287

Industria y Comercio
SUPERINTENDENCIA

SUPERINDUSTRIA Y COMERCIO
Radicacion : 04061816 00000003 Folios: 48
Fecha (AMD): 2006-07-27 17:25:05
Tramite : 011 PCT-PATENT 379 PASE NACI 400 OPOSICION
Dependencia: 2000 DIVISION DE NUEVAS CREACIONES

FORMULARIO ÚNICO DE OPOSICIÓN

① OPOSICIÓN A :

- [x] Patente de Invención.
- [ ] Patente de Modelo de Utilidad.
- [ ] Diseño Industrial.
- [ ] Esquema de Trazado para Circuitos Integrados.
- [ ] Marcas de Productos o Servicios.
- [ ] Marca Colectiva.
- [ ] Marca de Certificación.
- [ ] Lema Comercial.

SOLICITUD PUBLICADA

Número del expediente: 04-061816
Solicitante: NOVACEA, INC
Apoderado: DILIA MARIA RODRIGUEZ D` ALEMÀN
Título de la nueva creación o denominación del signo: COMPOSICIONES FARMACEUTICAS QUE COMPRENDEN COMPUESTOS ACTIVOS DE VITAMINA D.
Gaceta: 559

③ OPOSICIÓN PRESENTADA POR:

SOLICITANTE	Nombre: **PROCAPS S.A** Dirección: **CALLE 80 No. 78b-201** Nacionalidad o Domicilio: **COLOMBIA** Lugar de Constitución: **BARRANQUILLA** Teléfono: **371 91 61** Fax: **371 91 62** E-mail:	**IDENTIFICACIÓN** C.C. ☐ NIT ☒ C.E. ☐ Otro ☐ Cual ____ Número **890.106.527-5**
REPRESENTANTE O APODERADO	Nombre: **REINALDO PINILLA MORENO** Dirección: **TRANVERSAL 13 A 119-95 OFICINA 104** Teléfono: **213 12 13** Fax: **612 19 79** E-mail: **negocios@optimum-co.com**	**IDENTIFICACIÓN** C.C. ☒ NIT ☐ C.E. ☐ Otro ☐ Cual ____ Número **80425609** TP **91563**

④ FUNDAMENTOS DE LA OPOSICIÓN:

Art.14, 16, 18, 20 y 30 de la Decisión 486 de la CAN

Causal: **No cumple con los requisitos de patentabilidad.**

Signo opositor:

Justificación:

La presente solicitud no cumple con los requisitos de patentabilidad dado que lo revelado, ya se encontraba en el estado de la técnica o es posible derivarlo de la misma tal y como se demostrará en el capítulo "Fundamento de Hechos".

⑤ **Comprobante de pago No.** _____ **Fecha** _____

⑥ **ANEXOS**

- [X] Comprobante de pago de la tasa.
- [X] Poderes, si fuere el caso.
- [X] Pruebas de los fundamentos de la oposición.
- [ ] Documentos que acrediten el legítimo interés, si fuere el caso.
- [X] Documento que acredita la existencia y representación legal de las partes, cuando sean personas jurídicas.

⑦

NOMBRE: REINALDO PINILLA MORENO

FIRMA: _____

C.C. 80425609 **T.P.** 91563 CSJ

COMPARECENCIA PERSONAL Y AUTENTICACIÓN DE FIRMA[2]

El Notario Treinta y Nueve (E) de Bogotá, da fé que el anterior escrito dirigido a: Superintendencia Industria y Comercio fué presentado personalmente por: Reinaldo Pinilla Moreno quien exhibió la C.C. No. 80425609 de Usaquén y T.P. No. 91563 CSJ y manifestó que la firma y huella que aparecen en el presente documento son suyas, y que acepta el contenido del mismo.

FIRMA

27 MAR 2006

FRANCISCO ULLOA RIAÑO
NOTARIA TREINTA Y NUEVE (E)

Instrucciones para el diligenciamiento del presente formulario, ver reverso de esta página.

Optimum

Derecho de la C mpetencia, Propiedad Industrial y Comercio Exterior

Señora Jefe,

DIVISIÓN DE NUEVAS CREACIONES

Superintendencia de Industria y Comercio

E. S. D.

Referencia	: Expediente N° 04-061816
Asunto	: Oposición a la solicitud de patente de Invención titulada *"COMPOSICIONES FARMACÉUTICAS QUE COMPRENDEN COMPUESTOS ACTIVOS DE VITAMINA D."*.
Solicitante	: NOVACEA, IC.
Opositora	: PROCAPS S.A.

Publicada en la Gaceta de la Propiedad Industrial

N° 559 del 30 de Diciembre de 2005

Yo, *REINALDO PINILLA MORENO*, abogado con tarjeta profesional N° 91563 del consejo superior de la judicatura, en mi condición de apoderado de la sociedad *PROCAPS S.A.* conformada bajo las leyes de la República de Colombia y domiciliada en la ciudad de Barranquilla, Colombia, atentamente manifiesto que estando dentro del término de ley y por orden expresa de mi representada, presento oposición a la concesión del registro de la patente de Invención titulada *"COMPOSICIONES FARMACÉUTICAS QUE COMPRENDEN COMPUESTOS ACTIVOS DE VITAMINA D"*, solicitada por *NOVACEA, IC.*, y pido a Usted declarar fundada esta oposición y en consecuencia negar el registro de la patente solicitada.

Transversal 13ª N° 119-95, Of. 104 Tel. (571) 2 13 12 13 – 6 20 50 21 – Fax. (571) 6 12 19 79
E-mail: negocios@optimum-co.com - www.optimum-co.com
Bogotá Colombia

1. INTERÉS PARA ACTUAR

Conforme a lo dispuesto en el artículo 42 de la Decisión 486 de 2000 de la CAN, estando dentro del término estipulado por la ley, manifiesto el legitimo interés de nuestra representada debido a que la solicitud pretendida no cumple con los requisitos dispuestos en la ley como se analizará más adelante y de ser concedida atentaría directamente a la comercialización que pretende mi representada, en materia de CALCITRIOL CBG en particular. Por lo tanto, solicito respetuosamente a ese Despacho se sirva tener en cuenta la presente oposición dentro del trámite administrativo que se surte en esta Superintendencia.

2. FUNDAMENTOS DE HECHOS

2.1. La solicitud objeto de la presente fue presentada el 29 de junio de 2004, reivindicando prioridad bajo las solicitudes US60/334.554 de 03/12/2001 y fue publicada en Colombia, en la Gaceta de la Propiedad Industrial Nº 559 del 30 de diciembre de 2005, página 381, Nº publicación 905, cuya publicación internacional corresponde a la WO03/047595 del 12 de junio de 2003.

2.2. La solicitud colombiana 04-061816 objeto de la presente oposición, se refiere a una composición farmacéutica que comprende: (a) un componente en fase lipofílica, (b) uno o más tensoactivos, y (c) un compuesto activo de vitamina D; en donde la composición es una emulsión preconcentrada, la cual, al ser diluida con agua en una relación de agua con respecto a la composición de aproximadamente 1: 1 ó más de agua, forma una emulsión que tiene una

absorbancia mayor de 0.3 a 400 nanómetros; donde, la emulsión que se forma al diluir la emulsión preconcentrada con agua, es una emulsión con glóbulos de tamaño submicrónico; donde además comprende un componente de fase hidrofílica; donde el componente de fase hidrofílica comprende 1,2-propilenglicol; donde el componente de fase hidrofílica además comprende un alcanol de 1 a 5 átomos de carbono; donde el alcanol de 1 a 5 átomos de carbono es etanol; donde el componente de fase lipofílica comprende un monoglicérido, diglicérido o triglicérido; y, donde el componente de fase lipofílica comprende un triglicérido; donde el triglicérido se selecciona del grupo que consiste de aceites vegetales, aceites de pescado, grasas animales, aceites vegetales hidrogenados, aceites vegetales parcialmente hidrogenados, triglicéridos sintéticos, triglicéridos modificados, triglicéridos fraccionados y mezclas de los mismos.

2.3. En primera instancia, se debe advertir que las reivindicaciones 49 a 51 y 101 a 103 se refieren al método terapéutico y según la legislación aplicable, Decisión 486 de la Comunidad Andina de Naciones, Artículo 20- "*No serán patentables (...) d) los métodos terapéuticos o quirúrgicos para el tratamiento humano o animal, así como los métodos de diagnóstico aplicados a los seres humanos o a animales*".

2.4. De otra parte el artículo 14 de la Decisión 486 de la CAN consagra lo siguiente: "*Los países miembros otorgarán patentes para las invenciones, sean de producto o procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial*". Así las cosas, las invenciones objeto de patente deben ser nuevas y tener nivel inventivo. Bien, pues antes de la fecha de prioridad de esta solicitud se encuentran los siguientes documentos que afectan la novedad y el nivel inventivo de la presente solicitud:

- D1: US5486509, 23-01-1996.
- D2: US5502224, 26-03-1996

2.5.Ahora bien, el documento D1, particularmente en la descripción, ejemplo VIII, relacionan cuatro formulaciones que incluyen dihidroxivitamina D_3 como ingrediente activo y sus métodos de manufactura. Según dicho ejemplo, se define claramente una composición farmacéutica que comprende un componente en fase lipofílica; un componente de fase hidrofílica; uno o más tensoactivos, y un compuesto activo de vitamina D. De hecho, en la columna 10 línea 20 del ejemplo VIII-3 de dicho documento D1, se define una loción de uso tópico de agua en aceite que comprende Gliceril Monostearato; Propilen Glicol; tensoactivos; Agua; y, dihidroxivitamina D_3, entre otros. Como se nota, el documento anterior D1 divulgó con anterioridad la misma solución que lo pretendido en el presente documento objeto de oposición. De esta manera, a los ojos de este documento D1, la presente solicitud no es novedosa.

2.6.Por su lado, el documento D2 según la descripción, particularmente en la columna 7 líneas 14 a 38 y columna 16 líneas 58 a columna 22 línea 48, se refiere a concentrados que comprenden esteres o fosfátidos de vitamina D o vitamina E compuestos o combinados entre sí; solventes o mezclas de los mismos; surfactantes o mezclas de los mismos; una vitamina o provitamina; y, ácidos libres de grasa. Según este documento D2 anterior, se divulga con anterioridad el hecho de usar dichos concentrados para inhibir el crecimiento de células tumorales o como agentes para el tratamiento del cáncer según la columna 12 líneas 38 a 47. Igualmente en el documento anterior D2, también se refiere a varias rutas de administración según la columna 9 líneas 12 a 37. Según lo anterior, cualquier persona versada en la materia, obviamente puede llegar a una composición farmacéutica que comprende un componente en fase lipofílica; un componente de fase hidrofílica; uno o más tensoactivos, y un compuesto activo de vitamina D, adecuado para el tratamiento del cancer o para inhibir el crecimiento de células

tumorales. Así las cosas, el presente documento objeto de oposición, a la vista del documento D2 combinado con el documento D1, no comprende nivel o altura inventiva toda vez que cualquier persona versada en la materia obviamente a partir de dichas anterioridades combinadas puede llegar a dicha composición farmacéutica.

2.7. En consecuencia y de acuerdo a lo descrito anteriormente, se deriva que la solicitud colombiana **04061816** no puede pretender un monopolio por patente toda vez que lo que pretende no cumple con los tres requisitos necesarios para su protección.

2.8. Que por consiguiente, respecto a la solicitud pretendida se tiene que:

2.8.1. CARECE DE NOVEDAD: Según la decisión 486 de la Comunidad Andina de Naciones, Artículo 16.- *"Una invención se considerará nueva cuando no está comprendida en el estado de la técnica. El estado de la técnica comprenderá todo lo que haya sido accesible al público por una descripción escrita u oral, utilización, comercialización o cualquier otro medio antes de la fecha de presentación de la solicitud de patente o, en su caso, de la prioridad reconocida."*. A la fecha de prioridad, el 3 de diciembre de 2001, ya era conocido, pues como se demostró, era parte del arte anterior con base en el documento D1, una composición farmacéutica que comprende un componente en fase lipofílica; un componente de fase hidrofílica; uno o más tensoactivos; y, un compuesto activo de vitamina D sobre el cual busca protección en el capítulo reivindicatorio.

2.8.2. CARENCIA DE ALTURA INVENTIVA. Según la Decisión 486 de la Comunidad Andina de Naciones, Artículo 18.- *"Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia*

técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica.". A la fecha de prioridad, el 3 de diciembre de 2001, el documento D2 en conjunto con lo especificado en el documento D1, es posible deducir la conformación de una composición farmacéutica que comprende un componente en fase lipofílica; un componente de fase hidrofílica; uno o más tensoactivos; y, un compuesto activo de vitamina D sobre el cual busca protección en el capítulo reivindicatorio.

2.8.3. CARENCIA DE APLICACIÓN INDUSTRIAL La Decisión 486 de la CAN consagra en su artículo 14 lo siguiente: "*Los países miembros otorgarán patentes para las invenciones, sean de producto o procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial*". La presente solicitud carece de aplicación Industrial ya que como se demostró anteriormente, algunas cláusulas reclaman el método terapéutico y estos no son aplicables industrialmente porque primero no se obtiene un producto o proceso; y segundo no es reproducible o repetible a escala industrial. Por lo tanto, el objeto de la presente invención referido a los métodos terapéuticos, no cumple con el requisito de la aplicación industrial.

2.9. Dado lo anterior, es posible deducir que la solicitud y lo reivindicado no es objeto de patentabilidad pues es contrario a lo que dicta la Decisión 486 de la Comunidad Andina de Naciones, Artículo 14.- "*Los Países Miembros otorgarán patentes para las invenciones, sean de producto o de procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial.*". La solicitud pretendida no cumple con los tres requisitos de patentabilidad.

2.10. Por todo lo anterior, atentamente solicito a usted que se sirva declarar fundada la presente oposición y negar el registro Patente de invención denominada *COMPOSICIONES FARMACÉUTICAS QUE COMPRENDEN COMPUESTOS ACTIVOS DE VITAMINA D"*, solicitada por *NOVACEA, IC.,,* solicitud Colombiana que apareció publicada en la Gaceta de la Propiedad Industrial N° 559.

3. PRUEBAS

De conformidad con lo dispuesto por las normas vigentes, solicito a usted tener como prueba en este asunto los siguientes:

- D1: US5486509, 23-01-1996.
- D2: US5502224, 26-03-1996

4. FUNDAMENTO DE DERECHO

Fundamento esta observación en las normas legales siguientes:

4.1. Numeral 1 del artículo 586 del Código de Comercio.

4.2. Artículo 42 de la Decisión 486 de la Comisión del Acuerdo de Cartagena.

4.3. En las demás normas concordantes.

5. ANEXOS

Optimum

Derecho de la Competencia, Propiedad Industrial y Comerci Exterior

Para los efectos correspondientes anexo al presente escrito los siguientes documentos:

- D1: US5486509, 23-01-1996.
- D2: US5502224, 26-03-1996

6. NOTIFICACIONES

Para efectos de notificaciones que por disposición del artículo 50 del Decreto 01 de 1984 debe hacer su Despacho, informo que mi representada y la suscrita recibiremos notificaciones en la Secretaría de su Despacho o en mis oficinas situadas en la Transversal 13ª Nº 119-95, Of. 104 de esta ciudad de Bogotá.

Señor Superintendente,

REINALDO PINILLA MORENO

C.C. 80'425.609 de Bogotá

T.P.A. 91563 del C.S. de la J.

COMPARECENCIA PERSONAL Y AUTENTICACIÓN DE FIRMA[2]
El Notario Treinta y Nueve (E) de Bogotá, da fé que el anterior escrito dirigido a Superintendencia Industria y Comercio fué presentado personalmente por: Reinaldo Pinilla Moreno quien exhibió la C.C. No. 80 425 609 de Bogotá y T.P. No. 91563 CSJ y manifestó que la firma y huella que aparecen en el presente documento son suyas, y que acepta el contenido del mismo.
FIRMA
27 MAR. 2006
Notaría 39
FRANCI NATALLOA RIAÑO
NOTARIA 39 (E)

Transversal 13ª No 119-95 Of. 104 Tel. (571) 2 13 12 ... 6 70 ... 21 Fax (571) 6 ... 19 79
E-mail: negocios@optimum-co.com - www.optimum-co.com
Bogotá Colombia

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 06 - 24,194
FECHA : MARZO 27 DE 2006

SUPERINDUSTRIA Y COMERCIO
Radicacion : 04061816 00000003 Folios: 48
Fecha (AMD): 2006-03-27 17:26:05
Tramite : 011 PCT-PATENT 379 FASE NACI 400 OPOSICION
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

***** C O N S I G N A C I O N *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
LMB. PROCAPS	CONSIGNACION	BANCO POPULAR	050-00110-6	44744453	10/03/2006	260,500.00

***** C O N C E P T O *****

CANT.	RENTISTICO	CONCEPTO		TOTAL CONCEPTO
1	50005-01-04 PRESENTACION DE OBSERVACIONES	12 MARCAS Y LEMAS COMERCIALES		246,000.00
		TOTAL :	$	246,000.00

SON: DOSCIENTOS CUARENTA Y SEIS MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No._____

PROCAPS

PODER ESPECIAL

Yo, **WALTER TANAKA TATEKAWA,** mayor de edad y vecino de la ciudad de Barranquilla (Colombia), identificado como aparece al pie de mi firma, quien en el presente documento actúa en calidad de Representante Legal de la sociedad **PROCAPS S.A.** sociedad legalmente constituida y domiciliada en la ciudad de Barranquilla (Colombia), por el presente documento otorgo a **REINALDO PINILLA MORENO,** abogado titulado, identificado como aparece al pie de su firma, con tarjeta profesional No. 91563 expedida por el Consejo Superior de la Judicatura, poder especial, amplio y suficiente, para formular oposiciones u observaciones a la solicitud de patente No. 04-061816, de título: **"COMPOSICIONES FARMACÉUTICAS QUE COMPRENDEN COMPUESTOS ACTIVOS DE VITAMINA D"**, publicada en la gaceta No. 559 del 30 de Diciembre de 2005.

Nuestro apoderado queda facultado, de manera expresa, para realizar todo tipo de actos necesarios para llevar a cabo el trámite de la oposición, incluyendo, sin limitación, preparar y presentar solicitudes, hacer declaraciones, pagar tasas, contribuciones e impuestos, recabar los títulos o certificados. Asimismo quedan autorizados para impugnar administrativa y judicialmente todo lo que fuere resuelto en el expediente a que se hace expresa referencia en el presente poder, con todas las facultades generales y específicas que fuera requerido para ella.

De la misma manera, además de las facultades que le confiere la naturaleza del presente mandato, se encuentran facultados para recibir, desistir, transigir, sustituir, y reasumir el presente poder, incoar acciones, interponer recursos y demás facultades conferidas por la ley.

Dado y Firmado en Barranquilla (Colombia) a los 23 días del mes de Marzo de 2006.

PROCAPS S.A.
WALTER TANAKA TATEKAWA
C.C. N° 16.251.923
Representante Legal

Acepto,

REINALDO PINILLA MORENO
C.C. 80'425.609 de Bogotá
T.P.A. 91563 del C.S. de la J.

ADY ISABEL NAMEN SEGURA

NOTARIA SEGUNDA DEL CIRCULO DE BARRANQUILLA

DILIGENCIA DE PRESENTACION PERSONAL Y RECONOCIMIENTO

La suscrita Notaria Certifica que este escrito fué presentado Personalmente por: Walter Tovilla Toro Luis

Identificado con: 16251923

Expedida en: Palmira

Quien declaró que su contenido es cierto y que la firma puesta en el es suya.

Firma

24 MAR 2006

Huella del Otorgante

NOTARIA SEGUNDA
ADY ISABEL NAMEN SEGURA
BARRANQUILLA

03-08008498 024802 PROCAPS S.A. No.10054281
Barranquilla, 24 de Marzo de 2006 Hr:10:02:26

CAMARA DE COMERCIO DE BARRANQUILLA

********** Pag. 1
C A M A R A D E C O M E R C I O
D E B A R R A N Q U I L L A
08**********

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.
PROCAPS S.A.----------
NIT: 890.106.527-5.

EN JUNIO DE ESTE AÑO SE ELEGIRÁ JUNTA DIRECTIVA DE LA CÁMARA DE COMERCIO. LAS INSCRIPCIONES DE CANDIDATOS DEBEN HACERSE EN LA PRIMERA QUINCENA DE MAYO. PARA INFORMACIÓN DETALLADA DIRIGIRSE A LA SEDE PRINCIPAL O COMUNICARSE AL SIGUIENTE TELEFONO: 3303911.

EL SUSCRITO SECRETARIO DE LA CAMARA DE COMERCIO DE BARRANQUILLA,

C E R T I F I C A

Que por Escritura Pública No. 1.190 del 22 de Julio de 1976, otorgada en la Notaria Tercera de Barranquilla, inscrito(as) en esta Cámara de Comercio, el 28 de Julio de 1976 bajo el No. 6.070 del libro respectivo, fue constituida la sociedad----------
limitada denominada "PRODUCTORA DE CAPSULAS DE GELATINAS LIMITADA "PROCAPS LIMITADA".----------

C E R T I F I C A

Que por Escritura Pública No. 1.307 del 28 de Junio de 1979, otorgada en la Notaria Tercera de Barranquilla, inscrito(as) en esta Cámara de Comercio, el 23 de Julio de 1979 bajo el No. 10.039 del libro respectivo, la sociedad antes mencionada----------
se transformo en sociedad anonima bajo la denominacion social de "PRODUCTORA DE CAPSULAS DE GELATINA S.A." PROCAPS S.A.".----------

C E R T I F I C A

Que por Escritura Pública No. 3.810 del 31 de Dic/bre de 1980, otorgada en la Notaria Primera de Barranquilla, inscrito(as) en esta Cámara de Comercio, el 30 de Enero de 1981 bajo el No. 12.705 del libro respectivo, la sociedad antes mencionada----------
se transformo en sociedad de responsabilidad limitada bajo al razon social de "PRODUCTORA DE CAPSULAS DE GELATINA LIMITADA "PROCAPS LI - MITADA".----------

C E R T I F I C A

Que por Escritura Pública No. 3.755 del 10 de Nov/bre de 1983, otorgada en la Notaria Unica de Soledad, inscrito(as) en esta Cámara de Comercio, el 22 de Nov/bre de 1983 bajo el No. 17.922 del libro respectivo, la sociedad antes mencionada----------
se transformo en sociedad anonima bajo la denominacion social de "PRODUCTORA DE CAPSULAS DE GELATINA S.A. "PROCAPS S.A.".----------

C E R T I F I C A

********** C O N T I N U A **********

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.----------
NIT: 890.106.527-5.

Que por Escritura Pública No. 3.615 del 06 de Dic/bre de 1996, otorgada en la Notaria 2a. de Barranquilla, inscrito(as) en esta Cámara de Comercio, el 20 de Dic/bre de 1996 bajo el No. 67.218 del libro respectivo, la sociedad antes mencionada----------
cambio de razon social, por la denominacion LABORATORIOS PROCAPS S.A

C E R T I F I C A

Que por Escritura Pública No. 3.775 del 18 de Dic/bre de 1996, otorgada en la Notaria 2a. de Barranquilla, inscrito(as) en esta Cámara de Comercio, el 23 de Enero de 1997 bajo el No. 67.704 del libro respectivo, la sociedad antes mencionada----------
cambio de razon social, por la denominacion PROCAPS S.A. ----------

C E R T I F I C A

Que dicha sociedad ha sido reformada por las siguientes escrituras y/o documentos privados:

Numero	aaaa/mm/dd	Notaria	No. Insc o Reg	aaaa/mm/dd
39	1979/01/18	Notaria 3a. de Barranquilla.	9.452	1979/01/24
992	1981/04/30	Notaria 1a. de Barranquilla.	13.112	1981/05/06
1.464	1982/08/27	Notaria Unica de Soledad	15.533	1982/09/08
2.744	1983/08/18	Notaria Unica de Soledad	17.416	1983/08/26
3.826	1984/07/16	Notaria Unica de Soledad	19.554	1984/07/27
3.835	1985/08/26	Notaria Unica de Soledad	22.230	1985/09/04
2.260	1986/09/10	Notaria Unica de Soledad.	24.995	1986/09/15
1.849	1989/07/11	Notaria 2a. de Barranquilla.	33.909	1989/07/13
444	1990/02/21	Notaria 2a. de Barranquilla.	36.101	1990/03/16
3.090	1991/12/17	Notaria 2a. de Barranquilla	43.993	1992/01/28
269	1993/02/01	Notaria 2a. de Barranquilla	48.428	1993/02/16
2.342	1993/07/28	Notaria 2a. de Barranquilla	50.582	1993/08/10
3.652	1993/11/09	Notaria 2a. de Barranquilla	51.798	1993/11/22
3.615	1996/12/06	Notaria 2a. de Barranquilla	67.218	1996/12/20
89	1997/01/16	Notaria 2a. de Barranquilla	67.704	1997/01/23
2.070	1997/07/17	Notaria 2a. de Barranquilla	70.627	1997/07/31
2.281	1997/08/04	Notaria 2a. de Barranquilla	70.813	1997/08/14
765	1999/04/22	Notaria 3a. de Barranquilla	80.628	1999/04/23
459	2001/02/21	Notaria 2a. de Barranquilla	91.502	2001/02/23
2.608	2001/10/05	Notaria 2a. de Barranquilla	95.771	2001/11/08
1.730	2004/07/29	Notaria 2. de Barranquilla	112.999	2004/08/26
416	2005/03/03	Notaria 2. de Barranquilla	116.364	2005/03/10
1.613	2005/07/28	Notaria 2. de Barranquilla	119.322	2005/08/18

C E R T I F I C A

Que de acuerdo con la(s) escritura(s) arriba citada(s), la sociedad se rige por las siguientes disposiciones:
DENOMINACION O RAZON SOCIAL:
PROCAPS S.A.----------
SIGLA: PROCAPS.
DOMICILIO PRINCIPAL: Barranquilla.
NIT No: 890.106.527-5.

********** C O N T I N U A **********

CAMARA DE COMERCIO DE BARRANQUILLA

CAMARA DE COMERCIO
DE BARRANQUILLA

08**********

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.
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NIT: 890.106.527-5.

MATRICULA MERCANTIL: 24.802.

C E R T I F I C A

Direccion para notificaciones judiciales:
CL 80 No 78 B - 201.
De la ciudad de Barranquilla.

C E R T I F I C A

DURACION: El término de duración de la sociedad se fijó hasta el 18 de Agosto de 2073.

C E R T I F I C A

OBJETO SOCIAL: El objeto principal de la sociedad sera: a)-La fabricacion de capsulas y otros productos similares a partir de materiales tales como gelatinas u otros de distinta naturaleza, que puedan ser utilizados como envases o recipientes de productos y sustancias quimicas, farmaceuticas y cosmeticas, para uso y consumo humano y veterinario; b)-La investigacion, desarrollo y fabricacion de toda clase de productos y sustancias quimicas, farmaceuticas y cosmeticas para uso y consumo humano, animal y vegetal; c)-La compra y venta de materias primas, insumos y productos intermedios necesarios para los procesos de fabricacion de sustancias quimicas, farmaceuticas,alimenticias y cosmeticas, asi como la comercializacion interna y externa de los productos terminados, d) La compra, venta, importacion, exportacion y distribucion de toda clase de elementos instrumentales y equipos medicos,quirurgicos,odontologicos,y hospitalarios para la salud humana y animal. e)La compra,venta,exportacion y disdistribucion de dulces, confites, galletas y todo tipo de golosinas o alimentos para el consumo humano. f) Actuar como representante o agente de personas naturales y juridicas, nacionacionales y extranjeras en la realizacion de operaciones o tr nsacciones relacionadas con la industria farmaceutica y en especial con cualquiera de las actividades descritas en los literales a), b), c). d) y e) antes descritos; g) Prestar los servicios de asesoria y consultoria en las areas administrativas, de mercadeo, analisis clinico, asistencia tecnica, investigacion, costos y produccion, exportaciones, importaciones, ventas, relacionadas con iguales o similares actividades a las que constituyen el objeto de PROCAPS S.A., asi como los de publicidad y promocion de productos quimicos, cosmeticos y farmaceuticos para uso humano, animal o industrial; h)-Adquirir, operar, administrar y explotar, a cualquier titulo, empresa o establecimientos dedicados a la investigacion, de-

********** C O N T I N U A **********

PROCAPS S.A.----------
NIT: 890.106.527-5.

sarrollo, fabricacion o comercializacion de productos quimicos, farmaceuticos, hospitalarios o cosmeticos. En desarrollo de su objeto principal, la sociedad podra tomar interes social como accionista, socio o fundador de empresas que tengan igual o similar objeto, comprar, vender, importar y exportar maquinarias, insumos y repuestos requeridos para el desarrollo de sus actividades; tomar dinero en prestamo, financiar y garantizar las operaciones comerciales que celebre con terceros relativas a su objeto; celebrar y ejecutar todo tipo de actos y contratos necesarios para desarrollar sus operaciones; girar, aceptar, otorgar y protestar todo tipo de titulos valores e instrumentos negociables; comprar, vender y constituir gravamenes sobre bienes muebles e inmuebles; designar representantes o apoderados en el pais y en el exterior y, en general, realizar cualquier acto o negocio que sea necesario o conveniente para el desarrollo de su objeto social y para la mejor proteccion de sus intereses corporativos o la de sus socios. La sociedad podra garantizar el cumplimiento de obligaciones adquiridas por terceros o por sus socios, para lo cual podra inclusive constituir gravamenes reales sobre sus bienes de cualquier naturaleza.----------

C E R T I F I C A

CAPITAL	Nro Acciones	Valor Acción
Suscrito		
$**********1.220.000.000	**********1.220.000	**********1.000
Pagado		
$**********1.220.000.000	**********1.220.000	**********1.000
Autorizado		
$**********2.000.000.000	**********2.000.000	**********1.000

C E R T I F I C A

ADMINISTRACION: La direccion y administracion de la sociedad corresponderа a la Asamblea General de Accionistas, a la Junta Directiva, al Presidente, al Vicepresidente y a un Gerente General. Corresponde a la Junta Directiva las siguientes funciones y atribuciones entre otras: Autorizar cualquier acto o contrato cuya cuantia sea superior al equivalente en pesos Colombianos a doscientos cincuenta mil dolares de los Estados Unidos de America (US$250.000), liquidados a la tasa de cambio representativa del mercado vigente el dia en que se surta la transacion. Cuando se trate de operaciones en virtud de las cuales la sociedad garantice obligaciones de terceros o de los socios, se requerira que la operacion sea aprobada con el voto de por lo menos 2/3 partes de los integrantes de la Junta Directiva.
La sociedad tendra un Presidente , quien ejercera la representacion legal de la sociedad y tendra las siguientes atribuciones entre otras: Ejecutar las decisiones de la Asamblea General de Accionistas y de la Junta Directiva. Ejercer la Representacion Legal de la sociedad en todos los actos y contratos que se requieran para el desarrollo del objeto social, de conformidad con lo previsto en las leyes y en los presentes estatutos. Constituir apoderados judiciales y extrajudiciales, recibir notificaciones, absolver interrogatorios de parte, confesar obligaciones de la

********** C O N T I N U A **********

03-08008498 024802 PROCAPS S.A. No.10054283
Barranquilla, 24 de Marzo de 2006 Hr:10:02:26

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.
PROCAPS S.A.----------
NIT: 890.106.527-5.

sociedad, transigir, disistir y allanar a la sociedad en cualquier asunto judicial. Aprobar y celebrar en nombre de la socieda todo acto o contrato cuya cuantia sea igual o inferior al equivalente en pesos colombianos a doscientos cincuenta mil dolares de los Estados Unidos de America (US$250.000), liquidados a la tasa de cambio representativa del mercado vigente el dia en que se surta la transacion o celebracion del acto. Las demas funciones que le correspondan como Representante Legal por disposicion de la ley, de estos estatutos o de la Junta Directiva. El Presidente tendra un suplente, designado por la Junta Directiva, quien reemplazara al Presidente en sus faltas temporales, absolutas o meramente accidentales, con entidades facultades y sin que sea necesario para ello acreditar la ausencia del principal. La sociedad tendra un Vicepresidente designado por la Junta Directiva, quien tambien ejercera la representacion legal de la sociedad, pudiendo ejercerla conjunta o separadamente con el Presidente. El vicepresidente tendra las siguentes facultades entre otras. Ejecutar dentro de su competencia las decisiones de la Asamblea General de Accionistas, de la Junta Directiva y del Presidente. Ejercer la representacion legal de la sociedad en todos los actos y contratos que se requieran para el desarrollo del objeto social, de conformidad con lo previsto en las leyes y en los presentes estatutos. Constituir apoderados judiciales y extrajudiciales, recibir notificaciones, absolver interrogatorios de parte, confesar obligaciones de la sociedad, transigir, desistir y allanar a la sociedad en cualquier asunto judicial. Aprobar y celebrar en nombre de la sociedad todo acto o contrato cuya cuantia sea igual o inferior al equivalente en pesos colombianos a ciento cincuenta mil dolares de los Estados Unidos de America (US$150.000), liquidados a la tasa de cambio representativa del mercado vigente el dia en que se surta la aprobacion o celebracion del acto, siempre que no se trate de enajenar bienes inmuebles o cualquier otro activo fijo de la sociedad, inclusive de naturaleza muebles, acciones, cuotas o partes de interes inversiones y todo tipo de derechos de la sociedad, o de constituir gravamenes sobre cualquiera de los anteriores, facultades que radican exclusiva y privativamente en cabeza del Presidente. Las demas funciones que le corresponda como Representante legal por disposicion de la ley, de estos estatutos o de la Junta Directiva. El Vicepresidente tendra (1) suplente, designado por la Junra Directiva, quien lo reemplazara conjunta o separadamente en sus faltas temporales, absolutas o meramente accidentales, con identicas facultades y sin que sea necesario para ello acreditar la ausencia del principal. La sociedad tendra un (1) Gerente General, con su respectivo Suplente, designados por la Junta Directiva. El Suplente reemplazara al Gerente General, en sus faltas temporales, absolutas o meramente accidentales, quien junto con el

********** C O N T I N U A **********

PROCAPS S.A.----------
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gerente general, llevaran la representacion legal de la sociedad, con identicas facultades, pudiendolas ejercer conjunta o separadamente con el Presidente y Vicepresidente, con identicas facultades que el Gerente General y sin que sea necesario para ello acreditar la ausencia del titular. El Gerente General tendra las siguientes facultades entre otras: Ejercer la representacion legal de la sociedad, en todos los actos y contratos que se requieran para el desarrollo del objeto social, de conformidad con lo previsto en las leyes y en los presentes estatutos. Aprobar y celebrar en nombre de la sociedad todo acto o contrato cuya cuantia sea igual o inferior al equivalente en pesos Colombianos a Cincuenta Mil dolares de los Estados Unidos de America (US$50.000), liquidados a la tasa de cambio representativa del mercado vigente el dia en que se surta la aprobacion o celebracion del acto, siempre que no se trate de enajenar a cualquier titulo bienes inmuebles o cualquier otro activo fijo de la sociedad, inclusive de naturaleza mueble, acciones, cuotas o partes de interes, inversiones y todo tipo de derechos de la sociedad o de constituir gravamenes sobre cualquiera de las anteriores facultades que radican exclusiva y privativamente en cabeza del Presidente. ----------

C E R T I F I C A

Que según Acta No. 116 del 27 de Junio de 1997 correspondiente a la Asamblea de Accionistas en Barranquilla, de la sociedad:
PROCAPS S.A.----------
cuya parte pertinente se inscribió en esta Cámara de Comercio, el 15 de Agosto de 1997 bajo el No. 70.838 del libro respectivo, y según Acta No. 127 del 22 de Marzo de 2000 correspondiente a la Asamblea de Accionistas en Barranquilla, de la sociedad:
PROCAPS S.A.----------
cuya parte pertinente se inscribió en esta Cámara de Comercio, el 29 de Mayo de 2000 bajo el No. 87.250 del libro respectivo, fueron hechos los siguientes nombramientos:

CLASE: JUNTA DIRECTIVA

Principales

1. Minski Gontovnik Ruben	CC.*******7.463.982
2. Minski Gontovnik Meyer	CC.*******7.461.324
3. Minski Gontovnik Jose	CC.*******8.671.834

Suplentes

1. Minski Zilberblum Elliot	CC.******72.219.541
2. Minski Deitel Daniel	.****************
3. Minski Sharon	PA.*990.212.082.805

C E R T I F I C A

Que según Acta No. 116 del 27 de Junio de 1997 correspondiente a la Asamblea de Accionistas en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio, el 15 de Agosto de 1997 bajo el No. 70.838 del libro respectivo,

********** C O N T I N U A **********

03-08008498 024802 PROCAPS S.A.
Barranquilla, 24 de Marzo de 2006 Hr:10:02:26

No.10054284

C A M A R A D E C O M E R C I O
D E B A R R A N Q U I L L A
08**********

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.
PROCAPS S.A.----------
NIT: 890.106.527-5.

fueron hechos los siguientes nombramientos:

Cargo/Nombre	Identificación
Presidente.	
Minski Gontovnik Ruben	CC.*******7.463.982
Suplente del Presidente	
Minski Minski Abraham	CC.*********802.575
Vicepresidente	
Minski Gontovnik Meyer	CC.*******7.461.324
Suplente del vicepresidente	
Minski Gontovnik Jose	CC.*******8.671.834

C E R T I F I C A

Que según Acta No. 11 del 01 de Febrero de 2001 correspondiente a la Junta Directiva en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio, el 12 de Febrero de 2001 bajo el No. 91.273 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre	Identificación
Gerente General.	
Uribe Ochoa Guillermo Luis	CC.******70.073.754

C E R T I F I C A

Que según Acta No. 406 del 02 de Sep/bre de 2004 correspondiente a la Junta Directiva en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio, el 09 de Sep/bre de 2004 bajo el No. 113.263 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre	Identificación
Suplente del Gerente.	
Tanaka Walter	CC.******16.251.923

C E R T I F I C A

Que según Acta No. 124 del 29 de Marzo de 1999 correspondiente a la Asamblea de Accionistas en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio, el 04 de Agosto de 1999 bajo el No. 82.226 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre	Identificación
Revisor Fiscal Ppal.	
Vargas Pertuz Ana Isabel	CC.******32.696.645

C E R T I F I C A

********** C O N T I N U A **********

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.----------
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Que según Acta No. 127 del 22 de Marzo de 2000 correspondiente a la Asamblea de Accionistas en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio, el 29 de Mayo de 2000 bajo el No. 87.250 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre	Identificación
Suplente del Revisor Fiscal.	
Lozada Villareal Milena	CC.******32.748.111

C E R T I F I C A

Que por escritura publica No. 2.608 del 5 de Octubre del 2.001,otorgada en la Notaria 2a. de Barranquilla, inscrita en esta Camara de Comercio el 8 de Noviembre del 2.001, bajo los Nos. 95.771, 2.056, 2.057, 2.058, 2.059, 2.060, 2.061, 2.062 y 2.064 del libro respectivo, se reforma totalmente el articulo 65, contenido en la escritura publica No. 3.705 de Noviembre 6 de 1.998, de la Notaria Segunda del Circuito de Barranquilla, y se constituyen los siguientes Apoderados Generales, quienes tendran las funciones que a continuacion se indican en razon de su cargo en la sociedad: 1.-Se con fiere Poder Especial, Amplio y Suficiente a los senores: WALTER TANAKA TATEKAWA, C.C. No. 16.251.923; WILIIAM ALBERTO BARROS CERRA, C.C. No. 8.715.941, para que cada uno en forma independiente en nombre y representacion de PROCAPS S.A., suscriba todas las declaraciones tributarias, aduaneras y cambiarias a que este obligada la sociedad, asi como para que responda todo tipo de requerimiento o solicitudes de informacion de las autoridades tributarias, aduaneras o cambiarias,en los ordenes Distrital, Departamental y Nacional. 2.-Se confiere Poder Especial,amplio y suficiente,a los siguientes senores que conforman el Grupo A y el Grupo B para que conjuntamente sus criban contratos de suministros de mercancias y servicios con entidades oficiales, hasta por la suma de US$800.000.oo (OCHOCIENTOS MIL DOLARES DE LOS ESTADOS UNIDOS DE AMERICA), liquidados a la tasa representativa del mercado vigente en el dia que se realice la respectiva transaccion. GUPO A: GUILLERMO LUIS URIBE OCHO C.C. No. 70.073. 754, LINO LAZALA SILVA VARGAS C.C. No. 19.260.410, JAIRO MOLINA MEJIA C.C. No. 8.706.380, CLAUDIA TANGARIFE CASTILLO C.C. No. 41.660.-293. BRUPO B: WALTER TANAKA TATEKAWA C.C. No. 16.251.923, HENRY CARO DIAZ C.C. No. 19.395.298, CARMEN ALICIA FLOREZ C.C. No. 22.434.044, CARMEN ALICIA HERRERA DIAZGRANADOS C.C. No. 22.377.540. Para el ejer cicio de estas facultades se requerira por lo menos la firma de uno (1) de los apoderados especiales del Grupo A y uno de los apoderados especiales del Grupo B; 3.-Se otorga Poder General, Amplio y Suficiente a la Doctora JOSEFINA MIELES BUELVAS C.C. No. 33.138.497, a la Doctora MARCELA CARVAJALINO PAGANO C.C. No. 22.579.748, y al Doctor DANIEL MORENO VILLALBA, C.C. No. 19.113.210; para que cada uno representen a PROCAPS S.A., ante todo tipo de autoridades administrativas y judiciales, en asuntos de naturaleza cambiaria y fiscal, tales como: Contestar e interponer demandas y requerimientos, comparecer en juicios,pedir la practica de pruebas,recibir notificaciones,interponer recursos y ejercer acciones contencioso administrativ s y de tutela,transigir,conciliar,recibir en nombre de la sociedad

********** C O N T I N U A **********

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desistir, sustituir y reasumir este mandato. Para la celebracion de cualquier acuerdo conciliatorio o transaccional, judicial o extrajudicial, cualquiera que sea su cuantia,los Apoderados deberan aportar la autorizacion escrita del Representante Legal de la sociedad. Los Apoderados podran presentar solicitudes de devolucion y/o compensacion de impuestos y recibir los pagos por devoluciones de impuestos, firmar, notificarse y realizar todo acto necesario para cumplir con el mandato encomendado.----------

C E R T I F I C A

Que segun documento privado de fecha 7 de junio de 2000, inscrito en esta Camara de Comercio el 8 de junio de 2000, bajo el No.1.786 del libro respectivo, consta que RUBEN MINSKI GONTOVNIK, C.C. No. 7.463.982 de Barranquilla, actuando como Presidente y Representante Legal de la sociedad PROCAPS S.A, confiere poder especial, amplio y suficiente a KAREN FIGUEROA GARCIA, C.C.No.32.732.070 de Barranquilla, para que en su nombre y representacion de la sociedad suscriba todas declaraciones cambiarias a que este obligada PROCAPS S.A, en el giro normal de sus negocios y operaciones sociales.--

C E R T I F I C A

Que segun Documento Privado del 13 de diciembre del 2.002, inscrito en esta Camara de Comercio el 15 de enero del 2.003 bajo el No.2.290 del libro respectivo, el senor RUBEN MINSKI GONTOVNIK, C.C.No.7.463. 982, en calidad de REPRESENTANTE LEGAL, y en condicion de PRESIDENTE de PROCAPS S.A., mediante el presente documento otorgo PODER ESPECIAL al Sr. MARLON TATIS PAREDES, C.C.No.72.176.874, para que en representacion de PROCAPS S.A., suscriba declaraciones, reportes y cualquier tipo de documentos relacionados con tramites o registros de Comercio Exterior ante el Ministerio de Comercio Exterior y/o cualquier entidad del Estado que haga sus veces. En consecuencia, el Sr. MARLON TATIS PAREDES, podra suscribir en representacion de PROCAPS S.A. declaraciones, reportes y cualquier tipo de documentos relacionados con tramites o registros de Comercio Exterior ante el Ministerio de Comercio Exterior y/o cualquier entidad del Estado que haga sus veces.----------

C E R T I F I C A

Que segun documento privado del 10 de noviembre del 2004, inscrito en esta Camara de Comercio el 1 de diciembre del 2.004, bajo el No. 2.753 del libro respectivo consta que RUBEN MINSNSKI GONTOVNIK, C.C. 7.463.982 de Barranquilla, actuando como Presidente y Representante

********** C O N T I N U A **********

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.----------
NIT: 890.106.527-5.

Legal de la sociedad PROCAPS S.A, confiere poder especial, amplio y suficiente a ANTONIO VARELA DIAZ, C.C.No.72.004.669 de Barranquilla, para que en nombre y representacion de la sociedad firme las declaraciones cambiarias a que este obligada PROCAPS S.A., a suscribrir en el giro normal de sus negocios y operaciones sociales. Por lo anterior, el senor ANTONIO VARELA DIAZ, queda facultado para firmar las declaraciones comabiarias y demas documentos relacionados con las declaraciones, notificaciones, aportar y solicitar las pruebas que sean necesarias, desistir en general a realizar todo acto necesario de tal forma que pueda cumplir con el mandato dado.-

C E R T I F I C A

Que segun documento privado del 22 de Febrero de 2005, inscrito en esta Camara de Comercio el 28 de Marzo de 2.005, bajo el No. 2.820 del libro respectivo, consta que WALTER TANAKA TATEKAWA C.C. 16.251-923 de Palmira, quien actua como representante legal de la sociedad PROCAPS S.A, otorga Poder Especial, Amplio y Suficiente al senor MARLON TATIS PAREDES C.C. No. 72.176.874, para que en representacion de PROCAPS S.A., suscriba declaraciones aduaneras, reportes y cualquier tipo de documentos relacionados con los tramites o registros de comercio exterior ante la direccion de impuestos y aduanas nacionales y/o cualquier entidad que haga sus veces, y para que responda todo tipo de requerimientos y/o solicitudes de informacion de las autoridades aduaneras en los ordenes distrital, departamental y nacional.----------

C E R T I F I C A

Que por Escritura Publica No.1.613 del 28 de Junio del 2.005, otorgada en la Notaria Segunda de Barranquilla, inscrita en esta Camara de Comercio el 18 de agosto del 2.005 bajo el No.2.913 del libro respectivo, consta que RUBEN MINSKI GONTOVNIK, C.C.No.7.463.928, en su condicion de Presidente y Representante Legal de la sociedad PROCAPS S.A., confiere poder especial amplio y suficiente a los siguientes senores que conforman el Grupo A y el Grupo B, para que conjuntamente suscriban contratos de suministro de mercancias y servicios con entidades oficiales, hasta por la suma de OCHOCIENTOS MIL DOLARES DE LOS ESTADOS DE AMERICA (US$800.000), liquidados a la tasa representativa del mercado vigente en el dia que se realice la respectiva transaccion. GRUPO A: MIRIAM RODRIGUEZ DIAZ, C.C.No. 41.473.569. GRUPO B: NELSON SUAREZ CHAVEZ, C.C.No.79.417.971.------.

C E R T I F I C A

Que en esta Cámara de Comercio no aparecen inscripciones posteriores de documentos referentes a reforma, disolución, liquidación o nombramientos de representantes legales de la expresada sociedad.

C E R T I F I C A

Que su última Renovación fue el: 23 de Marzo de 2005.

********** C O N T I N U A **********

03-08008498 024802 PROCAPS S.A.
Barranquilla, 24 de Marzo de 2006 Hr:10:02:26

No.10054286

CAMARA DE COMERCIO
DE BARRANQUILLA

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.
PROCAPS S.A.----------
NIT: 890.106.527-5.

La información sobre embargos de establecimiento se suministra en Certificados de Matrícula, la de contratos sujetos a registro, en Certificados Especiales.

ANEXO 1
PATENTE US5486509

US005486509A

United States Patent [19]

Jimenez et al.

[11] Patent Number: 5,486,509

[45] Date of Patent: Jan. 23, 1996

[54] **METHOD OF PREVENTING AND TREATING CHEMOTHERAPY-INDUCED ALOPECIA**

[75] Inventors: **Joaquin J. Jimenez; Adel A. Yunis,** both of Miami, Fla.

[73] Assignee: **University of Miami, Miami, Fla.**

[21] Appl. No.: **250,646**

[22] Filed: **May 27, 1994**

Related U.S. Application Data

[63] Continuation of Ser. No. 903,829, Jun. 24, 1992, abandoned, which is a continuation-in-part of Ser. No. 810,412, Dec. 20, 1991, abandoned, which is a continuation-in-part of Ser. No. 786,788, Nov. 1, 1991, abandoned, which is a continuation-in-part of Ser. No. 722,500, Jun. 28, 1991, abandoned.

[51] Int. Cl.[6] A61K 31/59; C07C 401/00

[52] U.S. Cl. 514/167; 552/653; 514/880; 514/922

[58] Field of Search 424/45; 514/711, 514/12, 21, 167–171; 530/399; 552/653

[56] **References Cited**

U.S. PATENT DOCUMENTS

Re. 33,107	11/1989	Dikstein et al.	514/46
3,711,602	1/1973	Herschler	424/45
4,863,902	9/1989	Amagase et al.	514/12
4,871,723	10/1989	Makino et al.	514/167
4,883,791	11/1989	Pak et al.	514/167
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Primary Examiner—Stephen G. Walsh
Assistant Examiner—Lorraine M. Spector
Attorney, Agent, or Firm—Morgan & Finnegan

[57] **ABSTRACT**

The present invention relates, in general, to a method of inhibiting alopecia, or hair loss, and, in particular, to a method of inhibiting alopecia induced by chemotherapeutic agents. The invention further relates to a composition suitable for use in such a method. Active agents for use in the above method include proteinaceous growth factors, such as EGF, and/or vitamin D_3 or metabolites thereof.

37 Claims, 9 Drawing Sheets

FIG. 1

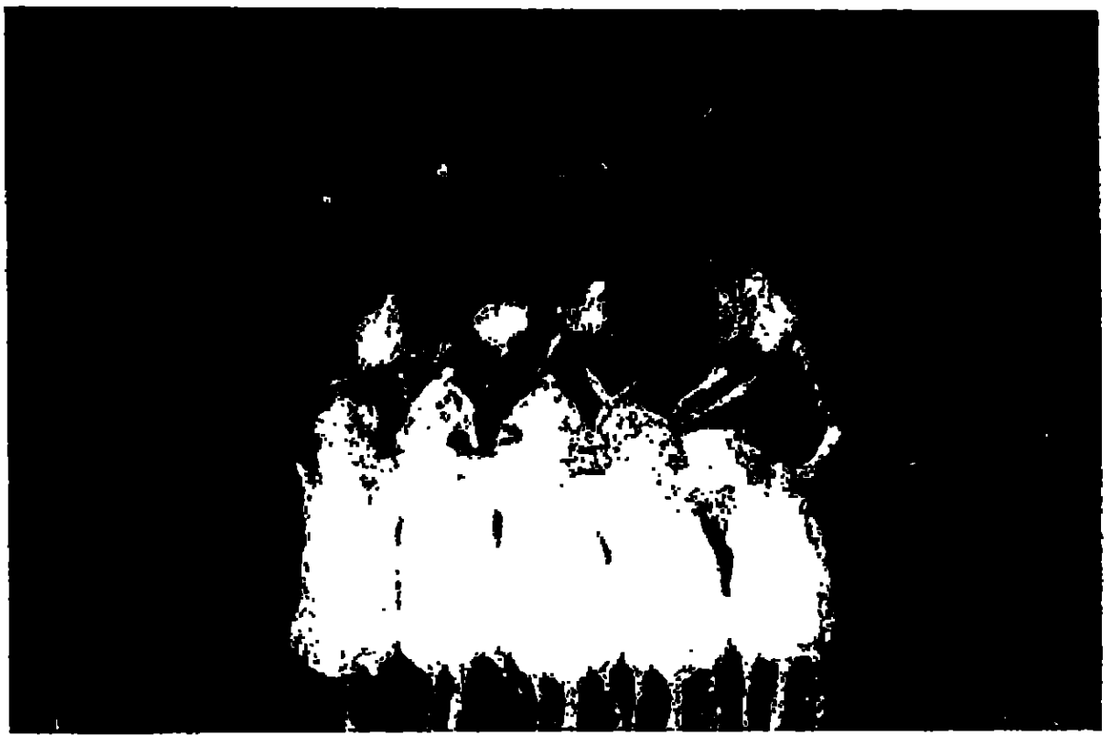


FIG. 2

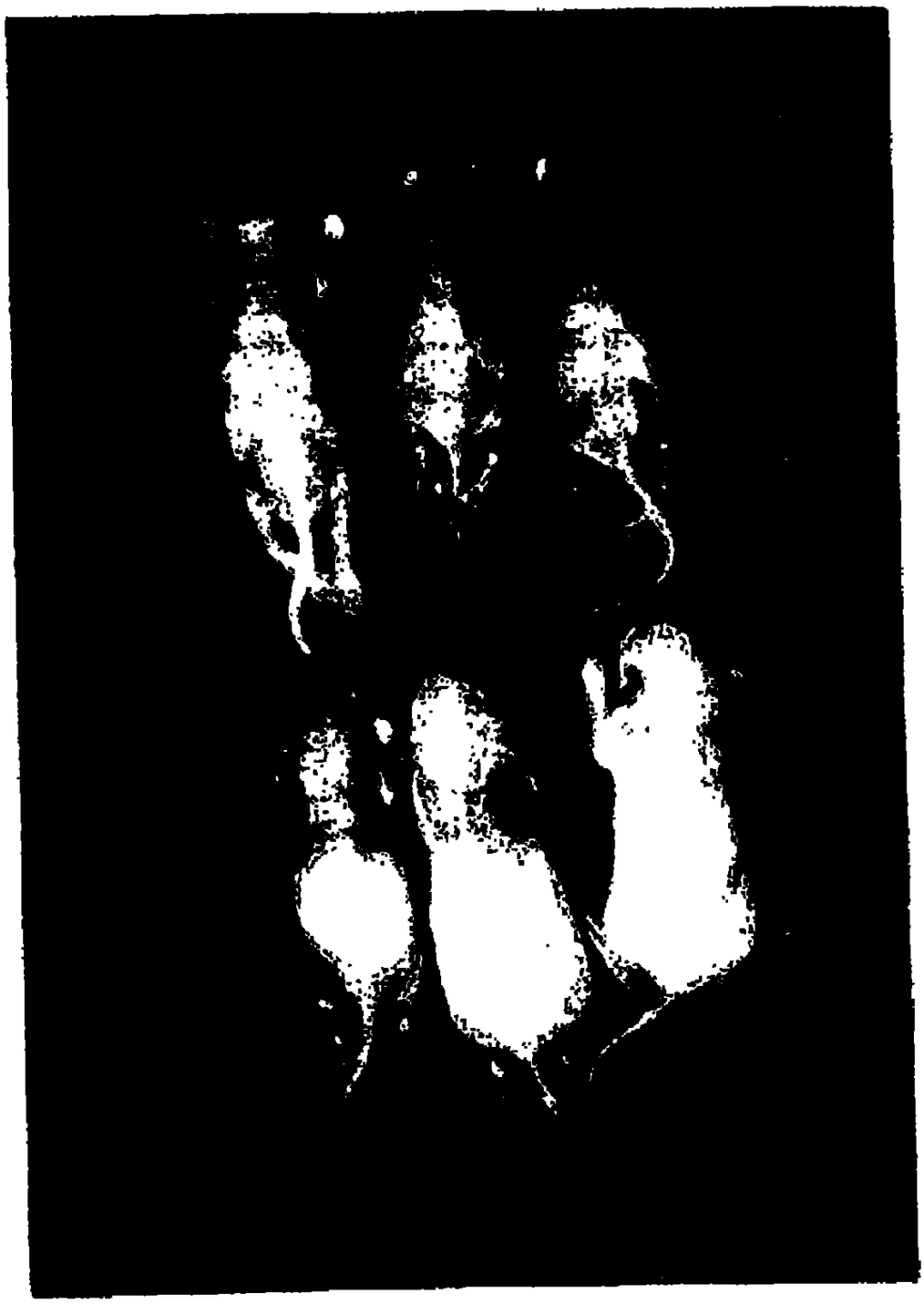


FIG. 3

FIG. 4

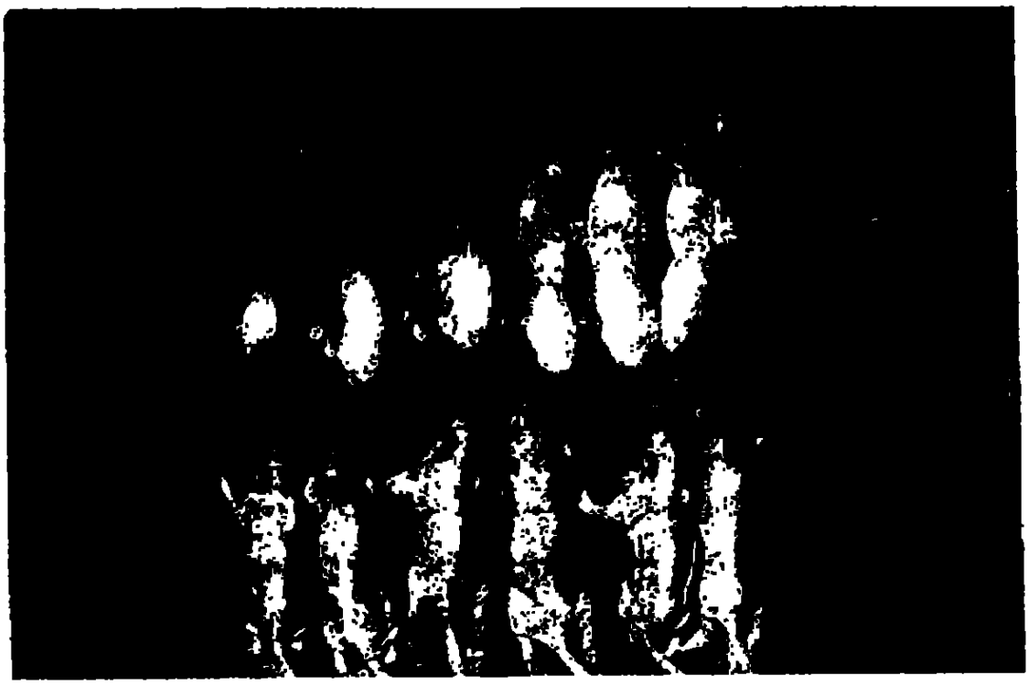


FIG. 5

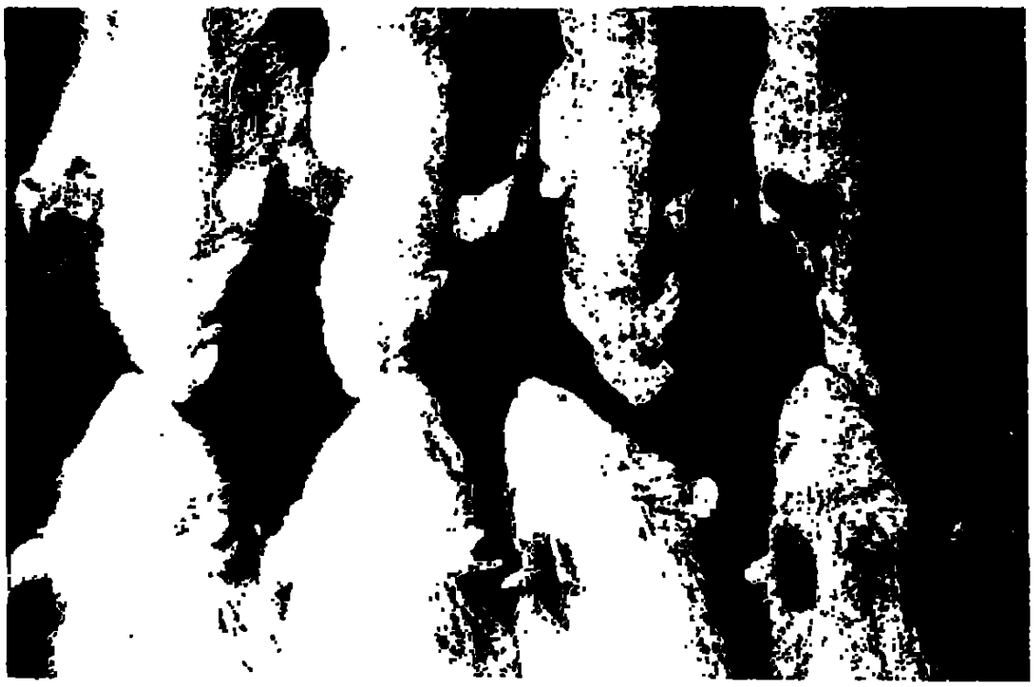


FIG. 6

FIG. 7

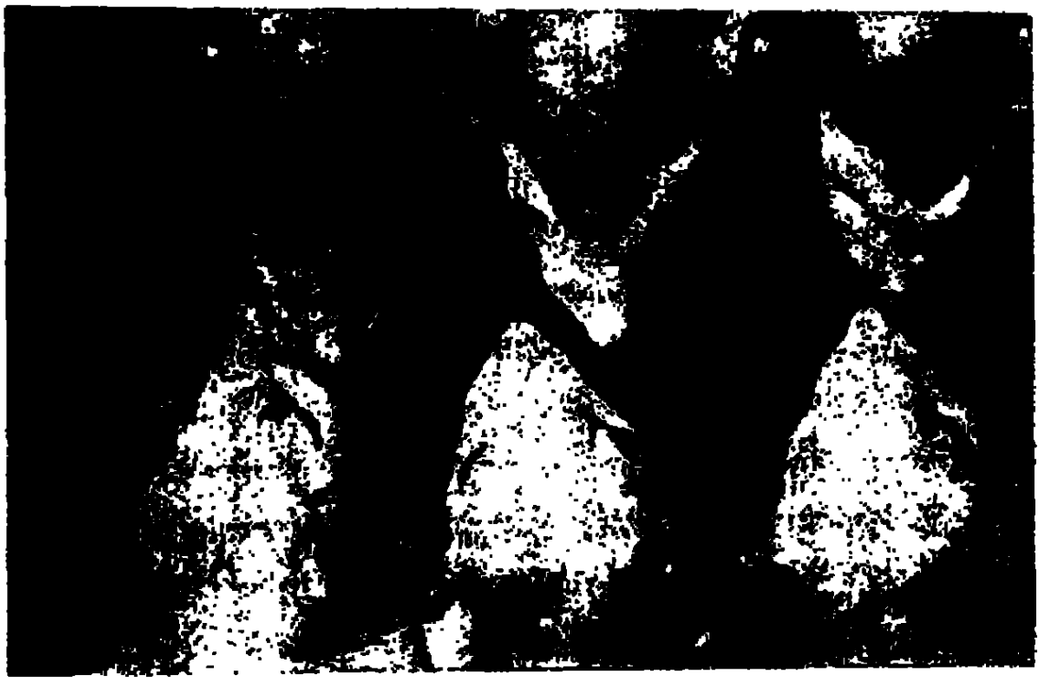


FIG. 8a

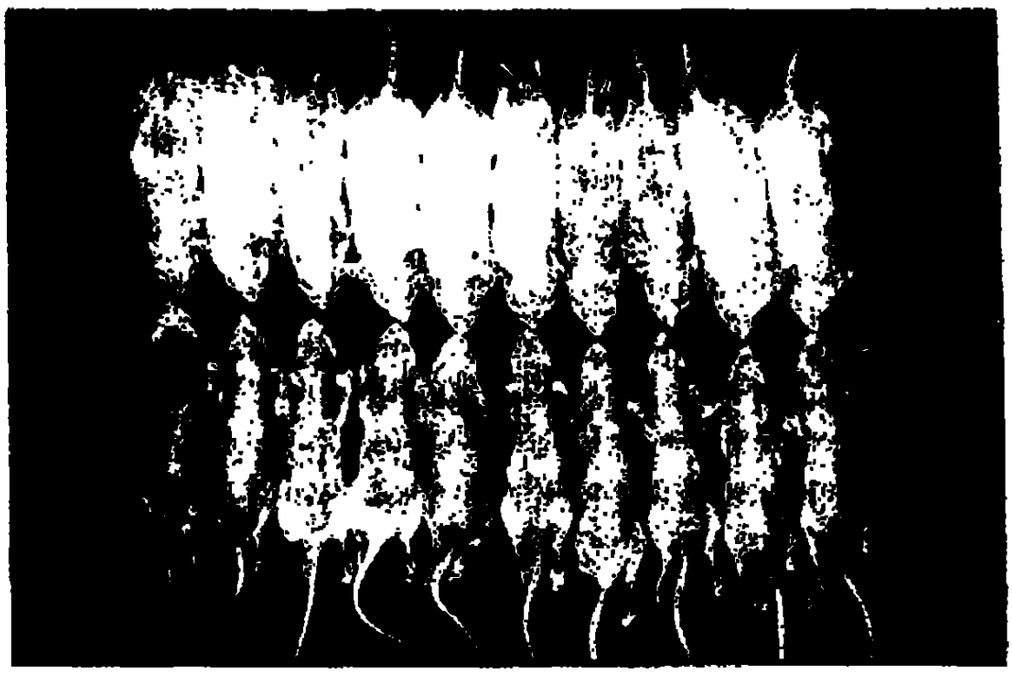


FIG. 8b

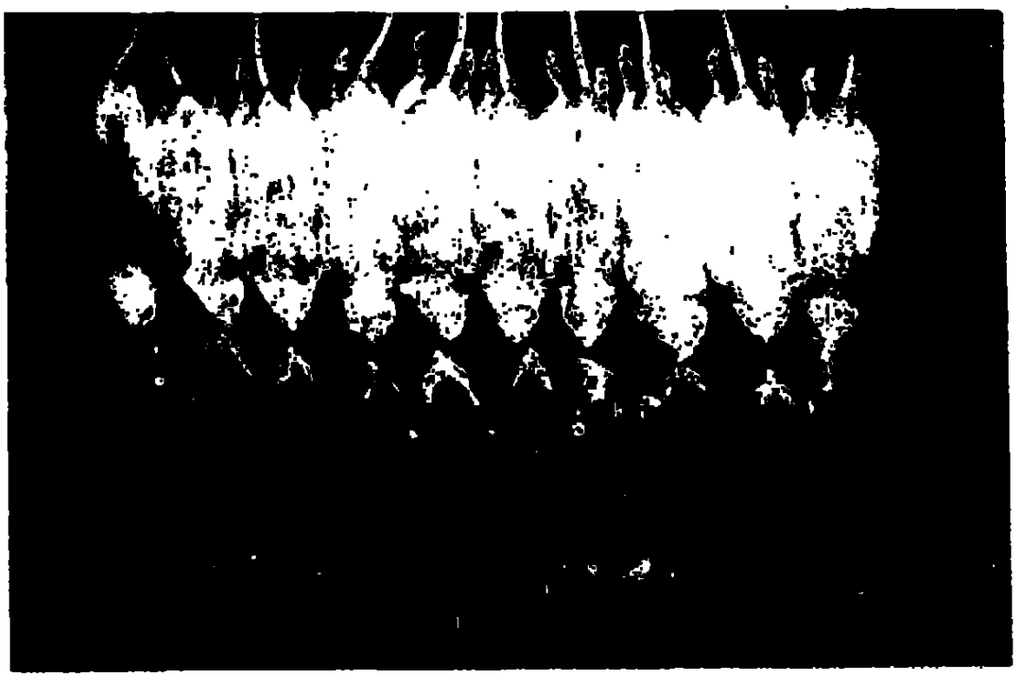


FIG. 8c

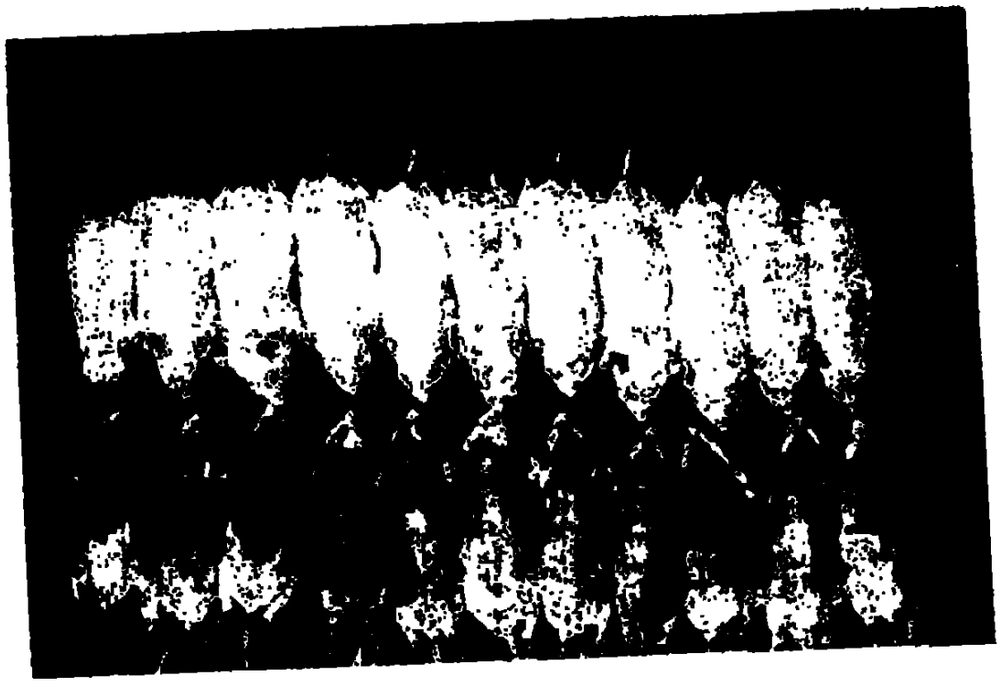


FIG. 9

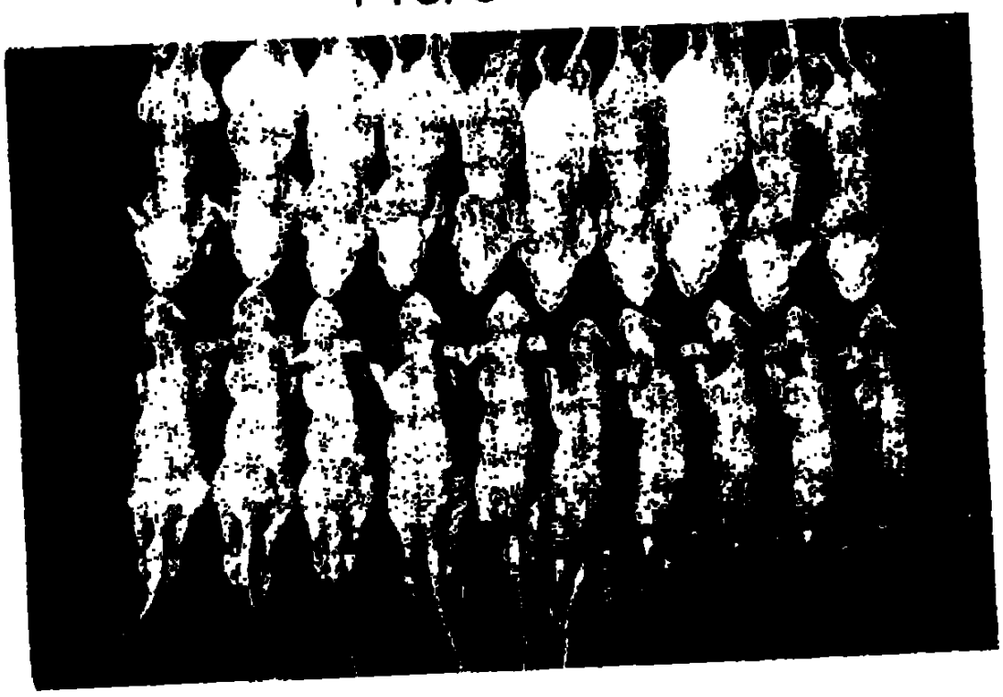
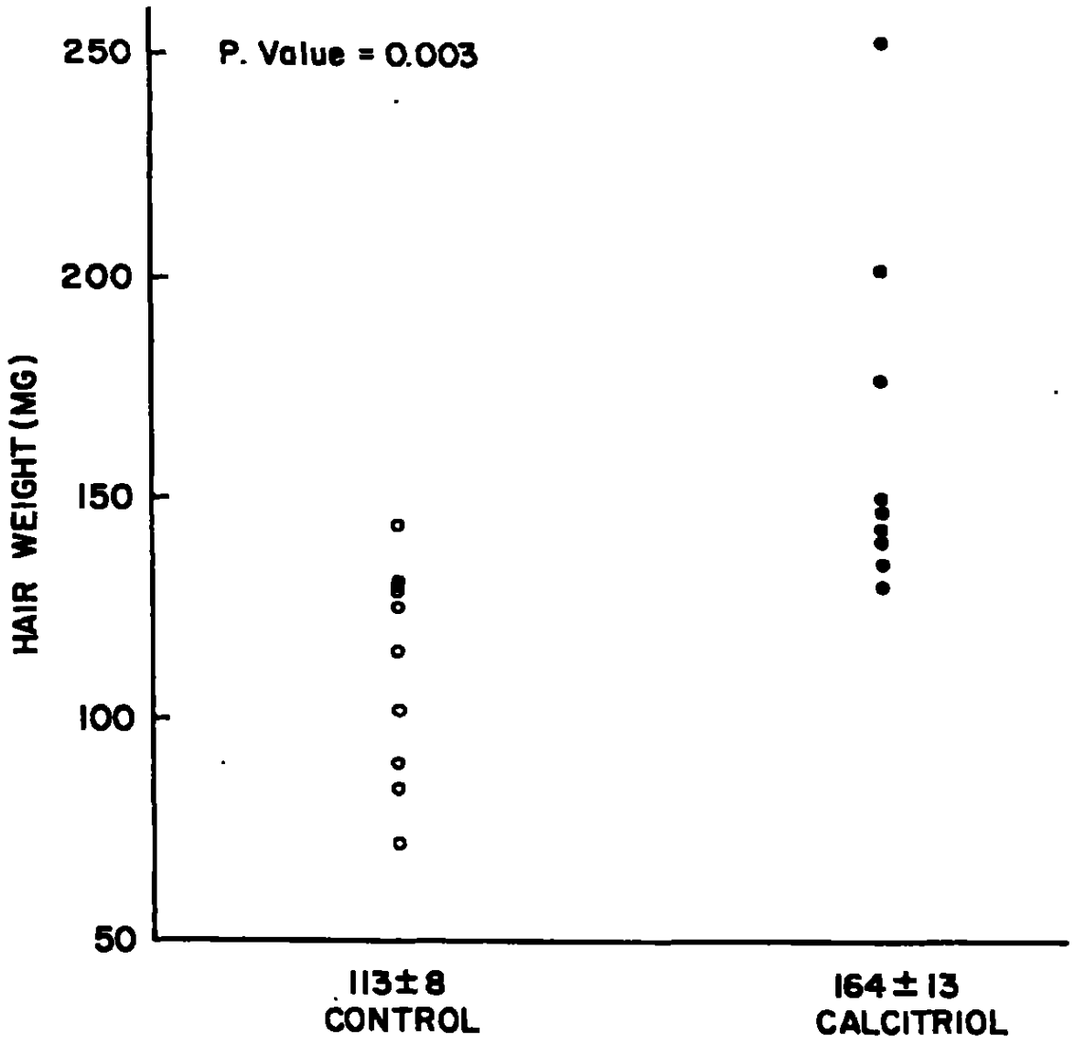


FIG. 10

FIG. 11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200
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CONTROL
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CALCITRIOL

METHOD OF PREVENTING AND TREATING CHEMOTHERAPY-INDUCED ALOPECIA

This is a continuation of application Ser. No. 07/903,829, filed on Jun. 24, 1992, now abandoned, which is CIP of Ser. No. 07/810,412 filed Dec. 20, 1991, now abandoned, which is a CIP of Ser. No. 07/786,788, filed Nov. 1, 1991, now abandoned, which is a CIP of Ser. No. 07/722,500, filed Jun. 28, 1991, now abandoned.

TECHNICAL FIELD

The present invention relates, in general, to a method of preventing or treating alopecia induced by chemotherapeutic agents.

BACKGROUND

Alopecia is a common and distressing side effect of many chemotherapeutic agents and for which there is currently no effective preventive measure. In a recent study, thirty-five of forty-six patients receiving chemotherapy ranked alopecia as more important than vomiting (Tierney et al, B. J. Cancer, 62:527–528, 1990).

Recently, using the young rat model, Applicants demonstrated that ImuVert, a biologic response modifier prepared from the bacterium *Serratia marcescens*, protected the animals from alopecia induced by cytosine arabinoside or adriamycin (Hussein et al, Science 249: 1564–1566, 1990). In subsequent studies, similar protection from ARA-C-induced alopecia was observed from recombinant interleukin-1 (IL-1) beta (Jimenez et al FASEB J. 1991).

The present invention provides an independent method of preventing and treating chemotherapy-induced alopecia. This method involves the use of a growth factor, such as epidermal growth factor (EGF) or fibroblast growth factor (FGF). It should be noted that, as far as Applicants are aware, ImuVert has not been shown to stimulate the production of EGF or FGF, nor has it been proposed to stimulate such production.

The present invention also relates to the use of Vitamin D_3, or a metabolite thereof, alone or in combination with EGF to prevent or treat alopecia. Vitamin D_3 is absorbed after ingestion of fish liver oils or irradiated yeast. Plants and animal sources contain only the inactive vitamin D precursors, 7 -dehydrocholesterol or ergosterol. 7-Dehydrocholesterol is stored in the skin and can be converted by sunlight into vitamin D_3. However, whether ingested or formed by ultraviolet irradiation in the skin, Vitamin D has to be transformed into active metabolites. Vitamin D_3 is converted to 25-hydroxycholecalciferol by liver enzymes. Then in the kidneys two compounds 1,25-dihydroxycholecalciferol and 24,25-dihydroxycholecalciferol are formed. The vitamin D active metabolites play an important role in the absorption of calcium from the intestinal tract, bone deposition and bone reabsorptiono

1,25-Dihydroxyvitamin D_3, an active metabolite of Vitamin D_3, has been shown to increase EGF receptors on breast cancer cells (Falene et al, Molec. and Cell. Endocrinol., 63 (1–2):189–198, 1989) and on a cell line established from rat calvaria (Petkovich et al, J. Biol. Chem. 262 (28): 13424–13428, 1987). However, as far as Applicants are aware, the effect of Vitamin D_3 or a metabolite thereof, on alopecia has not been shown or proposed.

SUMMARY OF THE INVENTION

It is a general object of the invention to provide a method of treating or preventing alopecia. It is a specific object of the invention to prevent or treat alopecia in patients undergoing treatment with chemotherapeutic agents, including cycle specific agents (such as cytosine arabinoside (ARA-C)) and non cycle specific agents (such as Cytoxan), individually or in combination.

Further objects and advantages of the invention will be clear from the description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a photograph of 10 rats from Experiment I, Table I (see below). All rats received ARA-C 50 mg/kg×7 days. Five rats on top received buffer solution s.c. Five rats on bottom received murine EGF 2 μg s.c. daily×7 days.

FIG. 2 is a photograph of 6 rats from Experiment III, Table I (see below). All rats received ARA-C 50 mg/kg×7 days. Three rats on top received buffer solution S.C. Three rats on bottom received rHu-EGF 2 μg s.c. daily×7 days.

FIG. 3 is a photograph of 4 rats from topical murine-EGF experiment (see below). All rats received ARA-C 50 mg/kg×7 days. Two rats on the left received murine EGF 10 μg in DMSO daily×7 days rubbed topically between the shoulder blades over an area of 1 cm^2. Two rats on the right received buffer solution topically.

FIG. 4 is a photograph of 12 rats from ARA-C-aFGF experiment (see below). All rats received ARA-C 50 mg/kg×7 days. Six rats on top received buffer solution s.c. Six rats on bottom received aFGF 2 μg s.c. daily×7 days.

FIG. 5 is a photograph of 8 rats treated with combination chemotherapy Cytoxan and Adriamycin. Four rats on top treated in addition with murine EGF. Four rats on bottom treated with buffer solution.

FIG. 6 is a photograph of 10 rats treated with VP-16. All rats received 1.5 mg/kg×3 days i.p. of VP-16. Five rats on top received buffer solution for four days prior to treatment. Five rats on bottom received Vitamin D_3 50 μg/day for 4 days for four days prior to treatment.

FIG. 7 is a photograph of 6 rats treated with combination chemotherapy Cytoxan and Adriamycin (25 mg/kg i.p.×1 day and 2.5 mg/kg i.p.×3 days, respectively). Three rats on top received buffer solution for four days prior to treatment. Three rats on bottom received Vitamin D_3 50 μg/day for four days prior to treatment.

FIG. 8(A–C). For each experiment, five day old rats were randomly divided into equal numbers. The experimental group of rats (top group) received 0.2 μg of 1,25-dihydroxyvitamin D_3 in 0.15 ml of absolute ethanol daily over the head and neck for 5 days. Control rats (bottom group) were similarly treated with 0.15 ml of absolute ethanol. One day after the last topical treatment, the rats from FIG. 8A were treated with Cytoxan (CTX), rats from FIG. 8B with the Etoposide (VP-16) regimen and rats from FIG. 8C with CTX+Adriamycin (ADM) regimen.

FIG. 9. Twenty 5-day old rats were randomly divided into two groups of 10 rats each. The experimental group of rats (top group) received 0.1 μg of 1,25(OH)2D_3 in 0.1 ml of absolute ethanol daily over the head only for 5 days. Control rats (bottom group) were similarly treated with 0.1 ml of absolute ethanol. One day after the last topical treatment, all rats were treated with the VP-16 regimen.

FIG. 10. Thirteen 9-day old rats were randomized into two groups. Experimental, 7 rats (top group), received 1 μg of RO 23-7553 in 0.2 ml absolute ethanol daily topically over the neck and back for 6 days. Control, 6 rats (bottom group), were similarly treated with 0.2 ml of absolute ethanol. One

day after the last treatment all rats were treated with the VP-16 regimen.

FIG. 11, shows the effect of 1,25-dihydroxyvitamin D_3 on hair growth.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates generally to a method of preventing or reducing alopecia, particularly in patients undergoing chemotherapy. Applicants have shown that a growth factor, such as EGF, and Vitamin D_3 appear to render the hair follicle resistant to the toxic effect of chemotherapeutic agents thus preventing hair loss.

In one embodiment of the present method, a growth factor is administered to a patient undergoing chemotherapy in an amount sufficient to prevent or reduce the hair loss that normally accompanies this treatment regimen.

Growth factors suitable for use in the present method include EGF, FGF, transforming growth factors (TGF), and platelet-derived growth factor (PDGF). The growth factors can be derived from natural sources (for example, human tissue or rodent tissue); however, recombinant production is preferred as large quantities can be produced at relatively low cost. Chemically synthesized factors can also be used. The use of portions or derivatives of growth factors, such as EGF and FGF, is also contemplated as long as those portions or derivatives can effect the same result observed with the factor itself.

In another embodiment of the present invention, Vitamin D_3 or metabolite, analog, derivative or structural variant thereof (for example 1,25-dihydroxy-16-ene-23-yne-cholecalciferol; 1 α-hydroxyvitamin D_3; 1 α-24-dihydroxyvitamin D_3, MC 903, etc.) is administered to a warm blooded animal, for example, a human, in an amount sufficient to prevent or reduce the hair loss or stimulate hair growth. Hair loss treatable or preventable using vitamin D_3 can be due to chemotherapy or other cause. Examples of Vitamin D_3 metabolites suitable for use in the present method include, but are not limited to, 1,25-dihydroxyvitamin D_3 and 1,25-dihydroxy-16-ene-23-yne cholecalciferol.

Compositions suitable for use in the claimed method include as an active agent a growth factor, Vitamin D_3 (or a metabolite or analog thereof) or a combination of both. Such compositions can be formulated by combining an active agent together with a pharmaceutically acceptable vehicle (carrier, diluent or excipient), in an amount sufficient to effect the preventative effect when administered in accordance with an appropriately designed treatment protocol. The composition can be in dosage unit form.

Though not limiting the present method to a particular mode of action, it is suggested that Vitamin D_3 protects against alopecia by increasing the receptors for EGF at the hair follicle level. Accordingly, administering a combination of a growth factor and Vitamin D_3 can be expected to provide for greater protection.

Compositions suitable for use in the method to which the invention relates can be in a form suitable for topical administration. In that event, the composition can take the form of a solution, lotion, cream, gel or ointment. When the composition is to be administered by injection, it advantageously takes the form of a solution. The vehicle used, regardless of the form taken by the composition, can be inert or can itself possess a physiologically or pharmaceutically beneficial effect.

Various additives can be included in the composition. In this regard, inclusion in the composition of an agent that stimulates production of the patients' own growth factor is contemplated. Inclusion in compositions suitable for topical administration of penetration enhancing agents, such as DMSO or ethanol, is preferred. Stabilizers that extend shelf life can also be included in the composition, regardless of the manner in which it is formulated.

One skilled in the art will appreciate that various concentrations of growth factor and/or Vitamin D_3 can be used in the above-described composition. Optimum concentrations can be readily determined by one skilled in the art.

As noted above, the method to which the invention relates can involve either topical application of the active agent (a growth factor and/or Vitamin D_3 or metabolite thereof) or administration by injection. The amount of the active agent and the frequency of administration can vary depending on the individual and can readily be optimized by one skilled in the art. As an example, however, a solution of 2–100 μg/ml of 1,25-dihydroxyvitamin D_3 in absolute ethanol can be prepared and 3–5 ml of that solution applied directly to the scalp at various points with a dropper followed by scalp massage for 3–5 min to ensure even distribution. When chemotherapy is involved, this treatment is, advantageously, administered once or twice daily beginning 5–8 days prior to initiation of chemotherapy and continued through the course of chemotherapy. However, it is also contemplated that the active agent can be administered substantially simultaneously with, or subsequent to, the administration of the chemotherapeutic agent.

The method to which the invention relates is shown in the Examples that follow to be effective when the cell cycle specific drug, ARA-C, is the chemotherapeutic agent used and when the combination of Adriamycin (cell cycle specific) and Cytoxan (non cell cycle specific) is used. It is contemplated, however, that, using the present method, hair loss resulting from treatment with other chemotherapeutic agents can be prevented.

The alopecia preventative effect observed by Applicants was wholly unexpected. Growth factors, such as EGF, are presumed stimulants of skin cell growth. Accordingly, these agents would be expected to induce the hair follicle to enter the cell cycle thus rendering the follicle more susceptible to chemotherapeutic agents, particularly cell cycle specific drugs, such as ARA-C. Thus, administration of growth factors to patients receiving chemotherapy would have been expected to aggravate hair loss. The reverse effect, however, was achieved. It should be noted therefore that the observations recorded herein with EGF and FGF are novel and have not been proposed or described in the literature. Similarly, nothing about the role of Vitamin D_3 in the body suggested that the vitamin would provide such excellent protection against alopecia, chemotherapeutically induced or otherwise.

The following non-limiting Examples describe certain aspects of the invention in greater detail.

EXAMPLES

The following experimental details relate to Examples I–IV set forth below.

Sprague Dawley rats were purchased from Charles River Laboratories, Wilmington, Mass. Cytosar-U (ARA-C) was from the Upjohn Company, Kalamazoo, Mich. Receptor grade EGF from mouse submaxillary glands, human recombinant EGF, dimethyl sulfoxide (DMSO) and Vitamin D_3

were purchased from Sigma Chemical Co., St. Louis, Mo. aFGF was purchased from AMGEN Corp., Thousand Oaks, Calif.

All rats from each experiment were treated with ARA-C 50 mg/kg intraperitoneally (i.p.) daily for 7 days. For subcutaneous (s.c.) injections, EGF and FGF were prepared in PBS 1% BSA. Alopecia was always recorded on day 12 of experiment, and scored as previously described (Hussein et al, *Science*, 249:1564–1566, 1990; Jimenez et al, FASEB J., 1991).

For topical treatment, murine EGF was prepared as follows: One vial of EGF (100 μg) was dissolved in 0.2 ml of PBS 1% BSA and 0.12 ml of this solution was added to 0.48 ml of DMSO. Three hours prior to ARA-C injection, 0.1 ml of the EGF-DMSO mixture was applied to each rat over a 1 cm^2 area between the shoulders using a rubber tip applicator. Rats were then kept individually separated for a period of three hours, following which the treated area was carefully washed with soap and water and dried. Treatment was continued for 7 days. Control animals were similarly treated using DMSO without EGF.

Example I

Protective Effect of EGF

Two separate experiments were conducted to test the ability of murine EGF to protect from ARA-C-induced alopecia. In Experiment I, twenty-two 7-day old rats were randomized in two groups of eleven rats each. In addition to ARA-C, Group I received 2 μg of mouse EGF s.c. in the back between the two hind legs 3 hours prior to ARA-C injections daily for 7 days. Group II, received buffer solution similarly and served as control. Ten of eleven rats in Group II developed virtually total body alopecia and one rat developed more than 50% hair loss. In contrast, in Group I, 5 rats had no detectable hair loss and 6 rats had mild hair loss (Table I, Experiment I (FIG. 1)). In Experiment II, twelve 7-day old rats were randomized in two groups of 6 rats each. In addition to ARA-C, Group I received mouse EGF 1 μg s.c. daily for 7 days. Group II received buffer s.c. All 6 rats in Group II developed moderately severe to severe alopecia, whereas in Group I, one rat had no detectable hair loss and 5 rats developed only minimal hair loss (Table I, Experiment II).

For the next experiment, rHu-EGF was used. Twelve 7-day old rats were randomized in two groups of six rats each. In addition to ARA-C, Group I received rHu-EGF 2 μg s.c. in the flank area daily for 7 days. Group II received buffer s.c. All 6 rats in Group II developed total body alopecia, whereas in Group I none of the rats had total body alopecia, one rat had no detectable hair loss, four rats had mild alopecia and one rat had moderate alopecia (Table I, Experiment III, (FIG. 2)).

TABLE I

OCCURRENCE OF ALOPECIA IN RATS TREATED WITH ARA-C. EFFECT OF MURINE EGF AND rHU-EGF.

	Alopecia*			
	0	1+	2+	3+
	Experiment I			
ARA-C	0	0	1	10
ARA-C + Murine EGF 2 μg	5	6	0	0
	Experiment II			
ARA-C	0	0	3	3
ARA-C + Murine EGF 1 μg	1	5	0	0
	Experiment III			
ARA-C	0	0	0	6
ARA-C + rHu-EGF 2 μg	1	4	1	0

Seven day old rats were used for all experiments.
All rats received ARA-C 50 mg/kg × 7 days I.P. in 0.1 ml.
Murine EGF and rHu-EGF in PBS 1% BSA were given 3 hours prior to ARA-C once daily in 0.1 ml s.c. × 7 days.
Controls received PBS 1% BSA 0.1 ml s.c. × 7 days.
Data recorded on day 12.

*No detectable alopecia, 0; mild alopecia defined as less than 50% hair loss, 1+; moderately severe alopecia with more than 50% hair loss, 2+; and total or virtually total (>90%) hair loss, 3+.

In the next experiment, twelve 7-day old rats were randomized in two groups of six rats each. Group I, in addition to ARA-C, received murine-EGF 10 μg in DMSO daily×7 days rubbed topically with a cotton tip applicator between the shoulder blades over an area of 1 cm^2. Group II received control solution topically. In Group II, all six rats developed complete body alopecia. In Group I, all rats developed complete body alopecia except where the EGF was applied topically (FIG. 3).

Example II

Protective Effect of aFGF

Fourteen 7-day old rats were randomized in two groups. All rats received ARA-C 50 mg/kg/day for seven days. In addition, Group I received aFGF 2 μg s.c. on back of head daily for seven days. Group II received buffer injections and served as controls. Alopecia was recorded on day 12 of experiment. All rats in Group II developed complete body alopecia. In contrast, all rats in Group I were protected locally at the site of injection (FIG. 4).

Example III

Protection from Cytoxan/Adriamycin-Induced Alopecia

Eight 4-day old Sprague Dawley rats were randomized in two groups of 4 rats each. Group I, received EGF 2μ s.c. on the head daily for 7 days. Group II received buffer injections and served as controls. One day after stopping EGF or buffer injections all rats received Cytoxan 25 mg/kg i.p.×1 day and Adriamycin 2.5 mg/kg i.p.×3 days. In Group II, all rats had 3+ alopecia over head and neck area. In contrast, in Group I one rat had mild alopecia, one rat minimal alopecia, and two rats no detectable alopecia over head and neck. (FIG. 5). ImuVert when used under similar conditions did not protect from alopecia caused by the Cytoxan/Adriamycin combination.

Example IV

Protective Effect of Vitamin D_3

Twelve 7 day old rats were randomized in two groups. Group I was treated daily with buffer 0.1 ml s.c. for four days. The second Group was treated daily with Vitamin D_3

50μg s.c. over head for four days. After stopping the buffer or Vitamin D_3 treatment, all rats received 1.5 mg/kg i.p. of VP-16 (Etoposide) daily for three days. All rats in Group I developed complete body alopecia while the rats in Group II were protected (FIG. 6 shows 4 rats from each group).

In other experiments, rats pretreated with Vitamin D_3 demonstrated excellent protection against alopecia produced by Etoposide, Cytoxan, Cytarabine and the combination of Cytoxan and Adriamycin (FIG. 7). The results are set forth below in Table II.

TABLE II

PROTECTION FROM CHEMOTHERAPY-INDUCED ALOPECIA BY PRETREATMENT WITH VITAMIN D_3.

Alopecic drug tested	Total No. of experiments	Total No. of animals	Protection from alopecia
Etoposide (VP-16)	2	22	Yes*
Cytoxan (CTX)	7	89	Yes*
Cytarabine (ARA-C)	1	8	Yes*
Adriamycin + CTX Combination	6	77	Yes*

Chemotherapeutic agents were given as follows: VP-16 1.5 mg/kg i.p. daily for 3 days; Cytoxan 32.5 mg/kg as a single injection; ARA-C 50 mg/kg i.p. daily for 7 days; for combination (Adriamycin 2.5 mg/kg i.p. daily for 3 days plus Cytoxan 25 mg/kg as a single injection). Vitamin D_3 was given in 50 μg daily doses i.p. or s.c. for 4 days prior to chemotherapy.

*In these experiments, protection from chemotherapy-induced alopecia was uniformly observed in all animals treated with Vitamin D_3

In other experiments, 1,25-dihydroxyvitamin D_3 applied topically (0.5 μg daily) in 50% ethanol or DMSO also protected rats from VP-16-induced alopecia.

Example V

Protective Effect of Vitamin D_3 Pretreatment

Topical Application of 1,25-dihydroxyvitamin D_3:

1,25-Dihydroxyvitamin D_3 was dissolved in absolute ethanol and applied topically with an applicator. Control animals were similarly treated with the same amount of ethanol. Animals were then kept individually separated for a period of three hours following which the treated area was carefully washed with soap and water and dried. Treatment was given daily beginning on day 5 after birth and ending on day 10.

Chemotherapy:

All chemotherapies were given I.P. and started at 11 days of age. CTX, 35 mg/kg, was given for one day only. VP-16, 1.5 mg/kg, was given for three days. For CTX and ADM combination, CTX, 25 mg/kg was given for one day and ADM, 2.5 mg/kg, for three days. At these doses neither CTX nor ADM alone will produce alopecia. Alopecia was recorded on the tenth day from beginning chemotherapy.

A total of 4 experiments were carried out. In the first experiment, protection from Cytoxan-induced alopecia was examined. The experimental group was pretreated with 0.2 μg of 1,25-dihydroxyvitamin D_3 in 0.15 ml of absolute ethanol applied topically over the head and neck and the control group received 0.15 ml of alcohol. All 10 rats in the control group became totally alopecic. In contrast, all animals in the experimental group were protected (FIG. 8A). The second experiment was carried out under similar conditions to examine protection from VP-16-induced alopecia. All 10 rats in the control group developed total body alopecia. In contrast, all rats in the experimental group were protected (FIG. 8B). The third experiment was designed to examine protection from alopecia induced by Cytoxan-Adriamycin combination. There were 11 rats in each group. Six rats in the control group developed alopecia over the head and neck and 5 rats developed total body alopecia. In contrast, all rats in the experimental group were protected (FIG. 8C). In the fourth experiment, protection from VP-16-induced alopecia was similarly examined except that the dose of 1,25-dihydroxyvitamin D_3 was reduced to 0.1 μg in 0.1 ml absolute ethanol applied topically over the head area only. All 10 rats in the control group became completely alopecic. In contrast, all rats in the experimental group were protected primarily at the site of 1,25-dihydroxyvitamin D_3 application (FIG. 9).

It is noteworthy that protection from 0.2 μg 1,25-dihydroxyvitamin D_3 was not limited to the site of application but involved the entire body, suggesting systemic absorption. When the dose was reduced to 0.1 μg applied to the head area only, protection from VP-16-induced alopecia was less generalized and was more limited to the site of application.

Example VI

Protection from VP-16-Induced Alopecia by Topical Application of RO 23-7553

RO 23-7553 (1,25 dihydroxy-16-ene-23-yne-cholecalciferol) was dissolved in absolute ethanol and applied topically with an applicator. Control animals were similarly treated with the same amount of ethanol. Animals were then kept individually separated for a period of three hours following which the treated area was carefully washed with soap and water and dried. Treatment was given daily beginning on day 9 after birth and ending on day 14.

On day 15 all animals received VP-16, 1.5 mg/kg i.p., for three days. Alopecia was recorded on day 25.

Thirteen rats were randomized in two groups. Experimental group, 7 rats; control group, 6 rats. The experimental group was pretreated with 1 μg of RO 23-7553 in 0.2 ml absolute ethanol applied topically over the neck and back and the control group received 0.2 ml of absolute ethanol. All six rats in the control group became totally alopecic over the neck and back. In contrast, all animals in the experimental group were protected (FIG. 10). It should be noted that when the chemotherapy is started at the age of 14 days, the head area does not become alopecic.

Example VII

Stimulation of Hair Growth by Vitamin D_3

During the course of the above-described studies on the protection from chemotherapy-induced alopecia by Vitamin D_3 and its active analog, 1,25-dihydroxyvitamin D_3, it was noted that rats treated with 1,25-dihydroxyvitamin D_3 not only were protected from chemotherapy-induced alopecia, but these rats had a better coat of hair and longer hair in the treated area. These observations prompted the following further experiments on the stimulation of hair growth by 1,25-dihydroxyvitamin D_3.

The backs of nineteen 25 day old Sprague Dawley-rats were shaven and randomized in two groups.

Group I (control 10 rats) received 0.1 ml of ethanol applied topically once daily to the shaven area for 14 days.

Group II (Calcitriol 9 rats) received 50 ng of 1,25-dihydroxyvitamin D_3 in 0.1 ml of ethanol applied topically once daily to the shaven area for 14 days.

On day 15 stimulation of hair regrowth was assessed by reshaving an area 6 cm×6 cm in diameter. The hair was collected and weighed. The difference between two groups was highly statistically significant. P. value 0.003 (see Table III and FIG. 11).

TABLE III

STIMULATION OF HAIR GROWTH BY CALCITRIOL IN RATS
Hair Weight in Mg.

Control	Calcitriol
98	202
131	143
72	150
84	253
102	130
144	177
115	140
129	147
125	135
130	
Mean S.E.M.	Mean S.E.M.
113 ± 8	164 ± 13

Based on these data showing stimulation of hair growth by 1,23-dihydroxyvitamin D_3 administered topically in the rat, it is expected that 1,25-dihydroxyvitamin D_3 can be used as a stimulant of hair growth in cases of alopecia of any cause. Additionally, the data suggest that vitamin D_3 and its metabolites is/are necessary for optimal hair growth and therefore can be used to prevent hair loss from any cause, including male pattern baldness.

Example VIII

Formulations

The following are four formulations that include 1,25-dihydroxyvitamin D_3 as active ingredient, and the methods of their manufacture.

1. TOPICAL SOLUTION

Ingredients	% (W/W)
1,25-Dihydroxyvitamin D_3	0.0002–0.10
Propylene Glycol	10.00
Propylene Glycol Dicaprylate/Dicaprate*	30.00
Butylated Hydroxytoluene (BHT)	0.05
Butylated Hydroxyanisole (BHA)	0.05
Ethyl Alcohol, Absolute q.s. to	100.00

Manufacturing Procedure

i. Weigh the appropriate amount of propylene glycol dicaprylate/dicaprate, ethyl alcohol, propylene glycol in a stainless steel container.
ii. Dissolve BHT and BHA into the solution from step (i).
iii. Add the 1,25-dihydroxyvitamin D_3 into the mixture from step (ii) and stir until dissolved.

2. BUFFERED TOPICAL SOLUTION

Ingredients	% (W/W)
1,25-Dihydroxyvitamin D_3	0.0002–0.10
Propylene Glycol	50.00
Hydroxypropyl cellulose (Klucel MF)	0.50
Methylparaben	0.20
Butylated Hydroxytoluene (BHT)	0.05
Butylated Hydroxyanisole (BHA)	0.05
Sodium Phosphate, Monobasic	0.43
Sodium Phosphate Dibasic	0.70
Sodium Hydroxide (q.s. to pH = 7)	0.04
Ethyl Alcohol, 95% Proof	30.00
Water q.s. to	100.00

Manufacturing Procedure

i. Dissolve the sodium phosphate, monobasic, sodium phosphate, dibasic, and sodium hydroxide in the water in a stainless steel container. Measure the pH of the solution. The pH of the solution should be 7.0; if not adjust the pH.
ii. Add the propylene glycol and ethyl alcohol to the solution from step (i).
iii. Dissolve the 1,25-dihydroxyvitamin D_3, methylparaben, BHT and BHA to the solution from step (ii).
iv. Dissolve Klucel MF to the solution from step (iii).

3. OIL-IN WATER BUFFERED TOPICAL LOTION

Ingredients	% (W/W)
1,25-dihydroxyvitamin D_3	0.0002–0.10
Cetyl Alcohol	0.25
Stearyl Alcohol	0.50
Sorbitan Monostearate	2.00
Glyceryl Monostearate and Polyoxyethylene Stearate Blend (Arlacel 165)	4.00
Polysorbate 60	1.00
Mineral Oil	4.00
Propylene Glycol	5.00
Butylated Hydroxyanisole	0.05
Propylparaben	0.05
Buffering Agent q.s. to pH	7.00
Sorbitol Solution	2.00
Edetate Disodium	0.10
Methylparaben	0.18
Water q.s. to	100.00

Manufacturing Procedure

i. Prepare the buffer solution (pH 7.0) in a stainless steel container.
ii. In a stainless steel vessel, at 70° C., melt the cetyl alcohol, stearyl alcohol, sorbitan monostearate, Arlacel 165, Polysorbate 60, mineral oil, butylated hydroxyanisole, propylparaben, and 50% propylene glycol together.
iii. Add the sorbitol solution to step (i) and heat the solution to 70° C.
iv. Add the edetate disodium and methylparaben to the solution from step (iii).
v. Dissolve the 1,25-dihydroxyvitamin D_3 in approximately 40% propylene glycol in a beaker and add this to the material from step (ii) while mixing. Rinse the container from 1st propylene glycol and add this to the mixture from step (ii).
vi. Add step (v) to step (iv) when both phases are at 70° C. and homogenize. Cool the emulsion to 200 m temperature.

4. TOPICAL GEL

Ingredients	% (W/W)
1,25-dihydroxyvitamin D_3	0.0002–0.10
Butylated Hydroxytoluene (BHT)	0.05
Butylated Hydroxyanisole (BHA)	0.05
Hydroxypropyl Cellulose	3.00
Ethyl Alcohol, 95% Proof	30.00
Water q.s. to	100.00

Manufacturing Procedure

i. Weigh the ethyl alcohol and water in a stainless steel container.

-continued

ii. Dissolve the 1,25-dihydroxyvitamin D_3, BHT and BHA to the solution from step (i).
iii. Dissolve the hydroxypropyl cellulose to the solution from step (ii).

*Can be substituted by the following materials (1) medium chain triglycerides; 2) dimethyl isosorbide; (3) polyethylene glycols; (4) ethoxydiglycol

The entire contents of all references cited above are incorporated herein by reference.

While the present invention has been described in some detail for purposes of clarity and understanding, one skilled in the art will appreciate that various changes in form and detail can be made without departing from the true scope of the invention.

What is claimed is:

1. A method of reducing chemotherapy-induced alopecia comprising administering to a host treated with at least one chemotherapeutic agent which induces such alopecia an effective amount of vitamin D_3 or derivative or analog or active metabolite thereof.

2. The method according to claim 1 wherein said derivative, analog or active metabolite is selected from the group consisting of 1,25-dihydroxyvitamin D_3 and 1,25-dihydroxy-16-ene-23-yne-cholecalciferol.

3. The method according to claim 1 wherein said vitamin D_3, derivative analog or active metabolite thereof is administered topically.

4. The method according to claim 1 wherein said at least one chemotherapeutic agent is cell cycle specific.

5. The method according to claim 4 wherein said at least one chemotherapeutic agent is cytosine arabinoside.

6. The method according to claim 1 wherein said at least one chemotherapeutic agent is non-cell cycle specific.

7. The method according to claim 6 wherein said at least one chemotherapeutic agent is Cytoxan.

8. The method according to claim 1 wherein said at least one chemotherapeutic agent is a cell cycle specific agent in combination with a non cell cycle specific agent.

9. The method according to claim 1 wherein vitamin D_3, or derivative or analog or active metabolite thereof, is administered prior to the initiation of said chemotherapy.

10. The method according to claim 1, wherein the vitamin D_3, derivative or analog or active metabolite thereof is administered by topically applying the same to the host prior to administering said chemotherapy.

11. The method according to claim 1 wherein said derivative, analog or active metabolite is selected from the group consisting of 1,25-dihydroxyvitamin D_3 and 1,25-dihydroxy-16-ene-23-yne-cholecalciferol and is administered topically.

12. The method according to claim 1 wherein said at least one chemotherapeutic agent is cell cycle specific and said derivative, analog or active metabolite is selected from the group consisting of 1,25-dihydroxyvitamin D_3 and 1,25-dihydroxy-16-ene-23-yne-cholecalciferol.

13. The method according to claim 1, wherein said at least one chemotherapeutic agent is cell cycle specific and said vitamin D_3 or derivative, analog or active metabolite thereof is administered topically.

14. The method according to claim 1, wherein said at least one chemotherapeutic agent is cell cycle specific and comprising administering vitamin D_3, 1,25-dihydroxy-16-ene-23-yne-cholecalciferol, 1α-hydroxyvitamin D_3, 1α, 24-dihydroxyvitamin D_3 or 1, 25-dihydroxyvitamin D_3.

15. The method according to claim 1 wherein said at least one chemotherapeutic agent is non cell cycle specific and said derivative, analog or active metabolite is selected from the group consisting of 1,25-dihydroxyvitamin D_3 and 1,25-dihydroxy-16-ene-23-yne-cholecalciferol.

16. The method according to claim 1 wherein said at least one chemotherapeutic agent is non cell cycle specific and said vitamin D_3 or derivative, analog or active metabolite thereof is administered topically.

17. The method according to claim 1 wherein said at least one chemotherapeutic agent is non cell cycle specific and comprising administering vitamin D_3, 1,25-dihydroxy-16-ene-23-yne-cholecalciferol, 1α-hydroxyvitamin D_3, 1α, 24-dihydroxyvitamin D_3 or 1, 25-dihydroxyvitamin D_3.

18. The method according to claim 1 wherein said at least one chemotherapeutic agent is a cell cycle specific agent in combination with a non cell cycle specific agent and said derivative, analog or active metabolite is selected from the group consisting of 1,25-dihydroxyvitamin D_3 and 1,25-dihydroxy-16-ene-23-yne-cholecalciferol.

19. The method according to claim 1 wherein said at least one chemotherapeutic agent is a cell cycle specific agent in combination with a non cell cycle specific agent and said vitamin D_3 or derivative, analog or active metabolite thereof is administered topically.

20. The method according to claim 1 wherein said at least one chemotherapeutic agent is a cell cycle specific agent in combination with a non cell cycle specific agent and comprising administering vitamin D_3, 1,25-dihydroxy-16-ene-23-yne-cholecalciferol, 1α-hydroxyvitamin D_3, 1α, 24-dihydroxyvitamin D_3 or 1, 25-dihydroxyvitamin D_3.

21. The method according to claim 1, wherein the vitamin D_3, or derivative or analog or active metabolite thereof is administered by topically applying the same to the host prior to administering said chemotherapy and said derivative, analog or active metabolite is selected from the group consisting of 1,25-dihydroxyvitamin D_3 and 1,25-dihydroxy-16-ene-23-yne-cholecalciferol.

22. The method according to claim 1, wherein the vitamin D_3, or derivative or analog or active metabolite thereof is administered by topically applying the same to the host prior to administering said chemotherapy and comprising administering vitamin D_3, 1,25-dihydroxy-16-ene-23-yne-cholecalciferol, 1α-hydroxyvitamin D_3, 1α, 24-dihydroxyvitamin D_3 or 1, 25-dihydroxyvitamin D_3.

23. The method according to claim 1 comprising administering vitamin D_3, 1,25-dihydroxy-16-ene-23-yne-cholecalciferol, 1α-hydroxyvitamin D_3, 1α, 24-dihydroxyvitamin D_3 or 1,25-dihydroxyvitamin D_3.

24. The method according to claim 1, comprising topically administering vitamin D_3, 1,25-dihydroxy-16-ene-23-yne-cholecalciferol, 1α-hydroxyvitamin D_3, 1α, 24-dihydroxyvitamin D_3 or 1,25-dihydroxyvitamin D_3.

25. The method according to claim 1, comprising topically administering vitamin D_3, 1,25-dihydroxy-16-ene-23-yne-cholecalciferol, 1α-hydroxyvitamin D_3, 1α, 24-dihydroxyvitamin D_3 or 1,25-dihydroxyvitamin D_3 and wherein said chemotherapeutic agent is cell cycle specific.

26. The method according to claim 1, comprising topically administering vitamin D_3, 1,25-dihydroxy-16-ene-23-yne-cholecalciferol, 1α-hydroxyvitamin D_3, 1α, 24-dihydroxyvitamin D_3 or 1,25-dihydroxyvitamin D_3 and wherein said at least one chemotherapeutic agent is non cell cycle specific.

27. The method according to claim 1, comprising topically administering vitamin D_3, 1,25-dihydroxy-16-ene-23-yne-cholecalciferol, 1α-hydroxyvitamin D_3, 1α, 24-dihydroxyvitamin D_3 or 1,25-dihydroxyvitamin D_3 and wherein said at least one chemotherapeutic agent is a cell cycle specific agent in combination with a non cell cycle specific agent.

28. The method according to claim 1, comprising administering vitamin D_3, 1,25-dihydroxy-16-ene-23-yne-cholecalciferol, 1α-hydroxyvitamin D_3, 1α, 24-dihydroxyvitamin D_3 or 1,25-dihydroxyvitamin D_3 prior to the initiation of said chemotherapy.

29. A method of reducing chemotherapy-induced alopecia comprising administering to a patient undergoing chemotherapy with at least one chemotherapeutic agent 1,25-dihydroxyvitamin D_3 in an effective amount.

30. The method according to claim 29 wherein said 1,25-dihydroxyvitamin D_3 is administered topically.

31. The method according to claim 29 wherein said at least one chemotherapeutic agent is cell cycle specific.

32. The method according to claim 31 wherein said at least one chemotherapeutic agent is cytosine arabinoside.

33. The method according to claim 29 wherein said at least one chemotherapeutic agent is non cell cycle specific.

34. The method according to claim 33 wherein said at least one chemotherapeutic agent is Cytoxan.

35. The method according to claim 31 wherein said at least one chemotherapeutic agent is a cell cycle specific agent in combination with a non cell cycle specific agent.

36. The method according to claim 31 wherein the 1,25-dihydroxyvitamin D_3 is administered prior to the initiation of said chemotherapy.

37. The method according to claim 31, wherein the 1,25-dihydroxyvitamin D_3 is administered by topical application to the host prior to administering said chemotherapy.

* * * * *

ANEXO 2
PATENTE US5502224

US005502224A

United States Patent [19]

Eugster et al.

[11] Patent Number: 5,502,224

[45] Date of Patent: Mar. 26, 1996

[54] **BIOTENSIDE ESTERS AND PHOSPHATIDES WITH VITAMIN-D AND VITAMIN-E COMPOUNDS**

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[73] Assignee: **Marigen, S.A., Riehen, Switzerland**

[21] Appl. No.: **312,980**

[22] Filed: **Sep. 30, 1994**

Related U.S. Application Data

[63] Continuation of Ser. No. 42,251, filed as PCT/CH92/00072, Apr. 16, 1992, abandoned.

[30] **Foreign Application Priority Data**

Jun. 4, 1991 [CH] Switzerland 01662/91

[51] Int. Cl.[6] C07C 401/00

[52] U.S. Cl. 552/653

[58] Field of Search 552/653

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Primary Examiner—Nicky Chan

Attorney, Agent, or Firm—Foley & Lardner

[57] **ABSTRACT**

New biotenside esters and phosphatides formed with Vitamin-D and Vitamin-E compounds possessing a pronounced antitumor activity, processes for their production as well as for the preparation of concentrates and pharmaceutical compositions containing these new esters and phosphatides, and their use for treating tumors are described.

4 Claims, No Drawings

BIOTENSIDE ESTERS AND PHOSPHATIDES WITH VITAMIN-D AND VITAMIN-E COMPOUNDS

This application is a continuation of application Ser. No. 08/042,251, filed Apr. 2, 1993, now abandoned, which is a continuation of PCT/CH92/00072, filed Apr. 16, 1992.

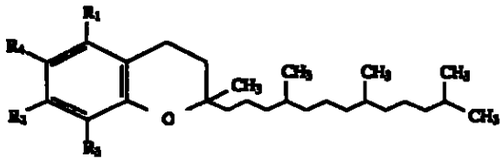
INTRODUCTION

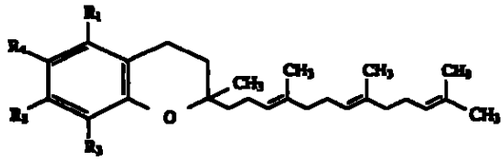
The present invention concerns new Esters and Phosphatides with Vitamin-D and Vitamin-E compounds, processes for their production as well as for the preparation of spontaneously dispersible concentrates and pharmaceutical compositions containing these compounds, and their use for the treatment of tumours.

Surprisingly it has been found that the newly synthetized Esters and Phosphatides with Vitamin-D and Vitamin-E compounds possess outstanding antitumour properties, particularly if these compounds are being incorporated into spontaneously dispersible concentrates.

DESCRIPTION OF THE INVENTION

The new Esters and Phosphatides with Vitamin-D and Vitamin-E compounds correspond to the general formulae (I) to (VI):


(I)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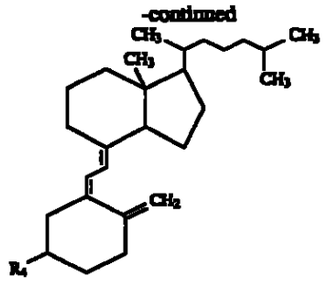
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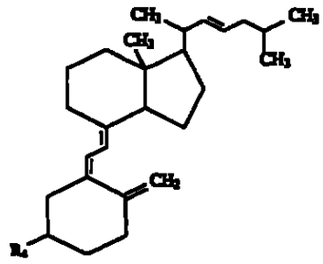
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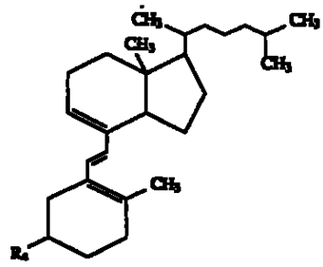
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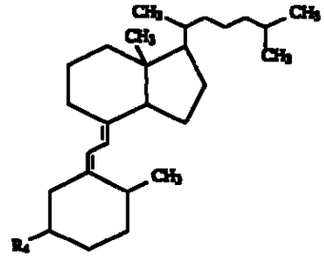
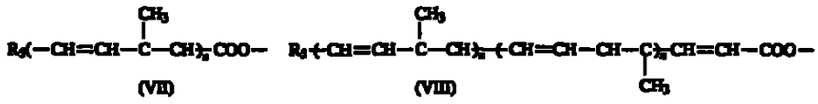
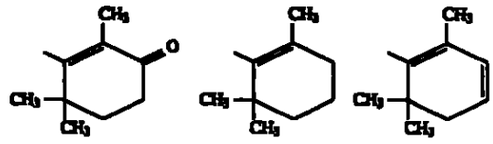

(VI)

whereby in the formulae (I) and (II) the radicals R_1, R_2 and R_3 stand for hydrogen or methyl and the radical R_4 of the formulae (I) to (VI) determines a C_1 to C_{22}-alkylcarbonyloxy, and a C_7- to C_{22}-alkylcarbonyloxy or alkapolyencarbonyloxy group respectively or a group of the formulae (VII) and (VIII) respectively:


(VII) (VIII)

in which n designates the number 1, 2, 3, 4 or 5 and R_5 stands for a radical of the following formulae:



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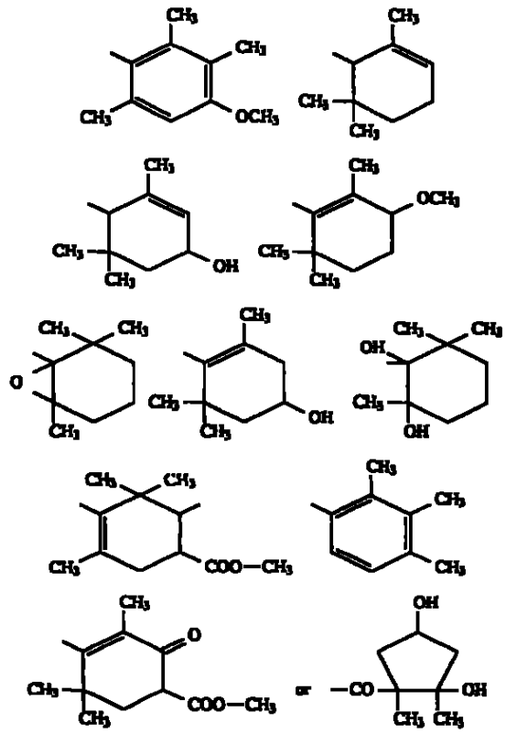
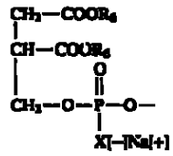


or represents the group of the formula (IX):



(IX)

in which R_6 denotes a C_1- to C_{32}-alkyl or a C_2- to C_{32}-alkenyl group and X represents an oxygen, sulfur or selenium atom, with the proviso that α-tocopheryl-esters formed with saturated or unsaturated carbonic acids having 14 to 22 carbon atoms are excluded.

The group of the formulae (VII) and (VIII) may have different stereoisomeric or rotational forms.

The class of the Vitamin-D compounds comprises the following chemical compounds:

Cholecalciferol: (5Z, 7E)-(3S)-9,10-seco-5,7,10 (19)-cholestatrien-3-ol

25-hydroxycholecalciferol [Calcidiol]: (5Z, 7E)-(3S)-9, 10-seco-5,7,10 (19)-cholestatrien-3,25-diol

1α-dehydroxy-cholecalciferol [Calcitriol]: (5Z, 7E)-(1S, 3R)-9,10-seco-5,7,10 (19)-cholestatrien-1,3,25-triol

Ergocalciferol [Calciol]: (5Z, 7E, 22E)-(3S)-9,10-seco-5, 7,10 (19),22-ergostatetraen-3-ol

Tachysterol: (6E)-(3S)-9,10-seco-5, (10)-6,8-cholestatrien-3-ol

Dehydrotachysterol: (5E, 7E)-(3S, 10S)-9,10-seco-5,7-cholestatrien-3-ol

The class of the Vitamin-E compounds comprises all tocol- and tocotrienol-compounds, such as, e.g.:

Tocol [2-methyl-2(4,8,12-trimethyltridecyl)chroman-6-ol] α-Tocopherol [5,7,8-trimethyltocol],

which can take on the following configurations:

12,12,12-α-tocopherol

2-epi-α-tocopherol

2-ambo-α-tocopherol

all-rac-α-tocopherol

4-ambo-8-ambo-α-tocopherol

β-Tocopherol [5,8-dimethyltocol]

γ-Tocopherol [7,8-dimethyltocol]

δ-Tocopherol [8-methyltocol]

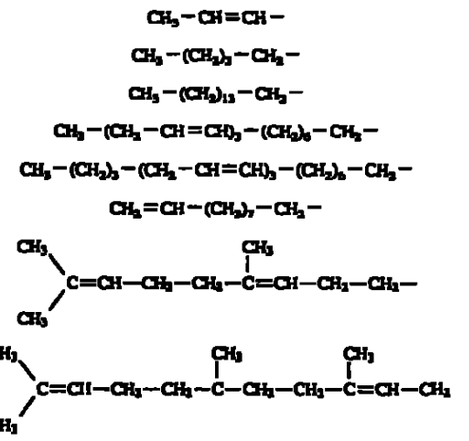
Tocotrienol [2-methyl-2-(4,8,12-trimethyldeca-3.7,11-trianyl)chroman-6-ol]

ρ1- oder ρ2-Tocopherol [5,7,8-trimethyltocotrienol] and

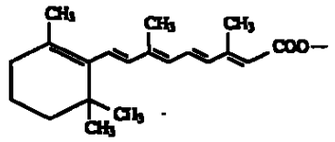
ε-Tocopherol [5,8-dimethyltocotrienol or ε-tocotrienol]

The alkylcarbonyloxy, alkenylcarbonoloxy or alkancoly-enocarbonyeloxy groups at R_4, as well as the alkyl, alkenyl or alkapolyen groups at R_8 can be straight chained or branched. Alkapolyen means the corresponding alkadiene, alkatriene, alkatetraene, alkapentaene, alkahoxaene and alkaheptaene compounds respectively.

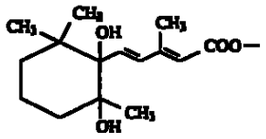
Examples of such groups are, e.g.:



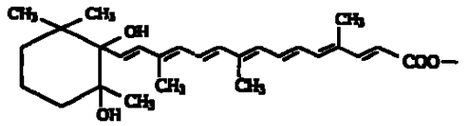
Important groups according to formula (VII) are characterized by the formulae (X), (XI) and (XII) respectively:



(X)



(XI)



(XII)

The group of the formula (X) may have different stereoisomeric forms, such as e.g. the all trans, the 9-cis or the 13-cis form.

Examples of the inventive new esters and phosphatides with vitamin-D and vitamin-E compounds are, inter alia:

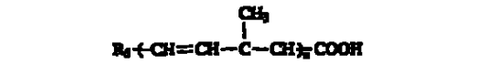
Cholecalciferol-caproylate

Ergocalciferol-caproylate
Cholecalciferol-10-undecenoate
Ergocalciferol-10-undecenoate
Cholecalciferol-laurate
Ergocalciferol-laurate
Cholecalciferol-palmitate
Ergocalciferol-palmitate
Cholecalciferol-linoleate
Ergocalciferol-linoleate
Cholecalciferol-linolenate
Ergocalciferol-linolenate
Cholecalciferol-all trans-retinate
Ergocalciferol-all trans-retinate
Cholecalciferol-3-ol-1,2,-dipalmitoylglycero-phosphatide
Cholecalciferol-3-ol-1,2,-dipalmitoylglycero-thiophosphatide
Cholecalciferol-geranyl-phosphatide
Cholecalciferol-farnesyl-phosphatide
Ergocalciferol-3-ol-1,2,-dipalmitoyl-glycero-phosphatide
Ergocalciferol-3-ol-1,2,-dipalmitoyl-glycero-thiophosphatide
Ergocalciferol-geranyl-phosphatide
Ergocalciferol-farnesyl-phosphatide
DL-α-Tocopherol-10-undecenoate
DL-α-Tocopherol-palmitate
DL-α-Tocopherol-all trans-retinate
DL-α-Tocopherol-13 cis-retinate

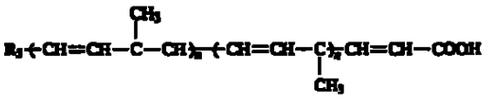
PREPARATION

The new sterolesters and sterolphosphatides with vitamin-D and vitamin-E compounds of the formulae (I) to (VI) may generally be prepared in semi-synthetic procedure by the following processes, which are known per se:

a) Reaction of a compound of the formula (XIV) or (XV):

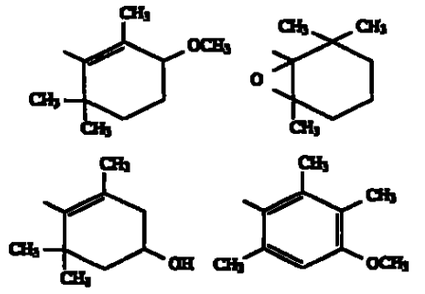


(XIV)



(XV)

wherein n represents the numbers 1, 2, 3, 4 or 5 and R_5 stands for one of the following radicals:



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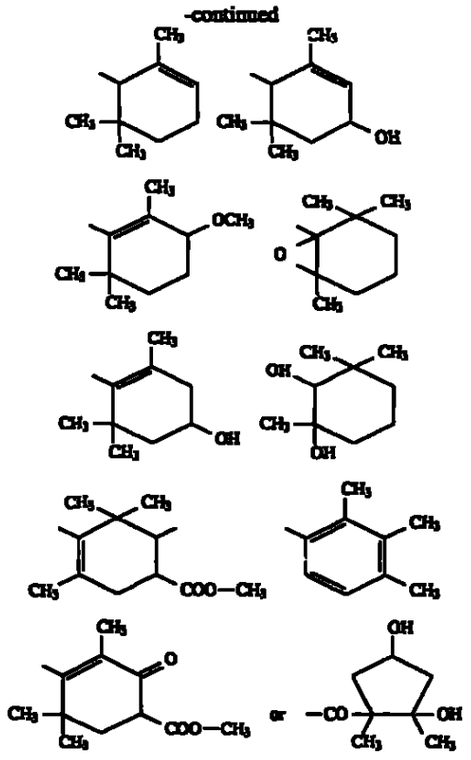
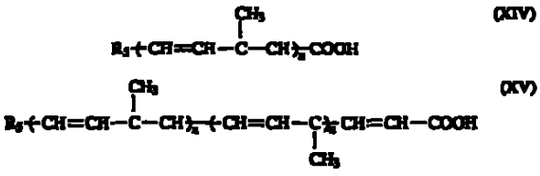


with N,N'-carbonyl-diimidazole at 25° to 70° C. with the addition of a catalytic amount of an alcoholate in tetrahydrofurane, benzene, chloroform or dimethylformamide or in a similar indifferent solvent, followed by alcoholysis of the imidazolides formed with a vitamin-D or vitamin-E compound.

b) Formation of the chloride or the bromide of a compound of the formulae (XIV), (XV) or (XVI):



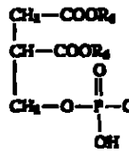
(XIV)

(XV)

(XVI)

in which R_6 means a C_1- to C_{32}-alkyl or a C_2- to C_{32}-alkenyl and a C_2- to C_{22}-alkapolyene group respectively, with a chlorination or bromination agent such as thionylchloride, oxalylchloride or oxalylbromide, followed by the reaction of the chloride or bromide formed with a vitamin-D or a vitamin-E compound at a temperature of between 40° and 120° C. in an indifferent solvent such as toluene or xylene, and in the presence of a catalyst such as dimethylformamide or p-dimethylaminopyridine.

d) Esterification of a compound of the formula (XVII):



(XVII)

in which R_6 signifies a C_1- to C_{22}-alkyl group or a C_2- to C_{22}- alkenyl/alkapolyene group, in an inert solvent such as pyridine, tetrahydrofurane or chloroform, at a temperature of 20° C. with pivaloylchloride, followed by the reaction of the product in the same solvent with a vitamin-D or a vitamin-E compound.

The novel esters and phosphatides with vitamin-D and vitamin-E compounds possess, surprisingly, excellent anti-tumour activity, most notably when these compounds have been incorporated into spon-taneously dispersible concentrates. For this reason, spontaneously dis-persible concentrates made up with the new esters and phosphatides of vitamin-D and vitamin-E compounds of the formulae (I) to (VI) are also a subject matter of the present invention. When treated with water, such concentrates render microemulsions having excellent phase stability as well as much improved membrane permeability and spreading properties.

These spontaneously dispersible concentrates prepared in accordance with the invention contain

- 0.001 to 25% by weight of individual esters or phosphatides of vitamin-D or vitamin-E compounds or combinations of these compounds
- 0.001 to 40% by weight of a solvent or solvent mixture which is pharmaceutically acceptable and acts as a hydrotropic or coemulsifier
- 0.001 to 90% by weight of a pharmaceutically acceptable surfactant or surfactant mixture, and optionally
- up to 10% by weight of a vitamin or provitamin
- up to 10% by weight of a free fatty acid, and if appropriate, customary excipients and/or diluents.

The surfactants or surfactant mixtures to be employed according to the invention can be anionic, cationic, amphoteric or non-ionic. Ideally, they are non-ionic and have an HLB-value (i.e. a hydrophilic-lipophilic balance) of between 2 and 18; preferably, it is between 2 and 6 on the one hand and 10 and 15 on the other hand. HLB values describe the hydrophilic and lipophilic properties of an emulsifier. In this context see "Hydrophile-Lipophile Balance: History and recent Developments" by Paul Becher in Journal of Dispersion Science and Technology, 5 (1), 81–96 (1984).

Suitable anionic surfactants can be both so-called water-soluble soaps and water-soluble synthetic compounds.

Suitable soaps are the alkali metal salts, alkaline earth metal salts or optionally substituted ammonium salts of higher fatty acids (C_{12} to C_{22}), for example the natural Na or K salts of oleic or stearic acids, or of natural mixtures of fatty acids which can be obtained, inter alia, from coconut oil or tallow oil. Other surfactants which may be mentioned are fatty acid methyltaurine salts, and modified and non-modified phospholipids.

However, more frequently used surfactants are so-called synthetic surfactants, in particular fatty sulfonates, fatty sulfates, sulfonated benzimidazole derivatives or alkylaryl-sulfonates.

The fatty sulfonates and fatty sulfates are usually present in the form of alkali metal salts, alkaline earth metal salts or optionally substituted ammonium salts and generally have an alkyl radical containing 8 to 22 C atoms, alkyl also encompassing the alkyl moiety of acyl radicals. Examples are the Na or Ca salt of ligninsulfonic acid, of dodecylsulfuric ester and sulfonic acids of fatty alcohol/ethylene oxide adducts. The sulfonated benzimidazole derivatives preferably contain two sulfonyl groups and one fatty acid radical containing about 8 to 22 C atoms. Alkylarylsulfonates are, for example, the Na, Ca or triethanolamine salts of dodecylbenzenesulfonic acid, of dibutylnaphthalenesulfonic acid or of a naphthalenesulfonic acid/formaldehyde condensation product.

The non-ionic surfactants are mainly chosen from amongst the polyglycol ether derivatives of aliphatic or cycloaliphatic alcohols, saturated or unsaturated fatty acids and alkylphenols which can contain 3 to 30 glycol ether groups and 8 to 20 C atoms in the (aliphatic) hydrocarbon radical and 6 to 18 C atoms in the alkyl radical. Other suitable non-ionic surfactants are the water-soluble polyethyleneoxy-adducts onto polypropylene glycol and alkyl polypropylene glycol with 1 to 10 C atoms in the alkyl chain, which adducts contain 20 to 250 ethylene glycol ether groups and 10 to 100 propylene ether groups. The compounds which have been mentioned customarily contain 1 to 5 ethylene units per propylene glycol unit.

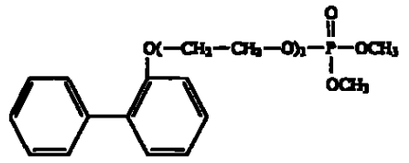
The following may be mentioned as examples of non-ionic surfactants: nonylphenol polyethoxyethanols, castor oil polyglycol ethers, polypropylene/polyethylene oxide adducts, tributylphenoxy-polyethoxy-ethanol, polyethyleneglycol and octylphenoxy-polyethoxyethanol. Moreover, fatty acid esters of polyoxyethylene-sorbitan, such as polyoxyethylene sorbitan trioleate, are also suitable.

The cationic surfactants are mainly quaternary ammonium salts which contain at least one alkyl radical having 8 to 22 C atoms as the N-substituent and which have lower, optionally halogenated alkyl radicals, benzyl radicals or lower hydroxyalkyl radicals as further substituents. The salts are mainly present in the form of halides, methylsulfates or ethylsulfates, for example stearyltrimethylammonium chloride or benzyldi-(2-chloroethyl)-ethylammonium bromide.

When preparing the inventive spontaneously dispersible concentrates, special preference is given to phosphoric acid ester tensides, such as:

Tristyrylphenolpolyoxyethylene-18-mono/dimethyl-phosphoric-acid-ester (Soprophore® FL, Rhône-Poulenc);

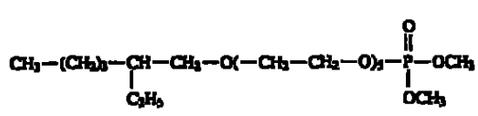
Nonylphenol-10-polyoxyethylene-mono/dimethylphosphoric-acid-ester (Diphasole® 3873, CIBA-GEIGY);



(Tensid 508, CIBA-GEIGY);

Tinovetin® JU (CIBA-GEIGY), a hydroxybiphenyl-10-ethoxy-phosphoric acid ester

Butyl-mono-4-ethoxy-phosphoric acid ester (Zerostate® AT, CIBA-GEIGY), and



(Zerostat® AN, CIBA-GEIGY), respectively.

The following compounds may be employed as the pharmaceutically acceptable solvent which acts as the hydrotropic, or coemulsifier, for example: esters of an aliphatic alcohol (C_3 to C_{18}) with an aliphatic carboxylic acid (C_{10} to C_{22}), such as isopropyl laurate, hexyl laurate, decyl laurate, isopropyl myristate and lauryl myristate; hydrocarbons having a straight carbon chain (C_{12} to C_{32}) which is substituted by 6 to 16 methyl groups and which can have up to 6 double bonds, examples which may be mentioned being terpenes, such as polymethylbutanes and polymethylbutenes.

Monoesters of ethylene glycol or propylene glycol with an aliphatic carboxylic acid (C_6 to C_{22}), such as propylene glycol monolaurate and propylene glycol monomyristate.

Esters of an aliphatic alcohol (C_{12} to C_{22}) with lactic acid, such as, for example, myristyl lactate or, preferably, lauryl lactate. Monoesters or diesters of glycerol with an aliphatic carboxylic acid (C_6 to C_{22}), such as, for example, glyceryl caprylate or Miglyol® 812 neutral oil (Oleum neutrale).

Esters of a poly(2-7)ethylene glycol glycerolether having at least one free hydroxyl group with an aliphatic carboxylic acid (C_6 to C_{22}), such as, for example, aliphatic alcohols (C_{12} to C_{22}), thus, inter alia, dodecanol, tetradodecanol, oleyl alcohol, 2-hexyldecanol and 2-octyldecanol.

Esters containing at least one free hydroxyl group, of poly-(2-10)glycol with an aliphatic carboxylic acid (C_6 to C_{22}), monoethers of a polyethylene glycol with an aliphatic alcohol (C_{12} to C_{18}), such as, for example, polyoxyethylene-(C_{10}) octylether.

Heterocyclic compounds such as 1-methyl-2-pyrrolidon.

Before their application in the spontaneously dispersible concentrates all technical tensides have been cleaned by filtration or by chromatography over aluminum-oxide with an inert solvent as eluent, such as tetrahydrofurane, ethylalcohol or dichlormethane.

Suitable additives for the spontaneously dispersible concentrates according to the invention are vitamins and provitamins [such as, for example, vitamin A (retinoic acid), retinol, carotanes, tocopherols], and also free fatty acids, such as: valeric acid, isovaleric acid, sorbic acid, isocaproic acid, pelargonic acid, lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, behenic acid, hexacosanoic acid, octacosanoic acid, pentadecanoic acid, decenylic acid, undecylenic acid, dodecenylic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid, erucic acid, etc.

The daily dose required for pharmaceutical administration is 0.001 to 25 mg/kg of body weight, if possible split into 2–3 individual doses. For this purpose, the new vitamin-sterol esters sterol phosphatides, or the spontaneously dispersible concentrates with these compounds, can be incorporated into the conventional pharmaceutical preparations and dosage forms, such as coated tablets, tablets, capsules, powders, granules, pellets, solutions, ampula, emulsions, creams or suppositories together with the customary excipients and/or diluents and stabilizers.

The active substances or mixtures of active substances which form the subject-matter of the invention, and the spontaneously dispersible concentrates which contain these active substances or mixtures of active substances, can be administered to humans orally, by injection (intravenously, subcutaneously or intramuscularly) or in other ways. If they are presented as solid dosage forms for oral administration, this can be in the form of tablets, granules, pellets, powders or capsules, etc. The preparations can contain additives, for example a pharmaceutical excipient, such as a saccharide or cellulose base, a binder, such as starch paste or methylcellulose, a filler, or a disintegrant, etc., with additives being employed which are customarily used in the preparation of medicinal or pharmaceutical formulations. When the active substances or mixtures of active substances according to the invention are administered orally in the form of liquid dosage forms, they can be present in any form selected from amongst aqueous preparations for internal use, from suspensions, emulsions and syrups, etc., and they can also be present in the form of dried preparations which are dissolved or emulsified prior to use.

When the active substances or mixtures of active substances according to the invention are processed in the form of aqueous solutions, suspensions or oily or aqueous emulsions, preferably microemulsions, from the spontaneously dispersible concentrates according to the invention, they can also be injected. However, it is customary to prepare the injection solutions shortly before administration, by dissolving or suspending the extracts or concentrates in aqueous, liquid media, such as sterile water or physiological sodium chloride solution or glucose solution.

If required, conventionally used solvents, stabilizers, preservatives and additives for the preparation of isotonic solutions can be added to a preparation for injection. The preparations for injection obtained in this manner are administered intravenously, intramuscularly, subcutaneously or in any other suitable way.

The present invention also relates to pharmaceutical preparations which contain the active substances, or mixtures of active substances, or the spontaneously dispersible concentrates, which have been above described, for controlling the growth of tumour cells. The pharmaceutical preparations according to the invention are those which can be used for enteral (such as oral or rectal) or for parenteral or topical administration to warm-blooded animals, which preparations contain the spontaneously dispersible concentrate on its own or together with a pharmaceutically acceptable excipient.

The dosage of the concentrates according to the invention depends on the warm-blooded species, on the age and on the individual condition, and on the mode of administration. For example, doses in the range of about 0.1–50 mg/kg of body weight are administered subcutaneously, and doses in the range of 0.05–5 mg/kg of body weight are administered intraperitoneally to warm-blooded animals having a low body weight, such as, for example, mice, rats and hamsters, to achieve an effect of tumour cell destruction.

The oral and rectal forms of the novel pharmaceutical preparations contain between 1 and 95%, preferably between 10 and 95%, and in particular between 20 and 95%, of the spontaneously dispersible concentrate according to the invention. For example, they can be present in unit-type dosage forms, i.e., as coated tablets, micropellets, tablets, suppositories or ampuls and, in particular, as capsules.

Suitable pharmaceutically acceptable excipients for the oral forms are mainly fillers, such as sugars (for example lactose, sucrose, mannitol or sorbitol), cellulose preparations and/or calcium phosphates (for example tricalcium phosphate or calcium hydrogen phosphate), furthermore binders, such as starch paste, with the use of, inter alia, corn starch, wheat starch, rice starch or potato starch, gelatine, tragacanth, methylcellulose, hydroxymethylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone and/or disintegrants (if desired), such as the above mentioned starches, furthermore carboxymethyl starch, crosslinked polyvinylpyrrolidone, agar, alginic acid or a salt thereof, for example sodium alginate.

Examples of suitable flow-control agents are the polyethylene glycols Nos. 200–600 and above.

The gelatine capsules, which are still the preferred dosage form for humans, are provided with suitable coatings, con-

centrated sugar solutions [which can optionally contain gum arabic, talc, polyvinylpyrrolidone, polyethylene glycol and/or titanium dioxide], lacquer solutions (aqueous or those which have been prepared using organic solvents), or enteric coatings of solutions of suitable cellulose preparations, such as microcrystalline cellulose (Avicelä), acetylcellulose phthalate, hydroxy-methylcellulose-phthalate, Metolose®, AQOAT® or a copolymer, such as Eudragit® L 30 D, being used, inter alia.

Pharmaceutical dosage forms for oral use which are particularly suitable according to the invention are two-piece gelatine capsules with a plasticizer, such as glycerol or sorbitol. The soft-gelatine or hard-gelatine capsules and the capsules made of AQOAT® hydroxypropyl methylcellulose respectively can contain the spontaneously dispersible concentrate according to the invention as a mixture with fillers, such as lactose, binders, such as starch, and/or glidants, such as talc or magnesium stearate, and, if appropriate, together with stabilizers and antioxidants, such as, for example, α-, β- or γ-tocopherol. It may be expedient to employ suitable liquids, such as liquid polyethylene glycols Nos. 200 to 600 as diluents, to which stabilizers and antioxidants can also be added.

For parenteral administration, distilled water is added to the concentrates according to the invention. To the aqueous microemulsion for injection which then forms, there can be added viscosity-increasing substances, for example Na-carboxymethyl-cellulose, sorbitol, mannitol and/or dextran, and if appropriate also stabilizers and antioxidants.

The pharmaceutical preparations for parenteral administration preferably contain between 0.1 and 60%, especially between 1 and 40%, of the spontaneously dispersible concentrate according to the invention.

Suitable preparations for topical use, which are particularly suitable for the prophylaxis and the treatment of cancers of the skin, are, for example, creams, ointments, pastes, foams, tinctures and solutions, which contain between 0.001 and 70% of the concentrate according to the invention.

Oily bases which are used for creams and oil-in-water emulsions which contain more than 50% water, are mainly fatty alcohols, for example lauryl alcohol, cetyl alcohol or stearyl alcohol, waxes of liquid to solid consistency, for example isopropyl myristate, wool wax or beeswax and/or hydrocarbons, such as, for example, petroleum jelly (petrolatum) or paraffin oil. Substances which are mainly suitable for emulsifying these oily bases are surface-active, pharmaceutically acceptable substances having predominantly hydrophilic properties, such as, for example, non-ionic emulsifiers, in particular fatty acid esters of polyalcohols or ethylene oxide adducts (such as polyglycerol fatty acid esters or polyethylene sorbitan fatty acid esters) having an HLB value of less than 8. Additives which are added to the water phase are, inter alia, agents which prevent desiccation of the creams, for example polyalcohols, such as glycerol, sorbitol, propylene glycol and/or polyethylene glycols Nos. 200 to 600, and furthermore preservatives, odor-imparting substances, etc.

Ointments are water-in-oil emulsions which contain up to 70%, but preferably between 20 and 50%, water or aqueous phases.

Substances which are suitable as the lipid phase are mainly hydro-carbons, for example petroleum jelly, paraffin oil and/or solid paraffins, which contain hydroxy compounds suitable for improving the water-binding capacity, for example fatty alcohols or esters, such as cetyl alcohol or wool wax alcohols.

In some cases, emulsifiers having an HLB-value of 8 to 16, such as, for example, sorbitan fatty acid esters (such as sorbitan isostearol) are also added. Additives which are added to the water phase are, inter alia, humectants, such as polyalcohols (glycerol, propylene glycol, sorbitol and/or polyethylene glycols No. 200, 400, 600); and furthermore preservatives, odor-imparting substances, etc.

Fatty ointments are anhydrous and chiefly contain hydrocarbons as the base, for example paraffin, petroleum jelly and/or liquid paraffins; moreover natural or partially-synthetic fats, such as, for example, coconut fatty acid triglycerides, furthermore: fatty acid partial esters of glycerol, such as, for example, the fatty alcohols, emulsifiers and/or additives which increase the water-absorption capacity, all of which have been mentioned in connection with the ointments.

Pastes are creams and ointments containing powder constituents which absorb secretions, such as, for example, metal oxides (such as titanium oxide or zinc oxide), and furthermore talc and/or aluminum silicates whose task it is to bind any moisture or discharge which may be present.

Foams are administered from pressurized containers and are oil-in-water emulsions of the spontaneously dispersible concentrates according to the invention which are present in aerosol form, with halogenated hydrocarbons (such as, for example, lower chloro-fluoroalkanes; such as dichlorodifluoromethane and dichlorotetrafluorethane) being added as propellants. Other substances which may be added are the customary additives, such as preservatives, etc.

The present invention also relates to the use of the active substances, mixtures of active substances and spontaneously emulsifiable concen-trates according to the invention for inhibiting the growth of tumour cells or as prophylactic agents against oncoses in humans and animals, administration preferably being carried out in the dosage forms which correspond to the pharmaceutical preparations described above. For use as dietary foods and as food additives, the optimum compositions must be established for every individual case.

Processing examples for inventive esters and phosphatides with vitamin-D and Vitamin-E compounds

1. Process for the preparation of DL-α-Tocopherol-palmitate

To a solution of 450 mg DL-α-Tocopherol and 50 mg dimethylformamide in 30 ml Toluene at 20° C. 350 mg palmitoylchloride (ca. 20% excess) are being added dropwise. The reaction mixture is heated to 80° C. and refluxed for 3 hours. Subsequently, the solvent is distilled off in vacuo and the residue is recrystallized in acetonitrile. DL-α-Tocopherolpalmitate is obtained in the form of crystals having a melting range of 53,8° to 55,2° C.

The following compounds are prepared in analogous manner:

DL-α-Tocopherolpivaloylate	RI 1.50102
DL-α-Tocopherolvalerate	RI 1.49328
DL-α-Tocopherolcaprylate	UV 296,8 733,6 RI 1.48018

-continued

DL-α-Tocopherolcrotonate	RI 1.50378
DL-α-Tocopherol-10-undecenoate	UV 296,6 233,6 RI 1,49188
DL-α-Tocopherol-2-dodecenoate	UV 296,4 233,0 RI 1,49388
DL-α-Tocopherololeate	RI 1,48158
Ergocalciferolpivaloylate	RI 1.52148
Ergocalciferolvalerate	RI 1.53924
Ergocalciferolcaprylate	UV 302,4 290,4 RI 1.53010
Ergocalciferolbenzoate	UV 301,6 289,0
Ergocalciferolpalmitate	RI 1.50316
Ergocalciferolcrotonate	UV 300,4 288,6 RI 1.55342
Ergocalciferol-10-undecenoate	UV 302,4 292,4 RI 1,50360
Ergocalciferololeate	RI 1.52620
Cholecalciferolpivaloylate	RI 1,51292
Cholecalciferolvalerate	RI 1.52714
Cholecalciferolcaprylate	RI 1,51756
	IR 2931 cm^{-1} ν(CH)
	2870 cm^{-1} ν(CH)
	1722 cm^{-1} ν(C—O) Ester.
	1467 cm^{-1} δ(CH)
	1370 cm^{-1} δ(CH_3)
	1171 cm^{-1} ν(C—O)
	959 cm^{-1} δ(CH) Vinyliden
Cholecalciferolpalmitate	RI 1.51418
	IR 2928 cm^{-1} ν(CH)
	2855 cm^{-1} ν(CH)
	1723 cm^{-1} ν(C—O) Ester
	1467 cm^{-1} δ(CH)
	1370 cm^{-1} δ(CH_3)
	1184 cm^{-1} ν(C—O)
	959 cm^{-1} δ(CH) Olefinic CH
Cholecalciferolcrotonate	RI 1,55444
	IR 2935 cm^{-1} ν(CH)
	1717 cm^{-1} ν(C—O) Ester
	1467 cm^{-1} δ(CH)
	1369 cm^{-1} δ(CH_3)
	1189 cm^{-1} ν(C—O)
	959 cm^{-1} δ(CH) Olefinic CH
Cholecalciferol-10-undecenoate	RI 1,51796
	IR 2931 cm^{-1} ν(CH)
	1725 cm^{-1} ν(C—O) Ester
	1458 cm^{-1} δ(CH)
	1371 cm^{-1} δ(CH_3)
	1179 cm^{-1} ν(C—O)
	δ(CH) } 974 cm^{-1} {δ(CH)
	trans } 913 cm^{-1} {δ(CH) of $CH{=}CH_2$
Cholecalciferololeate	RI 1.52092
	IR 2931 cm^{-1} δ(CH)
	1723 cm^{-1} ν(C—O) Ester
	1458 cm^{-1} δ(CH)
	1372 cm^{-1} δ(CH_3)
	1187 cm^{-1} ν(C—O)
	973 cm^{-1} δ(CH) trans
Cholecalciferol-all trans-retinate	NIR 1627 cm^{-2} (C=C) [D_3]
	1583 cm^{-1} C=C)
	[Retinate]
Cholecalciferolcrotonate	RI 1,55444
Cholecalciferol-10-undecenoate	RI 1,53796
Cholecalciferololeate	RI 1.52092

2. Preparation of Ergocalciferollinolenate

To a solution of 300 mg linolenic acid in 20 ml toluene at 10° C. 200 mg oxalylchloride in 30 ml toluene are being added dropwise. Subsequently, 10 ml toluene are being distilled off in vacuo and then 400 mg ergocalciferol and 50 mg dimethylformamide are added. Heat to 90° C. and reflux for 2 hours, then distill off the solvent on a Rotavapor. The residue is being chromatographed on a silicagel column with 80:20 cyclohexane/ethyl acetate.

Ergocalciferollinolenate is obtained, characterized by a Refractory Index (RI) n_D 20° C. of 1,53986 and a UV-absorption maximum lmax at 291,8 nm.

IR 2931 cm^{-1} δ(CH)
1725 cm^{-1} ν(C—O) Ester

-continued

1458 cm^{-1} δ(CH)
1372 cm^{-1} δ(CH_3)
1187 cm^{-1} ν(C—O)
973 cm^{-1} δ(CH) trans

In analogous manner the following products can be prepared:

DL-α-Tocopherololeate	RI 1,49302
Cholecalciferollinolenate	RI 1.54090
DL-α-Tocopherollinolenate	RI 1,49290
Ergocalciferollinolenate	RI 1.53274
Cholecalciferollinolenate	RI 1.52138
DL-α-Tocopherollinolenate	RI 1.49288
Ergocalciferol-all trans-	UV 291,5 RI 1,49226

-continued

retinate	IR	2958 cm^{-1} ν(CH) 2871 cm^{-1} ν(CH) 1701 cm^{-1} ν(C=O) Ester 1608 cm^{-1} ν(C=C) 1583 cm^{-1} ν(C=C) 1457 cm^{-1} δ(CH) 1239 cm^{-1} ν(C=O) 1153 cm^{-1} ν(C=O) 1016 cm^{-1} ν(C=O) 970 cm^{-1} δ(CH) } trans C=C)
Cholecalciferol-all trans-retinat	RI	1.48774
	IR	2958 cm^{-1} ν(CH) 2871 cm^{-1} ν(CH) 1701 cm^{-1} ν(C=O) Ester 1608 cm^{-1} ν(C=C) 1583 cm^{-1} ν(C=C) 1457 cm^{-1} δ(CH) 1239 cm^{-1} ν(C=O) 1153 cm^{-1} ν(C=O) 1016 cm^{-1} ν(C=O) 970 cm^{-1} trans C=C δ(CH)
	NIR	1627 cm^{-1} (C=C) [D_2] 1583 cm^{-1} (C=C) [Ret.]
DL-α-Tocopherol-all trans-retinate	RI	1.55350
	NIR	1583 cm^{-1} (C=C) [Ret.]
Ergocalciferol-13 cis-retinate	NIR	1627 cm^{-1} (C=C) [D_2] 1583 cm^{-1} (C=C) [Ret.]
Cholecalciferol-13 cis-retinate	NIR	1627 cm^{-1} (C=C) [D_3] 1583 cm^{-1} (C=C) [Ret.]
DL-α-Tocopherol-13 cis-retinate	NIR	1583 cm^{-1} (C=C) [Ret.]

*)N.B.: RI = Refraction Index, measured on a DUR Refractometer Schmidt + Haensch, Berlin.
IR = Infrared Spectra measured on a Spectrophotometer Perkin-Elmer 683G
NIR = Near Infrared (FT RAMAN) Spectra measured on a spectrometer YAG Laser, Excitation at 1064 nm.
UV — Spectra measured on a Spectrophotometer Shimadzu UV-160A

3. Preparation of Ergocalciferol-azafrinate

To a solution of 80 mg azafrinate, formula (XII) [process for preparing this compound vide Helv. Chim. Acta 58 (1975) 1722–1727 and Helv. Chim Acta 65 (1982) 353–354] in 50 ml chloroform 65 mg N,N'-carbonyl-diimidazol are added. The mixture is laft standing for 12 hours at room temperature, then 40 mg ergocalciferol are added. After further standing for 12 hours at a temperature of 30° C. the solvent is distilled off and the residue diluted in 50 ml ethyl acetate. The solution is washed once with 1/10 N hydrochloric acid and once with 1/10 N sodium hydroxide and then the solvent is distilled off. The residue is chromatographed on a silicagel column; eluent: n hexane/ethyl acetate 9:1.

Ergocalciferol-azafrinate is obtained with a melting point of 171° to 173° C. and an UV absorption maximum of 429,5 nm.

4. Process for preparing ergocalciferol-1,2-dipalmitoyl-glycero-phosphatide and ergocalciferol-1,2-dipalmitoyl-glycero-thiophosphatide

1,2-dipalmitoyl-glycero-3-H-phosphonate-triethylammonium salt is prepared according to the method of I. Lindth and J. Stawinski in J. Org. Chem 54, 1338–1342 (1989): "Synthesis of glycerophospholipids and their analogues via H-phosphonate".

The reaction product can be used for the next chemical step without any intermediary cleaning operation by chromatography.

600 mg of the reaction product and 600 mg ergocalciferol are dissolved in pyridine and brought to dryness in vacuo. The residue is again dissolved in 15 ml pyridine. After adding 0,2 ml pivaloylchloride, this solution is stirred 30 minutes in dry athmosphere. After further adding 0,1 ml pivaloyl-chloride the solution is again stirred for 30 minutes at room temperature.

The reaction solution is then divided into two parts: To the first part of this solution 150 mg Jodine, dissolved in 2 ml pyridine-water 98:2, are added. After 30 minutes stirring at 20° C. the reaction mixture is poured in 50 ml chloroform. This solution is washed once with 20 ml 5% sodium-bisulfitsolution and twice with 20 ml water. After separation the chloro-form-phase is distilled off to dryness on a Rotavapor.

To the other part of the solution 150 mg sulfur in 2 ml pyridine-toluene 1:1 are added. The solution is stirred for 3 hours at a temperature of 20° C. 50 ml chloroform are added and then the solution is washed twice with 20 ml water. After the separation has taken place the chloroform-toluene-phase is distilled off to dryness on a Rotavapor.

The two raw products are chromatographed on a silicagel column with chloroform/hexane 2:1 and 8:1 as eluent.

The following products are obtained:

Ergocalciferol-1,2-dipalmitoyl-glycero-phosphatide, with a melting poim of 168° to 170° C. and

Ergocalciferol-1,2-dipalmitoyl-glycero-thiophosphatide, with a (smectic) melting range of ca. 145° to 150 ° C.

In a corresping way the following compounds are also prepared:

Ergocalciferol-geranyl-phosphatide

Ergocalciferol-farnesyl-phosphatide

Cholecalciferol-geranyl-phosphatide

Cholecalciferol-farnesyl-phosphatide

DL-α-Tocopherol-geranyl-phosphatide

DL-α-Tocopherol-farnesyl-phosphatide

Rf-values of inventive compounds which are being prepared according to the examples 1 to 4:

1% solution in CH_2Cl_2, applied in bands 2 cm/2 µl
Linomat III CAMAC 10 cm run
UV 366 after 1:1 H_2SO_4/MeOH
2 min. 120° C.

	SYSTEM						
COMPOUND	1	2	3	4	5	6	7
TOCO-C 8:0	0.04	0.02	0.09	0.28	0.59	0.36	0.18
TOCO-C 11:1	0.04	0.25	0.09	0.28	0.59	0.36	0.18
TOCO-C 16:0	0.14	0.08	0.33	0.78	0.96	0.83	0.59
TOCO-trans-RET	0.08	0.04	0.26	0.76	0.93	0.85	0.54
TOCO-C 18:1	0.06	0.09	0.40	0.90	1.0	0.95	0.74
D_2 - C 16:0	0.25	0.13	0.35	0.89	1.0	1.0	0.88
D_2 - trans-RET.	0.21	0.1	0.33	0.85	0.97	1.0	0.83
D_2 - C 11:1	0.22	0.13	0.42	0.87	0.88	0.92	0.75
D_2 - trans-RET	0.15	0.14	0.41	0.89	1.0	0.94	0.79

EXPLANATION
System 1 Plate Merck Art. 5715 petrolether/diethylether 98:2
System 2 Plate Merck Art. 5715 petrolether/diethylether 97:3
System 3 Plate Merck Art. 5715 cyclohexane/ethylacetate 97:3
System 4 Plate Macherey Nagel RP 18 Art. 811'071 petrolether/diethylether 95:5
System 5 Plate Macherey Nagel RP 18 Art. 811'071 n hexane/t.butylmethylether/acetone 90:5:5
System 6 Plate Macherey Nagel RP 18 Art. 811'071 petrolether/cyclohexane/ethylacetate/H_2O 48:48:3:1
System 7 Plate Macherey Nagel RP 18 Art. 811'071 petrolether/diethylether 97:3

Composition examples of spontaneously dispersible agents which contain as substances possessing antitumour activity vitamin sterolesters and/or vitamin-sterolphosphatide compounds according to the formulae (I) to (VI):

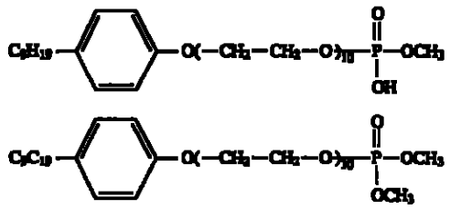
a) 0,5 to 25% by weight of one or several of the vitamin-sterolesters and/or vitamin-sterolphosphatide compounds of the formulae (I) to (VI) 0,1 to 40% by weight of isopropylmyristate, isopropylpalmitate or Miglyol® 812 (Dynamit Nobel) 20 to 45% by weight of emulsifier mixture Diphasol® 3873 (CIBA-GEIGY) 20 to

45% by weight of Invadin® JFC 800% (CIBA-GEIGY)

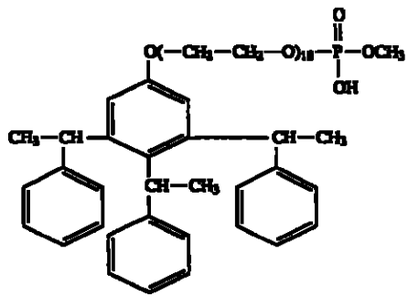
b) 0,5 to 25% by weight of one or several of the vitamin-sterolesters and/or vitamin-sterolphosphatide compounds of the formulae (I) to (VI) 0,1 to 40% by weight of isopropylmyristate, isopropylpalmitate or Miglyol® 812 (Dynamit Nobel) 20 to 45% by weight of Invadin® JFC 800% (CIBA-GEIGY) 20 to 45% by weight of Soprophor® FL (Rhône-Poulenc)

Miglyol® 812 is a neutral oil (oleum neutrale) of Dynamit Nobel, which is a triacylglycerol of the coconut fatty acids, the so-called fractionated, middle chained (C8 to C10) compounds [i.e. a caprylic/capric triglyceride in the CTFA classification].

Diphasol® 3873 (CIBA-GEIGY) (which is equivalent to SERMUL® EA188) is a surfactant mixture consisting of the following two compounds (50:50):



Invadin® JFC 800% (CIBA-GEIGY) is a tert. octylphenylpolyoxyethyleneether with 9 to 10 oxyethylene groups Soprophor® FL (Rhône-Poulenc) is a tristyrylphenolpolyoxy-ethylene-18-mono/dimethyl-phosphoracidester. with formula:



DEMONSTRATION OF THE SPREADING AND PERMEATION CAPACITY OF THE INVENTIVE CONCENTRATES AND OF EMULSIONS PREPARED WITH SUCH CONCENTRATES

Method: TL-Plate 0,25 mm Silicagel 60F254 Merck Art. No. 11798 with concentration zone.
Eluent: PBS Dulbecco's without Ca and Mg (= Ringer solution or physiological sodium chloride solution, buffered)

Rf.-Values	0,1% Substance, dissolved in dichloromethane Rf.-Values:	Concentrate in aquous emulsion 1000 ppm.
Ergocalciferol-C 8:0	0	21,21
Ergocalciferol-all trans retinate	0	48,39
Ergocalciferol-all trans retinate: (with DL-α-tocopherol-acetate as coemulgator)	0	45,45
DL-α-tocopherol-C 8:0	0	21,21

-continued

Method: TL-Plate 0,25 mm Silicagel 60F254 Merck Art. No. 11798 with concentration zone.
Eluent: PBS Dulbecco's without Ca and Mg (= Ringer solution or physiological sodium chloride solution, buffered)

Rf.-Values	0,1% Substance, dissolved in dichloromethane Rf.-Values:	Concentrate in aquous emulsion 1000 ppm.
DL-α-tocopherol-10-undecenoate	0	59,68
DL-α-tocopherol-C 16:0	0	48,48

P.S.: Microemulsions 1:1000 = 1'000 ppm active substances = 1 mg/ml.

The above indicated Rf.-Values illustrate the behaviour of the inventive concentrates in cell colonies and, more specifically, at the cell membrane. The reduced surface tension of the concentrates, with values of 30 to 32 mN/m, the small size of the generated micromicelles as well as the low viscosity of the microemulsion are all factors which influence the diffusion through the cell membrane and the spreading in the cell plasma in a very favourable manner. Control measurements, carried out at the Institute for Polymers, Swiss Federal Institute of Technology at Zurich, exhibited a radius for the micelles of around 1nm. (Prof. Dr. Pier Luigi LUISI and Prof. Dr. Peter SCHURTENBERGER).

Example for the pharmaceutical production of a system's preparation containing the inventive concentrates in the form of "multiple units".

a) Granulation (granules and pellets)

Metolose ® 90 SH-4000 (Shin-Etsu Chemical)	90.0 g
Avicel ® PH-101	80,3 g
Inventive CONCENTRATE	139,4 g
Aerosil ® 200	80,3 g
	390.0 g

Granulation in the high speed mixer or the fluidized bed, with the addition of 110 g ethanol, sieving on a 18 to 42 mesh screen with crushing, drying for 24 h at 40° C.

b) Enteric and sustained release coating in the fluidized bed with AQOAT® AS-HG (Shin-Etsu Chemical) and Talc

c) Composition of finished granules or micropellets

Core Material	44% by weight
Inventive CONCENTRATE	25% by weight
Enteric coating	31% by weight
	100%

N.B. The pellets or granules according to a) can also be filled without prior coating into capsules which are made of AQOAT® (HPMC-AS-M or HPMC-AS-N), have been sealed with acetone/ethanol 1:1 and can thus perform the functions of pH-control and slow release.

BIOLOGICAL ASSAYS.

The antitumour activity of spontaneously disperable concentrates containing active substances prepared according to the examples No. 1 to 4 is confirmed by the following test results:

1. In-vitro assays using suitable tumour cell lines

A biological assay system using microtiter plates and serial dilutions has been developed. Batches of 104 tumour cells per ml were set up in culture medium RPMI 1640 and inactivated with 10% of fetal calf serum (GIBCO); they are spread at a density low enough to enable them to grow

during the assay, in so-called non-confluent monolayers. Samples are added after 6–24 hours, with 100 µl per row, to which 100 µl of medium are added in the first well. Half of this mixture is withdrawn, transferred into the next well and again treated with 100 µl of medium, etc. This results in an n½ geometrical serial dilution.

In the plaque assay, the samples are incubated at 37° C. for 3 to 5 days under 3½% of CO_2. They are then stained and fixed using 0.1% crystal violet (Fluka, Buchs) in a solution of 70% of methanol, 1% of formaldehyde and 29% of water. The samples are evaluated under the microscope, magnification 300×. The greatest cytotoxic dilution is determined. The samples can also be evaluated quantitatively by means of scanning and absorption measurement in a spectrophotometer.

2. Evaluation of the results.

a) TS/A-Assay: greatest celltoxic dilution

PREPARATION	TUMOUR LINE: TS/A: murine adenocarcinoma 24 h	In dilution active up to 1: 72 h
D_2 - C 4:1	20'480'000	—
D_2 - C 8:0	2'000'000	4'000'000
D_2 - C 11:1	40'960'000	163'840'000
D_2 -all trans-RET.	16'000'000	64'000'000
DL-α-TOCO - C 8:0	4'000'000	32'000'000

TSA: murine adenocarcinoma (spontaneous mammacarcinoma) Prof.Dott. Guido FORNI, Istituto di Microbiologia, Università degli Studi di TORINO, Scuola di Medicina

b) Py 6-ASSAY: greatest celltoxic dilution

PREPARATION	TUMOUR LINE: Py 6 (Polyoma transformed 3T3 mouse cells) 20 h	In dilution active up to 1: 60 h
D_2 - C 4:1	10'240'000	—
D_2 - C 11:1	5'120'000	—
D_2 -all trans-RET.	16'000'000	32'000'000
D_2 - C 18:3	6'025'000	96'375'000
D_3 - C 18:3	6'000'000	24'000'000
DL-α-TOCO - C 8:0	8'000'000	16'000'000
DL-α-TOCO - C 11:1	12'000'000	48'000'000
DL-α-TOCO - C 18:1	6'000'000	24'000'000
DL-α-TOCO - C 18:3	6'025'000	48'200'000
TOCO-a.L-RETINAT	12'000'000	96'000'000

Py 6: Polyoma-virus-transformed 3T3 mouse cells

c) Other tumour-cell-lines

PREPARATION Exposure	TUMOUR LINES: Y T 43 In dilution active up to 1: 4 days	P 815 In dilution active up to 1: 4 days	L 929 In dilution active up to 1: 2 days
CALCIOL- C 4:1	48'000'000	16'000'000	64'000'000
CALCIOL- C 8:0	32'000'000	32'000'000	64'000'000
CALCIOL - C 11:1	32'000'000	16'000'000	32'000'000
CALC.-all trans-RET.	128'000'000	64'000'000	128'000'000
DL-α-TOCO-C 8:0	32'000'000	32'000'000	64'000'000
DL-α-TOCO-C 16:0	16'000'000	16'000'000	64'000'000

Y T 43: Ibridoma/anticorpi monoclonali (hybridoma cells)
P 815: mastocytoma
L 929: fibroblastoma (areolar and adipose tissue)

Prof. Guido Forni, Istituto di Microbiologia, Università degli Studi di Torino, Scuola di Medicina.

d) Cytotoxicity on human tumour cell lines Survival rate in % (MTT-method with tetrazol blue)

DILUTION 1: 1'000'000

PREPARATION	CELL-LINE: ST-4	RAJI	K 562	H L 60
CALCIOL-CROTONATE	—	8	3	9
CALCIOL-CAPROYLATE	1	7	8	7
CALCIOL-C 11:1				
CALCIOL-all tr.-RET.	—	9	3	8
	1	2	0	0
TOCO-CAPROYLATE	1	8	7	9
TOCO-10-UNDECEN.	—	1	1	0

DILUTION 1: 10'000'000

PREPARATION	CELL LINES: ST-4	RAJI	K 562	H L 60
CALCIOL-CROTONATE	57	83	63	93
CALCIOL-CAPROYLATE	39	88	71	35
CALCIOL - C 11:1				
CALCIOL-all tr.-RET.	56	91	56	88
	64	36	85	13
TOCO-CAPROYLATE	41	73	85	56
TOCO-10-UNDECEN.	67	10	85	100

ST-4 Linfoma convolto T
RAJI Leucemia linfoide B
K 562 Eritroleucemia
H L 60 Leucemia mieloide

e) Testing a Concentrate-Mixture with D_2- and D_3-Ester Components Antitumoral Activity on human cell-lines Killing rate in %

CONCENTRATION	CELL-LINES: ST-4	H L 60	K 562
1:10'000'000	100	100	100
1:100'000'000	73	68	70
1:1'000'000'000	47	6	54

Human Tumour cell-lines:
ST-4 Linfoma convolto T
HL 60 Leucemia mieloide
K 562 Eritroleucemia
CONCENTRATIONS: relative to the total content of Calciol- and Cholecalciol esters

Tests conducted at the Ospedale maggiore San Giovanni

Battista, LE MOLINETTE, Torino. Direction: Prof. Dott. Giorgio RIVARA. Additional Tests with human tumour cell-lines.

BATTELLE INSTITUTE, Frankfurt, FRG. (Dr. Matthias GIESE) assessed the cytotoxic effect on various selected cell lines of solid human tumours, which grow relatively slowly. The assay was conducted with 2%-concentrates (by weight) of:

A CALCIOL-CHOLECALCIOL-ESTER-MIXTURE 1*)

B DL-α-TOCOPHEROLESTER-MIXTURE 1*)

C CALCIOL-all trans-RETINATE

with the following tumour cell lines (provided by the German Cancer Research Center, DKFZ, Heidelberg, FRG):

1 EJ28 : carcinoma of bladder

2 LX-1 : carcinoma of lung

The following biological response modifiers (BRM) were used as controls:

a rhu Interferon-Gamma (Biozol, BRD)

b rhu Tumor-Necrosis Factor Alpha (Biozol, BRD)

Results are given in dilution series and using the IC_{50}-values as indicator. [Inhibitory concentration] Incubation of the cells for 24 h, and 72 h respectively, at 37° C.; vitality staining after 3 and 14 days. Testing range 1:106 to 1:109, calculated on the active substance content.

Smaller differences only between the individual cell lines became apparent, as well as relatively feeble differences between the the three concentrates. The IC_{50}-values are centered in the dilution range 1:107.

In summary, the findings of the tests demonstrate clearly and throughout a cytotoxic effect on the tumour cells and not merely a cytostatic effect.

The strongest cytotoxic effect was shown for the CALCIOL/CHOLECALCIOL-ESTER-MIXTURE. The control substances BRM, however, showed no measurable effect on either of the 2 cell lines included in the assay.

N.B. 1*) Composition of A: equal parts by weight of the following five Calciol-esters: calciol-C 11:1, C 12:1, C 16:0, C 183 and all trans retinate; plus equal parts by weight of the following five Cholecalciol-esters: Cholecalciol-C 4:1, C 11:1, C 16:0, C 18:3 and all trans retinate. Composition of B: equal parts by weight of the following five DL-α-tocopherol-esters: Toco-C 8:0, C 11:1, C 16:0, C 183 and all trans retinate.

Diagram 1/13 FIG. 1 and 2: Cytotoxicity on human solid tumours. Vide the technical appendix.

IN-VITRO ASSAYS CONDUCTED BY THE NATIONAL CANCER INSTITUTE, BETHESDA.

Broad systematic tests conducted by the NCI under their Developmental Therapeutics Program with 60 different cell-lines of human tumours covered 8 PANELS:

Leukemia

Non-small cell Lung Cancer

Small cell Lung Cancer

Colon Cancer

CNS Cancer

Melanoma

Ovarian Cancer

Renal Cancer

The following measuring rods were applied:

PG (Percentage Growth)

Dose-Response-Curves

Mean Graphs

The diagrams 2/13 to 13/13 with FIGS. 3.1 to 27 render the overall results in graphical summaries for

CALCIOL-10-UNDECENOATE-CONCENTRATE

CALCIOL-all trans-RETINATE-CONCENTRATE and

CALCIOL-LINOLENATE-CONCENTRATE

Cf. the Technical Annexures.

The principal result of the extensive assessment by NCI is quite comparable to the findings reached by BATTELLE INSTITUTE, namely that the INVENTIVE CONCENTRATES not only show an anti-proliferation effect, i.e. a growth inhibition, but a true and general cytotoxicity. This is particularly marked with those cell lines which possess short doubling times and are thus relatively more sensitive to antitumour agents than the slower growing (solid) tumour lines.

Cf. also: Michael R. Boyd: Status of the NCI Preclinical Antitumor Drug Discovery Screen, PPO updates, Vol. 3, October 1989, Number 10.

Anne Monks et al.: Feasibility of a High-flux Anticancer Drug Screen using a diverse Panel of cultured human Tumor Cell Lines. Journal of the National Cancer Institute, Vol. 83, No. 11, Jun. 5, 1991.

Proof of membrane-penetration at the single tumour cell.

It can be shown with light microscopy—and also with Laser scanning microscopy, that few hours after incubation there is forming a halo of vacuoles around the nucleus of the cell [example Py6-cells of 3T3-mice]; thinly disseminated, medium concentration (=dilution) of the active-substance containing concentrates].

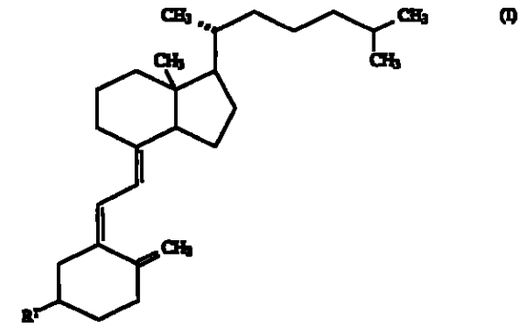
The analytical demonstration that these vacuoles in fact contain the VITAMIN-STEROLESTER active substances is quite clear and unequivocal: it involves cleaning the incubated tumour cells, extracting the cell plasma with 1% SDS, centrifuging, mixing the supernatant with a 0,05%-solution of Uvitex® CF conc. (CIBA-GEIGY) in acetone/water (85:15) or of Uvitex® EBF (CIBA-GEIGY) or of Tinopal GS (CIBA-GEIGY).

The VITAMIN-STEROLESTERS according to the invention extinguish the fluorescence in the longwave UV-segment which is normally occasioned by the markers Uvitex® CF conc. and Uvitex® EBF, and Tinopal® GS respectively. The thin-layer plate shows blue coloring.

UV-scanning at 366 nm. Microdialysis (Micellar electrokinetic capillary chromatography; MEEC) by means of capillary zone electrophoresis with a specialized buffer system and laser induced fluorescence detection (Beckman Instruments Pace 2000).

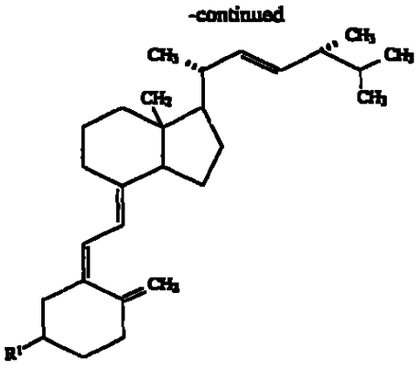
What we claim, is:

1. A purified ester of a vitamin-D compound according to one of formulae (I) to (IV):

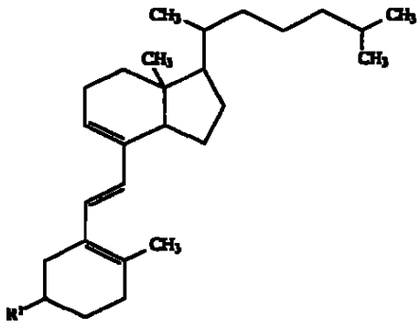


(I)

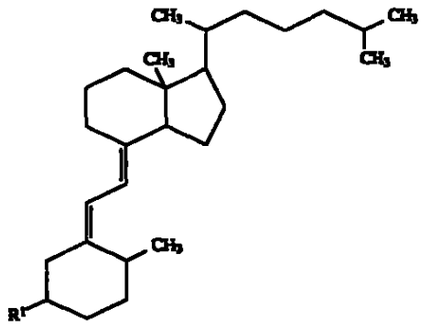
-continued



(II)

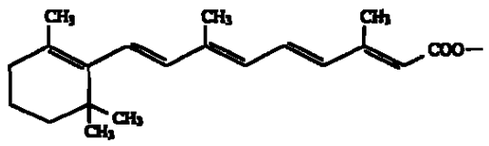


(III)



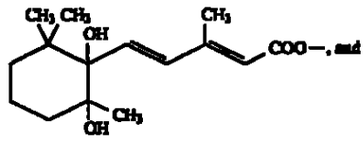
(IV)

wherein R^1 is selected from the group consisting of a group of formula (VII):



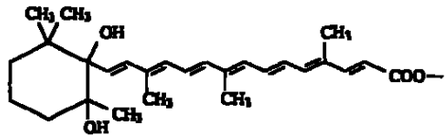
(VII)

a group of formula (VIII):



(VIII)

a group of formula (IX):



(IX)

2. The ester of claim 1 which is cholecalciferyl-all trans retinate.

3. The ester of claim 1 which is ergocalciferyl-all trans retinate.

4. The purified ester which is cholecalciferyl-10-undecenoate.

* * * * *

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ASUNTO:	Radicación PCT Nº	04-61816
	Trámite	011
	Evento	379
	Actuación	440
	Folios	009

Con fundamento en lo previsto por el artículo 43 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica que la oposición presentada por el doctor Reinaldo Pinilla Moreno, en su calidad de apoderado de Procaps S.A., reúne los requisitos formales exigidos por la ley al momento de su presentación.

En cumplimiento de lo dispuesto en la citada norma, se le concede el término de sesenta (60) días hábiles siguientes a la fecha de notificación del presente oficio, para que haga valer sus argumentaciones y presente pruebas.

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.

ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones.

21 ABR. 2006

El anterior requerimiento fue notificado por fijación en lista de fecha,_____.

202021

Sede Centro: Carrera 13 No. 27-00 Pisos 2, 5, 7 y 10
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