Veratrum alkaloids. XXIX.* Glucoveracintine, a new glycoalkaloid from *Veratrum album* subsp. *Lobelianum* (BERNH.) *Suessenguth*

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A mixture of substances, obtained from the aerial part of *Veratrum album* subsp. *Lobelianum* (BERNH.) *Suessenguth* by extraction with ethanol, was separated by column chromatography to yield glucoveracintine and veratroylamide. The isolated substances and products of hydrolysis were identified on the basis of spectral evidence.

Экстракцией надземной части растений *Veratrum album* subsp. *Lobelianum* (BERNH.) *Suessenguth* с этанолом была получена смесь веществ. Их разделяли при помощи хроматографии в колонках, причем был получен глюковерацинтин и вератроиламид. Выделенные вещества и продукты гидролиза были идентифицированы на основании их спектров.

Veracintine, 20-(2-methyl-1-pyrrolin-5-yl)-5-pregnen-3β-ol [1], 20-(2-methyl-1-pyrrolin-5-yl)-4-pregnen-3-one [2], and the ester alkaloid veratroylzygadenine [3, 4] have already been isolated from the benzene extract of the aerial part of *Veratrum album* subsp. *Lobelianum*.

The drug was after extraction with benzene dried and extracted with ethanol, which dissolved polar substances. The ethanolic extract was evaporated under reduced pressure and the residue dissolved in a mixture of 5% acetic acid and chloroform. The acid layer was extracted with chloroform and ethyl acetate, alkalized and extracted again with chloroform and ethyl acetate. The extracts were combined, the solvent removed *in vacuo* and the residue chromatographed over

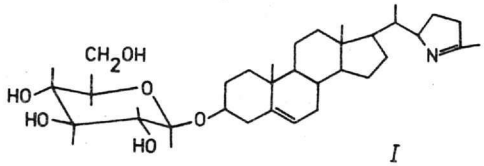
* For Part XXVIII see *Collect. Czech. Chem. Commun.* **42**, 3643 (1977).

silica gel. The substances thus obtained, provisionally designated *GR-8* and *GR-4*, gave a positive test with Dragendorf reagent and concentrated sulfuric acid, respectively.

The amorphous substance *GR-8* has, according to mass spectrum, molecular formula $C_{32}H_{51}NO_6$. This spectrum showed, in addition to the peak of molecular ion, fragments characteristic of the alkaloid veracintine [1]. Also some signals in the p.m.r. spectrum of the substance *GR-8* compared to those of the alkaloid veracintine. The bands of the i.r. spectrum in KBr were attributable to glycoalkaloids (1000—1100 cm^{-1}, broad), to C=N double bond (1650 cm^{-1}), and finally to OH groups (3400 cm^{-1}, broad).

Upon an enzymatic hydrolysis *GR-8* gave an aglycone identic, according to melting point, mixed melting point, and mass spectrum, with veracintine.

The sugar moiety in the hydrolyzed solution was proved to be glucose on the basis of paper and t.l.c. chromatography. It can be bound to veracintine exclusively through the 3β-hydroxyl group. The existence of the β-glycosidic bond was unambiguously proved by its cleavage by emulsine. The presented arguments allow to assign the structure of glucoveracintine (*I*) to the substance *GR-8*



I

The substance *GR-4* revealed in its mass spectrum the peak of molecular ion at *m/e* 181, which looses stepwise NH_2 (*m/e* 165, *m** 150.4) and carbonyl (*m/e* 137, *m** 113.8) groups. It is believed that substance *GR-4* is dimethoxybenzamide (veratroylamide). The isolated amount of this substance was insufficient for further examination.

Experimental

The melting points were determined on a Kofler micro hot-stage. The i.r. spectra were recorded with a Perkin—Elmer 477 spectrophotometer, the mass spectra with an AEI-MS 902 spectrometer, the optical rotation with a POLAMAT A apparatus, and the p.m.r. spectrum in CH_3OD with a Tesla BS 487 A instrument operating at 80 MHz, hexamethyldisiloxane being the internal reference substance. The occurence of alkaloids in

the respective fractions was monitored by t.l.c. chromatography on silica gel G (Merck) in the solvent system chloroform—methanol—benzene 8:1.5:0.5. Chromatograms were developed five times in the afore-mentioned system. Plates to be detected with concentrated sulfuric acid were heated at 120°C for 5 min. Silica gel for column chromatography No. 5 was floated according to [5].

Isolation of GR-4 and glucoveracintine

The dried drug after extraction with benzene (26 kg) was percolated with ethanol, the extract evaporated in a vacuum rotary evaporator at 45°C and the residue (5.4 kg) dissolved in portions (0.5 kg) in 5% acetic acid—chloroform 1:1. The acid layer was further extracted with chloroform (3 × 1 l) and ethyl acetate (3 × 1 l), organic layers were combined and deposited after evaporation to dryness. The acid layer was alkalified to pH 10 with dilute NaOH solution and gradually extracted with chloroform (5 × 1 l) and ethyl acetate till the latter gave a positive test with Dragendorf reagent. The combined chloroform and ethyl acetate extracts evaporated in a vacuum rotary evaporator at 45°C afforded a mixture (8.2 g) which was separated on a silica gel column [5]. The sample-to-support-weight ratio 1:100, the elution mixture composition chloroform—methanol—benzene 8:1:1, fractions 10 ml each. Compound *GR-4*, $C_9H_{11}NO_3$, m.p. 163°C, was found in fractions *15—18*.

Glucoveracintine *GR-8*, amorphous, obtained from fractions *42—64*, R_f 0.44, $[\alpha]_{546}^{19}$ 26 ± 2° (*c* 0.69, MeOH).

For $C_{32}H_{51}NO_6$ M^+ calculated: 545.3716; found: 545.3725; other species at *m/e* 530 (M − 15), 366 (M − $C_6H_{11}O_6$), *m/e* 83 (100%), 82. P.m.r. (in p.p.m. on the δ scale): 0.74 (s, C-18 methyl), 0.99 (s, C-19 methyl), 2.00 (s, C-25 methyl).

Enzymatic hydrolysis of GR-8

To the solution of *GR-8* (30 mg) in methanol (3 ml) and water (9 ml) emulsine (30 mg) was added and the solution was incubated at 35—37°C for 15 h. The solution was thereafter filtered, concentrated under diminished pressure, extracted with chloroform, the solvent removed and the obtained veracintine (7 mg) was crystallized from ether; m.p. 197—201°C, mixed m.p. 196—201°C, R_f 0.55 (chloroform—methanol 9:1).

For $C_{26}H_{41}NO$ M^+ calculated: 383.3188; found: 383.3198; other species at *m/e* 110, 91, 83, 82, 69, 61, 55, 41.

The aqueous layer resulting from the hydrolysis was evaporated to dryness, dissolved in ethanol (2 ml) and spotted on a chromatographic paper Whatman No. 4 together with glucose as standard; solvent system butanol—pyridine—water 10:3:3, spreading with anilinium phthalate, visualized after heating at 105°C for 5 min, R_f 0.30. The same solvent system and detection was employed for the identification of glucose on Lucefol; R_f 0.31.

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