Isolation and identification of alkaloids from *Fritillaria imperialis* L. var. *rubra maxima*

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Imperialine and further alkaloids of molecular formula \(C_{27}H_{41}N_2O\) and \(C_{27}H_{45}N_3O_3\) were obtained from bulbs of *Fritillaria imperialis* L. var. *rubra maxima*; the latter is identical with verticine. It has been isolated from the above-mentioned plant for the first time.

Imperialine was first isolated by Fragner [1] from *Fritillaria imperialis* L.; the presence of other two bases was reported by Indian authors [2]. Thin-layer chromatography of the alcoholic extract from bulbs indicated the occurrence of several alkaloids from which we were able to separate and characterize three bases.

Counter-current distribution of the alkaloid mixture afforded substance A having the \(R_t\) value identical with that of imperialine and differing in molecular formula. Infrared spectrum of this alkaloid revealed a strong absorption band at 1705 cm\(^{-1}\) diagnostic of a carbonyl group in a six-membered ring and bands at 1065 and 3400 cm\(^{-1}\) attributable to a hydroxyl group. No Bohlman bands, indicating the trans-quinolizidine arrangement in the heterocyclic moiety [3], were observed. The mass spectrum revealed the molecular ion coincident with the molecular formula \(C_{27}H_{41}N_2O_2\). The base peak at \(m/e\) 112 is diagnostic of alkaloids with cevanine skeleton [4]. On the basis of results so far known this alkaloid probably differs from other *Fritillaria* bases of cevanine type by a cis fusion of the quinolizidine ring.

Another base lettered B, separated from the alkaloid mixture by column chromatography was crystallized from chloroform. Infrared spectrum of this substance displayed vibrations at 1040 and 1050 cm\(^{-1}\), and at 3240 and 3400 cm\(^{-1}\) due to hydroxyl groups. No maximum in the carbonyl groups range was observed.

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The mass spectrum showed the molecular formula to be C$_{27}$H$_{45}$NO$_3$; fragmentation pattern at m/e 112, 111, and 98 evidences, similarly as in the first case, the cevanine backbone. The molecular formula of this base is identical with those of verticine (peimine) found in *Fritillaria verticillata* WILD. var. *Thunbergii* BAKER [5] and imperoline isolated by Paul and Boit from bulbs of *Fritillaria imperialis* var. *lutea* [6]. Verticine and alkaloid B have, in addition to the peak of molecular ion, equal fragmentation pattern and also $R_f$ values. Melting point and optical rotation of the isolated compound are identical with those given for verticine [7, 8] and therefore, the isolated alkaloid is verticine (I)

Further alkaloid designated C, separated by column chromatography, is the major base of molecular formula C$_{27}$H$_{43}$NO$_3$. Its i.r. spectrum revealed the presence of a carbonyl group, other physical data were in accordance with those reported for imperialine. Alkaloid C was shown to be identical with the authentic specimen of imperialine.

**Experimental**

Melting points were measured on a Kofler micro hot-stage, optical rotations with a Polamat A (Zeiss, Jena) polarimeter, infrared spectra in KBr discs with a Perkin—Elmer model 377 spectrophotometer, and mass spectra with an AEI MS 902 apparatus. Thin-layer chromatography on Silica gel G (Merck) was performed in solvent system benzene—acetone—methanol—diethylamine 90:4:4:2.

*Preparation of the extract from bulbs of Fritillaria imperialis* L.

The material was supplied by Sempra, Heřmanův Městec. Ground bulbs (12.65 kg) were macerated with ethanol (80 l total) at room temperature till the extract gave a positive Mayers' test. The extracts were filtered, the drug pressed by hand and ethanol removed on a vacuum rotary evaporator at 40°C outer temperature to a sirupy consistence.

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Work-up of the ethanol extract

The sirupy extract was made alkaline with a 10% solution of ammonium hydroxide to pH 11 and exhaustively extracted with chloroform. The organic layer (5 l) was dried with sodium sulfate and evaporated under reduced pressure to give 60 g of the alkaloid mixture (0.47%), 40 g of which was purified from the accompanying nonbasic compounds by dissolving in 5% acetic acid and extraction with chloroform and ethyl acetate. The acid layer was alkalified to pH 11 with a solution consisting of 5% sodium hydroxide and sodium hydrocarbonate solutions in a 1:1 ratio. The alkaloids were recovered by extraction with chloroform which was dried with sodium sulfate and distilled off.

Isolation of alkaloids from the chloroform portion

The mixture of purified alkaloids (5 g) was separated by a counter-current distribution in chloroform—10% acetic acid at 100 stages and 2 x 25 ml volume per one unit. The quality of alkaloids was evaluated in each fifth unit by t.l.c., and units containing the same substance were collected. Alkaloid A was obtained from units 21—29 in a usual way; yield 20 mg, amorphous, Rf 0.74, [α]25° +48.7° (c 1.0, CHCl3).

For C27H41NO3 M+ calculated: 411.3137; found: 411.3150. Other species at m/e 396 (M - CH3), 112 (C6H12N), 98 (C6H12N).

IR (cm⁻¹): 850, 970, 1010, 1065, 1205, 1240, 1380, 1705 (CO), 2860, 2920, 3440 (OH).

The work-up of the content of units 53—100 afforded a mixture of alkaloids (1.9536 g) which was separated by column chromatography on alumina (neutral, activity grade III) with a solvent system chloroform—benzene 8:2; the volume per one fraction being 25 ml. Alkaloid C found in fractions 25—39 crystallized from a mixture chloroform—methanol; m.p. 265°C, [α]25° -34.6° (c 0.16, CHCl3), Rf 0.74.

For C27H43NO3 M+ calculated: 429.3279; found: 429.3262. Other species at m/e 112, 111, 98.

IR (cm⁻¹): 805, 840, 895, 910, 925, 965, 1000, 1020, 1060, 1130, 1195, 1235, 1295, 1325, 1375, 1400, 1420, 1440, 1705, 2780, 2805, 2940, 3440.

Alkaloid B present in fractions 93—118 crystallized from chloroform, m.p. 218—220°C, [α]25° +14° (c 0.5, MeOH), Rf 0.46.

For C27H45NO3 M+ calculated: 431.3399; found: 431.3392. Other species at m/e 112, 98.

IR (cm⁻¹): 750, 815, 845, 890, 935, 965, 1040, 1060, 1095, 1130, 1175, 1215, 1290, 1340, 1385, 1415, 1445, 1580, 2870, 2920, 3240, 3400.

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References


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