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(±)-SEVERZINE FROM *CORYDALIS FLABELLATA*: INTERPRETATION OF NMR SPECTRA

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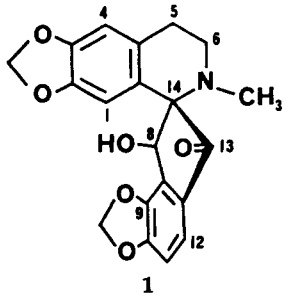
ABSTRACT.—The spirobenzylisoquinoline alkaloids (+)-sibiricine [**1**] and (±)-severzinine [**3**] have been isolated, for the first time, from the aerial parts of *Corydalis flabellata*. ^{1}H- and ^{13}C-nmr techniques, including ^{13}C{^{1}H} nOe, have been employed to establish the relative stereochemistry of severzinine at C-8 and C-13 and to identify unambiguously chemical shift values for all H and C nuclei. As a consequence of this study, the resonance assignments reported previously for H-8 and H-13 of the closely related alkaloid 13-*epi*-yenhusomine [**4**] should be reversed, as should those for C-9 and C-10..

Corydalis flabellata Edgew. (Fumariaceae) (syn. *Corydalis flavillata*) is an erect, profusely branched, perennial herb, common above 2500 m in the Himalayas (1). In the present study the dried aerial parts yielded two spirobenzylisoquinolines, a type of alkaloid known to occur primarily in *Corydalis* and the allied genus *Fumaria* (2). One of the alkaloids was identified as (+)-sibiricine [**1**], which has previously been found in other *Corydalis* species (2).

The second compound was characterized as an 8,13-dihydroxy compound, i.e., a dihydroderivative of **1**. Two such compounds are known (2), ochrobirine [**2**] ([α]D +36°), in which the hydroxy substituents are trans, and severzinine [**3**] ([α]D +109°), in which they are cis (2). The alkaloid isolated in the present study lacked optical activity, a feature not unusual in spirobenzylisoquinolines (2), and an analysis of ^{1}H- and ^{13}C-nmr chemical shifts revealed it to be significantly different from **2** (Table 1). The paucity of data available for **3** (3) prevented a rational comparison, and a comprehensive study had to be undertaken to establish that the isolated compound



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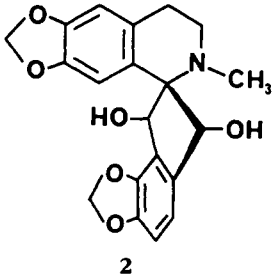

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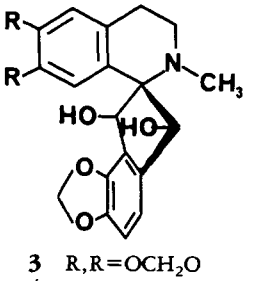

3 R,R=OCH_2O
4 R=OMe

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TABLE 1. ^{1}H-nmr and ^{13}C-nmr Chemical Shift Values and Coupling Constants (in parentheses) for **3** and Their Comparison with Previously Published Data for **2** (5) and **4** (4).

Atom	^{1}H			^{13}C		
	2	**3**	**4**	**2**	**3**	**4**
H-1/C-1	6.04 s	6.18 s	6.16 s	109.7 d	106.9 d	109.8 d
C-2				146.2 s	145.6 s	146.3 s
C-3				146.8 s	146.9 s	147.7 s
H-4/C-4	6.62 s	6.65 s	6.66 s	110.0 d	109.8 d	112.1 d
C-4a				126.0 s	131.7 s	130.6 s
H-5/C-5		2.78 t (6.3)	2.79 m	22.8 t	23.8 t	23.6 t
H-6/C-6		3.33 t (6.3) 3.34 t (6.3)	3.34 m	47.6 t	47.8 t	48.0 t
H-8/C-8	4.88 s	5.42 s	5.22 s	73.4 d	77.8 d	76.8 d
C-8a				121.5 s	122.4 s	122.6 s
C-9				144.7 s	143.0 s	147.9 s
C-10				148.6 s	148.5 s	142.5 s
H-11/C-11	6.85 s	6.82 d (7.8)	6.82 d (7.8)	107.1 d	109.0 d	106.5 d
H-12/C-12	6.85 s	6.87 d (7.8)	6.89 d (7.8)	116.1 d	116.2 d	115.6 d
C-12a				140.0 s	136.9 s	137.6 s
H-13/C-13	5.42 s	5.19 s	5.43 s	79.5 d	79.7 d	78.3 d
C-14				75.2 s	80.9 s	80.4 s
C-14a				129.5 s	121.8 s	120.2 s
N-Me	2.67 s	2.59 s	2.59 s	37.7 q	38.7 q	38.7 q
2,3-OCH_2O	5.87 s	5.83 s		101.0 t	100.8 t	
9,10-OCH_2O	6.00 s	5.98/6.04 AX (1.5)	5.96/6.00 AX	101.9 t	101.7 t	101.3 t
2-OMe			3.40 s			55.1 q
3-OMe			3.83 s			55.4 q

was indeed **3**. During the course of our investigation, reference was made to published data (4) for the closely related alkaloid 13-*epi*-yenhusomine [**4**]; this has led us to reassign H-8, H-13, C-9, and C-10 resonances in the latter.

The identity of the racemic alkaloid as an 8,13-dihydroxy *N*-methyl spirobenzylisoquinoline substituted at C-2/C-3 and C-9/C-10 with methylenedioxy groups could be established directly from the nmr spectra (Table 1). In the ^{1}H-nmr spectrum the methylenedioxy AX system (δ 5.98, 6.04) was assigned to ring D. The occurrence of a significant difference in shielding of the two protons of this ring-D substituent is to be anticipated if the C-8 and C-13 hydroxyls lie on the same side of the C,D-ring plane (cf. compound **4**), whereas, if they lie on opposite sides, then the environments of the two protons would be comparable (cf. compound **2**) (2,5).

The relative stereochemistry of the hydroxyl groups and the specific assignments of the protons were obtained through a series of homonuclear proton nOe difference spectra (6). Irradiation of the *N*-methyl resonance gave enhancements for ring-C proton resonances (8% each at δ 5.19 and 5.42) and for the H-6 multiplet at δ 3.33/3.34. The cis orientation of H-8 and H-13 was further substantiated by their reciprocal enhancement of each other (2%) and by the small (1%) enhancement both cause in the *N*-methyl resonance. Thus both hydroxyl groups lie on the same side of ring C and away from the *N*-methyl (i.e., towards ring A). Irradiation of the ring-A aromatic protons at δ 6.18 and 6.65 caused, respectively, enhancement of the hydroxyl resonance (2%) and of the 2H triplet for H-5 at δ 2.78; thus δ 6.18 must be H-1 and δ 6.65 must be H-4.

Irradiating the ring-D proton resonance at δ 6.87 enhanced the ring-C proton at δ 5.19 (1%), thus permitting assignment of the former to H-12 and the latter to H-13,

so requiring allocation of the δ 6.82 and 5.42 resonances to H-11 and H-8, respectively. These assignments for H-8 and H-13 are the reverse of those made for **4** (Table 1) by Mukhopadhyay *et al.* (4). These workers produced no direct evidence for their assignment of these two resonances. Their proposition relies on the assignment of long range heteronuclear couplings from H-8 and H-13 to the quaternary carbon atoms of ring D, which are also in conflict with our findings (see below). It seems likely that their assignments of these protons require reversal, in which case excellent agreement would be found between data for **3** and **4** (Table 1).

The ^{13}C-nmr spectrum revealed the anticipated 20 resonances and, as the ^{1}H-nmr spectrum was fully rationalized, a 2D C-H correlation experiment (7) optimized for one-bond couplings (Figure 1) allowed unambiguous assignment of C-1, C-4, C-5, C-6, C-8, C-11, C-12, C-13, and methylenedioxy resonances. In this study the C-H correlation links δ 5.19 to δ 79.7 for H-13/C-13 and δ 5.42 to δ 77.8 for H-8/C-8. This is again at odds with data reported (4) for 13-*epi*-yenhusomine where a HETCOR study appears to have associated the more shielded proton with the more shielded carbon. The aromatic quaternary carbon resonances were then assigned by a series of heteronuclear ^{13}C{^{1}H} nOe difference spectra (8). The results of these experiments, which show intensity increases for quaternary carbons close to irradiated protons, are set out in Table 2. The only unassigned quaternary aromatic resonance (δ 143.0) must be attributed to C-9. These chemical shifts again agree well with previously published (4) data for **4** except for C-9 and C-10, which must be reversed (Table 1). Mukhopadhyay *et al.* (4)

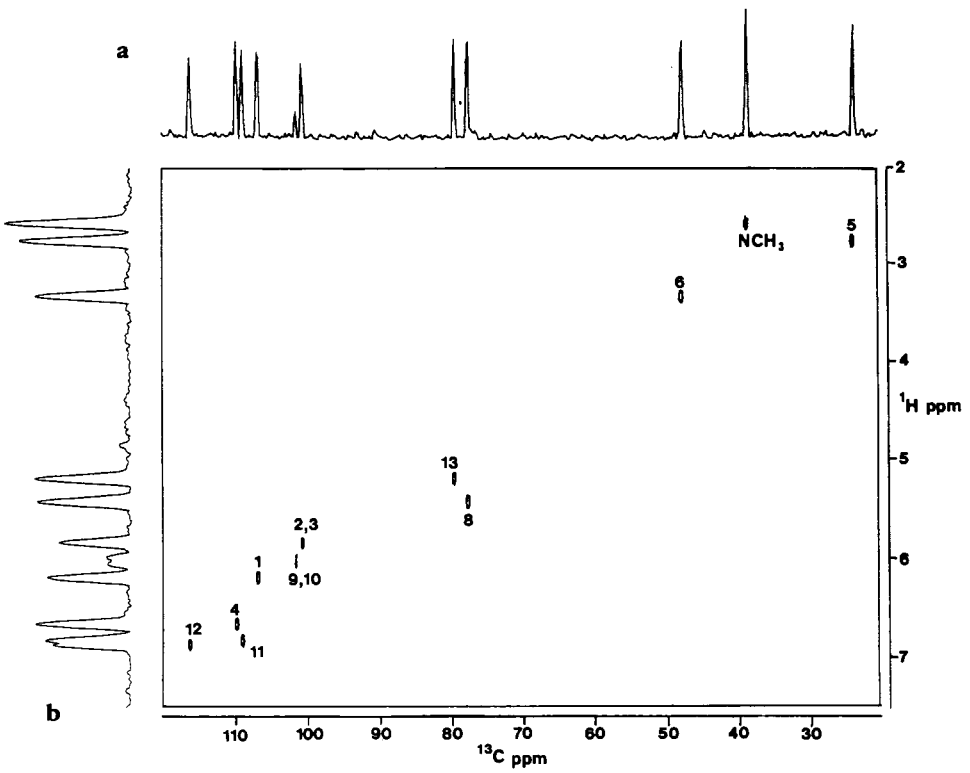

FIGURE 1. Contour plot of the 2D ^{13}C-^{1}H correlation spectrum of **3** together with (a) carbon and (b) proton projections. The sequence used removes all homonuclear proton couplings except those between shift-separated methylene protons attached to the same carbon. Thus the resonances in the proton projection appear as singlets except for the 9,10-methylenedioxy protons.

TABLE 2. $^{13}C\{^1H\}$-nOe Difference Spectra for **3**

Irradiated proton	Enhanced carbon	Enhancement (%)	Assignment
H-1	121.8	11	14a
	145.6	18	2
	122.4	6	8a
H-4	131.7	8	4a
	146.9	18	3
H-8	122.4	14	8a
H-11	148.5	15	10
H-12	136.9	10	12a
H-13	136.9	8	12a

based their assignment of C-9 and C-10 on a selective INEPT experiment (9) [not as they stated, a SINEPT (10) study], and while this clearly established the carbon resonances at C-9 and C-10 it does not distinguish between them. It remains unclear how their assignments of these carbons were achieved, and, in view of the evidence put forward here, it seems probable that they should be reversed for **4**.

These data clearly indicate that the isolated alkaloid was racemic severzinine [**3**]. This is the first report of severzinine existing in the racemic form.

EXPERIMENTAL

PLANT MATERIAL.—*C. flabellata* was collected from the Upshi region of Ladakh in the northeastern part of Jammu and Kashmir in June 1987. A voucher specimen (KASH 1107) is deposited at the Herbarium of Kashmir University.

EXTRACTION AND FRACTIONATION.—The dried aerial parts (5 kg) were cut into small pieces and extracted in EtOH at room temperature. The resulting extract was concentrated and extracted with 5% HOAc. The aqueous acid extract was then partitioned with $CHCl_3$. The $CHCl_3$-soluble portion was basified with 2% NaOH and, after removal of the base-soluble material, re-acidified with 2% H_2SO_4. The H_2SO_4-soluble fraction was then rebasified with NH_4OH (pH 9) and on extraction with Et_2O yielded **1**, mp 224–225° [identical to data in Preisner and Shamma (2)]. Similar treatment of the original HOAc-soluble fraction gave **3** (120 mg).

IDENTIFICATION OF (±)-SEVERZININE [**3**].—Needles from MeOH: mp 206°; $[\alpha]_D$ 0° ($c = 1.0$, $CHCl_3$); uv λ max nm 290; ^1H nmr and ^{13}C nmr see Table 1; eims *m/z* (rel. int.) $[M]^+$ 369 (5), 354 (18), 351 (59), 336 (38), 322 (100), 293 (34), 190 (58), 188 (19); calcd for $C_{20}H_{19}NO_6$, 369.1212, found (eims) 369.1204.

NMR SPECTROSCOPY.—Spectra were recorded at 25° on a Bruker WH360 spectrometer operating at 360.13 MHz for ^1H and at 90.56 MHz for ^{13}C nuclei. Standard and homodecoupled spectra were acquired with 32K data points over a spectrum width of 4000 Hz (11.1 ppm) and referenced to $CHCl_3$ at δ 7.25. Homonuclear ^1H nOe spectra were obtained with 16K data points over a spectral width of 3500 Hz (9.7 ppm). Secondary irradiation at 41 dB below 0.2 W was applied during an 8 sec delay followed by spin excitation with a 90° pulse. Blocks of 16 scans preceded by 2 dummy scans were accumulated for each irradiation site to give a total of 352 scans per site. Multiplets were irradiated by the technique (8) in which the irradiation frequency was cycled through each of the multiplet line positions in turn. The control spectrum was obtained with irradiation at δ 0.1. Line broadening of 1 Hz was applied before zero filling to 32K and Fourier transformation. Subtraction of the control spectrum from the selectively irradiated spectra gave the enhancements stated above.

^{13}C-nmr spectra were obtained on a 120-mg sample dissolved in 2 ml solvent with 32K data points over a spectrum width of 20,000 Hz and referenced to $CDCl_3$ at 76.9 ppm.

Heteronuclear ^1H-induced ^{13}C nOe spectra were obtained with 16K data points over a spectral width of 15,151 Hz. Secondary selective irradiation at 45 dB below 0.2 W was applied in the ^1H spectrum during a 10-sec delay followed by a 90° spin excitation pulse and data acquisition with broad band proton decoupling. A total of 1344 scans was accumulated for each irradiation site in the manner described for the homonuclear data. Line broadening of 4 Hz was applied to the data followed by Fourier transformation and subtraction of the control spectrum from each of the selectively irradiated spectra.

Two-dimensional H-C correlation spectra were obtained using the sequence proposed by Bax (7) in

which proton coupling is removed in both dimensions. A relaxation delay of 3 sec was used between scans; other parameters being SW(carbon) = 9259 Hz; 1K data points; SW(proton) = 2198 Hz; 128 FIDs each of 160 scans. The data was processed using sine bell squared window in both dimensions with zero filling the F1 data from 128 W to 512 W. ^{1}H résolution 8.6 Hz/point; ^{13}C resolution 18.1 Hz/point.

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