Carbon-13 Nuclear Magnetic Resonance: Aconitine-Type Diterpenoid Alkaloids from *Aconitum* and *Delphinium* Species

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Abstract: The ^{13}C NMR spectra of the aconitine-type diterpenoid alkaloids aconitine, mesaconitine, deoxyaconitine, delphinine, chasmanine, delphisine, neoline, condelphine, isotalatizidine, as well as 17 related derivatives, have been obtained by the Fourier transform technique at 25.03 MHz. The signal due to each carbon atom has been assigned, and some previously published assignments in the alkamine, delphonine, and the alkaloid, isotalatizidine, are corrected. The spectra are analyzed in terms of substituent effects. On the basis of chemical correlation of chasmanine-neoline-delphisine, and similar values for the resonances in the ^{13}C spectra of chasmanine, deoxyaconitine, delphinine, and delphonine, especially in the case of the ring A carbons, the configuration for the methoxyl at C-1 in chasmanine has been revised to an α-equatorial methoxyl group. The results of a study of the "pyrodelphonine chromophore" in the electronic ground state of the molecule, using ^{13}C and ^{1}H NMR techniques, are presented.

Introduction

Because the ^{13}C nuclear magnetic resonance (^{13}C NMR) spectra of organic molecules are markedly affected by changes in the steric and electronic environment, we have used this technique to examine some of the aconitine-type diterpenoid alkaloids from *Aconitum* and *Delphinium* species.[1] In contrast with other methods, ^{13}C NMR studies are faster and the conclusions are more reliable than those obtained from ir and proton NMR studies, provided that ^{13}C spectra of closely related compounds are available. Moreover, such spectra can be obtained easily for compounds which are not available in crystalline form. Jones and Benn[2] made a preliminary contribution in the ^{13}C NMR spectrometry of diterpenoid alkaloids, and on the basis of ^{13}C NMR data assigned structures for two newly isolated unknown bases. The significance of ^{13}C NMR data for the comparison of synthetic and naturally occurring substances or degradation products, especially in the case of the complex aconitine-type alkaloids, is obvious. Many of these natural products were isolated many years ago; however, the correct structures have been assigned only recently.[3,4] Even "identical ir spectra and x-ray powder patterns" have been shown to be unsafe for the establishment of identity of compounds (e.g., lycoctonine-browniine-chasmanine-neoline).[4] Similarly, small amounts of impurities may strongly influence ir spectra, leading to the incorrect conclusion about the identity of compounds (e.g., aconitine-hypaconitine).[5] ^{13}C NMR is a more powerful tool than proton NMR and ir since most of the carbon atoms in diterpenoid alkaloids give rise to separate signals. For example, 24 separate signals were resolved in the spectrum of natural neoline (**16**) ($C_{24}H_{39}NO_6$) and showed identity (±0.05 ppm) with the ^{13}C spectrum of a sample of neoline derived from the alkaloid delphisine.[4] On the basis of this observation we revised the structure of neoline. This work concerns the application of ^{13}C spectroscopy to diterpene alkaloids with special attention being given to chemical shift data.

Experimental Section

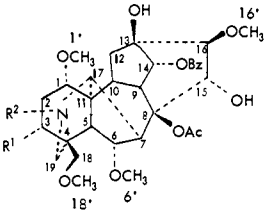
Carbon-13 spectra were determined at 25.03 MHz in the Fourier mode using a JEOL PFT-100 spectrometer in conjunction witl an EC-100 20K memory computer. The spectrometer features a deuterium lock system, a JNM-SD-HC random noise (2500 Hz bandwidth) proton decoupler, and JNM-DP-1 digital pulse programmer. Spectra of the compounds were determined in deuteriochloroform solutions (which also provided the lock signal) with 5% Me_4Si added as internal reference. All samples were contained in precision ground 10-mm o.d. tubes. The spectrometer was used in the crosscoil configuration. On the average, a 12 μs pulse, corresponding to an approximate tilt angle of 45°, was employed. For the average spectral width of 5000 Hz the delay between pulses was 3 s. Acquisition times averaged 1–2 h over 8K data points for concentrations of the order of 0.5–0.8 M. For off-resonance spectra this time was 4–8 h.

The bases were purified and/or synthesized by procedures given in the literature cited.

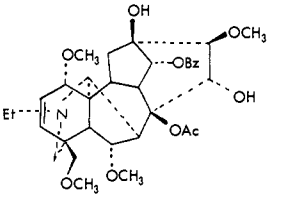
Results

The general procedure for carbon-13 data acquisition and initial interpretation involved determination of (a) the noise decoupled ^{13}C NMR spectrum, and (b) the single frequency off-resonance decoupled spectrum. The multiplicities generated in (b) enable distinction between the methyl (quartet), methylene (triplet), and methine (doublet) resonances. Having established the degree of substitution for the individual carbon resonances, assignments were made on the basis of chemical shifts calculated using additivity relationships and confirmed where possible by comparing the effects of specific structural changes.

Assignments for Aconitine Group Bases (Table I). Aconitine (1),[6] Mesaconitine (2),[6] Anhydroaconitine (3),[7] and Deoxyaconitine (4).[7] Oxygen substitution of quaternary C-8 and C-13 in bases **1–4** differentiates these carbons from C-4 and C-11. In delphinine (**5**) a C-15 oxygen function is absent resulting in the expected upfield shifts on C-8 (85.4 ppm) compared with **4** (92.0 ppm). Consequently, the resonance at 74.1 ± 0.2 in bases **1–4** is assigned to C-13. The quaternary resonances at 40.9 and 39.0 ppm in **3** and **4** may be assigned to c-4 since these are shifted downfield in **1**



1, R^1 = OH, R^2 = Et
2, R^1 = OH, R^2 = Me
4, R^1 = H, R^2 = Et



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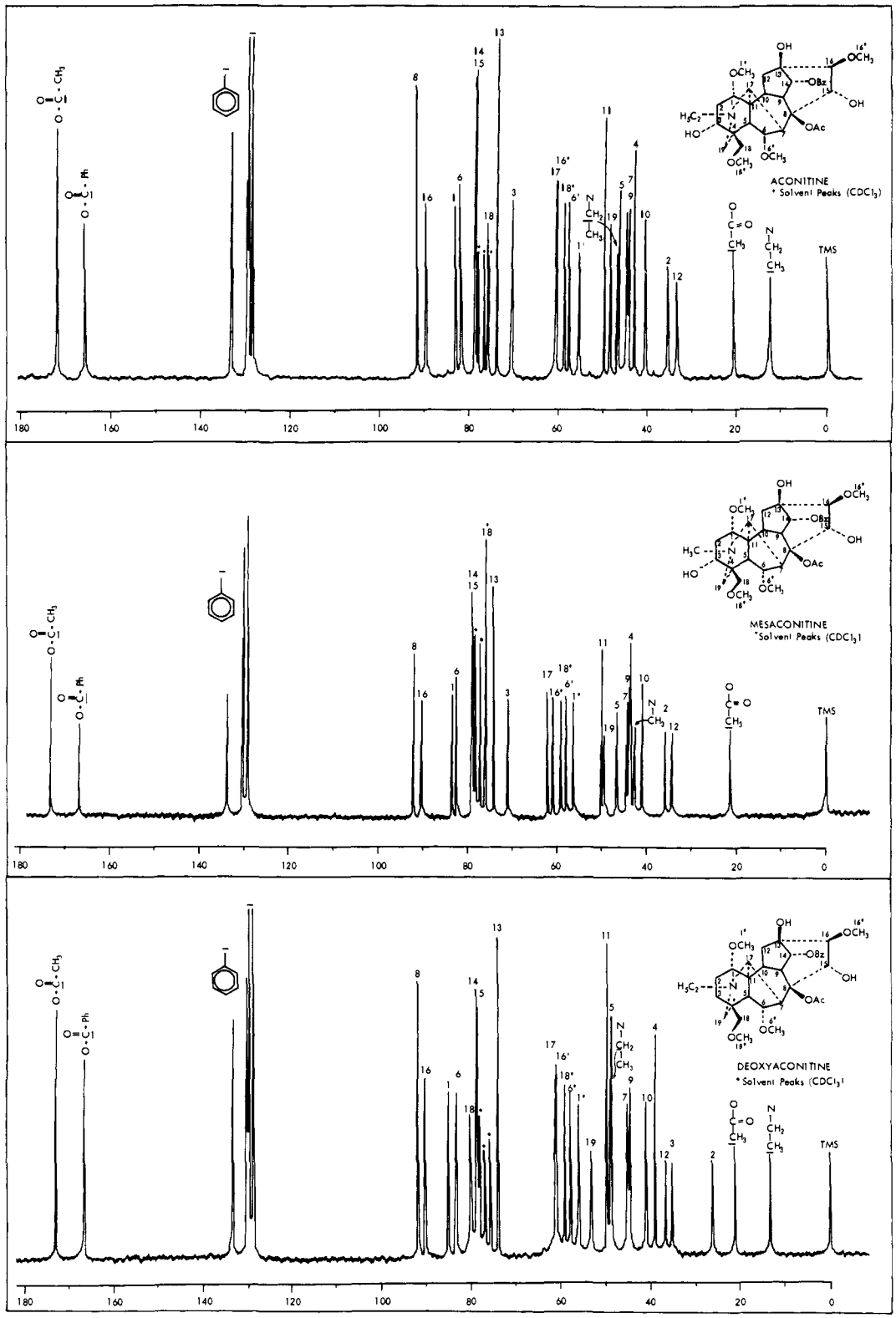


Figure 1. ^{13}C spectra of aconitine, mesaconitine, and deoxyaconitine.

(43.2 ppm) and **2** (43.5 ppm) as a result of the β-hydroxy effect. The structural features at the quaternary center C-11 are similar in bases **1–4** and the resonance at 49.3 ± 0.7 ppm is assigned to this carbon (see Figure 1).

The methylene carbon resonance at 75.6 ppm in aconitine (**1**) and similar low-field resonances at 75.8, 78.5, and 80.2 ppm in bases **2–4** are assigned to C-18, which represents the only oxygen substituted methylene in the molecule. The other remaining methylene carbons may be considered as of the *N*-alkyl type (C-19 and $N-CH_2$), which are predicted to resonate at lower field than the cycloalkane type (C-2, C-3, and C-12). Consequently, the methylene resonance at 48.8 and 46.9 ppm in **1** is assigned to C-19 and $N-CH_2$ on the basis that the higher field resonance disappears in the spectrum of **2** when *N*-methyl group replaces the *N*-ethyl group. The resonances at 34.0 ppm in **1** and 34.2 ppm in **2** are assigned to C-12 on the basis of comparison with the only cycloalkane-type methylene resonance at 34.2 ppm in **3**. The highest field methylene carbon resonance in **4** at 26.3 ppm is assigned to C-2 on the basis that

Table I. Carbon-13 Chemical Shifts and Assignments for Aconitine Group Bases[a]

Carbon	1	2	3	4
1	83.4	83.2	83.9	85.0
2	36.0	35.9	125.3	26.3
3	70.4	70.8	137.6	35.2
4	43.2	43.5	40.9	39.0
5	46.6	46.5	47.5	49.1
6	82.3	82.4	81.3	83.3
7	44.8[b]	44.3[b]	42.6[b]	45.1[b]
8	92.0	91.8	92.5	92.0
9	44.2[b]	43.8[b]	44.1[b]	44.6[b]
10	40.8	40.9	41.2	41.0
11	49.8	50.0	48.7	49.9
12	34.0	34.2	34.2	36.7
13	74.0	74.1	74.3	74.0
14	78.9	78.9	79.1	78.8[c]
15	78.9	78.9	79.1	79.0[c]
16	90.1	90.1	89.9	90.2
17	61.0	62.2	59.2	61.2
18	75.6	75.8	78.5	80.2
19	48.8	49.4	52.2	53.3
N—$CH_{2(3)}$	46.9	42.4	48.1	49.1
CH_3	13.3		12.6	13.4
1′	55.7	56.2	56.0	56.0
6′	57.9	57.9	57.9	57.9
16′	60.7	61.0	61.2	60.9
18′	58.9	59.0	59.0	59.0
C=O	172.2	172.3	172.2	172.2
CH_3	21.3	21.4	21.4	21.3
C=O	165.9	166.0	165.9	165.9
C_6H_5	129.8	129.9	130.0	129.9
	129.6	129.6	129.6	129.5
	128.6	128.6	128.6	128.5
	133.2	133.2	133.2	133.1

[a] Chemical shifts in ppm downfield from Me_4Si. Solvent deuteriochloroform. [b,c] Values in any vertical column may be interchanged.

hydroxyl substitution at C-3 in **1** produced the expected β shift (9.7 ppm). The remaining two methylene carbon resonances at 35.2 and 36.7 ppm in **4** can be assigned to C-3 and C-12 compared to those observed in the delphinine group bases [e.g., chasmanine (**14**)], since the structural features about the C-3 methylene center are similar.

The methine carbon resonances can be classified in two groups; a low-field group between 90 and 70 ppm, and a high-field group between 50 and 40 ppm. The resonance at 60.7 ± 1.5 ppm in **1–4** is assigned to C-17 (the only methine which is N-substituted).

The low-field group consists of hydroxyl- or methoxyl-substituted methine resonances. Methine carbons C-6, C-14, C-15, and C-16 in compounds **1–4** have relatively constant chemical shifts despite large structural changes and are assigned on the basis of the following observation: the lowest methine resonance of 90.0 ± 0.2 ppm in **1–4** corresponds to C-16. Both the neighboring C-15 and C-13 are hydroxy substituted, which can explain the low shift (double β-hydroxy effect). However, the β-hydroxy effects are not equal. The difference (6.5 ppm) in shift for C-16 in **4** and **5** is due to the β-hydroxy effect of the C-15 hydroxy group. The shift caused by the 13-OH group is much smaller, 1.5 ppm (comparing **5** with **14**). The resonance at 82.3 ± 1.0 ppm is assigned to C-6 because of its positional invariance in the bases studied (**1–4**). Two doublets exhibited by C-14 and C-15 methine carbons show virtual identity in position in the bases **1, 2** (78.9 ppm), and **3** (79.1 ppm), and because the same signals are only separated by 0.2 ppm in **4,** the assignments must remain tentative in this compound. The two remaining doublets are assignable to C-1 and C-3. One doublet in compounds **1** and **2** occurs at a similar position (70.4 and 70.8 ppm) which disappears (moved upfield, α-hydroxyl effect 35.6 ppm) in the spectrum of base **4,** and can be assigned to C-3. Consequently, the remaining doublets at 83.4, 83.2, 83.9, and 85.0 ppm in **1–4,** respectively, are assigned to C-1.

The assignments of the remaining four methine resonances (the upfield group) is the most difficult, because bases with specific structural modifications near to these centers are not easily available. Three doublets have a similar position in **4** relative to **1** and on the basis of this observation, the doublet which is shifted from 46.6 ppm in **1** to 49.1 ppm in **4** may be assigned to C-5. The C-5 is the nearest to C-3 from which the hydroxyl group is removed (**1** → **4**). A similar downfield shift is observed for another carbon β- to C-3 (C-1, 1.6 ppm). The resonance at 41.0 ± 0.2 ppm may be assigned to C-10 since the structural features of this center in the bases **1–4** are similar. However, methine signals for C-7 and C-9 are separated only by 0.6, 0.5, 1.5, and 0.5 ppm in **1–4,** respectively, and these assignments should be regarded as tentative.

Four different types of methyl carbons, acyl, *O*-methyl, *N*-methyl, and *N*-ethyl, on the one hand, and highly constant chemical shifts despite large structural changes, on the other hand, made the chemical shift assignments straightforward for methyl carbons. The methyl resonance of 21.3 ± 0.1 ppm in **1–4** can be assigned to an acyl-type methyl. The narrow range (±0.3 ppm) of chemical shifts exhibited by methoxyl carbons of 56.0 ppm in aconitine (**1–4**) and delphinine-type bases (**5, 6, 8, 9, 13–15**) suggests assignment to the C-1 methoxyl. The methyl resonance at 59.1 ± 0.2 ppm in aconitine-type (**1–4**), delphinine-type (**5–15**), and neoline-type (**16–27**) bases and the absence of this resonance in bases A and B examined by Jones[2] (where C-18 is a methyl at 27.4 ppm) suggest the assignment of this resonance to C-18′. A C-6 methoxyl group is common to aconitine (**1–4**), delphinine (**5–8, 10–13, 15**), and neoline-type bases (**16–25**), except for condelphine (**26**) and isotalatizidine (**27**), and the methyl resonance at 57.9 ± 0.3 may be assigned to this carbon. Consequently, the methyl resonance at 61.0 ± 0.3 in the bases **1–4** may be assigned to C-16′. In the aconitine group bases, the only *N*-methyl group is present in the alkaloid mesaconitine (**2**) and resonance at 42.4 ppm may be assigned to this carbon. In the bases **1, 3,** and **4** the nitrogen is ethyl substituted and methyl resonance at 13.0 ± 0.4 ppm corresponds to the methyl carbon of the *N*-ethyl group.

The assignments for the aromatic carbons of the benzoxy group attached at C-14 can be easily determined by comparison of observed resonances at 130.0 ± 0.2 (singlet), 129.6 ± 0.1 (twin doublet), 128.6 ± 0.1 (twin doublet), and 133.2 ± 0.1 ppm (doublet) in **1–4** with the values observed for methyl benzoate: 130.3 for C-1, 129.5 for ortho, 128.3 for meta, and 132.8 ppm for the para carbon.[8] The acetyl and benzoyl carbonyl carbons are found in well-defined regions of the ^{13}C spectrum,[9] and resonances at 172.2 ± 0.1 and 166.0 ± 0.1 ppm in **1–4** may be assigned to these carbons, respectively.

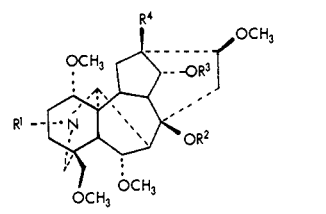
Assignments for Delphinium Group Bases (Table II). All carbons in ring A as well as C-6, C-6′, C-14, C-17, C-18, C-18′, C-19, *N*-methyl, acetoxy, and benzoxy were assigned by comparison of their ^{13}C spectra with that of the aconitine group bases (**1–4**). These assignments were also supported by the single frequency off-resonance decoupled (SFOCD) spectra (see Figure 2).

(a) **Delphinine (5)[10] and Delphinine 13-Acetate (6).[11]** The two unassigned singlets in compound **5** (C-8 and C-13) are recognized in the SFOCD spectrum at 85.4 and 74.8 ppm, respectively. Acetylation of the C-13 OH group shifts the C-13 resonance in **6** downfield (82.0 ppm) as a result of the greater electron-withdrawing power of the acetoxy group

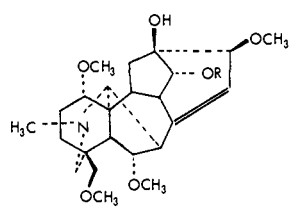
Table II. Carbon-13 Chemical Shifts and Assignments for Delphinine Group Bases[a]

Carbon	5	6	7	8	9	10	11	13	14	15
1	84.9	84.7	86.1	86.0	85.1	86.6	86.1	85.7	86.1	85.4
2	26.3	26.3	25.3	25.2	26.2	26.4	26.0	25.9	26.0	26.3
3	34.7	35.3	35.3	35.2	34.9	35.1	34.3	34.9	35.2	35.2
4	39.3	39.2	40.0	40.0	39.6	39.6	39.4	39.5	39.5	39.0
5	48.8	48.5	48.5	49.4	48.8	48.7	47.9	49.4	48.8	48.4
6	83.0[c]	83.1	83.6	83.5[c]	82.3	84.9	84.6	82.3	82.5[c]	84.1[c]
7	48.2	48.0	50.4	50.5	44.6[c]	44.6	44.4	51.5	52.8	48.4
8	85.4	85.3	146.6	148.7	83.7	40.1	—	72.8	72.6	78.3
9	45.1	43.6	47.6	47.9	44.2[c]	42.0	41.7	50.4	50.3	45.6
10	41.0	41.7	46.7	47.6	42.2	40.8	40.8	42.3	38.4	38.1
11	50.2	50.3	51.9	51.8	50.1	50.9	50.9	50.2	50.4	50.9
12	35.7	34.8	38.4	37.2	39.2	42.9	42.8	36.4	28.6	30.2
13	74.8	82.0	77.7	77.0	76.0	77.2	77.1	76.7	45.7	46.1
14	78.9	77.5	79.1	79.5	78.2	77.7	77.6	79.3	75.5	83.6[c]
15	39.3	39.2	116.3	114.3	137.4	134.3	134.7	40.3	39.2	34.7
16	83.7[c]	80.0	83.6	83.8[c]	125.2	128.7	128.3	84.4	82.2[c]	83.6[c]
17	63.3	63.2	78.6	77.5	64.4	62.1	62.6	63.5	62.4	60.8
18	80.2	80.1	80.3	80.3	80.5	80.6	80.4	80.6	80.8	80.4
19	56.1	56.1	56.5	56.6	56.1	56.4	56.7	56.2	54.0	54.3
N—CH_3(2)	42.3	42.4	42.7	42.6	42.5	42.4	42.4	42.3	49.3[b]	48.9[b]
1′	56.1	56.1	56.5	56.3	56.2	56.4	56.4	56.2	56.3	56.3
6′	57.6	57.7	58.1	58.1	57.2	57.8	57.9	57.8	57.2	57.6
16′	58.6	58.0	57.1	56.6	—	—	—	57.2	55.9	56.1
18′	58.9	59.0	59.2	59.1	59.1	59.2	59.2	59.1	59.2	59.1
C=O	169.4	170.0			169.5					58.5 (14′)
|		169.4								48.0 (8′)
CH_3	21.4	21.2			21.6					
		21.4								
C=O	166.0	166.0	168.0		166.7					
|	130.4	130.1	130.5		130.1					
C_6H_5	129.6	129.8	130.0		129.7					
	128.4	128.4	128.1		128.4					
	132.8	133.0	132.7		133.1					

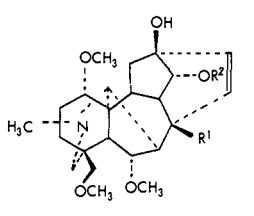
[a] In ppm downfield relative to Me_4Si. Solvent deuteriochloroform. [b] The ^{13}C methyl resonance in *N*-ethyl of chasmanine (14) and 8,14-di-*O*-methylchasmanine (15) occurs at 13.6 and 13.4 ppm, respectively. [c] Values within any vertical column may be interchanged.



5, R¹ = Me, R² = Ac, R³ = Bz, R⁴ = OH
6, R¹ = Me, R² = Ac, R³ = Bz, R⁴ = OAc
14, R¹ = Et, R² = R³ = R⁴ = H
15, R¹ = Et, R² = R³ = CH_3, R⁴ = H



7, R = Bz
8, R = H,



9, R¹ = OAc, R² = Bz
10, R¹ = R² = H
11, R¹ = D, R² = H

relative to the hydroxyl group. It has been observed that the carbons nearest the hydroxyl groups can be readily identified from chemical-shift changes attendant acetylation as a result of steric interaction with the acyl group. The resonance of carbons 12, 14, and 16 was shifted upfield 0.9, 1.4, and ~3 ppm in compound **6** as compared with **5**, as the result of steric effect caused by replacing hydrogen with acetyl on C-13 OH. However, the C-6 and C-16 resonances in **5** are separated only by 0.7 ppm; therefore, the assignments given in Table II are only tentative. The triplets in SFOCD spectra of **5** and **6** at 39.3 and 39.2 ppm, respectively, are new (compared to **4**) and can be assigned to C-15. The three remaining unassigned doublets in **5** at 48.2, 45.1, and 41.0 ppm must be assigned to C-7, C-9, and C-10, respectively. C-7 should be affected by the removal of the C-15 OH of deoxyaconitine (**4**)[12] in contrast with C-9 and C-10 which are unaffected. Similarly, the resonances at 48.0, 43.6, and 41.7 ppm in **6** are assigned to C-7, C-9, and C-10, respectively.

(b) Pyrodelphinine (7)[13] and Pyrodelphonine (8).[13] The two new (compared to **5**) low-field resonances at 116.3 and 146.6 ppm in **7** and 114.3 and 148.7 ppm in **8** are singlets and doublets in SFOCD spectra and can be assigned to C-8 and C-15, respectively. Most unusual are the large downfield shifts at C-17, 15.3 ppm in **7**, and 14.2 ppm in **8** (compared with **5**) caused by the C-8–C-15 double bond (will be discussed in more detail later). Because C-13 represents the only oxygen-substituted quaternary carbon in **7** and **8**, the resonances at 77.7 and 77.0 ppm must be assigned to this carbon. The C-6 and C-16 resonances in **7** are at 83.6 ppm and because they are separated only by 0.3 ppm in **8**, assignments for these carbons are only tentative.

(c) 8-Acetoxydemethoxyisopyrodelphinine (9),[13] Demethoxyisopyrodelphonine (10),[13] and 8-Deuteriodemethoxyisopyrodelphonine (11).[14] Isomerization of the double bond between C-8 and C-15 in **7** to give **9** (with C-15, C-16) results in several chemical-shift changes. Again the C-17 resonances shows the most unusual upfield shift (14.2 ppm), which proves that the lower chemical shift for this carbon in **7** is caused by the C-8–C-15 double bond. The 8-acetoxy group is absent in **10**, causing the resonance for C-15 to move upfield (3.1 ppm relative to **9**). Consequently, the other low-field doublet at 125.2 ppm in **9** which is moved downfield (3.5 ppm) in **10** can be assigned to C-16. The

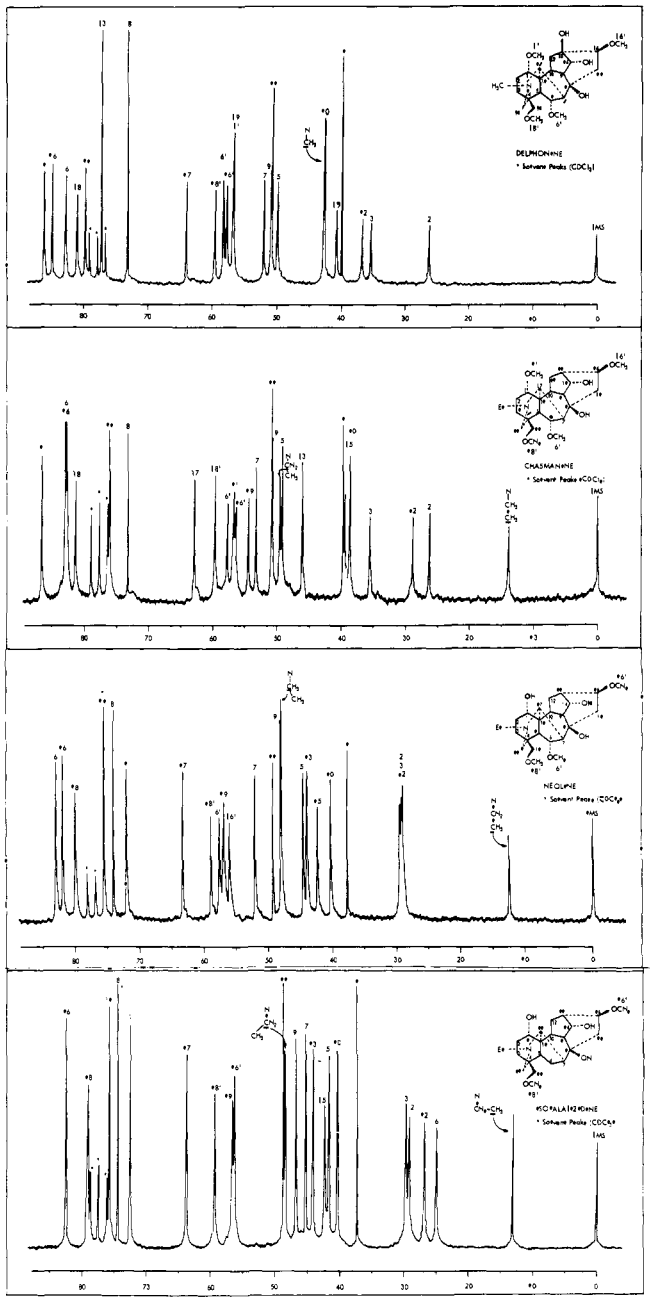
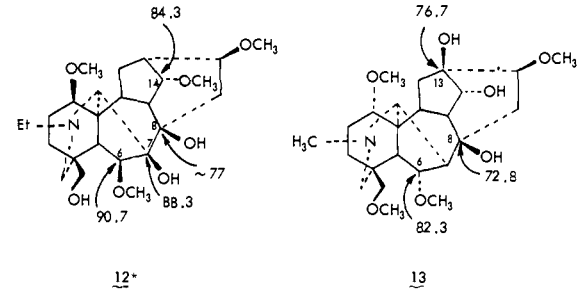


Figure 2. ^{13}C spectra of delphonine, chasmanine, neoline, and isotalatizidine.



*Data from ref. 2.

doublet at 40.1 ppm in **10** is assigned to C-8 on the basis of comparison with **11** where this carbon is deuterated and the C-8 signal in the ^{13}C spectra is absent.

(d) Delphonine (13),[10] Chasmanine (14),[4] and 1,8,14-Tri-*O*-methylneoline (15).[15] In contrast to **5** and **6** the determination of assignments of C-8 and C-13 in **13** is somewhat more complex. The ^{13}C spectra of **13** were first studied by Jones,[2] and the quaternary resonances at 76.9 and 72.8 ppm were assigned to C-8 and C-13, respectively, on the basis of comparison with the spectra of lycoctonine (**12**) type bases. The argument which has been used is the following: the low-field quaternary carbon resonances at 88.3 and ~77 ppm in compound **12** are assigned to C-7 and C-8, respectively. In **13** the "new" quaternay resonance at 72.8[16] ppm is assigned[2] to C-13. This cannot be correct, however, because the C-8 resonance in **12** and **13** is expected to be different ("new") as a result of the β-hydroxy effect (~4–8 ppm) of the C-7 hydroxy group in **12** which is absent in **13**. Our assignments for delphonine were derived by comparison with values of the alkaloid chasmanine (**14**). In **14** only one low-field quaternary resonance was observed at 72.6 ppm which is assigned to C-8 in this molecule. Since the structural features at the quaternary center C-8 are similar in the bases **13** and **14**, the resonance at 72.8 ppm in **13** is assigned to this carbon. Consequently, the remaining low-field singlet at 76.7 ppm in **13** can be assigned to C-13. The fact that the resonance at 72.8 ppm disappears after pyrolysis of delphinine (**5**) to the pyro compound (**7**), and hydrolysis of the latter to **8**, supports these assignments. The C-8 and C-13 resonances assigned by Jones[2] are not the only ones in **13** which should be corrected. In this place, however, we will discuss only the most important carbon assignments. Thus resonances in **13** at 79.3, 80.6, 82.3, 84.4, and 85.7 ppm are assigned[2] to C-18, C-6, C-1, C-14, and C-16, respectively. A careful study of the SFOCD spectrum of **13** has shown the signal at 79.3 ppm is a doublet, and that at 80.6 ppm is a triplet. Since C-18 represents the only oxygen substituted methylene in the molecule, the resonance at 80.6 ppm must be assigned to this carbon. The resonance at 85.7 ppm is assigned on the basis of comparison of **13** with **14** and **16** to C-1. The only difference in the carbons in compounds **14** and **16** is at C-1, which bears a methoxy and hydroxy substituent, respectively. The constant chemical shift for C-14 in compounds **1–11** (78.5 ± 1 ppm) and **14, 16–24,** and **27** (75.5 ± 0.5 ppm) suggests the assignment of the 79.3 ppm resonance in **13** to C-14. The remaining low-field methine resonances in **13** at 84.4 and 82.3 ppm are assigned to C-16 and C-6 on the basis of comparison with **14** where the C-13 hydroxy group is absent. This absence causes a small upfield shift (2.2 ppm) on C-16. However, the C-6 and C-16 resonances in **14** and **15** are separated only by 0.3 and 0.5 ppm, respectively, and therefore, the assignments given in Table II are tentative.

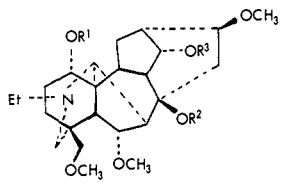
All of the assignments in **15** except C-7, C-8, C-9, C-14, and C-15 were made by comparison with **14.** The two hydroxyl groups (C-8 and C-14) in **14** are changed to methoxyl, producing an expected downfield shift of 8.1 ppm for the secondary carbinol carbon (C-14) and a smaller shift of 5.7 ppm for the tertiary (C-8). The β effects for substitution of methoxyl for hydroxyl have been reported as negative (upfield shift).[17] The C-7, C-9, and C-15 resonances in **15** were found in a higher field (4.4, 4.7, and 4.5 ppm), respectively, relative to **14.** The large difference (~10 ppm) exhibited by the primary and secondary methoxyl carbons in contrast to tertiary methoxyls can be very useful for investigation of the natural products which contain these groups.[17] Thus the C-14 methoxyl resonance in **15** was found at 58.5 ppm and the C-8 methoxyl resonance at 48.0 ppm.

Neoline Group Bases Assignments (Table III). (a) Neoline (16)[18] and 1-*epi*-Neoline (17).[18] The following carbons were assigned directly from the assignments in chasmanine (**14**): C-4, C-6, C-7, C-8, C-9, C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17, C-18, C-19, *N*-ethyl, and *O*-methyls. In compound **16,** C-1 of the ring A is α-hydroxy substituted and exerts a downfield shift (β effect) on C-2 (~3.5 ppm) in comparison with compound **14** where the group at C-1 is an

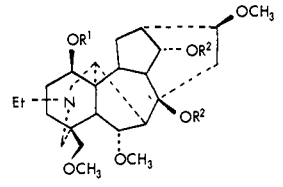
Table III. Carbon-13 Chemical Shifts and Assignments for the Neoline Group Base[a]

Carbon	16	17	18	19	20	21	22	23	24	25	26	27
1	72.3	69.0	72.0	72.1	68.6	77.5	72.6	212.7	213.8	213.2	72.1	72.3
2	29.5[b]	30.3	29.5[b]	29.5[b]	30.3	27.9	27.2	41.5	41.4	41.5	29.1[b]	29.2[b]
3	29.9[b]	31.2	29.9[b]	30.1[b]	31.2	34.6	32.0	38.4	38.8	38.9	29.7[b]	29.7[b]
4	38.2	40.1	38.2	38.1	39.4	39.0	39.1	39.4	39.5	39.5	37.3	37.3
5	44.9	39.7	46.1	44.1	39.2	49.3	39.3	52.9	53.5	53.0	41.4	41.7
6	83.3	82.8	84.1	84.2	83.7	83.5	83.6	83.2	82.2	82.8	25.1	25.0
7	52.3	51.7	48.2	48.3	47.9	49.4	47.8	48.9	52.9	53.0	45.8	45.3
8	74.3	73.9	85.4	85.8	86.0	85.6	85.5	85.9	74.0	80.3	74.5	74.3
9	48.3	48.4	44.0	43.3	43.6	44.3	43.5	43.5	48.4	54.8	44.6	46.7
10	40.7	39.4	40.8	38.5	38.4	38.5	38.4	38.6	39.8	37.6	37.0	40.4
11	49.6	50.5	49.9	49.8	50.9	49.4	49.3	61.0	60.9	61.0	49.0	48.7
12	29.8[b]	28.9	29.5[b]	29.5[b]	29.3	29.5	29.3	34.2	33.5	31.9	26.7	26.8
13	44.3	45.5	44.0	43.3	44.7	44.1	45.4	39.1	40.4	47.2	43.5	44.1
14	75.9	75.7	75.0	75.5	75.5	75.0	75.2	75.6	75.8	216.5	76.9	75.6
15	42.7	42.1	38.4	38.5	38.2	37.7	38.1	39.2	42.3	41.8	42.4	42.3
16	82.3	82.4	82.4	82.7	83.1	83.1	82.8	82.8	82.2	86.8	82.2	82.4
17	63.6	63.3	63.0	62.7	62.3	60.7	62.2	63.2	64.1	64.0	63.5	63.7
18	80.3	80.4	79.8	79.8	80.0	80.1	79.9	78.7	79.2	79.0	79.0	79.0
19	57.2	53.8	56.8	56.8	53.6	54.3	53.6	54.7	55.0	54.8	56.6	56.6
N—CH_2	48.2	48.8	48.4	48.0	48.9	48.6	48.8	48.6	48.6	48.6	48.4	48.4
CH_3	13.0	13.5	12.7	12.9	13.2	13.5	13.1	13.3	13.4	13.4	13.0	13.0
6′	57.8	57.5	58.1	58.0	57.8	58.1	57.9	58.2	57.9	58.1		
16′	56.3	56.2	56.6	56.5	56.4	56.5	56.5	56.4	56.3	56.0	55.9	56.2
18′	59.1	59.2	59.1	59.0	59.0	59.1	59.1	59.1	59.2	59.2	59.3	59.3
						170.1	169.7					
C=O			169.9	169.3	169.4	169.3	169.2	169.4				
				170.4	170.4	170.5	170.4	170.4			170.3	
						22.0	22.3					
CH_3			22.5	22.2	22.3	22.4	22.3	22.3				
				21.1	21.2	21.2	21.3	21.2			21.2	

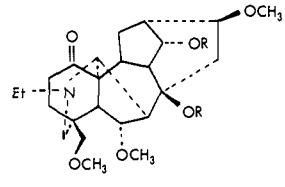
[a] In ppm downfield relative to Me_4Si. Solvent deuteriochloroform. [b] Values within any vertical column may be interchanged.



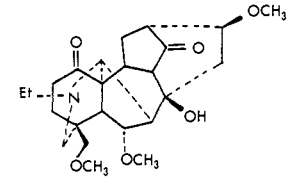
16, $R^1 = R^2 = R^3 = H$
18, $R^1 = R^3 = H$, $R^2 = Ac$
19, $R^1 = H$, $R^2 = R^3 = Ac$
21, $R^1 = R^2 = R^3 = Ac$



17, $R^1 = R^2 = H$
20, $R^1 = H$, $R^2 = Ac$
22, $R^1 = R^2 = Ac$



23, R = Ac
24, R = H



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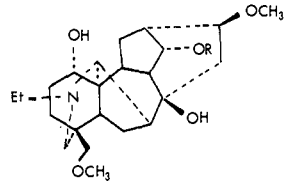


26, R = Ac
27, R = H

α-methoxy group. It has been reported[19] that the β effect produced by replacing the hydroxy group in cholesterol with methoxyl is 4.0 ppm, and the γ effect was reported as small (0.6 ppm). In the case of **16** → **14** the γ effect (on C-3) is 5.3 ppm and can be explained only by a change of conformation of ring A from boat in **16** to chair in **14** (see Discussion). An almost identical γ effect (on C-3) was observed (4.7 ppm) upon acetylation of the C-1α OH (see compound **21**). The other γ carbon (relative to C-1) C-5 resonance in **16** was found 3.9 ppm more upfield relative to **14**, or 4.4 ppm relative to **21**.[20] The resonances for C-2, C-3, and C-12 in **16** are separated only by 0.4 ppm, and the assignments must remain tentative for this compound. The C-1 resonance in **17** (C-1β OH) is at higher field (3.3 ppm) relative to **16**, and the difference is a little small in comparison with the reported difference exhibited by the epimeric 4-*tert*-butylcyclohexanols.[19]

(b) Neoline 8-Acetate (18),[18] Delphisine (Neoline 8,14-Diacetate) (19),[18] 1-*epi*-Delphisine (20),[18] Delphisine 1-Acetate (21),[18] and 1-*epi*-Delphisine 1-Acetate (22).[18] All of the assignments in **18** except C-7, C-8, and C-15 were made by comparison with **16**. The substitution of an acetoxy for a hydroxy group on C-8 produced an expected downfield shift (11.1 ppm α effect). The neighboring carbons (to C-8) C-7, C-9, and C-15 in **18** are shifted upfield 4.1, 4.3, and 4.3 ppm (β effect), respectively, relative to **16**. Acetylation of the second hydroxy group at position C-14 in **16** has almost no effect on the chemical shifts and the assignments for **19** were made by comparison with **18**. Compounds **19** and **20** are different only in configuration of the C-1 OH group (same as **16** and **17**), and once again it was found that C-1 with a quasi-equatorial hydroxy group is at lower field (72.1 ppm) than the carbon with an axial hydroxyl (68.6). Similarly, a difference of 4.9 ppm was found between the epimeric C-1 acetates **21** and **22**.

(c) 1-Ketodelphisine (23),[18] 1-Ketoneoline (24),[18] and 1,14-Diketoneoline (25).[18] The introduction of a keto group at C-1 (**23**) results in a number of chemical-shift changes. The resonance for C-1 is shifted to the ketone carbonyl region (212.7 ppm); C-11 and C-2 moves downfield 11.2 and 12.0 ppm, respectively, as expected, relative to **19**. The signal for C-3 also moves downfield 8.3 ppm; this is a larger

Table IV. High-Field Methine Resonances[a]

Carbon	Compounds					
	13	14	24	25	16	27
5	49.4	48.8	53.5	53.0	44.9	41.7
7	51.5	52.8	52.9	53.0	52.3	45.3
9	50.4	50.3	48.4	54.8	48.3	46.7
10	42.3	38.4	39.8	37.6	40.7	40.4
13		45.7	40.4	47.2	44.3	44.1

[a] In ppm downfield relative to Me_4Si.

γ-keto effect than that observed in the case of 3-cholestanone relative to cholestanol.[19] The δ-keto effect (4.7 ppm) on C-12 is probably a result of the steric interaction with the carbonyl group. The assignments for **24** were made on the basis of comparison with **23,** with the exception of the chemical-shift changes caused by the C-8 and C-14 acetoxy groups which were discussed earlier (see above). The resonance at 216.5 ppm in **25** is assigned to C-14 by comparison with **24,** and it is in agreement with reported differences between the resonances of six- and five-membered ketones.[19] The other effects on the chemical-shift changes caused by the C-14 keto group will be discussed below.

(d) Condelphine (26)[21] and Isotalatizidine (27).[21] A total of 21 out of 23 carbon resonances in **27** showed identity (±0.2 ppm or less) with those published by Jones.[2] The C-11 and N-CH_2 (from *N*-ethyl) are separated by only 0.3 ppm and the higher field resonance was assigned by Jones[2] to C-11. However, a careful study of the SFOCD spectrum of **27** has shown that the signal at 48.7 ppm is a singlet and that at 48.4 ppm is a triplet; these signals must thus be assigned to C-11 and N-CH_2, respectively. Most unusual are the large differences between the published values[2] and our assignments for C-14 (1.4 ppm) and C-8 (4.6 ppm). The only possibility in this situation can be an error in assignments. Thus resonances at 78.9 and ~77 ppm assigned by Jones[2] to C-8 and C-14, respectively, are solvent peaks ($CDCl_3$). The ^{13}C spectrum of **27** is presented in Figure 2, and assignments for the three solvent peaks can be easily made from signal intensity. The singlet at 74.3 ppm in the SFOCD spectrum of **27** is assigned to C-8 and the doublet at 75.6 ppm to C-14. The assignments for C-6 and C-15 in **27** should also be corrected. The resonance at 42.3 ppm is assigned to C-15 by comparison with **16** and the new resonance at 25.0 ppm to C-6.

The hydroxy group difference between **13** and **14,** the keto group difference between **24** and **25,** and the methoxyl difference between **16** and **27** are most valuable for high-field methine carbon assignments. From comparison of **13** and **14,** it is clear that the C-13 methine resonance in **14** is at 45.7 ppm, because the introduced hydroxy group in **13** at C-13 has no large effect on the C-5, C-7, C-9, and C-10 resonances. Two of the five low-field methine resonances in **24** (C-9 and C-13) have moved downfield in **25** as a result of the β effect of the C-14 keto group. In **27** the C-6 methoxy group is missing; thus by comparison with **16,** C-5 and C-7 can be distinguished from C-9, C-10, and C-13. From these comparisons we have been able to make satisfactory assignments to C-9, C-10, and C-13. The C-5 and C-7 resonances are assigned by comparison of **16** with **18** and **19** with **21** (see above). The individual low-field methine resonance assignments in the remaining bases studied were made on the basis of chemical-shift calculations using additivity relationships and confirmed where possible by comparing the effects of specific structural changes.

Discussion and Application

The similar pattern of carbon-13 shifts exhibited by the series of aconitine-type alkaloids investigated made possible self-consistent and reasonably unambigous assignment of nearly all the resonances for these compounds. A particularly important feature is the constant pattern of shifts exhibited by the quaternary carbons except where major stuctural or substitution changes occur. The different degree of oxygen substitutions in these molecules (5 in **27** compared to 9 in **1**) made possible the correct determination of cycloalkane type methylene and oxygen-substituted methine carbon assignments. In most cases the effects are of the correct order but in several instances their magnitudes differ from those expected on the basis of previous reports on simple systems, perhaps the consequence of possible electronic and steric contributions in the compact ring system of the diterpene alkaloid molecule.

Data presented in Table V demonstrate that the hydroxyl (for hydrogen), acetoxyl (for hydoxyl), and methoxyl (for hydroxyl) substituents at various positions in the alkaloid molecule (lycoctonine skeleton) cause the chemical shifts of the α and β carbons. The designations, α and β effects, refer to changes in chemical shifts caused by directly attached substituents on the α-carbon atom and those on the β-one, respectively.

The smaller α-hydroxy effect for tertiary alcohols relative to secondary alcohols is as expected.[22] The α effect caused by substitution of hydrogen at C-6 by methoxyl in **27** appears high (58.3), but substitution of H by OH results in a shift of 48 ppm and substitution of OH with OCH_3 causes an additional shift of ~10 ppm.[17]

The α and β effects for substituting acetoxy for hydroxy

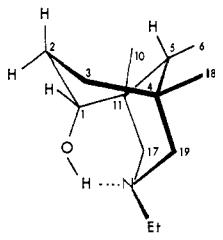
Table V. α and β effects Caused by Substitution of Hydroxyl for Hydrogen, Acetoxyl for Hydroxyl, and Methoxyl for Hydroxyl at Various Positions in Diterpene Alkaloids Possessing a Lycoctonine Skeleton[a]

Compared compounds	α-Effect (C-*i*)[b]	β-Effect (C-*i*)[b]		
4-H → 1α-OH	35.2 (C-3)	9.7 (C-2);	4.2 (C-4)	
10-H → 13-OH[c]	32.7 (C-8)	6.9 (C-7);	8.4 (C-9)	
14-H → 13-OH	31.0 (C-13)	7.8 (C-12);	3.8 (C-14);	2.2 (C-16)
5-H → 4α-OH	39.7 (C-15)	6.5 (C-16);	6.6 (C-8)	
27-H → 16-OCH_3	58.3 (C-6)	3.2 (C-5);	7.0 (C-7)	
19α-OH → 21α-OAc	5.4 (C-1)	−1.6 (C-2);	−0.4 (C-11)	
20β-OH → 22β-OAc	4.0 (C-1)	−3.1 (C-2);	−1.6 (C-11)	
16-OH → 18-OAc	11.1 (C-8)	−4.1 (C-7);	−4.3 (C-9);	−4.3 (C-15)
5-OH → 6-OAc	7.2 (C-13)	−0.9 (C-12);	−1.4 (C-14);	−3.7 (C-16)
27α-OH → 26α-OAc	1.3 (C-14)	−2.1 (C-9);	−0.6 (C-13)	
16α-OH → 14α-OCH_3	13.8 (C-1)	−3.5 (C-2)	0.8 (C-11)	
14-OH → 15-OCH_3	5.7 (C-8)	−4.4 (C-7);	−4.7 (C-9)[d];	−4.5 (C-15)
14-OH → 15-OCH_3	8.1 (C-14)	−4.7 (C-9)[d];	0.4 (C-13)	

[a] In ppm upfield relative to Me_4Si; a minus sign denotes an upfield shift on substitution. [b] The carbon atoms indicated in parentheses are those on which the effects are observed. [c] Under the condition that the effect of the C-16 methoxy group on the C-8 resonance in 13 is similar to that caused by the C-15–C-16 double bond in 10 (compare C-8 resonance in 5 with 9). [d] Double β effect.

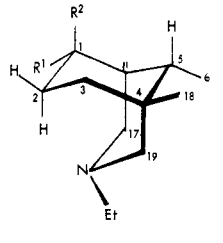
in the case of 1β-OH (axial) in **20** are normal relative to those observed for *cis*-4-*tert*-butylcyclohexanol/acetate.[19] The α effect upon acetylation of the C-1α OH (equatorial (?)) in **19** is 5.5 ppm, and it is almost double compared with that of *trans*-4-*tert*-butylcyclohexanol acetate[19] (2.9 ppm) or 3β-cholestanol acetate[19] (2.8 ppm) The β effects (**19** → **21**) in contrast to the α effect are smaller than expected

A



16, 18, 19, 26, and 27

B



17 and 20, R^1 = H, R^2 = OH
14, R^1 = OCH_3, R^2 = H
21, R^1 = OAc, R^2 = H
22, R^1 = H, R^2 = OAc

(see Table V). A similar anomaly has been observed upon methylation of the C-1α OH. The α effect caused by substitution of the C-1α OH by methoxyl in **16** is 13.8 ppm relative to **14** and it is larger than the published[17,19] α effects for secondary OH/OCH_3 (~10 ppm). These anomalies in α and β effects in the case of epimeric C-1 OH/OR alkaloids suggest that the simple electronic (OH)/OAc),[17] or steric (OH)/OCH_3)[17] effects are not the only ones operating. The ring A in alkaloids with C-1α OH is in a boat conformation (A) in contrast to the chair conformation (B) in the case where the C-1α substituents is acetoxyl or methoxyl.[23] Thus, the C-1 resonance in the C-1α OH compounds is shifted upfield, and the β carbons downfield as the result of conformation A, which is stabilizedy a hydrogen bond relative to C-1α OH compounds in the chair conformation. We have no values for C-1, C-2, and C-3 resonances in the case where the C-1α OH group is present in the chair conformation of ring A; however, a comparison of the epimeric acetates at C-1 in **21** and **22** (in which the conformation of the ring A is same) has shown that the C-1, C-2, and C-3 resonance are in lower field, 4.9, 0.7, and 2.6, respectively, if the acetoxy group is equatorial. In contrast to these results, when the hydroxyl is equatorial, comparison of the epimeric hydroxyls at C-1 in compounds **16** and **17** (in which the conformation of the ring A are boat (A) and chair (B), respectively) shows that C-1 resonance is at lower field (but now only 3.3 ppm) and the C-2 and C-3 resonances are at higher fields (0.2 and 1.3 ppm, respectively). In contrast, investigation of the epimeric 4-*tert*-butylcyclohexanols and their acetates has shown that the relative chemical shifts are similar in the case of equatorial OH and axial OH on one hand, and equatorial OAc and axial OAc on another.[19] The conclusion that we might reach is that, if the boat conformation (A) is keeping the C-1, C-2, and C-3 resonances high, then essentially the full value of this resonance will be seen on acetylation.

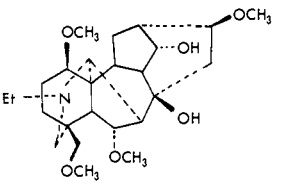
Structure of Chasmanine and Homochasmanine. Chasmanine, an alkaloid isolated from *Aconitum chasmanthum* Stapf, has been assigned[24] structure **28,** and this assignment was apparently firmly established[25] by correlation of the alkoloid with browniine (**29**) which in turn has been correlated with lycoctonine (**12**).[26]

Chasmanine has also been correlated[15] with neoline (**16**) by treatment of each alkaloid with sodium hydride and methyl iodide in refluxing dioxane for 12 and 24 h, respectively. Recently, we converted delphisine (**19**), an alkaloid whose molecular structure and absolute configuration have been established by an x-ray crystallographic study of its hydrochloride,[27] to neoline.[4] Consequently, on the basis of the correlation of chasmanine with neoline (**16**) chasmanine must also have a 1α-substituent and accordingly be assigned structure **14.** A ^{13}C study of chasmanine as compared with deoxyaconitine (**4**), delphinine (**5**), delphonine (**13**), and 1,8,14-tri-*O*-methylneoline (**15**), especially the ring A carbons (Table VI), confirms this assignment. Because **14** diacetate has been converted to homochasmanine by treatment with methanol under pressure, followed by saponification, homochasmanine may be assigned structure **30.**

Table VI. Carbon-13 Chemical Shifts for Ring A Carbons in Deoxyaconitine, Delphinine, Delphonine, and 1,8,14-Tri-*O*-methylneoline[a]

Compounds	Carbons						
	1	1'	2	3	4	5	11
Deoxyaconitine (4)	−1.1	−0.3	0.3	0.0	−0.5	0.3	−0.5
Delphinine (5)	−1.2	−0.2	0.3	−0.5	−0.2	0.0	−0.2
Delphonine (13)	−0.4	−0.1	−0.1	−0.3	0.0	0.6	−0.2
1,8,14-Tri-*O*-methylneoline (15)	−0.7	0.0	0.3	0.0	−0.5	0.4	0.5

[a] Chemical shifts in ppm relative to the corresponding carbon in chasmanine (14).



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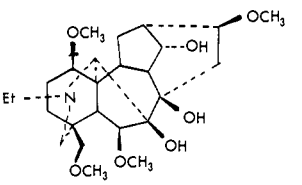

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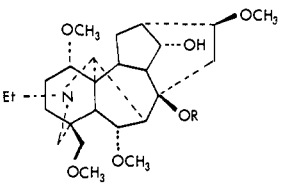
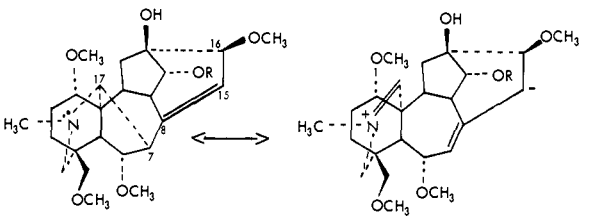


14, R = H
30, R = CH_3

Further Study on the "Pyrodelphonine Chromophore". In order to explain the unexpected ultraviolet absorption of pyrodelphonine (**8**) (λ_{max} 245 mμ, ϵ_{max} 6300), which disappears upon acidification, Wiesner, et al.,[28] have postulated the participation of the free electron pair on the nitrogen, the C-7–C-17 σ bond, and the π system of the double bond between C-8 and C-15 in an excited state, resembling **8a.** Later, Cookson, et al.,[29] explained the same phenomenon using valence-bond language. Seven years ago Wiesner, et



7, R=Bz
8, R=H

7a, R=Bz
8a, R=H

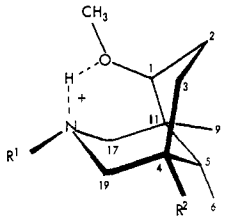
Table VII. Carbon-13 Chemical Shifts and Assignments[a] for Pyrodelphinine Free Base and after Acidifications, along with the Pyrodelphinine Oxide[b]

Carbon	Pyrodelphinine	1:1.3[c]	1:2[c]	1:3[c]	1:5[c]	1:8[c]	Pyrodelphinine in CD_3COOD	Pyrodelphinine oxide	Pyrodelphinine hydrochloride
1	86.1	84.8	84.1	83.2	82.0	81.6	82.0	85.8	81.2
2	25.3	24.7	24.5	24.1	23.7	23.6	23.7	22.8	23.8
3	35.3	34.5	34.1	33.4	32.5	31.8	30.9	34.3	32.7
4	40.0	40.0	40.1	40.1	40.0	40.0	40.2	41.2	40.3
5	48.5	48.7	48.6[d]	49.0	48.9	48.7	48.0	**50.9**	49.6
6	83.6	83.4[d]	83.3[e]	83.2	82.9	82.9[d]	83.4	82.8	82.9[d]
7	50.4	49.4	48.7[d]	48.4	47.5	47.0	46.6	48.7[d]	47.6
8	146.6	145.3	144.6	143.7	142.7	142.4	143.7	142.4	142.5
9	47.6	47.4	47.3	47.3	47.2	47.0	48.0	51.7[d]	47.3
10	46.7	46.2	46.1	45.8	45.5	45.4	45.9	45.8	45.5
11	51.9	51.6	51.6	51.5	51.4	51.5	52.3	52.2	51.4
12	38.4	37.8	37.6	37.3	36.8	36.6	36.9	38.6	36.5
13	77.7	77.6	77.6	77.6	77.6	77.8	77.9	77.3	77.5
14	79.1	78.3	78.2	78.0	77.6	77.8	78.3	77.7	77.5
15	116.3	117.9	118.8	120.0	121.6	122.3	123.3	120.6	121.8
16	83.6	83.5[d]	83.4[e]	83.2	82.8	82.8[d]	83.4	82.8	83.2[d]
17	78.6	77.8	77.4	76.9	76.3	76.5	78.3	91.2	76.7
18	80.3	79.8	79.7	79.4	79.1	78.9	79.8	79.7	78.9
19	56.5	56.7	57.0	57.2	57.9	58.6	60.4	75.7	58.0
$N\text{-}CH_3$	42.7	42.9	43.1	43.3	43.5	43.5	43.5	62.7	45.1
1'	56.5	56.0	55.9	55.7	55.5	55.5	56.3	56.1	55.4
6'	58.1	58.2	58.3	58.4	58.6	58.6	58.9	58.4	58.9
16'	57.1	57.1	57.2	57.2	57.3	57.4	57.8	57.4	57.7
18'	59.2	59.2	59.2	59.2	59.2	59.2	59.5	59.2	59.3

[a] In ppm downfield relative to Me_4Si. Solvent deuteriochloroform. [b] Without acetoxy and benzoxy carbons. [c] Pyrodelphinine:acetic acid molar ratio. [d, e] Values within any vertical column may be interchanged.

al.,[30] reported an unusual photoreduction of the C-17–C-7 bond of the 16-demethoxypyrodelphonine by borohydride to support his original views about the nature of the chromophoric system in the excited state of the pyro derivatives of the delphinium alkaloids. We focused our studies on the pyrodelphinine (**7**) "chromophore" in the electronic ground state of the molecule.[31] Our results are based on the differences in the ^{13}C NMR and proton NMR spectra of **7** caused by the protonation of the nitrogen atom. The observed carbon-13 chemical shifts and the assignments for pyrodelphinine as well as for its protonated forms, along with the pyrodelphinine oxide, are presented in Table VII. Protonation of the nitrogen in a base of such complexity by a strong acid-like HCl produces such a large influence on the carbon-13 chemical shifts that carbon assignments for the protonated alkaloid become difficult. In the case of pyrodelphinine hydrochloride resonances of 15 carbon atoms indicate larger shifts than 1 ppm compared with the pyrodelphinine free base, perhaps as a consequence of possible steric and electronic contributions of the compact ring structure. This observation indicates that the sole use of a strong acid such as HCl and $HClO_4$ is not the best choice for a carbon-13 study of these alkaloids. As indicated by the results given in Table VII, acetic acid is much more useful as a shift reagent, because the "step by step" manner of protonation of the nitrogen makes the interpretation of the differences easier. Using pyrodelphinine and acetic acid in a 1:8 molar ratio in chloroform solution, an almost identical effect on the carbon-13 shifts is produced as with HCl (pyrodelphinine hydrochloride). Acetic acid was selected because its dielectric constant ($\epsilon = 6.15$) is similar to that of chloroform ($\epsilon = 4.8$) and thus maintains the solvent effects relatively constant. Carbon-13 chemical shifts obtained for **7** in pure CD_3COOD are essentially the same as those observed in the acetic acid–chloroform mixture.

The carbon-13 chemical shift changes caused by the protonation of the nitrogen in **7** can be classified in three groups. Because quaternization changes the charge at the nitrogen by one electron, the observed effects of protonation are remarkably small.[32,33] The electrostatic effect of the positive nitrogen center will tend to withdraw electrons from the carbon framework. The carbons which represent the first group (inductive effect) are C-17, C-19, and the *N*-methyl carbons. The effect of the positive nitrogen center on the neighboring carbons in carbon-13 spectroscopy has been thoroughly studied in simple systems of *N*-methylpiperidines.[32] The conclusion of that investigation is that a methyl substituent strongly influences the direction of the resonance change. An upfield shift (3.4 ppm) at C-2 and C-6 in *N*-methyl-4-piperidone and a downfield shift (2.5 ppm) at C-2 and C-6 in *N*-methyl-2,6-dimethylpiperidine are observed upon protonation. By comparison, in pyrodelphinine the C-19 is unsubstituted and the C-17 is substituted. However, upon protonation with HCl the C-19 resonance is shifted downfield (1.4 ppm) and so is the $N\text{-}CH_3$ carbon resonance (2.4 ppm). The C-17 resonance is shifted upfield (1.9 ppm). Our results suggest that no simple relationships exist between these two systems. An explanation for this is, perhaps, that in contrast to the simple protonation of the *N*-methylpiperidines (in which case the quaternizing substituent takes up the axial position on the nitrogen in the favored chair conformation of most piperidines), quaternization of the aconitine-type alkaloids by acids is much more complex, because formation of an intramolecular hydrogen bond between the nitrogen and the C-1α oxygen also influences the carbon-13 shifts. Such a conformation is found in lappaconine (**31**) and heteratisine (**32**) hydrobromides.[34] If the conformation of the A ring (C-1, C-2, C-3, C-4, C-5, and C-11) is a chair in the free base with a very close contact between C-1–O and C-9–C-12 bonds (syn-axial 1,3 steric interaction), then a conformation change upon protonation of the nitrogen can explain the upfield shifts of the C-1, C-2, C-3, and C-12 carbon resonances. Therefore, we classify this change in the second group (steric effect). The C-12 resonance change in the pyrodelphinine upon protonation can be explained only by a conformation change of ring A. Since the C-12 is the δ carbon which is more than 4 Å away from the nitrogen, the in-



31, R^1 = Et, R^2 = OH
32, R^1 = Et, R^2 = CH_3
7, R^1 = Me, R^2 = CH_2OCH_3

ductive effect caused by the positive nitrogen, or the direct steric (through space) effect caused by the quaternization agent, is less probable. However, the explanation for the upfield shifts of C-1, C-2, and C-3 carbons is not as simple. In the change of the ring conformation from the chair to the strained and flattened boat upon protonation, C-2 is moving more than C-1 and C-3. In contrast to this, the C-2 resonance is shifted less upfield (1.5 ppm) compared to C-1 (4.9 ppm) and C-3 (2.6 ppm), probably as a result of syn-axial 1,3-steric interactions between the axial substituent at the nitrogen and the C-11–C-1 and C-4–C-3 bonds. An additional through-bond effect on the C-1 carbon resonance can be caused by hydrogen bonding.

Oxidation of the pyrodelphinine by means of *m*-chloroperbenzoic acid (1:1.1 molar ratio) in chloroform solution overnight at room temperature yields pyrodelphinine oxide. In this compound again the free electron pair of the nitrogen is removed ($N^+ \rightarrow O^-$); however, no hydrogen bond is possible. As a result of these conditions, the conformation of ring A is the same as in the free base, and no large shift changes (over 1 ppm) are observed for C-1, C-3, and C-12 upon oxidation. The C-2 carbon position in the chair conformation of ring A, however, is the nearest to the *N*-oxide group, and can explain the 2.5-ppm upfield shift. In the case of pyrodelphinine oxide the electrostatic effect of the positive nitrogen center is larger on the neighboring carbons than in the protonated one. The carbon-13 resonances of C-17, C-19, and the *N*-methyl carbon are shifted downfield 12.6, 19.1, and 20.0 ppm, respectively, compared with **7**, and the shifts are in good agreement with those found in scopolamine/oxide (11.4 ppm) and quinuclidine/oxide (15.1 ppm).[35]

Most unusual are the large upfield shift at C-15 (5.5 ppm) and downfield shift at C-8 (4.1 ppm) of pyrodelphinine upon acidification or N-oxidation. These carbons are γ and δ to the nitrogen and have the common feature that they lie in a plane approximately 4 Å away from the nitrogen center. According to published values of quaternary and methine carbon resonances in the six-membered ring double bond of terpenes[36] and steroids,[19] the resonance at 146.6 ppm for C-8 is shifted strongly downfield, and at 116.3 ppm for C-15 is shifted strongly upfield. A difference of 30.3 ppm exhibited by these carbons is higher than that found for isolated double bonds (10–20 ppm).[36,19] When a double bond is in conjugation with another double bond or keto group, the differences can be as high as 40 ppm. On the basis of these observations we can postulate that the free electron pair of the nitrogen, the C-17–C-7σ bond, and the π-electron pair of the C-8–C-15 double bond are conjugated in the electronic ground state of the molecule, and the structure of **7** may be portrayed as a resonance hybrid between the limiting structures **7** and **7a**. Thus, no uv/visible irradiation is necessary for the establishment of the **7** ↔ **7a** resonance; therefore, it represents the electronic ground state of the molecule. Delocalization of the free electron pair of the nitrogen will build up a negative charge on C-15,

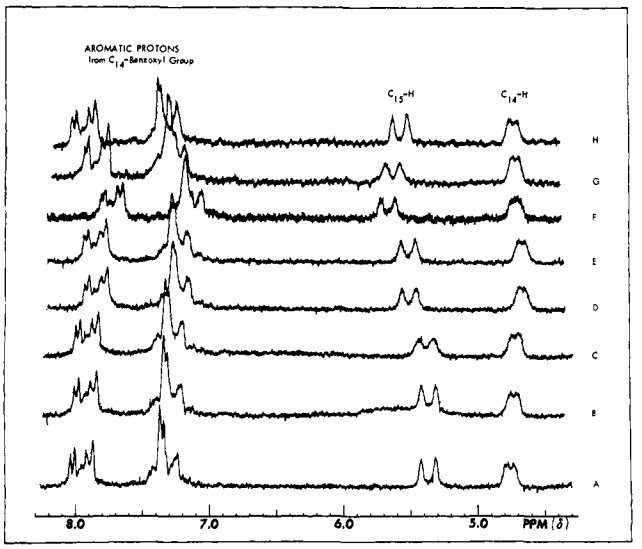


Figure 3. NMR spectra of pyrodelphinine (**7**) in the region between 4 and 8 ppm (δ) with tetramethylsilane as internal standard. Curves A, B, C, D, E, G, H all in $CDCl_3$. A (compound **7**); B (1:1 compound **7**/HOAc); C (1:2 compound **7**/HOAc); D (1:5 compound **7**/HOAc); E (1:8 compound **7**/HOAc); F (compound **7** in HOAc); G (compound **7**·HCl) and H (compound **7**·*N*-oxide).

and result in an upfield shift of its resonance, which can be seen upon protonation or oxidation of the nitrogen. It was found that some correlation of the proton screening with the electronegativity of the group to which hydrogen is bonded is not unexpected. Because identification of the C-15 proton signal is easy in the proton NMR spectrum of pyrodelphinine we have used this method to examine the unusual electron delocalization. The results of these investigations are presented in Figure 3. The C-15 proton resonance at 5.36 ppm in pyrodelphinine is shifted downfield to δ 5.63 ppm upon acidification with HCl (G). Similar shifts (0.30 ppm) are observed in acetic acid solution (F) and after oxidation with *m*-chloroperbenzoic acid (H) (0.26 ppm). This resonance change can also be followed by decreasing the pyrodelphinine:acetic acid molar ratio in $CDCl_3$ solution, in a series of 1:1 (B), 1:2 (C), 1:5 (D), and 1:8 (E). The C-15 proton resonance shift upon protonation, or oxidation of the nitrogen confirm the previous ^{13}C NMR conclusions that there is a direct interaction between the free electron pair of the nitrogen and the π electrons of the C-8 and C-15 double bond in the electronic ground state of the pyrodelphinine molecule.

Acknowledgment. The authors gratefully acknowledge a matching grant to the Chemistry Department from the National Science Foundation for purchase of the carbon-13 NMR spectrometer. We thank Mr. Courtney Pape for technical assistance in this work.

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A Carbon-13 Nuclear Magnetic Resonance Study of Mollisin and Its Biosynthesis

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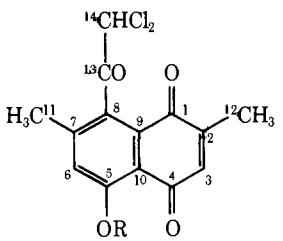
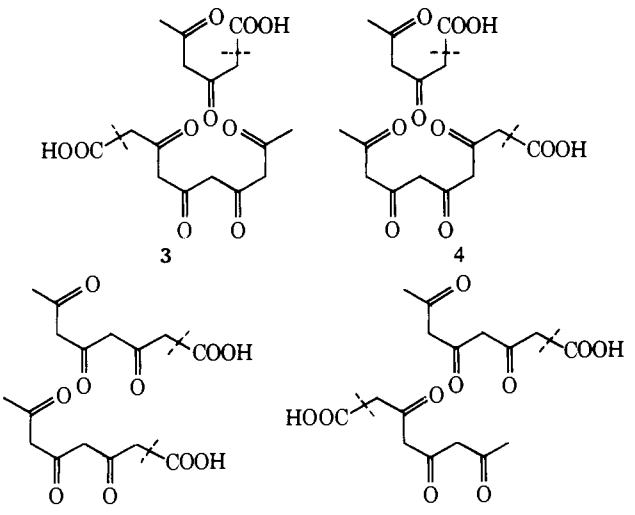
Abstract: The biosynthesis of mollisin (**1**) has been studied, utilizing both sodium [1-^{13}C]- and [2-^{13}C]acetate and sodium [1,2-^{13}C]acetate. The results confirm the biosynthetic scheme proposed by Tanabe and Seto. High levels of acetate incorporation and conversion of mollisin to the heretofore unknown mollisin acetate (**2**) made possible assignment of all ^{13}C resonance frequencies and ^{13}C–^{13}C coupling constants.

The work reported herein was undertaken with the aim of elucidating the acetate connectivity pattern in the biosynthesis of mollisin (**1**). During the course of our work a similar study of this molecule appeared[1] wherein it was concluded that the biosynthesis of mollisin had an acetate connectivity pattern consistent with biosynthesis **3** rather than alternatives such as **4**, **5**, or **6**.[2] As the carbon NMR spectra reported[1] suffered from low signal-noise (S-N) ratios, a number of important resonances and couplings germane to the problem were not observed. Since we were able to surmount the S-N difficulties (traceable to a combination of factors including low acetate incorporation, low isolated



1, R = H
2, R = $COCH_3$



3 **4**

5 **6**