Veratrum alkaloids
XXXIII.* Rhamnoveracintine — a new glycoalkaloid from *Veratrum album* ssp. *Lobelianum* (BERNH.) *Suessenguth*

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Received 8 May 1985

A mixture of alkaloids obtained by ethanolic extraction of the aerial part of *Veratrum album* ssp. *Lobelianum* (BERNH.) *Suessenguth* was separated by countercurrent distribution followed by column chromatography to afford a new alkaloid; its structure was proposed by spectral means from the product of hydrolysis. It was denoted rhamnoveracintine ((20*S*)-(2-methyl-1-pyrrolin-5-yl)-5-pregnen-3β-rhamnoside); it is the first veratrum glycoalkaloid containing rhamnose.

Смесь алкалоидов, полученная в результате экстракции этиловым спиртом воздушной части *Veratrum album* ssp. *Lobelianum* (BERNH.) *Suessenguth*, была разделена с помощью противоточного распределения с последующим хроматографическим делением на колонне, в результате чего был получен новый алкалоид, структура которого была предложена на основании спектроскопического изучения продуктов его гидролиза. Новый алкалоид был назван рамноверацинтином ((20*S*)-(2-метил-1-пирролин-5-ил)-5-прегнен-3β-рамнозид); это первый вератриновый гликоалкалоид, содержащий рамнозу.

The benzene extract of the aerial part of *Veratrum album* ssp. *Lobelianum* (BERNH.) *Suessenguth* was reported to contain alkaloids veracintine ((20*S*)-(2-methyl-1-pyrrolin-5-yl)-5-pregnen-3β-ol [1]), 20-(2-methyl-1-pyrrolin-5-yl)-4-pregnen-3-one) [2], veratroylzygadenine [3, 4], and glucoveracintine [5].

The benzene extracted drug was dried, the constituents were taken into ethanol, which dissolved the polar compounds. The ethanolic extract was evaporated under diminished pressure and temperature up to 45 °C, the residue was dissolved in the system diluted acetic acid ($\varrho = 50$ g dm^{-3})—chloroform, (volume ratio

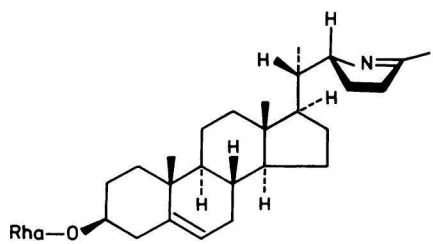
* For Part *XXXII* see Ref. [7].

(r_V) = 1 : 1). The portion soluble in acetic acid was extracted with chloroform and ethyl acetate, made alkaline with ammonia and repeatedly extracted with chloroform and ethyl acetate. The chloroform and ethyl acetate extracts were combined, the solvents were evaporated *in vacuo* and the residue was chromatographed on a silica gel-packed column to give compound GR-10, staining with the Dragendorff reagent.

Compound GR-10 is amorphous, [α] (546 nm, 19 °C) = −40° (ϱ = 7.0 g dm^{-3}, MeOH) and transparent in the range λ = 220—350 nm. Its IR spectrum displayed a broad absorption band at $\tilde{\nu}$ = 1000—1100 cm^{-1} characteristic of glycoalkaloids; bands at $\tilde{\nu}$ = 1380 cm^{-1}, 1460 cm^{-1}, and 2940 cm^{-1} are typical of a CH_3 group, at $\tilde{\nu}$ = 1650 cm^{-1} of a C=N double bond, and a broad band at $\tilde{\nu}$ = 3400 cm^{-1} of a hydroxyl group. This compound did not evaporate under conditions for mass spectrometric measurement and therefore its mass spectrum could not be taken. This fact together with the presence of the characteristic band in the IR spectrum indicate this compound to be a glycoalkaloid. This presumption was verified by an enzyme and acid hydrolyses both liberating the aglycone.

The enzyme-hydrolysis freed aglycone was proved to be identical with the alkaloid veracintine on the basis of its mass spectrum and comparison with the specimen (melting point, mixed melting point, R_f values).

The saccharide moiety was identified by paper and thin-layer chromatographies as rhamnose, which could be attached to veracintine through the 3β-hydroxyl group. The existence of probably β-glycosidic binding was proved by its fissility with emulsine. These results make it possible to ascribe the structure of rhamnoveracintine to compound GR-10.



Experimental

Melting points were determined on a Kofler micro hot-stage, the IR spectra of compounds in KBr pellets were measured with Perkin—Elmer spectrophotometer, model 477, the UV spectra with Specord UV VIS, the mass spectra with an AEI-MS 902 apparatus, and the ^{1}H NMR spectra of deuteriochloroform solutions containing hexamethyldisiloxane with a Tesla BS 487 A instrument operating at 80 MHz. Optical rotation was measured with Polamat A apparatus.

Separation of alkaloids by the column was monitored by thin-layer chromatography on silica gel G sheets (Merck) in chloroform—methanol—benzene, ($r_V = 8 : 1.5 : 0.5$). The chromatograms were developed five times and detected with Dragendorff reagent or with concentrated sulfuric acid and heating to 120 °C for 5 min. For column chromatography floated silica gel No. 5 was used [6].

Isolation of rhamnoveracintine

The air-dried and ground drug (26 kg) was stepwise extracted with benzene and ethanol. The ethanolic extract was concentrated under diminished pressure to 20 dm^3 at a temperature not exceeding 45 °C. The concentrated ethanolic extract (4 dm^3) was poured into diluted acetic acid ($\varrho = 50$ g dm^{-3}) (4 dm^3) and this mixture was repeatedly extracted with chloroform (3×1 dm^3) and ethyl acetate (3×1 dm^3). The chloroform and ethyl acetate extracts were separately concentrated to dryness and stocked. The acid layer was made alkaline to pH = 11 with dilute ammonia and subjected to an exhaustive extraction with chloroform (5×1 dm^3) and with ethyl acetate (3×1 dm^3) (negative reaction with Dragendorff reagent). The obtained extracts were combined, filtered, concentrated to dryness (10 g) and chromatographed on a silica gel column. The sample to carrier mass ratio was 1 : 100, chloroform—methanol—benzene ($r_V = 8 : 1 : 1$) being the eluent. Fractions (10 cm^3 each) were combined according to results of thin-layer chromatography. Rechromatography of fractions *69—85* afforded rhamnoveracintine GR-10 (25 mg), $R_f = 0.49$ (chloroform—methanol, $r_V = 8 : 2$). ^{1}H NMR spectrum, δ/ppm: 0.74 (s, C-18—methyl), 0.99 (s, C-19—methyl), 2.00 (s, C-25—methyl).

Enzymic hydrolysis

Rhamnoveracintine (10 mg) was dissolved in a mixture of methanol (1 cm^3) and distilled water (4 cm^3). The enzyme preparation (10 mg) was added to this solution and the mixture was incubated at 35—37 °C for 15 h, filtered, concentrated and extracted with chloroform. The solvent was removed, the residue was dissolved in ether from which veracintine crystallized (4 mg). M.p. = 197—201 °C, mixed melting point with the specimen 196—201 °C, $R_f = 0.55$ (chloroform—methanol, $r_V = 9 : 1$). For $C_{26}H_{41}NO$ M_r(calc.) = 383.3198, M_r(found by high resolution mass spectrometry) = 383.3188; other fragment ions, *m/z:* 110, 91, 83, 82, 69, 61, 55, 41.

The aqueous portion was evaporated and the residue was dissolved in ethanol (1 cm^3); this sample was spotted on the Whatman No. 4 paper and Lucefol, rhamnose being the reference and butanol—pyridine—water, $r_V = 10 : 3 : 3$ the solvent system. The spots were detected with aniline hydrogenphthalate and heated at 105 °C for 5 min; $R_f = 0.54$.

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Translated by Z. Votický