ALKALOIDS OF Merendera Iolantae

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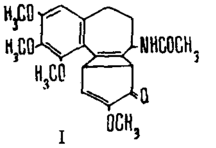
Merendera Ram., family Liliaceae, is a widely distributed but insufficiently studied genus of plants. Some colchicine-containing species of this genus growing within the boundaries of our country have been described [1-5]. They contain tropolone alkaloids of both neutral and basic nature (colchicine, colchamine, and their analogs), and also β- and γ-lumicolchicines.

In the USSR, Merendera is represented by eight species [6-7]. Only in Merendera robusta Bge has the composition of the alkaloids been studied in fairly detailed fashion [8-10]. This plant proved to be a producer of tropolone alkaloids.

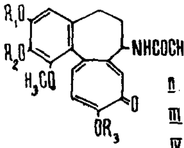
Studying Merendera iolantae E. Czerniak by chromatographic methods, we have found that it contains, in addition to tropolone compounds, bases with other carbon skeletons.

The seeds are the richest parts of the plant in alkaloids (1.11%). The leaves and stems contain a smaller amount of alkaloids (0.35%), and the corms and their hulls an extremely small amount of alkaloids (0.12-0.09%).

To isolate the alkaloids, 48 kg of the epigeal parts of the plant was extracted with methanol. By a method described previously [11], fractions of alkaloids of neutral and basic nature were obtained which, in their turn, were separated into phenolic and nonphenolic compounds. The chromatography on alumina of the mixture of neutral nonphenolic substances yielded β-lumicolchicine (I) and colchicine (II), and that of the mixture of phenolic substances yielded an unknown compound with mp 268-270°C. It does not contain a tropolone ring and from its color reactions it belongs to the lumi derivatives. The substance was named alkaloid MI-3.



I



II. $R_1=R_2=R_3=CH_3$

III. $R_1=R_3=H$; $R_2=CH$

IV. $R_1=R_2=CH_3$; $R_3=H$

From the phenolic-acid part of the neutral fraction by chromatography on silica gel we isolated 2-demethylcochiceine (III) and showed the presence of colchiceine (IV).

The phenolic-basic substances could not be separated by adsorbent. Colchameine (V) was isolated by partial separation according to the basic strength of the components; the presence of 3-demethylcolchamine (VI) and of two unknown nontropolone bases with R_f 0.49 and 0.53 (system 2) was established by thin-layer chromatography.

The bases of nonphenolic nature amounted to half the total alkaloids of M. iolantae and consisted of six substances with R_f 0.38, 0.40, 0.45, 0.54, 0.61, and 0.82 (system 2). By preparative chromatography and the use of buffer solutions, four of them – with R_f 0.38 (colchamine), 0.45, 0.61, and 0.82 – were isolated in the individual state.

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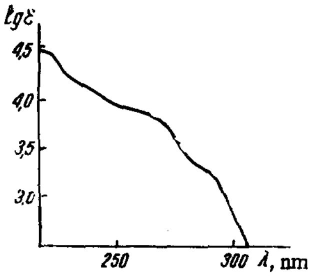
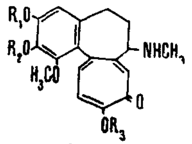


Fig. 1. IR spectrum of iolantamine (CH_3OH).



V. $R_1=R_2=CH_3$, $R_3=H$

VI. $R_1=R_3=CH_3$; $R_3=H$

The bases with R_f 0.61, mp 179-180°C, and R_f 0.82 (amorphous) did not contain tropolone rings. Their physicochemical constants differed from those of known compounds. We have called these alkaloids MI-2 and MI-4.

The base with R_f 0.45 (MI-1) has been studied in more detail. This base, which we have called iolantamine, is a white crystalline substance with the composition $C_{19}H_{23}O_3N$, mp 215-216°C, $[\alpha]_D^{20}+112°$ (c 0.95; chloroform). It gives a well-crystallized hydrochloride with mp 274-275°C and a methiodide with mp 257-258°C.

There are no characteristic maxima in the UV spectrum of iolantamine (Fig. 1), but there are inflections at 225, 275, and 288 nm. They are possibly due to the presence of a conjugated carbonyl group in the base. This is confirmed by the IR spectrum (1650 cm^{-1}). The IR spectrum (Fig. 2) also has the absorption band of a hydroxy group (3350 cm^{-1}). The NMR spectrum of iolantamine (Fig. 3) shows the signal of protons of an aromatic methoxy group (δ 3.78 ppm) and of an N-methyl group (2.38 ppm).

The following developed formula may be proposed for iolantamine on the basis of the facts presented: $C_{16}H_{16}(OH)(OCH_3)(CO)(NCH_3)$.

The NMR spectrum of the base under consideration has the resonance signals of three aromatic protons (6.49, 6.75, and 6.95 ppm) and of no less than four or five methylene groups.

A number of alkaloids isolated from Merendera iolantae have been identified from their R_f values, melting points, and specific rotations. The main substances of each fraction of the alkaloids have been studied additionally by color reactions, spectral methods, and some chemical reactions.

EXPERIMENTAL

The substances of phenolic and phenolic-acidic nature were chromatographed on paper with a density of 85 g/m^2 of Leningrad paper mill No. 2 in system 1) butan-1-ol saturated with 12% aqueous ammonia (100:50), and the other substances were chromatographed on a nonfixed thin layer of alumina in system 2) chloroform–methanol (24:1). The chromatograms were treated with 7% aqueous ferric chloride solution (system 1) and with iodine vapor (system 2). Neutral alumina (activity grade II) and type MSM silica gel (200-279 mesh) were used for thin-layer and preparative chromatography.

The UV spectra were taken 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 an H-60 Hitachi instrument (with tetramethylsilane as internal standard).

Determination of the Fractions of Alkaloids in Samples of the Plant. Weighed samples of the previously dried and ground plant material were placed in conical flasks and covered with methanol. Extraction was carried out five or six times at room temperature. Treatment of the methanolic extracts by the procedure described previously [12] gave fractions of alkaloids of neutral and basic nature (Table 1).

Isolation of the Fractions of Alkaloids. The comminuted air-dry epigeal part, 48 kg (without seeds) of Merendera iolantae collected in the fruit-bearing phase (March, 1965) in the Kara-Kala region of the Turkmen SSR was extracted with methanol. The following fractions were isolated:

I. Neutral-phenolic alkaloids (0.11%):	
neutral	34.30 g (0.07%)
phenolic	11.69 g (0.02%)
phenolic-acidic	7.70 g (0.02%)
II. Basic-phenolic alkaloids (0.28%):	
bases (nonphenolic)	90.65 g (0.19%)
phenolic bases	41.60 g (0.09%)
Total:	185.94 g (0.39%).

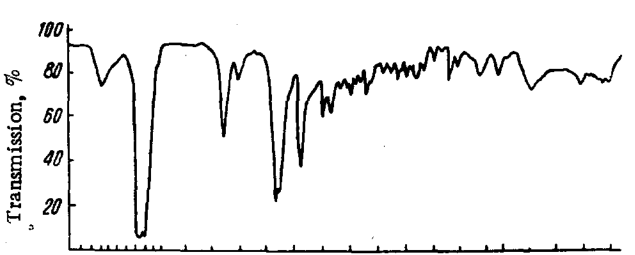


Fig. 2. IR spectrum of iolantamine (paraffin oil).

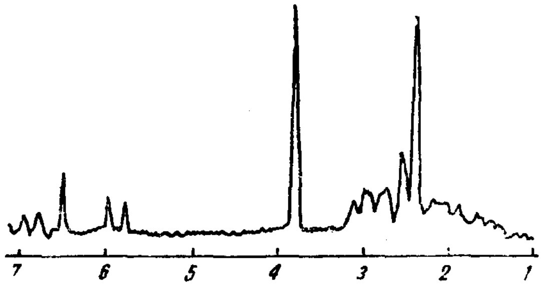


Fig. 3. NMR spectrum of iolantamine ($CHCl_3$).

TABLE 1

Part of the plant	Fraction of alkaloids, %		Total alkaloids, %
	neutral	basic	
Epigeal*	0,11	0,28	0,39
Seed†	0,80	0,31	1,11
Leaves and stems	0,15	0,20	0,35
Corms	0,07	0,05	0,12
Hulls of the corms	0,05	0,04	0,09

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*The epigeal part was collected in the fruit-bearing period and the others in the seed-ripening period.

†The seeds were previously defatted with petroleum ether.

For separation into individual compounds, the fractions of the alkaloids were chromatographed on columns of adsorbent by the method described previously [13].

Colchicine and β-Lumicolchicine. The mixture of neutral alkaloids (8.0 g) was chromatographed on a column of 200 g of alumina. A mixture of ether and chloroform (2: 1) eluted 0.46 g of a substance with R_f 0.92 (system 2), and chloroform and chloroform–methanol (99: 1) eluted 3.39 g of a substance with R_f 0.59.

The alkaloid with R_f 0.92 had mp 184-185°C (from ethyl acetate) $[\alpha]_D^{20}+307°$ (c 0.97; chloroform), UV spectrum: λ_{max} 226, 264, 342 nm. It gave no coloration with ferric chloride. From the constants given, the substance corresponded to β-lumicolchicine. It was shown to be identical with an authentic sample by means of a mixed melting point (mp 183-185°C).

The alkaloid with R_f 0.59 had 155-156°C (from ethyl acetate), $[\alpha]_D^{20}-120°$ (c 1.0; chloroform), UV spectrum: λ_{max} 246, 350 nm. After acid hydrolysis, it gave a green coloration with ferric chloride. The substance was shown to be identical with colchicine by a mixed melting point (mp 155-157°C).

Colchicinic Acid. A solution of 0.60 g of colchicine in 10 ml of methanol was treated with 25 ml of 5% methanolic sodium methoxide, and the mixture was boiled for 50 min. A product with mp 262-264°C was isolated in the usual way [13]. Yield 0.42 g (70%).

The Alkaloid MI-3. The mixture of alkaloids with a phenolic nature (9.35 g) was chromatographed on 200 g of alumina. In the first fractions (chloroform) 0.27 g of the alkaloid MI-3 with R_f 0.36 (system 2), mp 268-270°C, was eluted. The subsequent fractions [chloroform and chloroform–methanol (99: 1 and 97: 3)] contained 4.71 g of a mixture of substances with R_f 0.36 and 0.18.

The alkaloid MI-3 did not give the Oberlin-Zeisel reaction for a tropolone ring and it dissolved in conc. sulfuric acid, coloring it faintly red.

2-Demethylcochiceine (Alkaloid L-5). The mixture of compounds with a phenolic-acidic nature (3.0 g) was separated on a column of 290 g of silica gel. The substances were eluted with ether and with ether–chloroform (98: 2; 97: 3). The latter yielded 1.63 g of a substance with R_f 0.21, mp 180-183°C (amorphous). In solution with ferric chloride it gave an olive-green coloration (reaction for a nonmethylated tropolone hydroxyl). The acetylation product, with mp 119-122°C, was identified as the diacetyl derivative of alkaloid L-5 [14].

The mother solutions after the isolation of alkaloid L-5 contained 1.8 g of a mixture of substances with R_f 0.20 and 0.43 (system 1), which were identified as 2-demethylcolchiceine and colchiceine, resptively.

Colchameine. A solution of 2.75 g of the mixture of bases of phenolic nature in 5% hydrochloric acid was extracted with chloroform to eliminate resinous substances, after which the solution was brought to pH 7 with ammonia and was extracted with chloroform (extract I). Then the aqueous solution was brought to pH 8 and was reextracted with chloroform (extract II).

A chromatographic investigation showed that extract (I) contained one tropolone alkaloid (5.78 g) with mp 132-134°C (from a mixture of acetone and methanol), $[\alpha]_D^{18} -206°$ (c 0.62; chloroform). From the constants shown the alkaloid was identical with colchameine.

TABLE 2

Fraction	pH of the solution	R_f of the bases in the buffer extracts					
		0.38	0.40	0.45	0.54	0.61	0.82
I	6.5	—	+	+++	++	—	—
IIa	6.0	—	—	++	+++	+	—
IIb	6.0	++	—	—	—	+++	—
III	5.5	+++	—	—	—	—	++

Methylation of diazomethane was added to 0.1 g of colchameine in methanol, and the mixture was left for 12 h. Then the solvent was distilled off, and the residual substance was studied by chromatography (system 2). The reaction product gave two spots; one of them had the same R_f value as colchamine (R_f 0.38) and the other was apparently isocolchamine (R_f 0.53).

3-Demethylcolchamine. Chloroform extract II of the phenolic bases (12.68 g) consisted of three substances with R_f 0.49, 0.53, and 0.65 (system 2). The alkaloid with R_f 0.65 is a tropolone compound. From its R_f value it is identical with 3-demethylcolchamine (system 2).

Attempts to separate the mixture of alkaloids by column chromatography on alumina and on cellulose were unsuccessful.

Colchamine. During isolation and storage, the mixture of bases resinified strongly. After purification by preextraction it consisted of six substances with R_f 0.38, 0.40, 0.45, 0.54, 0.61, and 0.82 (system 2). To separate them, 20 g of the mixture was chromatographed on 500 g of alumina. Ether eluted all the compounds (4.65 g) except the substance with R_f 0.38. The subsequent eluates from ether–chloroform (1:1) and from chloroform contained 8.0 g of the alkaloid with R_f 0.38.

The alkaloid with R_f 0.38 had mp 185-186°C (from ethyl acetate), $[\alpha]_D^{22} -127°$ (c 0.99; chloroform). UV spectrum: λ_{max} 350 nm. From the constants given, the substance corresponded to colchamine.

Separation of the Fractions of Bases by Means of Buffer Solutions. A solution of 20 g of the mixture of bases in 300 ml of chloroform was extracted successively with 30-50 ml portions of buffer solutions with pH 5.5, 6.0, and 6.5 prepared from 0.1 M citric acid and 0.2 M monosodium phosphate [15]. Each fraction was treated repeatedly until the extraction of the bases ceased, as monitored by thin-layer chromatography (Table 2).

To isolate the bases, the buffer extracts were made alkaline with ammonia and extracted with chloroform. After drying, the solvent was distilled off.

Iolantamine. Fraction I (1.96 g) was separated on a column containing 47 g of alumina. The initial eluates contained an alkaloid with R_f 0.54 (0.35 g) and the subsequent ones a mixture of substances with R_f 0.54 and 0.45 (0.12 g). The subsequent ethereal eluates yielded iolantamine with R_f 0.45 (0.76 g). After recrystallization from ether containing 2-5% of acetone, mp 215-216°C.

Found %: C 72.04; 71.71; H 7.46; 7.59; N 4.11; 4.33; OCH_3 9.98; 9.12. $C_{19}H_{23}O_3N$. Calculated %: C 71.87; H 7.52; N 4.22; OCH_3 9.03.

The hydrochloride was obtained by mixing acetone solutions of the base and of hydrogen chloride.

The methiodide was precipitated by the addition of an excess of methyl iodide to an acetone solution of the base.

The Base MI-2. The mixture represented by fraction IIb (0.94 g) was chromatographed on 22 g of alumina. Elution of the substances with petroleum ether–acetone (10:1, 5:2) yielded the nontropolone base MI-2 with R_f 0.61 (0.24 g). It crystallized in the form of colorless needles with mp 179-180°C when the solvent evaporated from the concentrated eluate.

The petroleum ether–acetone (2:1) and acetone eluates yielded colchamine with mp 182-184°C (0.14 g).

The Base MI-4. The mixture of bases (3.8 g), after the isolation of colchamine, was rechromatographed on 90 g of alumina. Elution with ether gave 0.15 g of a nontropolone base with R_f 0.82 (amorphous).

The chloroform eluted 1.36 g of colchamine (R_f 0.38).

SUMMARY

The alkaloids of Merendera iolantae E. Czerniak have been studied for the first time. The total alkaloids consist of tropolone compounds and bases without a tropolone ring. From this plant we isolated the known alkaloids colchicine, colchamine, β-lumicolchicine, colchameine, and 2-demethylcolchiceine, and four new, nontropolone, bases – MI-1 (iolantamine), MI-2, MI-3, and MI-4. The presence of colchiceine, 3-demethylcolchamine, and two unknown bases with R_f 0.49 and 0.53 was shown by chromatographic methods.

A developed formula has been put forward for iolantamine on the basis of its spectral characteristics.

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