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HYDROLYZED TANNINS FROM *Geranium pusillum*

K. B. Kobakhidze and M. D. Alaniya

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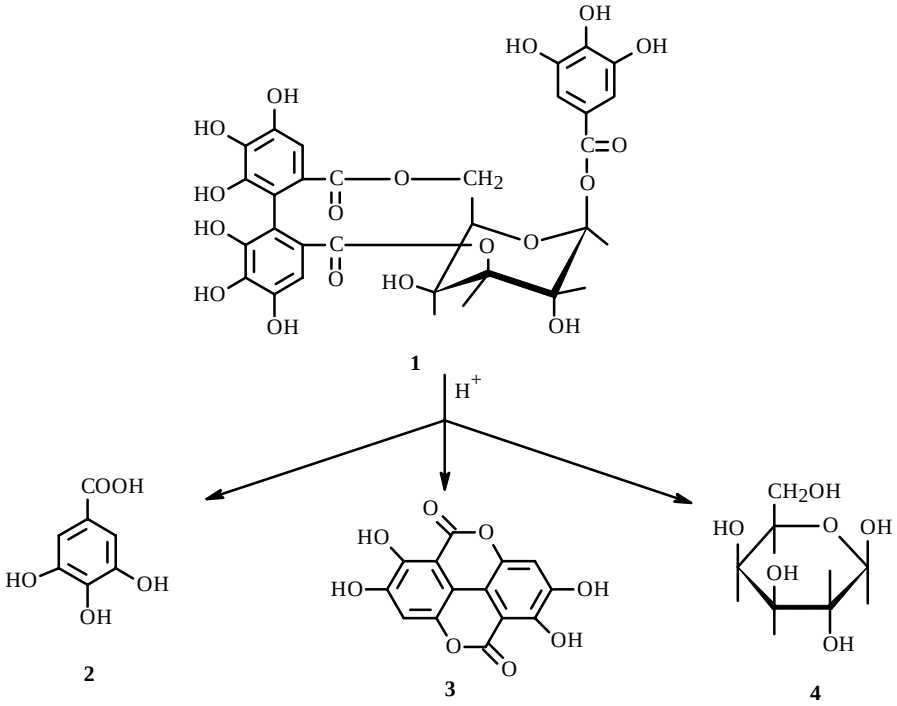
The polyphenolic compound 1-O-galloyl-3,6-hexahydroxybiphenyl-D-galactopyranoside (pusilagin) was isolated from the aerial part. The tannin structure was established using acid hydrolysis and IR, PMR, and ^{13}C NMR spectral methods.

Key words: *Geranium pusillum* L., tannin monomer, pusilagin.

The genus *Geranium* L. is represented by 23 species in the flora of Georgia. These are widely used in folk medicine as hemostatic and wound-healing agents [1].

Species of *Geranium* are known to be rich in flavonoids and hydrolyzed tannins [2]. We previously reported the isolation and identification of phenolic acids and flavonoids from the aerial part [3, 4]. This article presents results from a study of polyphenols of *G. pusillum*. The air-dried aerial part was exhaustively extracted with ethanol (70%). Phenolic compounds were extracted from the aqueous residue by ethylacetate. The ethylacetate fraction was separated over a polyamide column with subsequent purification over Sephadex LH-20 to afford **1**, which was an ellagotannin [5].

Compound **1** forms platelike light-brown crystals, mp 202-206°C. The UV spectrum exhibits maxima at 218 and 270 nm. The IR spectrum has absorption bands at 3200 (OH), 1695 (>C=O), and 1610 and 1580 cm^{-1}, indicative of its phenolic nature.



The PMR spectrum contains signals at 7.04 ppm for galloyl and at 6.65 and 6.68 ppm for hexahydroxybiphenyl (HHBP). Resonances from 6.35 to 4.15 ppm were assigned to carbohydrate protons. The PMR and ^{13}C NMR spectra confirmed the structure of **1** (Table 1).

I. G. Kutateladze Institute of Pharmaceutical Chemistry, Academy of Sciences of Georgia, fax (99532) 25 00 26.
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TABLE 1. ^{13}C NMR Data for Pusilagin (CD_3OD)

C	CS, ppm	C	CS, ppm
Galactose		HHBP	
1	95.06	ring B	
2	69.47	1	125
3	71.64	2	110.22
4	65.04	3	145.66
5	76.22	4	138.20
6	62.50	5	145.33
Galloyl		6	117.23
1	120.65	7 (C=O)	168.55
2.6	111.0	ring C	
3.5	146.41	1	125.54
4	140.43	2	108.37
7 (C=O)	166.72	3	145.24
		4	137.70
		5	146.08
		6	116.72
		7 (C=O)	170.14

Compound **1** was hydrolyzed by H_2SO_4 (5%) to give gallic (**2**) and ellagic (**3**) acids and D-galactose (**4**) in a 1:1:1 ratio. Quantitative determination of gallic acid used a colorimetric method [6]; of ellagic, gravimetric [7]; of D-galactose, semimicro sugar determination [8].

The experimental data and a comparison with the literature leads to the conclusion that the tannin isolated by us has a structure analogous to corilagin but with D-galactose instead of D-glucose in the carbohydrate part. An analogous compound has not been described in the literature. We named it pusilagin.

EXPERIMENTAL

Tannin was isolated and purified using polyamide prepared by the literature method [9] and Sephadex LH-20 (Pharmacia, Sweden). Paper chromatography (PC) used FN012 grade paper (Germany) and the following solvent systems: *n*-butanol:acetic acid:water (4:1:2, 1), pyridine:benzene:butanol:water (3:1:5:3, 2), acetic acid (5%, 3; 15%, 4). The melting point was determined on a Kofler block. The UV spectrum was recorded on a SF-16 spectrophotometer; IR spectrum, on a UR-20 apparatus in KBr disks; PMR and ^{13}C NMR, on a Bruker Avance DRX-400 (^1H, 400 MHz; ^{13}C, 100.6 MHz) in CD_3OD.

Isolation. Air-dried and ground raw material (500 g) from the aerial part of *G. pusillum* L. collected during massive flowering in Goriisk region of Georgia was exhaustively extracted with ethanol (70%, 1:10 ratio). After alcohol was removed, the aqueous fraction was purified of lipophilic compounds with $CHCl_3$ (1:1 ratio) until the $CHCl_3$ layer was colorless. Phenolic compounds were extracted from the aqueous fraction with ethylacetate (1:1). The combined fractions were evaporated to dryness in vacuum to afford the total polyphenols (16 g, 3.2%). A portion of these (2.6 g) was chromatographed over a polyamide column (d = 3 cm, h = 53 cm) with elution first by water and then by aqueous methanol (with increasing methanol concentration). The course of the separation was monitored by PC using system 1. Fractions eluted by 15% methanol contained mainly one compound with R_f 0.39 ± 0.1. The fractions were combined, concentrated, and rechromatographed over a Sephadex LH-20 column with elution by water to afford the pure compound (0.094 g, 0.116% yield based on air-dried raw material).

Compound **1** forms light-brown plates; mp 202-206°C; soluble in diluted methanol, ethanol, and acetone; R_f 0.39 ± 0.1 (system 1); 0.41 (system 3); 0.50 (system 4).

Found (%): C 49.7, H 3.45, O 43.9; $C_{27}H_{22}O_{18}$.

Calc. (%): C 51.1, H 3.47, O 45.42; MW 634.

UV spectrum (λ, nm): 218, 270. IR spectrum (ν, cm^{-1}): 3200 (OH), 1695 (>C=O), 1610 (phenyl), 1580 (–C=C–), 1200 (–C–O–C–).

PMR spectrum (δ, ppm, J/Hz): 6.35 (1H, d, J = 1.6, H-1 of D-galactose), 3.98 (1H, br.s, H-2), 4.80 (1H, br.s, H-3), 4.46 (1H, br.s, H-4), 4.51 (1H, t, J = 9.9, H-5), 4.95 and 4.15 (2H, t, J = 11, dd, J = 8, J = 11, CH_2-galactose), 7.04 (1H, s, H-2 of galloyl), 6.68 (1H, s, H-5 of HHBP), 6.65 (1H, s, H-5 of HHBP).

Table 1 contains the ^{13}C NMR data.

Acid Hydrolysis. A solution of **1** (0.25 g) was treated with H_2SO_4 (10 mL, 5%) and boiled for 3 h. The reaction mixture was cooled. The resulting precipitate of ellagic acid was filtered off, washed with water, and dried over anhydrous $CaCl_2$. Yield 0.1 g (83.3% of its content in $C_{27}H_{22}O_{18}$). Gallic acid was determined photocolorimetrically as 0.052 g or 86.7% of its content in $C_{27}H_{22}O_{18}$. Galactose in the hydrolysate was determined by a semimicro sugar analysis [8]. Treatment of the hydrolysate with $KMnO_4$ formed copper oxide (141 mg), which corresponds to galactose (62 mg, 88.83% of its content in $C_{27}H_{22}O_{18}$).

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