

Isoquinoline alkaloids of *Isopyrum thalictroides* L.

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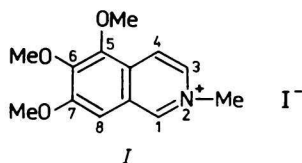
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A new simple isoquinoline alkaloid, *N*-methyl-5,6,7-trimethoxyisoquinolinium iodide, an aporphine alkaloid magnoflorine, and bisbenzylisoquinoline alkaloids berbamine and isotetrandrine were isolated from the methanolic extract of *Isopyrum thalictroides* L. Berbamine was found in this plant species for the first time.

Из метанольного экстракта *Isopyrum thalictroides* L. были выделены новый простой изохинолиновый алкалоид, йодид *N*-метил-5,6,7-триметоксиизохинолия, апорфиновый алкалоид магнофлорин, а также бис-бензилизохинолиновый алкалоид бербамин и изотетрандрин. Бербамин был в растениях этого рода обнаружен впервые.

Isoquinoline alkaloids present in *Isopyrum thalictroides* L. (family *Ranunculaceae*) could be classified into pseudoberberines (pseudoprotoberberine, pseudocoptisine, pseudocolumbamine, and dehydrocheliantipholine), aporphines (magnoflorine), and bisbenzylisoquinolines ((*S,S*)-tetrandrine, (*R,S*)-tetrandrine, (\pm)-tetrandrine, and *O*-methylrepandine) [1—3].

The molecular radical ion of the new isoquinoline alkaloid appeared at $m/z = 234.1126$ (for $C_{13}H_{16}N^+O_3$ calculated 234.1130); other characteristic peaks were found at $m/z M - 1$ and $M - 15$. The 1H NMR spectrum showed signals of three OCH_3 and one N^+-CH_3 groups, three protons of a nitrogen-containing aromatic ring and one singlet at $\delta = 7.87$ ppm. After presaturation of this signal a NOE effect was observed at H-1 ($\delta = 9.93$ ppm) in the differential spectrum [4]. This finding unambiguously indicated the presence of a proton resonating at $\delta = 7.87$ ppm at C-8. Consequently, the methoxyl groups have to be located in positions C-5, C-6, and C-7.



A like isoquinoline alkaloid, *N*-methyl-6,7-dimethoxyisoquinolinium chloride, by one methoxyl group at C-5 poorer, has already been isolated from *Thalictrum revolutum* DC. (family *Ranunculaceae*) [5].

Berberamine and isotetrandrine were obtained as amorphous compounds from the tertiary alkaloid fraction in a small amount. Their IR, UV, and mass spectral data and their optical rotations were in accordance with the respective values reported in [6].

As found, the principal alkaloid of this plant species is the quaternary aporphine derivative magnoflorine iodide.

Experimental

The melting points were determined on a Kofler micro hot-stage, the UV spectra of methanolic solutions and IR spectra of KBr pellets were measured with a Specord UV VIS (Zeiss, Jena) and Perkin—Elmer spectrophotometers, respectively. The optical rotation was taken with a Polamat A polarimeter, the mass spectra were run with an AEI MS-902 apparatus and the ^1H NMR spectra of deuterioacetone solutions were traced with a Bruker AM-300 instrument, tetramethylsilane being the internal reference. Solvent systems for thin-layer chromatography on Silufol sheets were: methanol—water—conc. ammonia ($\phi_r = 15:3:1$) S_1 , chloroform—methanol—acetic acid ($\phi_r = 47.5:47.5:5$) S_2 , and benzene—acetone—ammonia ($\phi_r = 4:3:1$) S_3 . The spots were detected either by UV λ_{254} and λ_{366} lights, or by spraying with Dragendorff reagent.

Isolation and identification of alkaloids

Isopyrum thalictroides (the aerial part and the roots) collected in Bratislava in May 1983 was air-dried and ground to give 1900 g of the drug. This was first defatted with light petroleum, then extracted with methanol in a Soxhlet apparatus and the solvent was removed under reduced pressure to yield 142 g of the methanolic extract. The latter was dissolved in hydrochloric acid (1 mol dm^{-3}), and the solution made alkaline with ammonia to pH = 8—10 was taken into ether. The evaporated ethereal portion represented the mixture of tertiary alkaloids (6.35 g), which was chromatographed on a silica gel-packed column (Silpearl) with chloroform—methanol mixtures of various ratios. The content of fractions (30 cm^3 each) was monitored by thin-layer chromatography. Fractions 4—8 ($\text{CH}_3\text{OH—CHCl}_3$, $\phi_r = 5:95$) afforded 8.2 mg of a compound of $R_f = 0.72$ (S_3). UV spectrum (CH_3OH), λ/nm ($\log(\epsilon/(\text{m}^2 \text{mol}^{-1}))$): 282 (2.94); $[\alpha](578 \text{ nm}, 23^\circ \text{C}, \rho = 1 \text{ g dm}^{-3}, \text{CHCl}_3) = 150^\circ$. Electron impact mass spectrum, m/z : 622.3040 (M^+) (for $\text{C}_{38}\text{H}_{42}\text{N}_2\text{O}_6$ calculated 622.3043), 485 ($M - 137$), 431 ($M - 191$). Comparisons with an authentic sample and with the reported data [6] show this compound to be isotetrandrine.

Evaporation of fractions 23—28 ($\text{CH}_3\text{OH—CHCl}_3$, $\phi_r = 1:4$) yielded 3.6 mg of a compound of $R_f = 0.45$ (S_3), $[\alpha](578 \text{ nm}, 23^\circ \text{C}, \rho = 1.0 \text{ g dm}^{-3}, \text{CHCl}_3) = 102^\circ$. UV

spectrum, λ/nm ($\log(\epsilon/(\text{m}^2 \text{mol}^{-1}))$): 212 (3.3), 282 (2.8); IR spectrum, $\tilde{\nu}/\text{cm}^{-1}$: 2840, 2920, 3560 (OH); mass spectrum, m/z : 608.2870 (M^+) (for $\text{C}_{37}\text{H}_{40}\text{N}_2\text{O}_6$ calculated 608.2886), 485 ($M - 123$), 417 ($M - 191$), 395, 379, 198, 175, 174. These and the reported data [6], and comparison with an authentic specimen disclose this compound to be berbamine.

After separation of tertiary bases the quaternary iodides were extracted with chloroform from the aqueous layer, which was acidified with concentrated hydrochloric acid and saturated with potassium iodide. Chloroform was evaporated and the residue was crystallized from methanol to give 0.970 g of crystals, m.p. = 263°C , $R_f = 0.33$ (S_1), $[\alpha]_D^{25}$ (578 nm, 21°C , $\rho = 1.0 \text{ g dm}^{-3}$, CH_3OH) = 200° . UV spectrum, λ/nm ($\log(\epsilon/(\text{m}^2 \text{mol}^{-1}))$): 228 (2.6), 271 (2.5), 310 (2.3). Identification as above employing [7] revealed this compound to be magnoflorine iodide.

The mother liquor after separation of magnoflorine was evaporated under diminished pressure to afford 0.980 g of a residue, which was chromatographed over silica gel (Silpearl) with chloroform—methanol in various volume ratios. The content of 10-cm^3 fractions was monitored by thin-layer chromatography. Fractions 51–63, eluted with chloroform—methanol, $\phi_r = 9:1$ were collected and the solid was crystallized from benzene—chloroform to yield 8.5 mg of a compound of m.p. = $162\text{--}165^\circ\text{C}$, $R_f = 0.29$ (S_2). UV spectrum, λ/nm ($\log(\epsilon/(\text{m}^2 \text{mol}^{-1}))$): 262 (3.8), 315 (2.85) was characteristic of a simple isoquinoline alkaloid [8]. IR spectrum, $\tilde{\nu}/\text{cm}^{-1}$: 980, 1000, 1040, 1115 ($\nu(\text{OCH}_3)$), 1270, 1358, 1417, 1472, 1602 ($\nu(\text{C}=\text{C})$), 2948. Mass spectrum, m/z : 234 (M^+), 233 ($M - 1$), 219 ($M - 15$), 204 ($\text{C}_{12}\text{H}_{14}\text{NO}_3$), 201, 200, 190–188 (diffuse), 176. ^1H NMR spectrum, δ/ppm : 9.93 (s, 1H, $J_{1,3} = 1.5 \text{ Hz}$, $J_{1,\text{NCH}_3} = 1 \text{ Hz}$, H-1), 8.62 (dd, 1H, $J_{3,4} = 7.0 \text{ Hz}$, H-3), 8.51 (d, 1H, H-4), 7.87 (s, 1H, H-8), 4.68 (s, 3H, $^+\text{NCH}_3$), 4.16, 4.14, 4.12 (3s, 9H, $3 \times \text{OCH}_3$). These data allow to ascribe the structure of *N*-methyl-5,6,7-trimethoxyisoquinolinium iodide (*I*) to this compound.

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