

Isolation and identification of verticine *N*-oxide from *Fritillaria* L.

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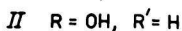
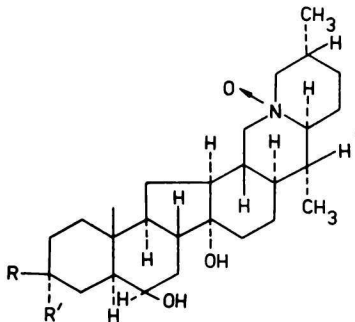
Received 10 April 1985

The structure of verticine *N*-oxide, isolated for the first time from the genus *Fritillaria* L., family *Liliaceae*, was solved by diffraction methods.

Из растений рода *Fritillaria* L., семейства *Liliaceae* впервые выделена *N*-окись вертицина, строение которой было установлено с помощью дифракционных методов.

Only few *N*-oxides of cevanine alkaloids appearing in genera *Petilium* and *Korolkowia*, family *Liliaceae*, have been reported. Thus, imperialine *N*-oxide was isolated from the aerial part of *Petilium eduardi* in 1976 [1]. The same compound was found in *Petilium raddeana* in 1981; authors of this paper state, however, that both alkaloids are not identical, but isomeric. These results were backed by physicochemical and spectral data of both alkaloids [2].

A new alkaloid denoted severine *N*-oxide (*I*) was isolated from the underground part of *Korolkowia sewerzowii* REGEL [3]; another *N*-oxide separated from the same plant was sevedine *N*-oxide (*II*) of molecular formula $C_{27}H_{45}NO_4$ [4].



It has been shown that severine *N*-oxide and sevedine *N*-oxide are identical with the partially synthetically prepared substances [3, 4].

Imperialine, verticinium chloride, and a small amount of a base HIP-17 have now been isolated from bulbs of *Fritillaria imperialis* L. cv. *aurora* in our research program on cevanine alkaloids.

Experimental

The dried sum of alkaloids (11.2 g) obtained by extracting the drug (19.5 kg) with ethanol was hot dissolved in methanol containing a small amount of chloroform and left to crystallize. Recrystallization from the same solvent afforded imperialine, the main alkaloid of the bulbs of *Fritillaria imperialis* L. cv. *aurora*.

Mother liquors yielded a mixture of minor alkaloids (5.2 g), which was separated by column chromatography on alumina (activity grade II, neutral, Lachema, Brno). The solvent system used was tetrachloromethane—benzene—methanol (volume ratio = 40:50:3); the separation was monitored by thin-layer chromatography on silica gel sheets by a double development in benzene—methanol—acetone—diethylamine (volume ratio = 90:4:4:2) and the alkaloids were spotted by Dragendorff reagent. The combined fraction (denoted HIP) (2.3725 g) was rechromatographed on a silica-gel-packed column (Silpearl, floated, No. 4, deactivated with water (10 mass %)) by elution with chloroform—methanol—benzene (volume ratio = 8:1:1).

Fraction HIP-17 (eluates 1—17) revealed one spot when monitored by thin-layer chromatography, was crystallized from methanol to give some crystals.

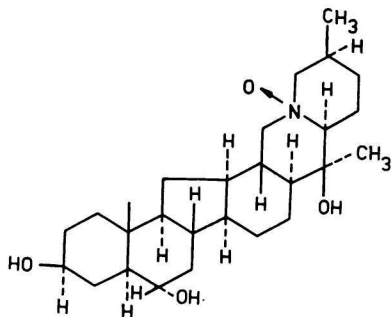
Fraction HIP-54 (eluates 28—54) (0.7621 g) was evaporated and rechromatographed through an alumina column with benzene—tetrachloromethane—methanol (volume ratio = 50:40:6) to afford, after crystallization from acetone—chloroform, an alkaloid (0.4937 g), which was shown to be identical with verticinium chloride [5, 6].

A total diffraction analysis was employed for structure elucidation of the alkaloid denoted as HIP-17. Dimension of the crystal was 0.5 mm × 0.15 mm × 0.1 mm. The refined crystal parameters $a = 10.830(2) \times 10^{-10}$ m, $b = 5.859(1) \times 10^{-10}$ m, $c = 20.187(4) \times 10^{-10}$ m, $\beta = 97.26(2)^\circ$ were obtained by the least-square method from twelve reflections precisely centred on a diffractometer Syntex P2₁; the systematic extinctions of reflections of $0k0$ type for $k = 2n + 1$ unequivocally showed the P2₁ space group. The diffraction intensities were measured with a Syntex P2₁ diffractometer using the graphite-monochromatized light radiation CuK α ($\lambda = 1.5418 \times 10^{-10}$ m) Θ — 2Θ by a scan technique in the $3 < 2\Theta \leq 110^\circ$ interval; 1448 reflections with $I \geq 1.96 \sigma(I)$ of the totally 1785 measured reflections were employed to elucidate and refine the structure. Selection of the enantiomorph in the polar group P2₁ was ambiguous and therefore solution of the structure was made possible by combination of the calculation procedures MULTAN [7] and MAGIC [8]. The structure was in the final stage refined by the least-square method to $R = 0.047$ ($R = \sum ||F_o| - |F_c|| / \sum |F_o|$, where $|F_o|$ and $|F_c|$ are the respective observed and calculated structural amplitudes); anisotropic thermal vibrations for the nonhydrogen atoms and isotropic vibrations for hydrogen atoms were presumed; calculation $|F_c|$ was based on the tabulated scattering

curves of the neutral atoms [9]; the final map of the residual electron density contained randomly distributed maxima below the $0.15 (10^{-10} \text{ m})^{-3}$ limit.

Results and discussion

Results of the diffraction analysis unequivocally showed alkaloid HIP-17 to be $3\beta,6\alpha,20\beta$ -trihydroxy-5 α -cevanine *N*-oxide (verticine *N*-oxide) (*III*).



III

As evident from the formula *III*, the presence of an *N*-oxide arrangement did not change the usual *trans* configuration at the E/F ring junction as already observed with quaternization of verticinone with CH_3Br [10]; this fact can be rationalized by a greater steric volume of the methyl group when compared with the oxygen atom. The bond lengths, valence and torsion angles in the 5 α -cevanine skeleton of the alkaloid under investigation are very close to those of other cevanine alkaloids [11—14].

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Translated by Z. Votický