ALKALOIDS OF *Glaucium fimbrilligerum*. II

S. U. Karimova, I. A. Israilov,
F. Vezhnik, M. S. Yunusov,
Yu. Slavik, and S. Yu. Yunusov

UDC 547.943

The alkaloids of the epigeal part and roots of the plant *Glaucium fimbrilligerum* have been studied. A total of 18 alkaloids has been isolated, of which epiglaufidine proved to be new, and its structure has been established. Columbamine has been isolated from the genus *Glaucium* for the first time, and magnoflorine and stilopine α-hydroxymethylate from this species of plant for the first time.

Continuing an investigation of *Glaucium fimbrilligerum* from various growth sites [1], we have studied the alkaloid composition of the epigeal part and roots of this plant collected in the Tashkent province at the end of flowering and beginning of fruit-bearing.

The chloroform extraction of the epigeal part yielded 0.28% of total alkaloids. The combined ethereal material was separated into phenolic and nonphenolic fractions. The nonphenolic fraction of alkaloids yielded dihydrosanguinarine, sanguinarine, chelerythrine, corydine, protopine, and allocryptopine [2], and the phenolic fraction yielded scoulerine, corydine, glaufidine, isocorytuberine, norisocorydine, isoboldine, and reticuline [1], and also a new base (I). The quaternary fraction of the total material yielded magnoflorine [3], corytuberine [4], stilopine α-hydroxymethylate [5], and columbamine [3]. All the known alkaloids isolated were identified by direct comparison with authentic samples and, in the case of columbamine, by conversion into isocorypalmine [1].

Base (I) is optically active, $[\alpha]_D$ +198° (c 0.5; methanol) and amorphous. The UV spectrum contains absorption maxima at 224, 270, and 305 nm (log ε 4.23, 3.72, and 3.37), which are characteristic for 1,2,10,11-tetrasubstituted aporphine alkaloids [6]. The mass spectrum of (I) has the peaks of ions with m/z 357 (M^+, 100%), 356 (M − 1.15%), 342 (M − 15, 60%), 340 (M − 17, 40%), 326 (M − 31, 90%), 314 (M − 43, 45%), 285 (M − 72, 40%), 178.5 (M^{++}), which also shows that the base belongs to the aporphine alkaloids of the corydine type. The NMR spectrum of (I) taken in $CDCl_3$ shows the signals of protons at (ppm) 2.50 (3 H, s, N—CH_3); 3.63 (3 H, s, OCH_3); 3.82 (6 H, s, 2 OCH_3); 6.75 and 6.92 (doublets, 1 H each, J = 8 Hz); 7.04 (1 H, s); and 3.0-4.0 (m, 5 H), in addition to which a one-proton multiplet with a half-width of 15 Hz can be seen at 4.93 ppm.

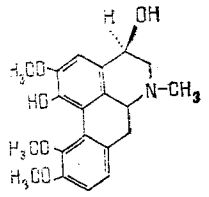
The spectral characteristics given are close to those of the pentasubstituted apophine alkaloid glaufidine with an alcoholic hydroxy group at C_4 [7].

The acetylation of (I) gave a diacetyl derivative (II), confirming the presence of two hydroxy groups. The NMR spectrum ($CDCl_3$) of (II) contains signals in the form of three-proton singlets of two acetoxy groups at 2.08 and 2.19 ppm, from an N-methyl group at 2.58 ppm, and from three methoxy groups at 3.40, 3.76, and 3.81 ppm; signals of three aromatic protons at 6.70-6.69 ppm and multiplets from five protons in the 3.0-3.5 ppm region, and also a one-proton multiplet with a half-width of 15 Hz at 6.12 ppm from a proton geminal to a hydroxy group.

Under the action of PCl_3 followed by Clemmensen reduction, base (I) was converted into corydine. This confirms the identical positions of the substituents in the aromatic rings of glaufidine and (I). Thus, (I) is 1,4-dihydroxy-2,10,11-trimethoxyaporphine. The appearance of the signal of the geminal proton at C_4 in the form of a multiplet with W = 15 Hz shows the β orientation of the hydroxy group [8, 9]; consequently, base (I) is epiglaufidine and has the following structure:

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Purkinje University, Brno, Czechoslovakia. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 493-496, July-August, 1983. Original article submitted June 3, 1982.

 0009-3130/83/1904-0464$07.50



The methanolic extraction of the roots of *G. fimbrilligerum* gave 0.15% of total alkaloids. The nonphenolic fraction of the ethereal total gave sanguinarine, chelerythrine, corydine, protopine, and allocryptine, and the phenolic fraction yielded corydine, glaufidine, isocorytuberine, and glaufine. The quaternary fraction of the roots contained magnoflorine, corytuberine, stilopine hydroxymethylate, and columbamine.

EXPERIMENTAL

For chromatography we used type KSK silica gel. The individualities of the individual alkaloids were checked by TLC in the following solvent systems: 1) benzene–ethanol (9:1); 2) chloroform–ethanol (9:1); 3) chloroform–methanol (4:1); 4) methanol–water–ammonia (15:3:1); and 5) methanol–water–acetic acid (15:3:1).

UV spectra were taken on a Hitachi spectrophotometer in ethanol, IR spectra on a UR-20 spectrometer (tablets with KBr), mass spectra on a MKh-1303 instrument, and NMR spectra in $CDCl_3$ on a JNM-4H-100/100 MHz instrument with HMDS as internal standard (δ, scale).

Isolation and Separation of the Total Alkaloids from the Epigeal Part. The air-dry ground plant *G. fimbrilligerum* (7.05 kg) was moistened with 6% ammonia solution and extracted with chloroform eight times. The combined and concentrated extracts were treated with 10% sulfuric acid. The acid solution was washed with ether and, with cooling, was made alkaline with 25% ammonia. The alkaline solution was shaken with ether (A) and then with chloroform. The chloroform extract was evaporated to dryness, giving 1.52 g of combined alkaloids (B). The alkaline solution was acidified with 10% sulfuric acid to pH 3-4, and a saturated solution of potassium iodide was added. After this, the solution was shaken with chloroform (5 × 0.5 liter). The combined chloroform extract was evaporated. This gave a fraction of iodides (I) of quaternary bases (0.2245 g).

The ethereal extracts (A) after concentration were treated with 0.1 N KOH solution. The ethereal layer was dried with sodium sulfate and evaporated, giving 17.46 g of ethereal nonphenolic bases (A_1). The alkaline solution was acidified to pH 5-6, alkalinized with ammonia solution, and extracted with ether and then with chloroform. Both extracts, after drying, were evaporated to dryness. This gave 1.66 g of ethereal phenolic bases and 0.10 g of chloroformic phenolic bases, which proved to be chromatographically identical and were combined (A_2 1.76 g).

Fraction A_1 was treated with methanol. This gave a mixture of crystals (9.43 g) from which, by fractional crystallization from methanol–chloroform, were isolated protopine (4.7 g), allocryptonine (3.5 g), and corydine (0.91 g).

Mother solution A_1 (8.03 g) and the combined material A_2 were separated by chromatography on a column of silica gel (1:30) as described in [1]. In this way, A_1 yielded 0.052 g of dihydrosanguinarine, 0.022 g of sanguinarine, 0.024 g of chelerythrine, 4.7 g of corydine, 0.30 g of protopine, and 1.52 g of allocryptopine, and A_2 yielded 0.23 g of isocorypalmine, 0.024 g of scoulerine, 0.83 g of corydine, 0.12 g of glaufidine, 0.052 g of epiglaufidine, 0.092 g of isocorytuberine, 0.02 g of norcorydine, 0.035 g of isoboldine, and 0.045 g of reticuline.

Combined materials B, on treatment with acetone, yielded 0.5 g of corydine.

Treatment of fraction I with methanol gave 23 mg of columbamine. The mother liquor (0.16 g) was chromatographed on a column of silica gel. The alkaloids were eluted with chloroform–methanol mixtures of various polarities. From the fractions eluted with the (95:5) mixture 3.2 mg of stilopine hydroxymethylate and 1.0 mg of magnoflorine were isolated. The (4:1) and (2:1) fractions yielded 14 mg of corytuberine.

Magnoflorine (iodide). This had mp 263-265°C.

Stilopine α-Hydroxymethylate. This had mp 265-266°C.

Columbamine (iodide). This had mp 226-227°C.

Reduction of Columbamine. A mixture of 10 mg of columbamine and 15 mg of sodium tetrahydroborate in methanol was stirred at room temperature for 10 min. The solvent was evaporated off, the dry residue was dissolved in 5% sulfuric acid, the solution was made alkaline with concentrated ammonia, and the reaction product was extracted with chloroform. After the solvent had been distilled off, a product was obtained which was identical with isocorypalmine (melting point, mass spectrum, TLC).

O,O-Diacetylepiglaufidine. A solution of 20 mg of epiglaufidine in 5 ml of acetic anhydride and 1 ml of pyridine was left at room temperature for a day. The solvent was evaporated off to dryness, the residue was dissolved in 5% sulfuric acid, the solution was made alkaline with 25% ammonia, and the reaction product was extracted with ether. The extract was evaporated, to give amorphous O,O-diacetylepiglaufidine. Mol. wt. 441 (mass spectrometrically).

Passage from Epiglaufidine to Corydine. A solution of 20 mg of the base in 3 ml of PCl_3 was boiled for 2 hours. Then 3 ml of water and concentrated ammonia to pH 8-9 were added to the mixture and the reaction product was extracted with chloroform. After the solvent had been distilled off, the residue was dissolved in 10% sulfuric acid, and zinc dust was added. The mixture was boiled on the water bath for 4 h. After cooling, it was filtered, and the filtrate was made alkaline with ammonia and extracted with chloroform. When the solvent was distilled off a product identical with corydine (melting point, mass spectrum, TLC) was obtained.

Isolation and Separation of the Total Alkaloids from the Roots. The comminuted dry roots (380 g) were extracted ten times with methanol. The concentrated methanolic extract was dried, and the residue was dissolved in 10% sulfuric acid. The acid solution was washed with ether and was then worked up by the method described above, giving 0.593 g of combined alkaloids (0.407 g of fraction A_1, 0.123 g of A_2, and 0.063 g of B), and 0.33 g of iodide fraction.

Treatment of fraction A_1 with methanol gave a mixture of crystals (0.18 g), from which protopine (0.021 g), allocryptopine (0.082 g), and corydine (0.067 g) were isolated by fractional crystallization. Mother liquor A_1 (0.23 g) and combined material A_2 were separated as described above. From A_1 were obtained sanguinarine (21 mg), chelerythrine (25 mg), corydine (100 mg), protopine (12 mg), and allocryptopine (22 mg), and from A_2 corydine (34 mg), glaufidine (22 mg), isocorytuberine (21 mg), and glaufine (5 mg).

The combined iodides were treated with methanol giving 68 mg of magnoflorine. The mother liquor after the separation of the magnoflorine (0.246 g) was chromatographed on a column of silica gel. The eluents used consisted of mixtures of chloroform and methanol in various proportions, and the (97:3) and (95:5) fractions yielded 1.0 mg of stilopine hydroxymethylate. The (9:1) and (4:1) fractions yielded 19 mg of corytuberine and 2 mg of columbamine.

SUMMARY

The alkaloids of the epigeal part and the roots of the plant *Glaucium fimbrilligerum* have been studied. A total of 18 alkaloids was isolated, of which epiglaufidine proved to be new, and its structure has been established. This is the first time that columbamine has been isolated from the genus *Glaucium* and the first time that magnoflorine and stilopine α-hydroxymethylate have been isolated from this species of plant.

LITERATURE CITED

1. S. U. Karimova, I. A. Israilov, M. S. Yunusov, and S. Yu. Yunusov, Khim. Prir. Soedin., 224 (1980).
2. R. A. Konovalova, S. Yu. Yunusov, and A. P. Orekhov, Zh. Obshch. Khim., 9, 1939 (1939).
3. A. Karimov, M. V. Telezhenetskaya, K. L. Lutfullin, and S. Yu. Yunusov, Khim. Prir. Soedin., 433 (1975).
4. A. Kh. Yunusov, I. A. Israilov, and M. S. Yunusov, Khim. Prir. Soedin., 681 (1973).
5. M. Alimova, I. A. Israilov, M. S. Yunusov, and S. Yu. Yunusov, Khim. Prir. Soedin., 874 (1979).

6. A. W. Sangster and K. L. Stuart, Chem. Rev., 65, 69 (1965).
7. I. A. Israilov, S. U. Karimova, M. S. Yunusov, and S. Yu. Yunusov, Khim. Prir. Soedin., 104 (1979).
8. O. Hoshino, H. Hara, M. Ogawa, and B. Umezawa, Chem. Pharm. Bull., 23, 2578 (1975).
9. H. Guinaudeau, M. Leboeuf, M. Debray, A. Cave, and R. R. Paris, Planta Med., 27, 304 (1975).

POTENTIOMETRIC ANALYSIS OF MIXTURES OF AMINO ACIDS WITH THEIR N-tert-BUTOXYCARBONYL DERIVATIVES IN MIXED SOLVENTS

T. Niyazkhonov, A. Ya. Veveris, and L. B. Kuznetsova

UDC 543.257:547.466

Potentiometric methods are proposed for the quantitative analysis of a mixture of a number of amino acids with their N-tert-butoxycarbonyl derivatives in mixed solvents. These methods can be used for the analysis of industrial samples of N-tert-butoxycarbonyl derivatives of amino acids.

N-tert-Butoxycarbonyl derivatives of amino acids, BOC-AAs, are a comparatively new class of derivatives of amino acids, AAs. They are obtained by replacing a hydrogen atom of the amino group of an AA by a N-tert-butoxycarbonyl group ($-\overset{\overset{O}{\|}}{C}-O-C(CH_3)_3$). BOC-AAS are used as protective groups in peptide synthesis, in increasing the molecular mass of biopolymers, in the production of physiologically active biochemical preparations, etc. [1, 2]. Reactions with the participation of BOC-AAs are performed in aqueous organic and organic solvents, and therefore the investigation of their acid–base properties in these media is of theoretical and practical interest. BOC-AAs are soluble in glacial acetic acid, dimethyl sulfoxide, dimethylformamide, and aliphatic nitriles and, with gentle heating, in water; on heating above 50°C, the action of strong acids and bases leads to the splitting out of the BOC group, which complicates the quantitative determination of the BOC-AAs.

There is information on the determination of individual amino acids and the analysis of their mixtures by acid–base titration of the NH_2 groups in protogenic solvents [3-5]. However, these solvents are unsuitable for the analysis of mixtures of BOC-AAs and AAs, since the majority of BOC-AAs do not contain NH_2 groups and, consequently, cannot be titrated as bases. The possibility of the differential titration of mixtures of BOC-AAs and AAs has scarcely been considered. There is likewise no information in the literature on the study of the acid–base properties of BOC-AAs and the values of their dissociation constants.

We have determined the dissociation constants, pK_a, of BOC-AAs in water and acetonitrile (AN) by Henderson's method using benzoic acid as standard:

	H_2O	AN
BOC-Glycine	3.74	20.44
BOC-Leucine	3.76	21.88
BOC-Valine	3.68	20.61
BOC-Serine	3.62	22.05
BOC-Glutamine	3.66	22.13
BOC-Proline	3.42	19.96
BOC-β-Phenyl-α-alanine	—	20.81
BOC-Tryptophan	3.62	21.96

As we see, in water, BOC-AAs exhibit approximately the same acidic properties and are stronger acids than benzoic. In AN, some differentiation of the acid properties of the BOC-AAs is observed: $\Delta pK_{(AN)}$ amounts to 1.5-2.0 units, in contrast to $\Delta pK_{(H_2O)}$, which is 0.25-0.3 units.

D. I. Mendeleev Moscow Institute of Chemical Technology. "Biokhimreaktiv" Scientific-Production Cooperative, Olaine. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 496-499, July-August, 1983. Original article submitted June 27, 1982.

0009-3130/83/1904-0467$07.50