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^{13}C-NMR STUDIES ON THE CEVANINE ALKALOIDS : THE APPLICATION OF ^{13}C-NMR SPECTRUM FOR STRUCTURE ELUCIDATION OF NEW ALKALOIDS, BAIMONIDINE AND ISOVERTICINE

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Summary: The ^{13}C-NMR spectra of seven cevanine alkaloids isolated from *Veratrum* and *Fritillaria* plants were measured and their signals were assigned, and these results were applicated for structure elucidation of two new cevanine alkaloids, baimonidine and isoverticine, isolated from mature *Fritillaria verticillata*.

Many steroidal alkaloids, including C-nor-D-homo steroidal alkaloids, have been isolated from *Veratrum* and *Fritillaria* plants, and their structures have been elucidated by means of spectral and chemical procedures. However, ^{13}C-NMR spectroscopy has not been utilized for their structure elucidations up to the present. Among these C-nor-D-homo steroidal alkaloids, only ^{13}C-NMR spectra of jervine and veratramine have been reported[1] and no pertinent assignments of carbon resonances on cevanine alkaloids have yet been reported. On the other hand, it is expected that new representatives are still being isolated from natural sources. Based on such facts, we have measured the proton noise and off-resonance decoupled ^{13}C-NMR spectra[2] of cevanine alkaloids, shinonomenine[3] (1), veraflorizine[3] (2), veramarine[4] (3), verticine[5] (4), and verticinone[5] (5), which have been isolated during our biogenetic studies on *Veratrum* and *Fritillaria* alkaloids. The assignments for these alkaloids were presented in Table 1.

The carbon resonances of A and B rings in 1-4 were assigned according to those of cholesterol[6] and cholestane-3β,6α-diol,[7] and those in 5 were accomplished by comparison with the known data of 3β-androstanol[8] and 6-androstanone,[9] after due consideration of substituent effects of hydroxyl and carbonyl groups. The data of jervine and veratramine were not referred because these spectra have been measured in C_5D_5N solution. This assignment shows some tendency for C-nor-D-homo steroidal skeleton to make a slight upfield shift at C-7 and the relatively large downfield shifts at C-8 and C-9, compared with normal steroids.

Of several low field resonances arising from the carbons substituted by electronegative atoms, two methylene resonances in each spectrum are due to C-18 and C-26, but could not be individually assigned. The resonance at 68.0 ppm in 1 was assigned to C-22, since this resonance shifted downfield upon introduction of an axial hydroxyl group at C-20 in 2-5. Carbon-20 in each of 2-5 was easily assignable by off-resonance decoupling. Carbon-16 in 3 remained as unassigned carbinyl carbon.

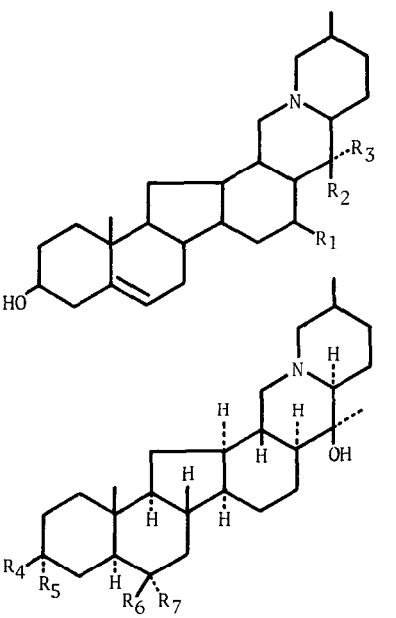
Three methyl resonances in each spectrum were easily resolved. The resonance at C-21 is apparently assigned by comparison of the spectra of 1-2. Namely, the methyl resonance at 8.6 ppm in 1 shifted downfield upon introduction of a hydroxyl group with the reversed configuration of methyl group at C-20 and its resonance in 2 is present in each spectrum of 3-5 which have the same situation at C-20. The resonance of C-19 in 1-3 shifted upfield by saturation of the double bond between C-5 and C-6 in 4 and 5, same as the shift observed between cholesterol and cholestanol.[6] Terefore, the remaining unaffected methyl resonance is assigned to C-27.

The structural change from 1 to 2 led to some assignments in addition to C-21 and C-22. One of two methine resonances at near 45.5 ppm in 1 is suitable for C-17, since the β-effect of the axial 20-hydroxyl group should deshield this carbon as well as C-22, and this assignment is further supported from the fact that C-17 in 3 resonates at lower field than that in 2 by the additional β-effect of the axial 16-hydroxyl group.[4] Moreover, the equatorial 20-methyl group is expected to shield C-16 and C-23 because of its γ-effect. In fact, two high field methylene resonances in 1 shifted upfield by about 4 ppm, although it is impossible to distinguish them from each other. However, it is possible to assign the higher field resonance, 19.9 ppm, to C-23 in 2, since this carbon in 3 resonates at 18.7 ppm without an appreciable change and C-16 is greatly deshielded by the α-effect of a hydroxyl group. The resonance at 36.2 ppm in 1 must be

Table 1. ^{13}C Chemical Shifts of Cevanine Alkaloids[a]

carbon	1	2	3	4	5	6a	7a
1	38.1	38.2	38.2	37.9	37.1	35.1	38.8
2	31.4*1	31.5	31.5*1	30.8	30.5*1	28.7	31.2
3	72.0	71.9	71.9	71.4	70.9	66.9	71.9
4	41.8	41.9	42.0	32.5	30.1*1	32.8	35.0
5	142.4	142.0	141.7	52.1	56.5*2	42.6	48.3
6	122.3	122.3	122.6	70.3	211.0	72.6	72.6
7	31.2*1	31.5	31.3*1	40.5	46.0	39.1	39.1
8	38.6	38.7	38.7	39.1	42.1	35.6	35.8
9	54.4	54.3	54.6	56.8	56.7*2	57.6	57.5
10	37.0	37.0	37.0	35.2	38.4	36.2	35.5
11	30.3*2	29.5*1	29.2*2	29.4	29.4*3	29.5*1	29.6*1
12	41.5	41.7	41.5	41.1	41.1	41.0	41.0
13	37.9	37.6	32.7	39.3	39.3	39.1	39.3
14	45.3*3	44.7	43.7	44.0	43.5	43.8	43.8
15	25.1*4	25.2	30.8	24.8	24.7	24.8	24.9
16	24.9*4	20.8	66.1	20.8	20.6	20.9	20.9
17	45.5*3	49.0	50.4	49.0	48.8	49.0	49.0
18	62.6*5	61.9*2	61.6*3	61.8*1	61.8*4	62.0*2	61.9*2
19	19.1	19.0	19.1	13.0	12.8	14.1	15.0
20	36.2	71.1	73.2	71.1	71.0	71.1	71.1
21	8.6	20.4	19.9	20.3	20.4	20.6	20.5
22	68.0	70.4	70.0	70.3	70.3	70.6	70.5
23	24.3*4	19.2	18.7	19.1	19.1	19.1	19.1
24	28.9*2	29.3*1	28.8*2	29.4	29.2*3	29.3*1	29.5*1
25	28.3	27.8	27.6	27.7	27.7	27.8	27.8
26	63.9*5	62.7*2	62.2*3	62.5*1	62.3*4	62.5*2	62.6*2
27	17.9	17.4	17.3	17.3	17.3	17.5	17.4

[a] In ppm relative to TMS in $CDCl_3$. Assignment of chemical shifts marked with the same number of asterisk may be reversed.



	R_1	R_2	R_3
Shinonomenine (1)	H	CH_3	H
Veraflorizine (2)	H	OH	CH_3
Veramarine (3)	OH	OH	CH_3

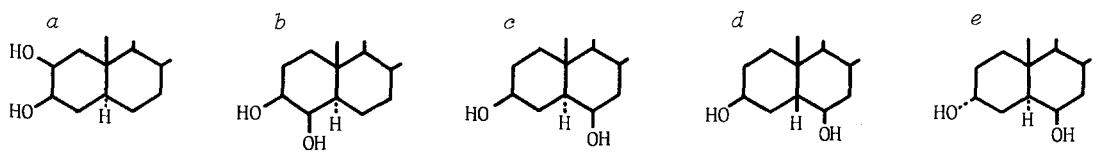
	R_4	R_5	R_6	R_7
Verticine (4)	OH	H	H	OH
Verticinone (5)	OH	H	=O	
Baimonidine (6a)	H	OH	OH	H
Isoverticine (7a)	OH	H	OH	H

assigned to C-20 which is the only remaining carbon expected to be appreciably affected. The introduction of a hydroxyl group at C-16 in 3 caused the shift, which is characteristic for an axial hydroxyl group, at C-13 (γ-effect) and C-15 (β-effect), as well as C-17 as mentioned above. These five resonances in 2 were commonly observed in 4 and 5.

Of the remaining unassigned resonances in each spectrum, the assignment of C-24 and C-25 was based on the data of 3-methylquinolizidine.[10] From the off-resonance decoupled spectra of these alkaloids, carbon-11 is the only unassigned methylene carbon. Although the assignment of C-12 and C-14 was difficult, these were tentatively assigned from the data of substituent effects produced by a methyl group on cyclopentane.[11]

Two new cevanine alkaloids which were isolated from the mature *Fritillaria verticillata* in our course of biogenetic studies on *Fritillaria* alkaloid, baimonidine (6a) from the aerial part, and isoverticine (7a) from the bulb, in addition to solanidine and hapepunine[12] which were first isolated from *F. verticillata*, were assigned their structures to stand on the data of ^{13}C-NMR spectra of cevanine alkaloids 1-5, and their absolute configurations were chemically determined.

Baimonidine (6a), named after the Japanese name "Baimo" for the original plant, mp 179-181.5° $C_{27}H_{45}NO_3$ (elementary analysis), $[\alpha]_D$ -36.4° (*c* 1.0, $CHCl_3$), MS: m/e 431 (M^+) and 112 (base peak), ^{1}H-NMR: δ 1.02 and 1.05 (3H each, s, H-19, this signal shifted downfield compared with that of cholesterol because of 1,3-diaxal interaction with β-axial hydroxyl group at C-2, C-4, or C-6, and H-21 which is methyl protons on carbon bearing oxygen), 1.11 (3H, d, J=7 Hz, H-27), 3.84 and 4.24 (1H each, m, $W_{1/2}$=8 Hz, H-3β and -6α, both signals shifted downfield to δ 4.94 and 5.11 on acetylation, respectively), IR: $\nu_{max}^{CHCl_3}$ 2750 (*trans*-quinolizidine) cm^{-1}, afforded O,O-diacetate (6b), mp 139-141°, δ 2.03 [6H, s, $(OAc)_2$], $\nu_{max}^{CHCl_3}$ 3550-3100, 1720, 1250 cm^{-1}. These narrow half-height widths of hydrogen bearing hydroxyl group, in addition to downfield shift of H-19, suggest following five structures (*a*, *b*, *c*, *d* and *e*) for the structure of 6a.



The ^{13}C-NMR spectrum of 6a exhibited four signals in the carbinyl carbon region at 66.9 (d), 70.6 (d), 71.1 (s), and 72.6 (d) ppm and two of these signals at 71.1 and 70.6 ppm were assigned to C-20 and C-22, respectively, in comparison with those of cevanine alkaloids 1-5. The remaining signals at 66.9 and 72.6 ppm in 6a correspond to carbinyl carbon in question. Djerassi *et al.*[8, 13] reported the chemical shifts in the ^{13}C-NMR spectrum of C-3 and C-4 in 5α-cholestane-3β,4β-diol at 72.2 and 74.8 ppm, respectively, and C-2 in 2β-cholestanol at 67.9 ppm. Also, in the ^{1}H-NMR spectrum of 6a, signals of two hydrogens adjacent to hydroxyl group did not change by decoupling. Rf value on tlc of isoverticine (7a), which was synthesized from 5 with $NaBH_4$ in EtOH, was not identical with that of 6a. Gough *et al.*[14] observed that the resonance of C-19 in A/B-*cis* steroids shifted downfield by 12 ppm than that in A/B-*trans* steroids in the ^{13}C-NMR spectrum. The chemical shift of C-19 of 6a at 14.1 ppm shifted downfield only by 1.2 ppm compared with that of 4. These data of the ^{13}C-NMR spectra of 6a are inconsistent with the structures *a*, *b*, *c*, and *d*. The ^{13}C-NMR spectrum of 6a provides a convincing evidence for the

remaining structure *e*. The chemical shifts of C-3 in 3α-cholestanol[8] at 66.6 ppm and of C-6 in 6β-androstanol[8] at 72.5 ppm are consistent with those of C-3 and C-6 in 6a. On the basis of ^{13}C-NMR spectral evidences, 6a was assumed as (22S,25S)-5α-cevanine-3α,6β,20β-triol.

To confirm the absolute configuration of 6a, 5 was converted to 6a, according to the method of Bose *et al.*[15] The physical constants of 6a agreed completely with those of the natural product, and the melting point of 6a was not depressed by admixture with the natural product.

Isoverticine (7a), mp 135-137°, $[\alpha]_D$ -45.0° (*c* 1, $CHCl_3$), $C_{27}H_{45}NO_3$ (elementary analysis), MS: m/e 431 (M^+), and 112 (base peak), ^{1}H-NMR: δ 1.03 (6H, s, H-19 and H-21), 1.09 (3H, d, J=7 Hz, H-27), 3.63 (1H, m, $W_{1/2}$= 23 Hz, H-3α, this signal shifted downfield to δ 4.73 on acetylation), 3.85 (1H, m, $W_{1/2}$= 8 Hz, H-6α, this signal shifted downfield to δ 5.00 on acetylation), IR: $\nu_{max}^{CHCl_3}$ 3500-3350, 2770 (*trans*-quinolizidine) cm^{-1}, afforded 0,0-diacetate (7b), amorphous, $\nu_{max}^{CHCl_3}$ 3500-3350, 2770, 1250 cm^{-1}. In the ^{13}C-NMR spectrum of 7a, carbon-19 is deshielded because of 1,3-diaxal interaction compared with that of 4. Furthermore, the carbon resonances of A and B rings agreed with the data of cholestane-3β,6β-diol,[7] after due consideration of the shielding trends of C-nor-D-homo steroidal skeleton, as mentioned above. The physical properties of 7a suggest that 7a possesses the same ring juncture at A/B and hydroxyl substitution as 6a, except for the configuration at C-3, and the structure of 7a was assumed as (22S,25S)-5α-cevanine-3β,6β,20β-triol.

The physical constants of isoverticine (7a) synthesized from verticinone (5) agreed completely with those of the natural product, and the melting point of 7a was not depressed by admixture with the natural product.

Two new cevanine alkaloids, 6a and 7a, possess 5α-cevanine skeleton, same as 4 and 5, and the isolation of these four alkaloids, in addition to solanidine, from *F. verticillata* suggests the biogenesis of *Fritillaria* alkaloids from solanidine via 2. From the fact that 6a, 7a and 4 have the same fundamental structure, 5α-cevanine skeleton, and that they possess different orientations of hydroxyl substitution at C-3 and C-6, it seems reasonable to conclude that they are synthesized through quite a similar pathway. It appears of interest to investigate further mechanism of hydroxylation, especially the orientations of these hydroxyl groups.

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