ALKALOIDS OF Oxytropis. I

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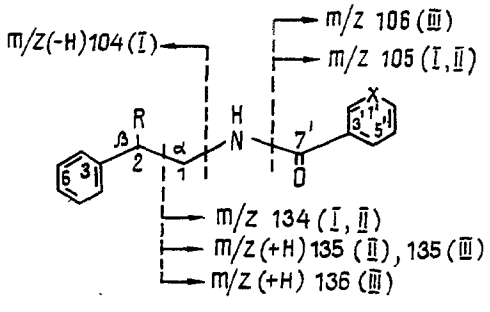
From the epigeal parts of two species of Oxytropis growing in Mongolia we have isolated the new alkaloids (+)-N-benzoyl-2-hydroxy-2-phenylethylamine and (−)-N-nicotinoyl-2-hydroxy-2-phenylethylamine, and also N-benzoyl-2-phenylethylamine, this being the first time this substance has been found in nature. The structures of the alkaloids were established on the basis of a comparative analysis of their spectral characteristics. Features of the mass-spectrometric behavior of this compound have been studied.

The oxytropis (crazyweed) genus Oxytropis DC., numbering 320 species, is one of the most widespread genera of the family Fabaceae [1]. A considerable number of species of this genus is widely used in Tibetan and the Mongolian medicines and is also employed in the folk medicines of Siberia, central Asia, and Kazakhstan as effective drugs [2, 3]. However, there are no representatives of Oxytropis in the arsenal of modern drugs which, in the opinion of Blinova et al. [2, 3] is connected with an inadequate study of the genus in the chemical aspect.

The majority of publications on oxytropis alkaloids are of indicative nature and are limited to establishing their presence either by a qualitative test or by a quantitative test with the isolation of the total alkaloids. Depending on the species, the amount of alkaloids ranges from 0.07 to 0.82% of the weight of the dry plant [3, 4].

The alkaloids of only two species – O. muricata (Pall.) DC. [5] and O. pseudoglandulosa Gontsch. ex Grub. [6] – have been subjected to a more profound study. In total, four bases belonging to the group of 2(β)-phenylethylamine and being derivatives of the latter and of the aromatic acids benzoic and cinnamic were isolated from the epigeal parts of these two plants.

We have studied the alkaloid compositions of the epigeal parts of two species of the genus Oxytropis growing in the Mongolian Peoples' Republic; O. muricata (Pall.) DC and O. trichophysa Bunge. We used ethanol to extract the raw material. The alkaloids were isolated both with the use of an acid and without it. From the plant O. trichophysa we isolated alkaloids (I) and (II), and from the epigeal part of O. muricata (II) and (III).



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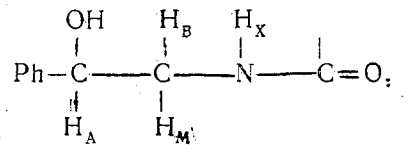
Alkaloid (I) had mp 117-118°C S(from acetone). The UV spectrum of (I) [$\lambda_{max}^{CH_3OH}$ (nm): 208, 226 (shoulder)] was characteristic for substances containing an aromatic ring [7], and its IR spectrum showed the absorption bands of a NHCO group at 3390 and 1650 cm^{-1}. The mass spectrum of (I) showed the peaks of ions with m/z 225 (M^+), 134, 105, 104, 91, and 77. The formation of the ions with m/z 105 and 77 showed the presence of a benzoyl residue in the (I) molecule and that of the ions with m/z 104 and 91 the possible presence of a phenethyl radical in it, which, in the light of the IR spectrum and an ion with m/z 134 (C_8H_8NO), was linked to the benzoyl group through a NH group.

This hypothesis was completely confirmed by the ^1H NMR spectrum of the alkaloid (500 MHz, DMSO-d_6, ppm): δ 2.84 (t, 2H, J = 6.5 Hz; C_6H_5–CH_2–); 3.48 (m, 2H; –CH_2–NH–); 7.12 (t, 1H, J_{ortho} = 7.5 Hz; H-6); 7.24 (d, 2H, J_{ortho} = 7.5 Hz; H-4; H-8); 7.29 (t, 2H, J_{ortho} = 7.5 Hz; H-5, H-7); 7.44 (t, 2H, J_{ortho} = 7.5 Hz; H-1', H-5'); 7.51 (t, 1H, J_{ortho} = 7.5 Hz; H-6'); 7.81 (d, 2H, J_{ortho} = 7.5 Hz; H-2', H-4'); 8.55 (br. s, 1H; NH).

Thus, (I) was N-benzoyl-2-phenylethylamine. It was known previously as a synthetic product [8], but this is the first time that it has been found in a plant.

Compound (II) had mp 154-155°C (from acetone) and was optically active $[\alpha]_D^{30}$ + 35.2° (c 0.68; methanol). Its IR spectrum contained the absorption bands of active hydrogen (3350 cm^{-1}), and of an amide carbonyl group (1650 cm^{-1}), while its UV spectrum [$\lambda_{max}^{CH_3OH}$ (nm): 207, 224 (shoulder)] almost coincided with that of (I).

The ^1H NMR spectrum of (II) differed from that of (I) only by the fact that in place of the pattern typical for Ph–CH_2–CH_2–NH–C(=O)–, grouping it contained the signals characteristic for the protons of the structure



They appeared in the spectrum in the form of an ABMX system [9] at (ppm) δ 3.51 (ddd., 1H, J_{BX} = 5.0 Hz, J_{BA} = 8.0 Hz,; J_{BM} = 14 Hz; H_B); 3.91 (ddd, 1H, J_{MA} = 3.5 Hz; J_{MX} = 7.0 Hz, J_{MB} = 14 Hz; H_M; 4.95 (dd, 1H, J_{AM} = 3.5 Hz; J_{AB} = 8.0 Hz; H_A).

These facts, together with the difference of 16 m.u. in the masses of the molecular ions of (I) and (II) showed that a hydroxy group was present in the (II) molecule at C-2. Consequently, (II) had the structure of (+)-N-benzoyl-2-hydroxy-phenylethylamine, agreeing well with the mass-spectrometric fragmentation of the alkaloid (see below). The racemate of (II) has been obtained previously from the epigeal part of the plant O. muricata growing in Eastern Siberia and its optical antipode from O. pseudoglandulosa growing in Mongolia [6]. The spectral characteristics and physicochemical constants of (II), except for $[\alpha]_D$, agreed with the corresponding characteristics of the antipode given in [6].

Alkaloid (III) – an optically active compound, just like (II) – had mp 157-158°C (from acetone), $[\alpha]_D^{30}$ – 25.3° (c 0.79; methanol). Its UV and IR spectra were similar to those of (I) and (II). Its mass spectrum had a low-intensity peak of the molecular ion with m/z 242, composition $C_{14}H_{14}N_2O_2$ (HRMS) and intense peaks of ions with m/z 136, 135, 107, 106, 79, and 78.

The close analogies between the mass spectra of (II) and (III) with shifts in the majority of peaks in the latter by 1 m.u. in the direction of high masses as compared with that of (III), and the presence of an additional nitrogen atom in the composition of these ions (for example, with m/z 106, C_6H_4NO) indicated that the benzene ring in the acyl part of the (II0 had been replaced by a heteroaromatic ring in (III). Its structure, and also a confirmation of the proposed structure of the whole molecule followed from a combined analysis of the ^1H NMR spectra of (II) and (III).

In the spectrum (III) (500 MHz, DMSO-d_6), as in the spectrum of (II) there were the signals of the protons of a pH–CH(OH)–CH_2–NH–grouping at (ppm) δ 3.30 (m, 1H, H–C–H): 3.48 (m, 1H; H–C–H); 4.77 (m, 1H; H–C–OH); 5.49 (d, 1H; J = 2.5 Hz; OH) and 8.67 (t, 1H; J = 5.0 Hz; NH). However, the spectra of these compounds differed in the region of resonance

of aromatic protons. The signals of the proteins of the phenyl radical present in the spectrum of (II) appeared in the spectrum of (III) at (ppm) δ 7.22 (t, 1H, J_{ortho} = 7.0 Hz; H-6); 7.31 (t, 2H, J_{ortho} = 7.0 Hz, H-5; H-7); 7.35 (d, 2H, J_{ortho} = 7.0 Hz; H-4, H-8). So far as concerns the signals of the benzoyl residue, they were absent from the spectrum of (III). In place of them, four one-proton signals were detected at (ppm) δ 7.45 (dd, 1H, J'_{ortho} = 5.0 (Hz, J''_{ortho} = 8.0 Hz; H-5'); 8.13 (dt, 1H, J''_{ortho} = 8.0 Hz; J_{meta} = 1.3 Hz; H-4'); 8.65 (dd, 1H, J'_{ortho} = 5.0 Hz; J_{meta} = 1.3 Hz; H-6'); 8.95 (d, 1H, J_{meta} = 1.3 Hz; H-2'), the chemical shifts and multiplicites of which were characteristic of the protons of a 3-substituted pyridine ring [10].

Consequently, (III) differed from (II) by the fact that its acyl moiety consisted not of a benzoic acid but of a nicotinic acid residue. From this followed the structure of (−)-N-nicotinoyl-2-hydroxy-2-phenylethylamine, which was confirmed by the presence in the mass spectrum of (III) of intense peaks of nicotinoyl and pyridine ions with m/z 106 and 79, respectively.

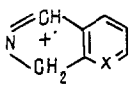
The alkaloids (II) and (III) were new, while we are the first to have detected (I) in nature. It is interesting to note that alntough they all belong to the alkamides of the 2(β)-phenylethylamine group, their properties differ greatly. Compounds (I) and (II) possess the properties of typical amides. They are insoluble in dilute acids and do not give the reactions for alkaloids with tungstosilicic acid and the Dragendorff reagent. At the same time, (III), thanks to the presence of the pyridine ring, exhibits the properties of typical bases and gives positive reactions with the reagents mentioned.

The importance of the mass-spectrometric information in the structural investigations of alkamides of the 2(β)-phenylethylamine group impelled us to make a more detailed study of the behavior of compounds (I-III) under electron impact. The features of the mass-spectrometric fragmentation of compounds (I-III) were determined by the nature of the acyl residue, i.e., by the presence of a benzamide (I, II) or a nicotinamide (III) grouping, and also by the presence of a hydroxy group at the β-carbon atoms of the phenethyl group (II), (III). The latter circumstance led to an instability of the molecular ions of (II) and (III) in view of the high tendency to cleavage of the $CH(OH)-CH_2$ bond. In addition to the simple cleavage of this bond, a rearrangement took place with the migration of the H atom of the OH group to the α-carbon atom, which gave peaks of maximum intensity with m/z 135 (II) and 136 (III). The origin of these ions was confirmed by accurate measurement of their masses and by metastable defocusing spectra. Some of the products of the breakdown of the M^+ ions of compounds (II) and (III) were stabilized in the form of the $(M - H_2O)^+$ ions with m/z 223 and 224, respectively.

The M^+ ion of compound (I) was the most stable. The ion with m/z 134 (C_8H_8NO) in its spectrum had a medium intensity, no rearrangement ion with m/z 135 was oberved, and the maximum peak was that of the benzoyl cation with m/z 105. In the spectrum of compound (II) the peak of this ion was the second in intensity, while in the spectrum of (III) the nicotinoyl cation produced analogously, with m/z 106, was of medium height. Another indication of the presence of a heterocyclic ring was the peak of a pyridine ion, C_5H_5N (m/z 79), the height of which exceeded that of the phenyl cation with m/z 77.

So far as concerns the fragments formed from the phenethyl part of the molecule, the most characteristic was the C_8H_8 ion with m/z 104 appearing in the spectrum of compound (I). The intensity of the tropylium ion with m/z 91 considerably exceeded the intensity of this ion. This is explained by the stability of the fragment − a benzamide molecule − on the formation of the ion with m/z 104.

The peaks of the hydroxybenzyl cations $C_6H_5CH = \overset{+}{O}H$ with m/z 107 (II and III) were also low. The spectra of (II) and (III) were additionally characterized by the peaks of ions with m/z 122 (C_7H_8NO, II) and 123 ($C_6H_7N_2O$, III) consisting of protonated benzamide and protonated nicotinamide, respectively. Furthermore, these spectra contained the peaks of ions with m/z 117 (C_8H_7N, II) and 118 ($C_7H_6N_2$, III), the analysis of the origin of which required an additional experiment. The MD spectrum of the ion with m/z 117 (II) and 118 (III) showed as precursors the ions $(M - H_2O)^+$ with m/z 135 (II) and 136 (III). It follows from this that the ions under consideration arose as the result of the successive alternative elimination of H_2O from the amide grouping and the cleavage of the $CH(OH)-CH_2$ bond. The following is the most probable structure for them:



EXPERIMENTAL

Melting points were determined on a Boëtius instrument (Germany). UV spectra were taken on a Hitachi spectrophotometer, IR spectra on a UR-20 spectrometer (tablets with KBr), and mass spectra on a MKh 1310 instrument at an ionizing voltage of 90 V, an emission current of 40 μA, and a direct-introduction temperature of 100°C. The conditions of obtaining the HRMS and MD spectra are given in [11]. The ^{1}H and ^{13}C NMR spectra were obtained on Bruker WP-200 SY and Varian VXR-500S spectrometers with TMS as internal standard.

For column chromatography we used brand L silica gel (Czechoslovakia) and for thin-layer chromatography the same brand of silica gel with the addition of 5% of gypsum in the solvent system chloroform–methanol (9:1), the revealing agents being a 1% ethanolic solution of ninhydrin and the Dragendorff reagent.

Isolation of Compounds (I) and (II) from O. trichophysa Bunge. The air-dry epigeal part (10 kg) collected in the Khasag zhargalan ula mountains on the territory of the Gobi-Altai aimak in the period of vigorous flowering was extracted with ethanol. The extracts were combined, concentrated to 7 liters, diluted with water in a ratio of 1:1, and extracted successively with hexane, chloroform, ethyl acetate, and butanol. On standing, the concentrated hexane and chloroform fractions deposited crystals of (II) (25 g). The chloroform fraction after the separation of (II) was chromatographed on a column of silica gel (1:20). The column was eluted with hexane and then with hexane–chloroform with increasing concentrations of the latter. The hexane–chloroform (2:3) eluates yielded compound (I) (0.2 g) and the 1:4) eluates yielded compound (II) (0.1 g).

Isolation of Compounds (II) and (III) from O. muricata (Pall.)DC. The raw material, collected in the flowering period in the mountains located along the northern shore of Lake Khubsugul was extracted with ethanol, and the ethanolic extracts were combined and evaporated. The residue was distributed between 5% aqueous sulfuric acid and chloroform. The acid solution was made alkaline with sodium carbonate (pH 9). This led to the precipitation of compound (II) (0.9 g), which was purified by repeated crystallization from acetone. The alkaline solution after the separation of the (II) was extracted with chloroform. The chloroform extracts were combined, dried over sodium sulfate, and evaporated. The dry residue was chromatographed on a column of silica gel (1:20). Petroleum–chloroform (3:7) eluates yielded compound (II) (0.1 g), and chloroform–methanol (3% of methanol) compound (III) (0.1 g).

N-Benzoyl-2-phenylethylamine (I). $C_{15}H_{15}NO_2$ (HRMS), mp 117-118°C (from acetone), mass spectrum: m/z (%), 225 (M^+, 39), 207(3), 134(18), 105(100), 104(44), 91(9), 84(11), 77(29).

(+)-N-Benzoyl-2-hydroxy-2-phenylethylamine (II). $C_{15}H_{15}NO_2$ (HRMS), mp 154-155°C (from acetone), $[\alpha]_D^{30}$ + 35.2° (with 0.68; methanol).

^{1}H NMR spectrum (200 MHz), $CDCl_3$, ppm: δ 3.32 (br. s, 1H; OH); 3.51 (ddd, 1H, J_1 = 5.0 z; J_2 = 8.0 Hz; J_3 = 14 Hz; H–C–H); 3.91 (ddd, 1H; J_1 = 3.5 Hz; J_2 = 7.0 Hz; J_3 = 14 Hz; H–C–H); 4.95 (dd, 1H; J_1 = 3.5 Hz; J_2 = 8.0 Hz; H–C–OH); 6.59 (br. s, 1H; NH); 7.38 (m, 8H; Ar–H); 7.74 (dd, 2H; J_{ortho} = 8.0 Hz; J_{meta} = 2.0 Hz; H-2', H-4').

^{13}C NMR spectrum (200 MHz, DMSO–D_6): δ 166.56 (CO); 143.96 (C-3); 134.67 (C-3), 131.25 (C-6'); 128.39; 128.19; 127.39; 126.15 (C-4, C-5, C-7, C-8, C-1', C-2', C-4', C-5'); 127.20 (C-6); 71.33 (C-2); 47.84 (c-1).

Mass spectrum, m/z (%): 242 [$(M + H)^+$; 0.3], 241 (M^+; 0.4), 223 (2), 135(100), 134(84), 122(14), 117(11), 107(6), 105(95), 91(4), 79(11), 77(39).

(–)-N-Nicotinoyl-2-hydroxy-2-phenylethylamine (III). $C_{14}H_{14}N$ (HRMS), mp 157-158°C (from acetone); $[\alpha]_D^{30}$ – 25.3° (with 0.79; methanol).

IR spectrum, $\lambda_{max}^{CH_3OH}$ (nm): 210, 256 (shoulder), IR spectrum, ν_{max} (cm^{-1}): 3330 and 1650 (NHCO).

Mass spectrum, m/z (%): 242 (M^+; 0.5), 224(2), 136(100), 135(70), 123(16), 118(9), 107(28), 106(52), 105(11), 79(35), 78(30), 77(22).

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SYNTHESIS OF THE AMIDE OF THE C-TERMINAL TETRAPEPTIDE OF THE SEQUENCE OF OXYTOCIN

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The synthesis has been effected of the amide of the tetrapeptide forming the sequence 6—9 of oxytocin with the use of benzyl protection of the thiol function of cysteine by two main schemes 1+3 and 2+2. The advantageousness of performing the synthesis by the 2+2 scheme has been shown. The overall yield of tetrapeptide using the method of condensation with the formation of mixed anhydrides amounted to 51% by the scheme proposed.

The peptide HCys(R)ProLeuGlyNH_2 is the key fragment in the synthesis of oxytocin. In developing a method for obtaining the structure of the peptide we have studied the possibility of its synthesis by two main schemes; 1+3 and 2+2. The development of the variants of the scheme was carried out with the use of benzyl protection of the thiol function of cysteine (R = $C_6H_5CH_2$—).

In the development of the 1+3 scheme for the synthesis of the tetrapeptide we took into account the fact that in the preparation of the amide of the 7—9 tripeptide (melanostatin; MIF) in spite of information [1] on the high yields of di- and tripeptides, these compounds were obtained by the activated-ester (AE) method in far worse quality and their yields were lower than in their preparation by the mixed-anhydride (MA) method [2]. The use of the amide in place of its ester is associated with the necessity for performing the condensation reaction in an aqueous medium because of the poor solubility of glycinamide in organic solvents and also with the difficulty of isolating the amide of the dipeptide in the pure form because of its increased solubility in water. In view of this, we have performed several variants of the synthesis of the tripeptide by a 1+2 scheme (scheme A) using mixed anhydrides with ethyl and butyl chloroformates and also with pivaloyl chloride. It was found that the yield of peptides on the use of pivaloyl chloride as condensing agent was somewhat lower in the sage of obtaining the dipeptide (67%) than on the use of ethyl or butyl chloroformate (75%). At the stage of obtaining the tripeptide the best yield of product was obtained with

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