CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTRAL ASSIGNMENTS FOR PYRROLIZIDINE ALKALOIDS

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ABSTRACT.—The natural abundance carbon-13 nmr spectra of some pyrrolizidine alkaloids, and their derivatives: retronecine, retronecine diacetate, retronecine hydochloride, platynecine, heliotrine, heliotrine acetate, supinine, lasiocarpine, crispatine, monocrotaline and maduresine, have been determined at 15.03 MHz. Several important chemical shift trends have been described which identify the basic pyrrolizidine alkaloid skeleton and the various ester groups in these alkaloids. On the basis of ^{13}C nmr data for crispatine, monocrotaline and madurensine, the chemical shift assignments reported earlier for C-3, C-9, C-12' and C-16, in retrorsine have been revised.

Pyrrolizidine alkaloids occur in several genera belonging to the families *Boraginaceae*, *Compositae*, *Leguminosae*, and *Apocynaceae* (1, 2). These alkaloids also have been identified in the hair-pencil secretions and scent organs of certain butterflies (3). Some of these compounds possess antitumor, carcinogenic, and/or hepatotoxic activities (1, 4). The wide range of biological activities exhibited by these alkaloids makes them interesting subjects of study. The structures of pyrrolizidine alkaloids have been elucidated by conventional spectroscopic methods and extensive chemical studies. Certain of the structures have been confirmed by X-ray crystallography or total synthesis. Except for two alkaloids, (5, 6) there has been no systematic, detailed study of the ^{13}C nmr spectra of members of this class of alkaloids. In this paper, we report ^{13}C nmr spectral assignments for pyrrolizidine alkaloids of varying substitution patterns and stereochemistry. Because structure elucidation of these alkaloids is frequently so difficult as to require X-ray crystallography, this ^{13}C nmr analysis should be of considerable value in solving structure problems.

RESULTS AND DISCUSSION

The chemical shifts for various pyrrolizidine alkaloids and their derivatives (table 1) were assigned with the help of noise decoupled spectra, single-frequency proton off-resonance decoupling (SFORD) techniques, direct analysis of nonprotonated carbons, application of known chemical shift rules for hydroxyl substitution, esterification shifts, and from comparisons of spectra from compound to compound.

The ^{13}C nmr spectrum of retronecine (**1**) exhibits 8 signals for 8 carbons in the molecule (table 1). The SFORD spectrum of retronecine shows 1 singlet, 3 doublets, and 4 triplets. The singlet at 137.9 ppm and the doublet at 127.1 ppm are assigned to the double bond at C-1 and C-2, respectively. Of the remaining two doublets at 71.1 and 79.5 ppm, the former is assigned to the hydroxyl group at C-7 by comparison with the corresponding chemical shift of the acetoxyl group at C-7 in retronecine diacetate (**2**). Assignment of the doublet at 79.5 ppm to C-8 is supported by the fact that the chemical shift of C-8 in **2** is shifted 3.7 ppm upfield. The triplet at 61.9 ppm in **1** is assigned to the oxymethylene unit at C-9 because this chemical shift moved downfield to 62.9 ppm in diacetate **2**. The upfield triplet at 35.3 ppm must be assigned to C-6 as it shifted further upfield in compound **2** because of the α-effect of the C-7 acetate. The remaining two triplets at 58.7 and 54.2 ppm in retronecine are assigned to C-3 and C-5,

TABLE 1. Carbon-13 chemical shifts and assignments for pyrrolizidine alkaloids and their derivatives.[a, b]

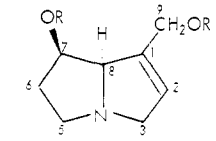
Carbon	1	2	3[c]	4[c]	5	6	7	8[e]	Carbon	10	11	12	13[f]	13[g]
1	137.9	133.8	135.7	45.8	136.4	135.3	137.9	135.0	1	132.9	132.8	129.9	132.4	132.4
2	127.1	127.7	120.5	30.4	127.4	127.6	125.6	128.6	2	135.5	134.3	136.1	134.7	134.7
3	58.7	61.2	56.8	57.2	62.0[h]	61.6[h]	61.9[h]	62.3	3	60.9	60.4	59.3	34.7	61.0
5	54.2	53.7	53.1	55.6	54.2	54.0	56.9	54.3	5	53.2	53.7	66.4	52.9	52.9
6	35.3	34.4	34.2	38.3	34.3	32.9	25.9	30.5	6	33.6	33.5	74.7[d]	37.9	37.9
7	71.1	74.0	68.4	75.0	75.6	76.8[d]	30.2	76.9	7	76.3	76.7	73.7[d]	77.4	77.4
8	79.5	75.8	77.8	73.1	78.6	78.4[d]	69.3	78.9	8	75.3	75.0	75.2	75.0	75.0
9	61.9	62.9	60.5	63.4	62.8[h]	62.0[h]	62.4[h]	62.3	9	61.3	61.3	61.5	66.9	62.7
1'	—	171.0	—	—	175.1	174.7	175.2	173.9	10	175.6[d]	173.5[d]	176.9	175.7	175.7
2'	—	20.8	—	—	82.6	83.2	83.1	83.8	11	37.6	78.7	76.3	81.3	81.3
3'	—	—	—	—	80.1	78.8[d]	71.5	78.9	12	76.3	76.8	40.5	35.7	35.7
4'	—	—	—	—	12.5	11.5	17.3[d]	13.0	12'	—	—	27.6	61.0	34.7
5'	—	—	—	—	57.0	56.8	—	56.5	13	48.1	44.2	135.6	131.2	131.2
6'	—	—	—	—	31.8	30.4	33.1	73.0	14	27.1	22.0	142.5	136.6	136.6
7'	—	—	—	—	16.4[d]	17.1	17.0[d]	24.6[d]	15	18.4	17.7	15.0	14.9	14.9
8'	—	—	—	—	17.1[d]	17.1	17.1[d]	26.5[d]	16	11.3	13.6	24.6	62.7	66.9
C-C=O	—	171.0	—	—	—	171.2	—	—	17	174.7[d]	174.1[d]	10.8	11.6	11.6
CH3	—	21.2	—	—	—	21.2	—	—	18	—	—	167.0	167.3	167.3

[a]Chemical shifts in ppm downfield from TMS. The solvent is $CDCl_3$ unless otherwise specified.
[b]Carbon-13 NMR spectra were taken at 15.03 MHz in the Fourier mode using a JEOL FX-60 spectrometer.
[c]The spectrum was taken in D_2O, using TMS as an external standard.
[d, h]These assignments may be interchanged in any vertical column.
[e]The carbon-13 chemical shift assignments of the angelic ester group in lasiocarpine are shown separately on structure **8**.
[f]The Italian worker's assignments for retrorsine. See Reference 5.
[g]Our assignments for retrorsine.

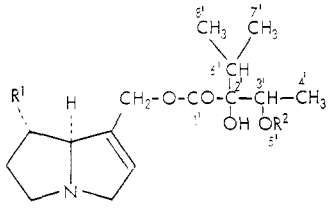
respectively. When the spectrum of **1** was compared with that of **2**, a significant shielding effect of the primary acetoxyl group on C–1 of diacetate **2** was observed. Comparison of the spectrum of retronecine hydrochloride (**3**) in D_2O with that of retronecine revealed that the chemical shifts of all carbons in **3** shifted upfield ($\Delta\delta$1.1–6.6 ppm). The doublet at 45.8 ppm and the triplet at 30.4 ppm are assigned to C–1 and C–2, respectively, in platynecine (**4**).

The ^{13}C nmr spectrum of heliotrine (**5**) shows 16 signals corresponding to 16 carbons in the molecule. The chemical shift of C–7 (α-hydroxyl group) in **5** is observed downfield (4.5 ppm) as compared with that of C–7 (β-hydroxyl group) in retronecine. A similar pattern also was observed for C–7 in the case of heliotrine acetate (**6**) and retronecine diacetate (**2**). The assignment of the doublet at 75.6 ppm to C–7 was confirmed by acetylation of heliotrine to heliotrine acetate (**6**). Comparison of the spectrum of supinine (**7**) with that of heliotrine revealed the absence of the chemical shift due to the hydroxy group at C–7 and changes in the chemical shifts of the basic skeleton of supinine. The chemical shift of C–3 is observed upfield at 71.5 ppm in supinine as compared with the corresponding carbon in heliotrine because of the substitution of methoxy by hydroxyl functionality. As a consequence of this substitution, the chemical shifts of the neighboring carbons C–2' and C–4' also are shifted downfield in supinine.

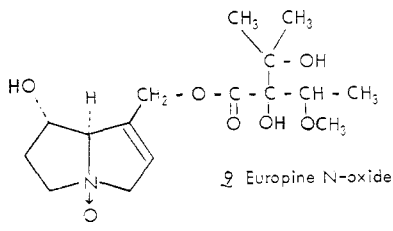
The ^{13}C nmr spectrum of lasiocarpine (**8**) exhibits six singlets. The singlets at 173.9 and 167.8 ppm are assigned to C–1' and C–9', respectively, the former being in the downfield region because of the pronounced α-effect of the hydroxyl group at C–2'. Of the two olefinic singlets, the signal at 135.0 ppm is assigned to C–1 by comparison with the corresponding chemical shift of C–1 in heliotrine



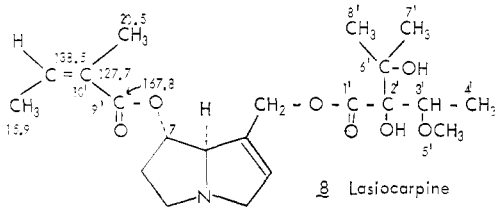
1 R = H Retronecine
2 R = Ac
4 Dihydro 1; 1β-CH_2OH



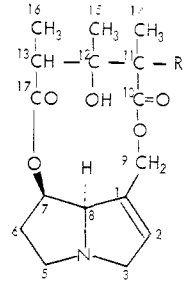
5 R^1 = OH; R^2 = CH_3 Heliotrine
6 R^1 = OAc; R^2 = CH_3
7 R^1 = H; R^2 = H Supinine



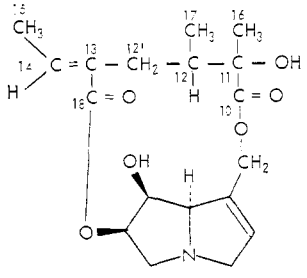
9 Europine N-oxide



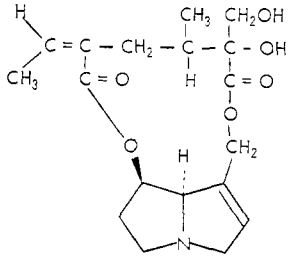
8 Lasiocarpine



10 R = H Crispatine
11 R = OH Monocrotaline



12 Madurensine



13 Retrorsine

acetate (135.3 ppm); therefore, the remaining singlet at 127.7 ppm may be assigned to C–10′. By comparison of the chemical shifts of C–2′ in heliotrine (**5**) and heliotrine acetate (**6**), the singlet at 83.8 ppm is assigned to C–2′, and thus the singlet at 73.0 ppm must be assigned to C–6′. In view of the values for C–7 (75.6 ppm) in heliotrine and C–9 (62.3 ppm) in lasiocarpine, the chemical shift assignments reported (6) for C–7 (97.1 ppm) and C–9 (77.9 ppm) in europine N-oxide (**9**) may be in error.

The SFORD spectrum of crispatine (**10**) shows 4 singlets, 5 doublets, 4 triplets and 3 quartets. We have observed that in the macrocyclic diesters, crispatine (**10**) and monocrotaline (**11**), the chemical shifts of C–1 are at a relatively higher field, whereas those of C–2 are at a lower field as compared with the chemical shifts of corresponding carbons in open-chain diesters. In crispatine (**10**) the quartet at 27.1 ppm is assigned to C–14 since it shifts upfield (22.0 ppm) in monocrotaline (**11**). Similarly, the doublet at 48.1 ppm is assigned to C–13 in crispatine. Of the remaining two quartets at 18.4 and 11.3 ppm, the former is assigned to C–15 because of the presence of an α-hydroxyl group. Comparison of the spectrum on monocrotaline (**11**) with that of crispatine (**10**) revealed the presence in **11** of an additional singlet at 78.7 ppm which must be assigned to C–11.

The spectrum of madurensins (**12**) shows 18 signals corresponding to 18 carbons in the molecule. The pattern of chemical shifts in madurensine is similar to that of retrorsine (**13**). (5) The chemical shifts for C–15 and C–18 are almost the same in **12** as observed for retrorsine, although the methyl group is trans to the C–18 carbonyl in madurensine. However, the chemical shifts for the olefinic carbons C–13 and C–14 are different in these two alkaloids. Assignments of chemical shifts to C–7 and C–8 in compounds **1** and **2** provided guidance for assignments of these carbons in alkaloids **10** to **13**. On the basis of ^{13}C nmr data of alkaloids **10** to **12**, we have revised the chemical shift assignments reported (5) for C–3, C–9, C–12′ and C–16 in retrorsine (**13**).

EXPERIMENTAL

Carbon-13 nmr spectra were determined at 15.03 MHz in the Fourier mode using a FX–60 spectrometer in conjunction with a JEC–980 computer. The spectra were determined at 30° in deuterochloroform solutions (which also provided the lock signal) with 5% tetramethylsilane as an internal reference. Samples were contained in precision ground 5-mm o.d. tubes. The spectrometer was used in the crosscoil configuration. On the average a 5 μs pulse, corresponding to an approximate tilt angle of 45°, was employed. For the average spectral width of 4000 Hz, the delay between pulses was 2s. Acquisition times averaged 1–2 h over 8K data points for concentrations of the order of 0.4–1.0M. For off-resonance spectra this time was 5–10 hours.

The alkaloids and their derivatives used here were isolated and/or synthesized by procedures given in the literature cited.

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LITERATURE CITED

1. F. L. Warren in *The Alkaloids*, Ed. R. H. F. Manske, Academic Press, New York, 1970, Vol. 12, Chapter 14.
2. C. K. Atal and R. S. Sawhney, *Indian J. Pharm.*, **35**, 1 (1973).
3. B. Tursch, J. C. Braekman and D. Daloze, *Experientia*, **32**, 401 (1976).
4. D. H. G. Crout in *The Alkaloids* (*Specialist Periodical Reports*), The Chemical Society, London, Vol. 6, Chapter 4.
5. H. Casal, J. Altamirano and P. Moyna, *Gazzeta Chimica Italiana*, **107**, 361 (1977).
6. L. H. Zalkow, L. Gelbaum and E. Keinan, *Phytochemistry*, **17**, 172 (1978).