Chemistry of Natural Compounds, Vol. 32, No. 2, 1996

Oxytropis ALKALOIDS. IV. STRUCTURE, SYNTHESIS, AND POSSIBLE ROUTES OF BIOSYNTHESIS

V. I. Akhmedzhanova

UDC 547.944/945

The results of an investigation of new alkaloids from plants of the Oxytropis *genus are generalized, the most common properties of alkaloids of the 2(β)-phenylethylamine series and possible routes for their biosynthetic formation are discussed, and material is presented on the structural study of the new alkaloid trichophysine and on the synthesis of (±)-oxytryphine and of trichophysine.*

The genus *Oxytropis* (fam. Fabaceae) has attracted attention thanks to the wide use of individual representatives of it in Tibetan medicine [1], the unusal narcotic type of action of one of them, and the small degree to which the plants have been studied in the chemical respect, particularly for their alkaloid content [2].

Since 1991, we have isolated from plants of this genus three known and five new bases belonging to three different classes: the 2(β)-ethylamines, the 2-oxazolines, and the indoles. The 2-oxazolidine derivatives (**1**) represent a new class of plant alkaloids [3]. Representatives of the indole series have been detected in plants of the Fabaceae family for the first time [4]. A peculiar alkaloid of the 2-phenylethylamine series with a nicotinoyl acyl fragment (**2**) has been found [2].

The plant *O. trichophysa* has proved to be richest in its variety of substances. Another new alkaloid, trichophysine (**3**), has been obtained from its epigeal part.

The UV spectrum of (**3**) is typical for alkaloids of the 2-phenylethylamine series, and its PMR spectrum [δ (ppm) 3.94 (t, 2H, J = 6.5 Hz, 2 × H-1); 6.20 (t, J = 6.5 Hz, H-2); 6.69 (br.s., 1H, NH); 7.38 (m, 11H, Ar-H); 8.05 (m, 4H, Ar-H)] differs from that of N-benzoyl-2-hydroxy-2-phenylethylamine by the fact that in the region of resonance of aromatic protons there are the signals not of 10 but of 15 protons, and the H-2 signal is shifted downfield by more than 1 ppm, which is characteristic for a proton geminal to an acylated secondary hydroxy group [5]. These facts, and also the presence in the IR spectrum of trichophysine of the absorption band of an ester carbonyl (114 cm^{-1}) permitted the assumption that it was the O-benzoyl derivative of (**4**). In actual fact, the latter has been detected in an alkaline hydrolysate of (**3**).

Thus, trichophysine has the structure (**3**) (Scheme 1), which was confirned by its formation from (**4**) by benzoylation.

Of the nine alkaloids isolated from plants of the *Oxytropis* genus, seven belong to the 2(β)-phenylethylamine series. A study of the structures and properties of these compounds and their comparison with those of other representatives known in the literature has revealed the most general laws characteristic for this series of alkaloids. They are all N-, O-, or N,O-acyl derivatives of 2-hydroxy-2-phenylethylamine a common property of which is their capacity for being hydrolyzed with the formation of an amine or an amide and an acid. They have also been synthesized independently from these components [6, 7].

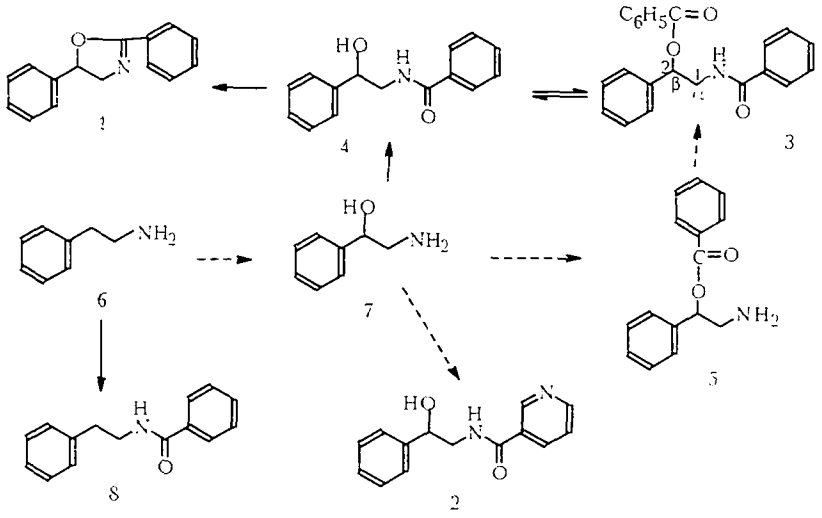
As typical amides, the N-acyl derivatives, possess no basicity and do not give the reaction for alkaloids with the Dragendorff reagent, except for (**2**), which is due to the presence of a pyridine ring in its molecule. With tungstosilicic acid they give a turbidity only if the initial treatment of the alkaloids was carried out not with dilute aqueous solutions of mineral acids, as is usual [8], but with stronger (~60%) acids.

The UV spectra of alkaloids of the 2-phenylethylamine series have an absorption maximum in the 200-215 nm interval and a maximum or shoulder in the 220-235 nm region, which undergoes a bathochromic shift in the spectrum of (**2**) [2]. The above-mentioned groups of derivatives can be distinguished by the presence or absence in their IR spectra of the absorption bands of ester, amide, and carbonyl groups and of active hydrogen. Features of the mass-spectrometric behavior of the N-acyl compounds of this series have been studied [2].

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 89 14 75. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 212-216, March-April, 1996. Original article submitted January 16, 1995.

0009-3130/96/3202-0187$15.00

In the PMR spectra of the N-acylated alkaloids, the protons of an unsubstituted ethylamine fragment are revealed in the form of a two-proton triplet (δ 2.60-3.00 ppm, Ph−C<u>H</u>$_2$−) and a two-proton quartet (3.30-3.80 ppm, −C<u>H</u>$_2$−NH−), owing to additional splitting of the triplet signal on the proton attached to nitrogen, with identical spin-spin coupling constants (A_2M_2X spin system) [2, 9]. As a result of deuterium exchange, the NH signal disappears from the spectrum and the quartet is converted into a triplet. The introduction of a hydroxy group into the C-2 position leads to a downfield shift of the signal of the geminal benzyl proton (δ 4.70-5.00 ppm), which appears in the spectrum in the form of a doublet of doublets because of the nonidentity of the neighboring methylene protons that has arisen. These methylene protons give two signals in the spectrum in the δ 3.30-4.00 ppm region with three spin-spin coupling constants (ddd) as the result of one geminal and two vicinal splittings (ABMX system) [2, 10].



Scheme 1. Possible routes in the biosynthesis of *Oxytropis* alkaloids (the full arrows show transitions achieved *in vitro*).

As was to be expected [5], acylation of the hydroxy group leads to a further downfield shift of the signal of the benzyl proton, which, in the spectra of the alkaloids of this series, is found in the δ 5.90-6.30 ppm interval. The fine structure of the signals that is observed in the spectra of O-acylated derivatives [11] is then close to that described above for the hydroxy-substituted compounds. Thus, the benzyl proton resonates in the form of a doublet of doublets, while the methylene protons give a multiplet. In the spectra of the N,O-acylated alkaloids [10], however (see also the PMR spectrum of trichophysine) the signals of these protons appear in the form of one- and two-proton triplets (AM_2X system with identical constants of vicinal spin-spin interactions) which shows the equivalence of the protons of the methylene group. The differences noted in the resonance of the methylene protons on passing from one type of derivative to another are apparently connected with stereochemical features of these groups of compounds.

As is known, the signals of the protons of a benzyl radical that are present in the *ortho* positions to a carbonyl group resonate in a weaker field. However, only in the spectra of N- and O-benzyl derivatives [2, 11] are they clearly separated from the signals of the other aromatic protons and are located in the δ 7.70-8.20 ppm region. The presence and the fine structure of these signals enable us to judge the nature of the substitution in this fragment.

The above-described laws of the PMR-spectroscopic behavior of alkaloids of the 2(β)-phenylethylamine series that have been found have diagnostic value in determining their structures.

The formation of oxytryphine (**1**) in the plant *O. trichophysa* during the period of the maximum accumulation of N-benzoyl-2-hydroxy-2-phenylethylamine (**4**), which is the main alkaloid quantitatively, and also an analysis of the literature [12], has enabled us to put forward a possible scheme of the successive biosynthesis of these two alkaloids from phenylalanine [3]. The other alkaloids isolated (**2**, **3**, **5**, and **8**) fall satisfactorily within the scheme proposed. Their formation can be represented as the result of the acylation of the same intermediates (**6**) and (**7**) (see Scheme 1). The transitions achieved *in vitro* (synthesis of (**4**) and (**8**) in [7] and [13], respectively) are evidence in favor of this biosynthetic scheme.

EXPERIMENTAL

UV spectra were taken on a UV/VIS Lambda-16 spectrometer, IR spectra on a Perkin-Elmer System 2000 FT-IR, PMR spectra on a Tesla BS-567 A (100 MHz) in $CDCl_3$ (0 — HMDS), and mass specta on a MKh 1310 with a system for direct injection into the ion source.

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

Isolation of Trichophysine (3). The air-dry epigeal part of the plant *O. trichophysa* (1 kg), gathered in Mongolia by D. Batsurén in the vegetation period, was wetted with an 8% solution of ammonia and was extracted with chloroform. After evaporation of the solvent the chloroform-extracted material was treated with hexane. The hexane extract was chromatographed on a column of silica gel (1:100) with elution by benzene–methanol (4:1). The eluates containing a substance with R_f 0.67 (revealed by iodine vapor) were combined and twice recrystallized from hexane–ether (7:3). This gave 20 mg of trichophysine, mp 100-103°C.

UV spectrum (ethanol, λ_{max}, nm): 201, 229.

IR spectrum (ν_{max}, cm^{-1}): 3346 (NH), 1714 (OCO), 1641 (NCO).

Mass spectrum, *m/z* (%): 240 (M-C_6H_5CO, 3), 223 (M-C_6H_5COOH, 2), 135 (93), 134 (90), 105 (100), 77 (89).

Saponification of Trichophysine. A mixture of 10 mg of trichophysine and 3 ml of a 5% alcoholic solution of KOH was boiled under reflux for 15 min. The solvent was evaporated off, the residue was treated with chloroform, and the resulting extract on TLC in various solvent systems showed a spot identical in terms of R_f with the spot of the marker N-benzoyl-2-hydroxy-2-phenylethylamine (revealed by iodine vapor).

Synthesis of Trichophysine. Benzoyl chloride (4 drops) was added to 50 mg of N-benzoyl-2-hydroxy-2-phenylethylamine (**4**) in 1 ml of pyridine, and the mixture was left at room temperature for 48 h. After the solvent had been eliminated, the dry residue was treated with 4% aqueous alkali and filtered off. The residue was washed with water, dried in the air, and treated with a mixture of ether and hexane (7:3). This yielded 10 mg of trichophysine, the melting point and IR and PMR spectra of which coincided with those of the natural compound.

Synthesis of Oxytryphine (1). N-Benzoyl-2-hydroxy-2-phenylethylamine (50 mg) was treated with conc. sulfuric acid (2 ml), and the mixture was left at room temperature for 10 min. Then it was poured into a cooled saturated solution of $NaHCO_3$ (2 ml). With cooling, dry $NaHCO_3$ was added to give a weakly alkaline reaction and the mixture was extracted with chloroform. Distillation of the solvent left 40 mg of the racemate of oxytryphine (oily substance, $[\alpha]_D \pm 0$) identical with the natural product in all its parameters except for its specific rotation [3]. Yield 81%.

REFERENCES

1. K. F. Blinova and E. I. Sakanyan, Rast. Res., **22**, 266 (1986).
2. 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).
3. V. I. Akhmedzhanova, D. Batsurén, and R. Sh. Shakirov, Khim. Prir. Soedin., 873 (1993).
4. V. I. Akhmedzhanova, M. A. Moldagulov, and R. Sh. Shakirov, Khim. Prir. Soedin., 90 (1993).
5. R. Silverstein, G. Bassler, and T. Morrill, The Spectromeric Identification of Organic Compounds, 3rd edn, Wiley, New York (1974) [Russian translation, Mir, Moscow (1977), p. 307].
6. S. M. Albonico, A. M. Kuck, and V. Deulofeu, J. Chem. Soc. (*C*), No. 13, 1327 (1967).
7. Z. N. Duboshina and N. F. Proskurnina, Zh. Obshch. Khim., **33**, 2071 (1963).
8. G. V. Lazur'evskii, I. V. Terent'eva, and A. A. Shamshurin, Practical Work in the Chemistry of Natural Compounds [in Russian] Vyssha Shkola, Moscow (1966), p. 319.
9. A. Karimov, M. G. Levkovich, N. D. Abdullaev, and R. Shakirov, Khim. Prir. Soedin., 424 (1993).
10. S. Huneck, J. D. Connolly, and T. Khaidov, Fitoterapia, **57**, No. 6, 423 (1986).
11. V. I. Akhmedzhanova, Khim. Prir. Soedin., 414 (1994).
12. R. H. F. Manske, The Alkaloids, Academic Press, New York, Vol. 3 (1953), p. 315.
13. Dictionary of Organic Compounds, Eyre and Spottiswoode, London, Vol. 1 (1953), p. 92.