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Berberis ALKALOIDS

XXXIX. NEW ALKALOIDS FROM *B. densiflora*

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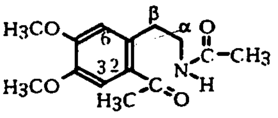
The alkaloid composition of the leaves of Berberis densiflora *has been studied. Berberine, β-allocryptopine, oxyacanthine, glaucine, thalicmidine, isocorydine, O-methylcorypalline and the new bases densinine and densiberine have been isolated. The structures of the new alkaloids have been established by a study of spectral characteristics and chemical transformations. This is the first time that any of the known alkaloids, apart from berberine, have been isolated from a plant of this species and it is the first time that β-allocryptopine and O-methylcorypalline have been isolated from the* Berberis genus.

Berberis densiflora Boiss. et Buhse (*Berberis turcomania densiflora*) (fam. Berberidceae) is a spiny bush 2-4 m tall growing in the Trancaucasus and also in the south of Turkmenistan [1] and is a good honey plant. Berberine has been extracted from the roots of this plant previously [2].

Continuing investigations of plants of the *Berberis* genus, we have studied the alkaloid composition of the leaves of *B. densiflora* gathered at the end of the vegetation phase in the Aidary gorge, south-western Kopet Dagh (Turkmenistan).

Chloroform extraction of the raw material yielded 0.15% of total bases: 0.1% of ether fraction and 0.05% of chloroform fraction. By chromatography on a column of alumina, glaucine, thalicmidine, isocorydine, oxyacanthine, O-methylcorypalline [3], and densinine (1) were isolated from the ether fraction, and berberine, β-allocryptopine [4] and densiberine in the form of the chloride (2) from the chloroform fraction.



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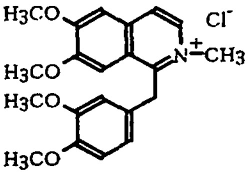

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Densinine (1), a base of nonphenolic character, crystallized from alcohol. Its UV spectrum had the maxima characteristic for substituted phenethylamines [5]. The IR spectrum showed absorption bands of active hydrogen at 3319 cm^{-1}, and of an amide carbonyl group and an aromatic ketone at 1635 and 1673 cm^{-1}, respectively [6]. The mass-spectrometric breakdown of densinine resembled that of N-acetylhomovetratrylamine, isolated from *B. oblonga* [7]. On analyzing the mass spectra of phenethylamines, Ferrigni et al. [8] established that the main direction of their breakdown under electron impact is the cleavage of the $C_\alpha - C_\beta$ and $C_\alpha - N$ bonds. Cleavage of the latter bond gives an idea of the substituent at the nitrogen atom.

In the case of densinine, the same breakdown was observed, with the formation of ions having *m/z* 194 ($C_\alpha - C_\beta$) and 206 ($C_\alpha - N$).

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In the PMR spectrum of densinine taken in $CDCl_3$, a three-proton singlet at 1.86 ppm related to an N-acetyl group, while a second singlet with an intensity of 3H at 2.56 ppm could be assigned the protons of a $CH_3-CO-Ar$ group [6]. The signals of two methylene groups appeared at 2.94 and 3.49 ppm, the first in the form of a two-proton triplet with $^3J = 6$ Hz ($H-C_\beta$) and the second in the form of two one-proton triplets with $^3J = 6$ Hz ($H-C_\alpha$) having a total integral intensity of two proton units. The appearance of the signals of the $C-\alpha$ protons in the form of two triplets can be explained by a hindered inversion of the unshared electron pair on the nitrogen. The protons of two methoxy groups appeared at 3.88 ppm in the form of a six-proton singlet. In the aromatic region of the spectrum, two one-proton singlets from *para*-located aromatic protons were observed at 6.73 and 7.14 ppm (H-6 and H-3), while the proton of an NH group gave a broad singlet at 6.66 ppm.

The facts given above permitted the assumption that compound (1) had the structure of N,2-diacetyl-4,5-dimethoxyphenethylamine. Thus, densinine (1) is a representative of a new subgroup of phenethylamine bases with a substituent in position 2.

Compound (1) is probably a biogenetic precursor of the simple isoquinoline alkaloids. An alternative route for its biogenesis is not excluded, either. This compound may be considered as a secotetrahydroquinoline, i.e., an oxidized product of the N-acetyl-1-methyltetrahydroisoquinoline alkaloids. From this point of view, (1) is a representative of a new type of isoquinoline bases of the secoisoquinoline type.

The UV spectrum of densiberberine (2) had maxima at 222, 256, and 317 nm. In the mass spectrum, the peak of the molecular ion was insignificant, while the peaks of the $(M - 1)^+$ and $(M - 16)^+$ ions with *m/z* 353 and 338, respectively, were intense. In the PMR spectrum of base (2) taken in $CDCl_3$, the signal of an N-methyl group appeared in the weak field at 4.49 ppm in the form of a three-proton broadened singlet. This showed the quaternary nature of the nitrogen atom. The spectrum also included the signals of four methoxy groups and a two-proton singlet of the methylene group of the benzyl component of the molecule. In the aromatic region of the spectrum, we observed the signals of seven aromatic protons. Two one-proton doublets in the weak field at 8.24 and 8.68 ppm with an SSCC $J = 6.0$ Hz were characteristic for the H-4 and H-3 protons of 1-benzylisoquinoline alkaloids [9].

The totality of the facts given permitted the assumption that base (2) was the N-methyl derivative of the 1-benzylisoquinoline alkaloid papaverine. This compound, in the form of the iodide, has been obtained synthetically [10, 11]. In the later paper, N-methylpapaverine chloride is mentioned but not characterized.

We have synthesized N-methylpapaverine iodide with mp 132-134°C and, by its passage through a column filled with an anion-exchanger in the Cl form, obtained N-methylpapaverine chloride, with mp 154-156°C from $CH_3OH-(CH_3)_2CO$ (1:1) and 156-157°C from C_2H_5OH. The product formed proved to be identical with the natural compound that we had isolated.

It must be mentioned that differing melting points have been given for N-methylpapaverine iodide: 134-135°C [11] and 155-157°C [12]. According to the results of our experiments, the melting point of N-methylpapaverine iodide is 132-134°C.

The reduction of (2) with $NaBH_4$ in alcohol gave a product identical with laudanosine [13].

Consequently, densiberine (2) is the new quaternary alkaloid N-methylpapaverine chloride and this is the first time that it has been found in Nature.

EXPERIMENTAL

For general observations and the isolation of the total bases, see [9].

Separation of the Total Alkaloids. Chloroform extraction of 1.07 kg of leaves yielded 1.61 g of total bases. The ether fraction (1.09 g) was chromatographed on a column of alumina in a ration of 1:30. Elution was conducted with chloroform and with mixtures of chloroform and methanol in various ratios. The chloroform eluates provided 0.01 g of glaucine, 0.16 g of thalicmidine, 0.09 g of isocorydine, and 0.02 g of O-methylcorypalline. From the fractions eluted by chloroform–methanol (98:2) we obtained oxyacanthine (0.06 g) and densinine (0.02 g).

Densinine (1) — $C_{14}H_{19}NO_4$, mp 128-129°C (EtOH).

UV spectrum (EtOH, λ_{max}, nm): 231, 274, 310.

IR spectrum (KBr, ν, cm^{-1}): 3319, 1673, 1635, 1451, 1300.

Mass spectrum (EI, 70 eV; m/z; I_{rel}, %): 265 (M^+, 40), 206 (100), 194 (90), 192 (70), 179 (90), 164 (50), 151 (40), 149 (40).

The chloroform fraction (0.52 g) was separated on a column with 15 g of silica gel. Elution with chloroform–methanol (96:4) permitted the isolation of 0.01 g of berberine in the form of the chloride, 0.02 g of β-allocryptopine, and 0.04 g of densiberine in the form of the chloride.

Densiberine Chloride (2) — $C_{21}H_{24}N^+O_4$ [sic], mp 156-157°C (EtOH).

UV spectrum (EtOH, λ_{max}, nm): 222, 256, 317.

Mass spectrum (EI, 70 eV, m/z; I_{rel}, %): 354 (M^+, 8), 353 ($[M - 1]^+$, 50), 340 (15), 339 (75), 338 ($[M - 16]^+$, 100), 325 (25), 324 (90), 151 (60), 142 (50).

PMR (100 MHz; $CDCl_3$, δ, ppm, J, Hz, 0–HMDS): 3.74, 3.79, 3.96, 4.10 (each 3H, s, $4OCH_3$), 4.49 (3H, br.s, N^+-CH_3), 5.05 (2H, s, CH_2), 6.19 (1H, dd, J=9.0, 2.0, H-6′), 6.65 (1H, d, J=9.0, H-5′), 6.95 (1H, d, J=2.0, H-2′), 7.46, 7.61 (each 1H, s, H-5, H-8), 8.24, 8.68 (each 1H, d, J=6.0, H-4, H-3).

Preparation of N-Methylpapaverine Iodide. A mixture of 0.65 g of papaverine, 4 ml of MeOH, and 2 ml of CH_3I was boiled in the water bath for 1.5 h. Evaporation under vacuum left a residue (0.85 g) which was crystallized from methanol–acetone (1:1). This gave 0.63 g of crystalline N-methylpapaverine iodide with mp 132-134°C.

Preparation of N-Methylpapaverine Chloride. A solution of 0.5 g of N-methylpapaverine iodide in 10 ml of methanol was passed through a column filled with 2 g of IRA-400 anion-exchanger (Cl form), prepared by the method of [14]. This gave 0.45 g of N-methylpapaverine chloride (yield ~90%), mp 154-156°C ($MeOH-(Me)_2CO$, 1:1); 156-157°C (EtOH). The product obtained was identical with (**2**) according to a mixed melting point and also to TLC (chloroform–methanol (9:1) system, SiO_2).

Preparation of Laudanosine. A solution of 35 mg of (**2**) in 2 ml of alcohol was treated with 0.1 g of $NaBH_4$ and the reaction mixture was boiled for 1 h. After evaporation of the alcohol, the residue was dissolved in water and extracted with ether. The ethereal extract was evaporated, and the residue was crystallized from alcohol. A product with mp 89-90°C, identical with laudanosine (mixed melting point and TLC in the $C_6H_6-C_2H_5OH$ (9:1) system in a layer of Al_2O_3) was obtained.

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