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ROBUSTAMINE — A NEW HOMOPROAPOPHINE BASE FROM *Merendera robusta*

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The new alkaloid robustamine has been isolated from the epigeal parts of Merendera robusta *Bge., fam. Liliaceae, and its structure has been established by spectral methods and chemical transformations as 11-hydroxy-2,12-dimethoxy-1,12-epoxyhexahydrohomoproaporphine, the suggested configuration being 6aS,8aR, 11S,12R.*

Merendera robusta has previously been characterized as a plant containing tropolone alkaloids exclusively. With respect to the qualitative composition of its alkaloids it proved to be close to industrial colchicine-containing species of autumn crocus — *Colchicum autumnale* L. [5] and *Colchicum speciosum* Stev. [6] —, and it has therefore been regarded and studied as a potential raw material for the production of colchamine and colchicine. With this aim, A. S. Sadykov et al. [1-4, 7-10] have investigated the alkaloid complexes of plants from various growth regions and have isolated a number of tropolone alkaloids and their photochemical isomers. The main *Merendera* alkaloids proved to be colchicine and colchamine. Only in plants gathered in the Kyzylkum area was the presence of unknown alkaloids without a tropolone ring detected [3].

In order to study these nontropolone alkaloids we have extracted the epigeal parts of the plant and have obtained alkaloid fractions by the procedure of [2, 4]. Their compositions were analyzed by the TLC and PC methods [11, 12]. In the alkaloid fractions of neutral, phenolic, and acid nature we detected the tropolone alkaloids and their photochemical isomers known for *Merendera robusta* (colchicine, N-formyl-N-deacetylcolchicine, β-and γ-lumicolchicines, 3-demethylcolchicine, 2-demethyl-β-lumicolchicine, colchicine, 3,10-didemethylcolchicine, and an unidentified compound with R_f 0.52). The fraction of alkali-insoluble bases contained colchamine (R_f 0.47) and nontropolone compounds with R_f 0.03, 0.23, 0.35, 0.41, and 0.52 (system 4), and the fraction of alkali-soluble bases contained 2-demethylcolchamine (R_f 0.50) and compounds with R_f 0.18, 0.27, 0.33, 0.40, and 0.51 (system 5).

To isolate the compounds without a tropolone ring, colchamine was elimated from the alkali-insoluble fraction by the nitrosation reaction, and the mixture of substances remaining was chromatographed on alumina. Two crystalline compounds were isolated, with R_f 0.41 and 0.52, having physicochemical constants differing from those of known alkaloids. We have determined the structure of the first of them, which has been called robustamine.

Robustamine, $C_{20}H_{27}NO_4$, mp 251-252°C $[\alpha]_D$ −40°, had in its IR spectrum absorption bands of a hydroxy group (3500-3300 cm^{-1}), of the C=C bonds of a benzene ring (1608 cm^{-1}), and of methylene groups (2955, 2858, 1470, 1456 cm^{-1}). Its PMR spectrum showed three-proton signals at 3.75 and 3.32 ppm due to two methoxy groups located in benzene and alicyclic rings. The protons of an N-methyl group resonated at 2.36 ppm, and the H-3 proton of a benzene ring at 6.45 ppm.

In the mass spectrum of the compound, peaks were observed of ions with *m/z* 345 (M^+, 50%), 344 $(M - 1)^+$ (100%), 323, 317, 312, 302 $(M - 43)^+$, 244, and 242, the nature of its fragmentation having no substantial differences from that of kesselringine and its analogs [13, 14].

From its PMR and mass-spectral characteristics, robustamine was assigned to the group of homoproaporphine bases with a ketal grouping that have been isolated from colchicine-containing species of plants [15]. The qualitative and quantitative compositions of the carbon atoms in the ^{13}C NMR spectrum also corresponded to the skeleton of autumn crocus alkaloids with a ketal grouping [16].

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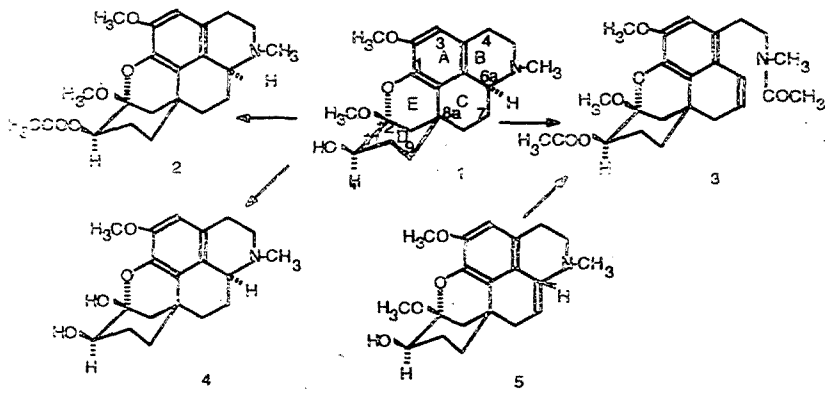
TABLE 1

Alkaloid	N–CH_3	ar.-OCH_3	al.-OCH_3	H-3	H-11(OAc)
	Chemical shifts, ppm				
Robustamine	2.36	3.75	3.32	6.40	4.97
Regeline	2.36	3.74	3.34	6.42	5.17
Regelinine	2.36	3.74	3.32	6.44	5.00
Luteicine	2.73	3.73	3.28	6.43	5.20

Acetylation of the base with acetic anhydride in the presence of a catalytic amount of sulfuric acid led to a monoacetyl derivative (**2**, scheme), which showed the presence of one hydroxy group in it. When the substance was acetylated with acetyl chloride, a N,O-diacetyl derivative (**3**) was obtained. The neutral character of the compound formed showed that, as in other tetrahydroisoquinoline alkaloids, this reaction took place with the opening of the isoquinoline ring. The IR spectrum of (**3**) showed absorption bands of O- and N-acetyl groups.



On being heated in dilute acid, robustamine was hydrolyzed to a demethylated derivative having M^+ 331, with a PMR spectrum showing no signal of an alicyclic methoxy group. On being heated in methanol with a catalytic amount of sulfuric acid the hydrolysis product was reconverted into the initial compound, which showed the location of this methoxy group in the ketal grouping, and the hemiketal nature of the product of the hydrolysis of the base — 12-demethylrobustamine (**4**).

With respect to its elementary composition and developed formula robustamine is identical with three homoproaporphine alkaloids with a ketal grouping — regeline [16], regelinine [17], and luteicine [18], but its IR and PMR spectra differed from those of these compounds, although the chemical shifts of the protons of the N-methyl and methoxy groups and of the single aromatic proton in their PMR spectra were very close. Only the signal of the N-methyl group in the spectrum of luteicine was appreciably shifted downfield.

It was assumed that the difference in the structures of robustamine and the above-mentioned compounds may reside in the asymmetric centers C-6a, C-8a, and C-11. In view of the fact that the pathway for the biosynthesis of the homoproaporphine alkaloids with a ketal gouping lies through dienone compounds [19, 20] the ketalization of which takes place only when there is a methoxy group at C-12, the above-mentioned bases should not differ with respect to the configuration at C-8a. Robustamine, being a levorotatory compound, differs from regeline, regelinine, and luteicine by its configuration at C-6a, and from the last of them also by the position of the ketal grouping.

To determine the configuration of the hydroxy group at C-11 in robustamine, we studied the signal of the H-11 proton geminal to it in the PMR spectra of this base and its analogs. In the spectra of robustamine and the bases mentioned above, in the main, the signal of this proton overlaps the three-proton singlet of a methoxy group in a benzene ring (3.8-4.0 ppm region). In the spectra of their acetyl derivatives the signal of the H-11 proton is shifted downfield by ~1.2-1.4 ppm and, since it appears in a region free from the signals of other protons, can easily be interpreted.

The doublet–doublet splitting of the signal of the H-11 proton in the PMR spectrum of robustamine, as in regelinine, exhibits two spin–spin coupling constants, with J_1 = 11.0 and J_2 = 5.5 Hz. The first of them witnesses the axial–axial arrangement of the methine proton in relation to one of its vicinal partners, and the other shows the axial–equatorial arrangement in relation to the other. The splitting of the H-11 signal with two coupling constants showed the presence of only two protons in the α-positions, i.e., of one methylene group adjacent to it. The nature of the multiplicity of the signal from this methine proton showed its axial orientation in the spirocyclohexane ring *D*. Consequently, the hydroxy group at C-11 is oriented equatorially.

Robustamine differs from regeline, regelinine, and luteicine with respect to the multiplicity of the H-11 signal of the acetyl derivative. At the same time, from the half-width (18 Hz) and multiplicity of the signal of this proton, robustamine is closer to regelinine. It was assumed that these two compounds have identically oriented H-11 protons in their rings *D*, and the slight difference in their signals is due to a difference in the configurations of these bases at the C-6a atom. This was confirmed by the identity of the N,O-diacetyl derivatives obtained from robustamine and from regelinine (**5**).

Thus, it has been established that robustamine has the structure 11-hydroxy-2,12-dimethoxy-1,12-epoxyhexahydrohomoproaporphine (**1**). From the facts given above and by analogy with other homoproaporphine alkaloids [15, 21] the 6aS,8aR,11S,12R configuration is proposed for it.

Robustamine is the first homoproaporphine alkaloid isolated from *Merendera robusta*.

EXPERIMENTAL

For general observations, see [10]. The following solvent systems were used for the chromatographic analysis of the fractions of alkaloids of neutral, phenolic, and acid nature: 1) chloroform—methanol (24:1), in a thin layer of alumina; 2) chloroform—isopropyl alcohol—acetone—benzene (20:4:4:5); and 3) chloroform—isopropyl alcohol—acetone—benzene—acetic acid (15:3:3:3:1), in a thin layer of type LS 5/40 silica gel with 13% of gypsum. Analysis of the fractions of alkali-soluble bases was effected by radial PC on paper from the Leningrad paper mill No. 2 (density 85 g/m^2) with mobile phase 4) *n*-butyl alcohol—5% acetic acid (50:50), and that of the alkali-soluble bases under the same conditions with the mobile phase 5) *n*-butyl alcohol—water (50:50).

Isolation of the Alkaloid Fractions. The dried and comminuted epigeal parts (without seeds) of *Merendera robusta* gathered during the seed-ripening period in the Tamdynskii region, Bukhara province (Kyzylkumy) (4.7 kg) were extracted with methanol, and alkaloid fractions were obtained by our usual method, g (%):

Neutral alkaloids	4.47 (0.11)
Phenolic alkaloids	0.52 (0.01)
Acidic alkalids	0.40 (0.01)
Alkali-insoluble bases	4.00 (0.09)
Alkali-soluble bases	7.42 (0.16)
Total	16.81 (0.38).

The separation of the fraction of alkali-insoluble bases into tropolone and isoquinoline compounds was achieved by the nitrosation method. The crude mixture of alkaloids (3.6 g) was treated with 10% acetic acid (3 × 40 ml). The resinous mass that had not passed into the acid was dissolved in chloroform, and the solution was likewise extracted with acetic acid. The cooled combined acid extract of alkaloids was treated with 7.0 g of sodium nitrite. The resulting precipitate of N-nitrosocolchamine was separated off and washed with water. The acid mother solution and the wash-waters were acidified with sulfuric acid to pH 1 and were extracted with chloroform twice, after which they were made alkaline with ammonia and were again exhaustively extracted with chloroform.

The second chloroform extract was dried, and the solvent was distilled off. The resulting mixture of nontropolone bases (2.1 g) was separated by chromatography on alumina (50 g). The substances were eluted with acetone (I), acetone—chloroform (9:1) (II), (8:2) (III), (7:3) (IV), and (5:5) (V), chloroform (VI), and chloroform—methanol (VII). Fractions (I)-(III) yielded 0.96 g of a base with R_f 0.41 (robustamine), and fraction (VI) 0.12 g of a base with R_f 0.52.

Robustamine, composition $C_{20}H_{27}NO_4$ (mass-spectrometrically), mp 251-252°C (from acetone) and $[\alpha]_D$ −40° (*c* 0.50; $CHCl_3$).

IR spectrum (cm^{-1}): 3500-3300, 2955, 2858, 2800, 1608, 1495, 1470, 1456, 1380, 1273, 1250, 1144, 1100, 1004, 982, 945, 895, 858, 760.

The base was readily soluble in a mixture of methanol and chloroform, moderately soluble in methanol and chloroform separately, sparingly soluble in water and acetone, and insoluble in ether and hexane.

The O-Acetyl Derivative (2). One drop of concentrated sulfuric acid was added to 0.1 g of robustamine in 1 ml of acetic anhydride. After 2 h, the excess of anhydride was eliminated by the addition of methanol and evaporation in vacuum.

A solution of the residue in water was made alkaline with ammonia, and the reaction product was extracted with chloroform. This led to the isolation of O-acetylrobustamine with mp 205-207°C (from acetone).

PMR spectrum ($CDCl_3$, ppm): 6.45 (1H, s, H-3), 3.78 (3H, s, OCH_3), 3.30 (3H, s, OCH_3), 2.38 (3H, s, N-CH_3), 2.06 (3H, s, $OCOCH_3$), 4.97 (1H, m, H-11).

The N,O-diacetyl derivative (3) was obtained by dissolving 0.1 g of (**1**) in 1 ml of acetyl chloride. The excess of the reagent was evaporated off, the residue was dissolved in water, and the reaction product was extracted with chloroform. N,O-Diacetylrobustamine was isolated.

IR spectrum (cm^{-1}): 1740 ($OCOCH_3$), 1680 ($NCOCH_3$).

12-Demethylrobustamine (4). A solution of 0.1 g of the base in 5 ml of 5% sulfuric acid was heated in the water bath for 2 h. The completeness of hydrolysis of the (**1**) was monitored by TLC. After the end of the reaction, the solution was made alkaline with ammonia and extracted with chloroform. The solvent was distilled off, and the hydrolysis product was crystallized from acetone. Compound (**4**) was isolated, with mp 150-152°C and $[\alpha]_D$ −46° (*c* 0.22; $CHCl_3$).

PMR spectrum ($CDCl_3$, ppm): 6.40 (1H, s, H-3), 3.73 (3H, s, OCH_3), 2.36 (3H, s, N-CH_3).

Methylation of (4). A solution of 0.05 g of (**4**) in 3 ml of 5% methanolic hydrogen chloride was boiled for 1 h and the solvent was distilled off. The residue was dissolved in water, and the solution was made alkaline with ammonia and extracted with chloroform.

The reaction product was identified chromatographically and by its PMR spectrum as robustamine. Its spectrum showed a signal of the protons of a ketalic methoxy group (3.32 ppm).

N,O-Diacetylregelinine (3) was obtained from regelinine (**5**) by a method analogous to that for N,O-diacetylrobustamine. From their chromatographic behaviors, these compounds proved to be identical.

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