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HYDROLYZED TANNIN FROM *Euphorbia glareosa* LEAVES

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Hydrolyzed tannin (I), the structure of which was established using chemical investigations and UV, IR, PMR and ^{13}C NMR spectra, was isolated from Euphorbia glareosa *leaves.*

Key words: *Euphorbia glareosa*, hydrolyzed tannin, glarein B.

We reported previously that hydrolyzed tannins were isolated from *Euphorbia glareosa* Pall. ex Bieb. leaves [1, 2]. In the present article, we establish the structure of one of them. Tannin (**1**) isolated by the previous method [1] is a yellowish crystalline substance, mp 156-158°C. The UV spectrum of this compound had maxima at 220, 270, and 360 nm; the IR spectrum, absorption bands at 3300 cm^{-1} (OH), 1675 (esterified carbonyl), and 1615 (phenyl), which is consistent with its phenolic nature [3].

The PMR spectrum showed signals that belong to phenol hydroxyls at 8.50-7.50 ppm; gallic acid, 7.60-6.40 ppm, and one D-glucose molecule, 6.40-3.20 ppm (Table 1) [1, 4].

Compound **1** was hydrolyzed by H_2SO_4 (5%) to give gallic acid (**2**) and D-glucose (**3**) in a 3:1 ratio. The gallic-acid content was calculated colorimetrically [4, 5]; D-glucose, by semimicro determination of the sugars [6].

Methanolysis of **1** by the literature method [7] produced **4** and methylgallate **5** in a 1:1 ratio.

Signals in the ^{13}C NMR spectrum at 166.43, 166.83, and 167.09 ppm are consistent with the presence of three esterified carbonyls belonging to gallic acid. This was confirmed by proton signals for H2′,6′; H2″,6″; and H2‴,6‴ in the PMR spectrum at 7.11, 7.26, 7.27, and 7.42 ppm (Table 1) [8].

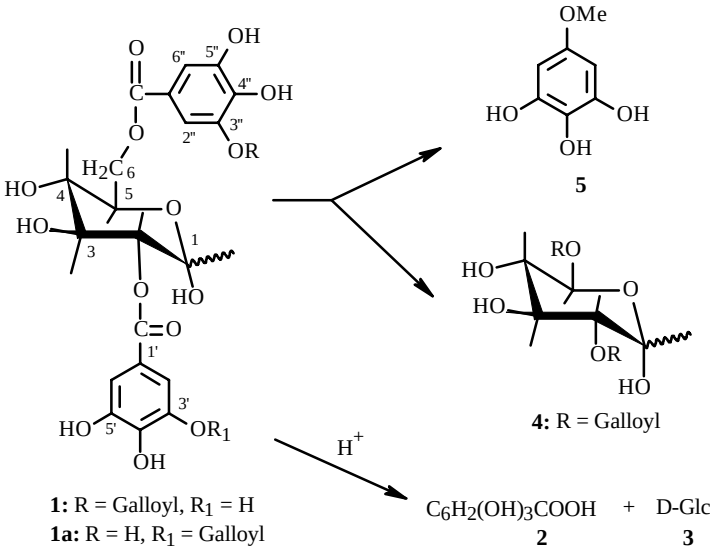

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TABLE 1. PMR and ^{13}C NMR Spectra of Tannin and Its Permethylate (δ, ppm, J/Hz)

C atom	PMR of **1**	C atom	**1**	Permethylate of **1** [9]	C atom	**1**	Permethylate of **1**
		D-glucose				Digalloyl	
123456	5.32 (d, J = 4.2)	123456	95.5	96.1 (96.2)	1″	122.25	126.11
	5.02 (m, J = 8.5)		73.8	73.8 (75.4)	2″	119.24	117.76
	3.70 (m, J = 8.5)		73.8	73.8 (77.1)	3″	135.99	144.94
	3.50 (m, J = 8.5)		69.7	69.7 (71.0)	4″	141.06	146.46
	3.4-3.8 m		72.8	72.8 (77.1)	5″	145.90	154.35
	4.43 (d, J = 13.0)		62.9	62.9 (61.3)	6″	113.57	112.33
	4.30 (dd, J = 13; 5)				7″	167.09	164.71
					1‴	121.50	125.76
		Galloyl			2‴	109.93	108.53
26	7.11 s	1′	121.22	124.68	3‴	145.95	154.03
		2′	109.93	108.53	4‴	138.90	144.30
		3′	145.95	154.26	5‴	145.90	144.30
		4′	138.77	143.73	6‴	109.77	108.21
		5′	145.90	154.16	7‴	166.83	165.71
		6′	109.77	108.21			-OCH_3
		7′	166.43	165.97			60.98
		Digalloyl					60.71
2,6′	7.20 s						60.45 (3×CH_3)
2,6″	7.52 s, 7.62 s						56.65 (5×CH_3)

The chemical shifts of the D-glucose indicate that the C2 and C6 hydroxyls are esterified by gallic acid units. Substitution at C1 is precluded because the C chemical shift in this instance, by analogy with previous data [9], would be 94.7 or 92.2. In our instance, it was 95.5 ppm, which may be due to the influence of the C2 substitution (β-effect) [10]. In fact, the chemical shift of C2 at 73.8 ppm (α-effect) indicates that it is substituted. The chemical shift of C6 at 62.3 ppm is also indicative of substitution [9, 11].

We elucidated the site of addition of the third gallic acid. According to the PMR spectrum, it is clear that it is bound directly to glucose and should be located in the chain as a dehydrodigallic (DHDG) or digallic (DG) acid [11]. The DHDG cannot be present because all three signals belonging to H2,H6×3 are present in the PMR spectrum; signals for C2 and C6, in the ^{13}C NMR spectrum (Table 1). Analysis of the chemical shifts for C-3″(3′) and C-2″(2′) leads to the assumption that gallic acid is bonded to C-3″(3′) of the terminal gallic acid. The change of the chemical shift for C-2″ compared with the others may be due to the β-effect of C-3″ substitution. A comparison of the chemical shifts for C_2, C_3, and C_4 of gallic acids of glycoside **1** and its methylated product also unambiguously indicate a 3→1-bond between galloyl units.

Thus, based on UV, IR, and NMR spectra, hydrolysis results, and comparison with literature data [3, 7, 8, 12], we propose the most probable structure **1** or **1a** for the investigated substance. This compound has not been reported in the literature.

EXPERIMENTAL

General comments [1, 2] and the isolation procedure [1] have been published.

Compound **1**, yellowish crystals, mp 156-158°C, λ_{max} (nm): 220, 270, 360. ν_{max} (KBr, cm^{-1}): 3300 (OH), 1675 (C=O), 1615 (phenyl).

For PMR and ^{13}C NMR data, see Table 1.

Hydrolysis. Tannin (**1**, 15 mg) was hydrolyzed as before [1, 2]. Paper chromatography of the reaction mixture after the appropriate workup detected D-glucose and gallic acid.

Methanolysis [7]. Compound **1** (15 mg) was dissolved in petroleum-ether(bp 85-120°C):$CHCl_3$:acetone (2 mL, 4:6:3, v/v) and in a mixture of CH_3OH and acetic-acid:sodium-acetate buffer (0.5 M, pH 6) and held at 37°C for 7 h. GC detected **4** and **5**.

Methylation of 1. Tannin (100 mg) was methylated by dimethylsulfate (0.3 mL) and K_2CO_3 (0.8 g) in acetone (10 mL) at room temperature (24 h) and then heated for 1 h. The insoluble part was filtered off. The filtrate was condensed and separated by preparative TLC using petroleum-ether(bp 85-120°C):$CHCl_3$:acetone (4:6:3) and benzene:acetone (4:1). The compound was a white powder after purification.

IR spectrum (KBr, ν_{max}, cm^{-1}): 1730 (esterified C=O), 1595. PMR specrum (acetone-d_6, δ, J/Hz): 7.60 (d, J = 2, H6 PMDG), 7.54 (d, J = 2, PMDG H-2), 7.50 (2H, s, PMDG H2′,H6′), 7.3 (H2, TMG, 2H, H6), 5.22 (d, J = 4.2, H1), 4.02 (3H, s, CH_3), 3.91 (6H, s, 2×CH_3), 3.89 (3H, s, CH_3), 3.88 (6H, s, 2×CH_3), 3.85 (3H, s, CH_3), 3.79 (3H, s, CH_3). For the ^{13}C NMR spectrum, see Table 1.

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