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^{13}C-NMR ASSIGNMENTS FOR LACTONE-TYPE NORDITERPENOID ALKALOIDS

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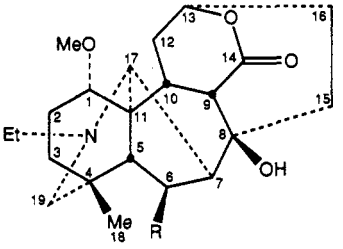
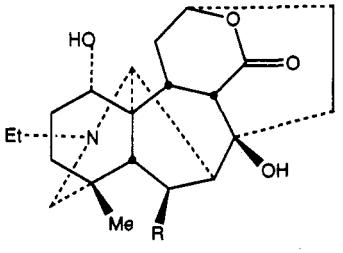
ABSTRACT.—^{13}C-nmr chemical shift assignments for the lactone-type diterpenoid alkaloids heteratisine [**1**], 6-acetylheteratisine [**3**], and 6-benzoylheteratisine [**2**] have been revised on the basis of the study of their 1D, 2D, and selective INEPT nmr spectra. Unambiguous ^{13}C-nmr chemical shift assignments for the related alkaloids heterophyllidine [**4**], heterophylline [**5**], and heterophyllisine [**6**], which have not been reported previously, are reported in this study. Complete ^{1}H-nmr chemical shift assignments are also reported.

The first lactone-type norditerpenoid alkaloids, heteratisine [**1**] and 6-benzoylheteratisine [**2**], were isolated by Jacobs and Craig (1,2) from *Aconitum heterophyllum* Wall. (Ranunculaceae) in 1942. The structure **1** for heteratisine was established through a number of chemical degradation studies (3,4) and confirmed by X-ray crystal structure determination of its hydrobromide salt (5,6). The absolute configuration of **1** was determined (7), through pyrolysis and oxidation experiments and studying the ord curves of the products.



1 R=OH
2 R=OCOPh
3 R=OAc
6 R=H



4 R=OH
5 R=H

^{13}C-nmr chemical shift assignments for **1** were first reported from this laboratory in 1976 (8). The assignments were based on the determination of the noise-decoupled and single-frequency off-resonance decoupled (SFORD) spectra. ^{13}C-nmr signals were assigned with the help of the single frequency proton off-resonance decoupling technique, application of the known chemical shift rules for -OH substitution and acetylation shifts, steric effects, and from comparison of spectra from compound to compound. ^{13}C-nmr assignments for 6-acetylheteratisine [**3**], prepared from heteratisine, were also reported from this laboratory (8,9) and by G. de la Fuente *et al.* (10). Recently, isolation of **3** from *Aconitum palmatum* Don. and revised ^{13}C-nmr chemical shifts were reported from this laboratory (11). ^{13}C-nmr chemical shift assignments for 6-benzoylheteratisine [**2**] were also reported (12). ^{13}C-nmr chemical shift assignments for the lactone-type norditerpenoid alkaloids heterophyllidine [**4**], heterophylline [**5**]. and heterophyllisine [**6**] are being reported for the first time in this work.

A growing need for the complete and unambiguous nmr chemical shift assignments for the naturally occurring complex diterpenoid alkaloids prompted us to examine the chemical shift assignments with the aid of modern 2D nmr techniques. In some cases the 2D nmr

spectra along with 1D DEPT studies resulted in minor revisions in the assignments (11,13,14). So far, complete and unambiguous nmr assignments for the lactone-type norditerpenoid alkaloids have not been reported. We now report the unambiguous nmr assignments for lactone-type norditerpenoid alkaloids **1–6**, through a study of their 1D (DEPT) and 2D (COSY, HETCOR) nmr spectra and selective INEPT experiments. We have found selective INEPT experiments to be helpful for these assignments. Recently the usefulness of selective INEPT results for the unambiguous nmr shift assignments for the norditerpenoid alkaloids containing 7,8-methylenedioxy and 10β-OH groups has been reported from this laboratory (15). Selective INEPT procedures have also been successfully applied to the elucidation of structures of other natural products (16,17).

RESULTS AND DISCUSSION

A study of the 2D-nmr spectra i.e., COSY and HETCOR including 1D DEPT experiments, of heteratisine [**1**] indicated that some of the previously assigned (8) ^{13}C-nmr chemical shift assignments should be revised. This revision was supported by the results of selective INEPT experiments on **1**. Revised and previously reported (8) ^{13}C nmr chemical shift assignments and complete ^{1}H nmr chemical shift assignments for heteratisine [**1**] are given in Table 1. Complete ^{1}H-nmr assignments for **1** were obtained through its COSY nmr plot, and these assignments were supported by HETCOR and selective INEPT experiments.

The previous assignment for C-19 of **1** at 58.3 ppm was changed to 57.8 (t) ppm on the basis of DEPT and selective INEPT experiment results. In the DEPT-

TABLE 1. ^{13}C- and ^{1}H-nmr Chemical Shift Assignments[a] of Heteratisine [**1**].

Position	New	Previous[b]	δ_H	Multiplicity
	δ_C	δ_C		J=Hz
1	83.5 d	83.5	3.11	t, 9.4
2	26.9 t	26.9	1.90; 2.10	m; m
3	36.8 t	36.8	1.20; 1.50	m; m
4	34.7 s	34.7	—	—
5	58.3 d	50.9	1.33	br.s, W1/2=5.0
6	72.9 d	72.9	4.47	m
7	50.7 d	49.3	2.38	d, 8.9
8	75.4 s	75.4	—	—
9	49.4 d	57.8	4.01	d, 7.5
10	42.8 d	42.8	2.25; 2.40	m; m
11	49.3 s	49.3	—	—
12	29.2 t	33.1	1.70; 2.30	m; m
13	75.8 d	75.8	4.71	m
14	176.0 s	176.0	—	—
15	33.1 t	29.2	1.67; 1.87	m; m
16	29.0 t	29.2	2.10; 3.22	m; m
17	62.2 d	62.2	3.45	d, 0.3
18	26.2 q	26.2	0.96	s
19	57.8 t	58.3	1.93; 2.53	d, 11.8; 11.8
N-CH_2	49.0 t	49.0	2.40	m
Me	13.5 q	13.5	1.02	t, 7.3
1-OMe	55.2 q	55.2	3.25	s
6-OH	—	—	5.19↓D_2O	d, 3.9
8-OH	—	—	5.11↓D_2O	s

[a]Chemical shifts in ppm downfield from TMS.

[b]Assignments in this column are from Pelletier *et al.* (8).

135 spectrum the signal at 58.3 ppm was found to be a methine carbon and that at 57.8 ppm a methylene carbon. In selective INEPT experiments, when H-18 (0.96 ppm) was selectively pulsed, a strong response to the signal at 34.7 ppm, assigned to C-4 (2 bonds away), was observed along with two other comparatively weaker responses at 58.3 and 57.8 ppm for C-5 and C-19, respectively, both being 3 bonds away from C-18. In the same experiment a weak response to a signal at 36.8 ppm is assigned to C-3. In the previous assignments a signal at 50.9 ppm was assigned to C-5 which is now assigned to C-7 on the basis of HETCOR and selective INEPT experiments. Thus, the signal at 50.7 ppm showed a correlation to H-7 (2.38 ppm) in the HETCOR contour plot, and in the selective INEPT experiment when H-6 (4.47 ppm) was selectively pulsed a weak response to a signal at 50.7 ppm was observed. Selectively pulsing the 6-OH proton (5.19 ppm) also showed a strong response to the signal at 50.7 ppm which is for C-7 being 3 bonds away.

The shift at 49.4 ppm previously assigned to C-7 is now assigned to C-9 on the basis of HETCOR results, where it showed a correlation to H-9 (4.01 ppm). The most interesting revision in this study was interchanging the signals at 29.2 (t) and 33.1 (t) ppm previously assigned to C-15 and C-12, respectively (8). The signal at 33.1 ppm showed a correlation to H-15 (1.67, 1.87 ppm) in the HETCOR plot. When H-13 (4.71 ppm was selectively pulsed, the signals showing strong responses were 176.0, 42.8, and 33.1 ppm. The signals at 176.0 and 42.8 ppm can be easily assigned to C-14 (3 bonds away) and C-10 (3 bonds away), respectively, but the signal at 33.1

TABLE 2. ^{13}C-nmr Chemical Shifts and Assignments[a] for Heterophyllidine [**4**], Heterophylline [**5**], and Heterophyllisine [**6**] Revised ^{13}C-nmr Assignments for 6-Benzoylheteratisine [**2**][b] and 6-Acetylheteratisine [**3**].

Carbon	5	4	6	2	3
C-1	72.6 d	72.7 d	84.4 d	82.3	82.2 d
C-2	29.4 t	29.5 t	27.0 t	26.7	26.7 t
C-3	30.8 t	31.7 t	37.2 t	36.4	36.3 t
C-4	33.1 s	33.1 s	34.8 s	34.9	34.7 s
C-5	44.5 d	54.1 d	46.5 d	55.9	55.4 d
C-6	26.2 t	72.4 d	27.2 t	74.5	74.0 d
C-7	45.7 d	50.3 d	49.4 d	49.9	49.5 d
C-8	75.6 s	76.2 s	75.4 s	75.2	75.1 s
C-9	50.0 d	49.3 d	50.4 d	48.9	48.5 d
C-10	39.8 d	40.0 d	42.7 d	42.7	42.7 d
C-11	50.1 s	49.8 s	49.6 s	48.7	48.7 s
C-12	30.0 t	30.3 t	28.7 t	29.4	29.3 t
C-13	75.0 d	75.1 d	75.5 d	75.8	75.2 d
C-14	173.6 s	175.1 s	174.3 s	173.5	173.8 s
C-15	34.1 t	33.5 t	33.9 t	35.3	35.7 t
C-16	29.4 t	29.9 t	29.3 t	28.8	28.8 t
C-17	62.2 d	63.9 d	60.9 d	62.6	62.4 d
C-18	27.7 q	27.6 q	26.5 q	26.0	25.9 q
C-19	60.5 t	62.1 t	56.6 t	57.5	57.3 t
N-CH_2	48.4	48.5 t	49.0 t	48.9	48.8 t
NMe	13.1 q	13.0 q	13.5 q	13.4	13.4 q
C-1′	—	—	55.6 q	54.9	54.9 q
C=O	—	—	—	—	170.8 s
Me	—	—	—	—	21.6 q

[a]Chemical shifts in ppm downfield from TMS.

[b]Shifts for C-6 benzoyl group in **2**: 166.6 s, 130.2 s (1), 128.5 d (3,5), 129.6 d (2,6), 132.8 d (4).

ppm can be assigned either to C-12 (2 bonds away) or C-15 (3 bonds away). A choice was made by selectively pulsing the proton of the 8-OH group (5.11 ppm); a strong response to the signal at 33.1 ppm was observed, and thus this signal was assigned to C-15 (3 bonds away). Similar results were obtained in the case of 6-acetylheteratisine [**3**]; the acetylation effect was observed on C-5 and C-7 signals which moved upfield as compared with those in heteratisine [**1**] (Tables 1 and 2). The assignments for 6-benzoylheteratisine [**2**] were revised on the basis of comparison with the revised assignments for 6-acetylheteratisine [**3**] and are presented in Table 2.

The unambiguous ^{13}C-nmr and complete ^{1}H-nmr chemical shift assignments for heterophyllidine [**4**], heterophylline [**5**], and heterophyllisine [**6**] are presented for the first time in Tables 2 and 3, respectively. These assignments are based on the results of 1D, 2D, DEPT, and selective INEPT nmr experiments on compounds **4–6**. The ^{13}C nmr assignments for heterophyllisine [**6**] compare well with the revised assignments for heteratisine [**1**].

EXPERIMENTAL

SAMPLES.—The samples of heteratisine [**1**], 6-acetylheteratisine [**3**], heterophyllidine [**4**], heterophylline [**5**], and heterophyllisine [**6**] used for this study were authentic and were of 30–40 mg (**1** and **3**) and 2–3 mg (**4–6**) size. Compounds **1**, **2** and **4–6** were isolated from *Aconitum helerophyllum* Wall. Compound **3** was isolated from *Aconitum palmatum* Don.

INSTRUMENTS.—All the nmr spectra were recorded in $CDCl_3$ on a Bruker AC 300 spectrometer operating at 300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C. The residual $CHCl_3$ and $^{13}CDCl_3$ in $CDCl_3$ were used as internal references (^{1}H δ 7.27; ^{13}C δ 77.0 ppm). The pulse sequences employed in the 1D and 2D nmr experiments were those of the standard Bruker software. The pulse sequence for the selective INEPT experiments was obtained by modifying the Bruker standard INEPT sequence according to Bax (18). A long range coupling value (^{lr}J) for the selective INEPT experiments was 6 Hz. In the selective INEPT experiments the decoupling powers used were S1=45L (for soft pulse) and S2=0L (for decoupling with CPD). The power 0L in the Bruker AC 300 spectrometer is approximately one watt. The following delays were utilized: D1=3 sec (Relaxation delay for ^{1}H, prepare decoupler power for soft pulse); D2=1/4J LR–0.015 sec (Refocusing delay). D3 was variable depending on the protons being selected: D3=1/4J LR–0.0075 sec (For polarization transfer from a C*H*); D3=1/8J LR–0.0075 sec (For polarization transfer from a CH_2); D3=1/10J LR–0.0075 sec (For polarization transfer from a

TABLE 3. ^{1}H-nmr Shifts and Assignments[a] for Compounds **3–6**.

Proton	3	4	5	6
H-1	3.11 (τ 9.5)	3.81 (br s, w 1/2=7.1)	3.78 (br s, w1/2=7.0)	3.16 (dd, 10.1)
H-2	1.86; 2.19 (m; m)	1.65; 1.95 (m; m)	1.65; 2.25 (m; m)	2.02; 2.22 (m; m)
H-3	1.24; 1.57 (m; m)	1.63; 1.81 (m; m)	1.25; 1.58 (m; m)	1.19; 1.52 (m; m)
H-5	1.45 (br.s,)	1.59 (br s)	1.63 (br s)	2.31 (m)
H-6	5.31 (d, 7.5)	4.62 (m)	1.85; 2.45 (m; m)	1.99; 2.33 (m: m)
H-7	2.51 (m)	2.49 (d, 7.9)	2.22 (m)	1.40 (d, 7.6)
H-9	3.81 (d, 7.8)	3.97 (d, 6.8)	2.93 (d, 7.3)	2.98 (d, 6.9)
H-10	2.21; 2.45 (m; m)	2.35; 245 (m; m)	2.21; 2.42 (m; m)	2.15; 2.25 (m; m)
H-12	1.68; 2.31 (m; m)	1.68; 2.33 (m; m)	1.78; 2.95 (m; m)	1.70; 2.31 (m; m)
H-13	4.72 (m)	4.86 (t, 7.2)	4.87 (t, 7.1)	4.72 (m)
H-15	1.75; 1.83 (m; m)	1.79; 1.95 (m; m)	1.75; 2.01 (m; m)	1.66; 1.97 (m; m)
H-16	2.12; 3.19 (m; m)	1.75; 2.48 (m; m)	1.70; 2.40 (m; m)	2.08; 3.20 (m; m)
H-17	3.80 (br.s)	3.44 (d, 0.2)	3.28 (d, 0.2)	3.38 (d, 0.2)
H-18	0.87 (s)	1.08 (s)	0.92 (s)	0.80 (s)
H-19	2.10; 2.56 (d, AB 11.7)	2.01; 2.47 (AB d, 11.8)	2.10; 2.48 (AB d, 11.7)	2.06; 2.45 (AB d, 12.1)
N-CH_2	2.62 (m)	2.47 (m)	2.52 (m)	2.42 (m)
$\overset{1}{M}e$	1.04 (t, 7.1)	1.11 (t, 7.1)	1.13 (t, 7.2)	1.06 (t, 7.1)
1-OH	—	1.66 (br s)	1.63 (br s)	—
6-OH	—	4.89 (d, 3.5)	—	—
8-OH	3.58 (s)	2.31 (s)	2.28 (s)	1.62 (br s)
1-OMe	3.26 (s)	—	—	3.28 (s)
OAc	2.06 (s)	—	—	—

[a]Chemical shifts in ppm downfield from TMS, multiplicities and J(Hz) in parentheses. These shifts were assigned on the basis of COSY, HETCOR, and selective INEPT experiments.

CH_3); D5=0.0075 sec (For allowing evolution of antiphase magnetization); D6=0.015 sec (To refocus shifts, and to set decoupler power).

The protons of the -OH groups were assigned on the basis of the deuterium exchange studies with D_2O.

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