

BERBERIS ALKALOIDS.

XXV. STRUCTURES OF BERNUMIDINE AND BERNUMICINE

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UDC 547.944/945

The alkaloid compositions of young shoots and leaves of *Berberis nummularia* Bge. have been studied. The new alkaloids bernumidine (II) and bernumicine (III) have been isolated from the leaves, and their structures have been established.

Continuing an investigation of alkaloids of plants of the genus *Berberis*, we have studied the alkaloid composition of young shoots and leaves of *B. nummularia* growing in the environs of Sarikurgan (Fergana province), in the flowering phase. Various organs of these plants have been investigated for the presence of alkaloids previously [1].

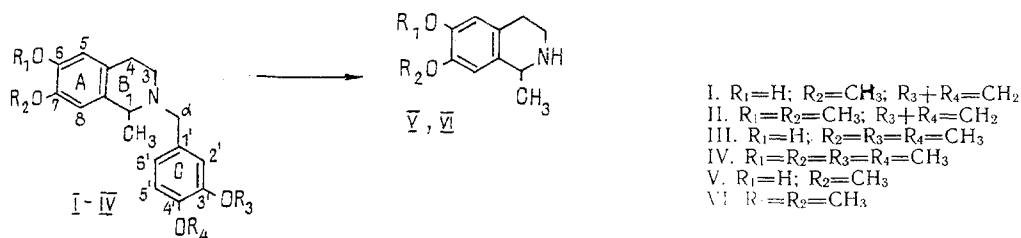
By ethanol extraction, 1.43% of total alkaloids was isolated from the young shoots. When this material was separated according to solubility and by chromatography on a silica gel column, berberine, palmatine, columbamine, magnoflorine, oxyacanthine, berbaminine, and aromoline were isolated.

By chloroform extraction, the leaves yielded 0.32% of total alkaloids: 0.18% of ether fraction and 0.14% of chloroform fraction. Chromatography of the ether fraction on a silica gel column led to the isolation of thalictidine, glaucine, oxyacanthine, N-methylcoclaurine, and reticuline and the new bases bernumidine (II) and bernumicine (III). The known alkaloids were identified by comparison with authentic samples and also from their spectral characteristics.

Bernumidine, an optically active base, has the composition $C_{20}H_{23}NO_4$ and crystallizes in the form of the hydrochloride. The UV spectrum of (II) had the maximum in the 286 nm region ($\log \epsilon$ 3.78) that is characteristic for tetrahydroisoquinolines [3]. The mass spectrum of bernumidine contained peaks of ions with m/z 341 (M^+), 326 ($M - 15$), 207, 206, 192, and 135. The nature of the mass fragmentation of (II) under electron impact resembled that of bernumine, isolated previously from this plant [1]. In the PMR spectrum of (II), taken in deuteriochloroform, signals were observed from a methyl group in the form of a three-proton doublet at (ppm) 1.48 with $J = 7.0$ Hz, from three methylene protons at 2.75 (2H, m), 3.24 (2H, m), and 3.70 (2H, q), from two methoxyls at 3.84 (3H, s) and 3.86 (3H, s), and from a methylenedioxy group at 5.90 (2H, s). The region of aromatic protons revealed signals in the form of two one-proton singlets at 6.51 and 6.59 and also one-proton signals in the form of doublets at 6.75 (1H, d, $J = 8.2$ Hz), 6.86 (1H, dd, $J_o = 8.2$ Hz, $J_m = 1.8$ Hz), and 7.02 (1H, d, $J_m = 1.8$ Hz).

The methylation of bernumine (I) [1] with diazomethane in absolute methanol gave bernumidine (II). The hydrogenation of (II) over Pd black led to (+)-salsolidine (VI) [2], shown to be identical with an authentic specimen from the absence of a depression of the melting point of a mixed sample, and from its IR spectrum.

Thus, according to its spectral characteristics and chemical transformations bernumidine belongs to the alkaloids of the bernumine (I) type and has structure (II).



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Bernumidine (III), an optically active base with the composition $C_{20}H_{25}NO_4$, isolated in the form of a colorless oil, crystallized in the form of the hydrochloride. The IR spectrum of (III) had an absorption band from active hydrogen at ν_{\max}^{KBr} 3400 cm^{-1} . The UV spectrum [$\lambda_{\max}^{C_2H_5OH}$ 286 nm ($\log \epsilon$ 3.84)] was characteristic for tetrahydroisoquinolines [3]. The mass fragmentation of (III) under electron impact resembled those of berberine (I) and of bernumidine (II). Signals were observed with m/z (J , %) 343 (M^+ ; 4), 328 ($M - 15$; 56), 192 (3), 178 (9), and 151 (100).

The presence in the mass spectrum of the ions with m/z 192 and 178 and the maximum ion with m/z 151 showed that in the (III) molecule there were methoxy and hydroxy groups in ring *A* and two methoxy groups in ring *C*.

The PMR spectrum of the hydrochloride of (III) contained signals from a CH_3 group at 1.61 (3H, d, $J = 6.6\text{ Hz}$), from three methylene groups at 2.97 (2H, m), 3.40 (2H, m), and 4.26 (2H, s), and from three methoxyls at 3.80 (6H, s, 2 OCH_3) and 3.77 (3H, s, OCH_3). A signal from a methine proton in the form of a one-proton quartet was observed at 4.44 ppm.

In the region of aromatic protons two one-proton singlets were detected at 6.65 and 6.72 ppm, and two broadened singlets at 7.01 (2H) and 7.12 (1H).

The methylation of (III) with diazomethane gave the O-methyl derivative (IV) in the form of an oil (M^+ 357). The hydrogenation of (III) over palladium black led to (+)-salsoline (V), shown to be identical with an authentic specimen by its IR spectrum and a mixed melting point.

Thus, in the (III) molecule the hydroxy and methoxy groups occupy positions 6 and 7, respectively, and there are two methoxy substituents in positions 3' and 4' of ring *C*.

On the basis of these results, structure (III) is proposed for bernumicine.

EXPERIMENTAL

For General Observations, See [4].

Isolation and Separation of the Alkaloids from Young Shoots of *B. nummularia*. The comminuted young shoots of *B. nummularia* (700 g) were extracted with ethanol by the procedure of [5]. From the total quaternary alkaloids, on the basis of their solubilities in the form of hydrochlorides, we isolated berberine (2.17 g), palmatine (0.17 g), columbamine (0.24 g), and magnoflorine (0.45 g). The total tertiary alkaloids (3.27 g) were chromatographed on a column of silica gel. Elution with chloroform—methanol (98:2) yielded oxyacanthine (1.18 g), a (96:4) ratio yielded aromoline (0.14 g), and a (95:5) ratio berbaminine (0.64 g).

Isolation and Separation of the Alkaloids from the Leaves of *B. nummularia*. a) The extraction of 800 g of air-dry comminuted *B. nummularia* leaves was carried out by the method of [4]. This gave 1.44 g of ether fraction and 1.12 g of chloroform fraction. The total yield of alkaloids was 0.32%.

b) **Separation of the Ether Fraction of Alkaloids.** The ether fraction (1.44 g) was chromatographed on a column of silica gel (1:40), and the alkaloids were eluted with chloroform and with mixtures of chloroform and methanol in various ratios. The chloroform eluate gave 0.05 g of bernumidine (II), 0.11 g of isotetrandrine, and 0.12 g of glaucine. Elution with chloroform—methanol (99:1) yielded 0.08 g of oxyacanthine, a (98:2) ratio gave 0.62 g of thalicmidine, a (97:3) ratio 0.06 g of bernumicine (III), a (96:4) ratio 0.04 g of N-methylcoclaurine, and a (95:5) ratio 0.03 g of reticuline.

Bernumidine (II) — oil, $[\alpha]_D +21^\circ\text{C}$ (c 0.01; $CHCl_3$), crystallizing in the form of the hydrochloride with mp $180-181^\circ\text{C}$. IR spectrum ν_{\max}^{KBr} , cm^{-1} : 2920, 2439, 1610, 1540, 1500, 1450, 1270, 1110, 1030, 930, 810, 780. UV spectrum ($\lambda_{\max}^{C_2H_5OH}$, nm): 286 ($\log \epsilon$ 3.78). Mass spectrum, m/z (J , %): 341(3), 326(52), 207(11), 206(8), 192(14), 135(100).

PMR spectrum of the hydrochloride of (II) in $CD_3OD + CDCl_3$ (ppm): 1.71 (3H, d, $J = 7.0\text{ Hz}$), 3.00 (2H, m), 3.32 (2H, m), 3.76 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 4.06 (2H, br.s), 4.15 (1H, m), 5.91 (2H, s, OCH_2O), 6.35 (1H, s), 6.56 (1H, s), 6.70 (1H, d, $J_o = 8.5$), 6.96 (1H, dd, $J_o = 8.5$; $J_m = 1.8$), 7.20 (1H, m, $J = 1.8$).

Bernumicine (III) — amorphous substance crystallizing in the form of the hydrochloride with mp $211-212^\circ\text{C}$, $[\alpha]_D +14^\circ$ (c 0.02; $CHCl_3$).

IR spectrum, ν_{\max}^{KBr} : 3400 cm^{-1} . UV spectrum, $\lambda_{\max}^{C_2H_5OH}$: 286 nm ($\log \epsilon$ 3.84).

Formation of Bernumidine (II) from Bernumine (I). An ethereal solution of diazomethane was added to a solution of 0.04 g of berberine in absolute methanol, and the mixture was left in the refrigerator. It was monitored by TLC on a plate with silica gel in the benzene—ethanol (9:1) system. After two days the solution was evaporated, and a few drops of alcoholic

HCl was added to the residue. The crystals of (II) that deposited were washed with acetone and dried. mp 180-181°C. From its IR spectrum and a mixed melting point, the product obtained was identified as bernumidine.

Debenzylation of Bernumidine (II) over Palladium Black. Bernumidine (0.04 g in 10 ml of methanol) was hydrogenated over palladium black. The course of the reaction was followed by chromatography in a thin layer of silica gel [benzene—ethanol (9:1) and chloroform—methanol (4:1) systems]. After 8 h, the catalyst was separated off, and the reaction mixture was evaporated. The dry residue was treated with 5 ml of 5% sulfuric acid. The acid solution was washed with ether, made alkaline with NH_4OH , and extracted with chloroform.

The chloroform solution was dried over Na_2SO_4 , filtered, and evaporated. The residue was treated with an alcoholic solution of HCl. When acetone was added to the resulting solution, fine crystals of (VI)·HCl precipitated, and these were separated off, washed with ether, and dried. mp 232-233°C (lit.: 234-235°C [2]).

Mass spectrum, m/z (J, %): 207 (M^+ , 1), 206 (12), 192 (100), 163 (7), 153 (17), 149 (9).

Debenzylation of Bernumicine (III) over Palladium Black. Bernumicine (0.04 g in 10 ml of methanol) was hydrogenated over palladium black in a similar way to bernumidine. This gave 12 mg of (V) in the form of the hydrochloride, mp 175-176°C (lit.: 174-175 [2]).

Mass spectrum: 193 (M^+ , 3), 192 (11), 178 (100), 164 (5), 163 (16), 149 (12).

IR spectrum ($\nu_{\text{max}}^{\text{KBr}}$, cm^{-1}): 3540, 3320, 1625, 1535, 1040.

PMR spectrum of the hydrochloride in CD_3OD : 1.68 (3H, d, $J = 6.5$ Hz), 2.87 (2H, m), 3.15 (2H, m), 3.75 (3H, s), 4.37 (1H, q), 6.50 (1H, s), 6.53 (1H, s).

Methylation of Bernumicine (III). An ethereal solution of diazomethane was added to a solution of 0.01 g of bernumicine in absolute methanol, and the mixture was left in the refrigerator for two days. The course of the reaction was followed in a thin layer of silica gel with the system benzene—ethanol (9:1).

After the completion of the reaction, the solvent was driven off, and the residue was chromatographed on a column of silica gel. Elution with benzene yielded 5 mg of (V) in the form of an oil. Mass spectrum: m/z 357.

Methylation of (V). An ethereal solution of diazomethane was added to a solution of 0.01 g of (V) in 10 ml of absolute methanol. The mixture was left in the refrigerator overnight, and then the solvent was eliminated under reduced pressure, and the residue was treated with alcoholic HCl. The crystals of the hydrochloride of (VI) that deposited were separated off, washed with ether and acetone, and dried. mp 231-232°C.

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