

**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES**

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Bogotá, D.C.,

Abogado

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ASUNTO	Radicación	09-10461
	Trámite	011
	Evento	378
	Actuación	519
	Oficio	1 6 6 7 1
	Folios	043

Patente de Invención



Patente de Modelo de Utilidad



Vía Nacional

Vía PCT (fase nacional)

Cap. I



Cap. II



No. Solicitud Internacional PCT/US2007/072919

Fecha de Presentación Internacional 06 de julio de 2007

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

1. Documentos estudiados

Descripción:	Folios 127 a 246	04 de febrero de 2009
Reivindicaciones:	Folios 247 a 259	04 de febrero de 2009
Prioridad:	US 60 819, 509	07 de julio de 2006
	US 60 886, 825	26 de enero de 2007

2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque:

Esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486).

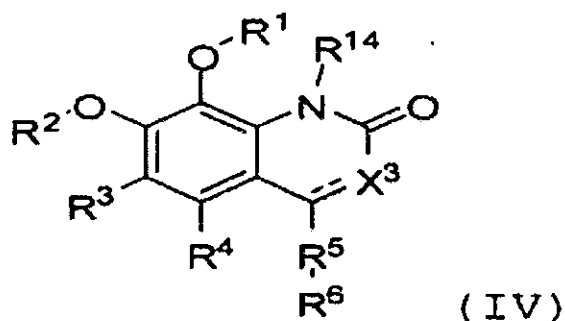
Y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

Título de la solicitud: "Inhibidores heteroaril bicíclicos de PDE4, métodos para su síntesis y métodos para inhibir PDE4" (modificado en el folio 272).

El problema planteado en esta solicitud consiste en la necesidad de proporcionar compuestos que tengan actividad inhibitoria de la fosfodiesterasa PDE4.

Para resolver este problema técnico la solicitud en estudio proporciona un compuesto de la fórmula (IV) y métodos para su preparación



El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A61K 31/4704; A61K 45/06; A61P 27/02

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

El objeto de las reivindicaciones 1-6 no es patentable de acuerdo con el Artículo citado.

5. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El uso de las reivindicaciones 29-32 no es patentable, de acuerdo con el artículo 14.

6. De la Solicitud de Patente

Titulo

Se sugiere modificar el título puesto que el mismo infiere un uso el cual es una característica no patentable. Se sugiere cambiarlo por "Inhibidores heteroaril bicíclicos de PDE4, métodos para su síntesis".

Reivindicaciones (Art 30 D 486, Art 22 PCT)

El capítulo reivindicatorio no es claro ni conciso cuando se usa los términos:

- Heterocicloalquilo, heterocicloalquilalquilo, heteroarilo, heteroaralalquilo: porque no se define el número de miembros presentes en el anillo, y para el caso de lo heteroátomos no se define el número y tipo de heteroátomos, además para los compuestos cíclicos que contienen una porción alquilo, no se define el número de carbonos que contiene la cadena.
- Aralalquilo, arilo, cicloalquilo, ciclo alquilalquilo: porque no se define el número de miembros que contiene el anillo, además para los compuestos cíclicos que contienen una porción alquilo, no se define el número de carbonos que contiene la cadena.
- Aminoácido, fosfonatos, fosfoamidas: no se definen sus estructuras, abarca todos aquellos compuestos que tengan esa característica química
- Acilo, acilalquilo, carboxialquilo, cianoalquilo, , alcoxi, alcoxialquilo, amidoalquilo, amino, alquilo, alquilalcoxi, aminoalquilo, alquenilo, alquinilo, cianoalquilo, carboxialquilo, éter, heteroalquilo, hidroxialquilo, haloalquilo, alquilo inferior, amino alquilo, aminoalcoxi: puesto que no se define el número de carbonos presentes en la cadena.

- El uso de expresiones como "alguno de los cuales puede estar opcionalmente sustituido" no son concisas ni claras porque no limitan el alcance la reivindicación, ni la definen de manera adecuada.
- El uso los términos "m", "n" "q" y "p" que se define común número entero 0, 1, 2, no es conciso, porque dan lugar a una innumerable combinación de compuestos que no encuentran soporte en la descripción porque no limitan el alcance la reivindicación, ni la definen de manera adecuada.

De acuerdo a lo anterior, se obtienen un número indefinido de compuestos que no encuentran soporte en la descripción.

Se sugiere al solicitante, definir cada término de manera apropiada y restringir la solicitud a una generalización razonable de los compuestos ejemplificados.

Para este efecto esta Oficina manifiesta que en la descripción se encuentran soportados los siguientes compuestos a los cuales el solicitante puede tener en cuenta para la respectiva restricción:

(IV)

R1	Ciclopentil, metilciclopropil, 6-hexanoico, isobutoxi, dimetilamino butyl, dimetilaminopentil,
R2	Metil,
R3	H
R4	H
R5	-O-, -NH-,
R6	3,5-dicloropiridina, 3,5-dimetilpiridina,
R14	H, metil, hidroxietil, dimetilaminoetil,
X3	CH ₂ , CHBr,

- La reivindicación la reivindicación 28 hace referencia a la descripción, según la expresión "seleccionado del grupo que consiste en los ejemplos 1 a 14", esta manera de reivindicar solo es aceptable cuando es estrictamente necesario, lo cual no es el caso, por lo tanto se sugiere al solicitante eliminar la alusión a los ejemplos.

7. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en la fecha de Presentación de la Solicitud de Prioridad: 07 de julio de 2006

Y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Item	Documento	Título	Fecha de Publicación*
D1	Vetrivel Nadaraj et al Arkivoc	Microwave-assisted synthesis of quinoline alkaloids: 4-Methoxy-1-methyl-2-quinolinone and its analogs Páginas 82-89	05 de marzo de 2006

D2	F. O'donnell et al <u>Analytica Chimica</u> Acta 572	A study of the analytical behaviour of selected synthetic and naturally occurring quinolines using electrospray ionisation ion trap mass spectrometry, liquid chromatography and gas chromatography and the construction of an appropriate database for quinoline characterization (page 63-76)	13 de mayo de 2006
D3	Platonov V V Et Al Russian Journal Of Applied Chemistry	Genetic relationship of organic bases of the quinoline and isoquinoline series from lignite semicoking tars with the initial biological material	2002
D4	Hisashi Ishii et al	Studien on the alkaloids of rutaceous plants. XIV syntheses of some Hydrogenated Derivates of Skimmianine	23 de junio de 1960
D5	WO9932449	Benzazine Derivatives Phosphodiesterase 4 Inhibitors	01 de julio de 1999

D1 Folios (358-365, pages 82-89)
Microwave-assisted synthesis of quinoline alkaloids:4-Methoxy-1-methyl-2-quinolinone and its analogs

Divulga procesos de síntesis de alcaloides de quinolina los cuales tienen actividad antiinflamatoria.

D2 Folios (366-379, pages 63-76)
A study of the analytical behaviour of selected synthetic and naturally occurring quinolines using electrospray ionisation ion trap mass spectrometry, liquid chromatography and gas chromatography and the construction of an appropriate database for quinoline characterization (page 63-76)

Divulga métodos analíticos para quinolonas alcaloides por cromatografía de gases, cromatografía de masas y espectrometría de masas.

D3 Folios (380-386, pages 649-655)
Genetic relationship of organic bases of the quinoline and isoquinoline series from lignite semicoking tars with the initial biological material

Divulga la relación genética de compuestos de quinolina e isoquinolina presentes en plantas nativas rusas. Se sugieren vías de transformación de los compuestos.

D4 Folios (387-392, pages 248-253)
Alkaloids of Rutaceous plants. XIV. Syntheses of some hydrogenated derivatives of skimmianine

Divulga rutas de síntesis de alcaloides derivados de la skimmianina.

D5
<http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=WO&NR=9932449A2&KC=A2&FT=D&ND=&date=19990701&DB=&locale=en> EP

Divulga compuestos de fórmula (I) en la que A es un heterociclo ortocondensado los cuales tienen actividad inhibitoria PDE 4 y alfa TNF.

8. Examen de Patentabilidad (Art 14 D486)

Novedad frente a D2

Las características **esenciales** de la composición de la reivindicación 7 habían sido divulgadas previamente en D2, el cual divulga compuestos de derivados de quinolina (folio 374, compuesto 34), donde R4 el equivalente de R3 en la actual solicitud es H, R3 el equivalente de R2 en la actual solicitud es OCH3, R2 el equivalente de R1 en la actual solicitud es, OCH3, R5 el equivalente de R4 es H, R6 equivalente de R5 y R6 es OH, OCH3, OCH2CH3, OCOCH3, y la posición X3 es CR7 donde R7 es H, CH3, halógeno, R1 el equivalente de R14 es H, CH3 (folio 374, compuesto 34).

En consecuencia, la reivindicación 7 no es nueva ó carece de novedad, según lo divulgado en el documento D2.

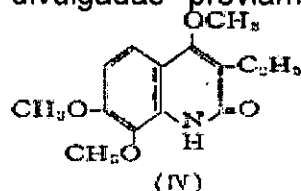
Novedad frente a D3

Las características **esenciales** de la composición de la reivindicación 7 habían sido divulgadas previamente en D3, el cual divulga compuestos de derivados de quinolina, donde El equivalente de R1 es OCH3, l equivalente de R2 es OCH3, R3 el equivalente de R1 es H, La posición de R4 es sustituida por un H, la posición de R5 es un -O-, la posición de R6 es un metilo y la posición de X3 es -CH- (folio 384, página 653)

En consecuencia, la reivindicación 7 no es nueva o carece de novedad, según lo divulgado en el documento D3.

Novedad frente a D4

Las características **esenciales** de la composición de la reivindicación 7 habían sido divulgadas previamente en D4, el cual divulga compuestos de formula IV



de derivados de quinolina (folio 388, donde El equivalente de R1 es OCH3, acetil, l equivalente de R2 es OCH3, acetil, R3 el equivalente de R1 es H, a posición de R4 es sustituida por un H, la posición de R5 es un -O-, la posición de R6 es un metilo, acetil y la posición de X3 es =CC2H5- y la posición de R14 es H, CH3.

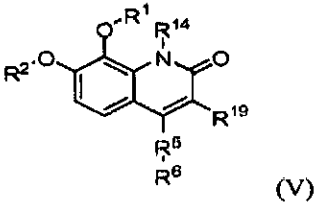
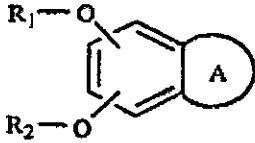
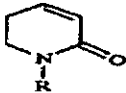
En consecuencia, la reivindicación 7 no es nueva o carece de novedad, según lo divulgado en el documento D4.

La reivindicación dependiente 8 no incluye características que hagan nueva o inventiva la reivindicación 7 por lo tanto no se considera nueva.

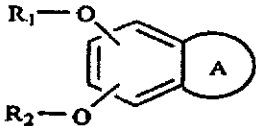
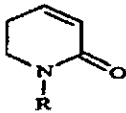
Nivel Inventivo (Art18 D486)

La reivindicación 7 consiste en un compuesto de fórmula I

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D5
Un compuesto de fórmula V	Un compuesto de fórmula I
 (V)	 donde A es entre  otros un grupo
R14 es H, halógeno, alquilo inferior, halo alquilo, alcoxi	Donde R14 es H, alquilo C1-4, alcoxi C1-4
R1 y R2 acilo, alquilacilo, carboxialquilo, alcoxi, alcoxi alquilo. R19 es H	R1 es un alquilo C1-4 o polifluoro alquilo C1-6 alquilo; R2 es arilo, aril-(C1-10)alquilo o (C1-7) cicloalquilo que opcionalmente puede tener un átomo de oxígeno.

El documento D5 se considera el estado de la técnica cercano a la invención definida en la reivindicación 7 porque divulga Un compuesto de fórmula I


donde A es entre otros un grupo . Donde R, el equivalente de R14 es H, alquilo C1-4, alcoxi C1-4, R1 es un alquilo C1-4 o polifluoro alquilo C1-6 alquilo; R2 es arilo, aril-(C1-10)alquilo o (C1-7) cicloalquilo que opcionalmente puede tener un átomo de oxígeno, para el tratamiento de patologías que implican la PDE4 tales como el enfisema enfermedades alérgicas e inflamatorias, EPOC, bronquitis crónica, asma y rinitis alérgica (página 5, líneas 1-31 ; página 8, líneas 20-27), un compuesto específico divulgado es 8-ciclopentiloxi-4-(3,5-dicloro-piridin-4-il-metil)-7-metoxi-2-oxo-1,2-dihidro-quinolina-ácido carboxílico metiléster (página 23, ejemplo 35 (compuesto 8)).

La diferencia entre el documento D5 y las presentes reivindicaciones es que el compuesto utilizado para el tratamiento de una patología que implica PDE4 tiene un sustituyente alternativo a R14 (un grupo carboxilo en lugar de un sustituyente seleccionado entre hidrógeno, halógeno, hidroxilo, alquilo inferior, hidroxialquilo, aminoalquilo y haloalquilo).

El efecto que logra el incluir no es adecuadamente demostrado puesto que en el ensayo in vivo (inflamación de la glándula lagrimal - ojo seco inducido en conejos) en los folios 244-246 de la descripción del expediente administrativo, se muestra que todos los compuestos ensayados son inactivos. A pesar de que se muestra en el ensayo de inhibición de PDE4 in vitro en folios 240 a 244 muestra que todos los compuestos ensayados inhiben la PDE4, estos datos sólo pueden ser considerado como un elemento adicional de conocimiento a menos que se compruebe que los compuestos reivindicados puedan tener un efecto técnico inesperado. Puesto que los únicos ejemplos en la aplicación que trataron una condición patológica en la presente solicitud no son activos, no es creíble que los compuestos reivindicados en la presente puedan encontrar dicho efecto.

Por lo tanto, el Problema Técnico Objetivo que pretende resolver esta invención se puede formular así: "cómo proporcionar compuestos alternativos a los conocidos en D5 y que sean útiles para el tratamiento de patologías que implican PDE4".

De lo que se concluye que, en ausencia de pruebas de que los compuestos reivindicados en la presente solicitud pueden encontrar un efecto técnico inesperado, el problema a ser resuelto por la presente solicitud sólo puede ser considerado como la provisión de compuestos químicos alternativos.

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría cambios menores de entre las posibilidades que se establecen en D5 en los compuestos de D5 para llegar así al objeto de la reivindicación 7 de la solicitud. Por lo cual se considera obvia.

Conclusión:
La reivindicación 7 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

Las reivindicaciones 8-32 no incluyen características que hagan nueva o inventiva la reivindicación 7, por lo que también carecen de nivel inventivo.

9. Conclusión

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

Item	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	Microwave-assisted synthesis of quinoline alkaloids: 4-Methoxy-1-methyl-2-quinolinone and its analogs Páginas 82-89	7 – 8	Novedad
D2	A study of the analytical behaviour of selected synthetic and naturally occurring quinolines using electrospray ionisation ion trap mass spectrometry, liquid chromatography and gas chromatography and the construction of an appropriate database for quinoline characterization (page 63-76)	7 – 8	Novedad
D3	Genetic relationship of organic bases of the quinoline and isoquinoline series from lignite semicoking tars with the initial biological material	7 – 8	Novedad
D4	Studies on the alkaloids of rutaceous plants. XIV syntheses of some Hydrogenated Derivates of Skimmianine	7 – 8	Novedad
D5	WO9932449	8-32	Nivel inventivo

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

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


JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha 12.5 SET. 2012

Elaborado por: Harvey Jaramillo *orlt*
Revisado Por: Jacqueline Alfonso

Microwave-assisted synthesis of quinoline alkaloids: 4-Methoxy-1-methyl-2-quinolinone and its analogs

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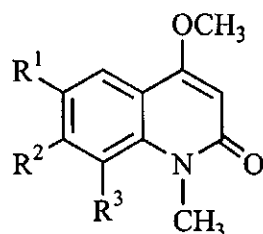
Abstract

The microwave-induced synthesis of some quinoline alkaloids of the type 4-methoxy-1-methyl-2-quinolinone including folimine is reported. The precursors 4-hydroxy-2-quinolinone derivatives were effectively prepared in a single step from aniline and diethylmalonate using *p*-toluenesulfonic acid as a catalyst and these intermediates were converted to the titled quinoline alkaloids by treatment with dimethyl sulfate, N,N-dimethylformamide and potassium carbonate. The presented synthetic procedure is a convenient, simple and fast alternative for synthesizing 4-methoxy-1-methyl-2-quinolinone and its derivatives.

Keywords: Quinolines, microwave irradiation, 4-hydroxy-2-quinolinones, 4-methoxy-1-methyl-2-quinolinones

Introduction

The root stock of quinoline alkaloids is specific to the plant family *Rutaceae* embodying about hundred and fifty genera with sixteen hundred species. The presence of quinoline nucleus in the framework of various pharmacologically active compounds with antiasthmatic,¹ antibacterial,² antifungal,³ antimalarial,⁴ anti-viral,⁵ anti-inflammatory⁶ activities continue to promote their synthetic efforts. Among the quinolines, some facile precursors are available which could be transformed into desired quinoline alkaloids using various reactions.⁷⁻⁹ For instance, 4-hydroxy-2-quinolinone is an important biosynthetic⁷ and synthetic^{8,9} precursor of quinoline alkaloids. Methylated compounds of this type like 4-methoxy-1-methyl-2-quinolinone (1), folimine (2), 4,6-dimethoxy-1-methyl-2-quinolinone and 4,7,8-trimethoxy-1-methyl-2-quinolinone (Scheme 1) are widely distributed in nature.¹⁰⁻¹³ Though these alkaloids are natural products, they are also used as synthetic intermediates for the preparation of other quinoline alkaloids¹⁴ and polyheterocycles.



- 1 $R^1=R^2=R^3=H$; 4-Methoxy-1-methyl-2-quinolinone
- 2 $R^1=R^2=H$, $R^3=OCH_3$; Folimine
- 3 $R^2=R^3=H$, $R^1=OCH_3$; 4,6-Dimethoxy-1-methyl-2-quinolinone
- 4 $R^1=H$, $R^2=R^3=OCH_3$; 4,7,8-Trimethoxy-1-methyl-2-quinolinone

Scheme 1

The classical synthetic protocols for the above quinoline intermediates and natural products suffer from some disadvantages such as low yield,¹⁵ lack of easy availability/preparation of the reagent,¹⁶⁻¹⁸ prolonged reaction time, multiple steps, requirement of excess of reagents/catalysts, need for special apparatus and harsh reaction conditions.^{16,17} In this connection and by knowing the advantages of microwave reactions,¹⁹⁻²¹ we felt that the synthesis of the above intermediates and quinoline alkaloids using microwave irradiation in an unaltered domestic microwave oven would make the task much easier than the thermal synthesis. Hence we wish to report a convenient and efficient synthesis of 4-hydroxy-2-quinolinone and 4-methoxy-1-methyl-2-quinolinone derivatives under microwave irradiation.

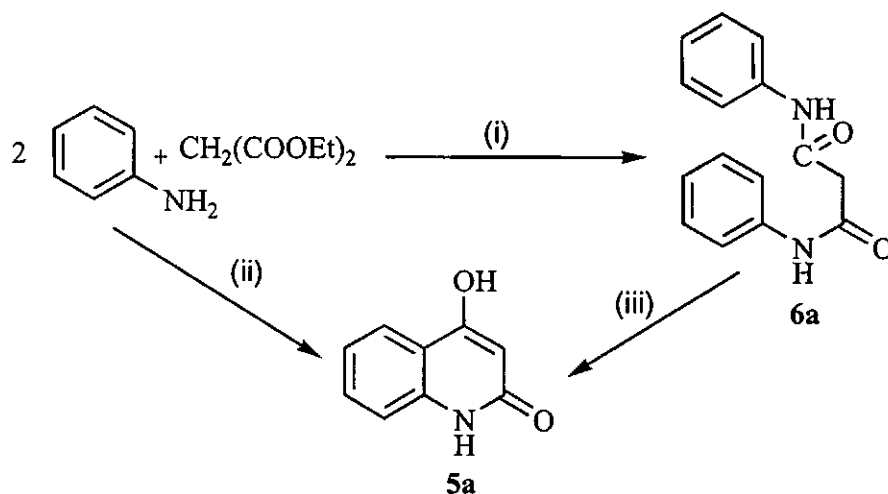
Results and Discussion

In an initial study, a mixture of aniline and diethylmalonate (2:1 molar ratio) was taken in a beaker (without adding any support, i.e., neat conditions), and irradiated under microwaves. This type of reaction is expected to be the most economical method since neither catalyst nor solvent is used. We failed to obtain 4-hydroxy-2-quinolinone (**5a**); but the *N,N'*-diphenyl malonamide (**6a**) was formed in 20 min (90% yield) (Scheme 2). This result agreed with the report given by Lange *et al*²² in their synthesis of 3-phenyl substituted 4-hydroxy-2-quinolinones. By contrast, in the presence of a catalytic amount of *p*-toluenesulfonic acid, the quinolinone **5a** was isolated in 89% yield in 6 min under the same reaction conditions (Scheme 2). IR spectrum of **5a** exhibited the absorption bands at 1660, 1606 cm^{-1} ascribable to carbonyl groups and a broad NH absorption in the region 3400-2900 cm^{-1} . The $^1\text{H-NMR}$ spectrum represented a singlet at δ 5.80 for the C_3 -proton, a multiplet in the region δ 7.05-7.65 for aromatic protons and a doublet at δ 7.70 for C_5 -proton. The singlet at δ 10.35 is accounted for NH proton and a broad singlet at δ 10.55 is due to the -OH group.

Table 1. Effect of solid support for the synthesis of **5a** under microwave irradiation (Power = 320W)

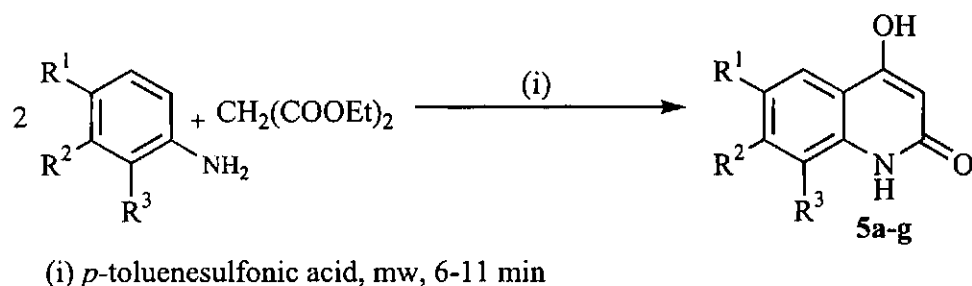
Support	Time (min)	Yield (%)
No	20	-
Silica gel	12	55
Acidic alumina	12	46
K ₁₀ clay	12	41
<i>p</i> -Toluenesulfonic acid	6	89

Different solid supports, including silica gel, acidic alumina and montmorillonite (K₁₀ clay) were also checked to define the most effective catalyst (Table 1). From these results, it is obvious that *p*-toluenesulfonic acid is the most adaptable and simplest catalyst for the synthesis of **5a**, as work-up is simply reduced to treatment with ice-cold water. Thus, we have extended these conditions to the synthesis of various 4-hydroxy-2-quinolinones (**5a-g**) with high yields (Scheme 3; Table 2). It was also attempted to convert *N,N'*-diphenyl malonamide (**6a**) to 4-hydroxy-2-quinolinone (**5a**) by microwave irradiation, in which the reaction was (Scheme 2) completed with in 3 min, in presence of the catalyst *p*-toluenesulfonic acid.



(i) neat condition, mw, 20 min (ii) *p*-toluenesulfonic acid, mw, 6 min
(iii) *p*-toluenesulfonic acid, mw, 3 min

Scheme 2



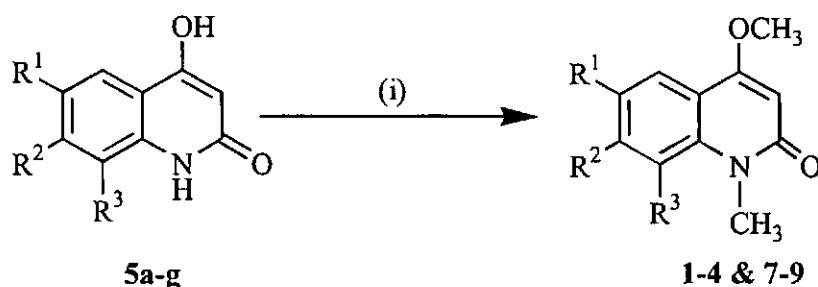
Scheme 3

Table 2. Microwave Synthesis of 4-Hydroxy-2-quinolinones under the catalyst *p*-toluenesulfonic acid

Compound	R ¹	R ²	R ³	Reaction Time (min)	Yield (%)	mp (°C)	Lit. mp (°C)
5a	H	H	H	6	89	>300	>300 ²³
5b	H	H	OCH ₃	6	90	248	245-248 ²³
5c	OCH ₃	H	H	8	89	300	298-300 ²³
5d	H	OCH ₃	OCH ₃	10	85	246	244-246 ¹³
5e	OCH ₃	H	OCH ₃	11	84	290	288-291 ¹⁶
5f	H	H	CH ₃	7	92	>300	360 ¹⁸
5g	CH ₃	H	H	8	96	>300	>300 ²⁴

Reaction of 4-hydroxy-8-methyl-2-quinolinone (**5f**) with dimethyl sulfate, *N,N*-dimethylformamide and potassium carbonate under microwave irradiation afforded methylated quinolinone **8** in 70% yield in 3 min (Scheme 4). The formation of 1,8-dimethyl-4-methoxy-2-quinolinone (**8**) is supported by the observation of peak in the expected chemical shifts of –OCH₃ and N-CH₃ protons as singlets at δ 3.99 and 3.81 respectively in the ¹H-NMR spectrum. Similar reaction of **5e** and **5g** with the same reagents yielded 4,6,8-trimethoxy-1-methyl-2-quinolinone (**7**) and 1,6-dimethyl-4-methoxy-2-quinolinone (**9**) in good yield.

The synthesis of natural products demonstrates an interesting application of this synthetic methodology. Treatment of 4-hydroxy-2-quinolinone (**5a**) with *N,N*-dimethylformamide, dimethyl sulfate and potassium carbonate gave 4-methoxy-1-methyl-2-quinolinone (**1**) in 66% yield in 3 min. Similarly, treatment of **5b-d** with the above reagents afforded the natural products **2**, **3**, and **4** in good yield. The spectroscopic properties of our synthetic materials **1** to **4** agreed well with those reported in the literature.¹⁰⁻¹³ In addition to its simple reaction conditions, this procedure has the advantage of very short reaction times, easy experimental and work-up procedures.



(i) DMF, dimethyl sulfate, K_2CO_3 , mw, 3-6 min

Scheme 4

Conclusions

In conclusion, we have demonstrated an efficient, mild method for the synthesis of 4-hydroxy-2-quinolinones and in parallel, we have also developed an easy and fast synthetic methodology for naturally occurring 4-methoxy-1-methyl-2-quinolinone derivatives.

Table 3. Microwave Synthesis of 4-Methoxy-1-methyl-2-quinolinones

Compound	R ¹	R ²	R ³	Reaction Time (min)	Yield (%)	mp (°C)	Lit. mp (°C)
1	H	H	H	3	66	100	100-101 ¹⁰
2	H	H	OCH ₃	3	69	140	139-140 ¹¹
3	OCH ₃	H	H	4	70	148	145-146 ¹²
4	H	OCH ₃	OCH ₃	5	65	146	143-148 ¹³
7	OCH ₃	H	OCH ₃	6	67	163	161-162 ¹⁶
8	H	H	CH ₃	3	70	236	-
9	CH ₃	H	H	4	69	241	-

Experimental Section

General Procedures. Melting points were recorded on Boetieus microheating table and are uncorrected. IR (KBr) spectra were recorded on a Shimadzu-8201 FT spectrophotometer. ¹H-NMR spectra were recorded on AMX-400 (400MHz) spectrophotometer, using TMS as an internal reference, and Mass spectra were recorded at 70eV on a Joel JMS-D-300 instrument. The reactions were carried out in a domestic microwave oven (KENSTAR –OM-20ESP, 2450 MHz).

General procedure for the synthesis of 4-hydroxy-2-quinolinones (5a-g). Respective aniline (10 mmol), diethyl malonate (5 mmol) and *p*-toluenesulfonic acid (120mg) were taken in a 50 ml beaker and properly mixed with the help of a glass rod. The so-obtained paste was irradiated in a microwave oven at the power of 320 W for the specified time (Table 2). After irradiation, cold water was added to the reaction mixture. The solid obtained was filtered, washed with water, dried and recrystallised (Ethanol-Acetic acid) to afford the desired product in good yield.

4-Hydroxy-2-quinolinone (5a). $\nu_{\max}$ (KBr)/ cm^{-1} : 3400-2900, 1660, 1606, 1514; $^1\text{H-NMR}$ (DMSO- d_6) δ (ppm): 5.80 (s, 1H, C₃-H), 7.05-7.65 (m, 3H, Ar-H), 7.70 (d, 1H, C₅-H), 10.35 (s, 1H, NH), 10.55 (brs, 1H, OH); MS (m/z): 161 (M^+). Anal. Found. C 67.05; H 4.40; N 8.68. Calcd. For C₉H₇NO₂; C 67.08; H 4.38; N 8.69.

4-Hydroxy-8-methoxy-2-quinolinone (5b). $\nu_{\max}$ (KBr)/ cm^{-1} : 3400-2900, 1661, 1605, 1514; $^1\text{H-NMR}$ (DMSO- d_6) δ (ppm): 3.90 (s, 3H, C₈-OCH₃), 5.86 (s, 1H, C₃-H), 7.14-7.40, (m, 2H, C₆-H & C₇-H), 7.78 (d, 1H, C₅-H), 10.32 (s, 1H, NH), 10.54 (brs, 1H, OH); MS (m/z): 191 (M^+). Anal. Found. C, 62.79; H, 4.76; N, 7.30. Calcd. For C₁₀H₉NO₃; C 62.82; H 4.75; N 7.33.

4-Hydroxy-6-methoxy-2-quinolinone (5c). $\nu_{\max}$ (KBr)/ cm^{-1} : 3450-2950, 1660, 1606, 1515; $^1\text{H-NMR}$ (DMSO- d_6) δ (ppm): 3.91 (s, 3H, C₆-OCH₃), 5.90 (s, 1H, C₃-H), 7.21-7.54, (2d, 2H, C₇-H & C₈-H), 7.81 (s, 1H, C₅-H), 10.34 (s, 1H, NH), 10.53 (brs, 1H, OH); MS (m/z): 191 (M^+). Anal. Found. C 62.83; H 4.78; N 7.35. Calcd. For C₁₀H₉NO₃; C 62.82; H 4.75; N 7.33.

7,8-Dimethoxy-4-hydroxy-2-quinolinone (5d). $\nu_{\max}$ (KBr)/ cm^{-1} : 3390-2900, 1660, 1606, 1515; $^1\text{H-NMR}$ (DMSO- d_6) δ (ppm): 3.80 (s, 3H, OCH₃), 3.92 (s, 3H, OCH₃), 6.03 (s, 1H, C₃-H), 7.08-7.62 (2d, 2H, C₅-H & C₆-H), 10.35 (s, 1H, NH), 10.55 (brs, 1H, OH); MS (m/z): 221 (M^+). Anal. Found. C 59.75; H 5.05; N 6.30. Calcd. For C₁₁H₁₁NO₄; C 59.72; H 5.02; N 6.33.

6,8-Dimethoxy-4-hydroxy-2-quinolinone (5e). $\nu_{\max}$ (KBr)/ cm^{-1} : 3400-2900, 1661, 1606, 1513; $^1\text{H-NMR}$ (DMSO- d_6) δ (ppm): 3.52 (s, 3H, OCH₃), 3.62 (s, 3H, OCH₃), 6.27 (s, 1H, C₃-H), 7.58 (s, 1H, C₇-H), 7.62 (s, 1H, C₅-H), 10.33 (s, 1H, NH), 10.54 (brs, 1H, OH); MS (m/z): 221 (M^+). Anal. Found. C 59.71; H 5.00; N 6.31. Calcd. For C₁₁H₁₁NO₄; C 59.72; H 5.02; N 6.33.

4-Hydroxy-8-methyl-2-quinolinone (5f). $\nu_{\max}$ (KBr)/ cm^{-1} : 3390-2900, 1661, 1605, 1515; $^1\text{H-NMR}$ (DMSO- d_6) δ (ppm): 2.40 (s, 3H, C₈-CH₃), 5.78 (s, 1H, C₃-H), 7.06-7.37 (m, 2H, C₆-H & C₇-H), 7.67 (d, 1H, C₅-H), 10.31 (s, 1H, NH), 10.53 (brs, 1H, OH); MS (m/z): 175 (M^+). Anal. Found. C 68.58; H 5.15; N 8.09. Calcd. For C₁₀H₉NO₂; C 68.57; H 5.18; N 8.00.

4-Hydroxy-6-methyl-2-quinolinone (5g). $\nu_{\max}$ (KBr)/ cm^{-1} : 3400-2900, 1662, 1605, 1515; $^1\text{H-NMR}$ (DMSO- d_6) δ (ppm): 2.43 (s, 3H, C₆-CH₃), 5.81 (s, 1H, C₃-H), 7.10-7.40, (2d, 2H, C₇-H & C₈-H), 7.68 (s, 1H, C₅-H), 10.33 (s, 1H, NH), 10.54 (brs, 1H, OH); MS (m/z): 175 (M^+). Anal. Found. C 68.52; H 5.17; N 8.00. Calcd. For C₁₀H₉NO₂; C 68.57; H 5.18; N 8.00.

Synthesis of 4-methoxy-1-methyl-2-quinolinones (3a-g). Respective 4-hydroxy-2-quinolinone (1 mmol) was taken in 100 ml conical flask and to this, dimethyl sulfate (2 mmol), potassium carbonate (0.8 g) and N, N-dimethyl formamide (5 ml) were added, mixed well and irradiated for the specified time (Table 3). The reaction was monitored for every 30 seconds by the tlc. After irradiation, the reaction mixture was poured into ice water and extracted well with chloroform. The extract was dried (Na₂SO₄) and column chromatographed to yield the pure compound. The

spectroscopic properties of our synthetic materials **1** to **4** and **7** agreed well with those reported in the literature¹⁰⁻¹³.

1,8-Dimethyl-4-methoxy-2-quinolinone (8). $\nu_{\max}$ (KBr)/cm⁻¹: 1642, 1580, 1459; ¹H-NMR (DMSO-d₆) δ (ppm): 2.40 (s, 3H, C₈-CH₃), 3.75 (s, 3H, -NCH₃), 3.95 (s, 3H, -OCH₃), 6.01 (s, 1H, C₃-H), 7.06-7.68 (m, 2H, C₆-H & C₇-H), 8.01 (d, 1H, C₅-H); MS (m/z): 203 (M⁺). Anal. Found. C 70.85; H 6.46; N 6.86. Calcd. For C₁₂H₁₃NO₂; C 70.93; H 6.45; N 6.89.

1,6-Dimethyl-4-methoxy-2-quinolinone (9). $\nu_{\max}$ (KBr)/cm⁻¹: 1643, 1580, 1460; ¹H-NMR (DMSO-d₆) δ (ppm): 2.42 (s, 3H, C₆-CH₃), 3.81 (s, 3H, -NCH₃), 3.99 (s, 3H, -OCH₃), 6.02 (s, 1H, C₃-H), 7.20 – 7.62 (2d, 2H, C₇-H & C₈-H), 7.99 (s, 1H, C₅-H); MS (m/z): 203 (M⁺). Anal. Found. C 70.91; H 6.49; N 6.89. Calcd. For C₁₂H₁₃NO₂; C 70.93; H 6.45; N 6.89.

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References

1. Doube, D.; Blouin, M.; Brideau, C.; Chan, C.; Desmarais, C.; Ethier, D.; Falgoutret, J. P.; Friesen, R. W.; Girard, M.; Girard, Y.; Guay, J.; Tagari, P.; Young, R.N. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1255.
2. Kidwai, M.; Bhushan, K. R.; Sapra, P.; Saxena, R. K.; Gupta, R. *Bioorg. Med. Chem.* **2000**, *8*, 69.
3. Moissev, I. K.; Zemtsova, M. N.; Trakhtenberg, P. L.; Kulikova, D. A.; Pskobkina, I.; Neshchadim, G. N.; Ostapchuk, N. V. *Khim. Farm. Zh.* **1998**, *22*, 1448.
4. Craig, J. C.; Person, P. E. *J. Med. Chem.* **1971**, *14*, 1221.
5. Narsinh, D.; Anamik, S. *Ind. J. Pharm. Sci.* **2001**, *63*, 211.
6. Dillard, R. D.; Pavey, D. E.; Benslay, D. N. *J. Med. Chem.* **1973**, *16*, 251.
7. Cobet, M.; Luckner, M. *Phytochemistry* **1971**, *10*, 1031.
8. Majumdar, K. C.; Mukhopadhyay, P. P. *Synthesis* **2003**, 97.
9. Yong, R. L.; Hyuk, K.; Wha, S. K.; Kyung, R. M.; Youngsoo, K.; Seung, H. L. *Synthesis* **2001**, 1851.
10. Nayar, M. N. S.; Sutar, C. V.; Bhan, M. K. *Phytochemistry* **1971**, *10*, 2843.
11. Razzakova, D. M.; Bessonova, I. A.; Yunusov, S. Yu. *Khim. Prir. Soedin.* **1972**, *8*, 133; *CA* **77**, 72563.
12. Lamberton, J. A.; Price, J. R. *Aus. J. Chem.* **1953**, *6*, 173.
13. Storer, R.; Young, D. W.; Taylor, D. R.; Warner, J. M. *Tetrahedron* **1973**, *29*, 1721.
14. Naito, T.; Kaneko, C. *Chem. Pharm. Bull.* **1978**, *31*, 366.

15. Coppola, G. M. *J. Heterocycl. Chem.* **1983**, *20*, 1217.
16. Storer, R.; Young, D. W. *Tetrahedron* **1973**, *29*, 1215.
17. Manandhar, M. D.; Hussaini, F. A.; Dapil, R.S.; Shob, A. *Phytochemistry* **1985**, *24*, 199.
18. Ziegler, E.; Wolf, R.; Kappe, J. *Monatsche* **1965**, *96*, 418.
19. Kabalka, G. W.; Pagni, R. M. *Tetrahedron* **1997**, *53*, 7999.
20. Roberts, B. A.; Strauss, C. R. *Acc. Chem. Res.* **2005**, *38*, 653.
21. Thamarai Selvi, S.; Nadaraj, V.; Mohan, S.; Sasi, R.; Hema, M. *Bioorg. Med. Chem.* In Press.
22. Lange, J. H. M.; Verveer, P. C.; Osnaburg, S. J. M.; Visser, G. M. *Tetrahedron Lett.* **2001**, *42*, 1367.
23. Shobana, N.; Yeshoda, P.; Shanmugam, P. *Tetrahedron* **1989**, *45*, 757.
24. Subramanian, M.; Mohan, P. S.; Shanmugam, P.; Rajendra Prasad, K. J. *Z. Naturforsch* **1992**, *47b*, 1016.



A study of the analytical behaviour of selected synthetic and naturally occurring quinolines using electrospray ionisation ion trap mass spectrometry, liquid chromatography and gas chromatography and the construction of an appropriate database for quinoline characterisation

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Abstract

Mass spectral fragmentation of quinoline alkaloids of significance in plants has been investigated using electrospray ionisation ion trap mass spectrometry (ESI-MSⁿ) with a view to characterisation of molecules of unknown structure isolated from these natural sources. This investigation has led to the generation of an appropriate database incorporating data from ESI-MSⁿ and also from gas liquid chromatography (GLC) and liquid chromatography (HPLC) for these low molecular mass quinolines. This has been put to practical application in the identification of quinoline alkaloids in a plant extract. Thus, an acid extraction of the leaves of *Choisya ternata* containing such tertiary alkaloids was analysed by liquid chromatography–electrospray ionisation mass spectrometry (HPLC–ESI-MS) and the resulting behaviour of the quinolines was compared with that of the quinoline alkaloids in the database.

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Keywords: Quinolines; Electrospray ionisation ion trap mass spectrometry; Fragmentation patterns; High performance liquid chromatography; Gas liquid chromatography; Quinoline database; *Choisya ternata*

1. Introduction

The Rutales represents an order of flowering plants containing more than 3000–3500 species. Rutaceae is one of the six families belonging to Rutales. Several plant extracts of rutaceous species have been reported to possess pharmacological activity and hence there is an interest in the isolation and characterisation of the active principles present in rutaceous plants. Compounds with a variety of chemical structures have been isolated from these plants. Of the alkaloids present in these plants, those possessing the basic quinoline skeleton are the largest in number [1]. Electron impact mass spectrometry (EI-MS) [2] has to date played an important part in the structural elucidation of quinoline alkaloids but electrospray ionisation ion trap mass spectrometry (ESI-MSⁿ) fragmentation studies have not as yet

been reported. Only limited studies have been carried out on the liquid chromatography (HPLC) and gas liquid chromatography (GLC) of quinoline derivatives [3,4].

Natural products research at The University of Ulster for the last 35 years has been particularly concerned with quinoline alkaloids, their isolation, structural elucidation, synthesis and biosynthesis. Thus, a variety of natural and synthetic quinolines were available for the purposes of this paper, i.e. a study of certain analytical chemical properties of such quinolines and the construction of an appropriate database.

This paper therefore deals with the ESI-MSⁿ, HPLC and GLC behaviour of naturally occurring quinoline alkaloids and synthetic derivatives that were available. Their analytical characterisation in terms of their ESI-MSⁿ behaviour, their GLC and HPLC retention times has been documented and used to create a database. This database has been put to practical application for the identification of quinoline alkaloids in a plant extract. The acid soluble extract of *Choisya ternata* was investigated in this study and comparisons were made between the analytical results

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of the plant extract examination and the analytical information on the database in order to identify different quinoline alkaloids present in the plant extract.

2. Experimental

2.1. Chemicals

HPLC grade acetonitrile, water, methanol and ethyl acetate were the solvents used. They were purchased from Fischer Chemicals (Loughborough, Leicester, UK). The AnalaR formic acid was 98–100% pure and was from BDH Laboratory Supplies (Poole, Dorset, UK).

2.2. Quinolines

The quinoline substances investigated were either isolated from natural sources or synthesised in the laboratory according to methods previously reported in the literature [5–7]. They had a purity of at least 95%.

$1.0 \times 10^{-3} \text{ mol L}^{-1}$ stock solutions for HPLC and GLC analysis were prepared in methanol. Similar stock solutions for ESI-MSⁿ analysis were prepared in 0.05% formic acid/methanol.

2.3. Instrumentation

The ESI-MSⁿ behaviour of the quinolines was investigated using the Finnigan MAT LCQTM quadrupole ion trap mass spectrometer. The samples were introduced by direct infusion in a 0.05% formic acid/methanol solution via a syringe pump at a flow rate of $5 \mu\text{L min}^{-1}$. In the ESI source nitrogen sheath gas flow was maintained at 50 and refers to an arbitrary value set by the software. The heated capillary temperature was at 200 °C and the spray voltage was set to 4.25 kV.

HPLC analysis was carried out using a Thermo Products HPLC system consisting of a Spectra System UV2000 detector, a Spectra System AS3000 autosampler, a Spectra System P4000 pump and Spectra System SN4000 data relay system. The system was controlled from a computer with a Spectra System PC1000 computer program that was also used for evaluating chromatograms. The column used was a Luna $5 \mu\text{C}_{18}$ (250 mm, 4.6 mm). The mobile phase was 60% acetonitrile + 40% water using isocratic elution and detection was performed at 214 nm. The flow rate was set at 0.5 mL min^{-1} .

The GLC instrument was a Perkin-Elmer 8600 series Gas Chromatograph. The column used was the low polarity Zebron ZB-5 (30 m, 0.25 mm). A gradient temperature program was employed. The starting temperature was 60 °C with a ramp rate of $10^\circ\text{C min}^{-1}$ with a final temperature of 280 °C for 5 min. The carrier gas pressure was 20 psi. The injector and detector temperatures were set at 250 and 300 °C, respectively.

2.4. Extraction of plant alkaloids

Leaves and stems of *C. ternata* were dried in an oven at 40 °C for 5 days. The plant material was then ground in a

blender and left to stand at room temperature for 2 weeks in MeOH. The MeOH solution was filtered from the plant residue and the solvent removed from the filtrate using rotary evaporation. The residue was diluted with H₂O (200 mL) and extracted with EtOAc (200 mL). The EtOAc solution was washed through with H₂O ($3 \times 200 \text{ mL}$) using a separating funnel. The EtOAc layer was further extracted with aqueous HCl (1 M, $4 \times 150 \text{ mL}$). The acid soluble fractions were combined and basified with dilute NaOH. The resultant basic solution was extracted with EtOAc ($4 \times 250 \text{ mL}$), dried over anhydrous MgSO₄ and the solvent removed to yield acid soluble components as a gum.

3. Results and discussion

This paper deals with the ESI-MSⁿ, HPLC and GLC behaviour of 67 quinoline compounds (both naturally occurring alkaloids and synthetic compounds). The analytical data gathered are presented in Tables 1 and 2 and have been used to generate a database on quinoline derivatives. The chemical structures of these quinolines are given in Fig. 1 and for the purpose of this study they have been placed in sub-groups according to their characteristic chemical structures. The molecular masses, molecular ions and fragment ions from the ESI-MSⁿ analysis of the quinolines are tabulated in Table 1. Table 2 lists the HPLC and GLC retention times of these molecules. Figs. 2–4 show some of the ESI-MSⁿ signals of selected quinolines and some proposed fragmentation processes.

3.1. Flindersine compounds

3.1.1. ESI-MSⁿ behaviour

Quinolines in this group have the pyrano-(2,3-*c*)-quinol-5-one [8] structure in common. These molecules generally show a loss of 42 u, corresponding to $\text{CH}_2=\text{CH}-\text{CH}_3$ from the pyrano ring, mostly at the MS² stage but for some at MS³ and MS⁴ stages. *N*-Methylflindersine (1) [9], 7-hydroxy-*N*-methylflindersine (3) and 7-hydroxyflindersine (4) all lose 42 u at the MS² stage. A loss of 28 u at the MS³ stage is then seen, which is likely to be due to the loss of CO from the 5-C position. *N*-Methylflindersine and 7-hydroxyflindersine both lose a further 28 u at the MS⁴ stage, due to another CO molecule, this time due to the loss of oxygen from the pyrano ring or in the case of 7-hydroxyflindersine (4) it could be due to the 7-hydroxyl group oxygen being lost as CO followed by ring contraction. The proposed fragmentation pattern for (1) is given in Fig. 2.

7-Dimethylallyloxy-*N*-methylflindersine (7) initially loses 68 u from the dimethylallyloxy side-chain presumably due to the loss of pentadiene (C₅H₈). The next fragmentation losses are similar to the previously discussed molecules, with a loss of 42 u followed by 28 u. Veprisine (5) [10] loses two CH₃ groups before losing C₃H₆ from the dimethylpyrano ring. The behaviour of 7-tetrahydropyranyloxyflindersine (6) is anomalous and it is the only compound in this group not to lose 42 u. There is an initial loss of 84 u due to the fragmentation of the tetrahydropyran ring from the side-chain of flindersine (6). The loss of the tetrahydropyran ring would leave 7-hydroxyflindersine as

Table 1
ESI-MSⁿ behaviour of quinolines

Compound name	MM	[M + H] ⁺	[M + Na] ⁺	MS ²	MS ³	MS ⁴	MS ⁵	MS ⁶
Flindersine compounds								
<i>N</i> -Methylflindersine (1)	241	242		200	172	144		
7,8-Dimethoxyflindersine (2)	287	288						
7-Hydroxy- <i>N</i> -methylflindersine (3)	257	258		216	188	147		
7-Hydroxyflindersine (4)	243	244		202	174	146		
Veprisine (5)	301	302		287	272	230		
7-Tetrahydropyranyloxy flindersine (6)	327		350	266				
7-Dimethylallyloxy- <i>N</i> -methyl flindersine (7)	325	326		258	216	188		
3-C-Dimethylallylquinol-2-one								
4-Hydroxy-6-methoxy-3-(3'-methylbut-2-enyl)-quinol-2-one (8)	259	260		204	176			
4-Hydroxy-6,8-dimethoxy-3-(3'-methylbut-2-enyl)-quinol-2-one (9)	303	304		248	217	202	174	
4-Hydroxy-8-methoxy-3-(3'-methylbut-2-enyl)-1-methylquinol-2-one (10)	273	274		218	164	149	121	
4-Hydroxy-3-(3'-methylbut-2-enyl)-quinol-2-one (11)	229	230		174	146			
4-Hydroxy-8-methoxy-3-(3'-methyl-2'-hydroxybutyl)-1-methylquinol-2-one (12)	291	292		274	218	164	149	120
4-Hydroxy-7,8-methylenedioxy-3-(3'-methylbut-2-enyl)-quinol-2-one (13)	273	274		218	188	160	131	
4,6-Dimethoxy-3-(3'-methylbut-2-enyl)-quinol-2-one (63)	273	274		218	203	188		
Linear and angular furoquinolines								
5-Methylfuro-(3,2- <i>c</i>)quinol-4-one (15)	199	200		172	144			
4,6-Dimethoxyfuro(3,2- <i>c</i>)-quinoline (16)	229							
γ-Fagarine (17)	229	230		215	187			
Skimmianine (18)	259	260		245	227	199	184	156
Dictamnine (19)	199	200		185	129			
Evoxine (20)	347	348		330	246	231	216	188
Choisyine (21)	329	330		256	241	213	185	157
7- <i>O</i> -Dimethylallyl-γ-fagarine (22)	313	314		246	231	216	188	
4-Chloro-6,7-methylenedioxyfuroquinoline (23)	247	248		213				
	249	250						
6,7-Methylenedioxyfuro-[2,3- <i>b</i>]quinol-4-one (24)	229	230		188	160			
Dehydroplatydesmine (25)	257	258						
Dihydrofuroquinolines								
2-Hydroxyisopropyl-6-methoxy-5-methyldihydro-furo(3,2- <i>c</i>)quinol-4-one (14)	289	290		272	230	215		
Ifllaiamine (26)	243	244		188	160	132	106	
Balfourodine (27)						257		238
				272 ⁺		254 ⁺		224
	289	290		218	254	230	239	210
Lemobiline (28)	243	244		188	160	132	106	
Araliopsine (29)	259	260		242	200	172	144	
Araliopsine acetate (30)	301	302		242	200	172	144	
<i>O</i> -Methylbalfourodinium (31) cation				286 ⁺		271		224
		304		232	254	254 ⁺	239	210
4-Chloro-6-methoxydihydrofuro-(3,2- <i>c</i>)-quinoline (40)	235	236		221	193			
	237	238		223	195			
Dihydropyranoquinol-4-ones								
Ribalinine (32)	259	260		242	200	172	144	
Isobalfourodine (33)	289	290		272	240	225	197	169
Quinol-2-ones								
7,8-Dimethoxy-4-hydroxy-quinol-2-one (34)	221	222		207	189	179		
7,8-Dimethoxy-1,3-dimethyl-4-hydroxy-quinol-2-one (35)	249	250		235	220	192		
4,7,8-Trimethoxy-3-iodo-quinol-2-one (36)	361	362		334	306	278	250	222
8-Methoxy-4-hydroxy-quinol-2-one (37)	191	192		177	135			
4-Hydroxy-1-methylquinol-2-one (38)	175	176		134				
4-Epoxygeranyloxyquinol-2-one (39)	313		336	184				
4-Ethoxy-8-methoxyquinol-2-one (41)	219	220		192	177	135	107	
4-Acetyloxy-3-(1',1'-dimethylallyl)-1-methyl-quinol-2-one (42)	285	286		244	188	134	106	
3-(2'-Hydroxy-3'-methylbut-3'-enyl)-4-methoxy-1-methylquinol-2-one (43)	273		296	256	224	196	181	
4,8-Dimethoxy-3-(2',3'-dihydroxy-3'-methylbutyl)-1-methylquinol-2-one (44)	321		344	326	254	226		
4,6-Dimethoxy-3-(3'-methyl-2'-oxobutyl)-1-methyl-quinol-2-one (45)	303	304		232	204	188		
Dictamninc acid (52)	219	220						

Table 1 (Continued)

Compound name	MM	[M+H] ⁺	[M+Na] ⁺	MS ²	MS ³	MS ⁴	MS ⁵	MS ⁶
4-Methoxyquinol-2-one (53)	175	176						
4-Methoxy-1-methylquinol-2-one (54)	189	190		175	147			
3-Bromo-4-hydroxyquinol-2-one (55)	239	240		161				
4-Dimethylallyloxy-1-methylquinol-2-one (56)	243	244		176	134	106		
1,3-Dimethyl-4-hydroxyquinol-2-one (57)	189	190		134				
1-Ethyl-4-hydroxy-3-methylquinol-2-one (61)	203	204		176				
3-Geranyl-4-hydroxyquinol-2-one (62)	297	298		174	146			
Lunacridine (64)	305	306		288	232	217		
Edulitine (65)	205	206		191				
Other quinoline derivatives								
Orixine (46)	335	336		246	216	188	160	131
5-Methoxy-2,2-dimethyldihydropyrano-(2,3- <i>b</i>)-quinoline (47)	243	244		188	158	130	103	
3,5-Diacetyloxy-2,2-dimethyldihydropyrano(2,3- <i>b</i>)quinoline (48)	329		352	292	250			
2,4-Dichloroquinoline (49)	197	198/200/202						
2,4-Quinolinediol (50)	161	162						
2,4-Dimethoxyquinoline (51)	189	190		175	147			
2,4-Dimethoxyquinoline-3-carboxylic acid (58)	233	234		216	186	158		
2,4,8-Trimethoxyquinoline (59)	219	220		205	176	161		
2,4,7-Trimethoxyquinoline (60)	219	220		205	176			
Vepridimerine-A (66)	602	603		368	326	311	296	
Ketoquinolonedimer (67)	428	429		242	200	172		

* The fragment ion in bold is the one chosen for the next fragmentation step.

a fragment ion but no further losses were observed as seen for 7-hydroxyflindersine (4).

3.1.2. HPLC and GLC behaviour

The seven flindersines were analysed by HPLC and GLC. From the results in Table 2, the most polar compound of the group with both the OH and the NH groups acting as weak acids was seen to be 7-hydroxyflindersine (4) which had, as expected, the lowest reversed phase HPLC retention time of 8.17 min. 7-Hydroxyflindersine (4) is a relatively non-volatile and high melting point compound and is found to be retained on the GLC column. Tetrahydropyranyloxyflindersine (6) is an oil and it behaves similarly to (4) with no GLC signal in the time scale of the experiment. *N*-Methylation of 7-hydroxyflindersine effectively reduces its polarity by removing the weakly acidic NH group and hence 7-hydroxy-*N*-methylflindersine (3) has an HPLC R_t of 10.60 min. This substance, isolated as an oil, was also retained on the GLC column. *N*-Methylflindersine (1) lacks both weakly acidic OH and NH groups and is thus less polar than the above compounds with an R_t of 17.77 min using HPLC.

7,8-Dimethoxyflindersine (2) possesses the weakly acidic NH group but also two relatively hydrophobic methoxy groups. As a result it gives an HPLC R_t of 12.04 min and GLC elution at 22.57 min. Veprisine (5) has lost the weakly acidic NH group by methyl substitution but retains the two relatively hydrophobic methoxy groups. This results in an increased HPLC R_t of 18.06 min.

It was expected that 7-dimethylallyloxy-*N*-methylflindersine (7), with its hydrocarbon side-chain and an *N*-methyl group, would be least polar compound in this group of quinolines but it gave no signal using GLC. A possible reason for the compound not being eluted may be due to a Claisen rearrangement under the elevated temperature conditions of GLC. The ally-

loxybenzenes are known to undergo this rearrangement yielding 2-allylphenols. If compound (7) undergoes this rearrangement it will produce a 7-hydroxy derivative which may not be eluted from a GLC column as with 7-hydroxyflindersine (4).

3.2. 3-*C*-Dimethylallylquinol-2-ones

3.2.1. ESI-MSⁿ behaviour

These quinolones [11,12] are structurally similar except for the positions of the methyl and methoxyl groups. These molecules behave similarly at the initial MS² stage in that all the compounds except (12) give a loss of 56 u which is due to C₄H₈ (i.e. 2-methylprop-1-ene) breaking off from the dimethylallyl side-chain leaving a =CH₂ functionality at the 3-*C* position. 4-Hydroxy-3-(3'-methylbut-2'-enyl)-quinol-2-one (11) then loses 28 u at the MS³ stage due to CO loss from the C-4 position and ring contraction. 4-Hydroxy-7,8-methylenedioxy-3-(3'-methylbut-2'-enyl)-quinol-2-one (13) loses 30 u after the initial loss of 56 u. This could be due to the loss of CH₂=O from the 7,8-methylenedioxy group. A 28 u loss then occurs as with (11).

4-Hydroxy-8-methoxy-3-(3'-methyl-2'-hydroxybutyl)-1-methylquinol-2-one (12) is not a 3-*C*-dimethylallylquinol-2-one but behaves like one after an initial loss of H₂O. The loss of H₂O forms a double bond in the side-chain creating a structure like the molecule 4-hydroxy-8-methoxy-3-(3'-methylbut-2'-enyl)-1-methylquinol-2-one (10). Both molecules have the same fragmentation pattern after that stage in that they both lose the C₄H₈ group from the side-chain in the 3 position and then proceed to lose 54 u (CH₂=C=C=O) followed by loss of a methyl group (15 u). 4,6-Dimethoxy-3-(3'-methylbut-2-enyl)-quinol-2-one (63) showed an initial loss of 56 u, due to C₄H₈. Further losses of 15 and 15 u from the two methoxyl groups were observed.

Table 2
HPLC and GLC behaviour of quinolines

Compound name	<i>R_t</i> values (min)	
	HPLC	GLC
Flindersine compounds		
7-Hydroxyflindersine (4)	8.17	–
7-Tetrahydropyranyloxyflindersine (6)	8.20	–
7-Hydroxy- <i>N</i> -methylflindersine (3)	10.60	–
7,8-Dimethoxyflindersine (2)	12.04	22.57
<i>N</i> -Methylflindersine (1)	17.77	18.83
Veprisine (5)	18.06	–
7-Dimethylallyloxy- <i>N</i> -methylflindersine (7)	–	–
3-<i>C</i>-Dimethylallylquinol-2-ones		
4-Hydroxy-6,8-dimethoxy-3-(3'-methylbut-2'-enyl)-quinol-2-one (9)	7.36	22.42
4-Hydroxy-3-(3'-methylbut-2'-enyl)-quinol-2-one (11)	7.52	20.33
4,6-dimethoxy-3-(3'-methylbut-2'-enyl)-quinol-2-one (63)	9.81	21.34
4-Hydroxy-7,8-methylenedioxy-3-(3'-methylbut-2'-enyl)-quinol-2-one (13)	10.33	21.10
4-Hydroxy-6-methoxy-3-(3'-methylbut-2'-enyl)-quinol-2-one (8)	12.20	23.55
4-Hydroxy-8-methoxy-3-(3'-methylbut-2'-enyl)-1-methylquinol-2-one (10)	12.40	21.61
4-Hydroxy-8-methoxy-3-(3'-methyl-2'-hydroxybutyl)-1-methylquinol-2-one (12)	12.76	23.07
Linear and angular furoquinolines		
Evoxine (20)	5.70	23.42
Choisyine (21)	6.97	–
7- <i>O</i> -Dimethylallyl- γ -fagarine (22)	8.08	21.43
Skimmianine (18)	8.55	20.77
Dehydroplatydesmine (25)	9.49	–
γ -Fagarine (17)	9.79	18.99
5-Methylfuro-(3,2- <i>c</i>)quinol-4-one (15)	10.07	16.28
Dictamnine (19)	10.31	18.22
4,6-Dimethoxyfuro(3,2- <i>c</i>)-quinoline (16)	10.73	16.14
6,7-Methylenedioxyfuro-[2,3- <i>b</i>]quinol-4-one (24)	–	18.61
4-Chloro-6,7-methylenedioxy-furoquinoline (23)	–	–
Dihydrofuroquinolines		
Balfordine (27)	5.21	28.20
Araliopsine (29)	5.93	22.71
Lemobline (28)	6.89	–
Iffaamine (26)	7.18	21.62
Araliopsine acetate (30)	9.40	23.78
2-Hydroxyisopropyl-6-methoxy-5-methyldihydro-furo(3,2- <i>c</i>)quinol-4-one (14)	10.72	22.56
4-Chloro-6-methoxydihydrofuro-(3,2- <i>c</i>)-quinoline (40)	12.82	18.34
<i>O</i> -Methylbalfourodinium (31)	–	21.38
Dihydropyranoquinol-4-ones		
Ribalinine (32)	5.37	22.63
Isobalfourodine (33)	–	24.35
Quinol-2-ones		
Dictamninc acid (52)	4.21	21.33
3-Bromo-4-hydroxy-quinolin-2-one (55)	4.37	20.16
4-Methoxy-1-methyl-quinolin-2-one (54)	4.49	21.36
1,3-Dimethyl-4-hydroxy-quinolin-2-one (57)	5.61	18.20
4-Methoxy-quinolin-2-one (53)	5.67	18.61
4-Hydroxy-1-methylquinol-2-one (38)	5.98	16.63
1-Ethyl-4-hydroxy-3-methyl-quinolin-2-one (61)	6.00	18.84
Edulitine (65)	6.04	20.46
7,8-Dimethoxy-4-hydroxy-quinol-2-one (34)	6.50	19.55
7,8-Dimethoxy-1,3-dimethyl-4-hydroxy-quinol-2-one (35)	6.68	19.98
4,8-Dimethoxy-3-(2',3'-dihydroxy-3'-methylbutyl)-1-methylquinol-2-one (44)	7.37	–
4-Ethyl-8-methoxyquinol-2-one (41)	7.64	19.37
4,7,8-Trimethoxy-3-iodo-quinol-2-one (36)	8.32	21.41
8-Methoxy-4-hydroxy-quinol-2-one (37)	–	–
3-Geranyl-4-hydroxy-quinolin-2-one (62)	10.16	–
4-Epoxygeranyloxyquinol-2-one (39)	10.33	–
3-(2'-Hydroxy-3'-methylbut-3'-enyl)-4-methoxy-1-methylquinol-2-one (43)	10.33	21.10
4,6-Dimethoxy-3-(3'-methyl-2'-oxobutyl)-1-methyl-quinol-2-one (45)	10.71	22.21
4-Dimethyl-allyl-oxy-1-methyl-quinolin-2-one (56)	12.56	19.43

Table 2(Continued)

Compound name	<i>R_t</i> values (min)	
	HPLC	GLC
Lunacridine (64)	13.28	24.80
4-Acetyloxy-3-(1',1'-dimethylallyl)-1-methyl-quinol-2-one (42)	–	19.38
Other quinoline derivatives		
2,4-Dimethoxyquinoline-3-carboxylic acid (58)	4.24	17.47
2,4-Quinoline diol (50)	4.33	21.39
3,5-Diacetyloxy-2,2-dimethyldihydropyrano(2,3- <i>b</i>)quinoline (48)	6.47	–
Orixine (46)	10.74	23.13
2,4,8-Trimethoxy quinoline (59)	11.32	17.67
2,4-Dichloroquinoline (49)	15.17	13.65
2,4-Dimethoxyquinoline (51)	15.18	14.78
2,4,7-Trimethoxyquinoline (60)	15.34	17.91
5-Methoxy-2,2-dimethyldihydropyrano-(2,3- <i>b</i>)-quinoline (47)	–	18.40
Vepridimerinc-A (66)	–	–
Ketoquinolinedimer (67)	20.19	20.19

3.2.2. HPLC and GLC behaviour

Seven 3-*C*-dimethylallylquinol-2-ones were submitted to chromatographic analysis. All of the compounds eluted with GLC retention times in excess of 20 min (Table 2), illustrating that the effects of the weakly acidic OH and NH groups were counterbalanced by the effect of the hydrophobic side-chain. For example, 4-hydroxy-3-(3'-methyl-but-2'-enyl)-quinol-2-one (11) is unsubstituted in the benzene ring and had a GLC retention time of 20.33 min. Its relatively low HPLC retention time of 7.52 min shows the importance of the weakly acidic 4-OH and 1 N-H groups in its elution. If the hydrogens in these groups are substituted by methyl as in (63) and (10), respectively, then the HPLC retention times increase to 9.81 and 12.40 min, respectively. The hydroxyl group in the 3-*C* side-chain increases the overall polarity of the molecule and results in an increase in the GLC retention time when compounds (10) (21.61 min) and (12) (23.07 min) are compared.

Substitution of a methoxyl group in (11) in position 6 increases the *R_t* in HPLC from 7.52 to 12.20 min as observed for 4-hydroxy-6-methoxy-3-(3'-methylbut-2'-enyl)-quinol-2-one (8) suggesting that the +R mesomeric/resonance effect of OMe has reduced the acidity of the 1 N-H group. Compound (8), however, stays on the GLC column longer than compound (11).

3.3. Linear and angular furoquinolines

3.3.1. MS behaviour

This group possesses the basic quinoline structure with an attached furan ring as shown in Fig. 1. The molecules differ only in the positions of substituent groups such as methoxyl groups. 5-Methylfuro-(3,2-*c*)quinol-4-one (15) loses 28 u at both the MS² and MS³ stages. This would be due to a loss of CO from both position 4 and the furan ring. γ -Fagarine (17) [8] has a methoxyl group present on both the 4-*C* and 8-*C* atoms. The initial loss of 15 u by this molecule is due to the loss of a methyl group from one of these methoxyl groups leaving the oxygen behind to form a carbonyl group. A further loss

of 28 u is due to the loss of this oxygen atom with a carbon atom in the form of CO. The MS^{*n*} signals for (17) are given in Fig. 3.

Skimmianine (18) behaves similar to γ -fagarine (17) losing 15 u in MS² stage and a loss of 18 u in the MS³ stage. This loss is likely to be due to H₂O from an OH group which would have to be generated by furan ring opening under the MS conditions. A total of 28 u (CO) is lost at MS⁴ followed by a loss of 15 u (CH₃) and 28 u (CO) at MS⁵ and MS⁶ levels. Dictamnine (19), which has only one –OCH₃ group, loses 15 u due to the loss of CH₃ at the MS² stage. At the MS³ stage a loss of 56 u is observed and this may be due to the simultaneous loss of two CO molecules coming from the two oxygen atoms.

Evoxine (20), 7-*O*-dimethylallyl- γ -fagarine (22) and choisyine (21) all lose a large side-chain but at different MS stages. Evoxine initially loses H₂O after which a loss of 84 u is observed. This is due to a loss of the C₅H₈O side-chain leaving a carbonyl group at the 7-*C* position. This ion fragment further loses 15, 15 and 28 u successively. 7-*O*-Dimethylallyl- γ -fagarine (22) initially loses the dimethylallyl side-chain as C₅H₈ (68 u). Choisyine (21) loses 74 u at the MS² stage, presumably due to ring opening and loss of part of the dihydrofuran side-chain.

The presence of chlorine in 4-chloro-6,7-methylenedioxy-furoquinoline (23) is seen by the isotopic cluster at *m/z* 248 and 250 for the [M+H]⁺ ion. The loss of the chlorine radical occurs at the MS² stage and further fragmentation was not observed. 6,7-Methylenedioxyfuro-[2,3-*b*]quinol-4-one (24) loses 42 u initially, possibly due to the loss of C₂H₂O from the furan ring. Further loss of 28 u at MS³ stage may be due to the loss of CO from the 4-carbonyl group. For dehydroplatydesmine (25) only the [M+H]⁺ ion is observed at *m/z* 258.

3.3.2. HPLC and GLC behaviour

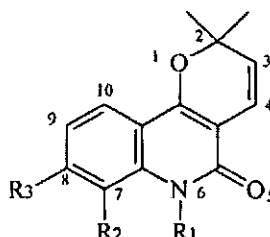
Dictamnine (19) has one methoxyl group in the 4 position, γ -fagarine (17) has methoxyl groups in the 4 and 8 positions and skimmianine (18) has methoxyl groups in the 4, 7

and 8 positions. The HPLC retention times for these compounds are 10.31, 9.79 and 8.55 min and their GLC retention times are 18.22, 18.99 and 20.77 min, respectively. The HPLC results appear to indicate that, in the absence of –OMe +R effects on weakly acidic groups as is observed for 4-OH and 1-NH quinolines, the more –OMe groups these quinolines possess the more polar their retention behaviour is. The GLC behaviour is explained by the relationship of increased

polarity to increased retention time. 4,6-Dimethoxyfuro(3,2-c)-quinoline (16) is structurally similar to γ -fagarine (17) but is in an angular form. It stays on the HPLC column longer than γ -fagarine and elutes faster using GLC. It appears that the linear compound behaves in a more polar manner than the angular compound.

Evoxine (20) has a similar structure to 7-O-dimethylallyl- γ -fagarine (22), except it is more polar, containing two hydroxy

Flindersine Compounds

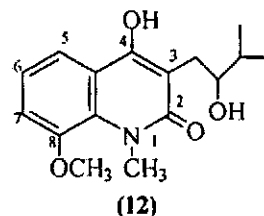
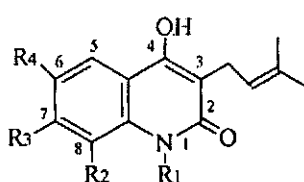


(1) $R_1=CH_3$ $R_2=R_3=H$ (2) $R_1=H$ $R_2=R_3=OCH_3$ (3) $R_1=CH_3$ $R_2=OH$ $R_3=H$

(4) $R_1=R_3=H$ $R_2=OH$ (5) $R_1=CH_3$ $R_2=R_3=OCH_3$

(6) $R_1=R_3=H$ $R_2=\text{tetrahydropyranyloxy}$, (7) $R_1=CH_3$ $R_2=\text{Dimethylallyloxy}$ $R_3=H$

3-C-Dimethylallylquinol-2-ones



(8) $R_1=R_2=R_3=H$, $R_4=OCH_3$ (9) $R_1=CH_3$ $R_2=R_4=OCH_3$ $R_3=H$

(10) $R_1=CH_3$, $R_2=OCH_3$, $R_3=R_4=H$ (11) $R_1=R_2=R_3=R_4=H$

(13) $R_1=R_4=H$ $R_2=R_3=\text{Methylenedioxy}$

(63) $R_1=R_2=R_3=H$, $R_4=OCH_3$, OH in 4-position replaced by OCH_3

Linear and Angular Furoquinolines

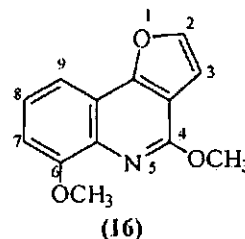
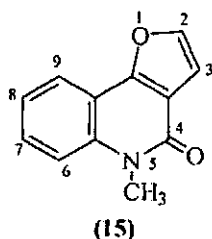
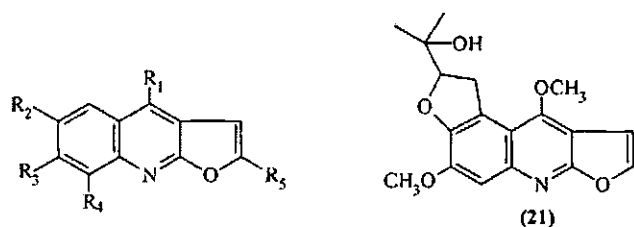


Fig. 1. Chemical structures of quinolines.



Dihydrofuroquinolines

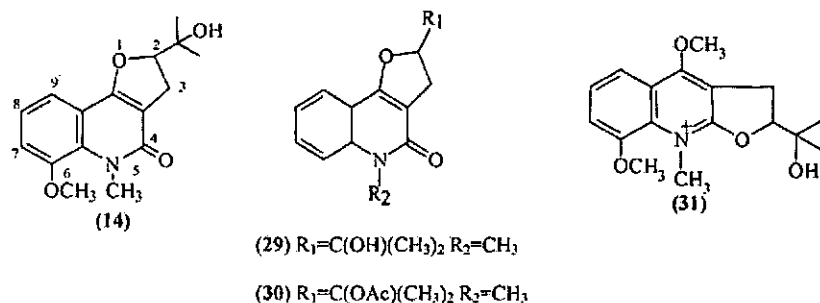
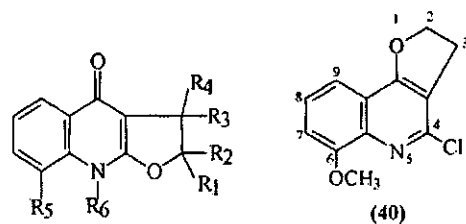


Fig. 1. (Continued)

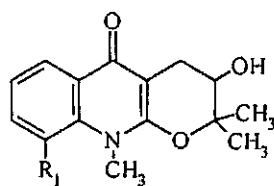
groups on the dimethylallyl side-chain. Evoxine therefore behaves as a more polar molecule with an HPLC retention time of 5.70 min compared to 8.08 min for (22). It also has the longest GLC retention time of this group of compounds with a value of 23.42 min. Dehydroplatydesmine (25) has a similar structure to dictamnine (19) except it has a hydroxyisopropyl side-chain. The more polar dehydroplatydesmine therefore elutes faster than dictamnine using HPLC with retention times of 9.49 and 10.31 min, respectively. Using GLC dehydroplatydesmine did not elute in the time scale of the experiment. Choisyine (21), which has a hydroxyl group in the side-chain, behaves like a polar molecule and elutes early on HPLC with a retention value of 6.97 min. But it is retained on the GLC column and does not elute within the run time.

3.4. Dihydrofuroquinolines

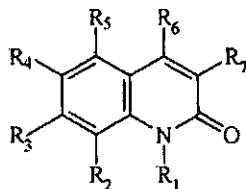
3.4.1. MS behaviour

Both linear and nonlinear forms of this group are present in nature. Ifflaiamine (26) [8] and lemobiline (28) [9] adopt the linear form and have the same molecular masses. The molecules are structurally similar and the fragmentation patterns are the same. However, they can be differentiated by their retention times using GLC as discussed in Section 3.4.2. From the structures of these isomers, the initial loss of 56 u cannot be easily accounted for. Loss of 56 u may be due to loss of butene (C_4H_8). To lose C_4H_8 from structures (26) and (28) as a single mass loss, the opening of the dihydrofuran ring followed by retro Claisen rearrangement of the 5-C chain to produce

dihydropyranoquinol-4-ones

(32) $R_1=H$ (33) $R_1=OCH_3$

quinol-2-ones



- (34) $R_1=R_4=R_5=R_7=H$, $R_2=R_3=OCH_3$, $R_6=OH$ (35) $R_4=R_5=H$, $R_2=R_3=OCH_3$, $R_1=R_7=CH_3$, $R_6=OH$ (36) $R_1=R_4=R_5=H$, $R_2=R_3=R_6=OCH_3$, $R_7=I$ (37) $R_1=R_3=R_4=R_5=R_7=H$, $R_2=OCH_3$, $R_6=OH$ (38) $R_1=CH_3$, $R_2=R_3=R_4=R_5=R_7=H$, $R_6=OH$ (39) $R_1=R_2=R_3=R_4=R_5=R_7=H$, $R_6=$ Epoxygeranyloxy (41) $R_1=R_3=R_4=R_5=R_7=H$, $R_2=OCH_3$, $R_6=OC_2H_5$ (42) $R_1=CH_3$, $R_2=R_3=R_4=R_5=H$, $R_6=OCOCH_3$, $R_7=1',1'$ -dimethylallyl (43) $R_1=CH_3$, $R_2=R_3=R_4=R_5=H$, $R_6=OCH_3$, $R_7=2'$ -hydroxy-3'-methylbut-3'-enyl (44) $R_1=CH_3$, $R_2=R_6=OCH_3$, $R_3=R_4=R_5=H$, $R_7=2',3'$ -dihydroxy-3'-methylbutyl (45) $R_1=CH_3$, $R_2=R_3=R_5=H$, $R_4=R_6=OCH_3$, $R_7=3'$ -methyl-2'-oxobutyl (52) $R_1=R_2=R_3=R_5=H$, $R_4=R_6=OCH_3$, $R_7=COOH$ (53) $R_1=R_2=R_3=R_4=R_5=R_7=H$, $R_6=OCH_3$ (54) $R_1=CH_3$, $R_2=R_3=R_4=R_5=R_7=H$, $R_6=OCH_3$ (55) $R_1=R_2=R_3=R_4=R_5=H$, $R_6=OH$, $R_7=Br$ (56) $R_1=CH_3$, $R_2=R_3=R_4=R_5=R_7=H$, $R_6=$ dimethylallyloxy (57) $R_1=R_7=CH_3$, $R_2=R_3=R_4=R_5=H$, $R_6=OH$ (61) $R_1=CH_2CH_3$, $R_2=R_3=R_4=R_5=H$, $R_6=OH$, $R_7=CH_3$ (62) $R_1=R_2=R_3=R_4=R_5=H$, $R_6=OH$, $R_7=$ Geranyl (64) $R_1=CH_3$, $R_2=R_6=OCH_3$, $R_3=R_4=R_5=H$, $R_7=2$ -Hydroxy-3-methylbutyl (65) $R_1=R_3=R_4=R_5=R_7=H$, $R_2=R_6=OCH_3$

Fig. 1. (Continued)

a 3,3-dimethylallyl side-chain which can lose a 4-carbon unit as butene has to be considered. The resulting ions each lose CO at the MS^3 and MS^4 stages followed by a loss of 26 u possibly due to a loss of acetylene as a neutral molecule at the MS^5 stage. 2-Hydroxyisopropyl-6-methoxy-5-methyldihydrofuro(3,2-*c*)quinol-4-one (14) shows an initial loss of 18 u. This is due to the loss of H_2O from the hydroxyisopropyl side-chain on the furan ring. A loss of 42 u at the MS^3 stage indicates that C_3H_6 is lost from this side-chain. The further loss of 15 u is due to the loss of a CH_3 group from the methoxyl group on the 6-*C* atom. Araliopsine (29) [12], which is also in the angular form, initially loses 18 u, which is due to loss of H_2O from the

hydroxyl group in the side-chain on the furan ring. The loss of 42 u at the MS^3 level is likely to be due to the loss of C_3H_6 from this side-chain. The loss of 28 u at MS^4 and MS^5 stages indicates further losses of CO. Araliopsine acetate (30) loses acetic acid initially, which has a mass of 60 u. The fragmentations thereafter are identical to araliopsine (29). Balfourodine (27) and the methylated quaternary derivative balfourodinium cation (31), as expected, behave similarly in the ESI- MS^n study. Both compounds have a hydroxyisopropyl side-chain at position 2. Hence, at the MS^2 stage, an initial loss of H_2O has been observed. Losses of propene, CH_3 and CO are also common to both molecules.

other quinoline derivatives

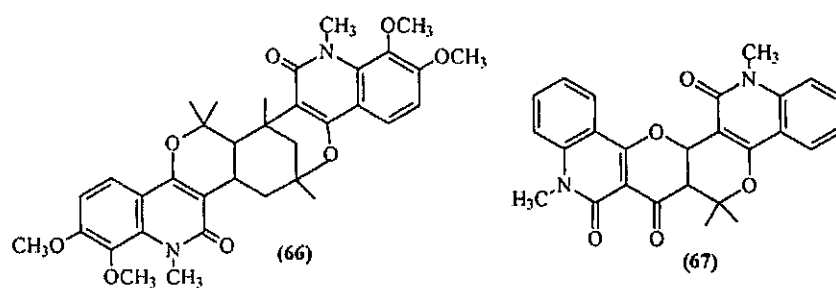
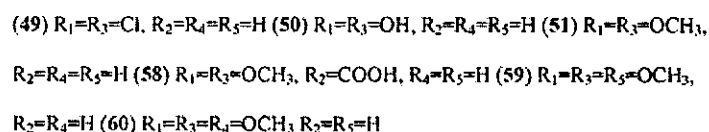
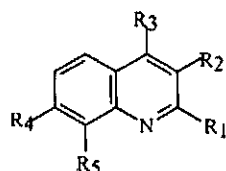
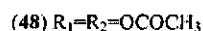
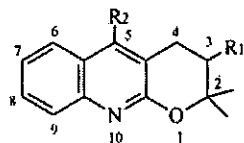
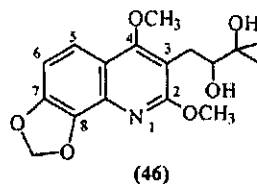


Fig. 1. (Continued).

The $[M+H]^+$ ions reported for 4-chloro-6-methoxydihydrofuro-(3,2-*c*)-quinoline (40) account for both isotopes of Cl. The data obtained from this molecule show a loss of CH_3 from a methoxyl group present on the 6-*C* atom. A further loss of 28 u at MS^3 indicates the loss of CO from this position. The proposed fragmentation pattern for (40) is given in Fig. 4.

3.4.2. HPLC and GLC behaviour

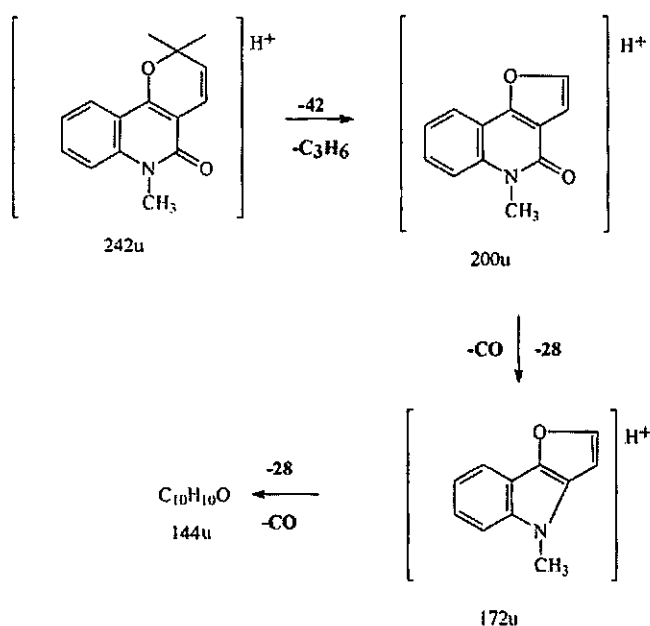
Araliopsine acetate (30) should behave in a less polar manner than (29) due to the loss of a free $-OH$ group on the side-chain. The retention times using HPLC are 9.40 and 5.93 min, respectively, as expected. 2-Hydroxyisopropyl-6-methoxy-5-methyldihydro-furo(3,2-*c*)quinol-4-one (14) has an HPLC retention time of 10.72 min in comparison to its linear form, balfourodine (27), which has an HPLC retention time of 5.21 min. Their GLC retention times are 22.56 and

28.20 min, respectively. This suggests that the linear form of the compound is more polar than the angular form. The isomeric lemobiline (28) and ifflaiamine (26) give relatively low HPLC retention times of 6.89 and 7.18 min illustrating their relative polarity. 4-Chloro-6-methoxydihydrofuro(3,2-*c*)quinol-4-one (40) behaves in the least polar manner for this group of dihydrofuroquinolines in that it has an HPLC R_t of 12.82 min and a GLC R_t of 18.34 min. *O*-Methylbalfourodinium (31) has a GLC retention time of 21.38 min but is not detected by HPLC, presumably being eluted as a cation with the solvent front.

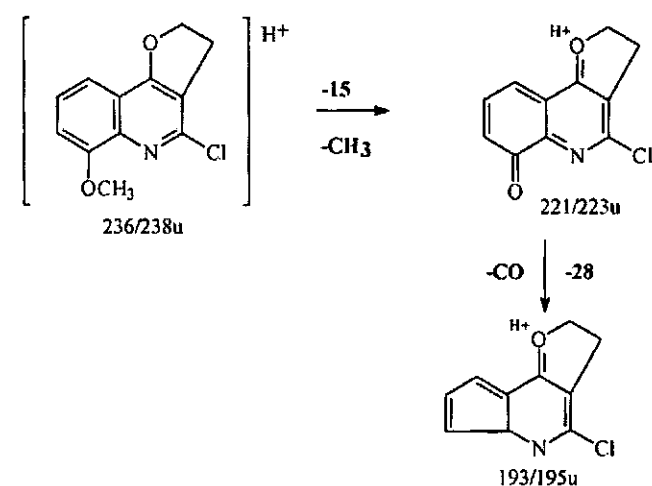
3.5. Dihydropyranoquinol-4-ones

3.5.1. MS behaviour

Both linear and angular forms of pyranoquinolines are present in nature with ribalinine (32) and isobalfourodine (33) both pos-

Fig. 2. ESI-MSⁿ fragmentation of *N*-methylflindersine.

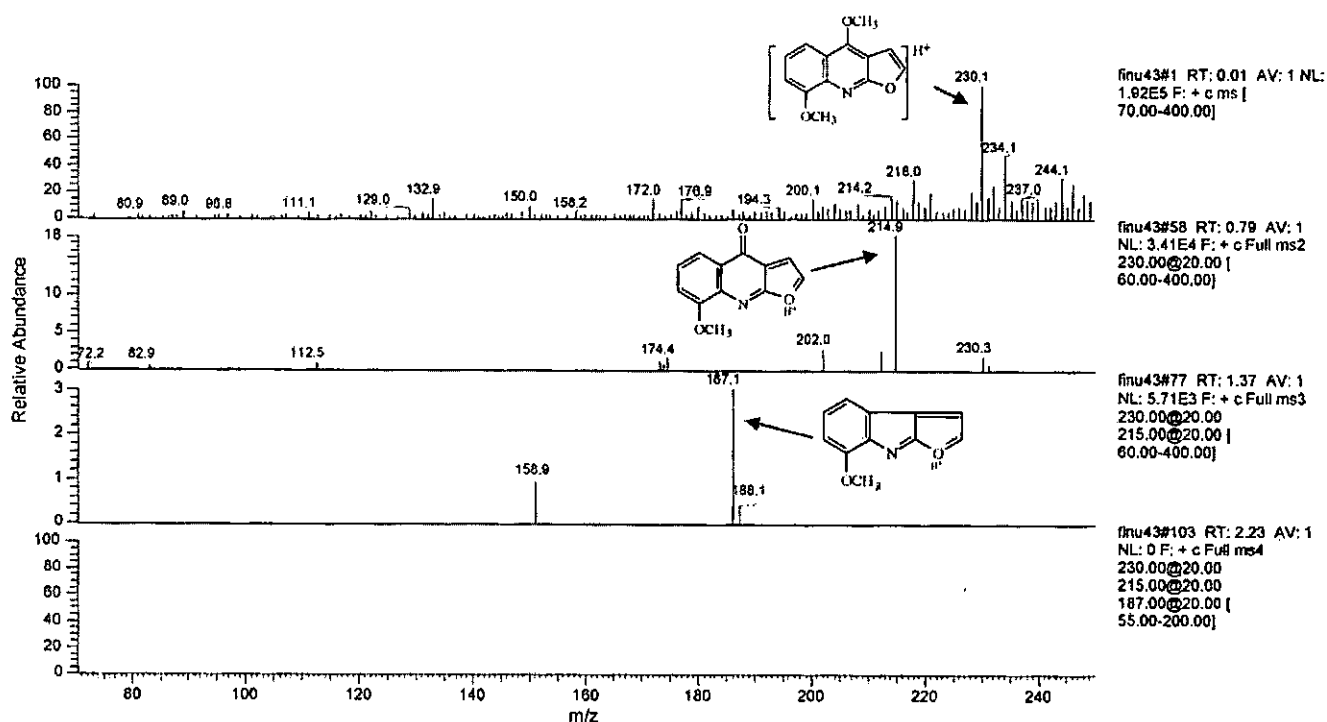
sessing the linear form. The only difference in structure between the two compounds is the occurrence of a methoxyl group in the 9-C position of isobalfourodine. Ribalinine (32) [13] and isobalfourodine (33) initially lose a molecule of water. This would be due to loss of the aliphatic hydroxyl group. Ribalinine then loses 42 u, which is probably a neutral mass loss of propene. There are two further 28 u losses due to CO with corresponding ring contractions. Isobalfourodine has an extra methoxy group in its

Fig. 4. ESI-MSⁿ fragmentation of 4-chloro-6-methoxydihydrofuro-(3,2-c)-quinoline.

primary structure but does not show the loss of 42 u as in the case of ribalinine. Instead, it loses 32 u which could be due to loss of methanol. A loss of 15 u at MS⁴ would then be due to a methyl group. However, it does show further losses of CO, which is similar to ribalinine.

3.5.2. HPLC and GLC behaviour

GLC results for isobalfourodine (33) and ribalinine (32) showed their retention times at 24.35 and 22.63 min, respectively, indicating that they are relatively polar compounds having the -OH group in the pyran ring. Such an effect was also observed for other quinolines which have an -OH group in

Fig. 3. ESI-MSⁿ spectra of γ -fagarine.

the 3-*C* dimethylallyl side-chain. The HPLC retention time of 5.37 min for (32) again illustrates the polarity of such –OH substituted compounds.

3.6. Quinol-2-ones

3.6.1. MS behaviour

Almost all of the quinol-2-ones show signals due to their $[M+H]^+$ ions using ESI–MS. A few of these molecules due to their contact with glass vials also show signals due to $[M+Na]^+$ adduct ions. ESI–MSⁿ fragmentations are dependent on the substituent groups attached to these molecules. If methoxyl groups are present, loss of 15 u at the MS² stage is a common feature as illustrated by quinol-2-ones of structures (34), (35), (37) and (65) in Table 1. Quinol-2-ones, with alkyl substituents such as dimethylallyl and geranyl, represented by a structure such as (62), tend to fragment at the alkyl side-chains at the MS² stage, e.g., 3-geranyl-4-hydroxy-2-quinolinone (62) showed an initial loss of 124 u. This was possibly C₉H₁₆, which would have been part of the side-chain breaking off. Similar to many aromatic *C*-alkyl derivatives some of these *C*-alkyl quinol-2-ones can fragment at the carbon α–β bond leaving the α-carbon attached to the quinolone nucleus. So, a 5-carbon side-chain loses a 4-carbon unit either as butene or butadiene depending on the presence of a double bond in the side-chain. A substituent such as a geranyl group loses a 9-carbon side-chain. 4,6-Dimethoxy-3-(3'-methyl-2'-oxobutyl)-1-methyl-quinol-2-one (45) shows a loss of 72 u at the MS² stage due to a loss of C₄H₈O from the 3-*C* side-chain, leaving a CH₂ group attached to the 3-*C* position. The second loss of 28 u at MS² indicates a loss of CO from the 2 position. Those quinol-2-ones with alkyl groups on an oxygen function tend to lose the entire alkyl unit in the fragmentation stages, e.g., 4-dimethylallyloxy-1-methyl-2-quinolinone (56) showed an initial loss of 68 u, which corresponds to the loss of C₅H₈. Further losses of 42 and 28 u were then observed. Ethyl substituents either on oxygen or nitrogen atoms have been found to lose ethene at the MS² stage as indicated by fragmentations of quinol-2-ones (41) and (61). 4-Epoxygeranyloxyquinol-2-one (39) shows an initial loss of 152 u due to the side-chain at position 4 breaking off. There were no further losses observed for this molecule at MS³ and MS⁴ stages.

Compound (42) loses 42 u at MS² level due to a possible loss of ketene (CH₂=C=O) resulting in a formation of an angular dihydrofuroquinoline by cyclisation with the neighbouring dimethylallyl group. Further fragmentation of this ion is similar to compounds (26) and (28).

Bromo- and iodo-quinol-2-ones behave differently on ESI–MSⁿ analysis. The bromo compound (55) loses the Br radical at the MS² stage, whereas with the iodo compound (36) the loss of the I radical was not observed.

4,8-Dimethoxy-3-(2',3'-dihydroxy-3'-methylbutyl)-1-methylquinol-2-one (44) shows an initial loss of 18 u which is due to H₂O from one of the hydroxyl groups on the 3-*C* side-chain. Another neutral mass loss of 72 u due to C₄H₈O was then lost from the side-chain. A further loss of 28 u would be due to the loss of CO from the 2 position. 4-Methoxy-1-methyl-2-quinolinone (54) showed an initial loss

of 15 u, due to CH₃, and a second loss of 28 u, due to CO. 1,3-Dimethyl-4-hydroxy-2-quinolinone (57) showed a loss of 56 u at MS² which may be two losses of 28 u, due to loss of two CO molecules. Lunacridine (64) [8] initially loses H₂O, then loses part of the prenyl side-chain and finally loses 15 u. Dictamninc acid (52) [7] showed the $[M+H]^+$ ion at *m/z* 220 as expected but did not give any fragmentation at the MS² level even by increasing the collision energy.

3.6.2. HPLC and GLC behaviour

The relatively polar and weakly acidic 2,4-quinoline diol (50) is discussed in the next subsection under other quinoline derivatives. However, it can be named 4-hydroxyquinol-2-one and has a retention time of 4.33 min using HPLC and 21.39 min using GLC. A number of quinol-2-ones chromatographically analysed are structurally similar to (50) but differ in the substituent groups present. In general, the substitution of alkyl and alkoxy groups gave rise to increased HPLC retention times as shown in Table 2. 3-Geranyl-4-hydroxy-quinolin-2-one (62) and 4-epoxygeranyloxyquinol-2-one (39) are retained longer on the HPLC column at 10.16 and 10.33 min, respectively, due to the effect of their hydrophobic side-chains. Similarly, 4-dimethylallyloxy-1-methyl-quinolin-2-one (56) is retained on an HPLC column significantly longer than (54) with retention time 12.56 min as compared to 4.49 min due to the hydrophobic/non-polar nature of its dimethylallyloxy side-chain.

1,3-Dimethyl-4-hydroxy-quinolin-2-one (57) differs from 4-hydroxy-1-methylquinol-2-one (38) by having an extra methyl group in the 3 position. (57) behaves in a more polar manner as it has a shorter retention time using HPLC and a longer retention time using GLC. But the +I effect of CH₃ group in the 3 position would decrease the acidity of the 4-OH group and hence make it less polar. Therefore, the more polar behaviour of (57) using HPLC and GLC is difficult to explain on these electronic grounds. However, due to tautomerism of the 4-OH group, (38) may be more affected than (57) by this process and behave more like a 2,4-diketone and hence be less polar.

In comparison to 4-hydroxy-1-methylquinol-2-one (38), 4-methoxy-1-methyl-quinolin-2-one (54) would be expected to stay on the HPLC column longer due to having a methoxy group in place of the weakly acidic hydroxyl group. However, this is not what is observed. (54) behaves in a more polar manner using both HPLC and GLC with retention times of 4.49 and 21.36 min, respectively, in comparison to 5.98 min (HPLC) and 16.63 min (GLC) for (38). Compound (38), as has been stated above, has a structure which can undergo keto-enol tautomerism. It may be possible that (38) behaves more like a 2,4-diketone and hence is less polar.

Dictamninc acid (52) has a weakly acidic carboxyl group present in the 3 position. 4-Methoxy-quinoline-2-one (53) shares the same structure as dictamninc acid except it does not have this carboxyl group. Due to this dictamninc acid gives rise to a particularly short HPLC retention time (4.21 min) and a relatively long GLC retention time (21.33 min) in comparison to 4-methoxy-quinoline-2-one (53) with retention times of 5.67 and 18.61 min, respectively.

3.7. Other quinoline derivatives

3.7.1. MS behaviour

Molecules in this group have a variety of structures, substituent groups and side-chains. Orixine (**46**) [8] has a methylenedioxy group in the C-7 and C-8 positions. Orixine initially loses 90 u from the 3-C side-chain. This molecule is similar to 4-hydroxy-7,8-methylenedioxy-3-(3'-methylbut-2-enyl)-quinol-2-one (**13**), discussed in Section 3.2.1, since they both lose CH₂O from the methylenedioxy group in the second stage after the initial loss of the 3-C side-chain. 5-Methoxy-2,2-dimethyldihydropyrano-(2,3-*b*)-quinoline (**47**), like the flindersine molecules, loses a mass unit of 56 u initially. This corresponds to a loss of C₄H₈ from the pyrano ring. Then there is a loss of 30 u, which may be a loss of CH₂O from the 2-C position. For 3,5-diacetyloxy-2,2-dimethyldihydropyrano-(2,3-*b*)-quinoline (**48**) there is an initial loss of 60 u. This is due to the loss of acetic acid, which may be from either acetyloxy side-chain at the 3 or 5-C position. A loss of 42 u is seen at the MS² level, which indicates a loss of C₃H₆ from the 2-C position. 2,4-Dimethoxyquinoline (**51**) showed a loss of 15 u due to the loss of a CH₃ radical at the MS² level and a loss of 28 u at the MS³ level due to the loss of CO. For 2,4-dimethoxyquinoline-3-carboxylic acid (**58**), there was an initial loss of H₂O and then possibly CH₂O. 2,4,8-Trimethoxyquinoline (**59**) and 2,4,7-trimethoxyquinoline (**60**) behave similarly on application of ESI-MSⁿ. These compounds can however be differentiated by HPLC as seen in Table 2. Both vepridimerine-A (**66**) and the unsubstituted dimer (**67**) lose large side-chains initially. Compound (**66**) loses 235 u and compound (**67**) loses 187 u.

Only the [M+H]⁺ ions were observed for 2,4-dichloroquinoline (**49**) and 2,4-quinolinediol (**50**). The [M+H]⁺ ions reported for (**49**) account for the presence of two chlorine atoms.

3.7.2. HPLC and GLC behaviour

The weakly acidic 2,4-dimethoxyquinoline-3-carboxylic acid (**58**) is shown to be the most polar compound in this group with an HPLC retention time of 4.24 min. 2,4-Quinoline diol (**50**), with weakly acidic properties, has a very similar retention time to (**58**) using HPLC but is retained longer on the GLC column than (**58**). This can possibly be explained by a significant difference in melting points, i.e. (**58**) has a melting point of 162 °C and (**50**) has a melting point of >300 °C.

2,4-Dichloroquinoline (**49**) and 2,4-dimethoxyquinoline (**51**) have a very similar retention time using HPLC suggesting comparable polarities but the dimethoxy derivative, (**51**), is retained longer on the GLC column possibly due to their slight difference in melting points (65 and 72 °C, respectively). An extra methoxyl group at the 8 position, as with 2,4,8-trimethoxy quinoline (**59**), appears to confer increased polarity and the HPLC retention time is reduced considerably to 11.32 min. This is reflected in an increased GLC retention time to 17.67 min. The structural isomers (**59**) and 2,4,7-trimethoxyquinoline (**60**), differing only in the positioning of one of the methoxyl groups, behave quite differently in HPLC (11.32 and 15.34 min, respectively) indicating that the 2,4,8-trimethoxyquinoline (**59**) is more polar than 2,4,7-trimethoxyquinoline (**60**).

Table 3

ESI-MS data following HPLC of acid soluble extract of *Choisya ternata*

Peak number	HPLC retention time	[M+H] ⁺
1	3.48	330
2	4.93	330
3	5.79	348
4	6.43	348
5	6.95	330
6	7.80	260
7	8.96	330
8	9.48	366
9	12.95	344
10	15.06	313
11	16.64	296
12	20.54	328

3.8. Plant extract examination by HPLC-ESI-MS

The analytical database, as collated in Tables 1 and 2, has found application in comparing these data with those of a plant extract. *C. ternata* [8], a rutaceous plant known to contain quinoline alkaloids, was chosen. The extract, containing tertiary alkaloids of this plant and obtained by acid extraction of its leaves, was analysed by HPLC-ESI-MS and the results were compared with compound information on the database.

In all, 12 HPLC-ESI-MS peaks were obtained. Mass spectral data for all these peaks are shown in Table 3. For the peaks numbered 1, 2, 5 and 7 the molecular ion is 330, which indicates a molecular mass of 329. This corresponds with the molecular mass of choisyine (**21**), known to be present in this plant. The HPLC retention time of choisyine from the database, under the same HPLC conditions, is 6.97 min. Peak 5 gives an HPLC *R*_t value at 6.95 min indicating that this peak could be choisyine. Peaks 1, 2 and 7 may be due to isomeric structures of choisyine. This would account for the same molecular mass but different retention times. Examples of isomeric structures of choisyine which may account for these peaks are shown in Fig. 5.

The molecules corresponding to peaks 3 and 4 have a molecular mass of 347. Comparison with information on the database indicates that peak 3 may be due to evoxine (**20**) as the retention times correspond. Peak 4 again could be due to an isomer of evoxine. Similarly, peak 6 could be due to skimmianine (**18**) with reference to the database.

7-*O*-Dimethylallyl-γ-fagarine (**22**) has a molecular mass of 312 and is known to be present in the plant. The molecule corresponding to peak 10 has the same molecular mass but the retention times do not correspond, again suggesting the presence of an isomer.

3.9. Database and web interface

The database contains the analytical results from ESI-MSⁿ, HPLC and GLC for 67 quinolines. For each compound the following additional information: molecular weight, molecular formula and structure, is available. The compounds are categorised as follows: flindersine compounds, 3-*C*-dimethylallylquinol-2-ones, linear and angular furoquinolines, dihydrofuroquinolines,

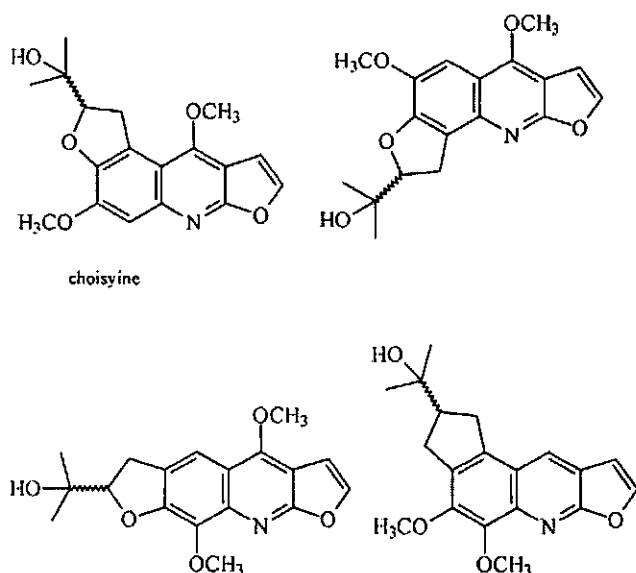


Fig. 5. Some quinolines isolated from *Choisy ternata*.

dihydropyranoquinol-4-ones, quinol-2-ones and other quinoline derivatives. The database is implemented in MySQL and run on a central server in the Bioinformatics Research Group at the University of Ulster, <http://bioinformatics.Ulster.ac.uk/~kay/>. The database may be browsed via a web interface, or interrogated using standard web forms.

4. Conclusions

The analytical behaviour of a selection of 67 naturally occurring and synthetic quinolines using the techniques ESI-MSⁿ, HPLC and GLC has been studied. This information will aid in the identification of unknown naturally occurring quinolines as borne out by the identification of certain quinolines in the acid extract of the leaves of *C. ternata*. This study has also provided an insight into the effect of substituent groups on the analytical

behaviour of these quinoline derivatives. It has been shown that the nature and the position of the substituent groups in a particular quinoline contribute to its ESI-MSⁿ and chromatographic behaviour. Some synthetic derivatives have been included as they could be biogenic precursors and could be found in plant extracts in small concentrations.

The analytical information from this paper has been uploaded into a database and has been made available on the University Intranet for use initially within the University of Ulster Pharmaceutical Biotechnology Research Group. Ultimately the complete database will be available on the Internet when this information is in the public domain.

Acknowledgement

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References

- [1] M.F. Grundon, *The Alkaloids: Quinoline Alkaloids Related to Anthranilic Acid*, vol. 32, Academic Press, London, 1988, pp. 341–436.
- [2] A. Sturaro, P. Traldi, P. Bravo, F. Viani, G. Resnati, *Org. Mass Spectrom.* 22 (1987) 462.
- [3] W.N. Setzer, B. Vogler, R.B. Bates, J.M. Schmidt, C.W. Dicus, P. Nakkiew, W.A. Haber, *Phytochem. Anal.* 14 (2003) 54.
- [4] I. Jacuemon-Collet, J.M. Bessiere, S. Hannedouche, C. Bertrand, I. Fouraste, C. Moulis, *Phytochem. Anal.* 12 (2001) 312.
- [5] W.H. Watters, D.Phil. Thesis, University of Ulster, Coleraine, 1996.
- [6] J.L. Gaston, D.Phil. Thesis, University of Ulster, Coleraine, 1979.
- [7] S.A. Surgenor, D.Phil. Thesis, University of Ulster, Coleraine, 1978.
- [8] I. Mester, *Fitoterapia* 44 (1973) 123.
- [9] L. Jurd, R.Y. Wong, *Aust. J. Chem.* 34 (1981) 1625.
- [10] S.A. Khalid, P.G. Waterman, *J. Nat. Prod.* 45 (1981) 343.
- [11] B.P. Das, D.N. Chowdhury, *Chem. Ind.* (1978) 272.
- [12] I. Mester, *Fitoterapia* 48 (1977) 268.
- [13] R. Torres, B.K. Cassels, *Phytochemistry* 17 (1978) 838.

CHEMISTRY OF FOSSIL FUEL

Genetic Relationship of Organic Bases of the Quinoline and Isoquinoline Series from Lignite Semicoking Tars with the Initial Biological Material

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Abstract—The genetic relationship of quinoline and isoquinoline compounds present in semicoking tars of Kimovsk lignites (near-Moscow fields) with the initial vegetable material is discussed. Transformation pathways of the native compounds in the course of lignite formation are suggested.

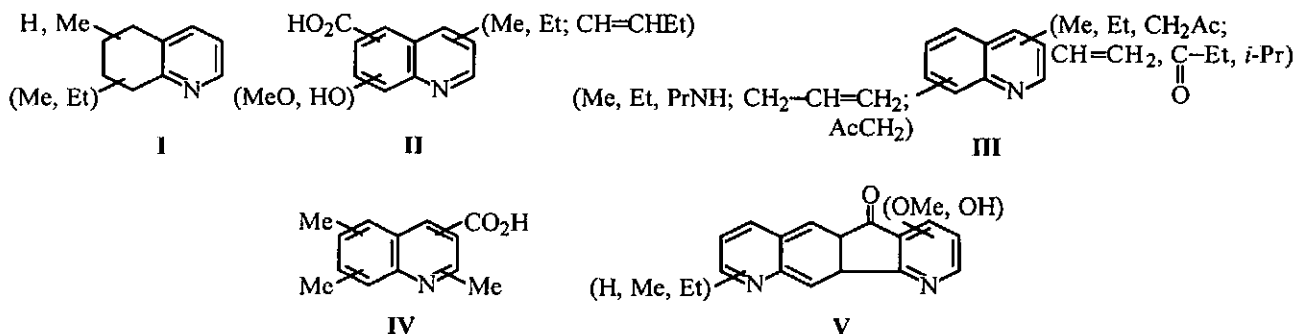
Recently there has been a considerable progress in detailed studies of biological markers bearing information about the initial biomaterial, primary sedimentation, and hence about further diagenesis and catagenesis. Such studies formed a new direction in geo-organic chemistry. Alkaloids form one of the most important groups of lignite biomarkers. Structural studies of individual nitrogen-containing heterocycles isolated from lignites give insight into their genesis. Extensive data have been obtained [1] on the composition of various extracts and tars formed in low-temperature semicoking of lignite from the Kimovsk open pit, near-Moscow fields.

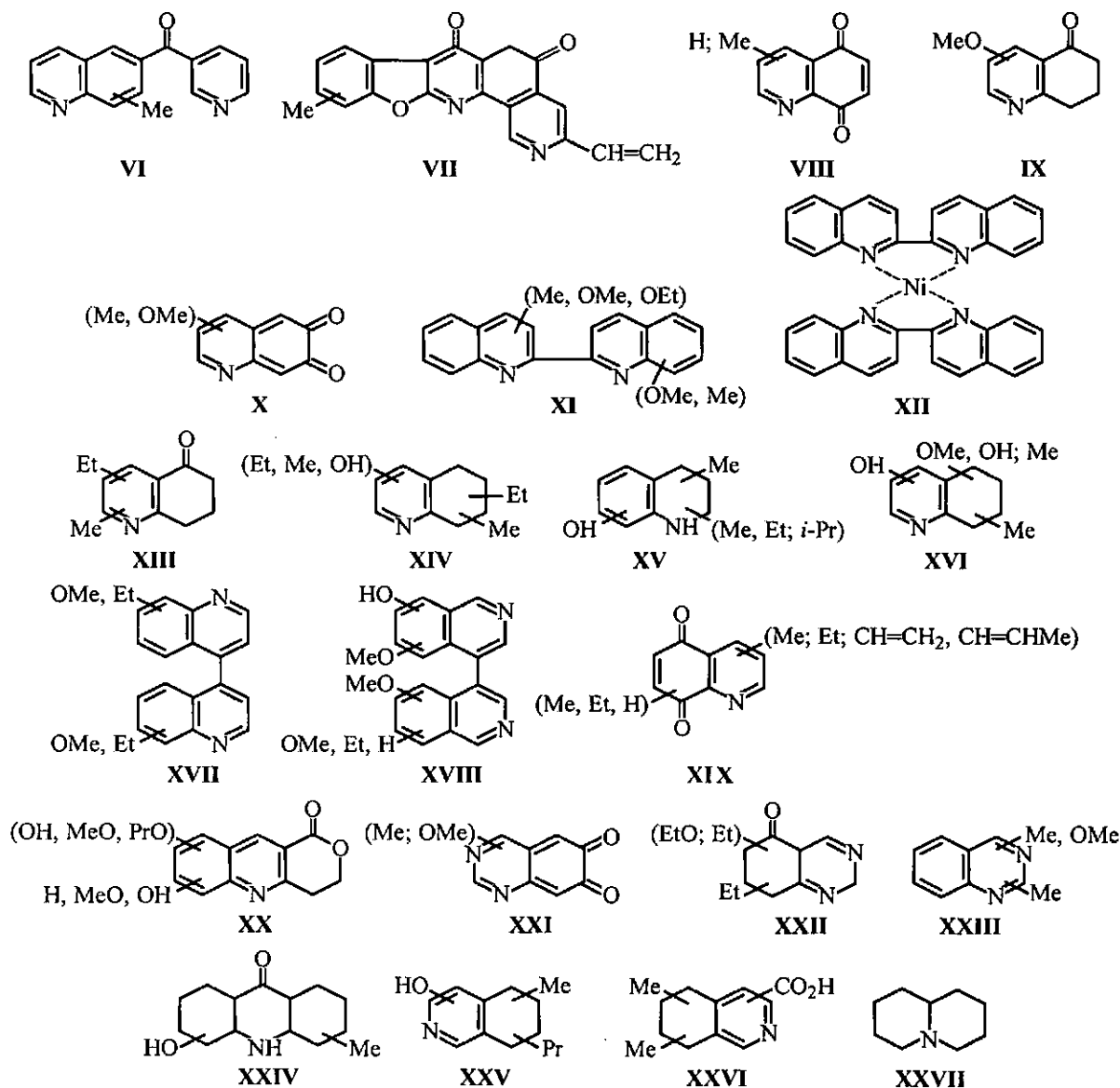
It was found that the major organic bases of semicoking tars (SCTs) are nitrogen-containing heterocycles boiling below 300°C (wt % of fraction): pyridine (1.2), C₁-alkylpyridines (2.1), C₂-alkylpyridines (5.8), C₃-alkylpyridines (6.5) >C₄-alkylpyr-

idines (8.9), alkylquinolines (5.0), alkylisoquinolines (10.4), hydrogenated quinolines (10.2), polycyclic aromatic compounds (5.5), indoles (5.6), alkylindoles (5.4), C₃-alkylindoles (5.0), >C₄-alkylindoles (1.2), carbazoles (3.9), and hydrogenated benzopyrroles (12.3).

The share of quinoline and isoquinoline derivatives in nitrogen-containing compounds is fairly high; therefore, it seemed of interest to study the genesis of these structures in the organic matter of lignites (OML). A detailed study of organic bases from lignite SCT by IR, UV, ¹H NMR, and ¹³C NMR spectroscopy, cryoscopy, preparative thin-layer chromatography, GC-MS, and elemental and quantitative functional analyses allowed identification of compounds I–XXVII characterizing structural fragments of OML and elucidation of the features of nitrogen distribution (see scheme 1).

Scheme 1.





In this connection, it was necessary to pay attention to biomarkers that give insight into the relationship between the nitrogen-containing lignite fragments and the biological material. Structures I–XXVII in OML should be associated with vegetable alkaloids, because so strong enrichment of the initial organic matter with oxygen-containing and alkyl groups by other pathways of biogeochemical degradation seems improbable. Alkaloids occur most widely in Angiospermae, especially in *Papaveraceae*, *Ranunculaceae*, *Leguminosae*, *Rubiaceae*, *Solanaceae*, and some other families. In algae, fungi, mosses, ferns, and Gymnospermae, alkaloids occur relatively seldom; therefore, as noted in numerous papers [2], the content of heterocyclic nitrogen in sapropelic caustobiotoliths is relatively low. The fact that alkaloids are distributed among

botanic families nonuniformly gives reason to consider them as fairly selective biological markers. It should be noted that the group of quinoline and especially isoquinoline alkaloids is characterized by the largest number of representatives and diversity of their structures. This is confirmed by a large content of quinolines and isoquinolines in SCT [1].

To reveal a genetic relationship between this group of nitrogen-containing compounds and natural alkaloids, we performed their structural classification based on the structure of the heterocycle. Structures I–XVII, XIX, and XX were classed with the quinoline series; structures XVIII, XXV, and XXVI, with the isoquinoline series; XXI–XXIII, with the quinazoline series; and structure XXVII belongs to belong to the group of natural alkaloids of the quinolizidine series.

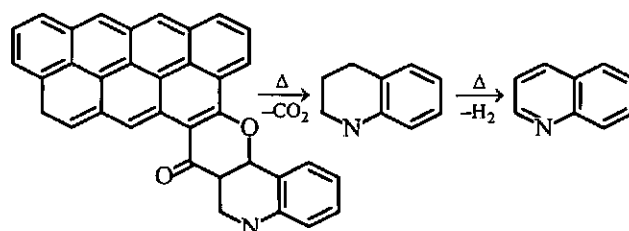
Distribution of various groups of alkaloids in the biological material

Plant family	Quinolines	Isoquinolines	Quinazolines	Quinolizines
<i>Rutaceae</i>	+	+	++	+
<i>Rubiaceae</i>	++	+		
<i>Papaveraceae</i>		++		
<i>Ranunculaceae</i>		+		+
<i>Acantaceae</i>		+	+	
<i>Leguminosae</i>		+		++
<i>Chenopodiaceae</i>		+		+
<i>Berberidaceae</i>		+		+
<i>Solanaceae</i>		+		+
<i>Menispermaceae</i>		+		+
Fungi producing alkaloid precursors in OML		+	+	+

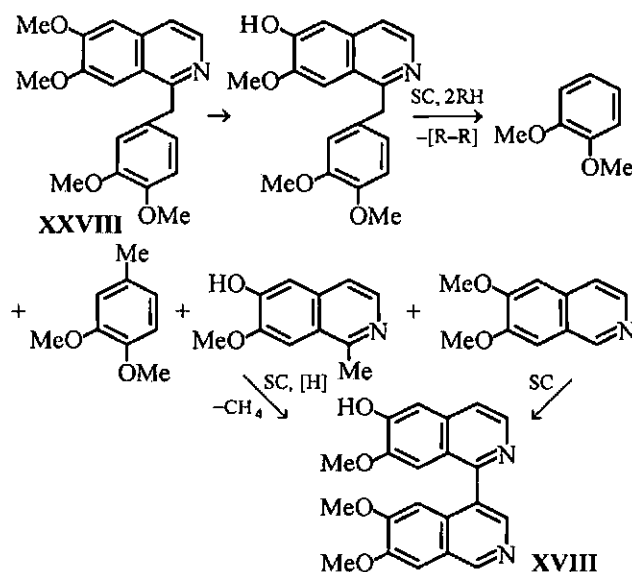
A comparison of the molecules isolated from OML with various natural compounds allowed the former compounds to be classed with biomarkers of the second kind, in which the thermodynamically stable molecular core is preserved, but substituents have underwent changes in the course of biogeochemical transformation of the organic matter of the initial vegetable material, or in the course of semicoking (SC). The structures of the organic bases allow fairly accurate identification of families, genera, and even species of plants from which lignite was formed (see table).

A large number of quinolines and isoquinolines are produced by the *Rutaceae*, *Rubiaceae*, *Leguminosae*, and *Solanaceae* families. Alkaloids of the quinoline series occur in almost all the families, but in *Papaveraceae* their content is the highest. The theory of the biological conservatism of plants gives grounds to state that evolutionary precursors of these plant families were involved in the primary sedimentations. Also, a very important part in the stage of peat genesis was played by fungi which produced various aliphatic amines and polypeptides. Under the action of microbial enzymes, these compounds transformed into various nitrogen-containing heterocycles, including those of the classes under consideration.

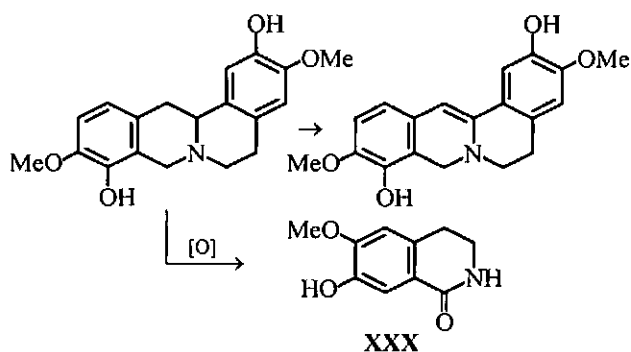
The preservation of the quinoline and isoquinoline cores in OML is due to their fairly high thermodynamic stability. It has been noted that these structures are linked to the peripheral part of the coal macromolecular network; however, owing to increased stability, they are also involved in formation of the polymeric core of a caustobiolith macromolecule. That is why data on recovery of nitrogen-containing heterocycles by nondestructive methods (e.g., extraction) are scarce [1]. A large number of such structures, detected in SCT, were released in the course of thermal degradation of the polymeric matrix. For example, the following mechanism can be suggested for formation of quinoline in pyrolysis of coal:



Quinolines formed in thermal degradation of caustobioliths and occurring in the nature contain, as a rule, a reduced (di- or tetrahydro) heteroring. This fact gives grounds to state that, in chemical transformations occurring in the course of semicoking or of biosynthesis in the initial vegetable material, isoquinolines are formed by decarboxylation of aromatic amino acids, many of which have been identified in aqueous extracts of coals. One of the major producers of isoquinolines in the modern flora is the *Papaveraceae* family. Representatives of this family contain such alkaloids as, e.g., morphine, codeine, papaverine, etc. One of possible pathways of biochemical transformations of native compounds is as follows:



Papaverine **XXVIII** undergoes enzymatic demethoxylation. In the course of semicoking, one phenyl radical undergoes radical elimination, and two isoquinoline fragments are coupled to form product **XVIII** identified in SCT. In the course of biogeochemical and subsequent thermochemical transformations, such a structure can be yielded by degradation of polymethylene bridges in such compounds as salsoline (*Solanaceae*) and laudanine (*Papaveraceae*). It has been found previously [3] that the organic matter of some lignites contains isoquinoline lactams (isoquinolones), which are presumably formed by amination in aqueous solutions of the corresponding isocoumarins. Substituted isoquinolones were also isolated from SCTs of lignites from the near-Moscow fields. The involvement of plants producing alkaloids in the primary sedimentation suggests that isocoumarin lactams could also be formed by oxidative degradation of the corresponding substituted isoquinolines. For example, skulerine **XXIX**, which belongs to the subclass of diisoquinolines occurring mainly in the *Papaveraceae*, *Menispermaceae*, and *Berberidaceae* families in both hydrogenated and dehydrogenated forms, gives hydrogenated isoquinolone **XXX**:



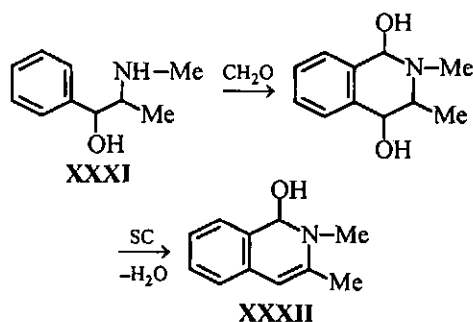
Hydrogenated rings in nitrogen-containing alkaloids can act as hydrogen donors in various coal treatment processes. Correspondingly, the higher the content of hydrogenated isoquinolines in OML, the more facile is the destructive thermal hydrogenation of this caustobiolith.

A more detailed comparative analysis of the chemical structures identified in SCT showed that the nitrogen-containing organic bases in OML do not always correspond to the natural isoquinoline and quinoline alkaloids; hence, biogeochemical transformation does not always account for the formation of particular structures in the organic matter of a caustobiolith.

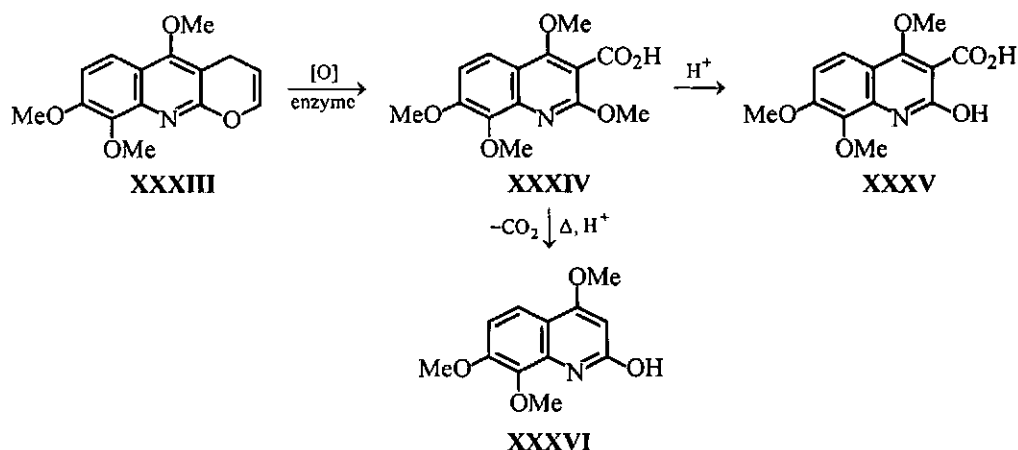
It is widely believed that alkaloids are formed from amino acids under the action of microbial enzymes in the stage of peat genesis. Changes in the composition

of the organic matter initially buried in sediments and the degree of decomposition of the organic matter supplied to the sediments will depend on the extent and nature of microbial activity [2]. Microbial oxidation can cease in any stage, and the selective effect of microorganisms can result in concentration of biologically more stable organic residues and of nitrogen-containing heterocyclic systems. Enzymes are either accumulated in the organic matter, increasing its weight, or favor transformation of organic substances into water-soluble or colloidal forms. Thus, isoquinolines of OML can be formed *in vitro* from the corresponding amino acids containing a phenylethylamine residue under the action of various microbial enzymes. It is believed that an important part is played in the process by formaldehyde (the primary product of CO_2 assimilation), which acts as a methylating and ring-closing agent, as shown for isoquinolines. Furthermore, in the course of lignite formation, isoquinolines can also be formed from acyclic alkaloids. This group of alkaloids consists of aliphatic amines formed in the course of life activity of fungi (degradation of polypeptide fungal toxins) in the peat genesis stage.

Gymnospermae can also be involved in OML formation. Below we show as example the scheme of formation of isoquinoline **XXXII** from ephedrine **XXXI**, suggesting participation of coniferous plants in primary sedimentation. As far as modern coniferous plants are concerned, ephedrine was isolated from plants of the *Ephedra* (*Gnetaceae* family) genus and from *Taxus laccata* (*Taxaceae*). The reaction occurs *in vitro* and is effected by enzymes:

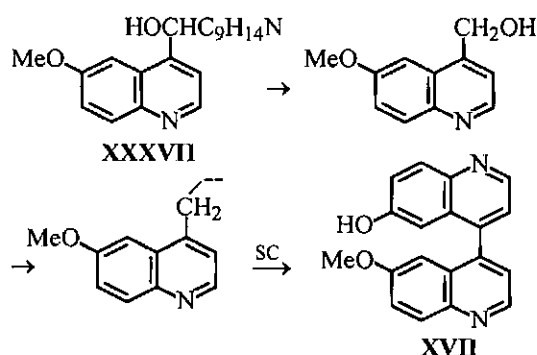


It is important that, as compared to isoquinolines, quinoline alkaloids are considerably less abundant in the flora and occur in a very narrow group of families. For example, two structurally related alkaloids, skimmianine and dictamnine, have been isolated from two closely related *Rutaceae* plants, namely, *Skimmia japonica* and *Dictamnus albus* [4]. Below is given the scheme of biogeochemical transformations of skimmianine **XXXIII** under conditions of lignite formation:



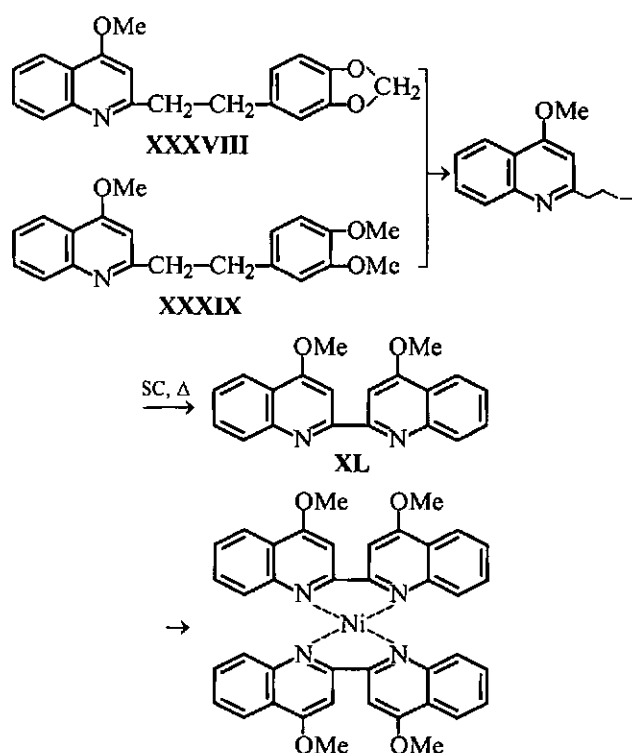
The reaction occurs via skimmianic acid **XXXIV** and yields two substituted isoquinoline structures **XXXV** and **XXXVI**, identified in lignite SCT.

It is interesting that more than half of natural compounds containing a quinoline core belong to the group of quinine alkaloids, which is inherent in certain species, namely, *Cinchona* and *Remija* (*Rubiaceae* family). Presumably, the evolutionary precursors of these species, producing a large number of quinine alkaloids (quinoline group), such as cinchonine, quinine, quinidine, hydrocinchonine, hydroquinine, hydroquinidine, homoquinine, quinallene, aricine, cusconine, paricine, etc., could be the suppliers of aromatic and hydroaromatic substituted isoquinolines to OML. Below is given the scheme of possible biogeochemical transformations of quinine **XXXVII**:



The first stage of microbiological degradation of quinine is accompanied by elimination of the quinuclidine core. After that, the structure becomes incorporated via the methylol substituent in the periphery of the lignite macromolecular network. In the course of SC, the quinoline fragments may undergo radical coupling to give structure **XVII** identified in SCT of near-Moscow lignites.

Of particular interest is formation of substituted 2,2'-biquinoline **XL**. Firstly, this structure was presumably formed from the natural quinolines cusparine **XXXVIII** and galipine **XXXIX**, which are produced in the modern flora by *Calipea cusparia*:



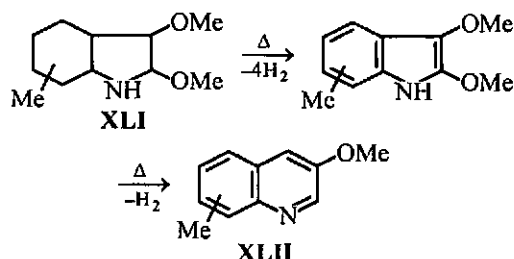
After structures **XXXVIII** and **XXXIX** become incorporated in the lignite matrix via alkyl radicals in 2-position, the 2,2'-biquinoline structure is formed by free-radical thermochemical degradation. Under the conditions of degradation of the polymeric molecule, two molecules of such a dimer react with the mineral matter of coal to form chelates with various metals.

For example, a similar nickel complex **XII** was identified in SCT. Presumably, nickel and other elements pass into such chelates from metal porphyrins, which results in metal migration in the course of thermochemical reactions.

Secondly, some authors note that 2,2'-biquinoline is formed in 15% yield by heating of quinoline with nickel on alumina at 325°C. Thus, the quinoline dimer is synthesized either from molecules that have been already eliminated from the matrix or from biogeochemically transformed quinoline alkaloids conserved in lignite pores.

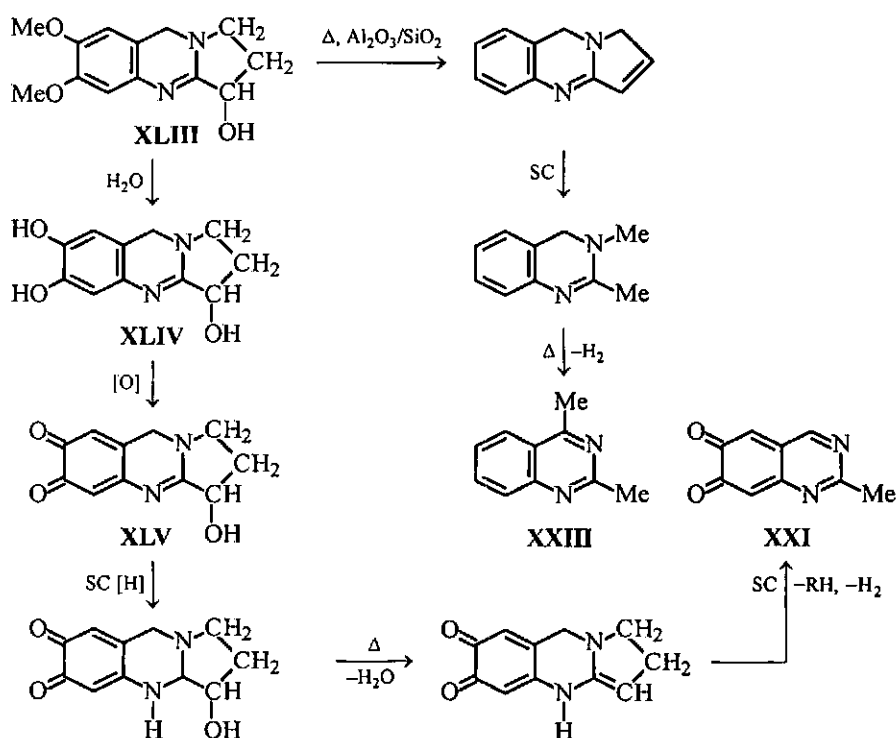
Detailed analysis also reveals inconsistency between the relatively small number of natural quinoline alkaloids and fairly large number and diversity of nitrogen-containing organic bases incorporating the quinoline core. This fact is attributable to the formation of quinolines, similarly to isoquinolines, from amino acid residues in the biochemical stage *in vitro* of lignite formation under the action of enzymes. Also, as noted in [5], pyrolysis of lignite SCT is accompanied by ring extension: The content of nitrogen atoms incorporated in six-membered aromatic heterocycles increases, whereas the content of five-membered rings decreases. Hence, one of the pathways of thermochemical transformations of nitrogen is the formation

of six-membered heterocycles in general, and of quinoline cores in particular. For five-membered heterocyclic systems, ring extension under conditions of thermal degradation of lignites is quite possible. The scheme of thermochemical transformation of indoles **XLI** into quinolines **XLII** is shown below:



Compounds structurally similar to **XLII** were identified in lignite SCT in a fairly large amount. Thus, a significant part of quinolines could be formed from indole compounds in the course of semicoking. Furthermore, these reactions are promoted by carbonyl compounds whose content in primary tars is fairly high, because the caustobiolith under consideration has a low degree of metamorphism and high degree of oxidation (with oxygen mainly occurring in keto and quinoid groups). Taking into account the degree of metamorphism of caustobiolith, we find that the

Scheme 2.

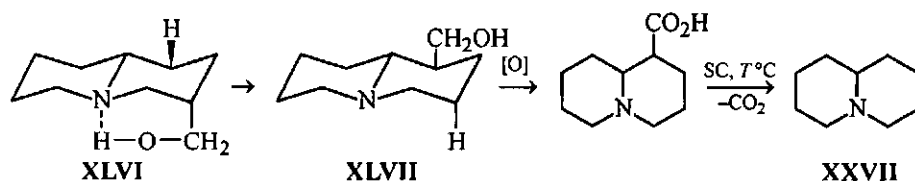


yield of indoles from coals is considerably higher than that from lignites, i.e., indoles exhibit increased thermodynamic stability. Comparison of the compositions of SCTs from coals and lignites shows that the content of quinolines in coals is higher than that in lignites, which confirms the assumption that quinolines are formed from substituted indoles. This assumption is also supported by the fact that indole alkaloids prevail in modern flora, and isoquinolines are present in a considerably smaller amount, being produced by a fairly narrow group of plants. It is significant that indoles are produced in large amounts by fungi; therefore, we can make an important conclusion that substituted quinolines originate from the activity of fungi that produce indole compounds undergoing subsequent thermochemical transformations.

Structures **XXI–XXIII** in OML are attributable to participation of plants producing a relatively small group of quinazoline alkaloids in the primary sedimentation. These compounds are produced by *Acanthaceae* and *Rutaceae* plants; also, they are microbial metabolites. For example, peganine (vasicine) **XLIII** was isolated from two species, *Peganum harmala* and *Adhatoda vasica*. The process can occur by two dif-

ferent pathways yielding structures **XXI** and **XXIII**. The pathway of formation of *o*-quinoid compound **XXI** is of particular interest. Vasinine, most probably, undergoes enzymatic hydroxylation in the *o*-position in the stage of peat genesis to form structure **XLIV**, which is oxidized by atmospheric oxygen under aerobic conditions with the participation of bacteria, yielding quinone **XLV**. It was noted previously that the introduction of hydroxy groups into an aromatic substrate is effected by bacteria of the genera *Pseudomonas* and *Nocardia*, and also by *Aspergillus niger* fungus (see scheme 2).

Quinolizine compounds **IV**, **VII**, and **VIII** are still more specific alkaloids than quinazolines. The involvement of the biological material producing compounds of this class is responsible for the presence in the organic matter of near-Moscow lignites of substances with structure similar to **XXVII**. The quinolizine core usually occurs in alkaloids in the fully reduced quinolizidine form, e.g., in *Leguminosae* family and in lupines, in particular. When heated in aromatic hydrocarbons, lupinine **XLVI** isomerizes in the course of enzymatic processes into epilupinine **XLVII**:



This fact suggests that the primary alcoholic group in lupinine occurs in the less sterically favorable axial position owing to hydrogen bonding. The hydrogenated quinolizidine rings are hydrogen donors in thermal transformations of OML; therefore, the presence of structures similar to **XXVII** in the organic matter positively affects various processes of coal treatment.

CONCLUSIONS

(1) A genetic relationship was revealed between quinolines and isoquinolines detected among organic bases of semicoking tars of near-Moscow lignites, on the one hand, and vegetable alkaloids and fungal metabolites, on the other.

(2) Pathways of possible biogeochemical transformations of the initial nitrogen-containing compounds in the course of lignite formation and semicoking were suggested.

REFERENCES

1. Polovetskaya, O.S., Chemical Composition of Organic Bases from Semicoking Tar of Near-Moscow Lignites and Thermochemical Transformations of These Bases, *Cand. Sci. Dissertation*, St. Petersburg, 1999.
2. *Organic Geochemistry. Methods and Results*, Eglington, G. and Murphy, M.T.J., Eds., Berlin: Springer, 1969.
3. Podshibyakin, S.I., Genetic Relationship between the Pyrone Group Compounds in the Organic Matter of Kansk-Achinsk Lignite and the Initial Biological Material, *Cand. Sci. Dissertation*, St. Petersburg, 2000.
4. Orekhov, A.P., *Khimiya alkaloidov* (Alkaloid Chemistry), Moscow: Akad. Nauk SSSR, 1955.
5. Bronshtein, A.P., Makarov, T.N., Akulov, P.V., and Grigor'ev, N.P., *Khim. Tverd. Topl.*, 1979, no. 6, pp. 65–74.

り再結晶を繰り返せば無色針状品 mp 263°(decomp) 2 mg を得.

本研究に際し終始御懇篤なる御指導を賜った京都大学 富田真雄教授に厚く感謝致します. また本研究における旋光度は日本新薬研究所 小泉淳二, 三森信夫氏, 赤外線吸収スペクトルは京都大学薬学部 町田勝之輔, 内田郁子氏により測定, 元素分析は京都大学微量元素分析センター 穂積啓一郎博士ならびにその協力者によって施行せられたものでありここに記して謝意を表します.

京都大学薬学部

UDC 547.944.8

55. 石井 永: Skimmianine の還元成績体の合成 (ミカン科植物
アルカロイド研究 第 14 報^{*1)})

Hisashi Ishii: Studies on the Alkaloids of Rutaceous Plants. XIV.
Syntheses of Some Hydrogenated Derivatives of Skimmianine.

(Faculty of Pharmacy, Kyoto University^{*2)})

In order to obtain ultraviolet and infrared spectra of the reduction products of skimmianine, syntheses were carried out according to the route shown in Charts 1~3 for isoskimmianine (II), dihydroskimmianine (III), tetrahydroskimmianine (IV), tetrahydroisoskimmianine (V), tetrahydronorskimmianine (VI), norskimmianine (VII), tride-methyltetrahydroskimmianine (VIII), isodictamnine (XIII), and dihydrodictamnine (XIV). New compounds prepared in the present series of work included dihydroisoskimmianine (IX), dihydronorskimmianine (X), N-methyltetrahydroskimmianine (XV), and N-methyl-hexahydroskimmianine methiodide (XIX). Absorption of these compounds, which lacked the lactam and carbonyl bands in their infrared spectra, in the range of 1700 to 1600 cm^{-1} was examined (Table I).

(Received June 23, 1960)

著者は前報¹⁾において京都市天ヶ嶽産ツルシキミ *Skimmia japonica* THUNB. var. *intermedia* KOMATSU form. *repens* (NAKAI) HARA の含有塩基を検索中水溶性塩基として base C (仮称) を得, 本物質がその UV スペクトルならびに元素分析値よりみて furoquinoline 核より誘導される骨格を持つものではないかと推定した. 今回著者はミカン科植物にもつとも分布の広いアルカロイドである skimmianine (I) の還元成績体と上記の base C との UV ならびに IR スペクトルを比較するために skimmianine について種々の還元を追試するとともに数種の還元成績体を合成することが出来た.

すでに太田等^{2,3)}は skimmianine (I) に対して PtO_2 を触媒として接触還元を行なえば furan 環の開裂した tetrahydro 体を与え, これらが 3-ethylcarbostyryl 体であることを明らかにした. また別に Price 等⁴⁾はミカン科に属する植物 *Acronychia baueri* SCHOTT および *Melicope fareana* ENGL. 中に含まれる furoquinoline アルカロイドの acronycidine の構造研究中 Raney Ni を触媒として接触還元を行ない tetrahydro 体である 3-ethylcarbostyryl 体と同時に dihydro 体を得た. ついで太田等⁵⁾は skimmianine (I) を PdO 触媒として還元して furan 環の 2,3 位の二重結合にのみ水素を付加した dihydro 体のみを得て, Price 等が得た dihydro

*¹ 第 13 報 石井: 本誌 81, 243(1961). 本研究は富田真雄: ミカン科植物アルカロイド研究 (M. Tomita: Studies on the Alkaloids of Rutaceous Plants) の一部である.

*² Yoshida-konoe-cho, Sakyo-ku, Kyoto.

1) 富田, 石井: 本誌 78, 1180(1958).

2) 太田: 本誌 73, 63(1953).

3) T. Ohta, T. Miyazaki: Pharm. Bull. (Japan) 1, 184(1953).

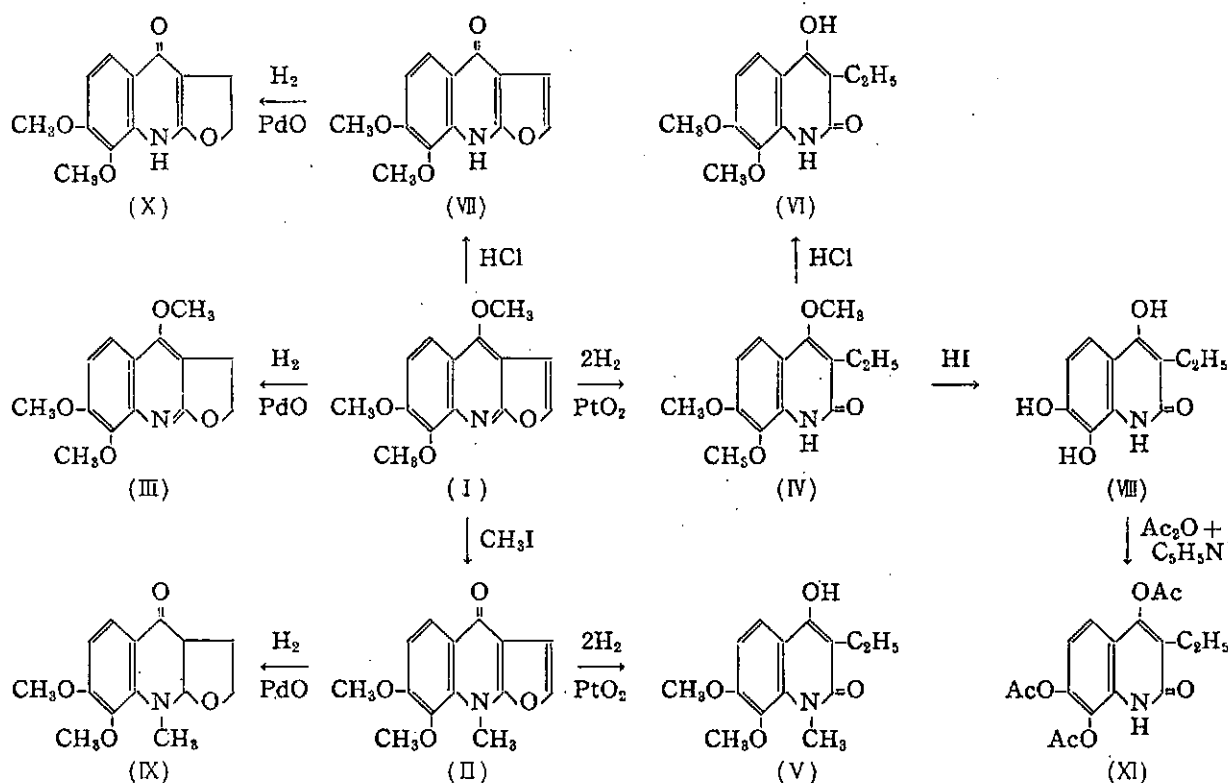
4) F.N. Lahey, J.A. Lamberton, J.R. Price: Australian J. Sci. Research A3, 155(1950).

5) 太田, 宮崎, 森: 本誌 74, 708(1954).

体の水素の付加した位置を明らかにするとともに furoquinoline アルカロイドを PdO で還元すれば dihydro 体のみを与え、PtO₂ で還元すれば tetrahydro 体のみを一方向的に与えるが、Raney Ni を触媒として還元を行なえば両者の混合物を与えること、ならびに PdO で還元して得られた dihydro 体は最早さらに PtO₂ で還元しても tetrahydro 体は得られず還元強く抵抗することを明らかにした。

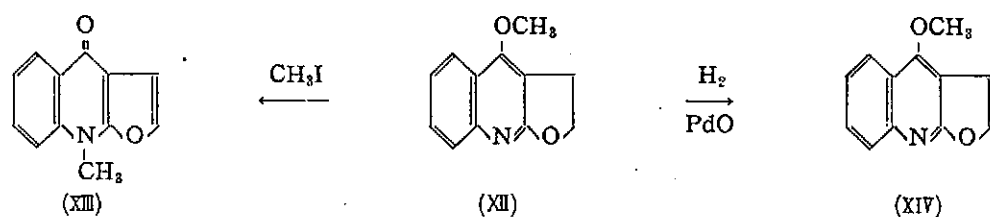
著者は Chart 1 に示すように skimmianine^{*6} (I), isoskimmianine⁷⁾ (II) を原料として対応する dihydro-skimmianine^{5,7)} (III), tetrahydroskimmianine^{2,3,7)} (IV) tetrahydroisoskimmianine⁷⁾ (V), tetrahydronor-skimmianine⁸⁾ (VI), norskimmianine⁸⁾ (VII), tridemethyltetrahydroskimmianine⁸⁾ (VIII) を合成し、さらに今回新たに dihydroisoskimmianine (IX), dihydronorskimmianine (X) を合成した。

Chart 1.



ついで著者は比較研究のため Chart 2 にしたがって dictamnine^{*4} (XII) より isodictamnine⁹⁾ (XIII) ならびに dihydrodictamnine⁵⁾ (XIV) の合成も追試した。

Chart 2.



*3 本研究に使用した skimmianine は京都市天ヶ嶽産ツルシキミならびに伊吹山および京都市西芳寺谷産イヌザンショウ *Xanthoxylum schinifolium* (Sieb. et Zucc.) ENGL. の樹皮 (富田, 石井: 未発表) より得られたものを使用した。

*4 本研究に使用した dictamnine はカラスザンショウ木部 (富田, 石井: 本誌 78, 1442(1958) より得られたものを使用した。

6) 朝比奈, 犬伏: 本誌 50, 1133(1930); Ber. 63, 2052(1930).

7) 太田, 宮崎, 森: 東薬年報 4, 7(1954).

8) J. A. Lamberton, J. R. Price: Australian J. Chem. 6, 66(1953).

応条件下においては 2(1*H*)-quinolone 型より他の tautomer である 2-hydroxyquinoline 型 (IV') が安定になるため反応が進行しないのであつて 2-hydroxyquinoline 型をとり得ない 1-methyl-2-quinolone 型のものなら反応が進行するのではないかと推定し、さきに得られた N-methyltetrahydroskimmianine (XV) を LiAlH₄ で還元した。ここに得られる反応生成体は種々取り扱つても結晶化しないがヨウ化メチルと処理すれば mp 155~157°(decomp) の無色柱状晶を与える。本物質は Beilstein 反応陽性で元素分析値は N-methylhexahydroskimmianine methiodide (XX) に一致し、また UV スペクトルは Fig. 1 に示すように 274 mμ にベンゼン核の吸収を示し、さらに 298~318 mμ にかけて巾の広いベンゼン核に共役する二重結合を有する吸収を示す。以上の結果よりみて著者の得たメチル化体は N-メチル化体であることは確実である。

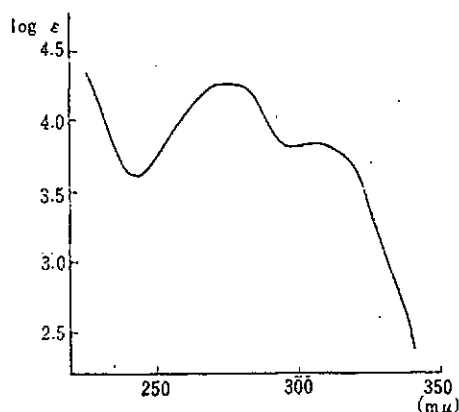


Fig. 1.
Ultraviolet Absorption Spectrum (in EtOH)
of N-Methylhexahydroskimmianine
Methiodide (XX)

さきに Witkop 等¹³⁾ は 2(1*H*)-quinolone と 4(3*H*)-quinolone の区別に IR スペクトルならびに UV スペクトルを用いることを提唱し、その中で 2(1*H*)-quinolone は 1660~1650 cm⁻¹ に強い lactam による carbonyl band が存在するのに反して、4(3*H*)-quinolone は幾分低波数の 1630~1620 cm⁻¹ に吸収を示すことを明らかにした。ついで Grundon 等^{14,15)} は 4-alkoxy-2(1*H*)-quinolone 誘導体もまた同じ吸収を示すことを明らかにし、

Table I. Infrared Absorption (Nujol Paste) in the Region 1700~1600 cm⁻¹
of Some Hydrogenated Derivatives of Skimmianine

	2(1 <i>H</i>)-Quinolone amide (I) (cm ⁻¹)	4(3 <i>H</i>)-Quinolone amide (I) (cm ⁻¹)	Other 1700~1600 cm ⁻¹ bands
Isoskimmianine (II)		1627 (s)	
Dihydroisoskimmianine (IX)		1629 (s)	
Norskimmianine (VIII)		1626 (s)	
Dihydronorskimmianine (X)		1617 (s)	
Isodictamnine (XIII)		1623 (s)	
Tetrahydroskimmianine (IV)	1638 (s)		1621 (m), 1602 (m)
N-Methyltetrahydroskimmianine (XV)	1634 (s)		
Tridemethyltetrahydro- skimmianine triacetate (XI)	1660 (s)		1615 (m)
Derivatives of 2,4-Dihydroxyquinoline			
Tetrahydronorskimmianine (VI)		1631 (s)	
Tetrahydroisoskimmianine (V)		1625 (m)	
Tridemethyltetrahydroskimmianine (VIII)		1630 (s)	
Some Related Compounds			
Skimmianine (I)		1617 (m), 1619 (m)	
Dihydroskimmianine (III)		1628 (s), 1606 (m)	
Dictamnine (XII)		1622 (m)	
Dihydrodictamnine (XIV)		1634 (s)	

13) B. Witkop, J.B. Patrick, M. Rosenblum: J. Am. Chem. Soc. 73, 2641(1951).

14) M.F. Grundon, N.J. McCorkindale, M.N. Rodger: J. Chem. Soc. 1955, 4284.

15) M.F. Grundon, N.J. McCorkindale: Ibid. 1957, 2177.

2(1*H*)-quinolone の吸収範囲を幾分拡張し 1660~1640 cm^{-1} であるとした。しかしながら一方 carbonyl 基を持たない物質も dihydrodictamnine (XIV) IR (KBr) 1631 cm^{-1} , dihydro- γ -fagarine IR (KBr) 1645 cm^{-1} を初めとしてその他のものにもまたこの領域に吸収を持つことを明らかにし、必ずしも furoquinoline 類においては 1660~1620 cm^{-1} に存在する吸収は lactam の存在することを表わさないことを明らかにした。今回著者の合成した種々の還元成績体の 1700~1600 cm^{-1} の範囲における IR スペクトルは Table I の通りである。すなわちこれらの結果を概観すると 2(1*H*)-quinolone 体は 4(3*H*)-quinolone 体より高波数側に吸収がみられるが必ずしも Grundon 等がいうように carbonyl band による 2(1*H*)-quinolone 体と 4(3*H*)-quinolone 体の区別は簡単ではなく、さらにかなり多くの carbonyl を持たない quinoline 誘導体が quinoline 核の吸収をこの付近に有することが判明した。

実 験 の 部^{*5}

Dihydroskimmianine (III) skimmianine (I) (mp 176.5~178.5°) 0.6 g を EtOH 280 cc に熱時溶解、一夜放置し結晶の析出しないことを確認してから、PdO を加えて接触還元を行ない H_2 を吸収させる。反応液は Pd を濾取した後減圧下石炭ガス気流中溶媒を留去。残渣を EtOH より再結晶。無色菱状晶 mp 163~165.5° (文献⁵⁾ 値 mp 162° (158° で収縮)。 $\text{C}_{14}\text{H}_{15}\text{O}_4\text{N}$ 計算値 C 64.36, H 5.79, 実験値 C 64.66, H 5.70。

Tetrahydroskimmianine (IV) skimmianine (I) 0.3 g を AcOH 90 cc に溶解し PtO_2 を加えて接触還元を行なう。 H_2 吸収後 Pt を濾去し減圧下石炭ガス気流中溶媒を留去。残渣を EtOH より再結晶。無色柱状晶 mp 182~184° (文献²⁾ 値 mp 183°)。 $\text{C}_{14}\text{H}_{17}\text{O}_7\text{N}$ 計算値 C 63.86, H 6.51, 実験値 C 64.10, H 6.70。

Isoskimmianine (II) skimmianine (I) 2.5 g に MeI 3 cc を加え封管中沸騰水浴上 3 hr 反応。冷後 MeI を留去し残渣を EtOH より再結晶。無色針状晶。mp 187~189° (文献⁶⁾ 値 mp 185°) 収量 2.15 g。 $\text{C}_{14}\text{H}_{18}\text{O}_4\text{N}$ 計算値 C 64.86, H 5.05, 実験値 C 64.69, H 5.04。

Tetrahydroisoskimmianine (V) isoskimmianine (II) 0.3 g を EtOH 45 cc に溶解し PtO_2 を加えて接触還元。 H_2 飽和後触媒を濾取し減圧下石炭ガス気流中溶媒を留去。残渣を aq EtOH より再結晶。無色羽毛状晶。mp 120°(decomp) (文献⁷⁾ 値結晶水 1 分子を有するもの mp 120° (109° で収縮)。本物質を 0.5 mmHg に 90° で P_2O_5 上 10 hr 乾燥すれば結晶は微細粉末化する。mp 155~157° (文献⁷⁾ 値無水物 mp 157°)。 $\text{C}_{14}\text{H}_{17}\text{O}_4\text{N}$ 計算値 C 63.86, H 6.51, 実験値 C 64.03, H 6.59。

Tetrahydronorskimmianine (VI) tetrahydroskimmianine (IV) 0.3 g に conc HCl 0.6 cc を加えアスベスト上直火にて還流すれば次第に結晶が析出。12 min 後反応を中止し、放冷後 dil NaOH を加えて溶解し、不溶性物質を濾去。母液に HCl を滴下し酸性とし析出する結晶を濾取。水洗後 EtOH より再結晶。無色菊花状晶。mp 203~205° (文献³⁾ 値 mp 202°) 収量 0.2 g。 $\text{C}_{13}\text{H}_{15}\text{O}_4\text{N}$ 計算値 C 62.64, H 6.07, 実験値 C 62.36, H 6.01。

Norskimmianine (VII) skimmianine (I) 1 g を EtOH 40 cc に溶解しこれに conc HCl 8 cc を加え沸騰水浴上 10 hr 還流。反応終了後一夜放置し析出する粗結晶 0.35 g を濾取。ついで dil NaOH に加温溶解した後濾過し濾液に AcOH を滴下し酸性とし析出する沈殿を濾取。析出物を EtOH に溶解した後、水を滴下して再結晶を繰り返せば無色針状晶 mp 232~233° (文献⁸⁾ 値 mp 235~236°) 0.3 g を得。 $\text{C}_{13}\text{H}_{11}\text{O}_4\text{N}$ 計算値 C 63.67, H 4.52, 実験値 C 63.53, H 4.60。

Tridemethyltetrahydroskimmianine (VIII) tetrahydroskimmianine (IV) 0.3 g に HI (bp 126°) 2 cc を加え油浴上 1 hr 還流し、放冷後析出する結晶を濾取。ベンゼンで洗浄 10% EtOH に溶解氷室に一夜放置。ここに析出する結晶を 10% EtOH で再結晶を繰り返せば無色針状晶 0.2 g を得。本物質は mp 140° で発泡し結晶は微粉状となり、さらに加熱を続けければ mp 245~246° で熔融 (文献³⁾ 値 mp 243~244°^{*6})。本物質を 0.5 mmHg 60°, 10 hr P_2O_5 上乾燥すれば最早 140° にて発泡せず mp 245~246° を示す。 $\text{C}_{11}\text{H}_{11}\text{O}_4\text{N}$ 計算値 C 59.71, H 5.01, 実験値 C 59.53, 59.30, H 5.08, 5.04。

Tridemethyltetrahydroskimmianine triacetate (XI) tridemethyltetrahydroskimmianine (VIII) 0.2 g に Ac_2O 0.7 cc およびピリジン数滴を加え水浴上 1 hr 加熱。反応終了後反応液を水中にあげ析出する沈殿を濾取。EtOH より再結晶。無色絹糸状晶。mp 258~259° (文献³⁾ 値 mp 264°) 収量 0.2 g。 $\text{C}_{17}\text{H}_{17}\text{O}_7\text{N}$ 計算値 C 58.79, H 4.93, 実験値 C 59.03, H 5.08。

Dihydroisoskimmianine (IX) isoskimmianine (II) 0.3 g を EtOH 45 cc に溶解し、PdO を加えて接触還元。 H_2 飽和後触媒を濾取。反応液は減圧下石炭ガス気流中溶媒を留去。残渣を Me_2CO より再結晶。無色針

^{*5} 融点はすべて未補正、接触還元の出量はすべて定量的。

^{*6} 文献³⁾ によれば本物質は mp 243~244° と記載され、その分析値 $\text{C}_{11}\text{H}_{11}\text{O}_4\text{N} \cdot 1\frac{1}{2}\text{H}_2\text{O}$ に一致している。著者の乾燥後のものは無水物に元素分析値が一致することからみれば、再結後未乾燥のまま測定した場合の mp 140° は $1\frac{1}{2}\text{H}_2\text{O}$ の結晶水をもつたものであり、無水物の mp は mp 245~246° を示すものと思われる。

状晶 mp 174~175°. $C_{14}H_{15}O_4N$ 計算値 C 64.36, H 5.79, 実験値 C 64.41, H 5.62.

Dihydronorskimmianine (X) norkimmianine (VII) 0.32 g を EtOH 45 cc に溶解. PdO を加え接触還元を行ない H_2 飽和後触媒を濾取. ついで減圧下石炭ガス気流中溶媒を留去. 残渣を Me_2CO より再結晶. 無色微細針状晶. mp 198~200°. $C_{13}H_{13}O_4N$ 計算値 C 63.15, H 5.30, 実験値 C 63.15, H 5.35.

Isodictamnine (XIII) dictamnine (XII) (mp 130~132.5°) 0.2 g に MeI 1.5 cc を加え封管中沸騰水浴上加熱 3 hr. 反応終了後過剰の MeI を留去し残渣を aq EtOH より再結晶. 無色針状晶. mp 187~188° (文献⁹⁾ 値 mp 188°). $C_{12}H_{11}O_2N$ 計算値 C 72.35, H 4.55, 実験値 C 72.43, H 4.77.

Dihydrodictamnine (XIV) dictamnine (XII) 0.2 g を EtOH 10 cc に溶解し PdO を加え接触還元を行ない H_2 飽和後触媒を濾去. EtOH 溶液は減圧下 EtOH を留去. 残渣を aq MeOH より再結晶. 無色絹糸状晶. mp 94~97° (文献⁹⁾ 値 mp 96~97°). $C_{12}H_{11}O_2N$ 計算値 C 71.62, H 5.51, 実験値 C 71.56, H 5.55.

Tetrahydrokimmianine (IV) の $LiAlH_4$ による還元を試み 微粉化した $LiAlH_4$ 1 g を無水 Et_2O 40 cc に浮遊させておき, tetrahydrokimmianine (IV) 0.5 g を無水 tetrahydrofuran 50 cc に溶解したものを 25 min にわたり滴下. 全量を加えた後水浴上 3 hr 還流. 反応液を放冷後 aq Et_2O を加えて $LiAlH_4$ を分解し. さらに微量の $AcOEt$ を加えて完全に分解. ついで Na_2SO_4 飽和水溶液を少量加えた後 Et_2O 層を分取. 水層は Et_2O で数回洗浄. Et_2O 洗液は Et_2O 溶液と合して $MgSO_4$ で乾燥後 Et_2O を留去. 残渣を Me_2CO より再結晶. 無色柱状晶. mp 184~185°. 収量 0.45 g. 本物質は原料の tetrahydrokimmianine (IV) と混融しても融点降下を認めず. また IR スペクトルも全く一致. $C_{14}H_{17}O_4N$ 計算値 C 63.86, H 6.51, 実験値 C 63.57, H 6.20.

N-Methyltetrahydrokimmianine (XV) KOH 0.3 g を MeOH 8 cc に溶解し. これに tetrahydrokimmianine (IV) 0.76 g ならびに MeI 0.5 cc を加えた後. MeOH を加えて全量 20 cc とし水浴上加熱還流. 2 hr 後 KOH 0.2 g ならびに MeI 0.5 cc を加えて加熱還流を続ける. さらにこの操作を 2 回行なう. 反応終了後析出した KI を濾取した後. 少量の MeOH で洗浄し MeOH に合併して減圧下石炭ガス気流中溶媒を留去. 残渣を $CHCl_3$ で抽出. $CHCl_3$ 抽出液は減圧下石炭ガス気流中溶媒を留去し残渣をベンゼンより再結晶. 微細針状晶. 収量 0.18 g. mp 182~184°. 本物質は原料の tetrahydrokimmianine (IV) と混融して融点降下を示さない. 母液は減圧下溶媒を留去. 残渣をリグロイン (bp 83~96°) で抽出し不溶性の tetrahydrokimmianine を除去. 抽出液は再び減圧下完全に溶媒を留去した後無水ベンゼンに溶解. Al_2O_3 (14×75 mm) のカラムクロマトグラフィーにかける. 比較的吸着の弱い部分を取りベンゼンを留去. 残渣を hexane より再結晶. 無色針状晶. mp 94~95°. 収量 0.56 g N-methyltetrahydrokimmianine (XV). $C_{12}H_{13}ON(OCH_3)_2$ 計算値 C 64.96, H 6.91, OCH_3 33.57, 実験値 C 64.96, H 6.91, OCH_3 34.51, 33.27.

Tetrahydrokimmianine の CH_2N_2 によるメチル化 tetrahydrokimmianine (IV) 0.2 g を可及的に少量の EtOH に溶解したものに. nitrosomethylurea 2 g より製した CH_2N_2 の Et_2O 溶液を加え二日放置. さらに nitrosomethylurea 12 g より製した $CH_2N_2 \cdot Et_2O$ 溶液を二日毎に加えて. 脱色しなくなったことを確認してから減圧下 Et_2O を完全に留去. 残渣を無水ベンゼンに溶解. Al_2O_3 (10×50 mm) のカラムクロマトグラフィーにかければ留液の各部より得られる結晶は mp 90~96° を示す. 全量を合して hexane より再結晶. mp 95~96°. 無色柱状晶. 本物質はさきに得られた N-methyltetrahydrokimmianine (XV) と混融融点降下を示さず. また IR スペクトルも全く一致.

N-Methylhexahydrokimmianine methiodide (XX) N-methyltetrahydrokimmianine (XV) 0.31 g を無水 Et_2O 50 cc に溶解し. 氷冷下 $LiAlH_4$ 0.6 g を無水 Et_2O 40 cc に混濁させたものに滴下. 全量を加えた後水浴上 7 hr 還流. 反応終了後放冷し少量の $AcOEt$ を滴下して過剰の $LiAlH_4$ を分解. さらに Na_2SO_4 飽和水溶液を滴下し壁面に沈殿が析出し始めたら滴下を止め. 暫時放置し無機塩の析出を完全にする. 無機塩は濾去し. 濾液を分液して Et_2O 層を取り. 無機塩は少量の Et_2O で洗浄. 洗液は元の Et_2O に合併し $MgSO_4$ で乾燥後減圧下溶媒を留去. 残渣に MeOH 2 cc および MeI 1 cc を加え. 3 hr 水浴上加熱還流させる. 反応終了後減圧下溶媒を留去. $Me_2CO \cdot AcOEt$ 混液より再結晶. 無色柱状晶. mp 155~157° (decomp). 収量 0.3 g. $C_{16}H_{24}O_3NI$ 計算値 C 47.41, H 5.93, 実験値 C 47.48, 47.50 H 6.01 6.19.

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