to dissolve the ingredients [6]. A 0.05% solution of strophanthidin acetate was prepared in the following way. To a mixture of 25 ml of 95% ethanol and 37.5 ml of glycerol was added 0.125 g of strophanthidin acetate and the resulting mixture was stirred at 80°C (in a water bath). After dissolution was complete, the volume was made up to 220 ml with buffer solution having the appropriate pH value (4.0-5.0). After careful stirring and filtration through a mushroom-type filter the solution was distributed into 1-ml neutral-glass ampuls, which were sealed and were sterilized at 100°C for 30 min.

The stability of the strophanthidin acetate was studied under conditions of "accelerated aging" at an elevated temperature (60°C).

SUMMARY

1. A method has been developed for obtaining stable 0.05% injection solutions of the cardiac glycoside strophanthidin acetate in 1-ml ampuls. It has been established that the optimum pH is 4.0-5.0.

2. The use of citrate–phosphate or acetic-acid–acetate buffers has been proposed for stabilizing strophanthidin acetate.

LITERATURE CITED

1. K. N. Khodzhaev, R. I. Shamsutdinov, G. L. Genkina, T. T. Shakirov, R. Sh. Yamatova, M. B. Gorovits, N. K. Abubakirov, and S. S. Azizova, USSR Inventor's Certificate No. 487,881; Byull. Izobret., No. 38, 57 (1975).
2. K. N. Khodzhaev, R. I. Shamsutdinov, G. L. Genkina, T. T. Shakirov, R. Sh. Yamatova, M. B. Gorovits, N. K. Abubakirov, and S. S. Azizova, USSR Inventor's Certificate No. 487,882; Byull. Izobret., No. 38, 58 (1975).
3. E. I. Zatula, F. A. Konev, N. A. Bugrim, and N. V. Tinoshchenko, Farmatsiya, No. 2, 23 (1977).
4. E. I. Zatula and N. A. Bugrim, Med. Prom. SSSR, No. 12, 35 (1964).
5. State Pharmacopoeia of the USSR [in Russian], IXth ed., Moscow (1961), p. 705.
6. State Pharmacopoeia of the USSR [in Russian], Xth ed., Moscow (1968), p. 108.

ALKALOIDS OF *Haplophyllum latifolium*

THE STRUCTURE AND SYNTHESIS OF HAPLAMIDE AND HAPLAMIDINE

E. F. Nesmelova, I. A. Bessonova, and S. Yu. Yunusov

UDC 547.944/945

The plant *Haplophyllum latifolium* Kar. et Kir. (family Rutaceae) is found in Uzbekistan [1], Kirghizia [2], and Kazakhstan [3]. It has been shown previously that it contains alkaloids [4]. We have investigated the alkaloids of the epigeal part of this plant collected by R. S. Sakhibiddinov in the Fergana valley of KirgSSR (basin of the R. Kugart, village of Mikhailovka) among the wheat crops in the flowering period on May 14, 1974. The raw material was extracted with methanol. The extract obtained was separated into basic, acidic, and neutral fractions. A study of the basic and acidic fractions showed that they contained no alkaloids. By chromatography on alumina, the neutral fraction gave a crystalline mixture (0.05% on the weight of the dry plant) from which we have isolated alkaloids that we have called haplamide and haplamidine.

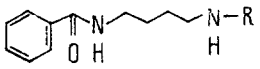
On the basis of spectral characteristics we have previously put forward the structure of N,N'-dibenzoylputrescine for haplamine (I) [5]. The correctness of formula (I) was confirmed by the synthesis of N,N'-dibenzoylputrescine, which was performed by the reaction between putrescine, obtained by the action of bromine and alkali on adipic acid diamide [6], and benzoyl chloride [7]. In its melting point, spectral characteristics, and TLC behavior

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 749-752, November-December, 1978. Original article submitted June 16, 1978.

0009-3130/78/1406-0637$07.50

the synthetic dibenzoylputrescine was identical with haplamide. Consequently, haplamide has the structure (I).

Haplamidine (II) is a new compound differing from haplamide by a CH=CH group. As the result of a study of its spectral characteristics and a comparison of them with those of haplamide, the structure of N-benzoyl-N'-cinnamoylputrescine has been suggested for haplamidine [8]. We have confirmed this structure by the synthesis of (II). As the starting material we used the dibenzoylputrescine that we had synthesized, from which, by alkaline cleavage, we obtained monobenzoylputrescine (III) [7]. The reaction of the latter with cinnamoyl chloride, prepared from cinnamic acid [9], formed N-benzoyl-N'-cinnamoylputrescine, which was identical with natural haplamidine.



I. $R = COC_6H_5$

II. $R = COCH{=}CHC_6H_5$

III. $R = H$

By the method described above we also investigated the epigeal part of *H. latifolium* collected by K. Taidzhanov in the flowering–beginning of fruit-bearing phase on May 26, 1975, in the Alchamaidan area (Fergana range, Mount Baubash-ata, KirgSSR). A mixture of alkaloids (0.04% on the weight of the dry plant) consisting of haplamide and haplamidine was again obtained only from the neutral fraction of the methanolic extract.

Thus, the alkaloid composition of the epigeal part of *H. latifolium* collected from different sites at different times within Kirghizia proved to be very similar both qualitatively and quantitatively. However, the samples investigated do not contain the furanoquinoline alkaloids that are characteristic of this plant [4, 10].

EXPERIMENTAL

For general observations, see [11], the homogeneity of the substances was determined by chromatography in a thin layer of silica gel in the systems 1) toluene–ethyl acetate–formic acid (5:4:1), and 2) benzene–methanol (4:1). The spots were revealed with Dragendorff's reagent.

Isolation and Separation of the Total Alkaloids. The dry comminuted epigeal part (5100 g) collected on May 14, 1974, was extracted with methanol. The concentrated methanolic extract was treated with chloroform. The chloroform solution was extracted with 10% sulfuric acid and then with 4% caustic soda solution. The acid and alkaline extracts contained no alkaloids. The residue obtained after the chloroform had been distilled off was chromatographed on alumina. Ether–chloroform (9:1) eluates gave a black-green crystalline mixture (2.72 g) which was dissolved in ethanol and boiled with activated carbon. The ethanolic solution was passed through silica gel. The crystals obtained from the ethanolic eluates were rechromatographed on silica gel. Ethyl acetate eluates yielded successively crystals with mp 166-167°C (I) and 139-140°C (II).

Haplamide (I), after chromatographic purification, was crystallized repeatedly from ethanol; mp 172-173°C, $[\alpha]_D^{20}$ ±0° (c 0.1; pyridine).

Haplamidine (II) formed a white crystalline substance with mp 139-140°C (ethanol).

Synthesis of Haplamide and Haplamidine. Dibenzoylputrescine. In portions, with stirring, bromine (1.06 ml) and adipic acid diamide (1.44 g) were added to a solution of caustic soda (3.2 g) in water (20 ml) at 14°C. The mixture was heated for 15 min and cooled, and a solution of caustic potash (3 g in 3 ml of water) was added. With shaking, benzoyl chloride (3.5 ml) was added to the reaction mixture. A precipitate deposited, which was chromatographed on alumina. The first ethereal eluates yielded crystals (1.8 g) with mp 177°C (ethanol). UV spectrum: $\lambda_{max}^{ethanol}$ 226 nm (log ε 4.42); λ_{min} 214 nm (log ε 4.26). IR spectrum, cm^{-1}: 3330, 1634 (NH–CO). Mass spectrum, m/e, %: 296 (6, M^+), 191 (8), 175 (11), 162 (11), 148 (10), 105 (100), 77 (45). A mixture with haplamide gave no depression of the melting point.

Monobenzoylputrescine. A mixture of 8.8 g of dibenzoylputrescine with 132 ml of 89% ethanol and 44 ml of 50% aqueous caustic soda was boiled for 4 h. Then the mixture was cooled, the crystals of the starting material that deposited (6.1 g) were filtered off, the ethanol was distilled off, and the aqueous solution was extracted with chloroform. The

chloroform extract, carefully dried over sodium sulfate, was concentrated and chromatographed on alumina. Dibenzoylputrescine (0.22 g) was obtained from the first chloroform eluates, and a mixture of dibenzoylputrescine and monobenzoylputrescine from the subsequent ones. Chloroform–ethanol (9:1) eluates gave monobenzoylputrescine (0.22 g) in the form of an oil that crystallized on standing. Repeated chromatography on alumina and silica gel yielded crystals with mp 125-132°C (methanol), readily soluble in water, less readily in ethanol and methanol, and sparingly in chloroform.

UV spectrum: λ_{max} 224 nm (log ε 4.00), λ_{min} 215 nm (log ε 3.89). IR spectrum: 3600-3050 cm^{-1} (broad maximum, NH and NH_2), 1650 cm^{-1} (amide carbonyl). Mass spectrum, m/e (%): 192 (2, M^+), 163 (22), 162 (7), 148 (12), 135 (13), 134 (28), 105 (100), 77 (67). NMR spectrum (CF_3COOH), ppm: 1.79 (1 H, broadened signal, NH); 2.50-2.95 (5 H, multiplet, aromatic protons); 3.54 (2 H, broadened signal, NH_2); 6.65 (2 H, broadened signal, CO–NH–CH_2); 7.05 (2 H, broadened signal, –CH_2–NH_2); 8.44 (4 H, broadened singlet, C–CH_2–CH_2–C).

N-Benzoyl-N'-cinnamoylputrescine. When solutions of monobenzoylputrescine (0.08 g) in chloroform (1 ml) and of cinnamoyl chloride (0.07 g) in chloroform (1 ml) were mixed, crystals deposited (0.12 g) and these were chromatographed on a column of silica gel. From chloroform–ethanolic eluates crystals of N-benzoyl-N'-cinnamoylputrescine (0.04 g) deposited with mp 139-140°C. UV spectrum, nm: λ_{max} 218, 224, 273, 300, inflection (log ε 4.31, 4.34, 4.13, 3.69); λ_{min} 214, 220, 255 (log ε 4.24, 4.30, 4.04). IR spectrum, cm^{-1}: 3320, 1631 (NH–CO). Mass spectrum, m/e (%): 322 (7, M^+), 201 (25), 191 (11), 175 (32), 174 (38), 162 (12), 160 (5), 148 (12), 146 (7), 131 (50), 105 (100), 103 (24), 77 (30). A mixture with haplamidine gave no depression of the melting point.

The isolation and separation of the alkaloids of the epigeal part of a plant collected on May 26, 1975 (7 kg) was carried out in the same way as described above. This gave 2.8 g of a crystalline mixture of haplamide and halpamidine.

SUMMARY

The alkaloids haplamide and haplamidine have been isolated from the epigeal part of the plant *Haplophyllum latifolium* growing in Kirghizia. It has been shown that haplamide has the structure of N,N'-dibenzoylputrescine and haplamidine is N-benzoyl-N'-cinnamoylputrescine.

LITERATURE CITED

1. Flora of Uzbekistan [in Russian], Vol. IV, Tashkent (1959), p. 74.
2. Flora of the Kirghiz SSR [in Russian], Vol. VII, Frunze (1957), p. 503.
3. Flora of Kazakhstan [in Russian], Vol. VI, Alam-ata (1963), p. 58.
4. E. F. Nesmelova and G. P. Sidyakin, Khim. Prirodn. Soedin., 584 (1973).
5. E. F. Nesmelova, I. A. Bessonova, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 289 (1977).
6. C. Buehler and D. Pearson, A Survey of Organic Syntheses, Wiley-Interscience, New York (1970).
7. A. A. Ryabinin and E. M. Il'ina, Dokl. Akad. Nauk SSSR, 76, 689 (1951).
8. E. F. Nesmelova, I. A. Bessonova, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 427 (1977).
9. Preparative Organic Chemistry [in Russian], Moscow–Leningrad (1964), p. 437.
10. E. F. Nesmelova, I. A. Bessonova, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 666 (1975).
11. V. I. Akhmedzhanova, I. A. Bessonova, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 320 (1976).