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The total alkaloids of the roots of *Convolvulus subhirsutus* collected in the period of vigorous growth close to the village of Dzhilga, Chimkent province, have been studied. From the phenolic fraction of the total alkaloids of this species of plant we have isolated phyllalbine and convolidine for the first time. From the nonphenolic fraction of the combined bases we isolated convolvine, which has been found previously, the new alkaloid confoline, and an unidentified base (III). On the basis of spectral characteristics and some spectral transformations it has been established that confoline has the structure of (+)-N-formylconvolvine.

Eighteen species of plants of the genus *Colvolvulus* (family *Convolvulaceae*) grow in the territory of Central Asia [1]. Of them, five species have been investigated for their alkaloid content: *C. erinaceus* [2]; *C. hamade* [3], *C. linneatus* [4], *C. subhirsutus* (*pseudo-canthabrica*) [5], and *C. krausenus* [6]. The species *C. subhirsutus* proved to contain the largest amount of alkaloids with 4.1% of total alkaloids in the roots at the end of the vege-tation period and 2.08% in the epigeal part at the beginning of the vegetation period [7]. The two bases present in largest amount (convolvine and convolamine) and also the unidentified liquid base convolvicine have been isolated from the roots and epigeal part of *C. subhirsutus* [5]. In view of the pharmacological activity of the tropane alkaloids, we have continued to study the alkaloid composition of this plant.

From the roots of *C. subhirsutus* collected in the environs of the village of Dzhilga, Chimkent province, Kazakh SSR, in the phase of vigorous growth of the plant, by the usual method we isolated the total bases, which amounted to 2.68% of the weight of the air-dry roots. The combined material was separated into phenolic and nonphenolic fractions.

The separation of the nonphenolic fraction led to the isolation of two bases (I and II) which had not been found previously in C. subhirsutus, with mp 209-210°C (methanol) and 214-215°C (methanol), Rf 0.6 and 0.32 (system 2), respectively. The mass spectrum of base (I) contained, in addition to the peak of the molecular ion with m/e 291, the peaks of ions with m/e 124 (100%), 108, 97, 96, and 82, which are characteristic for alkaloids of the tropane series. According to its IR spectrum, alkaloid (I) contained an OH group (3400 cm⁻¹), and aromatic ester carbonyl group (1720 cm⁻¹), and a 1,2,4-trisubstituted benzene ring (820, 890 cm^{-1}). The NMR spectrum of (I) taken in CDCl₃ confirmed that it is a tropine ester. Thus, a characteristic signal of the proton a C_3 of the tropane nucleus is observed in 5.18 ppm region in the form of a triplet (in contrast to a quintet in the case of pseudotropine [8]). The protons of the N-CH3 group give a signal at 2.30 ppm (3 H, s). The acid moiety of the molecule appears in the following way: the signal of an aromatic Hc proton at 6.87 ppm (d, Jortho = 9 Hz), the signals of H_a and H_b protons at 7.40-7.55 ppm, and the signal of the proton of an OH group at 8.27 ppm (it disappears on deuteration). A comparison of the NMR and mass spectra of (I) and of convolamine showed that (I) differed only by the presence of a hydroxy group in place of one of the methoxy groups in the benzene ring.

To elucidate the position of the hydroxy group, compound (I) was subjected to alkaline hydrolysis, leading to the isolation of the amino alcohol tropine and an acid with mp 208-209°C which, from its melting point and R_f value in comparison with an authentic sample, corresponded to vanillic acid. Thus, it may be concluded that base (I) is (±) 3α -vanilloyloxy-tropane. An alkaloid with the same structure has been isolated from the roots of *Phyllantus discoides* (family *Euphorbiaceae*) and has been called phyllalbine. In its physicochemical properties and spectral characteristics, base (I) was identical with phyllalbine [8].

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 672-676, September-October, 1980. Original article submitted June 16, 1980.

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Base (II) differed from phyllalbine only by the presence of a proton in place of the methyl group attached to nitrogen. Such an alkaloid, which is called convolidine, has previously been isolated from the roots of *C. krauseanus* [6]. A comparison of base (II) with convolidine showed their identity (TLC, mixed melting point). This is the first time that convolidine has been isolated from the species *C. subhirsutus*.

When the nonphenolic fraction of the alkaloids was treated with citrate-phosphate buffer solution having pH 6.8, a fraction was obtained which consisted of technical convolvine. Then the alkaloids were extracted with 10% sulfuric acid (fraction A). However, part of the bases was not extracted by the acid (fraction B). The mixture of bases of fraction B was chromatographed on a column of silica gel. Ethereal eluates yielded two more alkaloids (III and IV). Base (III) with mp 190-191°C (acetone), Rf 0.57 (in system 1) dissolved readily in chloroform and less readily in acetone and did not dissolve in 5% sulfuric acid solution. Molecular weight 608 (mass-spectrometrically).

We have called base (IV) with mp 140-141°C (acetone), Rf 0.47 (system 1) confoline. The mass spectrum of the base contains the peaks of ions with m/e 319 (M⁺) 290 (M - 29), 182, 165, and 137, and also the peaks of ions that are characteristic for the decomposition of the tropane nucleus. The IR spectrum of confoline showed absorption bands of conjugated ester and amine carbonyls at 1710 and 1660 cm⁻¹, respectively, and also the absorption bands of a 1,2,4-trisubstituted benzene ring at 825 and 875 cm⁻¹. Substitution in the benzene ring was confirmed by the presence in the NMR spectrum of (IV) of the signals from the H_a and H_b protons in the 7.45-7.60 ppm region (2 H, m) and from the H_c proton at 6.78 ppm (1 H, d). The spectrum also shows the signals of an aldehyde proton (1 H, s, 8.04 ppm), of methine protons at C₃ (1 H, t, 5.28 ppm), C₁ (1 H, m, 4.58 ppm), and C₅ (1 H, m, 4.08 ppm), of the protons of two methoxy groups (6 H, s, 3.86 ppm), and of methylene protons at 2.0-2.1 ppm.

On the basis of the spectral characteristics, it may be assumed that (IV) is an ester of of a substituted tropine and an aromatic acid. Subsequent chemical transformations confirmed this hypothesis.

When confoline was hydrolyzed, the amino alcohol moiety yielded a substance the IR and mass spectra of which were identical with those of convolvine. From the acid fraction of the hydrolysate an acid was isolated which was identified by its Rf value and a mixed melting point as veratric acid.

A comparison of the NMR, IR, and mass spectra of (IV) with the spectra of convolvine revealed that the IR spectrum of (IV) lacked the absorption band of active hydrogen but contained the absorption band of an amide carbonyl group. The mass spectrum of (IV) differs from that of convolvine by 28 m/e, which corresponds to a formyl radical. In actual fact, the NMR spectrum of confoline showed the signal of an aldehyde proton, and the signal of the proton at C₁ had undergone a paramagnetic shift by 0.5 ppm due to the influence of an amide carbonyl. We assumed that confoline is $(\pm)-(N-formylconvolvine)$.



This hypothesis was confirmed by the semisynthesis of confoline from convolvine and methyl formate. The synthetic product was identified with confoline with respect to its R_f value and the absence of a depression of the mixed melting point.

EXPERIMENTAL

IR spectra were taken on a UR-20 spectrophotometer (molded tablets with KBr), NMR spectra on a JNM-4H 100/100 MHz instrument with hexamethyldisiloxane as internal standard (δ scale), and mass spectra on a MKh-1303 mass spectrometer.

For chromatography we used KSK silica gel, alumina (Brockmann activity grade II), and the following solvent systems: 1) cyclohexane-chloroform-diethylamine (5:4:1), and 2) chloro-form-methanol-25% ammonia (8:2:0.2).

Isolation of the Total Alkaloids. Air-dry comminuted roots of C. subhirsutus (16 g) were moistened with 10% ammonia solution and, after 2 h, the alkaloids were exhaustively

extracted with chloroform. The chloroform extract was treated with 10% sulfuric acid. From the acid solution 217.6 g of crystalline colvolvine sulfate was isolated. The acid solution was washed with ether and, with cooling, it was made alkaline with 25% ammonia solution. The alkaloids were extracted with chloroform. The combined chloroform extracts were dried over anhydrous potassium carbonate and evaporated. This gave 428.8 g of combined alkaloids.

Separation of the Combined Alkaloids. The combined bases were dissolved in three liters of chloroform and the solution was treated with 4% KOH solution. The alkaline extracts were acidified with 20% sulfuric acid and then, with cooling, they were made alkaline with 25% ammonia solution and the alkaloids were exhaustively extracted with chloroform. The chloroform solution was dried with anhydrous sodium sulfate. The solvent was distilled off to give 21.5 g of combined phenolic alkaloids.

After the treatment with alkali, the chloroform solution was washed with water and was treated with citrate-phosphate buffer solution having pH 6.8 until the convolvine had been extracted completely. The buffer extract was acidified with 10% sulfuric acid and it was then made alkaline, with cooling, with 25% ammonia and the alkaloids were extracted with chloroform. This gave 199.5 g of bases of a pH 6.8 fraction (technical convolvine).

From the combined chloroform-extracted material the alkaloids were extracted with 5% sulfuric acid. After being washed with ether, the acid extracts were made alkaline, with cooling, with 25% ammonia and the alkaloids were extracted with chloroform. After drying with anhydrous potassium carbonate and the distillation of the solvent, the combined alkaloids of fraction A were obtained. The chloroform solution remaining after the acid treatment was made alkaline, washed with water, and evaporated. This gave 25.5 g of combined bases (fraction B).

Isolation of Phyllalbine and of Convolidine. The phenolic fraction of the combined alkaloids (21.5 g) was boiled in acetone (5×200 ml). The concentrated acetone mother liquor yielded 10.4 g of technical phyllalbine.

Base (III). Fraction B (25.5 g) was chromatographed on a column of silica gel. From ethereal eluates (fractions 11-72) by treatment with acetone a crystalline base with mp 190-191°C (acetone) was isolated (2.65 g).

<u>Confoline</u>. Ethereal fractions 73-92 yielded a crystalline mixture of two alkaloids (0.9 g) with Rf 0.57 and 0.47 (system 1). It was separated on a column of alumina (1:25). Ethereal eluates (fractions 4-9) yielded base (III), and the subsequent fractions (10-16) base (IV) contaminated with a small amount of (III). The individual base (IV) with mp 140-141°C was isolated by fractional recrystallization from hexane.

Acid Hydrolysis of Confoline. The base (40 mg) was boiled with 3 ml of 16% hydrobromic acid solution for 3 h. The reaction mixture was made alkaline with 25% ammonia. The alkaline solution was evaporated to dryness and the residue was treated with dry chloroform. After the solvent has been distilled off and the residue had been dried in vacuum, 28 mg of a crystalline base with mp 113-114°C, R_f 0.2 (system 1) (convolvine), was isolated.

Alkaline Hydrolysis of Confoline. A mixture of 30 mg of confoline and 5 ml of 10% methanolic KOH was boiled for 5 h. After the end of the reaction (TLC monitoring), the solvent was driven off in vacuum, the residue was dissolved in 4 ml of 20% sulfuric acid, and the solution was extracted with chloroform. After the extract had been dried with anhydrous calcium chloride and the solvent had been distilled off, a substance was obtained with mp 181°C, Rf 0.1 (system 1, revealed with a 1% solution of Bromocresol Green). A mixture with an authentic sample of veratric acid gave no depression of the melting point.

<u>Preparation of Confoline from Convolvine</u>. A solution of 0.2 g of convolvine in 5 ml of methyl formate was boiled on the water bath for 5 h. The reaction mixture yielded a base identical with convoline in R_f value and from the absence of depression of the melting point of a mixture with an authentic sample.

SUMMARY

1. Five bases have been isolated from the roots of *Convolvulus subhirsutus* collected in the Chimkent province: convolvine, which has been isolated previously, and phyllalbine and convolidine, the new alkaloid confoline, and an unidentified base (III), all found for the first time in this plant.

2. On the basis of spectral characteristics and some chemical transformation it has been established that confoline has the structure of (\pm) -N-formylconvoline.

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FRACTIONAL AND AMINO ACID COMPOSITIONS OF THE PROTEINS OF THE

NEEDLES OF Pinus sylvestris

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UDC 581.134.4:630.892

Four fractions of soluble protein have been isolated by the method of successive extraction from the needles of *Pinus sylvestris*, and their quantitative ratios have been established. The quantitative amino acid compositions of the fractions isolated and of the unextracted protein, which amounts to about 55% of the total amount of protein in the needles, have been determined. The amount of crude protein in the initial material was 11% on the absolutely dry needles.

The degree to which the proteins have been studied in various plant tissues, and, in particular, in coniferous tissues, is far from commensurate with their physiological and practical importance [1-7].

In the present investigations we have considered the fractional composition of the proteins present in the needles of *P. sylvestris*, the amino acid composition of the total protein and of each protein fraction, and the amounts of the various forms of nitrogen in the needles.

The results that we have obtained on the total nitrogen, the protein nitrogen, and the nonprotein nitrogen permit the conclusion that the amount of protein (more than 11%) of pine needles is not inferior to many varieties of fodder herbs and cereals:

Form of nitrogen	Nitrogen content, %	
	on the dry substance	on the total nitrogen
Total	1.88	100
Protein	1.25	66.5
Nonprotein	0.63	33.5
Crude protein	11.72	-

The fractional (group) composition of the proteins of *P. sylvestris* needles is as follows:

Siberian Technological Institute, Krasnoyarsk. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 676-678, September-October, 1980. Original article submitted March 31, 1980.

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