Chemistry of Natural Compounds, Vol. 36, No. 2, 2000

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

L. N. Gvazava and M. D. Alaniya

UDC 547.982/83/84

A new dimeric hydrolyzed tannin called glarein A (1) is isolated from Euphorbia glareosa *Pall. ex Bieb. leaves. Glarein A consists of two D-glucose residues, two valoneaylic groups, and two gallic acids. The structure is established by chemical reactions and UV, IR, and PMR spectra.*

Key words: *Euphorbia glareosa*, dimeric tannin, glarein A.

Plants of the *Euphorbia* genus are widely distributed over the whole world. A total of 800 species have been counted, 45 of which grow in Georgia [1]. The chemical composition and pharmacological properties of various spurge species have been reported. Plants of this genus contain triterpenes, steroids, sesquiterpenes, phenols, and others [2, 3]. Various biological activities, mainly hypotensive and cardiotonic [2-6], have been observed for the plant preparations.

We found that spurge species growing in Georgia are rich in flavonoids and hydrolyzed tannins.

Phenolic compounds were extracted from an aqueous extract of *E. glareosa* leaves by a butanol—butylacetate mixture (1:1). The evaporated extract was repeatedly fractionated on the ion-exchanger EDE-10p (HCO_3^-) and chromatographed on an LH-20 Sephadex column (eluent was ethylacetate-saturated water) to give free gallic (**2**) and ellagic (**3**) acids and three pure compounds A, B, and C, which give positive reactions with alcohol solutions of $FeCl_3$, typical of phenols. They turned out to be hydrolyzed tannins [7, 8]. Acid decomposition showed that compounds A and B contain D-glucose; compound C, L-rhamnose, as monosaccharides.

The structure of tannin A (**1**) is established in the present article. Compound **1** forms transparent platelike crystals with mp 190-196°C. The UV spectrum of **1** exhibits maxima at 220, 270, and 360 nm. The IR spectrum contains absorption bands of OH groups (3300 cm^{-1}), esterified carbonyl (1675), and phenyl radical (1610), consistent with a phenolic compound [7, 8].

The PMR spectrum contains signals belonging to phenolic hydroxyls at 8.50-7.52 ppm. Signals appearing in the range 7.20-6.40 ppm are assigned to gallic-acid protons. Signals in the range 6.40-3.20 ppm belong to the two D-glucose molecules (Table 1) [10, 11].

Compound **1** is hydrolyzed by 5% H_2SO_4 to give gallic (**2**) and ellagic (**3**) acids and D-glucose (**4**) in the ratio 2:1:1. The content of gallic acid was determined colorimetrically [12, 13]; of ellagic acid, gravimetrically [13]; of glucose, using a semimicromethod of sugar determination [14]. The most probably position of the glucosidic ether was found by comparing the chemical shifts of the C=O carbon atom in gallic acid and hexahydroxydiphenyl with those of vuudfruticoside.

Compound **1** was methylated [15] to give the permethylate. Purification and hydrolysis with methanolic sodium methoxide gave methyltri-O-methylgallate (**5**), trimethylocta-O-methylvalonate (**6**), and methyl-D-glucose (**7**). Monomethyl-D-glucose was identified by comparing it with known samples using paper chromatography and GLC. We synthesized methyltri-O-methylgallate from gallic acid for comparison. Tri-O-methylocta-O-methylvalonate was identified by comparing its specific rotation and UV and PMR spectra with the literature values [9, 15, 16]. The production of **3** by acid hydrolysis of **1** and **6** after cleavage of the permethylate suggests that the tannin does not contain a euphorbinoyl group [16], which is characteristic of spurge phenols.

A comparison of the results with the literature data [9, 15, 16] for **1** suggests that the most probable structure is **1**. This compound has not been described in the literature. We called it glarein A.

I. G. Kutateladze Institute of Pharmacochemistry, Academy of Sciences of Georgia. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 112-114, March-April, 2000. Original article submitted October 26, 1998.

 0009-3130/00/3602-0140$25.00

TABLE 1. PMR Spectrum of Glarein A (1)

C atom	ppm	Lit. [9]	C atom	ppm	Lit. [9]
1	4.60 d	4.49 d	2-6	6.32 br. s	6.48 br. s
2	6.26 m	6.22 m	3-2	7.06 s	7.03 s
3	6.22 m	6.21 m	3-6	7.06 s	7.03 s
4	5.81 br. t	5.81 br. t	4-6	6.54 s	6.53 s
5	4.67 dd	4.67 dd	6-6	6.32 s	6.64 s
6	4.30 d	3.72 d	2'-6	6.70 s	6.71 s
	5.04 dd	5.33 dd			
1'	5.98 d	7.27 d	3'-2	7.11 s	7.31 s
2'	5.02 dd	5.19 dd	3'-6	7.16 s	7.31 s
3'	6.17 t	5.49 t	4'-6	6.98 s	7.09 s
4'	4.90 t	4.91 t	6'-6	6.64 s	6.68 s
5'	4.20-	4.15 dd			
	4.28 dd				
6'	4.28 d	3.88 d			
	6.49 dd	5.04 dd			

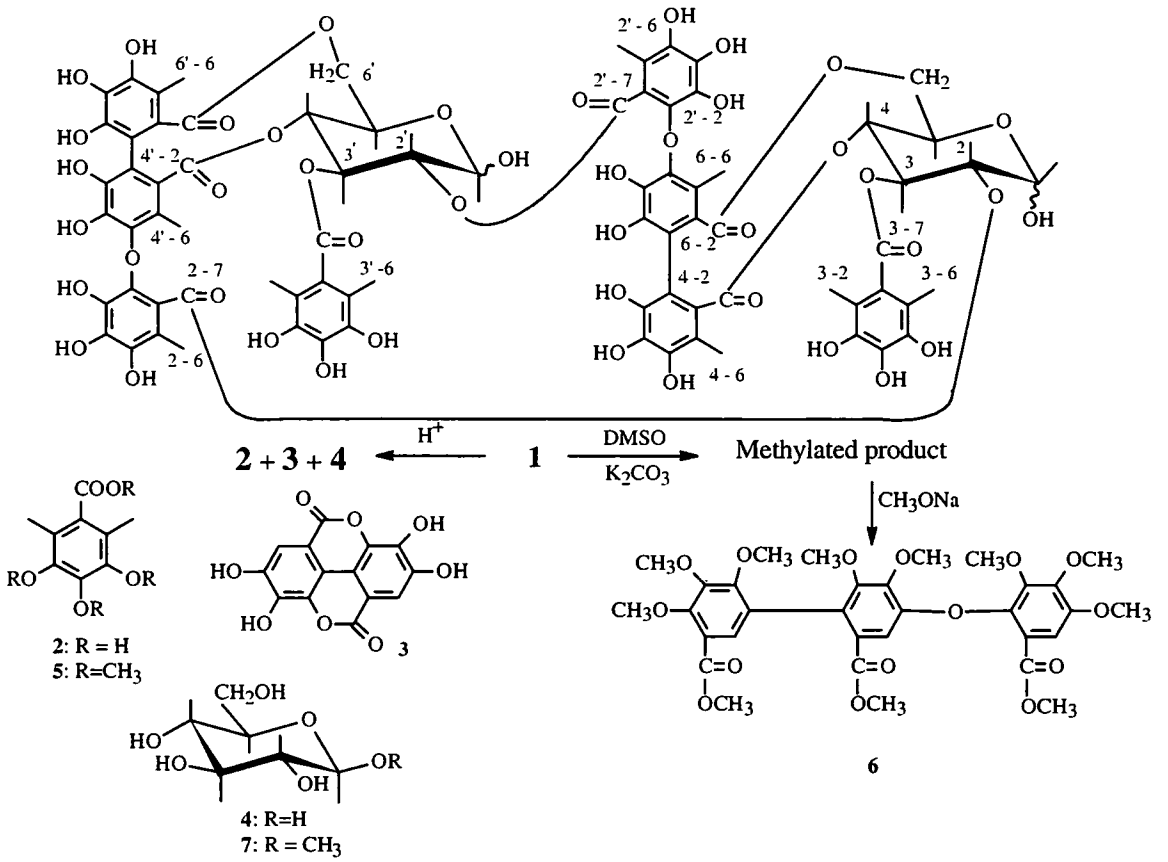

Fig. 1. Chemical transformation of compound A.

Compounds **2** and **3** give a positive reaction for phenolic acids. The mobility on paper chromatography in various systems is analogous to that for gallic and ellagic acids individually. The IR spectrum contains absorption bands characteristic of phenolic hydroxyl at 3400, 3300 cm^{-1}, and of carbonyl (C=O) at 1712 cm^{-1}. The melting point was not depressed by mixing with an authentic sample. According to our results, **2** is gallic acid and **3** is ellagic acid [7].

EXPERIMENTAL

Tannins were purified and separated using Sephadex LH-20 and ion-exchanger EDE-10p (HCO_3^--form). Chromatography used grade M (St. Petersburg) and FN-12 paper (Germany), plates with layers of silica gel, silufol UV-254, and the following solvent systems: 1) 1-butanol—CH_3CO_2H—H_2O (4:1:2), 2) C_5H_5N—C_6H_6—butanol-H_2O (4:1:5:3), 3) C_6H_6—$(CH_3)_2CO$ (3:1), 4) $CHCl_3$ (neat), 5) C_6H_6—CH_3OH—CH_3CO_2H (glacial) (45:8:15), 6) 5% acetic acid, and 7) 60% aqueous formamide buffered to pH 3 with formic acid [18]. Melting points were determined on a Kofler block; optical rotation, on a SU-2 polarimeter; elemental composition, by a semimicromethod [17].

UV spectra were recorded on an SF-16 spectrophotometer; IR spectra, on a UR-20 apparatus in KBr pellets; PMR, on a BC-497 (100 MHz) spectrometer with HMDS internal standard in deuteropyridine; mass spectra, on a MAT-112 instrument (Varian). GLC was carried out on a Chrom-5 instrument in a column packed with 5% Silicone XE-60 on chromaton N-AN-HMDS using a flame-ionization detector, He carrier gas at 40 ml/min, column temperature 210°C, vaporizer 250°C, and detector 270°C.

Isolation. Ground air-dried *Euphorbia glareosa* leaves (500 g) were extracted with hot water (5 l). The aqueous extract was treated with a butanol—butylacetate mixture (1:1, 2 l). The combined extracts were evaporated to dryness to give tannins (15 g). A portion (5 g) of these was dissolved in water (25 ml) and purified of accompanying substances on a column of EDE-10p ion-exchanger (53 × 1.7 cm). The column was washed with water (1 l) to give a fraction enriched with tannins that was dried and chromatographed on a column packed with Sephadex LH-20 (36 × 2 cm). The eluent was water saturated with ethylacetate (1.5 l). Fractions were collected as colored bands eluted. The composition of these was monitored by paper chromatography using system 1. Fractions containing two components were rechromatographed after condensing on a column packed with Sephadex LH (36 × 2 cm) and eluted with the same solvent. Fractions with one component were purified analogously. The yields of pure compounds were: **1** (1.67 g, 1.03%), **2** (0.12 g, 0.07%), and **3** (0.08 g, 0.048%).

Compound 1 (glarein A): transparent platelike crystals, mp 190-196°C, $[\alpha]_D$ +108.5 ± 0.5° (*c* 1.0; acetone—methanol, 8:2).

Found (%): C 4.29, H 3.05, O 2.81. $C_{68}H_{48}O_{44}$. Calc. (%): C 4.336, H 3.06, O 2.806. M = 1568.

UV spectrum (λ_{max}, nm): 220, 270, 360 (log ε 3.49, 5.29, 4.50).

IR spectrum (ν, cm^{-1}): 3300 (OH), 1675 (=CO), 1610 (phenyl), 1580 (–C=C–), 1200 (–C–O–C–).

PMR (δ, ppm, J, Hz): 4.60 (1H, d, J = 8, 1-H), 6.26 (1H, m, 2H), 6.22 (1H, m, 3-H), 5.81 (1H, br. t, J = 10, 4-H), 4.67 (1H, dd, J = 10, J = 7, 5-H), 5.04 (dd) and 4.30 (1H, d, J = 13, J = 7, 6-H), 5.98 (1H, d, J = 3, 1′-H), 5.02 (1H, dd, J = 9.5, J = 8, 2′-H), 6.17 (1H, t, J = 9.5, J = 5, 3′-H), 4.90 (1H, t, J = 10, 4′-H), 4.20-4.28 (1H, dd, J = 10, J = 5.5, 5′-H), 6.49 and 4.28 (1H, dd and d, J = 13, 6′-H), 6.32 (1H, br. s, 2-6-H), 7.06 (2H, s, 3-2 and 3-6-H), 6.54 (1H, s, 4-6-H), 6.64 (1H, s, 6-6-H), 6.70 (1H, s, 2′-6-H), 7.11 (1H, s, 3′-2-H), 7.16 (1H, s, 3′-6-H), 6.98 (1H, s, 4′-6-H), 6.64 (1H, s, 6′-6-H).

Acid Hydrolysis. A solution of **1** (0.2 g) was treated with 5% H_2SO_4 (40 ml) and boiled for 9 h. The reaction mixture was cooled. The precipitate of ellagic acid was filtered off, washed with water, and dried over anhydrous $CaCl_2$. Yield 0.070 mg, 90.8% of its content in $C_{68}H_{48}O_{44}$. The content of gallic acid was determined photocolorimetrically. Yield 0.081 g, 94.18% of its content in $C_{68}H_{48}O_{44}$. The hydrolysate contained glucose, the content of which was determined by a micromethod for determining sugars. Treatment of the analyzed solution (4 ml) with $KMnO_4$ produced 4.9 mg of cupric oxide, which is equivalent to 4.11 mg of glucose according to a calibration table. Therefore, 40 ml of solution contained 41.10 mg of glucose, 89.6% of its content in $C_{68}H_{48}O_{44}$.

Methylation. Compound **1** (10 mg) in dimethylsulfate (90 ml) was treated with K_2CO_3 (100 mg) in acetone (2 ml), left to stand at room temperature for 12 h, and heated for 3 h. The resulting permethylate was analyzed by TLC in system 3. The unreacted material was filtered off. The filtrate was dried under vacuum and purified by preparative chromatography on a thin layer of silica gel in system 3. The permethylated derivative (18.5 mg) was obtained as a bright yellow powder. It was dissolved in absolute methanol (1 ml), treated with methanolic sodium methoxide (1 ml, 1%), and left at room temperature for one day with intermittent stirring. The reaction mixture was neutralized with acetic acid and concentrated under vacuum to a small volume. The solid was extracted with $CHCl_3$. The extract was concentrated and placed on a thin layer of silica gel in system 4. Two bands were cut from the TLC at the position of methyltri-O-methylgallate (3 mg) and trimethylocta-O-methylvalonate (10 mg). According to TLC in system 2 and GLC of authentic samples, the aqueous fraction contained methyl-β-D-glucose.

Methyltri-O-methylgallate: white powder, $C_{11}H_{14}O_5$. PMR (δ, ppm): 7.34 (2H, s) and 3.85 (12H, CH_3 × 4, s).

Trimethylocta-O-methylvalonate: $C_{32}H_{36}O_{15}$, $[\alpha]_D$ -15.4° (*c* 0.3, acetone).

UV spectrum (λ_{max}, nm): 222, 303 (log ε 4.14, 3.85). PMR (δ, ppm): 6.92 (1H, s), 7.30 (1H, s), 7.35 (1H, s), 3.50-4.07 (33H, CH_3 × 11).

Compound 3. $C_{14}H_6O_8$, transparent needlelike crystals, mp 358-360°C, R_f 0.58 ± 0.02 [18].

REFERENCES

1. *Flora of Georgia* [in Russian], Metsniereba, Tbilisi (1983), Vol. 8, p. 147.
2. A. I. Shreter, *Medicinal Plants of the Soviet Far East* [in Russian], Nauka, Moscow (1975).
3. *Plant Resources of the USSR* [in Russian], Nauka, Leningrad (1986), Vol. 2, p. 202.
4. V. V. Telyat'ev, *Medicinal Plants of Eastern Siberia* [in Russian], Irkutsk (1971).
5. E. D. Petryaev, *Medicinal Plants of Zabaikal'* [in Russian], Nauka, Chita (1952).
6. N. K. Fruetov and G. N. Kadaev, *Poisonous Plants* [in Russian], Khabarovsk (1971).
7. G. Nonaka, K. Ishimaru, K. Mihashi, et al., *Chem. Pharm. Bull.*, **36**, No. 4, 857 (1988).
8. Sh. Yu. Islambekov, A. K. Karimdzhanov, S. M. Mavlyanov, and A. I. Ismailov, *Khim. Prir. Soedin.*, 293 (1990).
9. T. Yoshida, T. Chou, Y. Maruyama, and T. Okuda, *Chem. Pharm. Bull.*, **38**, No. 10, 2681 (1990).
10. N. K. Kochetkov, A. F. Bochkov, et al., *Carbohydrate Chemistry* [in Russian], Khimiya, Moscow (1967).
11. *Progress in Carbohydrate Chemistry* [in Russian], Nauka, Moscow (1985).
12. S. M. Mavlyanov, Sh. Yu. Islambekov, A. K. Karimdzhanov, A. I. Ismailov, and N. I. Iskhakov, *Khim. Prir. Soedin.*, 506 (1981).
13. S. M. Mavlyanov, Sh. Yu. Islambekov, F. G. Kamaev, U. A. Abdullaev, A. K. Karimdzhanov, and A. I. Ismailov, *Khim. Prir. Soedin.*, 238 (1997).
14. A. I. Ermakov, V. V. Arasimovich, M. I. Smirnova-Ivannikova, and I. K. Murri, *Methods of Biochemical Plant Research* [in Russian], Gosizdat Sel.-Khoz. Lit., (1952), p. 170.
15. S. Kadota, Y. Takamori, N. N. Khin, et al., *Chem. Pharm. Bull.*, **38**, No. 19, 2687 (1990).
16. T. Okuda, T. Yoshida, and T. Hatano, *Heterocycles*, **30**, No. 2, 1195 (1990).
17. V. S. Bostoganashvili, D. G. Turabelidze, and D. G. Narchemashvili, *Biologically Active Compounds of the Flora of Georgia* [in Russian], Metsniereba, Tbilisi (1973).
18. Yu. V. Roshchin and S. F. Dzhumyrko, *Khim. Prir. Soedin.*, 610 (1969).