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ALKALOIDS OF GENTIAN

T. U. Rakhmatullaev, S. T. Akramov, and S. Yu. Yunusov

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We gathered Gentiana olgae Rgl. et Schmalh. in the eastern part of Alai (Karagai-Tugai) in the flowering period (August 20). Its epigeal part contains 0.31% of total alkaloids on the weight of the dry plant from which we isolated three bases: two known ones—gentiananine and gentianadine [2] and one new one—with mp 149–150° C (acetone). The latter is readily soluble in water, methanol, and ethanol, and sparingly soluble in ether, chloroform, acetone, and benzene; it gives no precipitate with silicotungstic acid and is not shown up by Dragendorff's reagent and by iodine. It is a weak optically inactive base and does not titrate with acids. Its empirical composition—$C_6H_7O_3N$—was confirmed by the presence in its mass spectrum of the molecular ion with m/e 141.

The physicochemical properties of the base differ from those of alkaloids described in the literature. We have named it gentianaine. The substance forms an O-acetyl derivative of mp 164–165° C with difficulty. The IR spectrum of gentianaine has absorption bands at 1640 cm^{-1} (amide carbonyl), 1718 cm^{-1} (carbonyl group), and 3260 and 3390 cm^{-1} (active hydrogen). The presence of a carbonyl group is shown by the formation of a 2,4-dinitrophenylhydrazone. The carbonyl group in gentianaine is an aldehyde group giving the silver mirror reaction with silver oxide in an ammoniacal medium. Gentianaine decolorizes bromine water and its IR spectra has a band at 1690 cm^{-1} which shows the presence of a double bond in its molecule.

The UV spectrum of gentianaine [λ_{max} 231, 238 mμ (log ε 4.1, 4.16, respectively)] is similar to the spectra of the α-pyridones [3, 4] (Fig. 1). On the basis of the above information, the following developed formula may be written for gentianaine:

$$C_4H_4(-NH-CO-)(-OH)(-CHO)(=).$$

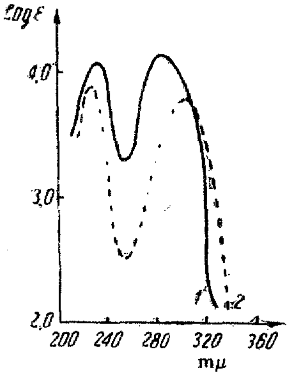
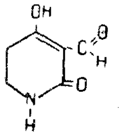


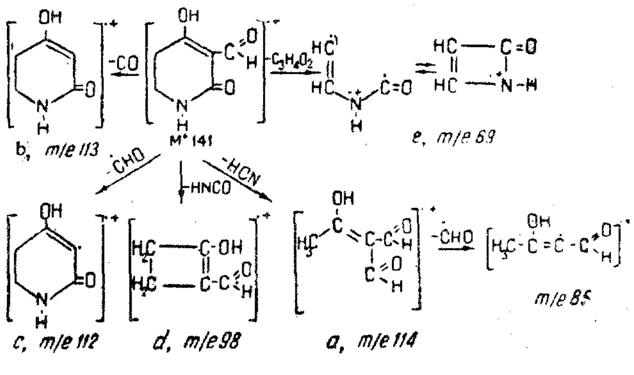
Fig. 1. UV spectrum of gentianaine (1) and of 6-methyl-α-pyridone (2).

Taking into account the physicochemical properties found in our study of the structure of gentianaine, and also biogenetic considerations on the arrangement of the functional groups, the following most probable structure (I) may be proposed for it:



I

A mass spectrometric study of the fragmentation of gentianaine has shown that the ionization of the molecule probably takes place through the loss of one electron of the unshared pair of electrons of the nitrogen atom; a fairly stable peak of the molecular ion with m/e 141 corresponding to 85% of the maximum peak (Fig. 2, a) is formed, as a result of which α- and β-ruptures of a C—C bond take place with the formation of the maximum peak of an ion with m/e 69. The mass spectrum also has peaks with m/e 114, 113, 112, and 98, which are formed from the molecular ion with the elimination of HCN, CO, CHO, and HNCO groups, respectively. In the case of the deutero analogs of gentianaine (Fig. 2, b) we observed peaks with m/e 142 and 143 appearing because of the replacement, respectively, of one and two atoms of hydrogen by deuterium in the —OH and >NH groups. The sequence of fragmentation of the deutero analog of gentianaine remained the same, but the m/e ratios of the fragments formed were one unit greater than for gentianaine itself (table).



Continuing the separation of the mother liquors from the total alkaloids of Gentiana turkestanorum Gand. and Gentiana olivieri Griseb [2], we again isolated gentianaine.

Gentiana caucasica M. B. The plant was collected by S. Yu. Yunusov in the flowering phase in the Kislovodsk region. The usual chloroform extraction of the epigeal part of this plant yielded 0.91% of total alkaloids. Chromatography of these on paper showed the presence of two bases: with R_f 0.71 (UV light) and 0.79 (Dragendorff's reagent); butanol–acetic acid–water (20 : 1 : 20) system. The separation of the total alkaloids yielded gentianaine [1] and gentiananine [2].

Substance	M^+	a	b	c	d	e
Gentianaine	141	114	113	112	98	69
Deutero analog of gentianaine	143	115	114	—	99	70

Gentiana kaufmanniana Rgl. et Schmalh. The plant was collected in the flowering period in the region of the Trans-Alai range (Laisu, KirgSSR); its epigeal part contained 1.7% of total alkaloids, from which we isolated three bases: gentianine [1], gentiananine [2], and gentianaine.

Experimental

The UV spectra were recorded on an SF-4 spectrophotometer (in ethanol), the IR spectra on a UR-10 double-beam spectrometer, and the mass spectra on an MKh-1303 instrument fitted with a system for introducing the sample directly into the ion source at 120° C with an ionization energy of 50 eV.

Alkaloids of G. olgae. Forty-three kilograms of the epigeal part was moistened with 8% ammonia and extracted with chloroform. The concentrated chloroformic extract was repeatedly treated with 10% sulfuric acid. The acid solution was shaken with ether. The ethereal extract amounted to 3.85 g. Then the acid solution was made alkaline with ammonia and extracted with chloroform, yielding 120.0 g of a mixture of alkaloids. From the dry residue of the chloroform extract, an additional 12.0 g of total alkaloids was obtained. The combined total was 135.85 g. When 3.85 g of the ethereal extract was treated with acetone, 0.2 g of gentianaine with mp 149–150° C (from acetone) was isolated.

Gentiananine. The acetone treatment of the chloroformic alkaloids gave a precipitate of gentiananine with mp 375–380° C (decomp.). Yield 27.0 g (20.4% of the total alkaloids).

Gentianadine. The acetone was evaporated and the residue was treated with benzene. Benzene-soluble and benzene-insoluble fractions were obtained. After the elimination of the benzene, a semicrystalline substance was formed which crystallized on trituration with a mixture of ether and acetone (1 : 1). The crystals were filtered off with suction and recrystallized from ether and then from petroleum ether, mp 77–78° C. Yield 45.0 g (34.7% of the total alkaloids).

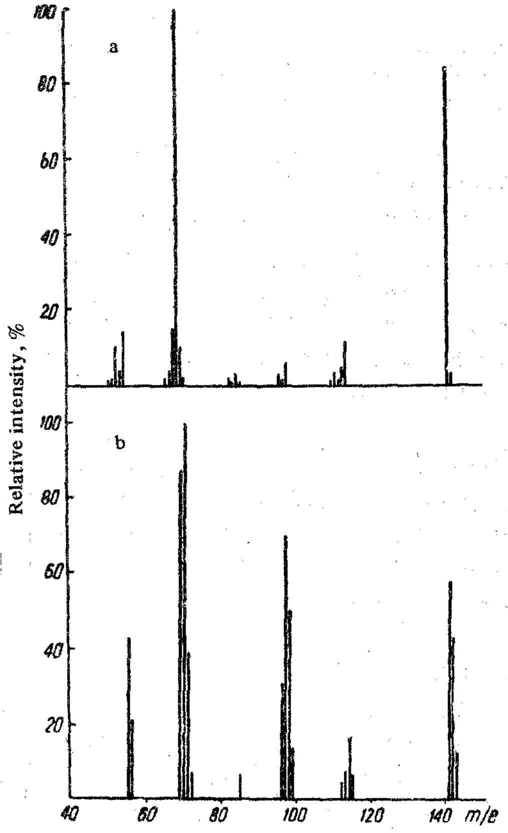


Fig. 2. Mass spectra of gentianaine (a) and the deutero analog of gentianaine (b).

Gentianaine. The benzene-insoluble fraction was treated with 5% hydrochloric acid. The acid solution was shaken with ether, and then the evaporation of the ether yielded gentianaine. The acid solution was made alkaline with ammonia and shaken with chloroform. The chloroform was distilled off to give the bulk of the gentianaine. The total yield of this alkaloid was 10.0 g (7.5% of the total alkaloids). Found, %: C 51.5; H 5.03; N 9.96; mol. wt. 141 (mass spectrometry). Calculated for $C_6H_7O_3N$, %: C 51.01; H 4.99; N 9.99; mol. wt. 141.12.

Preparation of the deutero analog of gentianaine was performed as follows: a solution of 0.1 g of gentianaine in 5 ml of deuterated ethanol was heated on the water bath for 30 min, and the ethanol was evaporated off. This operation was repeated several times. The mass spectrum of the deutero analog of gentianaine had a molecular peak with m/e 143, and in the IR spectrum bands appeared at 2560 and 2400 cm^{-1}.

O-Acetylgentianaine was prepared from a mixture of 0.25 g of gentianaine, 10 ml of acetyl chloride, and two drops of pyridine which was left in a closed vessel for several days. O-Acetylgentianaine was produced in the form of silky yellow needles with mp 164–165° C (ethanol). Yield 0.15 g.

The 2,4-dinitrophenylhydrazone of gentianaine was obtained by the addition to an aqueous solution of gentianaine of 2,4-dinitrophenylhydrazine hydrochloride. It melted with decomposition at 221–222° C (from water).

The alkaloids of G. olivieri. The plant was collected in the basin of the Kara-Tag, TadzhSSR on May 20, 1964, in the flowering stage. The epigeal part (410 g) was extracted with chloroform. The ether-soluble fraction amounted to 2.13 g and the chloroform-soluble fraction to 0.65 g, total 2.78 g (0.67% of total extract on the weight of the dry plant).

The chloroform extract was treated with benzene. When the benzene-insoluble fraction was treated with acetone, 0.2 g of gentianaine was obtained (7% of the total bases).

Alkaloids of G. turkestanorum. The plant was collected in the eastern part of the Alai on the watershed between the rivers Nura and Karagai-Tugai, KirgSSR on July 26, 1965 in the budding phase. The alkaloids were extracted from 375 g of the epigeal part with chloroform. The ether-soluble fraction amounted to 2.0 g and the chloroform-soluble fraction to 0.5 g, total 2.5 g (0.66% of the weight of the dry plant).

By the method described above, 0.2 g of gentianaine (8% of the mixture of bases) was isolated.

Alkaloids of G. caucasica. The usual chloroform extraction of 1.8 kg of the plant yielded 3.5 g of total alkaloids. These did not dissolve completely on treatment with 5% hydrochloric acid. The acid-insoluble fraction was treated with 4% caustic soda and the extract was precipitated by acidification. This gave 0.26 g of gentiananine.

The acid extract was made alkaline with ammonia and extracted with chloroform. The chloroform extract was separated on a column of alumina. From 1.8 g of the ethereal eluate was obtained 0.6 g of gentiananine with mp 76–80° C (from ethanol).

Alkaloids of G. kaufmanniana. The chloroform extraction of 0.65 g of the plant yielded 8.74 g of ethereal and 2.16 g of chloroformic total alkaloids. After the elimination of the solvent, the whole of the ethereal alkaloids crystallized, and from them was obtained 7.4 g of gentiananine (67.7% of the total alkaloids). Treatment of the chloroformic extract with acetone precipitated 0.65 g of gentianaine (5.9% of the total alkaloids). From the residue of the mother liquor insoluble in 5% hydrochloric acid, 0.1 g of gentiananine (1% of the total alkaloids) was isolated.

Conclusions

From Gentiana olgae have been isolated gentiananine, gentianadine, and the new base gentianaine $C_6H_7O_3N$. The structural formula for the latter has been given. G. caucasica contains gentianine and gentiananine; and G. kaufmanniana contains gentianine, gentianaine, and gentiananine; gentianaine has been found in the mother liquors from G. turkestanorum and G. olivieri.

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Institute of the Chemistry of Plant Substances, AS UzSSR