

A NEW BISBENZYLISOQUINOLINE ALKALOID FROM *Mahonia aquifolium* (PURSH) NUTT.

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Baluchistine and a new phenolic bisbenzylisoquinoline alkaloid aquifoline were isolated from a mixture of tertiary alkaloids from the underground part of *Mahonia aquifolium* (PURSH) NUTT. On the basis of thin-layer chromatography these compounds also occur in the stem and seeds of the investigated plant.

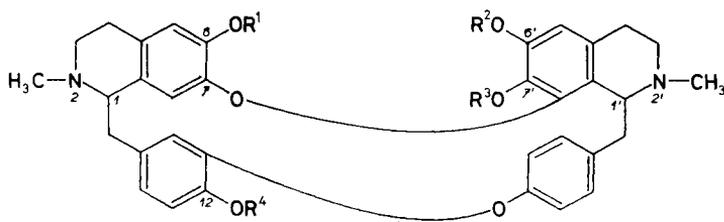
So far we have isolated from the plant *Mahonia aquifolium* (PURSH) NUTT. alkaloids of the protoberberine group – berberine, palmatine, jatrorrhizine, columbamine¹, oxyberberine², further aporphine alkaloids corytuberine, isocorydine, corydine, isoboldine and magnoflorine³. Of alkaloids of the bisbenzylisoquinoline group we isolated oxyacanthine, aromoline, berbamine, obamegine and isotetrandine².

In this paper we describe the isolation and identification of alkaloids from the roots of this plant. Using countercurrent distribution and preparative thin-layer chromatography two substances could be obtained.

The first alkaloid displays in its mass spectrum a molecular ion at m/z 594, corresponding to $C_{36}H_{38}N_2O_6$. Further fragmentation indicates that the alkaloid is of the oxyacanthine type, m/z 487 ($M - 107$, $M - C_7H_7O$). The base peak belongs to the ion m/z 381 ($C_{22}H_{25}N_2O_4$); other peaks of ions were at m/z 367 ($C_{21}H_{23}N_2O_4$), 191, 174 ($C_{11}H_{12}NO$), and 168. The results of these measurements are indicative of the structure of demethyloxyacanthine. In the 1H NMR spectrum two signals of two NCH_3 groups are present at δ 2.57 and 2.65 ppm, a further two OCH_3 groups at δ 3.24 and 3.58 ppm and ten aromatic protons in the δ 5.42 to 7.47 ppm region. The signal δ 3.58 is proof that one methoxy group is in position $C_{(6)}$ and the two asymmetric centres of this alkaloid have opposite configurations^{4,5}. The signal δ 3.24 belongs to the methoxyl group in position $C_{(7)}$. On the basis of these measurements, the IR spectra and optical rotation and on the basis of a comparison with an authentic sample the isolated substance was identified as baluchistine (6'-O-demethyloxyacanthine). So far this alkaloid was isolated only from a repre-

representative of the *Berberidaceae* family, *i.e.* from the species *Berberis baluchistanica* AHRENDT⁶. Methylation of baluchistine with diazomethane at 0°C gives di-O-methyl derivative which was identified on the basis of thin-layer chromatography and the mass spectra as obaberine⁷.

The second alkaloid displays in its mass spectrum a molecular ion at m/z 594, for $C_{36}H_{38}N_2O_6$. According to another peak, m/z 471 ($M - 123$, $M - C_7H_7O_2$), the alkaloid belongs to the berbamine type. The base peak has m/z 381 while other peaks are at m/z 367, 192, 191, 174 and 168. In the 1H NMR spectrum of this substance the signals of protons of two NCH_3 groups are present at δ 2.24 and 2.59 ppm, further of two OCH_3 groups at 3.16 and 3.52 ppm and of ten aromatic protons in the δ 6.06–7.33 ppm region. The signal at 3.16 ppm can be assigned to the methoxyl group in position $C_{(7)}$ and the signal at 3.52 ppm to the methoxy group in position $C_{(6')}$. The signal at 3.52 ppm is proof that two chiral centres have opposite configurations, because in the case of an equal configuration this signal would have a value of $\delta \sim 3.35$ ppm (refs^{4,5}). On the basis of a comparison of the CD spectra of berbamine (nm, $\Delta\epsilon$): 194, -35.03 ; 209, $+30.92$; 218, 0; 230, $+27.07$; 246, -13.79 ; 260, -0.87 ; 269, -1.52 ; 288, $+4.98$, and of this alkaloid it follows that the configuration 1*R*, 1'*S* may be assigned to the isolated product, which is in agreement with the literature⁷ for bisbenzylisoquinoline alkaloids of the berbamine type, isolated from the plants of the *Berberidaceae* family. According to the interpretation of the spectral measurements and the physical constants of the second alkaloid it represents a new bisbenzylisoquinoline alkaloid with the structure of 6-O-demethylberbamine (6',7-dimethoxy-2,2'-dimethylberbamane) for which we propose the trivial name aquifoline.



configuration at

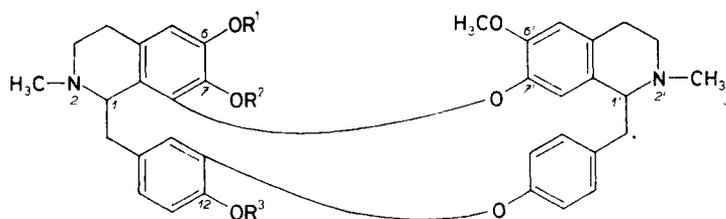
$C_{(1)}$, $C_{(1')}$

Oxyacanthine	$R^1 = R^2 = R^3 = CH_3$; $R^4 = H$
Aromoline	$R^1 = R^2 = CH_3$; $R^3 = R^4 = H$
Baluchistine	$R^1 = R^3 = CH_3$; $R^2 = R^4 = H$
Obaberine	$R^1 = R^2 = R^3 = R^4 = CH_3$

R, *S*
R, *S*
R, *S*
R, *S*

In addition to baluchistine the isomeric alkaloid aromoline was found² in the investigated plant, containing phenolic groups in positions $C_{(7')}$ and $C_{(12)}$ and having

the same absolute configuration. In addition to aquifoline an isomeric alkaloid was also detected², *i.e.* obamegine, with phenolic groups in positions C₍₇₎ and C₍₁₂₎ and with the same absolute configuration. Table I gives the values of the ¹H NMR spectra of bisbenzylisoquinoline alkaloids which we isolated from *Mahonia aquifolium* (PURSH) NUTT.^{2,3}. The measured values agree with the data from literature^{6,8}.



configuration at
C₍₁₎, C_(1')

Berbamine	R ¹ = R ² = CH ₃ ; R ³ = H
Obamegine	R ¹ = CH ₃ ; R ² = R ³ = H
Isotetrandrine	R ¹ = R ² = R ³ = CH ₃
Aquifoline	R ¹ = R ³ = H; R ² = CH ₃

R, S
R, S
R, S
R, S

TABLE I

Chemical shifts (δ , ppm) of oxyacanthine alkaloids in CDCl₃ solutions

Alkaloid	NCH ₃		NCH ₃			
	2	2'	6	6'	7'	12
Oxyacanthine	2.56	2.65	3.62	3.78	3.19	—
Aromoline	2.54	2.53	3.58	3.80	—	—
Baluchistine	2.57	2.65	3.58	—	3.24	—

Chemical shifts (δ , ppm) of berbamine alkaloids in CDCl₃ solution_e

Alkaloid	NCH ₃		NCH ₃			
	2	2'	6	7	6'	12
Berbamine	2.26	2.58	3.75	3.12	3.58	—
Obamegine	2.29	2.51	3.78	—	3.89	—
Isotetrandrine	2.26	2.57	3.75	3.13	3.61	3.91
Aquifoline	2.24	2.59	—	3.16	3.52	—

EXPERIMENTAL

The melting points were measured on a Kofler block and they are not corrected. The UV spectrum was measured on a Specord UV VIS (Jena) instrument, in methanol. The IR spectrum was measured on a Perkin-Elmer spectrophotometer, in KBr pellets. Optical rotation was measured on a Polamat A polarimeter, while the mass spectra were measured on an AEI MS 902 spectrometer. The ^1H NMR spectrum was recorded on a Bruker AM 300 instrument, in CDCl_3 , at 30°C , using tetramethylsilane as internal reference. The values are given in δ scale. The CD spectrum was measured on a Roussel Jouan CD 185 dichrographe in methanol.

For thin-layer chromatography and preparative chromatography silica gel G (Merck) was used. The following systems were used as eluents: ethyl acetate-methanol-ammonia 27 : 4 : 1 and cyclohexane-chloroform-diethylamine 4 : 5 : 1. Detection was carried out in UV light (254 and 366 nm) and with Dragendorff's reagent.

Extraction and Isolation of Alkaloids

The plants were collected in March 1979 in Bratislava. The roots were separated from the aerial parts and dried at room temperature. After grinding the roots (200 g) were extracted with methanol in a Soxhlet extractor. Methanol was evaporated in a vacuum and the residue was dissolved in $0.1 \text{ mol} \cdot \text{dm}^{-3}$ sulfuric acid. Further, the solution was alkalized with ammonia to pH 8–10 and extracted with ether. After evaporation of ether a mixture of tertiary alkaloids was obtained. This was separated by countercurrent distribution (100 stages, ratio of phases 25 : 25 ml) between chloroform and acetate buffer of pH 5. The alkaloids were extracted from the aqueous phase with chloroform, after alkalization with sodium carbonate solution. On the basis of thin-layer chromatography the contents of corresponding tubes were combined to 6 fractions altogether. The alkaloids present in fraction 6 (tubes 84–98) were separated by preparative thin-layer chromatography on silica gel. Crystallization gave two substances which were identified by physico-chemical methods.

Baluchistine: crystallized from acetone (0.0961 g, 0.004%), m.p. $212\text{--}214^\circ\text{C}$, $[\alpha]_{578}^{25} + 177.9^\circ$ (c 0.1, methanol). UV spectrum (methanol): λ_{max} nm (log ϵ): 228 (4.8) shoulder, 284 (3.9). IR spectrum (cm^{-1}): 3 560 (hydroxyl), 2 940, 2 840, 1 580, 1 490, 1 370, 1 230, 1 082, 1 010, 938, 755. Mass spectrum, m/z : 594.2695 (M^+). For $\text{C}_{36}\text{H}_{38}\text{N}_3\text{O}_6$ calculated 594.2730 (70), 487 (2.5), 382 (32), 381 (100), 367 (45), 191.5 (21), 191 (100), 174, 168 (40). ^1H NMR spectrum: 2.57, 2.65 (3 H, s, 2 NCH_3), 3.25, 3.58 (3 H, s, 2 OCH_3), 5.42–7.47 (10 arom. H).

Methylation of baluchistine: 28 mg of baluchistine were methylated with diazomethane for 48 h at 0°C . After distilling off of diazomethane and thin-layer chromatography and crystallization from methanol a substance (14.2 mg) was obtained with m.p. 152°C . Mass spectrum, m/z : 622 (M^+), 621, 607, 591, 515 ($\text{M} - 107$), 395, 381, 379, 198, 175, 174. On the basis of comparison with an authentic sample of obaberine the substances were found identical.

Aquifoline (6-O-demethylberbamine): crystallized from benzene-chloroform (0.0729 g, 0.003%), m.p. 168°C , $[\alpha]_{578}^{25} + 80^\circ$ (c 0.1, methanol). UV spectrum (methanol), λ_{max} (log ϵ): 229 (4.6) (shoulder, 282 (3.9)). IR spectrum (cm^{-1}): 3 460 (hydroxyl), 2 940, 2 840, 1 570, 1 495, 1 475, 1 370, 1 230, 1 080, 1 020, 930, 752. Mass spectrum, m/z : 594.2726 (M^+) for $\text{C}_{36}\text{H}_{38}\text{N}_2\text{O}_6$, 593, 471, 385, 381, 367, 192, 174, 168. ^1H NMR spectrum: 2.24, 2.59 (3 H, s, 2 NCH_3), 3.16, 3.52 (3 H, s, 2 OCH_3), 6.06–7.33 (10 arom. H). CD spectrum (nm, $\Delta\epsilon$): 193, -26.39 ; 207, $+16.51$; 218, 0; 229, $+19.27$; 247, -10.57 ; 260, -0.62 ; 269, -1.13 ; 288, $+2.87$.

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