

## Berberis ALKALOIDS

### XXXV. THE STRUCTURE OF NUMMULARINE

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*The new N-benzyltetrahydroisoquinoline alkaloid nummularine has been isolated from the leaves of Berberis nummularia. On the basis of a study of its spectral characteristics (PMR, IR, and mass spectra), the structure of nummularine has been established as 6-hydroxy-N-(3'-hydroxy-4'-methoxybenzyl)-7-methoxy-1-methyltetrahydroisoquinoline.*

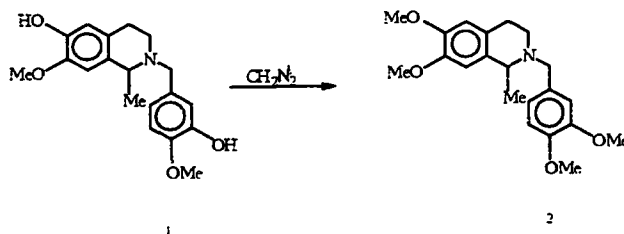
Continuing an investigation of alkaloids of plants of the *Berberis* genus, we have studied the alkaloid composition of the leaves of *B. nummularia* gathered in the Naukatskii region of the Oshskaya oblast in the phase of incipient fruit formation. We have previously isolated a number of new alkaloids in an investigation of the epigeal organs of this plant [3, 4].

By chloroform extraction of the leaves we obtained 0.24% of total alkaloids. The chromatography of this material on a column of silica gel led to the isolation of noroxyhydrastinine, glaucine, isoboldine, corypalline, oxyacanthine, isocorydine, obaberine, aromoline, and berbaminine, and the new base nummularine (1). The known alkaloids were identified from their spectral characteristics and also by comparison with authentic specimens.

Nummularine is an optically active crystalline base with the composition  $C_{19}H_{23}NO_4$ . Its UV spectrum showed the maximum in the 284 nm region that is characteristic for tetrahydroisoquinolines. The mass spectrum revealed peaks of ions with  $m/z$  329 ( $M^+$ ), 314 ( $M - 15$ ), 192, 178, 137 (100%). The mass-spectral fragmentation of nummularine under electron impact was characteristic for alkaloids of the 1-methyl-N-benzylisoquinoline series [3, 4]. The appearance of an ion with  $m/z$  314 ( $M - 15$ ) showed the presence of a methyl substituent at C-1, and that of an ion with  $m/z$  192 and of the maximum ion with  $m/z$  137 showed that there are methoxy and hydroxy substituents in rings A and C.

The methylation of (1) with diazomethane gave the O,O-dimethyl ether (2), identical, according to TLC and its IR spectrum, with O-methylbernumicine [3]. The passage to O-methylbernumicine showed that the substituents in rings A and C occupy the 6,7- and 3',4'- positions.

The PMR spectrum of nummularine (1) showed four one-proton multiplets at 2.75, 3.25, 2.88, and 2.96 ppm, which are characteristic for the protons of the interconnected methylene groups of tetrahydroisoquinoline alkaloids, two one-proton doublets at 3.76 and 3.87 ppm with the SSCC  $J = 13.0$  Hz, a three-proton doublet at 1.55 ppm ( $J = 6.5$  Hz), a one-proton quartet at 3.96 ppm ( $J = 6.5$  Hz), and two singlets from the protons of methoxy groups at 3.98 and 4.03 ppm in the aliphatic part of the spectrum.



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In the aromatic part of the spectrum there were two one-proton singlets at 6.71 and 6.78 ppm, which are characteristic for *para*-located protons, and also the signals of an aromatic system split in the *ABC* manner: doublet at 7.05 ppm with the *ortho* constant  $J = 8.2$  Hz, doublet at 7.05 ppm with the *meta* constant  $J = 2.1$  Hz, and a doublet of doublets at 6.79 ppm with the constants  $J = 8.2$  and  $J = 2.1$  Hz.

To establish the mutual positions of the methoxy and hydroxy groups in rings *A* and *C*, we performed experiments to measure intramolecular nuclear Overhauser effects. When an H-4 proton (2.88 or 2.96 ppm) was irradiated with an additional radiofrequency field, a NOE (17%) was observed on the singlet at 6.71 ppm, and when the nuclear resonance transition of the protons of one of the methoxy groups — namely that at 4.03 ppm — was saturated a NOE (17%) was observed on the singlet at 6.78 ppm. Irradiation of the protons of the second methoxy group did not give rise to a NOE on the signals at 6.71 and 6.78 ppm. It follows from this that the three-proton singlets at 3.98 and 4.03 ppm must be assigned to the methoxy groups of rings *A* and *C*, and the one-proton singlets at 6.71 and 6.78 ppm to H-5 and H-8, respectively.

When the protons of the secondary methyl group (doublet at 1.55 ppm) were irradiated, a NOE (6%) was observed on the H-8 proton, which showed its position at C-1. A proton in the geminal position to this methyl group resonated at 3.96 ppm and was partially masked by the base of the signal of the methoxy functions.

To determine the positions of the methoxy and hydroxy groups in ring *C*, we measured the spatial interaction of the protons of the methoxy group at 4.03 ppm and of the methylene group of the benzyl radical with the aromatic protons. Irradiation of the protons of this methoxy group showed a NOE (~10%) on the doublet at 7.05 ppm with  $J = 8.2$  Hz of an *ABC* system, while, through suppression of small unresolved SCCs and a possible NOE, irradiation of the protons of the benzyl methylene group increased by 20% the intensity of the signals of the second doublet at 7.05 ppm with  $J = 2.1$  Hz and that of the doublet of doublets at 6.97 ppm with  $J = 8.2$  Hz and  $J = 2.1$  Hz. From this it is possible to draw the conclusion that in ring *C* the methoxy group is located at C-4' and the hydroxy group at C-3'.

For a more accurate determination of the chemical shifts and coupling constants of the protons of the methylene groups of the isoquinoline nucleus, we made use of a theoretical calculation of the multiplicities of the signals with iteration optimization of the constants by the LAOCON-5 program. After optimization we obtained spin-spin coupling constants with an accuracy of not less than  $\pm 0.05$  Hz. The optimized values of the chemical shifts did not differ appreciably from those obtained experimentally:  $\pm 0.004$  ppm.

## EXPERIMENTAL

For general observations, see [1]. PMR spectra were taken on Tesla BS-567A/100 MHz and Bruker WM-500 instruments.

**Isolation and Separation of the Alkaloids from the Leaves of *Berberis nummularia*.** a) **Extraction.** The dry comminuted leaves (900 g) were extracted by the procedure of [2]. This gave 1.75 g of ether fraction and 0.41 g of chloroform fraction.

b) **Separation of the Ether Fraction of Alkaloids.** The ether fraction of alkaloids (1.75 g) was chromatographed on a column of silica gel (1:30). The alkaloids were eluted with chloroform and with mixtures of chloroform and ethanol in various ratios. The chloroform fraction yielded 0.025 g of noroxyhydrastinine and 0.71 g of glaucine. Elution with chloroform—ethanol (98:2) gave 0.1 g of isoboldine and 0.017 g of corypalline, and a (97:3) mixture gave 0.12 g of oxyacanthine, 0.064 g of nummularine (1) and 0.035 g of isocorydine.

c) **Separation of the Chloroform Fraction of Alkaloids.** The chloroform fraction (0.41 g) was separated in a similar way to the ether fraction, which led to the isolation of 0.075 g of obaberine, 0.12 g of oxyacanthine, 0.036 g of aromoline, and 0.025 g of berbaminine.

**Nummularine (1),**  $C_{19}H_{23}NO_4$ , crystalline substance, mp 134–135°C,  $[\alpha] +21^\circ\text{C}$  ( $c$  0.03,  $\text{CH}_3\text{OH}$ ).

IR spectrum:  $\nu_{\text{max}}(\text{KBr})$ : 3480  $\text{cm}^{-1}$  (OH).

UV spectrum:  $\lambda_{\text{max}}(\text{C}_2\text{H}_5\text{OH})$ : 284 nm ( $\log \epsilon$  3.97).

Mass spectrum:  $m/z$  ( $I$ , %) 329 ( $M^+$ , 3), 314 ( $M-15$ , 57), 192 (4), 178 (8), 137 (100).

PMR: 3.96 (1H, dd,  $J=6.5$ , H-1), 2.75 (1H, m,  $J=12.44$ , 5.90, 4.20, H-3e), 3.25 (1H, m,  $J=12.44$ , 9.50, 5.00, H-3a), 2.88 (1H, m,  $J=16.33$ , 4.20, 5.00, H-4e), 2.96 (1H, m,  $J=16.33$ , 9.50, 5.90, H-4a), 6.71 (1H, s, H-5), 6.78 (1H, s, H-8), 3.76 (1H, d,  $J=13.0$ , H-9), 3.87 (1H, d,  $J=13.0$ , H-9), 3.98 (3H, s, 7-OCH<sub>3</sub>), 1.55 (3H, d,  $J=6.5$ , 1-CH<sub>3</sub>), 7.05 (1H, d,  $J=2.1$ , H-2'), 7.05 (1H, d,  $J=8.2$ , H-5'), 6.97 (1H, dd,  $J=8.2$ , 2.1, H-6'). 4.03 (3H, s, 4'-OCH<sub>3</sub>).

**Methylation of Nummularine (1).** An ethereal solution of diazomethane was added to a solution of 0.02 g of nummularine in absolute methanol, and the mixture was left in a refrigerator for four days. The course of the reaction was monitored in a thin layer of silica gel with the benzene—ethanol (9:1) system. After the reaction was complete, the solvent was evaporated off, and the residue was chromatographed on a column of silica gel. Elution with benzene gave 0.01 g of O-methylbernumicine, identical with an authentic specimen according to TLC and IR spectra [3]. Mass spectrum:  $m/z$  357 ( $M^+$ ).

## REFERENCES

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