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CARBON-13 AND PROTON NUCLEAR MAGNETIC RESONANCE SPECTRA OF VERATRUM ALKALOIDS[1]

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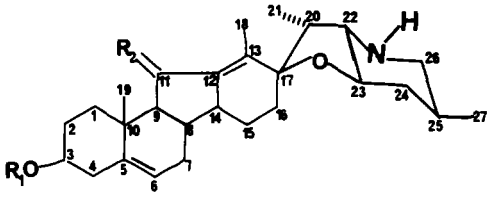
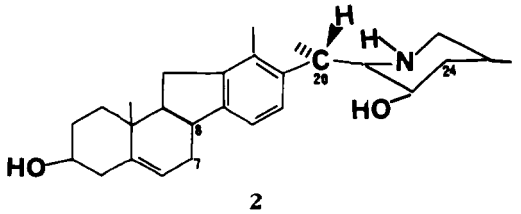
ABSTRACT.—^{1}H- and ^{13}C-nmr assignments are reported for the steroidal alkaloids jervine, veratramine, cyclopamine, and cycloposine. Eleven of the jervine and at least four of the veratramine carbon resonances, reported earlier by others, have been reassigned. ^{1}H- and ^{13}C-nmr spectra independently confirm the structure of cycloposine as 3-glucosyl-11-deoxojervine. The lesser shielding shown by C-7 and C-8 of the jerveratrum alkaloids, compared to the cholestane framework, suggests a general feature of the C-nor-D-homo skeleton.

The veratrum alkaloids occupy a position of prominence among steroidal alkaloids because some possess an unusual C-nor-D-homo skeleton (1, 2) and some are hypotensive (3), insecticidal (4), or teratogenic (5). ^{13}C-nmr data have been reported for two members of the jerveratrum group (6) and for several ceveratrum alkaloids (7-9). In particular, the ^{13}C-nmr spectra of jervine (**1**) and veratramine (**2**) were of interest in our attempts to assign carbon resonances in the spectra of the mammalian teratogens cyclopamine (11-deoxojervine) (**3**) and cycloposine (3-glucosyl-11-deoxojervine) (**4**). Based upon ^{13}C-nmr assignments reported (6) for jervine and veratramine, attempts to reconcile the structure of cyclopamine with its spectrum showed several points of disagreement. For example, an appropriate resonance for C-24 was not apparent in the cyclopamine spectrum and resonances assigned earlier to carbons 14, 16, and 20 of jervine appeared to be at variance with signals observed for cyclopamine. Inasmuch as the structure and stereochemistry of both jervine (10, 11) and cyclopamine (10-13) appeared to be firmly anchored on both physical and chemical grounds, the ^{13}C-nmr



1 R_1=H, R_2=O
3 R_1=H, R_2=H
4 R_1=β-D-Glc, R_2=H_2



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assignments of jervine and veratramine were the most likely source of disagreement. This study reports the ^{1}H- and ^{13}C-nmr spectra of several jerveratrum alkaloids, which have led to the reassignment of certain resonances in the ^{13}C-nmr spectra of jervine and veratramine.

RESULTS

The natural abundance ^{13}C-nmr spectra of jervine and veratramine in C_5D_5N were originally determined by Sprague *et al.* (6) at 15.08 MHz in one of the first ^{13}C-nmr investigations of steroidal alkaloids (14). Chemical-shift correlations between these alkaloids and degradation products of jervine together with proton-decoupling techniques were employed to make most of the assignments. Our proton spectra, in C_5D_5N and $CDCl_3$, have been analyzed with the aid of proton-proton decoupling and, to some extent, two-dimensional proton-proton correlations. Carbon resonances in the same solvents have been assigned by single-frequency irradiation of the proton resonances.

JERVINE.—*Proton spectra.*—Compared to the spectrum of cyclopamine (see below), the presence of an 11-keto group in jervine greatly simplified its ^{1}H-nmr spectrum (Table 1). Besides the absence of protons at C-11, the keto group of jervine exerted a discriminating effect on both H-1 protons and allowed H-9 to be perceived as a readily observable doublet in a rather congested portion of the spectrum. At 200 MHz, several proton signals that were clearly recognizable in the C_5D_5N spectrum of jervine were

TABLE 1. 200 MHz ^{1}H-nmr Data of Jervine, Cyclopamine, and Veratramine

Proton	Chemical Shift in ppm (J in Hz)		
	Jervine (C_5D_5N, 90°)	Cyclopamine (C_5D_5N, 90°)	Veratramine ($CDCl_3$)
1 eq	2.81 dt (13.6, 3.7)	[a]	[a]
1 ax	1.32 td (4.0, 13.6)	[a]	1.27 td (4.0, 12.8)
2 eq	~2.0	~2.05	2.24 dq (13.7, 2.5)
2 ax	~1.75-1.85	~1.75	[a]
3	3.73 tt (11.1, 5.0)	3.74 tt (5.4, 11.7)	3.58 tt (4.8, 11.0)
4 eq	2.57 ddd (2.3, 13.2, 4.9)	2.59 ddd (13.5, 5.4, 2.7)	2.36 ddd (5.0, 1.9)
4 ax	2.44 ddd (10.9, 2.0, 13.0)	~2.5	[a]
6	5.37 dt (5.6, 1.4, 1.4)	5.36 dt (5.4, 2.7, <2)	5.49 br d (6.0)
7 eq	~2.25-2.30 ddd (2.5, 11.8)[b]	~2.25	2.58 dtd (14.5, 5.0, 2.5)
7 ax	~1.80-1.90	~1.8	~2.0
8	~1.55	~1.45	2.92 ddd (4.5, 12.2, 2.2)
9	1.77 d (13.0)	[a]	~1.85
11 eq	—	[a]	2.61 dd (15.0, 2.0)
11 ax	—	[a]	2.79 dd (8.0, 14.7)
14	~2.0	[a]	[a]
15	~1.40-1.45	[a]	[a]
16	[a]	[a]	[a]
18	2.36 d (2.2)	1.73 br s	2.32 br s
19	1.12 s	0.94 s	1.14 s
20	2.46 qd (8.8, 7.8)	~2.45	3.49 qd (7.2, 5.6)
21	0.99 d (7.8)	1.03 d (7.8)	1.39 d (7.1)
22	2.72 t (9.3)	2.72 t (9.3)	2.48 dd (4.8, 9.0)
23	3.35 td (3.9, 9.8, 9.4)	3.32 td (11.1, 4.8)	3.27 td (4.8, 10.2)
24 eq	2.14 dtd (11.5, 1.4, 3.8)	~2.2	~2.0
24 ax	1.18 dt (11.7, 12.5)	1.21 dt (12.6)	0.98 dt (12.5, 11.0)
25	~1.6	~1.6	~1.6
26 eq	3.02 ddd (12.2, 4.7, 1.2)	3.05 dd (12.3, 4.8)	3.00 dd (5.2, 11.9)
26 ax	2.27 dd (11.2, 12.2)	2.29 dd (12.0)	2.11 dd (11.9)
27	0.83 d (6.9)	0.83 d (6.6)	0.82 d (7.0)

[a]Not assigned.

[b]Multiplet partially visible. Additional coupling of 5.6 Hz was established by collapse of H-6 to a broad singlet upon irradiation at δ 2.3.

due to protons 6, 3, 23, 26 eq, 1 eq, 22, 4 eq, 26_{ax}, 9, 1_{ax}, 24_{ax}, and all methyl group resonances. Signals that were partially visible were due to H-20, H-4_{ax}, and H-7 eq. The positions of several other resonances were located by mutual decoupling of protons to which they were coupled. Thus, irradiation of the readily assigned H-27 and H-6 resulted in observable changes in the regions of obscured signals H-25 ($\delta\sim 1.6$) and H-7_{ax} ($\delta\lesssim 1.8$-1.9), respectively, while irradiation of the latter signals resulted in the collapse of H-27 and H-6 as required. The approximate positions of both H-2's, H-14, and H-8 were derived by mutual decoupling of H-3, H-18, and both H-7's, respectively. An assignment of at least one H-15 was made by observing the collapse of the proton-coupled carbon signal assigned to C-15 to a doublet upon irradiation at δ 1.4 and by sharpening in the δ 1.4-1.5 region upon irradiation at the chemical shift of H-14. The other assignments for the ring E and ring F protons were confirmed by mutual decoupling. For example, irradiation of the H-24 resonances decoupled H-23 and vice versa. For all proton-bearing carbons except C-16, at least one proton was located in the ^{1}H-nmr spectrum. In addition, four long-range couplings were observed in the 90° jervine proton spectrum due to interactions between H-2 eq and H-4 eq, H-4 ax and H-6, H-18 and H-14, and H-24 eq and H-26 eq.

Carbon spectra.—^{13}C-nmr resonances of jervine previously reported (6) for C-5 and C-13 were found to be reversed since conversion of jervine to 12,13-dihydrojervine resulted in loss of the olefinic signal earlier assigned to C-5. Reduction of jervine also shifted the methyl carbon resonance at 12.4 ppm upfield to 10.5 ppm (see Experimental section). Because C-18 should be more greatly affected by reduction of the C-12, C-13 double bond, the assignments of C-18 and C-21 were reversed from those made earlier (6); single frequency on resonance decoupling (SFORD) of H-18 and H-21 confirmed these reassignments. Confirmation or reassignment of all proton bearing carbon resonances in the jervine spectrum was made by sford, except for C-16 which was assigned by default. Decoupling of the respective proton resonances required reassignment of the carbon signals due to C-1, C-4, C-8, C-14, C-16, C-20, and C-24. Thus, 11 carbon resonances mentioned in this section required reassignment and are noted in Table 2. Comparison of jervine spectra in C_5D_5N at 20° and 90° showed very slight chemical shift differences, but no signals appeared to be interchanged. In $CDCl_3$, C-16 shifted upfield $\sim$1.5 ppm.

VERATRAMINE.—*Proton spectra.*—Because better resolution of several proton resonances in $CDCl_3$ allowed assignments which could not be made in C_5D_5N, both the ^{1}H- and ^{13}C-nmr spectra of veratramine were measured in $CDCl_3$. Examination of the veratramine ^{1}H-nmr spectrum in $CDCl_3$, utilizing homonuclear H-H decoupling and 2-D proton-proton J-coupled correlation experiments, allowed assignment of all aliphatic protons except H-1 eq, H-2_{ax} and H-4_{ax}.

Carbon spectra.—Slight chemical shift differences (<1 ppm) between literature spectra of veratramine in C_5D_5N (6) and our data in $CDCl_3$ were observed. With the exception of the C-19 signal, all proton-bearing carbon resonances of veratramine were assigned by SFORD. The carbon resonances of only C-7 and C-8 and of C-20 and C-24 clearly required reversal from their earlier assignments.

CYCLOPAMINE AND CYCLOPOSINE.—*Proton spectra.*—Due to overlapping signals, several proton resonances which were readily identified for jervine (H-1 eq, H-1_{ax}, and H-9) were not assignable in the cyclopamine ^{1}H-nmr spectrum. Except for this additional complexity, the cyclopamine spectrum was similar to that observed for jervine. The attachment of the glucose residue to C-3 in cycloposine was indicated by a downfield shift of H-3 from δ 3.74 in cyclopamine to δ 4.05.

Carbon spectra.—Comparison with the jervine and veratramine ^{13}C-nmr spectra

TABLE 2. 50 MHz ^{13}C-Chemical Shift Assignments for Jervine, Cyclopamine, Cycloposine, and Veratramine

Carbon No.	^{13}C-Chemical Shift[a]				
	Jervine (C_5D_5N, 90°)	Jervine[b] ($CDCl_3$)	Cyclopamine (C_5D_5N, 20°)	Cycloposine[c] (C_5D_5N, 90°)	Veratramine ($CDCl_3$)
1	37.8[d]	36.8	38.8	38.6	38.0
2	31.1	31.1[e]	32.3[e]	30.2	31.3
3	71.5	71.6	71.4	78.0[e]	71.8
4	42.7[d]	41.4	43.0	39.2	41.9
5	143.2 (20°)[d]	142.3	142.8[f]	142.2[f]	142.4[g]
6	120.9 (20°)	120.9	121.5	122.2	122.0[g]
7	39.7	38.9	39.7	39.9	44.1[d]
8	39.0[d]	37.9	42.3	42.6	41.2[d]
9	63.2	62.5	52.5	52.7	56.9
10	37.8	37.0	36.9	37.2	36.9
11	206.8 (20°)	206.8	29.2	29.3	30.3[e]
12[h]	137.2 (20°)	137.1	127.7	127.7	140.3[g]
13[h]	146.4 (20°)[d]	145.8	142.0[f]	141.9[f]	132.6[g]
14	45.1[d]	44.8	49.5	49.6	143.9[g]
15	24.9	24.3	25.2	25.2	119.8[g]
16	32.2[d]	30.7[e]	32.4[e]	32.4	125.2[g]
17	85.8	85.5	85.3	85.5	143.0[g]
18	12.4[d]	12.1	13.6	13.4	15.8
19	18.9	18.4	19.2[i]	18.9[i]	19.4
20	41.4[d]	40.3	40.5	40.7	36.1[d]
21	11.0[d]	10.8	11.3	11.0	19.2
22	67.6	66.5	67.3	67.3	67.1
23	77.1	76.4	76.0	76.2	70.8
24	31.4[d]	30.9[e]	31.5	31.5	30.5[d,e]
25	31.8	31.5	31.7	31.8	32.2
26	55.4	54.6	55.4	55.5	54.0
27	18.9	18.8	18.8[i]	18.7[i]	18.8

[a] δ in ppm from TMS.

[b] Tentatively reported in footnote (c) of the Table [Gaffield (26)]. The value listed in this footnote for C-14 should be changed to 44.8.

[c] Signals due to the 3-O-glucosyl residue were observed at: δ 102.8 (C-1′), 75.3 (C-2′), 78.7[e] (C-3′), 72.3 (C-4′), 78.6[e] (C-5′), and 63.4 (C-6′).

[d] Reassigned from earlier work [Sprague *et al.* (6)].

[e,f] Signals may be reversed.

[g] Assigned by analogy to earlier work [Sprague *et al.* (6)].

[h] Assigned for **1**, **3**, and **4** on the basis of olefinic chemical shifts in α,β-unsaturated ketones [Levy and Nelson (27)].

[i] Signals may be reversed.

permitted assignment of most ^{13}C resonances of cyclopamine although assignment of signals due to C-8, C-9, C-11, and C-14 remained in doubt. Irradiation at δ~2.2, a value typical of allylic protons, caused decoupling of the ^{13}C resonance at 29.2 ppm which was then assigned to C-11. Although H-24 eq also occurred at δ~ 2.2, decoupling of the 31.5 ppm resonance upon irradiation of H-24$_{ax}$ (δ 1.21) permitted assignment of this resonance to C-24 (see also assignment of C-24 in jervine, Table 2). Shielding of C-11 may be due to its eclipsed γ-interaction with C-18 through an unsaturated system. Decoupling of the carbon signal at 49.5 ppm upon irradiation at δ 1.8-1.9 allowed assignment of this resonance to C-14 since H-14 was known to occur at δ~2.0 in jervine. Furthermore, decoupling of the carbon resonance at 42.3 ppm by irradiation at δ~1.4 suggested that this signal belonged to C-8 since in the spectrum of cyclopamine the H-8 signal occurred at δ~1.45. The remaining signal (52.5 ppm) was assigned to C-9 in agreement with its upfield shift (~10 ppm) upon removal of the adjacent C-11 keto group.

The ^{13}C-nmr spectrum of cycloposine resembled a summation of the ^{13}C-nmr spectra of cyclopamine and D-glucose. In accordance with glycosidation induced shift rules (15, 16), a downfield shift of C-3 (~7 ppm) and upfield shifts of C-2 (~2 ppm) and C-4 (~4 ppm) established the attachment of the sugar at C-3 in cycloposine as in

other steroidal alkaloids such as khasianine, solasonine, and solamargine (17). These results provided independent confirmation of the cycloposine structure as 3-glucosyl-11-deoxojervine, as previously assigned by Keeler (18) utilizing chemical and spectral methods.

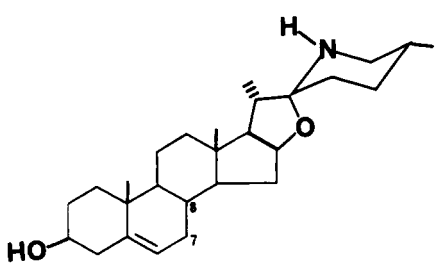
DISCUSSION

With determination of the ^{13}C-nmr assignments for the jerveratrum alkaloids completed, comparison was made with literature data for other C-nor-D-homo steroids and for steroids containing the cholestane skeleton. C-7 and C-8 of the C-nor-D-homo steroid structure in cyclopamine and cycloposine are deshielded by approximately 8 and 11 ppm, respectively, compared with data for cholesterol and its derivatives (19) and for steroidal alkaloids such as solasodine (**5**) (20), which contain the cholestane skeleton (Table 3). Loss of the shielding environment provided by C-18 and other portions of the β-face of the steroidal framework could be responsible for these differences.

TABLE 3. Comparison of the C-7 and C-8 Resonances of Cyclopamine and Cycloposine to those of Related Ring Systems

Compound	C-7	C-8	Reference
Cyclopamine (**3**)	39.7	42.3	—
Cycloposine (**4**)	39.9	42.6	—
Cholesterol	31.9	31.9	19
Solasodine (**5**)	32.1	31.5	20
Shinonomenine (**6**)	31.2	38.6	8
Veraflorizine (**7**)	31.5	38.7	8

^{13}C-nmr data have been reported (8) for the cevanine alkaloids shinonomenine (**6**) and veraflorizine (**7**), both of which contain A, B, and C rings similar to the jerveratrum alkaloids. Whereas most of the ^{13}C-nmr assignments to carbons of the A and B rings of these C-nor-D-homo steroidal alkaloids are nearly identical to those determined for cyclopamine and cycloposine, values assigned (8) to C-7 (≃31 ppm) and C-8 (≃39 ppm) of **6** and **7** are ~8 and 3.5 ppm upfield (Table 3). If it is assumed that these assignments are correct, a major factor which could contribute to the differences in C-8 resonances is the lack of a C-12, C-13 double bond in **6** and **7** which forces the A, B, and C rings closer in shape to those of the cholestane framework. Other possible factors include the presence of additional rings fused onto the D-ring of the cevanine alkaloids and their attendant conformational consequences or solvent effects. Recent X-ray studies of isobaimonidine have indicated (21) that an appreciable amount of flexibility exists in the cevanine skeleton. However, these explanations appear to be less satisfactory in resolving the discrepancy in C-7 assignments.



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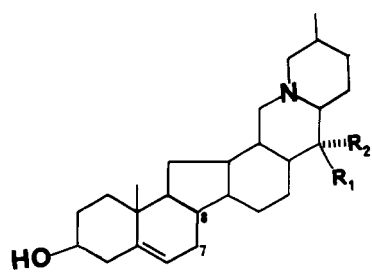

6 R_1=CH_3, R_2=OH
7 R_1=OH, R_2=CH_3

EXPERIMENTAL

ALKALOIDS.—The isolation, purification procedures, and physical properties of jervine (22), veratramine (23), cyclopamine (23), and cycloposine (18) have been described previously. All samples were exchanged with D_2O before examination by nmr techniques.

A sample of 12β, 13α-dihydrojervine, mp 242-244° [lit. (24) mp 247-248.5°], was provided by Dr. D. Brown (25). Its tentative ^{13}C-nmr assignments, with superscripts denoting assignments that may be interchanged, are as follows. ^{13}C nmr [$CDCl_3$] δ 10.5, q, C-21; 10.5, q, C-18; 18.8, q, C-19; 18.8, q, C-27; 25.5, t, C-15; 30.0, t, C-24[a]; 31.2, t, C-2; 31.6, d, C-25; 32.2, t, C-16[a]; 37.1, s, C-10; 37.1, t, C-1; 37.5, d, C-8; 38.9, t, C-7; 39.6, d, C-20; 41.5, t, C-4; 42.6, d, C-14; 46.6, d, C-13; 54.6, t, C-26; 57.6, d, C-12; 64.3, d, C-9[b]; 65.0, d, C-22[b]; 71.7, d, C-3; 76.1, d, C-23; 86.5, s, C-17; 121.4, d, C-6; 142.3, s, C-5; 217.7, s, C-11.

PROTON NMR SPECTROSCOPY.—^1H-nmr were measured at 200 MHz on a Nicolet NTC-200 spectrometer. Spectra were acquired with a 60° pulse angle at a 4 s repetition rate and a 3000 Hz spectral width twofold zero-filled into 32K of memory, giving a digital resolution of 0.19 Hz. All signals were referenced to TMS. The intensity of homonuclear irradiation was performed at several power levels to effectively cover different multiplet widths being irradiated, and the irradiation frequency was automatically stepped through 20 Hz intervals over the proton regions of interest.

CARBON-13-NMR SPECTROSCOPY.—^{13}C nmr were measured at 50.3 MHz on the same instrument by using 45-60° angles (90° for multiplicity separation sequences) at a 4 s repetition rate and a 12,000 Hz spectral width twofold zero-filled into 32K of memory giving a digital resolution of 0.75 Hz. Gated spectra (decoupler off only during acquisition) were obtained by square wave modulation of the decoupler frequency. In order to correlate carbon chemical shifts with the known proton chemical shifts, SFORD experiments were conducted by irradiation at a power of 640 Hz with the proton frequency set at resonances of interest and by stepping through the proton region of interest in increments of 0.1 ppm. Sharpening of affected carbon signals was observed. Although programmed SFORD was the major method used to determine complex coupling patterns, two-dimensional techniques using the Nicolet 1180 version of COSY were employed on occasion. The non-first-order behavior of many multiplets limited the utility of the two-dimensional method.

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