Alkaloids of *Berberis julianae* SCHNEID.

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So far, the alkaloids of *Berberis julianae* SCHNEID. have not been investigated. We isolated berberine, magnoflorine, and glaucine from the aboveground part of this plant directly. Jatrorrhizine was identified as tetrahydro derivative. The presence of palmatine and further unidentified alkaloids was evidenced chromatographically. The alkaloid glaucine has not been found in plants of this genus.

Berberis julianae SCHNEID. is an evergreen shrub, belonging to family *Berberidaceae*, home-grown in Central China. It is cultivated in our country in parks and botanical gardens.

As yet, quaternary bases of protoberberine type, berberine, palmatine, jatrorrhizine, columbamine, and epiberberine have been isolated from plants of the genus *Berberis* [1—4], whereas magnoflorine represents most frequently the aporphine type of alkaloids [5]. Additional two new alkaloids of the latter type, bervulcine and its isomer vulracine were recently isolated [6].

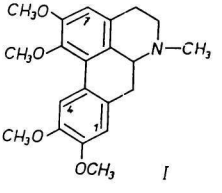
The aboveground part was collected in flower (April 1972) in Bratislava. The dried milled material was extracted with methanol, the solvent was removed and the residue dissolved in 1% acetic acid. The solution was worked up employing the method of fractional isolation of alkaloids [7]. The acid solution was filtered and basified with a saturated sodium carbonate solution and extracted with ether. Alkaloids obtained after removal of ether were separated into phenolic and non-phenolic fractions; the former was not investigated because of the lack of material, the latter afforded, when chromatographed over alumina, two substances of R_F 0.64 and 0.78 (system B_2), respectively.

The compound of R_F 0.64 was found to be identical with glaucine (*I*) on the basis of the following findings. The mass spectrum revealed a molecular ion peak at 355.1370 corresponding to the molecular formula $C_{21}H_{25}NO_4$. Fragments at M −15 and M −31 were indicative of the loss of methyl and methoxy groups. Unsubstantial fragmentation suggested the aporphine backbone of the alkaloid.

This substance displayed 4 singlets of three protons each at δ 3.85, 3.83, 3,81, and 3.59 p.p.m. (4 methoxy groups). The singlet at δ 2.51 p.p.m. was ascribed to three protons of the $-NCH_3$ grouping. Aromatic protons were seen as singlets at δ 8.00 (proton at C-4), 6.60 (proton at C-1), and 6.51 p.p.m. (proton at C-7). The u.v. spectrum was identical with that of glaucine [10]. The identity of both substances was also corroborated by chromatographic comparison with an authentic specimen of glaucine.

Another nonphenolic base of R_F 0.78 had, according to mass spectroscopy (M+ 353.1214), molecular formula $C_{21}H_{23}NO_4$. The unsubstantial fragmentation is also characteristic of an aporphine alkaloid. It could be assumed that dehydroglaucine might be involved. Neverthelles, the slight amount of the alkaloid under investigation avoided the identification.

The aqueous solution remaining after the extraction with ether was alkalified with sodium hydroxide and anew extracted with ether. The ethereal solution was evaporated and the residue afforded *via* citrate a yellow chloride, m.p. 196°C. Both the i.r. spectrum and the R_F value were identical with those of berberine chloride. Mother liquors furnished a mixture of berberine chloride and an alkaloid, which showed the same R_F value in various systems as palmatine chloride. The water solution was acidified with hydrochloric acid and treated with potassium iodide solution [8]. The quaternary bases were extracted with chloroform, the solvent was removed and the residue dissolved in hot methanol, from which the little soluble magnoflorine iodide (m.p. 268°C) crystallized. Its i.r. spectrum and R_F value compared with those of the authentic specimen [9]. Mother liquors contained a substance, the R_F value of which was identical with jatrorrhizine. Attempts to obtain this base in crystalline form failed. Upon reduction with zinc in hydrochloric acid the sample afforded the crystalline tetrahydro derivative whose i.r. and u.v. spectra were identical with tetrahydrojatrorrhizine.



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Experimental

Melting points were measured on a Kofler micro hot-stage. Infrared spectra were recorded with a Zeiss UR-10 spectrometer, ultraviolet spectra with a UNICAM SP-1800 apparatus, mass spectra with an AEI MS spectroscope. The p.m.r. spectra were taken with a TESLA 487 spectrometer, tetramethylsilane being an internal reference substance. The optical rotation was measured with a Zeiss POLAMAT S polarimeter. Solvent systems for thin-layer chromatography on silica gel G (Stahl): ethanol—water—ammonia 15 : 9 : 1 (*A*) cyclohexane—chloroform—diethylamine 4 : 5 : 1 (B_1), 7 : 2 : 1 (B_2), 5 : 4 : 1 (B_3), on Silufol UV 254 methanol—diethylamine 4 : 1 (*C*), on alumina (Stahl) benzene—methanol 9.9 : 0.1 (*D*), on Whatman No. 1 paper butanol—acetic acid—water 10 : 1 : 3 (S_1), ethanol—water 3 : 2 (S_2). Visualization with 254 nm u.v. light, Dragendorf reagent and potassium iodoplatinate solution.

Extraction of alkaloids

The drug (700g) was extracted in a Soxhlet apparatus with methanol (6000 ml), which was then removed and the residue dissolved in 1% acetic acid (150 ml). The solution was filtered, made alkaline with a saturated sodium carbonate solution and extracted with

ether (5 × 100 ml). The ethereal solution was purified with charcoal and evaporated to dryness. The yield of tertiary bases was 0.76 g.

The aqueous solution after extraction with ether was basified with 40% NaOH and extracted with ether to which solid citric acid was added. The precipitated citrates were filtered off and converted to chlorides with hydrochloric acid. Berberine chloride (0.13 g, m.p. 196°C; R_F 0.43 (S_2), 0.16 (C)). Further amount of berberine chloride was obtained from mother liquors together with palmatine chloride (R_F 0.38 (S_2), 0.14 (C)).

Isolation of magnoflorine and identification of jatrorrhizine

The solution after removal of the quaternary nonphenolic bases was acidified with concentrated hydrochloric acid and filtered. Potassium iodide solution (80 g KI, 100 ml H_2O) was added and the iodides of phenolic protoberberines and other quaternary bases were extracted with chloroform (6 × 500 ml). The residue remaining after the evaporation crystallized from methanol to afford magnoflorine iodide (0.68 g, m.p. 266—270°C, R_F 0.44 (A)). The mixed melting point with the authentic specimen did not show any depression.

Less pure portion of magnoflorine iodide was obtained from the mother liquor which contained, according to paper chromatography in the solvent system S_1, jatrorrhizine iodide (R_F 0.39). The mixture obtained after evaporation of the mother liquor was reduced with zinc and dilute hydrochloric acid for 1 1/2 hr at elevated temperature, alkalified with ammonia and extracted with ether. The residue after evaporation of the solvent and crystallization from methanol gave tetrahydrojatrorrhizine [8.8 mg, m.p. 203—204°C (no depression with authentic specimen), R_F 0.39 (B_3)].

The work-up and separation or tertiary bases

The mixture of tertiary bases (0.76 g) was dissolved in hydrochloric acid (1.8%, 50 ml), filtered, and made alkaline (pH 14) with 40% NaOH. The ethereal extract yielded after removal of the solvent nonphenolic bases (0.33 g).

The aqueous solution was acidified with acetic acid and then basified with ammonia. Extraction with ether gave 0.10 g of phenolic tertiary bases.

The nonphenolic portion of tertiary bases contained two substances, which were separated on an alumina (grade IV) column employing a gradient elution (benzene, benzene—methanol (1 : 1), methanol); fractions (2 ml each) 6—14 (benzene—methanol) furnished glaucine (29.6 mg, R_F 0.64 (B_2), m.p. 116°C, mixed melting point with authentic specimen 118—120°C, M^+ 355.1370, species at m/e 340 and 324), λ_{max} 242, 283, and 301 nm (log ε 4.59, 4.18, and 4.16).

Glaucine dissolved in methanol, acidified with hydrochloric acid and treated with ether furnished glaucine hydrochloride [14.4 mg, $[\alpha]_{546}^{26}$ +94° (c 0.164, ethanol)].

Fractions 15—21 (benzene—methanol) afforded an amorphous base [5.5 mg, R_F 0.78 (B_2), M^+ 353.1214, m/e 338 and 322, λ_{max} 261 and 330 nm (log ε 3.99 and 3.40)].

The mixture of phenolic bases A_2 consisted of 5 substances of R_F 0.22, 0.54, 0.63, 0.76, 0.89 (B_1). These bases could not be separated and identified because of the lack of material. None of the spots revealed an identical R_F value with specimens of berbamine or oxyacanthine.

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