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HIGH RESOLUTION NMR STUDIES OF PANICULINE AND RELATED *LYCOPODIUM* COMPOUNDS

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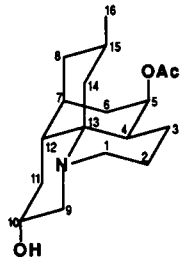
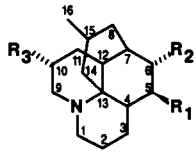
ABSTRACT.—Signals in the ^{1}H- and ^{13}C-nmr spectra of paniculine [**1**] were assigned by ^{1}H-^{13}C (HCCORR) and ^{1}H-^{1}H (COSY) 2D shift correlations. Deacetylpaniculine [**2**], acetyldihydrolycopodine [**3**], and deacetyllycoclavine [**4**] were also characterized by comparison with **1**.

Over the past two decades considerable information has been gathered about new structures, biosynthesis, and synthesis (1–5) of *Lycopodium* alkaloids. A review has been published recently (6), and the isolation of some new alkaloids with strong pharmacological activity has also been reported (7,8).

As part of our continuing studies of Chilean species of *Lycopodium* (9–13), we reported the revised structure of paniculine [**1**] (13), a lycopodine-related alkaloid with a hydroxy group at C-10α, which is an unusual position for functionalization in the lycopodane-type alkaloids. This structure was confirmed by an X-ray study (14).



1



2 $R_1=R_3=OH$, $R_2=H$
3 $R_1=OAc$, $R_2=R_3=H$
4 $R_1=R_2=OH$, $R_3=H$

A necessary step towards the examination of the biosynthesis of this type of alkaloid was the complete assignment of the ^{1}H and ^{13}C nmr spectra. A detailed study of these spectra for paniculine helped to characterize the related alkaloids deacetylpaniculine [**2**], acetyldihydrolycopodine [**3**], and deacetyllycoclavine [**4**].

^{1}H-^{13}C and ^{1}H-^{1}H shift correlated 2D nmr spectra were obtained to confirm (13) or complete (15) previous assignments. The ^{1}H homonuclear correlation spectrum (COSY-90, 500 MHz) displayed observable correlations for nearly all the protons of the alkaloid.

The signal at 24.1 ppm is assigned to C-16 (on the basis of its correlation with the H-16 protons). The H-16 signal correlated with the proton absorbing near 2.6 ppm, which in turn must be attached to C-15 (23.6 ppm). H-15 correlates with methylene protons at C-8 and C-14, which are easily distinguished by the further coupling observed between H-8 and the methine H-7. Consequently, peaks at 41.8, 42.8, and 35.0 are variously assigned to C-8, C-14, and C-7. The equatorial or axial disposition for each proton of methylene carbons 8 and 14 can readily be deduced from their coupling patterns and values.

The methines CH-5 and CH-10 are

TABLE 1. ^{1}H nmr Data for Compounds **1**, **2**, **3**, and **4**.[a]

Proton	Compound			
	Paniculine[b] [**1**]	Deacetylpaniculine[c] [**2**]	Acetyldihydro-lycopodine[c] [**3**]	Deacetyllycoclavine[c] [**4**]
H-1_{ax}	3.42 ddd (13.5, 13.5, 4.8)	3.44 ddd (13.5, 13.5, 3.8)	3.43 ddd (13.5, 13.5, 3.5)	3.42 ddd (14.0, 14.0, 3.5)
H-1_{eq}	2.49 dd (13.5, 5.9)	2.61 dd (13.5, 6.5)	2.30 dd (13.5, 4.9)	2.51 dd (14.0, 3.5)
H-2_{ax}	1.93 ddddd (13.8, 13.5, 13.5, 5.9, 5.9)	1.98 ddddd (13.5, 13.5, 13.5, 4.6, 4.6)	1.94 dddd (13.5, 13.5, 13.5, 4.9)	2.01
H-2_{eq}	1.34 br d (13.8)	—	—	1.36
H-3_{ax}	1.68 dddd (13.5, 13.5, 13.0, 2.1)	1.85 dddd (13.5, 13.0, 11.9, 4.1)	1.70 dddd (13.5, 13.5, 13.5, 2.1)	1.84
H-3_{eq}	1.42 br d (13.5)	1.48 m	—	1.48
H-4	2.42 ddd (13.0, 6.0, 2.4)	2.30 ddd (11.9, 5.7, 3.5)	2.45 ddd (13.5, 6.5, 3.0)	2.60
H-5	5.07 dd (6.0, 6.0)	3.95 dd (5.7, 5.7)	5.08 dd (6.5, 6.2)	3.83 br d (6.0)
H-6_{ax}	2.09 ddd (16.4, 6.4, 6.4)	2.08 ddd (11.9, 5.7, 5.7)	2.08 ddd (16.2, 6.5, 6.5)	3.72 s
H-6_{eq}	1.50 d (16.0)	1.51 dd (11.9, 5.7)	1.48 d (16.2)	—
H-7	1.78 br s	1.77 br s	1.74 br s	1.84 br s
H-8_{ax}	1.25 ddd (13.6, 13.6, 5.0)	1.20 ddd (13.5, 13.5, 6.5)	1.25 ddd (13.5, 13.5, 5.1)	1.24 ddd (13.2, 13.5, 5.1)
H-8_{eq}	1.66 ddd (13.5, 6.0, 2.4)	1.71 ddd (13.5, 6.5)	1.63 ddd (13.5, 13.5, 2.1)	1.72 ddd (13.2, 6.0, 2.4)
H-9_{ax}	2.95 dd (10.8, 10.8)	2.96 dd (10.8, 10.8)	3.16 ddd (11.6, 11.6, 3.0)	3.18 ddd (12.4, 12.4, 3.0)
H-9_{eq}	2.75 ddd (10.8, 5.2, 1.8)	2.75 ddd (10.8, 5.1, 1.6)	2.55 dd (11.6, 2.0)	2.51
H-10_{ax}	3.80 dddd (10.8, 10.8, 5.2, 5.2)	3.81 dddd (10.8, 10.8, 5.1, 5.1)	1.70	1.55
H-11_{ax}	1.48 m	1.50 m	—	—
H-11_{eq}	1.62 dddd (11.1, 4.6, 3.8, 1.6)	1.71 dddd (11.6, 4.6, 3.8, 1.6)	—	—
H-12	1.37 m	—	—	1.68 br d
H-14_{ax}	0.75 dd (14.3, 14.3)	0.71 dd (13.5, 12.9)	0.96 dd (13.5, 13.5)	0.90 dd (13.5, 13.5)
H-14_{eq}	2.64 dd (13.0, 6.0)	2.61 dd (13.5, 6.5)	2.65 dd (13.5, 6.0)	2.61 dd (13.5, 6.0)
H-15	2.63 q dddd (13.5, 13.5, 6.0, 6.0, 6.0)	2.89 q dddd (6.5, 12.9, 6.5, 12.9, 6.5)	2.62 q dddd (6.0, 6.0, 6.0, 13.5, 13.5)	2.62 q dddd (6.0, 13.5, 13.5, 6.0, 6.0)
Me	0.91 d (6.5)	0.88 d (6.5)	0.91 d (6.0)	0.85 d (6.0)
OAc	2.02 s	—	2.01 s	—

[a] *J* values (in Hz) are given in parentheses.
[b] Taken at 200 MHz amd 500 MHz.
[c] Taken at 400 MHz.

units that can also be assigned directly to signals at 5.08 and 3.43 ppm, respectively, in the ^{1}H-nmr spectrum and to signals at 70.2 and 68.5 ppm in the ^{13}C-nmr spectrum. H-5 correlates in the COSY spectrum with the methine H-4 (2.45 ppm) and is also coupled to H-6_{ax} (2.08 ppm). By reference to the heteronuclear correlation, the peaks at 31.1 and 31.2 ppm can then be assigned to C-4 and C-6, respectively.

The signal for H-10 (3.81 ppm) correlates with the protons of methylene carbons 9 and 11. The equatorial or axial nature of each proton of carbons 9 and 11 can be ascertained from the coupling patterns. Accordingly, in the ^{13}C-nmr spectrum, peaks at 54.7 and 34.4 ppm are assigned to C-9 and C-11, respectively.

Of the remaining methylene carbons 1, 2, and 3, the first, attached to the nitrogen atom, corresponds to the peak at 47.0 ppm in the ^{13}C-nmr spectrum. Its H-1_{ax} and H-1_{eq} protons absorb at 3.43 and 2.48 ppm, respectively, and in the COSY spectrum correlate with signals at 1.94 (H-2_{ax}) and 1.34 ppm (H-2_{eq}), which in turn correlate with those of H-3_{ax} (1.68 ppm) and H-3_{eq} (1.42 ppm). Examination of the ^{1}H-^{13}C spectrum enables the peaks at 20.4 and 23.1 ppm to be assigned to C-2 and C-3, respectively.

The assignment of the remaining signals, namely those corresponding to the methine CH-12, the quaternary C-13, and the acetate group, is quite obvious.

The ^{1}H-nmr spectrum of deacetylpaniculine [**2**] (Table 1), when compared with that of **1**, shows the changes to be expected for the substitution of the acetoxy group by a hydroxy.

Similarly, the ^{1}H- and ^{13}C-nmr data for acetyldihydrolycopodine [**3**] (Tables 1 and 2) can be assigned through comparison with those of **1**. The lack of functionality at C-10 causes greater overlapping of the ^{1}H-nmr signals, which makes their assignment difficult. Apart from the expected shielding by 2.1 ppm for H-10_{ax}, other minor changes (ca. 0.2 ppm) in the chemical shifts of H-9_{ax}, H-9_{eq}, H-1_{eq}, and H-14_{ax} are observed. In the ^{13}C-nmr spectrum, the changes affect the peaks assigned to C-10 (−42.1 ppm), C-9 (−12.0 ppm), C-11 (−9.6 ppm), C-12 (+1.9 ppm), and C-13 (−3.9 ppm), whereas the remaining signals are practically unaffected.

TABLE 2. ^{13}C-Nmr Data for Compounds **1**, **2**, **3**, and **4**.

Carbon	1	2	3	4
C-1	47.0	47.2	47.0	47.4
C-2	20.4	20.6	20.4	20.4
C-3	23.1	23.4	23.2	23.1
C-4	31.1	32.5	31.3	23.2
C-5	70.2	68.7	70.4	75.0
C-6	31.2	33.9	31.3	78.2
C-7	35.0	35.4	35.0	44.2
C-8	41.8	41.6	41.9	40.4
C-8	54.7	54.8	42.7	47.3
C-10	68.5	68.4	26.4	27.0
C-11	34.4	34.6	24.8	26.8
C-12	43.5	43.8	45.4	45.0
C-13	53.9	54.4	50.0	55.1
C-14	42.8	42.8	43.0	42.9
C-15	23.6	23.5	23.6	24.0
C-16	24.1	23.9	24.2	24.2

The ^{1}H-nmr spectrum of deacetyllycoclavine [**4**] (Table 1) can be assigned through comparison with those of **1** and **3**. Signals at 3.83 and 3.72 ppm in the ^{1}H-nmr spectrum of **4** correspond to H-5 and H-6, respectively, and some minor changes (ca. 0.14–0.20 ppm) with respect to data for **3** are also detected for the signals for H-3_{ax}, H-4, and H-1_{eq}. On the other hand, the presence of the hydroxy group at C-6_{ax} produces extensive changes in the ^{13}C-nmr spectrum of **4** compared with **1** and **3**. The shifts (Table 2) affected not only the carbons lying close to C-6 such as C-7 (+9.2 ppm with respect to the same signals for **3**) and C-5 (+4.6 ppm) but also C-4 (−7.9 ppm), C-9 (+5.4 ppm), and C-13 (+5.1 ppm), and to a lesser extent C-8 and C-11. These changes could be accounted for by the axial disposition of the hydroxy group at C-6 and its spatial relationship with the shifted carbons due

to the steric compression of the molecule. In this case the assignment is also based on previous off-resonance and selective decoupling experiments.

EXPERIMENTAL

^{1}H-nmr (200 MHz) and ^{13}C-nmr (50 MHz) were measured on a Bruker WP 200 SY in $CDCl_3$ with TMS as internal standard. ^{1}H (400 MHz) and ^{13}C (100 MHz) spectra were run on a Bruker WM-400 and ^{1}H-nmr spectra (500 MHz) on a Bruker WM-500 spectrometer.

^{1}H-^{13}C 2D nmr correlation was obtained through the HCCORR. AU Bruker pulse sequence using a 1k × 256w matrix. Response was tuned for $^1J_{CH}$ = 140 Hz with a recycle delay of 1.5 sec. Fourier transformation was made after sine-bell filtration in both time domains.

The ^{1}H-^{1}H-COSY 90 spectrum was obtained using the COSY. AU pulse sequence with a 1k × 256w matrix and a recycle delay of 1 sec. Fourier transformation took place after sine-bell filtration in both time domains.

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