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APPLICATION OF CARBON-13 NMR SPECTROSCOPY TO THE STRUCTURAL ELUCIDATION OF C_{19}-DITERPENOID ALKALOIDS FROM ACONITUM AND DELPHINIUM SPECIES

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The carbon-13 nuclear magnetic resonance spectra of the aconitine-type diterpenoid alkaloids pseudaconitine, indaconitine, veratroylpseudaconine, falaconitine, mithaconitine as well as the lycoctonine-type diterpenoid alkaloids browniine, 14-acetylbrowniine, delphatine, delcosine, 14-acetyldelcosine, delsoline, lycoctonine, tricornine, anthranoyllycoctonine, ajacine, methyllycaconitine and "delsemine" have been determined at 25.03 and 15.03 MHz in the Fourier mode. The signal due to each carbon atom in these alkaloids has been assigned with the help of proton decoupling techniques, additivity relationships and the effects induced by certain structural changes. Previously published carbon-13 chemical shift assignments for the alkaloids browniine and lycoctonine are corrected. Carbon-13 nmr data have also been applied in the structural determination of several previously unknown alkaloids isolated from Aconitum falconeri, Delphinium ajacis and Delphinium tricorne.

The recent application of carbon-13 nuclear magnetic resonance spectroscopy to alkaloids and other natural products has generated a large amount of substituent and additivity data for various series of simple and complex organic compounds. The carbon-13 nmr spectra of diterpenoid alkaloids reveal not only the number and type of carbons in the molecule but also indicate close structural similarities and give detailed information about the degree and sites of oxygen substitution. We anticipate that these data will be of critical importance in the structure determination of newly isolated alkaloids from various Aconitum, Delphinium and Garrya species. In 1972 Jones and Benn[1,2] published a useful contribution in the application of carbon-13 nmr spectroscopy to C_{19}-diterpenoid alkaloids. They utilized the carbon-13 nmr data in determining the structure of two new C_{19}-diterpenoid alkaloids designated as alkaloid A and alkaloid B. Recently we revised[3] the chemical shift assignments of alkaloid A and B as well as the structure of alkaloid A mainly on the basis of carbon-13 nmr analyses of a series of C_{19}-diterpenoid alkaloids. Later we reported[4] a comprehensive carbon-13 nmr study of twenty-six aconitine-type alkaloids and derivatives.

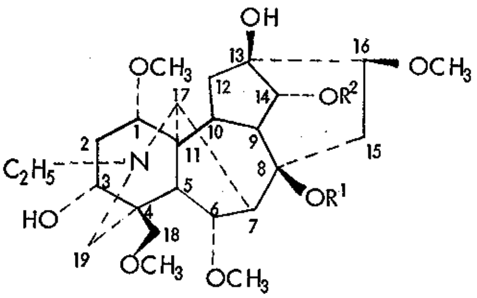
Recently we used[5,6] carbon-13 nmr spectroscopy in the identification and elucidation of the structures of several known alkaloids and two new C_{19}-diterpenoid alkaloids isolated from the roots of Aconitum falconeri. During a reinvestigation of the alkaloidal constituents of the seeds of Delphinium ajacis, we detected[7] the presence of previously unreported alkaloids by the aid of this technique. Carbon-13 nmr spectroscopy has also proven valuable in showing that "delsemine" is a mixture of two closely related alkaloids which differ only in the side chain of the anthranilic acid moiety.[8] In this paper the detailed application of the carbon-13 nmr technique to difficult problems of complex C_{19}-diterpenoid alkaloids will be presented.

The general procedure for carbon-13 nmr data acquisition and assignment of the resonances for each of the compounds reported here involved determination of the noise-decoupled and the single-frequency off-resonance decoupled (SFORD) spectra. The signals were assigned by means of the single-frequency proton off-resonance decoupling technique,[9,10] direct analysis of non-protonated carbon centers, application of known chemical shift rules for hydroxyl substitution and acetylation shifts,[4] steric effects and from comparisons of spectra from compound to compound.

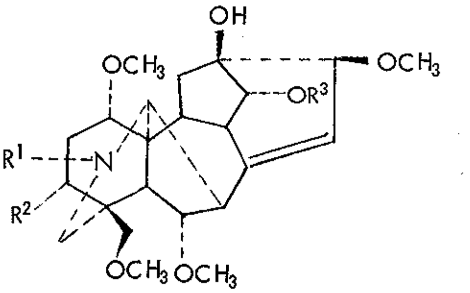
ASSIGNMENTS FOR ACONITINE-TYPE ALKALOIDS (TABLE 1).

Pseudaconitine (1), Indaconitine (2), Veratroylpseudaconine (3), Falaconitine (4), and Mithaconitine (5).

We have reported[5,6] the isolation of three known alkaloids, pseudaconitine (1), indaconitine (2), and veratroylpseudaconine (3), and two new alkaloids, falaconitine (4) and mithaconitine (5) from the roots of *A. falconeri*. The structures of these alkaloids were elucidated mainly by the aid of carbon-13 nmr spectroscopy. The identity of these alkaloids was confirmed by comparison with authentic samples. We report in Table 1, the carbon-13 nmr chemical shift data for alkaloids 1 to 6. The chemical shift assignments for pseudaconitine (1) and indaconitine (2) were made by correlation with previously published[4] carbon-13 nmr spectra of a variety of model *Aconitum* and *Delphinium* alkaloids e.g., aconitine, delphinine, etc. The presence of the veratroyl group in pseudaconitine was confirmed by a comparison of observed resonances in 1 with the values observed for methyl veratrate. The chemical shifts of alkaloids 1 and 2 are in good agreement with their respective structures. The pattern of carbon-13 chemical shifts in alkaloid 3 was similar to that of pseudaconitine. However, comparison of the spectrum of 3 with that of 1 revealed the absence of chemical shifts due to the C-8 acetyl group and changes in chemical shifts of C-7, C-8 and C-15 in alkaloid 3, leading to the conclusion that the C-8 acetyl group is absent.



1 R^1=Ac R^2=Vr Pseudaconitine
2 R^1=Ac R^2=Bz Indaconitine
3 R^1=H R^2=Vr Veratroylpseudaconine



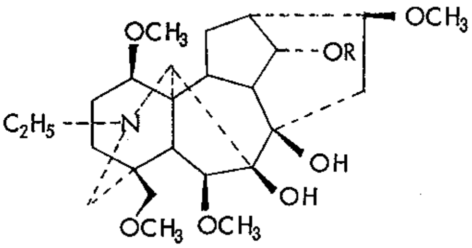
4 R^1=C$_2$H$_5$ R^2=OH R^3=Vr Falaconitine
5 R^1=C$_2$H$_5$ R^2=OH R^3=Bz Mithaconitine
6 R^1=CH$_3$ R^2=H R^3=Bz Pyrodelphinine

Correlation of two newly isolated alkaloids, falaconitine (4) and mithaconitine (5) with alkaloids 1 to 3 was also made through a study of their carbon-13 nmr spectra. This comparison afforded evidence for the presence of a veratroyl group and a benzoyl group in alkaloids 4 and 5, respectively, as well as the basic C_{19}-diterpenoid skeleton in these alkaloids. The presence of a double bond between C-8 and C-15 in alkaloids 4 and 5 was established by comparison with the carbon-13 chemical shifts of pyrodelphinine (6). These data indicate that alkaloids 4 and 5 are similar to pseudaconitine (1) and indaconitine (2), respectively, except for the presence of the double bond between C-8 and C-15.

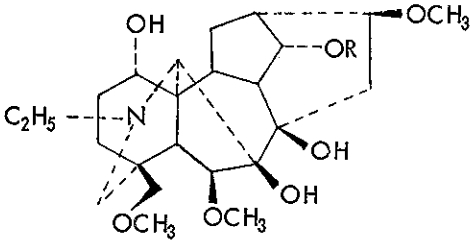
ASSIGNMENTS FOR BROWNIINE AND DELCOSINE GROUP ALKALOIDS (TABLE 2).

Browniine (7), 14-Acetylbrowniine (8), Delphatine (9), Delcosine (10), 14-Acetyldelcosine (11) and Delsoline (12).

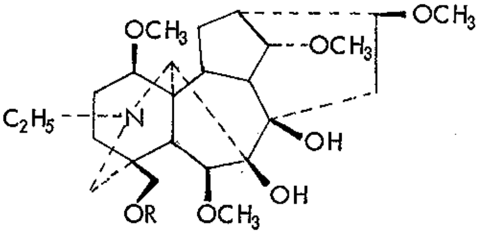
During a reinvestigation of the alkaloids of the seeds of *D. ajacis*, we have isolated[7] two previously unreported alkaloids, browniine (7) and delphatine (9), and a new alkaloid (8), $C_{27}H_{43}NO_8$, mp 123-124°C, along with the previously reported alkaloids delcosine (10), 14-acetyldelcosine (11), and delsoline (12). A recent study of the carbon-13 nmr spectra of more than fifty *Aconitum* and *Delphinium* alkaloids and derivatives in our laboratory has provided a foundation for unambiguous assignments of all carbon resonances for the lycoctonine-type alkaloids. The observed carbon-13 chemical shifts and assignments for alkaloids 7 to 12 are presented in Table 2. The resonances which we observed for browniine (7) are in agreement with those reported[2] by Jones and Benn, although several of our assignments are different. The carbon-13 nmr spectrum of the new alkaloid (8) shows twenty-seven signals corresponding to twenty-seven carbon atoms in the molecule. The spectrum closely resembles that of browniine with the addition of a quartet at 21.5 ppm and a singlet at 171.9 ppm. The structure of 14-acetylbrowniine (8) can thus be assigned to this new alkaloid. Comparison of the carbon-13 nmr spectrum of 14-acetylbrowniine with that of the new alkaloid confirmed its structure as 8. The spectrum of delphatine (9) reveals the presence of an extra methoxyl group at C-14 in comparison with browniine. The carbon-13 chemical shifts of delphatine are in agreement with its structure (9).



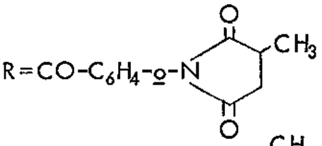
7 R = H Browniine
8 R = Ac 14-Acetylbrowniine
9 R = CH_3 Delphatine

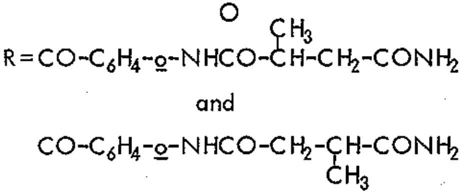


10 R = H Delcosine
11 R = Ac 14-Acetyldelcosine
12 R = CH_3 Delsoline



13 R = H Lycoctonine
14 R = Ac Tricornine
15 R = CO-C_6H_4-o-NH_2 Anthranoyllycoctonine
16 R = CO-C_6H_4-o-$NHCOCH_3$ Ajacine

17  Methyllycaconitine

18  "Delsemine"

$R = CO{-}C_6H_4{-}\underline{o}{-}NHCO{-}\overset{CH_3}{\overset{|}{C}}H{-}CH_2{-}CONH_2$

and

$CO{-}C_6H_4{-}\underline{o}{-}NHCO{-}CH_2{-}\underset{CH_3}{\underset{|}{C}}H{-}CONH_2$

The conformation of the C-1 hydroxyl group in delcosine and related alkaloids was determined by their carbon-13 nmr analysis. Examination of the spectra of delcosine, 14-acetyldelcosine and delsoline confirmed the presence of the C-1 hydroxyl group in the α-position by observing chemical shifts at ~72 ppm in these alkaloids. Additional semilarities in the carbon-13 nmr spectra of alkaloids 10 - 12 confirmed their skeleton and substitution pattern.

ASSIGNMENTS FOR LYCOCTONINE-TYPE ALKALOIDS (TABLE 3).

Lycoctonine (13), Tricornine (14), Anthranoyllycoctonine (15), Ajacine (16), Methyllycaconitine (17) and "Delsemine" (18).

Recently we reported[8,11] the isolation from D. tricorne of various known lycoctonine-type alkaloids, lycoctonine, methyllycaconitine, "delsemine" and a new alkaloid designated as tricornine. Table 3 shows the carbon-13 chemical shifts and assignments for lycoctonine (13), tricornine (14), anthranoyllycoctonine (15), ajacine (16), methyllycaconitine (17) and "delsemine" (18). On the basis of carbon-13 chemical shift assignments of browniine (7) and other lycoctonine-type alkaloids, we have revised several assignments for lycoctonine which were reported[2] earlier.

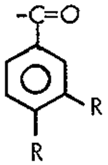
The carbon-13 nmr spectrum of tricornine, $C_{27}H_{43}NO_8$, mp 187-189°C, exhibited twenty-seven signals corresponding to twenty-seven carbons in the molecule. The spectrum of tricornine closely resembles that of lycoctonine except for the presence of two additional peaks, a quartet at 20.8 ppm and a singlet at 170.9 ppm, which suggests the presence of an acetyl group. The chemical shift of the C-18 carbon in tricornine appeared at 69.2 ppm — significantly downfield compared with lycoctonine. Tricornine is thus the C-18 acetate of lycoctonine, a fact confirmed by its preparation from lycoctonine by acetylation with acetic anhydride and pyridine at room temperature.

Examination of the carbon-13 nmr spectra of anthranoyllycoctonine (15), ajacine (16), methyllycaconitine (17) and "delsemine" (18) revealed the presence of an anthranilic acid moiety and the basic lycoctonine skeleton in these alkaloids. The presence of the anthranoyl group in the alkaloids 15 to 18 was confirmed by a comparison of the observed resonances with those observed for methyl anthranilate. The spectra of anthranoyllycoctonine (15), ajacine (16) and methyllycaconitine (17) are in good agreement with their respective structures, but that of "delsemine" showed 45 peaks of which 24 peaks correspond with the 24 carbons of lycoctonine. The twin peaks at 141.9 and 141.7, 114.8 and 114.7, 51.9 and 51.7, 41.4 and 39.0 and 17.9 and 17.1 ppm along with six downfield singlets for six carbonyl groups indicate that "delsemine" is actually a mixture of two closely related bases which differ only in the side chain of the anthranilic acid moiety. Thus, the structure of "delsemine" can be represented only as **18**. We have also established[8] that "delsemine" is formed from methyllycaconitine during isolation in the presence of NH_4OH. This problem and others mentioned earlier represent examples of the great value of carbon-13 nmr spectroscopy in solving complex structural problems which would otherwise be extremely difficult to solve.

Table 1. Carbon-13 Chemical Shifts and Assignments for Aconitine-type Alkaloids[a,b]

Carbon	1	2	3	4	5	6
1	83.6	83.2	83.4	83.8[c]	83.6	86.1
2	35.1	35.1	35.8	38.0	38.2	25.3
3	70.9	71.2	71.3	71.4	71.8	35.3
4	43.1	43.0	43.3	44.0	44.1	40.0
5	48.7	48.6	47.5	48.0	48.3	48.5
6	82.1	82.0	82.5	83.7[c]	83.6	83.6
7	48.7	48.6	53.8	49.5	49.6	50.4
8	85.3	85.3	73.6	146.6	146.5	146.6
9	47.2	47.2	47.5	48.2	48.3	47.6
10	40.7	40.7	41.9	46.2	46.4	46.7
11	50.1	50.0	50.2	51.6	51.7	51.9
12	33.7	33.5	33.7	33.4	33.4	38.4
13	74.7	74.5	75.8	77.4	77.6	77.7
14	78.4	78.5	79.8	78.1	78.3	79.1
15	39.6	39.4	42.4	116.1	116.4	116.3
16	83.0	82.8	82.5	83.1	83.1	83.6
17	61.4	61.4	61.6	77.8	78.5	78.6

Table 1 – continued

Carbon	1	2	3	4	5	6
18	76.2	76.5	76.7	76.1	76.4	80.3
19	48.7	48.6	48.9	49.7	49.9	56.5
N-CH_2(3)	47.2	47.2	47.5	47.7	47.9	42.7
CH_3	13.3	13.3	13.5	13.5	13.5	-
1'	55.7	55.6	55.8	56.2	56.3	56.5
6'	57.6	57.5	57.5	58.0	58.1	58.1
16'	58.7	58.5	58.3	57.3	57.2	57.1
18'	58.9	58.9	59.1	59.2	59.2	59.2
-C=O	169.4	169.2	-	-	-	-
CH_3	21.5	21.5	-	-	-	-
-C=O	165.6	165.7	166.2	167.5	168.0	168.0
 R R	122.5	129.7	122.5	122.9	130.2	130.5
	110.2	129.2	110.5	110.3	130.0	130.0
	152.8	128.1	153.1	153.0	128.2	128.1
	148.4	132.7	148.6	148.5	132.8	132.7
	111.8	-	112.3	112.5	-	-
R=OCH_3	55.7	-	55.8	55.9	-	-

[a] Chemical shifts in ppm downfield from TMS. Solvent deuterochloroform.

[b] *Carbon-13 nmr spectra were taken at 25.03 MHz in the Fourier mode using a* JEOL-PFT-100 spectrometer in conjunction with a EC-100-20K memory computer.

[c] These assignments may be interchanged in any vertical column.

Table 2. Carbon-13 Chemical Shifts and Assignments for Browniine and Delcosine Group Alkaloids[a,b]

Carbon	7[c]	7	8	9	10	11	12
1	82.1	85.2	84.2	83.9[d]	72.7	72.6	72.6
2	33.4	25.5	26.2	26.2	27.5	27.2	27.2
3	32.6	32.5	32.4	32.4	29.4	29.9[d]	29.3
4	38.6	38.4	38.1	38.1	37.6	37.5	37.4
5	45.3	45.1	42.6	43.3	44.0	43.5[e]	43.9
6	90.5	90.1	90.3	90.6	90.1	90.2	90.4
7	89.2	89.1	88.3	88.4	87.9	87.6	87.8
8	76.6	76.3	77.1	77.5	78.1	78.4	78.5
9	50.2	49.6	51.2	49.8	45.3	44.9	44.9
10	46.3	36.4	38.1	38.1	39.4	38.0	37.7
11	48.4	48.2	49.5	48.9	48.9	49.2	49.3
12	27.8	27.5	28.2	28.7	29.4	29.4[d]	30.5
13	37.0	46.1	45.7	46.1	45.3	42.6[e]	43.3
14	78.4	75.3	76.0	84.3[d]	75.8	76.3	84.5
15	25.6	33.1	33.7	33.5	34.5	33.8	33.5
16	85.3	81.7	82.4	82.6	82.0	82.7	82.9
17	65.3	65.4	64.8	64.8	66.3	66.1	66.0
18	75.5	78.0	78.0	78.1	77.4	77.3	77.3
19	53.2	52.7	52.7	52.8	57.1	57.2	57.2
N-CH_2	51.2	51.3	48.8	51.1	50.4	50.3	50.3
CH_3	14.1	14.3	14.2	14.2	13.7	13.6	13.5
1'	57.7	56.0	55.8	55.7	-	-	-
6'	55.8	57.5	57.3	57.3	57.4	57.2	57.2
14'	-	-	-	57.8	-	-	57.9
16'	56.4	56.5	56.2	56.3	56.4	56.3	56.3
18'	59.2	59.1	59.0	59.0	59.1	59.1	59.1

Table 2 - continued

Carbon	7[c]	7	8	9	10	11	12
-C=O	-	-	171.9	-	-	171.4	-
CH_3	-	-	21.5	-	-	21.4	-

[a] Chemical shifts in ppm downfield from TMS. Solvent deuterochloroform.

[b] Carbon-13 nmr spectra were taken at 15.03 MHz in the Fourier mode using a FX-60 spectrometer in conjunction with a JEC-980 computer.

[c] Jones's and Benn's tentative assignments for browniine (Ref. 2).

[d,e] These assignments may be interchanged in any vertical column.

Table 3. Carbon-13 Chemical Shifts and Assignments for Lycoctonine-type Alkaloids[a,b]

Carbon	13[c]	13	14	15	16	17	18
1	83.0	84.2[d]	84.0	84.0	83.9	83.9	83.9
2	33.8	26.1	26.1	26.2	26.1	26.0	26.1
3	31.2	31.6	31.9	32.3	32.2	32.0	32.2
4	38.6	38.6	37.2	37.6	38.2	37.6	37.6
5	43.4	43.3	43.3	43.3	43.3	43.2	43.3
6	90.7	90.6	90.9	91.0	91.0	90.8	91.0
7	88.3	88.3	88.5	88.6	88.6	88.5	88.6
8	77.0	77.5	77.5	77.6	77.5	77.4	77.5
9	49.7	49.7	50.4	50.4	50.5	50.3	50.5
10	46.1	38.0	38.1	38.3	37.6	38.0	38.2
11	49.1	48.9	49.0	49.1	49.1	49.0	49.1
12	29.0	28.8	28.7	28.8	28.6	28.7	28.7
13	38.0	46.1	46.1	46.2	46.1	46.1	46.1
14	84.2	84.0[d]	84.0	84.0	83.9	83.9	83.9
15	25.7	33.7	33.7	33.7	33.8	33.6	33.8
16	84.2	82.7	82.6	82.6	82.6	82.5	82.6

Table 3 - continued

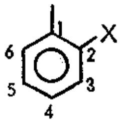
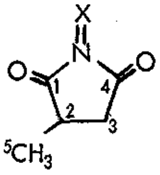
Carbon		13^{c}	13	14	15	16	17	18
17		64.8	64.8	64.6	64.6	64.5	64.5	64.5
18		67.4	67.6	69.1	68.7	69.8	69.5	69.8
19		53.5	52.9	52.4	52.6	52.5	52.3	52.4
N-CH_2		51.1	51.1	51.0	51.0	51.0	50.9	50.9
CH_3		13.7	14.1	14.1	14.1	14.0	14.0	14.0
1'		57.7	55.7	55.7	55.8	55.8	55.7	55.7
6'		55.6	57.7	57.8	57.9	57.8	57.8	57.8
14'		58.0	58.0	58.0	58.0	58.1	58.2	58.1
16'		56.2	56.2	56.3	56.3	56.3	56.3	56.3
C=O		-	-	170.9	-	-	-	-
CH_3		-	-	20.8	-	-	-	-
-C=O		-	-	-	167.9	168.1	164.1	168.1
	1	-	-	-	110.4	114.5	127.1	114.7,114.8
	2	-	-	-	150.9	141.9	133.1	141.9,141.7
	3	-	-	-	116.9^{e}	120.6^{e}	129.4	120.7^{e}
	4	-	-	-	134.4^{d}	135.0^{d}	133.6	134.9^{d}
	5	-	-	-	116.4^{e}	122.5^{e}	131.0	122.5^{e}
	6	-	-	-	130.8^{d}	130.3^{d}	130.0	130.3^{d}
X=NH-C=O		-	-	-	-	169.0	-	-
CH_3		-	-	-	-	25.5	-	-
	1	-	-	-	-	-	179.8	-
	2	-	-	-	-	-	37.0	-
	3	-	-	-	-	-	35.3	-
	4	-	-	-	-	-	175.8	-
	5	-	-	-	-	-	16.4	-

Table 3 - continued

Carbon	13[c]	13	14	15	16	17	18
X=HN-C=O	-	-	-	-	-	-	174.1
CH-CH_3	-	-	-	-	-	-	51.9,17.1
CH_2	-	-	-	-	-	-	39.0
H_2N-C=O	-	-	-	-	-	-	172.4
X=HN-C=O	-	-	-	-	-	-	170.0
CH_2	-	-	-	-	-	-	41.4
CH-CH_3	-	-	-	-	-	-	51.7,17.9
H_2N-C=O	-	-	-	-	-	-	176.0

[a] Chemical shifts in ppm downfield from TMS. Solvent deuterochloroform.

[b] Carbon-13 nmr spectra were taken at 15.03 MHz in the Fourier mode using a FX-60 spectrometer in conjunction with a JEC-980 computer.

[c] Jones's and Benn's tentative assignments for lycoctonine (Ref. 2).

[d,e] These assignments may be interchanged in any vertical column.

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