

BERBERIS ALKALOIDS.

XXIV. STRUCTURE OF BERNUMINE

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Bernumine, which belongs to a new group of N-benzyltetrahydroisoquinoline alkaloids, has been isolated from the leaves of Berberis nummularia Bge. Its structure has been established on the basis of spectral characteristics and chemical transformations.

We have studied the alkaloid composition of the leaves of *Berberis nummularia* Bge. growing in the Chilisaiskii Gorge (Naukatskii region, Kirghizia). A number of isoquinoline alkaloids have been isolated from this plant previously [1-3]. Chloroform extraction of the leaves of this plant yielded 0.28% of total alkaloids. By chromatography of the ether fraction on a column of silica gel we isolated isotetrandrine, glaucine, laudanosine, oxyacanthine, thalicmidine, and a new base with the composition $C_{19}H_{21}NO_4$, which we have called bernumine (I). The known alkaloids were identified by their physicochemical constants and spectral characteristics, and also by comparison with authentic specimens.

Bernumine (I) is an optically active base possessing phenolic properties and readily soluble in organic solvents. Its IR spectrum has an absorption band due to active hydrogen ($\nu_{\max}^{KBr} 3450 \text{ cm}^{-1}$), and its UV spectrum [$\lambda_{\max}^{C_2H_5OH} 285 \text{ nm}$ ($\log \epsilon 3.84$)] is characteristic for the tetrahydroisoquinoline alkaloids [4]. In the mass spectrum of (I) the ions with m/z 312 ($M - 15; 55$) and 135 (100) formed on cleavages *a* and *b* are intense, while the ions formed from the tetrahydroisoquinoline part of the molecule, with m/z 192, 190, and 178, are weak. Thus, the mass fragmentation of (I) under electron impact differs sharply from that of known benzyltetrahydroisoquinolines [7]. The appearance of ions with m/z 192, 190, 178, and 135 shows the presence of hydroxy and methoxy groups in ring *A* and also of a methylenedioxy group in ring *C*.

Parameters of the 1H and ^{13}C NMR spectra and also values of the direct C—H constants are given in Table 1.

From the multiplicities of the signals of the aromatic protons in the PMR spectrum of (I) it is possible to conclude that the substituents in ring *A* are present in the 6,7-positions, while in ring *C* the methylenedioxy group occupies the 3',4'-position.

The mutual positions of the hydroxy and methoxy groups in ring *A* were established by measuring the intramolecular nuclear Overhauser effect (NOE). Thus, on the irradiation with a saturated radiofrequency field of the resonance transitions of the methoxy group at 3.75 ppm, the integral intensity of the singlet at 6.42 ppm increased by more than 10%, and when the signal of the methyl group at 1.30 ppm was irradiated, an increase in the intensity of the signal of the same aromatic proton at 6.42 ppm by 6% was observed. This shows that the methoxy group is present in the C-7 position and, consequently, the hydroxy group is at C-6, which corresponds to a broadened signal at 4.86 ppm in the PMR spectrum.

The ^{13}C NMR spectrum of bernumine (I) consists of 19 signals well separated into groups of signals of characteristic fragments. There are 13 signals in the weak-field part of the spectrum: 12 from the carbons of the two aromatic rings and one from the methylenedioxy carbon. The latter was unambiguously identified from the triplet splitting ($^1J_{CH} = 173.4 \text{ Hz}$) of the signal in the spin-locked spectrum at 100.77 ppm. The carbons of the aromatic rings can be divided into three characteristic groups: oxy-quaternary carbons, quaternary carbons, and tertiary carbons. The first group is distinguished by the characteristic values of the chemical shifts in the 147.6-143.9 ppm range, and the last by doublet splitting in the spin-locked spectrum.

Thus, the signals at 147.55, 146.49, 145.04, and 143.92 ppm may be assigned to the four oxy-quaternary carbons.

The group of quaternary carbons C-4a, C-8a, and C-1' is represented by signals at 126.11, 132.24, and 130.51 ppm, and the group of tertiary aromatic carbons by five signals: 121.93, 114.28, 109.44, 109.26, and 107.76 ppm. Four of these

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TABLE 1. Characteristics of the ^1H and ^{13}C NMR Spectra of Bernumine in CDCl_3

Proton	Chemical shifts, ppm (J, Hz)	Carbon	chemical shifts, ppm	J_{CH} , Hz
H-1	3,74 (ddd, 6,6)	C-3'	147,55(a)	—
H ₃ C-1	1,30 (d, 6,6)	C-4'	146,49	—
		C-7	145,04(a)	—
		C-6	143,92	—
2H-2	2,30—3,20 (m)	C-8a	132,24	—
2H-3		C-1'	130,51	—
H-5	6,56(1H, s)	C-4a	126,11	—
H ₃ CO-7	3,75(3H, s)	C-6'	121,93	162,3
H-8	6,42 (1H, s)	C-5	114,28(b)	156,8
2H- α	3,64 (d, $^2J=13,5$)	C-2'	107,76(c)	163,7
	3,58 (d, $^2J=13,5$)	C-8	109,44(c)	155,4
H-2'	6,87 (d, $J_m=1,5$)	C-5'	109,26(b)	162,3
		OCH ₂ O	100,77	173,4
		C- α	57,34	134,6
OCH ₂ O	5,92 (s)	OCH ₃	55,84	144,3
		C-1	55,51	133,2
H-5'	6,65 (d, $^3J=8,0$)	C-3	43,25	134,6
		C-4	25,74	127,6
H-6'	6,74 (d, $^3J=8,0$, $J_m=1,5$)	H ₃ C	19,96	126,2

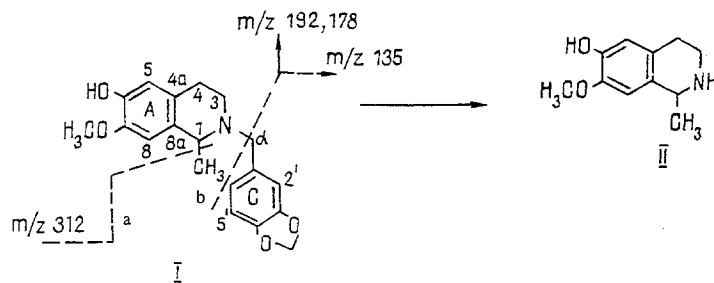
a, b, c) Possible alternative assignments of the signals.

carbons (C-5', C-2', C-5, and C-8) experience the combined action of oxy-substitution increments in the α - and β -positions, and their signals are therefore shifted upfield by 13-14 ppm. Because of β - and γ -oxy-substitutions in the aromatic ring, the carbon in the C-6' position experiences an upfield shift by only 6-7 ppm. Consequently, the weakest-field signals of this group (121.93 ppm) must be assigned to the C-6' carbon. The doublet of doublets of this carbon in the spin-locked spectrum exhibits the large constant of 162.3 Hz with the directly bound proton, and a constant of 4.2 Hz with the β -located H-2' proton. The small coupling constant with the α -located H-5' proton, ≤ 2.0 Hz, appears only in the form of a slight distortion of the form of the signal.

Two well-resolved doublets of this group of signals in the spin-locked spectrum, with $^1J_{\text{CH}} = 156.8$ Hz and 155.4 Hz at 114.28 and 109.44 ppm, respectively, relate to the C-5' and C-2' carbons, respectively. The signal at 109.26 likewise appears in the form of doublets with $^1J_{\text{CH}} = 155.4$ and 163.2 Hz, but with broadened components. This agrees well with the existence of long-range $^nJ_{\text{CH}}$ constants for the C-5' carbon. A doublet of narrow components with $^1J_{\text{CH}} = 163.7$ Hz at 107.76 ppm is assigned to C-8.

In the region of the signals of carbons of saturated chains there are six signals distinguished by the type of proton substitution: one signal for a CH carbon, three signals for CH₂ carbons, and two signals for CH₃ carbons. The signal of a tertiary carbon with $^1J_{\text{CH}} = 133.2$ Hz at 55.51 ppm naturally relates to the C-1 carbon. The pronounced difference in the values of the chemical shifts of the signals of the CH₂ carbons makes their assignment to the corresponding atoms obvious. A weak-field triplet at 57.34 ppm with $^1J_{\text{CH}} = 134.6$ ppm must be assigned to the bridge (α) methylene, since it experiences two increments of the downfield shift: from the directly bound nitrogen and from the aromatic ring of the substituent. The signal of the methylene carbon in the C-3 position is shifted downfield only as the result of the action of the nitrogen and resonates at 43.25 ppm, $^1J_{\text{CH}} = 134.6$ Hz. The strongest-field signal of a methylene carbon, at 25.74 ppm relates to C-4, with $^1J_{\text{CH}} = 127.6$ Hz.

The assignment of the signals of the methyl carbons is obvious: the signal at 55.84 ppm with $^1J_{\text{CH}} = 144.3$ ppm relates to the methoxy carbon, and the signal at 19.96 ppm ($^1J_{\text{CH}} = 126.2$ Hz) to the methyl carbon at C-1. In the quartet signal of this carbon in the spin-locked spectrum the small constant $^2J_{\text{CH}} = 2.8$ Hz of its interaction with the H-1 methine proton also appears.



The hydrogenation of bernumine (I) over Pd black led to (+)-salsoline, which was identified by comparison with an authentic sample from the absence of a depression of the melting point of a mixture and from its IR spectrum. Thus, on the basis of an analysis of spectral characteristics and the formation of salsoline on hydrogenation, structure (I) has been established for bernumine, representing a new group — 1-methyl-*N*-benzyltetrahydroisoquinoline alkaloids.

EXPERIMENTAL

For General Observations, See [5]. PMR spectra were taken on a BS-567 A/100 Hz Tesla instrument. HMDS was used as internal standard. ^{13}C NMR spectra were recorded on a WM-400 instrument (Bruker) with a working frequency for the isotopic ^{13}C nuclei of 100.65 MHz. The spectrum with broad-band ^{13}C —[H] decoupling from protons and the spectrum with a complete set of J_{CH} constants — the spin-locked spectrum — were studied.

Isolation of the Alkaloids from the Leaves of *B. nummularia* and Their Separation. a) **Extraction.** The dry comminuted leaves (1.2 kg) were extracted by the method of [6]. This gave 1.84 g of ether fraction and 1.52 g of chloroform fractions. The total yield of alkaloids was 0.28% of the weight of the air-dry leaves.

b) **Separation of the Ether Fraction of Alkaloids.** The total ether fraction (1.84 g) was chromatographed on a column of silica gel (1:40). The alkaloids were eluted with chloroform and mixtures of chloroform with methanol in various ratios. The chloroform-eluted fraction yielded 0.07 g of bernumine in the form of an oil, 0.21 g of isotetrandrine, and 0.25 g of glaucine, while elution with chloroform—methanol (99:1) gave 0.11 g of laudanosine, and chloroform—methanol (97:3), 0.17 g of oxyacanthine and 0.74 g of thalicmidine.

Bernumine (I), composition $\text{C}_{19}\text{H}_{21}\text{NO}_4$, oil $[\alpha]_D +33^\circ$ (c 0.04; CHCl_3). IR spectrum, $\nu_{\text{max}}^{\text{KBr}}$: 3450 cm^{-1} (OH). UV spectrum, $\lambda_{\text{max}}^{\text{C}_2\text{H}_5\text{OH}}$: 285 nm ($\log \varepsilon$ 3.84). Mass spectrum, m/z (J, %) 327 (M^+ , 2), 312 ($\text{M} - 15$, 55), 192 (2.5), 190 (2), 178 (5.5), 135 (100).

Debenzylation of Bernumine (I) over Palladium Black. Bernumine (0.05 g in 10 ml of methanol) was hydrogenated over palladium black for 6 h. Then the catalyst was separated off and was washed with methanol, and the solvent was evaporated off. The dry residue was treated with 5 ml of 5% H_2SO_4 , the acid solution was washed with ether and made alkaline with ammonia, and the reaction product was extracted with chloroform. The chloroform solution was dried over Na_2SO_4 , filtered, and evaporated, and the residue was treated with alcoholic HCl. The crystals that deposited were filtered off, washed with acetone, and dried. This gave (+)-salsoline, mp $199\text{--}200^\circ\text{C}$. IR spectrum $\nu_{\text{max}}^{\text{KBr}}$: 3470 cm^{-1} . Mass spectrum, m/z : 193 (M^+ , 6), 192(14), 178(100), 163(16). PMR spectrum (hydrochloride, δ , ppm, CD_3OD): 1.68 (3H, d, $J = 6.5$), 2.87 (m, 2H-3), 3.15 (m, 2H-4), 3.75 (3H, s, OCH_3), 4.57 (1H, q), 6.50 (1H, s), 6.53 (1H, s).

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