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HYDROLYZED TANNINS OF *Euphorbia glareosa* LEAVES. STRUCTURE OF GLAREIN C

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The structure of glarein, a new hydrolyzed tannin from Euphorbia glareosa *Pall. ex Bieb. leaves, was established using physicochemical properties of the compound itself, its hydrolysis products, and IR, UV, and PMR spectra.*

Key words: tannins, glarein C, *Euphorbia glareosa*.

We previously reported the isolation of glareins A-C and the structure of glarein A from *Euphorbia glareosa* (Euphorbiaceae) leaves [1]. We now report the structure determination of glarein C (**1**).

Compound **1** is a yellowish-brown amorphous powder, mp 172-174°C, $[\alpha]_D^{20}$ +80.1° (*c* 1.0, MeOH). Acid hydrolysis produces L-rhamnose (**2**) and gallic acid (**3**) in a 1:2 ratio. The content of gallic acid was established colorimetrically [2, 3]; of L-rhamnose, using a semimicro method of sugar determination [4].

The UV spectrum has absorption maxima at 220 and 280 nm. The IR spectrum exhibits absorption bands at 3250 (OH), 1700 (ester carbonyl), 1650 (conjugated ketone), and 1615 cm^{-1} (phenol) [5, 6].

The PMR spectrum of **1** has three groups of signals: 1) at strong field (1.08 ppm) for CH_3 groups; 2) at 3.8-6.5 ppm including a multiplet centered at 4.0, the fine structure of which is typical of a pentet. The spectral data indicate that glycoside **1** contains L-rhamnose (or a related compound, for example, fucose). In this instance, the multiplet is due to spin—spin coupling of H-5 (δ 4.00 ppm) with a methyl group and H-4 (δ 4.30 ppm) with approximately equal SSCCs. The experimental results from decoupled NMR confirm this hypothesis. Saturation of the doublet decomposes the pentet into a doublet. Saturation of the pentet changes the doublet at 1.08 ppm into a singlet. The weak-field part of the spectrum (third group of signals at 7.0-8.5 ppm) contains strong signals of aromatic protons as complicated and overlapping multiplets. They are partially overlapped by strong and broad (half-width 75-100 Hz) lines that can be assigned to resonances of the acidic OH groups of gallic acid.

GC analysis of the hydrolysis products indicates that the ratio of L-rhamnose to gallic acid is 1:2.

Methylation of **1** according to the literature method [7] gave the permethylate, which gave after purification and hydrolysis by methanolic NaOMe the O-methyl-tri-O-methylgallate (**4**) and dimethylrhamnose (**5**). The preparation of **4** indicates that the molecule lacks digallic acid [8]. The hydrolysate of methylation product **1** contains 3,4-O-dimethyl-L-rhamnose according to TLC and GC [9].

Compared with the literature values, protons H-1 [6.5 ppm (5.9 [10]*), J = 8 Hz] and H-2 [5.85 ppm (5.17 [11]), J = 8 Hz] of L-rhamnose experience the greatest shifts in the PMR spectrum of **1**. Therefore, it can be assumed that the phenol substituents are located at positions C-1 and C-2.

The results show that **1** is a monomeric hydrolyzable tannin of gallic acid that contains L-rhamnose. The structure of this compound, called glarein C, is shown in the diagram.

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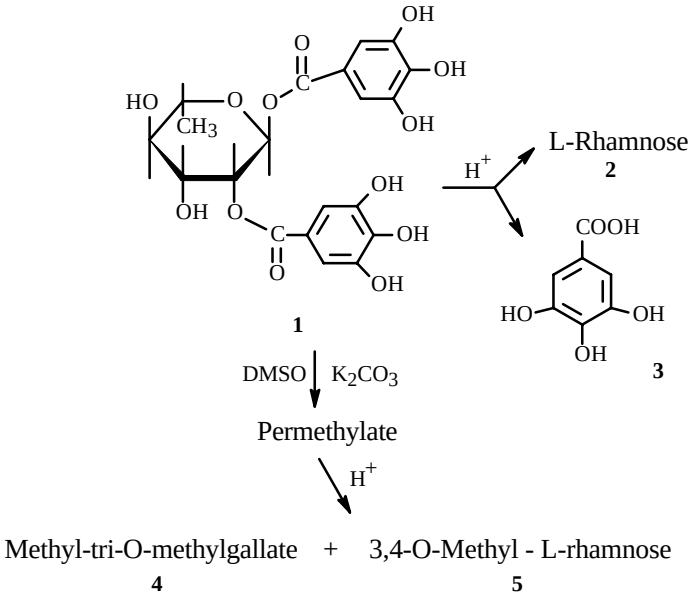

EXPERIMENTAL

Sephadex LH-20 and ion-exchanger EDE-10p (HCO_3^- form) were used to purify and separate tannins; papers "M" (St. Petersburg) and FN-12 (East Germany) and Silufol UV-254 plates with a fixed silica-gel layer and solvent systems *n*-BuOH—CH_3CO_2H—H_2O (4:1:2) (1) and pyridine—benzene—butanol—water (4:1:5:3) (2), for chromatography. Melting points were determined on a Kofler block; optical rotation, on a SU-2 polarimeter; elemental composition, by a semimicro method [12]. UV spectra were recorded on a SF-16 spectrophotometer; IR spectra, on a UR-20 apparatus in KBr pellets; PMR, on a BC-497 spectrometer (100 MHz) with HMDS internal standard and C_5D_5N solvent. GC was performed on a Chrom-5 instrument using a column with XE-60 Silicone (5%) on chromaton N-AN-HMDS, flame detector, He carrier-gas flow rate 40 mL/min, column temperature 210°C, vaporizer 230°C, and detector 270°C.

The isolation of **1** has been reported [1].

Glarein C (**1**) is a yellowish-brown amorphous powder, mp 172-174°C, $[\alpha]_D^{20}$ +80.1°C (*c* 1.0, MeOH). Found (%): C 50.98, H 4.12, O 44.12. Calc. (%): C 53.09, H 4.42, O 42.48. MW 468, $C_{20}H_{20}O_{13}$, λ_{max} (EtOH, nm, log ε): 220 (5.4), 280 (2.1). ν_{max} (KBr, cm^{-1}): 3250 (OH), 1700 (ester carbonyl), 1650 (conjugated ketone), 1615 (phenol) [2, 3]. PMR spectrum (δ, ppm, J/Hz): 1.08 (3H, s, CH_3, L-rhamnose), 4.0 (pentet, H-5), 4.3 (m, H-4), 4.85 (m, H-3), 5.85 (d, J = 8, H-2), 6.50 (d, J = 8, H-1), 7.00, 7.04 and 7.42, 7.85 (s, 1H×4, gallic acid 2-2, 2-6, 1-2, and 1-6).

Hydrolysis of Glarein C. A solution of **1** (20 mg) was treated with H_2SO_4 (5%, 40 mL) and boiled for 5 h. The gallic-acid content as determined photocolorimetrically was 0.010 g, which corresponds to 70% of its content in $C_{20}H_{20}O_{13}$.

Paper chromatography of the aqueous part of the hydrolysate using systems 1 and 2 detected L-rhamnose. The content of L-rhamose was calculated using a micro method for determining sugars [1]. Treatment of the analytical solution (40 mL) with $KMnO_4$ formed cuprous oxide (14.5 mg), which corresponds according to a conversion table to 6.2 mg of L-rhamnose or 89.5% of its content in $C_{20}H_{20}O_{13}$.

Methylation of glarein C and hydrolysis were performed as before [1]. Two sections were cut from the TLC with a compound appearing at the level of methyl-tri-O-methylgallate (5 mg). TLC in system 1 and GC analysis detected 3,4-O-methyl-L-rhamnose in the aqueous part.

Methyl-tri-O-methylgallate is a white crystalline powder, $C_{11}H_{14}O_5$, PMR (δ, ppm): 7.34 (2H, s), and 3.85 (12H, CH_3×4, s) [1].

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