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PROTON AND CARBON-13 NMR STUDIES OF DELPHELINE, 8,9-METHYLENEDIOXYLAPPACONITINE AND DICTYZINE

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Abstract Unambiguous proton and carbon-13 nmr assignments for the norditerpenoid alkaloids (**1**), 8,9-methylenedioxylappaconitine (**2**) and dictyzine (**3**) were accomplished through detailed analysis of the DEPT, COSY, fixed evolution HETCOR, NOESY and selective INEPT techniques This work corrects previous assignments for **1-3**

Introduction

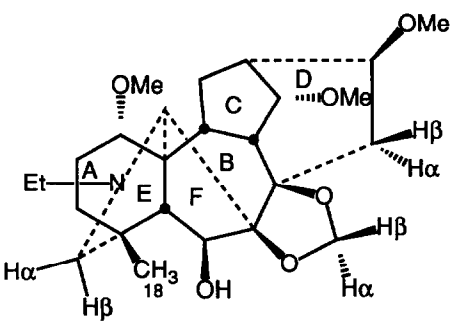
A variety of norditerpenoid alkaloids have been isolated from *Aconitum* and *Delphinium* species [1] During the past five years, more than one hundred and fifty new alkaloids of this class have been isolated, and this trend continues unabated In contrast to earlier degradation methods employed for the structure determination of these complex alkaloids,[2] during the past 15 years, most of the structures have been derived from proton and carbon-13 nmr studies On the basis of the ^{13}C nmr data for a large number of norditerpenoid alkaloids, we have tabulated the ^{13}C nmr chemical shift ranges of various functional groups in this class of compounds [1] Although correct structures have been deduced in most of the cases of known or newly isolated alkaloids making use of the values cited in these tables, it is likely that errors have been made in the chemical shift assignments for the individual carbon atoms We had pointed out such errors in the chemical shift assignments for C-10 and C-13 of some C-9 oxygenated alkaloids [3] Since determination of substitution sites (usually due to oxygenated substituents) relies heavily on the ^{13}C chemical shift analyses, it is imperative that these assignments be made unambiguously

With the help of proton decoupling techniques, additivity relationships and effects owing to specific structural changes in a number of closely related lycoctonine-type alkaloids, Jones and Benn published in 1973, the general pattern of ^{13}C shifts in norditerpenoid alkaloids [4] At that time, the C-10 and C-13 resonances were distinguished by assigning the lower field signal around 43–46 ppm to C-10 and the higher field signal around 37–43 ppm to C-13 for the lycoctonine-type alkaloids bearing no hydroxyl group at C-9 A later study, however, reversed the previously assigned values for browniine and lycoctonine [5] Consequently, the C-10 and C-13 chemical shifts for 14-acetylbrowniine, delphatine, delcosine, 14-acetyldelcosine, delsoline, tricornine, anthranoyllycoctonine, ajacine, methyllycaconitine and delsemine were assigned the values ~37 6–39 4

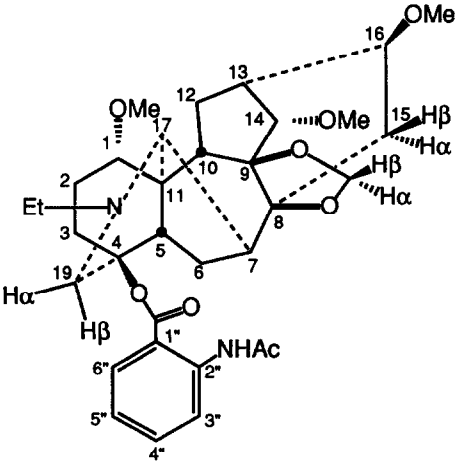
and ~43 6–46 1 ppm, respectively In a ^{13}C nmr study of aconitine-type alkaloids, chasmanine, 1,8,14-tri-*O*-methylneoline, neoline, 1-*epi*-neoline, 8-acetylneoline, delphisine, 1-*epi*-delphisine, 1-acetyldelphisine, 1-acetyl-1-*epi*-delphisine, condelphine and isotalatizidine were also assigned similar values [6] No explanation was given for changing the previously assigned chemical shifts [4]

These new assignments, however, still derived from chemical shift rationales and not from directly observed scalar or dipolar spin interactions Nevertheless, they were subsequently accepted and many newly isolated alkaloids were assigned closely similar shifts for C-10 and C-13 These assignments, however, showed inconsistency of the β- and γ-effects for C-10 and C-13 in alkaloids bearing a hydroxyl group at C-9, as in monticoline[3] and similarly C-9 substituted alkaloids [7]

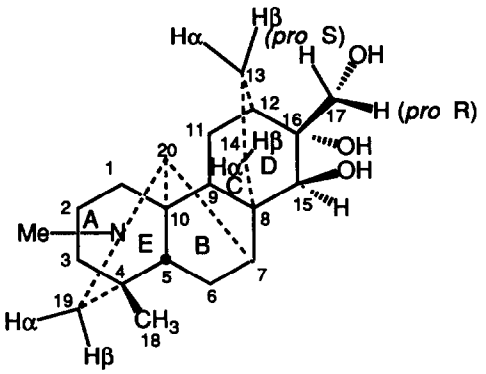
We now report the complete and unambiguous assignments of the ^{13}C nmr spectra of delpheline (**1**), 8,9-methylenedioxylappaconitine (**2**) and dictyzine (**3**) Delpheline was first isolated from *D elatum* L [8] and its structure **1**[9] was established by formal interconversion with lycoctonine [10,11] Tatsiensine, a norditerpenoid alkaloid isolated from *D. tatsienense* Franch was transformed into delpheline and the carbon-13 nmr signal assignments (for **1**) published in 1983 were based upon chemical shift rationale [12] Dictyzine was first isolated from *D dictyocarpum* DC in 1978[13] and its structure (**3**) was based on an X-ray crystal structure determination [14] The alkaloid (**3**) was also isolated from *D. brunonianum* Royle[15] and *D tatsienense* Franch [3,16] The ^{13}C chemical shift assignments were again based on chemical shift rationales [3] The alkaloid **2** was prepared from lappaconitine in order to study the ^{13}C methylene chemical shifts of the dioxymethylene group in **2** and tatsidine [17] The chemical shift assignments of **1** and **2** agree with our revised general assignments for C-10 and C-13 [3] As a result of these studies, several assignments for the ^{13}C chemical shifts of **1-3** have been corrected



1 Delpheline



2 8,9-Methylenedioxylappaconitine



3 Dictyzine

For the convenience of depiction in diagrams **1** and **2**, H-19α and H-19β indicate the pseudo-axial and pseudo-equatorial protons, respectively, in the chair conformation of the E ring formed by C-4, C-5, C-11, C-17, N and C-19 Similarly in **3** these protons in ring E are formed by C-4, C-5, C-10, C-20, N and C-19 Also, in **3**, H-α and H-β in ring C are designated for the pseudo-axial and pseudo-equatorial protons of the twist boat conformation of the ring formed by C-8, C-9, C-11, C-12, C-13 and C-14

Table 1 ^{13}C and ^{1}H nmr chemical shift assignments of delpheline (**1**) (in $CDCl_3$)

Carbon	δ (ppm)	Proton	δ(ppm)	J (Hz)
1	82 9	1β	2 99	dd, $J_{1\beta,2\alpha}$=9 9, $J_{1\beta,2\beta}$=7 3
2	26 9	2β*	2 02	m, $J_{2\beta,1\beta}$=7 3, $J_{2\alpha,2\beta}$=12 4
3	36 9			$J_{2\beta,3\beta}$=4 8, $J_{2\beta,3\alpha}$=2 4
4**	33 9	2α*	2 13	m
5	56 7	3β*	1 21	m
6	79 2	3α	1 56	br d, $J_{3\alpha,3\beta}$=13 4, $J_{3\alpha,2\beta}$=2 4
7**	92 7			$J_{3\alpha,2\alpha}$=5 1
8**	84 1	5	1 19	s, $W_{1/2}$=7 0
9	40 4	6	4 17	s, $W_{1/2}$=3 0
10	47 8	9	3 62	m, $J_{9,14}$=4 9
11**	50 4	10*	2 10	m
12	28 1	12β*	1 81	m
13	37 7	12α*	2 50	dd, $J_{12\alpha,12\beta}$=14 5, $J_{12\alpha,10}$=4 9
14	83 0	13	2 35	dd, $J_{13,14}$=6 9, $J_{13,12\beta}$=4 5
15	33 4	14	3 67	m, $J_{9,14}$=4 9
16	81 8	15β*	1 83	m, J_{gem}=14 9, $J_{15\beta,16}$=7 3
17	63 6	15α	2 47	dd, $J_{15\alpha,16}$=9 0, J_{gem}=14 9
18	25 4	16*	3 21	m
19	57 3	17	3 04	s, $W_{1/2}$=6 2
NCH_2	50 6	18	0 90	s
NCH_2CH_3	14 0	19β	2 21	AB J_{gem}=11 6
1-OCH_3	56 3	19α*	2 64	AB J_{gem}=11 6
14-OCH_3	57 8	-OCHβO-	5 02	s
16 -OCH_3	55 6	-OCHαO-	5 10	s
-OCH_2O-	92 9	-NCH_2	2 76,2 64	m,m
		-NCH_2CH_3	1 02	t, 7 0
		14-OCH_3	3 40	s
		16-OCH_3	3 33	s
		1-OCH_3	3 23	s
		6-OH	3 34	s

* The chemical shift was obtained from cross section of 2D-nmr spectra
** The assignment was done by selective INEPT

Table 2 ^{13}C and ^{1}H nmr assignments for 8,9-methylenedioxylappaconitine (2)

Carbon	δ (ppm) ($CDCl_3$)	δ (ppm) (C_6D_6)	Proton	δ (ppm) ($CDCl_3$)	J (Hz)	δ (ppm) (C_6D_6)	J (Hz)
1	84 4	84 1	1β	3 17	dd, $J_{1\beta,2\beta}$=6 8, $J_{1\beta,2\alpha}$=9 9	2 82	dd, $J_{1\beta,2\beta}$=5 4, $J_{1\beta,2\alpha}$=9 9
2	26 7	27 1	2β	2 13*	m	1 93	m, $J_{2\beta,1\beta}$=5 4, $J_{2\alpha,2\beta}$=13 0
3	31 8	32 1					$J_{2\beta,3\beta}$=2 5, $J_{2\beta,3\alpha}$=5 0
4	84 6	84 8	2α	2 24*	m	2 34*	m
5	47 5	48 1	3β	1 84	br t, $J_{3\beta,2\alpha}$=13 3, $J_{3\beta,2\beta}$=4 9	1 75*	m
6	27 1	25 3			$J_{3\alpha,3\beta}$=11 3		
7	45 8	48 6	3α	2 56*	m	2 69*	m
8	82 9	83 2	5	2 30*	m	2 45	d, $J_{5,6\beta}$=7 8
9	86 1	86 6	6β	1 99*	dd, $J_{6\alpha,6\beta}$=14 9, $J_{6\beta,5}$=7 6	2 36*	m
10	48 1	46 3	6α	2 60*	m	1 77*	m
11	51 0	51 4	7	2 17*	m	2 32*	m
12	24 7	27 3	10	2 27*	m	2 11	dd, $J_{10,12\alpha}$=4 3, $J_{10,12\beta}$=12 0
13	35 6	36 4	12β	1 98	dd, $J_{12\alpha,12\beta}$=15 7, $J_{12\beta,10}$=8 4	1 69*	m
14	88 4	88 9	12α	1 62*	m	2 69*	m, $J_{12\alpha,10}$=4 3, $J_{12\alpha,12\beta}$=14 9
15	38 6	39 3	13	2 29*	m	2 24	dd, $J_{13,14}$=4 5, $J_{13,12\beta}$=7 5
16	83 0	83 5	14	3 56*	d, $J_{14,13}$=4 5	3 55	d, $J_{14,13}$=4 5
17	60 1	60 0	15β	2 40	dd, $J_{15\alpha,15\beta}$=13 6, $J_{16,15\beta}$=8 7	2 90	dd, $J_{15\alpha,15\beta}$=13 7, $J_{15\beta,16}$=8 8
19	55 8	56 3	15α	2 21*	m	2 29*	m, $J_{15\alpha,16}$=8 3
N-CH_2	49 0	48 9	16*	3 32*	m	3 33	br t, $J_{16,15\beta}$=8 8, $J_{16,15\alpha}$=8 3
NCH_2CH_3	13 5	13 6	17	2 98	s, $W_{1/2}$=4 2	3 08	s
1-OCH_3	56 7	56 0	19β	2 50*	d, Jgem=11 2	2 56	d, Jgem=11 2
-OCH_2O-	96 6	97 1	19α	3 50	d, Jgem=11 2	3 64	d, Jgem=11 2
14-OCH_3	57 4	57 1	-OCHαO-	5 46	dd, Jgem=2 2	5 78	d, Jgem=2 1
16-OCH_3	56 3	56 1	-OCHβO-	5 12	dd, Jgem=2 2	5 19	d, Jgem=2 1
-OCO-	167 4	167 9	-NCH_2	2 50	m	2 32*	m
1"	115 7	115 5	NCH_2CH_3	1 10*	t, J=7 3	1 02	t, 7 2
2"	141 7	143 2	14-OCH_3	3 33	s	3 27	s
3"	120 3	120 6	16-OCH_3	3 30	s	3 04	s
4"	134 4	134 9	1-OCH_3	3 28	s	2 99	s
5"	122 3	122 1	3"	8 65	dd, $J_{3",4"}$=7 2, $J_{3",5"}$=1 4	9 27	dd, $J_{3",4"}$=8 5, $J_{3",5"}$=1 1
6"	131 0	131 3	6"	7 89	dd, $J_{6",5"}$=8 2, $J_{6",4"}$=1 7	8 05	dd, $J_{6",5"}$=8 0, $J_{6",4"}$=1 7
-$COCH_3$	169 0	168 3	4"	7 49	ddd, $J_{4",5"}$=7 7, $J_{4",3"}$=7 2	7 22	dt, $J_{4",5"}$=7 2, $J_{4",3"}$=8 5
-$COCH_3$	25 6	25 0			$J_{4",6"}$=1 7		$J_{4",6"}$=1 7
			5"	7 02	ddd, $J_{5",6"}$=8 2, $J_{5",4"}$=7 7	6 75	dt, $J_{5",4"}$=7 2, $J_{5",6"}$=8 0
					$J_{5",3"}$=1 4		$J_{5",3"}$=1 1
			-$COCH_3$	2 21	s	1 77	s
			-NH-	11 04	s	11 17	br s

* The chemical shift was obtained from cross section of 2D-nmr spectra

Table 3 ^{13}C and ^{1}H nmr chemical shift assignments of dictyzine (3) (in CD_3OD)

Carbon	δ (ppm)	Proton	δ(ppm)	J (Hz)
1	27 6	1β	1 40*	m, $J_{1\alpha,1\beta}$=14 4, $J_{1\beta,2\alpha}$=8 8**
2	21 8	1α	1 88*	m, $J_{1\beta,1\alpha}$=12 5
3	41 2	2β	2 23*	m, $J_{2\alpha,2\beta}$=12 7**
4	35 3	2α	1 43*	m, $J_{2\beta,2\alpha}$=12 7**, $J_{2\alpha,1\beta}$=8 8**
5	54 0	3β	1 20*	m, $J_{3\beta,2\alpha}$=7 5**
6	24 0	3α	1 54*	m, $J_{3\alpha,3\beta}$=12 0, $J_{3\alpha,2\alpha}$=4 1 $J_{3\alpha,2\beta}$=2 2
7	44 0	5	1 10	br d, $J_{5,6\beta}$=7 8
8	43 0	6β	2 68	dd, $J_{6\beta,6\alpha}$=13 2, $J_{6\beta,5}$=7 8
9	42 5	6α	1 18*	m
10	46 9	7	2 10	br d, $J_{7,6\alpha}$=5 4
11	24 7	9	1 82*	m
12	36 5	11β	1 60*	m
13	23 0	11α	1 23*	m
14	29 0	12	1 61*	m
15	87 1	13β	1 27*	m
16	81 1	13α	1 96*	m
17	67 9	14β	1 13*	m
18	27 0	14α	1 96*	m
19	60 8	15	3 88	s
20	74 7	17-*pro* R	3 98	d, J_{gem}=11 7
$-NCH_3$	44 5	17-*pro* S	3 58	d, J_{gem}=11 7
		18	0 70	s
		19β	2 29	AB, J_{gem}=11 2
		19α	2 42	AB, J_{gem}=11 2
		20	3 30*	s
		21(NCH_3)	2 26	s

* The chemical shift was obtained from the cross-section of fixed evolution HETCOR
** The J value was read from the cross-section of HOMO 2D-J

Table 4 ^{1}H–^{1}H correlations and nOe's of delpheline (1)

Observed H	nOe's (NOESY)	Correlations (COSY)
-OCHβO-	6-OH, -OCHαO-	-OCHαO-
-OCHαO-	-OCHβO-	-OCHβO-
H6	6-OH, H19β, H18	H5(w), H18
H14	14-OCH_3, H13	H13, H9
H9	H6-OH, H10	H14, H10
H16	H13, H12α, H17, H15α	H15α, H15β
H17	NCH_2, H16, H15α	H5
H1	H10, H2β, 1-OCH_3	H2α, H2β
NCH_2	H17	CH_3CH_2N
H19α	H19β, CH_3CH_2N	H19β
H12α	H12β, H16, 1-OCH_3	H10(w), H12β(s)
H15α	H15β, H16, H17	H15β, H16
H13	H12β, H16, 16-OCH_3, H14, 14-OCH_3	H12β, H14
H19β	H19α, H6	H19α
H10	H5, H9, H1	H12α, H12β, H9
H2α	H2β	H2β, H3β, H3α, H1
H2β	H2α, H1, 1-OCH_3	H2α, H3β, H1
H15β	H15α	H15α, H16
H12β	H12α, H13	H10, H13, H12α
H3α	H3β	H3β, H2α
H3β	H3α	H3α, H2β, H2α
H5	H18, H10	H6, H17
H18	H5, H6	H6
6-OH	H6, H9, -OCHβO-	
CH_3CH_2N	H19α	CH_3CH_2N
14-OCH_3	H13, H14	
16-OCH_3	H13, H16	
1-OCH_3	H1, H2β, H12α	

Table 5a nOe's and correlations of 8,9-methylenedioxylappaconitine (2) (in $CDCl_3$)

Observed H	nOe's (NOESY)	Correlations (COSY)
-OCHαO-		-OCHβO-
-OCHβO-	H6β	-OCHαO-
H14	H13, H12α, 14-OCH_3	H13
H19α	H19β, CH_3CH_2N	H19β
H16	H17	H15β, H15
H1	H5, H2α	H2α, H2β
H17	H16, H15α, CH_3CH_2N	
H6α	H6β	H6β, H7
H3α	H3β	H3β
H19β	H19α	H19α
-CH_2N-	CH_3CH_2N	CH_3CH_2N
H15β	H15α	H15α, H16
H5	H1, H6β, H3β	H6β
H13	H14, 16-OCH_3, 14-CH_3	H14, H12β
H10	H12β	H12β
H15α	H16, H15β	H16, H15β
H7		H6β
H6β	H5, H6α	H5, H6α
H12α	H14, H12β	H12β
H3β	H3α, H5	H3α, H2β
H12β	H12α, H10	H12α, H13, H10
CH_3CH_2N	-CH_2N, H17, H19α	-CH_2N
H3"	H4"	H4"
H6"	H5"	H5"
H4"	H3", H5"	H3", H5"
H5"	H4", H6"	H4", H6"

Table 5b nOe's and correlations of 8,9-methylenedioxylappaconitine (2) (in C_6D_6)

^{1}H	nOe's (NOESY)	Correlations (COSY)
-OCHαO-	14-OCH_3	-OCHβO-
-OCHβO-	H6β	-OCHαO-
H19α	H19β, CH_3CH_2N	H19β
H14	14-OCH_3, H13, H10, H12β	H13
H16	H17, H12α, H15α, H13	H15α, H15β
14-OCH_3	H14, H13, -OCHαO-	
H17	H16, H12α, CH_3CH_2N	
16-OCH_3	H16, H13	
1-OCH_3	H2β	
H15β	H15α	H15α, H16
H1	H10, H2β, H3β	H2β
H12α	H12β, H16, H17	H12β, H10
H3α	H3β	H3β
H19β	H19α, H7, H6α	H19α
H5	H10, H3β	
H6β	H6α, -OCHβO-	H6α
CH_2N	CH_3CH_2N	CH_3CH_2N
H2α	H2β	H2β
H7	H19β	
H15α	H15β, H16	H15β, H16
H13	H12β, H14, 14-OCH_3, H16, 16-OCH_3	H14
H10	H14, H1, H5, H12β	H12α, H12β
H2β	H2α, H1, 1-OCH_3	H2α, H1
H3β	H3α, H1, H5	H3α
H6α	H6β, H19β	H6β
H12β	H12α, H10, H13, H14	H12α, H10
CH_3CH_2N	CH_2N, H17, H19α	CH_2N
H3"	H4"	H4"
H6"	H5"	H5"
H4"	H3", H5"	H3", H5"
H5"	H4", H6"	H4", H6"

Results and Discussion

The ^1H and ^{13}C nmr assignments for **1–3** are summarized in Tables 1, 2 and 3, with important homonuclear scalar and dipolar couplings compiled in Tables 4 through 6 The ^{13}C signal assignments rely upon the initial delineation of the individual proton spin systems within each molecule using COSY, and when necessary, relayed coherence transfer (RCT),[18] and NOESY spectra In order to optimize spectral dispersion, studies on **2** were performed in both $CDCl_3$ and C_6D_6 which gave complementary signal resolution, C_6D_6 as the solvent gave the spectrum with the minimum overlap Discussion of the nmr data for **2** refer to spectra run using $CDCl_3$ as the solvent unless stated otherwise Overlap in the ^1H nmr of **1** in $CDCl_3$ was sufficiently minimal for all assignments, while the limited solubility of **3** in $CDCl_3$ and CD_2Cl_2 required the use of CD_3OD as a solvent With the proton spin systems mapped, the protonated carbons were unambiguously assigned using the HETCOR and fixed evolution HETCOR spectra [19] Assignments of the quaternary carbon resonances (multiplicities were determined by DEPT spectra) relied upon detection of long range (two and three bond) heteronuclear couplings ($^1H/^{13}C$) Since insufficient sample was available to employ either the FLOCK[20] or COLOC[21] techniques, and hardware limitations excluded the use of inverse detection,[22] these assignments were accomplished using selective INEPT (SINEPT) experiments [23] For all three alkaloids, the carbon assignments were completed without reliance upon chemical shift rationales with the exception of those carbons bearing heterosubstituents (nitrogen and oxygen) The only skeletal conformational freedom in alkaloids (**1**) and (**2**) exists in the A- and D-rings The results of the nmr studies revealed that the A-ring adopts a chair conformation and the D-ring a boat conformation for all three alkaloids Molecular mechanics modelling (QUANTA/CHARMm) studies were in agreement with these results

Table 6 nOe's and correlations of dictyzine (**3**) (in CD_3OD)

Observed H	nOe's (NOESY)	Correlations (COSY)
H17-*pro* R	H17-*pro* S, H9	H17-*pro* S
H15	H14β	
H17-*pro* S	H17-*pro* R, H9, H11β	H17-*pro* R
H20	H7, H14α, NCH_3	
H6β	H6α, H5	H6α, H5
H19α	H19β	H19β
H19β	H19α, H6α, H18	H19α
NCH_3	H20, H7	
H2α	H2β	H1α, H3α, H2β
H7	H6α, NCH_3, H20	H6α
H13α	H13β	H13β, H14β
H14α	H14β, H20	H14β, H13β
H1α	H1β	H2α, H1β
H9	H11β, H5, H17-*pro* R,S	H11β, H11α
H12		
H11β	H11α, H9, H17-*pro* S	H9, H11α, H12
H3α	H3β, H18	H3β, H2α
H2β	H2α	H2α, H1β
H1β	H1α	H1α, H2β
H13β	H13α	H13α, H14α, H14β
H11α	H11β	H11β, H9
H3β	H3α, H18	H3α, H2β
H6α	H6β, H7, H19β	H6β, H7
H14β	H15, H14α	H13α, H14α, H13β
H5	H9, H6β, H18	H6β
H18	H5, H3α, H3β, H19β	

Assignment of the quaternary carbon signals

The ^{13}C nmr spectrum of **1** showed four quaternary carbon signals at 33 9, 50 4, 84 1 and 92 7 ppm, which were assigned to C-4, C-11, C-8 and C-7, respectively, on the basis of selective INEPT studies Thus, polarization transfer from the methyl singlet (H-18, δ 0 90) to the quaternary carbon at δ 33.9 confirmed this carbon as C-4 (two-bond polarization transfer) The signal at δ 50 4 must therefore belong to C-11 as the only remaining non-oxygenated quaternary carbon The C-8 and C-7 resonances were distinguished on the basis of a polarization transfer from H-5 (δ 1 19, bs, assignment as H-5 discussed below) to the quaternary carbon at δ 92 7, which must therefore by C-7 (three bond polarization transfer) In this latter experiment, polarization transfers from H-5 to C-6 (δ 79 2), C-11, C-4 and C-18 (δ 25 4) were also observed The remaining oxygenated quaternary carbon, C-8, can therefore be assigned to the signal at δ 84 1

Four quaternary carbons were observed in the ^{13}C nmr spectrum of **2** at 51 0, 82 9, 84 6 and 86 1 ppm, apart from the resonances of the anthranoyl group discussed later Polarization transfers from H-17 and H-3β were the key to distinguish these carbons The broad singlet at 2 98 ppm ($W_{1/2}$ = 4 2 Hz) is typical for H-17 and was assigned as such The multiplet at 1 84 ppm (*ddd*, J = 13 3, 11 3, 4 9 Hz) showed coupling in the COSY spectrum to an overlapped multiplet at 2 56 ppm, which the fixed evolution HETCOR revealed to be its gem partner of a methylene pair This signal at 1 84 ppm was also coupled to overlapped multiplets at δ 2 13 and 2 24, also shown by the HETCOR and DEPT spectra to be a methylene pair Both protons of this second methylene pair were in turn coupled to a methine at 3 17 ppm (*dd*, J = 6 8, 9 9 Hz), typical for the relatively shielded C-1 oxygenated carbinol proton This sequence, CH(O-)-CH_2-CH_2-, must therefore be the C-1 through C-3 spin system The signal at 1 84 ppm was assigned to H-3β based upon the size of the coupling constants (J = 13 3 Hz, trans diaxial coupling with H-2α) and an nOe with H-1 observed in the difference nOe spectrum A similar analysis was even easier in C_6D_6 where the protons of this spin system were more cleanly resolved, with the exception of H-2α In this solvent, nOe's between H-1 and H-5 with H-3β were observed in the 2D-nOe spectrum

In the selective INEPT experiments, polarization transfers from H-17 to the quaternary carbons at 82 9 and 51 0 ppm were observed, which therefore can be assigned to C-8 (via three bond polarization transfer) and C-11 (via two bond polarization transfer), respectively Enhancements were also observed in this experiment for the methine carbons at 84 4 and 47 5 ppm The former resonance must therefore correspond to C-1 which correlated with the proton resonance at 3 17 ppm in the fixed evolution HETCOR spectrum, confirming this assignment as H-1 Enhancement of the signal at 47 5 ppm was also observed upon polarization transfer from H-3β optimized for heteronuclear coupling of 3 Hz, so this methine must be C-5 Another enhancement upon saturation of H-3β was observed for the quaternary carbon at 84 6 ppm optimized for heteronuclear coupling of 6 Hz, so this resonance can be assigned to C-4 The remaining quaternary carbon signal at 86 1 ppm must therefore by C-9

Dictyzine (**3**) has four quaternary carbons, C-4, C-8, C-10 and C-16 with resonances for non-protonated carbons found at δ 35 3, 43 0, 46 9 and 81 1 The low field signal could unambiguously be assigned to C-16 due to its chemical shift since C-16 is the only oxygenated quaternary carbon in **3** In selective INEPT studies, saturation of the H-18 methyl singlet led to an enhance-

ment of the quaternary carbon at δ 35 3, which could therefore be assigned to C-4, two bonds removed from H-18 In addition to the enhancement of C-4, a methine (δ 54 0) and two methylene carbons were also enhanced The methine can therefore be assigned to C-5, while the methylenes can be assigned to C-3 (δ 41 2) and C-19 (δ 60 8), all three bonds removed from H-18 The two methylenes were easily distinguished on the basis of their chemical shifts since C-19 is nitrogen substituted

Both H-7 (δ 2 10, b*d*, J = 5 4 Hz) and H-6β (δ 2 68, *dd*, J = 13 2, 7 8 Hz) were well resolved in the ^{1}H nmr spectrum Assignment of these resonances are discussed in detail below Polarization transfer from H-7 to a quaternary carbon at δ 46 9 enabled assignment of this signal as C-10 (three bonds removed from H-7) In addition, enhancements were also observed for two methines at δ 54 0 and 42 5 The former methine carbon was assigned to C-5 based on the polarization transfer from H-18, thus the methine at δ 42 5 must be C-9 Both C-5 and C-9 are three bonds removed from H-7 Enhancements upon saturation of H-6β in a selective INEPT experiment were observed to quaternary carbons at δ 35 3 (previously assigned to C-4 based upon polarization transfer from H-18) and 43 0 This latter signal must therefore be the remaining quaternary carbon, C-8 (three bonds removed from H-6β) Also enhanced in this experiment were methines at δ 74 7 and 44 0 The methine resonance at δ 74 7 was assigned to C-20 (three bonds removed from H-6β) and the resonance at 44 0 to C-7 (two bonds removed from H-6β) This latter carbon also showed the expected one bond correlation with H-7 in the HETCOR spectrum

Assignment of the secondary carbinol signals

Delpheline (**1**) showed four methines with directly bonded oxygens at δ 79 2, 81 8, 82 9 and 83 0 which were one bond coupled to protons at δ 4 17 (b*s*, $W_{1/2}$ = 3 Hz), 3 21 (*m*), 2 99 (dd, J = 9 9, 7 3 Hz) and 3 67 (*m*), respectively The two higher field methines (δ 3 21 and 2 99) were both adjacent to methylene groups as indicated by the COSY and HETCOR spectra, and thus must be C-1 and C-16 The proton spin system which included the highest field carbinol proton was shown to be –CH(O–)CH_2CH_2–, with two methylene groups in sequence adjacent to the secondary carbinol proton The δ 2 99 and 82 9 resonances must therefore belong to H-1 and C-1 The carbinol methine at δ 3 21 showed coupling only to an isolated methylene pair, which itself showed no further coupling, and therefore can be assigned to H-16 with the C-16 resonance assigned to the signal at δ 81 8 It is of interest to note that H-16 did not show coupling to H-13 in the COSY spectrum as would be expected if the D-ring was in a boat conformation, which would result in a 90° dihedral angle between H-16 and H-13 The calculated dihedral angle between H-16 and H-13 from molecular modelling studies was 92 8° for the D-ring boat conformation The two remaining carbinol methine protons were easily distinguished from the COSY spectrum Thus, H-14 (δ 3 67, *m*, $J_{9,14}$ = 4 9 Hz) was readily identified as a member of the C-ring cyclopentane spin system which showed all the expected couplings in the COSY spectrum with the exception of coupling between H-13 and H-12α due to a dihedral angle of 90° (calculated from molecular modelling 83 7°) The HETCOR spectrum enabled the assignment of the C-14 resonance at δ 83 0, with the remaining C-6 methine assigned to the signal at δ 79 2, correlating to the proton singlet at δ 4 17 (*s*, $W_{1/2}$ = 3 Hz) in the HETCOR spectrum The H-6 proton showed weak coupling with H-5

in the COSY spectrum, and selective INEPT experiments had revealed a two bond polarization transfer from H-5 to the methine carbon at δ 79 2, supporting this assignment

Alkaloid **2** showed three signals due to oxygenated methine carbons 83 0, 84 4 and 88 4 ppm, with the second resonance already assigned to C-1, leaving only C-14 and C-16 to be assigned The signal at 88 4 ppm correlated with a proton doublet (δ 3 56, J = 4 5 Hz) in the fixed evolution HETCOR spectrum Thus this proton must be H-14 (coupled only to H-13) and the lower field carbon signal was assigned to C-14 The resonance at 83 0 showed one-bond coupling with a multiplet at 3 32 which was overlapped by a methoxyl singlet Nevertheless, this multiplet showed coupling to the two H-15 methylene protons in the COSY spectrum and can be assigned to C-16 [The dihedral angle between H-16 and H-13 being ~90° – (calculated from molecular modelling 95 8°) – H-16 shows no coupling with H-13] This was confirmed in C_6D_6, H-16 was a well resolved triplet (δ 3 33, $J_{16,15\alpha}$ = 8 3, $J_{16,15\beta}$ = 8 8 Hz) coupled only to the C-15 methylene protons

Dictyzine (**3**) has only a single, secondary carbinol, C-15, but two low field methines appeared in the ^{13}C nmr spectrum at δ 87 1 and 74 7, one bond coupled to proton singlets at δ 3 88 and 3 30, respectively The lower field proton and carbon resonances were assigned to H-15 and C-15 The higher field carbon signal was ultimately assigned to C-20 as the COSY revealed that the δ 3 30 singlet was part of the H-5 through H-20 spin system (–CH–CH_2–CH–CH– system) and could be assigned to H-20 as discussed below

Assignment of the methine carbons

The three methine carbons of the cyclopentane C-ring of **1**, C-9, C-10 and C-13, were routinely assigned at δ 40 4, 47 8, and 37 7 from the HETCOR spectrum, once the C-ring proton spin system was detailed in the COSY spectrum (Table 1) The broad singlet at δ 1 19 ($W_{1/2}$ = 7 0 Hz) showed weak coupling with H-6 in the COSY spectrum and was assigned to H-5, supported by the observations of nOe's with the H-18 methyl protons and H-10 (1,3-diaxial-type dipolar coupling) These observations and W-coupling between H-5 and H-17 (δ 3 04 b*s*, $W_{1/2}$ = 6 2 Hz) enabled assignment of C-5 and C-17 to the resonances at 56 7 and 63 6, respectively, from the HETCOR spectrum

In the ^{13}C nmr spectrum of **2**, five non-oxygenated methines appeared at 35 6, 45 8, 47 5, 48 1 and 60 1 ppm As previously described, C-5 was assigned the signal at 47 5 based upon selective INEPT experiments, while the signal at 60 1 was assigned to C-17 due to its low field shift and one bond correlation with H-17 in the fixed evolution HETCOR spectrum Polarization transfers upon saturation of H-17 were also observed to the signals at 45 8 and 48 1, which therefore must be C-7 and C-10 The H-7 methine (δ 2 17, *m*) was distinguished by coupling to both C-6 methylene protons (δ 1 99 and 2 60, H-6β and H-6α, respectively, both *m*), which were in turn identified by the coupling of the upper field proton to H-5 No coupling was observed between H-5 and H-6α in the COSY spectrum due to the 90° dihedral angle (calculated 104 4°) between these protons The C-5 methine (δ 2 30, *m*) was itself unambiguously located from the HETCOR spectrum one bond coupled with C-5 The HETCOR spectrum thus enabled identification of the 45 8 ppm resonance as C-7, and the 48 1 ppm resonance must therefore belong to C-10 The re-

maining methine carbon, δ 35 6, must consequently be assigned to C-13 This assignment was confirmed by the coupling of H-14 to H-13 (δ 2 29), with the fixed evolution HETCOR experiment revealing the one bond coupling from H-13 to C-13

In C_6D_6, H-5 was a well separated broadened doublet (δ 2 45, J = 7 8 Hz) again showing coupling only to H-6β (δ 2 36, *m*) It is interesting to note that in C_6D_6, the relative positions of H-6α and H-6β are reversed in comparison to the chemical shifts recorded in $CDCl_3$ (Table 2) Presumably this large solvent effect is due to a reorientation of the anthranoyl group in C_6D_6 relative to that in $CDCl_3$

The five nonoxygenated methines of **3**, δ 74 7, 54 0, 44 0, 42 5 and 36 5 were assigned to C-20, C-5, C-7, C-9 and C-12, respectively, from the HETCOR spectrum after defining the proton spin systems in the COSY spectrum Thus, C-12 (δ 36 5, H-12 δ 1 61) was easily assigned as the central methine carbon member of the –CH–CH_2–CH–CH_2–CH_2– spin system identified in the COSY spectrum belonging to C-9 through C-14 The terminal methine member of this spin system correlated with the methine carbon resonance at δ 42 5 in the HETCOR spectrum, enabling assignment of this signal as C-9 This was confirmed by selective INEPT experiments previously described upon polarization transfer from H-7 The three remaining methines were all members of the same spin system –CH–CH–CH_2–CH–, with one of the three methine protons appearing as a singlet (δ 3 30) with only weak coupling to a second methine proton (δ 2 10, b*d*, J = 5 4 Hz) The remaining methine proton also appeared as a broad doublet (δ 1 10 b*d*, J = 7 8 Hz), and these latter two protons must therefore be the methines which flank the methylene carbon (C-6) with the methine singlet assignable to H-20 The low field methine carbon (δ 74 7) was thus assigned to C-20 from the HETCOR spectrum Selective INEPT experiments (previously described) confirmed the assignments of C-5, C-7 and C-20 upon polarization transfers from H-18 (to C-5), H-7 (to C-5 and C-9), and H-6β (to C-7 and C-20)

While an all vicinal coupling pattern would predict this spin system to be C-20/C-7/C-6/C-5, potential W-coupling between H-5 and H-20 with no observable coupling between H-20 and H-7 (analogous W-coupling between H-5 and H-17 was observed in **1**) means the C-20/C-5/C-6/C-7 alternative must also be considered These two possibilities were resolved by assigning H-7 and H-5 on the basis of the 2D-nOe spectrum (Table 6) The lower field methine doublet showed an nOe with the *N*-methyl singlet and H-20 (as well as with H-6α) and thus can be assigned to H-7 The higher field methine doublet had nOe's with the C-18 methyl singlet and H-6β as well as with the proton ultimately assigned as H-9, and thus must be H-5 The corresponding carbon resonances were therefore assigned from the HETCOR spectrum These nOe's as well as the coupling constants also distinguished H-6α and H-6β Thus the couplings between H-5 and H-6α and between H-6β and H-7 were very small due to dihedral angles approaching 90° (calculated for H-5/H-6α 114 7°, calculated for H-6β/H-7 88 0°)

Assignment of methylene carbons

With the assignment of the C-2, C-3, C-12 and C-15 methylene protons in the COSY spectrum of **1** completed as described above, the carbon resonances for these methylene groups were easily assigned from the fixed evolution HETCOR spectrum as δ 26 9, 36 9, 28 1 and 33 4, respec-

tively The three remaining methylenes C-19, the *N*-ethyl methylene and the dioxymethylene were similarly assigned to δ 57 3, 50 6 and 92 9, respectively, from the HETCOR spectrum as their directly bonded protons were quite distinct in the ^{1}H nmr spectrum

Eight methylene carbons are present in **2** δ 24 7, 26 7, 27 1, 31 8, 38 6, 49 0, 55 8 and 96 6 The low field resonance is routinely assigned to the methylenedioxy carbon The *N*-ethyl methylene carbon was easily located at δ 49 0 from the fixed evolution HETCOR spectrum The 55 8 ppm resonance can be easily assigned to C-19 due to the directly attached nitrogen, selective INEPT experiments also showed a polarization transfer from H-17 to this carbon Location of the C-2, C-3, C-6 and C-15 methylene protons in the COSY spectrum as discussed above enabled easy assignment of the corresponding carbons from the fixed evolution HETCOR spectrum at 26 7, 31 8, 27 1 and 38 6 ppm, respectively The remaining resonance at 24 7 ppm must therefore be C-12 Coupling from H-13 to one of the C-12 methylene protons (δ 1 98, *dd*, J = 15 7, 8 4 Hz, H-12β) enabled location of the C-12 methylene protons and confirmed the C-12 assignment Coupling was not observed between H-13 and H-12α in the COSY spectrum due to a dihedral angle of ~90° (calculated 88 4°) between these two protons A similar analysis followed for the assignments in C_6D_6

The methylene carbons of **3** were easily assigned from the fixed evolution HETCOR spectrum once the separate proton spin systems were mapped from the COSY spectrum the C-1 through C-3 system as well as the C-5 through C-20 and the C-9 through C-14 systems The C-1 and C-3 termini of the C-1 through C-3 adjacent methylene system were distinguished on the basis of nOe's between both H-3α and H-3β and the C-18 methyl protons Selective INEPT experiments previously described, confirmed the assignment of C-3 upon a polarization transfer via three bonds from H-18 The C-17 (δ 67 9) and C-19 (δ 60 8) methylene carbons were assigned from the fixed evolution HETCOR spectrum as well, based on the lower field signal for the protons on the oxygenated carbon (δ 3 98 and 3 58 for H-17, δ 2 42 and 2 29 for H-19) Polarization transfer from H-18 to C-19 via three bonds in a selective INEPT experiment confirmed the assignment of C-19 The assignments of C-19 and C-17 were reversed in the original work [3]

Assignment of the methyl carbons

The high field ^{13}C methyl resonance of **1** was assigned to the methyl group of the *N*-ethyl chain, correlating with the methyl triplet (δ 1 02, *t*, J = 7 0 Hz) in the HETCOR spectrum The C-18 methyl singlet correlated with the methyl carbon at δ 25 4 in the HETCOR spectrum, and was thus assigned The remaining methyl resonances belong to methoxyl groups, and these were distinguished on the basis of nOe studies (Table 4) Thus, each methyl of the methoxy group showed an nOe with the corresponding carbinol methine proton in the 2D-nOe spectrum The lowest methoxyl singlet (δ 3 40, nOe's with H-14 and H-13) was assigned to the C-14 methoxyl group, the highest field methoxyl singlet (δ 3 23, nOe's with H-1, H-2α, and H-12α) to the C-1 methoxyl group, and the intermediate methoxyl resonance (δ 3 33, nOe's with H-13 and H-16) to the C-16 group The corresponding carbon resonances were then routinely determined by the HETCOR spectrum (Table 1)

In **2**, the acetyl (δ 25 6) and *N*-ethyl methyl (δ 13 5) groups were easily assigned on the basis of their chemical shifts with the expected one bond correlations in the HETCOR spectrum as well The three methoxyl groups at C-1, C-14 and C-16 were distinguished by nOe's An nOe between H-14 and the low field methoxy singlet (δ 3 33) identifies this as the C-14 methoxyl group, correlating with the methoxyl carbon at 57 4 ppm in the HETCOR spectrum The high field methoxy singlet (δ 3 28) showed an nOe with H-2α, identifying this as the C-1 methoxyl group, correlating with the methoxyl carbon at δ 56 7 The remaining methoxy singlet (δ 3 30) must therefore be the C-16 methoxyl group, which showed an nOe with H-13 This last methoxy resonance correlates with the carbon at δ 56 3

The assignment of the two methyl group carbons of **3** was routine based upon the expected chemical shift differences (δ 27 0 for C-18, δ 44 5 for the *N*-methyl group, C-21)

Assignment of aromatic resonances of 2

The ^{1}H nmr spectrum showed a clear 1,2-disubstituted aromatic ring pattern δ 8 65 (*dd* J = 7 2, 1 4 Hz), 7 89 (*dd*, J = 8 2, 1 7 Hz), 7 49 (*ddd*, 7 7, 7 2, 1 7 Hz) and 7 02 (*ddd*, J = 8 2, 7 7, 1 4 Hz) Polarization transfers in the selective INEPT experiments upon saturation of the low field resonance showed enhancements of the 115 7 ppm nonprotonated carbon assigned to C-1" and 122 3 ppm protonated carbon assigned to C-5" The low field proton resonance (δ 8 65) also showed *meta* coupling to H-5" (δ 7 02), and can therefore be assigned to H-3", the remaining proton assignments follow from the COSY spectrum, and the protonated carbon assignments from the HETCOR spectrum Saturation of the resonance at 7 89 led to enhancements of the carbonyl carbon at δ 167 4, and the nonprotonated carbon at δ 141 7, assigned to C-2" Thus the anthranoyl carbonyl carbon could be assigned to the resonance at δ 167 4, and the *N*-acetyl carbonyl carbon must be the remaining carbonyl resonance at δ 169 0

Assignment of diastereotopic methylene protons

The diastereotopic C-2 and C-3 protons were assigned on the basis of coupling constants (when discernible) and nOe's Thus in **1**, H-2α showed trans-diaxial coupling with H-1 (J = 9 9 Hz), while H-2β had nOe's with both H-1 and the C-1 methoxyl group One H-3 proton was severely overlapped by the H-5 broad singlet, but the other H-3 proton was assigned to the equatorial α-position on the basis of the relatively small couplings with the C-2 methylene protons ($J_{2\beta,3\alpha}$ = 2 4 Hz, $J_{2\alpha,3\alpha}$ = 5 1 Hz), giving H-3α the appearance of a broadened doublet in the ^{1}H nmr spectrum The A-ring of **1** therefore exists in a chair conformation, which was also predicted to be the lowest energy conformation in molecular modelling experiments using the QUANTA/CHARMm program

The C-12 protons were easily assigned as previously discussed H-12α (δ 2 50) showed no coupling with H-13 due to a 90° dihedral angle, coupling between H-12β and H-13 was 4 5 Hz Furthermore, H-12α showed nOe's with H-16 and the C-1 methoxyl group and must lie on the α-face of the molecule The C-15 methylene protons were identified by the nOe between H-15α and H-17 The C-19 and methylenedioxy protons were also assigned on the basis of nOe's H-19β showed an nOe with H-6 while H-19α had nOe's only with H-19β and the *N*-ethyl methyl protons The α-proton of the methylenedioxy group had an nOe with the C-6 hydroxyl proton while the β-

proton of this methylene pair showed an nOe only with its gem-partner, thereby distinguishing the remaining diastereotopic methylene protons of **1**

In alkaloid **2**, the stereochemical assignment of the diastereotopic methylene protons was made on the basis of observed couplings as discussed previously for the C-3, C-6 and C-12 methylene groups The C-2 methylene proton resonances in $CDCl_3$ were too overlapped with near identical chemical shifts to identify unambiguously on the basis of coupling constants Nevertheless, these protons were distinguished on the basis of an nOe between the H-1β (axial) and H-2β (equatorial) protons, with no nOe detected between H-1 and H-2α protons (trans diaxial relationship) In C_6D_6, H-2β was a cleanly resolved multiplet and was easily assigned as the equatorial proton based upon the coupling constants (Table 2) The methylenedioxy and C-19 methylene protons were distinguished on the basis of nOe's from either the 2D-nOe spectrum or difference nOe spectra (Table 5) Thus, the high field proton of the methylenedioxy pair (δ 5 12) showed an nOe to H-6β, distinguishing the methylenedioxy pair

In $CDCl_3$, only one H-19 proton was resolved (δ 3 50, J_{AB} = 11 2 Hz) The remaining H-19 proton was in a heavily overlapped region of the spectrum (δ 2 5–2 6) which included H-6α, precluding the possibility of observing an unambiguous nOe between these two protons (as well as the possibility of observing an nOe with H-7 since this resonance could not be definitively assigned to H-19 or H-6α The resolved H-19 proton did show an nOe with the methyl triplet of the *N*-ethyl chain Since this methyl group should adopt a conformation oriented away from the B-ring, the low field resonance (δ 3 50) was tentatively assigned to the H-19 proton oriented away from the B-ring and the higher field resonance (δ 2 50) was assigned to the H-19 proton directed under the B-ring These assignments were supported by the nmr studies in C_6D_6 in which both H-19 protons were well resolved (δ 3 64 and 2 56, both *d*, J = 11 2 Hz) In the 2D-nOe spectrum, the lower field resonance again showed an nOe with the methyl group of the *N*-ethyl chain (as well as an nOe with the methylene protons of this chain), while the higher field resonance showed nOe's with H-6α and H-7

An nOe between H-17 and H-15α (δ 2 21) identified the C-15 diastereotopic methylene pair This nOe was observed in both solvents ($CDCl_3$ and C_6D_6) The large coupling between H-15β and H-16 (J = 8 7 Hz) as well as H-15α and H-16 (J = 8 3 Hz) indicates that this ring exists in the boat conformation

Assignment of the diastereotopic methylene protons on the C-1 through C-3 fragment of **3** was complicated by the severe overlap in the ^1H nmr spectrum Nevertheless, a difference nOe experiment upon saturation of H-19α, which was well resolved, revealed an nOe to one of the C-3 protons which therefore must be H-3α (δ 1 54, *m*) Furthermore, this proton (H-3α) showed W-coupling to one of the C-1 diastereotopic protons, suggesting that both of these protons are in an equatorial orientation Confirming this assignment, the gem/partner H-3β, located at δ 1 20 (*m*) from the fixed evolution HETCOR experiment, showed trans-diaxial coupling ($J_{2\alpha,3\beta}$ = 7 5 Hz) with one of the C-2 methylene protons at δ 1 43, which therefore must be the axial H-2α The gem partner of H-2α was located from the fixed evolution HETCOR spectrum at δ 2 23 (H-2β) The H-2α proton in turn showed trans-diaxial coupling with one of the C-1 methylene protons ($J_{1\beta,2\alpha}$ = 8 8 Hz), which must therefore be H-1β The gem partner of H-1β was again located from the fixed

evolution HETCOR experiment at δ 1 88 (H-1α) While all the coupling constants of this spin system composed of three methylenes were not completely resolved due to overlap, these sequential trans-diaxial couplings indicate that the A-ring of **3** exists in a chair conformation Molecular mechanics calculations discussed below showed that the boat conformation of the A-ring is 6 4 kcal/mol higher in energy than the chair conformation in the absence of solvent interactions

The C-6 methylene protons were distinguished on the basis of their couplings with H-5 and nOe's One of the H-6 protons was only very weakly coupled to H-5 ($J < 1$ Hz), though coupled with H-7 ($J = 5$ 4 Hz) This proton was assigned to H-6α since a 90° dihedral angle exists between H-5 and H-6α The H-6β proton showed only very weak coupling with H-7 ($J < 1$ Hz), but strong coupling with H-5 ($J = 7$ 8 Hz) This proton was assigned to H-6β which has a 90° dihedral angle with H-7 (calculated 88 0°), but is nearly eclipsed with H-5 (8 2° dihedral angle from molecular mechanics calculations) Confirming these assignments was the observation of an nOe between one of the H-19 protons and H-6α This in turn enabled assignment of the H-19 protons that which showed the nOe with H-6α must be H-19β while that which had the previously mentioned nOe with H-3α must be H-19α

Only one H-11 proton showed coupling with H-12, and this proton was assigned to H-11β, H-11α has a 90° dihedral angle with H-12 Furthermore, H-11β showed an nOe with H-9 and one of the H-17 protons Similarly, only one of the H-13 protons showed coupling with both H-14 protons, and this was assigned as H-13β The remaining H-13 proton, H-13α, has a 90° dihedral angle with H-14β and shows coupling only with H-14α The assignment of the H-14 protons were further confirmed by nOe's H-14α has an nOe with H-20, while H-14β shows an nOe with H-15

The significant chemical shift difference between the *pro*-R and *pro-S* H-17 protons suggested that a dominant rotamer about the C-16/C-17 bond may exist This rotameric dominance could easily be enforced by hydrogen bonding between the C-17 hydroxyl and either the C-15 or C-16 hydroxyl groups The 2D-nOe spectrum showed a clear distinction in their orientations, thus, one of the H-17 protons showed nOe's with both H-9 and H-11β, while the other H-17 proton showed an nOe only with H-9 This nOe pattern can be easily explained if the dominant conformation is controlled by hydrogen bonding with the C-16 hydroxyl group Under this constraint, the H17-*pro-S*/H9 and H17-*pro-S*/H11β distances were calculated to be 1 96 Å and 2 30 Å, respectively, while the H17-*pro-R*/H9 and H17-*pro-R*/H11β distances were calculated to be 3 14 Å and 3 94 Å, in accord with the nOe results with the latter distance being too large to observe an nOe under these conditions[24] If hydrogen bonding between the C-15 and C-17 hydroxyl groups was dominant, the anticipated nOe results would be that both H-17 protons would show nOe's with H-11β, while only the *pro-R* proton would show an nOe with H-9 For this rotamer the H17-*pro-S*/H9 and H17-*pro-S*/H11β distances were calculated to be 3 76 Å and 3 71 Å, respectively, while the H17-*pro-R*/H9 and H17-*pro-R*/H11β distances were calculated to be 2 54 Å and 2 18 Å These distances are not in accord with the observed nOe's, and the H-17 protons were therefore assigned as shown in Table 3, albeit somewhat tentatively

Figure 1A

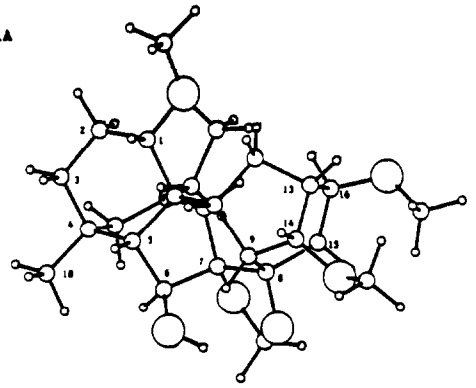


Figure 1C

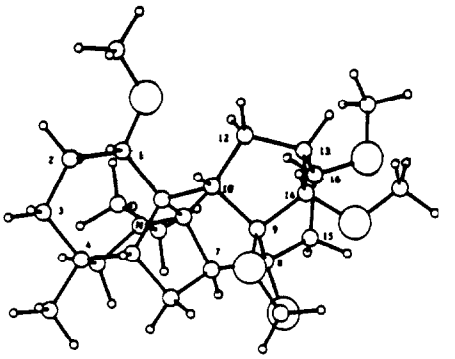


Figure 1B

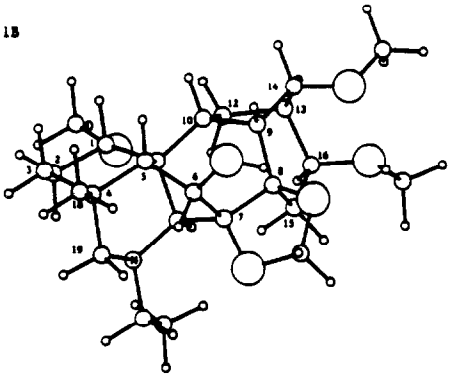


Figure 1D

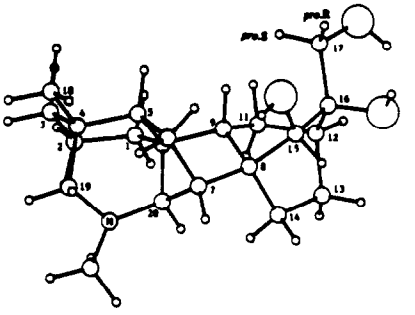


Figure 1 Perspective drawings from molecular mechanics calculations (A) Delpheline (**1**) with the A-ring in a chair and the D-ring in the boat conformation, (B) **1** with the D-ring in the half chair conformation predicted to be 3 3 kcal/mol higher in energy than the conformation shown in Figure 1A (D-ring in boat) (C) Minimum energy conformation of **2** with the A-ring in a chair and the D-ring in a boat conformation (D) Minimum energy conformation of **3** with the A-ring in a chair conformation and hydrogen bonding between the C-17 and C-16 hydroxyl groups

Conformational analyses

As a result of these nmr studies, the A-ring of all three alkaloids was indicated to exist predominantly in a chair conformation as was previously suggested for these diterpene alkaloids in the absence of a C-1α hydroxyl group In this latter case, hydrogen bonding between the C-1 hydroxyl proton and the nitrogen will favor the boat conformation for the A-ring [6,25] While the remaining rings of **3** are quite rigid, the D-ring of **1** and **2** can adopt a chair (more accurately, a flattened or half-chair) or a boat conformation As has been previously established for tatsidine [24], the boat conformation is preferred for the D-ring of both **1** and **2** Finally, the nOe's between the methyl of the *N*-ethyl group and H-19α in **1** and **2** indicate that this ethyl chain is oriented away from the α-face of the B-ring, as expected on the basis of steric considerations

Molecular modelling studies using the QUANTA/CHARMm program[26] supported these conclusions The dominant conformations of the A-rings of all three alkaloids were predicted to be the chair forms, while the D-rings of **1** and **2** in the boat conformations were clear energy minima (Figure 1) For delpheline (**1**), the conformation in which the D-ring adopts a half-chair form was also found as a global minimum and was predicted to be 3 3 kcal/mol higher in energy (in the absence of solvent interactions) than the conformation with the D-ring in the boat form as found by nmr (Figure 1) In the higher energy D-ring/chair conformation, the H-15β/H-16 dihedral angle was predicted to be 87 8°, while the H-15α/H-16 dihedral angle was predicted to be 21 53° Clearly this former dihedral angle is incompatible with the observed coupling between these two protons, $J_{15\beta,16}$ = 7 3 Hz The dihedral angles predicted for the lower energy D-ring/boat conformation were more in accord with the observed couplings H-15β/H-16 dihedral angle of 152 85°, H-15α/H-16 dihedral angle of 39 74°, $J_{15\alpha,16}$ = 9 0 Hz A chair or half-chair D-ring conformation was not found for **2** It is also of interest to note that in both **1** and **2**, the minimum energy conformation shows an orientation of the methyl terminus of the *N*-ethyl chain pointing away from the B-ring, as expected from the nmr studies

Experimental

General Procedures

Delpheline **1** Tatsiensine, isolated from *D tatsienense*, was hydrolysed to afford 6-deacetyl tatsiensine which when hydrogenated gave delpheline, m p 217–219°, as described earlier [12] Delpheline has been also isolated in our laboratory from *D occidentale*, [27] and *D elatum* [28] 8,9-Methylenedioxylappaconitine **2** was prepared from lappaconitine as described earlier [17] Dictyzine **3** was obtained from the polar alkaloidal fractions of *D tatsienense* as previously described [16] The ^1H and ^{13}C nmr spectra were recorded on a Varian XL–400 (93 94 kG, 400 MHz for ^1H, 100 MHz for ^{13}C) Spectra recorded in $CDCl_3$ used the 7 24 ppm resonance of residual $CHCl_3$ and the 77 0 ppm resonance of $^{13}CDCl_3$ as internal references of ^1H and ^{13}C, respectively Spectra recorded in C_6D_6 used the 7 15 ppm resonance of residual C_6HD_5 and the 128 0 ppm resonance of 1-[^{13}C]-C_6D_6 as internal references for ^1H and ^{13}C, respectively Spectra recorded in CD_3OD used the 3 30 ppm resonance of residual CHD_2OD and the 49 0 ppm resonance of $^{13}CD_3OD$ as internal references for ^1H and ^{13}C, respectively

Nmr multipulse sequences

All nmr studies on **1** were run using 12 mg, on **2** using 12 mg, and on **3** using 5 mg of sample, (spherical 125μL nmr tube) All 1D and 2D pulse sequences were run using standard Varian software, version 6 1c, except the fixed evolution HETCOR experiment which was added to the sequence library according to Reynolds' program [19] The fixed evolution HETCOR experiment was

utilized to enhance the sensitivity for detecting correlations between methylene carbons and their one bond coupled, magnetically nonequivalent protons ^{13}C-Multiplicities were assigned with the DEPT experiment and ^{13}C assignments were completed using the fixed evolution HETCOR experiment for one bond heteronuclear couplings (^{1}H, ^{13}C), and the FLOCK and selective INEPT sequences for two and three bond heteronuclear couplings (^{1}H, ^{13}C) The evolution time in the fixed evolution HETCOR experiment was set at 19 ms with a refocusing interval of 23 8 ms [19] Selective INEPT experiments were recorded with the excitation and refocusing delays optimized for different coupling constants according to the formulae $\Delta 1 = 1/2J$ and $\Delta 2 = 1/3J$, respectively [23]

Molecular mechanics

Molecular modelling studies were performed using the QUANTA/CHARMm program on a Silicon Graphics work station The Boltzmann jump technique (to 3000°C) with subsequent minimization was applied to each conformation to confirm that the structure was in a global minimum

References

1 Pelletier, S W , Mody, N V , Joshi, B S , Schramm, L C in *Alkaloids Chemical and Biological Perspectives*, vol 2, ed by S W Pelletier, John Wiley & Sons, New York, **1984**, pp 205-462, Pelletier, S W , Joshi, B S in *Alkaloids Chemical and Biological Perspectives*, vol 7, ed by S W Pelletier, Springer-Verlag, New York, 1990, pp 297–564
2 Pelletier, S W , Keith, L H in *The Alkaloids*, vol 12, ed R H F Manske, Academic Press, New York, **1970**, pp 1-134
3 Joshi, B S , Wunderlich, J K , Pelletier, S W *Can J Chem* **1987**, *65*, 99
4 Jones, A J , Benn, M H *Can J Chem.***1973**, *51*, 486
5 Pelletier, S W , Mody, N V , Sawhney, R S , Bhattacharyya, J *Heterocycles* **1977**, *7*, 327
6 Pelletier, S W , Djarmati, Z *J Am. Chem Soc* **1976**, *98*, 2626
7 Pelletier, S W , Srivastava, S K , Joshi, B S , Olsen, J D *Heterocycles* **1985**, *22*, 331
8 Goodson, J A *J Chem Soc* **1943**, 139
9 Cookson, R C , Trevett, M E *J Chem Soc* **1956**, 3121
10 Carmack, M , Ferris, J P , Harvey, J , Magat, P L , Martin, E W , Mayo, D W *J Am Chem Soc* **1958**, *80*, 497
11 Edwards, O E , Marion, L , Palmer, K H *Can J Chem* **1958**, *36*, 1097
12 Pelletier, S W , Glinski, J A , Joshi, B S , Chen, S -Y *Heterocycles* **1983**, *20*, 1347
13 Salimov, B T , Abdullaev, N D , Yunusov, M S Yunusov, S Yu *Khim Prir Soedin.* **1978**, 235
14 Tashkhodzhaev, B *Khim Prir Soedin* **1982**, 230
15 Deng, W , Sung, W L *Heterocycles* **1986**, *24*, 869
16 Zhang, X , Snyder, J K , Joshi, B S , Glinski, J A , Pelletier, S W *Heterocycles*, in press
17 Joshi, B S , Desai, H K , Pelletier, S W , Snyder, J K , Zhang, X , Chen, S -Y *Phytochemistry* **1990**, *29*, 357
18 (a) Eich, G , Bodenhausen, G , Ernst, R R *J Am Chem Soc* **1982**, *104*, 3731 (b) Bax, A , Drobny, G *J Magn Reson* **1985**, *61*, 306
19 (a) Reynolds, W F , Hughes, D W , Perpick-Dumont, M , Enriquez, R G *J Magn Reson* **1985**, *64*, 304 (b) Perpick-Dumont, M , Reynolds, W F , Enriquez, R G *Magn Reson Chem* **1988**, *26*, 358
20 Reynolds, W F , McLean, S , Perpick-Dumont, M , Enriquez, R G. *Magn Reson. Chem* **1989**, *27*, 162
21 (a) Kessler, H , Griesinger, C , Zarbock, J , Loosli, H R *J Magn Reson* **1984**, *57*, 331 (b) Kessler, H , Bermel, W , Griesinger, C *J Am Chem Soc* **1985**, *107*, 1083
22 Bax A , Summers, M F *J Am Chem Soc* **1986**, *108*, 2093
23 Bax, A *J Magn Reson* **1984**, *57*, 314
24 We've assumed that it would be difficult to observe nOe's in our spectra across distances of more than 3 5Å given the large number of alternative relaxation pathways available for most protons and the relative rigidity of the molecule resulting in correlation times dominated by isotropic tumbling
25 Snyder, J K , Zhang, X , Joshi, B S , Pelletier, S W *Magn Reson Chem* **1989**, *27*, 1057
26 Polygen Corporation, 200 Fifth Avenue, Waltham, MA 02254
27 Kulanthaivel, P , Pelletier, S W , Olsen, J D *Heterocycles* **1988**, *27*, 339
28 Pelletier, S W , Ross, S A , Kulanthaivel, P *Tetrahedron* **1989**, *45*, 1887