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Contribuição à Química de Produtos Naturais.

Análise Estrutural e  
Estereoquímica de Alcalóides

( Francisco de Assis Machado Reis )

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BIBLIOTECA CENTRAL

A autocrítica é uma das qualidades mais difíceis de se exercitar. A tarefa de escrever um resumo crítico da nossa própria contribuição científica foi ao mesmo tempo difícil e constrangedora.

O fato deste ser um dos primeiros trabalhos na UNICAMP por essa nova modalidade de concurso, criou-nos dificuldades na sua preparação, pela ausência de tradição, mas por outro lado propiciou-nos a oportunidade de inovar.

Os candidatos, com maturidade científica para fazerem o concurso de Livre-Docência, na sua maioria não dispõem de tempo para conduzir um trabalho de pesquisa pessoal com vistas à preparação de uma tese, dentro dos padrões de originalidade, e uso de recursos nacionais para este fim. Este novo tipo de procedimento é mais condizente com a nossa realidade, além de evitar que os docentes entrem em competição com seus próprios orientados.

## PREFÁCIO

Ao elaborarmos este trabalho procuramos dar uma visão global da nossa produção científica de forma sintética sem prejudicar sua clareza.

No Capítulo I resumimos nossos resultados na área de isolamento e determinação estrutural de produtos naturais. No Capítulo II resumimos as aplicações da ressonância magnética nuclear de próton e carbono-13.

Procuramos evitar a discussão extensa do que havia sido publicado na literatura e para facilitar a compreensão, anexamos cópias dos trabalhos no apêndice. Os resultados ainda não publicados foram discutidos com mais detalhes. No entanto, em nenhum momento buscamos aprofundar a discussão nem tampouco acrescentamos informações experimentais. Isto se deve ao fato de não se tratar de dados oriundos de um trabalho pessoal, mas sim da somatória de resultados de nossos orientados e colaboradores e até mesmo de orientados de colegas que nos convidaram a dar nossa contribuição.

Gostaríamos de deixar registrado o nosso reconhecimento sincero a todos aqueles que contribuíram para a nossa formação e aperfeiçoamento científico:

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## INTRODUÇÃO

A flora brasileira dispendo de aproximadamente 120.000 espécies vegetais é o maior estímulo ao desenvolvimento da Química de Produtos Naturais no país. Para se ter uma idéia, basta verificarmos os Congressos Anuais da Sociedade Brasileira para o Progresso da Ciência. A maioria dos trabalhos se concentra nesta área a ponto dela ter sido desde o início desmembrada da Química Orgânica, constituindo-se em "disciplina" a parte.

No seu desenvolvimento podemos distinguir tres fases principais: farmacognóstica; fitoquímica e integrada.

A fase farmacognóstica teve seu início em 1847 com a vinda de Theodoro Peckolt, farmacêutico alemão e verdadeiro precursor da química de produtos naturais no Brasil. Sua notável contribuição consistiu na descrição e estudo de cerca de seis mil plantas usadas em medicina popular, trabalho este continuado por seu filho Gustavo Peckolt e neto Oswaldo Peckolt. Destaquemos também a contribuição do botânico Manuel Pio Correa que nos deixou uma obra notável que é o "Dicionário de Plantas Úteis do Brasil" de grande importância para quem se dedica à Química de Produtos Naturais (Q. P. N.).

A fase fitoquímica teve seu início no final da década de 50, no Rio de Janeiro, por iniciativa de Walter Mors. Inicialmente, o grupo de químicos incluía Otto R. Gottlieb e Mauro T. Magalhães no Instituto de Química Agrícola, tendo na época como Diretor Fausto Aita Gai. Posteriormente, o grupo de Mors se transferiu para a Faculdade de Farmácia da UFRJ com o apoio de Paulo Lacaz. Este grupo congregou, Benjamin Gilbert, Roderick Barnes, Keith Brown, Afonso P. Seabra, e no Instituto Nacional de Tecnologia,

Líbero Antonaccio, tendo Otto Gottlieb logo se transferido para a Universidade de Brasília criando um núcleo independente. Estes pesquisadores do maior quilate foram os responsáveis pela formação da maioria dos químicos que hoje se dedicam a esta disciplina.

A terceira fase, que chamamos integrada, começou a ser articulada desde a década de 70. Seu objetivo seria de integrar à química, a botânica e a farmacologia. Seria uma consequência natural, após o estágio avançado que cada uma destas disciplinas alcançou individualmente. Um programa interdisciplinar propiciaria benefícios inegáveis ao país. Se a nossa colocação é feita no condicional é porque consideramos que esta fase, de fato, ainda não foi alcançada. A pesquisa interdisciplinar necessita de um programa de apoio financeiro que seria o catalizador da integração. Todas as iniciativas neste sentido foram decididamente apoiadas pela comunidade científica envolvida mas bloqueadas pela incompreensão da tecno-burocracia que tem dominado os organismos de fomento à pesquisa neste país.

Não fosse a insistência de alguns grupos que com os poucos recursos disponíveis insistem em realizar este trabalho integrado, diríamos que a terceira fase não existe. De fato na situação presente estamos ainda na fase fitoquímica engatinhando para a integração.

A nosso ver, a grande limitação da Q.P.N. no Brasil é a ausência de uma infraestrutura básica para testes biológicos o que permitiria o cumprimento de um de seus maiores objetivos, qual seja o de aplicar o grande potencial de suas descobertas em benefício da nossa comunidade. Os custos de implantação de um laboratório para triagem de atividade biológica são elevados mas se justificam plenamente por fechar este elo de trabalho interdisciplinar



de importância fundamental neste país. De nada adianta falar de potencialidade das aplicações de nossas plantas como fonte de medicamentos sem que tenhamos meios de testá-las até para proteger nossas populações de um possível mau uso.

Por razões até culturais, via de regra, nossa atenção é voltada para as aplicações farmacêuticas. No entanto não podemos perder de vista que a química de produtos naturais tem muitas outras aplicações e que economicamente são até mais importantes, como na perfumaria, alimentos, corantes, resinas agentes tensoativos, etc.

Ao iniciarmos nossas atividades de pesquisa nesta Universidade procuramos, antes, avaliar as áreas de nosso interesse, que fossem complementares aos grupos de pesquisa existentes e que permitisse trazer nossa contribuição. Os alcalóides constituem uma grande classe de produtos, com aproximadamente 5.000 compostos conhecidos, que atraiu pouco interesse dos químicos no Brasil à exceção de Líbero Antonaccio, Benjamin Gilbert e seu grupo até abandonarem esta área de pesquisa no início da década de 70. Por serem derivados de aminoácidos, estas substâncias possuem um grande potencial de atividade biológica constituindo-se num excelente campo de pesquisa.

Desde o início as nossas pesquisas objetivaram o isolamento, a determinação estrutural, a síntese, a aplicação de métodos rápidos de determinação estrutural e o estudo estereoquímico dos produtos naturais, principalmente de alcalóides indólicos e isoquinolínicos de diversos tipos.

Isolamos cerca de tres dezenas de alcalóides indólicos dentre os quais seis estruturas novas<sup>1,2</sup>, alguns alcalóides isoquinolínicos<sup>3</sup>, furoquinolínicos<sup>4,5</sup> e outras substâncias não al-

caloídicas<sup>6</sup>.

Em nosso trabalho estivemos sempre preocupados com a atividade biológica dos extratos e produtos isolados. Neste sentido em colaboração com o Dr. Urbano M. F. Meirelles<sup>3,7-9</sup> e Dr<sup>a</sup> Nilce Meirelles estudamos a ação sobre a musculatura lisa e efeitos bioquímicos<sup>10</sup> de vários compostos benzilisoquinolínicos; e com Dr<sup>a</sup> Marilda M. de Oliveira a atividade anticancerígena de alcalóides indólicos<sup>11</sup>. Infelizmente as dificuldades em continuar este trabalho conjunto com os farmacologistas foram crescentes até descontinuarmos momentaneamente esta parte do nosso projeto científico.

A contribuição mais significativa que pudemos trazer situa-se no estudo de Ressonância Magnética Nuclear de <sup>13</sup>C de produtos naturais. Tivemos oportunidade de analisar: ácidos graxos<sup>12</sup>; ácidos diidrocinâmicos<sup>6</sup>; alcalóides peptídicos<sup>13</sup>; alcalóides indólicos<sup>14,15</sup> e alcalóides isoquinolínicos de diversos tipos<sup>16-22</sup>. Neste último grupo de substâncias, isoquinolínicos, poucas referências constavam da literatura e nos dedicamos com afinco para localizar fontes destes produtos ou conseguir coleções de substâncias que nos permitiram estudá-las sistematicamente e publicar diversos trabalhos<sup>16-22</sup>. Estudamos cerca de 80 compostos que certamente são hoje de grande valia para pesquisadores atuando nesta área de produtos naturais.

A análise dos alcalóides bisbenzilisoquinolínicos foi certamente o trabalho mais complexo que realizamos, tendo sido nosas as primeiras referências na literatura sobre Ressonância Magnética Nuclear de <sup>13</sup>C dessas substâncias, apesar de outros grupos fora do país estarem dedicados também a este mesmo objetivo.

## CAPÍTULO I

### ISOLAMENTO E DETERMINAÇÃO ESTRUTURAL

A química dos alcalóides é muito atraente, seja pelo potencial de atividade biológica ou pela grande diversidade de estruturas que se constitui num desafio acadêmico a qualquer pesquisador atuando na área de produtos naturais. Tratam-se de substâncias freqüentemente frágeis e seu manuseio exige metodologia cuidadosa que permita sua separação e purificação. Por outro lado estas bases são convenientes para a preparação de sais hidrossolúveis que são mais apropriados para os ensaios de atividade biológica.

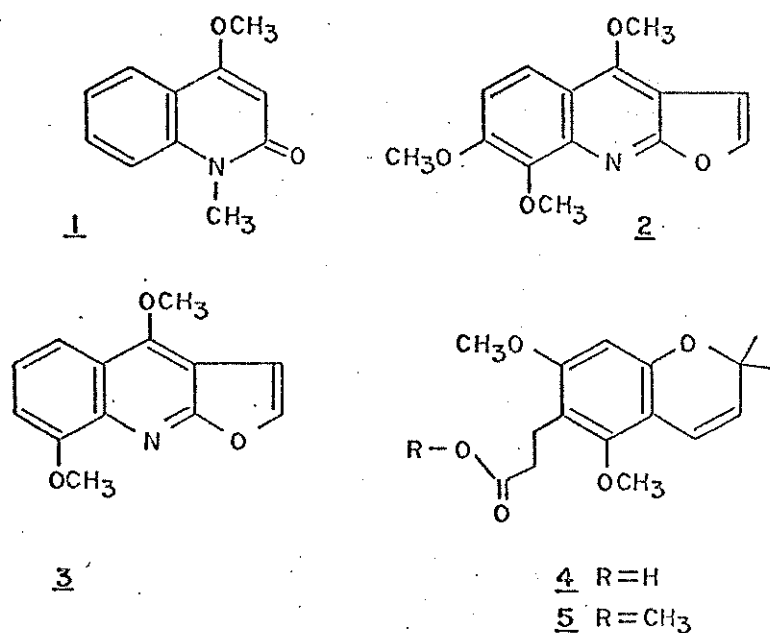
Concentramos nossos esforços no estudo dos alcalóides indólicos e por consequência nosso interesse voltou-se para a família Apocinaceae. Desta tivemos a oportunidade de estudar duas espécies de Aspidosperma: Aspidosperma pruinosum e Aspidosperma ramiflorum e uma espécie do genero Peschiera - Peschiera fuchsiaeifolia. Mais de duas dezenas de compostos foram isolados sendo seis deles novos. Tivemos também oportunidade de estudar uma Rutaceae - Adiscanthus fusciflorus, uma Lauraceae - Ocotea sp, uma Moniaceae - Daphnandra dielsii e uma Menispermaceae - Chondodendron tomentosum.

#### 1. Adiscanthus fusciflorus

Esta espécie Rutaceae foi o objeto de trabalho <sup>4-6</sup> de Maria de Nazare V. McDougal, ex-aluna, procedente do Instituto Nacional

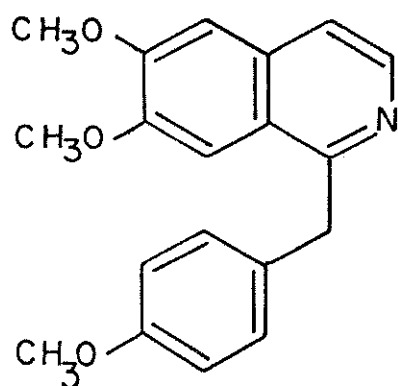
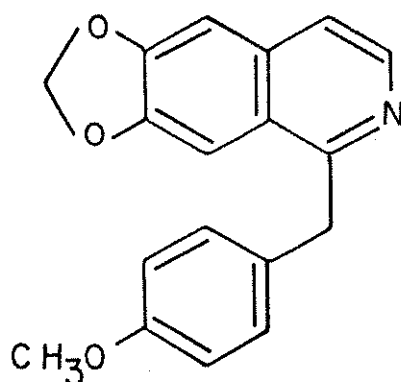
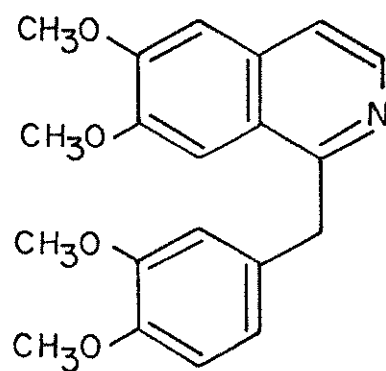
de Pesquisa da Amazônia. Os extratos desta planta foram simultaneamente enviadas ao nosso laboratório e ao do Prof. Gottlieb, sem o nosso conhecimento recíproco, tendo levado ao isolamento praticamente das mesmas substâncias.

Obtivemos alcalóides N-metil-4-metoxi-2-quinolona 1, skimianina 2 e  $\gamma$ -fagarina 3 além de dois produtos novos derivados do ácido diidrocinâmico, 3-[2', 6'-dimetoxi-6'', 6''-dimetilpirano(2'', 3'': 4', 3')fenil]-propiónico 4 e seu éster metílico 5.



## 2. Ocotea sp

Desta espécie de Lauraceae isolamos dois alcalóides benzilisoquinolínicos: 1-(p-metoxibenzil)-6,7-dimetoxi isoquinolina (DMP) 6 e 1-(p-metoxibenzil)-6,7-metilenodioxi isoquinolina (MMIQ) 7 com o objetivo de manter um trabalho de colaboração com o Departamento de Farmacologia consistindo de um estudo de estrutura versus atividade biológica destes compostos muito semelhantes à papaverina 8 <sup>3,7-10</sup>.

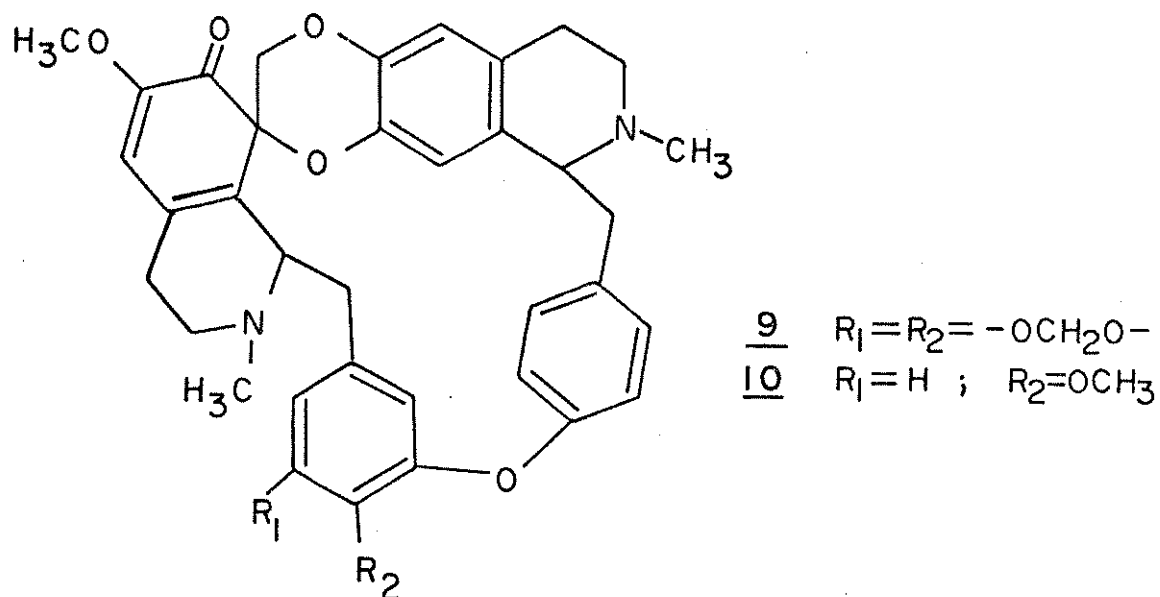
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O isolamento destes compostos nos permitiu comparar atividade biológica do DMP 6 e MMIQ 7 com a papaverina cuja ação é tradicionalmente conhecida. Por outro lado nos permitiu o início do estudo de Ressonância Magnética Nuclear de  $^{13}\text{C}$  (RMN de  $^{13}\text{C}$ ) dos alcalóides isoquinolínicos.

### 3. Daphnandra dielsii

Uma espécie australiana da família Menispermaceae, cujo extrato nos foi enviado pelo Prof. Ian Ralph C. Bick, foi estudada por Luzia Koike<sup>22</sup> visando isolar alcalóides bisbenzilisoquinolínicos com o objetivo de analisá-los por RMN de  $^{13}\text{C}$ .

Foram isoladas deste extrato a repandulina 9 e dielina 10 com estruturas não confirmadas. Foi desenvolvido um trabalho de reações químicas conjuntamente com análise de RMN de  $^{13}\text{C}$  destes compostos e seus derivados, tendo sido confirmada quase que totalmente a estrutura destes dois alcalóides.



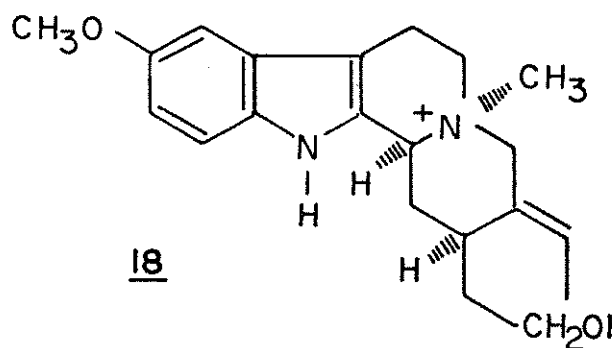
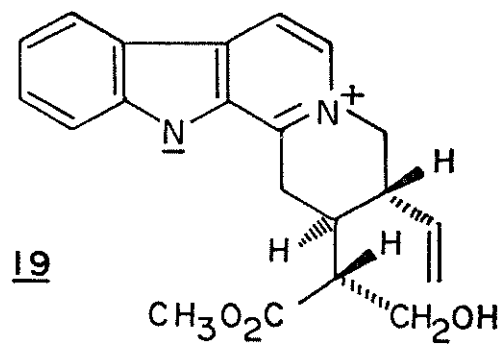
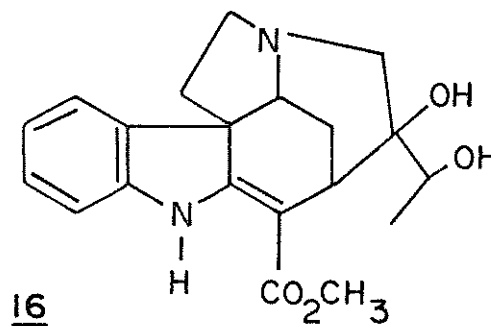
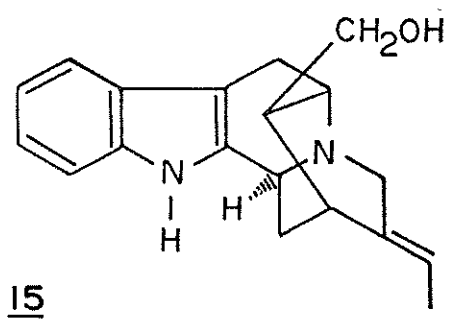
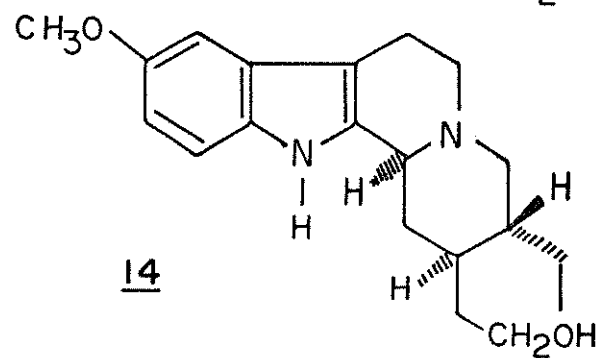
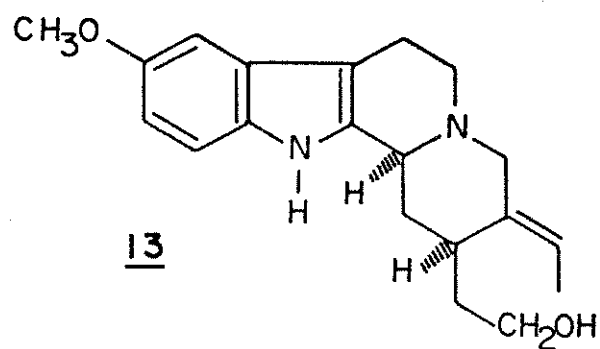
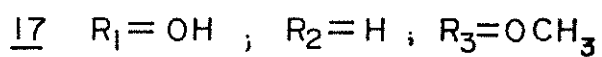
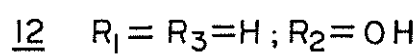
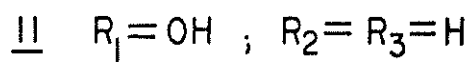
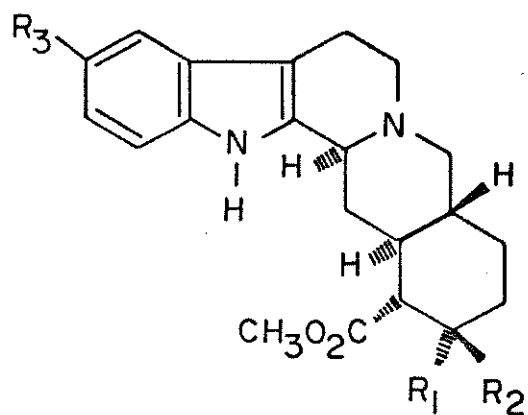
#### 4. Aspidosperma pruinorum - Aspidosperma ramiflorum

As espécies do gênero Aspidosperma foram distribuídas em oito séries tendo sido sistematicamente estudadas por B. Gilbert<sup>23</sup> e seu grupo que então reuniu as substâncias isoladas em tres grupos distintos (ver quadros 1 e 2 pag. 13 e 14) procurando dessa forma estabelecer uma correlação entre critérios químicos e morfológicos. Este trabalho foi interrompido antes que todas as espécies tivessem sido estudadas.

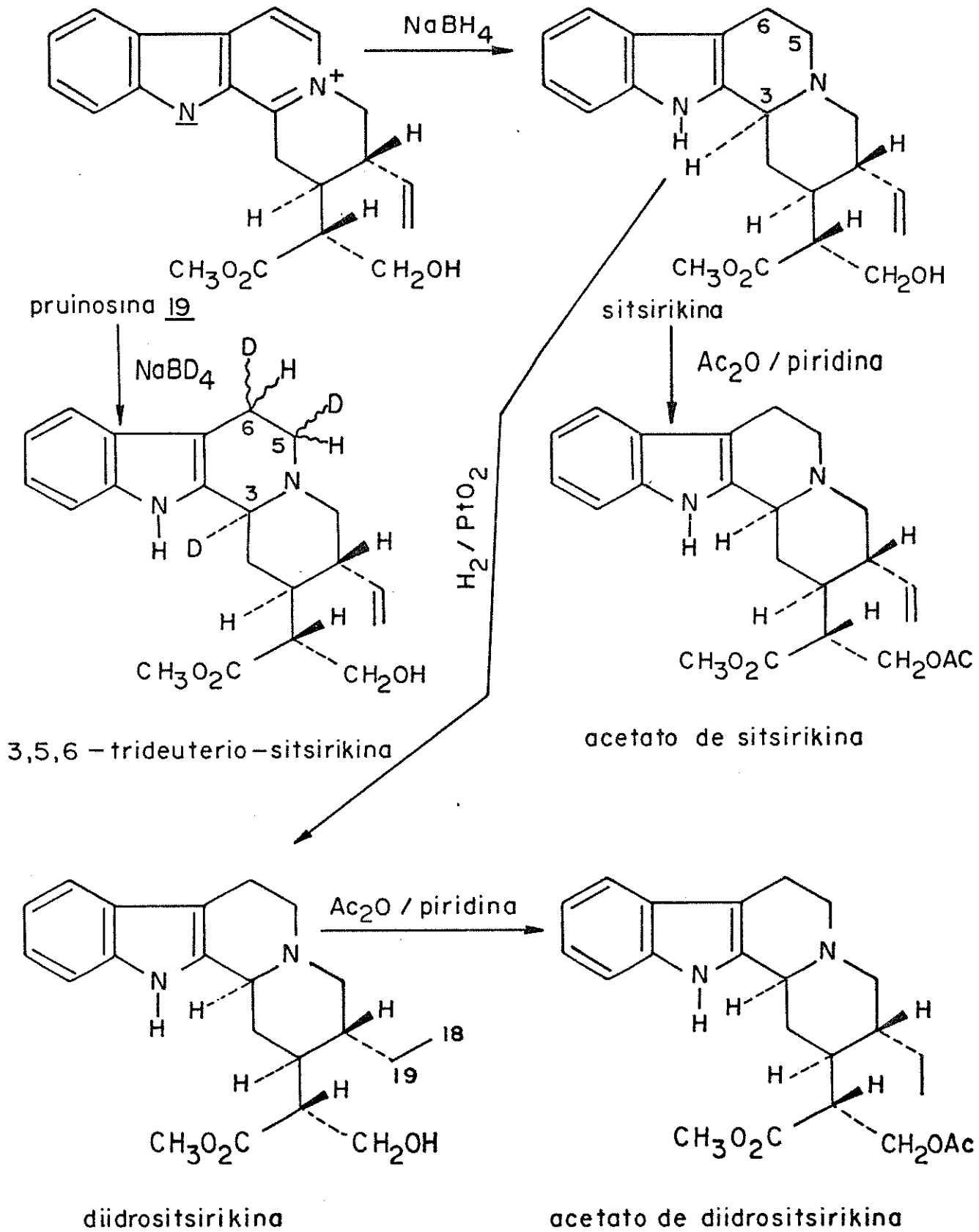
Decidimos então estudar as espécies que não tivessem sido analisadas e verificar se enquadravam no esquema proposto.

Aspidosperma pruinorum é uma espécie extra amazônica que foi estudada por Domingos Savio Nunes em sua Tese de Mestrado. Foram isolados nove alcalóides: yoimbina 11;  $\beta$  yoimbina 12; 10-metoxigeissoschizol 13; 10-metoxi-diidrocorinanteol 14; normacusi-na-B 15; compactinervina 16; 10-metoxiyoimbina 17; pruinósídina 18 e pruinósina 19. Os tres últimos alcalóides são compostos não descritos na literatura.

A determinação estrutural da 10-metoxiyoimbina 17 foi



## Esquema 1



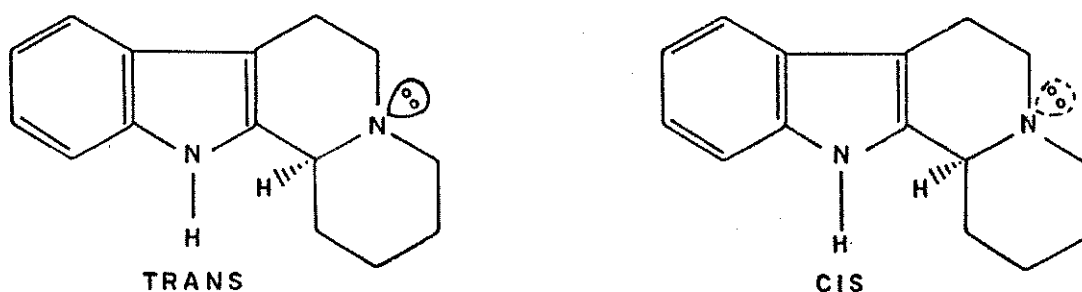


relativamente simples e feita baseada apenas em análise de espectros usuais obtidos sem precisar recorrer a reações químicas. Por RMN de  $^{13}\text{C}$  foi possível confirmar completamente a estereoquímica deste composto.

A pruinósídina 18 também não apresentou grandes dificuldades. Os métodos físicos usuais foram empregados tendo o espectro de massa do composto apresentado muita semelhança ao do 10-metoxigeissoschizol 13. A estrutura e também a estereoquímica foram confirmadas fazendo-se a metilação do composto 13 com iodeto de metila e posterior comparação, principalmente por RMN de  $^{13}\text{C}$ .

A determinação da estrutura da pruinósina 19 demandou uma série de reações químicas associado às medidas espectroscópicas que comparadas com dados da literatura permitiram a fixação total da estrutura inclusive sua estereoquímica (Esquema 1).

O isolamento de indolquinolizidinas se constituiu numa excelente oportunidade de analisar suas diferentes conformações. Até hoje persiste a polêmica em torno da definição entre cis e trans quinolizidinas com diversos autores adotando técnicas (IV, RMN de  $^1\text{H}$  e de  $^{13}\text{C}$ ) e critérios diversos. O problema é ainda mais complexo porquanto se sabe que a rápida inversão do nitrogênio pode interconverter os dois isômeros. Teremos oportunidade de discutir o assunto no Capítulo II quando tratamos de RMN de  $^{13}\text{C}$ .



INDOLQUINOLIZIDINAS

Com exceção da compactinervina 16 que foi isolada em pequena quantidade, todas as demais substâncias pertencem ao grupo II (quadro 1) o que vem a confirmar a proposta de inclusão dessa espécie na série Nitida<sup>23</sup>.

O estudo de Aspidosperma ramiflorum foi interrompido<sup>24</sup> e somente há pouco tempo reiniciado tendo sido isolados apenas dois compostos: yoimbina 11 e  $\beta$  yoimbina 12. Apesar dessas substâncias pertencerem ao grupo II é prematuro incluir Aspidosperma ramiflorum como espécie da série Nitida.

Este trabalho com Aspidosperma foi uma boa contribuição ao conhecimento deste gênero de grande dispersão no Brasil. Gostaríamos de frisar que apesar da correlação entre critérios químicos e botânicos ser boa, achamos que é um trabalho a ser retomado, principalmente se considerarmos que a biossíntese dos alcalóides indólicos é atualmente bem conhecida e os grupos corinano, ibogano e aspidospermano, perfeitamente definidos, são diferentes dos grupos propostos por B. Gilbert.

É fundamental que seja feita uma revisão taxonômica do gênero e que o trabalho químico seja revisto com mais uniformidade nas técnicas de isolamento dos alcalóides. Do nosso ponto de vista a cromatografia líquida de alta eficiência deveria ser usada na separação e quantificação dos alcalóides.

Por outro lado, o isolamento de tres novas substâncias por si só se constitui numa contribuição enriquecedora no campo destes produtos derivados dos aminoácidos, além de aumentar nosso conhecimento sobre a química deste gênero de Apocinacea.



Quadro 2  
CLASSIFICAÇÃO DAS ESPÉCIES ASPIDOSPERMA COM BASE  
EM SEU CONTEÚDO ALCALOÍDAL

SÉRIES	ESPÉCIES	GRUPO I	GRUPO II	GRUPO III
Polyneura	I quebracho-blanco polyneuron cylindrocarpon chakensis	A B G A B B A B	J A G	C
Polyneura	II eburneum olivaceum nigricans	B B		D E F D E F F
Pyricolla	ulei pyricollum gomezianum multiflorum australe parvifolium	B C	D	D F A D D E D E E F F
Tomentosa	subincanum dasycarpon tomentosum		G H	D F D E F C D
Macrocarpa	macrocarpon verbascifolium ducke	F F F		
Macroloba	pyrifolium refractum populifolium	B C C C		C
Nobiles	album spruceanum obscurinervium neblinae limae fendleri sandwithianum megalocarpon exaltum	A D D B E E B D B D A D B E D		
Nitida	marcgravianum auriculatum oblongum discolor rigidum carapanauba pruinatum	B	B C B C B D C F E E B D G	B
	compactinervium			B C

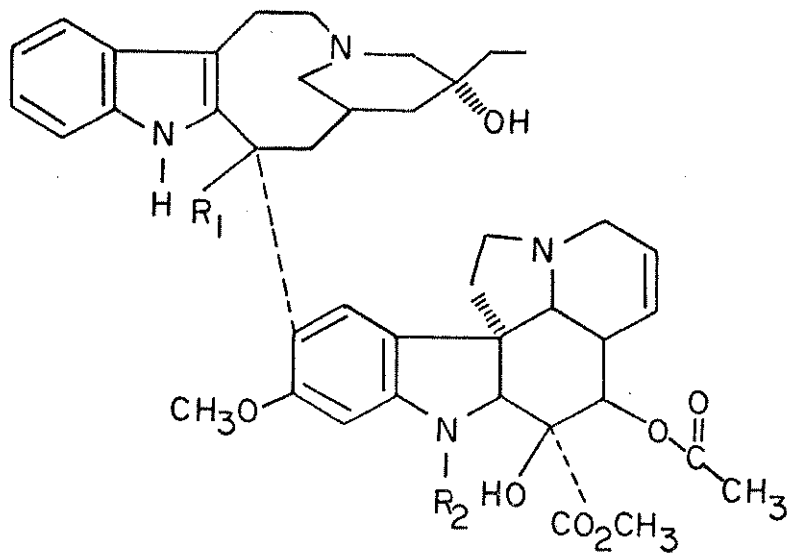
## 5. Peschiera fuchsiaefolia

Esta espécie de Apocinaceae foi objeto da Tese de Mestrado da Sr<sup>a</sup> Raquel Marques Braga<sup>1, 15</sup>.

A atividade anticancerígena evidenciada por alguns alcalóides bisindólicos, dos quais os mais significativos são a vimblastina - VLB 20 e vincristina - VCR 21, nos interessou pelo estudo dessa espécie que sabíamos haver sido isolada a voacamina 22. O trabalho foi dirigido para o isolamento dessas substâncias dimericas e foram isolados: voacamina 22; descarbometoxivoacamina 23; desmetilvoacamina 24 e voacamidina 25 (bisindóis); voacanginaidroxiindolenina 26; perivina 27; 16-epiafinina 28; voacangina 29; voacalotina 30 e afinisina 31.

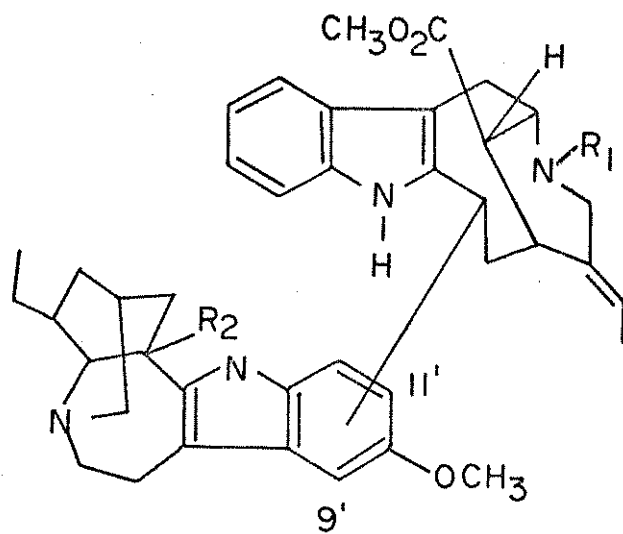
Os diferentes extratos dessa planta foram submetidos a ensaios farmacológicos no Instituto Biológico em São Paulo com a Dr<sup>a</sup> Marilda M. de Oliveira e verificou-se que as frações polares (Extrato G) eram ativas (pag. 18). Tais frações foram estudadas tendo sido isolados tres alcalóides indólicos quaternários de estrutura até então desconhecida: fuchsiaefolina 32; 12-methoxi-Nb-metilvoacalotina 33 e o éster etílico do 12-metoxi-Nb-metilvoacalotina 34.

A determinação da estrutura da fuchsiaefolina 32 foi feita baseada nos dados espectroscópicos usuais (IV, UV, EM e RMN de <sup>1</sup>H) além da RMN de <sup>13</sup>C. O 12-metoxi-Nb-metilvoacalotina 33 e seu respectivo ester etílico 34 tiveram sua estrutura também determinada pelos métodos acima referidos e por um estudo de RMN de <sup>13</sup>C comparativo com o composto 35 preparado por reação da voacalotina 30 com iodeto de metila.



20  $R_1 = \text{CO}_2\text{CH}_3$  ;  $R_2 = \text{CH}_3$

21  $R_1 = \text{CO}_2\text{CH}_3$  ;  $R_2 = \text{CHO}$

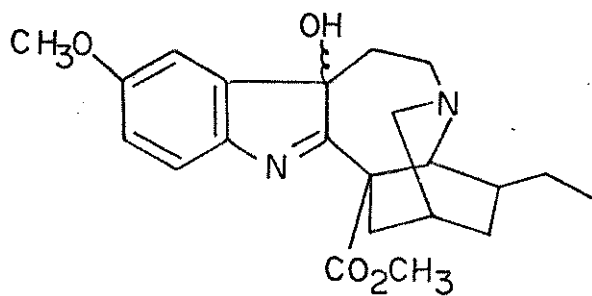


22  $R_1 = \text{CH}_3$  ;  $R_2 = \text{CO}_2\text{CH}_3$  ligação no  $\text{C}_{11'}$

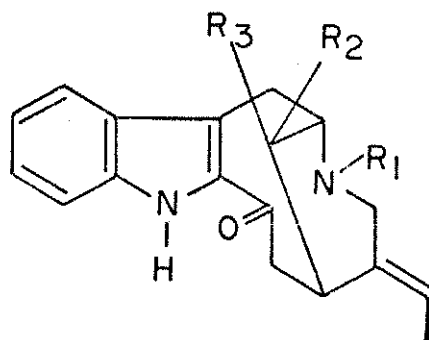
23  $R_1 = \text{CH}_3$  ;  $R_2 = \text{H}$  ligação no  $\text{C}_{11'}$

24  $R_1 = \text{H}$  ;  $R_2 = \text{CO}_2\text{CH}_3$  ligação no  $\text{C}_{11'}$

25  $R_1 = \text{CH}_3$  ;  $R_2 = \text{CO}_2\text{CH}_3$  ligação no  $\text{C}_9$

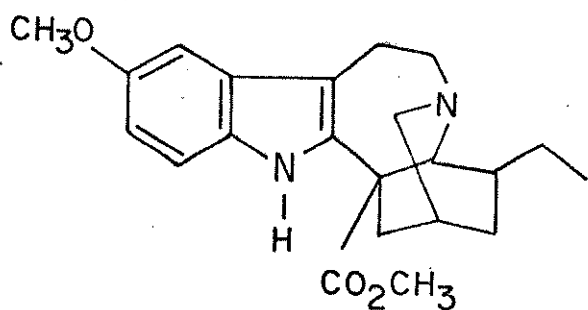


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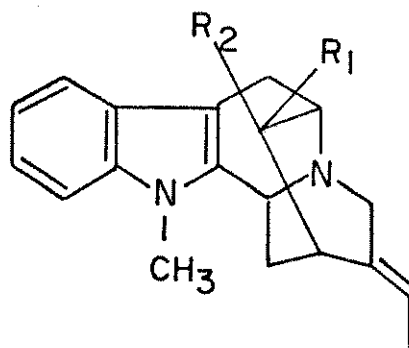


27  $R_1 = R_2 = \text{H}$  ;  $R_3 = \text{CO}_2\text{CH}_3$

28  $R_1 = \text{CH}_3$  ;  $R_2 = \text{CH}_2\text{OH}$  ;  $R_3 = \text{H}$

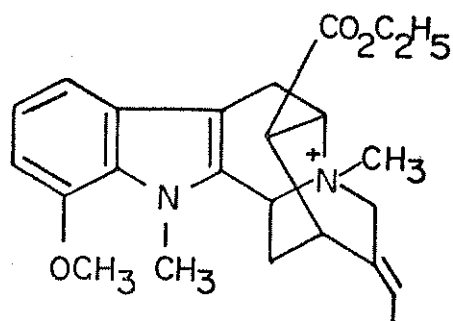
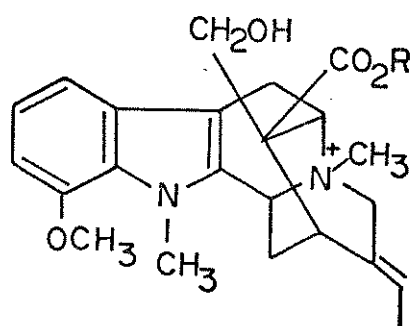
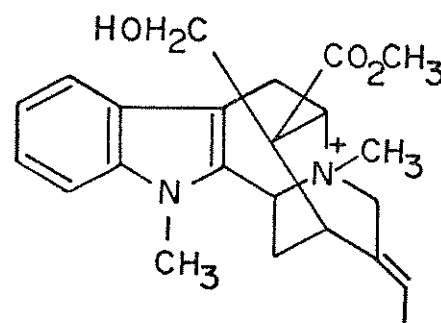


29



30  $R_1 = \text{CO}_2\text{CH}_3$  ;  $R_2 = \text{CH}_2\text{OH}$

31  $R_1 = \text{CH}_2\text{OH}$  ;  $R_2 = \text{H}$

3233 R = CH<sub>3</sub>34 R = C<sub>2</sub>H<sub>5</sub>35

Não nos foi possível submeter estes sais a ensaios biológicos mas o seu isolamento tem como aspecto positivo a descoberta de novos alcalóides quaternários. Estes compostos são incomuns pela presença de uma metoxila na posição 12. Outro aspecto relevante deste trabalho foi o isolamento de perivina 27, composto  $\alpha$  acilindólico que juntamente com voacangina 29 são consideradas precursores dos bisindólicos do tipo voacamina 22.

ENSAIOS BIOLÓGICOS

Os trabalhos experimentais foram realizados com leucemia linfocítica P 388 em camundongos DBA/2, pesando de 15 a 18 gr. O tratamento foi feito por nove dias consecutivos, através da inoculação intraperitoneal da droga. A avaliação dos resultados foi feita marcando-se diariamente o número de animais mortos e calculando-se ao término da experiência o tempo médio de sobrevivência do controle (C) e dos tratados (T). Valores de T/C maiores que 125 % indicam atividades significante.

Os resultados dos testes até agora realizados são:

Subst.	Dose mg/Kg	T/C %	Avaliação
Extr. C	50	121	não ativo
Extr. C	70	257	ativo
Extr. C	70	116,6	não ativo
Extr. C	70	92	não ativo
Extr. G	47,5	125	ativo
Extr. G	60	138	ativo
Extr. G	75	146	ativo
24	32	85	tóxico
24	16	183	ativo
24	16	111	não ativo

A análise dos resultados obtidos até agora são:

Extr. C inativo

Extr. G ativo (compostos polares)

24 demonstra ter atividade, porém em vista dos resultados não concordantes, deveria ser submetido a novos testes <sup>11</sup>.



## CAPÍTULO II

### RESSONÂNCIA NUCLEAR MAGNÉTICA DE $^{13}\text{C}$

A diversidade de estruturas elaboradas pelos organismos vivos sempre trouxe para os químicos desafios que são ao mesmo tempo difíceis e fascinantes a superar. Os compostos orgânicos são disseminados aos milhares e na elucidação de suas estruturas são usados inúmeros métodos químicos e físico-químicos obrigando os que atuam na área de química de produtos naturais a diversificarem seus conhecimentos a fim de poderem trazer uma contribuição científica considerável.

A evolução desta disciplina é cheia de contribuições de outras áreas, notadamente da física, eletrônica e ultimamente da computação sem as quais estaríamos hoje ainda gastando anos de trabalho para determinações estruturais.

O advento da espectroscopia de infravermelho, de ultravioleta-visível, da espectrometria de massa e da Ressonância Magnética Nuclear (RMN) foram decisivas. A difração de Raios X principalmente com os novos difratômetros automáticos colocaram em nossas mãos instrumentais poderosíssimos e capazes de solucionar a grande maioria dos problemas estruturais. A única limitação do Raios X é a exigência de cristais nem sempre disponíveis o que algumas vezes podem ser obtidos através de derivados dos produtos a serem analisados.

A RMN de protons através da análise de deslocamentos químicos e constantes de acoplamento fornece informações conclusivas sobre a estrutura de muitos compostos orgânicos e também teve um progresso considerável com o advento de novos aparelhos de alto

campo magnético (imãs supercondutores) que além de permitirem uma ótima resolução de moléculas complexas traz consigo novas técnicas como INDOR, ressonância diferencial e outras.

Apesar de todas estas vantagens a RMN de  $^1\text{H}$  apresenta como limitação maior o fato de nada revelar sobre o esqueleto de átomos de carbono.

A análise de RMN de  $^{13}\text{C}$  sempre foi possível pois este núcleo possui Spin 1/2 e portanto teoricamente observável. Na prática trata-se de um problema difícil. O  $^{13}\text{C}$  possui um tempo de relaxação longitudinal mais longo do que o  $^1\text{H}$ , sua abundância natural é de apenas 1,1% exigindo grande quantidade de amostra e devido a pequena razão giromagnética do  $^{13}\text{C}$  a sua sensibilidade em abundância natural é menor ( $1,59 \times 10^{-2}$  do que de  $^1\text{H}$ ).

Além das dificuldades acima se sobrepunham outras de ordem instrumental. A baixa razão sinal/ ruído (S/R) poderia ser superada pela acumulação do espectro obtido na memória de um computador, no entanto a estabilidade do aparelho de RMN convencional é baixa para permitir a obtenção de espectro de  $^{13}\text{C}$  em boas condições.

O desenvolvimento da técnica de RMN pulsada com transformada de Fourier permitiu a um só tempo superar todas as limitações acima, transformando a RMN de  $^{13}\text{C}$  num método de rotina no estudo de moléculas orgânicas.

A técnica consiste em irradiar a amostra com um pulso curto de alta potência de forma a excitar ao mesmo tempo todas as frequências de Larmor dos  $^{13}\text{C}$  da molécula. O interferograma obtido contém toda a informação de RMN. Este interferograma é uma função de onda complexo, resultante da superposição de todas as frequências de Larmor estimuladas pelo pulso, que é convertida tematicamente em um espectro de frequências de Larmor e no

espectro de RMN propriamente dito, pela transformada de Fourier. A transformada de Fourier do interferograma do pulso, pode ser feita em segundos por um computador, obtendo-se um espectro com uma relação sinal/ruído (S/R) muito maior do que em um espectrômetro convencional. A relação S/R pode ser aumentada de  $\sqrt{n}$  (n= número de acumulações) se acumularmos os interferogramas na memória do computador antes de procedermos a transformada de Fourier. Dessa forma, a partir de 1972 observamos uma disseminação de equipamentos de RMN com TF e um progresso constante no uso da RMN de  $^{13}\text{C}$ .

Conforme assinalamos anteriormente, a partir do desenvolvimento de aparelhos operando com transformada de Fourier a RMN de  $^{13}\text{C}$  tomou um impulso muito grande e seu potencial aplicável na química de produtos naturais foi detectado. Atribuimos ao Prof. Ernest Wenkert uma grande contribuição nesta área, notadamente no campo dos alcalóides indólicos. Moléculas extremamente complexas foram analisadas e, além da atribuição de deslocamentos químicos, a estereoquímica e conformações foram detectadas numa gama muito ampla de compostos naturais.

A RMN de  $^{13}\text{C}$  atualmente é uma alternativa válida, em muitos casos, à difração de Raios X, principalmente por ser este último instrumento caro e pouco acessível a muitos Laboratórios de Produtos Naturais.

Todos estes aspectos despertaram nosso interesse e decidimos empreender um estudo sistemático de produtos naturais através de RMN de  $^{13}\text{C}$ . Nosso trabalho neste campo se estendeu aos:

Ácidos graxos

Ácidos diidrocinâmicos

Alcalóides peptídicos

Alcalóides indólicos

Alcalóides isoquinolínicos: benzilisoquinolínicos  
oxoaporfínicos  
aporfínicos  
bisbenzilisoquinolínicos

### 1. Ácidos Graxos

A análise de ácidos <sup>12</sup>graxos se restringiu aos E-2 insaturados de seis a nove carbonos envolvendo cálculos teóricos de deslocamento químico baseado em parâmetros de Bus <sup>25</sup> e um estudo dos efeitos eletrônicos na transformação em seus respectivos sais sódicos (Tabela 1). Também foram analisados ácidos graxos insaturados E,E-2,4 de seis a nove carbonos, e seus sais (Tabela 1).

Este trabalho permitiu que se evidenciasse a isomerização da dupla ligação do cloreto do ácido E,E-2,4-octadienóico na preparação do éster ciclopentenílico deste ácido. Observou-se como produto desta reação uma mistura de ésteres do ácido E,E-3,5 e E,Z-3,5-octadienóico na proporção de 1:1.

### 2. Ácidos Diidrocinâmicos

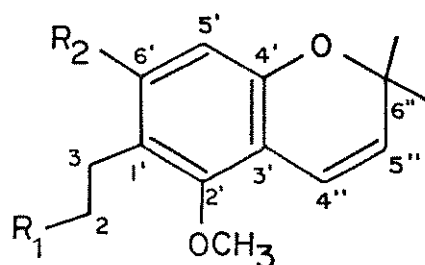
Quando do estudo de Adiscanthus fusciflorus (ver cap. I) foram isolados dois compostos novos derivados do ácido diidrocinâmico: 3-[2', 6'-dimetoxi-6", 6"-dimetilpirano (2", 3"= 4', 3') fenil]-propiónico 36 e seu éster metílico 37.

Fizemos um estudo de RMN de <sup>13</sup>C destes compostos <sup>6</sup> comparando-os com o derivado diidro 38 e o composto 39 que é um produto natural isolado <sup>26</sup> de outra espécie de Rutaceae.

TABELA 1

	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9
Ac. E-2-Hexenoic	171,9	120,7	151,9	34,3	21,1	13,6			
Sal sódico	176,5	128,4	146,4	35,1	22,8	14,9			
Ac. E-2-Heptenoic	172,1	120,7	152,1	32,0	30,0	22,2	13,7		
Sal sódico	175,9	128,5	145,4	32,6	31,7	23,4	15,0		
Ac. E-2-Octenoic	172,2	120,5	152,2	32,2	27,5	31,3	22,4	14,0	
Sal sódico	176,0	128,6	145,0	33,1	32,7	29,4	23,0	14,9	
E-2-Octeno				33,1	32,08	29,97	23,09	14,13	
Ac. E-2-Nonenoic	172,0	120,6	151,9	32,2	27,8	28,8	31,5	22,5	13,8
Sal sódico	175,8	128,7	144,8	33,1	32,8	30,1	29,7	23,8	15,0
E-2-Noneno				33,17	32,43	30,24	29,48	23,24	14,16
Ac. E,E-2,4-Hexadienoic	169,9	118,3	145,8	129,8	139,4	18,0			
Sal sódico	176,0	127,3	142,1	131,4	138,0	19,6			
Ac. E,E-2,4-Heptadienoic	172,6	118,3	147,3	127,2	147,2	26,0	12,7		
Sal sódico	176,6	127,3	142,6	129,1	144,9	27,0	14,2		
Ac. E,E-2,4-Octadienoic	172,5	118,3	147,1	128,2	145,5	35,0	21,8	13,5	
Sal sódico	175,6	128,0	141,7	130,4	141,5	35,9	23,1	14,7	
Ac. E,E-2,4-Nonamienoic	172,8	118,2	147,3	128,0	145,9	32,7	30,7	22,2	13,8
Sal sódico	175,4	128,2	141,6	130,3	141,3	33,4	32,0	23,2	14,9

Este trabalho permitiu confirmar totalmente a estrutura dos produtos naturais isolados

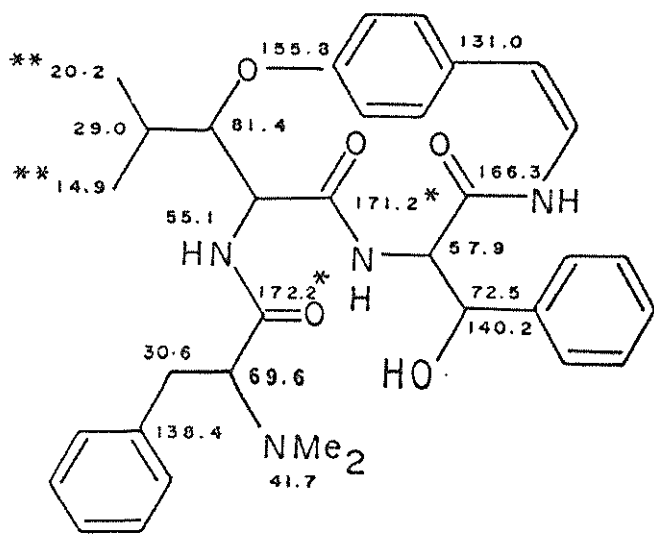


<u>36</u>	$R_1 = \text{CO}_2\text{H}$	$R_2 = \text{OCH}_3$
<u>37</u>	$R_1 = \text{CO}_2\text{CH}_3$	$R_2 = \text{OCH}_3$
<u>38</u>	$R_1 = \text{CO}_2\text{H}$	$R_2 = \text{OCH}_3$ 4'', 5'' dihid
<u>39</u>	$R_1 = \text{CH}_2\text{OH}$	$R_2 = \text{H}$

### 3. Alcalóides Peptídicos

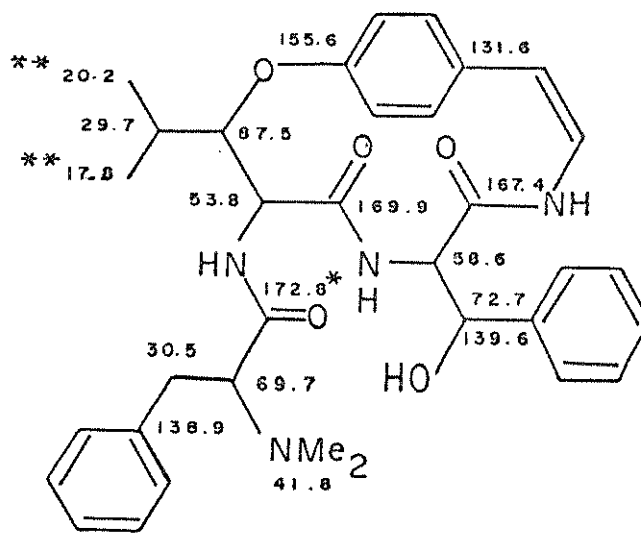
Os alcalóides ciclopeptídicos são compostos macrocíclicos com um anel de 14 membros possuindo interações espaciais muito interessantes de serem estudadas por RMN de  $^{13}\text{C}$ . Analisamos a scutianina D 40, scutianina E 41, seus respectivos o-acetil derivados 42 e 43 e algumas moléculas modelo<sup>13</sup>. Direcionamos nosso trabalho para os carbonos  $\text{sp}^3$  procurando identificar interações que fossem diagnóstico da configuração de determinados aminoácidos presentes nestes alcalóides.

Observamos diferenças marcantes nos carbonos da  $\beta$  hidroxileucina que diferenciavam claramente as configurações L-eritro da scutianina D 40 e D-eritro da scutianina E 41. Este trabalho permitiu, por analogia, atribuir a configuração L-eritro para as discarinas A 44, B 45 e lasiodina B 46 cujos deslocamentos químicos haviam anteriormente sido atribuídos<sup>27</sup>.



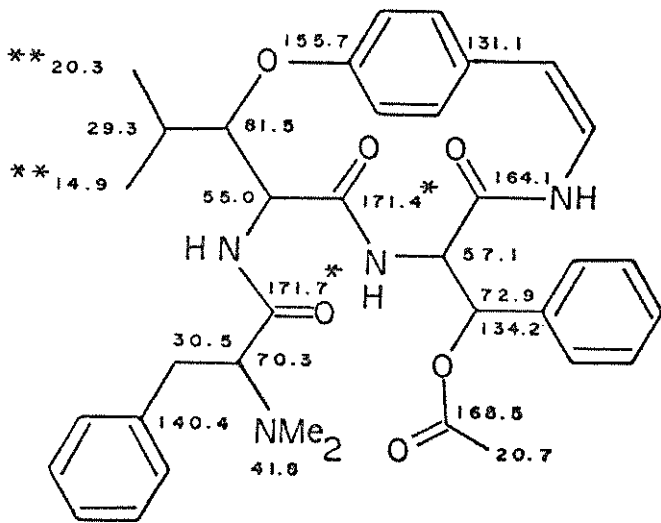
40 Scutianina D

L-eritro-β-hidroxi-leucina-  
-L-treo-β-fenilserina

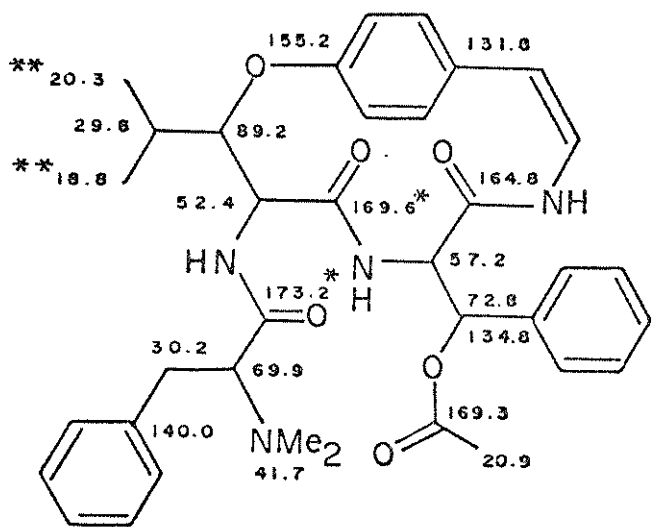


41 Scutianina E

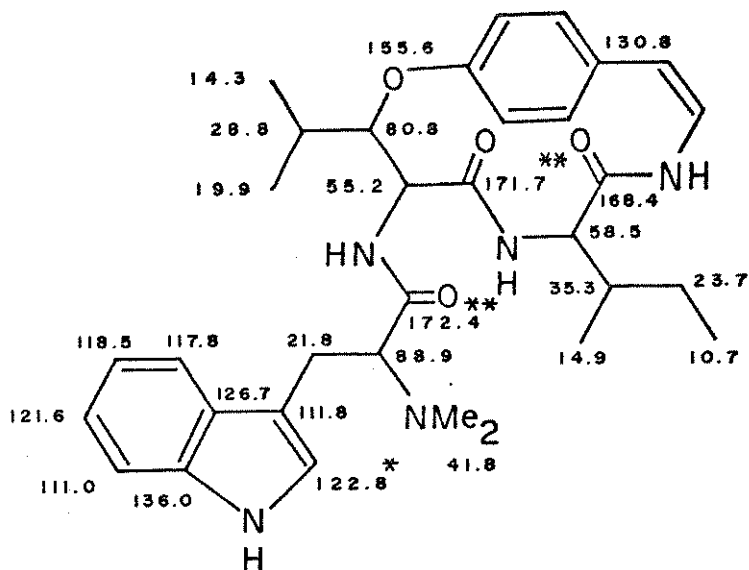
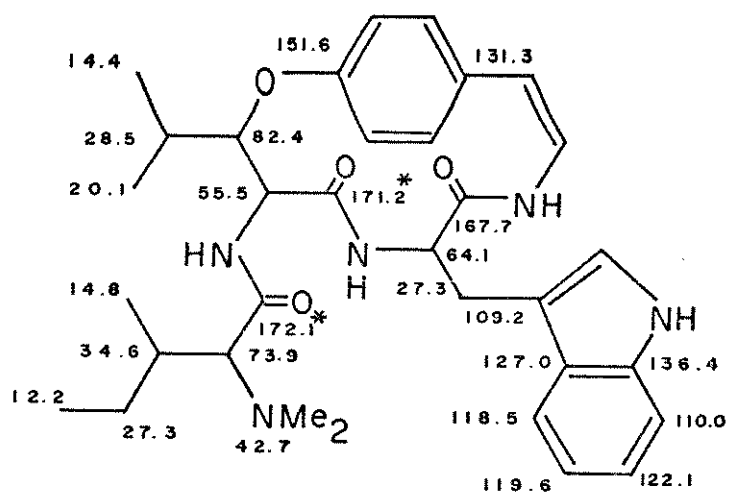
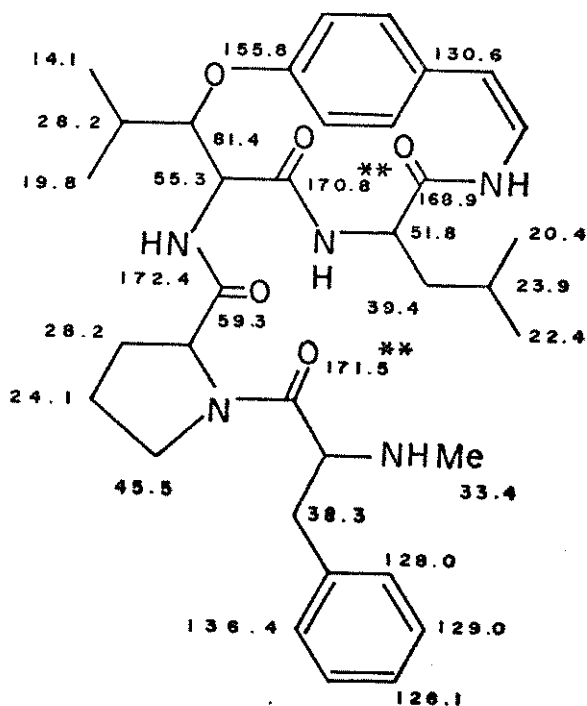
D-eritro-β-hidroxi-leucina-  
-D-treo-β-fenilserina



42 O-acetil Scutianina D



43 O-acetil Scutianina

44 discarina A45 discarina B46 lasiodina B

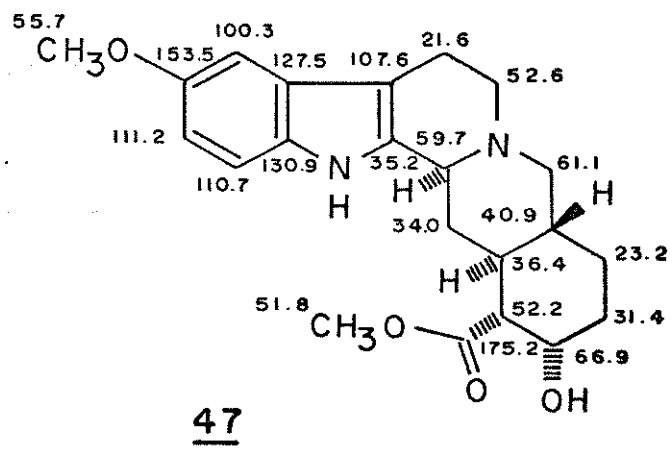


#### 4. Alcalóides Indólicos

Analizamos várias dezenas de alcalóides indólicos entre produtos naturais, seus derivados e compostos sintéticos. Este trabalho abrange vários tipos de indóis, incluindo mono e bis-indóis. Nosso interesse esteve sempre voltado para a análise estereoquímica e conformacional além da simples atribuição de deslocamentos químicos.

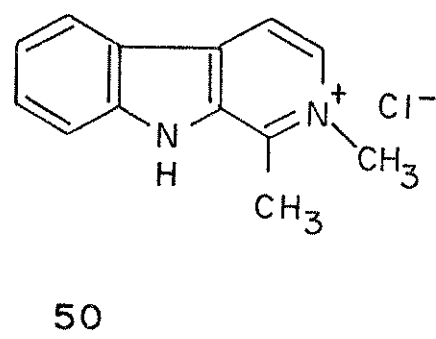
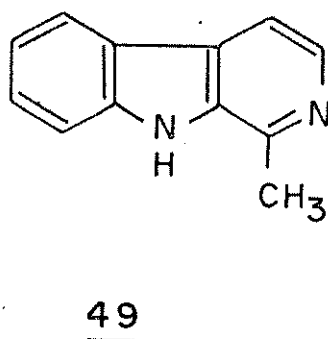
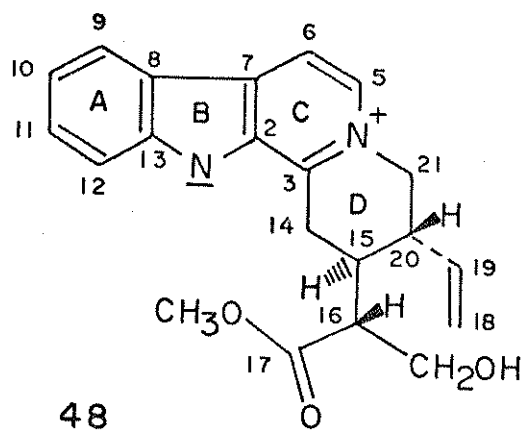
##### a- yoimbinóides

A estrutura da 10-metoxiyuibina 47 foi determinada<sup>2</sup> e sua estereoquímica completamente resolvida sem maiores dificuldades por comparação com dados da literatura<sup>28</sup>.



##### b- Pruinósina, seus derivados e moléculas modelo

Para determinar a estrutura<sup>2</sup> da pruinósina 48, tivemos que recorrer a uma análise completa de seus derivados e moléculas modelo que foram sintetizadas para este fim. Dessa forma analisamos os deslocamentos químicos de <sup>13</sup>C do harmano 49 e Nb-metil-har-

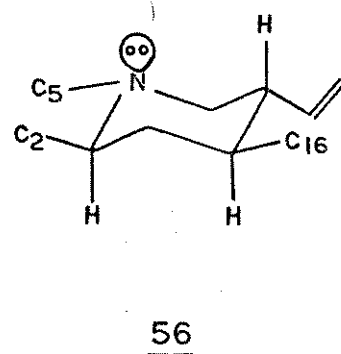
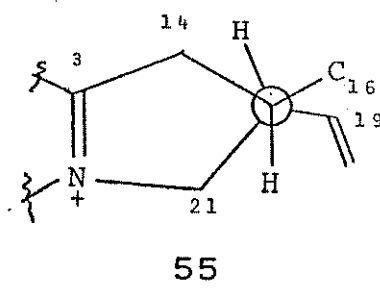
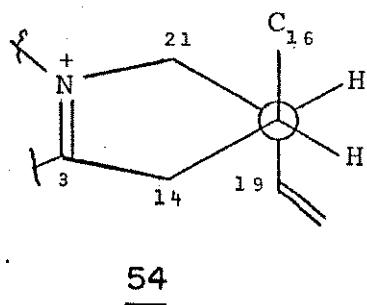


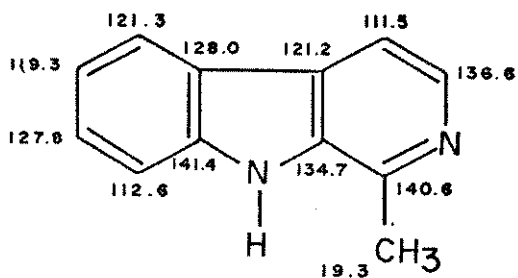
mano 50 previamente ao estudo da pruinósina.

Os deslocamentos químicos dos carbonos dos anéis "A", "B" e "C" da pruinósina foram muito semelhante aos atribuídos ao composto 50.

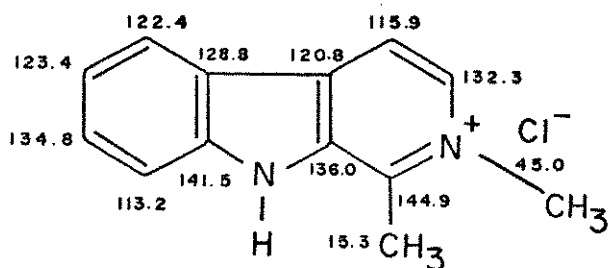
A presença de dois carbonos quirais no anel D, nos levaram a uma análise mais acurada dos carbonos  $sp^3$  procurando identificar as conformações possíveis.

Nas fórmulas 54 e 55 encontram-se as duas semi-cadeiras possíveis. Ambas apresentam barreiras termodinâmicas consideráveis. Quando comparamos as duas semi-cadeiras, observamos que os  $C_{16}$  e  $C_{19}$  na fórmula 54 estão em posição trans-diaxial com interação

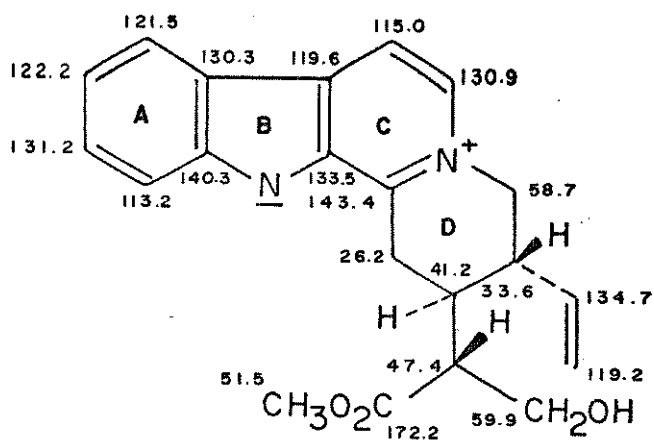




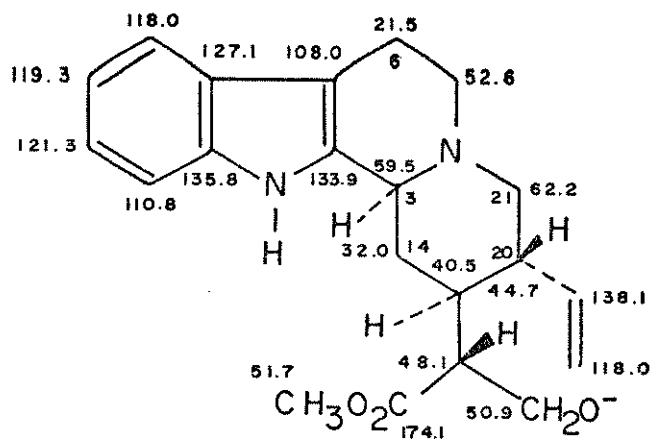
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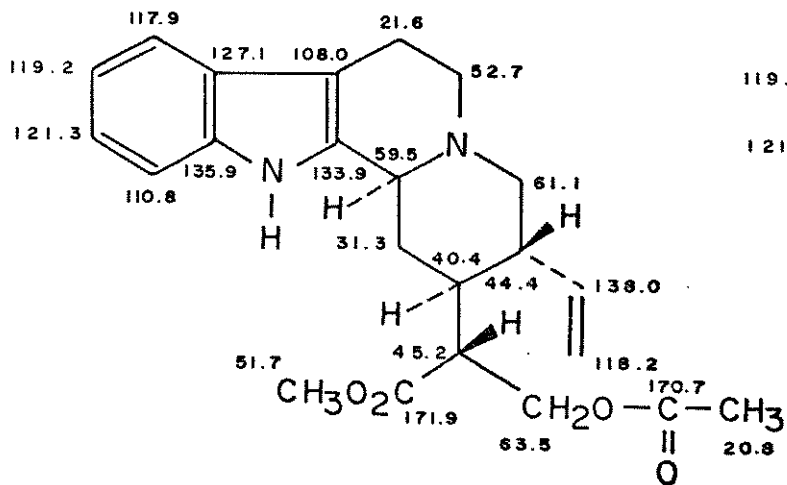
50



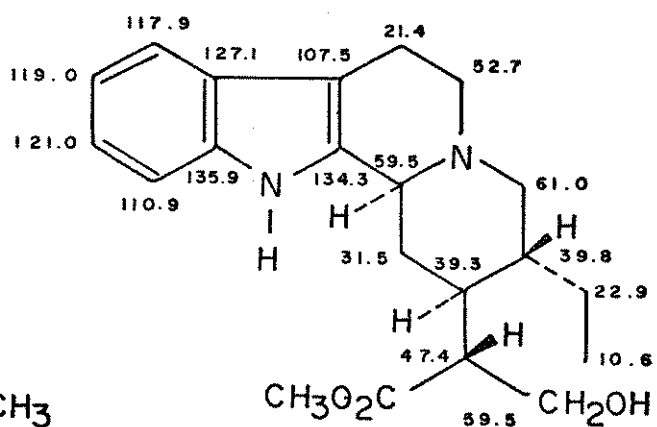
48



51



52



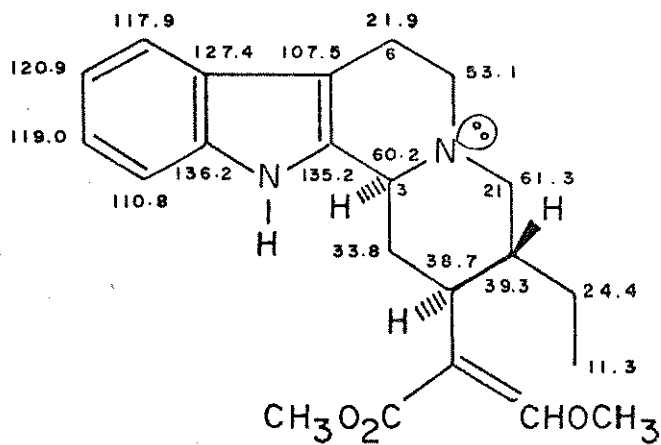
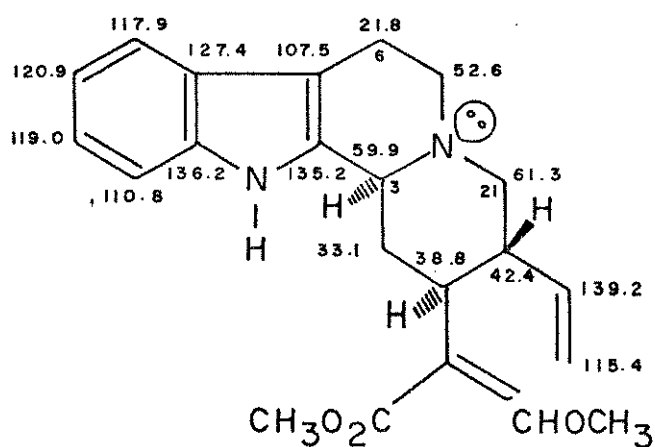
53

butano gauche  $C_{19} - C_{14}$  e  $C_{16} - C_{21}$ . Na semi-cadeira 55 os  $C_{16}$  e  $C_{19}$  tem apenas uma interação butano gauche entre si, com ambos os substituintes em posições equatoriais.

Consideramos que a conformação mais estável é a 55. Comparando-se a pruinósina 48 com seu produto de redução 51 (sitsirikina), cuja conformação mais estável para o anel D é a 56, observamos algumas alterações significativas que atribuímos a efeitos eletrônicos causados pela perda de aromaticidade do anel C. Desproteção no:  $C_{19}$  de  $134,7 \rightarrow 138,1$  ppm ( $\Delta\delta=3,4$  ppm);  $C_{14}$  de  $26,2 \rightarrow 32,0$  ppm ( $\Delta\delta=5,8$  ppm) e  $C_{20}$  de  $33,6 \rightarrow 44,7$  ppm ( $\Delta\delta=11$  ppm).

A atribuição dos deslocamentos químicos dos derivados da pruinósina: sitsirikina 51, acetil-sitsirikina 52 e diidrositsirikina 53 foi feita por comparação com a corinanteína 57 e diidrocoringanteína 58<sup>29</sup>.

Os deslocamentos químicos dos carbonos  $C_3$ ,  $C_6$ ,  $C_{14}$ ,  $C_{15}$ ,  $C_{20}$  e  $C_{21}$  dos compostos 51, 52 e 53 serviram de diagnóstico para o esclarecimento da estereoquímica nos centros quirais  $C_3$ ,  $C_{15}$  e  $C_{20}$ . Os valores em torno de  $C_3$  ~59,5 ppm,  $C_{21}$  ~61,0 ppm e  $C_6$  ~21,5 ppm tanto nos compostos 51, 52 e 53, como em 57 e 58 estudados por



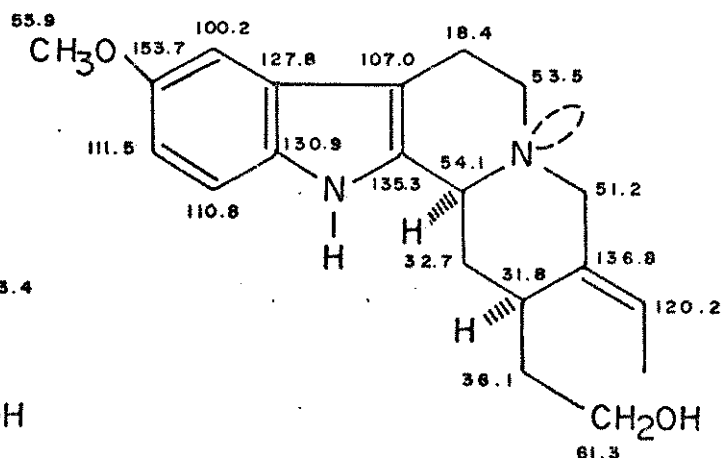
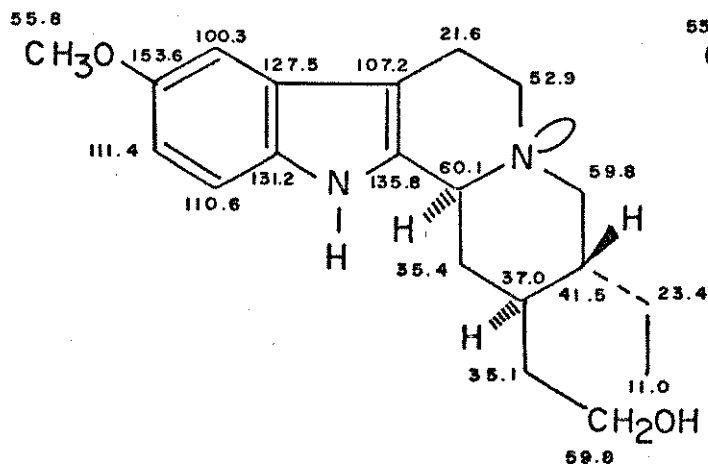
Wenkert, indicam uma junção C/D trans-quinolizidina. Os  $C_{14}$  de 51, 52 e 53, da mesma forma que em 57 e 58, tiveram deslocamentos químicos de  $\sim 32,0$  ppm, e os  $C_5$  de  $\sim 52,5$  ppm.

Os dados obtidos para os carbonos  $C_{14}$   $\sim 32,0$  ppm e  $C_{21}$   $\sim 61$  ppm dos compostos 51, 52 e 53, indicaram que os substituintes em  $C_{15}$  e  $C_{20}$  são equatoriais como na estrutura 56 em comparação com os mesmos carbonos dos compostos 57 e 58.

Todos os derivados da pruinosa 48, possuem sistema quinolizidínico. A maioria dos indóis são trans-quinolizidínicos e vários métodos foram desenvolvidos para diferenciar entre cis e trans. A RMN de  $^{13}C$  é um método eficiente para fazer esta diferenciação.

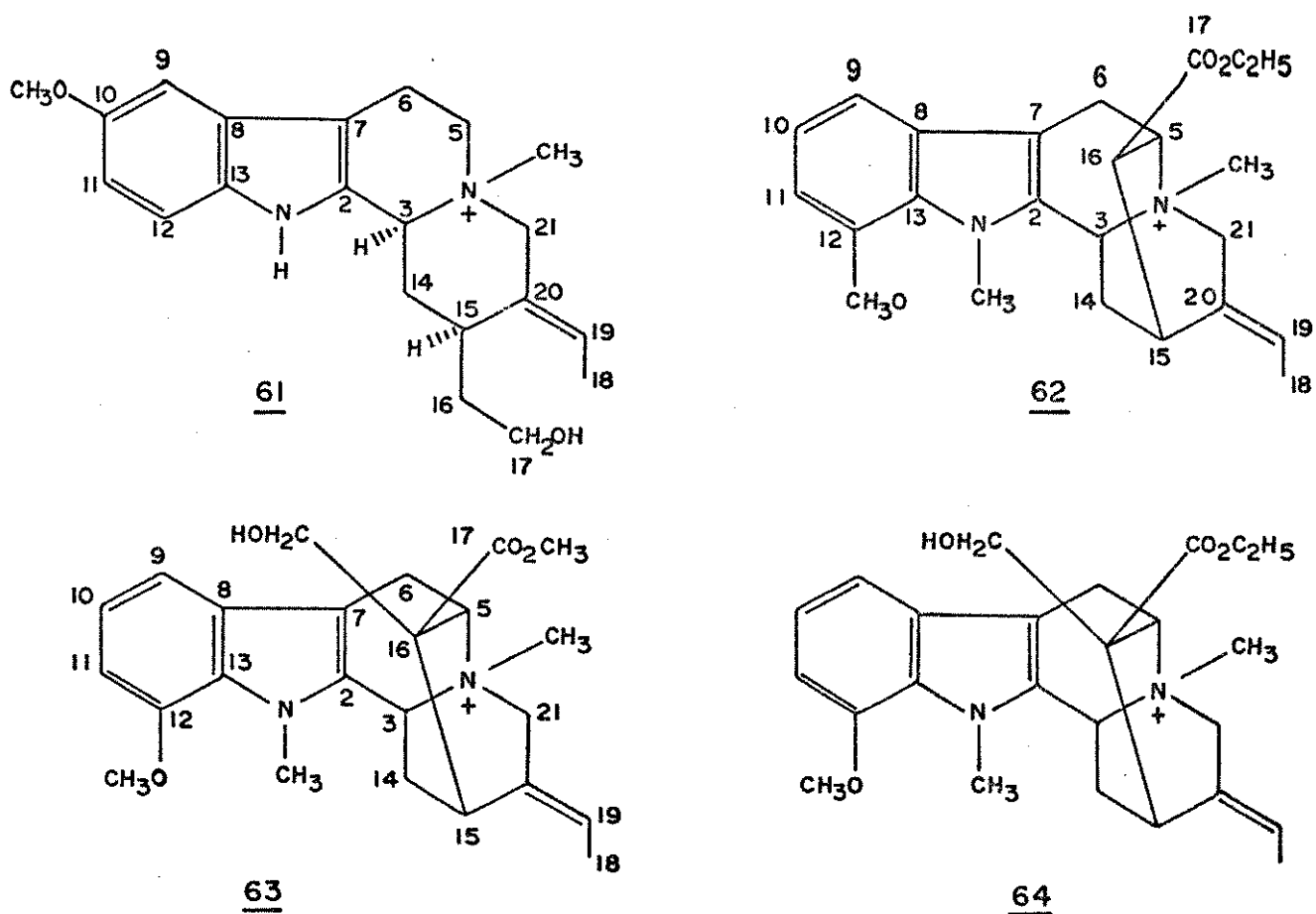
Os deslocamentos do  $C_3$ ,  $C_{21}$  e  $C_6$  são usados como diagnóstico, sendo os valores de  $\sim 59,5$  ppm,  $\sim 61,0$  ppm e  $21,5$  ppm respectivamente, são característicos de junção C/D trans 58 enquanto  $C_3$   $\sim 53$  ppm  $C_6$   $\sim 17$  ppm e  $C_{21}$   $\sim 51$  ppm identificam junção C/D cis (60)<sup>30,31</sup>.

Estudamos ainda dois produtos naturais 10-metoxi-diidrocorinanteol 59 e o 10-metoxi-geissoschizol 60. Os deslocamentos químicos indicam claramente que 59 é um alcalóide trans-quinolizidínico e que 60 é cis-quinolizidínico



c) Alcalóides Quaternários

O isolamento da pruinósídina 61, fuchsiaefolina 62, 12-metoxi-Nb-metilvoacalotina 63 e o éster etílico do 12-metoxi-Nb-metilvoacalotina 64, todos sais de amônio quaternário, nos levaram a estudar estas substâncias por RMN de  $^{13}\text{C}$  e comparar os efeitos da quaternarização do Nb em comparação com estruturas semelhantes dos alcalóides isoquinolínicos.



Este estudo exigiu uma análise mais extensa incluindo algumas moléculas sintéticas e produtos de metilação de substâncias naturais relacionadas. Dessa forma foram incluídas: o tetraidroarmano 65, Nb-metil-tetraidroarmano 66, afinisina 67, Nb-metil-afinisina 68, voacalotina 69, Nb-metil-voacalotina 70 e 10-metoxi-geissoschizol 71 (Quadro 3, pag.35).

A maioria destes indóis são do tipo sarpagina que possuem uma quinuclidina na sua parte terpênica. Esta estrutura é rígida o que permite uma análise da estereoquímica sem as dificuldades de sistemas que permitem trocas de conformações.

Antes de abordarmos os alcalóides quaternários atribuímos os carbonos da normacusina-B 72, afinisina 67 e voacalotina 69 que são alcalóides terciários. Não existem grandes diferenças nos carbonos  $sp^2$  destes compostos exceto no anel de cinco membros de 72 o que é explicado pela ausência da metila e o  $C_{12}$  que está desprotegido pela perda da interação  $\gamma$  com o  $N-CH_3$ .

O aspecto mais relevante deste trabalho é a observação dos efeitos causados nos carbonos  $sp^3$  em função da configuração do  $C_{16}$ . Comparando-se os deslocamentos do  $C_6$  e  $C_{14}$  nos tres compostos observamos que nos compostos 72 e 67 estes carbonos absorvem a -27 e -32 ppm respectivamente enquanto no 69 que possui um substituinte endo em relação ao indol, os valores são 22,2 e 28,2 ppm. Esta proteção observada é consequência do substituinte que na posição endo está eclipsado em relação ao  $C_6$  e gauche em relação ao  $C_{14}$  (Figura 1). A observação destes carbonos ( $C_6$  e  $C_{14}$ ) pode ser usada como diagnóstico para a configuração do  $C_{16}$  destes sistemas.

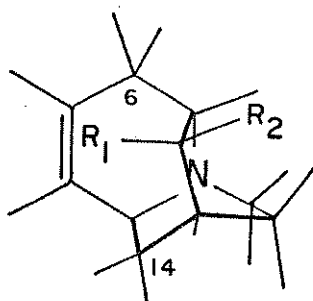


FIGURA 1

Nb-metil-tetraidro-harmano 66

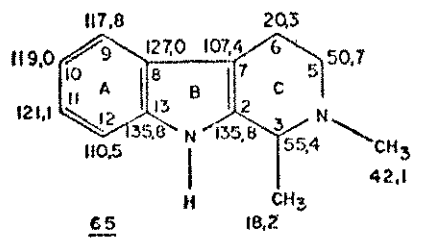
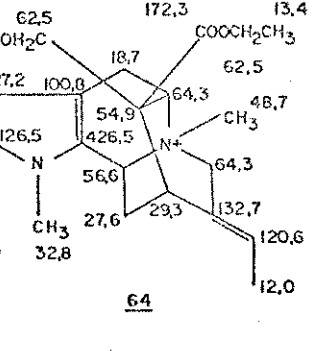
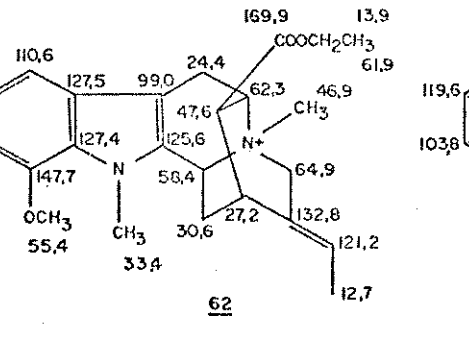
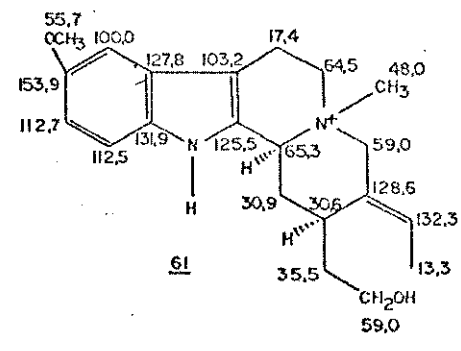
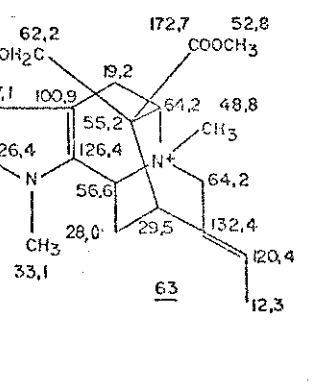
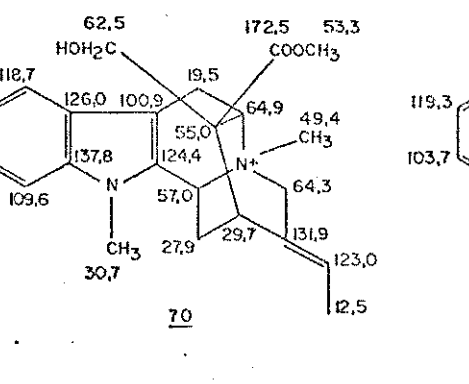
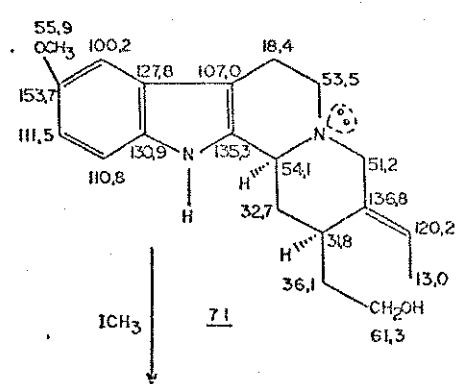
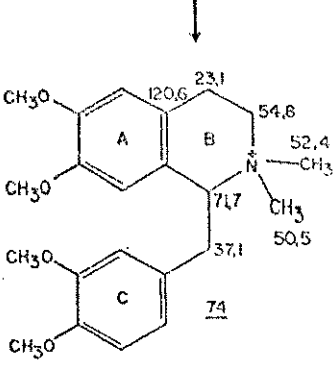
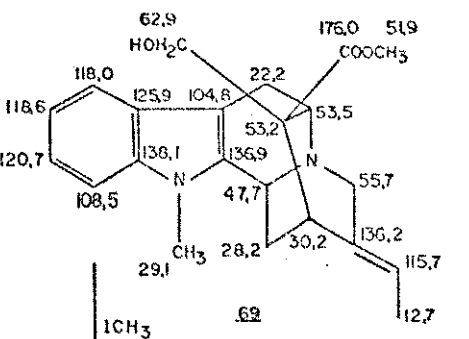
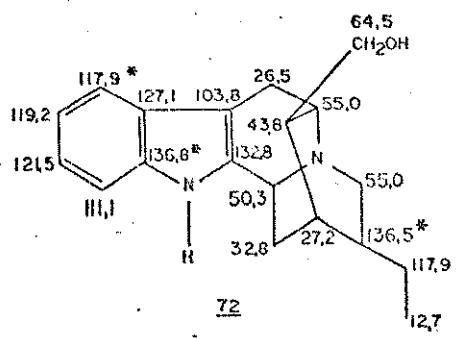
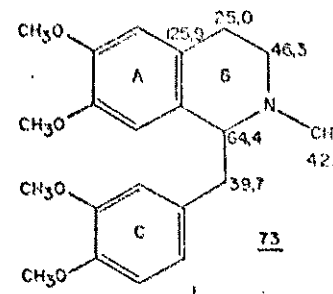
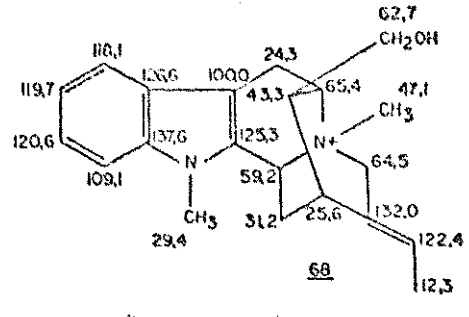
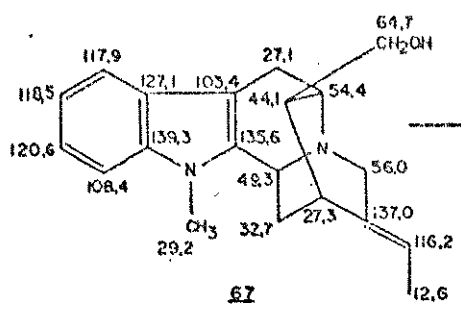
As atribuições dos deslocamentos químicos deste composto não apresentou maiores dificuldades e se encontram diretamente sobre a formula 65. A transformação desta base em seu respectivo sal 66 produziu alterações no anel C, semelhantes às observadas no anel B dos alcalóides benzil-tetraidro-isoquinolínicos 73 e 74<sup>16</sup>. Os carbonos situados em posição  $\beta$  em relação ao novo Nb-CH<sub>3</sub> foram desprotegidos de ~10 ppm: C<sub>3</sub> a 65,6 ppm ( $\Delta\delta = 10,2$  ppm), C<sub>5</sub> a 60,3 ( $\Delta\delta = 9,6$  ppm) e CH<sub>3</sub> a 51,4 ppm ( $\Delta\delta = 9,3$  ppm). Os carbonos  $\gamma$  foram protegidos: C<sub>6</sub> a 17,6 ppm ( $\Delta\delta = -2,7$  ppm), C<sub>3</sub>-CH<sub>3</sub> a 15,1 ppm ( $\Delta\delta = -3,1$  ppm) e C<sub>2</sub> a 124,9 ppm ( $\Delta\delta = -9,1$  ppm).

A proteção de -9,1 ppm do C<sub>2</sub> seria demasiadamente grande para ser causada somente por uma interação  $\gamma$ . Também foi observada a proteção do C<sub>7</sub> a 102,9 ppm ( $\Delta\delta = -4,5$  ppm). Possivelmente a proteção do C<sub>7</sub> e parte da proteção do C<sub>2</sub> seriam efeitos eletrônicos devidos à existência da carga positiva sobre o N<sub>b</sub>, sendo que este último (C<sub>2</sub>) sofre uma proteção adicional em consequência de uma interação  $\gamma$  gauche com o Nb-metil adicional. Um efeito semelhante foi observado em carbonos correspondentes aos alcalóides 73 e 74.

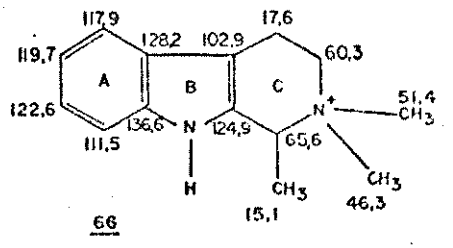
A preocupação suplementar de reagir os alcalóides 67, 69 e 71 com iodeto de metila para obtermos 68, 70 e 61 respectivamente se revelou de grande utilidade na interpretação dos alcaloides quaternários novos (62, 63, 64 e 61). Estas transformações apresentaram as mesmas alterações observadas nos deslocamentos químicos do composto 66, com pequenas variações de  $\Delta\delta$ , obtido por iodometilação 65 + 66 (ver quadro 3).



Quadro 3



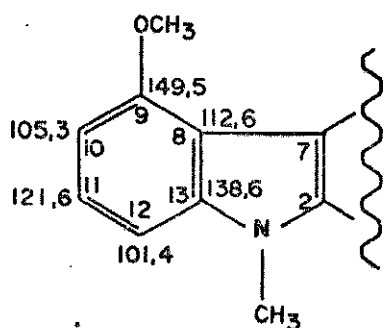
ICH<sub>3</sub>



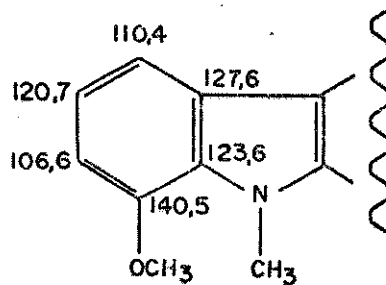
Fuchsiaefolina 62, 12-metoxi-Nb-metil-voacalotina 63, éster etílico do 12-metoxi-Nb-metil-voacalotina 64 e pruinósidina 61

Tres destes produtos naturais (62, 63 e 64) são indóis substituídos por uma metoxila. A posição desta foi confirmada por RMN de  $^{13}\text{C}$  isto porque 12-metoxi-indóis são raros e os espectros de ultravioleta disponíveis na literatura para este cromóforo são contraditórios.

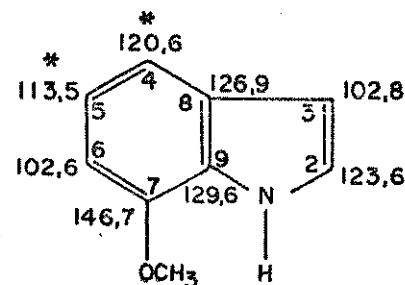
Para atribuir os carbonos  $\text{sp}^2$  da porção indólica utilizamos cálculos empíricos considerando a fixação da metoxila nos  $\text{C}_9$  e  $\text{C}_{12}$ , usando valores de:  $\text{C}_{\text{íps}} \sim +31,4$  ppm,  $\text{C}_{\text{orto}} \sim -14$  ppm,  $\text{C}_{\text{meta}} \sim +1,0$  ppm e  $\text{C}_{\text{para}} \sim -7,7$  ppm. Estes valores aplicados no sal da afinisina 68 nos conduziu a duas possibilidades a ou b.



a



b



c

Dentre estas duas a que mais se aproxima de nossos dados é a estrutura b, pois a estrutura a deve ser desprezada pela falta do sinal de carbono quaternário à 112 e 138 ppm dos C-8 e C-13, além da falta de CH próximo à 101 ppm. Atribuímos à interação peri entre os grupos metoxila e Na-metil as discrepâncias observadas nos  $\text{C}_{12}$  e  $\text{C}_{13}$ . Estes cálculos estão bem coerentes com o 7-metoxi-indol<sup>32</sup> (c) admitindo-se uma inversão nas atribuições dos  $\text{C}_4$  e  $\text{C}_5$ .

Os carbonos  $sp^3$  da fuchsiaefolina 62, apresentaram deslocamentos químicos semelhantes ao sal de afinisina 68. A estereoquímica do  $C_{16}$  foi determinada como sendo exo, baseando-se nos deslocamentos dos  $C_6$  e  $C_{14}$  com valores de 24,4 ppm e 30,6 ppm, respectivamente, coerentes com os do sal da afinisina 68, (Quadro 3).

A diferença básica do 12-metoxi-N<sub>b</sub>-metil-voacalotina 63, seu éster etílico 64 em relação à fuchsiaefolina 62 é que neste último o  $C_{16}$  é monossustituído e nos dois primeiros é dissustituído.

A dissustituição dos sais naturais observada por espectrometria de massa é confirmada pelos deslocamentos químicos dos  $C_6$  e  $C_{14}$  que estão protegidos de 5,7 ppm e 3,0 ppm em relação à fuchsiaefolina 62, variações estas coerentes com interações eclipsada e gauche que o grupo adicional introduz nestes carbonos.

Nosso objetivo ao estudar a RMN- $^{13}C$  destes compostos foi o de determinar a configuração do  $C_{16}$ , para o 12-metoxi-N<sub>b</sub>-metilvoacalotina 63. A configuração do  $C_{16}$  no composto 64 foi proposta em analogia ao 12-metoxi-N<sub>b</sub>-metilvoacalotina 63, pois acreditamos que o primeiro sal é um artefato do último.

Este objetivo foi alcançado, através da comparação direta dos deslocamentos químicos dos carbonos destes sais, com os do iodeto de N<sub>b</sub>-metilvoacalotina 70 obtido a partir de voacalotina.

De fato, quando comparamos os deslocamentos do  $CH_2OH$  e da carbonila (Quadro 3) nos dois sais naturais 63 e 64, com os do sal da voacalotina 70, verificamos que a atribuição da estereoquímica dos sais naturais está coerente.

O fato da carbonila ser protegida de 3,5 ppm quando passamos da voacalotina 69 para seu sal 70, nos dá mais uma evidên-

cia para que a atribuição dos carbonos no sal da afinisina 68 esta correta, isto é, que o grupo  $CH_2OH$  neste último composto seja protegido de 2 ppm (Quadro 3).

Desta forma e por esta técnica pudemos estabelecer definitivamente a estrutura dos tres alcalóides indólicos novos, tendo inclusive determinado toda a sua estereoquímica.

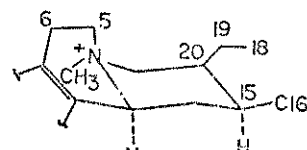
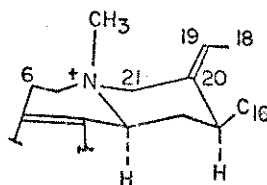
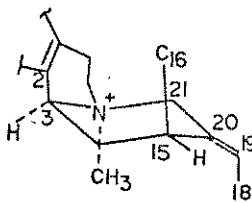
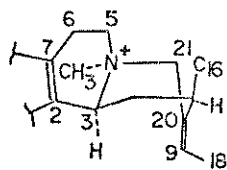
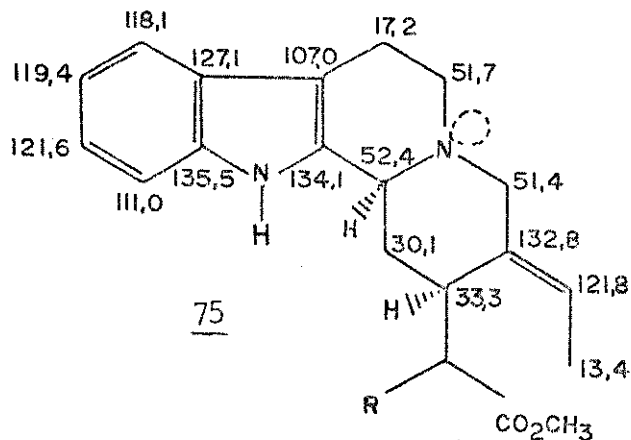
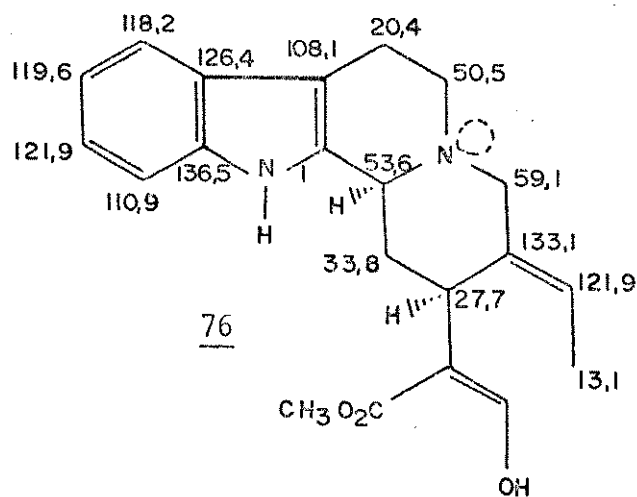
A pruinósidina 61 ao contrário dos alcalóides anteriores, que por sua estrutura quinuclidínica são rígidos, possui um sistema quinolizidínico que permite mudanças conformacionais. Temos que considerar diversas conformações possíveis (a, b, c e d) e interpretar os valores dos deslocamentos químicos observados. As atribuições se encontram diretamente sobre a fórmula(Quadro 3).

Observando-se a estrutura da pruinósidina 61, vê-se que este alcalóide se diferencia do 10-metoxi-gessoschizol 71 apenas pela presença de um grupo metila ligado ao Nb. Isto foi confirmado através da reação de 71 com iodeto de metila, obtendo-se um produto cujos espectros apresentaram total identidade com a pruinósidina.

Quando analisamos a estrutura do 10-metoxi-geissoschizol 71, concluímos que aquele composto possui uma junção dos aneis C/D do tipo cis quinolizidina tendo sua conformação preferida ilustrada na fórmula b. Esta conclusão é fundamentada nos deslocamentos químicos dos  $C_3 = 54,1$  ppm,  $C_6 = 18,4$  ppm e  $C_{21} = 51,2$  ppm, semelhantes aos observados <sup>31</sup> na porção quinolizidínica do alcalóide bisindólico geissospermina 75. Apesar da orientação axial dos  $C_2$  e  $C_{16}$  serem termodinamicamente desfavorecidas estudos de Raios X confirmaram esta conformação (b) para a geissospermina 75.

A tentativa de aplicação na fórmula b dos parâmetros empíricos desenvolvidos para os alcalóides quaternários anteriores

apresentou desvios consideráveis que nos levaram a sugerir a con-  
 formação a para a pruinósidina 61 que é semelhante à proposta pa-  
 ra <sup>30, 31</sup> a geissoschizina 76



#### d) Alcalóides Bis-indólicos

15

Foram analisados seis compostos bisindólicos do tipo voa-  
 camina (ver tabela 2): voacamina 77; descarbometoxi-voacamina 78;  
 desmetilvoacamina 79; voacamidiana 80; 19,20-diidrovoacamina 81  
 e diidro-desmetilvoacamina 82.

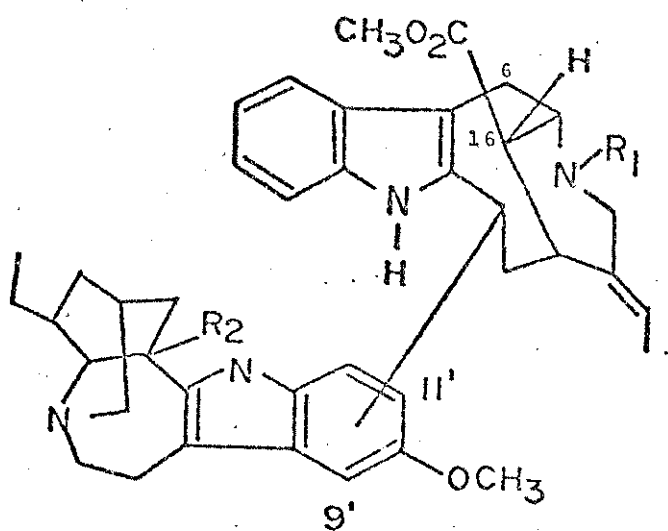
Comparando-se a desmetil-voacamina e voacamina, observa-  
 mos que a introdução do metila no Nb produz uma proteção nos  $C_6$   
 ( $\Delta\delta = -4,8$  ppm) e  $C_{16}$  ( $\Delta\delta = -6,3$  ppm). Esta proteção simultânea é

TABELA 2

CARBONO	<u>77</u>	<u>78</u>	<u>79</u>	<u>81</u>	<u>80</u>	<u>82</u>
2	135,5	135,5	135,5	135,6	134,9	135,7
3	37,4	37,6	37,1	37,2	37,1	37,3
5	59,9	59,7	53,3	59,2	59,8	53,0
6	19,8	19,4	24,6	19,2	19,0	25,5
7	109,6	110,0	110,2	109,8	110,8	110,9
8	129,4	129,4	129,7	129,6	129,9	129,5
9	117,2	117,0	117,1	117,2	116,9	117,3
10	119,8*	118,6	118,7	118,6	118,5	118,7
11	121,3	121,2	121,3	121,3	120,5	121,3
12	110,1	110,0	110,6	110,5	109,4	110,4
13	136,2	137,5	137,1	136,9	137,4	137,1
14	36,6	36,1	36,3	31,4	37,1	31,6
15	33,3	33,4	34,1	32,8	32,1	33,4
16	46,0	46,7	52,3	49,7	46,9	52,3
18	12,3	12,2	12,0	11,4	12,2	11,1
19	118,7*	118,6	117,3	23,5	118,5	23,5
20	137,7	137,8	140,1	43,7	137,9	45,9
21	52,3	52,2	44,1	51,9	52,3	40,8
COOMe	170,7	171,1	171,0	171,5	171,4	171,3
COOCH <sub>3</sub>	49,8	49,7	49,8	49,5	49,8	49,7
NMe	41,9	42,1		42,4	42,2	
CH <sub>2</sub> OH						
2'	136,9	142,1	137,6	137,5	138,8	137,5
3'	52,0	49,7	51,8	52,3	51,0	51,9
5'	53,0	54,1	53,0	53,0	53,9	53,0
6'	22,1	20,6	22,2	22,2	24,9	22,2
7'	109,6	108,4	109,6	110,3	109,0	109,8
8'	129,4	128,4	129,7	130,0	126,2*	130,1
9'	99,1	98,5	99,1	99,0	126,4*	99,2
10'	150,6	150,7	150,7	150,8	152,4	150,9
11'	127,1	127,9	127,1	127,0	112,5	127,1
12'	109,5	109,6	109,7	109,6	109,4	109,6
13'	130,0	129,0	130,1	130,3	131,8	130,2
14'	27,2	26,1	27,3	27,3	27,2	27,3
15'	31,8	31,7	31,9	31,9	31,8	31,9
16'	54,8	40,9	54,8	54,8	55,8	54,9
17'	36,2	33,9	36,3	36,4	33,5	36,4
18'	11,5	11,8	11,6	11,6	11,6	11,5
19'	26,6	27,6	26,7	26,7	26,7	26,7
20'	38,8	41,7	38,9	38,9	38,9	38,9
21'	56,9	57,8	57,0	57,0	58,2	57,0
COOMe	174,9		174,9	175,0	175,5	174,9
COOCH <sub>3</sub>	51,8		50,0	49,0	52,5	50,6
OMe	56,0	55,9	56,0	56,0	57,8	56,0

Obs: \* o valor pode ser interconvertido dentro da mesma coluna.

explicada pela rápida inversão do Nb, com o Nb-metil num rápido equilíbrio entre as posições axial e equatorial induzindo a proteção observada. As experiências feitas com variação de temperatura na aquisição dos espectros de RMN de  $^{13}\text{C}$  para detectar os diferentes conformêros, foram infrutíferas.



- 77  $\text{R}_1=\text{CH}_3; \text{R}_2=\text{CO}_2\text{CH}_3$  ligação no  $\text{C}_{11'}$
- 78  $\text{R}_1=\text{CH}_3; \text{R}_2=\text{H}$  ligação no  $\text{C}_{11'}$
- 79  $\text{R}_1=\text{H}; \text{R}_2=\text{CO}_2\text{CH}_3$  ligação no  $\text{C}_{11'}$
- 80  $\text{R}_1=\text{CH}_3; \text{R}_2=\text{CO}_2\text{CH}_3$  ligação no  $\text{C}_9'$
- 81  $\text{R}_1=\text{CH}_3; \text{R}_2=\text{CO}_2\text{CH}_3$ ; 19,20 diidro; ligação no  $\text{C}_{11'}$
- 82  $\text{R}_1=\text{H}; \text{R}_2=\text{CO}_2\text{CH}_3$ ; 19,20 diidro; ligação no  $\text{C}_{11'}$

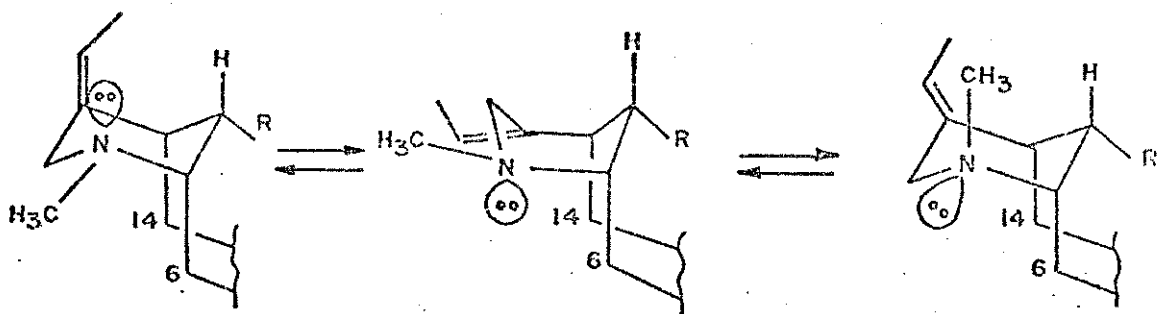


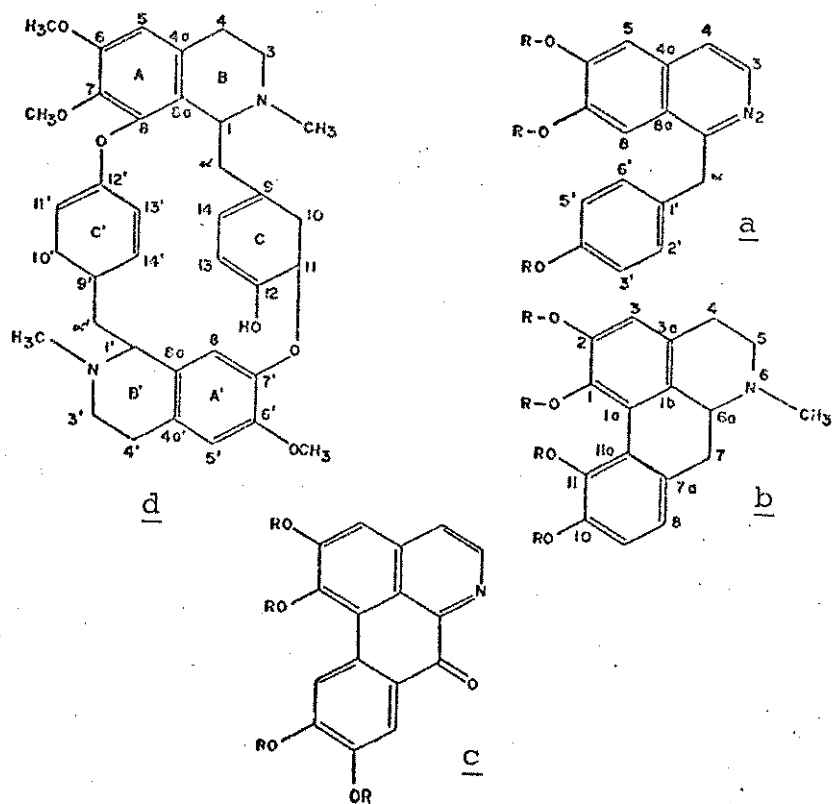
Figura 2 INVERSÃO DO "N" NA VOACAMINA

## 5. Alcalóides Isoquinolínicos

Os isoquinolínicos são considerados como o maior grupo dentre os demais alcalóides. Coincidentemente o primeiro alcalóide descoberto, a morfina, pertence ao grupo dos isoquinolínicos.

O desenvolvimento de novos métodos analíticos, e a aplicação de novas técnicas espectrofotométricas é o caminho natural para facilitar o trabalho daqueles que se dedicam à química de produtos naturais. Entre os alcalóides isoquinolínicos existem vários exemplos de compostos que levaram décadas para terem sua estrutura conhecida.

Ao vislumbrarmos a potencialidade da RMN de  $^{13}\text{C}$  na química de produtos naturais, nos surpreendemos que justamente no campo dos alcalóides isoquinolínicos poucos pesquisadores haviam se interessado. Haviam poucos trabalhos publicados<sup>33-37</sup> e muito trabalho a ser feito. Estudamos mais de sete dezenas de alcalóides isoquinolínicos: benzilisoquinolínicos (a)<sup>16</sup>, aporfínicos (b)<sup>17</sup>, oxoaporfínicos (c)<sup>19</sup> e bisbenzilisoquinolínicos (d)<sup>18, 20-22, 38</sup>.



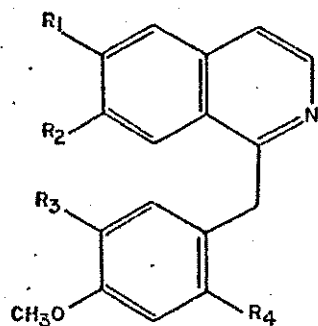
a) Benzilisoquinolínicos

Analizamos a papaverina 83, 1-(p-metoxibenzil)-6,7-dimetoxiisoquinolina 84, 1-(p-metoxibenzil)-6,7-metilenodioxisoquino



lina 85, nitro papaverina 86, N-metil-papaverina 87, N-metil-1-(p-metoxibenzil)-6,7-dimetoxiisoquinolina 88, N-metil-1-(p-metoxibenzil)-metilenodioxisoquinolina 89, as tetra-hidrobenzilisoquinolinas 90 e 91 e os sais quaternários 92, 93 e 94. Os deslocamentos químicos destes compostos se acham na tabela 3.

Este trabalho <sup>16</sup> permitiu observar os efeitos eletrônicos e espaciais causados pela quaternarização destas bases e que foram muito úteis nos estudos de vários alcalóides naturais quaternários isoquinolínicos. Tais estudos puderam ainda ser usados e comparados com compostos indólicos quaternários e foram fundamentais nas determinações estruturais dos produtos naturais novos, conforme vimos anteriormente.

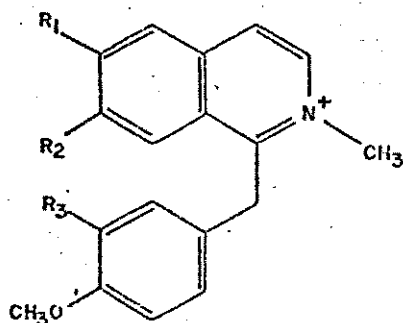


83  $R_1 = R_2 = R_3 = \text{OCH}_3$ ;  $R_4 = \text{H}$

84  $R_1 = R_2 = \text{OCH}_3$ ;  $R_3 = R_4 = \text{H}$

85  $R_1 = R_2 = -\text{OCH}_2\text{O}-$ ;  $R_3 = R_4 = \text{H}$

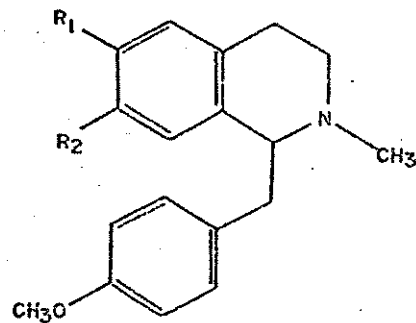
86  $R_1 = R_2 = R_3 = \text{OCH}_3$ ;  $R_4 = \text{NO}_2$



87  $R_1 = R_2 = R_3 = \text{OCH}_3$

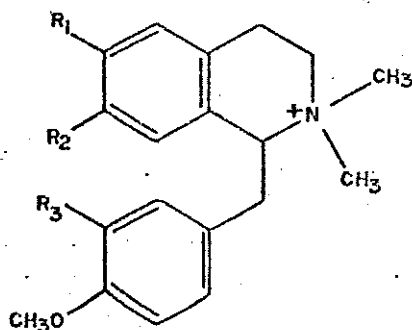
88  $R_1 = R_2 = \text{OCH}_3$ ;  $R_3 = \text{H}$

89  $R_1 = R_2 = -\text{OCH}_2\text{O}-$ ;  $R_3 = \text{H}$



90  $R_1 = R_2 = \text{OCH}_3$

91  $R_1 = R_2 = -\text{OCH}_2-$



92  $R_1 = R_2 = R_3 = \text{OCH}_3$

93  $R_1 = R_2 = \text{OCH}_3$ ;  $R_3 = \text{H}$

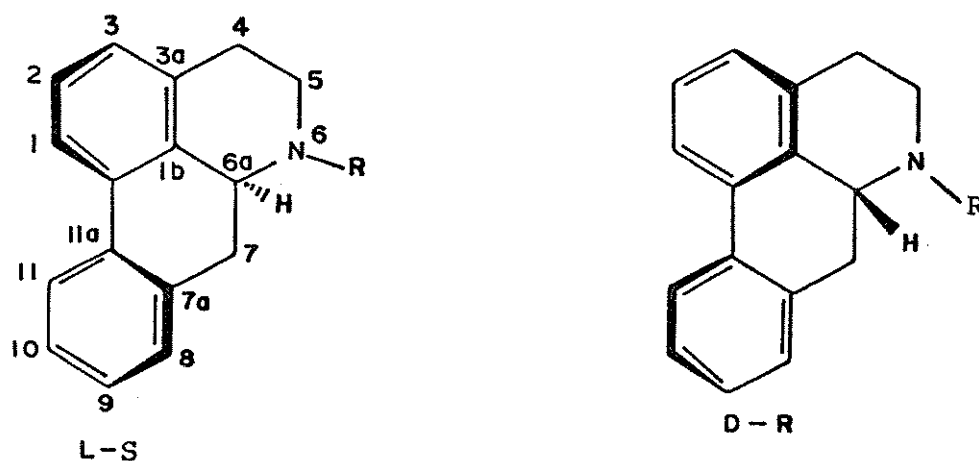
94  $R_1 = R_2 = -\text{OCH}_2\text{O}-$ ;  $R_3 = \text{H}$

Tabela 3

Carbono	83	84	85	86	90	91	87	88	89	92	93	94
1	157,4	157,7	158,0	155,4	64,4	65,0	152,7	151,7	151,7	71,3	71,7	71,9
3	140,6	140,8	141,0	140,2	46,3	46,6	135,6	134,3	136,1	54,7	54,8	54,4
4	118,3	118,4	119,1	118,3	25,0	25,6	122,5	121,5	123,1	23,1	23,1	23,3
4a	133,0	133,2	134,8	132,6	125,3	127,3	135,4	134,7	137,5	120,6	120,6	122,0
5	104,9	105,1	102,9	104,9	113,0	108,0	106,1	105,4	103,5	111,0	111,1	107,8
6	152,0	152,1	150,0	152,5	146,6	145,0	156,7	155,7	155,0	148,9	148,5	147,5
7	149,7	149,5	148,0	149,8	145,7	145,4	154,2	153,6	154,7	146,6	146,1	145,5
8	103,8	104,0	101,7	102,8	110,7	107,0	104,6	104,0	102,0	110,5	110,2	107,5
8a	122,5	122,6	124,6	122,4	128,6	130,4	123,9	123,0	125,6	119,1	119,2	120,9
$\alpha$	42,0	41,7	41,4	37,9	39,7	40,5	34,8	33,6	34,6	37,4	37,1	37,0
1'	131,9	131,5	131,3	128,7	131,3	131,6	125,4	124,1	124,4	126,3	125,8	125,5
2'	111,5	129,2	129,2	113,4	130,1	130,1	111,5	127,8	128,6	113,1	131,1	130,5
3'	148,6	113,7	113,7	152,2	113,0	113,2	149,0	113,4	114,3	148,9	113,4	113,4
4'	147,0	157,7	157,7	146,9	157,3	157,5	147,9	157,3	158,3	147,9	158,2	158,1
5'	110,5	113,7	113,7	107,7	113,0	113,2	111,1	113,4	114,3	110,1	113,4	113,4
6'	120,1	129,2	129,1	141,0	130,1	130,1	118,9	127,8	128,6	122,3	131,1	130,5
-OCH <sub>3</sub>	55,5	55,2	55,1	55,6	54,6	54,9	56,6	56,6	54,9	56,4	55,4	54,6
		55,7		55,7	55,0		56,5	56,0		55,4	54,7	
		55,8			55,2		56,0	55,1		54,9		
							55,8			54,7		
-OCH <sub>2</sub> O			101,3			100,2			102,5			100,8
-NCH <sub>3</sub>					42,1	42,4						
<sup>+</sup> NCH <sub>3</sub>							46,9	45,8	46,8	52,3	52,4	52,2
										50,3	50,5	50,6

b) Aporfínicos

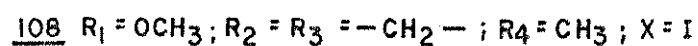
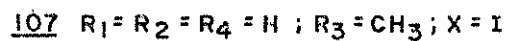
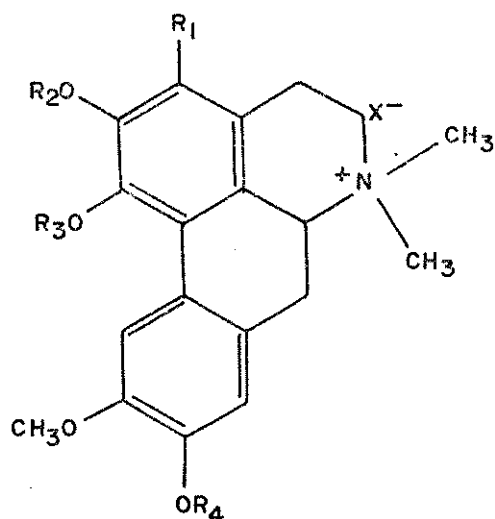
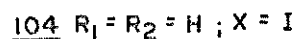
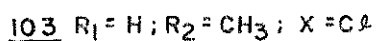
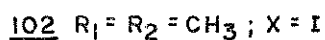
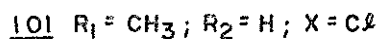
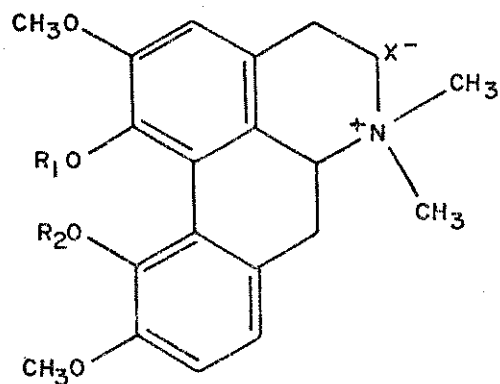
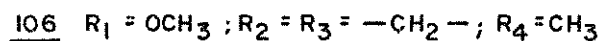
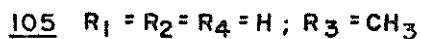
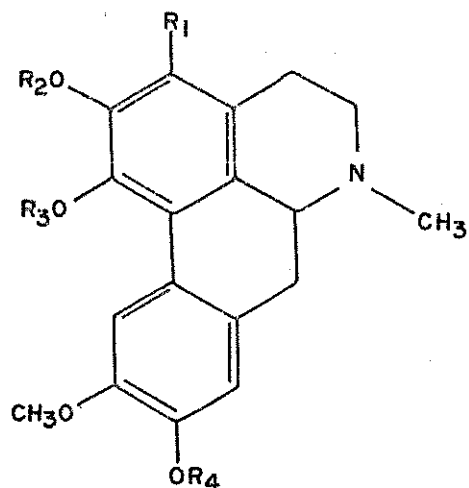
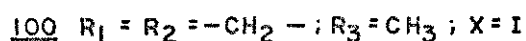
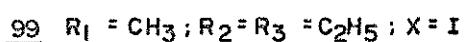
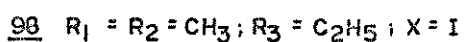
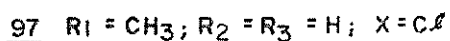
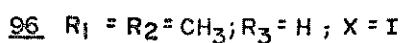
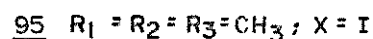
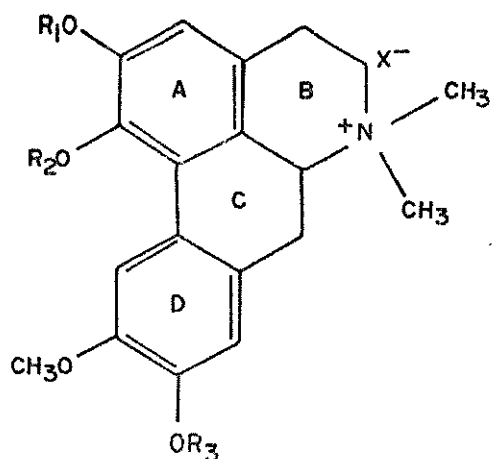
Os aporfínicos se constituem na segunda maior família do grupo dos alcalóides isoquinolínicos com mais de 85 compostos conhecidos. Por outro lado a presença de um sistema bifenílico permite dois arranjos espaciais, configuração L-S e configuração D-R extremamente interessantes de serem estudadas por RMN de  $^{13}\text{C}$  pelos efeitos que os diversos tipos de substituintes podem causar em tais sistemas.



Os diversos métodos físicos de análises foram empregados na determinação estrutural destes compostos, mas nenhum deles, exceto a difração de Raios X, resultou na fixação dos substituintes sempre presentes nestes alcalóides.

Analisamos <sup>17</sup> quatorze alcaloides, bases e sais, com padrões de substituição diferentes: N-metil-glaucina 95; xantoplanina 96; laurifolina 97; O-etil-xantoplanina 98; O,O'-dietil-laurifolina 99; N-metil-dicentrina 100; N-metil-isocoridina 101; O,O-dimetil-magnoflorina 102; N-metil-coridina 103; magnoflorina 104; boldina 105; ocoteina 106; N-metil-boldina 107 e N-metil-ocoteina 108.

Os dados se encontram nas tabelas 4 e 5 (pag. 48 e 49).



Através da observação dos efeitos da metilação de fenóis produzidos nas posições *ipso*, *orto* e *para* podemos determinar cla-

ramente posições das metoxilas muito comuns nestes alcalóides. Outra observação feita no decorrer deste trabalho é a observação da alteração do ângulo do sistema bifenílico dos compostos substituídos nas posições  $C_1$ ,  $C_2$ ,  $C_{10}$  e  $C_{11}$ .

Sabe-se que os carbonos de metoxilas *ortho* substituídas são progressivamente desprotegidas à medida em que aumenta o volume destes substituintes<sup>39</sup>. Entretanto os valores para os carbonos das metoxilas fixadas nos  $C_1$  e  $C_{11}$  da O,O-dimetil-magnoflorina 102 estão mais protegidos do que os mesmos carbonos da N-metil-isocoridina 101 e N-metil-coridina 103. Por outro lado observou-se também proteção dos  $C_{3a}$  e  $C_8$  de 102 quando comparados aos mesmos carbonos de 101 e 103. Estas alterações são explicadas pelo aumento da conjugação do oxigênio com os sistemas  $\pi$  dos anéis aromáticos correspondentes. Este aumento de conjugação é possível pelo aumento do ângulo de torção do sistema bifenílico destes compostos.

Tabela 4

Carbono	95	96	97	98	99	100	105
1	145,6	145,9	142,2	145,6	144,5	143,1	141,9
1a	127,7	127,6	118,2†	127,7	128,0	116,7	126,6
1b	118,6	118,4	118,3†	118,5	118,6	117,9	125,8
2	153,9	153,6	148,0	153,9	153,9	148,5	147,9
3	110,2	109,8	108,0	110,1	110,0	106,4	113,2
3a	124,1†	124,4	119,8	123,9	124,0†	121,8†	129,7†
4	24,3	24,0	23,5	24,2	24,2	24,0	28,8
5	61,4	61,5	61,5	61,3	61,4	61,8	53,3
6a	70,3	69,9	69,7	70,4	70,3	69,8	62,5
7	29,5	28,9	28,6	29,6	29,5	28,7	34,1
7a	124,0†	123,9	123,7	123,9	123,8†	123,3	130,1†
8	111,4	114,5	114,3	112,3	112,3	111,6	114,1
9	148,8	145,9	145,4	148,4	148,1	148,8	144,9
10	148,2	146,5	146,2	148,2	148,1	148,2	145,4
11	111,4	111,4	112,1	111,6	111,8	110,2	110,1
11a	123,0	122,0	122,5	122,8	123,1	122,0†	123,5
-N <sup>+</sup> Me <sub>2</sub>	44,0;54,8	43,6;54,3	42,6;53,4	43,5;54,7	43,5;54,7	43,6;54,3	60,2
-OMe (C-1)	60,5	60,1		60,4			
-OMe (C-2)	55,7	55,8	55,7	55,9	55,9	55,9	
-OMe (C-9)	56,1						
-OMe (C-10)	56,1	55,8	55,7	55,9	55,9	55,9	56,1
-OCH <sub>2</sub> O-						101,3	
-OEt (C-1)					15,7;69,0		
-OEt (C-9)				14,8;64,5	14,5;64,5		

Obs: † Os valores podem ser interconvertidos dentro da mesma coluna.

Tabela 5

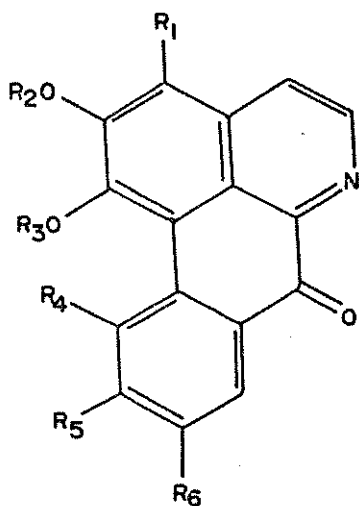
Carbono	101	102	103	104	106	107	108
1	143,0	146,9	143,6	140,2	143,2	143,9	144,7
1a	126,0	124,5	119,2†	118,9	110,4	127,1	110,7
1b	118,3	121,0	119,2†	117,7	127,4	117,1	118,7
2	152,9	153,0	150,9	148,8	134,8	151,1	135,3
3	110,6	111,0	110,6	109,6	139,1	113,6	138,6
3a	125,2	122,9	119,6†	120,3	119,1	124,1†	114,3
4	23,8	23,4	23,9	23,4	23,6	23,4	19,3
5	60,3	60,9	61,0	61,5	53,2	61,4	61,5
6a	69,1	69,3	69,8	69,7	62,3	69,7	69,5
7	30,6	30,4	30,7	30,3	34,1	28,7	28,6
7a	124,3	125,2	124,7	123,8	127,4	123,8†	122,6
8	119,6	122,6	125,3	120,8	111,1	114,5	111,6
9	111,5	112,3	111,8	110,9	147,5	145,8	148,0
10	149,7	152,4	152,5	147,6	147,5	146,6	148,0
11	143,5	147,1	143,6	140,2	110,0	110,9	109,5
11a	102,2	123,8	124,6	119,2	123,5	121,8	121,9
-N <sup>+</sup> Me <sub>2</sub>	42,9;53 5	43,1;53 9	44,0;54 2	43,4;54 2		43,2;53 7	43,6;54 4
-OMe (C-1)	62,1	60,7				59,6	
-OMe (C-2)	55,8	55,9	56,1	55,8	59,3		59,4
-OCH <sub>3</sub> (C <sub>3</sub> )					56,0		56,0
-OCH <sub>3</sub> (C <sub>9</sub> )					55,8		55,9
-OMe (C-10)		55,9	56,1	55,8			
-OMe (C-11)	55,8	60,7	62,2				
-OCH <sub>2</sub> O-					100,4		101,1

Obs: †, ||, † os valores podem ser interconvertido dentro da mesma coluna.

## c) Oxoaporfínicos

Este foi o primeiro trabalho<sup>19</sup> publicado de RMN de  $^{13}\text{C}$  de alcalóides oxoaporfínicos. Foram analisados a oxo-O-metil-pukateína 109, O-metil-moscatolina 110 e oxo-glaucina 111. Para facilitar a atribuição dos oxoaporfínicos tivemos que analisar previamente as metanonas: papaveraldine 112, (6,7-dimetoxiisouquinolinil)-(4'-metoxifenil)metanona 113 e (6,7-metilenodioxisouquinolinil)-(4-metoxifenil)-metanona 114. Os deslocamentos químicos se encontram nas tabelas 6 e 7.

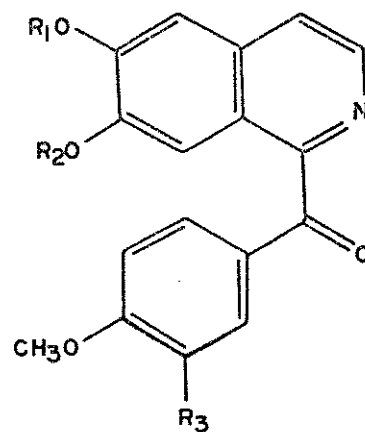
ID 6880



109  $R_1 = R_5 = R_6 = \text{H}$ ;  $R_2 = R_3 = -\text{CH}_2-$ ;  $R_4 = \text{OCH}_3$

110  $R_1 = \text{OCH}_3$ ;  $R_2 = R_3 = \text{CH}_3$ ;  $R_4 = R_5 = R_6 = \text{H}$

111  $R_1 = R_4 = \text{H}$ ;  $R_2 = R_3 = \text{CH}_3$ ;  $R_5 = R_6 = \text{OCH}_3$



112  $R_1 = R_2 = \text{CH}_3$ ;  $R_3 = \text{OCH}_3$

113  $R_1 = R_2 = \text{CH}_3$ ;  $R_3 = \text{H}$

114  $R_1 = R_2 = -\text{CH}_2-$ ;  $R_3 = \text{H}$



Tabela 6

Carbono	<u>109</u>	<u>110</u>	<u>111</u>	<u>111<sup>o</sup></u>
1	147,3	148,2	148,9	155,9
1a	104,4	115,4	119,1	120,1
1b	121,6	122,5	121,1	123,4
2	151,9	147,0	156,1	162,9
3	102,1	156,2	105,7	106,5
3a	135,2	130,8	134,8	135,3
4	123,3	118,9	122,9	126,2
5	144,0	144,3	144,3	133,1
6a	144,3	145,0	144,9	141,7
7	182,0	182,3	180,7	174,8
7a	133,2	131,4	126,3	133,1
8	120,2	127,9	109,2	110,1
9	129,1	128,7	150,2	150,2
10	116,1	134,1	153,2	156,7
11	156,0	127,4	109,7	111,3
11a	123,1	134,3	128,7	130,4
-OCH <sub>3</sub>	55,7	61,7	60,2*	61,6*
		61,3	55,8	57,3
		60,9	55,8	56,3
			55,8	56,3
-OCH <sub>2</sub> O-	101,5			

Obs:  $\rho$  CDCl<sub>3</sub> + gotas de F<sub>3</sub>CCOOH

\* sinal atribuido à metoxila no C<sub>1</sub>

Tabela 7

Carbono	<u>112</u>	<u>113</u>	<u>114</u>
1	153,5	153,5	154,7
3	139,7	139,9	140,2
4	120,9	121,1	121,7
4a	133,6	133,8	133,3
5	104,6	104,7	102,6
6	152,9	153,0	151,0
7	150,7	150,9	149,1
8	103,8	104,0	102,1
8a	122,5	122,6	123,7
1'	129,6	129,7	129,7
2'	111,7	133,1	133,0
3'	148,7	113,6	113,6
4'	153,5	163,0	163,8
5'	109,7	113,6	113,6
6'	126,6	133,1	133,0
-OCH <sub>3</sub>	55,9	56,0	55,0
	55,9	56,0	
	55,9	55,6	
	55,9		
C=O	186,4	187,0	*
-OCH <sub>2</sub> O-			101,7

Obs: \* devido à baixa concentração da amostra, o sinal não foi detectado.

d) Bisbenzilisoquinolínicos

A família dos bisbenziltetrahydroisoquinolínicos (BBI) é a maior dentre os compostos isoquinolínicos estando subdividida em vinte e oito tipos estruturais diferentes. Estas substâncias são resultantes do acoplamento de duas unidades benziltetrahydroisoquinolínicos (BI) e possuem, na maioria dos casos, estruturas bastante complexas. Estes compostos tricíclicos (BI) ao se associarem formam um macrociclo com geralmente 18 átomos e interações estruturais ao mesmo tempo atraentes e desafiadoras para uma análise conformacional.

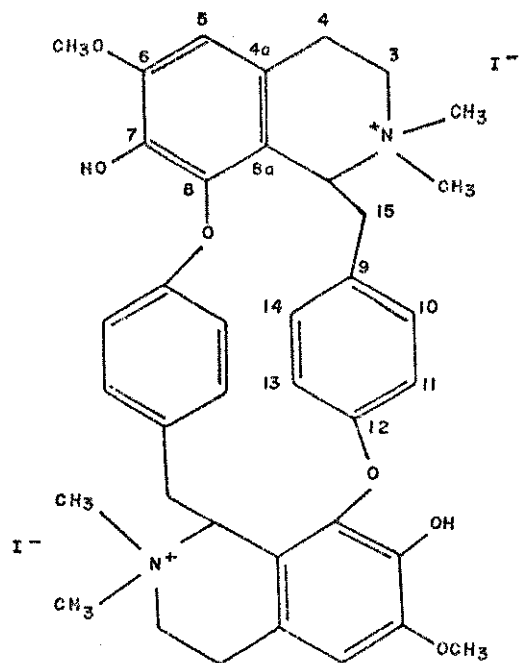
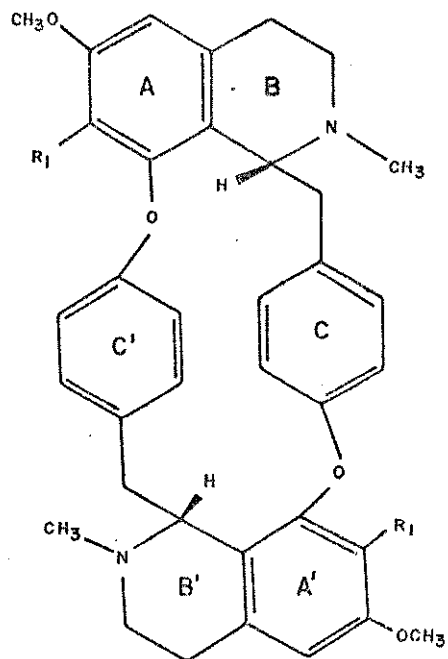
É indiscutível a importância destes alcalóides que tem um largo espectro de ação biológica sendo no entanto a mais difundida e popular a atividade curarizante.

Os trabalhos anteriores com alcalóides isoquinolínicos mais simples serviram de suporte para a tarefa árdua de analisar pela primeira vez estas moléculas altamente complexas.

O estudo desta família de alcalóides, que é parte integrante da Tese de Doutorado da Profa. Luzia Koike, exigiu uma associação de RMN de <sup>1</sup>H e <sup>13</sup>C e diferentes técnicas de obtenção dos espectros. Os compostos estudados pertencem a seis tipos: isocondondendrina, bebeerina, berbamina, repandine, micrantina e repandulina.

I) Alcalóides tipo Isocondondendrina e bebeerina

Os compostos do tipo isocondondendrina 115 são simétricos e por isto foram mais simples de serem analisados<sup>16</sup>. Foram observados os efeitos de metilação → 116 e acetilação → 117 e quaternarização → 118 destes alcalóides, cujas atribuições se encontraram nas tabelas 8 e 11 (pag. 53 e 61).

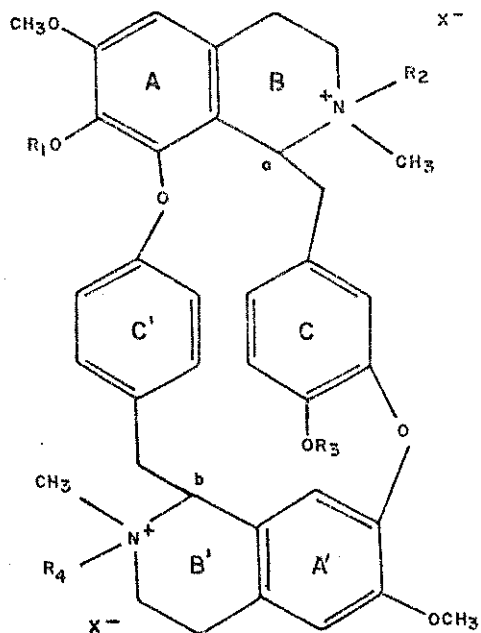
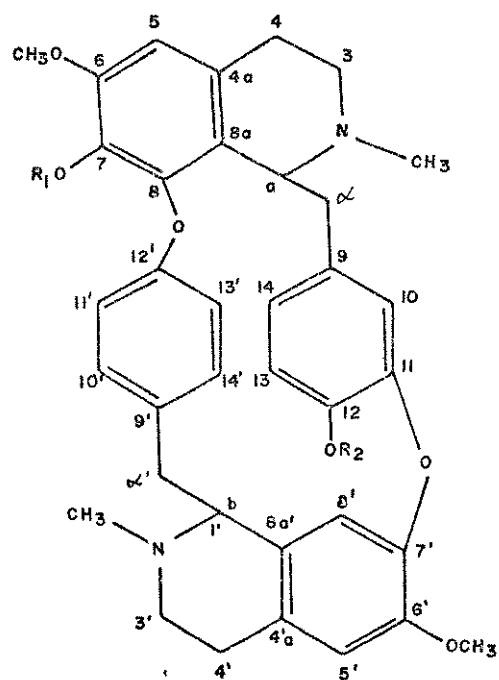


115  $R_1 = \text{OH}$

116  $R_1 = \text{OCH}_3$

117  $R_1 = \text{O}-\text{C}(=\text{O})-\text{CH}_3$

118



119  $R_1 = R_2 = \text{H}$  ( $a, b = R, R$ )

120  $R_1 = R_2 = \text{CH}_3$  ( $a, b = R, R$ )

121  $R_1 = \text{CH}_3$ ;  $R_2 = \text{H}$  ( $a, b = R, R$ )

122  $R_1 = \text{H}$ ;  $R_2 = \text{CH}_3$  ( $a, b = R, R$ )

123  $R_1 = \text{CH}_3$ ;  $R_2 = \text{Ac}$  ( $a, b = R, R$ )

124  $R_1 = \text{Ac}$ ;  $R_2 = \text{CH}_3$  ( $a, b = R, R$ )

125  $R_1 = \text{Ac}$ ;  $R_2 = \text{Ac}$  ( $a, b = R, R$ )

126  $R_1 = R_2 = \text{H}$  ( $a, b = R, S$ )

127  $R_1 = R_3 = R_4 = \text{H}$ ;  $R_2 = \text{CH}_3$ ;  $X = \text{Cl}^-$  ( $a, b = R, S$ )

128  $R_1 = R_3 = \text{H}$ ;  $R_2 = R_4 = \text{CH}_3$ ;  $X = \text{I}^-$  ( $a, b = R, S$ )

129  $R_1 = R_2 = \text{H}$ ;  $R_3 = R_4 = \text{CH}_3$ ;  $X = \text{I}^-$  ( $a, b = R, R$ )

130  $R_1 = R_2 = R_3 = R_4 = \text{CH}_3$ ;  $X = \text{I}^-$  ( $a, b = R, R$ )

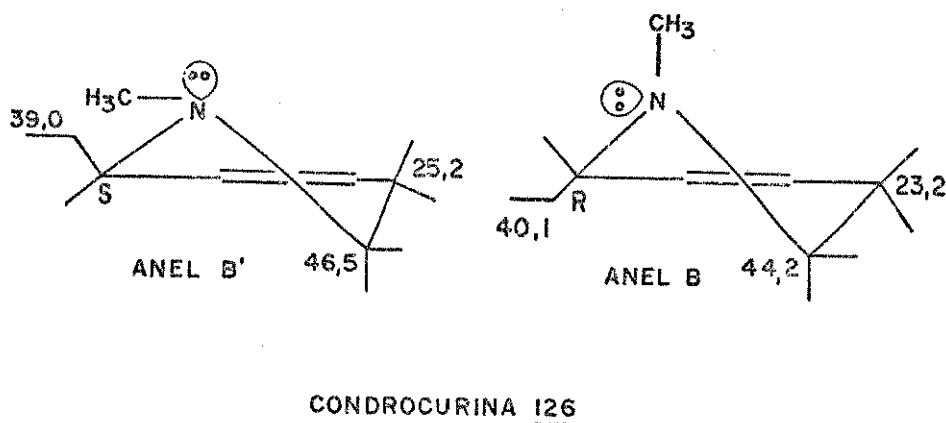
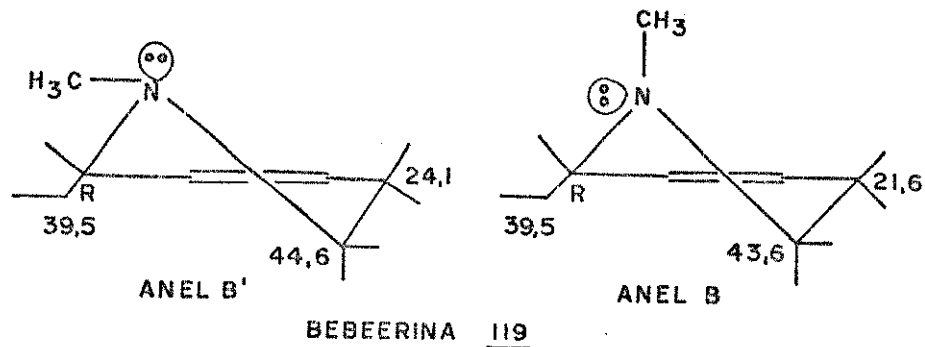
131  $R_1 = R_2 = R_3 = R_4 = \text{H}$ ;  $X = \text{Cl}$  ( $a, b = R, R$ )

Estes dados foram muito importantes na análise dos compostos do tipo bebeerina 119. Nestes tivemos oportunidade de analisar não só diversos derivados metilados e acetilados seletivamente, 120 121 122 123 124 e 125 mas também a condrocurina 126 que é o diastereoisômero da bebeerina 119. Foram também analisados os sais quaternários derivados da condrocurina (127 e 128) e da bebeerina (129 130 e 131). Estes compostos se caracterizam por apresentarem as unidades BI unidas por ligações etéreas do tipo "cabeça-cauda". Os deslocamentos químicos se encontram nas tabelas 9 e 11 (pag.59 e 61).

Da análise dos compostos tipo bebeerina ressaltaram importantes informações do ponto de vista conformacional.

Os valores de  $\delta$  dos carbonos  $C_4$  ou  $C_4'$ , revelaram-se diagnósticos da posição relativa do  $N-CH_3$  ou  $N'-CH_3$ . Assim, considerando-se o anel B' da bebeerina, o  $C_4'$ , a 24,1 ppm indica que o  $N'-CH_3$  está em posição preferencialmente equatorial. Por outro lado, o  $C_3'$ , a 44,6 ppm é resultado de uma interação " $\gamma$  gauche" entre o  $C\alpha'$  e  $C_3'$ . O  $C_4$  a 21,6 ppm indica claramente uma maior interação com o  $N-CH_3$ , que assim deve estar em posição axial, dessa forma propomos as semicadeiras para os anéis B' e B, respectivamente.

Comparando-se a bebeerina 119 com seu diastereoisômero condrocurina 126 nota-se que os deslocamentos químicos dos carbonos são muito semelhantes, exceto para  $C_8'$  que sofre proteção de  $\Delta\delta = -1,6$  ppm ( $\delta = 119,5$  ppm em 119 para  $\delta = 117,9$  ppm em 126), e para  $C_3'$ , que sofre desproteção de  $\Delta\delta = +1,9$  ppm ( $\delta = 44,6$  ppm em 119 para  $\delta = 46,5$  ppm em 126). A conformação preferida para o anel B' de ambos os compostos é uma semi-cadeira com o  $C\alpha$  pseudo-axial em 119 e pseudo-equatorial em 126. Esta conformação para a condrocurina implica uma interação peri entre o  $C_8$ , e o  $C\alpha'$ , e uma eliminação da interação " $\gamma$  gauche" entre  $C\alpha'$  e  $C_3'$ , causando proteção no  $C_8'$ .



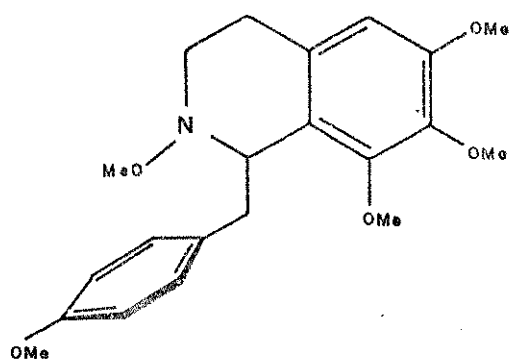
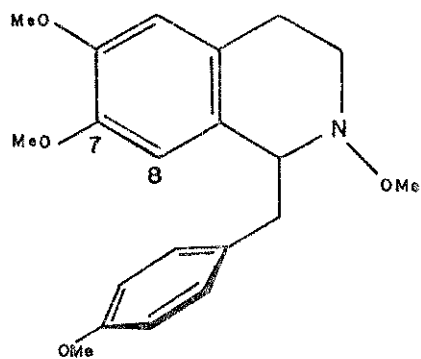
( $\Delta\delta = -1,6$  ppm) e desproteção no  $C_3$ , ( $\Delta\delta = +1,9$  ppm), respectivamente.

Por outro lado a análise por RMN de  $^1H$  e sua correlação com os dados de  $^{13}C$  nos permitiu detectar a conformação preferida destes compostos. Os anéis A', B' e C' adotam uma conformação dobrada semelhante a laudanosina 132. Os anéis A, B e C uma conformação estendida, em virtude da substituição no  $C_8$ , que afasta o anel C de forma semelhante ao que se observa no composto 133. Em função das análises desenvolvidas sugere-se que em solução estes alcalóides adquirem conformações preferidas apresentada na figura 3 (pag. 62) para a série bebeerina 119 e na figura 4 (pag. 63) para a condrocurina 126.

Em função da conformação proposta para a bebeerina 119 (fig. 3) observou-se que quando se introduz um substituinte maior na posição  $C_{12}$  o anel C sofre rotação projetando o  $C_{12}$  para a parte externa da molécula a fim de aliviar a compressão espacial exist

tente no interior desta. Esta rotação aproxima o  $H_{8,1}$ , ao mesmo tempo que afasta o  $H_{14}$ , da região de proteção anisotrópica do anel  $C'$ . Por exemplo na transformação da bebeerina 119 em 7,12,0,0-dimetil-bebeerina 120, nota-se proteção de próton  $H_8$ , ( $\Delta\delta = -0,45$  ppm) e desproteção do  $H_{14}$ , ( $\Delta\delta = +0,20$  ppm). A rotação altera também a conjugação do éter difenílico  $A'-O-C$  destes alcalóides aumentando a conjugação do oxigênio com o anel  $A'$  e causando uma proteção nos deslocamentos químicos dos carbonos  $C_{8,1}$ ,  $C_6$ , e  $C_{4a'}$ . O  $C_6$ , é o menos afetado, devido a efeitos secundários relativos à presença da metoxila.

Comparando-se as conformações propostas para a bebeerina 119 (figura 3) e condrocurina 126 (figura 4) observa-se que neste último o  $H_8$ , está mais direcionado para o interior da zona de proteção do anel  $C'$ . A maior proteção no deslocamento químico do  $H_8$ , da condrocurina 126 ( $\delta = 5,90$  ppm) em relação ao  $H_8$ , da bebeerina 119 ( $\delta = 6,04$  ppm) confirma esta observação e suporta a conformação proposta para os compostos desta série.

133132Tabela 8

Carbono	<u>115</u>	<u>116</u>	<u>117</u> <sup>†</sup>
1 (1')	58,0	59,2	59,4
3 (3')	44,0	44,4	44,2
4 (4')	25,8	24,6	24,5
4a (4a')	122,9	129,4	132,6
5 (5')	107,3	108,9	108,7
6 (6')	149,9	151,4	150,5
7 (7')	135,7	138,5	129,3
8 (8')	139,4	143,3	142,8
8 (8a')	124,8	125,2	124,8
9 (9')	129,0	130,1	130,8
10 (10')	127,2§	127,7§	128,0§
11 (11')	114,3	113,6	113,9
12 (12')	153,3	153,7	153,5
13 (13')	117,4	117,0	117,3§
14 (14')	128,6§	128,3§	128,2
15 (15')	33,8	37,6	39,1
OCH <sub>3</sub>	55,2	59,7	55,8
		55,7	
NCH <sub>3</sub>	40,5	42,1	42,1

Obs: §; || os valores podem ser interconvertidos dentro da mesma coluna.

<sup>†</sup> os deslocamentos químicos do acetil C=O e CH<sub>3</sub> são respectivamente 166,9 e 19,6 ppm.



Tabela 9

 $\delta$  (ppm) de  $^{13}\text{C}$  da série bebeerina-condrocurina

C	119	120	121	122	123	124	125	126
1	59,8	60,3	60,2	60,2	60,4	60,6	60,2	59,3
3	43,6	43,1	43,5	43,4	43,1	43,2	42,9	44,2
4	21,6	21,6	21,9	21,4	21,6	22,1	21,5	23,2
4a	*123,9	129,4	129,2	124,3	129,5	132,0	132,0	124,6
5	107,7	108,7	108,8	107,7	108,8	108,7	108,4	107,7
6	146,8	151,5	151,7	146,5	151,7	150,5	150,2	146,7
7	137,3	140,1	140,4	137,0	140,3	131,2	130,9	137,2
8	138,5	145,9	144,8	138,5	145,0	144,4	144,0	137,9
8a	*124,0	124,2	124,2	124,3	124,1	124,3	123,7	125,4
$\alpha$	39,5	38,9	39,4	39,5	39,2	39,3	38,7	40,1
9	133,2	133,9	133,4	134,1	140,5	134,0	140,1	132,6
10	120,2	122,1	120,7	121,4	121,8	122,5	121,5	121,0
11	+142,8	143,9	143,1	143,6	146,5	144,2	146,4	143,1
12	145,9	148,9	146,1	148,8	139,9	149,2	139,9	145,9
13	115,2	111,7	115,2	112,8	122,4	112,0	122,2	115,4
14	125,8	125,0	126,3	124,6	125,2	125,4	124,9	125,7
1'	64,7	64,9	65,2	64,7	64,6	65,1	64,8	64,4
3'	44,6	45,6	45,4	45,0	45,0	45,7	45,4	46,5
4'	24,1	25,2	24,9	24,4	24,6	25,2	25,2	25,2
4a'	128,4	126,5	128,4	126,5	127,1	126,5	127,7	128,6
5'	112,0	112,1	112,0	111,9	112,2	112,4	112,0	111,9
6'	148,2	147,8	148,4	148,3	148,3	148,1	147,9	148,0
7'	+143,5	143,2	143,5	143,3	143,2	143,3	142,8	143,1
8'	119,5	116,2	119,3	117,2	117,7	116,3	117,4	117,9
8a'	128,4	127,9	128,3	127,5	127,2	127,2	128,0	128,1
$\alpha'$	39,5	39,4	39,7	39,5	40,1	39,6	39,9	39,0
9'	131,5	131,5	131,3	131,5	131,2	132,7	132,3	131,7
10'	131,3	131,9	132,0	131,5	131,9	132,0	131,7	132,2
11'	114,7	114,7	115,2	114,3	114,8	115,0	114,6	113,2
12'	155,2	155,2	155,6	155,0	155,4	154,7	154,4	155,4
13'	113,1	112,9	113,0	113,5	113,2	113,6	113,6	114,9
14'	129,2	129,0	129,6	129,3	129,3	129,3	129,0	129,9
NMe	41,3	* 41,3	* 41,5	* 41,4	41,4	* 41,5	* 41,1	+ 42,2
	41,3	* 42,1	* 41,8	* 41,8	41,4	* 42,1	* 41,9	+ 42,5
OMe	55,7	55,7	55,8	56,0	55,7	55,9	55,5	* 55,8
	55,7	55,7	55,8	56,0	55,7	55,9	55,5	* 56,0
		60,8	61,0	56,0	60,9	55,9		
		(C-7)	(C-7)		(C-7)			
C=O					168,3	168,2	167,7	
							168,1	
CO-CH <sub>3</sub>		55,7			20,3	20,1	19,6	
		(C <sub>12</sub> OCH <sub>3</sub> )					20,0	

Obs: Os sinais \* e + significam que os dados podem estar invertidos.

Tabela 10 - Dados de RMN-<sup>1</sup>H da bebeerina e seus derivados.

Substância	δ (ppm)									
	OMe	NMe	H-5	H-5'	H-8'	H-10	H-13	H-14		
<u>119</u>	3,97 (s)	2,55 (s), 2,35 (s)	6,75 (s)	6,61	6,04	6,66 (d, J = 2,0)	6,87 (d, J = 8,3)	7,03 (dd, J = 2,0; 8,3)		
<u>120</u>	3,93; 3,91, 3,76; 3,74	2,56; 2,34	6,77	6,64	5,59	6,62 (d, J = 2,0)	6,87 (d, J = 8,3)	7,23 (dd, J = 2,0; 8,3)		
<u>121</u>	3,95; 3,92, 3,80	2,60; 2,32	6,77	6,64	6,00	6,71 (d, J = 2,0)	6,88 (d, J = 8,3)	7,03 (dd, J = 2,0; 8,3)		
<u>122</u>	3,92; 3,90 3,74	2,51; 2,34	6,74	6,60	5,60	6,54 (d, J = 2,0)	6,86 (d, J = 8,3)	7,23 (dd, J = 2,0; 8,3)		
<u>123</u>	3,85; 3,82 3,70	2,52; 2,30 2,12	6,53	6,63	5,60	6,58 (d, J = 2,0)	6,91 (d, J = 8,5)	7,18 (dd, J = 2,0; 8,5)		
<u>124</u>	3,90; 3,85, 3,70	2,55; 2,30 2,07	6,70	6,57	5,50	6,60 (d, J = 2,0)	6,78 (d, J = 8,5)	7,15 (dd, J = 2,0; 8,5)		
<u>125</u>	3,83	2,30; 2,51 2,11 2,06	6,58	6,61	5,58	m	6,91 (d, J = 8,3)	7,13 (dd, J = 2,0; 8,3)		

Tabela 11.

$\delta$  (ppm) de  $^{13}\text{C}$  dos alcalóides BBI quaternários das séries bebeerina-condrocurina-isocondodendrina

<u>C</u>	<u>127</u>	<u>128</u>	<u>129</u>	<u>130</u>	<u>131</u>	<u>118</u>
1	68,7	68,5	65,9	66,5	61,9	69,2
3	54,5	54,7	55,0	55,3	45,0	54,3
4	23,6	23,6	23,6	24,3	21,1	23,8
4a	120,1	125,4	120,4	125,6	120,0	121,2
5	108,7	109,7	108,8	110,3	107,9	109,8
6	149,6	154,3	149,0	154,4	148,9	149,8
7	138,8	140,4	137,5	140,4	137,7	137,5
8	137,4	143,8	138,2	144,9	138,2	138,6
8a	119,8	119,9	119,9	121,2	120,0	119,2
$\alpha$	38,6	39,7	36,8	37,4	40,2	37,7
9	129,0	130,8	129,1	130,2	128,0	127,4
10	124,0	123,6	123,3	124,0	120,0	129,8
11	142,4	142,5	142,0	143,0	142,1	118,2
12	148,8	149,2	147,7	149,4	147,7	154,6
13	116,7	114,5	117,1	114,1	116,5	114,9
14	127,4	127,8	123,9	124,6	126,0	129,4
1'	65,1	72,7	72,5	73,4	64,7	69,2
3'	45,9	54,2	55,0	55,5	44,4	54,3
4'	22,6	23,6	23,6	24,3	21,1	23,8
4a'	124,4	123,2	123,1	123,6	123,5	121,2
5'	112,3	112,9	113,0	113,7	112,3	109,8
6'	150,3	150,9	149,9	151,4	149,2	149,8
7'	146,4	146,0	145,2	145,4	144,2	137,5
8'	118,4	116,7	117,1	117,9	116,8	138,6
8a'	121,0	121,3	122,7	123,1	121,9	119,2
$\alpha'$	40,0	37,0	38,0	38,8	39,7	37,7
9'	129,9	129,5	128,6	129,7	127,3	127,4
10'	134,0	134,1	131,6	132,3	131,9	129,8
11'	115,3	114,5	115,5	115,5	114,3	118,2
12'	156,4	156,5	155,7	156,6	155,1	154,6
13'	113,1	112,9	113,0	113,7	113,7	114,9
14'	130,8	131,2	129,9	130,9	130,1	129,4
+NMe	40,5 (N')	*51,0,	51,1,	*51,4,	40,4,	51,8
		*51,2,	51,1,	*51,6,	40,4,	51,8
	* 51,3 (N),	+52,9,	*52,4,	+52,8,		53,0
		+54,7	*52,9	+53,5		53,0
	* 54,5 (N)					
OMe	56,4,	§56,1,	+56,4,	§56,7,	55,9,	57,0
	56,4	§56,5,	+56,7	§56,9,	55,9	57,0
		56,5,		57,3,		
$\text{C}_7\text{OCH}_3$		60,7		61,4		

Obs: Os sinais \*, + e § significam que os dados podem estar invertidos.

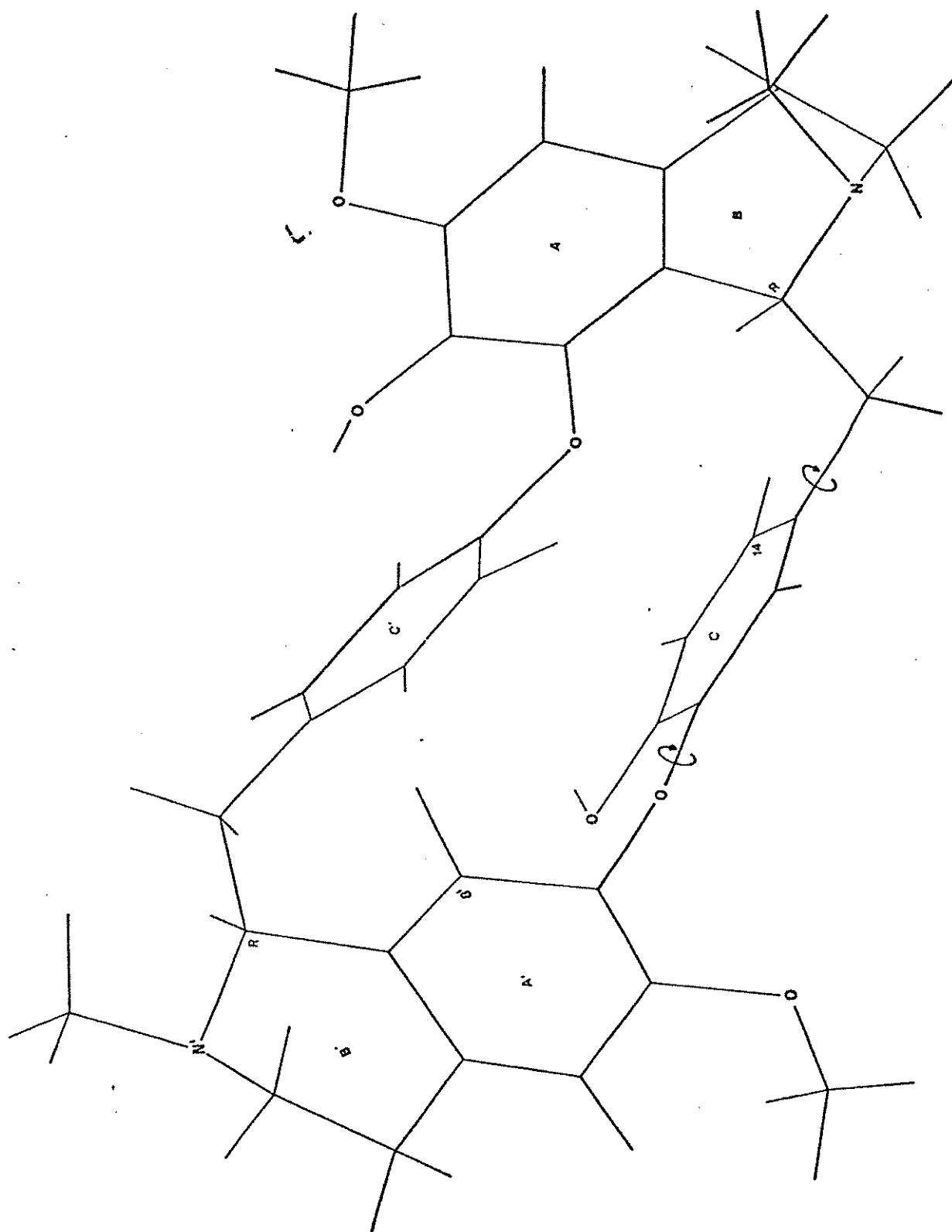


Figura 3 - Conformação preferida de bebeerina 119

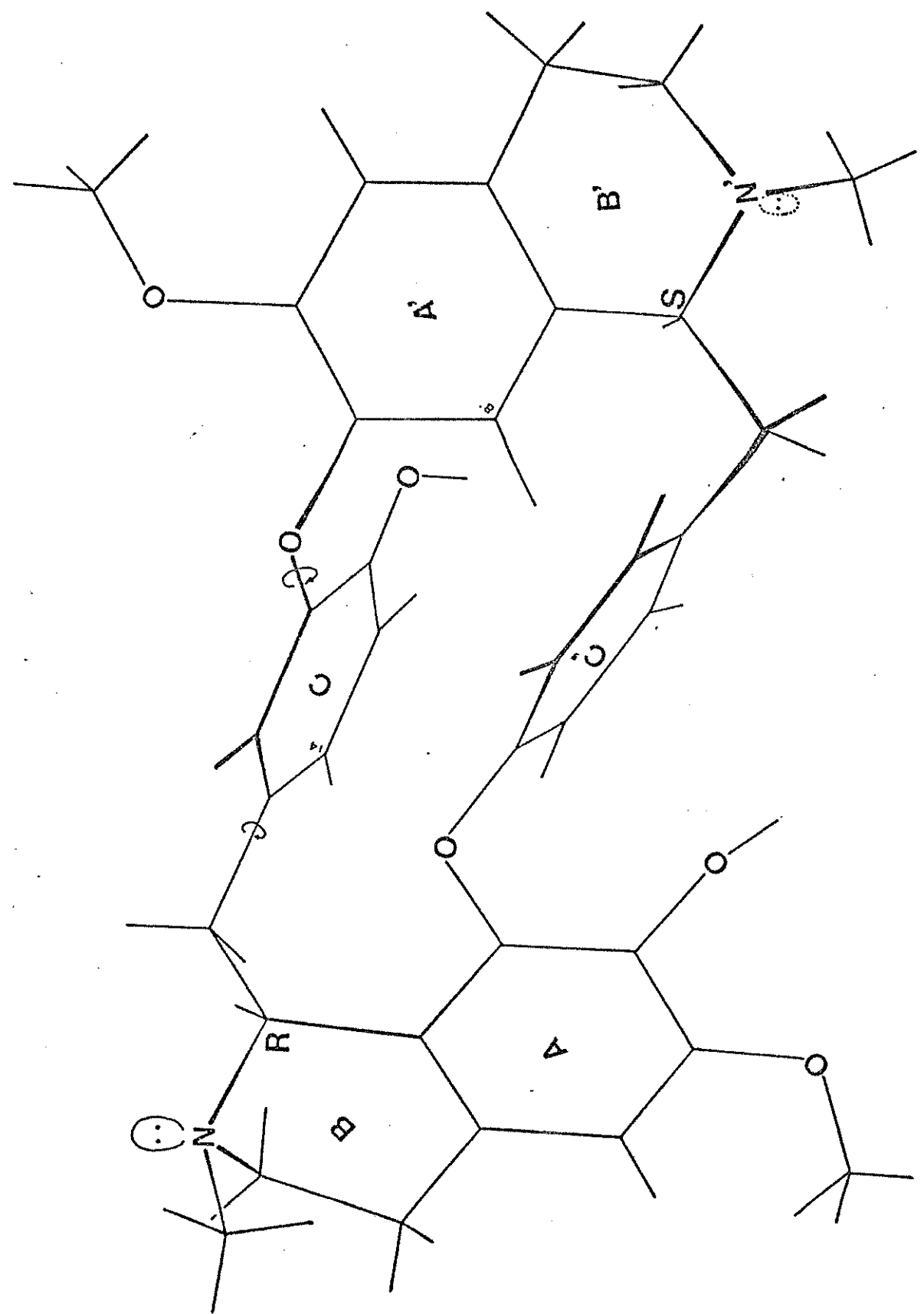
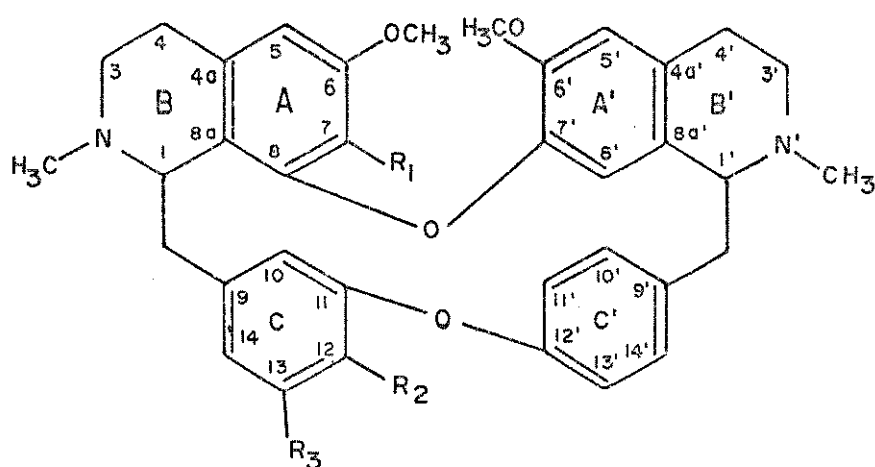


Figura 4 - Conformação preferida de condrocurina 126

## II. Alcalóides tipo Berbamina

Os alcalóides BBI do tipo berbamina possuem suas unidades BI unidas "cauda-cauda".

A atribuição dos deslocamentos químicos de  $^{13}\text{C}$  da fean-  
tina 134; tenuipina 135; nortenuipina 136; berbamina 137; O-acetilnortenui-  
pina 138 e O-acetil berbamina 139 se encontram na tabela 12<sup>18</sup> (pag. 67).



134  $R_1=R_2=\text{OMe}$  ;  $R_3=\text{H}$  (R, R)

135  $R_1=\text{OMe}$  ;  $R_2=R_3=-\text{OCH}_2\text{O}-$  (S, S)

136  $R_1=\text{OH}$  ;  $R_2=R_3=-\text{OCH}_2\text{O}-$  (S, S)

137  $R_1=\text{OCH}_3$  ;  $R_2=\text{OH}$  ;  $R_3=\text{H}$  (R, S)

138  $R_1=\text{OCOCH}_3$  ,  $R_2=R_3=-\text{OCH}_2\text{O}-$  (S, S)

139  $R_1=\text{OCH}_3$  ;  $R_2=-\text{OCOCH}_3$  ;  $R_3=\text{H}$  (R, S)

140  $R_1=R_2=\text{OMe}$  ;  $R_3=\text{H}$  (S, S)

Analisando os deslocamentos químicos dos carbonos do anel B' e examinando as possíveis conformações, podemos dizer que o anel B' de 134 tende para uma semi cadeira onde o grupo N'-CH<sub>3</sub> ocupa uma posição equatorial com pouca interação com o C<sub>4</sub>, ( $\delta = 25,3$  ppm) mas com uma interação "γ gauche" com C<sub>α</sub>'. O anel B tende para uma semi-cadeira com o grupo N-CH<sub>3</sub> na posição axial com interação "γ gauche" com o C<sub>4</sub> ( $\delta = 22,1$  ppm) e sem interação com C<sub>α</sub> ( fig. 5 )

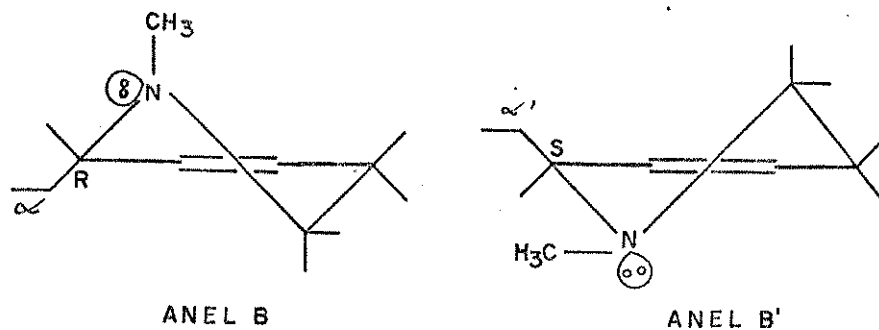


FIGURA 5

Comparamos os deslocamentos químicos dos carbonos  $sp^3$  do par feantina (R,R) 134 e berbamina (R,S) 137 e verificamos que, quando os centros quirais  $C_1$  e  $C_1'$ , do produto natural têm a mesma configuração (RR ou SS), o  $\Delta\delta = [C\alpha - C\alpha']$  é grande. Quando as configurações dos carbonos  $C_1$  e  $C_1'$ , são diferentes (RS ou SR), os valores de  $\Delta\delta = [C\alpha - C\alpha']$  são menores, tendendo para zero.

No composto 137, cujos centros assimétricos  $C_1$  e  $C_1'$ , possuem configuração R e S, respectivamente, os valores de  $\delta$  são próximos ( $C\alpha = 37,5$  ppm e  $C\alpha' = 38,2$  ppm) com um  $\Delta\delta = 0,7$  ppm. No composto 134, cujos centros assimétricos  $C_1$  e  $C_1'$ , possuem configuração R e R, têm ( $C\alpha = 41,9$  ppm e  $C\alpha' = 38,3$  ppm) o  $\Delta\delta = 3,6$  ppm. O valor extremo nesta série foi encontrado na tenuipina 135 ( $\Delta\delta = 4,0$  ppm). Estes dados nos indicam que existe maior interação espacial na série R,R e S,S do que na série R,S ou S,R. Neste tipo de alcalóide BBI, os deslocamentos químicos do  $C\alpha$  e  $C\alpha'$  são sensíveis à configuração dos centros quirais, e estes valores podem ser indicativos de alcalóides que tenham configuração idêntica (R,R ou S,S) ou diferentes (RS ou SR) nos centros quirais  $C_1$  e  $C_1'$ .

Fizemos uma análise do anel macrocíclico com modelos moleculares "Dreiding" associando-a com a cristalografia<sup>40</sup> de raios-X

da tetrandrina 140 e os deslocamentos químicos da feantina 134, que é enantiômero da tetrandrina 140. Concluimos que também em solução essa molécula adquire uma conformação "dobrada" para os anéis A', B' e C' e "estendida" para os anéis A, B e C. O grupo N-CH<sub>3</sub> do anel B' desta série ocupa posição preferencialmente equatorial e o N-CH<sub>3</sub> do anel B ocupa posição preferencialmente axial de forma semelhante à estrutura cristalina<sup>40</sup>.

As conformações preferidas para a feantina 134 e berbamina 137 estão propostas nas figuras 6 e 7, respectivamente. Observa-se que a mudança da configuração do C<sub>1</sub>, produz modificações conformacionais não apenas na sua vizinhança como também em todo o anel macrocíclico, e em consequência a conformação do anel B é alterada. Nota-se que o deslocamento químico do C<sub>4</sub> da feantina 134 é 22,1 ppm e na berbamina 137 é 23,9 ppm indicando uma redução da população conformacional da semi-cadeira na qual o metila de N-CH<sub>3</sub> é axial.



Tabela 12

Dados de RMN-<sup>13</sup>C da série berbamina

<u>C</u>	<u>134</u>	<u>135</u>	<u>136</u>	<u>137</u>	<u>138</u>	<u>139</u>
C-1	61,4	61,4	61,5	62,0	61,4	62,0
C-3	44,1	44,0	44,0	44,7	43,8	44,8
C-4	22,1	22,0	21,7	23,9	22,0	23,8
C-4a	*128,0	*127,5	123,0	129,0	134,9	127,1
C-5	105,8	105,6	*104,7	105,4	105,6	105,4
C-6	151,2	151,2	145,6	151,7	149,7	151,7
C-7	137,9	136,6	134,3	136,8	130,9	137,8
C-8	148,2	148,3	141,6	147,7	147,0	147,6
C-8a	123,0	122,4	123,0	120,1	122,4	120,3
C-α	41,9	42,2	42,2	37,5	42,7	37,6
C-9	134,9	136,4	136,5	134,0	136,5	137,8
C-10	116,2	110,3	110,3	115,3	110,5	116,9
C-11	146,9	143,1	143,1	143,8	143,1	151,4
C-12	149,3	133,4	133,3	147,3	133,4	141,5
C-13	111,6	148,1	148,2	114,6	148,4	121,2
C-14	122,6	104,5	*104,5	123,5	104,6	123,3
C-1'	63,9	63,7	63,5	63,4	64,1	63,7
C-3'	45,3	45,0	45,0	45,2	45,5	45,9
C-4'	25,3	24,8	25,2	24,8	24,9	25,5
C-4α'	*128,1	*127,9	+128,2	127,9	128,9	128,5
C-5'	112,7	112,5	112,8	111,1	112,5	111,0
C-6'	148,5	148,5	148,6	149,9	148,7	149,7
C-7'	143,7	143,6	143,3	143,4	142,6	143,3
C-8'	120,0	120,0	120,4	119,7	120,5	119,6
C-8a'	*127,8	*127,2	+127,7	126,3	127,7	126,6
C-α'	38,3	38,2	37,5	38,2	39,7	38,1
C-9'	134,9	134,9	135,1	134,6	134,9	135,2
C-10'	+129,9	+129,9	§129,9	*130,0	*130,0	*130,1
C-11'	121,7	121,5	121,4	121,2	121,6	121,9
C-12'	153,6	153,3	153,1	153,9	153,4	153,9
C-13'	121,7	121,5	121,4	121,4	121,6	121,7
C-14'	+132,4	+132,4	§132,3	*132,0	*132,4	*132,1
NMe	§ 42,3	42,0	42,2	+ 42,6	+ 42,2	42,7
NMe'	§ 42,6	42,0	42,2	+ 42,0	+ 42,1	42,7
OMe	55,8	55,5	56,0	55,7	55,9	55,7
OMe (C-7)	56,1 55,8	55,5	56,0	55,7	55,7	55,5
OMe (C-7)	60,1	60,0		60,3		60,4
OCH <sub>2</sub> O		101,2	101,2		101,3	
C=O					167,5	169,0
COCH <sub>3</sub>					19,7	20,8

Obs: Os sinais \*, + e § significam que os dados podem estar invertidos.

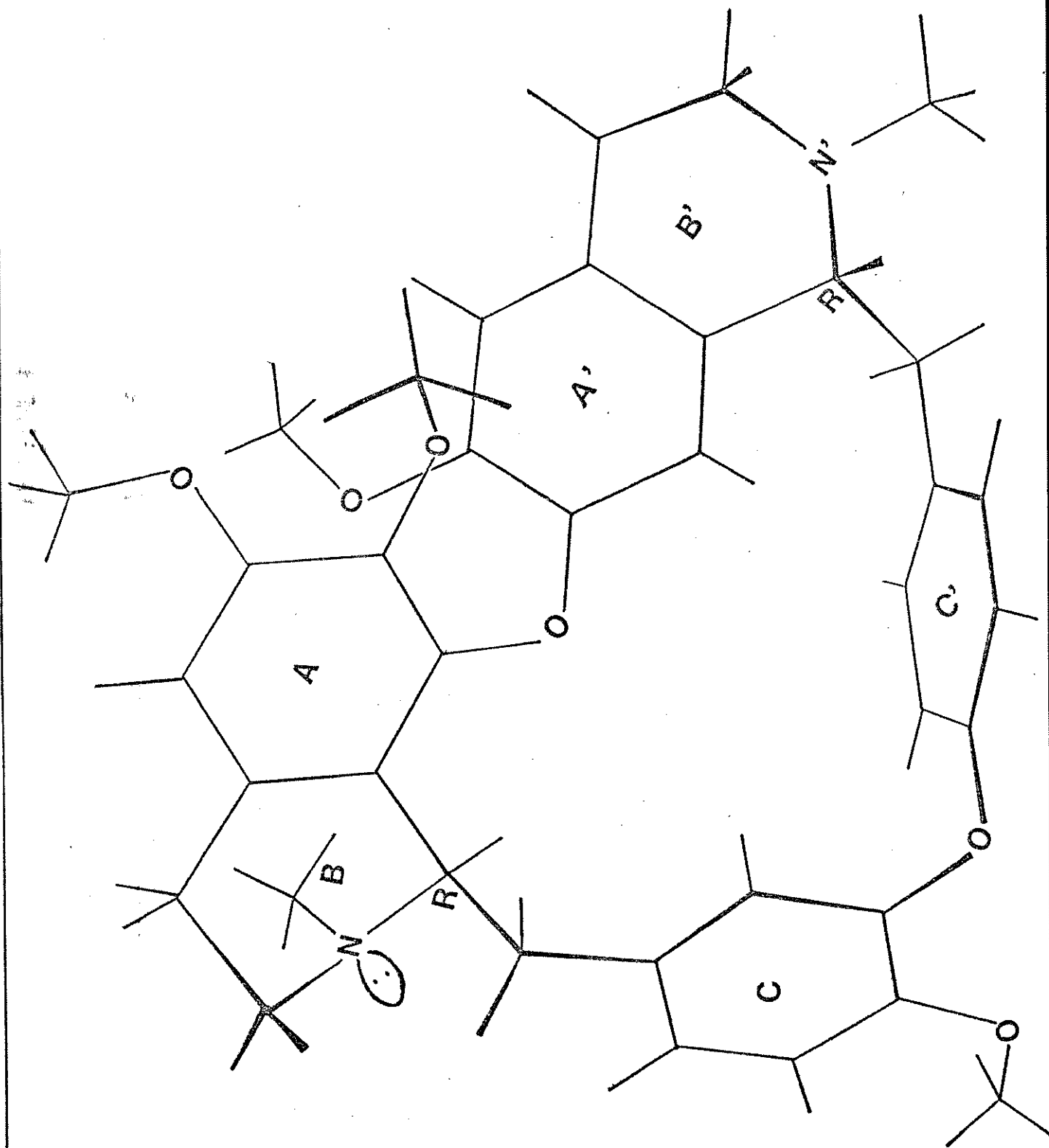


Figura 6 - Conformação preferida da feantina 134

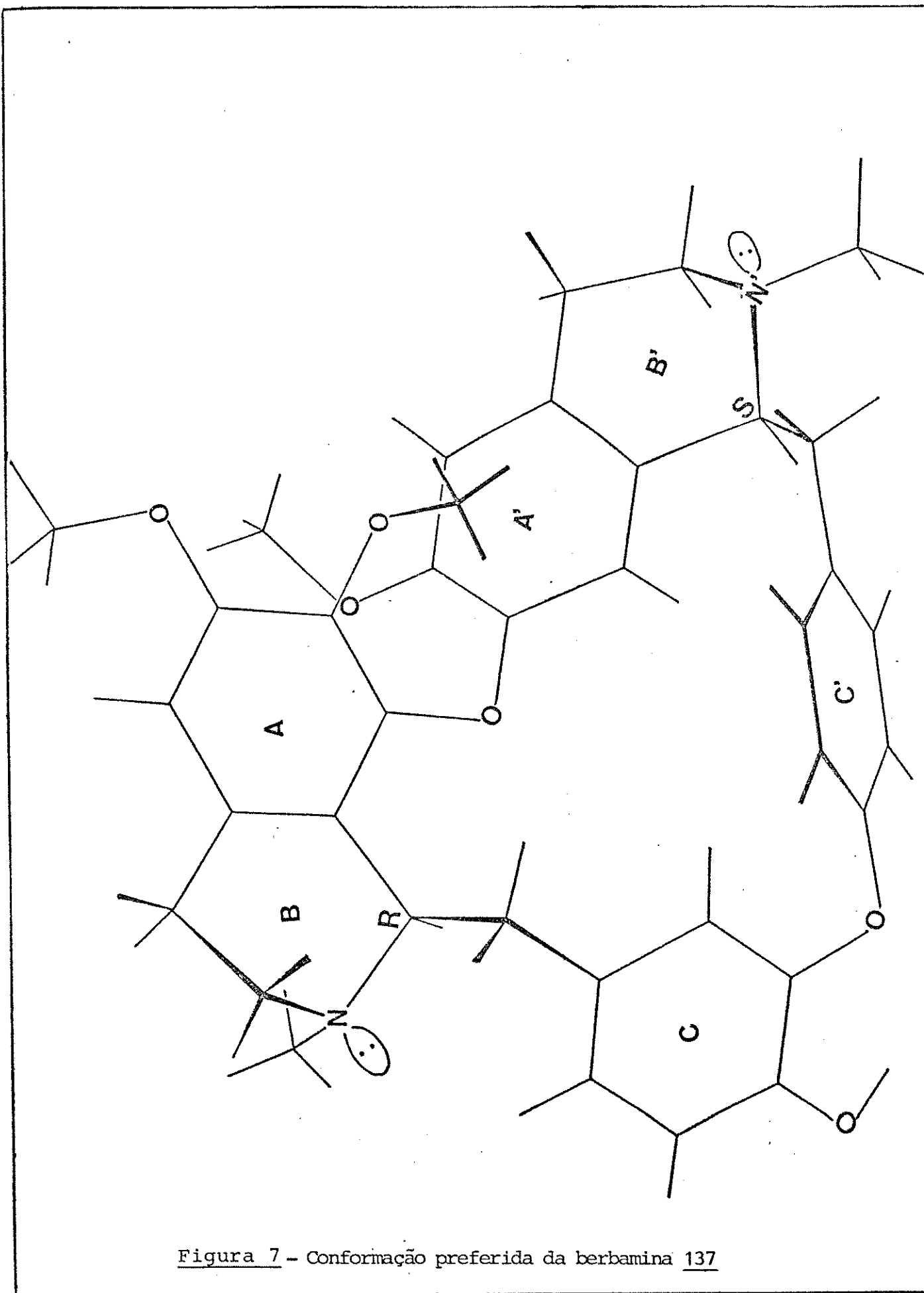
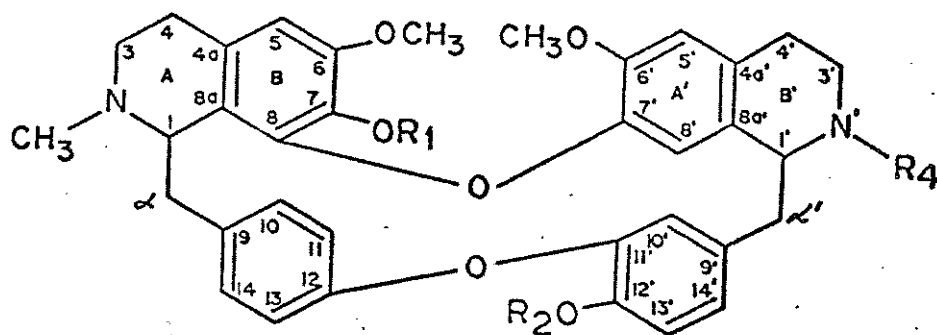


Figura 7 - Conformação preferida da berbamina 137

### III- Alcalóides do tipo dafnolina - repandina

Os compostos do tipo dafnolina - repandina se caracterizam por funções das unidades BI "cauda - cauda". Eles se diferenciam dos compostos vistos anteriormente, por apresentarem um padrão de substituição diferente nos anéis C e C'.

Analizamos <sup>21</sup> os espectros da dafnolina 141, N-metil-dafnolina 142, dafnandrina 143, O-metil-dafnandrina 144 (todos de configuração  $C_1-S; C_1,-R$ ), repandina 145 e O-metil-repandina 146 (ambos de configuração  $C_1-S; C_1,-S$ ).



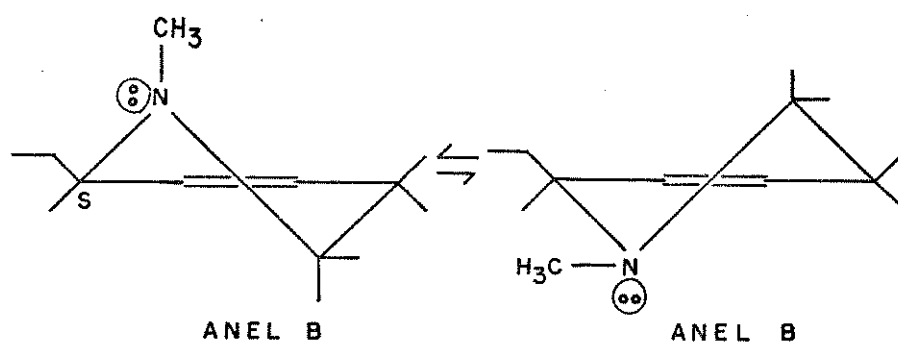
<u>141</u>	$R_1=R_2=R_4=H$ (S,R)
<u>142</u>	$R_1=R_2=H; R_4=CH_3$ (S,R)
<u>143</u>	$R_1=H; R_2=CH_3; R_4=H$ (S,R)
<u>144</u>	$R_1=R_2=CH_3; R_4=H$ (S,R)
<u>145</u>	$R_1=R_4=CH_3; R_2=H$ (S,S)
<u>146</u>	$R_1=R_2=R_4=CH_3$ (S,S)

Na N-metilação da dafnolina 141 → 142 observamos uma desproteção nos  $C_3$  ( $\Delta\delta = +5,1$  ppm) e  $C_1$  ( $\Delta\delta = +9,6$  ppm), e proteção  $\gamma$  no  $C_{8a}$  ( $\Delta\delta = -2,7$  ppm) e  $C_{a'}$  ( $\Delta\delta = -3,7$  ppm), alterando-se muito pouco  $C_{4'}$ .

O deslocamento químico do  $C_4$ , (28,4 ppm) indica que o grupo metila sobre o nitrogênio de 142 entrou na posição equato-

rial, sendo que os demais compostos desta série, os deslocamentos químico do carbono  $C_4$  variou entre 23,6 a 26,4 ppm. Estes valores intermediários indicam que o anel B está sujeito a maiores mudanças conformacionais, aumentando o equilíbrio entre as populações de semi-cadeiras com N-CH<sub>3</sub> axial e equatorial. A interação  $\gamma$  entre o grupo N-CH<sub>3</sub> com o  $C_4$  sofre o efeito destas mudanças (figura 8), apresentando valores de deslocamentos químicos intermediários.

Figura 8



Equilíbrio entre as semi-cadeiras do anel B da série dafnolina-repandina.

A comparação feita entre a N-metildafnolina 142 (S,R), e repandina 145 (S,S), confirmou que também nesta série BBI o valor de  $\Delta\delta$  entre o  $C\alpha$  e  $C\alpha'$  de um mesmo composto dá indicações sobre a configuração dos centros quirais  $C_1$  e  $C_1'$ . Quando estes centros quirais têm a mesma configuração (S,S ou R,R) como por exemplo na repandina 145, o valor de  $\Delta\delta = [C\alpha - C\alpha'] = 3,1$  ppm, e quando os centros quirais têm configurações diferentes (RS ou S,R), como em 142, o  $\Delta\delta [C\alpha - C\alpha'] = 1,7$  ppm.

Uma análise dos deslocamentos químicos de 145 e de 142, que pertencem a séries diastereoisômeras, mais precisamente dos carbonos  $C_5$ ,  $C_{4a'}$ ,  $C_9$  e  $C_{14'}$ , os quais estão na posição *para* à ligação éster difenílica entre os BI nos leva a concluir que na série repandina (S,S) os ângulos entre os planos dos anéis A e A' e

C e C' são maiores em relação à série dafnolina (SR).

Subst.	C <sub>5</sub>	C <sub>4a'</sub>	C <sub>9</sub>	C <sub>14'</sub>
<u>142</u>	104,7	128,7	130,6	124,5
<u>145</u>	106,5	130,9	132,3	123,7
$\Delta\delta$	+1,8	+2,2	+1,7	-0,8

O deslocamento químico do C<sub>8</sub>, pode confirmar as modificações angulares existentes nas ligações etéreas dos anéis AA' e CC' das séries SR e SS. Na série SR o C<sub>8</sub>, está sujeito a maior compressão espacial do anel C', apresentado um  $\delta = 117,3$  em 142, enquanto que na série SS, o composto 145 tem o C<sub>8</sub>, a  $\delta = 119,8$  ppm. Isto é notado quando se examina o modelo molecular de ambas as séries diastereoisoméricas (figuras 9 e 10).

Nos alcalóides do tipo dafnolina o anel C é *para* dissustituído o que normalmente influencia a conformação dobrada. Essa possibilidade no entanto é impedida pelo substituinte ligado ao C<sub>8</sub>. Por outro lado, a porção A' B' C', onde o C<sub>8</sub> não tem substituinte que facilitaria uma conformação dobrada, apresenta um padrão *meta* de substituição no anel C' que impede esta possibilidade. Concluimos que para haver conformação dobrada é necessário a relação *para* dissustituído no anel C ou C' associada à ausência de substituinte no carbono C<sub>8</sub> ou C<sub>8</sub>'. Não havendo a combinação desses dois fatores nesta série, prevalece a conformação estendida para ambas as porções BI.

A análise das conformações propostas para os alcalóides das séries S,R (O-metildafnandrina 144) e SS (repandina 145) evi-

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dência claramente as alterações nos ângulos interplanares entre os éteres A-O-A' e C-O-C' em consequência da mudança de configuração no C<sub>1</sub>. Estas alterações são comprovadas, pelos deslocamentos químicos dos carbonos sp<sup>2</sup> já comentadas e são confirmadas pela RMN-<sup>1</sup>H tomando-se o δ do próton H<sub>8</sub>' nos diferentes compostos das duas séries.

Nos compostos de configurações S,R: dafnolina 141, N-metil-dafnolina 142, dafnandrina 143, e O-metildafnandrina 144 o próton H<sub>8</sub>, ressona à 5,76; 5,73; 5,66 e 5,70 ppm, respectivamente. Examinando-se a conformação proposta para a O-metildafnandrina 144 (figura 9) observa-se que o H<sub>8</sub>, está direcionado para a região de proteção anisotrópica dos anéis C e C', razão pela qual sofre ressonância a campo mais alto dos demais prótons aromáticos.

Nos alcalóides de configuração S,S repandina 145 e O-metilrepandina 146 os H<sub>8</sub>, sofrem ressonância à δ = 6,33 e 6,38 ppm. Na conformação proposta para a repandina 145 (figura 10) este próton (H<sub>8</sub>,) está direcionado na região de desproteção anisotrópica do anel C', compensando dessa forma a proteção causada pelo anel C, conseqüentemente o H<sub>8</sub>, sofre ressonância a campo mais alto que os demais prótons aromáticos e ao mesmo tempo mais desprotegido quando comparado com o mesmo próton na dafnolina 141 e em seus derivados 142, 143 e 144. Estas diferenças observadas nos δ do H<sub>8</sub>, que são ≥ 0,6 ppm nos compostos S,R e SS são ilustrativas das modificações acentuadas que se produzem nestes compostos macrociclos pela mudança de configuração no centro quiral C<sub>1</sub>.

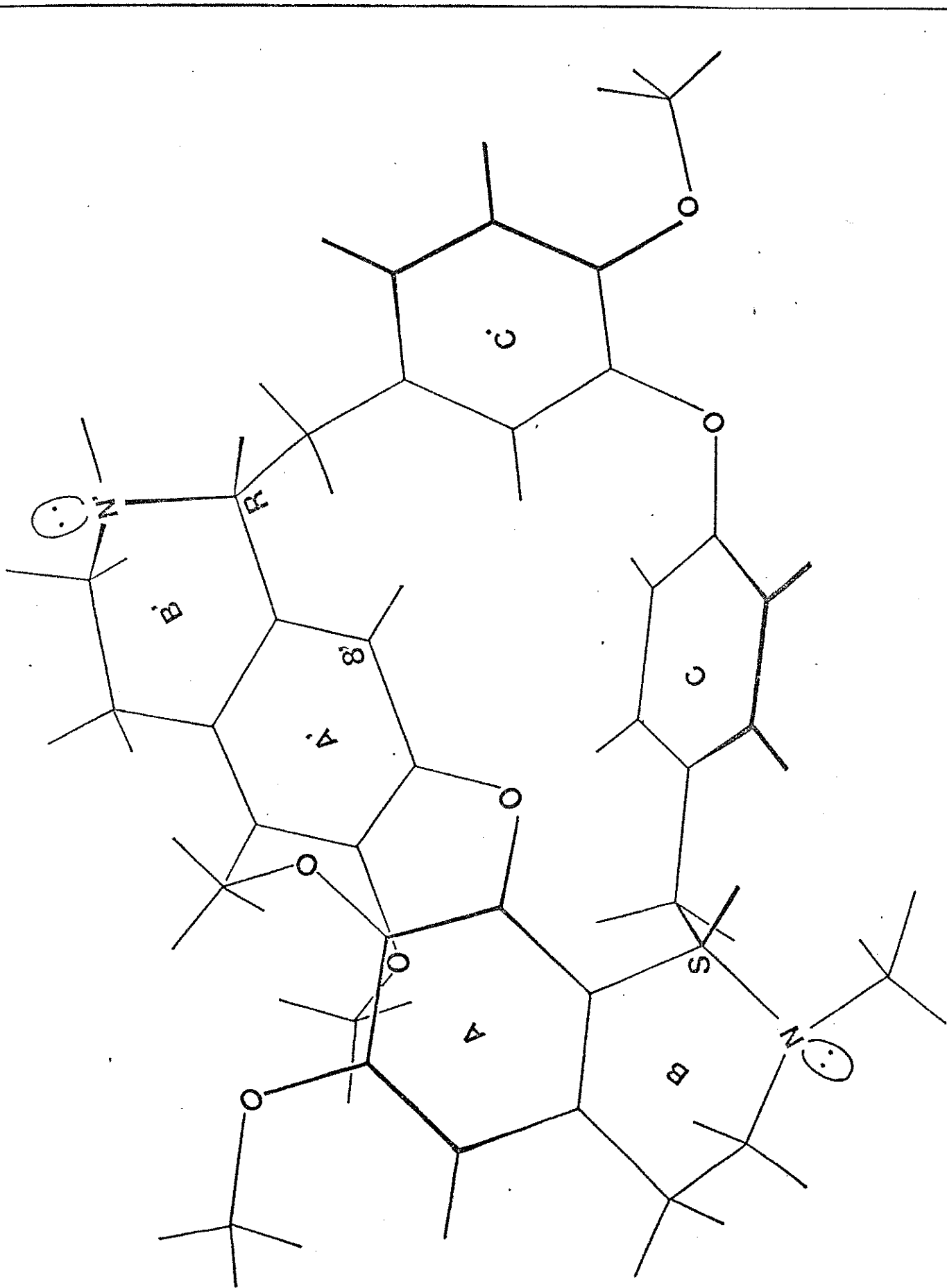


Figura 9 - Conformação preferida de O-metildafnandrina 144



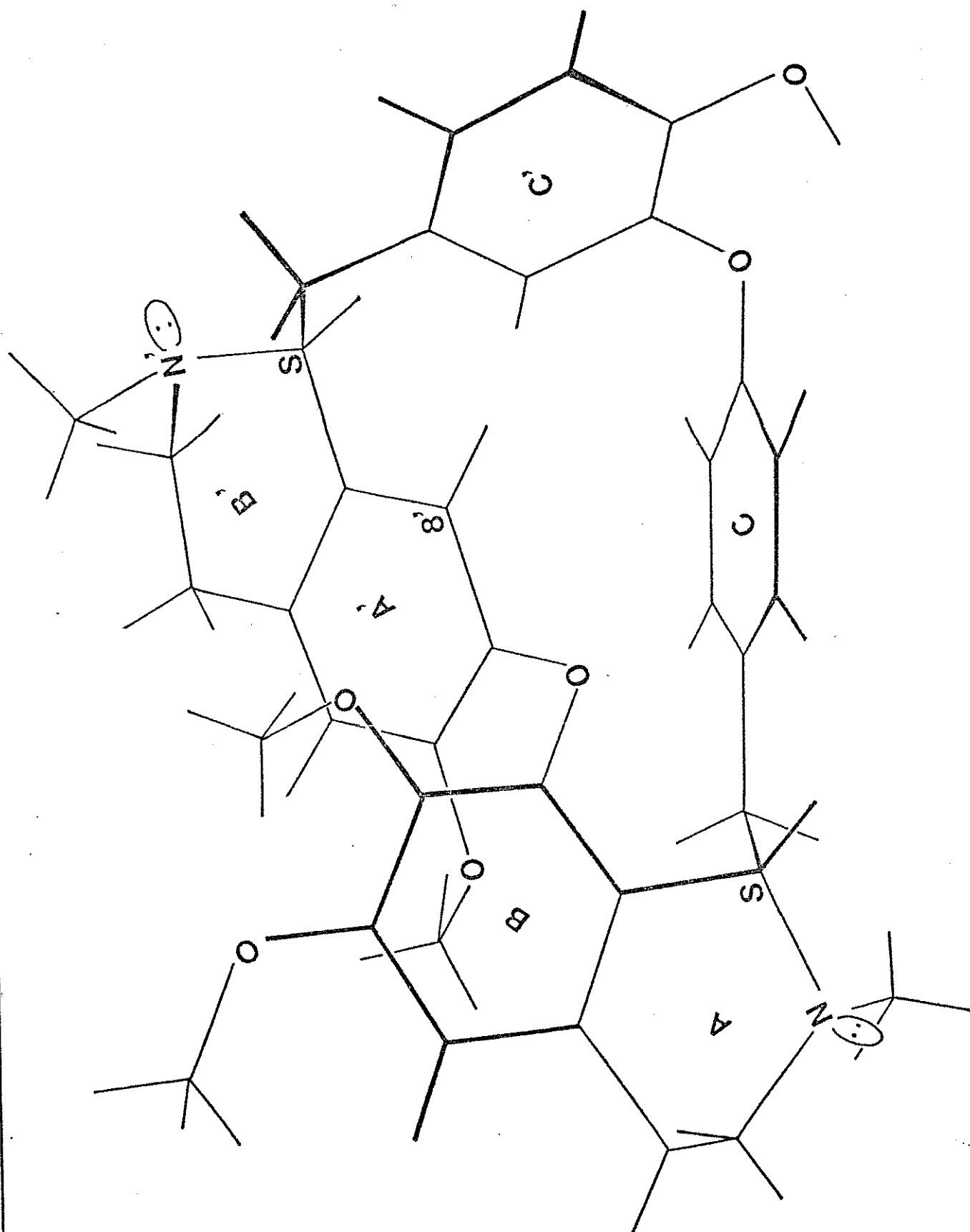


Figura 10 - Conformação preferida da repandina 145

#### IV- Alcalóides do tipo micrantina

Na série micrantina a junção das duas unidades benzilisoquinolinas formam um anel suplementar (anel D) do tipo dibenzodioxínico, o que torna estes compostos mais rígidos quando comparados com os das séries precedentes. Serão analisadas, além da micrantina 147, a O-metilmicrantina 148 e a N,O-dimetilmicrantina 149.

Os deslocamentos químicos dos  $^{13}\text{C}$  da micrantina 147 evidenciaram, de imediato, valores para os deslocamentos químicos dos carbonos  $^{13}\text{C}$   $\text{sp}^2$  diferentes dos demais BBI analisados. Em função desta diferença foi sintetizado o dibenzodioxina 150.

Aos carbonos  $\text{sp}^2$  de 147 totalmente substituídos os valores (ppm): 138,6; 137,7; 139,0 e 139,6 foram atribuídos aos carbonos  $\text{C}_8$ ,  $\text{C}_7$ ,  $\text{C}_7'$  e  $\text{C}_6$ , respectivamente, por comparação com os deslocamentos químicos dos carbonos semelhantes no modelo 150. Os carbonos  $\text{C}_5 = 106,7$  ppm,  $\text{C}_{4a} = 126,7$  ppm,  $\text{C}_{8a} = 120,0$  ppm e  $\text{C}_6 = 147,7$  ppm foram atribuídos por comparação com os mesmos carbonos do anel A da feantina (134) que é o modelo mais próximo, sendo confirmada pela correlação com os demais compostos desta série. A faeantina também serviu de modelo para a seguinte atribuição dos deslocamentos químicos dos carbonos  $^{13}\text{C}$  dos anéis C e C':  $\text{C}_9 = 135,0$  ppm;  $\text{C}_{10} = 129,2$  ppm;  $\text{C}_{11} = 120,6$  ppm;  $\text{C}_{12} = 154,8$  ppm;  $\text{C}_{13} = 121,9$  ppm;  $\text{C}_{14} = 131,2$  ppm (para o anel C);  $\text{C}_{9'} = 132,5$  ppm;  $\text{C}_{10'} = 115,9$  ppm;  $\text{C}_{11'} = 144,5$  ppm;  $\text{C}_{12'} = 145,8$  ppm;  $\text{C}_{13'} = 116,5$  ppm e  $\text{C}_{14'} = 122,2$  ppm (anel C'). Os demais carbonos  $\text{sp}^2$  foram assim atribuídos:  $\text{C}_{5'} = 112,6$  ppm;  $\text{C}_{8'} = 117,6$  ppm;  $\text{C}_{4a'} = 130,7$  ppm e  $\text{C}_{8a'} = 128,6$  ppm, por comparação com os carbonos  $\text{C}_{5'}$ ,  $\text{C}_{8'}$ ,  $\text{C}_{4a'}$  e  $\text{C}_{8a'}$  da dafnandrina 143.

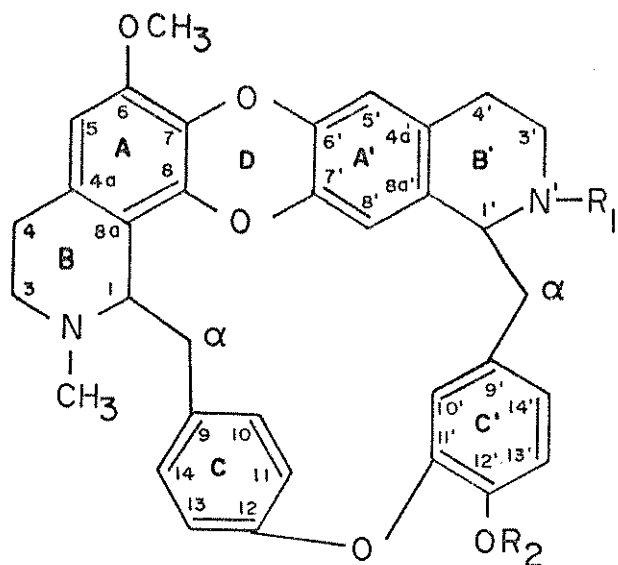
A análise dos dados da O-metilmicrantina 148 permitiram

confirmar as atribuições acima, principalmente as dos carbonos do anel C, pois aqui também notamos os efeitos que a metilação do fenol sobre o C<sub>12</sub>, provoca nos deslocamentos químicos deste anel, ou seja, desproteção em *ipso* C<sub>12</sub>, ( $\Delta\delta = +3,9$  ppm), *orto* C<sub>11</sub>, ( $\Delta\delta = +1,2$  ppm) e *para* C<sub>9</sub>, ( $\Delta\delta = +1,7$  ppm), e proteção no carbono C<sub>13</sub>, *orto* ( $\Delta\delta = -4,1$  ppm), este último devido à interação " $\gamma$  gauche" entre a metila sobre o fenol e o C<sub>13</sub>.

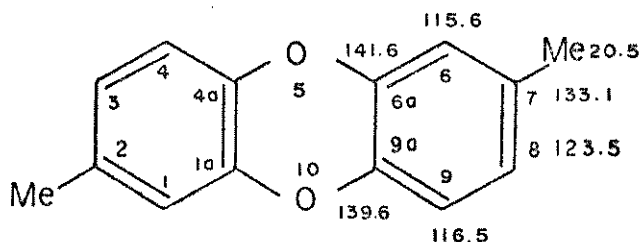
Os deslocamentos químicos dos carbonos alifáticos de 147 foram divididos em dois grupos, e foram atribuídos ao C<sub>4</sub>  $\delta = 23,5$  ppm, N -CH<sub>3</sub>  $\delta = 41,2$  ppm; C $\alpha$   $\delta = 42,2$  ppm, C<sub>3</sub>  $\delta = 44,3$  ppm, C<sub>1</sub>  $\delta = 60,2$  ppm do anel B, carbonos C<sub>4</sub>,  $\delta = 28,0$  ppm, C<sub>3</sub>,  $\delta = 41,2$  ppm; C $\alpha$ ,  $\delta = 42,3$  ppm e C<sub>1</sub>,  $\delta = 59,5$  ppm do anel B', por analogia aos anéis B e B' da dafnolina 141.

A N'-metilação da O-metil-micrantina 148 produzindo 149, comprovou, pela semelhança dos deslocamentos químicos apresentados com relação à 147 e 148 que a atribuição destes compostos está correta. A única exceção são os carbonos do anel B', o que já era esperado, que pela reação de N'-metilação ocasionou um efeito  $\beta$  de desproteção nos carbonos C<sub>3</sub>, ( $\Delta\delta = +3,9$  ppm) e C<sub>1</sub>, ( $\Delta\delta = +7,1$  ppm) e fraca interação  $\gamma$  no C<sub>4</sub>, ( $\Delta\delta = -1,2$  ppm) e no C $\alpha'$  ( $\Delta\delta = -0,3$  ppm).

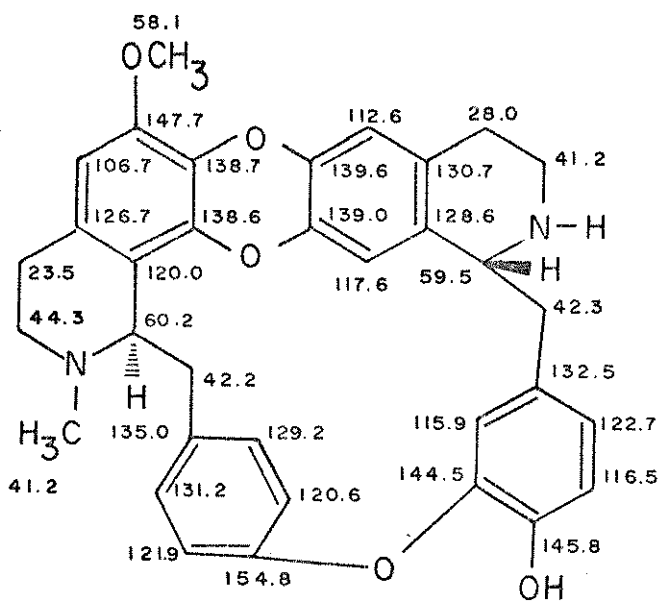
A existência do sistema dibenzodioxínico do anel D altera enormemente a conformação do macrociclo dos compostos desta série. Na unidade BI, composta pelos anéis A, B e C, o anel C, apesar de apresentar a relação *para* entre o C $\alpha$  e a ligação éter difenílica, não pode tomar uma conformação dobrada. Por outro lado, a unidade BI, formada pelos anéis A' B' e C', não apresenta substituição no carbono C<sub>8</sub>', e no entanto o anel C' tem o carbono C $\alpha'$  e a ligação éter difenílica em relação *meta*, tendo que adotar a



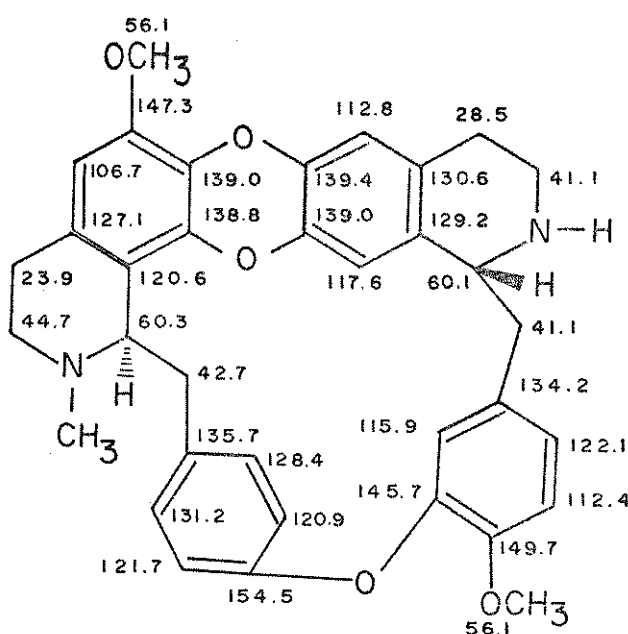
Tipo micrantina



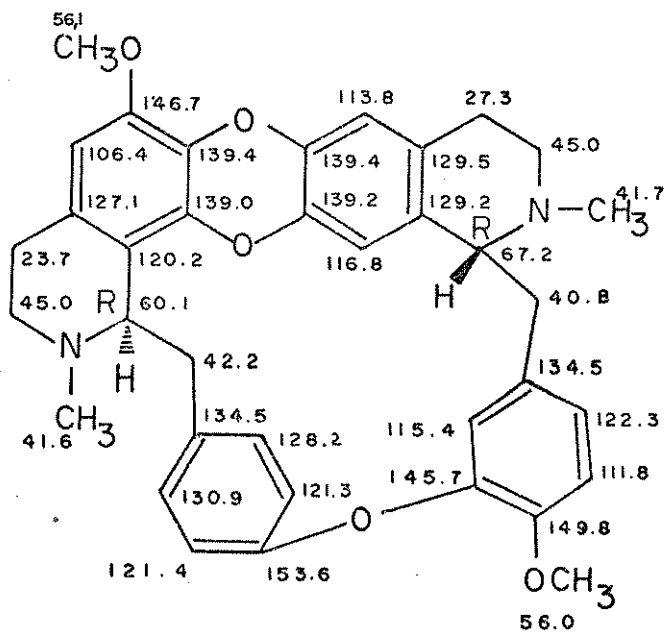
150



(R,R) Micrantina 147



(R,R) O, metil, micrantina 148



(R,R) O, metil, N, metil, micrantina 149

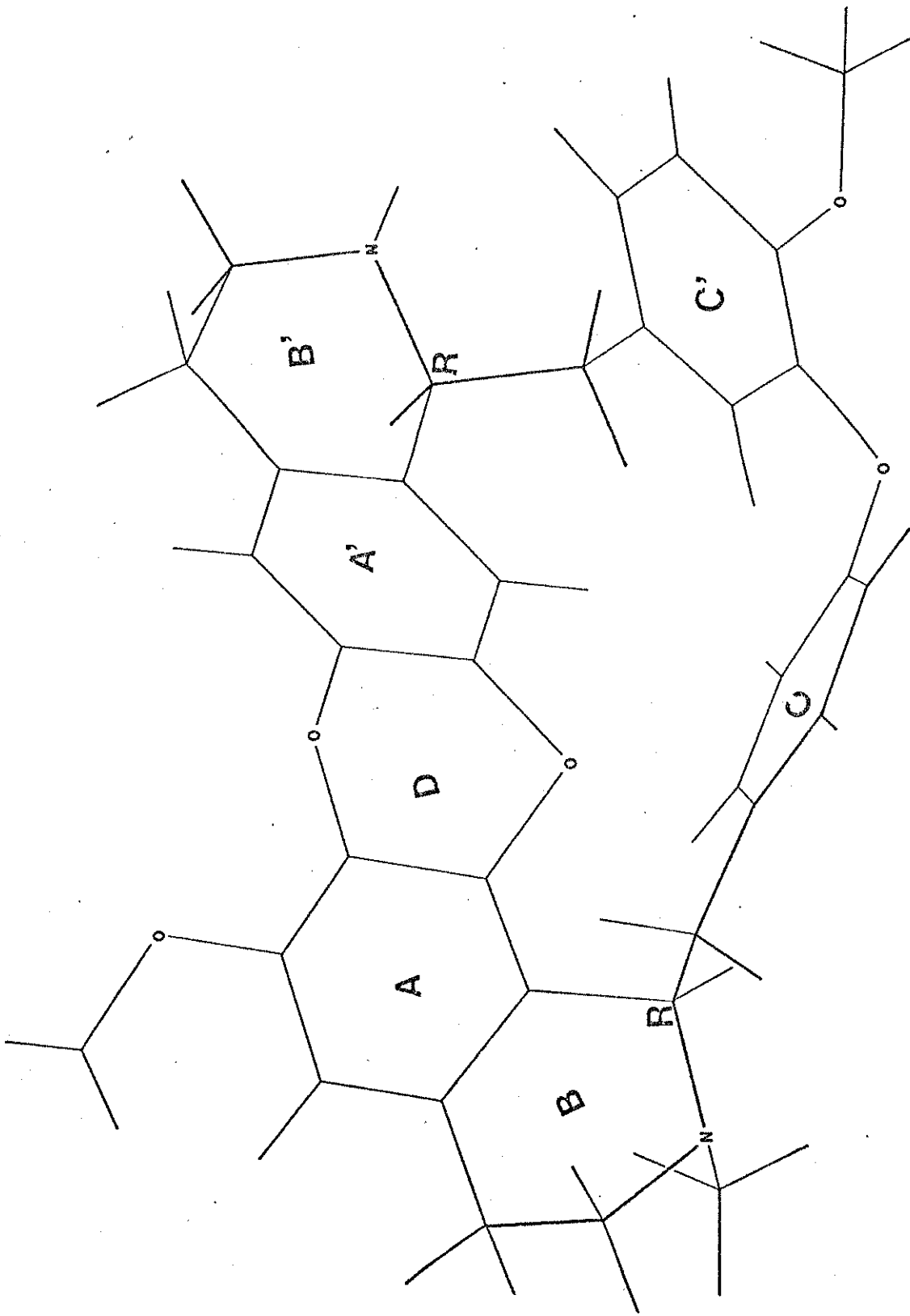
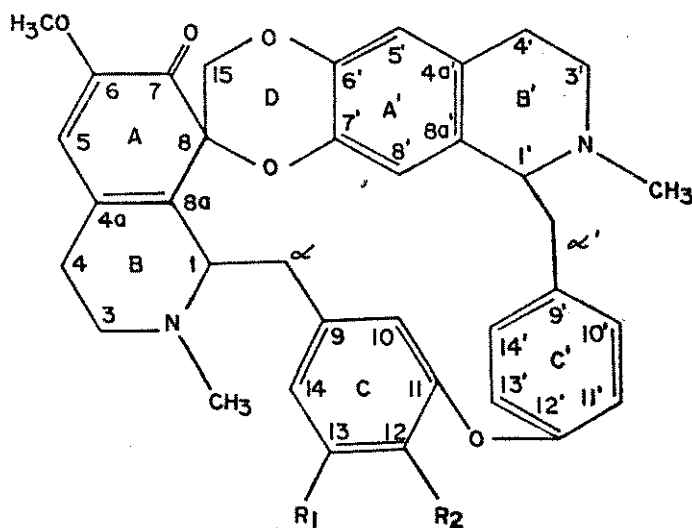


Figura 11- Conformação preferida da micrantina 147

conformação estendida. Por outro lado o sistema dibenzodioxínico formado pela junção dos anéis A e A' forçam ambas as porções BI a adotarem conformações estendidas. Conseqüentemente a flexibilidade destes compostos é consideravelmente mais crítica do que a dos compostos das séries precedentes, devendo apresentar, em solução, uma predominância da conformação apresentada na figura 11.

#### V- Alcalóides do tipo Repandulina

Os componentes desta série possuem estrutura bem distinta dos demais alcalóides bisbenzilisoquinolinas. Essas diferenças consistem principalmente na presença de um anel D que liga os anéis A e A', e de uma ciclohexadienona no lugar do anel totalmente aromático (anel A) encontrado em todas as séries anteriores. Foram feitas as atribuições dos deslocamentos químicos dos espectros de RMN-<sup>13</sup>C da repandulina 151 e pseudo-repandulina (dielina) 152 e seus respectivos produtos de redução, repandulinol 153 e dielinol 154<sup>41</sup>.



TIPO REPANDULINA

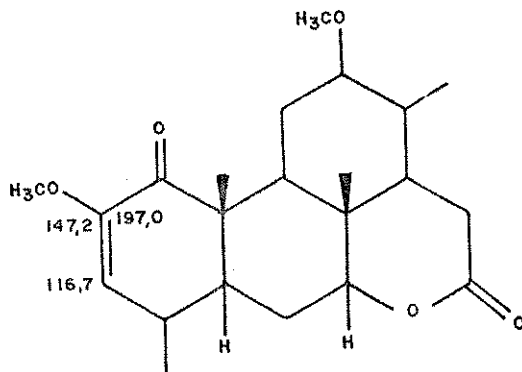
Na atribuição dos deslocamentos químicos dos carbonos dos anéis C e C' de 151 usou-se como modelo os deslocamentos químicos dos carbonos dos anéis C e C' da tenuipina 135. Desse modo, foram atribuídos aos carbonos do anel C os respectivos deslocamentos químicos:  $C_{14} = 103,8$ ;  $C_{10} = 111,9$ ;  $C_{12} = 133,4$ ;  $C_9 = 136,1$ ;  $C_{11} = 142,8$  e  $C_{13} = 147,9$  ppm e ainda  $\delta = 101,0$  ppm ao carbono pertencente ao substituinte metilenodioxí.

Para o anel C' foram atribuídos aos carbonos  $C_{11'}$ ,  $C_{13'}$ ,  $C_{10'}$ ,  $C_{9'}$ ,  $C_{14'}$  e  $C_{12'}$ , na ordem, os deslocamentos químicos 123,2; 122,1; 134,9; 132,4; 129,3; e 152,6 ppm.

Os carbonos do anel A' foram atribuídos levando-se em consideração o anel A' do composto que mais se assemelha, isto é, a micrantina 147, portanto foram atribuídos aos carbonos  $C_{5'}$ ,  $C_{8'}$ ,  $C_{8a'}$ ,  $C_{4a'}$ ,  $C_{7'}$ ,  $C_{6'}$ , os deslocamentos 114,9; 116,3; 127,8; 129,0; 139,4; 140,1 ppm, respectivamente.

Quanto aos carbonos  $sp^2$  remanescentes, atribue-se 193,7 ppm à carbonila, e os deslocamentos químicos ( $\delta$ ) a 148,0; 131,8; 125,7 e 112,9 ppm aos carbonos  $C_6$ ,  $C_{8a}$ ,  $C_{4a}$  e  $C_5$ , respectivamente. O deslocamento do  $C_5$  foi confirmado pela observação do espectro totalmente acoplado, comparação com o espectro da dielinol 154, e da irradiação seletiva na frequência de próton no espectro de  $^{13}C$  de 152, usando-se o deslocamento químico do próton  $H_5$ . Com efeito, irradiando na frequência de 549 Hz o sinal duplo do  $C_5$  na dielina 152 coalesce (figura 12) Por outro lado podemos observar no composto 155, deslocamentos <sup>42</sup> químicos semelhantes para carbonos  $C_7$ ,  $C_6$  e  $C_5$ . A atribuição da  $C_{4a} = 125,7$  ppm e  $C_{8a} = 131,7$  ppm foi feita considerando as formas de ressonância da cetona conjugada. Observando a figura 13 vê-se que a posição relativa ao  $C_{8a}$  é a que mais deve sentir os efeitos da retirada de elétrons por resso

nância, e por conseguinte deve estar mais desprotegida.



155

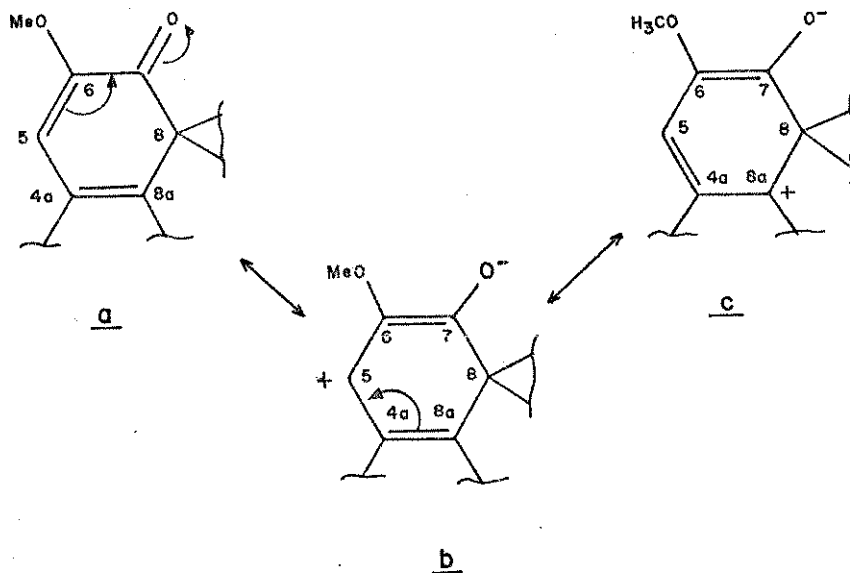
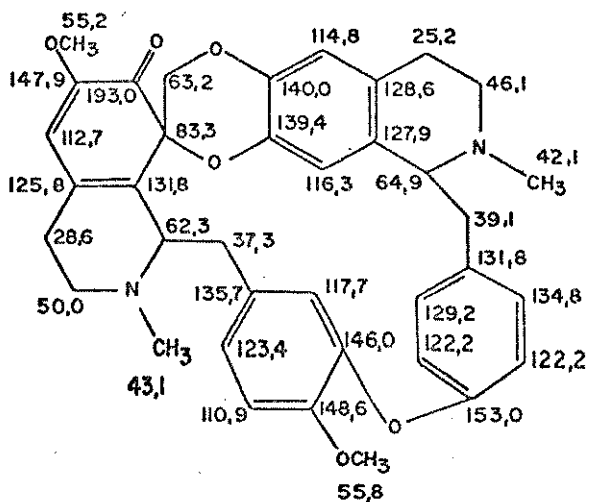


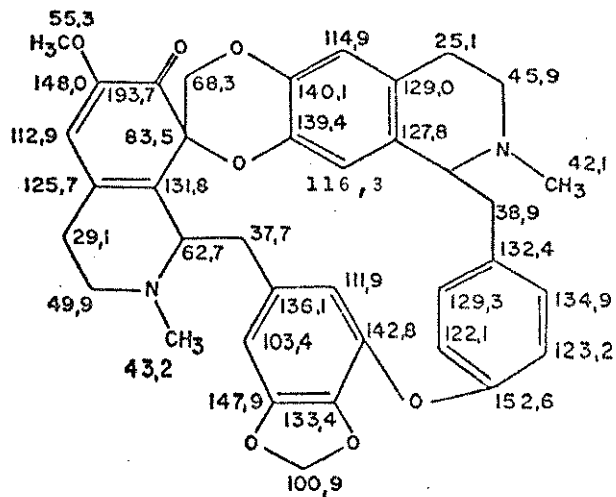
Figura 13

Aos carbonos alifáticos de 151 foram atribuídos os seguintes deslocamentos químicos:  $C_{1'}$  = 64,9;  $C_{3'}$  = 45,9;  $C_{4'}$  = 25,1 e  $C_{\alpha'}$  = 38,9 ppm (para o anel B'), os quais permaneceram inalterados após a redução para fornecer o álcool 153. Os carbonos do anel B foram atribuídos comparando-se com os carbonos situados na mesma posição no composto 152 e no produto de redução 153; sendo atri-

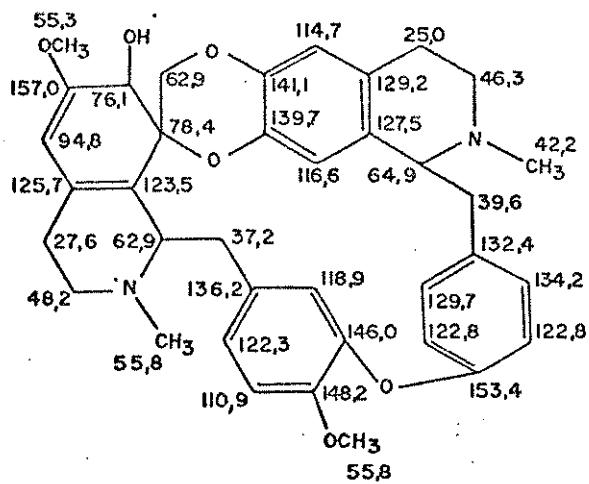




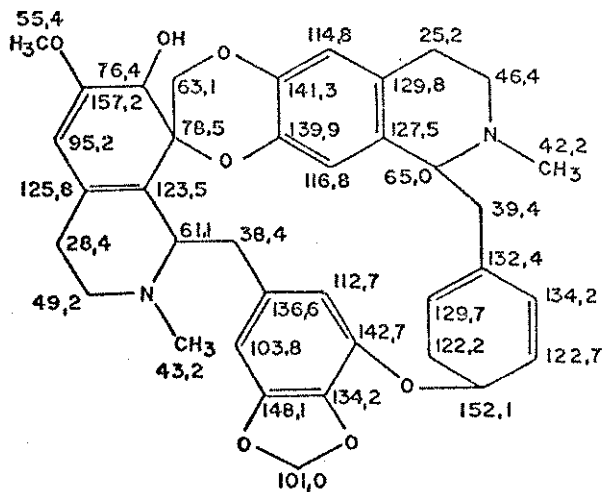
DIELINA 152



REPANDULINA 151



DIELINOL 154



REPANDULINOL 153

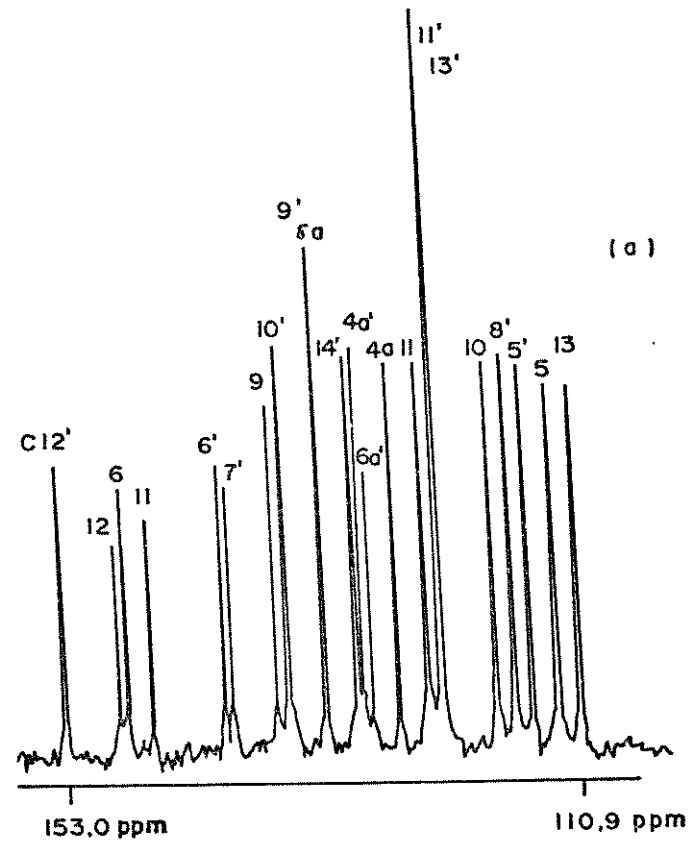
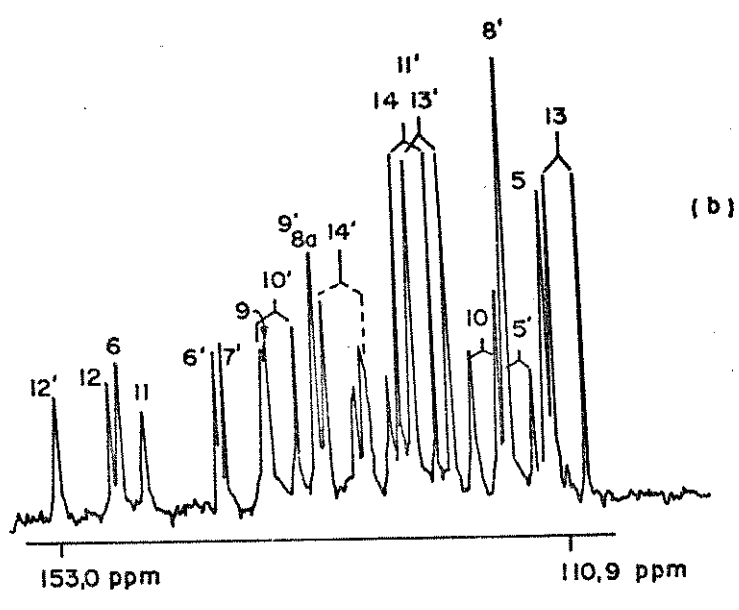


Figura 12 - Espectro de RMN-<sup>13</sup>C da dielina 152

- a) - totalmente desacoplado
- b) - com desacoplamento seletivo de próton H<sub>5</sub> e H<sub>8</sub>,

buídos  $C_1 = 62,7$ ;  $C_3 = 49,9$ ;  $C_4 = 29,1$ ;  $C\alpha = 37,7$  ppm, respectivamente.

A análise espectral do repandulíno 153 confirma a atribuição feita para a repandulina 151. Os carbonos dos anéis C, C', B' e A' não apresentaram diferenças nos deslocamentos químicos. No anel A, os carbonos posicionados  $\beta$  ( $C_5$ ;  $\Delta\delta = -17,7$  ppm) e  $\delta$  ( $C_{8a}$ ;  $\Delta\delta = -8,3$  ppm) sofreram proteção devido à retirada da conjugação, pois a eliminação das formas de ressonância (figura 13) cessa o efeito de desproteção observada no produto natural. O carbono carbonílico ao se transformar em álcool secundário sofreu proteção de  $\Delta\delta = -117,3$  ppm e induziu proteção no carbono  $C_8$  ( $\Delta\delta = -5,0$  ppm) e desproteção no  $C_6$  ( $\Delta\delta = +9,2$  ppm). Os carbonos do anel B também sofreram ligeiras modificações nos seus deslocamentos químicos e assim foram atribuídos:  $C_1 = 61,1$ ;  $C_3 = 49,2$ ;  $C_4 = 28,4$  e  $C\alpha = 38,4$  ppm.

A principal diferença entre a pseudo-repandulina e a repandulina é o substituinte do anel C, que em 152 é metoxi e em 151 metilenodioxí. Na atribuição da dielina os carbonos pertencentes ao anel C foram assinalados levando-se em consideração o anel da feantina 134, portanto foram assinalados  $C_9 = 135,7$ ;  $C_{10} = 117,7$ ;  $C_{11} = 146,0$ ;  $C_{12} = 148,6$ ;  $C_{13} = 110,9$  e  $C_{14} = 123,4$  ppm. Os demais anéis não apresentaram diferenças com relação à repandulina 151. A redução de 152 forneceu 154, que apresentou os mesmos efeitos ao passar 151  $\rightarrow$  153, confirmando as atribuições feitas para a repandulina. Devemos ressaltar que o espectro de RMN de  $^{13}C$  da dielina obtido com irradiação na frequência do próton  $H_5$  [(549 Hz) figura 12] confirmou a atribuição feita para o  $C_5$ .

Este trabalho permitiu confirmar a quase totalidade da estrutura da dielina e repandulina. Restou a ser confirmada a junção dos anéis D e A' que formam uma benzodioxina. A estrutura desta parte das moléculas possuem dois éteres formados por:  $C_{15}-O-C_6$ ;  $C_8-O-C_7$ , como nas fórmulas 151 e 152. Na estrutura alternativa estes éteres seriam formados por:  $C_{15}-O-C_7$ , e  $C_8-O-C_6$ . Usando-se modelos concluímos que não havia distinção entre ambos que fosse detectável por RMN de  $^{13}C$ .

REFERÊNCIAS BIBLIOGRÁFICAS

01. "Alcalóides indólicos de Peschiera fuchsiaefolia (DC) Miers", R. M. Braga, H. F. Leitão Filho e F. de A. M. Reis, Ciência e Cultura (Suplemento) 32, 463 (1980).
02. "Alcalóides indólicos novos de Aspidosperma pruinatum", D. S. Nunes, L. Koike, J. J. Taveira e F. de A. M. Reis, Ciência e Cultura (Suplemento), 32, 466 (1980).
03. "Ações biológicas do 1-(p-metoxibenzil)-6,7-metilenodioxiquinolina", U. M. F. Meirelles, L. R. Simioni, A. F. Magalhães e F. de A. M. Reis, Acta Amazônica, 7, 39 (1977).
04. "Estudo químico de Adiscanthus fusciflorus", M. N. V. McDougal e F. de A. M. Reis, trabalho não publicado.
05. "Análise de RMN de  $^{13}\text{C}$  de alcalóides de Adiscanthus fusciflorus", P. C. Vieira, M. A. Alvarenga, O. R. Gotlieb e F. de A. M. Reis, Ciência e Cultura (Suplemento), 32, 464 (1980).
06. "Structural confirmation of dihydrocinnamic acids from Adiscanthus fusciflorus by  $^{13}\text{C}$  NMR", P. C. Vieira, M. A. Alvarenga, O. R. Gotlieb, M. N. V. McDougal e F. de A. M. Reis, Phytochemistry, 19, 472 (1980).
07. "Efeito do 1-(p-metoxibenzil)-6,7-metilenodioxiquinolina (MMIQ) sobre as respostas do músculo reto abdominal isolado de sapo, à acetilcolina (Ach) e ao McN-A-343", U. M. F. Meirelles, P. M. R. Silva, L. R. Simioni e F. de A. M. Reis, Ciência e Cultura (Suplemento), 29, 459 (1977).
08. "Effect of 1-(p-methoxybenzil)-6,6 methylenedioxyisoquinoline (MMIQ) on skeletal muscle. A mechanical and eletrophysiological study", U. M. F. Meirelles, M. D. Fontana e F. de A. M. Reis, Seventh International Congress of Pharmacology, Paris, junho de 1978.

09. "Ações de papaverina sobre diafragma isolado de ratos", U. M. F. Meirelles, M. D. Fontana e F. de A. M. Reis, 7º Congresso Latinoamericano de Farmacologia, São Paulo, dezembro de 1978.
10. "Efeito do D. M. P. (desmetoxipapaverina) sobre a respiração de partículas sub-mitochondriais", N. C. Meirelles e F. de A. M. Reis, *Ciência e Cultura (Suplemento)*, 30, 819 (1978).
11. M. M. de Oliveira, R. M. Braga e F. de A. M. Reis, trabalho não publicado.
12. "Dienoic acids synthesis and  $^{13}\text{C}$  NMR spectral analysis", N. Frighetto, C. L. P. Silveira, F. de A. M. Reis, E. G. Magalhães e E. A. Ruveda, *Chem. Phys. Lipids*, 22, 115 (1978).
13. "Peptide alkaloids of *Scutia buxifolia*", A. F. Morel, R. V. F. Bravo, F. de A. M. Reis e E. A. Ruveda, *Phytochemistry*, 18, 473 (1979).
14. "Análise estereoquímica de alcalóides indólicos tipo quinolizidina e quinuclidina por RMN de  $^{13}\text{C}$ ", R. M. Braga, D. S. Nunes, L. Koike e F. de A. M. Reis, *Ciência e Cultura (Suplemento)*, 32, 464 (1980).
15. " $^{13}\text{C}$  NMR analysis of alkaloids from *Peschiera fuchsiaeifolia*", R. M. Braga, H. F. Leitão Filho e F. de A. M. Reis, *Phytochemistry*, 23, 175 (1984).
16. " $^{13}\text{C}$  NMR spectral analysis of some isoquinoline alkaloids", A. J. Marsaioli, E. A. Ruveda e F. de A. M. Reis, *Phytochemistry*, 17, 1655 (1978).
17. " $^{13}\text{C}$  NMR analysis of aporphine alkaloids", A. J. Marsaioli, F. de A. M. Reis, A. F. Magalhães, F. A. Ruveda e A. M. Kuch, 18, 165 (1979).
18. "Stereochemical aspects and  $^{13}\text{C}$  NMR spectroscopy of the berbamine class of bisbenzylisoquinoline alkaloids", L. Koike, A. J. Marsaioli, E. A. Ruveda, F. de A. M. Reis e I. R. C. Bick,

- Tetrahedron Lett., 3765, (1979).
19. "<sup>13</sup>C NMR analysis of some oxoaporphine alkaloids", A. J. Marsaioli, A. F. Magalhães, E. A. Ruveda e F. de A. M. Reis, *Phytochemistry*, 19, 955 (1980).
  20. "Proton and carbon-13 nuclear magnetic resonance spectroscopy and conformational aspects of bis(benzylisoquinoline) alkaloids", L. Koike, A. J. Marsaioli e F. de A. M. Reis, *J. Org. Chem.*, 46, 2385 (1981).
  21. "Carbon-13 nuclear magnetic resonance spectroscopy and conformational analysis of the daphnoline - repandine class of bis(benzylisoquinoline) alkaloids", L. Koike, A. J. Marsaioli, I. R. C. Bick e F. de A. M. Reis, *J. Org. Chem.*, 47, 4351 (1982).
  22. "Estudos de RMN de <sup>13</sup>C na determinação estrutural de repandulina e pseudorepandulina", L. Koike, I. R. C. Bick e F. de A. M. Reis, *Ciência e Cultura (Suplemento)*, 32, 465 (1980).
  23. "Um estudo fitoquímico do genero Aspidosperma", Benjamin Gilbert, *An. Acad. Bras. Ciênc.*, (Suplemento), 38, 315 (1966).
  24. "Alcalóides de Aspidosperma ramiflorum", F. de A. M. Reis, trabalho não publicado.
  25. "<sup>13</sup>C NMR of methyl, methylene and carbonyl carbon atoms of methyl alkenoates and alkynoates", J. Bus, I. Sies e M. S. F. Lie Ken Jie, *Chem. Phys. Lipids*, 17, 501 (1976).
  26. "Dihydrocinnamic acids from Hortia badinii", D. B. Correa, O. R. Gotlieb e A. P. de Pádua, *Phytochemistry*, 14, 2059 (1975).
  27. "Carbon-13 NMR analysis of cyclic peptide alkaloids", M. País, F. X. Jarreau, M. Conzalez Sierra, O. A. Mascaretti, E. A. Ruveda, C. J. Chang, E. W. Hagaman e E. Wenkert, *Phytochemistry*, 18, (1979).

28. "General methods of synthesis of indole alkaloids. Short routes of construction of yohimboid and ajmalicinoid alkaloid systems and their  $^{13}\text{C}$  nuclear magnetic resonance spectral analysis", E. Wenkert, C. J. Chang, H. P. S. Chawla, D. W. Cochran, E. W. Hagaman, J. C. King e K. Orito, *J. Am. Chem. Soc.*, 98, 3645 (1976).
29. "Carbon-13 NMR spectroscopy of naturally occurring substances. Alkaloids", E. Wenkert, J. S. Bindra, C. J. Chang, D. W. Cochran e F. M. Schell, *Acc. Chem. Res.*, 7, 46 (1974).
30. "Structure d'un alcaloïde indolique ancien: la geissoschizine", M. Damak, A. Ahond, P. Potier e M. M. Janot, *Tetrahedron Lett.*, 4731 (1976).
31. " $^{13}\text{C}$  NMR analysis of geissospermine and its indole alkaloids monomers fragments", R. Goutarel, M. Pais, H. E. Gottlieb, e E. Wenkert, *Tetrahedron Lett.*, 1235 (1978).
32. " $^{15}\text{N}$ -nuclear magnetic resonance spectroscopy - Natural abundance  $^{15}\text{N}$  NMR of monosubstituted indoles", E. Rosemberg, K. L. Willianson e J. D. Roberts, *Org. Magn. Reson.*, 8, 177 (1976).
33. "Carbon-13 nuclear magnetic resonance spectroscopy of naturally occurring substances", E. Wenkert, B. L. Buckwalter, I. R. Burfitt, M. J. Gasic, H. E. Gottlieb, E. W. Hagaman, F. M. Schell e P. M. Woukulich, "Topic in Carbon-13 NMR spectroscopy", G. C. Levy ed., vol. 2 Wiley-Interscience, N. York (1976).
34. " $^{13}\text{C}$  magnetic resonance spectra of some isoquinoline alkaloids and related model compounds", D. W. Hughes, H. L. Holland e D. B. MacLean, *Can. J. Chem.* 54, 2252 (1976).
35. "Studies on the alkaloids of papaveraceous plants. XXIX. Conformational analysis of tetrahydroprotoberberines by carbon-13 nuclear magnetic resonance spectroscopy", N. Takao, K. Iwasa, M. Kamigauchi e M. Sugiura, *Chem. Pharm. Bull.*, 25, 1426 (1977).



36. "Conformational analysis of quaternary protoberberine alkaloids by Carbon-13 NMR spectroscopy", K. Yoshikawa, I. Morishima, J. Kunitomo, M. Ju-ichi e Y. Yoshida, Chem. Lett. 961 (1975).
37. "PH-Abhängigkeit der  $^{13}\text{C}$  - chemischen verschiebungen sechsgliedriger stickstoff - heteroaromaten", E. Beitmaier e K. H. Spohn, Tetrahedron, 29, 1145 (1973).
38. "Estudo de RMN de  $^{13}\text{C}$  de alcalóides bisbenzilisoquinolínicos", L. Koike, A. J. Marsaioli, I. R. C. Bick e F. de A. M. Reis, Ciência e Cultura (Suplemento), 32, 466 (1980).
39. " $^{13}\text{C}$  NMR studies. VIII.  $^{13}\text{C}$  spectra of some substituted anisoles", K. S. Dhami e J. B. Stothers, Can. J. Chem., 44, 2855 (1966).
40. "Conformation and reactivity of the macrocyclic tumor-inhibitory alkaloid tetrandrine", C. J. Gilmore, R. F. Bryan e M. S. Kupchan, J. Am. Chem. Soc., 98, 1947 (1976).
41. "Alkaloids of Daphnandra Species. Part VII. Chemical evidence for the structure of repanduline", J. Harley-Mason, A. S. Howard, W. I. Taylor, M. J. Vernengo, I. R. C. Bick e P. S. Clezy, J. Chem. Soc. (C), 1948 (1967).
42. "The use of Carbon-13 nuclear magnetic resonance in natural products chemistry", F. W. Werli e T. Nishida, Fortschr. Chem. Org. Natursffe, 36, 78 (1979).

A P Ê N D I C E

# Algumas ações biológicas do 1-(p-metoxibenzil)-6,7-metilenodioxiisoquinolina

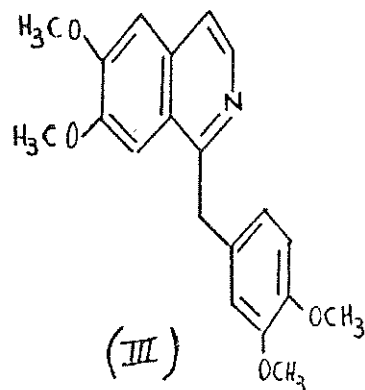
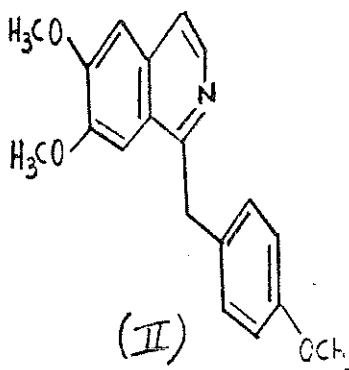
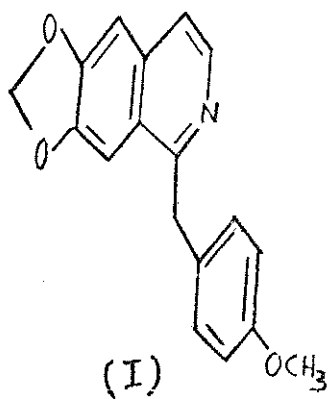
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## Resumo

São descritos resultados preliminares obtidos pela análise de propriedades biológicas do 1-(p-metoxibenzil)-6,7-metilenodioxiisoquinolina (MMIQ), extraído de uma Lauraceae, gênero *Ocotea*, das redondezas de Manaus, AM.

Dois alcalóides do tipo benzilisoquinolina foram isolados (Franca *et al.*, 1975) por cromatografia do extrato benzênico bruto, de uma

espécie de *Ocotea* (Lauraceae) colhida e identificada por W. Rodrigues nas redondezas de Manaus: 1-(p-metoxibenzil)-6,7-metilenodioxiisoquinolina (I) e 1-(p-metoxibenzil)-6,7-dimetoxiisoquinolina (II) (DMP). As estruturas destes alcalóides foram determinadas pelos métodos físicos usuais (RMN<sup>1</sup>H, EM, IV, e UV) e por comparação dos dados espectrais com os da papaverina (III) e outros análogos (Shamma, 1972).



Numa segunda etapa, outro processo de isolamento foi realizado. Partindo de 1600 g de madeira previamente extraída com éter de petróleo durante 72 horas para eliminar substâncias graxas, o material foi alcalinizado com hidróxido de amônio e extraído por 72 horas com clorofórmio sob refluxo.

Depois de evaporado o solvente foram obtidos 80 g de extrato dos quais 50 g foram cromatografados em coluna de sílica e eluída com benzeno e clorofórmio. Das frações 13 a 16 foram isolados 1,5 g de MMIQ e 3 g de DMP.

Resultados deste laboratório (em preparação) mostram que o MMIQ exerce nítido efeito inibitório sobre as respostas contráteis a vários agonistas como o cloreto de bário, a

acetilcolina, a fenilefrina, a histamina, adrenalina e serotonina, bem como efeito espasmolítico quando adicionado na vigência de plena contração das preparações. Estes resultados foram obtidos em aorta e duodeno isolados de coelho e em cadeia traqueal, taenia caecum e íleo isolados de cobaia.

Triner *et al.* (1970) estudando o relaxamento de músculo liso provocado pela papaverina verificaram um consistente decréscimo da atividade da fosfodiesterase do 3',5'-AMP cíclico e admitiram possível relação causa-efeito entre estes fenômenos.

Na mesma ordem de idéias, estudamos a influência do MMIQ sobre a atividade da fosfodiesterase de cérebro de coelho e constata-

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mos que a partir de 10  $\mu\text{M}/1$  já se manifesta inibição, que atinge os valores de 50% e 67% para concentrações de 100 e 200  $\mu\text{M}/1$  do inibidor, respectivamente (Vercesi *et al.*, 1975).

Analizamos também no músculo reto abdominal isolado de sapo possível influência do MMIQ sobre as respostas contráteis à acetilcolina (Ach, Fig. 1). Verificamos que o alcalóide produz inibição do tipo não-competitivo. Assim as curvas dose-resposta correspondentes às concentrações de 10, 30 e 100  $\mu\text{g}/\text{ml}$  do inibidor não atingem mais o nível de resposta máxima com o aumento da concentração do

agonista. O valor médio do  $\text{pD}'2$  é de 3,9 com uma variação aparentemente maior que a prevista (Grana, 1972), o que ocorre também com a papaverina nas mesmas condições. Em ambos os casos observa-se que a intensidade do bloqueio é função não apenas da concentração do inibidor como também de uma condição inerente ao animal o que se depreende de resultados de quatro experimentos simultâneos usando-se músculos isolados de dois ou de quatro animais. A natureza desta variação comparada com a de outros antagonistas é assunto em investigação neste laboratório.

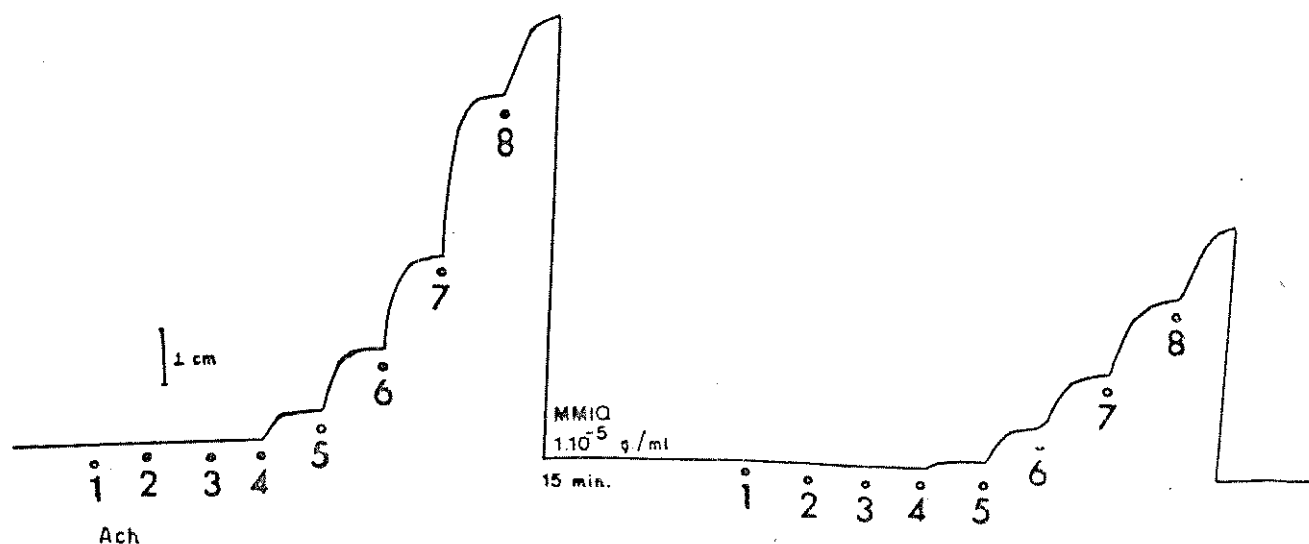


Fig. 1 — Registro experimental das respostas à adição cumulativa de acetilcolina sobre o músculo reto abdominal de sapo "in vitro", em presença ou não do MMIQ. Os números indicam os locais de adição do agonista e, ao ponto 1 corresponde a concentração final de  $1 \cdot 10^{-7}$   $\text{g}/\text{ml}$ . Razão = 3.

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#### SUMMARY

In this report we describe preliminary results about some biological properties of 1-(p-Methoxybenzyl) - 6,7 - methylenedioxyisoquinoline extracted from a species of *Ocotea* (Lauraceae) which grows near Manaus, AM.

#### BIBLIOGRAFIA CITADA

- FRANCA, NÍDIA C.; GIESBRECHT, ASTRÉA M.; GOTTLIEB, O.R.; MAGALHÃES, A.F. & MAGALHÃES, E.G.  
1975 — Benzylisoquinolines from *Ocotea* species. *Phytochemistry*, 14 : 1671-1672.

GRANA, E.

1972 — *Appunti dalle lezioni di Farmacologia Molecolare*. Pavia, La Goliardica Pavese.

SHAMMA, M.

1972 — *The Isoquinoline Alkaloids*. Chemistry and Pharmacology. New York. Acad. Press.

TRINER, L.; VULLIEMOZ, Y.; SCHWARTZ, I. & NAHAS, G.G.

1970 — Cyclic Phosphodiesterase Activity and the Action of Papaverine. *Biochem. Biophys. Res. Comm.*, 40 : 64-69.

VERCESI, A.E.; MAGALHÃES, A.F. & MEIRELLES, U.M.F.

1975 — The action of 1-(p-methoxybenzyl)-6,7-methylenedioxyisoquinoline, a structural analogue of papaverine on adenosine cyclic 3', 5'-monophosphate phosphodiesterase. *IRCS Medical Science*, 3 : 492.

## DIENOIC ACIDS, SYNTHESIS AND $^{13}\text{C}$ NMR SPECTRAL ANALYSIS

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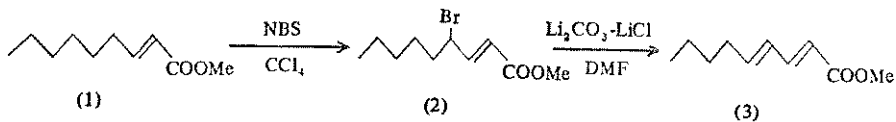
A preparation of E,E-2,4-dienoic acids, together with the assignments of their  $^{13}\text{C}$  NMR signals and the shifts observed after transformation into their sodium salts, is described. The stereochemistry of the double bonds of 3,5-dienoic esters, obtained from 2,4-dienoic acids, on the basis of  $^{13}\text{C}$  NMR data, is also presented.

### I. Introduction

Recently, effort has been devoted to the synthesis of E,E-2,4-dienoic acids [1]. In this connection, and as part of a project on the synthesis of unsaturated carboxylic acids, we wish to report a simple preparation of 2,4-dienoic acids, their  $^{13}\text{C}$  NMR analysis and their transformation into 3,5-dienoic acid derivatives.

### II. Methods

The synthetic procedure is illustrated with the preparation of methyl E,E-2,4-nonadienoate\*. By treatment of methyl E-2-nonenolate (1) – readily prepared from



heptanal and malonic acid in pyridine [2], followed by esterification – with *N*-bromosuccinimide in carbon tetrachloride, the corresponding bromo ester (2) was obtained in 71% yield\*\*. The dehydrohalogenation reaction of (2) proved to be more difficult than expected, even if very effective amines such as 1,5-diazabicyclo-(4.3.0)-nonene-5

\* For another procedure for preparation of (3), see R.A. Gato, M.Sc. Dissertation, Universidade de São Paulo, 1976.

\*\* All compounds have been fully characterized (IR,  $^1\text{H}$  NMR, mass spectra).

(DBN) and 1,5-diazabicyclo-(5.4.0)-undecene-5 (DBU) were used under a variety of reaction conditions. It was found, however, that the reaction could be successfully carried out (85% yield) by using lithium carbonate–lithium chloride in dimethylformamide\*, a well-known method for dehydrobromination of bromoketones. The stereochemistry of (3) was assigned on the basis of the  $^1\text{H}$  NMR spectrum and by using lanthanide shift reagents [3].

### III. Results and Discussion

The availability of fatty acids with relatively short carbon chains, with one and two conjugated double bonds, and the considerable interest focused on the  $^{13}\text{C}$  NMR spectral analysis of this type of compound [4,5], prompted us to undertake an analysis of these substances.

The  $^{13}\text{C}$  NMR chemical shifts of several unsaturated fatty acids, presented in Table 1, were assigned on the basis of previous results [6] and on parameters proposed by Bus et al. [7]. Although we were dealing with carboxylic acids, instead of methyl esters, a good correlation was obtained between experimental and calculated values. The interesting result that emerges from this analysis is the assignment of the 27.5 and 27.8 ppm signals to the homoallylic carbons of E-2-octenoic and E-2-nonenic acids. Since these values are within the normal range for an allylic carbon shift of a Z-disubstituted double bond, care should be taken with a double configuration assignment in unsaturated carboxylic acids on the basis of  $^{13}\text{C}$  NMR data alone.

In order to extend the study of the effect of ionization on chemical shifts of unsaturated carboxylic acids [8], the  $^{13}\text{C}$  NMR spectra of the corresponding sodium salts were also analyzed. As expected, C-2 and C-3 are deshielded and shielded respectively, while the remaining carbons are deshielded in all salts, in comparison with similar sites of the corresponding acids, as shown in table 1. Part of these effects, however, could be attributed to the change of solvent ( $\text{CDCl}_3 \rightarrow \text{D}_2\text{O}$ ) used for recording the spectra of both series of compounds [8,9].

Table 2 shows the shifts of four E,E-2,4-dienoic acids and the effect observed on the  $\text{sp}^2$  carbons by ionization. The changes are similar to the previously reported ones [8].

As part of the work on dienoic acids and in connection with another project, esterification reactions with sterically hindered alcohols were also studied. We have found that, by reaction of sorboyl chloride with 1-ethynylcyclopentanol in triethyl-

\* A magnetically stirred mixture of 2 (11.28 g), lithium carbonate (4.48 g) and lithium chloride (3.16 g) in dry dimethylformamide (50 ml) was heated in an oil bath at 125–130°C for 1.5 hr. The reaction mixture was then poured into water and thoroughly extracted with ether. The combined ether extracts were washed twice with 10% aqueous hydrochloric acid, with water, dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated. The residue gave (3) (6.47 g) after distillation, b.p. 75–78°C/0.5 torr. Compound (3) has approximately 90% purity on GLC, carbowax 20 M at 200°C; the remaining 10% is probably a stereoisomer since by catalytic hydrogenation only methyl nonanoate was detected on GLC.

Table 1

	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9
E-2-Hexenoic acid*	171.9	120.7	151.9	34.3	21.1	13.6			
Sodium salt***	176.5	128.4	146.4	34.30	21.15	13.55**			
E-2-Heptenoic acid*	172.1	120.7	152.1	35.1	22.8	14.9			
Sodium salt***	175.9	128.5	145.4	31.90	30.0	22.2	13.7		
E-2-Octenoic acid*	172.2	120.5	152.2	32.6	30.30	22.20	13.70**		
Sodium salt***	176.0	128.6	145.0	32.2	31.7	23.4	15.0	14.0	
E-2-Nonenoic acid*	172.0	120.6	151.9	32.20	27.5	31.3	22.4	13.95**	
Sodium salt***	175.8	128.7	144.8	33.1	27.80	31.35	22.20	14.9	
				32.2	29.4	32.7	23.0	22.5	13.8
				32.20	27.8	28.8	31.5	22.60	14.05**
				33.1	28.05	28.85	31.50	23.8	15.0

\* The spectra were recorded on a Varian XL-100-15 spectrometer at 25.2 MHz in the Fourier transform mode and in CDCl<sub>3</sub> solutions. The  $\delta$  values are in ppm downfield from TMS.

\*\* Calculated values according to ref. 17.

\*\*\* In D<sub>2</sub>O solutions having 0.1% of DMSO as internal reference,  $\delta$ (TMS) =  $\delta$ (DMSO) + 40.5 ppm.

Table 2

	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9
E,E-2,4-Hexadienoic acid***	169.9	118.3	145.8	129.8	139.4	18.0			
Sodium salt**	176.0	127.3	142.1	131.4	138.0	19.6			
E,E-2,4-Hepadienoic acid*	172.6	118.3	147.3	127.2	147.2	26.0	12.7		
Sodium salt**	176.6	127.3	142.6	129.1	144.9	27.0	14.2		
E,E-2,4-Octadienoic acid*	172.5	118.3	147.1	128.2	145.5	35.0	21.8	13.5	
Sodium salt**	175.6	128.0	141.7	130.4	141.5	35.9	23.1	14.7	13.8
E,E-2,4-Nonadienoic acid*	172.8	118.2	147.3	128.0	145.9	32.7	30.7	22.2	14.9
Sodium salt*	175.4	128.2	141.6	130.3	141.3	33.4	32.0	23.2	14.9

\* The spectra were recorded on a Varian XL-100-15 spectrometer at 25.2 MHz in the Fourier transform mode and in CDCl<sub>3</sub> solutions. The  $\delta$  values are in ppm downfield from TMS.

\*\* In D<sub>2</sub>O solutions having 0.1% of DMSO as internal reference;  $\delta(\text{TMS}) = \delta(\text{DMSO}) + 40.5$  ppm.

\*\*\* In CDCl<sub>3</sub> solution containing some methanol.



amine at room temperature, the corresponding 3,5-hexadienoic ester was obtained as sole reaction product in 72% yield. By using cyclopentanol and isopropanol the respective unconjugated esters were also obtained, although in lower yield (46%). With methanol, however, a 1 : 1 mixture of conjugated and unconjugated methyl esters was shown to be the product in 54% yield (GLC and  $^1\text{H}$  NMR).

In order to study this reaction\*, which can be explained by an addition of the alcohol to an intermediate unsaturated ketene, from the point of view of the stereochemistry of the isomerized double bonds, a careful analysis of the methylene region of the  $^{13}\text{C}$  NMR spectrum of the ester obtained from *E,E*-2,4-octadienoic acid and cyclopentanol was carried out.

By reaction of *E,E*-2,4-octadienoyl chloride with cyclopentanol in triethylamine an approximately 1 : 1 mixture of stereoisomeric  $\Delta$  3,5-esters (GLC and  $^1\text{H}$  NMR) was obtained in 85% yield. The  $^{13}\text{C}$  NMR spectrum of this mixture showed methylene signals at 20.9, 25.4, 38.2 and 38.4 ppm. The signals at 38.4 and 38.2 ppm, which were assigned to C-2 and the ones at 20.9 and 25.4 ppm assigned to C-7, show that it was a mixture of *E,Z*-3,5- and *E,E*-3,5- octadienoic esters, respectively, on the basis of the known shielding effect of an allylic carbon of a *Z*-disubstituted double bond. This was further confirmed since isomerization with iodine [10] gave a product where mainly the signals corresponding to the *E,E*-isomer were observed\*\*.

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#### References

- [1] J. Tsuji, H. Nagashima, T. Takahasi and K. Masaoka, *Tetrahedron Lett.* (1977) 1917
- [2] M. Jacobson, I. Keiser, D.L. Chambers, D.H. Miyashita and C. Harding, *J. Med. Chem.* 14 (1971) 236
- [3] J.H. MacMillan and S.S. Washburne, *Org. Magn. Resonance* 6 (1974) 250

\* For other procedures of deconjugation of methyl sorbate see K. Torssell, *Tetrahedron Lett.* (1974) 623, and for deconjugation of unsaturated acids P.E. Pfeffer and L.S. Gübert, *J. Org. Chem.* 36 (1971) 3290.

\*\*The  $^{13}\text{C}$  NMR spectrum of the stereoisomeric mixture of cyclopentyl-3,5-octadienoates in  $\text{CDCl}_3$  solution showed the following signals: 13.4, 14.1, 20.9, 23.6, 25.4, 32.5, 38.2, 38.4, 77.0, 122.5, 124.7, 127.1, 128.5, 133.1, 133.5, 135.5, 170.9 and 171.0 ppm. After isomerization, and recording the spectrum also in  $\text{CDCl}_3$  solution, the following signals were observed: 13.4, 23.6, 25.4, 32.5, 38.2, 77.0, 122.5, 128.5, 133.6, 135.6 and 171.0 ppm downfield from TMS.

- [4] F.D. Gunstone, M.R. Pollard, C.M. Scrimgeour and H.S. Vedanayagam, *Chem. Phys. Lipids* 18 (1977) 115, and references cited herein
- [5] J. Bus, I. Sies and M.S.F. Lie Ken Jie, *Chem. Phys. Lipids* 18 (1977) 130, and references cited therein
- [6] E. Lippmaa, T. Pehk, K. Anderson and C. Happe, *Org. Mag. Resonance* 2 (1970) 109
- [7] J. Bus, I. Sies and M.S.F. Lie Ken Jie, *Chem. Phys. Lipids* 17 (1976) 501
- [8] E. Wenkert, B.L. Buckwalter, I.R. Burfitt, M.J. Gasic, H.E. Gottlieb, E.W. Hagan, F.M. Schell and P.M. Wovkulich, *Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Naturally Occurring Substances*, in: *Topics in Carbon-13 NMR Spectroscopy*, Vol. 2 (G.C. Levy, ed.) Wiley-Intersciences, New York, 1976
- [9] J.G. Batchelor, J.H. Prestegard, R.J. Cushley and S.R. Lipsky, *J. Am. Chem. Soc.* 95 (1973) 6358
- [10] W.D. Celmer and I.A. Solomons, *J. Am. Chem. Soc.* 75 (1973) 3430.

## STRUCTURAL CONFIRMATION OF DIHYDROCINNAMIC ACIDS FROM *ADISCANTHUS FUSCIFLORUS* BY $^{13}\text{C}$ NMR\*

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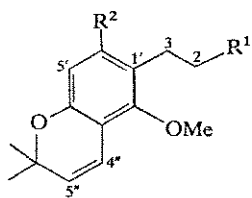
(Received 17 May 1979)

**Key Word Index**—*Adiscanthus fusciflorus*; Rutaceae; dihydrocinnamic acids; alkaloids;  $^{13}\text{C}$ NMR spectra.

**Abstract**—In the wood of *Adiscanthus fusciflorus* six known alkaloids 4-methoxy-2-quinolone, 1-methyl-4-methoxy-2-quinolone, dictamine, skimmianine,  $\gamma$ -fagarine and *N*-methylflindersine and two new dihydrocinnamic acids 3-[2',6'-dimethoxy-6'',6''-dimethylpyrano(2'',3'':4',3')phenyl]-propionic acid and its methyl ester were identified. The structures of the dihydrocinnamic acid derivatives were confirmed by  $^{13}\text{C}$ NMR.

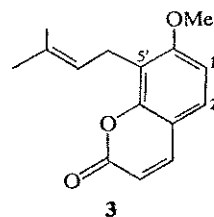
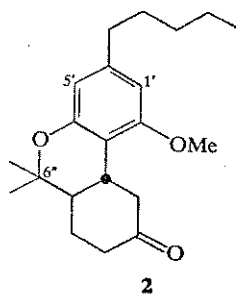
*Adiscanthus fusciflorus* Ducke, an arboreal Rutaceae species from the Amazon region, contains in a section of its trunk (bark included) 4-methoxy-2-quinolone, 1-methyl-4-methoxy-2-quinolone, dictamine and skimmianine [3],  $\gamma$ -fagarine [4] and *N*-methylflindersine. All these alkaloids have been separated previously from other rutaceous species, the first four inclusively from another Amazonian species, *Hortia longifolia* Spr. ex Engl. [3], and the last one by *N*-methylation of natural flindersine [5]. Their identification was based on spectra and direct comparison with authentic samples.

Two additional constituents, isolated from the same extract of *A. fusciflorus*, were the dihydrocinnamic acid derivatives **1a** and **1b**, which, jointly with dihydrocinnamyl alcohols such as **1c** [6], had been previously found in *Hortia badinii* M.A. Lisboa. The structural elucidation of these natural products had been based chiefly on the interpretation of  $^1\text{H}$ NMR spectra. Their re-isolation was therefore an opportunity to test the proposed formulae by  $^{13}\text{C}$ NMR.



**1a**  $\text{R}^1 = \text{CO}_2\text{Me}$ ,  $\text{R}^2 = \text{OMe}$   
**1b**  $\text{R}^1 = \text{CO}_2\text{H}$ ,  $\text{R}^2 = \text{OMe}$   
**1c**  $\text{R}^1 = \text{CH}_2\text{OH}$ ,  $\text{R}^2 = \text{H}$

substituted C-5' must indeed be vicinal to only one methoxyl ( $\delta$  55.3),  $^{13}\text{C}$ NMR showing the other one ( $\delta$  62.0) to be flanked by two *ortho* substituents. The signals due to the corresponding *ipso*-carbons (C-6'  $\delta$  158.5, C-2'  $\delta$  154.9) can be easily assigned due to their complex secondary splitting in the fully proton-coupled C spectrum. In this same spectrum, signals of the *ipso*-carbons C-2'' and C-3'' show a contrastingly simpler splitting pattern which can be eliminated by decoupling respectively at the resonance frequencies of H-4'' (double irradiation at  $\delta$  6.48) and H-5'' (double irradiation at  $\delta$  5.45). These decoupling experiments were additionally very useful in the confirmation of the respective assignment of signals to C-4'' and C-5'' since the first order C—H couplings are eliminated. Finally, double irradiation at the frequency of the gem-dimethyl protons ( $\delta$  1.38) collapsed the fine splitting of the C-5'' signal, confirming the vicinality of this carbon and  $\text{CMe}_2$ . At this stage, since correlation of signals to carbons of the propionic acid moiety is trivial, only the signals at  $\delta$  113.7 and 75.5 remained to be assigned, a task performed by comparison with model compounds **2** [7] and **3** [8], respectively.



The phloroglucinol type substitution for **1a** can be safely accepted from  $^1\text{H}$ NMR evidence for the sole aromatic proton ( $\delta$  6.18). The corresponding un-

\* Part IV in the series "The Chemistry of Brazilian Rutaceae". For Part III see ref. [1]. Based on part of the M.S. thesis presented by P.C.V. to Universidade de São Paulo (1978). Also part of a project on the  $^{13}\text{C}$ NMR spectroscopy of natural products. For the preceding paper see ref. [2].

The spectrum of **1a** served as the basis in the interpretation of the spectra of **1b**, dihydro-**1b** and **1c**. The suppression of the 4'',5''-double bond of **1b** in dihydro-**1b** caused a paramagnetic shift of the C-2'' signal ( $\Delta\delta$  4.4), an expression of the endocyclic homoallylic effect [9]. Most significantly, from the

Table 1. Carbon shifts of the natural dihydrocinnamic acids and dihydro derivative **1**, and the model compounds **2** and **3**\*

C	1a	1b	Dihydro-1b	1c	2[7]	3[8]
MeO-1	51.2					
1	173.5	176.5	179.5	61.4		
2	34.0	33.8	34.2	33.5		
3	19.0	18.7	19.0	24.9		
1'	113.7	113.5	113.0	125.7		
2'	154.9	154.4	153.4	153.4	158.1	
MeO-2'	62.0	61.8	60.6	62.1		
3'	107.4	107.2	106.5	114.4	109.7	117.6
4'	152.9	152.5	156.9†	152.0	153.9	152.5
5'	95.7	95.6	96.0	112.5	109.9	112.5
6'	158.5	158.2	157.2†	130.2		159.9
MeO-6'	55.3	55.0	55.3			
4''	117.2	116.7	17.1	117.1		
5''	126.7	126.7	32.5	126.9		
6''	75.5	75.6	74.1	75.4	76.3	
2Me-6''	27.7	27.6	26.7	27.6	27.8	18.6

\* The numbering system of the model compounds was selected to facilitate comparison of analogous carbons in **1**, **2** and **3**.

† Interchangeable.

point of view of structural confirmations, the C-6' ( $\delta$  158.5) and C-3' ( $\delta$  19.0) peaks of **1a** appear at higher ( $\delta$  130.2) and lower ( $\delta$  24.9) field, respectively, in the spectrum of **1c**. This is due to the absence in this compound of MeO-6' which thus fails to shield C-3 through a  $\gamma$ -effect.

#### EXPERIMENTAL

*Isolation of the constituents.* *Adiscanthus fusciflorus* Ducke was collected near Manaus, AM and identified by W. A. Rodrigues, botanist, Instituto Nacional de Pesquisas da Amazônia. Powdered trunkwood and bark (2 kg) were extracted with EtOH. The extract (20 g) was suspended in hexane, filtered, the hexane evapd and the residue (7 g) submitted to dry column chromatography (Si gel deactivated with 10% H<sub>2</sub>O, C<sub>6</sub>H<sub>6</sub>-CHCl<sub>3</sub>, 9:1). The column was extruded and divided into 8 equal parts which gave from bottom to top 8 fractions. Fraction 4 was purified by TLC (Si gel, C<sub>6</sub>H<sub>6</sub>-EtOAc, 7:3) giving **1a** (30 mg). Fractions 5 and 6 were washed with hexane to give **1b** (200 mg). Fraction 7 contained 1-methyl-4-methoxy-2-quinolone. The hexane insol. part of the extract was suspended in CHCl<sub>3</sub> and filtered. The CHCl<sub>3</sub> was evapd and the residue (6 g) submitted to Si gel (120 g) column chromatography, elution with CHCl<sub>3</sub>-MeOH, 100:0 to 95:5, giving fractions A to G. Fraction B was purified by TLC (Si gel, C<sub>6</sub>H<sub>6</sub>-EtOAc, 4:1) to give **1a** (60 mg). Fraction C was separated by TLC (Si gel, C<sub>6</sub>H<sub>6</sub>-EtOAc, 7:3) into dictamine (20 mg) and  $\gamma$ -fagarine (60 mg). Fraction D was purified similarly to give *N*-methylflindersine (50 mg). Fraction E was separated by TLC (Si gel, C<sub>6</sub>H<sub>6</sub>-EtOAc, 3:2) into skimmianine (20 mg) and 1-methyl-4-methoxy-2-quinolone. Fraction G was washed and cryst. from EtOH to give 4-methoxy-2-quinolone. Fractions A and F did not lead to pure compounds.

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#### REFERENCES

1. Corrêa, D. de B., Gottlieb, O. R. and Pádua, A. P. de (1979) *Phytochemistry* **18**, 351.
2. Morel, A. F., Bravo, R. V. F., Reis, F. de A. M. and Rúveda, E. A. (1979) *Phytochemistry* **18**, 473.
3. Corrêa, D. de B., Gottlieb, O. R., Pádua, A. P. de and Rocha, A. I. da (1976) *Rev. Latinoam. Quim.* **7**, 43.
4. Robertson, A. V. (1963) *Aust. J. Chem.* **16**, 451.
5. Iriarte, J., Kincl, F. A., Rosenkranz, G. and Sondheimer, F. (1956) *J. Chem. Soc.* 4170.
6. Corrêa, D. de B., Gottlieb, O. R. and Pádua, A. P. de (1975) *Phytochemistry* **14**, 2059.
7. Archer, R. A., Johnson, D. W., Hagaman, E. W., Moreno, L. N. and Wenkert, E. (1977) *J. Org. Chem.* **42**, 490.
8. Wenkert, E., Buckwalter, B. L., Burfitt, I. R., Gašić, M. J., Gottlieb, H. E., Hagaman, E. W., Schell, F. M. and Wovkulich, P. M. (1976) *Topics in Carbon-13 NMR Spectroscopy* (Levy, G. C., ed.) Vol. 2. Wiley-Interscience, New York.
9. Wenkert, E., Cochran, D. W., Hagaman, E. W., Schell, F. M., Neuss, N., Katner, A. S., Potier, P., Kan, C., Plat, M., Koch, M., Mehri, H., Poisson, J., Kunesch, N. and Rolland, Y. (1973) *J. Am. Chem. Soc.* **95**, 4990.

## PEPTIDE ALKALOIDS OF *SCUTIA BUXIFOLIA*

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**Key Word Index**—*Scutia buxifolia*; Rhamnaceae; peptide alkaloids; scutianine H; MIKES;  $^{13}\text{C}$  NMR spectra; scutianine D; scutianine E.

**Abstract**—From the bark extract of *S. buxifolia*, the known alkaloids scutianines B, C, D and E, and the new one, scutianine H, have been isolated. The structure of scutianine H, based mainly on its MS fragmentation and on the MIKES (mass-analysed ion kinetic energy spectrum) of the base ion peak, is suggested. From the  $^{13}\text{C}$  NMR spectral analysis of the diastereoisomeric scutianines D and E, information for the assignment of the stereochemistry of the  $\beta$ -hydroxyleucine unit in related alkaloids was also obtained.

Continuing our chemical study of the constituents of *Scutia buxifolia* Reiss., we report on work which is being carried out in our laboratories and, in view of the recent publication of Tschesche and Hillebrand [1], we also make some explanatory comments about the designations of the cyclopeptide alkaloids isolated from this interesting plant.

The first member of the scutianines, isolated by Tschesche *et al.* in 1967 [2], was named scutianine A, 1a, and its structure elucidation represented a pioneer contribution to the field. Later on, in 1971, the same group also isolated scutianine B, 2 [3].

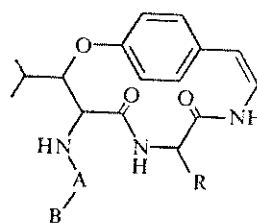
In 1974 we determined the stereochemistry of all the chiral centres of scutianine A, 1a [4], with the exception of those of the  $\beta$ -hydroxyleucine unit, which had been previously determined on other alkaloids through special degradations [5, 6]. We also reported the isolation and the structure of two new alkaloids, scutianine D, 3, and scutianine C, 4, as well as a neutral product, called scutianene C, 5, because of its structural similarity with 4 [4, 8]. By analysis of the 220 MHz  $^1\text{H}$  NMR spectrum, the *erythro* form of the  $\beta$ -hydroxyleucine unit was shown to be present in 4 and the *threo* form of the  $\beta$ -phenylserine moiety was identified amongst the hydrolysis products of dihydro-4 [8]. The 220 MHz  $^1\text{H}$  NMR spectrum of 5 showed that the signals corresponding to the  $\alpha$  and  $\beta$  protons of the hydroxyleucine unit had  $\delta$  values different than those observed for the corresponding protons of 4, indicating some change in the stereochemistry of this unit in 5 in relation to alkaloid 4. Thus, 5—whether or not an artefact—should be related to an alkaloid stereoisomeric with 4.

Simultaneously, Tschesche *et al.* [7] published the isolation, from the same source, of three alkaloids named scutianine C, 3, and two diastereoisomers, scutianine D and scutianine E, 4. The stereochemistry of the  $\beta$ -hydroxyamino acid units of the latter compounds was shown to be *L-erythro-β*-hydroxyleucine, *L-threo-β*-phenylserine and *D-erythro-β*-hydroxyleucine and *D-threo-β*-phenylserine, respectively. By comparison of the spectral properties it can be deduced that the previously reported scutianines C and D [8, 4] are identical

to the scutianines D, 4, and C, 3, reported by the Bonn group [7]. Consequently, we suggest that these latter designations be adopted, leaving the scutianene 5 undetermined, until a correlation with an alkaloid of known stereochemistry is carried out.

More recently, the isolation of scutianine F, 1b, a desmethyl derivative of scutianine A, and of scutianine G, another diastereoisomer of 4 was reported [9, 1]. Further, by re-examination of the bark extract of *S. buxifolia*, scutianine B, 2, the now-named scutianines C, 3, D and E, 4, and the new cyclopeptide alkaloid, scutianine H were isolated.

Scutianine H, 6, mp 242–243°, showed  $\text{M}^+$  at *m/e* 550 indicating a formula  $\text{C}_{31}\text{H}_{42}\text{N}_4\text{O}_5$ . The IR spectrum exhibited bands corresponding to peptide linkages and the UV spectrum and the MS fragmentation were



- 1a A = Pro; B = Dimethyl Phe; R =  $\text{CH}_2\text{C}_6\text{H}_5$ , Scutianine A [2].  
 1b A = Pro; B = Methyl Phe; R =  $\text{CH}_2\text{C}_6\text{H}_5$ , Scutianine F [9].  
 2 A = Dimethyl Phe; R =  $\text{CH}_2\text{C}_6\text{H}_5$ , Scutianine B [3].  
 3 A = Dimethyl Ileu; R =  $\text{CH}_2\text{C}_6\text{H}_5$ , Scutianine D [4]; Scutianine C [7].  
 4 A = Dimethyl Phe; R =  $\text{CH}(\text{OH})\text{C}_6\text{H}_5$ , Scutianine C [8]; Scutianine D\* [7]; Scutianine Et† [7]; Scutianine G [1].  
 5 A =  $\text{C}_6\text{H}_5\text{CH}=\text{CH}-\text{CO}$ ; R =  $\text{CH}(\text{OH})\text{C}_6\text{H}_5$ , Scutianene C [4].  
 6 A = Dimethyl Ileu; R =  $\text{CH}(\text{OH})\text{C}_6\text{H}_5$ , Scutianine H (This work).  
 \* *L-erythro-β*-hydroxyleucine; *L-threo-β*-phenylserine.  
 † *D-erythro-β*-hydroxyleucine; *D-threo-β*-phenylserine.

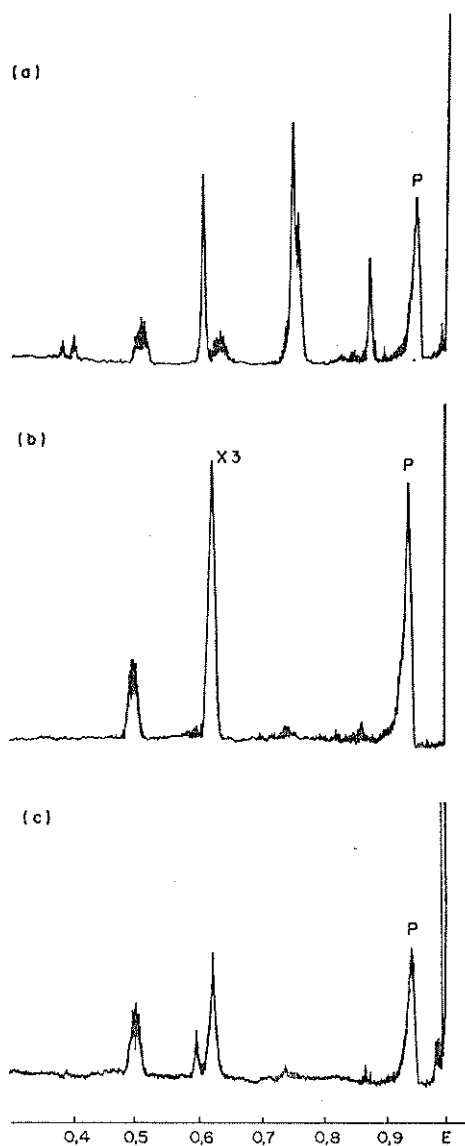


Fig. 1. (a) MIKES of  $m/e$  114,  $(\text{CH}_3)_2\text{N}=\text{CH}-\text{CH}(\text{CH}_3)-\text{CH}_2\text{CH}_3$ , from *N,N*-dimethylisoleucine. (b) MIKES of  $m/e$  114,  $(\text{CH}_3)_2\text{N}=\text{CH}-\text{CH}_2-\text{CH}(\text{CH}_3)_2$ , from *N,N*-dimethylleucine. The peak at 0.63 E should be multiplied by a factor of three to obtain the actual height. (c) MIKES of  $m/e$  114,  $(\text{CH}_3)_2\text{N}=\text{CH}-\text{CH}_2-\text{CH}_2\text{CH}_2\text{CH}_3$ , from *N,N*-dimethylnorleucine. P is a phantom peak typical of the instrument.

typical of a 14-membered peptide alkaloid. The base peak at  $m/e$  114 and the fragments at  $m/e$  507, 493, 444, 394, 190, 135, 107, 106, 105 and 97 indicated that the basic terminal, the  $\beta$ -hydroxy and the ring amino acids were *N,N*-dimethylleucine or *N,N*-dimethylisoleucine,  $\beta$ -hydroxyleucine and  $\beta$ -phenylserine, respectively. The peak at  $m/e$  105, typical of the benzoyl group, in the MS of oxidihydroscutianine H and the identification of *p*-tyramine amongst the hydrolysis products of dihydroscutianine H, confirm the presence of the  $\beta$ -phenylserine and styrylamine units in **6**.

Although differentiation between *N,N*-dimethylleucine and *N,N*-dimethylisoleucine has been successfully carried out by high resolution  $^1\text{H}$  NMR [10, 4],

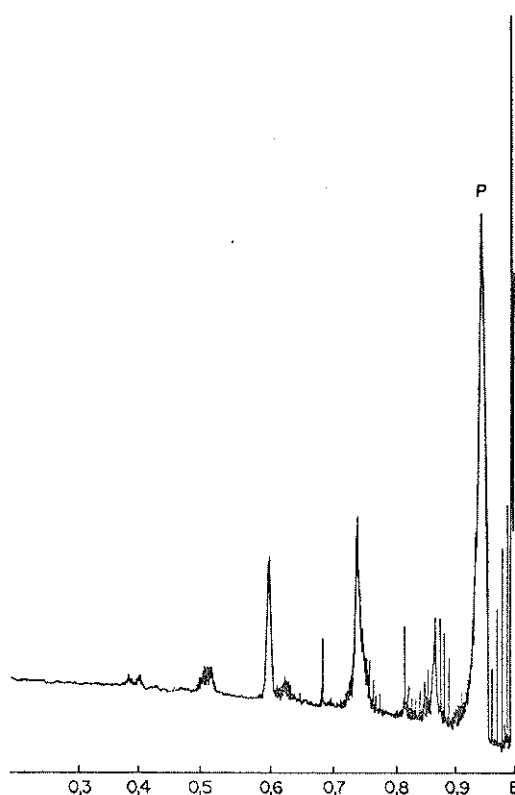


Fig. 2. MIKES of  $m/e$  114,  $(\text{CH}_3)_2\text{N}=\text{CH}-\text{CH}(\text{CH}_3)-\text{CH}_2\text{CH}_3$ , from the EI fragmentation of scutianine C.

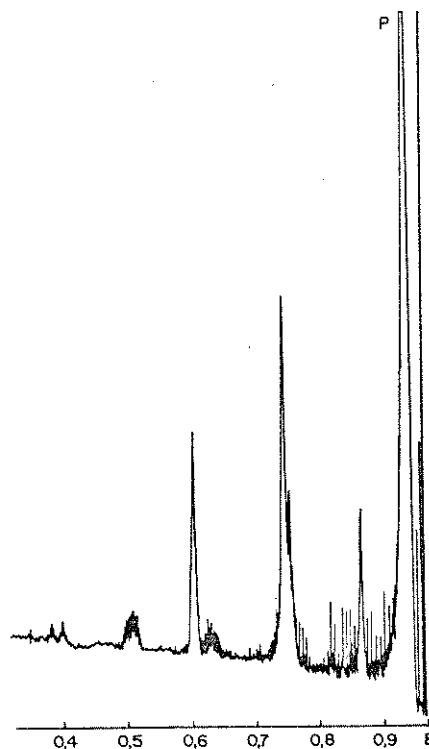


Fig. 3. MIKES of  $m/e$  114,  $(\text{CH}_3)_2\text{N}=\text{CH}-\text{C}_6\text{H}_5$ , from the EI fragmentation of scutianine H.

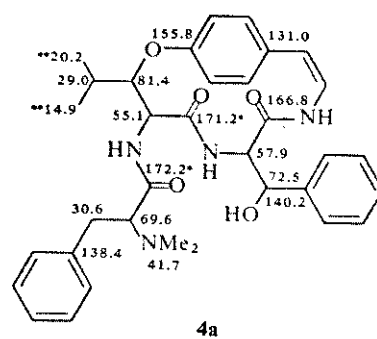
it was interesting to find a method which can differentiate unambiguously the possible isomers of  $m/e$  114, including *N,N*-dimethylnorleucine, using only minute quantities of natural product.

In 1974, McLafferty *et al.* reported the application of metastable ion (MI) and collisional activation (CA) spectra to the determination of leucine and isoleucine residues in peptides [11]. Therefore, we felt that perhaps the MI spectra or more correctly the mass-analysed ion kinetic energy spectra (MIKES) could provide fingerprints of the various isomers of  $m/e$  114 permitting the unequivocal differentiation between the isomers. Indeed, MIKES of  $m/e$  114 from *N,N*-dimethylisoleucine (Fig. 1a), from *N,N*-dimethylleucine (Fig. 1b) and from *N,N*-dimethylnorleucine (Fig. 1c) are dramatically different, showing that there is no equilibration of ion structures and that the fragmentation of these ions is very structure dependent. Since the basic terminal amino acid of scutianine C has been previously determined as *N,N*-dimethylisoleucine [4, 7], we used it as a model alkaloid, and the MIKES of its  $m/e$  114 ion is shown in Fig. 2. Except for the appearance of spikes in the spectrum due to thermal effects and interfering fragments from the first field free region, the aspect of this spectrum is the same shown in Fig. 1a and is significantly different than those shown in Figs. 1b and 1c. Thus, it seems that, independent of its interpretation, the MIKES can be very useful to the natural products chemist as a fingerprint tool, much like IR spectroscopy. Indeed, we used this technique to establish the structure of the basic terminal amino acid of scutianine H (Fig. 3).

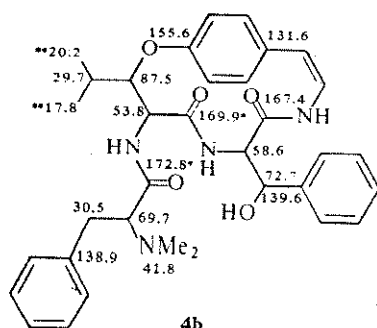
The availability of the diastereoisomeric cyclopeptide alkaloids, scutianines D and E, with  $\beta$ -hydroxyamino acid units of known stereochemistry [7], prompted us to study them by  $^{13}\text{C}$  NMR spectroscopy, hoping to detect features characteristic of a given configuration, since that information could be used as a complement of previous  $^1\text{H}$  NMR studies [5], for the configuration assignment of other members of this group of natural products.

For the analysis of the  $^{13}\text{C}$  NMR spectra we focused our attention mainly on the  $\text{sp}^3$  carbon shifts, and their assignments, based on previously reported data for related alkaloids [12, 13], on the multiplicity of signals in SFORD spectra and on the analysis of model compounds, are shown in 4a and 4b, for scutianines D and E, respectively. The shifts of the methyl groups, their non-equivalence ( $\Delta\delta = 5.3$  ppm) and the shifts of the  $\alpha$ ,  $\beta$  and  $\gamma$  carbons of the  $\beta$ -hydroxyleucine unit of 4a, are in good agreement with the reported ones for frangulanine, discarines A and B, and lasiodine B [12, 13]. The  $\alpha$  and  $\beta$  carbons of the phenylserine unit and those of the *N,N*-dimethylamino acid residue of 4a, were assigned by comparison with model compounds 7 and 8. The difference in  $\delta$  value between the  $\text{CH}_2$  group of the model compound 7, and the same carbon of 4a, unambiguously assigned on the basis of its multiplicity in the SFORD spectrum, can be attributed to a  $\gamma$  effect, probably as the result of a preferred conformation of the *N,N*-dimethylphenylalanine moiety in the alkaloid.

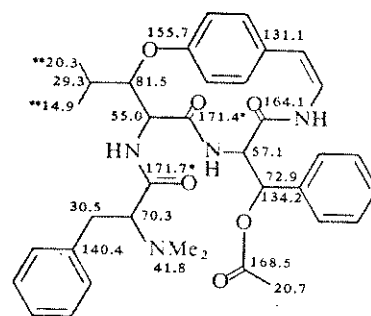
Scutianine E, 4b, shows shifts similar to the ones observed in 4a, except for the  $\beta$ -hydroxyleucine unit. The difference between the methyl groups is clearly less ( $\Delta\delta = 2.4$  ppm), indicating that they are less hindered than in 4a, and further, the  $\alpha$  and  $\beta$  carbons are shielded



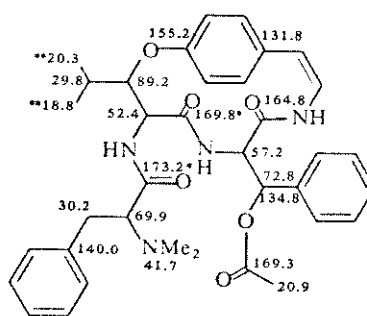
4a



4b



4c



4d

( $\Delta\delta = -1.2$  ppm) and deshielded ( $\Delta\delta = 6.1$  ppm), respectively. The difference in configuration of the phenylserine residue in 4a and 4b does not significantly affect the shifts of the  $\alpha$  and  $\beta$  carbons of this unit.

Acetylation of scutianine E, 4d, apart from producing shielding effects on the non-protonated aromatic carbon and weak effects on the  $\alpha$  and  $\beta$  carbons of the phenylserine unit, and shielding of one of the CO groups, as in scutianine D, 4c, induces shielding and deshielding of

the  $\alpha$  and  $\beta$  carbons, respectively, of the hydroxyleucine.

The clear difference in the  $\delta$  values of the  $\beta$  carbons and the different  $\Delta\delta$  between the methyl shifts of the hydroxyleucine residues of both alkaloids, **4a** and **4b**, together with previously reported  $^1\text{H}$  NMR data [5], could be used as an indication of the configuration of this unit. Discarines A and B, the major bases of *D. longispina* [14] for example, show signals at 82.4 ppm and  $\Delta\delta = 5.7$  ppm, and at 80.8 ppm and  $\Delta\delta = 5.6$  ppm, for the  $\beta$  carbons and for the difference between the chemical shifts of the methyl groups respectively [12, 13] indicating that the hydroxyleucine moiety of these alkaloids possess the *L-erythro* configuration.

#### EXPERIMENTAL

A double-focusing mass spectrometer with a reversed Nier-Johnson geometry was used to obtain the mass spectra and the MIKES. Measurements were carried out with an electron energy of 70 eV and an ion source temp. of 100–120°. The accelerating potential was 3 kV. All samples were introduced via a direct inlet system. The MIKES were obtained by focusing the precursor ion ( $m/e$  114 in this case) on the collector with the magnetic field and scanning the electric sector potential. The  $^1\text{H}$  NMR were recorded at 100 MHz and the  $^{13}\text{C}$  NMR spectra at 25.2 MHz in the Fourier transform mode and in  $\text{CDCl}_3$  solutions. Chemical shifts are expressed on the TMS scale according to:  $\delta\text{TMS} = \delta\text{CDCl}_3 + 76.9$  ppm. The asterisks on the formulae indicate possible signal reversal. The acetates and Me esters were all prepared by standard methods.  $\text{Ac}_2\text{O}$  and  $\text{C}_5\text{H}_5\text{N}$  and ethereal  $\text{CH}_2\text{N}_2$ , respectively, and the physical data were compared, when available, with those of the literature.

**Extraction of *Scutia buxifolia*.** The plant material was collected at Livramento (Rio Grande do Sul, Brazil) in July 1976. The powdered bark (5 kg) was extracted as before [8], yielding a mixture of alkaloids as a solid residue (4.1 g).

**Isolation of the alkaloids.** The alkaloid mixture was fractionated on a  $\text{SiO}_2$  (H type) chromatographic column using mixtures of  $\text{CHCl}_3$ -MeOH as solvent and on PLC, as described before [8], if further purification was required.

**Scutianine D (4a).** Crystallization of the resultant solid (320 mg) from  $\text{CHCl}_3$ -Et<sub>2</sub>O gave **4a**, mp 217–218°;  $[\alpha]_D^{20} - 202^\circ$  ( $\text{CHCl}_3$ ,  $c = 0.1$ ). (Lit. [7] mp 219–220°;  $[\alpha]_D^{20} - 196^\circ$  ( $\text{CHCl}_3$ ,  $c = 0.1$ )). No UV absorption. IR  $\nu_{\text{cm}^{-1}}$ : 3600; 3285; 2795; 1650; 1625; 1250. MS  $m/e$  (rel. int.) 584 (0.02); 493 (4.5); 478 (0.2); 435 (0.1); 387 (1.4); 342 (4.2); 331 (1.4); 190 (0.6); 148 (100); 135 (2.1); 120 (0.6); 107 (1.0); 106 (4.6); 105 (4.8); 97 (1.1); 91 (2.42); 77 (4.1).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  0.98 (3H, *d*,  $J = 6$  Hz); 1.24 (3H, *d*,  $J = 6$  Hz); 2.23 (6H, *s*).  $^{13}\text{C}$  NMR  $\delta$  [ $\text{sp}^2$ ] CH: 120.7; 121.2; 122.4; 125.0; 126.0; 126.4; 127.9; 128.2; 129.0; 129.6; 130.7.

**Scutianine D acetate (4e).** Mp 236°, from EtOH-H<sub>2</sub>O;  $[\alpha]_D^{20} - 163^\circ$  ( $\text{CHCl}_3$ ,  $c = 0.1$ ). (Lit. [7] amorphous;  $[\alpha]_D^{20} - 140^\circ$  ( $\text{CHCl}_3$ ,  $c = 0.1$ )). IR  $\nu_{\text{cm}^{-1}}$ : 3320; 2780; 1760; 1650; 1620; 1230.  $^{13}\text{C}$  NMR  $\delta$  [ $\text{sp}^2$ ] CH: 118.9; 121.4; 122.6; 124.9; 126.1; 126.4; 128.4; 129.0; 129.8.

**Scutianine E (4b).** Crystallization of the resultant yellowish oil (450 mg) from MeOH-H<sub>2</sub>O gave **4b**, mp 110–110.2°;  $[\alpha]_D^{20} - 21^\circ$  ( $\text{CHCl}_3$ ,  $c = 0.1$ ). (Lit. [7] mp 121°;  $[\alpha]_D^{20} - 22.2^\circ$  ( $\text{CHCl}_3$ ,  $c = 0.1$ )). No UV absorption. IR  $\nu_{\text{cm}^{-1}}$ : 3600; 3280; 2800; 1650; 1625; 1250. MS  $m/e$  (rel. int.) 584 (0.05); 493 (4.1); 478 (1.2); 387 (1.3); 190 (1.0); 148 (100); 135 (4.1); 120 (1.5); 107 (3.2); 106 (2.2); 105 (2.1); 97 (2.7); 91 (5.1); 77 (18.4).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.11 (6H, *d*,  $J = 6$  Hz); 2.34 (6H, *s*).  $^{13}\text{C}$  NMR  $\delta$  [ $\text{sp}^2$ ] CH: 121.0; 122.5; 123.1; 125.2; 126.2; 127.7; 128.1; 128.2; 129.0; 129.9; 130.3.

**Scutianine E acetate (4d).** Mp 105°; from EtOH-H<sub>2</sub>O;  $[\alpha]_D^{20} + 58^\circ$  ( $\text{CHCl}_3$ ,  $c = 0.1$ ). (Lit. [7] mp 178–180°;  $[\alpha]_D^{20} + 52.1^\circ$  ( $\text{CHCl}_3$ ,  $c = 0.1$ )). IR  $\nu_{\text{cm}^{-1}}$ : 3320; 2780; 1750; 1680; 1620; 1240. MS  $m/e$  (rel. int.) 626 (0.1); 583 (0.3); 567 (0.2); 535 (58); 475 (3.7); 190 (2.6); 148 (100); 135 (10.5); 120 (5.3); 107

(5.3); 106 (4.1); 105 (9.8); 97 (8.3); 91 (18.9); 77 (7.4).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.2 (6H, *d*,  $J = 6$  Hz); 2.04 (3H, *s*); 2.38 (6H, *s*).  $^{13}\text{C}$  NMR  $\delta$  [ $\text{sp}^2$ ] CH: 119.4; 121.5; 123.0; 125.2; 126.1; 126.6; 127.8; 128.3; 129.2; 130.0; 131.1.

**Scutianine B (2).** Crystallization of the resultant solid (50 mg) from  $\text{CHCl}_3$ -hexane gave **2**, mp 248°;  $[\alpha]_D^{20} - 290^\circ$  ( $\text{CHCl}_3$ ,  $c = 0.1$ ). Identical with an authentic sample (TLC, 3 solvents). MS  $m/e$  148 (base ion peak).

**Scutianine C (3).** Crystallization of the resultant solid (70 mg) from  $\text{CHCl}_3$ -Et<sub>2</sub>O gave **3**, mp 263–265°;  $[\alpha]_D^{20} - 182^\circ$  ( $\text{CHCl}_3$ ,  $c = 0.1$ ). MS  $m/e$  534 ( $M^+$ ); 114 (base ion peak). Identical with an authentic sample (TLC, 3 solvents).

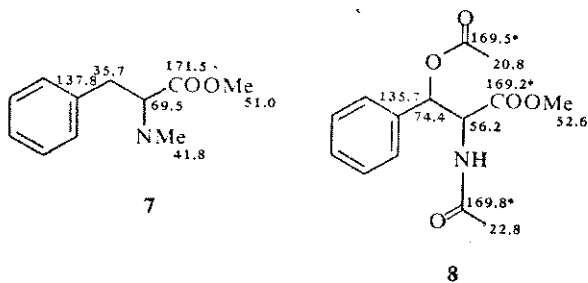
**Scutianine H (6).** The crystalline residue (50 mg), recrystallized from  $\text{CHCl}_3$ -Et<sub>2</sub>O gave **6**, mp 242–243°; homogeneous on TLC; MS (high resolution), found: 550.3184, calc. for  $\text{C}_{31}\text{H}_{42}\text{N}_4\text{O}_5$ ; 550.3155;  $[\alpha]_D^{20} - 233^\circ$  ( $\text{CHCl}_3$ ,  $c = 0.1$ ). No UV absorption. IR  $\nu_{\text{cm}^{-1}}$ : 3580; 3285; 2780; 1640; 1620; 1500; 1250. MS  $m/e$  (rel. int.) 550 (0.2); 535 (0.2); 507 (0.04); 493 (1.5); 444 (1.0); 401 (0.3); 394 (0.42); 353 (0.1); 342 (0.2); 331 (0.3); 329 (0.4); 190 (1.8); 177 (0.8); 135 (10.4); 120 (3.15); 114 (100); 107 (3.2); 106 (5.7); 105 (6.3); 97 (10.3); 85 (16.8); 77 (5.6).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  0.74–0.98 (9H, *m*); 1.22 (3H, *d*,  $J = 8$  Hz); 2.00 (6H, *s*); (*d*<sub>5</sub>-pyridine):  $\delta$  0.84 (3H, *t*,  $J = 6$  Hz); 0.94 (3H, *d*,  $J = 6$  Hz); 1.20 (3H, *d*,  $J = 8$  Hz); 1.28 (3H, *d*,  $J = 6$  Hz); 2.32 (6H, *s*).

**Dihydroscutianine H.** The hydrogenation of scutianine H under the conditions described for peptide alkaloids yielded dihydro-**6**, mp 276–277° (from Me<sub>2</sub>CO). IR  $\nu_{\text{cm}^{-1}}$ : 3580; 3290; 1640; 1500; 1250. MS  $m/e$  (rel. int.) 552 (0.1); 495 (0.2); 446 (0.1); 403 (0.3); 344 (0.3); 333 (0.3); 192 (0.2); 175 (0.7); 137 (0.2); 114 (100); 107 (2.5); 106 (3.1); 105 (3.3); 97 (2.2); 85 (3.9); 77 (3.3). Hydrolysis of dihydro-**6** was performed in a sealed tube at 110° with 6N HCl for 12 hr. the aq. acidic soln was concd and the residue was used to identify *p*-tyramine by a previously described procedure [14].

**Oxidihydroscutianine H.** A soln of dihydro-**6** (10 mg) was oxidized as described before [8]. Crystallization of the residue from Me<sub>2</sub>CO gave oxidihydroscutianine H, mp 288–290°. MS  $m/e$  (rel. int.) 550 (1.2); 535 (0.14); 521 (0.12); 507 (0.4); 493 (1.1); 446 (0.2); 353 (0.4); 218 (0.6); 195 (1.1); 190 (0.3); 175 (2.3); 155 (3.5); 135 (1.7); 114 (100); 107 (9.2); 105 (12.4); 97 (5.8); 85 (12.3); 77 (6.2).

**N,N-Dimethyl-norleucine.** It was prepared by reductive methylation of the corresponding amino acid [15], mp 165.6–166.5°. IR  $\nu_{\text{cm}^{-1}}$ : 2800; 1645.  $^1\text{H}$  NMR 60 MHz ( $\text{CDCl}_3$ ):  $\delta$  0.95 (3H, unresolved *t*); 1.2 (4H, *m*); 2.95 (6H, *s*); 3.5 (1H, *m*); 8.92 (1H, *br s*).

**N,N-Dimethylphenylalanine methyl ester (7).** Viscous oil.  $^1\text{H}$  NMR 60 MHz ( $\text{CDCl}_3$ ):  $\delta$  2.36 (6H, *s*); 2.8–3.5 (3H, *m*); 3.55 (3H, *s*); 7.35 (5H, *s*).



**O,N-Diacetyl- $\beta$ -phenylserine methyl ester (8).** From a commercial sample of  $\beta$ -phenylserine, mainly in the *threo* form— from  $^1\text{H}$  NMR analysis according to [6]—compound **8** was prepared. Mp 145°. IR  $\nu_{\text{cm}^{-1}}$ : 3280; 1760; 1750; 1680.  $^1\text{H}$  NMR 60 MHz ( $\text{CDCl}_3$ ):  $\delta$  1.9 (3H, *s*); 2.1 (3H, *s*); 3.6 (3H, *s*); 5.1 (2H, *m*); 6.2 (1H, *d*); 7.3 (5H, *s*). MS  $m/e$  (rel. int.) 279 (5.3); 220 (2.6); 173 (25); 160 (21); 149 (43.4); 130 (50); 113 (38.2); 107 (51); 89 (100); 79 (20); 43 (22).



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## REFERENCES

1. Tschesche, R. and Hillebrand, D. (1977) *Phytochemistry* **16**, 1817.
2. Tschesche, R., Welters, R. and Fehlhaber, H.-W. (1967) *Chem. Ber.* **100**, 323.
3. Tschesche, R., Ammermann, E. and Fehlhaber, H.-W. (1971) *Tetrahedron Letters* 4405.
4. González Sierra, M., Mascaretti, O. A., Merkuza, V. M., Tosti, E. L., Rúveda, E. A. and Chang, C.-J. (1974) *Phytochemistry* **13**, 2865.
5. González Sierra, M., Mascaretti, O. A., Diaz, F. J., Rúveda, E. A., Chang, C.-J., Hagaman, E. W. and Wenkert, E. (1972) *J. Chem. Soc. Chem. Commun.* 915.
6. Marchand, J., Rocchiccioli, F., Pais, M. and Jarreau, F.-X. (1972) *Bull. Soc. Chim. Fr.* 4699; Marchand, J., Pais, M. and Jarreau, F.-X. (1971) *Bull. Soc. Chim. Fr.* 3742.
7. Tschesche, R. and Ammermann, R. (1974) *Chem. Ber.* **107**, 2274.
8. Merkuza, V. M., González Sierra, M., Mascaretti, O. A., Rúveda, E. A., Chang, C.-J. and Wenkert, E. (1974) *Phytochemistry* **13**, 1279.
9. Tschesche, R., Hillebrand, D., Wilhelm, H., Ammermann, E. and Eckhardt, G. (1977) *Phytochemistry* **16**, 1025.
10. Chang, C.-J., Hagaman, E. W., Wenkert, E., González Sierra, M., Mascaretti, O. A., Merkuza, V. M. and Rúveda, E. A. (1974) *Phytochemistry* **13**, 1273.
11. Levsen, K., Wipf, H.-K., McLaferty, F. W. (1974) *Org. Mass. Spectrom.* **8**, 117.
12. Haslinger, H. (1978) *Tetrahedron* **34**, 685.
13. Pais, M., Jarreau, F.-X., González Sierra, M., Mascaretti, O. A., Rúveda, E. A., Chang, C.-J., Hagaman, E. W. and Wenkert, E., unpublished results.
14. Mascaretti, O. A., Merkuza, V. M., Ferraro, G. E., Rúveda, E. A., Chang, C.-J. and Wenkert, E. (1972) *Phytochemistry* **11**, 1133.
15. Bowman, R. E. and Stroud, H. H. (1950) *J. Chem. Soc.* 1342.

## $^{13}\text{C}$ NMR ANALYSIS OF ALKALOIDS FROM *PESCHIERA FUCHSIAEFOLIA*

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**Key Word Index**—*Peschiera fuchsiaefolia*; Apocynaceae; bisindole alkaloids; voacamine; decarbomethoxyvoacamine; demethylvoacamine; dihydrovoacamine; voacamidine; affinisine; 16-epiaffinine; perivine; voachalotine; voacangine; voacanginehydroxyindolenine;  $^{13}\text{C}$  NMR.

**Abstract**—Fractionation of an ethereal extract of *Peschiera fuchsiaefolia* resulted in the isolation of decarbomethoxyvoacamine, demethylvoacamine, voacamidine, perivine, 16-epiaffinine and voacanginehydroxyindolenine, together with the previously reported alkaloids voacamine, voacangine, voachalotine and affinisine. Analysis of the  $^{13}\text{C}$  NMR spectra of the bisindole alkaloids and of 16-epiaffinine is reported.

### INTRODUCTION

Reports on the anticancer activity of bisindole alkaloids of the voacamine type [1] led us to re-investigate the bark of *Peschiera fuchsiaefolia* [2, 3], directing our research on the isolation of these compounds.

### RESULTS AND DISCUSSION

After alkalization the ground bark of *P. fuchsiaefolia* was extracted with ether. The crude extract was dispersed in 10% acetic acid and the aqueous solution was extracted with chloroform and ether at different pHs, yielding fractions A, B and C. These fractions were monitored by mass spectrometry, which revealed that B was rich in dimeric compounds. This fraction was then submitted to Sephadex LH 20 column chromatography allowing a good MW separation, followed by preparative TLC purification leading to the isolation of the bisindoles voacamine (1a), decarbomethoxyvoacamine (1b), demethylvoacamine (1c) and voacamidine (1d), and the monomeric indoles affinisine (2) and voachalotine (3). Pure voacangine (4) voacanginehydroxyindolenine (5) and voacamine (1a) were obtained from fraction A, while fraction C furnished two  $\alpha$ -acylindoles, perivine (6) and 16-epiaffinine (7), which were not reported previously [2, 3].

The  $^{13}\text{C}$  NMR spectra were interpreted on the basis of standard chemical shift theory, comparison with reference compounds and mainly by analysis of the SFORD and fully coupled spectral data.

Table 1 presents the  $^{13}\text{C}$  NMR data of compounds 1a-1f. The shift assignments were made in conformity with the published data of voacangine (4) [4, 5] vobasinol (8) [6] and ibogaine (9) [4, 5]. The replacement of the C-3 hydroxyl group of 8 by a voacangine unit in voacamine (1a) produced the expected shielding at C-3 ( $\Delta\delta$  29.4) and

deshielding at C-14 and C-15 ( $\Delta\delta$  1.1 and 4.1, respectively). Analogous effects were observed in the tabernaegantines [7]. On the other hand, in the voacangine moiety the replacement of the hydrogen at C-11 by a vobasine unit induced a deshielding of the *ipso* ( $\Delta\delta$  15.7) and shielding of the *ortho* carbons ( $\Delta\delta_{\text{C-10}}$  3.0 and  $\Delta\delta_{\text{C-12}}$  1.4). The remaining carbons showed little or no modification.

The R configuration at C-20 of dihydrovoacamine (1e), the sole product of the catalytic hydrogenation of 1a, was deduced from the chemical shifts of C-14 and C-16. C-14 at  $\delta$  31.4 was shielded ( $\Delta\delta$  5.2) in 1e by comparison with the same site of 1a due to a  $\gamma$ -interaction with C-19, while C-16 was deshielded ( $\Delta\delta$  3.7) (The differences produced by the  $\alpha$  or  $\beta$  ethyl group orientation were previously discussed for tabernaegantines A and B [7], dregamine and tabernamontanine [6].)

Comparison of the  $^{13}\text{C}$  chemical shifts of demethylvoacamine (1c) and voacamine (1a) revealed some interesting conformational aspects concerning the  $N_b$ -methyl group. Introduction of the *N*-methyl group into 1c induced simultaneous shielding at C-6 ( $\Delta\delta$  4.8) and C-16 ( $\Delta\delta$  6.3). These facts can be explained by taking the inversion of  $N_b$  into consideration. Thus the rapid interconversion between the equatorial and axial  $N_b$ -methyl group leads to the observed shieldings. Analogous effects were observed at C-6 ( $\Delta\delta$  6.3) and C-16 ( $\Delta\delta$  2.6) of 1e for the introduction of the *N*-methyl group in demethyldihydrovoacamine (1f) (going from 1f to 1e). The larger  $\Delta\delta$  value at C-6 suggests a preferential equatorial position for the  $N_b$ -methyl group in the dihydro compound, thus avoiding an additional methyl  $\text{H}_{20}$  1,3-diaxial interaction.

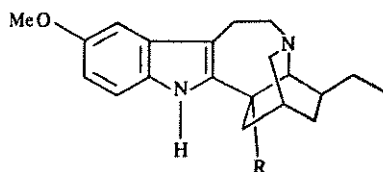
An interesting feature revealed in our work was the isolation of demethylvoacamine (1c) together with voacangine (4) and the  $\alpha$ -acylindole perivine (6), implying that both monomers 4 and 6 are precursors in the biosynthetic pathway of 1c. Though *in vitro* data [8] support the above suggestion, rigorous *in vivo* experiments would provide final confirmation.

### EXPERIMENTAL

Mps are uncorr. Specific rotations were measured in  $\text{CHCl}_3$ , UV spectra in EtOH and IR spectra in  $\text{CHCl}_3$ .  $^1\text{H}$  NMR spectra

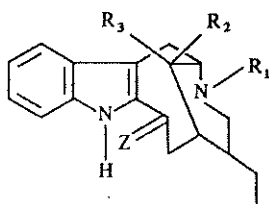
\* Present address: Departamento de Morfologia e Sistemática Vegetais, Instituto de Biologia, Universidade Estadual de Campinas, Campinas 13100, São Paulo, Brazil.

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4 R = COOMe

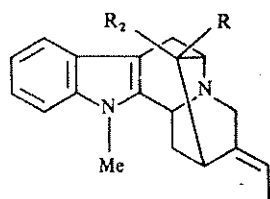
9 R = H



6 R<sub>1</sub> = H; Z = O; R<sub>2</sub> = H; R<sub>3</sub> = COOMe

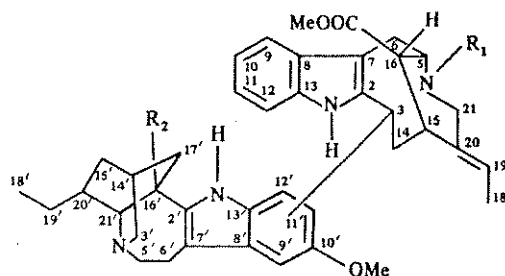
7 R<sub>1</sub> = Me; Z = O; R<sub>2</sub> = CH<sub>2</sub>OH; R<sub>3</sub> = H

8 R<sub>1</sub> = Me; Z = αH, βOH; R<sub>2</sub> = H; R<sub>3</sub> = CO<sub>2</sub>Me



2 R<sub>1</sub> = CH<sub>2</sub>OH, R<sub>2</sub> = H

3 R<sub>1</sub> = COOMe, R<sub>2</sub> = CH<sub>2</sub>OH



1a R<sub>1</sub> = Me; R<sub>2</sub> = COOMe;

LINKAGE at 11'

1b R<sub>1</sub> = Me; R<sub>2</sub> = H;

" " 11'

1c R<sub>1</sub> = H; R<sub>2</sub> = COOMe;

" " 11'

1d R<sub>1</sub> = Me; R<sub>2</sub> = COOMe;

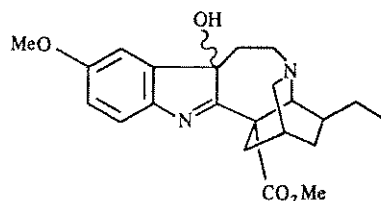
" " 9'

1e R<sub>1</sub> = Me; R<sub>2</sub> = COOMe; 19-20 DIHYDRO;

" " 11'

1f R<sub>1</sub> = H; R<sub>2</sub> = COOMe; 19-20 DIHYDRO;

" " 11'



5

at 60 and 100 MHz were obtained using TMS as int. standard. <sup>13</sup>C NMR spectra were recorded at 25.2 MHz with Fourier transform using CDCl<sub>3</sub> as solvent and TMS as int. standard. MS were determined at 70 eV. Silica gel 0.05-0.25 mesh (Carlo Erba) and silica gel HF<sub>254-366 nm</sub> (Merck) were used for CC and TLC, respectively. Detection of components was made by UV (254 and 305 nm) and spraying with Dragendorff's reagent followed by MeOH-H<sub>2</sub>SO<sub>4</sub> and heating the plates at 150° for 5 min.

**Plant material.** Stem bark of *P. fuchsiae* (DC.) Miers was collected in the Zeferino Vaz University City. The air-dried bark (2.929 g) was moistened with a saturated NaHCO<sub>3</sub> soln and extracted in a Soxhlet with Et<sub>2</sub>O. On concn, the Et<sub>2</sub>O extract gave a viscous oil which was added to a 10% HOAc soln and kept at 5° overnight. After filtration the aq. phase was extracted with Et<sub>2</sub>O (extract A 6.65 g) and CHCl<sub>3</sub> (extract B 18.14 g). The pH was then raised to 8 with a saturated NaHCO<sub>3</sub> soln and extracted with Et<sub>2</sub>O (extract C 8.52 g) and CHCl<sub>3</sub> (extract D 0.25 g).

**Extract A** (2.96 g) was fractionated on a silica gel column eluting with CHCl<sub>3</sub>, and CHCl<sub>3</sub> with increasing amounts of MeOH, yielding voacamine (1a) (0.076 g) [9], voacangine (4) (0.41 g) [10] and voacanginehydroxyindolenine (5) (0.1388 g) [11]. TLC also indicated the presence of demethylvoacamine (1c) [12] and affinisine (2) [13].

**Extract B.** A Sephadex LH 20 column (6 g) eluted with CHCl<sub>3</sub>-MeOH (9:1) permitted a crude separation of the dimeric from the monomeric compounds. The combined fractions were further purified using a silica gel column and/or prep. TLC, yielding the bisindoles voacamine (1a) (0.196 g) [9], demethyl-

voacamine (1c) (0.232 g) [12], decarbomethoxyvoacamine (1b) (0.178 g) [14] and voacamidine (1d) (0.206 g) [12], and the indoles vonchapotine (3) (0.221 g) [13] and affinisine (2) (0.329 g) [13].

**Extract C** (3 g) was fractionated on a silica gel column eluting with CHCl<sub>3</sub>, and CHCl<sub>3</sub> with increasing amounts of MeOH, leading to the isolation of perivine (6) (0.1399 g) [8, 10], 16-epiaffinisine (7) (0.2043 g) [15], affinisine (2) (0.2167 g) [13] and decarbomethoxyvoacamine (1b) (0.1295 g) [14].

**Dihydrovoacamine (1e).** An EtOH soln of voacamine (1a) (0.206 g) with a catalytic amount of PtO<sub>2</sub> was submitted to hydrogenation (45 psi H<sub>2</sub>) in a Parr apparatus for 30 min. Filtration through a Celite pad and evapn of solvent furnished 0.198 g of dihydrovoacamine, mp 210-212° (MeOH); [α]<sub>D</sub><sup>25</sup> + 40.9° (0.010 g/ml CHCl<sub>3</sub>); UV λ<sub>max</sub><sup>CHCl<sub>3</sub></sup> nm (log ε): 226.2 (4.75), 286.2 (4.28), 293.7 (4.28); IR ν<sub>max</sub><sup>CHCl<sub>3</sub></sup> cm<sup>-1</sup>: 3450, 1710; <sup>1</sup>H NMR: δ 0.93 (m), 2.47 (3H, s), 2.60 (3H, s), 3.63 (3H, s), 3.93 (3H, s), 7.48 (1H, s), 7.60 (1H, s); MS m/z (rel. int.): 720 (55), 706 [M]<sup>+</sup> (31), 511 (100).

**Demethyl-dihydrovoacamine (1f).** An EtOH soln of demethylvoacamine (1c) (0.206 g) was hydrogenated as above to furnish 0.149 g 1f. Mp 210° (MeOH); UV λ<sub>max</sub><sup>CHCl<sub>3</sub></sup> nm (log ε): 226.8 (4.68), 285 (4.23), 293 (4.22); IR ν<sub>max</sub><sup>CHCl<sub>3</sub></sup> cm<sup>-1</sup>: 3460, 1720; <sup>1</sup>H NMR: δ 0.93 (t), 2.47 (3H, s), 3.67 (3H, s), 3.97 (3H, s); MS m/z (rel. int.): 720 (62), 706 [M]<sup>+</sup> (44), 136 (100).

**Acknowledgements**—We wish to express our appreciation to Professor Anita J. Marsaioli for helpful suggestions, Dr. Norbert

Table 1. <sup>13</sup>C NMR data for voacamine alkaloids and their derivatives

Carbon	4	8	9	7	1a	1b	1c	1e	1d	1f
2		135.4		134.4	135.5	135.5	135.5	135.6	134.9	135.7
3		66.8		189.5	37.4	37.6	37.1	37.2	37.1	37.3
5		59.4		56.5	59.9	59.7	53.3	59.2	59.8	53.0
6		19.6		19.3	19.8	19.4	24.6	19.2	19.0	25.5
7		107.3		120.4	109.6	110.0	110.2	109.8	110.8	110.9
8		128.7		128.1	129.4	129.4	129.7	129.6	129.9	129.5
9		117.6		121.2	117.2	117.0	117.1	117.2	116.9	117.3
10		118.6		120.2	119.8*	118.6	118.7	118.6	118.5	118.7
11		121.4		126.5	121.3	121.2	121.3	121.3	120.5	121.3
12		110.0		112.1	110.1	110.0	110.6	110.5	109.4	110.4
13		136.7		136.3	136.2	137.5	137.1	136.9	137.4	137.1
14		35.5		43.4	36.6	36.1	36.3	31.4	37.1	31.6
15		29.2		31.2	33.3	33.4	34.1	32.8	32.1	33.4
16		47.1		38.5	46.0	46.7	52.3	49.7	46.9	52.3
18		12.2		12.0	12.3	12.2	12.0	11.4	12.2	11.1
19		118.6		120.2	118.7*	118.6	117.3	23.5	118.5	23.5
20		136.5		135.2	137.7	137.8	140.1	43.7	137.9	45.9
21		53.9		51.9	52.3	52.2	44.1	51.9	52.3	40.8
COOMe		174.3		—	170.7	171.1	171.0	171.5	171.4	171.3
COOCH <sub>3</sub>		50.3		—	49.8	49.7	49.8	49.5	49.8	49.7
NMe		42.1		41.7	41.9	42.1	—	42.4	42.2	—
CH <sub>2</sub> OH				66.7						
2'	137.3		142.9		136.9	142.1	137.6	137.5	138.8	137.5
3'	51.7		50.0		52.0	49.7	51.8	52.3	51.0	51.9
5'	53.1		54.2		53.0	54.1	53.0	53.0	53.9	53.0
6'	22.2		20.7		22.1	20.6	22.2	22.2	24.9	22.2
7'	110.0		109.1		109.6	108.4	109.6	110.3	109.0	109.8
8'	129.1		129.7		129.4	128.4	129.7	130.0	126.2*	130.1
9'	100.7		100.3		99.1	98.5	99.1	99.0	126.4*	99.2
10'	154.0		153.9		150.6	150.7	150.7	150.8	152.4	150.9
11'	111.9		110.8		127.1	127.9	127.1	127.0	112.5	127.1
12'	111.1		110.6		109.5	109.6	109.7	109.6	109.4	109.6
13'	130.6		130.0		130.0	129.0	130.1	130.3	131.8	130.2
14'	27.3		26.5		27.2	26.1	27.3	27.3	27.2	27.3
15'	32.0		32.0		31.8	31.7	31.9	31.9	31.8	31.9
16'	55.0		42.0		54.8	40.9	54.8	54.8	55.8	54.9
17'	36.5		34.2		36.2	33.9	36.3	36.4	33.5	36.4
18'	11.7		11.9		11.5	11.8	11.6	11.6	11.6	11.5
19'	26.7		27.8		26.6	27.6	26.7	26.7	26.7	26.7
20'	39.1		41.5		38.8	41.7	38.9	38.9	38.9	38.9
21'	57.6		57.5		56.9	57.5	57.0	57.0	58.2	57.0
COOMe	175.6		—		174.9	—	174.9	175.0	175.5	174.9
COOCH <sub>3</sub>	52.7		—		51.8	—	50.0	49.7	52.5	50.6
OMe	55.7		56.0		56.0	55.9	56.0	56.0	57.8	56.0

Spectra were obtained at 25.2 MHz in Fourier transform mode in CDCl<sub>3</sub> solutions. Chemical shifts are expressed on the TMS scale according to: δ TMS = δ CDCl<sub>3</sub> + 76.9 ppm.

\*Assignments for these signals within a vertical column may be reversed.

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REFERENCES

- Kingston, D. G. I. (1978) *J. Pharm. Sci.* 67, 272.
- Fernandez, M. E., Albonico, S. M. and Ruveda, E. A. (1967) *An. Asoc. Quim. Argent.* 55, 239.
- Achenbach, H. (1966) *Tetrahedron Letters* 4405.

- Damak, M., Poupat, C. and Ahond, A. (1976) *Tetrahedron Letters* 3531.
- Wenkert, E., Cochran, D. W., Gottlieb, H. E., Hagaman, E. W., Braz F<sup>o</sup>, R., Matos, F. J. A. and Madruga, M. I. L. M. (1976) *Helv. Chim. Acta* 59, 2437.
- Ahond, A., Bui, A. M., Potier, P., Hagaman, E. W. and Wenkert, E. (1976) *J. Org. Chem.* 41, 1878.
- Bombardelli, E., Bonati, A., Gabeta, B., Martinelli, E. M., Mustich, G. and Danieli, B. (1976) *J. Chem. Soc. Perkin Trans 1*, 1432.
- Büchi, G., Manning, R. E. and Monti, S. A. (1964) *J. Am.*

- Chem. Soc.* **86**, 4631.
9. Voticky, Z., Jahodar, L. and Cava, M. P. (1977) *Collect. Czech. Chem. Commun.* **42**, 1403.
  10. Holubek, J. and Strouf, O. (1965) *Spectral Data and Physical Constants of Alkaloids*, No. 295, Heyden, London.
  11. Thomas, D. W. and Biemann, K. (1968) *Tetrahedron* **24**, 4223.
  12. Achenbach, H. and Schaller, E. (1976) *Chem. Ber.* **109**, 3527.
  13. Achenbach, H. (1966) *Tetrahedron Letters* 4405.
  14. Thomas, D. W. and Biemann, K. (1965) *J. Am. Chem. Soc.* **87**, 5447.
  15. Naranjo, J., Pinar, M., Hesse, M. and Schmid, H. (1972) *Helv. Chim. Acta* **55**, 752.

## <sup>13</sup>C NMR SPECTRAL ANALYSIS OF SOME ISOQUINOLINE ALKALOIDS

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**Key Word Index**—<sup>13</sup>C NMR spectra; benzyloisoquinoline alkaloids; papaverine; 1-(*p*-methoxybenzyl)-6,7-dimethoxyisoquinoline; 1-(*p*-methoxybenzyl)-6,7-methylenedioxyisoquinoline; 1-(*p*-methoxybenzyl)-6,7-dimethoxytetrahydroisoquinoline; 1-(*p*-methoxybenzyl)-6,7-methylenedioxytetrahydroisoquinoline; *N*-methylpapaverine; *N*-methyllaudanosine; bisbenzyloisoquinoline alkaloids; isochondodendrine.

**Abstract**—The <sup>13</sup>C NMR spectra of some isoquinoline and tetrahydroisoquinoline alkaloids and their corresponding *N*-methosalts and of the bisbenzyloisoquinoline alkaloid isochondodendrine were recorded and the signals assigned. The substituent shielding effects and the <sup>13</sup>C-<sup>1</sup>H long range couplings were analysed and utilized in the spectral interpretation.

### INTRODUCTION

In recent years effort has been devoted to the <sup>13</sup>C NMR spectral analysis of isoquinoline alkaloids and valuable information regarding their structural features has been obtained [1-3]. In this connection, and as part of a project on the <sup>13</sup>C NMR spectroscopy of natural products [4], and in order to facilitate the shift assignments of related alkaloids, the analysis of the <sup>13</sup>C NMR spectra of some benzyloisoquinoline, benzyltetrahydroisoquinoline alkaloids, their corresponding *N*-methosalts and the bisbenzyloisoquinoline alkaloid isochondodendrine was carried out. To the best of our knowledge this is the first <sup>13</sup>C NMR spectral analysis of a bisbenzyloisoquinoline alkaloid.

### RESULTS AND DISCUSSION

Table 1 lists the carbon shifts of papaverine **1**, 1-(*p*-methoxybenzyl)-6,7-dimethoxyisoquinoline **2** and 1-(*p*-methoxybenzyl)-6,7-methylenedioxyisoquinoline **3** [5], assigned by standard chemical shift theory, comparison with reference compounds and mainly, by analysis of the SFORD and the fully coupled <sup>13</sup>C NMR spectra.

Amongst the proton-bearing carbons of **1**, clearly assigned from a SFORD spectrum, C-3 and C-4 show shifts at 140.6 and 118.3 ppm respectively, in agreement with reported values for isoquinoline and further, confirmed by analysis of their fine structure from a <sup>1</sup>H-coupled <sup>13</sup>C spectrum [<sup>2</sup>*J*(C<sub>4</sub>-H<sub>3</sub>) = 8 Hz, <sup>3</sup>*J*(C<sub>4</sub>-H<sub>3</sub>) = 4.9 Hz, <sup>2</sup>*J*(C<sub>3</sub>-H<sub>4</sub>) = 3.1 Hz] [6]. Of the remaining 5 methines, the lowest field signal at 120.1 ppm can be assigned to C-6, by its chemical shift and its complex coupling pattern due to a <sup>3</sup>*J*<sub>CH</sub> and two angle dependent couplings with the benzylic protons [7]. The distinction of the methines *ortho* to methoxyl groups is founded again on the analysis of their fine structure, the signal at 104.9 ppm showing a <sup>3</sup>*J*<sub>CH</sub> = 4.9 Hz was assigned to C-5 while the one at 103.8 ppm, with no long-range splittings to C-8. Carbon-5' at 110.5 ppm appears as a clean doublet with no discernible <sup>2</sup>*J*<sub>CH</sub> or <sup>3</sup>*J*<sub>CH</sub>, and C-2, at 111.5 ppm shows a very complex fine structure.

The signals corresponding to the oxygenated quaternary carbons at 152.0 and 149.7 ppm were assigned to C-6 and C-7 respectively, by comparison with related carbons of 6,7-dimethoxyisoquinoline **5** and the remaining ones at 148.6 and 147.0 ppm, to C-3, and C-4, respectively, by comparison with the benzyl moiety of laudanosine, **9** [1].

Based on their multiplicities, the non-oxygenated quaternary carbon signals at 133.0 and 122.5 ppm were assigned to C-4a and C-8a respectively, C-4a shows a clear triplet [<sup>3</sup>*J*(C<sub>4</sub>-H<sub>8,3</sub>) = 7.0 Hz] while C-8a appears as a complex multiplet, difficult to analyze because of

Table 1. <sup>13</sup>C NMR spectral data for compounds **1-4**, **7** and **8**\*

Carbon	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>7</b>	<b>8</b>
1	157.4	157.7	158.0	155.4	64.4	65.0
3	140.6	140.8	141.0	140.2	46.3	46.6
4	118.3	118.4	119.1	118.3	25.0	25.6
4a	133.0	133.2	134.8	132.6	125.3	127.3
5	104.9	105.1	102.9	104.9	113.0	108.0
6	152.0	152.1	150.0	152.5	146.6	145.0
7	149.7	149.5	148.0	149.8	145.7	145.4
8	103.8	104.0	101.7	102.8	110.7	107.0
8a	122.5	122.6	124.6	122.4	128.6	130.4
α	42.0	41.7	41.4	37.9	39.7	40.5
1'	131.9	131.5	131.3	128.7	131.3	131.6
2'	111.5	129.2	129.2	113.4	130.1	130.1
3'	148.6	113.7	113.7	152.2	113.0	113.2
4'	147.0	157.7	157.7	146.9	157.3	157.5
5'	110.5	113.7	113.7	107.7	113.0	113.2
6'	120.1	129.2	129.1	141.0	130.1	130.1
-OMe	55.5	55.2	55.1	55.6	54.6	54.9
		55.7		55.7	55.0	
		55.8			55.2	
-OCH <sub>2</sub> O-			101.3			100.2
-NME					42.1	42.4

\* The spectra were obtained at 25.2 MHz in Fourier transform mode in CDCl<sub>3</sub> solutions. The data for each carbon are shown in ppm downfield from TMS.

the overlap of signals. The remaining two signals at 157.4 and 131.9 ppm, which are, as expected, essentially unaffected in compounds **1**, **2** and **3**, were assigned to C-1 and C-1', respectively. Further, the  $\delta$  values of **6**, used as model for ring B of these compounds, support the above assignments [8]. The nonaromatic carbons of **1**, **2** and **3** were readily assigned on the basis of standard chemical shift theory and analysis of the SFORD spectrum.

Comparison of the shifts of C-5 and C-8 of **1** and **2** with the reported values for related carbons of 6,7-dimethoxyisoquinoline **5** [2], shows some interesting results. In **1** and **2** the mentioned carbons are clearly shielded and, at least on C-5, this is probably due to an electronic effect of the substituent on C-1, apparently the observed shielding effect is independent of the electron releasing capacity of the substituents on ring C, since the introduction of a nitro group, **4**, does not affect the chemical shift of C-5. On C-8 however, a  $\gamma$  effect produced by C<sub>2</sub>, similar to that observed on a similar carbon in the conversion of naphthalene to 1-methylnaphthalene [9], could be invoked to explain its chemical shift.

The replacement of the 6,7-dimethoxyisoquinoline in **1** and **2** by a methylenedioxyisoquinoline system in **3** causes the expected changes on the benzenoid carbons; C-5, C-6, C-7 and C-8 are shielded while C-4a and C-8a are deshielded [1]. The transformation of **1** into **4**, affects the carbons of ring C in a predictable way confirming the above assignment.

The analysis of **7** and **8** was greatly simplified by previous work [1] and their shifts are also listed in Table 1. As expected, the resonances due to the nonaromatic carbons and those of ring C of **7** and **8** are in good agreement with the reported values for laudanose **9** and for compounds **2** and **3** respectively; they were readily assigned and further confirmed by analysis of their multiplicities. Comparison of the carbon signals of the tetrahydroisoquinoline system of **9** with the corresponding ones of **7**, shows agreement for all except for the shift of 132.2 ppm assigned to C-8a, which should be reversed with the 129.0 ppm signal

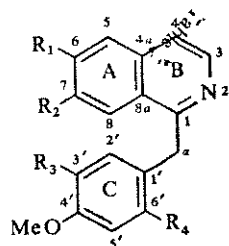
Table 2. <sup>13</sup>C NMR spectral data for compounds **10-15**\*

Carbon	<b>10</b> †	<b>11</b>	<b>12</b>	<b>13</b> †	<b>14</b>	<b>15</b>
1	152.7	151.7	151.7	71.3	71.7	71.9
3	135.6	134.3	136.1	54.7	54.8	54.4
4	122.5	121.5	123.1	23.1	23.1	23.3
4a	135.4	134.7	137.5	120.6	120.6	122.0
5	106.1	105.4	103.5	111.0	111.1	107.8
6	156.7	155.7	155.0	148.9	148.5	147.5
7	154.2	153.6	154.7	146.6	146.1	145.5
8	104.6	104.0	102.5	110.5	110.2	107.5
8a	123.9	123.0	125.6	119.1	119.2	120.9
$\alpha$	34.8	33.6	34.6	37.4	37.1	37.0
1'	125.4	124.1	124.4	126.3	125.8	125.5
2'	111.5	127.8	128.6	113.1	131.1	130.5
3'	149.0	113.4	114.3	148.9	113.4	113.4
4'	147.9	157.3	158.3	147.9	158.2	158.1
5'	111.1	113.4	114.3	110.1	113.4	113.4
6'	118.9	127.8	128.6	122.3	131.1	130.5
OMe	56.6	56.6	54.9	56.4	55.4	54.6
	56.5	56.0		55.4	54.7	
	56.0	55.1		54.9		
	55.8			54.7		
+ NMe	46.9	45.8	46.8	52.3	52.4	52.2
				50.3	50.5	50.6
-OCH <sub>2</sub> O-			102.5			100.8

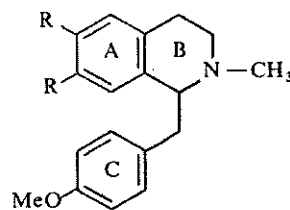
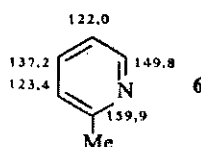
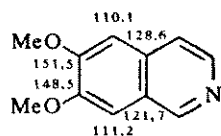
\* The spectra were obtained at 25.2 MHz in Fourier transform mode in CDCl<sub>3</sub> solutions. The data for each carbon are shown in ppm downfield from TMS.† Some methanol was added for better dissolution of the compound.

previously assigned to C-1 [10]. The replacement of the 6,7-dimethoxy groups of **7** by the methylenedioxy unit of **8**, produces again the expected changes confirming the above assignments.

The transformation of compounds **1**, **2** and **3** into their corresponding *N*-methosalts **10**, **11** and **12** respectively, produces similar changes on A and B rings to the ones observed on isoquinoline by protonation [11]. Carbon-1 and C-3 are shielded while C-4, C-6 and C-7 are deshielded. The remaining aromatic carbons of **10**, **11** and **12**, except C-1', which suffers a strong shielding effect, are



- 1**, R<sub>1</sub> = R<sub>2</sub> = R<sub>3</sub> = -OMe; R<sub>4</sub> = H  
**2**, R<sub>1</sub> = R<sub>2</sub> = -OMe, R<sub>3</sub> = R<sub>4</sub> = H  
**3**, R<sub>1</sub> = R<sub>2</sub> = -OCH<sub>2</sub>O-; R<sub>3</sub> = R<sub>4</sub> = H  
**4**, R<sub>1</sub> = R<sub>2</sub> = R<sub>3</sub> = -OMe; R<sub>4</sub> = -NO<sub>2</sub>



- 7**, R<sub>1</sub> = R<sub>2</sub> = -OMe  
**8**, R<sub>1</sub> = R<sub>2</sub> = -OCH<sub>2</sub>O-

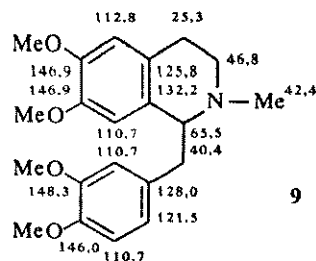
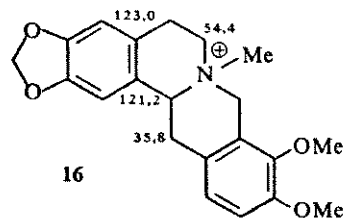


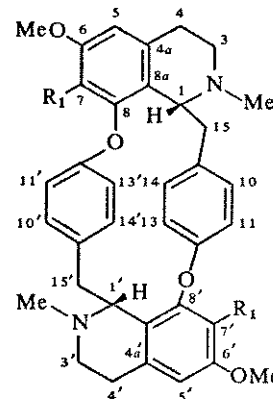
Table 3. <sup>13</sup>C NMR spectral data for compounds 17-19\*

Carbon	17†	18	19‡
1(1')	58.0	59.2	59.4
3(3')	44.0	44.4	44.2
4(4')	25.8	24.6	24.5
4a(4a')	122.9	129.4	132.6
5(5')	107.3	108.9	108.7
6(6')	149.9	151.4	150.5
7(7')	135.7	138.5	129.3
8(8')	139.4	143.3	142.8
8(8a')	124.8	125.2	124.8
9(9')	129.0	130.1	130.8
10(10')	127.2§	127.7§	128.0§
11(11')	114.3	113.6	113.9
12(12')	153.5	153.7	153.5
13(13')	117.4	117.0	117.3§
14(14')	128.6§	128.3§	128.2
15(15')	33.8	37.6	39.1
OMe	55.2	59.7	55.8
NMe	40.5	42.1	42.1

\* The spectra were obtained at 25.2 MHz in the Fourier transform mode in CDCl<sub>3</sub> solutions. The δ values are in ppm downfield from TMS. † Some methanol was added for better solution of the compound. ‡ The acetyl C=O and Me shifts are 166.9 and 19.6 ppm, respectively. §. || Signals within the vertical columns may be reversed. ¶ The numbering system follows the rules reported in C.A. 1967-71.



16



17, R<sub>1</sub> = OH  
18, R<sub>1</sub> = OMe

19, R<sub>1</sub> = OC(=O)Me

essentially unaffected. Carbon-α is also shielded in comparison with the related carbon of 1, 2 or 3 due to a γ effect imposed by the N-Me group.

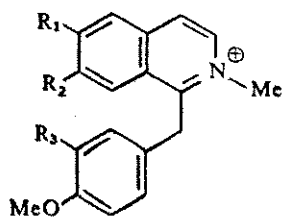
In view of the relatively wide distribution of quaternary alkaloids derived from tetrahydroisoquinoline, the N-methosalts 13, 14 and 15 were also analyzed. As expected, the most affected carbons are the ones at ring B; C-1 and C-3 are deshielded while C-4, C-4a and C-8a are shielded comparing with the related carbons of the tertiary alkaloids. As was observed above, the N atom carrying a positive charge induces a shielding effect on C-1. The δ values for all carbons of compounds 10-15 are listed in Table 2.

In an attempt to explain the shifts of the carbons of ring B of 13, 14 and 15, a comparison with like carbons of the α and β forms of quaternary protoberberine alkaloids was carried out [12]. The striking similarity of the δ values of C-3, C-4a, C-8a and C<sub>z</sub> of 13, 14 and 15 with the corresponding ones of 16 could be explained assuming that ring B with a rigid half-chair conformation has the benzylic carbon C<sub>z</sub> at a quasi-axial position [13].

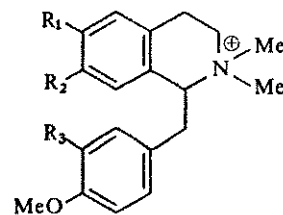
The shifts of the bisbenzylisoquinoline alkaloid isochondodendrine 17, its Me and acetyl derivatives

18 and 19 respectively, greatly simplified by the symmetry of these molecules, are listed in Table 3. The nonaromatic carbons of 17 were assigned by analysis of a SFORD spectrum, standard chemical shift theory and comparison with related carbons of 7 and 8. The introduction of a third oxygenated function on C-8 of the tetrahydroisoquinoline system produces a shielding effect on C-1 and C-15, similar to that observed on the protoberberine alkaloids [2].

The quaternary aromatic carbons, distinguished on the basis of the SFORD spectrum, show signals at 129.0 and 153.8 ppm which remain unchanged in the transformation of 17 into 18 and 19. Based on this result and on the similarity of their shifts with C-1' and C-4', of 7 and 8 they were assigned to C-9 and C-12 respectively. The assignment of the remaining quaternary carbons is founded on the known fact that in a 1,3-dimethoxy-2-hydroxy substituted benzene ring, the methylation of the OH group induces deshielding on ipso, ortho and para carbons, while the meta positions are much less affected [14]. Further support for the assignment of signals at 122.0 and 124.8 ppm to the nonoxygenated quaternary carbons C-4a and C-8a respectively, and



10, R<sub>1</sub> = R<sub>2</sub> = R<sub>3</sub> = -OMe  
11, R<sub>1</sub> = R<sub>2</sub> = -OMe; R<sub>3</sub> = H  
12, R<sub>1</sub> = R<sub>2</sub> = -OCH<sub>2</sub>O-; R<sub>3</sub> = H



13, R<sub>1</sub> = R<sub>2</sub> = R<sub>3</sub> = -OMe  
14, R<sub>1</sub> = R<sub>2</sub> = -OMe; R<sub>3</sub> = H  
15, R<sub>1</sub> = R<sub>2</sub> = -OCH<sub>2</sub>O-; R<sub>3</sub> = H



the signals at 149.9, 135.7 and 139.7 ppm to the oxygenated ones C-6, C-7 and C-8, was obtained by acetylation. As expected, C-7 suffers a shielding effect. C-6, C-8 and C-4a, *ortho* and *para* to the acetoxy group respectively, are deshielded, while C-5 and C-8a are unaffected [15].

Of the aromatic methine shifts, the assignment of the 107.3 ppm signal to C-5 is simple. The remaining 4 signals are split into two pairs of similar chemical shifts. Although the higher field pair, 114.3 and 117.4 ppm, can be assigned to carbons *ortho* to the oxygenated function of the *p*-substituted benzene ring, specific assignments become difficult. The non-equivalence of the protons attached to these carbons, previously observed in the PMR spectrum of 17, has been attributed to restricted rotation of both benzene rings [16].

#### EXPERIMENTAL

Papaverine was a commercial sample and compounds 2 and 3 were isolated from an *Ocotea* sp. kindly provided by Dr. U. M. F. Meirelles, according to ref. [17]. Compound 4 was prepared according to ref. [18]. The *N*-methosalts were prepared by refluxing with  $\text{CH}_3\text{I}$  in MeOH, their reductions were carried out with  $\text{NaBH}_4$  in MeOH solns. Methylation and acetylation of 17 was carried out under usual conditions; treatment with  $\text{CH}_2\text{N}_2$  in  $\text{CHCl}_3$ -MeOH soln and  $\text{Ac}_2\text{O}$ -Py, yielded 18 and 19, respectively.

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#### REFERENCES

1. Wenkert, E., Buckwalter, B. L., Burffit, I. R., Gasić, M. J., Gottlieb, H. E., Hagaman, E. W., Schell, F. M. and Wovkulich, P. M. (1976) in *Topics in Carbon-13 NMR Spectroscopy* (Levy, G. C., ed.) Vol. 2. Wiley-Interscience, New York.
2. Hughes, D. W., Holland, H. L. and Maclean, D. B. (1976) *Can. J. Chem.* **54**, 2252.
3. Takao, N., Iwasa, K., Kannigauchi, M. and Sigiura, M. (1977) *Chem. Pharm. Bull.* **25**, 1426.
4. Ferreira Fonseca, S., de Paiva Campello, J., Barata, L. E. S. and Rúveda, E. A. (1977) *Phytochemistry* (in press).
5. Compound 3 has a papaverine-like activity which is under study. Meirelles, U. M. F., Reis, F. A. M., Simioni, L. R. and Magalhães, A. F., unpublished results.
6. Johns, S. R. and Willing, R. I. (1976) *Australian J. Chem.* **29**, 1617.
7. Marshall, J. L., Müller, D. E., Conn, S. A., Seiwel, R. and Ihrig, A. M. (1974) *Accounts Chem. Res.* **7**, 333.
8. Levy, G. C. and Nelson, G. L. (1972) *Carbon-13 Nuclear Magnetic Resonance for Organic Chemists*. Wiley-Interscience, New York.
9. Clark, P. D., Ewing, D. F. and Scrowston, R. M. (1976) *Org. Magn. Resonance* **8**, 252.
10. Wenkert, E., pers. comm.
11. Breitmaier, E. and Spohn, K.-H. (1973) *Tetrahedron* **29**, 1145.
12. Yoshikawa, K., Morishima, I., Kunitomo, J., Ju-ichi, M. and Yoshida, Y. (1975) *Chem. Lett.* 961.
13. A  $^3J(\text{C}_8-\text{H}_1) = 5.0$  Hz observed in a fully coupled spectrum of 13, indicative of a close to  $45^\circ$  dihedral relationship, gives further support to this explanation.
14. Castelhão, J. F., Jr., Gottlieb, O. R., de Lima, A. R., Mesquita, A. A. L., Gottlieb, H. E. and Wenkert, E. (1977) *Phytochemistry* **16**, 735.
15. The fine structure of  $\text{C}_7$ - $^3J(\text{C}_7-\text{H}_5) = 7.4$  Hz of 17, which becomes a multiplet in 18 and again a doublet [ $^3J(\text{C}_7-\text{H}_5) = 8.0$  Hz] in 19, confirms the previous assignment. Methylation of 17 produces a crowded system in which the *o*-methyl group of  $\text{C}_7$  resonates, as expected, at lower field.
16. Galeffi, C., Marini-Bettolo, G. B. and Vecchi, D. (1975) *Gazz. Chim. Ital.* **105**, 1207.
17. Franca, N. C., Giesbrecht, A. M., Gottlieb, O. R., Magalhães, A. F. and Magalhães, E. G. (1975) *Phytochemistry* **14**, 1671.
18. Pschorr, R. (1904) *Chem. Ber.* **37**, 1926.

<sup>13</sup>C NMR ANALYSIS OF APORPHINE ALKALOIDS

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**Key Word Index**—<sup>13</sup>C NMR spectra; aporphine alkaloids; glaucine methiodide; xantoplanine iodide; laurifoline chloride; dicentrine methiodide; isocorydine methochloride; corydine methochloride; *O,O'*-dimethylmagnoflorine iodide; magnoflorine iodide; ocoteine; boldine; ocoteine methiodide; boldine methiodide.

**Abstract**—The <sup>13</sup>C NMR spectra of some tertiary and quaternary aporphine alkaloids are recorded and the signals assigned. The substituent shielding effects together with the effects of *N*- and *O*-methylation, and the twisting of the biphenyl system, are analysed and utilized in the spectral interpretation.

## INTRODUCTION

The presence of a twisted biphenyl system and the steric effects produced by substituents in the skeleton of aporphines, make them very attractive for spectral analysis. Evidence obtained by UV, ORD, PMR and MS studies [1, 2] were useful for distinguishing between the 1,2,9,10 and 1,2,10,11 oxygenated patterns [3], but the nature (MeO— or HO—) of a particular substituent at a determined position remained difficult to assign. Consequently, and in view of the striking sensitivity of carbon shifts to steric effects, it was decided to carry out an analysis of some representative members of this large group of isoquinoline alkaloids [4], as continuation of our project on <sup>13</sup>C NMR spectral analysis of natural products [5].

## RESULTS AND DISCUSSION

The assignment of glaucine methiodide **2a** was based on the reported data for glaucine **1** [6] and on the effects of *N*-methylation on the neighbouring carbon shifts. The quaternization of the *N*-atom of **1** causes deshielding of C-5 and C-6a and shielding of C-1b, C-3a, C-7 and C-7a by magnitudes similar to those previously observed in the 1-benzyltetrahydroisoquinoline alkaloid series [5].

Comparison of **2a** with the monophenolic and diphenolic alkaloids xantoplanine iodide **2b** and laurifoline chloride **2c**, respectively, shows that the replacement of the OMe ( $\delta = 56$  ppm) on C-9 of **2a** by an OH group as in **2b**, produces the normal effects on the carbons *ortho* to the phenolic function, while the *para* position (C-11a) is essentially unaffected. In **2c**, however, the replacement of the sterically hindered OMe ( $\delta = 60.4$

ppm) on C-1 of **2a** by an OH group, induces stronger shielding effects on C-1a, C-2 and C-3a, *ortho* and *para* respectively to the oxygenated function, as previously described for other crowded systems [7]. The carbon signals of *O*-ethylxantoplanine iodide **2d** and *O,O'*-diethylaurifoline iodide **2e** are similar to the ones observed in **2a**, the CH<sub>2</sub> of the *O*-ethyl group on C-1 of **2e**, being sterically hindered, resonates at lower field than the CH<sub>2</sub> of the *O*-ethyl group on C-9 of **2d** and **2e**.

It is known that the replacement of two OMe groups of an aromatic system by a methylenedioxy moiety induces shielding of the oxycarbons and their neighbours by 1–2 and 3–4 ppm, respectively, and deshielding of the remaining ones by 1–2 ppm [6]. Comparison of the ring A carbon shifts of **2a** with those of dicentrine methiodide **2f**, shows stronger shielding effects on C-2 and C-1a, while C-1b and C-3a are also shielded. These apparently anomalous observations are consequences of the fact that the interactions of the methoxyl oxygens with the aryl carbons are more easily altered than the corresponding ones of the planar and rigid methylenedioxy group in highly substituted systems. These data establish that the reported effects of changing two OMe by a —OCH<sub>2</sub>O— moiety on the benzene carbon shifts, are valid only for systems free of severe steric interactions.† In the aporphine series, for example, comparison of the reported shifts of ring D of **1**, carrying two OMe groups without steric interactions, with similar sites of nantenine **3**, shows the normal effects [6]. The remaining carbon shifts of **2f**, are practically unaffected by comparison with **2a**. The shifts of compounds **2a–2f** are listed in Table 1.

The shifts of the highly strained 1,2,10,11-tetra-substituted quaternary aporphines, isocorydine methochloride **5a**, *O,O'*-dimethylmagnoflorine iodide **5b**, corydine methochloride **5c** and magnoflorine iodide **5d**, are listed in Table 2. The assignments are based on the  $\delta$  values recorded for isocorydine **4** [6], on the effects of *N*-quaternization and *O*-methylation of sterically hindered OH groups and on comparison of the signals of the 4 compounds with each other.

\* Present address: Pfizer S.A.C.I., Buenos Aires, Argentina.

† In agreement with these observations, the transformation of (–)-*O*-methylcapaurine into 1-methoxy-2,3-methylenedioxy-tetrahydroprotoberberine and 1,2-methylenedioxy-3-methoxytetrahydroprotoberberine shows shielding of all ring A carbon shifts (Kametani, T., Fukumoto, K., Ihara, M., Ujii, A. and Koizumi, H. (1975) *J. Org. Chem.* **40**, 3280).

Table 1. \*§ <sup>13</sup>C NMR data of aporphine alkaloids and their derivatives

	2a	2b	2c	2d†	2e†	2f
1	145.6	145.9	142.2	145.6	144.5	143.1
1a	127.7	127.6	118.2‡	127.7	128.0	116.7
1b	118.6	118.4	118.3‡	118.5	118.6	117.9
2	153.9	153.6	148.0	153.9	153.9	148.5
3	110.2	109.8	108.0	110.1	110.0	106.4
3a	124.1‡	124.4	119.8	123.9	124.0‡	121.8‡
4	24.3	24.0	23.5	24.2	24.2	24.0
5	61.4	61.5	61.5	61.3	61.4	61.8
6a	70.3	69.9	69.7	70.4	70.3	69.8
7	29.5	28.9	28.6	29.6	29.5	28.7
7a	124.0‡	123.9	123.7	123.9	123.8‡	123.3
8	111.4	114.5	114.3	112.3	112.3	111.6
9	148.8	145.9	145.4	148.4	148.1	148.8
10	148.2	146.5	146.2	148.2	148.1	148.2
11	111.4	111.4	112.1	111.6	111.8	110.2
11a	123.0	122.0	122.5	122.8	123.1	122.0‡
—N <sup>+</sup> Me <sub>2</sub>	44.0; 54.8	43.6; 54.3	42.6; 53.4	43.8; 54.7	43.5; 54.7	43.6; 54.3
—OMe (C-1)	60.5	60.1		60.4		
—OMe (C-2)	55.7	55.8	55.7	55.9	55.9	
—OMe (C-9)	56.1					55.9
—OMe (C-10)	56.1	55.8	55.7	55.9	55.9	55.9
—OCH <sub>2</sub> O-						101.3
—OEt (C-1)					15.7; 69.0	
—OEt (C-9)				14.8; 64.5	14.5; 64.5	

\* Spectra were obtained at 25.2 MHz in Fourier transform mode in CDCl<sub>3</sub> solutions with some MeOH for better dissolution of the compounds. The  $\delta$  values are in ppm downfield from TMS.

† In CDCl<sub>3</sub> solution.

‡ Signals may be reversed.

§ Although the fully coupled spectra showed complex fine structures preventing unambiguous assignments, some of the carbon shifts listed in this and in Tables 2 and 3, were confirmed by <sup>13</sup>C-<sup>1</sup>H long-range couplings.

Table 2.\* <sup>13</sup>C NMR data for aporphine alkaloids and their derivatives

	5a†	5b‡	5c‡	5d§
1	143.0	146.9	143.6	140.2
1a	126.0	124.5	119.2¶	118.9
1b	118.3	121.0	119.2¶	117.7
2	152.9	153.0	150.9	148.8
3	110.6	111.0	110.6	109.6
3a	125.2	122.9	119.6¶	120.3
4	23.8	23.4	23.9	23.4
5	60.3	60.8	61.0	61.5
6a	69.1	69.3	69.8	69.7
7	30.6	30.4	30.7	30.3
7a	124.3	125.2	124.7	123.8
8	119.6	122.6	125.3	120.8
9	111.5	112.3	111.8	110.9
10	149.7	152.4	152.5	147.6
11	143.5	147.1	143.6	140.2
11a	120.2	123.8	124.6	119.2
—N <sup>+</sup> Me <sub>2</sub>	42.9; 53.5	43.1; 53.9	44.0; 54.8	43.4; 54.2
—OMe (C-1)	62.1	60.7		
—OMe (C-2)	55.8	55.9	56.1	55.8
—OMe (C-10)		55.9	56.1	55.8
—OMe (C-11)	55.8	60.7	62.2	

\* Spectra were obtained at 25.2 MHz in Fourier transform mode. The  $\delta$  values are in ppm downfield from TMS.

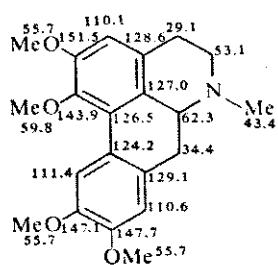
† In CDCl<sub>3</sub> solution with some MeOH.

‡ In CDCl<sub>3</sub> solution.

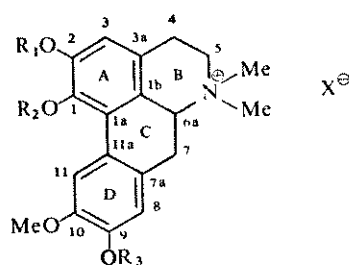
§ In CDCl<sub>3</sub> solution with some TFA.

|| Signals may be reversed.

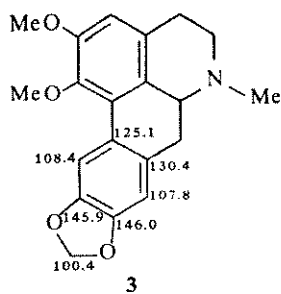
¶ Signals may be reversed.



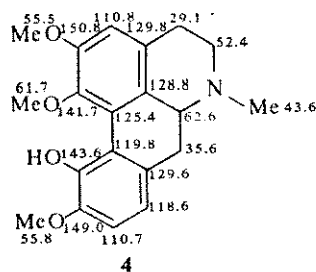
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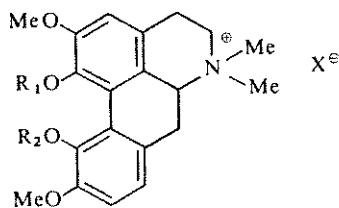
- 2a R<sub>1</sub> = R<sub>2</sub> = R<sub>3</sub> = Me; X = I
- 2b R<sub>1</sub> = R<sub>2</sub> = Me; R<sub>3</sub> = H; X = I
- 2c R<sub>1</sub> = Me; R<sub>2</sub> = R<sub>3</sub> = H; X = Cl
- 2d R<sub>1</sub> = R<sub>2</sub> = Me; R<sub>3</sub> = Et; X = I
- 2e R<sub>1</sub> = Me; R<sub>2</sub> = R<sub>3</sub> = Et; X = I
- 2f R<sub>1</sub> = R<sub>2</sub> = -CH<sub>2</sub>-; R<sub>3</sub> = Me; X = I



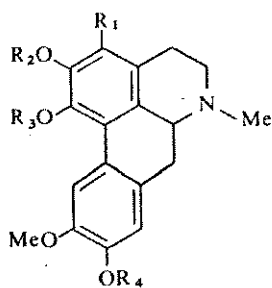
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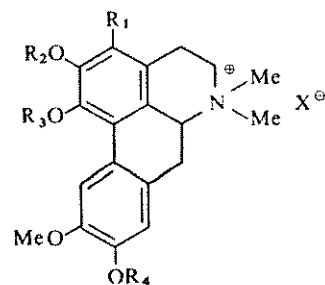
4



- 5a R<sub>1</sub> = Me; R<sub>2</sub> = H; X = Cl
- 5b R<sub>1</sub> = R<sub>2</sub> = Me; X = I
- 5c R<sub>1</sub> = H; R<sub>2</sub> = Me; X = Cl
- 5d R<sub>1</sub> = R<sub>2</sub> = H; X = I



- 6a R<sub>1</sub> = R<sub>2</sub> = R<sub>4</sub> = H; R<sub>3</sub> = Me
- 6b R<sub>1</sub> = OMe; R<sub>2</sub> = R<sub>3</sub> = -CH<sub>2</sub>-; R<sub>4</sub> = Me



- 7a R<sub>1</sub> = R<sub>2</sub> = R<sub>4</sub> = H; R<sub>3</sub> = Me; X = I
- 7b R<sub>1</sub> = OMe; R<sub>2</sub> = R<sub>3</sub> = -CH<sub>2</sub>-; R<sub>4</sub> = Me; X = I

- P. M., Swan, R. J. and Vernengo, M. J. (1966) *J. Chem. Soc. (C)* 1340.
3. For a recent review see: Guinaudeau, H., Leboeuf, M. and Cave, A. (1975) *Lloydia* 38, 275.
  4. Kuck, A. M., Albónico, S. M., Deulofeu, V. and Escalante, M. G. (1967) *Phytochemistry* 6, 1541; Baralle, F., Busch, A., Vernengo, M. J. and Kuck, A. M. (1972) *Lloydia* 35, 300.
  5. Marsaioli, A. J., Rúveda, E. A. and Reis, F. A. M. (1978) *Phytochemistry* 17, 1655.
  6. Wenkert, E., Buckwalter, B. L., Burfitt, I. R., Gasić, M. J., Gottlieb, H. E., Hagaman, E. W., Schell, F. M. and Wovkulich, P. M. (1976) *Topics in Carbon-13 NMR Spectroscopy* (Levy, G. C., ed.) Vol. 2. Wiley-Interscience, New York.
  7. Wenkert, E. and Gottlieb, H. E. (1977) *Phytochemistry* 16, 1811.
  8. Dhami, K. S. and Stothers, J. B. (1966) *Can. J. Chem.* 44, 2855.

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6a and shows the glaucine 1 methylene- of a third produces A carbon a could be uced by a and by the and *ortho* . On C-4 p on C-3, while the ected. The esponding e expected e shifts of

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<sup>13</sup>C NMR ANALYSIS OF SOME OXOAPORPHINE ALKALOIDS

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**Key Word Index**—<sup>13</sup>C NMR spectra; oxoaporphine alkaloids; oxo-*O*-methylpukateine; *O*-methylmoscatoline; oxoglauanine; papaveraldine; (6,7-dimethoxyisoquinolinyl)-(4'-methoxyphenyl)-methanone; (6,7-methylenedioxyisoquinolinyl)-(4-methoxyphenyl)-methanone.

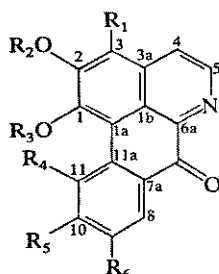
## INTRODUCTION

Recently we described the isolation and structure elucidation of oxo-*O*-methylpukateine (**1a**)\* and of the known alkaloid *O*-methylmoscatoline (**1b**) from *Dugetia eximia* [1]. In order to confirm the proposed structure for **1a**, its synthesis was carried out [2-4]. Following the sequence reported by Cohen *et al.* [4] and starting from 6'-nitropapaveraldine, oxoglauanine (**1c**) was also prepared. The absence of <sup>13</sup>C NMR spectral data of oxoaporphines prompted us to undertake the analysis of **1a**, **1b** and **1c** as an aid in the structure elucidation of new compounds and also as a continuation of our project on <sup>13</sup>C NMR spectroscopy of isoquinoline alkaloids [5, 6].

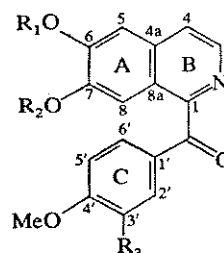
## RESULTS AND DISCUSSION

In order to facilitate the shift assignment of the oxoaporphine alkaloids **1a-1c**, the analysis of papaveraldine (**2a**), (6,7-dimethoxyisoquinolinyl)-(4'-methoxyphenyl)-methanone (**2b**), (6,7-methylenedioxyisoquinolinyl)-(4'-methoxyphenyl)-methanone (**2c**), was first carried out.

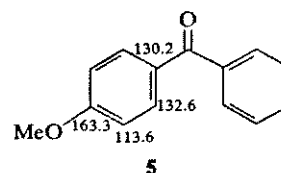
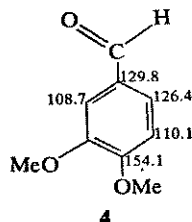
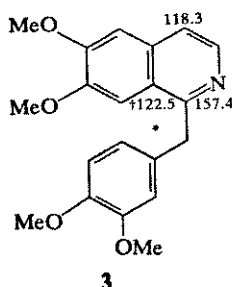
The dimethoxyisoquinoline system of **2a** shows carbon shifts and multiplicities, in the fully coupled spectrum, comparable to those observed in papaverine (3) [5], except for the C-1, C-4 and C-8a signals. Carbon-1 and C-4 are shielded and deshielded respectively in **2a**, due to the effect of the CO group, and C-8a appears as a clean triplet [<sup>3</sup>J(C-8a, H-4); <sup>3</sup>J(C-8a,



**1a** R<sub>1</sub> = R<sub>5</sub> = R<sub>6</sub> = H; R<sub>2</sub> = R<sub>3</sub> = —CH<sub>2</sub>—; R<sub>4</sub> = OMe  
**1b** R<sub>1</sub> = OMe; R<sub>2</sub> = R<sub>3</sub> = Me; R<sub>4</sub> = R<sub>5</sub> = R<sub>6</sub> = H  
**1c** R<sub>1</sub> = R<sub>4</sub> = H; R<sub>2</sub> = R<sub>3</sub> = Me; R<sub>5</sub> = R<sub>6</sub> = OMe



**2a** R<sub>1</sub> = R<sub>2</sub> = Me; R<sub>3</sub> = OMe  
**2b** R<sub>1</sub> = R<sub>2</sub> = Me; R<sub>3</sub> = H  
**2c** R<sub>1</sub> = R<sub>2</sub> = —CH<sub>2</sub>—; R<sub>3</sub> = H



\*After submission of our manuscript [1] we became aware of a publication by Hsu, C.C., Dobberstein, R. H., Cordell, G. A. and Farnsworth, N. R. (1977) *Lloydia* **40**, 152, in which the isolation of **1a** was reported.

† Complex multiplet.

H-5)], since further long range splittings produced by the methylene group were eliminated. A comparison of the carbon signals of ring C of **2a** with the corresponding ones of veratraldehyde (**4**) [7] shows good agreement allowing a preliminary shift assignment.

The analysis of the fine structure of these methines from the  $^1\text{H}$ -coupled  $^{13}\text{C}$  spectrum indicates, however, that the signals at 111.7 and 109.7 ppm should be assigned to C-2' and C-5' respectively, although the shifts of related carbons of **4** show the opposite trend. The assignment of rings A and B carbon shifts of **2b** is based on comparisons with the isoquinoline system of **2a**, and for ring C, the methoxybenzophenone **5** was used as model [8]. Finally, the replacement of the 6,7-dimethoxyisoquinoline in **2a** by a methylenedioxyisoquinoline in **2c** produces the expected changes on the benzenoid carbons [9], while the remaining carbon shifts are essentially unaffected. The non-aromatic carbons of **2a**, **2b** and **2c** were readily assigned by standard chemical shift theory. The shifts of compounds **2a-2c** are listed in Table 1.

The first oxoaporphine alkaloid analysed was oxoglaucine (**1c**) and its  $sp^2$  carbon signals were split into two groups on the basis of their signal multiplicities from a SFORD spectrum. Of the proton-bearing carbon shifts, the signals at 105.7, 122.9 and 144.3 ppm were assigned to C-3, C-4 and C-5, respectively, by analysis of their fine structure in the fully coupled spectrum, leaving those at *ca* 109 ppm to C-8 and C-11 or vice versa. Unambiguous assignment, however, was made by selective decoupling at 8 ppm which is the resonance frequency of H-8 [10]. The non-protonated carbon signals at 119.1, 121.1, 134.8 and 144.9 ppm were assigned, again on the basis of their fine structure from a fully coupled spectrum, to C-1a, C-1b, C-3a and C-6a, respectively, while for the assignments of the remaining signals at 126.3 and 128.7 ppm and those of the non-protonated, oxyge-

nated aromatic carbons, a comparison between the  $\Delta\delta$  values observed in the transformation of laudanosine (**6**) into glaucine (**7**) [9] with the ones observed going from papaveraldine (**2a**) into oxoglaucine (**1c**) was used. The OMe groups on C-2, C-9 and C-10 show signals at 55.8 ppm and the one on C-1, as expected, resonates at lower field, 60.2 ppm. The signal at 180.7 ppm was assigned to the CO group.

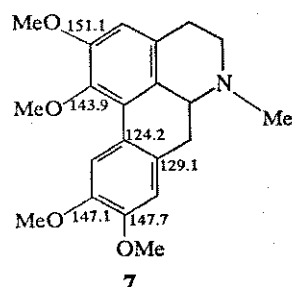
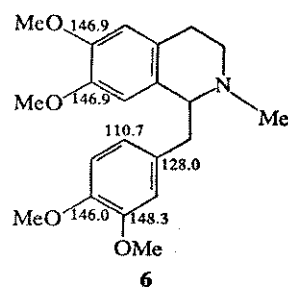


Table 1.  $^{13}\text{C}$  NMR data of compounds **2a-2c**

Carbon	<b>2a</b>	<b>2b</b>	<b>2c</b>
1	153.5	153.5	154.7
3	139.7	139.9	140.2
4	120.9	121.1	121.7
4a	133.6	133.8	133.3
5	104.6	104.7	102.6
6	152.9	153.0	151.0
7	150.7	150.9	149.1
8	103.8	104.0	102.1
8a	122.5	122.6	123.7
1'	129.6	129.7	129.7
2'	111.7	133.1	133.0
3'	148.7	113.6	113.6
4'	153.5	163.0	163.8
5'	109.7	113.6	113.6
6'	126.6	133.1	133.0
-OMe	55.9	56.0	55.0
	55.9	56.0	
	55.9	55.6	
	55.9		
C=O	186.4	187.0	*
-OCH <sub>2</sub> O-			101.7

The spectra were obtained at 25.2 MHz in Fourier transform mode in  $\text{CDCl}_3$  solutions. The data for each carbon are shown in ppm downfield from TMS.

\*Due to the lower sample concentration the CO signal was not detected.

The introduction of a third OMe group on C-3 produces expected changes on the isoquinoline carbons of *O*-methylmoscatoline (**1b**) compared with those of **1c**. Carbon-3 and its corresponding *para* and *ortho* positions, C-1a, C-2 and C-3a, are deshielded and shielded, respectively, and C-4, which suffers a  $\gamma$ -effect by the new oxygenated function, is also shielded. The assignment of C-7a, C-11a and C-10 is based on the effects produced on some of the aromatic carbons by the replacement of a benzylic methylene by a CO group (**8**→**9**) [9, 11] using nuciferine ring D shifts, **10** [9] as a reference compound, leaving C-8, C-9 and C-11 unresolved. The shifts of the OMe groups of **1b** at *ca* 61 ppm indicate that they are sterically hindered, but specific assignments become difficult.

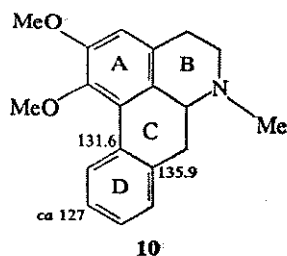


Table 2.  $^{13}\text{C}$  NMR data of oxoaporphines **1a**–**1c**

Carbon	<b>1a</b>	<b>1b</b>	<b>1c</b>	<b>1c*</b>
1	147.3	148.2	148.9	155.9
1a	104.4	115.4	119.1	120.1
1b	121.6	122.5	121.1	123.4
2	151.9	147.0	156.1	162.9
3	102.1	156.2	105.7	106.5
3a	135.2	130.8	134.8	135.3
4	123.3	118.9	122.9	126.2
5	144.0	144.3	144.3	133.1
6a	144.3	145.0	144.9	141.7
7	182.0	182.3	180.7	174.8
7a	133.2	131.4	126.3	133.1
8	120.2	127.9	109.2	110.1
9	129.1	128.7	150.2	150.2
10	116.1	134.1	153.2	156.7
11	156.0	127.4	109.7	111.3
11a	123.1	134.3	128.7	130.4
—OMe	55.7	61.7	60.2†	61.6†
		61.3	55.8	57.3
		60.9	55.8	56.3
			55.8	56.3
—OCH <sub>2</sub> O—	101.5			

The spectra were obtained at 25.2 MHz in the Fourier transform mode in  $\text{CDCl}_3$  to which some drops of MeOH were added for better solution of the compounds. The  $\delta$  values are in ppm downfield from TMS.

\*In  $\text{CDCl}_3$  solution with some TFA.

†Signal assigned to the OMe carried by C-1.

The signal assignments of oxo-*O*-methylpukateine (**1a**) were greatly simplified by the analysis of **1b** and **1c**. As expected, the effects of the replacement of two OMe groups by a methylenedioxy moiety in sterically hindered systems (**1c**→**1a**) are observed on the benzenoid carbons [6], while the remaining carbons are little affected. In agreement with these observations, C-3, C-4, C-5 and C-6a show the expected multiplicities in a fully coupled spectrum. The introduction of a OMe group on C-11 produces predictable changes on ring D carbon shifts, allowing its complete assignment.

In view of the low solubility generally observed in some oxoaporphines and of the comparative study of  $^1\text{H}$  NMR in  $\text{CDCl}_3$  and TFA solutions [10], the  $^{13}\text{C}$  NMR analysis of oxoglucine (**1c**) in  $\text{CDCl}_3$  solution with some TFA was also undertaken. As expected, rings A and B carbons suffer changes similar to those observed on isoquinoline by protonation [12] and further, the minor changes observed on ring D carbon shifts and on the CO group could be explained assuming a keto-enol tautomerism, as was previously suggested by UV spectroscopy [13]. The shifts of **1a**, **1b** and **1c** in  $\text{CDCl}_3$  solutions and those of **1c** in  $\text{CDCl}_3$  solution with some TFA are listed in Table 2.

**Acknowledgements**—We thank FINEP (Financiadora de Estudos e Projetos) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) (Grant No. 2222 1697/77) for financial support.

## REFERENCES

- Gottlieb, O. R., Magalhães, A. F., Magalhães, E. G., Maia, J. G. S. and Marsaioli, A. J. (1978) *Phytochemistry* **17**, 837.
- Zymalkowski, F. and Happel, K. H. (1969) *Chem. Ber.* **102**, 2959.
- Taylor, W. I. (1961) *Tetrahedron*, **14**, 42.
- Cohen, J., Von Langenthal, W. and Taylor, W. I. (1961) *J. Org. Chem.* **26**, 4143.
- Marsaioli, A. J., Rúveda, E. A. and Reis, F. de A. M. (1978) *Phytochemistry* **17**, 1655.
- Marsaioli, A. J., Reis, F. de A. M., Magalhães, A. F., Rúveda, E. A. and Kuck, A. M. (1979) *Phytochemistry* **18**, 165.
- Erasmuson, A., Ferrier, R. J., Franca, N. C., Gottlieb, H. E. and Wenkert, E. (1977) *J. Chem. Soc. Perkin Trans.* **1**, 492.
- Shapiro, M. J. (1977) *Tetrahedron* **33**, 1091.
- Wenkert, E., Buckwalter, B. L., Burfitt, I. R., Gasić, M. J., Gottlieb, H. E., Hagaman, E. W., Schell, F. M. and Wovkulich, P. M. (1976) *Topics in Carbon-13 NMR Spectroscopy* (Levy, G. C., ed.) Vol. 2. Wiley-Interscience, New York.
- Urzúa, A. and Cassels, B. K. (1976) *Heterocycles* **4**, 1881.
- Castelão J. F., Jr., Gottlieb, O. R., de Lima, R. A., Mesquita, A. A. L., Gottlieb, H. E. and Wenkert, E. (1977) *Phytochemistry* **16**, 735.
- Britmaier, E. and Spohn, K. H. (1973) *Tetrahedron* **29**, 1145.
- Senter, P. D. and Chen, C.-L. (1977) *Phytochemistry* **16**, 2015.



Proton and Carbon-13 Nuclear Magnetic  
 Resonance Spectroscopy and Conformational  
 Aspects of the Curine Class of  
 Bis(benzylisoquinoline) Alkaloids

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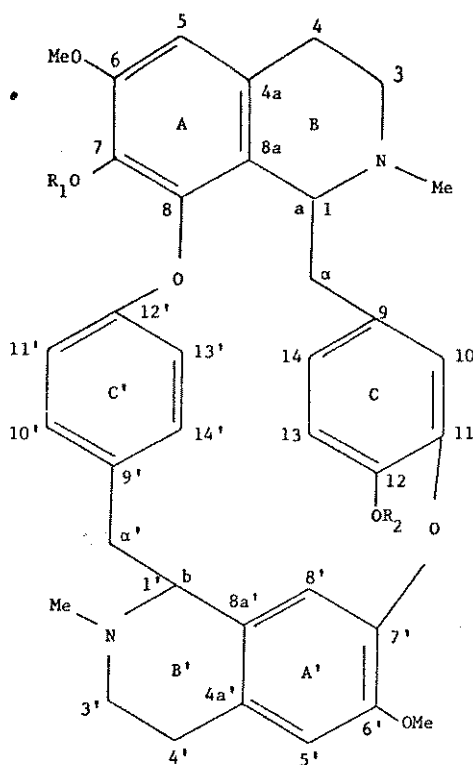
Due to the neuromuscular blocking activity shown by some members of the curine class of bis(benzylisoquinoline) alkaloids<sup>1</sup> much effort has been devoted to the elucidation of their structures, and more recently, by the application of modern spectroscopic techniques, data on their solution conformations have also been reported.<sup>2</sup>

The increasing use of <sup>13</sup>C NMR spectroscopy for structure elucidation of natural products and the lack of data on the bis(benzylisoquinolines) prompted us to analyze different classes of these alkaloids<sup>3,4</sup> in the hope of contributing to the determination of related but up to now unresolved structures<sup>5</sup> as well as to the detection, in combination with <sup>1</sup>H NMR, of conformational features of these interesting substances.

The <sup>13</sup>C NMR analyses were initiated with bebeerine (1) and its derivatives 2-7.

Table I lists their carbon shifts which were assigned by standard chemical shift theory and analysis of the SFORD and fully coupled spectra. Due to the overlapping of signals, the application of inversion recovery conditions was necessary to detect the sp<sup>2</sup> carbons nonbonded to hydrogen. The assignment was further supported by the known effects of alkylation and acetylation of phenols, analysis of model compounds, and selective irradiations.

The carbons of rings ABC and A'B'C' were assigned, taking isochondrodendrine (9) and 1-(p-methoxybenzyl)-6,7-dimethoxytetrahydroisoquinoline (10)<sup>3</sup> as models, respectively. The assignments of rings A and C' carbons of 1 were in accordance with rings A and C of 9, respectively,



1. R<sub>1</sub> = R<sub>2</sub> = H (a, b = R, R)
2. R<sub>1</sub> = R<sub>2</sub> = Me (a, b = R, R)
3. R<sub>1</sub> = Me, R<sub>2</sub> = H (a, b = R, R)
4. R<sub>1</sub> = H, R<sub>2</sub> = Me (a, b = R, R)
5. R<sub>1</sub> = Me, R<sub>2</sub> = Ac (a, b = R, R)
6. R<sub>1</sub> = Ac, R<sub>2</sub> = Me (a, b = R, R)
7. R<sub>1</sub> = Ac, R<sub>2</sub> = Ac (a, b = R, R)
8. R<sub>1</sub> = R<sub>2</sub> = H (a, b = R, S)

showing the same C-H nonequivalence in ring C as well. Confirmation of ring A chemical shifts was carried out by methylation, going from 1 to 2, 3, and/or 5, and acetylation, going from 1 to 6 and/or 7, of the phenols.<sup>4-6</sup> The

(1) Shamma, M. "The Isoquinoline Alkaloids"; Academic Press: New York, 1972; p 142.

(2) Egan, R. S.; Stanaszek, R. S.; Williamson, D. E. *J. Chem. Soc. Perkin Trans. 2* 1973, 716. Bick, I. R. C.; Harley-Mason, J.; Sheppard, N.; Vernengo, M. *J. Chem. Soc. C* 1961, 1896.

(3) Marsaioli, A. J.; Ruveda, E. A.; Reis F. de A. M. *Phytochemistry* 1978, 17, 1665.

(4) Koike, L.; Marsaioli, A. J.; Ruveda, E. A.; Reis, F. de A. M.; Bick, I. R. C. *Tetrahedron Lett.* 1979, 3765.

(5) Guha, K. P.; Mukherjee, B.; Mukherjee, R. *J. Nat. Prod.* 1979, 42, 1.



Table I.  $^{13}\text{C}$  NMR Data for Bebeerine (1) and Related Alkaloids<sup>a</sup>

carbons	chemical shift, ppm							
	1 <sup>b</sup>	2	3	4	5	6	7	8
1	59.8	60.3	60.2	60.2	60.4	60.6	60.2	59.3
3	43.6	43.1	43.5	43.4	43.1	43.2	42.9	44.2
4	21.6	21.6	21.9	21.4	21.6	22.1	21.5	23.2
4a	123.9 <sup>d</sup>	129.4	129.2	124.3	129.5	132.0	132.0	124.6
5	107.7	108.7	108.8	107.7	108.8	108.7	108.4	107.7
6	146.8	151.5	151.7	146.5	151.7	150.5	150.2	146.7
7	137.3	140.1	140.4	137.0	140.3	131.2	130.9	137.2
8	138.5	145.9	144.8	138.5	145.0	144.4	144.0	137.9
8a	124.0 <sup>d</sup>	124.2	124.2	124.3	124.1	124.3	123.7	125.4
$\alpha$	39.5	38.9	39.4	39.5	39.2	39.3	38.7	40.1
9	133.2	133.9	133.4	134.1	140.5	134.0	140.1	132.6
10	120.2	122.1	120.7	121.4	121.8	122.5	121.5	121.0
11	142.8 <sup>c</sup>	143.9	143.1	143.6	146.5	144.2	146.4	143.1
12	145.9	148.9	146.1	148.8	139.9	149.2	139.9	145.9
13	115.2	111.7	115.2	112.8	122.4	112.0	122.2	115.4
14	125.8	125.0	126.3	124.6	125.2	125.4	124.9	125.7
1'	64.7	64.9	65.2	64.7	64.6	65.1	64.8	64.4
3'	44.6	45.6	45.4	45.0	45.0	45.7	45.4	46.5
4'	24.1	25.2	24.9	24.4	24.6	25.2	25.2	25.2
4a'	128.4	126.5	128.4	126.5	127.1	126.5	127.7	128.6
5'	112.0	112.1	112.0	111.9	112.2	112.4	112.0	111.9
6'	148.2	147.8	148.4	148.3	148.3	148.1	147.9	148.0
7'	143.5 <sup>c</sup>	143.2	143.5	143.3	143.2	143.3	142.8	143.1
8'	119.5	116.2	119.3	117.2	117.7	116.3	117.4	117.9
8a'	128.4	127.9	128.3	127.5	127.2	127.2	128.0	128.1
$\alpha'$	39.5	39.4	39.7	39.5	40.1	39.6	39.9	39.0
9'	131.5	131.5	131.3	131.5	131.2	132.7	132.3	131.7
10'	131.3	131.9	132.0	131.5	131.9	132.0	131.7	132.2
11'	114.7	114.7	115.2	114.3	114.8	115.0	114.6	113.2
12'	155.2	155.2	155.6	155.0	155.4	154.7	154.4	155.4
13'	113.1	112.9	113.0	113.5	113.2	113.6	113.6	114.9
14'	129.2	129.0	129.6	129.3	129.3	129.3	129.0	129.9
NMe	41.3, 41.3	41.3, 42.1	41.5, 41.8	41.4, 41.8	41.4, 41.4	41.5, 42.1	41.1, 41.9	42.2, 42.5
OMe	55.7, 55.7	55.7, 55.7, 60.8 (C-7)	55.8, 55.8, 61.0 (C-7)	56.0, 56.0, 56.0	55.7, 55.7, 60.9 (C-7)	55.9, 55.9, 55.9	55.5, 55.5	55.8, 56.0
C=O					168.3	168.2	167.7, 168.1	
O    CCH <sub>3</sub>					20.3	20.1	19.6, 20.0	

<sup>a</sup> The spectra were obtained in  $\text{CDCl}_3$  solutions. Chemical shifts are expressed on the  $\text{Me}_4\text{Si}$  scale according to the following equation  $\delta^{\text{Me}_4\text{Si}} = \delta^{\text{CDCl}_3} + 76.9$  ppm. <sup>b</sup> Some drops of MeOH were added for better solution. <sup>c,d</sup> Signals within vertical column may be reversed.

Table II.  $^1\text{H}$  NMR Data for Bebeerine (1) and Relative Alkaloids<sup>a,b</sup>

compd	chemical shift, ppm							
	OMe	NMe	H-5	H-5'	H-8	H-10'	H-13'	H-14'
1	3.97 (s)	2.55 (s), 2.35 (s)	6.75 (s)	6.61	6.04	6.66 (d, $J = 2.0$ )	6.87 (d, $J = 8.3$ )	7.03 (dd, $J = 2.0, 8.3$ )
2	3.93, 3.91, 3.76, 3.74	2.56, 2.34	6.77	6.64	5.59	6.62 (d, $J = 2.0$ )	6.87 (d, $J = 8.3$ )	7.23 (dd, $J = 2.0, 8.3$ )
3	3.95, 3.92, 3.80	2.60, 2.32	6.77	6.64	6.00	6.71 (d, $J = 2.0$ )	6.88 (d, $J = 8.3$ )	7.03 (dd, $J = 2.0, 8.3$ )
4	3.92, 3.90 3.74	2.51, 2.34	6.74	6.60	5.60	6.54 (d, $J = 2.0$ )	6.86 (d, $J = 8.3$ )	7.23 (dd, $J = 2.0, 8.3$ )
5	3.85, 3.82 3.70	2.52, 2.30	6.53	6.63	5.60	6.58 (d, $J = 2.0$ )	6.91 (d, $J = 8.5$ )	7.18 (dd, $J = 2.0, 8.5$ )
6	3.90, 3.85, 3.70	2.55, 2.30	6.70	6.57	5.50	6.60 (d, $J = 2.0$ )	6.78 (d, $J = 8.5$ )	7.15 (dd, $J = 2.0, 8.5$ )
7	3.83	2.30, 2.51	6.58	6.61	5.58	overlap	6.91 (d, $J = 8.3$ )	7.13 (dd, $J = 2.0, 8.3$ )

<sup>a</sup> The spectra were obtained in  $\text{CDCl}_3$  solutions. The  $J$  values are given in hertz. <sup>b</sup> OAc group: 5, 2.12 ppm; 6, 2.07 ppm; 7, 2.06 and 2.11 ppm.

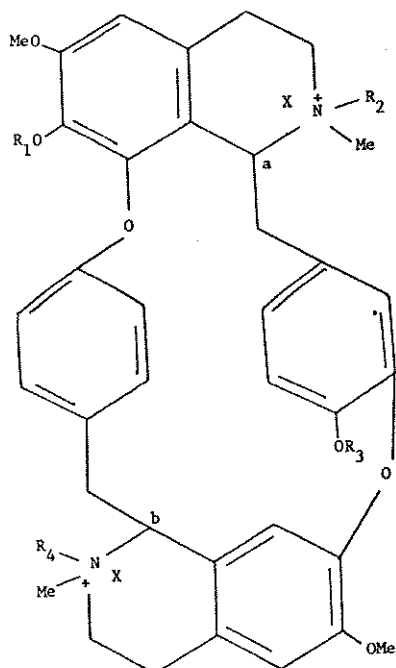
at the  $\gamma$ -position is completely free of its steric effect, showing an even more deshielded signal than C-4' of 1, supporting the above arguments.

At this point, the analysis of the diastereoisomeric alkaloid chondrocurine (8), greatly facilitated by the infor-

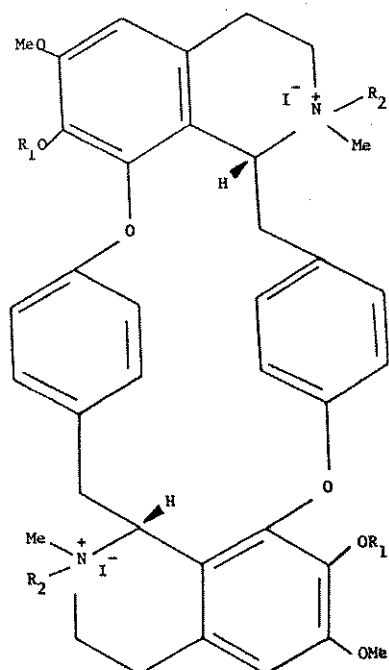
mation obtained with 1, was carried out. Comparison of both sites of chemical shifts reveals that all carbons have very similar values. For a conformational analysis we focused our attention on C-3' and C-8' which are slightly deshielded and shielded, respectively, in 8.

Accepting that in both diastereoisomers ring B adopts the same predominant half-chair conformation, one can see that C- $\alpha'$  would be pseudoaxial in bebeerine (1) and pseudoequatorial in chondrocurine (8). The latter situations releases, at least partially, the C- $\alpha'$ /C-3' interaction and increases at the same time that of C- $\alpha'$ /C-8'.

Quaternary bis(benzylisoquinoline) alkaloids are widely distributed in nature, and the onium function is supposed to play an important role in their pharmacological activities. In connection with problems of structure determination of compounds like (+)-tubocurarine chloride (13),



13.  $R_1 = R_3 = R_4 = H$ ,  $R_2 = Me$ ;  $X = Cl^-$  (a, b = R, S)  
 14.  $R_1 = R_2 = R_3 = R_4 = Me$ ;  $X = I^-$  (a, b = R, S)  
 15.  $R_1 = R_3 = H$ ,  $R_2 = R_4 = Me$ ;  $X = I^-$  (a, b = R, R)  
 16.  $R_1 = R_2 = R_3 = R_4 = Me$ ;  $X = I^-$  (a, b = R, R)  
 17.  $R_1 = R_2 = R_3 = R_4 = H$ ;  $X = Cl^-$  (a, b = R, R)



18.  $R_1 = H$ ,  $R_2 = Me$

a monoquaternary salt that was long thought a diquaternary salt,<sup>8,9</sup> two diastereoisomeric series of bis(benzylisoquinoline) *N*-metho salts were analyzed, and the <sup>13</sup>C NMR data are listed in Table III.

The transformation of the tertiary alkaloids into their corresponding *N*-metho salts affects all N vicinal carbons, and the observed chemical shifts can be interpreted as a net balance between electronic and steric effects. On the basis of the above arguments, it could be accepted that the second *N*-Me group (going from 1 to 15) will be pseudoaxial in ring B' of 15, while the observed shielding at C-4' is the result of a  $\gamma$  interaction between them (shielding) and the deshielding due to the quaternarization of the nitrogen. On the other hand, the absence of a net shielding effect ( $\Delta\delta_{1-15} = 2.00$  ppm) at C-4 suggests that the second *N*-Me group is introduced at the pseudoequatorial position.

In an attempt to differentiate both diastereoisomeric series of the curine class of bis(benzylisoquinoline) alkaloids a comparison of the *O,O'*-dimethylbebeerine and *O,O'*-dimethylchondrocurine *N*-metho salts (16 and 14) was carried out, and here again the differences in chemical shifts between both compounds are not significant.

Finally, the analysis of (+)-tubocurarine chloride (13), focused on the N-neighboring sp<sup>3</sup> carbons, was also carried out. Of the signals attributable to *N*-Me groups (C-1 and C-1', C-3 and C-3'), those at 51.3 and 54.5 ppm together with the ones at 68.7 and 54.5 ppm were assigned to the *N*-Me groups and to C-1 and C-3, respectively, of ring B, on the basis of the known effects produced by the transformation of a tertiary base into the corresponding quaternary salt<sup>3</sup> (1  $\rightarrow$  15, 2  $\rightarrow$  16, 9  $\rightarrow$  18). The remaining signals at 40.5, 65.1, and 45.9 ppm that were assigned to the *N*-Me, C-1', and C-3', respectively, of ring B' show that the transformation of a tertiary base into its hydrochloride produces practically no changes on the neighboring carbons. These observations were supported by a comparative analysis of bebeerine (1) and its corresponding hydrochloride 17, confirming that the N at ring B' is in fact a hydrochloride as was previously shown.<sup>8,9</sup>

### Experimental Section

The <sup>13</sup>C NMR spectra were obtained in a 10-mm spinning tube from solutions of approximately 0.5 mmol of compound in 1 mL of solvent. The instrument employed was a Varian XL-100 NMR spectrometer operating at 25.2 MHz and interfaced with a Varian 620-L Fourier transform computer with a 16K memory. The chemical shifts ( $\pm 0.05$  ppm) were measured at a 5-kHz spectral width, with an acquisition time of 0.8 s and a 15- $\mu$ s pulse width, by using an internal deuterium lock.

Inversion recovery spectra were measured by using the usual  $(\pi - \tau - \pi/2 - T)_n$  formula with  $\tau = 0.3$  and  $T = 20$  s. Off-resonance decoupled spectra were collected by setting the decoupler frequency a few parts per million outside the normal absorption range at a high power level. For specific H-8 decoupling the decoupler was set at the H-8 resonance frequency.

Proton magnetic resonance spectra were recorded on a Varian XL 100 spectrometer in the frequency sweep mode.

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The following compounds were prepared by standard procedures and gave satisfactory spectral and physical data: (*R,R*)-

(8) Everett, A. J.; Lowe, L. A.; Wilkinson, S. *J. Chem. Soc., Chem. Commun.* 1970, 1020.

(9) Codding, P. W.; James, M. N. G. *J. Chem. Soc., Chem. Commun.* 1972, 1174.

Table III.  $^{13}\text{C}$  NMR Data for Bebeerine *N*-Metho Salt 15 and Related Alkaloids<sup>a</sup>

carbons	chemical shift, ppm					
	13	14	15	16	17	18
1	68.7	68.5	65.9	66.5	61.9	69.2
3	54.5	54.7	55.0	55.3	45.0	54.3
4	23.6	23.6	23.6	24.3	21.1	23.8
4a	120.1	125.4	120.4	125.6	120.0	121.2
5	108.7	109.7	108.8	110.3	107.9	109.8
6	149.6	154.3	149.0	154.4	148.9	149.8
7	138.8	140.4	137.5	140.4	137.7	137.5
8	137.4	143.8	138.2	144.9	138.2	138.6
8a	119.8	119.9	119.9	121.2	120.0	119.2
$\alpha$	38.6	39.7	36.8	37.4	40.2	37.7
9	129.0	130.8	129.1	130.2	128.0	127.4
10	124.0	123.6	123.3	124.0	120.0	129.8
11	142.4	142.5	142.0	143.0	142.1	118.2
12	148.8	149.2	147.7	149.4	147.7	154.6
13	116.7	114.5	117.1	114.1	116.5	114.9
14	127.4	127.8	123.9	124.6	126.0	129.4
1'	65.1	72.7	72.5	73.4	64.7	69.2
3'	45.9	54.2	55.0	55.5	44.4	54.3
4'	22.6	23.6	23.6	24.3	21.1	23.8
4a'	124.4	123.2	123.1	123.6	123.5	121.2
5'	112.3	112.9	113.0	113.7	112.3	109.8
6'	150.3	150.9	149.9	151.4	149.2	149.8
7'	146.4	146.0	145.2	145.4	144.2	137.5
8'	118.4	116.7	117.1	117.9	116.8	138.6
8a'	121.0	121.3	122.7	123.1	121.9	119.2
$\alpha'$	40.0	37.0	38.0	38.8	39.7	37.7
9'	129.9	129.5	128.6	129.7	127.3	127.4
10'	134.0	134.1	131.6	132.3	131.9	129.8
11'	115.3	114.5	115.5	115.5	114.3	118.2
12'	156.4	156.5	155.7	156.6	155.1	154.6
13'	113.1	112.9	113.0	113.7	113.7	114.9
14'	130.8	131.2	129.9	130.9	130.1	129.4
*NMe	40.5 (N'), 51.3 (N), 54.5 (N)	51.0, 51.2, 52.9, 54.7	51.1, 51.1, 52.4, 52.9	51.4, 51.6, 52.8, 53.5	40.4, 40.4	51.8, 51.8, 53.0, 53.0
OMe	56.4, 56.4	56.1, 56.5, 56.5, 60.7	56.4, 56.7	56.7, 56.9, 57.3, 61.4	55.9, 55.9	57.0, 57.0

<sup>a</sup> The spectra were obtained in (a)  $\text{D}_2\text{O}$ -MeOH for 13, 14, and 16, (b)  $\text{D}_2\text{O}$ - $\text{Me}_2\text{SO}$  for 18 and 19, and (c)  $\text{CDCl}_3$ -MeOH for 15 and 17. The chemical shifts are expressed on the  $\text{Me}_4\text{Si}$  scale according to the following equations:  $\delta_{\text{Me}_4\text{Si}} = \delta_{\text{MeOH}} + 49.3 \text{ ppm}$ ,  $\delta_{\text{Me}_4\text{Si}} = \delta_{\text{Me}_2\text{SO}} + 40.4 \text{ ppm}$ , and  $\delta_{\text{Me}_4\text{Si}} = \delta_{\text{CDCl}_3} + 76.9 \text{ ppm}$  for systems a-c, respectively.

*O,O*-dimethylbebeerine (2),<sup>10</sup> (*R,R*)-12-*O*-methylbebeerine (4),<sup>10</sup> (*R,R*)-7-*O*-acetyl-12-*O*-methylbebeerine (6),<sup>10</sup> (*R,S*)-*O,O*-dimethylchondrocurarine iodide (14),<sup>11</sup> (*R,R*)-*N,N'*-dimethylbebeerine iodide (15),<sup>12</sup> (*R,R*)-*N,N',O,O*-tetramethylbebeerine iodide (16),<sup>12</sup> bebeerine hydrochloride (17),<sup>12</sup> *N,N'*-dimethylisochondrodendrine iodide (18).<sup>12</sup>

For better solution all iodide ions were exchanged by chloride by using freshly prepared silver chloride.<sup>11</sup>

(*R,R*)-7-*O*-Methylbebeerine (3) was obtained as follows. Monomethylation of 1 was carried out with  $\text{CH}_2\text{N}_2$  by using a standard procedure,<sup>13</sup> yielding 3: mp 119.2-124.0 °C;  $[\alpha]_{\text{D}}^{25} -249$  (c 0.10,  $\text{CHCl}_3$ ); mass spectrum, *m/e* (relative intensity) 608 ( $\text{M}^+$ , 10), 204 (14), 192 (86), 190 (46), 158 (100);  $\text{H}^1$  NMR ( $\text{CDCl}_3$ ), see Table II;  $^{13}\text{C}$  NMR, see Table I;  $\text{C}_{37}\text{H}_{40}\text{O}_6\text{N}_2$  requires *m/e* 608.2886, found *m/e* 608.2897 ( $\text{M}^+$ ).

Acetates 5 and 7 were prepared by standard methods. For (*R,R*)-12-*O*-acetyl-7-*O*-methylbebeerine (5): mp 95.7-99.0 °C;  $[\alpha]_{\text{D}}^{25} -318$  (c 0.12,  $\text{CHCl}_3$ ); mass spectrum, *m/e* (relative intensity) 650 ( $\text{M}^+$ , 73), 340 (100), 312 (90);  $\text{H}^1$  NMR ( $\text{CDCl}_3$ ), see Table II;  $^{13}\text{C}$  NMR, see Table I;  $\text{C}_{33}\text{H}_{42}\text{O}_7\text{N}_2$  requires *m/e* 650.2992, found *m/e* 650.3020 ( $\text{M}^+$ ). For (*R,R*)-*O,O*-diacetylbebeerine (7): mp 135.1-136.4 °C;  $[\alpha]_{\text{D}}^{25} -242$  (c 0.12,  $\text{CHCl}_3$ ); mass spectrum, *m/e* (relative intensity) 678 ( $\text{M}^+$ , 22), 340 (100);  $\text{H}^1$  NMR ( $\text{CDCl}_3$ ), see

Table II;  $^{13}\text{C}$  NMR, see Table I;  $\text{C}_{40}\text{H}_{42}\text{O}_8\text{N}_2$  requires *m/e* 678.2941, found *m/e* 678.2932 ( $\text{M}^+$ ).

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**Registry No.** 1, 436-05-5; 2, 1812-55-1; 3, 31944-97-5; 4, 59685-16-4; 5, 76947-03-0; 6, 59685-17-5; 7, 76947-04-1; 8, 477-58-7; 13, 57-94-3; 14, 7601-55-0; 15, 16202-76-9; 16, 16240-52-1; 17, 6059-41-2; 18, 32434-25-6.

(10) Galeffi, C.; Marini-Bettolo, G. B.; Vecchi, D. *Gazz. Chim. Ital.* 1975, 105, 1207.

(11) Naghaway, J. A.; Soine, T. O. *J. Pharm. Sci.* 1978, 67, 1204.

(12) Dutche, J. D. *J. Am. Chem. Soc.* 1946, 68, 419.

(13) Hultin, E. *Acta Chem. Scand.* 1961, 15, 1130 and references cited therein.

## Carbon-13 Nuclear Magnetic Resonance Spectroscopy and Conformational Analysis of the Daphnoline-Repandine Class of Bis(benzylisoquinoline) Alkaloids

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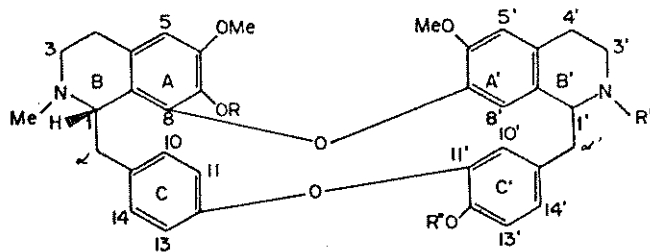
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The conformation of macrocyclic compounds are a challenge which has normally been solved by using X-ray crystallography. On the basis of previous works on bis(benzylisoquinoline) alkaloids<sup>1b,2</sup> and conscious that <sup>1</sup>H and <sup>13</sup>C NMR data are reliable tools for conformational analysis, we decided to carry out a careful analysis of the 18-membered macrocyclic alkaloids of the daphnoline-repandine class in order to contribute to the determination of related unknown compounds as well as to stimulate similar studies on other macrocyclic compounds.

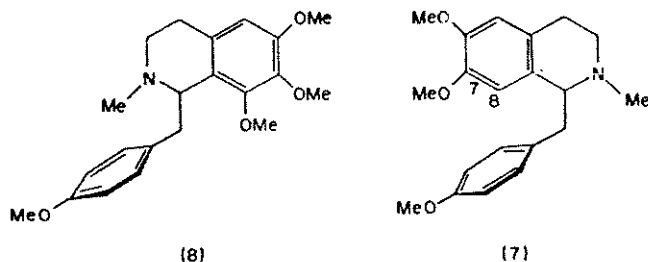
The <sup>13</sup>C NMR shifts for daphnoline and some of its derivatives (1-4) are listed in Table I. Assignments for



- (1) R=R'=R''=H, 1' = R
- (2) R=R''=H, R'=Me, 1' = R
- (3) R=R'=H, R''=Me, 1' = R
- (4) R=R''=Me, R'=H, 1' = R
- (5) R=R'=Me, R''=H, 1' = S
- (6) R=R'=R''=Me, 1' = S
- (13) R=R'=R''=Me, 1' = R

two alkaloids which belong to a diastereomeric series, repandine (5) and *O*-methylrepandine (6), are also listed. The values have been derived from standard chemical shift theory and from a study of the SFORD and the fully coupled <sup>13</sup>C NMR spectra. We have also taken into account analyses which have been reported previously for various mono-<sup>1</sup> and bis(benzylisoquinoline)<sup>1b,2</sup> alkaloids. It has been possible to resolve most of the uncertainties in assignment by selective heteronuclear irradiations and by a consideration of the known effects of *O*- and *N*-alkylation.

In the case of monobenzylisoquinoline alkaloids, it is known from <sup>1</sup>H NMR data<sup>3</sup> that a base such as *N,O,O*-trimethylcoclaurine (7) preferentially adopts a folded conformation in solution, but if a substituent such as methoxyl is inserted at C-8 as in 8, an extended conformation is preferred.



These facts can only be used for the bis(benzylisoquinoline) conformational analysis, taking into consideration that new steric interactions arise when the macrocycle is formed by the coupling of two benzylisoquinoline

(1) (a) Wenkert, E.; Buckwalter, B. L.; Burffit, I. R.; Gasić, M. J.; Gottlieb, H. E.; Hagaman, E. W.; Schell, F. M.; Wovkulich, P. M. In "Topics in Carbon-13 NMR Spectroscopy"; Levy, G. C., Ed.; Wiley-Interscience: New York, 1976, p 105. (b) Marsaioli, A. J.; Rúveda, E. A.; Reis, F. de A. M. *Phytochemistry* 1978, 17, 1655.

(2) (a) Koike, L.; Marsaioli, A. J.; Rúveda, E. A.; Reis, F. de A. M.; Bick, I. R. C. *Tetrahedron Lett.* 1979, 3765. (b) Koike, L.; Marsaioli, A. J.; Reis, F. de A. M. *J. Org. Chem.* 1981, 46, 2385.

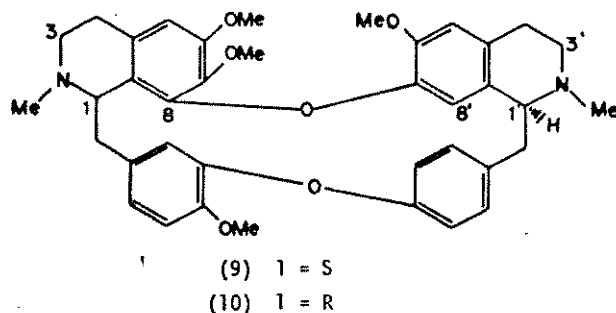
(3) (a) Tomita, M.; Shingu, T.; Fujitani, K.; Furukawa, H. *Chem. Pharm. Bull.* 1965, 13, 921. (b) Dalton, D. R.; Cava, M. P.; Buck, K. T. *Tetrahedron Lett.* 1965, 2687.

Table I<sup>a</sup>

carbons	Chemical shift, ppm					
	1	2	3	4	5	6
1	60.8	60.9	61.3	61.5	59.7	60.5
3	44.6	45.0	45.3	45.3	43.8	44.4
4	23.6	24.4	24.8	25.2	25.9	26.4
4a	121.9	122.9	122.9	127.5	127.0	127.6
5	104.5	104.7	104.7	105.7	106.5	106.8
6	147.9	146.7	147.0	151.3	151.2	151.9
7	133.6	133.4	133.6	137.5	136.0	136.0
8	141.2	141.3	141.7	147.9	148.3	148.5
8a	121.9	122.7	123.4	122.7 <sup>b</sup>	121.1	121.9
$\alpha$	39.9	40.0	39.8	39.8	40.7	40.6
9	130.2	130.6	130.9	130.7	132.3	133.9
10	127.7	128.6	127.9	127.6	129.9	129.9
11	121.7	121.4	121.9	122.1 <sup>b</sup>	120.1	120.3
12	151.6	152.6	151.9	151.6	155.0	155.4
13	120.4	120.5	120.8	120.8	121.6	121.7
14	130.9	131.3	131.0	131.1	131.4	131.4
1'	54.7	64.3	55.0	54.8	65.4	65.5
3'	38.5	43.6	39.3	38.9	46.1	46.6
4'	29.0	28.4	29.9	29.6	22.5	23.2
4a'	128.0	128.7	129.1	128.2	130.9	131.1
5'	111.8	111.2	112.2	111.7	112.1	112.5
6'	148.3	148.2	148.5	148.3	148.9	149.0
7'	144.0	143.3	144.1	144.7	144.2	144.5
8'	116.3	117.3	116.4	115.7 <sup>c</sup>	119.8	120.3
8a'	127.2	124.3	127.9	127.9	127.2	127.6
$\alpha'$	42.0	38.3	42.6	42.3	43.8	43.6
9'	138.7	138.6	139.3	139.3	137.5	137.9
10'	115.8	116.8	116.2	115.9 <sup>c</sup>	119.4	120.3
11'	144.0	143.7	146.2	147.0	146.5	148.5 <sup>b</sup>
12'	145.9	146.0	149.5	149.5	145.9	148.6 <sup>b</sup>
13'	114.7	114.2	110.9	110.8	116.7	113.3
14'	123.2	124.5	123.0	123.0	123.7	123.4
NCH <sub>3</sub>	41.8	41.8	41.9	41.3	41.8	41.6
N'-CH <sub>3</sub>		41.8			41.1	42.2
OCH <sub>3</sub>	55.9	55.3	56.1	55.9	54.9	56.3
C7-OCH <sub>3</sub>	55.0	56.1	55.2	54.8	55.4	55.7
				60.3	59.4	59.7

<sup>a</sup> The spectra were obtained in CDCl<sub>3</sub> solutions. Chemical shifts are expressed on the Me<sub>4</sub>Si scale according to the following equation:  $\delta(\text{Me}_4\text{Si}) = \delta(\text{CDCl}_3) + 76.9$  ppm. <sup>b,c</sup> Signals within a vertical column may be reversed.

units. In bis(benzylisoquinoline) alkaloids of the tetrandrine (9) series, one of the coclaurine units is substi-

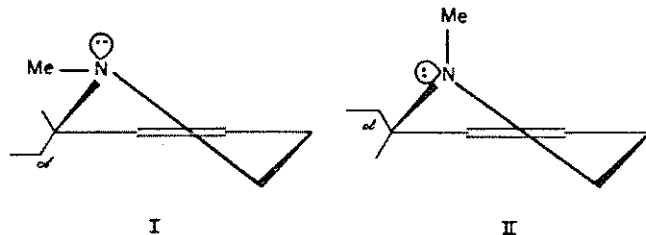


tuted at position 8 while the other is not, and here too the coclaurine residues adopt extended and folded conformations, respectively.<sup>2a,4,5</sup> This appears to be broadly true also in the enantiomeric isotetrandrine (10) series,<sup>2a</sup> as well

as for the curine class,<sup>2b</sup> although in some cases molecular crowding may result in the distinction between fully folded and fully extended conformations being less sharp.

The observation that O-7-methylation of daphnandrine (3; 3 to 4) affects in a predicted way<sup>1b</sup> the shifts of carbons belonging to ring A, while the remaining carbons show little or no effect, has led us to compare carbon shifts for rings AA', BB', and CC' of *N*-methyl daphnoline (2) and repandine (5) in order to detect different conformational aspects in these two diastereomeric compounds.

The methylenes and methine  $\Delta\delta$ s for 2 and 5 appear to be due to stereochemical and conformational effects associated with the B and B' rings. A half-chair conformation with an equatorial *N*-methyl group must be assigned to B' of 2 to account for the virtual absence of a  $\gamma$ -gauche effect at C-4' ( $\delta$  28.4) as compared to the corresponding carbons of daphnoline (1,  $\delta$  29.0). On the other hand, C- $\alpha'$  is considerably shielded in *N*-methyl daphnoline (2,  $\delta$  38.3) as compared to that in daphnoline (1,  $\delta$  42.0), and pseudoaxial conformation cis to the methylimino group is thus to be attributed to the benzyl substituent (see structure I). This in turn produces in the case of *N*-methyl-



daphnoline (2) a distinct shielding at C-3' ( $\delta$  43.6) as compared to C-3 ( $\delta$  45.0), and the latter value suggests that the benzyl group is substituted pseudoequatorially at C-1 (structure II).

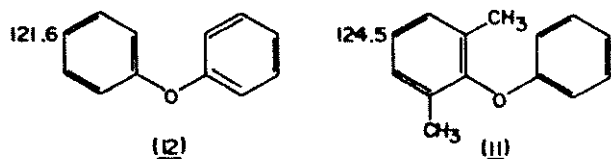
In the diastereomeric repandine (5) series, ring B carbon shifts show little difference as compared to those in 2, suggesting that ring B has the same conformation in both the repandine and daphnoline series. There are, however, differences between the two series in the signals associated with ring B'; thus C-4' is considerably shielded in repandine (5,  $\delta$  22.5), which indicates an axial conformation for the *N*'-methyl group. On the other hand, C-3' is not shielded ( $\delta$  46.1), suggesting that C-1' is substituted pseudoequatorially at C-1' and cis to the methylimino group (structure II).

From the experimental data on bis(benzylisoquinoline) alkaloids<sup>1b,2</sup> 21.4 and 28.4 ppm (for C-4 and C-4') are, so far, the extreme values observed when the *N*-methyl group is axial and equatorial, respectively. The intermediate values more frequently observed for C-4 and C-4' are indicative of the ratio between different conformations bearing axial and equatorial *N*-methyl groups.

Comparison between sp<sup>2</sup> carbon resonances in the daphnoline and repandine series discloses some interesting features concerning the diphenyl ether moieties. The deshieldings of C-9 ( $\Delta\delta$  1.7) and C-12 ( $\Delta\delta$  2.4) in 5 as compared to the same sites in 2, which are ipso and para to the ring C ether linkage, are most probably attributable to a modification in electron conjugation with the phenyl moieties. These facts are consistent with a deshielding of 2.9 ppm for C-4 in 2,6-dimethylphenyl phenyl ether (11) in comparison to a similar site in diphenyl ether (12), which is caused by an average interplanar angle modification from 33° (in 12) to 67° (in 11).<sup>6</sup>

(4) Gilmore, C. J.; Bryan, R. F.; Kupchan, S. M. *J. Am. Chem. Soc.* 1976, 98, 1947.

(5) (a) Bick, I. R. C.; Harley-Mason, J.; Sheppard, N.; Vernengo, M. *J. J. Chem. Soc.* 1961, 1896. (b) Monograph [on bis(benzylisoquinoline) alkaloids, in Japanese] commemorating the retirement of Professor Masao Tomita: Ageo, S., Ed.; Tomita Memorial Committee, Kyoto University Faculty of Pharmacy, Kyoto, Japan, 1967, p 44.



The above observation has led us to propose that analogous conformational changes occur with respect to rings C and C' of 2 (1*S*,1'*R*) and 5 (1*S*,1'*S*).

Dreiding model analysis of 2 and 5 reveals that the A and A' diphenyl ether moieties also suffer conformational changes in these two diastereomeric compounds. The fact that C-5 and C-4a' (para to ether linkages) resonate at lower fields in 5 than in 2 indicates the occurrence of wider interplanar angles between A and A' in the repandine series.

The lack of  $^{13}\text{C}$  NMR data for obaberine (13), which would be comparable to *O*-methylrepandine (6), was compensated for by  $^1\text{H}$  NMR spectroscopic observations. The 6'- and 7-methoxy groups of 13 resonate at  $\delta$  3.63 and 3.20, while those of 6 resonate at  $\delta$  3.42 and 3.03, respectively.<sup>5</sup> Molecular models of these two diastereomeric compounds, together with  $^1\text{H}$  NMR spectroscopy observations, reveal that the planes of rings A and A' are tilted at an angle of about 70° to one another in 13. As a result, the protons of the 7-methoxyl groups are brought above the shielding zone of ring A', but the 6'-methoxy group remains outside the shielding zone of A. On the other hand, in compound 6 the larger interplanar angle between A and A' brings both the 7- and 6'-methoxyl groups above the aromatic shielding zones.

The chemical shifts of the H-8' protons further confirm the angular modifications in both AA' and CC' ether linkages which occur in the 1*S*,1'*R* and 1*S*,1'*S* series. In

obaberine (13), H-8' appears at an abnormally high field ( $\delta$  5.48)<sup>5</sup> as a consequence of the anisotropic shielding of rings C and C', while H-8' of 6 lies in the shielding zone of ring C and deshielding zone, of C'; these two effects evidently cancel each other out, and the proton resonates further downfield ( $\delta$  6.38).

From the foregoing considerations, it is suggested that none of the benzylisoquinoline moieties in this series acquires a fully folded conformation in solution. This results in less flexible molecules, and the difference in absolute configuration at C-1' for the repandine series causes an increase in the interplanar angles between rings AA' and CC' as compared to those for the daphnoline series. It would appear from this and previous conformational studies<sup>2</sup> that in order to acquire a folded conformation, the coclaurine units of a bis(benzylisoquinoline) must be unsubstituted at C-8, and their benzyl groups should have a para diphenyl ether link. These conditions do not occur in the daphnoline-repandine series, so that the extended conformation prevails.

### Experimental Section

The  $^{13}\text{C}$  NMR spectra were obtained in a 10-mm spinning tube from solutions of approximately 0.5 mmol of compound in 1 mL of solvent. The instrument employed was Varian XL-100 NMR spectrometer operating at 25.2 MHz and interfaced with a Varian 620-L Fourier transform computer with a 16K memory. The chemical shifts ( $\pm 0.05$  ppm) were measured at a 5-kHz spectral width, with an acquisition time of 0.8 s and a 15- $\mu\text{s}$  pulse width, by using an internal deuterium lock.

Registry No. 1, 479-36-7; 2, 519-53-9; 3, 1183-76-2; 4, 24306-66-9; 5, 518-92-3; 6, 4021-17-4.

(6) Buchanan, G. W.; Montaudou, G.; Finocchiaro, P. *Can. J. Chem.* 1974, 52, 767.



STEREOCHEMICAL ASPECTS AND  $^{13}\text{C}$  NMR SPECTROSCOPY OF THE BERBAMINE CLASS OF  
BISBENZYLISOQUINOLINE ALKALOIDS<sup>1</sup>

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Summary: An analysis of the  $^{13}\text{C}$  NMR spectra of bisbenzylisoquinoline alkaloids of the berbamine type shows the dependence of spectroscopic properties on preferred conformation.

Apart from the interesting pharmacological activity shown by some members<sup>2</sup>, the bisbenzylisoquinoline alkaloids form a particularly attractive group for studying the interrelationship between preferred conformation and spectroscopic properties, as a result of the rather inflexible rings possessed by the majority of these bases. In continuation of our project on isoquinoline alkaloids<sup>3,4</sup>, we have accordingly selected the berbamine class for an exhaustive  $^{13}\text{C}$  NMR spectroscopic analysis. A correlation of the spectra of phaeanthine 1a, tenuipine 1b, nortenuipine 1c, O-acetylnortenuipine 1e, berbamine 1d, and O-acetylberbamine 1f has allowed an assignment of chemical shift to be made for each carbon in these bases (Table). The assignments were facilitated by comparisons with model compounds, by consideration of the known effects of electron-withdrawing groups (phenyl and acetyl) in a benzene ring<sup>5</sup>, and of the multiplicity of the signals in the coupled spectra.

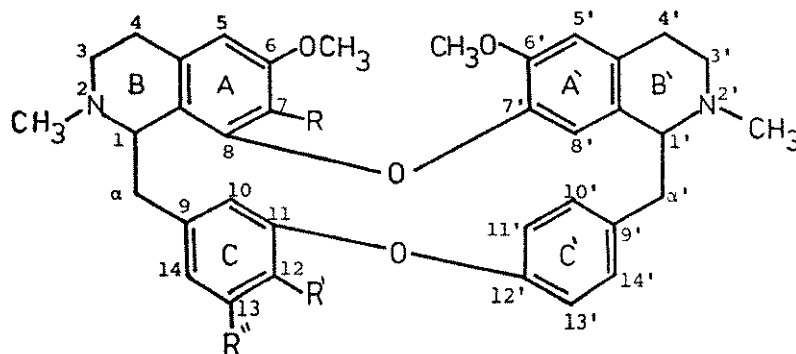
The values for the  $\text{sp}^3$  carbon shifts which result from this analysis are of particular interest. The methylene and methine shifts in the case of 1a can be divided into two groups: those with signals at 25.3, 38.3, 45.3 and 63.9 ppm (ring B') and those at 22.1, 41.9, 44.1 and 61.4 ppm (ring B). The first group can be attributed to C-4', C- $\alpha$ ', C-3' and C-1' respectively, which show slight shielding as compared to corresponding values for a monobenzyltetrahydroisoquinoline alkaloid<sup>3,8</sup>. It is assumed in these assignments that rings A', B' and C' have a folded conformation closely analogous to that of a monomeric N-methylbenzylisoquinoline alkaloid<sup>6</sup>, in which ring B' adopts a half-chair form with the N-methyl group pseudoequatorial, as established for the solid phase by X-ray crystallographic analysis<sup>2</sup> of the enantiomeric alkaloid tetrandrine 1g; that this conformation is largely maintained in solution is suggested by its properties and

TABLE

	<u>1a</u>	<u>1b<sup>n</sup></u>	<u>1c</u>	<u>1d</u>	<u>1e</u>	<u>1f</u>
C-1	61.4	61.4	61.5	62.0	61.4	62.0
C-3	44.1	44.0	44.0	44.7	43.8	44.8
C-4	22.1	22.0	21.7	23.9	22.0	23.8
C-4a	128.0 <sup>+</sup>	127.5 <sup>+</sup>	123.0 <sub>ξ</sub>	129.0	134.9	127.1
C-5	105.8	105.6	104.7 <sup>+</sup>	105.4	105.6	105.4
C-6	151.2	151.2	145.6	151.7	149.7	151.7
C-7	137.9	136.6	134.3	136.8	130.9	137.8
C-8	148.2	148.3	141.6	147.7	147.0	147.6
C-8a	123.0	122.4	123.0 <sub>ξ</sub>	120.1	122.4	120.3
C-α	41.9	42.2	42.2	37.5	42.7	37.6
C-9	134.9	136.4	136.5	134.0	136.5	137.8
C-10	116.2	110.3	110.3	115.3	110.5	116.9
C-11	146.9	143.1	143.1	143.8	143.1	151.4
C-12	149.3	133.4	133.3	147.3	133.4	141.5
C-13	111.6	148.1	148.2	114.6	148.4	121.2
C-14	122.6	104.5	104.5 <sup>+</sup>	123.5	104.6	123.3
C-1'	63.9	63.7	63.5	63.4	64.1	63.7
C-3'	45.3	45.0	45.0	45.2	45.5	45.9
C-4'	25.3	24.8	25.2	24.8	24.9	25.5
C-4α'	128.1 <sup>+</sup>	127.9 <sup>+</sup>	128.2 <sup>n</sup>	127.9	128.9	128.5
C-5'	112.7	112.5	112.8	111.1	112.5	111.0
C-6'	148.5	148.5	148.6	149.9	148.7	149.7
C-7'	143.7	143.6	143.3	143.4	142.6	143.3
C-8'	120.0	120.0	120.4	119.7	120.5	119.6
C-8a'	127.8 <sup>+</sup>	127.2 <sup>+</sup>	127.7 <sup>n</sup>	126.3	127.7	126.6
C-α'	38.3	38.2	37.5	38.2	39.7	38.1
C-9'	134.9	134.9	135.1	134.6	134.9	135.2
C-10'	129.9 <sub>ξ</sub>	129.9 <sub>ξ</sub>	129.9 <sub>ξ</sub>	130.0 <sup>+</sup>	130.0 <sup>+</sup>	130.1 <sup>+</sup>
C-11'	121.7	121.5	121.4	121.2	121.6	121.9
C-12'	153.6	153.3	153.1	153.9	153.4	153.9
C-13'	121.7	121.5	121.4	121.4	121.6	121.7
C-14'	132.4 <sub>ξ</sub>	132.4 <sub>ξ</sub>	132.3 <sub>ξ</sub>	132.0 <sup>+</sup>	132.4 <sup>+</sup>	132.1 <sup>+</sup>
NMe	42.3 <sup>n</sup>	42.0	42.2	42.6 <sub>ξ</sub>	42.2 <sub>ξ</sub>	42.7
NMe'	42.6 <sup>n</sup>	42.0	42.2	42.0 <sub>ξ</sub>	42.1 <sub>ξ</sub>	42.7
OMe	55.8	55.5	56.0	55.7	55.9	55.7
	56.1	55.5	56.0	55.7	55.7	55.5
OMe(C-7)	60.1	60.0		60.3		60.4
OCH <sub>2</sub> O		101.2	101.2		101.3	

## (TABLE continued)

The spectra were recorded in the Fourier transform mode on a Varian XL-100-15 spectrometer at 25.2 MHz in  $\text{CDCl}_3$  solution. The  $\delta$  values are in ppm downfield from TMS.  $\pi$ : some methanol was added for better solution of the compound.  $\dagger, \ddagger, \S$ : signals within the vertical columns may be reversed.  $\delta$   $\text{COCH}_3$  and  $\text{COCH}_3$  for 1e: 167.5 and 19.7; for 1f: 169.0 and 20.8 respectively.



1a, R = R' = OMe; R'' = H (*R,R*)

1b, R = OMe; R' = R'' =  $-\text{OCH}_2\text{O}-$  (*S,S*)

1c, R = OH; R' = R'' =  $-\text{OCH}_2\text{O}-$  (*S,S*)

1d, R = OMe; R' = OH; R'' = H (*R,S*)

1e, R =  $-\text{OCMe}$ ; R' = R'' =  $-\text{OCH}_2\text{O}-$  (*S,S*)

1f, R = OMe; R' =  $-\text{OCMe}$ ; R'' = H (*R,S*)

1g, R = R' = OMe; R'' = H (*S,S*)

reactions<sup>2</sup>. An extended conformation is attributed to rings A,B and C of 1a with ring B in a half-chair form, as for 1g; the N-methyl group and C- $\alpha$  are assigned pseudoaxial conformations, which produces a difference in shift of the methylene carbons associated with ring B as compared to those of B'. This is reflected in the shielding experienced by C-4, which now has a  $\gamma$ -gauche relationship to the N-methyl group; C-3 shows only slight shielding, whereas C- $\alpha$  is considerably deshielded. The shielding observed on C-1 is attributed to the  $\gamma$ -effect of the oxygenated function at C-8. The two N-methyl carbons are practically equivalent (42.3 and 42.6 ppm), and the methoxyl carbon signals appear at normal field (ca. 56 ppm), except for that attached to C-7, which being sterically hindered, resonates at 60 ppm. In the  $^1\text{H}$  NMR spectrum of 1a, however, the N-2 methyl group and the methoxyls at both C-7 and C-6' are clearly shielded<sup>7</sup>, showing the higher sensitivity of proton shifts to anisotropic effects.

The shifts of the  $\text{sp}^3$  carbons of tenuipine 1b, nortenuipine 1c, and its acetyl derivative 1e are in good agreement with those in corresponding positions in 1a. These bases belong to an enantiomeric series to 1a, and the main structural difference consists in replacement of the methoxyl in ring C by a methylenedioxy group; the carbon of the latter resonates at normal field

(101.2 ppm). In berbamine ld, which belongs to a diastereomeric series, the methoxyl and methylimino carbons show shifts corresponding to those for la. As far as the methylene and methine carbons are concerned, signals at 24.8, 45.2 and 63.4 ppm were assigned to C-4', C-3' and C-1' respectively, and those at 23.9, 44.7 and 62.0 ppm to C-4, C-3 and C-1. It is apparent that  $sp^3$  carbons in equivalent positions in the two benzylisoquinoline moieties give signals much closer together than in the case of la-lc; for C- $\alpha$  and C- $\alpha'$  of ld, the signals are very similar (37.5 and 38.2 ppm), whereas in the case of la,  $\Delta\delta$  for C- $\alpha$  and C- $\alpha'$  amounts to 3.6 ppm, while for lb with a methylenedioxy group in ring C, the difference is even greater (4.0 ppm); similarly,  $\Delta\delta$  for C-4 and C-4' of ld is 0.9 ppm as compared to 3.2 ppm for la. These data suggest that there is less steric crowding in the *R,S* than in the *R,R* or *S,S* series, an inference which is supported by the difference in shift of the C-6' methoxy groups (ca. 3.6 and 3.2 ppm) in their respective  $^1H$  NMR spectra<sup>7</sup>. It would appear, moreover, that the distinction shown in the *R,R* and *S,S* series between the completely extended and completely folded conformations of the two coclaurine units is less sharp in the *R,S* series.

It is evident that the chemical shifts of the benzylic methylene carbons in these types of bisbenzylisoquinoline alkaloids are especially sensitive to the configurations at the chiral centres, and together with the  $^1H$  NMR data, could be of diagnostic value in distinguishing alkaloids of different stereochemistry.

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#### REFERENCES

1. We have found our results are more easily described in terms of the classical system of numbering (c.f. reference 6, p. 115) rather than the more systematic scheme proposed by M. Shamma and J.L. Maniot in "Isoquinoline Alkaloid Research, 1972-1977", Plenum Press, New York, 1978, p. 71.
2. C.J. Gilmore, R.F. Bryan, and S.M. Kupchan, *J. Amer. Chem. Soc.*, 1976, 98, 1947.
3. A.J. Marsaioli, E.A. Rúveda, and F. de A.M. Reis, *Phytochemistry*, 1978, 17, 1655.
4. A.J. Marsaioli, F. de A.M. Reis, A.F. Magalhães, E.A. Rúveda, and A.M. Kuck, *Phytochemistry*, 1979, 18, 165.
5. H.E. Gottlieb, *Israel J. Chem.*, 1977, 16, 37.
6. M. Shamma, "The Isoquinoline Alkaloids", Academic Press, New York, 1972, p. 81.
7. I.R.C. Bick, J. Harley-Mason, N. Sheppard, and M.J. Vernengo, *J. Chem. Soc.*, 1961, 1896.
8. E. Wenkert, B.L. Buckwalter, I.R. Burfitt, M.J. Gasić, H.E. Gottlieb, E.W. Hagaman, F.M. Schell, and P.M. Wovkulich, in "Topics in Carbon-13 NMR Spectroscopy", (G.C. Levy, ed.), vol. 2, Wiley-Interscience, New York, 1976.

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