

After the separation of the gentianine (314 g), the ethereal fraction of the combined alkaloids (754 g) of *C. olivieri* (collected in May, 1969, in the basin of the river Karatag, Tadzhik SSR) was dissolved in chloroform and separated according to the strengths of the bases with sodium phosphate buffer. The initial buffer solutions were prepared by mixing a 5% solution of caustic soda with orthophosphoric acid to give definite pH values. The chloroform solution after being treated with the buffer and with 5% sulfuric acid was neutralized with 25% ammonia and was dried with anhydrous sodium sulfate, and the chloroform was distilled off.

| Fraction: | pH of buffer | Wt. of fraction, g |
|-----------|----------------------|--------------------|
| 1 | 6 | 9,2 |
| 2 | 4 | 8,6 |
| 3 | 2 | 28,5 |
| 4 | 5% H_3PO_4 | 8,9 |
| 5 | 5% H_2SO_4 | 125,5 |
| 6 | Chloroformic residue | 45,5 |

Fractions 1 and 2, by treatment with acetone, gave gentiobetine [1]. Fraction 5 was treated with ethanol, water, acetone, and methanol and was passed through a column of alumina. Gentianine [2], gentianan [3], oliverine [4], and a base with mp 253–254°C (ethanol) were isolated.

The chloroform residue was treated with methanol. The slow evaporation of the methanolic extract led to the deposition of crystals with the composition $C_{11}H_{10}NO_2Cl_3$ (I), mp 134–135°C, mol. wt. 293 (mass-spectrometrically for ^{35}Cl). The base is readily soluble in organic solvents and crystallizes from methanol. Its UV spectrum (λ_{max} 266 nm, $\log \epsilon$ 3.17) is very similar to that of dihydrogentianine (II). The IR spectrum has absorption bands for a lactone group at 1730 cm^{-1} and for a pyridine nucleus at 1590 cm^{-1} .

The oxidation of substance of (I) with potassium permanganate in acetone gave an acid identical with the acid (III) obtained under the same conditions from gentianine (IV).

The NMR spectrum of the base (Fig. 1) had two signals in the form of singlets at 0.94 and 1.16 ppm due to the two α protons of a pyridine ring. A two-proton triplet at 5.46 ppm ($J=6.0\text{ Hz}$) was due to the

methylene group of a lactone ring $CH_2-O-C=O$, and a six-proton multiplet the center of which was located at τ 7.00 ppm was due to three other methylene groups.

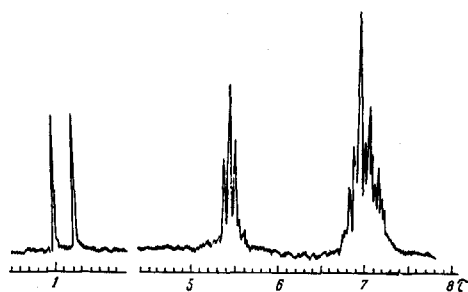
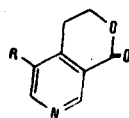


Fig. 1. NMR spectrum of the base with mp 134–135°C.



- I. $R - CH_2 \cdot CH_2 \cdot CCl_3$
- II. $R - CH_2 \cdot CH_3$
- III. $R - COOH$
- IV. $R - CH=CH_2$

These results permitted the assumption that the base that we had isolated was the product of the condensation of chloroform with gentianine and that the chloroform added to the vinyl group of (IV). In actual fact, by heating a chloroform solution of gentianine with benzoyl peroxide [5, 6] we obtained substance (I). The study of the other fractions is continuing.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from *Khimiya Prirodnkh Soedinenii*, No. 3, pp. 350–353, May–June, 1972. Original article submitted January 19, 1972.

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EXPERIMENTAL

The UV spectrum was taken on an SF-4 instrument (ethanol), the IR spectrum on a UR-10 instrument (molded tablets with KBr), the mass spectrum on an MKh-1303 mass spectrometer with a glass inlet system at 40 eV and 150 mA, and the NMR spectrum on a JNM-4H-100/100 MHz instrument in CDCl₃ with HMDS as the internal standard (τ scale), and the pH values of the buffer solutions were determined on an LPU-1 potentiometer.

Isolation of the Alkaloids. *G. olivieri* (530 kg) was extracted with chloroform in the usual way. This gave 754 g of ethereal alkaloids and 327 g of chloroformic alkaloids (0.2% of the weight of the dry plant). The material (240 g) from the mother solution of the ethereal fraction after the isolation of the gentianine was dissolved in 5 liters of chloroform, and the solution was treated with sodium phosphate buffer. The buffer solutions were made alkaline with gaseous ammonia, and then the alkaloids were extracted with chloroform.

Fraction 1 (9.2 g) and fraction 2 (8.6 g) were treated with acetone. They gave gentiotibetine - 1.26 and 0.58 g, respectively.

Fraction 5 (125.5 g) was dissolved in ethanol and the solution was left in the refrigerator for several days. Technical gentiananine deposited (7.7 g). The ethanol was evaporated off, and the residue was treated with hot water. The aqueous extract yielded 10.57 g of gentianine. By fractional crystallization and the passage of the mother solution through a column of alumina, oliverine (3.53 g) and a base with mp 253-254°C (0.98 g) were isolated.

The chloroform residue (45.0 g) was treated with methanol. The extract was separated off, and the methanol was slowly evaporated. Crystals deposited with mp 130-132°C (1.61 g). After recrystallization from methanol of the base with mp 134-135°C, the mother liquor was passed through a column of alumina. A benzene eluate gave an additional 0.68 g of the base with mp 134-135°C.

Oxidation of the Base with mp 134-135°C. To a solution of 0.1 g of the base in 50 ml of acetone was added (in portions) 0.25 g of an acetone solution of potassium permanganate. The mixture was heated in the water bath for 3 h. The precipitate of manganese dioxide was filtered off and washed with acetone and then with hot water. The aqueous filtrate was evaporated to dryness, and the residue was acidified with ethanolic hydrogen chloride. A precipitate (15 mg) deposited with mp 256-260°C (decomp., from water).

Condensation of Gentiananine with Chloroform. Benzoyl peroxide (0.05 g) was added to a chloroform solution of gentiananine (0.1 g). The mixture was heated in a sealed tube in the water bath for 10 h. Then the chloroform was evaporated off and the reaction mixture, in benzene solution, was passed through a column of alumina. The benzene eluate yielded a base with mp 134-135°C.

SUMMARY

From *G. olivieri* we have isolated gentiotibetine, gentiananine, gentianine, oliverine, and a base with mp 253-254°C. The product of the condensation of gentiananine with chloroform has been obtained from the chloroform residue.

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