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6″-GALLOYLPICEIN AND OTHER PHENOLIC COMPOUNDS FROM *Arctostaphylos uva-ursi*

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Phenolic compounds from leafy shoots of A. uva-ursi *(Ericaceae) were studied. The new phenolic glycoside 6″-galloylpicein and 40 known compounds were isolated. Roots of* A. uva-ursi *afforded 16 compounds. A C-glycoside of bergenin was found for the first time in the family Ericaceae. The dominant components of* A. uva-ursi *leaves according to HPLC were arbutin, (+)-catechin, and corilagin; of stems, picein and (+)-gallocatechingallate; of roots, (–)-epicatechin, (–)-epicatechingallate, and (+)-catechin.*

Keywords: *Arctostaphylos uva-ursi*, Ericaceae, 6″-galloylpicein, phenolic glycosides, catechins, tannins, HPLC.

Leaves of *Arctostaphylos uva-ursi* (L.) Spreng. (Ericaceae) are an official plant raw material and are recommended for use as a diuretic and disinfectant for inflammatory diseases of urinary pathways [1]. The leaves (*gya′kyi ma* in Tibetan) were used in Buryatiya Tibetan medical practice for diseases related to increased stomach acidity (gastritis, heartburn), as an antipyretic for measles and a sedative for neurasthenia, and as powders in agents for diseases of the endocrine system (Basedow's disease) [2]. Chemical studies showed the presence in the aerial part of *A. uva-ursi* of various groups of phenolic compounds (simple phenols, phenolic glycosides, flavonoids, procyanidins, tannins) [3–5], triterpenes [6], polysaccharides [7], lipids [8], and essential oil [9]. Data on the composition of the plant roots are limited to reports of the presence in them of unedoside [10]. Usable reserves of *A. uva-ursi* within the Republic of Buryatiya are greater than 1,000 t/year [11]. Therefore, this region was recommended for inclusion among the principal sites for collection of this plant species. Leafy plant shoots were proposed as medicinal raw material in addition to the traditionally used leaves. This necessitated additional chemical studies.

The goal of our work was to study the composition of phenolic compounds from leafy shoots and roots of *A. uva-ursi* growing in Buryatiya.

Fractionation and chromatographic separation of substances extracted from leafy shoots of *A. uva-ursi* by column chromatography (CC) over polyamide, SiO_2, Sephadex LH-20, and preparative HPLC isolated the new phenolic glycoside **1** and 40 known compounds that were identified as arbutin (**2**), methylarbutin (**3**), 6″-galloylarbutin (**4**), picein (**5**), pyroside (**6**), gallic acid (**7**), 1,6-di-*O*- (**8**), 3,4,6-tri-*O*- (**9**), and 1,2,3,4,6-penta-*O*-galloylglucoses (**10**), (+)-catechin (**11**), (–)-epicatechin (**12**), (–)-epigallocatechin (**13**), (+)-gallocatechingallate (**14**), (–)-epigallocatechingallate (**15**), (–)-epicatechingallate (**16**), (–)-epigallocatechinmethylgallate (**17**), (–)-epigallocatechindigallate (**18**), procyanidins B1 (**19**) and B2 (**20**), corilagin (**21**), chebulagic acid (**22**), caffeic acid (**23**), 2-*O*- (**24**), 3-*O*- (**25**), 4-*O*- (**26**), 1,3-di-*O*- (**27**), and 3,5-di-*O*-caffeylquinic acids (**28**), cinnamic (**29**), 2-hydrocinnamic (**30**), 2-methoxycinnamic (**31**), ferulic (**32**), isoferulic acids (**33**), quercetin (**34**) and its glycosides hyperoside (**35**), 6″-galloylhyperoside (**36**), isoquercitrin (**37**), avicularin (**38**), quercitrin (**39**), rutin (**40**), and quercetin-3-*O*-gentiobioside (**41**).

Compound **1** was an amorphous white powder. The molecular formula according to HR-ESI-MS was $C_{21}H_{22}O_{11}$ {*m*/*z* 473.361 ($[M + Na]^+$; calcd 473.389)}. The acid hydrolysis products of **1** contained an equimolar mixture of *p*-hydroxyacetophenone, gallic acid, and glucose. Treatment of the compound with tannase formed picein (**5**) and gallic acid. This indicated that the structure of **1** was probably a picein derivative substituted by gallic acid in the carbohydrate. The site of attachment of the gallic acid of **1** was determined by exhaustive methylation by dimethylsulfate, subsequent formolysis, and analysis of the methylation products by GC/MS. It was established that the hydrolysis products contained 2,3,4-tri-*O*-Me-Glc*p*, which indicated that the glucopyranose was acylated only in the C-6 position because the *p*-hydroxyacetophenone group was located on C-1. The PMR spectrum exhibited two resonances of a *p*-substituted benzene ring (A_2B_2-type) as 2H doublets at 7.15 and 8.03 ppm that were assigned to H-2, H-6 and H-3, H-5, respectively (Table 1).

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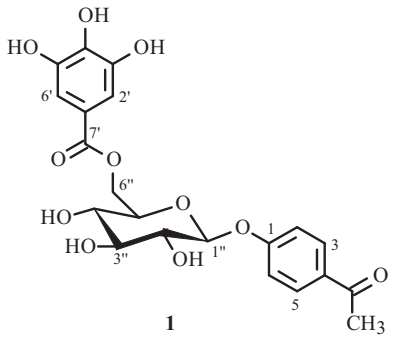
TABLE 1. PMR (500 MHz) and ^{13}C NMR (125 MHz) Spectra of **1** and **5** (DMSO-d_6, δ, ppm, J/Hz)

C atom	δ_H		δ_C	
	1	**5**	**1**	**5**
		p-Hydroxyacetophenone		
1	–	–	132.8	132.9
2	8.03 (2H, d, J = 9.2)	7.92 (2H, d, J = 9.2)	121.5	121.7
3	7.15 (2H, d, J = 9.2)	7.12 (2H, d, J = 9.2)	119.3	119.2
4	–	–	163.0	162.8
5	7.15 (2H, d, J = 9.2)	7.12 (2H, d, J = 9.2)	120.3	119.2
6	8.03 (2H, d, J = 9.2)	7.92 (2H, d, J = 9.2)	121.5	121.7
7	–	–	202.9	202.3
8	2.63 (3H, s)	2.60 (3H, s)	27.5	27.4
		Gallic acid		
1′	–	–	122.3	–
2′	7.25 (2H, s)	–	109.8	–
3′	–	–	145.3	–
4′	–	–	139.8	–
5′	–	–	145.3	–
6′	7.25 (2H, s)	–	109.8	–
7′	–	–	168.5	–
		β-Glucopyranose		
1″	4.78 (1H, d, J = 7.4)	4.81 (1H, d, J = 7.4)	102.7	102.5
2″			75.2	75.3
3″	3.31–3.69 (4H, m)	3.29–3.82 (4H, m)	78.9	78.8
4″			72.4	72.0
5″			76.0	78.2
6″	3.85 (1H, dd, J = 2.1, 12.2)	3.89 (1H, dd, J = 2.1, 12.2)	65.8	62.3
	4.04 (1H, dd, J = 6, 12.2)	4.02 (1H, dd, J = 6, 12.2)		



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The presence of a 3H singlet at 2.63 ppm showed that the acyl substituent on the benzene ring was an acetyl. Resonances of the carbohydrate part were located in the range 3.31–4.78 ppm and included a doublet at 4.78 ppm belonging to the anomeric proton. The positions and multiplicities of protons in the range 3.31–3.69 ppm were characteristic of glucosyl methine protons. The presence of a gallic acid group was confirmed by a 2H singlet at 7.25 ppm. The ^{13}C NMR spectrum was similar to that of **5** and contained additional resonances due to the presence of the galloyl group in the compound (109.8, 122.3, 139.8, 145.3, 168.5 ppm) (Table 1). A weak-field shift of the C-6″ resonance (+3.5 ppm) relative to that of **5** confirmed that the substituent was located in this position. A strong-field shift was observed for the resonance of C-5″ (–2.2 ppm), indicating that neighboring atom C-6″ was substituted. The position of the resonance for glucopyranose C-1″ (102.7 ppm) in the ^{13}C NMR spectrum and the size of the spin–spin coupling constant of the resonance for the anomeric proton (J = 7.4 Hz) were characteristic of the *β*-configuration of the carbohydrate.

Thus, the results indicated that **1** was 4-hydroxyacetophenone-4-*O*-*β*-(6″-galloyl)glucopyranoside (6″-galloylpicein), was a new natural compound, and was the first acylated picein derivative.

Shoots and leaves of *A. uva-ursi* had previously afforded **2**, **3**, **4** [12], **5** [10], **7**, **10** [13], **11** [5], **20** [14], **21** [15], **23** [16], **34**, **35**, **37**–**41** [4], and **36** [17]. Compounds **6**, **8**, **9**, **12**–**19**, **22**, **24**–**33** were found for the first time in this plant. Compounds **17**, **18**, **22**, **30**, **31**, and **33** were isolated for the first time from the family Ericaceae.

TABLE 2. Content of Phenolic Compounds in Morphological Groups of *A. uva-ursi*[a,b]

Compound	Leaves	Stems	Roots
	Simple phenols, mg/g		
Arbutin	82.40	18.69	0.81
6″-Galloylarbutin	+[c]	Tr.	–
Picein	12.32	68.12	1.07
6″-Galloylpicein	–	+	Tr.
Gallic acid	2.66	0.89	0.03
Bergenin	–	–	1.05
	Galloylglucoses, mg/g		
1,6-Di-*O*-galloylglucose	4.93	4.19	0.07
3,4,6-Tri-*O*-galloylglucose	10.09	3.22	0.18
1,2,3,4,6-Penta-*O*-galloylglucose	23.52	2.82	Tr.
	Catechins, mg/g		
(+)-Catechin	64.40	17.38	1.45
(–)-Epicatechin	Tr.	10.24	8.18
(–)-Epigallocatechin	15.76	9.19	–
(–)-Epicatechingallate	–	5.48	2.89
(+)-Gallocatechingallate	7.96	26.44	–
(–)-Epigallocatechingallate	23.92	5.59	–
(–)-Epigallocatechinmethylgallate	+	+	+
(–)-Epigallocatechindigallate	–	Tr.	–
	Tannins, mg/g		
Corilagin	57.51	15.03	0.66
Chebulagic acid	15.07	4.12	Tr.
	Phenylpropanoids, μg/g		
Caffeic acid	354.78	79.33	Tr.
2-*O*-Caffeylquinic acid	31.73	Tr.	–
3-*O*-Caffeylquinic acid	95.20	37.77	–
4-*O*-Caffeylquinic acid	23.57	141.87	–
1,3-Di-*O*-caffeylquinic acid	Tr.	245.63	–
3,5-Di-*O*-caffeylquinic acid	861.36	262.87	–
Cinnamic acid	3578.24	3152.60	Tr.
2-Hydroxycinnamic acid	202.55	Tr.	–
2-Methoxycinnamic acid	195.17	Tr.	–
Ferulic acid	Tr.	76.23	–
Isoferulic acid	497.69	Tr.	–
	Flavonoids, mg/g		
Quercetin	Tr.	Tr.	–
Hyperoside	2.92	1.12	Tr.
Isoquercitrin	0.62	0.64	–
Avicularin	0.91	0.50	–
Quercitrin	0.20	0.24	–
Rutin	4.69	2.82	–
Quercetin-3-*O*-gentiobioside	1.06	1.51	–
Total content of identified compounds, mg/g, including:	336.78	202.23	15.73
simple phenols	97.38	87.70	2.96
galloylglucoses	38.54	10.23	0.25
catechins	112.04	74.32	12.52
tannins	72.58	19.15	0.66
phenylpropanoids	5.84	4.00	Tr.
flavonoids	10.40	6.83	Tr.

[a]Of air-dried raw material mass; [b]raw material I; [c]–, absent; +, present; Tr.: trace < 10 μg/g.

TABLE 3. Content of Phenolic Compounds in Shoots of *A. uva-ursi*, mg/g, of Air-Dried Raw Material Mass

Compound	Raw material sample							
	II	III	IV	V	VI	VII	VIII	IX
Arbutin	52.28	80.59	79.61	81.29	88.13	86.56	67.86	64.34
Picein	8.68	7.47	12.32	7.18	7.12	6.92	8.65	8.55
Gallic acid	2.60	2.71	1.61	2.56	2.67	2.27	3.16	2.35
1,6-Di-*O*-galloylglucose	3.58	3.91	3.23	4.15	3.84	3.95	4.01	2.71
3,4,6-Tri-*O*-galloylglucose	9.80	11.16	7.49	11.56	11.95	11.46	8.49	7.87
1,2,3,4,6-Penta-*O*-galloylglucose	12.14	13.89	15.65	15.99	15.04	15.52	21.89	11.71
(+)-Catechin	29.77	34.31	48.35	35.71	34.78	35.91	31.87	20.00
(–)-Epicatechin	15.34	17.11	8.71	17.63	17.43	17.22	11.23	10.99
Corilagin	48.05	55.32	53.09	55.45	54.85	53.37	62.79	49.79
Chebulagic acid	14.18	14.46	14.01	14.30	14.55	13.49	16.65	8.05
Total content including:	196.42	240.93	244.07	245.82	250.36	246.67	236.60	186.36
simple phenols	63.56	90.77	93.54	91.03	97.92	95.75	79.67	75.24
galloylglucoses	25.52	28.96	26.37	31.70	30.83	30.93	34.39	22.29
catechins	45.11	51.42	57.06	53.34	52.21	53.13	43.10	30.99
tannins	62.23	69.78	67.10	69.75	69.40	66.86	79.44	57.84

HPLC was used to study the quantitative composition of phenolic compounds in stem and leaves of *A. uva-ursi* (Table 2). The dominant groups of compounds in leaves were catechins (112.04 mg/g), simple phenols (97.38 mg/g), and tannins (72.58 mg/g). Simple phenols (87.70 mg/g) and catechins (74.32 mg/g) typically dominated the stems. The nature of the accumulation of pure compounds in morphological groups showed that high concentrations of arbutin (**2**, 82.40 mg/g), (+)-catechin (**11**, 64.40 mg/g), and corilagin (**21**, 57.51 mg/g) were noted in leaves; picein (**5**, 68.12 mg/g) and (+)-gallocatechingallate (**14**, 26.44 mg/g), in stems. A feature of the phenylpropanoid complex was the dominance of cinnamic acid derivatives (**29**, **30**, **31**), the total contents of which were 3.98 and 3.15 mg/g for leaves and stems, respectively. The principal component of the caffeic acid derivatives was 3,5-di-*O*-caffeylquinic acid (**28**, 262.87–861.36 mg/g). The flavonoids of *A. uva-ursi* included mono- and biosides of quercetin and were dominated by rutin (**40**, 2.82–4.69 mg/g), hyperoside (**35**, 1.12–2.92 mg/g), and quercetin-3-*O*-gentiobioside (**41**, 1.06–1.15 mg/g). The total content of unidentified phenolic compounds in leaves was 336.78 mg/g; in stems, 202.23 mg/g.

Quantitative analysis of *A. uva-ursi* shoots for the presence of the dominant compounds in eight batches of raw material from various regions of the Republic of Buryatiya showed that the content of simple phenols in them could be 63.56–97.92 mg/g; of galloylglucoses, 22.29–34.39; of catechins, 30.99–57.06; and of tannins, 57.84–79.44 mg/g (Table 3). Arbutin (**2**, 52.28–88.13 mg/g), corilagin (**21**, 48.05–62.79), and (+)-catechin (**11**, 20.00–48.35) were the species marker components with the highest contents.

The study of phenolic compounds from *A. uva-ursi* roots led to the isolation of 16 compounds including **2**, **5**, **7**, **8**–**12**, **16**, **17**, **21**–**23**, **29**, **35**, and bergenin (**42**). The presence of C-glycoside **42**, which is characteristic of species from the family Saxifragaceae (*Bergenia*, *Saxifraga*, etc.), was established for the first time for the family Ericaceae. According to HPLC, the dominant components of *A. uva-ursi* roots were catechins (–)-epicatechin (**12**, 8.18 mg/g), (–)-epicatechingallate (**16**, 2.89 mg/g), and (+)-catechin (**11**, 1.45 mg/g) (Table 2). The total contents of phenolic glycosides, galloylglucoses, and tannins were 15.73, 0.25, and 0.66 mg/g, respectively.

EXPERIMENTAL

Leafy shoots of *A. uva-ursi* were collected near Ulan-Ude (28 Aug., 2011; 51°86′85″ N, 107°56′06″ E; raw material No. I); on Barsk ridge (Mukhorshibir Region; 3 Aug. 2009; 51°32′04″ N, 107°53′83″ E; II); in Goryachinsk village (Pribaikal Region; IGEB SB RAS experimental plantation; 7 Sept. 2010; 52°98′55″ N, 108°29′03″ E; III); in Goryachinsk village (Pribaikal Region, wooded zone; 7 Sept. 2010; 52°98′63″ N, 108°29′31″ E; IV); in Pykhta gap (near Ulan-Ude; 15 Sept. 2011; 51°82′37″ N, 107°70′00″ E; V); in Verkhnyaya Berezovka village (near Ulan-Ude; 9 Sept. 2012; 51°80′90″ N, 107°57′92″ E; VI); in Mostovka town (Pribaikal Region; 29 Aug. 2011; 52°07′70″ N, 107°00′08″ E, VII); in Kucheger town (Kurumkan Region; 27 Aug. 2011; 54°88′52″ N, 110°99′36″ E; VIII); and in Ust-Barguzin village (Barguzin Region; 29 Aug. 2012; 53°40′91″ N,

109°02′09″ E, IX). Roots of *A. uva-ursi* were collected near Ulan-Ude (28 Aug. 2011; 51°86′85″ N, 107°56′06″ E). The species was determined by Dr. Pharm. Sci. T. A. Aseeva (IGEB SB RAS). Samples of *A. uva-ursi* were preserved in the IGEB SB RAS Herbarium (No. AEr/ae-14/52-24/0811, -16/22-17/0809, -21/14-54/0910, -21/14-55/0910, -11/63-05/0911, -18/53-33/0912, -15/54-28/0811, -15/54-30/0811, and -20/47-25/0812).

Polyamide (Woelm), silica gel (SiO_2, Sigma), and Sephadex LH-20 (Pharmacia) were used for CC. Spectrophotometry studies were carried out on an SF-2000 spectrophotometer (OKB Spektr); MS analysis, in an MAT 8200 high-resolution mass spectrometer (Finnigan). NMR spectra were recorded on a VXR 500S NMR spectrometer (Varian). GC/MS analysis was performed on a 5973 N chromatography mass spectrometer with an MSD 5973 N mass-selective detector (Agilent Technologies) with a diffusion pump, an HP-5ms capillary column (30 m/250 μm/0.25 μm), He carrier gas (1 mL/min), vaporizer temperature 280°C, column 50°C (2 min), 50–200°C (4°C/min), 200–280°C (20°C/min), 280°C (isothermal, 5 min), ion source 170°C, interface between the GC and mass-selective detector 280°C, ionizing-electron energy 70 eV, sample volume 1 μL with stream division 20:1.

Extraction and Fractionation. Ground leafy shoots (980 g, raw material I) was extracted with EtOH (70%) on a boiling-water bath (1:20, 5×). The EtOH extract was concentrated to an aqueous residue that was extracted with hexane, $CHCl_3$, EtOAc, and BuOH to afford five fractions: hexane (30.97 g, 3.2% yield of air-dried raw material mass), $CHCl_3$ (32.73 g, 3.3%), EtOAc (167.22 g, 17.1%), BuOH (189.14 g, 19.3%), and aqueous residue (124.46 g, 12.7%). An interfacial solid (3.23 g, 0.33%) precipitated during treatment of the concentrated extract with hexane. It was identified as ursolic acid and α-amyrin in a 1:9.2 ratio according to GC/MS.

The EtOAc fraction (104 g) was separated beforehand over a preconditioned polyamide cartridge (300 g) [18] with elution by H_2O, EtOH (40% and 98%), and NH_3 (0.5%) in EtOH. The aqueous fraction was chromatographed over Sephadex LH-20 (2.5 × 70 cm) using $CHCl_3$:EtOH (100:0→70:30) and afforded arbutin (22.42 g, **2**), methylarbutin (21 mg, **3**), 6″-galloylarbutin (54 mg, **4**) [12, 15], pyroside (6″-acetylarbutin, 14 mg, **6**) [19], and gallic acid (42 mg, **7**) [20]. Fractions eluted by EtOH (40% and 98%) were separated over polyamide (4 × 80 cm, H_2O:EtOH, 100:0→4:98) and SiO_2 (3 × 60 cm; hexane:EtOAc, 100:0→70:30; EtOAc:Me_2CO, 100:0→70:30) and by preparative HPLC (conditions 1). The isolated compounds were identified as 1,6-di-*O*- (92 mg, **8**), 3,4,6-tri-*O*- (101 mg, **9**), and 1,2,3,4,6-penta-*O*-galloylglucoses (117 mg, **10**) [21]; (+)-catechin (57 mg, **11**) [20]; (–)-epicatechin (5 mg, **12**); (–)-epigallocatechin (62 mg, **13**); (+)-gallocatechingallate (37 mg, **14**) [22]; corilagin (2.87 g, **21**) [23]; chebulagic acid (219 mg, **22**) [24]; cinnamic (14 mg, **29**), *o*-coumaric (2-hydroxycinnamic, 3 mg, **30**), 2-methoxycinnamic (6 mg, **31**), ferulic (19 mg, **32**), and isoferulic acids (12 mg, **33**) [25]; quercetin (7 mg, **34**) [20]; hyperoside (quercetin-3-*O*-galactoside, 53 mg, **35**) [26]; 6″-galloylhyperoside [quercetin-3-*O*-(6″-galloyl)-galactoside, 7 mg, **36**] [27], isoquercitrin (quercetin-3-*O*-glucoside, 11 mg, **37**) [28]; avicularin (quercetin-3-*O*-arabinoside, 17 mg, **38**), and quercitrin (quercetin-3-*O*-rhamnoside, 5 mg, **39**) [26]. The fraction eluted by NH_3 (0.5%) in EtOH was chromatographed over polyamide (4 × 90 cm, H_2O:EtOH, 100:0→4:98) to isolate caffeic acid (14 mg, **23**) [20] and 2-*O*- (9 mg, **24**), 3-*O*- (8 mg, **25**), 4-*O*- (8 mg, **26**), 1,3-di-*O*- (5 mg, **27**), and 3,5-di-*O*-caffeylquinic acids (63 mg, **28**) [29].

The BuOH fraction (125 g) was separated analogously. The aqueous fraction obtained after elution of the polyamide cartridge underwent CC over SiO_2 (3 × 60 cm; hexane:EtOAc, 100:0→70:30; EtOAc:Me_2CO, 100:0→70:30) and preparative HPLC (conditions 1) to yield picein (7.56 g, **5**) [19] and **1** (14 mg). The alcohol-soluble eluates contained (–)-epigallocatechingallate (17 mg, **15**), (–)-epicatechingallate (21 mg, **16**), (–)-epigallocatechinmethylgallate (5 mg, **17**) [30], (–)-epigallocatechindigallate (9 mg, **18**) [31], procyanidins B1 (33 mg, **19**) and B2 (20 mg, **20**) [32], rutin (115 mg, **40**) [28], and quercetin-3-*O*-gentiobioside (51 mg, **41**) [33].

Fractionation and chromatographic separation of *A. uva-ursi* root (320 g) extract isolated **2** (22 mg), **5** (37 mg), **7** (5 mg), **8** (9 mg), **9** (14 mg), **10** (4 mg), **11** (22 mg), **12** (31 mg), **16** (15 mg), **17** (4 mg), **21** (37 mg), **22** (6 mg), **23** (4 mg), **29** (3 mg), **35** (2 mg), and bergenin (97 mg, **42**) [34].

6″-Galloylpicein (1). $C_{21}H_{22}O_{11}$. UV spectrum (MeOH, λ_{max}, nm): 216, 268, 280sh. HR-ESI-MS *m*/*z* 473.361 $[M + Na]^+$. FAB^+-MS *m*/*z* 451 $[M + H]^+$, 298 $[(M – galloyl) + H]^+$, 136 $[(M – galloyl – glucose) + H]^+$ (100%). Table 1 presents the PMR spectrum (500 MHz, DMSO-d_6) and ^{13}C NMR spectrum (125 MHz, DMSO-d_6).

Acid Hydrolysis. Compound **1** (5 mg) was dissolved in TFA (10 mL, 1%) and heated at 100°C for 6 h. The TFA was removed in vacuo in the presence of MeOH. The hydrolysate was dissolved in MeOH (5 mL) and analyzed by HPLC (conditions 2, phenolic compounds; conditions 3, carbohydrates).

Enzymatic Hydrolysis. Compound **1** (2 mg) and tannase (10 mg) from *A ficuum* (Sigma, 150 U/g) were dissolved in acetate buffer (5 mL, pH 5.0), incubated at 38°C for 6 h, heated on a boiling-water bath for 15 min, and centrifuged (6000 g,

20 min). The supernatant was extracted with EtOAc (5 × 5 mL). The organic extracts were combined and concentrated. The dry solid was dissolved in MeOH (5 mL) and analyzed by HPLC (conditions 2).

Methylation. A mixture of **1** (4 mg), Me_2SO_4 (100 μL), and anhydrous Na_2CO_3 (150 mg) in Me_2CO (2 mL) was heated at 90°C for 4 h. Solid Na_2SO_4 was filtered off. The mixture was poured into ice water. The resulting precipitate was centrifuged, washed until neutral, and dried in vacuo. Formolysis and hydrolysis of the permethylate were carried out as before [35]. The hydrolysate was analyzed by GC/MS.

HPLC. Conditions 1: 600E liquid chromatograph (Waters), Lichrospher RP-18 column (4.6 × 200 mm, ∅ 5 μm, Merck Millipore), mobile phase H_2O (A), MeCN (B), gradient regime (%B in A) 0–60 min linear gradient 7–40% B, 60–90 min 40–60% B, flow rate 1 mL/min, column temperature 40°C, λ 270 nm. Conditions 2: Milikhrom A-02 microcolumn liquid chromatograph (Ekonova), ProntoSIL-120-5-C18 AQ column (2 × 75 mm, ∅ 5 μm, Metrohm AG), mobile phase (4.1 M $LiClO_4$ in 0.1 M $HClO_4$):H_2O 5:95 (A), MeCN (B), gradient regime (%B in A) 0–7.6 min 7–22%, 7.6–8.6 min 22–25%, 8.6–12 min 25–27%, 12–17 min 27–100%, 17–20 min 100–7%, flow rate 150 μL/min, column temperature 35°C, sample volume 2 μL, λ 216, 278, 330, 360 nm. Conditions 3: Milikhrom A-02 microcolumn liquid chromatograph (Ekonova), Separon 5-NH_2 column (2 × 75 mm, ∅ 5 μm, Tessek Ltd.), mobile phase 75% MeCN, isocratic regime (0–20 min), flow rate 100 μL/min, column temperature 35°C, sample volume 4 μL, λ 190 nm.

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