

Tertiary alkaloids of *Mahonia aquifolium* (PURSH) NUTT. III*

D. KOŠŤÁLOVÁ, V. HROCHOVÁ, and J. TOMĚKO

Department of Pharmacognosy and Botany, Faculty of Pharmacy,
Komenský University, CS-832 32 Bratislava

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The bisbenzylisoquinoline alkaloids aromoline and obamegine and the protoberberine alkaloid oxyberberine were isolated for the first time from the roots of *Mahonia aquifolium* (PURSH) NUTT. in addition to further two bisbenzylisoquinoline alkaloids berbamine and oxyacanthine, which have already been found in this species. Seeds of this plant contained the bisbenzylisoquinoline alkaloid isotetrandrine besides of other above-mentioned bases.

Из корней *Mahonia aquifolium* (PURSH) NUTT. были впервые выделены бисбензилизохинолиновые алкалоиды аромолин, обамегин и протоберберин, кроме дальнейших двух бисбензилизохинолиновых алкалоидов бербамина и оксикантина уже раньше обнаруженных в этой породе. Семени этого растения содержали, кроме выше приведенных оснований, бисбензилизохинолиновый алкалоид изотетрандрин.

Aromoline was reported to be present in only one representative of the *Berberidaceae* family, in *B. orthobotrys* BIENERT ex. AITCH. [1]. Obamegine has been isolated from plants of the genus *Mahonia* NUTT. from the roots of *M. repens* (LINDL) G. DON only [2], oxyberberine exclusively from the species *M. morrisonensis* TAKEDA [3], berbamine, oxyacanthine, and isotetrandrine were separated from the roots of *M. aquifolium* (PURSH) NUTT. [4], berbamine from flowers [5], and tertiary alkaloids of the aporphine group — isocorydine, corydine, and isoboldine — from leaves [6].

The isolation of tertiary aporphine alkaloids corytuberine, isocorydine, corydine, and isoboldine and presence of trace amount of berbamine in the leaves of *M. aquifolium* (PURSH) NUTT. have been also described [7]. The roots, stems as well as seeds of this plant did not contain any of these tertiary aporphine alkaloids. From the roots the alkaloids of protoberberine group — berberine, palmatine, columbamine, and jatrorrhizine and one aporphine alkaloid magnoflorine, from the stems berberine, palmatine, and jatrorrhizine have been isolated [8].

These results are in line with those of the investigated species *M. repens* (LINDL)

*For Part II see Ref. [7].

G. DON, in the leaves of which tertiary aporphine alkaloids isocorydine, corydine, thaliporphine, and glaucine were found, nonetheless, these aporphine alkaloids were absent in the roots and stems. Bases isolated from the roots and stems were as follows: the protoberberine alkaloids berberine, jatrorrhizine, columbamine, and palmatine, the aporphine alkaloid magnoflorine, as well as bisbenzylisoquinoline bases obamegine, thalrugosine, and obaberine [2].

As a continuation of our research program, we investigated the constituents of plants of the *Mahonia* NUTT. family, and from the roots and seeds of *M. aquifolium* (PURSH) NUTT. we isolated tertiary alkaloids. Thin-layer chromatography of the extract showed that both parts contained the same compounds. Seeds, however, contained an additional alkaloid — isotetrandrine.

The bisbenzylisoquinoline alkaloids revealed a maximum in the UV region at $\lambda \sim 283$ nm and a minimum at about $\lambda = 260$ nm [9]. Alkaloids of the oxyacanthine type have a characteristic fragmentation pattern, in particular peaks at $m/z = (M_r - 191)$ and $(M_r - 107)$. That at $m/z = (M_r - 137)$ used to be of lower intensity than the $m/z = (M_r - 191)$ in alkaloids of the berbamine type, whilst the peak at $m/z = (M_r - 107)$ is of higher intensity than $m/z = (M_r - 191)$ of oxyacanthine type [10]

The sum of alkaloids obtained from the roots was fractionated by countercurrent distribution into five fractions, that from the seeds was separated by column chromatography.

From the first fraction of countercurrent distribution a compound was obtained the UV spectrum of which displayed absorptions at $\lambda = 225$ nm, 290 nm, and 345 nm characteristic of protoberberine alkaloids. Its IR spectrum contained stretching vibration of an amide group at $\tilde{\nu} = 1640$ cm^{-1} [11]. This compound was compared with a specimen of oxyberberine and both proved to be identical. For $\text{C}_{20}\text{H}_{17}\text{NO}_5$ (M^+) m/z calculated: 351, 1107; found: 351, 1114.

The UV spectra and further constants of alkaloids isolated from fraction 2 were identical with those reported for berbamine and oxyacanthine [12].

Fraction 4 afforded a crystalline substance showing in the high resolution mass spectrum the molecular radical ion at $m/z = 594.2719$ (for $\text{C}_{36}\text{H}_{38}\text{N}_2\text{O}_6$ calculated $m/z = 594.2728$) and further peaks at $m/z = 471$ ($M_r - 123$) and $m/z = 403$ ($M_r - 191$) characteristic of a berbamine type alkaloid. The ^1H NMR spectrum showed signals of two NCH_3 groups ($\delta = 2.29$ ppm and 2.50 ppm), two OCH_3 groups ($\delta = 3.78$ ppm and 3.91 ppm), and ten protons of aromatic rings at $\delta = 6.06$ —7.26 ppm. Positions of these signals indicate that the isolated compound could be identical with obamegine; this assignment was favoured also by comparisons with the specimen, its enantiomer *N*-methyl-7-*O*-demethylpeinamine, and with atherospermoline and its enantiomer krukovine. Signals of protons due to methylamine grouping for obamegine isolated from *M. repens* (LINDL) G. DON at $\delta = 2.33$ ppm and 2.55 ppm and those of two methoxy groups at $\delta = 3.79$ ppm and 3.94 ppm evidence [2] that both compounds are identical (Fig. 1).

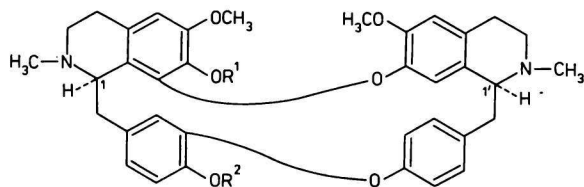


Fig. 1. Structure of the berbamine type bisbenzylisoquinoline alkaloids.

	R ¹	R ²
Berberamine	CH ₃	H
Obamegine	H	H
Isotetrandrine	CH ₃	CH ₃

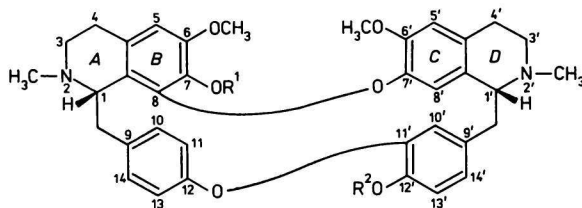


Fig. 2. Structure of the oxyacanthine type bisbenzylisoquinoline alkaloids.

	R ¹	R ²
Oxyacanthine	CH ₃	H
Aromoline	H	H

Crystallization of fraction 5 yielded a compound the high resolution mass spectrum of which displayed the peak of molecular radical ion at $m/z = 594.2718$ (for $C_{36}H_{38}N_2O_6$ calculated $m/z = 594.2728$). The peak at $m/z = 487$ ($M_r - 107$) is diagnostic of an oxyacanthine type of bisbenzylisoquinoline alkaloids. The presence of signals of two NCH_3 groups in the 1H NMR spectrum ($\delta = 2.56$ ppm and 2.54 ppm) indicates that the methyl group can be absent only in the ring B (Fig. 2). Further signals are associated with two OCH_3 groups ($\delta = 3.60$ ppm and 3.81 ppm) and ten protons of aromatic rings (a broad band at $\delta = 5.61$ —7.40 ppm). This and further arguments (melting point, optical rotation, IR spectra, and comparison of these data with those reported for aromoline [12, 13]) showed that both alkaloids are identical.

The UV spectrum of a base present in seeds only is characteristic of a bisbenzylisoquinoline alkaloid. Its mass spectrum showing a peak of molecular

radical ion at $m/z = 622.3037$, fitting the formula $C_{38}H_{42}N_2O_6$ and peaks at $m/z = (M_r - 137)$ and $(M_r - 191)$ is indicative of a compound of tetrandrine type [10]. These data together with the melting point, optical rotation, and IR spectrum proved this alkaloid to be isotetrandrine (Fig. 1).

Experimental

The melting points were measured with a Kofler micro hot-stage, the IR spectra of KBr pellets were recorded with a Perkin—Elmer, model 477 apparatus, the UV spectra of methanolic solutions were run with a Specord UV VIS (Zeiss, Jena) spectrophotometer, optical rotations of chloroform solutions were measured with a Polamat A instrument, mass spectra with an AEI MS 902 spectrometer, and 1H NMR spectra of deuteriochloroform solutions with Jeol FX 100 or Bruker AM 300 instruments, tetramethylsilane being the internal reference. Purity of the isolated compounds was checked by thin-layer chromatography on silica gel (Merck) using benzene—acetone—ammonia (volume ratio = 4:6:1) or benzene—chloroform—diethylamine (volume ratio = 4:5:1) as solvent systems. The alkaloids were detected by irradiation with short-wave light $\lambda = 254$ nm and 366 nm, by visualization with Dragendorff reagent or potassium iodoplatinate. The mixture of tertiary alkaloids from the roots was separated by countercurrent distribution between acetate buffer solution of pH = 5 and chloroform as a stationary phase. The mixture of tertiary alkaloids from seeds was separated by column chromatography on silica gel (Merck). The single alkaloids were eluted with chloroform, chloroform—methanol of increasing polarity, and with methanol.

Isolation and identification of alkaloids

Mahonia aquifolium (PURSH) NUTT. was collected in Bratislava in March 1978. The roots and stems and the fruits were separately dried at an ambient temperature, and finally the seeds were separated from fruits. All specimens are registered in the herbarium of the Department of Pharmacognosy and Botany, Faculty of Pharmacy, Komenský University, Bratislava.

The roots (2000 g) were ground and extracted with methanol. The solvent was removed under diminished pressure, the residue was dissolved in sulfuric acid ($c = 0.5$ mol dm^{-3}), filtered and the filtrate was basified with concentrated ammonia to pH = 8—10. The ethereal extract was worked up to give the mixture of tertiary bases (13.80 g).

A part of this mixture (3.5 g) was separated by 60-plate countercurrent distribution. Compounds present in the aqueous and chloroform layers were combined according to thin-layer chromatographic monitoring in both systems; alkaloids from the aqueous solutions were basified with concentrated ammonia and taken into ether prior to combination. Totally five fractions were obtained.

Oxyberberine

Fraction one (tubes 1—12; 0.52 g) was purified by column chromatography over alumina (Merck) using ether—chloroform (volume ratio = 1 : 3) as eluent. Oxyberberine was crystallized from ether—chloroform. Yield = 0.032 g (0.001 %), m.p. = 196—198 °C. UV spectrum; λ_{\max}/nm , ($\log (\epsilon/(\text{m}^2 \text{mol}^{-1}))$): 225 (3.70), 290 (2.95), 345 (2.98). IR spectrum; $\bar{\nu}/\text{cm}^{-1}$: 1640 (amide). Mass spectrum; m/z : 351 (M^+), 336 ($\text{C}_{19}\text{H}_{14}\text{NO}_5$), 322 ($\text{C}_{19}\text{H}_{16}\text{NO}_4$), 308 ($\text{C}_{18}\text{H}_{14}\text{NO}_4$), 292 ($\text{C}_{17}\text{H}_{10}\text{NO}_4$), 177.5, 168.

Oxyacanthine

Fraction two (tubes 13—25; 1.10 g) was dissolved in sulfuric acid ($c = 1 \text{ mol dm}^{-3}$); to this solution saturated sodium sulfate was added, the formed precipitate was dissolved in acetic acid ($c = 1 \text{ mol dm}^{-3}$), the solution was alkalinized with concentrated ammonia and extracted with ether. The concentrated extract was left to crystallize. Yield = 0.2050 g (0.01 %), m.p. = 210—212 °C, $[\alpha]$ (578 nm, 23 °C, $\rho = 2.0 \text{ g dm}^{-3}$, CHCl_3) = + 285°. UV spectrum; λ_{\max}/nm , ($\log (\epsilon/(\text{m}^2 \text{mol}^{-1}))$): 284 (2.8). IR spectrum; $\bar{\nu}/\text{cm}^{-1}$: 2840, 2940, 3450 (hydroxyl). Mass spectrum; m/z : 608.2876 (M^+) (for $\text{C}_{37}\text{H}_{40}\text{N}_2\text{O}_6$ calculated $m/z = 608.2886$), 607, 501, ($\text{M}_r - 107$), 417 ($\text{M}_r - 191$), 396, 395, 381, 379, 198, 175, 174.

Berberamine

Mother liquor after separation of oxyacanthine was basified with concentrated ammonia and extracted with ether. The solvent was distilled off and the residue was crystallized from benzene. Yield = 0.5000 g (0.02 %), m.p. = 145—146 °C, $[\alpha]$ (578 nm, 23 °C, $\rho = 2.5 \text{ g dm}^{-3}$, CHCl_3) = + 112°. UV spectrum; λ_{\max}/nm ($\log (\epsilon/(\text{m}^2 \text{mol}^{-1}))$): 284 (2.8). IR spectrum; $\bar{\nu}/\text{cm}^{-1}$: 2840, 2940, 3560 (hydroxyl). Mass spectrum; m/z : 608.2875 (M^+) (for $\text{C}_{37}\text{H}_{40}\text{N}_2\text{O}_6$ calculated $m/z = 608.2886$), 485 ($\text{M}_r - 123$), 417 ($\text{M}_r - 191$), 395, 379, 198, 175, 174.

Obamegine

Fraction four (tubes 38—45; 0.5928 g) was dissolved in acetic acid ($c = 1 \text{ mol dm}^{-3}$), the solution was filtered, the filtrate was made alkaline with concentrated ammonia and extracted with ether. The organic layer was concentrated and the residue was crystallized from benzene—chloroform. Yield = 0.1583 g (0.007 %), m.p. = 170—175 °C, $[\alpha]$ (578 nm, 21 °C, $\rho = 1.0 \text{ g dm}^{-3}$, CHCl_3) = + 275°. UV spectrum; λ_{\max}/nm ($\log (\epsilon/(\text{m}^2 \text{mol}^{-1}))$): 284 (2.6). IR spectrum; $\bar{\nu}/\text{cm}^{-1}$: 2810, 2920, 3560 (hydroxyl). Mass spectrum; m/z : 594 (M^+), 593, 471, 403, 402, 381, 192, 191, 175, 174, 168. ^1H NMR spectrum; δ/ppm : 2.29, 2.50 (s, 2NCH₃), 3.78, 3.91 (s, 2OCH₃), 6.06—7.26 (m, 10H, ArH).

Aromoline

Fraction five (tubes 46—60; 0.9820 g) was crystallized from chloroform—methanol. Yield = 0.0490 g (0.002 %), m.p. = 208—212 °C, $[\alpha]$ (578 nm, 23 °C, $\rho = 1.0 \text{ g dm}^{-3}$, CHCl_3) = + 320°. UV spectrum; λ_{\max}/nm ($\log (\epsilon/(\text{m}^2 \text{mol}^{-1}))$): 228 (3.8 sh), 284 (2.9). IR

spectrum; $\bar{\nu}/\text{cm}^{-1}$: 3450 (hydroxyl), 2940, 1620, 1580, 1440, 1382, 1318, 1205, 1082, 1015, 972, 932, 755. Mass spectrum; m/z : 594 (M^+), 591, 487, 402, 382, 381, 191, 174, 168. $^1\text{H NMR}$ spectrum; δ/ppm : 2.54 (s, 2NCH_3), 2.56 (s, $\text{C}-2'-\text{NCH}_3$), 3.60 (s, $\text{C}-6'-\text{OCH}_3$), 3.81 (s, $\text{C}-6-\text{OCH}_3$), 5.61—7.40 (m, 10H, ArH).

Isotetrandrine

The sum of tertiary alkaloids from seeds (2 g) was chromatographed through a column of silica gel. Combination of fractions 25—72 (V(chloroform): V(methanol)=95:5) and removal of solvents afforded a residue, which was crystallized from ether. Yield=0.335 g (0.007 %), m.p.=182—186 °C, $[\alpha]$ (578 nm, 23 °C, $\rho=2.0 \text{ g dm}^{-3}$, CHCl_3) = +160°. UV spectrum; $\lambda_{\text{max}}/\text{nm}$ ($\log(\epsilon/(\text{m}^2 \text{ mol}^{-1}))$): 284 (2.8). IR spectrum; $\bar{\nu}/\text{cm}^{-1}$: 2920, 2840, 1608, 1586, 1505, 1260, 1240, 1210, 1140, 1060, 1030, 880, 840. Mass spectrum; m/z : 622 (M^+), 485, 431, 395, 381, 198, 175, 174. $^1\text{H NMR}$ spectrum; δ/ppm : 2.25, 2.56 (s, 2NCH_3), 3.12, 3.61, 3.75, 3.91 (s, 4OCH_3), 5.99—7.25 (m, 10H, ArH).

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