2,10-DIDEMETHYLCOLCHICINE – A NEW ALKALOID FROM MERENDERA ROBUSTA

B. Ch. Chommadov, M. K. Yusupov, and Kh. A. Aslanov

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The alkaloid complex of Merendera robusta Bge. (family Liliaceae) growing in the Tashkent province of the Uzbek SSR has been investigated. The main alkaloids of this plant are colchicine and colchamine. Eleven tropolone alkaloids and their photochemical isomers have been isolated and identified. A new alkaloid of phenolic nature has been isolated for which the structure of 2,10-didemethylcolchicine has been established.

A series of investigations has been devoted to Merendera robusta Bge (family Liliaceae). This most widespread and largest colchicine-containing plant of Central Asia has been recommended as a promising source of the valuable tropolone alkaloids colchicine and colchamine [4]. The systematic analysis of the composition of the alkaloids of this plant from various growth sites is therefore of definite interest.

We have studied in detail the epigeal parts of M. robusta gathered in the Gulistan region of Tashkent province. Fractions of alkaloids of neutral, phenolic, acidic, basic, and phenolic-basic nature were obtained from the plant, and these were investigated for the concentrations of individual compounds by paper and thin-layer chromatographies. It was established that the fractions of neutral alkaloids consisted mainly of colchicine (R_f 0.45) with, as impurities very small amounts of N-formyl-N-deacetylcolchicine (R_f 0.36), β- and γ-lumicolchicines (R 0.79 and 0.54), and three unidentified tropolone alkaloids with R_f 0.73, 0.77, and 0.85 (system 1).

The fraction of phenolic alkaloids contained 2-demethyl- and 3-methylcolchicines (R_f 0.86 and 0.76) and their photochemical isomers – 2-demethyl- and 3-demethyl-β-lumicolchichines (R_f 0.92 and 0.80), and also an unidentified tropolone alkaloid with R_f 0.97 (system 2). This is the first time that 3-demethyl-β-lumicolchicine has been detected in Merendera species. The fraction of alkaloids of basic nature consisted mainly of colchamine (R_f 0.78) with, as impurities, small amounts of compounds having R_f 0.10 and 0.90, and 0.94 (system 3), while in the fraction of phenolic bases colchameine (R_f 0.76, main substance), and 2-demethylcolchameine (R_f 0.64, system 3) were detected.

The acid fraction of the alkaloids consisted mainly of one compound which, from its R_f value (0.26 in system 2) and color reaction differed from tropolone compounds of the colchicine series known previously [6, 7]. It must be mentioned that the amount of this fraction of alkaloids in the plant several times exceeded that of the fraction of phenolic substances.

By purification and crystallization from ethyl acetate, the fraction of acid compounds yielded a new alkaloid with the composition $C_{20}H_{21}O_6N$ (mass spectrometrically), mp 259-261°C, $[\alpha]_D$ −230° (c 2.1; $CHCl_3$). Its UV spectrum contained absorption maxima at 246 and 348 nm, which permitted this compound to be assigned to the group of tropolone alkaloids. Its IR spectrum contained the absorption bands of the tropolone carbonyl (1605 cm^{-1}), the amide carbonyl (1675 cm^{-1}), the hydroxy groups (3275 cm^{-1}), and the benzene ring (1600 cm^{-1}) of the colchicine alkaloids. The PMR spectrum showed the signals of four aromatic protons that are characteristic for the tropolone alkaloids: two one-proton singlets [H-4 and H-8 protons (6.51 and 7.55 ppm)] and two one-proton doublets – the H-11 and H-12 protons (7.26 and 7.52 ppm, J = 11 Hz). The signals of the protons of a N-acetyl group were observed at 1.96 ppm and those of two methoxy groups at 3.65 and 3.91 ppm. In addition, there were the signals of the protons of two methylene groups (4 H, m, 2.26 ppm), of two hydroxy groups (2 H, 5.8 ppm), of an amide proton (NH, 7.20 ppm), and of a methine proton (H-7, 4.60 ppm).

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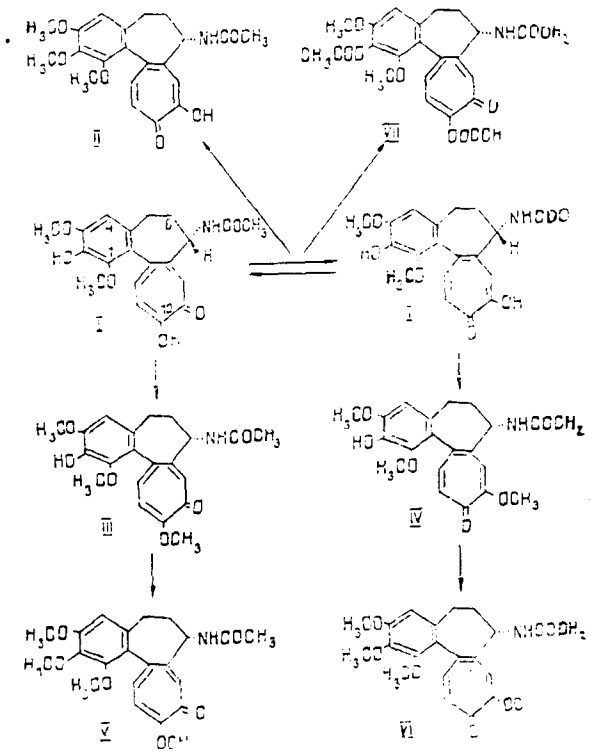
According to the facts given above, the alkaloid corresponded to the developed formula $C_{15}H_9(NHCOCH_3)(OH)_2(CO)(OCH_3)_2$. The mass spectrum, which contained ions with m/z 371 (M^+), 343, 328, 312, 300, 284, and 269, confirmed this formula. When the alkaloid was methylated with methyl iodide in the presence of potassium acetate, a monomethyl ether identical with colchiceine (II, scheme) was isolated. The incomplete methylation of the alkaloid with diazomethane led to a mixture of four compounds. Two of them, being dimethyl ethers, were identified as colchicine (V) and isocolchicine (VI). The other two, containing phenolic hydroxy groups, proved to be monomethyl ethers, and the structures of 2-demethylcolchicine (III) and 2-demethylisocolchicine (IV) have been established for them. This showed that one of the hydroxy groups of the alkaloid was a tropolone hydroxy group and the other was located in the C-2 position of the aromatic ring.

On the basis of the facts given above, the structure of 2,10-didemethylcolchicine (I) has been established for this alkaloid.



The alkaloid was readily acetylated by acetic anhydride, forming a diacetyl derivative. The small angle of rotation, the singlet nature of the signals of aromatic protons, and the large downfield shift of the signal of the H-proton in the PMR spectrum showed [8] that only one of the possible isomers had been formed – that of the normal series diacetyl-2,10-didemethylcolchicine (VII).

Santavy et al. [9, 10] have recorded the isolation of 2,10-didemethylcolchicine (2-demethylcolchiceine) from Gloriosa superba Levin, but this compound was not characterized. Furthermore, the same R_f values were given for (I) and for 3,10-didemethylcolchicine (3-demethylcolchiceine). The priority of the authors mentioned may therefore be disputed.

Two isomers of (I), each with a tropolone ring and two hydroxy groups – 2,3-didemethylcolchicine and 3,10-didemethylcolchicine (as numbered according to the IUPAC rules) – have been reported [7, 9, 10].

EXPERIMENTAL

The compositions of the alkaloid fractions and the individualities of the compounds obtained were studied by the methods of thin-layer and paper chromatographies (TLC and PC). For TLC we used glass plates with a fixed layer of LS 5/40 silica gel with 13% of gypsum and the mobile system chloroform–ethanol–acetone–benzene–25% aqueous ammonia (20:4:4:5:1) (system 1). Radial paper chromatography was conducted on Filtrak No. 2 paper in the

following solvent systems: n-butanol–12% aqueous ammonia (2:1) (system 2) and n-butanol–5% acetic acid (1:1) (system 5). To separate the alkaloids we used alumina for chromatography (activity grade II).

UV spectra were taken on a Beckman model 25 spectrometer in methanol solutions, IR spectra on a double-beam spectrometer in tablets with KBr, PMR spectra on a XL-100 instrument in $CDCl_3$ solutions, and mass spectra on a Varian MAT-311 spectrometer.

Isolation of the Alkaloid Fractions. The epigeal part of the M. robusta collected in the flowering period (2 kg) were extracted five times with methanol. The last two fractions were obtained with heating.

The solvent was distilled off, with the application of vacuum at the end of the process. The concentrated dark brown solution was diluted with water (1 liter) and filtered, and the resinous residue was washed with water and with 3% hydrochloric acid. The weakly acidic aqueous solution filtered from the resin was acidified with hydrochloric acid (1:1) to pH 1 and was extracted with ether to eliminate acidic substances and other ether-soluble products. After this, the acid solution (1.5 liter) was extracted six times with chloroform to separate the neutral-phenolic alkaloids. Then mixtures of basic and phenolic-basic substances were obtained by the alkalinization of the acid solution with ammonia (pH 8-9) and extraction with chloroform.

The chloroform extract of the neutral-phenolic alkaloids, after the bulk of the solvent had been distilled off, was extracted three times with 3% caustic soda solution and was washed twice with water. The chloroform reaction yielded 5.42 g (0.27%) of a neutral fraction of alkaloids. The alkaline and aqueous extracts were combined, were washed twice with small volumes of chloroform, and were saturated with carbon dioxide and extracted with chloroform. As a result, 0.82 g (0.04%) of phenolic alkaloids was isolated. The alkaline solution was then acidified with hydrochloric acid (1:1) to pH 1 and was extracted with chloroform. This gave 2.84 g (0.14%) of the fraction of acidic substances.

The chloroform extract of the basic and phenolic-basic alkaloids was extracted three times with a 3% solution of caustic soda and was washed with water. After this, the chloroform fraction yielded 3.44 g (0.17%) of bases. The combined alkaline and aqueous extracts, after the addition of an excess of ammonium sulfate, were extracted with chloroform, as a result of which 1.18 g (0.06%) of phenolic bases was isolated. The total amount of alkaloids was 13.70 g (0.68%).

In a study of the fraction of neutral alkaloids we found that on silica gel plates the β- and γ-lumicolchicines can be distinguished from tropolone compounds not only by R_f values but also by specific color reactions. In iodine vapor they all showed a brownish-yellow coloration on chromatograms. When the chromatograms were sprayed with a modified Dragendorff reagent, γ-lumicolchicine formed a dark blue spot which, on storage, changed to orange-red. β-Lumicolchicine exhibited an orange coloration with this reagent which then changed to dark graphite. Colchicine and N-formyl-N-deacetylcolchicine were detected only after 5-6 min with the usual orange coloration.

The change in the colors of the spots of the phenolic alkaloids on chromatograms have been described in [11].

2,10-Didemethylcolchicine. The alkaloid is readily soluble in chloroform, soluble in methanol and acetone, and sparingly soluble in ether and water. A methanolic solution of it is colored brownish green by ferric chloride, and on the addition of acids the brownish tinge intensifies. When an acid solution of the alkaloid is heated it becomes greenish brown and a precipitate deposits.

The methyl ether (II) of (I) was obtained by heating 0.06 g of the alkaloid (R_f 0.26), 0.1 g of anhydrous potassium carbonate, and 0.1 ml of methyl iodide in methanol solution. The solvent was distilled off, and the dry residue was extracted with chloroform. A compound was isolated which was identified by its R_f value and IR spectrum as colchiceine (R_f 0.86, system 2).

Dimethyl Ethers (V) and (VII) of (I). The methylation of 0.80 g of (I) in 10 ml of chloroform was performed by the addition of a saturated ethereal solution of diazomethane in small portions until the spot of (I) on a chromatogram (TLC monitoring) had disappeared. The mixture of solvents was distilled off, the residue was dissolved in chloroform, and

the solution was exhaustively extracted with 3% caustic potash solution. The chloroform solution was washed with water, the solvent was distilled off, and the resulting mixture, amounting to 0.22 g, was identified chromatographically as colchicine (V) and isocolchichine (VI) – dimethyl ethers of (I) (R_f 0.49 and 0.33, respectively, in system 1).

Monomethyl Ethers (III) and (IV) of (I). The alkaline extract of the products of the methylation of the alkaloid was treated with chloroform, after which it was acidified with sulfuric acid and extracted with chloroform. The resulting mixture, amounting to 0.54 g, was separated by chromatography on 20 g of alumina. Chloroform–methanol (98:2) eluted a compound with mp 177-178°C (from acetone) which was identified by color reactions, physical constants, and chromatographically as 2-demethylcolchicine (III) [2, 11]. The same mixture in ratios of 98:3 and 98:4 eluted 2-demethylisocolchicine (IV), the structure of which was confirmed by methylation with diazomethane to isocolchicine (VI).

Diacetyl Derivative (VII) of (I). A mixture of 0.20 g of (I), 0.5 g of freshly fused anhydrous sodium acetate, and 1 ml of acetic anhydride was heated at 50°C for 16 h. After cooling, the mixture was diluted with methanol and evaporated, and the residue was extracted with chloroform. The diacetyl derivative was isolated, with mp 220-221°C (from ethyl acetate and ether) and $[\alpha]_D$ –130° (c 0.95; chloroform).

IR spectrum: 1750 cm^{-1} (2 $OCOCH_3$).

LITERATURE CITED

1. G. V. Lazur'evskii and V. A. Maslennikova, Dokl. Akad. Nauk SSSR, 63, 443 (1948).
2. A. S. Sadykov and M. K. Yusupov, Nauchn. Tr. Tashk. Gos. Univ., Estestv. Nauki, No. 203, 15 (1962).
3. J. L. Kaul, B. K. Moza, F. Šantavy, and P. Vrublovsky, Collect. Czech. Chem. Commun., 29, 1689 (1964).
4. A. S. Sadykov, M. K. Yusupov, B. Chommadov, and Kh. Turdikulov, Khim.-farm. Zh., No. 6, 29 (1971).
5. Kh. Turdikulov, M. K. Yusupov, and A. S. Sadykov, Khim. Prir. Soedin., 247 (1972).
6. M. K. Yusupov and A. S. Sadykov, Nauchn. Tr. Tashk. Gos. Univ., 2, No. 286, 56 (1966).
7. B. Chommadov, M. K. Yusupov, and A. S. Sadykov, Khim. Prir. Soedin., 82 (1970).
8. V. Delaroff and P. Rathle, Bull. Soc. Chim. France, No. 6, 1621 (1965).
9. R. S. Thakur, H. Potešilova, and F. Šantavy, Planta Med., 28, 201 (1975).
10. H. Potešilova, L. Hruban, and F. Šantavy, Collect. Czech. Chem. Commun., 41, 3146 (1976).
11. A. S. Sadykov, M. K. Yusupov, and B. Chommadov, Rast. Res., 5, 441 (1969).