# ALKALOIDS OF Gentiana olivieri

T. U. Rakhmatullaev and S. Yu. Yunusov

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The chloroform fraction of the mixture of alkaloids of G. olivieri [1] was treated with chloroform, under which conditions it did not all dissolve. Gentiananine was isolated from the insoluble fraction [2]. The chloroform-soluble part of the combined alkaloids was separated according to basicities by means of a sodium phosphate buffer. The chloroform solution after treatment with the buffer and with 5% sulfuric acid was neutralized with 25% ammonia and dried with anhydrous sodium sulfate, and the chloroform was distilled off.

| Fraction | pH of the buffer | Wt. of the fractions, g |
|---|---|---|
| 1 | 6 | 11.0 |
| 2 | 4 | 7.52 |
| 3 | 2 | 28.45 |
| 4 | 5% $H_2SO_4$ | 69.0 |
| 5 | Chloroform residue | 63.0 |

On a paper chromatogram, fractions 1 and 2 gave similar spots. Consequently they were combined and were then passed through a column of alumina. The ethyl acetate eluate yielded gentioflavine [3]. Fraction 5, by treatment with benzene and acetone and chromatography through a column of alumina yielded a base with mp 144-145°C, composition $C_{20}H_{20}N_2O_4$ (ethanol), mol. wt. 352 (mass spectrometrically), $R_f$ 0.80. It dissolved readily in chloroform and sparingly in ethanol, acetone, and methanol. In its physicochemical properties, this base differed from the alkaloids described in the literature; we have called it oliveramine.

The UV spectrum of oliveramine – $\lambda_{max}$ 273 nm (log ε 3.48) – is similar to that of dihydrogentianine. Its IR spectrum has characteristic absorption bands at 1585 $cm^{-1}$ (pyridine) and 1720 $cm^{-1}$ ($\alpha,\beta$-unsaturated $\delta$-lactone) [4]. By measuring the integral intensity of the absorption of the lactone carbonyl, it was found that the molecule of oliveramine has two lactone groups.

The NMR spectrum of oliveramine (Fig. 1) shows four signals in the form of singlets at 1.00, 1.06, 1.34, and 1.57 ppm, which are due to four $\alpha,\alpha'$-aromatic protons of pyridine rings. This shows that the remaining three positions of the pyridine rings are substituted. The signal of a methyl group at 8.56 ppm is split into two with a spin–spin coupling constant J = 7 Hz. This shows that the methyl group is present in the form of a $>CH-CH_3$ group. The signal from the methine proton is masked by the signals of the methylene groups of the lactone ring, since the two multiplets the centers of which are located at 5.54 and 7.03 ppm correspond to two $-CH_2-CH_2-O-C=O$ groups [5]. The signals at 8.02 and 7.33 ppm are due to two methylene groups. The multiplicities of these signals can be explained by a virtual spin–spin coupling in a

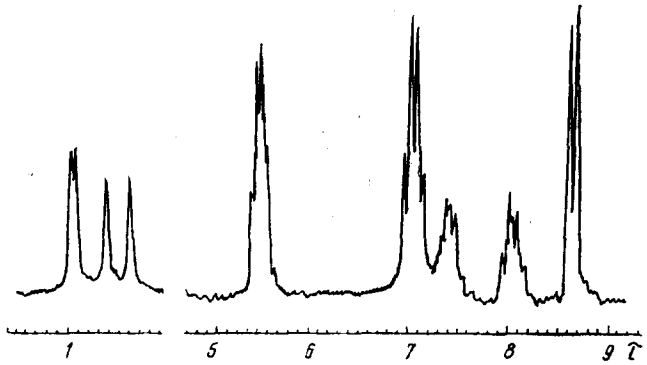


Fig. 1. NMR spectrum of oliveramine.

Ar—$CH_2$—$CH_2$—CH(|)—Ar system [6].

On the basis of what has been said, we propose (I) as the most probable structure for oliveramine. The structure agrees well with the features of its mass spectrum.

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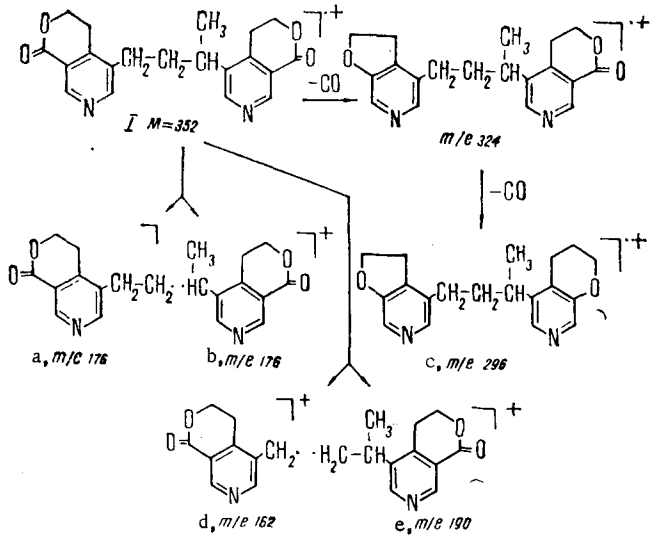
The results of a mass-spectrometric study of oliveramine showed that the main direction of fragmentation in it begins with $\alpha$ cleavage; the fairly stable ions a and b (100%) with m/e 176 are formed. One of the intense ions is c with m/e 296, which is formed from the molecular ion by the successive splitting out of two CO molecules. The mass spectrum of oliveramine also shows $\beta$ cleavage and the ions d and e corresponding to it. In addition, the mass spectrum has the peaks of lower mass numbers that are characteristic for pyridine derivatives [7].

The possible fragmentation pathways of oliveramine in the mass spectrum can be represented by the following scheme.




## EXPERIMENTAL

The UV spectrum was taken on an SF-4 instrument (in ethanol), the IR spectrum on a UR-10 instrument (molded tablets with KBr), the mass spectrum on an MKh-1303 with a glass inlet system at 40 eV and 150 mA, and the NMR spectrum on a JNM-4H-100/100 MHz instrument (in $CDCl_3$ with HMDS as internal standard, $\tau$ scale), and the pH values of the buffer solutions were determined on an LPU-1 potentiometer.

The combined alkaloids (250 g) were treated with 5 liters of chloroform. The insoluble fraction consisted of 62 g of technical gentiananine. The chloroform-soluble fraction was treated with sodium phosphate buffer.

Fractions 1 and 2 were combined and chromatographed through a column of alumina. The ethyl acetate eluate (12.0 g) yielded 1.0 g of gentioflavine.

Fraction 4 was treated with acetone. The insoluble residue consisted of 5.1 g of technical gentiananine. The acetone was evaporated in vacuum and the residue was boiled with benzene. The benzene-insoluble fraction was dissolved in acetone and chromatographed through a column of alumina. From the acetone eluate (1.63 g) treatment with ethanol gave 0.97 g of oliveramine.

## SUMMARY

The separation of the chloroform fraction of the combined alkaloids of G. olivieri according to basicities (pH 6.0 and 4.0) has yielded gentioflavine, gentiananine, and the new base oliveramine, $C_{20}H_{20}N_2O_4$. On the basis of its UV, IR, NMR, and mass spectra structure (I) has been put forward as the most probable for it.

## LITERATURE CITED

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