

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

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Imperialine and further alkaloids of molecular formula  $C_{27}H_{41}NO_2$  and  $C_{27}H_{45}NO_3$  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.

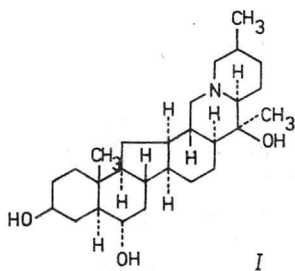
Из луковиц *Fritillaria imperialis* L. var. *rubra maxima* были получены империалин и дальнейшие два алкалоида общих формул  $C_{27}H_{41}NO_2$  и  $C_{27}H_{45}NO_3$ . Алкалоид  $C_{27}H_{45}NO_3$  является идентичным с вертицином. Этот алкалоид был впервые получен из вышеприведенного растения.

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  $R_f$  value identical with that of imperialine and differing in molecular formula. Infrared spectrum of this alkaloid revealed a strong absorption band at  $1705\text{ cm}^{-1}$  diagnostic of a carbonyl group in a six-membered ring and bands at  $1065$  and  $3400\text{ cm}^{-1}$  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  $C_{27}H_{41}NO_2$ . 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  $1050\text{ cm}^{-1}$ , and at  $3240$  and  $3400\text{ cm}^{-1}$  due to hydroxyl groups. No maximum in the carbonyl groups range was observed.

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.

### 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 alkalinized 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  $2 \times 25$  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,  $R_f$  0.74,  $[\alpha]_{546}^{26} - 48.7^\circ$  ( $c$  1.0,  $\text{CHCl}_3$ ).

For  $\text{C}_{27}\text{H}_{41}\text{NO}_2$ ,  $M^+$  calculated: 411.3137; found: 411.3150. Other species at  $m/e$  396 ( $M - \text{CH}_3$ ), 112 ( $\text{C}_7\text{H}_{14}\text{N}$ ), 98 ( $\text{C}_6\text{H}_{12}\text{N}$ ).

IR ( $\text{cm}^{-1}$ ): 850, 970, 1010, 1065, 1205, 1240, 1380, 1445, 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.  $265^\circ\text{C}$ ,  $[\alpha]_{546}^{24} - 34.6^\circ$  ( $c$  0.16,  $\text{CHCl}_3$ ),  $R_f$  0.74.

For  $\text{C}_{27}\text{H}_{43}\text{NO}_3$ ,  $M^+$  calculated: 429.3279; found: 429.3262. Other species at  $m/e$  112, 111, 98.

IR ( $\text{cm}^{-1}$ ): 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.  $218-220^\circ\text{C}$ ,  $[\alpha]_{578}^{27} - 14^\circ$  ( $c$  0.5, MeOH);  $R_f$  0.46.

For  $\text{C}_{27}\text{H}_{45}\text{NO}_3$ ,  $M^+$  calculated: 431.3399; found: 431.3392. Other species at  $m/e$  112, 98.

IR ( $\text{cm}^{-1}$ ): 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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