Alkaloids from *Berberis julianae* SCHNEID. II.

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Dimeric benzylisoquinoline alkaloids berbamine and oxyacanthine, and quaternary bases berberine, jatrorrhizine, and magnoflorine were isolated from roots of *Berberis julianae* SCHNEID. The presence of palmatine was evidenced chromatographically.

Из корней растения *Berberis julianae* SCHNEID. были выделены дибензилизохинолиновые алкалоиды: бербамин и оксиакантин. Из квартерных алкалоидов были получены берберин, ятроррицин и магнофлорин, а хроматографически было доказано присутствие пальматина.

The genus *Berberis* L. of the *Berberidaceae* family includes about 190 species of woody plants spread in Europe, North Africa, America, and East and Central Asia. The constituents of about 150 species have not been examined as yet [1]. The principal basic constituents of this genus are alkaloids of isoquinoline type. Concerning the structure, they can be divided into seven skeletal groups of protoberberine, aporphine, bisbenzylisoquinoline, phthalido-isoquinoline, protopine, proaporphine- and aporphine-benzylisoquinoline type [2]. The last two types were mentioned in 1973, when *Shamma et al.* reported the structure of two new dimeric tertiary alkaloids, pakistanamine and pakistanine, from *Berberis baluchistanica* AHRENDT. [3].

In our previous paper [4] we described the isolation and identification of alkaloids from the aboveground part of *Berberis julianae* SCHNEID. This paper concerns alkaloids isolated from roots.

The plant material was collected in the Botanical Garden of the Faculty of Natural Sciences, Komenský University, Bratislava in January 1974. The roots were air-dried, at room temperature, ground, extracted and the isolated mixture of bases was separated into tertiary and quaternary alkaloids by the praparative method according to *Slavík* [5, 6]. The tertiary alkaloid berbamine $C_{37}H_{40}O_6N$ was obtained by column chromatography on alumina, its isomer oxyacanthine by the method described by *Orechoff* [7]. The identity of both alkaloids was proved basing upon spectral evidence (mass, infrared), optical rotation and comparison with the authentic specimen [8]. From quaternary nonphenolic alkaloids we isolated the yellow coloured berberine hydrochloride $C_{20}H_{18}O_4NCl$ the identity of which was proved on the base of infrared spectrum. The mother liquor contained mixture of berberine chloride and palmatine chloride.

After conversion into iodides the fraction of phenolic alkaloids gave the orange coloured jatrorrhizine iodide $C_{20}H_{20}O_4NI$; its identity was evidenced by the ultraviolet spectrum measured in an alkaline medium in which this alkaloid could be distinguished from its isomer columbamine [9]. The mother liquors after separation of jatrorrhizine afforded magnoflorine iodide $C_{20}H_{24}O_4NI$ whose infrared spectrum was identical with that of the authentic specimen [8].

Experimental

Melting points were measured on a Kofler micro hot-stage. The infrared spectra were recorded in KBr discs with a UR-10 (Zeiss, Jena) spectrometer, the ultraviolet spectra of methanolic solutions with a UNICAM SP-700 apparatus, and the mass spectra with an AEI MS 902 spectrometer. The optical rotation was taken with a POLAMAT S (Zeiss, Jena) polarimeter. The purity of the isolated alkaloids was checked by thin-layer chromatography on silica gel G (Merck) in solvent systems ethanol—water—ammonia 15 : 9 1 (S_1), benzene—acetone—ether—25% ammonia 4 : 6 1 : 0.3 (S_2), cyclohexane—chloroform—diethylamine 4 : 5 : 1 (S_3) and on alumina G (Merck) in solvent systems benzene—methanol 9.9 : 0.1 (S_4) and 8 : 2 (S_5). Spots on the thin layers were visualized either with Dragendorff reagent or with potassium iodoplatinate solution.

Isolation of alkaloids

The root (474 g) was extracted with methanol in a Soxhlet apparatus for 40 hrs, then the solvent was distilled off, the residue dissolved in 1% acetic acid (500 ml) and filtered. The filtrate was alkalized with a saturated sodium carbonate solution, extracted with ether (5 × 200 ml), the ethereal solution was purified with charcoal and evaporated to dryness. The yield of tertiary bases: 0.56 g.

The aqueous layer was treated with 40% sodium hydroxide and extracted with ether. Addition of solid citric acid (5 g) to the ethereal solution resulted in separation of citrates of nonphenolic quaternary bases; they were filtered off and dissolved in a warm dilute hydrochloric acid from which, after cooling, chlorides of quaternary bases were obtained (4.08 g). Crystallization from methanol afforded berberine chloride (2.60 g), m.p. 196°C, R_F 0.34 (S_1), IR: 828, 1039, 1510, 1570, 1600, and 3350 cm^{-1}. Further portion of berberine chloride and possibly palmatine chloride (0.17 g), R_F 0.34 and 0.32 (S_1), respectively, were obtained by crystallization of mother liquors.

The aqueous layer after separation of nonphenolic quaternary bases was acidified with hydrochloric acid, filtered, treated with an aqueous KI solution (80 g in 100 ml) and extracted with chloroform. The organic layer was evaporated and the residue after crystallization from methanol yielded jatrorrhizine iodide (0.66 g), m.p. 228—232°C, R_F 0.54 (S_1), λ_{max} = 436, 340, and 260 nm (log ε = 3.76, 4.46, and 4.40). Magnoflorine iodide (0.05 g), m.p. 268°C, R_F 0.43 (S_1) was obtained from the mother liquor. Its i.r. spectrum was found to be identical with that of an authentic specimen.

Separation of tertiary bases

Tertiary bases (0.56 g) were dissolved in 4% hydrochloric acid and purified with charcoal. A 40% sodium hydroxide solution was added to the filtrate and the nonphenolic tertiary bases present in solution were removed by extraction with ether. The aqueous layer was acidified with acetic acid, alkalized with ammonia, and extracted with ether. A mixture of phenolic tertiary bases (0.37 g), which was obtained after removal of the solvent, was dissolved in 5% hydrochloric acid and filtered; a saturated solution of sodium nitrate was then added to the filtrate. The aqueous layer separated from the oily portion was made alkaline, extracted with ether, evaporated, and the residue, after crystallization from methanol, furnished oxyacanthine (12.5 mg), m.p. 209—211°C, R_F 0.33 (S_3) and 0.76 (S_5), $[\alpha]_{546}^{25}$ +280° (*c* 0.1, $CHCl_3$). IR: 2800 cm^{-1} (N—CH_3), 2950 cm^{-1} (O—CH_3), 3450 cm^{-1} (OH groups). λ_{max} = 284 nm (log ε 3.84). Mass: *m/e* 608, 607, 501, 395, 385, 379, 198 (*m/2e*), 174 (100%).

The alkaline aqueous layer was extracted with chloroform and the dry residue was chromatographed over an alumina (activity grade IV) column in systems benzene, benzene—methanol 2—5% (fractions 2 ml each). The benzene—methanolic fractions *1—11* afforded berbamine (11.2 mg) which was crystallized from benzene; m.p. 145—147°C, R_F 0.41 (S_4) and 0.64 (S_2), $[\alpha]_{546}^{23}$ +100° (*c* 0.1, MeOH). IR: 2800 cm^{-1} (N—CH_3), 2950 cm^{-1} (O—CH_3), 3450 cm^{-1} (OH groups). λ_{max} = 284 nm (log ε 3.84). Mass: *m/e* 608, 607, 485, 395, 381, 379, 198 (*m/2e*, 100%), 175 (*m/2e*), 174.

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