STRUCTURE OF ALKALOID L-5 FROM COLCHICUM LUTEUM

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In one of our papers [1] we reported a study of the dynamics of the contents of the alkaloids of Colchicum luteum Baker (yellow autumncrocus) growing in the gorge of the Sazan-Ata River, Chimkent region. We have subsequently investigated the isolation of the alkaloids from the epigeal part of the plant by the method described previously [2]. In plants collected in the flowering phase, they amounted to 1.58%, and in the fruit-bearing phase to 0.29%. In a comparative study of the total alkaloids obtained, a considerable difference in their composition was found. By adsorption chromatography on alumina, from the first combined extract we obtained cochlamine, 3-desmethyl cochlamine, colchicine, 2-desmethylcolchicine, and 3-desmethyl-β-lumicolchicine. The first two bases, with a tropolone ring, and 3-desmethyl-β-lumicolchicine have been isolated from yellow autumncrocus for the first time.

From the plants collected in the fruit-bearing phase we likewise extracted colchicine, β-lumicolchicine, N-desacetyl-N-formylcolchicine, 2-desmethylcolchicine, 3-desmethyl-β-lumicolchicine, luteidine (alkaloid L-2), and new compounds with R_f 0.21 and 0.76 (system 1) which we have called L-5 and L-6, respectively. Paper chromatography showed the presence of colchiceine and unknown bases with R_f 0.38, 0.40, and 0.86 (system 2). Kesselringine and luteine (alkaloid L-1) were absent.

The results obtained show that yellow autumncrocus from the gorge of the Sazan-Ata River differs considerably in respect of the composition of the combined alkaloids from the same plant species from the Parkent reserve [3].

Alkaloid L-5, with the composition $C_{20}H_{21}O_6N$, consisted of a yellow amorphous substance melting at 179–183° C. It forms the bulk of the phenolic-acidic fraction of the alkaloids. A spectroscopic study of this compound showed the presence of a tropolone ring in it: the UV spectrum (Fig. 1) has maxima at 244 and 350 mμ, and the IR spectrum (Fig. 2) has absorption bands at 1354 and 1280 cm^{-1} [4]. This was also established by the reactions with ferric chloride.

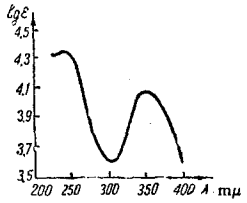


Fig. 1. UV spectrum of the alkaloid L-5 in methanol.

The IR spectrum of the alkaloid has other absorption bands at 1615 cm^{-1} (tropolone carbonyl), 1675 cm^{-1} (amide carbonyl), 3275 cm^{-1} (hydroxyl group), and 900–800 cm^{-1} (aromatic ring of colchicine alkaloids) [5].

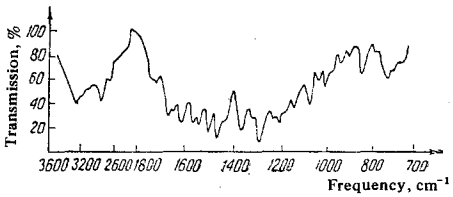


Fig. 2. IR spectrum of the alkaloid L-5 (tablets with KBr).

The NMR spectrum of the substance (Fig. 3) has the signals (δ scale) of the protons of a N-acetyl group (2.13 ppm) and the singlets of two aromatic methoxyl groups (3.57 and 3.96 ppm) and of a tropolone hydroxyl (3.83 ppm). In

the monomethyl ether of the alkaloid the latter signal is absent, and at 3.93 ppm a narrow signal of a tropolone methoxyl group appears. The identification of these signals was carried out on the basis of the results given by Delaroff and Rathle [6].

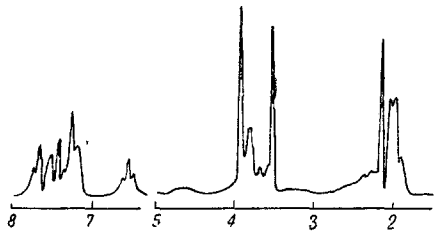


Fig. 3. NMR spectrum of the alkaloid L-5 (in $CDCl_3$).

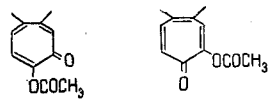
The above information and functional analysis show that the alkaloid L-5 may be represented by the following developed formula

$$C_{15}H_3(OCH_3)_2(OH)(CO, OH)(NHCOCH_3).$$

The NMR spectrum also has broad signals at 1.98 and 2.30 ppm corresponding to methylene protons. A proton attached to a tertiary carbon atom appears in the form of a very broad signal (because of spin-spin coupling) at 4.45–4.85 ppm. The shift of the signal into the weak field takes place under the influence of the descreening effect of an acetamido group.

The assignment of the lines in the aromatic part of the spectrum presents certain difficulties because of the presence in the sample of the two isomeric systems responsible for the tautomerism of the tropolone ring [7], which either broadens the lines because of the small difference in chemical shifts (for example, the proton of the aromatic nucleus at 6.58 ppm) or complicates the multiplicity of this part of the spectrum.

The acetylation of the alkaloid L-5 gave a crystalline diacetyl derivative. The relatively high angle of rotation and somewhat greater value of the main maximum in the UV spectrum [8, 9] of the diacetyl derivative permits the conclusion that in this case only one of the two possible products of the normal series (a) is formed:



a

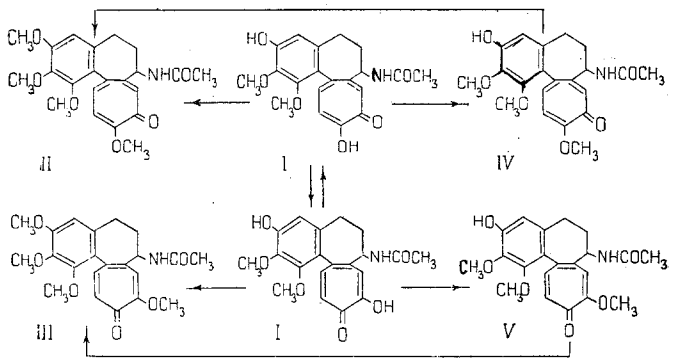
When the alkaloid was exhaustively methylated with diazomethane, we isolated colchicine (Scheme, II) and isocolchicine (III), which shows that the alkaloid is a substance of the colchiceine type and thereby confirms the tropolone nature of one of its hydroxyl groups. The second hydroxyl group must be at C_2, C_3, or C_4 of the aromatic ring. To determine its position, the methanolysis [8, 10] of the substance was carried out, which gave two isomeric monomethyl ethers. One of them was identified as 2-desmethylcolchicine (IV), and the other as 2-desmethyl-isocolchicine (V), which has not previously been reported in the literature. With acetic anhydride it gives an acetyl derivative. The further methylation of V gave isocolchicine (III). These two phenolic substances could also be separated when the alkaloid L-5 was incompletely methylated with diazomethane.

Thus, it has been established that the second hydroxyl group in L-5 is on C_2 of the aromatic ring. Consequently the substance has the structure of 2-desmethylcolchiceine (I). The reactions performed with it can be illustrated by the Scheme given below.

2-Desmethylcolchiceine is a compound which has not been described in the literature. It has been isolated from the plant for the first time. The fact that it is present in the native state was shown by treating IV under the conditions of the isolation of the alkaloids from the plant material. It was found that under these conditions I is not formed from IV.

An investigation of the phenolic-acidic fractions of the substances obtained from various parts of Merendera robusta Bge Merendera Iolantae E. Czerniak, Colchicum Kesselringii Rgl. (Kesselring's autumncrocus), and

Colchicum luteum Baker (yellow autumncrocus), from various growth sites showed that they contain 40–90% of 2-desmethylcolchicine. Thus, 2-desmethylcolchiceine is present in all colchicine-containing plants of Central Asia; it is possibly an intermediate in the biosynthesis of colchicine.



The alkaloid L-6, with the composition $C_{21}H_{23}O_6N$, consists of a white crystalline substance with mp 291–293° C (from acetone) and $[\alpha]$ −410° (c 0.88; methanol). UV spectrum, λ_{max}, mμ: 228, 264, 282, 342. It was isolated from the phenolic fraction. According to its spectra and color reaction, it belongs to the lumi- derivatives of the tropolone alkaloids.

EXPERIMENTAL

Radial chromatography was carried out on chromatographic paper (density 85 g/m^2) of the Volodarskii Leningrad No. 2 Mill in the following solvent systems (by volume): 1) butan-1-ol saturated with 12% aqueous ammonia (100 : 50) for the neutral substances, and 2) butan-1-ol saturated with water (100 : 40) for the basis substances. The substances on the chromatographs were revealed with 7% aqueous ferric chloride (system 1) and with Dragendorff's reagent (system 2). Neutral alumina (activity grade II) and type MSM silica gel (200–270 mesh) were used for preparative chromatography.

The UV spectra were recorded on an SF-4A spectrometer (in methanol), the IR spectra on a UR-10 double-beam spectrometer (tablets with KBr), and the NMR spectra on a H-60 Hitachi spectrometer (with tetramethylsilane as internal standard).

Isolation of the alkaloid fractions. 17.6 kg of the comminuted air-dry epigeal part of yellow autumncrocus collected in the fruit-bearing phase (June 3–13, 1964) in the gorge of the Sazan-Ata River was extracted with methanol. The alkaloid fractions were isolated from the residue obtained after the distillation of the solvent (table). The alkaloid fractions from 400 g of the leaves and stems of the plant collected in the flowering phase were isolated in the same way.

Fraction	Fruit-bearing phase		Flowering phase	
	g	%	g	%
Neutral	29.84	0.17	3.96	0.99
Phenolic	9.86	0.06	0.52	0.13
Phenolic-acidic	3.83	0.02	0.20	0.05
Basic	3.88	0.02	1.16	0.29
Basic-phenolic	2.72	0.02	0.48	0.12
Total	50.13	0.29	6.32	1.58

Isolation of the individual compounds. The fractions of the substances were subjected to adsorption chromatography on a column of alumina. The elution of the alkaloids was monitored by color reactions [11] and by paper chromatography [12].

The chromatography of 28.2 g of the neutral fraction of alkaloids from the plant (fruit-bearing phase) on 560 g of adsorbent by the method described previously [3] gave β-lumicolchicine (0.13 g), colchicine (12.32 g), and N-desacetyl-N-formylcolchicine (0.32 g), while 6.0 g of the phenolic fraction (on 140 g of adsorbent) yielded 3-desmethyl-β-lumicolchicine (1.10 g), alkaloid L-6 (0.68 g), and 2-desmethylcolchicine (1.35 g).

The fraction of alkaloids of basic nature consisted of three compounds with R_f 0.8, 0.56, and 0.86 (system 2). From it was extracted 0.27 g of luteidine (R_f 0.56). The basic-phenolic fraction contained a substance with R_f 0.40 (system 2), which it was impossible to obtain in the crystalline state.

By chromatography of the fractions of the alkaloids of the yellow autumncrocus in the flowering phase we isolated colchicine, 3-desmethyl-β-lumicolchicine, 2-desmethylcolchicine, colchamine, and 3-desmethylcholchamine.

The alkaloid L-5 (I). Four grams of the phenolic-acidic fraction of the alkaloids was chromatographed on 315 g of silica gel. The first eluates from ether contained 0.16 g of material. When the chromatogram was treated with ferric chloride it became colored brown; R_f 0.01 (system 1). The subsequent ethereal and ethereal-chloroformic (99 : 1) eluates yielded 2.1 g of substance I with R_f 0.21 which gave a yellow spot when the chromatogram was treated with a solution of ferric chloride. A mixture of ether and chloroform (97 : 3) eluted 0.32 g of colchiceine (R_f 0.62).

It was impossible to crystallize the eluted substances, and of them only the alkaloid L-5 was studied.

Found, %: C 67.01, 66.05; H 5.58, 5.49; N 3.92, 3.67; OCH_3 16.26, 16.01. Calculated for $C_{20}H_{21}O_6N$, %: C 66.68; H 5.69; N 3.78; 2 OCH_3 16.72.

Alkaloid L-5 is readily soluble in methanol, chloroform, and acetone, and sparingly soluble in water, ether, and petroleum ether. It dissolves in dilute alkalies, coloring the solution light yellow. The solutions in organic solvents are more highly colored. With ferric chloride, the neutral and acidic aqueous solutions give an olive-green coloration.

Acetylation. A mixture of 0.3 g of I, 0.38 g of anhydrous sodium acetate, and 4 ml of acetic anhydride was left at 45–50° C for a day. After cooling, the reaction mixture was diluted with small portions of methanol and was carefully evaporated. The residue was dissolved in water and the substance was extracted with chloroform. The solvent was distilled off, giving the acetyl derivative with mp 119–122° C (from ethyl acetate and ether), $[\alpha]_D^{20}$ −170° (c 0.64; chloroform). IR spectrum, ν_{max}, cm^{-1}: 1730, 1250. UV spectrum, λ_{max}, mμ: 246, 350.

Found, %: C 63.33, 63.18; H 5.21, 5.08; N 3.10, 3.06. Calculated for $C_{24}H_{25}O_8N$, %: C 63.29; H 5.20; N 3.07.

The compound obtained was not revealed on paper with ammonia and ferric chloride, which shows the acetylation of both hydroxyl groups.

Methylation of I to form colchicine (II) and isocolchicine (III). An excess of an ethereal solution of diazomethane was added to a methanolic solution of 1.0 g of the alkaloid. After the end of the reaction, the solvent was distilled off and the residue was chromatographed on 20 g of alumina. Substance II with mp 154–156° C [ether–chloroform (1 : 1) and chloroform eluates] and substance III with mp 220–222° C [chloroform and chloroform–ethanol (99 : 1) eluates] were isolated, and they were identified by a direct comparison with authentic samples.

Methylation of I to 2-desmethylcolchicine (IV) and 2-desmethylisocolchicine (V). With continuous shaking, an ethereal solution of diazomethane obtained from 0.70 g of nitrosomethylurea was added to a methanolic solution of 2.0 g of the alkaloid. The solvent was distilled off, and the residue was dissolved in chloroform and extracted with 3% caustic soda solution.

The chloroform solution contained 0.46 g of a mixture of substances II and III. By extraction with chloroform (after the addition of ammonium sulfate) the alkaline extract yielded 0.94 g of substances of phenolic nature. They were separated on 20 g of alumina, giving substances IV and V [chloroform–methanol (99 : 1) and (98 : 2) eluates].

The 2-desmethylcolchicine (IV) had mp 276–278° C (from acetone), $[\alpha]_D^{20}$ −132° (c 0.85: chloroform). NMR spectrum: 3.93 ppm.

The 2-desmethylisocolchicine (V) had mp 289–292° C (from acetone and ethyl acetate), $[\alpha]_D^{20}$ −418° (c 0.49; chloroform).

The alkaline solution after the removal of the neutral and phenolic substances was acidified with HCl and again extracted with chloroform. The residue after the solvent had been distilled off was found to contain unchanged starting material.

Methanolysis. A solution of 0.60 g of I in 10 ml of absolute methanol containing 3% of hydrogen chloride was boiled in a flask with a reflux condenser fitted with a calcium chloride tube for 6 hr. Then the solvent was distilled off, the residue was dissolved in 3% caustic soda solution, an excess of ammonium sulfate was added, and the solution was extracted with chloroform. This yielded 0.21 g of a mixture of substances IV and V. After acidification of the alkaline solution and extraction with chloroform, 0.37 g of unchanged starting material was obtained.

Acetyl-2-desmethylcolchicine. A mixture of 0.1 g of IV, 0.25 g of anhydrous sodium acetate, and 0.5 ml of acetic anhydride was left at 45–50° C for 12 hr. After cooling it was diluted with methanol, the solvent was evaporated off, and the residue was extracted with chloroform. This gave the acetyl derivative with mp 223–225° C (from ethyl acetate and ether), $[\alpha]_D^{20}$ −116° (c 0.84; chloroform). IR spectrum, ν_{max}, cm^{-1}: 1735, 1220.

Found, %: C 66.74, 66.60; H 5.90, 5.75; N 3.10, 3.03. Calculated for $C_{23}H_{25}O_7N$, %: C 66.69; N 5.82; H 3.07.

Acetyl-2-desmethylisocolchicine. The substance was obtained from V under the conditions of the preceding experiment; mp 244–246° C, $[\alpha]_D^{20}$ −306° (c 0.49; chloroform). IR spectrum, ν_{max}, cm^{-1}: 1735, 1220.

Found, %: C 66.76, 66.63; H 5.79, 5.73; N 3.12, 3.04. Calculated for $C_{23}H_{25}O_7N$, %: C 66.69; H 5.82; N 3.07.

Methylation of 2-desmethylisocolchicine (V) to form III. An excess of diazomethane in ether was added to a solution of 0.06 g of the substance in methanol. The solvents were distilled off and substance III with mp 221–223° C (from ethyl acetate) was isolated.

Confirmation of the native nature of the alkaloid L-5 (I). A solution of 0.2 g of IV in 1 ml of water was acidified to pH 1 with HCl. The acid solution was extracted with chloroform, and then the chloroform extract was extracted with 3% caustic soda solution. After the addition of ammonium sulfate, the extraction of the alkaline solution with chloroform gave IV in quantitative yield. Then the alkaline solution was acidified to pH 1 and extracted with chloroform. The chloroform extract contained no substance I, the product of the hydrolysis of IV.

The work was carried out with cooled acidic and alkaline aqueous solutions.

Alkaloid L-6. This was isolated by the adsorption chromatography on alumina of the fractions of phenolic compounds. It was eluted with a mixture of ether and chloroform (1 : 1) after the 3-desmethyl-β-lumicolchicine.

Found, %: C 65.77, 64.79; H 6.32, 5.89; N 3.96, 3.66. Calculated for $C_{21}H_{23}O_6N$, %: C 65.41; H 6.02; N 3.63.

The alkaloid is readily soluble in methanol, less readily in chloroform, and sparingly in acetone and water, and is insoluble in ether and petroleum ether. It dissolves in conc H_2SO_4 with a violet coloration. The Oberlin-Zeisel reaction for a tropolone ring is negative.

CONCLUSIONS

From Colchicum luteum Baker collected in the gorge of the Sazan-Ata River in the Chimkent region have been isolated the known alkaloids colchamine, 3-desmethylcolchamine, 3-desmethyl-β-lumicolchicine, colchicine, 2-desmethylcolchicine, β-lumicolchicine, N-desacetyl-N-formylcolchicine, and luteidine, and the new alkaloids L-5 and L-6. The first three bases have been obtained from this plant for the first time.

Paper chromatography showed the presence of another three new alkaloids with R_f 0.38, 0.40, and 0.86. On the basis of UV, IR, and NMR spectroscopy and chemical reactions, the structure of 2-desmethylcolchiceine has been proposed for the alkaloid L-5.

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