Alkaloids of *Mahonia aquifolium* (PURSH) NUTT. II*

[a]J. SLAVÍK, [a]J. BOCHOŘÁKOVÁ, [b]D. KOŠŤÁLOVÁ, and [b]V. HROCHOVÁ

[a]*Department of Medicinal Chemistry and Biochemistry, Faculty of Medicine, J. E. Purkyně University, CS-662 43 Brno*

[b]*Department of Pharmacognosy and Botany, Faculty of Pharmacy, Komenský University, CS-832 32 Bratislava*

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Corytuberine, isolated for the first time from the sum of alkaloids obtained from the leaves of *Mahonia aquifolium* (PURSH) NUTT., was identified from its spectral data and by comparison with the specimen; so far, it has not been found in any plant of the *Berberidaceae* family. Additional alkaloids identified from the above-mentioned sum of alkaloids were: magnoflorine, isocorydine, corydine, isoboldine, and berbamine. Berberine and palmatine were identified by thin-layer chromatography. Magnoflorine was shown to be the principal alkaloid of the above-ground bark; it was accompanied by berberine and jatrorrhizine. Further alkaloids from the bark identified by thin-layer chromatography were isocorydine, berbamine, corytuberine, and columbamine.

Из смеси алкалоидов, приготовленных из листьев *Mahonia aquifolium* (PURSH) NUTT. впервые был выделен коритуберин, идентичность которого была доказана спектроскопически сравнением с образцом стандарта. Выделение этого алкалоида из упомянутого растения является первым доказательством его нахождения в семействе *Berberidaceae*. Следующие содержащиеся в листьях алкалоиды – это магнофлорин, изокоридин, коридин, изоболдин и бербамин. Хроматографически было подтверждено наличие берберина и пальматина. Магнофлорин был также выделен в качестве основного алкалоида из коры надземной части растения. Далее, из коры были выделены берберин и иатроризин и хроматографически доказано присутствие изокоридина, бербамина, коритуберина и колумбамина.

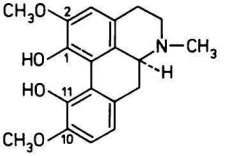
Our previous paper [1] reported the presence of magnoflorine and further bases in the roots of *Mahonia aquifolium* (PURSH) NUTT.; this paper deals with the isolation and identification of alkaloids present in the leaves, bark, and wood of this plant collected at two localities — namely in the cities of Brno and Bratislava.

* For Part I see Ref. [1].

Alkaloids of roots and leaves of a related species, *Mahonia repens* (LINDL.) G. DON, have already been studied [2]; 1,2,9,10-tetrasubstituted aporphine bases, thaliporphine and glaucine, as well as 1,2,10,11-tetrasubstituted aporphine alkaloids corydine and isocorydine were obtained from the leaves. Bases of this type have not been evidenced either in the underground or in the woody aerial part.

Corydine, isocorydine, and isoboldine were identified [3] when investigating exclusively the leaves of *M. aquifolium* (PURSH) NUTT., whilst isocorydine and berbamine were found in flowers [4].

Our examination of individual plant organs of aerial part of this plant showed the principal base of leaves to be corytuberine (*I*); its presence in the *Berberidaceae* family has not been reported as yet.



I

This highly polar alkaloid was isolated after its conversion to corytuberine hydrogen iodide [5] in a relatively high yield (0.86 %), when collected in Brno, or in a 0.43 % yield from Bratislava. It was accompanied by its *N*-methyl derivative — magnoflorine. Both alkaloids belong to 1,2,10,11-tetrasubstituted aporphines. Also a minute amount of isocorydine, corydine, and isoboldine, bases of this group, was isolated in addition to berbamine belonging to bisbenzylisoquinoline group. Thin-layer chromatography revealed the presence of berberine and palmatine in minute amounts. Leaves and the underground part of this plant species grown in Bratislava contain magnoflorine; other alkaloids of the aporphine group were found in the leaves only [1]. The presence of aporphine alkaloids in leaves is a dominant feature not only of *M. aquifolium*, but also of *M. repens*.

The UV spectrum of isocorydine, corydine, and magnoflorine is characteristic of 1,2,10,11-tetrasubstituted aporphines having the absorption maxima at 220 nm, 270 nm, and 305 nm [6, 7]. The UV spectrum of isoboldine in indicative of 1,2,9,10-tetrasubstituted aporphines with absorption maxima at 220 nm, 282 nm, and 305 nm [6, 7].

The mass spectrum of corytuberine reveals peaks at m/z 327.1471 ($M^{+\cdot}$, for $C_{19}H_{21}NO_4$ calculated 327.1470), 312 ($M_r - 15$), 310 ($M_r - 17$), 284 ($M_r - 43$); the peak at m/z 312 is greater than that at m/z 310, which provides evidence for substitution of OH groups at C-1 and C-11 of the aporphine backbone [8]. The ^1H NMR spectrum of this compound contains signals of two methoxyl groups at $\delta = 4.07$ ppm, three protons of an aromatic ring at $\delta = 6.98$ ppm (3-H) and 7.12 ppm (8.9-H). Signal at $\delta = 3.38$ ppm is diagnostic of an N—CH_3 group [9].

Distribution of alkaloids in the aerial part of this plant collected in the city of Brno is listed in Table 1. The greatest content of alkaloids is present in the bark (>2 %), in which the principal alkaloid is magnoflorine iodide (1 %), followed by berberine chloride (0.12 %) and jatrorrhizine iodide (0.06 %). Further alkaloids were identified by thin-layer chromatography (Table 1). Isolation of the afore-mentioned alkaloids has already been described in our previous paper [1]. The woody part was the poorest in alkaloids; also here the dominating alkaloid is magnoflorine. Occurrence of magnoflorine in the aerial part of *M. aquifolium* has so far not been reported. Table 2 presents the distribution of alkaloids in the leaves collected in the city of Bratislava.

Experimental

The melting points were determined on a Kofler micro hot-stage, the IR spectra were measured with a Perkin—Elmer spectrometer, model 477, in KBr, the UV spectra of methanolic solutions were recorded with a UV VIS (Zeiss, Jena) spectrophotometer. Optical rotations were recorded with a Polamat A instrument. The mass spectra were run with an AEI MS 902 apparatus. The ^{1}H NMR spectra of trifluoroacetic acid solutions containing tetramethylsilane as an internal reference were measured with a Jeol FX 100 spectrometer operating at 99.6 MHz. Purity of the alkaloids isolated was checked by thin-layer chromatography on silica gel G (Merck) using methanol—water—diethylamine (volume ratio $r_V = 15:3:1$) S_1, methanol—diethylamine ($r_V = 4:1$) S_2, and cyclohexane—chloroform—diethylamine ($r_V = 7:2:1$) S_3. The alkaloids were visualized by potassium hexaiodoplatinate, Dragendorff's reagent or at $\lambda = 254$ nm or 366 nm.

The complete above-ground part of the drug collected at the beginning of November 1982 in Brno was assorted into leaves, wood, and bark. In Bratislava, there were collected only leaves in December 1982. Both drugs were air-dried at room temperature and worked up in the same manner.

The ground leaves from Bratislava (200 g) were defatted by light petroleum and extracted with methanol in a Soxhlet apparatus. The methanolic solution was evaporated under reduced pressure and the residue (46.5 g), dissolved in 0.1 M-H_2SO_4, was filtered, made alkaline with sodium carbonate and extracted with ether. The ethereal solution was evaporated to give a mixture of tertiary alkaloids (0.16 g, 0.08 %, portion *A*).

The aqueous layer was made alkaline with 10 M-NaOH and extracted with ether. Evaporation of ether afforded 5.1 mg (0.003 %) of portion *B*. Thin-layer chromatography in S_2 indicated and comparison with the specimen proved the bases in the leaves to be palmatine ($R_f = 0.20$) and berberine ($R_f = 0.24$).

The aqueous layer after separation of portion *B* was acidified to pH = 6—7 with 2 M-H_2SO_4; addition of saturated potassium iodide solution followed by extraction with chloroform—methanol ($r_V = 8:2$) afforded portion *I* (iodides). Crystallization of this portion from methanol afforded corytuberine hydrogen iodide as a major alkaloid (0.86 g, 0.43 %), $R_f = 0.77$ (S_1), m.p. = 213 °C, $[\alpha]$ (578 nm, 25 °C) = +290° ($\varrho = 1.0$ g dm^{-3}, ethanol), UV spectrum—λ_{max}/nm (log (ε/(m^2 mol^{-1}))): 223 (3.6), 270 (3.0), 308 (2.8); IR spectrum—$\tilde{\nu}$/cm^{-1}: 3220 ν(OH), 1610, 1580 (aromatic ring). Mass spectrum—*m*/*z*: 327,

Table 1

Distribution of alkaloids in the aboveground part of *Mahonia aquifolium* (PURSH) NUTT. collected in Brno

Plant organ	*w*/% (of the total aerial part)	*w*(portion of alkaloids)/%		
		Portion *A*	Portion *B*	Portion *I* (iodides)
Wood	56	0.013 (isocorydine, berbamine, 2 unidentified alkaloids)	0.003 (berberine, palmatine)	0.12 (magnoflorine (0.04), corytuberine (traces))
Leaves	28	0.12 (isocorydine, isoboldine, corydine, berbamine, 2 unidentified alkaloids)	0.005 (berberine, palmatine)	0.96 (corytuberine (0.86), magnoflorine)
Bark	15	0.32 (isocorydine, berbamine, scoulerine, 2 unidentified alkaloids)	0.15 (berberine (0.12), palmatine)	1.72 (magnoflorine (0.99), jatrorrhizine (0.06), columbamine, corytuberine)

Table 2

Distribution of alkaloids in the leaves[a] of *Mahonia aquifolium* (PURSH) NUTT. collected in Bratislava

w (portion of alkaloids)/%		
Portion *A*	Portion *B*	Portion *I* (iodides)
0.08 (isocorydine (0.002), corydine (0.002), isoboldine (0.002), berbamine (0.003))	0.003 (palmatine, berberine)	(corytuberine (0.43), magnoflorine (0.16))

a) 23 % of the total mass.

326, 312, 310, 284, 269, 266, 163.5, 147.5, 128. ^{1}H NMR spectrum—δ/ppm: 3.38 (d), 4.07 (s, 6H), 6.98 (s), 7.12 (s).

Crystallization of mother liquors after separation of corytuberine hydrogen iodide from methanol yielded magnoflorine iodide (0.32 g, 0.16 %), m.p. = 261 °C, [α] (578 nm, 20 °C) = + 192.5° (ϱ = 2.0 g dm^{-3}, methanol), R_f = 0.24 (S_1). UV spectrum—λ_{max}/nm (log (ε/(m^2 mol^{-1}))): 225 (3.6), 270 (2.9), 310 (2.8). Its identity with the specimen [10] was corroborated by comparison.

Portion *A* (0.16 g) was chromatographed on a silica gel column (silica gel Merck), chloroform, chloroform—methanol, and methanol being the respective eluents. Fractions *1—15* (chloroform—methanol, r_v = 9 : 1) were collected, the solvent was evaporated and the residue was crystallized from methanol. Yield 5.2 mg, 0.002 %, R_f = 0.66 (S_3), m.p. = 184 °C, [α] (578 nm, 22 °C) = + 206° (ϱ = 1.0 g dm^{-3}, chloroform). UV spectrum—λ_{max}/nm (log (ε/(m^2 mol^{-1}))): 220 (3.3), 268 (3.1), 304 (2.8). These data together with comparison with the specimen proved this compound to be isocorydine [3, 10].

Fractions *16—19* (elution with chloroform—methanol, r_v = 9 : 1) were worked up to give corydine (5.8 mg, 0.002 %), R_f = 0.59 (S_3), [α] (578 nm, 22 °C) = + 196° (ϱ = 1.0 g dm^{-3}, ethanol), UV spectrum—λ_{max}/nm (log (ε/(m^2 mol^{-1}))): 220 (3.4), 270 (2.9), 305 (2.6). Its identity was verified by comparison with the specimen [3, 10].

Fractions *20—26* (chloroform—methanol, r_v = 8 : 2) gave upon the work-up berbamine (6.2 mg, 0.003 %), R_f = 0.30 (S_3). UV spectrum—λ_{max}/nm (log (ε/(m^2 mol^{-1}))): 284 (2.7), [α] (578 nm, 22 °C) = + 102° (ϱ = 1.0 g dm^{-3}, chloroform), IR spectrum—$\tilde{\nu}$/cm^{-1}: 3450 (OH). Its identity was proved by comparison with the specimen [11].

Fractions *27—35* (chloroform—methanol, r_v = 7 : 3) were collected and the solvent was evaporated. Yield 4.6 mg (0.002 %) of a compound of R_f = 0.11, (S_3), [α] (578 nm, 22 °C) = + 50° (ϱ = 1.0 g dm^{-3}, ethanol), UV spectrum—λ_{max}/nm, (log (ε/(m^2 mol^{-1}))): 219 (3.5), 268 (2.9), shoulder, 282 (3.0), 305 (3.1), IR spectrum—$\tilde{\nu}$/cm^{-1}: 1070, 1100, 1280, 1315, 1350, 1410, 1510, 1570, 1610, 3400—3450. These data together with those reported [3, 10] and comparison with the specimen showed this base to be isoboldine.

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