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ALKALOIDS OF *Doronicum macrophyllum*

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The genus *Doronicum* (family Compositae) has not previously been studied chemically. The roots of *Doronicum macrophyllum* collected in the stage of the withering of the epigeal part in October, 1974, in the Nakhichevan ASSR contained 0.03% of total alkaloids. These were separated on a column of silica gel, and three bases were isolated. Two of them were identified as otosenine (II) and floridanine (III) [1], and the third proved to be new, and we have called it *doronine* (I). Substance (I) crystallized from a mixture of benzene and cyclohexane with mp 113-114°C (decomp.), $[\alpha]_D$ +45.4° (c 1.1; chloroform); picrate, mp 235°C (decomp.). The IR spectrum of (I) has absorption bands of active hydrogen in the 2800-3500-cm^{-1} region and of carbonyl groups at 1750 and 1620 cm^{-1}, the latter having the position and shape that is characteristic for otonecine esters [2]. The presence in the mass spectrum of (I) of the peaks of ions with m/e 168, 151, 150, 122, and 110 confirms that this base belongs to the group of otonecine diesters. The molecular ion of the substance has the form of a doublet with m/e 459/461 in a ratio of 3:1, which shows the presence of one chlorine atom in the molecule. Ions with m/e 424 and 423 (M − Cl and M − HCl, respectively) confirm the presence of the halogen.

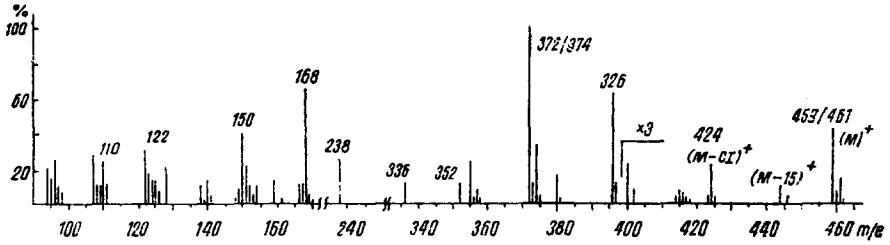
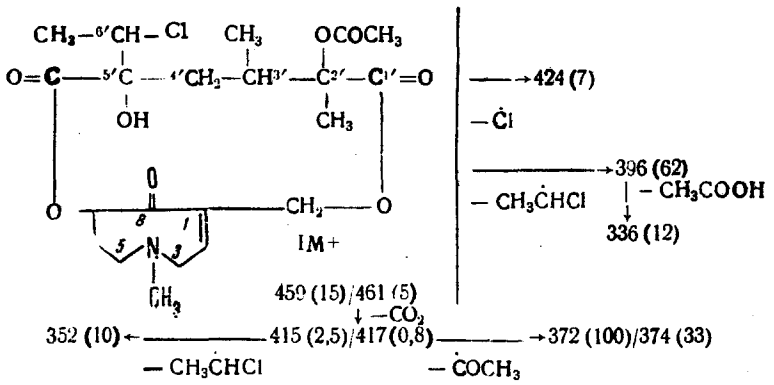


Fig. 1

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. N. Narimanov Azerbaidzhan State Medical Institute, Baku. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 194-196, March-April, 1976. Original article submitted April 28, 1975.



Analysis of the mass and NMR spectra permits the conclusion that the halogen is attached at C_6'. This is shown by the low intensity of the M − 44 ions (m/e 415/417) and by the presence of an intense peak of the ion M − CH_3CHCl with m/e 396.

The NMR spectrum of doronine ($CHCl_3$, δ scale) has a three-proton singlet at 1.65 ppm, which is characteristic for a CH_3 group at C_2' to which an $OCOCH_3$ group is attached [2]. The presence of the latter in this position is confirmed by the maximum ion with m/e 372/374 (M − CO_2 − $COCH_3$) and by an ion with m/e 336 (396 − CH_3COOH).

Both directions of fragmentation are characteristic for alkaloids of the floridanine type [3]. In addition to the ions mentioned, the mass spectrum of (I) has an ion with m/e 238 that is characteristic of otonecine bases (see Fig. 1).

On the basis of what has been said, doronine must have the structure (I) (see scheme). The NMR spectrum confirms the structure shown. In addition to the singlet of the CH_3 group at C_2' that has been mentioned, there is a six-proton singlet from N—CH_3 and $OCOCH_3$ groups at 2.03 ppm, two overlapping doublets from two CH_3 groups at C_3' and C_6' in the 1.21-ppm region, and the multiplet of a vinyl proton at 6.11 ppm.

A final proof of the structure of doronine is its correlation with otosenine, for which purpose the latter was acetylated with acetyl chloride under the conditions used for the conversion of otosenine into florosenine [2]. However, under these conditions we obtained doronine (I), i.e., in addition to acetylation of the C_2'—OH group, the epoxide ring of otosenine opened.

Since in the isolation of (I) no hydrochloric acid was used, i.e., the total bases were extracted with alkaline chloroform, and then sulfuric acid, we consider that doronine is, in all probability, a natural substance. The analogous alkaloid jakonine is known [4].

EXPERIMENTAL METHOD

Extraction. The comminuted raw material (4 kg) was moistened with 5% aqueous sodium carbonate solution, and after 2 h it was covered with chloroform. After a day, the chloroform extract was poured off and the plant was covered with a fresh portion of chloroform. The operation was repeated three times. The combined chloroform extracts were evaporated in vacuum to 0.6 liter, and this residue was extracted with 10% H_2SO_4 (6 × 10 ml). After a preliminary washing with ether, the acid solution was made alkaline with sodium carbonate, and the alkaloids were extracted with chloroform. This gave 1.35 g of a mixture of bases in the form of an oil.

Separation. The combined alkaloids (1.35 g) were placed in a column containing 42 g of silica gel. Fractions with a volume of 200 ml were collected. The separation was checked by thin-layer chromatography on plates of silica gel fixed with gypsum in the chloroform—methanol (9:1) and (8:2) systems and on plates with a nonfixed layer of alumina in the chloroform—benzene—methanol (5:4:1) system. On elution with chloroform, fractions 1-9 contained a very small amount of a mixture of substances. Elution with chloroform—methanol (99:1) gave 0.33 g of a mixture of bases with a predominance of (I) (fractions 10-14). Further elution of the column with the same mixture (fractions 15-35) gave 0.71 g of a mixture of alkaloids with a predominance of (II) and, finally, on elution with a mixture of chloroform and methanol (95:5), 0.25 g of a mixture with a predominance of (III) was obtained (fractions 36-42).

Isolation of Doronine. The material of fractions 10-14 (0.33 g) was passed through a column of silica gel. Elution was performed with chloroform and chloroform–methanol (99:1). After the corresponding fractions had been combined, 0.28 g of doronine was obtained in the form of an oil which still contained a small amount of impurity. Consequently, the alkaloid was repurified by passage through a column of alumina. Elution was performed with benzene, and then with chloroform. The chloroform fractions contained 0.15 g of a chromatographically homogeneous substance, which was dissolved in benzene and was induced to form crystals by the addition of cyclohexane until a turbidity was produced.

Isolation of Otosenine. The material of fractions 15-35 (0.71 g) was treated with ethanol. This gave 0.21 g of ethanol-insoluble residue, mp 226°C, a mixture of which with a sample of otosenine melted at 226°C. Its R_f coincided with that of an authentic sample.

Isolation of Floridanine. The material of fractions 36-42 (0.25 g) was treated with acetone, and the insoluble residue was recrystallized from chloroform. This gave 0.12 g of a substance with mp 190-191°C; a mixed melting point with a sample of floridanine (mp 194°C) melted at 192-193°C. The R_f value coincided with that of an authentic sample.

Conversion of Otosenine into Doronine. A mixture of 0.2 g of otosenine and 3.3 ml of acetyl chloride was heated for 10 min, and then it was left at room temperature for 2 h. On standing, darkening of the solution and the deposition of a precipitate were observed. The last traces of acetyl chloride were eliminated in vacuum. Then the residue was treated with 5 ml of cooled 5% H_2SO_4. Part of the black resinous mass did not dissolve. The decanted acid solution was made alkaline with ammonia and treated with chloroform. The residue after the elimination of the chloroform was dissolved in benzene, and cyclohexane was added. This gave 0.18 g of a crystalline product with mp 113°C (decomp.), $[\alpha]_D$ +46.6, (c 0.9; chloroform), which began to soften gradually from 98°C onwards. Its R_f value and IR spectra were identical with those of natural doronine; M^+ 459/461.

SUMMARY

1. Two known alkaloids, otosenine and floridanine, and a new alkaloid doronine have been isolated from the roots of *Doronicum macrophyllum*.

2. On the basis of its spectral and chemical characteristics, the structure of 6'-chlorodeoxyfloridanine has been established for doronine.

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