $[\alpha]_D^{26} + 98^\circ$  (c 0.26, CHCl<sub>3</sub>). UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 250 (3.91) nm. IR (KBr): 2943, 2866, 1727, 1689 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  7.22 (1H, s), 5.34 (1H, t,  $J = 3.5$  Hz), 3.62 (3H, s), 2.89 (1H, dd,  $J = 4.2, 13.7$  Hz), 1.203, 1.197 (each 3H, s), 1.14 (6H, s), 0.93, 0.90, 0.80 (each 3H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  197.4, 178.3, 155.0, 144.5, 129.8, 121.5, 53.3, 51.8, 46.9, 46.3, 45.8, 42.2, 42.1, 41.64, 41.57, 40.3, 34.0, 33.3, 32.4, 30.9, 28.4, 27.8, 26.0, 23.8, 23.5, 23.1, 22.1, 19.1, 18.8, 17.5. EIMS (70 eV)  $m/z$ : 500 [M]<sup>+</sup> (21), 262 (27), 247 (96), 203 (100). HREIMS: Calcd for C<sub>31</sub>H<sub>45</sub>O<sub>3</sub>Cl: 500.3057. Found: 500.3060. Anal. (Table 2).

**2-Chloro-3-oxooleana-1,12-dien-28-oic Acid (7).** **7** was prepared from **6** according to the same method as for **B-15** except that the reaction time was 4 h. The reaction mixture was subjected to flash column chromatography [hexanes–EtOAc (4:1) followed by hexanes–EtOAc (3:1)] to give **7** as an amorphous solid (77%):  $[\alpha]_D^{26} + 88^\circ$  (c 0.50, CHCl<sub>3</sub>). UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 252 (3.20) nm. IR (KBr): 3297, 2943, 2870, 1733, 1691, 1601 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  7.23 (1H, s), 5.35 (1H, t,  $J = 3.3$  Hz), 2.86 (1H, dd,  $J = 4.3, 13.8$  Hz), 1.22, 1.21, 1.16, 1.13, 0.94, 0.92, 0.84 (each 3H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  197.4, 184.4, 154.9, 144.3, 129.9, 121.8, 53.2, 46.8, 46.4, 45.8, 42.21, 42.16, 41.6, 41.3, 40.3, 34.0, 33.3, 32.5, 32.4, 30.9, 28.5, 27.8, 26.0, 23.7, 23.5, 23.0, 22.1, 19.0, 18.9, 17.7. EIMS (70 eV)  $m/z$ : 486 [M]<sup>+</sup> (25), 248 (100), 203 (96). HREIMS: Calcd for C<sub>30</sub>H<sub>43</sub>O<sub>3</sub>Cl: 486.2901. Found: 486.2898. Anal. (Table 2).

**Methyl 2-Bromo-3-oxooleana-1,12-dien-28-oate (8).** A solution of **22** (220 mg, 0.46 mmol) in AcOH including 1 M HBr (4.9 mL) and CHCl<sub>3</sub> (6.1 mL) was stirred at room temperature for 1 h. The mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub>. After it was washed with water three times, it was worked up according to the standard method to give a solid (260 mg). The solid was subjected to flash column chromatography [hexanes–

EtOAc (6:1)] to afford **8** as an amorphous solid (238 mg, 96%):  $[\alpha]_D^{26} + 88^\circ$  (c 0.51, CHCl<sub>3</sub>). UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 260 (3.69) nm. IR (KBr): 2943, 2870, 1733, 1691, 1601 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  7.49 (1H, s), 5.35 (1H, t,  $J = 3.5$  Hz), 3.63 (3H, s), 2.90 (1H, dd,  $J = 4.0, 13.8$  Hz), 1.20, 1.15 (each 6H, s), 0.94, 0.91, 0.81 (each 3H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  197.3, 178.3, 159.5, 144.6, 121.8, 121.5, 53.3, 51.8, 46.9, 46.5, 45.8, 43.1, 42.3, 42.1, 41.7, 40.3, 34.0, 33.3, 32.4, 30.9, 28.7, 27.8, 26.0, 23.8, 23.6, 23.2, 22.3, 19.1, 18.7, 17.5. EIMS (70 eV)  $m/z$ : 546 (5.0) and 544 (5.2) [M]<sup>+</sup>, 262 (8.5), 203 (24), 118 (100), 116 (100). HREIMS: Calcd for C<sub>31</sub>H<sub>45</sub>O<sub>3</sub>Br: 544.2552. Found: 544.2553. Anal. (Table 2).

**2-Bromo-3-oxooleana-1,12-dien-28-oic Acid (9).** **9** was prepared from **8** according to the same method as for **B-15** except that the reaction time was 4 h. The reaction mixture was subjected to flash column chromatography [hexanes–EtOAc (4:1) followed by hexanes–EtOAc (3:1)] to give **9** as an amorphous solid (76%):  $[\alpha]_D^{26} + 82^\circ$  (c 0.31, CHCl<sub>3</sub>). UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 260 (3.52) nm. IR (KBr): 3434, 2939, 2870, 1727, 1686, 1601 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  7.49 (1H, s), 5.35 (1H, t,  $J = 3.4$  Hz), 2.86 (1H, dd,  $J = 4.2, 13.7$  Hz), 1.21 (6H, s), 1.16, 1.14, 0.94, 0.92, 0.83 (each 3H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  197.2, 184.4, 159.3, 144.3, 121.84, 121.79, 53.3, 46.8, 46.5, 45.8, 43.1, 42.2, 42.0, 41.3, 40.3, 34.0, 33.2, 32.5, 32.4, 30.9, 28.7, 27.8, 26.0, 23.7, 23.5, 23.0, 22.2, 19.1, 18.7, 17.7. EIMS (70 eV)  $m/z$ : 532 (13) and 530 (14) [M]<sup>+</sup>, 285 (5.6), 283 (6.2), 248 (100), 235 (10), 233 (11), 203 (84). HREIMS: Calcd for C<sub>30</sub>H<sub>43</sub>O<sub>3</sub>Br: 530.2396. Found: 530.2383. Anal. (Table 2).

**Methyl 2-Cyano-3-oxooleana-1,12-dien-28-oate (10).** A solution of **27** (141 mg, 0.28 mmol) and DDQ (98%) (79 mg, 0.34 mmol) in benzene (10 mL) was heated under reflux for 4 h. After insoluble matter was removed by filtration, the filtrate was evaporated in vacuo to give a solid. The solid was subjected to flash column chromatography [benzene–acetone (20:1)] to give a crystalline solid (123 mg, 88%): mp 201–202 °C;  $[\alpha]_D^{26} + 67^\circ$  (c 0.53, CHCl<sub>3</sub>). UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 240 (3.65) nm. IR (KBr): 2945, 2874, 2232, 1724, 1686 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  7.75 (1H, s), 5.36 (1H, t,  $J = 3.5$  Hz), 3.64 (3H, s), 2.91 (1H, dd,  $J = 3.9, 13.9$  Hz), 1.22, 1.21, 1.15, 1.14, 0.94, 0.92, 0.83 (each 3H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  198.3, 178.3, 170.2, 144.8, 121.1, 115.2, 114.0, 52.8, 51.8, 46.9, 45.8, 45.1, 42.3, 41.7, 41.3, 40.8, 40.5, 34.0, 33.3, 32.4, 32.3, 30.9, 27.9, 27.8, 26.0, 23.8, 23.4, 23.1, 21.8, 18.9, 18.1, 17.6. EIMS (70 eV)  $m/z$ : 491 [M]<sup>+</sup> (35), 459 (13), 432 (27), 262 (22), 247 (24), 203 (100). HREIMS: Calcd for C<sub>32</sub>H<sub>45</sub>O<sub>3</sub>N: 491.3399. Found: 491.3391. Anal. (Table 2).

**2-Cyano-3-oxooleana-1,12-dien-28-oic Acid (11).** **11** was prepared from **10** according to the same method as for **B-15** except that the reaction time was 3 h. The reaction mixture was subjected to flash column chromatography [hexanes–EtOAc (3:1) followed by hexanes–EtOAc (2:1), then hexanes–EtOAc (1:1)] to give **11** as an amorphous solid (71%, 91% based on recovered **10**):  $[\alpha]_D^{26} + 61^\circ$  (c 0.66, CHCl<sub>3</sub>). UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 238 (3.87) nm. IR (KBr): 3387, 2947, 2870, 2233, 1729, 1691, 1609 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  7.75 (1H, s), 5.35 (1H, t,  $J = 3.3$  Hz), 2.86 (1H, dd,  $J = 4.0, 13.6$  Hz), 1.22, 1.21, 1.15, 1.12, 0.94, 0.92, 0.85 (each 3H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  198.2, 184.3, 170.1, 144.4, 121.4, 115.1, 114.1, 52.7, 46.8, 45.7, 45.0, 42.2, 41.3, 40.8, 40.5, 33.9, 33.2, 32.5, 32.2, 30.9, 27.9, 27.7, 25.9, 23.7, 23.4, 22.9, 21.8, 18.9, 18.1, 17.7. EIMS (70 eV)  $m/z$ : 477 [M]<sup>+</sup> (18), 462 (5.6), 431 (16), 416 (10), 248 (76), 235 (25), 203 (100). HREIMS: Calcd for C<sub>31</sub>H<sub>43</sub>O<sub>3</sub>N: 477.3243. Found: 477.3240. Anal. (Table 2).

**Methyl 2-Cyano-3-oxoursa-1,12-dien-28-oate (12).** **12** was prepared from **30** according to the same method as for **10** to give an amorphous solid (62%):  $[\alpha]_D^{26} + 53^\circ$  (c 0.35, CHCl<sub>3</sub>). UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 240 (3.74) nm. IR (KBr): 2973, 2926, 2870, 2229, 1723, 1686 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  7.77 (1H, s), 5.33 (1H, t,  $J = 3.7$  Hz), 3.62 (3H, s), 2.29 (1H, d,  $J = 11.2$  Hz), 1.23, 1.21, 1.14, 1.11 (each 3H, s), 0.96, 0.88 (each 3H, d,  $J = 6.3$  Hz), 0.86 (3H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  198.3, 178.1, 170.4, 139.3, 124.2, 115.2, 114.0, 53.2, 52.8, 51.7, 48.3, 45.1, 42.7, 41.2, 40.70, 40.65, 39.1, 39.0, 36.7, 32.6, 30.8, 28.1, 28.0, 24.3, 23.6, 23.4, 21.8, 21.3, 18.9, 18.2, 17.8, 17.2. EIMS (70

eV)  $m/z$ : 491 [ $M$ ]<sup>+</sup> (38), 431 (35), 262 (46), 249 (82), 203 (65), 84 (100). HREIMS: Calcd for  $C_{32}H_{45}O_3N$ : 491.3399. Found: 491.3395. Anal. (Table 2).

**2-Cyano-3-oxoursa-1,12-dien-28-oic Acid (13).** **13** was prepared from **12** according to the same method as for **B-15** except that the reaction time was 4 h. The reaction mixture was subjected to prep-TLC [hexanes–EtOAc (1.5:1)] to give **13** as an amorphous solid (74%):  $[\alpha]^{26}_D +48^\circ$  (c 0.50,  $CHCl_3$ ). UV (EtOH)  $\lambda_{max}$  (log  $\epsilon$ ): 238 (3.86) nm. IR (KBr): 3417, 2973, 2926, 2870, 2233, 1731, 1689  $cm^{-1}$ .  $^1H$  NMR ( $CDCl_3$ ):  $\delta$  7.77 (1H, s), 5.31 (1H, t,  $J = 3.2$  Hz), 2.24 (1H, d,  $J = 11.0$  Hz), 1.22, 1.20, 1.12, 1.11 (each 3H, s), 0.95, 0.88 (each 3H, d,  $J = 5.7$  Hz), 0.87 (3H, s).  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  198.2, 184.2, 170.2, 139.0, 124.4, 115.1, 114.1, 52.8, 52.7, 48.2, 45.0, 42.6, 41.2, 40.68, 40.65, 39.1, 39.0, 36.7, 32.5, 30.7, 28.1, 28.0, 24.1, 23.6, 23.3, 21.8, 21.3, 18.9, 18.2, 17.7, 17.2. EIMS (70 eV)  $m/z$ : 477 [ $M$ ]<sup>+</sup> (22), 431 (23), 248 (100), 203 (48). HREIMS: Calcd for  $C_{31}H_{43}O_3N$ : 477.3243. Found: 477.3240. Anal. (Table 2).

**Methyl 2-Formyl-3-oxooleana-1,12-dien-28-oate (14).** To a stirred solution of phenylselenenyl chloride (98%) (161 mg, 0.82 mmol) in  $CH_2Cl_2$  (7.2 mL) was added a solution of pyridine (75 mg, 0.95 mmol) in  $CH_2Cl_2$  (1.0 mL) in an ice bath. After 15 min, a solution of **25** (204 mg, 0.41 mmol) in  $CH_2Cl_2$  (2.0 mL) was added, and the mixture was stirred an additional 1 h. After the mixture was washed with 10% aqueous HCl solution (3 mL) twice, 30%  $H_2O_2$  (0.4 mL) was added to the stirred mixture in the ice bath. After an additional 40 min, the mixture was worked up according to the standard method to give a solid (199 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (5:1)] to afford **25** (20 mg) and **14** as an amorphous solid (144 mg; 71%, 79% based on recovered **25**):  $[\alpha]^{26}_D +12^\circ$  (c 0.60,  $CHCl_3$ ). UV (EtOH)  $\lambda_{max}$  (log  $\epsilon$ ): 238 (3.85) nm. IR (KBr): 2946, 2867, 1724, 1703, 1673, 1610  $cm^{-1}$ .  $^1H$  NMR ( $CDCl_3$ ):  $\delta$  10.00 (1H, s), 7.79 (1H, s), 5.37 (1H, t,  $J = 3.6$  Hz), 3.63 (3H, s), 2.90 (1H, dd,  $J = 4.2$ , 13.9 Hz), 1.18, 1.17, 1.16, 1.14, 0.94, 0.91, 0.85 (each 3H, s).  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  203.7, 190.7, 178.3, 165.2, 144.5, 131.2, 121.6, 52.8, 51.8, 47.0, 45.8, 45.1, 42.3, 41.7, 41.3, 40.5, 39.8, 34.0, 33.3, 32.44, 32.38, 30.9, 28.2, 27.8, 26.0, 23.8, 23.5, 23.2, 21.7, 19.2, 18.2, 17.6. EIMS (70 eV)  $m/z$ : 494 [ $M$ ]<sup>+</sup> (95), 435 (87), 262 (40), 203 (100). HREIMS: Calcd for  $C_{32}H_{46}O_4$ : 494.3396. Found: 494.3398. Anal. (Table 2).

**2-Formyl-3-oxooleana-1,12-dien-28-oic Acid (15).** **15** was prepared from **32** according to the same method as for **14**. The reaction mixture was subjected to flash column chromatography [hexanes–EtOAc (3:1) followed by hexanes–EtOAc (2:1)] to give **15** as an amorphous solid (71%, 84% based on recovered **32**):  $[\alpha]^{26}_D +26^\circ$  (c 0.95,  $CHCl_3$ ). UV (EtOH)  $\lambda_{max}$  (log  $\epsilon$ ): 240 (3.82) nm. IR (KBr): 2948, 2866, 1725, 1701, 1674, 1608  $cm^{-1}$ .  $^1H$  NMR ( $CDCl_3$ ):  $\delta$  10.00 (1H, s), 7.79 (1H, s), 5.36 (1H, t,  $J = 3.3$  Hz), 2.86 (1H, dd,  $J = 3.8$ , 13.9 Hz), 1.18, 1.17, 1.15, 1.14, 0.94, 0.92, 0.87 (each 3H, s).  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  203.7, 190.7, 184.3, 165.0, 144.2, 131.2, 121.8, 52.8, 46.8, 45.7, 45.1, 42.3, 41.4, 41.3, 40.5, 39.8, 34.0, 33.2, 32.5, 27.8, 26.0, 23.7, 23.5, 23.0, 21.6, 19.2, 18.2, 17.8. EIMS (70 eV)  $m/z$ : 480 [ $M$ ]<sup>+</sup> (5.5), 434 (3.1), 419 (3.4), 248 (56), 233 (27), 203 (100). HREIMS: Calcd for  $C_{31}H_{44}O_4$ : 480.3240. Found: 480.3237. Anal. (Table 2).

**Methyl 2-Carboxy-3-oxooleana-1,12-dien-28-oate (16).** **(1) From 14:** To a solution of **14** (357 mg, 0.72 mmol) in acetone (71 mL) was added Jones reagent (0.5 mL) dropwise in an ice bath. The mixture was stirred in the ice bath for 20 min. After excess Jones reagent was decomposed with MeOH, the acetone was evaporated in vacuo. After water was added to the resultant mixture, the aqueous mixture was extracted with EtOAc three times. The extract was washed with water (three times) and saturated aqueous NaCl solution (three times), dried over anhydrous  $MgSO_4$ , and filtered. The filtrate was evaporated in vacuo to give a residue (294 mg). The residue was subjected to flash column chromatography [hexanes–EtOAc (1:1) followed by EtOAc] to afford **14** (89 mg) and **16** as a crystalline solid (109 mg; 30%, 39% based on recovered **14**): mp 230–231  $^\circ C$ ;  $[\alpha]^{26}_D +85^\circ$  (c 0.61,  $CHCl_3$ ). UV (EtOH)  $\lambda_{max}$  (log  $\epsilon$ ): 234 (3.78) nm. IR (KBr): 3436, 2946, 2876, 1756,

1722, 1633  $cm^{-1}$ .  $^1H$  NMR ( $CDCl_3$ ):  $\delta$  8.43 (1H, s), 5.36 (1H, t,  $J = 3.5$  Hz), 3.64 (3H, s), 2.90 (1H, dd,  $J = 3.9$ , 13.7 Hz), 1.24, 1.21, 1.19, 1.13, 0.94, 0.91, 0.85 (each 3H, s).  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  209.2, 178.4, 173.4, 165.2, 144.5, 123.3, 121.4, 52.4, 51.8, 47.0, 45.7, 45.5, 42.3, 41.7, 41.1, 40.6, 40.4, 34.0, 33.3, 32.4, 32.3, 30.9, 28.3, 27.8, 26.0, 23.8, 23.5, 23.1, 22.0, 19.0, 18.3, 17.7. EIMS (70 eV)  $m/z$ : 510 [ $M$ ]<sup>+</sup> (16), 492 (15), 451 (14), 433 (14), 262 (27), 203 (100). HREIMS: Calcd for  $C_{32}H_{46}O_5$ : 510.3345. Found: 510.3347. Anal. (Table 2).

**(2) From 17:** A solution of **17** (500 mg, 0.95 mmol) in MeOH (29 mL) and aqueous KOH solution (KOH, 2.9 g; water, 10 mL) was heated under reflux for 15 min. After removal of MeOH in vacuo, the mixture was acidified with 5% aqueous HCl solution. It was extracted with EtOAc (three times). The extract was washed with water and saturated aqueous NaCl solution (each three times), dried over  $MgSO_4$ , and filtered. The filtrate gave **16** as a crystalline solid (470 mg, 97%). It was used for the next reaction without further purification.

**Methyl 2-Methoxycarbonyl-3-oxooleana-1,12-dien-28-oate (17).** **17** was prepared from **31** by the similar method as for **14**. The reaction mixture was subjected to flash column chromatography [hexanes–EtOAc (4:1)] to give **17** as an amorphous solid (83%, 90% based on recovered **31**):  $[\alpha]^{26}_D +63^\circ$  (c 0.78,  $CHCl_3$ ). UV (EtOH)  $\lambda_{max}$  (log  $\epsilon$ ): 230 (3.97) nm. IR (KBr): 2947, 2866, 1727, 1684, 1624  $cm^{-1}$ .  $^1H$  NMR ( $CDCl_3$ ):  $\delta$  7.73 (1H, s), 5.37 (1H, t,  $J = 3.5$  Hz), 3.79, 3.64 (each 3H, s), 2.90 (1H, dd,  $J = 3.9$ , 13.7 Hz), 1.16 (6H, s), 1.15, 1.12, 0.94, 0.91, 0.84 (each 3H, s).  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  201.2, 178.4, 166.0, 164.3, 144.5, 129.2, 121.7, 52.7, 52.4, 51.8, 47.0, 45.9, 45.8, 42.3, 41.8, 41.5, 40.3, 39.5, 34.1, 33.3, 32.4, 32.3, 30.9, 28.7, 27.8, 25.9, 23.8, 23.6, 23.2, 21.5, 19.4, 18.0, 17.5. EIMS (70 eV)  $m/z$ : 524 [ $M$ ]<sup>+</sup> (24), 492 (23), 465 (13), 262 (35), 203 (100). HREIMS: Calcd for  $C_{33}H_{48}O_5$ : 524.3502. Found: 524.3494. Anal. (Table 2).

**2-Methoxycarbonyl-3-oxooleana-1,12-dien-28-oic Acid (18).** A solution of **3** (52 mg, 0.10 mmol) in MeOH (5.2 mL) containing concentrated  $H_2SO_4$  (0.15 mL) was heated under reflux for 30 min. After saturated aqueous NaCl solution was added to the mixture, it was extracted with EtOAc three times. The extract was worked up according to the standard method to give a residue (53 mg). The residue was subjected to flash column chromatography [hexanes–EtOAc (2:1)] to give **18** as an amorphous solid (42 mg, 78%):  $[\alpha]^{26}_D +61^\circ$  (c 0.56,  $CHCl_3$ ). UV (EtOH)  $\lambda_{max}$  (log  $\epsilon$ ): 230 (3.83) nm. IR (KBr): 3323, 2947, 2866, 1733, 1695, 1622  $cm^{-1}$ .  $^1H$  NMR ( $CDCl_3$ ):  $\delta$  7.73 (1H, s), 5.37 (1H, t,  $J = 3.4$  Hz), 3.79 (3H, s), 2.86 (1H, dd,  $J = 4.1$ , 13.7 Hz), 1.16, 1.15, 1.14, 1.12, 0.94, 0.92, 0.86 (each 3H, s).  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  201.1, 184.2, 166.0, 164.2, 144.2, 129.2, 122.0, 52.7, 52.4, 46.9, 45.9, 45.8, 42.2, 41.5, 41.4, 40.3, 39.5, 34.0, 33.3, 32.5, 32.3, 30.9, 28.7, 27.8, 26.0, 23.7, 23.6, 23.0, 21.4, 19.4, 18.0, 17.7. EIMS (70 eV)  $m/z$ : 510 [ $M$ ]<sup>+</sup> (2.6), 495 (2.0), 478 (2.5), 432 (3.0), 263 (29), 248 (58), 231 (37), 203 (100). HREIMS: Calcd for  $C_{32}H_{46}O_5$ : 510.3345. Found: 510.3344. Anal. (Table 2).

**Methyl 2-Aminocarbonyl-3-oxooleana-1,12-dien-28-oate (19).** A solution of **17** (100 mg, 0.19 mmol) in saturated ammonia MeOH (10 mL) was kept at room temperature overnight. The mixture was evaporated in vacuo to give a solid (108 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (1.5:1)] to give **19** as an amorphous solid (94 mg, 96%):  $[\alpha]^{26}_D +77^\circ$  (c 0.60,  $CHCl_3$ ). UV (EtOH)  $\lambda_{max}$  (log  $\epsilon$ ): 236 (3.91) nm. IR (KBr): 3413, 2943, 2866, 1727, 1689  $cm^{-1}$ .  $^1H$  NMR ( $CDCl_3$ ):  $\delta$  8.45 (1H, brs), 8.27 (1H, s), 5.72 (1H, brs), 5.37 (1H, t,  $J = 3.4$  Hz), 3.64 (3H, s), 2.90 (1H, dd,  $J = 4.2$ , 13.9 Hz), 1.17, 1.16, 1.15, 1.14, 0.94, 0.92, 0.84 (each 3H, s).  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  205.8, 178.4, 169.0, 165.8, 144.3, 121.8, 52.2, 51.8, 47.0, 46.0, 45.7, 42.3, 41.8, 41.2, 40.4, 39.6, 34.1, 33.3, 32.5, 32.3, 30.9, 29.1, 27.8, 26.0, 23.8, 23.6, 23.2, 21.9, 19.4, 18.6, 17.6. EIMS (70 eV)  $m/z$ : 509 [ $M$ ]<sup>+</sup> (34), 492 (23), 450 (100), 262 (19), 203 (56). HREIMS: Calcd for  $C_{32}H_{47}O_4N$ : 509.3505. Found: 509.3500. Anal. (Table 2).

**Methyl 1 $\alpha$ ,2 $\alpha$ -Epoxy-3-oxoolean-12-en-28-oate (22).**<sup>9</sup> To a solution of **B-13** (223 mg, 0.48 mmol) in 2 N aqueous NaOH solution (1.7 mL) and THF (11 mL) was added a solution of

30% H<sub>2</sub>O<sub>2</sub> (1.4 mL) in MeOH (2.8 mL) in an ice bath. The mixture was stirred at room temperature for 4 h. To the mixture were added saturated aqueous NaHSO<sub>3</sub> and 5% aqueous NaOH solutions, successively. After removal of THF and MeOH, the resultant mixture was acidified with 6 M aqueous HCl solution. The acidic layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> three times. The extract was worked up according to the standard method to give **22** as a crystalline solid (228 mg, 99%). This material was used for the next reaction without further purification. An analytically pure sample was obtained by recrystallization from MeOH as colorless needles: mp 212–213 °C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +157° (c 0.80, CHCl<sub>3</sub>). IR (KBr): 2943, 2866, 1727, 1699 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  5.36 (1H, t, *J* = 3.3 Hz), 3.64 (3H, s), 3.50 (1H, d, *J* = 4.5 Hz), 3.37 (1H, d, *J* = 4.5 Hz), 2.90 (1H, dd, *J* = 4.2, 13.9 Hz), 1.21, 1.11, 1.01, 0.97, 0.94, 0.92, 0.80 (each 3H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  213.0, 178.4, 144.5, 121.8, 64.1, 57.1, 51.8, 47.0, 46.3, 45.9, 45.0, 42.1, 41.7, 40.8, 39.7, 38.8, 34.1, 33.3, 32.5, 32.3, 30.9, 28.2, 28.0, 26.0, 24.0, 23.8, 23.3, 21.1, 19.1, 17.4, 15.1. EIMS (70 eV) *m/z*: 482 [M]<sup>+</sup> (7.7), 422 (13), 262 (31), 249 (11), 203 (100). HREIMS: Calcd for C<sub>31</sub>H<sub>46</sub>O<sub>4</sub>: 482.3396. Found: 482.3391.

**Methyl 2-Methoxy-3-oxooleana-1,12-dien-28-oate (23).** A mixture of **22** (300 mg, 0.62 mmol) and Na (360 mg) in MeOH (36 mL) was heated under reflux for 48 h. After removal of MeOH in vacuo, the resultant mixture was diluted with water and then acidified with 6 M aqueous HCl solution. The aqueous mixture was extracted with a mixture of CH<sub>2</sub>Cl<sub>2</sub> and Et<sub>2</sub>O (1:2) three times. The extract was worked up according to the standard method to give a solid (320 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (4:1)] to afford **22** (31 mg) and **23** as an amorphous solid (270 mg; 87%, 98% based on recovered **22**): UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 266 (3.77) nm. IR (KBr): 2946, 2866, 1727, 1682, 1621 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  5.96 (1H, s), 5.36 (1H, t, *J* = 3.5 Hz), 3.64, 3.55 (each 3H, s), 2.90 (1H, dd, *J* = 4.1, 13.7 Hz), 1.17 (6H, s), 1.16, 1.13, 0.93, 0.90, 0.81 (each 3H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  200.1, 178.4, 149.0, 144.6, 126.3, 121.9, 54.9, 53.2, 51.8, 47.0, 45.9, 45.4, 43.3, 42.3, 41.7, 40.2, 38.4, 34.0, 33.3, 32.6, 32.5, 30.9, 28.5, 27.8, 26.0, 23.81, 23.76, 23.2, 22.0, 20.4, 19.2, 17.4. EIMS (70 eV) *m/z*: 496 [M]<sup>+</sup> (80), 436 (21), 328 (19), 262 (36), 203 (100). HREIMS: Calcd for C<sub>32</sub>H<sub>48</sub>O<sub>4</sub>: 496.3553. Found: 496.3544.

**Methyl 2-Hydroxy-3-oxooleana-1,12-dien-28-oate (24).** A suspension of **23** (100 mg, 0.20 mmol) in 3 M aqueous HCl solution (3 mL) and AcOH (3 mL) was heated under reflux for 5 h. The mixture was neutralized with saturated aqueous Na<sub>2</sub>CO<sub>3</sub> solution. The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> three times. The extract was worked up according to the standard method to give a solid (90 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (5:1)] to afford **24** as an amorphous solid (78 mg, 81%): UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 272 (3.63) nm. IR (KBr): 3426, 2939, 2870, 1725, 1667, 1648 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  6.35 (1H, s), 5.93 (1H, brs), 5.34 (1H, t, *J* = 3.5 Hz), 3.63 (3H, s), 2.89 (1H, dd, *J* = 4.0, 13.7 Hz), 1.22 (6H, s), 1.13, 1.12, 0.94, 0.91, 0.80 (each 3H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  201.3, 178.4, 144.4, 143.9, 128.4, 122.0, 54.1, 51.8, 47.0, 45.9, 44.1, 43.3, 42.2, 41.6, 40.2, 38.7, 34.1, 33.3, 32.7, 32.5, 30.9, 27.8, 27.4, 26.1, 23.8, 23.6, 23.2, 22.0, 19.8, 18.9, 17.5. EIMS (70 eV) *m/z*: 482 [M]<sup>+</sup> (26), 446 (68), 422 (25), 262 (35), 203 (100). HREIMS: Calcd for C<sub>31</sub>H<sub>46</sub>O<sub>4</sub>: 482.3396. Found: 482.3387.

**Methyl 2-Hydroxymethylene-3-oxoolean-12-en-28-oate (25).**<sup>19</sup> To a stirred mixture of **B-3** (1084 mg, 2.31 mmol) and ethyl formate (97%) (707 mg, 9.26 mmol) in benzene (12 mL) was added NaOMe (501 mg, 9.27 mmol). The mixture was stirred at room temperature for 1 h. After the mixture was washed with 5% aqueous HCl solution twice, it was worked up according to the standard method to give **25** as an amorphous solid (1095 mg, 95%). This material was used for the next reaction without further purification. An analytically pure sample was obtained by flash column chromatography [hexanes–EtOAc (7:1)] and subsequent recrystallization from MeOH as colorless needles: mp 199–201 °C. UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 296 (3.94) nm. IR (KBr): 3426, 2943, 2862, 1725, 1637,

1588 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  14.92 (1H, d, *J* = 3.1 Hz), 8.58 (1H, d, *J* = 3.1 Hz), 5.35 (1H, t, *J* = 3.7 Hz), 3.64 (3H, s), 2.90 (1H, dd, *J* = 4.2, 13.6 Hz), 2.29 (1H, d, *J* = 14.4 Hz), 1.92 (1H, d, *J* = 14.4 Hz), 1.20, 1.16, 1.12, 0.94 (each 3H, s), 0.91 (6H, s), 0.80 (3H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  190.9, 188.6, 178.4, 144.0, 122.3, 106.0, 52.3, 51.8, 47.0, 46.0, 45.9, 42.0, 41.6, 40.3, 39.4, 39.3, 36.5, 34.1, 33.3, 32.5, 32.1, 30.9, 28.6, 27.9, 25.9, 23.8, 23.6, 23.3, 21.1, 19.7, 16.8, 14.7. EIMS (70 eV) *m/z*: 496 [M]<sup>+</sup> (4.4), 437 (23), 262 (38), 233 (20), 203 (100). HREIMS: Calcd for C<sub>32</sub>H<sub>48</sub>O<sub>4</sub>: 496.3553. Found: 496.3550.

**Methyl Isoxazolo[4,5-b]olean-12-en-28-oate (26).** A mixture of **25** (994 mg, 2.0 mmol), hydroxylamine hydrochloride (1391 mg, 20 mmol) in water (1.8 mL) and EtOH (48 mL) was heated under reflux for 1 h. After EtOH was removed in vacuo, EtOAc was added to the resultant mixture. The EtOAc layer was washed with water (three times) and saturated aqueous NaCl solution (three times), dried over MgSO<sub>4</sub>, and filtered. The filtrate gave a solid (1086 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (6:1) followed by hexanes–EtOAc (5:1)] to give **26** as an amorphous solid (934 mg, 86%): UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 228 (3.65) nm. IR (KBr): 2940, 2864, 1725 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  7.98 (1H, s), 5.34 (1H, t, *J* = 3.5 Hz), 3.63 (3H, s), 2.89 (1H, dd, *J* = 4.4, 13.7 Hz), 2.42 (1H, d, *J* = 15.1 Hz), 1.30, 1.21, 1.15, 0.93, 0.90, 0.88, 0.79 (each 3H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  178.4, 173.2, 150.4, 144.0, 122.3, 109.0, 53.7, 51.7, 46.9, 46.3, 46.0, 42.0, 41.6, 39.5, 38.9, 35.5, 34.9, 34.0, 33.3, 32.5, 32.1, 30.9, 29.0, 27.9, 25.9, 23.8, 23.5, 23.2, 21.6, 19.0, 16.7, 15.4. EIMS (70 eV) *m/z*: 493 [M]<sup>+</sup> (11), 434 (18), 262 (28), 249 (16), 203 (100). HREIMS: Calcd for C<sub>32</sub>H<sub>47</sub>O<sub>3</sub>N: 493.3556. Found: 493.3556.

**Methyl 2-Cyano-3-oxoolean-12-en-28-oate (27).** To a stirred solution of **26** (887 mg, 1.80 mmol) in Et<sub>2</sub>O (50 mL) and MeOH (25 mL) was added NaOMe (3.2 g) in an ice bath. The mixture was stirred at room temperature for 1 h. The mixture was diluted with a mixture of CH<sub>2</sub>Cl<sub>2</sub> and Et<sub>2</sub>O (1:2) (50 mL). After the extract was washed with 5% aqueous HCl solution, it was worked up according to the standard method to afford **27** as an amorphous solid (879 mg, 99%). This material was used for the next reaction without further purification. An analytically pure sample was obtained by flash column chromatography [hexanes–EtOAc (5:1)] as an amorphous solid: UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 238 (3.88) nm. IR (KBr): 2946, 2870, 2202, 1724, 1633 cm<sup>-1</sup>. <sup>1</sup>H NMR of major tautomer **27a** (CDCl<sub>3</sub>):  $\delta$  6.15 (1H, brs), 5.31 (1H, t, *J* = 3.6 Hz), 3.63 (3H, s), 2.88 (1H, dd, *J* = 4.0, 13.6 Hz), 2.09 (1H, d, *J* = 15.0 Hz), 1.16, 1.13, 1.07, 0.95, 0.93, 0.90, 0.76 (each 3H, s). EIMS (70 eV) *m/z*: 493 [M]<sup>+</sup> (6.3), 434 (17), 262 (19), 249 (20), 203 (100). HREIMS: Calcd for C<sub>32</sub>H<sub>47</sub>O<sub>3</sub>N: 493.3556. Found: 493.3548.

**Methyl 2-Hydroxymethylene-3-oxours-12-en-28-oate (28).**<sup>23</sup> **28** was prepared from **B-4** according to the same method as for **25** to give an amorphous solid (quantitative). This material was used for the next reaction without further purification. An analytically pure sample was obtained by flash column chromatography [hexanes–EtOAc (7:1)] and subsequent recrystallization from MeOH as colorless needles: mp 170–171 °C. UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 294 (3.86) nm. IR (KBr): 3426, 2947, 2921, 2866, 1727, 1637, 1590 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  14.91 (1H, brs), 8.57 (1H, s), 5.31 (1H, t, *J* = 3.7 Hz), 3.62 (3H, s), 2.31 (1H, d, *J* = 14.4 Hz), 2.27 (1H, d, *J* = 12.5 Hz), 1.95 (1H, d, *J* = 14.4 Hz), 1.19, 1.12, 1.10 (each 3H, s), 0.96 (3H, d, *J* = 6.0 Hz), 0.92 (3H, s), 0.87 (3H, d, *J* = 6.6 Hz), 0.81 (3H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  191.0, 188.5, 178.2, 138.4, 125.6, 106.0, 53.2, 52.3, 51.7, 48.4, 45.7, 42.4, 40.3, 39.7, 39.5, 39.3, 39.1, 36.8, 36.4, 32.4, 30.9, 28.7, 28.2, 24.4, 23.7, 23.6, 21.4, 21.1, 19.7, 17.2, 17.0, 14.8. EIMS (70 eV) *m/z*: 496 [M]<sup>+</sup> (11), 437 (15), 262 (80), 233 (41), 203 (100). HREIMS: Calcd for C<sub>32</sub>H<sub>48</sub>O<sub>4</sub>: 496.3553. Found: 496.3547.

**Methyl Isoxazolo[4,5-b]urs-12-en-28-oate (29).** **29** was prepared from **28** according to the same method as for **26** to give an amorphous solid (84%): UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 228 (3.70) nm. IR (KBr): 2969, 2922, 2870, 1725 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  7.98 (1H, s), 5.31 (1H, t, *J* = 3.4 Hz), 3.62 (3H, s), 2.46 (1H, d, *J* = 15.0 Hz), 2.27 (1H, d, *J* = 11.1 Hz), 1.31, 1.22,

1.10 (each 3H, s), 0.96 (3H, d,  $J = 6.3$  Hz), 0.90 (3H, s), 0.88 (3H, d,  $J = 6.3$  Hz), 0.81 (3H, s).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  178.2, 173.2, 150.4, 138.4, 125.5, 109.1, 53.7, 53.2, 51.7, 48.3, 46.3, 42.3, 39.7, 39.3, 39.1, 38.8, 36.8, 35.8, 34.9, 32.4, 30.9, 29.1, 28.3, 24.4, 23.7, 23.5, 21.6, 21.4, 19.0, 17.2, 16.9, 15.6. EIMS (70 eV)  $m/z$ : 493  $[\text{M}]^+$  (9.1), 434 (20), 262 (65), 249 (33), 203 (100). HREIMS: Calcd for  $\text{C}_{32}\text{H}_{47}\text{O}_3\text{N}$ : 493.3556. Found: 493.3547.

**Methyl 2-Cyano-3-oxours-12-en-28-oate (30).** **30** was prepared from **29** according to the same method as for **27** to give an amorphous solid (quantitative). This material was used for the next reaction without further purification. An analytically pure sample was obtained by flash column chromatography [hexanes–EtOAc (5:1)] as an amorphous solid: UV (EtOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 238 (3.93) nm. IR (KBr): 2947, 2870, 2203, 1724, 1631  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR of major tautomer **30a** ( $\text{CDCl}_3$ ):  $\delta$  5.92 (1H, brs), 5.28 (1H, t,  $J = 3.5$  Hz), 3.61 (3H, s), 2.26 (1H, d,  $J = 11.0$  Hz), 2.13 (1H, d,  $J = 15.0$  Hz), 1.16, 1.13, 1.08, 1.07, 0.96 (each 3H, s), 0.95, 0.77 (each 3H, d,  $J = 6.3$  Hz). EIMS (70 eV)  $m/z$ : 493  $[\text{M}]^+$  (6.8), 434 (19), 262 (62), 249 (44), 203 (100). HREIMS: Calcd for  $\text{C}_{32}\text{H}_{47}\text{O}_3\text{N}$ : 493.3556. Found: 493.3558.

**Methyl 3-Hydroxy-2-methoxycarbonylolean-2,12-dien-28-oate (31).** A mixture of **B-3** (2.0 g, 4.27 mmol) and 1.8 M DMF solution of methoxymagnesium methyl carbonate (Stiles' reagent) (20 mL, 36 mmol) was heated under reflux for 2 h while a slow stream of  $\text{N}_2$  was bubbled through the mixture with a pipet. To the mixture were added 5% aqueous HCl solution and EtOAc. The aqueous layer was extracted with EtOAc (three times). The combined organic layers were washed with water (three times) and saturated aqueous NaCl solution (three times), dried over  $\text{MgSO}_4$ , and filtered. The filtrate was evaporated in vacuo to give a solid (2.26 g). To a solution of the solid in THF (30 mL) was added excessive amount of ethereal diazomethane. The mixture was kept at room temperature for 10 min. The mixture was evaporated in vacuo to give a solid (2.38 g). The solid was subjected to flash column chromatography [hexanes–EtOAc (7:1)] to give **B-3** (330 mg) and **31** as crystals (1.66 g; 74%, 89% based on recovered **B-3**): mp 160–162  $^\circ\text{C}$ . UV (EtOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 262 (4.01) nm. IR (KBr): 2948, 2858, 1737, 1660, 1615  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  12.51 (1H, s), 5.33 (1H, t,  $J = 3.7$  Hz), 3.74, 3.63 (each 3H, s), 2.89 (1H, dd,  $J = 4.2, 13.9$  Hz), 2.35 (1H, d,  $J = 15.7$  Hz), 1.18, 1.14, 1.10, 0.94 (each 3H, s), 0.91 (6H, s), 0.78 (3H, s).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  178.5, 177.9, 174.2, 143.8, 122.6, 94.3, 52.5, 51.8, 51.7, 47.0, 46.13, 46.09, 42.0, 41.7, 39.4, 38.6, 38.4, 35.7, 34.1, 33.3, 32.6, 32.1, 31.0, 28.8, 27.9, 26.0, 23.8, 23.6, 23.3, 20.4, 19.8, 16.8, 15.1. EIMS (70 eV)  $m/z$ : 526  $[\text{M}]^+$  (0.6), 494 (5.6), 479 (2.5), 466 (1.6), 435 (13), 262 (28), 203 (100). HREIMS: Calcd for  $\text{C}_{33}\text{H}_{50}\text{O}_5$ : 526.3658. Found: 526.3658.

**2-Hydroxymethylene-3-oxolean-12-en-28-oic Acid (32).** To a stirred mixture of oleanonic acid (**B-1**)<sup>10</sup> (540 mg, 1.19 mmol) and ethyl formate (97%) (357 mg, 4.66 mmol) in THF (12 mL) was added NaOMe (258 mg, 4.78 mmol). The mixture was stirred at room temperature overnight. The mixture was acidified with 10% aqueous HCl solution. The mixture was extracted with EtOAc three times. The extract was worked up according to the standard method to give a solid (600 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (5:1) followed by hexanes–EtOAc (4:1)] to afford **B-1** (168 mg) and **32** as a crystalline solid (260 mg; 45%, 66% based on recovered **B-1**): mp 200–203  $^\circ\text{C}$  dec. UV (EtOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 292 (3.93) nm. IR (KBr): 2946, 2654, 1732, 1694, 1644, 1587  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  14.91 (1H, brs), 8.59 (1H, s), 5.34 (1H, t,  $J = 3.5$  Hz), 2.86 (1H, dd,  $J = 4.5, 13.9$  Hz), 2.29 (1H, d,  $J = 14.6$  Hz), 1.93 (1H, d,  $J = 14.6$  Hz), 1.19, 1.16, 1.10, 0.94, 0.92, 0.91, 0.82 (each 3H, s).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  190.7, 188.8, 184.7, 143.8, 122.6, 105.9, 52.2, 46.8, 46.0, 45.9, 41.9, 41.2, 40.2, 39.34, 39.30, 36.5, 34.0, 33.3, 32.6, 32.0, 30.9, 28.6, 27.8, 25.9, 23.7, 23.5, 23.1, 21.0, 19.6, 17.0, 14.6. EIMS (70 eV)  $m/z$ : 482  $[\text{M}]^+$  (1.8), 438 (2.7), 436 (3.6), 248 (77), 203 (100). HREIMS: Calcd for  $\text{C}_{31}\text{H}_{46}\text{O}_4$ : 482.3396. Found: 482.3392.

**Evaluation Methods. 1. Reagents.** Recombinant mouse IFN- $\gamma$  (LPS content, <10 pg/mL) was purchased from Genzyme

(Cambridge, MA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Inhibitory test compounds were dissolved in DMSO before addition to cell cultures; final concentrations of DMSO were 0.1% or less. Controls with DMSO alone were run in all cases.

**2. Cell Culture.** To obtain primary macrophages, female CD-1 mice, 5–10 weeks of age (Charles River Breeding Laboratories, Wilmington, MA), were injected intraperitoneally with 2 mL of 4% thioglycollate broth (Difco Laboratories, Detroit, MI). Four days after injection, peritoneal macrophages were harvested and processed according to Nathan's procedure.<sup>7b</sup> Cells were seeded in 96-well plates at  $2 \times 10^5$  cells/well and incubated for 48 h with 20 ng/mL IFN- $\gamma$  in the presence or absence of inhibitory test compounds.

**3. Measurement of NO Production in Mouse Macrophages.** Nitrite accumulation was used as an indicator of NO production in the medium and was assayed by the Griess reaction.<sup>7a</sup> Griess reagent (100  $\mu\text{L}$ ) was added to 100  $\mu\text{L}$  of each supernatant from IFN- $\gamma$  or inhibitory test compound-treated cells in triplicate. The protein determination was performed by Bradford protein assay. The plates were read at 550 nm against a standard curve of sodium nitrite.

**Acknowledgment.** We thank Drs. Carl Nathan and Qiao-wen Xie for expert advice on the preparation of macrophages and the nitric oxide assay. We also thank Dr. Steven Mullen (University of Illinois) for the mass spectra and Professor David A. Evans and Mr. Brett D. Allison (Harvard University) for the optical rotation measurements. This investigation was supported by funds from NIH Grant 1 R01-CA78814; the Norris Cotton Cancer Center; U.S. Department of Defense Grants DAMD17-96-1-6163, DAMD17-98-1-8604, and DAMD17-99-1-9168; the Oliver and Jennie Donaldson Charitable Trust; the National Foundation for Cancer Research; and a Zenith Award from the Alzheimer's Association. M.B.S. is an Oscar M. Cohn Professor, F.G.F. is an Oscar M. Cohn Scholar, and Y.W. is a Howard Hughes Medical Institute Predoctoral Fellow.

## References

- (1) (a) Connolly, J. D.; Overton, K. H. *The Triterpenoids. In Chemistry of Terpenes and Terpenoids*; Newman, A. A., Ed.; Academic Press: New York, 1972; pp 207–287. (b) Nakanishi, K.; Ito, S. Biosynthesis of oleanane and ursene triterpenes in tissue culture of *Isodon japonicus*. In *Natural Products Chemistry*, Vol. 3; Nakanishi, K., Goto, T., Ito, S., Natori, S., Nozoe, S., Eds.; Kodansha: Tokyo, 1983; pp 185–187.
- (2) Devon, T. K.; Scott, A. I. *The Terpenes. In Handbook of Naturally Occurring Compounds*, Vol. 2; Academic Press: New York, 1972; pp 281–384.
- (3) (a) Nishino, H.; Nishino, A.; Takayasu, J.; Hasegawa, T.; Iwashima, A.; Hirabayashi, K.; Iwata, S.; Shibata, S. Inhibition of the tumor-promoting action of 12-O-tetradecanoylphorbol-13-acetate by some oleanane-type triterpenoid compounds. *Cancer Res.* **1988**, *48*, 5210–5215. (b) Hirota, M.; Mori, T.; Yoshio, M.; Iriye, R. Suppression of tumor promoter-induced inflammation of mouse ear by ursolic acid and 4,4-dimethylcholestanol derivatives. *Agric. Biol. Chem.* **1990**, *54*, 1073–1075. (c) Yu, L.; Ma, R.; Wang, Y.; Nishino, H.; Takayasu, J.; He, W.; Chang, M.; Zhen, J.; Liu, W.; Fan, S. Potent anti-tumorigenic effect of tubeimoside 1 isolated from the bulb of *Bolbostemma paniculatum* (Maxim) Franquet. *Int. J. Cancer* **1992**, *50*, 635–638. (d) Umehara, K.; Takagi, R.; Kuroyanagi, M.; Ueno, A.; Taki, T.; Chen, Y.-J. Studies on differentiation-inducing activities of terpenes. *Chem. Pharm. Bull.* **1992**, *40*, 401–405. (e) Singh, G. B.; Singh, S.; Bani, S.; Gupta, B. D.; Banerjee, S. K. Anti-inflammatory activity of oleanolic acid in rats and mice. *J. Pharm. Pharmacol.* **1992**, *44*, 456–458. (f) Huang, M.-T.; Ho, C.-T.; Wang, Z. Y.; Ferrao, T.; Lou, Y.-R.; Stauber, K.; Ma, W.; Georgiadis, C.; Laskin, J. D.; Conney, A. H. Inhibition of skin tumorigenesis by rosemary and its constituents carnosol and ursolic acid. *Cancer Res.* **1994**, *54*, 701–708. (g) Liu, J.; Liu, Y.; Mao, Q.; Klaassen, C. D. The effects of 10 triterpenoid compounds on experimental liver injury in mice. *Fundam. Appl. Toxicol.* **1994**, *22*, 34–40. (h) Liu, J. Pharmacology of oleanolic acid and ursolic acid. *J. Ethnopharmacol.* **1995**, *49*, 57–68 and references therein.

- (4) After we started this project, the following systematic studies of structure–activity relationships based on chemical modification of oleanane triterpenoids were reported: (a) Sakurawi, K.; Yasuda, F.; Tozoy, T.; Nakamura, M.; Sato, T.; Kikuchi, J.; Terui, Y.; Ikenishi, Y.; Iwata, T.; Takahashi, K.; Konoike, T.; Mihara, S.; Fujimoto, M. Endothelin receptor antagonist triterpenoid, myriceric acid A, isolated from *Myrica cerifera*, and structure activity relationships of its derivatives. *Chem. Pharm. Bull.* **1996**, *44*, 343–351. (b) Kashiwada, Y.; Wang, H.-K.; Nagao, T.; Kitanaka, S.; Yasuda, I.; Fujioka, T.; Yamagishi, T.; Cosentino, L. M.; Kozuka, M.; Okabe, H.; Ikeshiro, Y.; Hu, C.-Q.; Yeh, E.; Lee, K.-H. Anti-AIDS agents. 30. Anti-HIV activity of oleanolic acid, pomolic acid, and structurally related triterpenoids. *J. Nat. Prod.* **1998**, *61*, 1090–1095.
- (5) (a) Moncada, S.; Palmer, R. M. J.; Higgs, E. A. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* **1991**, *43*, 109–141. (b) Nathan, C. F.; Xie, Q.-W. Regulation of biosynthesis of nitric oxide. *J. Biol. Chem.* **1994**, *269*, 13725–13728. (c) Anggard, E. Nitric oxide: mediator, murderer, and medicine. *Lancet* **1994**, *343*, 1199–1206. (d) Nathan, C. F.; Xie, Q.-W. Nitric oxide synthases: roles, tolls, and controls. *Cell* **1994**, *78*, 915–918.
- (6) (a) Sporn, M. B.; Roberts, A. B. Peptide growth factors and inflammation, tissue repair, and cancer. *J. Clin. Invest.* **1986**, *78*, 329–332. (b) Ohshima, H.; Bartsch, H. Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. *Mutat. Res.* **1994**, *305*, 253–264.
- (7) (a) Ding, A.; Nathan, C. F.; Graycar, J.; Derynck, R.; Stuehr, D. J.; Srimal, S. Macrophage deactivating factor and transforming growth factors- $\beta$ 1, - $\beta$ 2, and - $\beta$ 3 inhibit induction of macrophage nitrogen oxide synthesis by IFN- $\gamma$ . *J. Immunol.* **1990**, *145*, 940–944. (b) Bogdan, C.; Paik, J.; Vodovotz, Y.; Nathan, C. Contrasting mechanisms for suppression of macrophage cytokine release by transforming growth factor- $\beta$  and interleukin-10. *J. Biol. Chem.* **1992**, *267*, 23301–23308.
- (8) Synthesis of **20** and **21** was already reported: Honda, T.; Gribble, G. W. Design and synthesis of 23,24-dinoroleanolic acid derivatives, novel triterpenoid-steroid hybrid molecules. *J. Org. Chem.* **1998**, *63*, 4846–4849.
- (9) Brieskorn, C. H.; Seifert, M. Methylgruppen-Umlagerungen an Triterpenoiden, 2. Mitt. Aromatisierung des Ringes A. *Arch. Pharm.* **1982**, *315*, 846–851.
- (10) Simonsen, J.; Ross, W. C. J. Oleanolic acid. In *The Terpenes*; Cambridge University: Cambridge, 1957; Vol. 5, pp 221–285.
- (11) Sharpless, K. B.; Lauer, R. F.; Teranishi, A. Y. Electrophilic and nucleophilic organoselenium reagents. New routes to  $\alpha,\beta$ -unsaturated carbonyl compounds. *J. Am. Chem. Soc.* **1973**, *95*, 6137–6139.
- (12) DDQ oxidation of **B-3** in benzene (reflux) did not give **B-13** at all.
- (13) Dean, P. D. G. Halogenolysis of methyl glycyrrhetate with lithium iodide-dimethylformamide. *J. Chem. Soc. (C)* **1965**, 6655.
- (14) Begum, S.; Adil, Q.; Siddiqui, B. S.; Siddiqui, S. Synthesis of 2 $\beta$ -hydroxyursolic acid and other ursane analogues from ursolic acid. *Aust. J. Chem.* **1993**, *46*, 1067–1071.
- (15) Simonsen, J.; Ross, W. C. J. Ursolic acid. In *The Terpenes*; Cambridge University: Cambridge, 1957; Vol. 5, pp 114–135.
- (16) Kurata, Y.; Hirota, H.; Honda, T.; Takahashi, T. Preparation of a tricyclic A-ring analog of quassin. *Chem. Pharm. Bull.* **1987**, *35*, 837–840.
- (17) In addition to **5**, these conditions also gave the completely demethylated product **4** (yield, 12%; 18% based on recovered **23**) and the other partially demethylated product **24** (yield, 16%; 23% based on recovered **23**) from **23**.
- (18) Shaw, J. I.; Stevenson, R. 4-Bromo- and 4-chloro-cholest-4-en-3-one. *J. Chem. Soc.* **1955**, 3549–3551.
- (19) Zaprutko, L. Triterpenoids. Part 9. [1] Structure elucidation of a new sodium dichromate oxidation product of methyl oleanolate and some of its derivatives. *Pol. J. Chem.* **1995**, *69*, 1003–1012.
- (20)  $^1\text{H}$  and  $^{13}\text{C}$  NMR of **25**, **28**, and **32** in  $\text{CDCl}_3$  showed that they are the single tautomer as depicted in Schemes 4 and 6 (see Experimental Section).
- (21) Clinton, R. O.; Manson, A. J.; Stonner, F. W.; Neumann, H. C.; Christiansen, R. G.; Clarke, R. L.; Ackerman, J. H.; Page, D. F.; Dean, J. W.; Dickinson, W. B.; Carabateas, C. Steroidal[3,2-c]-pyrazoles. II. Androstanes, 19-norandrostanes and their unsaturated analogs. *J. Am. Chem. Soc.* **1961**, *83*, 1478–1491.
- (22) Johnson, W. S.; Shelberg, W. E. A plan for distinguishing between some five- and six-membered ring ketones. *J. Am. Chem. Soc.* **1945**, *67*, 1745–1754.
- (23) Glen, A. T.; Lawrie, W.; McLean, J.; Younes, M. E.-G. Triterpenoid constituents of rose-bay willow-herb. *J. Chem. Soc. (C)* **1967**, 510–515.
- (24) Liotta, D.; Barnum, C.; Puleo, R.; Zima, G.; Bayer, C.; Kezar, H. S., III. A simple method for the efficient synthesis of unsaturated  $\beta$ -dicarbonyl compounds. *J. Org. Chem.* **1981**, *46*, 2920–2923.
- (25) Finkbeiner, H. L.; Stiles, M. Chelation as a driving force in organic reactions. IV. Synthesis of  $\alpha$ -nitro acids by control of the carboxylation-decarboxylation equilibrium. *J. Am. Chem. Soc.* **1963**, *85*, 616–622.
- (26) Albert, A. Table and discussion: electronic effects in molecules (Hammett and Taft sigma values). *Selective Toxicity*, 7th ed.; Chapman and Hall: London, 1985; pp 644–649.
- (27) Gagne, D.; Pons, M.; Philibert, D. RU38486: a potent antigluco-corticoid in vitro and in vivo. *J. Steroid Biochem.* **1985**, *23*, 247–251.
- (28) Narayanan, C. R.; Natsu, A. A. Synthesis of some bridged triterpene ethers. *J. Org. Chem.* **1974**, *39*, 2639–2641 and references therein.
- (29) Younes, M. E.-G. Chemical examination of local plants. XIV. triterpenoids from the leaves of Egyptian *Callistemon lanceolatus*. *Aust. J. Chem.* **1975**, *28*, 221–224.
- (30) Huneck, S. Triterpene-IV. Die Triterpensäuren des Balsams von *Liquidambar orientalis* Miller. *Tetrahedron* **1963**, *19*, 479–482.
- (31) Huneck, S.; Snatzke, G. Triterpene, IX. Über die Triterpene aus der Rinde von *Sambucus nigra* L. und die Darstellung von 3-epi-Ursolsäure. *Chem. Ber.* **1965**, *98*, 120–125.
- (32) Kitagawa, I.; Kitazawa, K.; Yoshioka, I. Photochemical transformation leading to eupteleogenin-I. Introduction of epoxy-lactone system. *Tetrahedron* **1972**, *28*, 907–921.
- (33) Kuwada, S.; Matsukawa, T. Ursolic acid. II. Oxidation of ursolic acid and its derivatives with chromic acid. *J. Pharm. Soc. Jpn. (Yakugaku Zasshi)* **1933**, *53*, 593–612.
- (34) Sahu, N. P.; Mahato, S. B.; Chakravarti, R. N. Effect of Raney nickel on some dihydroxy triterpenoids in high boiling solvents. *J. Indian Chem. Soc.* **1973**, *50*, 771–773.
- (35) Djerassi, C.; Thomas, D. B.; Livingston, A. L.; Thompson, C. R. Terpenoids. XXXI. The structure and stereochemistry of medicagenic acid. *J. Am. Chem. Soc.* **1957**, *79*, 5292–5297.
- (36) Sundaramaiah, T.; Ramraj, S. K.; Rao, K. L.; Bai, V. V. Synthesis of A-aza triterpenes-I: A-aza triterpenes from methyl oleanonate, methyl betulonate and lupenone. *J. Indian Chem. Soc.* **1976**, *53*, 664–665.
- (37) Finlay, H. J.; Honda, T.; Gribble, G. W.; Danielpour, D.; Benoit, N. E.; Suh, N.; Williams, C.; Sporn, M. B. Novel A-ring cleaved analogs of oleanolic and ursolic acids which affect growth regulation in NRP-152 prostate cells. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1769–1772.

JM000008J