Pakistanamine, the main alkaloid from seeds of *Berberis julianae* SCHNEID.

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Pakistanamine, a proaporphine benzylisoquinoline dimeric base, has been isolated from seeds of *Berberis julianae* SCHNEID. (*Berberidaceae*) as the principal alkaloid. Berberine and jatrorrhizine, belonging to quaternary bases, were separated from seeds in a smaller amount.

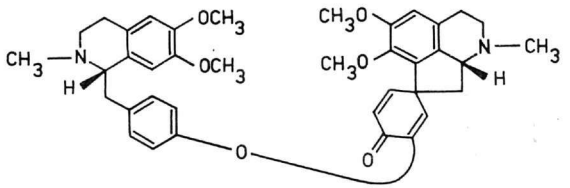
Из семян растения *Berberis julianae* SCHNEID. (*Berberidaceae*) была выделена проапорфиновая бензилизохинолиновая димерная база пакистанамин как главный алкалоид. Из квартерных алкалоидов были изолированы в малых количествах берберин и ятроррицин.

Seeds of the cultivated species *B. julianae* SCHNEID., collected in the Arboretum SAV in Mlyňany, were subjected to investigation, since alkaloids of this source have not been studied. Berberine was isolated from the fraction of quaternary nonphenolic alkaloids as hydrochloride. Its identity was confirmed on the basis of melting point, u.v. spectrum, R_f value, and by comparison with the authentic specimen [1]. A base, present in the fraction of quaternary phenolic alkaloids, afforded on reduction with zinc powder in acid medium tetrahydrojatrorrhizine [2]. Its identity was verified by comparison with authentic specimen. Tetrahydrojatrorrhizine upon oxidation with iodine furnished jatrorrhizinium iodide, the identity of which was proved on the basis of melting point, u.v. and i.r. spectra, R_f value, and by comparison with the authentic specimen [1, 3].

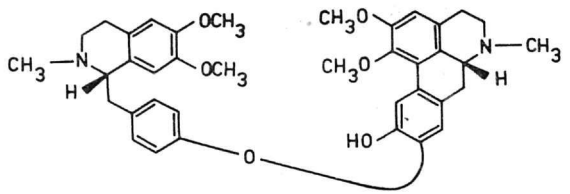
Another alkaloid was separated as picrate from the fraction of tertiary bases; it was converted into chloride by passing through an amberlite column, alkalified with ammonia and extracted with ether. The obtained amorphous base did not crystallize from organic solvents. The attempt to obtain a crystalline hydrochloride failed due to a considerable browning of the product during crystallization. The amorphous base, obtained after removal of ether, had R_f 0.44 (S_2). The i.r.

spectrum of the isolated alkaloid displayed bands at 1640 and 1668 cm^{-1} characteristic of the presence of a conjugated carbonyl group. The u.v. spectrum showed maxima indicative of alkaloids of aporphine and benzylisoquinoline structure [4]. The mass spectrum revealed a slight peak of molecular ion at *m/e* 622. The high resolution measurement of the much more abundant peak at *m/e* 620 ($M-2$) showed the molecular formula to be $C_{38}H_{40}N_2O_6$. All other species are in accordance with those reported for pakistanamine [4]. Two singlets in the ^{1}H-n.m.r. spectrum evidence the presence of two N—CH_3 groups, other four singlets the presence of four methoxyl groups in the alkaloid structure. These findings allow to assert this alkaloid to be pakistanamine (*I*) originally isolated from *Berberis baluchistanica* AHRENDT. by *Shamma* and coworkers [4].

To verify this statement pakistanamine was converted in acid medium into a more stable 1-*O*-methylpakistanine (*II*) by a dienon phenolic rearrangement. The latter displayed in its i.r. spectrum a band at 3420 cm^{-1} characteristic of the presence of a hydroxyl group, whereas bands belonging to the conjugated carbonyl group disappeared. Some maxima in the u.v. spectrum underwent a bathochromic shift in alkaline medium, this being diagnostic of the presence of a phenolic group. The u.v., i.r., and ^{1}H-n.m.r. spectra of pakistanamine were found to be identical with those of our sample.



I



II

Experimental

Melting points were measured on a Kofler hot-stage, the i.r. spectra with a Perkin—Elmer 377 spectrometer, the u.v. spectra with a Polamat Zeiss apparatus. The mass spectra were taken with an AEI MS-902 spectrometer, the ^{1}H-n.m.r. spectra with an HA 100 Varian instrument, tetramethylsilane being the internal reference substance. The development systems for thin-layer chromatography on Silica gel G (Merck) were: methanol—diethylamine 4:1 (S_1) and cyclohexane—chloroform—diethylamine 4:5:1 (S_2).

Extraction and isolation of alkaloids

Fruits were collected in March 1976 in Mlyňany; seeds were separated from the pericarp after drying at room temperature. Fats from the dry ground seeds (383 g) were removed by maceration with ether for some days and the remaining material was extracted with methanol in a Soxhlet apparatus. The residue after removal of methanol *in vacuo* (52 g) was dissolved in 1% acetic acid, the aqueous extract alkalified with sodium carbonate and extracted with ether. The ethereal extract was separated and the solvent distilled off. The residue (1.87 g) was a mixture of tertiary bases which on thin-layer chromatography on silica gel (S_2) had R_f 0.44, 0.30, 0.27, and 0.21. Alkaloid having R_f 0.44 was present in the greatest amount.

The aqueous layer was alkalified with 40% NaOH and extracted with ether; solid citric acid added to the ethereal extract caused separation of citrates, which were filtered off and converted into chlorides by dilute hydrochloric acid (1:1). Crystallization from methanol afforded berberinium chloride (15 mg), m.p. 204°C, R_f 0.27 (S_1). λ_{max}^{MeOH} 350, 266, 229 nm (log ε 4.40, 4.39, 4.40).

The aqueous layer after separation of berberine and addition of a saturated solution of potassium iodide was extracted with chloroform. Alkaloids remaining after removal of chloroform were reduced with zinc powder and hydrochloric acid at elevated temperature. The work-up with ammonia and extraction with ether furnished a mixture of tetrahydro derivatives (22 mg), which were dissolved in ether (2 ml) to which a 2% solution of KOH was added. The alkaline aqueous layer was separated, acidified with hydrochloric acid, alkalified with ammonia and extracted with ether. Tetrahydrojatrorrhizine, m.p. 202°C, R_f 0.20 (S_2), obtained after removal of the solvent and crystallization from methanol was quantitatively dissolved in methanol and oxidized with iodine to give jatrorrhizinium iodide (6 mg), m.p. 206°C, R_f 0.40 (S_1). Its u.v. and i.r. spectra accorded with those reported [1, 3].

To the mixture of tertiary bases (1.87 g) dissolved in methanol a solution of picric acid was added. Picrates thus obtained were converted into chlorides (0.47 g) by means of Amberlite IRA 402 (Cl^- form) column. The residue after removal of organic solvents showing on thin-layer chromatography the presence of one alkaloid was dissolved in dilute acetic acid, alkalified with ammonia and extracted with ether. Yield 86 mg of a light-yellow amorphous substance, R_f 0.44 (S_2), $[\alpha]_{578}^{26}$ +66° (*c* 0.33, MeOH). I.r. spectrum: 1640, 1668 cm^{-1}. λ_{max}^{MeOH} 230 (sh), 282, 310 (sh) nm (log ε 4.46, 3.98, 3.60). Mass spectrum (*m/e*):

622 (M^+, $C_{38}H_{42}N_2O_6$), 417, 416 ($C_{26}H_{26}NO_4$), 415 ($C_{26}H_{25}NO_4$), 326, 310 ($C_{19}H_{20}NO_3$), 206 (b.p., $C_{12}H_{16}NO_2$), 204 ($C_{12}H_{14}NO_2$), 192, 191, 190, 107. ^{1}H-n.m.r. spectrum (on the δ scale in p.p.m.): 2.33 and 2.51 (ss, two N—CH_3 groups), 3.53, 3.63, 3.79, 3.81 (ss, four O—CH_3 groups).

Dienone phenolic rearrangement of pakistanamine to 1-O-methylpakistanine (II)

Pakistanamine (10 mg) dissolved in 3 N-H_2SO_4 (5 ml) was heated at 70°C for 28 h, then cooled, treated with ammonia to pH 8 and extracted with chloroform. The residue after removal of solvent crystallized from ether to afford 1-*O*-methylpakistanine, m.p. 112°C, R_f 0.34 (S_2), $[\alpha]_{578}^{23}$ +86° (*c* 0.30, MeOH). I.r. spectrum: 3420 cm^{-1} (OH groups). λ_{max}^{MeOH} 225 (sh), 270 (sh), 280, 306 nm (log ε 4.55, 4.11, 4.17, 4.02). $\lambda_{max}^{MeOH-KOH}$ 230 sh, 260, 283 nm, 305 sh, 340 nm. Mass spectrum: 620 ($M-2$), 416, 311, 310, 206 (b.p.), 190.

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