Isolation of quaternary alkaloids from *Mahonia aquifolium* (PURSH) *Nutt. I.*

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Five alkaloids were isolated from the underground part of *Mahonia aquifolium* of domestic origin: magnoflorine, berberine, palmatine, jatrorrhizine, and columbamine. The aerial part of this plant contained only berberine, palmatine, and jatrorrhizine. The identity of the isolated bases was corroborated by colour reactions in the u.v. light, by i.r. and mass spectra, optical rotation and comparison with the authentic specimens.

Из подземной части растения *Mahonia aquifolium* (PURSH) *Nutt.* домашнего происхождения было изолировано пять алкалоидов: магнофлорин, берберин, пальматин, ятрорризин и колумбамин. В надземной части присутствуют только берберин, пальматин и ятрорризин. Изолированные соединения были идентифицированы на основании реакций окрашивания в УФ свете, измерением УФ, ИК и масс-спектров, оптического вращения и сравнением с автентическими образцами.

The investigation of 17 species of the genus *Mahonia Nutt. (Berberidaceae)* showed that the principal constituents are alkaloids of protoberberine, bisbenzylisoquinoline, and aporphine groups [1, 2]. One of the most frequented species of this genus is *Mahonia aquifolium* encountered as a cultivated garden plant. So far, roots and the aerial part were investigated and berberine (the protoberberine group), oxyacanthine, berbamine (the bisbenzylisoquinoline group), and isocorydine (the aporphine group) were isolated [2, 3]. Berberine, jatrorrhizine, palmatine (the protoberberine group), oxyacanthine, berbamine, and isotetrandrine (the bisbenzylisoquinoline group) were found in roots, while berberine was isolated from leaves and from the above-ground parts [4, 5]. Isocorydine and berbamine were found in fresh flowers of this species [6], isocorydine, corydine, and isoboldine in leaves [7].

This paper deals with the isolation and identification of quaternary alkaloids from roots and aerial part of *Mahonia aquifolium* of domestic origin.

Isomeric alkaloids of the protoberberine group berberine, palmatine, jatrorrhizine, and columbamine were isolated from the mixture of quaternary alkaloids;

moreover, the aporphine alkaloid magnoflorine was found in the roots. Their identity was corroborated by means of colour reactions in the u.v. light, by u.v. spectra characteristic of protoberberine and aporphine alkaloids, by i.r. spectra identic with those published, as well as by comparison with specimens. Alkaloids columbamine and magnoflorine had mass spectra in line with those already reported [8].

The u.v. spectrum of the quaternary phenolic aporphine alkaloid magnoflorine was indicative of 1,2,10,11-tetrasubstituted aporphines [9]. The spectrum, measured in an alkaline medium, displayed a bathochromic shift of the maximum, thus evidencing the presence of a phenolic group [9]. The mass spectrum of this alkaloid revealed a diagnostic peak at *m/z* 341 (M – HI, $C_{20}H_{23}NO_4$) and further fragments at *m/z* 327 (6%, $M - CH_3I$), 142 (80%, CH_3I), 128 (42%, HI), 58 (100%) in line with the spectrum of the specimen and the literature [8]. The presence of magnoflorine in this plant was proved for the first time. So far, this alkaloid was isolated from three species of this genus only: *M. fortunei, M. lomarifolia*, and *M. morrisonensis* [10, 11]. The identity of columbamine was evidenced on the basis of u.v. spectrum, characteristic of quaternary protoberberine alkaloids [12, 13]. Like jatrorrhizine, this alkaloid displayed a strong bathochromic shift in its u.v. spectrum measured in an alkaline medium, thus providing an evidence of a phenolic group. Mass spectrum of this alkaloid showed a characteristic peak at *m/z* 337 (M – HI, $C_{20}H_{19}NO_4$) and further fragments at *m/z* 142 (42%, CH_3I), 128 (100%, HI) in line with those reported [8]. The presence of columbamine has not been reported in the genus *Mahonia Nutt.* as yet.

Berberine, palmatine, and jatrorrhizine were the quaternary alkaloids isolated from the aerial part. The presence of columbamine and magnoflorine has not been proved in this part of the plant.

The ability of plants of *Berberidaceae* family, especially of the most abundant genus *Berberis* L. to produce alkaloids of several chemical groups is known [14, 15]. The presence of an alkaloid with an aporphine structure and isomeric protoberberine alkaloids in the plant under investigation is taxonomically significant and could be expected. All alkaloids shown in this paper were also present in the chemotaxonomically related genus *Berberis* L.

Experimental

Melting points were determined on a Kofler micro hot-stage. Infrared spectra were taken with a Perkin—Elmer spectrometer, model 477, in KBr tablets. Ultraviolet spectra were measured with a UV VIS (Zeiss, Jena) apparatus in methanol. Optical rotations of methanolic solutions were recorded with a Polamat A instrument, electron impact mass spectra with a Jeol JMS-D 100 apparatus.

The purity of the separate alkaloids was checked by thin-layer chromatography on silica gel LSL_{254} (Lachema, Brno) in methanol—diethylamine 4:1. The isolated compounds were visualized with Dragendorf reagent and in the u.v. light.

Extraction and isolation of quaternary alkaloids from the roots of Mahonia aquifolium (PURSH) *Nutt.*

Dried and ground roots (5000 g) were extracted with light petroleum and with methanol in the Soxhlet apparatus. The methanolic extract was concentrated and the residue dissolved in 5% HCl. The solution was filtered, alkalinized with a concentrated NH_4OH to pH 8 and extracted with ether. The mixture of tertiary alkaloids was obtained after removal of the solvent. The alkaline aqueous layer was acidified with concentrated HCl to pH 4; a saturated KI solution was added to it. Iodides of quaternary phenolic and nonphenolic bases were extracted with chloroform from which they were obtained after removal of the solvent (14.95 g).

Berberine iodide. The mixture of quaternary alkaloids (2.00 g) was separated on an Al_2O_3 column (Reanal, neutral, activity grade II) using chloroform, chloroform—methanol, and methanol (5 ml fractions) as eluents. The alkaloid obtained from the combined fractions *96—160* after evaporation of the solvent and crystallization from methanol (44 mg) was identical (R_f value, i.r. spectrum) with berberine iodide. M.p. 265°C, λ_{max}/nm (log ε): 265 (4.48), 350 (4.42), 420 (3.70) were in accordance with those reported [16].

Jatrorrhizine iodide (16 mg) was obtained from combined fractions *181—330.* M.p. 212°C, R_f 0.46, λ_{max}/nm (log ε): 228 (3.90), 268 (3.87), 275 sh (3.84), 352 (3.82), 436 (3.18). The quinoid structure formed in an alkaline medium was characterized by an absorption band at 485 nm, this being a diagnostic feature distinguishing this alkaloid from its isomer columbamine [8, 13].

Magnoflorine iodide (108 mg) separated after removal of berberine and jatrorrhizine was recrystallized from methanol. M.p. 251—252°C, $[\alpha]_D^{23} = +195°$, R_f 0.05, λ_{max}/nm (log ε): 225 (4.66), 269 (3.95), 315 (3.79) were identical with the data reported for this alkaloid [17]. It displayed a characteristic blue fluorescence at 254 nm.

The mother liquor after separation of magnoflorine iodide contained four alkaloids of R_f 0.12, 0.21, 0.27, and 0.33; they were purified on an alumina (Reanal, neutral, activity grade II) column using chloroform, chloroform—methanol, and methanol for elution (fractions 5 ml each).

Palmatine iodide (4.1 mg) was obtained from fractions *170—192* by crystallization from methanol. M.p. 225°C, R_f 0.12. Its identity with the specimen was proved on the basis of spectral and physicochemical data [18, 13].

Columbamine iodide (3.1 mg) was separated from the combined fractions *201—220* by crystallization from methanol. M.p. 207—209°C did not reveal depression with the specimen, R_f 0.27, λ_{max}/nm (log ε): 228 (3.90), 268 (3.87), 280 sh (3.83), 350 (3.82), 430 (3.16); $\lambda_{max}^{MeOH+NaOH}$/nm (log ε): 215 (4.07), 275 (4.08), 332 (3.78), 380 (3.74), 460 (2.87). On the basis of the u.v. spectrum measured in the alkaline medium this alkaloid can be distinguished from its isomer jatrorrhizine [8]. Columbamine has at 254 nm a noticeable yellow fluorescence.

Extraction and isolation of quaternary alkaloids from the aerial part of Mahonia aquifolium (PURSH) *Nutt.*

The dried and ground aerial part (3200 g) was worked up by the same procedure as the underground part. The tertiary alkaloids being removed, the aqueous layer was basified with 40% NaOH to pH 14 and extracted with ether. The residue after evaporation of ether was hot-dissolved in dilute (1:1) HCl.

Berberine chloride, separated as a yellow precipitate after cooling of the acidified solution, was crystallized from methanol (1.582 g); m.p. 204—205°C, R_f 0.21, λ_{max}/nm (log ε): 265 (4.46), 350 (4.44), and 420 (3.76) were in agreement with the published data [16].

A saturated KI solution was added to the acidified (concentrated HCl, pH 4), originally alkaline aqueous layer and iodides on quaternary alkaloids were extracted with chloroform. The mixture of three alkaloids (2.00 g, R_f 0.46, 0.16, 0.12), obtained after evaporation of the solvent, was separated on a column packed with Al_2O_3 (Reanal, neutral, activity grade II) by the same procedure as mentioned above.

Palmatine iodide (1.2 mg) was obtained from the chloroform—methanol (9:1) fraction. M.p. 225°C, R_f 0.12.

Berberine iodide (19.8 mg) was separated from the combined chloroform—methanol (4:1) fractions. M.p. 265°C, R_f 0.16.

Jatrorrhizine iodide (72 mg) was isolated from the combined chloroform—methanol (1:1) fractions. M.p. 212°C, R_f 0.46.

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