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ALKALOIDS AND FLAVONOIDS OF *Oxytropis muricata*

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From the epigeal part of Oxytropis muricata *growing on Mongolian territory we have isolated the new alkaloid muricatide, the known alkaloids N-benzoyl-2-hydroxyphenethylamine and N-nicotinoyl-2-hydroxyphenethylamine, and the flavonoids robinin and kaempferol. On the basis of spectral characteristics, the structure of muricatide has been established as N-benzoyl-2-acetoxyphenethylamine, and this has been confirmed by partial synthesis. Muricatide was known previously as a synthetic compound but this is the first time that it has been found in Nature.*

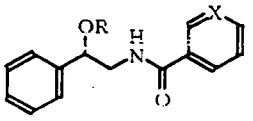
Oxytropis muricata (Pall.) DC is a perennial herbaceous plant of the Fabaceae family that is widely distributed in the territory of Mongolia and Eastern Siberia. It is used in folk medicine, particularly in Tibet, where it is called dag-shar-nag-bo [1]. It is employed in the individual form and in multicomponent mixtures as a wound-healing, diuretic, and cardiac agent and also in cancerous diseases and infections of the liver.

The plant has been little studied in the chemical respect [1, 2]. In a search for active principles we have continued the study of the alkaloids of the epigeal part of *O. muricata* growing in Mongolia [3]. We used methanol and chloroform for treating the raw material, the extraction of the alkaloids being performed both with and without the use of an acid. Individual bases were isolated by column chromatography and by their solubility in organic solvents. As a result, in addition to the bases that we had detected previously — N-benzoyl-2-hydroxyphenethylamine (**1**) and N-nicotinoyl-2-hydroxyphenethylamine (**2**) [3] — we isolated a new alkaloid which has been called muricatide (**3**),

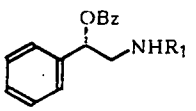
Muricatide (**3**) has mp 114-115°C. Its UV spectrum contains an absorption maximum at 226 nm, characteristic for aromatic substances. In the IR spectrum we observed absorption bands of ester and amide carbonyls (1732 and 1642 cm^{-1}, respectively). The mass spectrum contained a weak peak of the molecular ion with *m/z* 283, the maximum peak being that of a benzoyl cation with *m/z* 105. The presence of an acetyl group in the (**3**) molecule was shown by the peak of an ion with *m/z* 240 $(M - 43)^+$ and was confirmed by the ^1H NMR spectrum of the alkaloid, in which there was a signal of the protons of an acetyl group at δ 2.02 ppm (3H, s). Furthermore, in the spectrum we observed signals of methylene protons at 3.78 ppm (2H, t, J = 6.5 Hz), of a methine proton, geminal for the acetyl group at 5.92 ppm (1H, t, J = 6.5 Hz), of a NH group at 6.42 ppm (1H, br. s), and of 10 aromatic protons in the 7.10-7.50 ppm interval (8H, m) and at 7.65 ppm (2H, dd, J_o = 7 Hz, J_m = 3 Hz)

The facts given showed that muricatide was an N,O-diacyl derivative of phenethylamine containing, as the acyl radicals, benzoic and acetic acid residues and that two structures for it were possible — i.e., muricatide is the acetyl derivative either of (**1**) or of trichophydine (**5**), which we had earlier isolated from *O. trichophysa* [4].

As a result of the acetylation of these alkaloids with acetic anhydride in pyridine, it was found that compound (**3**) was identical (TLC) with the O-acetyl derivative of (**1**), which was obtained in the individual crystalline form and proved to be identical with muricatide according to a mixed melting point and IR spectra.



1. R=H, X=CH
2. R=H, X=N
3. R=Ac, X=CH



4. R_1=Ac
5. R_1=H

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Consequently, muricatide has the structure of N-benzoyl-2-acetoxyphenethylamine. This substance was known as a synthetic product [5], but this is the first time it has been found in Nature.

Earlier, on the basis of an analysis of literature information and our own experimental results, we proposed a probable scheme of the biosynthesis of the *Oxytropis* alkaloids from phenylalanine [6, 7] according to which the formation of muricatide can be represented as the result of the acetylation of (**1**).

In addition to alkaloids, we have isolated from this plant the known flavonoids kaempferol and robinin, identified by comparison with authentic specimens in terms of mixed melting points and IR spectra [8]. The native natures of kaempferol and the alkaloid (**1**) were confirmed by a supplementary experiment.

It is known that robinin possesses a diuretic and hypoazotemic action and, under the name of flaronin, has been approved by the [Uzbekistan] Ministry of Health (1985, reg. No. 85/1520/5) for use in chronic and acute renal insufficiency [9]. Thus one of the active principles of *O. muricata* responsible for its diuretic properties is robinin.

EXPERIMENTAL

General Observations. UV, IR, PMR, and mass spectra were taken, respectively, on the following instruments: UV/VIS Spectrometer Lambda-16, Perkin-Elmer System 2000 FT-IR, Tesla BS-567 A (100 MHz) in $CDCl_3$ (0–HMDS), and MKh 1310 with a system for the direct injection of the sample into the ion source.

For column chromatography we used type KSK silica gel, and for thin-layer chromatography the same silica gel with the addition of 5% of gypsum in the benzene–methanol (4:1) solvent system with iodine vapor as the revealing agent.

Isolation and Separation of the Substances. The air-dry epigeal part (860 g) was moistened with 8% ammonia solution and was exhaustively extracted with chloroform. The concentrated chloroform solution (A) was treated with 10% aqueous sulfuric acid. The robinin that precipitated at the boundary of the two layers was separated off and was twice crystallized from water. This gave 0.1 g of robinin, with mp 198-199°C. With cooling, the acid solution was made alkaline by the addition of concentrated ammonia and was extracted successively with ether (0.5 g) and chloroform (0.12 g). The mixture of alkaloids obtained amounted to 0.07% of the weight of the dry raw material. The ether fraction was chromatographed on a column of silica gel (1:100), using gradient elution. Ethereal eluates, with supplementary crystallization from ether, yielded (**1**) (50 mg), mp 151-152°C. The mother solution from (**1**) was rechromatographed on a column of silica gel (1:100) and was eluted with ether–hexane (3:7). Technical muricatide (7 mg) was isolated from the first eluates, and this was crystallized from ether–hexane to give 5 mg of pure muricatide. The chloroform solution (A), after treatment with acid, was washed with water, dried, and concentrated. The dry residue was treated successively with ether, chloroform, and ethyl acetate. The ethyl acetate fraction was separated by chromatography on a column of silica gel (1:60). Chloroform–methanol eluates yielded 10 mg of kaempferol with mp 276-278°C (from acetone).

Muricatide (3), mp 114-115°C (from hexane–ether).

UV spectrum (ethanol, λ_{max}, nm): 208 infl., 226.

IR spectrum (ν_{max}, cm^{-1}): 3065, 2982, 1732, 1642, 1579, 1533, 1488, 1453, 1421, 1367, 1313, 1245, 1050, 949, 905, 857, 723, 695.

Mass spectrum, m/z (%): 283 (M^+, 9), 240 (8), 177 (17), 164 (13), 135 (50), 134 (30), 117 (25), 105 (100), 77 (25).

^1H NMR spectrum: δ 2.02 (3H, s, $-COCH_3$), 3.78 (2H, t, J=6.5 Hz, 1-H_2), 5.92 (1H, t, J=6.5 Hz, 2-H), 6.42 (1H, br.s, –NH–), 7.10-7.50 (8H, m, Ar-H), 7.65 ppm (2H, t, J = 7 Hz, J_m = 3 Hz, Ar-H).

Synthesis of Muricatide (3). A solution of 50 mg of N-benzoyl-2-hydroxyphenethylamine in 3 drops of pyridine was treated with 0.5 ml of acetic anhydride. The mixture was left for two days and was then evaporated under vacuum. The dry residue was treated with hexane–ether (7:3), and, after concentration, the mixture was left in the cold. The crystals that deposited were separated off and recrystallized from hexane–ether (7:3). This gave 30 mg of muricatide in the form of colorless needles with mp 114-115°C. A mixture with an authentic specimen gave no depression of the melting point, and their IR spectra were identical.

Check of the Native Natures of (1) and Kaempferol. The raw material (200 g) was extracted with methanol. The dry extract was treated successively with ether, chloroform, and ethyl acetate. When the ethereal extract was concentrated, a precipitate deposited the crystallization of which from acetone–ether led to (**1**) (10 mg). The ethyl acetate fraction of the extract was separated by the method described above and yielded 3 mg of kaempferol.

REFERENCES

1. K. F. Blinova and E. I. Sakanyan, Rast. Resurs., **22**, No. 2, 266 (1986).
2. Z. N. Duboshina and N. F. Proskurnina, Zh. Obshch. Khim., **33**, 2071 (1963).
3. D. Batsurén, S. Tsétségmaa, N. Batbayar, D. Dungérdorzh, V. I. Akhmedzhanova, Yu. M. Mil'grom, Ya. V. Rashkes, and A. A. Ibragimov, Khim. Prir. Soedin., 388 (1992).
4. V. I. Akhmedzhanova, Khim. Prir. Soedin., 414 (1994);
5. S. Huneck, J. D. Connolly, and T. Khaidov, Fitoterapia, **57**, 423 (1986).
6. V. I. Akhmedzhanova, D. Batsurén, and R. Sh. Shakirov, Khim. Prir. Soedin., 873 (1993).
7. V. I. Akhmedzhanova, Khim. Prir. Soedin., 212 (1996).
8. V. I. Akhmedzhanova, Khim. Prir. Soedin., 638 (1986).
9. V. E. Sokolov and L. N. Lyubartseva, Vopr. Med. Khim., 379 (1979); Instruction on the Use of Flaronin, approved by the [Uzbekistan] Ministry of Health, November 26, 1985.