N-OXIDES OF HOMOAPORPHINE ALKALOIDS FROM MERENDERA RADDEANA

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The alkaloid complex of the leaves, stems, seeds, and seed pods of Merendera raddeana Rgl. (family Liliaceae) has been investigated. A number of known tropolone alkaloids and their photochemical isomers have been isolated together with the homoaporphine bases merenderine, kreysiginine, and O-methylkreysiginine and their N-oxides, which have not been described in the literature. One of them (merenderine N-oxide) has been characterized by its physical constants and has been investigated by IR, PMR, and mass-spectral methods. By a study of the PMR spectrum of the fraction of neutral alkaloids from the leaves of the plant it has been established that it consists mainly of a mixture of equal amounts of colchicine and cornigerine.

The alkaloids of the Merendera raddeana Rgl. have been reported in the literature [1, 2]. A number of tropolone alkaloids and their photochemical isomers have been isolated and identified, and the presence of unknown alkaloids in the plant has also been shown. For one of them (merenderine) the structure of (+)-1,10-dihydroxy-2,11,12-trimethoxyhomoaporphine has been established [3]. The same base, called bechuanine, has been isolated from Iphigenia bechuanica [4].

Continuing a study of the alkaloids of M. raddeana, we have extracted the leaves, stems, and seed pods of the plant. It was found that the leaves and the stems contained the largest amounts of alkaloids in the initial vegetation period (0.37%). At the end of vegetation, the amount of alkaloids, particularly those with a tropolone ring, decreased appreciably (0.22%). Furthermore, both at the beginning and, particularly, at the end of the vegetation period alkaloids containing phenolic hydroxy groups of both neutral and basic nature predominated in the plant.

At the end of the vegetation period the part of the plant containing the most alkaloids became the seeds (0.49%). The main alkaloid in them was colchicine (0.40%) and the amounts both of phenolic and of nonphenolic bases were very low. In the period of ripening of the seeds, the seed pods contained the same amount of alkaloids (0.21%) as the leaves and stems (0.22%). However, the amount of colchicine in them was twice as great and the amount of phenolic bases only half as much as in the leaves and stems.

By chromatographic methods, in the neutral fraction of the alkaloids from the leaves and stems we identified colchicine (I), β-lumicolchicine, and N-formyl-N-deacetylcolchicine and we also detected an unknown compound with R_f 0.23. The latter was present in the leaves and seed pods at the end of the vegetation period. In addition, the presence of cornigerine (II) in this fraction was established. Cornigerine has been isolated by Santavy et al. from a number of species of Liliaceae belonging to various genera, including Colchicum L. and Merendera ramond [5-8]. However, this is the first time it has been detected in a plant growing on the territory of our country. In view of its great closeness to colchicine with respect to its chemical nature and chromatographic mobility [5-8], cornigerine could remain undetected in some of the plants investigated. We detected it with the aid of the PMR spectrum of the neutral fraction of alkaloids from the leaves of the plant.

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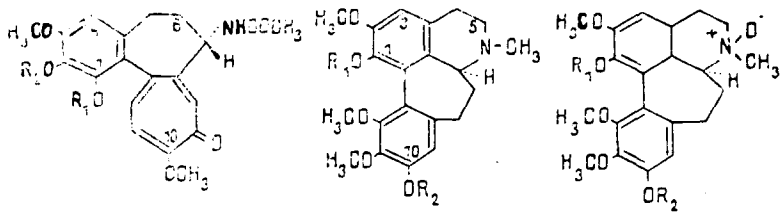

I. $R_1=R_2=CH_3$
II. $R_1+R_2=CH_2$
III. $R_1=R_2=H$
IV. $R_1=H$; $R_2=CH_3$
V. $R_1=R_2=CH_3$
VI. $R_1=H$; $R_2=CH_3$
VII. $R_1=R_2=CH_3$
VIII. $R_1=R_2=H$

The fraction of phenolic alkaloids from M. raddeana consisted of a mixture of approximately equal amounts of 2- and 3-demethylcolchicines (R_f 0.64 and 0.54). A third base, with R_f 0.37 (system 4), was detected only in the seed pods and could not be identified. The acid fractions of the alkaloids contained 2- and 3-demethylcolchiceines, likewise in equal amounts (according to PMR spectra).

The fraction of bases contained four substances: two of them, with R_f 0.74 and 0.79 (system 3), were identified as kreysigine (IV) and O-methylkreysigine (V), which are formed from merenderine by methylation with diazomethane. The other two spots, with R_f 0.62 and 0.68, obviously corresponded to kreysigine and O-methylkreysigine N-oxides (VI and VII), since on the reduction of the fraction of bases with zinc dust in hydrochloric acid these spots disappeared and the intensities of the spots with R_f 0.74 and 0.79 increased. The correctness of this hypothesis was confirmed by showing the identity of the compound having R_f 0.62 and 0.68 with kreysigine and O-methylkreysigine N-oxides obtained by methylating merenderine N-oxide with diazomethane.

The fraction of phenolic bases consisted mainly of merenderine (III) with R_f 0.57. It also contained a base with R_f 0.26 (system 1) in smaller amount, and other minor alkaloids. The presence of the first of them in the plant has been reported previously [2]. By the chromatographic separation of this fraction of alkaloids we succeeded in isolating, in addition to merenderine, a base with R_f 0.26 for which a structure has been established.

The base with R_f 0.26 had the composition $C_{21}H_{25}O_6N$, mp 251-252°C, $[\alpha]_D$ +128°, M 387 (mass-spectrometrically). Its UV spectrum had absorption maxima at 260 and 290 nm ($\log \varepsilon$ 4.16 and 3.77) which are characteristic for the homoaporphine bases of Colchicum species [3, 4]. The IR spectrum of the base showed the absorption bands of a pentasubstituted benzene ring (900-800 cm^{-1}), of methylene groups (1465 cm^{-1}), and of the C=C bonds of a benzene ring (1600 cm^{-1}). The PMR spectrum, taken in CF_3COOH, showed the signals of two aromatic protons, which appeared in the form of one-proton singlets (6.52 and 6.47 ppm), of three O-methyl groups (3.65, 3.59, and 3.46 ppm), and of a N-methyl group (3.18 ppm).

It follows from the facts presented that the base was a homoaporphine compound pentasubstituted in the benzene rings by oxygen functions, two of the positions being occupied by hydroxy groups and three by methoxy groups. The C-1 position in it, as in merenderine, was obviously occupied by a hydroxy group, as was confirmed by the high intensity of the peak of the $(M-17)^+$ ions in its mass spectrum [9]. In such a case, a methoxy group was located in the C-12 position, as was also shown by the upfield chemical shift of the signals of the protons of one of the methoxy groups in the PMR spectrum of the base (3.46 ppm) [10].

The characteristic downfield shift of the signal of the N-methyl group (3.18 ppm) in the PMR spectrum, the low intensity of the molecular ions, and the presence of the peak of a $(M-16)^+$ ion in the mass spectrum of the compound permitted the assumption that the sixth of the oxygen atoms formed the N-oxide group of a base. Furthermore, in contrast to other common, known, aporphine bases, the strongest peak in the mass spectrum was that of the $(M-59)^+$ ion, probably formed by the splitting out of an oxygen atom and of a CH_2-N-CH_3 fragment from the tetrahydroisoquinoline moiety of the base. The high solubility of the base in water and its low solubility in nonpolar organic solvents also indicated the presence of a N-oxide group in it.

On the basis of the assumption that this compound was the N-oxide of a homoaporphine base, we carried out its reduction with zinc dust. This led to the isolation of a tertiary

TABLE 1.

Part of the plant and vegetation period	Weight, g	Fraction, g/%					Sum of the alkaloids, g/%
		neutral	phenolic	acidic	basic	basic-phenolic	
Leaves and stems, flowering	970	0,79 0,08	0,97 0,10	0,26 0,03	0,16 0,02	1,43 0,14	3,61 0,37
Leaves and stems, ripening of the seeds	770	0,21 0,03	0,52 0,07	0,13 0,02	0,10 0,01	0,72 0,09	1,68 0,22
Seeds	116	0,468 0,40	0,058 0,05	0,012 0,01	0,014 0,01	0,018 0,02	0,570 0,49
Seed pods	40	0,025 0,06	0,027 0,07	0,005 0,01	0,011 0,03	0,017 0,04	0,085 0,21

TABLE 2.

Part of the plant and period of vegetation	Compositions of the fractions (% on the sum of the fractions) and Rf values of the spots of the alkaloids (system 1)					
	β-lumicolchicine (0.5)	colchicine (0.30)	N-formyl-N-deacetylcolchicine (0.26)	unidentified alkaloids		
				(0.3)	(0.20) (0.16)	(0.00)
Leaves and stems, flowering	5	70	20	1	1	3
Leaves and stems, ripening of the seeds	25	35	5	20	2	3
Seeds	2	84	13	—	—	1
Seed pods	30	40	Tr.	25	2	3

base which was identified as merenderine (III). Thus, the base corresponded to the structure of merenderine N-oxide (VIII).

EXPERIMENTAL

The alkaloids were extracted from the epigeal part of Merendera raddeana collected in the mountains of Agarats (Armenian SSR). The individuality and authenticity of the substances were checked by thin-layer and paper chromatographies. The former was carried out on glass plates with an unfixed layer of alumina (activity grade II), using the mobile system chloroform–methanol (24:1) (1) or (23:2) (2), and the second with the systems n-butanol–5% acetic acid (1:1) (3) and n-butanol–12% aqueous ammonia (1:1) (4).

The chromatograms on the plates were revealed with iodine vapor and those on paper with a modified Dragendorff reagent. The phenolic compounds were first examined after the action of ammonia vapor on them in a chamber, where they exhibited an intensely yellow coloration. For definitive identification, they were revealed with a 7% solution of ferric chloride [11].

UV spectra were taken in methanol solution on a SF-4A spectrometer, IR spectra in tablets with potassium bromide on a UR-10 double-beam spectrometer, PMR spectra in deuterochloroform on a XL-100 instrument, and mass spectra on a MKh-1330 spectrometer.

The fractions of alkaloids were isolated from the epigeal parts of M. raddeana by methanolic extraction using a procedure described previously [12]. For the amounts of the fractions, see Table 1.

The quantitative compositions of the alkaloid fractions were studied by chromatographic methods, the relative amounts of the individual compounds being estimated roughly from the areas of the spots and the intensities of the colorations.

The compositions of the fractions of neutral nature are given in Table 2.

It was impossible to crystallize colchicine from the fractions of neutral substances of the leaves and stems in the flowering period of the plant. After the separation of the accompanying substances (β-lumicolchicine, N-formyl-N-deacetylcolchicine, etc.), chromatography on alumina by a known procedure gave a fraction of alkaloids with R_f 0.36 corresponding to colchicine, but, unlike colchicine, incapable of being crystallized. The PMR spectrum of the substance differed from that of colchicine. On the basis of spectral characteristics it was assumed that it had a dimeric structure or consisted of a mixture of colchicine and another tropolone alkaloid. A comparative analysis of the PMR spectrum of the substance and literature information on colchicine an dother tropolone alkaloids [6, 13, 14] showed that it consisted of an equimolar mixture of colchicine and cornigerine. Details of the PMR spectrum of this mixture are given below, the protons corresponding to colchicine being marked with the letter c and those correpsonding to cornigerine with n.

PMR spectrum of the mixture of colchicine and cornigerine (in $CDCl_3$, ppm): 6.48 (1H, s, H-4)^c, 6.56 (1H, s, H-4)^n, 6.89 (2H, d, J = 11 Hz, 2H-11)^{c,n}, 7.30 (1H, d, J = 11 Hz, H-12)^n, 7.36 (1H, d, J = 11 Hz, H-12)^c, 7.59 (2H, s, 2H-8)^{c,n}, 3.68 (3H, s, CH_3O-1)^c, 3.82 (3H, s, CH_3O-3)^n, 3.94 (3H, s, CH_3O-3)^c, 3.98 (3H, s, CH_3O-2)^c, 4.04 (6H, s, $2CH_3O$-10)^{c,n}, 6.02 (2H, s, $-OCH_2O-$)^n, 1.98 (6H, s, $2COCH_3$)^{c,n}, 2.45 (8H, m, $4CH_3$)^{c,n}, 4.64 (2H, m, 2H-7), 7.80 (2H, 2NH)^{c,n}.

Merenderine N-Oxide (VIII). The fraction of the phenolic bases from the leaves and stems of M. raddeana (2 g) was separated by chromatography on a column containing 40 g of alumina. Ether–chloroform (9:1) and (2:1) eluted 920 mg of merenderine with R_f 0.57. The eluates obtained from pure chloroform contained mixtures of three substances (R_f 0.52, 0.38, and 0.26). Chloroform–methanol (98:2) and (95:5) eluates contained 240 mg of substance (VIII) with R_f 0.26, mp 251-252°C (from acetone) and $[\alpha]_D$ +125° (c 0.94; methanol).

Mass spectrum (m/z): 387 (M^+, 11%), 371 (53), 370 (17), 369 (11), 354 (89), 340 (42), and 328 (100).

Merenderine N-oxide is readily soluble in water, moderately soluble in methanol, less soluble in chloroform and acetone, and insoluble in ether. In concentrated sulfuric acid it forms a colorless solution. In aqueous solution it gives a faint green color with ferric chloride which disappears on acidification.

Reduction of Merenderine N-Oxide (VIII) to Merenderine (III). A solution of 100 mg of (VIII) in 20 ml of 20% hydrochloric acid was treated with 3 g of zinc dust, and the mixture was shaken for 3 h. After the end of the reaction, the product was isolated in the usual way and was identified by its R_f values and melting point (228-229°C) in comparison with an authentic sample of (III). Compounds (III) and (VIII) had, respectively, R_f 0.49 and 0.58 (system 3) and 0.90 and 0.41 (system 4).

Methylation of Merenderine N-Oxide (VII) to Kreysigenine and O-Methylkreysigenine N-Oxides (VI) and (VII). A saturated solution of diazomethane in petroleum ether was added in very small portions to a solution of 60 mg of (VIII) in 5 ml of methanol until the mixture of mono- and dimethyl ethers – the N-oxides of kreysigine and O-methylkreysigine – had been formed (TLC, system 1).

The reaction products were identical with the compounds having R_f 0.62 and 0.69 (system 3) present in the fraction of bases from the leaves and stems of the plant.

Reduction of the Fraction of Bases. A solution of 50 mg of the fraction of bases from the leaves and stems of the plant, consisting of compounds with R_f 0.62, 0.69, 0.74, and 0.79 (system 3) in 10 ml of 15% hydrochloric acid was treated with 2 g of zinc dust, and the mixture was shaken for 3 h. The solid matter was filtered off and the filtrate was extracted with chloroform. Two substances were detected in the filtrate – with R_f 0.74 and 0.79, these R_f values being identical with those of kreysigine and O-methylkreysigine.

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ALKALOIDS OF ACONITUM COREANUM

VI. STRUCTURE OF ACORIDINE

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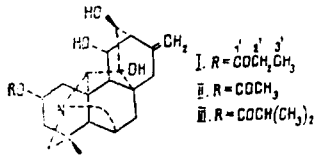
The new C_{20}-diterpene alkaloid acoridine has been isolated from the epigeal part of Aconitum coreanum (Levl.) Rapaics, and the structure of 14-hydroxy-2-propionylhetisine has been established for it on the basis of spectral characteristics. Acoridine is the first diterpene alkaloid esterified by propionic acid.

Continuing a study of the alkaloid composition of the plant Aconitum coreanum (Levl.) Rapaics, we have investigated the epigeal part of the plant collected in the withering phase in the environs of the village of Chernyatino, Maritime Territory. The concentration of alkaloids amounted to 1.05% on the weight of the air-dry raw material. From the total alkaloids we isolated by chromatography 13-acetyl-14-hydroxy-2-isobutyrylhetisine [1] and its N-oxide [2], 14-hydroxy-2-isobutyrylhetisine [3] and its N-oxide [4], and 2-acetyl-14-hydroxyhetisine (acorine) [3], and also an optically active base with mp 204-206°C (I). Base (I) had the composition $C_{23}H_{31}NO_5$ (HRMS).* Its IR spectrum showed absorption bands at 3370 cm^{-1} (OH) and 1730 cm^{-1} (C=O). In its PMR spectrum, signals appeared the chemical shifts and multiplicities of which almost coincided with those of acorine (II) and of 14-hydroxy-2-isobutyrylhetisine (III), with the only difference that the spectrum of (I) contained a quartet at 2.28 ppm (2 H, J = 7.5 Hz) and a triplet at 1.07 ppm (3 H, J = 7.5 Hz) in place of the signals of the protons of an acetoxy group in the spectrum of (II) (δ 1.99 ppm, 3 H) and the signals of the protons of an isobutyryloxy group in the spectrum of (III) (δ 2.45 ppm, 1 H and 1.12 ppm, 6 H). These facts showed that (I) was 14-hydroxy-2-propionylhetisine. We have called the new base acoridine.



*The high-resolution mass spectrum was taken by Yu. M. Mil'grom.

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