Berberis ALKALOIDS. XVI. STRUCTURE OF BERPODINE

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The alkaloid composition of young shoots of Berberis heteropoda has been studied. Together with known alkaloids, we have isolated the new dimeric base berpodine and have established its structure by a study of spectral characteristics and by chemical transformations. The known alkaloid isotetrandrine has also been isolated from this plant, for the first time.

Continuing an investigation of the alkaloids of plants of the genus Berberis, we have investigated young shoots of Berberis heteropoda Schrenk [1] (gathered in the fruit-bearing phase) growing in the environs of the village of Saty, Alma-Ata province. By extraction with ethanol we isolated 1.85% of total alkaloids, of which 0.27% was bererine.

By separating the total mixture obtained according to solubility and on a silica gel column we isolated isotetrandrine, oxyacanthine, berbamunine, palmatine, and columbamine, and a new dimeric base with mp 197-198°C (methanol), composition $C_{40}H_{38}N_2O_{13}$, which has been called berpodine.

The known alkaloids were identified by a study of their physicochemical constants and spectral characteristics, and by direct comparison with authentic samples.

In its UV spectrum, berpodine (I) showed absorption at $\gamma_{max}^{C_2H_5OH}$ 235 nm (shoulder) and 295 nm (log ε 4.34, 4.18). The IR spectrum contained absorption bands in the regions of (cm^{-1}) 3300 (OH); 1680 (C=O); 1510, and 1490.

In the PMR spectrum of (I), taken in Py-d_5, we observed the three-proton signals of four methoxy groups at (ppm) 3.44, 3.49, 3.98, and 4.01. Multiplets of eight protons of methylene groups at C-5,5' and C-6,6' appeared at 1.85, 2.39, 2.43, 2.55, 2.96, 3.07, 3.28, and 3.82. The protons of two methylendioxy groups resonated in the form of slightly broadened signals at 5.75 and 5.80 and at 5.84 and 5.91. In the region of aromatic protons there were pairs of doublet signals at 6.50 and 7.07, with ^3J = 8.1 Hz, and 6.60 and 7.32, with ^3J = 8.8 Hz, assigned to the ortho-interacting H-11,12 and H-11',12' atoms. In addition, another four singlets were observed in this region, at 6.82 and 7.74 and at 6.44 and 6.56, which may be assigned to the H-1',1 and H-4,4' (4',4) protons in the two protoberberine halves of compound (I).

In the ^{13}C NMR spectrum of compound (I) (Table 1) three doublet signals were observed at (ppm) 56.17, 66.97, and 78.21, and two singlets at 83.24 and 85.13. On the other hand, in the PMR spectrum there was a generalized and greatly broadened signal with an integral intensity of three proton units, which is characteristic for hydroxy groups. On analyzing these facts, we came to the conclusion that the berpodine molecule contained one secondary and two tertiary hydroxy groups. Three signals of 1H each, at 5.32, 5.91 (this one fused with the singlet at 5.91 from $-O-CH_2-O-$), and 6.11 in the spectrum of (I) remained unassigned. Of these, we assigned the singlet at 5.91 to the methyl geminal to the secondary hydroxy group at C-13, and the singlets at 5.32 and 6.11 ppm to H-14' and H-8, respectively.

The ^{13}C NMR spectra in Py-d_5 were investigated in two regimes: with complete suppression of spin–spin interaction of the carbon nuclei with protons and with its retention. The results of the assignment of the signals made on the basis of the characteristics of the ^{13}C NMR spectra in the two regimes and an analysis of literature information on monomeric protoberberine alkaloids [3-6] are given in Table 1.

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TABLE 1. Chemical Shifts (δ, ppm) of the Carbon Atoms in the ^{13}C NMR Spectrum of Berpodine in Py-d_5 and Values of the Spin–Spin Coupling Constants ^1J (C, H) (WM-400, 100.61 Mz for ^{13}C)*

Carbon atom	δ, ppm	^1J (C,H), Hz	Carbon atom	δ, ppm	^1J(C.H),Hz
1	109,05	159,5	1′	107,73	162,3
2	143,50	—	2′	146,07	—
3	147,28	—	3′	147,06	—
4	107,82	162,3	4′	107,82	162,3
4a	131,34		4a′	129,32	
5	29,74	129,0	5′	28,86	129,0
6	42,52	135,9	6′	45,44	136,9
8	78,21	165,1	8′	172,60	—
8a	125,16	—	8a′	129,09	—
9	151,98	—	9′	151,78	—
10	145,96	—	10′	145,69	—
11	111,51	156,8	11′	112,03	159,5
12	125,38	163,7	12′	123,97	162,3
12a	132,56	—	12a′	132,88	—
13	66,97	142,9	13′	83,24	—
14	85,13	—	14′	56,17	142,9
14a	133,57	—	14a′	130,78	—
O—CH$_2$—O	101,37	172,0	O—CH$_2$—O	101,83	173,4
9—OCH$_3$	61,66	144,3	9′—OCH$_3$	61,56	144,3
10-OCH$_3$	55,34	144,3	10′—OCH$_3$	55,51	144,3

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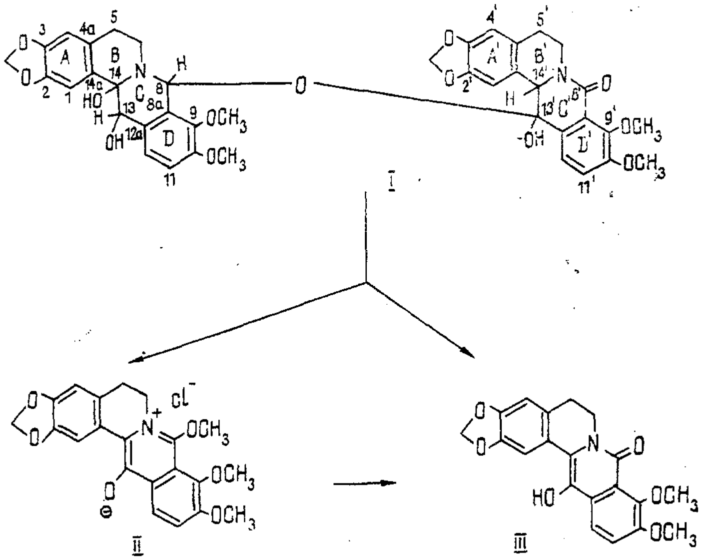
*The assignments of the signals of carbon atoms with similar symbols may be interchanged in the individual cases.



Chemical transformations of berpodine

The cleavage of berpodine (I) with dry HCl in anhydrous methanol (scheme) gave 8-methoxyberberine phenolbetaine (II) and 13-hydroxy-8-oxoberberine (III), which were shown to be identical with authentic specimens by their physicochemical constants, spectral characteristics, and the absence of depressions of mixed melting points. The yield of (III) in the cleavage products was almost twice as great as that of (II). Apparently, (II) was partially converted into (III) during the reaction. To confirm this, we subjected (II) to acid hydrolysis and obtained (III).

As the result of a complete analysis of all the spectral information in the light of the chemical transformations we came to the conclusion that berpodine is a bisprotoberberine alkaloid. Its two protoberberine moieties are linked to one another through an oxygen bridge between C-8 and C-13', while two methylenedioxy groups are located at C-2,3 and C-2',3' and four methoxy groups at C-9,10 and C=9,10'. The single secondary hydroxy group is attached to C-13 and the two tertiary hydroxy groups to C-13' and C-14, with an amide carbonyl group at C-8'.

Thus, the most probable structure (I) is proposed for the new alkaloid berpodine isolated from young shoots of B. heteropoda.

EXPERIMENTAL

Chromatography was conducted on type KSK silica gel and alumina (Brockmann activity grade II). For TLC we used the chloroform—methanol (95:5; 97:3; and 9:1) and chloroform—ethanol—conc. NH_4OH (3.5:3:1) systems. UV spectra were taken on a Hitachi EPS-3T spectrophotometer (ethanol), IR spectra on a UR-20 spectrophotometer (KBr tablets), mass spectra on an MKh-1310 mass spectrometer; and ^1H and ^{13}C NMR spectra on Bruker WM-500 and WM-400 spectrometers in Py-d_5, with TMS as internal standard. Elementary analysis was conducted on a Carlo Erba type EA-1108 automatic CHNSO analyzer.

Extraction of Young Shoots of B. heteropoda. The comminuted young shoots (4500 g) were extracted three times with ethanol in the cold. The combined alcoholic extracts were evaporated, and the viscous residue was dissolved in 3% acetic acid. The solution was filtered and was washed twice with ether. Then the filtrate was made alkaline with 25% ammonia solution to pH 9, and the alkaloids were extracted successively with ether (A), 14.1 g, chloroform (B), 36 g, and chloroform—ethanol (9:1) (C), 21 g. Acidification of the alkaline aqueous mother liquor with conc. HCl led to the separation of 12.1 g of berberine hydrochloride.

Separation of the Mixtures of Alkaloids. Part of the alkaloid mixture A (5.2 g) was chromatographed on a column of silica gel. The alkaloids were eluted with chloroform and with mixtures of chloroform and ethanol in various ratios. The chloroform—ethanol (98:2) eluate yielded 0.4 g of isotetrandrine, the (96:4) eluate 1.8 g oxyacanthine, the (95:5) eluate 0.75 g of berbamunine, the (93:7) eluate 0.27 g of reticuline, and the (9:1) eluate 0.15 g of berpodine, mp 197-198°C (methanol), composition $C_{40}H_{38}N_2O_{13}$, R_f 0.56 (TLC on silica gel in the chloroform—methanol (9:1) system).

Cleavage of Berpodine (I). a) Production of 13-Hydroxy-8-oxoberberine (III). A suspension of 120 mg of berpodine (I) in 10 ml of absolute pyridine was treated with 5 ml of anhydrous methanolic HCl, and the mixture was stirred at room temperature for 3 h. The precipitate that deposited was separated off, washed with methanol, and recrystallized from methanol, to give 55 mg of pale brownish crystals of (III) with mp 217-216°C [sic]. Lit. 216-217°C [2]. IR, ν_{max} (KBr) cm^{-1}: 1650 (amide carbonyl), 3300 (OH). Mass spectrum 367,10435 ($C_{20}H_{17}NO_6$).

b) 8-Methoxyberberine Phenolbetaine (II). The mother solution after the separation of the (III) was evaporated to dryness, and treated with 10 ml of ether. The resulting suspension was boiled under reflux and a few drops of chloroform were added to achieve complete dissolution. On cooling, the solution deposited orange crystals (25 mg) with mp 175-176°C. M^+ 381. PMR spectrum ($CDCl_3$): 2.87 (2H, t); 3.83 (3H, s); 3.97 (6H, s); 4.53 (2H, t); 5.94 (2H, s); 6.51 (1H, s); 7.32 (1H, d); 8.26 (1H, d); 8.76 (1H, s).

Acid Hydrolysis of 8-Methoxyberberine Phenolbetaine (II). This was carried out by the procedure of [2], giving 13-hydroxy-8-oxoberberine, identical, according to spectral characteristics, TLC, and the absence of a depression of a mixed melting point, with the (III) obtained by the cleavage of berpodine (I).

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