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HOMOAPORPHINE ALKALOIDS FROM *Merendera robusta*

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In an investigation of the total alkaloids of Merendera robusta *Bge, family Liliaceae from various growth sites as a promising raw material for obtaining colchamine and colchicine we have isolated for the first time merenderine and the new homoaporphine alkaloids merobustine and merobustinine, the structures of which have been establishd by chemical and spectral methods as 1,12-dihydroxy-2,10,11-trimethoxyhomoaporphine and 2,11-dihydroxy-1,10,12-trimethoxyhomoaporphine.*

Homoaporphine alkaloids with five oxygen-containing substituents in positions 1, 2, 10, 11, and 12 of the biphenyl nucleus have been isolated from representatives of a number of colchicine-containing plant genera of the family Liliaceae [1, 2]. They are present in only small amounts in these producing plants, with the exception of *Merendera radde*, in the epigeal parts of which the homoaporphine merenderine (bechuanine) and the tropolone alkaloid colchicine are the main components of the total alkaloids [3]. Other *Merendera* species, including *M. robusta* have hitherto been known exclusively as producers of tropolone alkaloids [4, 5]. The presence of nontropolone (isoquinolone) alkaloids in *M. robusta* growing in the Kyzylkum (Bukhara province) has been reported only in [6].

As a result of an investigation of the epigal parts of *M. robusta* gathered in the Ashgabat and Surkhan-Dar'ya provinces, we have detected the presence in the fraction of basic alkaloids of a mixture of tropolone and isoquinoline compounds. This was unexpected in view of results obtained previously [6-8].

We used 3% acetic acid for the extraction of the plant. In this process the extraction of the total alkaloids and their separation into neutral and basic compounds was considerably simplified, and their further separation into individual fractions was achieved by the methods used in the extraction of plants with alcohols [8]. On the use of this method, no appreciable decrease in the yields of alkaloids was observed.

By analysis of the composition of total alkaloids with methods developed previously it was established that the fractions of alkaloids of neutral, phenolic, and acidic nature consisted of the tropolone alkaloids and their photochemical isomers (colchicine, 3-demethylcolchicine, colchiceine, β-lumicolchicine, and 2-demethyllumicolchicine) that are customary for *M. robusta*. The fraction of alkali-insoluble bases consisted mainly of
colchamine (R_f 0.76) and minor nontropolone compounds (R_f 0.80 and 0.84, system 1). The fractions of alkali-soluble bases contained nontropolone
compounds having R_f 0.77 and 0.86, with very small amounts of colchameine nd 2-demethylcolchamine. We isolated merenderine by the chromatographic separation of this fraction from plants gathered in the environs of Ashgabat, while the plants from Surkhan-Dar'ya province yielded merenderine, merobustine,and merobustinine.

Merobustine (I), composition $C_{21}H_{25}O_5N$, had absorption maxima in its UV spectrum at 260 and 290 nm, and in its IR spectrum absorption bands in the 3420-3470 cm^{-1} region (OH). The PMR spectrum of the base showed the signals of the protons of a N-methyl group (3H, s, 2.40 ppm), of three methoxy groups in benzene rings (9H, 3.83-3.85 ppm, and of two aromatic protons (1H × 2, ss, 6.67 and 6.43 ppm, H-3 and H-9). The mass spectrum contained the characteristic peaks of ions with m/z 371 (M^+), 356 $(M - 15)^+$, 354 $(M - 17)^+$, and 340 $(M - 31)^+$. The peak of the $M - 17)^+$ ion was the maximum one. These facts, and the appearance of the signals of all the methoxy groups in a relatively weak field of the PMR spectrum

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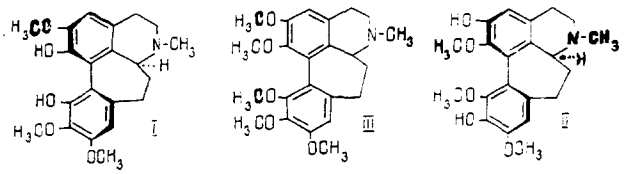
excluded positions 1 and 12 for them [11, 12]. On methylation with diazomethane, the base formed a di-O-methyl derivative, which was identified as O-methylkreysigine (III) [1]. Consequently, the hydroxy groups in merobustine are present in positions 1 and 12, and it is represented by the structure of 1,12-dihydroxy-2,10,11-trimethoxyhomoaporphine (I):



Thus, merobustine is an enantiomer of the alkaloid CC-24 isolated from *Colchicum cornigerum* [13].

Merobustinine (II), composition $C_{21}H_{25}O_5N$, was, according to its UV spectrum, with absorption maxima at 258 and 292 nm, close to merenderine and to merobustine. Its PMR spectrum contained signals of the protons of a N-methyl group (2.38 ppm), of three methoxy groups (3.85, 3.60, and 3.52 ppm) and of two aromatic protons (1H × 2, ss, 6.68 and 6.55 ppm, H-3 and H-9. The signals of two of the methoxy groups, being shifted into the strong-field region of the spectrum (3.60 and 3.52 ppm), showed that these groups were present in positions 1 and 12. The substitution of position 1 by a methoxy group was also confirmed by the mass spectrum of the alkaloid, which showed the peaks of ions with m/z 471 (M^+), 356 ($M - 15)^+$, 354 ($M - 17)^+$, and 340 ($M - 31)^+$, the last of them being the strongest.

The dimethyl ether of the alkaloid was identical with O-methylkreysigine. Consequently, the substituents in the biphenyl nucleaus of merobustine were arranged in the same way as in other, known, homoaporphine derivatives [1]. This base, having three methoxy and two hydroxy groups is isomeric with the known homoapoporphine alkaloids merenderine, multifloramine, szovitsinine, baytopine, and androbine [1] and with merobustine. In its physicochemical constants merobustinine differed from these compounds, and, obviously, the hydroxy and methoxy groups have different positions in them. In the PMR spectrum of its O,O-diacetyl derivative the signal of the H-3 proton had shifted downfield (6.68 → 6.76 ppm), which showed its α-position with respect to the hydroxy (C-3) group. The second hydroxy group was obviously in ring *D*, and it could be assigned only to C-11, since position 12 is occupied by a methoxy group. In position 10, it would be adjacent to the H-9 proton, which was excluded by the PMR spectrum of the diacetyl derivative. Thus, merobustinine is described by the structure 2,11-dihydroxy-1,10,12-trimethoxyhomoaporphine (II).

In view of the fact that merobustine and merobustinine are dextrorotatory homoaporphine alkaloids, they must each have the absolute (S)-configuration [14].

EXPERIMENTAL

UV spectra were taken on a SF-4A spectrometer in methanol, IR spectra on a UR-10 double-beam spectrometer in a thin layer, PMR spectra on a XL-100 instrument in $CDCl_3$, and mass spectra on a Varian MAT-311 spectrometer.

The individuality and authenticity of the substances were checked by PC and TLC. Radial PC was conducted on Filtrak paper using the mobile phases n-butyl alcohol–hydrochloric acid–water (50:7.5:13.3) (1) and n-butyl alcohol-5% acetic acid (50:50) (2), and TLC on glass plates with a fixed layer of LS 5/40 silia gel and 13% of gypsum, using the mobile phases chloroform–isopropyl alcohol–acetone–benzene–acetic acid (15:3:3:3:1) (3) and chloroform–isopropyl alcohol–acetone–benzene–25% aqueous ammonia (20:4:4:5:1) (4).

The alkaloids were isolated from the dried and comminuted epigeal parts of *Merendera robusta* gathered in the environs of Ashgabat on May 9, 1988 in the seed-ripening period (sample I), in the Dzhar-Kurgan region of the Surkhan-Dar'ya province on March 9 and 25, 1991 in the period of defloration and incipient fruit-formation (sample II), and on April 26, 1990, in the seed-ripening period (sample III).

Isolation of the Alkaloid Fractions. Samples (I-III) were extracted by steeping for a day with 3% acetic acid six times. Each of the combined acetic acid extracts was acidified with 49% sulfuric acid to pH 1 and was extrracted three times with ether. The strongly acidic aqueous extract was then extracted six times with chloroform. In this way, the total neutral, phenolic, and acidic alkaloids were obtained (extract A). After this, the solution was made alkaline with concentrated ammonia to pH 8 and was again extracted six times with chloroform. This gave the total basic and phenolic-basic compounds (extract B).

Chloroform extracts A and B were concentrated by evaporation and were worked up by a method described previously [8]. The alkaloid fractions (samples I-III) were obtained in the amounts (g/%) shown below.

Fraction	I (1.0 kg)	II (3.1 kg)	III (2.3 kg)
Neutral	1.72/0.17	3.72/0.12	1.91/0.08
Phenolic	0.24/0.02	2.48/0.08	1.18/0.05
Acidic	0.30/0.03	0.31/0.01	
Alkali-insoluble	1.12/0.12	2.54/0.08	1.36/0.07
Alkali-soluble	0.74/0.07	1.86/0.06	1.96/0.08
Sum of the alkaloids	4.12/0.41	10.91/0.35	6.41/0.28

Merenderine. The fraction of alkali-soluble bases from the plants of sample I (0.7 g) was chromatographed on 12 g of alumina, with elution by ether–chloroform (9:1) (1) and (2:1) (2), chloroform (3), and chloroform–methanol (98:2) (4) and (95:5) (5). The first fractions of eluate (1) contained a mixture of alkaloids, and the subsequent fractions 0.24 g of merenderine with mp 227-228°C (from acetone) [15]. It was impossible to crystallize substances from eluates 2-5.

Merobustine (I). A chloroform solution of 1.8 g of the fraction of alkali-soluble bases from sample II was purified by passage through 15 g of alumina. After the solvent had been distilled off, the residue was crystallized from acetone, giving 0.27 g of merenderine.

The mother solution from the crystallization of merenderine was evaporated, the residue was dissolved in 5% acetic acid, and the tropolone alkaloids were precipitated by the addition of sodium nitrite. After the precipitate had been separated off, the filtrate was made alkaline with ammonia and was extracted with chloroform. The solvent was distilled off, and the residue was chromatographed on 20 g of alumina with elution by acetone (1) and by acetone–methanol (98:2) (2) and (95:5) (3). From eluate 2 we isolated 42 mg of merobustine with mp 241-242°C (from acetone) and $[\alpha]_D$ + 76° (c 0.6; $CHCl_3$).

Merobustinine (II) was isolated from sample III by treating it in a similar way to sample II, with the working up of the fraction of alkali-soluble bases. This gave 0.18 g of merenderine and 42 mg of merobustinine with mp 216-218°C (from acetone) and $[\alpha]_D$ + 42° (c 0.50; $CHCl_3$).

Diacetylmerobustinine. One drop of concentrated sulfuric acid was added to a solution of 20 mg of (II) in 1 ml of acetic anhydride. After the end of the reaction, the excess of acetic anhydride was evaporated off in vacuum, and the residue was dissolved in water. The aqueous solution was made alkaline with ammonia, and the reaction product was extracted with chloroform. O,O-Diacetyl-(II) was isolated.

IR spectrum: 1740 cm^{-1} (2 × $OCOCH_3$). PMR spectrum (ppm): 3.82; 3.58; 3.48 (3 × OCH_3), 2.00 (2 × $OCOCH_3$), 2.40 ($N-CH_3$), 6.76 and 6.58 (1H × 2, ss, H-3 and H-9). Mass spectrum: m/z 455 (M^+).

O-Methylkreysigine (III). Diazomethane in n-hexane was added separately to methanolic solutions of (I) and (II). After the end of the reaction the solvent was distilled off, the residue was dissolved in chloroform, and the solution was washed with water. The reaction products were identified by chromatographic comparison with authentic sampels of O-methylkreysigine (systems 1 and 2).

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