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CONSTITUENTS OF *Arctostaphylos uva-ursi* LEAVES

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A new natural compound p-*digallic acid ethyl ester and 1,3,6-trigalloylglucose first time isolated from* Arctostaphylos uva-ursi*, and also known for this plant compounds tetragalloylglucose, gallic acid, arbutin, and hyperoside were characterized by PMR , ^{13}C NMR, and UV spectroscopy and mass spectrometry.*

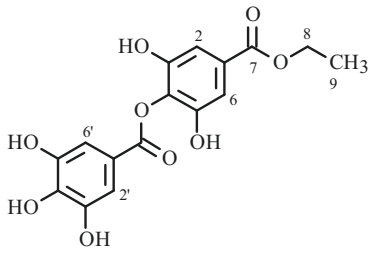
Keywords: *Arctostaphylos uva-ursi*, leaves, *p*-digallic acid ethyl ester, 1,3,6-trigalloylglucose, tetragalloylglucose, gallic acid, arbutin, hyperoside.

Arctostaphylos uva-ursi (L.) Spreng. (Ericaceae) is a medicinal plant indigenous to many countries including the Russian Federation [1–4]. Leaves of this plant have long been used in traditional medicine as a diuretic, antimicrobial, and anti-inflammatory agent for various diseases of the urogenital tract. The pharmacological activity is associated mainly with simple phenols (hydroquinone and its β-D-glucopyranoside arbutin) [2–4].

According to the literature [2–8], leaves of this plant contain simple phenols (hydroquinone, arbutin, methylarbutin, etc.), flavonoids (hyperoside etc.), coumarins, phenolcarboxylic acids (gallic acid and its derivatives), tanning agents, hydroxycinnamic acids, organic acids, saponins, vitamins, and other secondary and primary metabolites. Although the chemical composition of leaves of *A. uva-ursi* is very well studied, the constituent composition of the plant raw material remains interesting. In this respect, gallic-acid derivatives deserve attention because 1,6-digalloylglucose, 3,4,6-trigalloylglucose, tetragalloylglucose, 1,2,3,4,6-pentagalloylglucose, and corilagin are known from its leaves [5, 6].

The goal of the present work was to study the constituent composition of *A. uva-ursi* leaves.

The PMR spectrum of **1** had two 2H singlets belonging to digallic acid at 6.94 ppm (H-2′, H-6′) and 6.54 ppm (H-2, H-6). The singlets for the digallic acid protons indicated that the depside had the *p*-digallic acid structure. The C-2 and C-6 protons would have been observed as doublets for *m*-digallic acid. Furthermore, the PMR spectrum of **1** showed resonances for ethyl protons at 4.19 ppm (2H, q, J = 7 Hz, H-8) and 1.25 ppm (3H, t, J = 7 Hz, H-9). This was consistent with esterification of the C-1 carboxylic acid of gallic acid. The presence in **1** of ethyl gallate was confirmed by mass spectra in which base peaks were ions with *m*/*z* 221 $[M^+$ of ethyl gallate + Na$]^+$ and 199 $[M^+$ of ethyl gallate + H$]^+$. Thus, **1** had the structure *p*-digallic acid ethyl ester and was a new natural compound.



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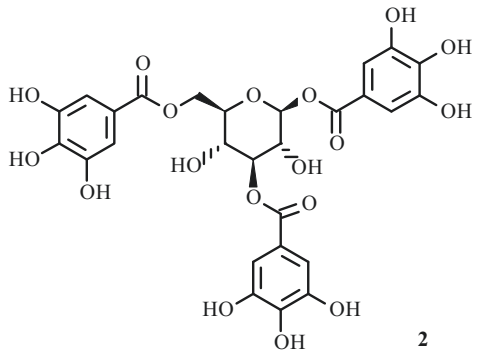

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Compound **2** with the structure 1,3,6-trigalloylglucose was especially interesting [9]. According to TLC, it was the dominant constituent. A doublet at 6.13 ppm with SSCC 7 Hz in the PMR spectrum of **2** belonged to the anomeric glucose proton (H-1) and indicated that one of the gallic acids was bonded to the glucose C-1 hydroxyl. Such an anomalous chemical shift (~6.5 ppm) for a glucose anomeric proton was observed by us earlier for other compounds, in particular, 1-*O*-*β*-D-glucopyranosides of *p*-coumaric and caffeic acids [10]. The PMR spectrum of **2** also exhibited three 2H singlets at 6.99, 6.85, and 6.78 ppm for six protons of three gallic acids. Compound **2** was isolated for the first time from *A. uva-ursi* leaves. Corilagin (trigalloylglucose, $C_{27}H_{22}O_{18}$), 3,4,6-trigalloylglucose, tetragalloylglucose, and 1,2,3,4,6-pentagalloylglucose were isolated earlier from them [5, 6].

The PMR spectrum of **3** showed four 2H singlets at 6.99, 6.85, 6.82, and 6.78 ppm for eight protons of four gallic acids. The compound was identified as tetragalloylglucose, which was known from leaves of this plant although without actual assignments of the gallic acids because that required additional research.

Compounds **4**, **5**, and **6** were identified using UV, PMR, and mass spectra as gallic acid [11], arbutin [12], and hyperoside [13, 14].

EXPERIMENTAL

General Comments. PMR spectra were taken on Bruker AM 300 instruments (300 MHz); mass spectra, Kratos MS-30 mass spectrometer. UV spectra were recorded using a Specord 40 spectrophotometer (Analytik Jena).

Air-dried leaves (100 g) of *A. uva-ursi* from a collection in 2016 in Perm Krai were extracted twice with EtOH (70%) at room temperature for 24 h and then with heating on a boiling-water bath for 30 min. The combined aqueous EtOH extracts were evaporated *in vacuo* to 50 mL, mixed with silica gel L 40/100 (30 g), and dried. The dried powder (dry extract + silica gel) was placed onto a layer of silica gel (8-cm diameter, 5-cm height) formed as a suspension in $CHCl_3$. The chromatography column was eluted by $CHCl_3$ and $CHCl_3$–EtOH mixtures in various proportions (99:1, 98:2, 97:3, 95:5, 93:7, 90:10, 85:15, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70). Separation of the compounds was monitored by TLC on Sorbfil PTSKh-AF-A-UF plates using $CHCl_3$–EtOH (9:1), $CHCl_3$–EtOH–H_2O (26:16:3), and *n*-BuOH–AcOH (glacial)–H_2O (4:1:2).

Fractions containing **1** were combined. The precipitate forming from them was separated and recrystallized from H_2O to afford **1** in 0.5% yield (of the air-dried raw material mass). Fractions containing dominant **2** were combined. The precipitate forming from them was separated and recrystallized from an Me_2CO–$CHCl_3$ mixture to afford **2** in 1.5% yield (of the air-dried raw material mass). Fractions containing dominant **3** were combined. The precipitate forming from them was separated and recrystallized from an Me_2CO–$CHCl_3$ mixture to afford **3** in 0.3% yield (of air-dried raw material mass). Fractions containing **4** were combined. The precipitate forming from them was separated and recrystallized from an EtOH–$CHCl_3$ mixture to afford **4** in 1.0% yield (of air-dried raw material mass).

Fractions containing **5** and **6** were placed onto Woelm polyamide for further purification. Dry powder (extract + polyamide) was placed onto a chromatography column (5.0-cm height, 4-cm diameter) that was eluted by H_2O and aqueous EtOH (20, 40, 70, 96%) to afford **5** (H_2O eluent) and **6** (70% EtOH eluent). The products were recrystallized from MeOH and aqueous EtOH, respectively.

Ethyl Ester of *p*-Digallic Acid (Ethyl Digallate) (1). White crystals with a grayish tint, mp 210–212°C (H_2O), $C_{16}H_{14}O_9$. UV spectrum (EtOH, λ_{max}, nm): 220, 276. Mass spectrum (ESI-MS), *m/z*: 221 [M^+ ethyl gallate + Na]$^+$, 199 [M^+ ethyl gallate + H]$^+$, 171 [M^+ gallic acid + H]$^+$. ^{1}H NMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 9.23 (3H, br.s, 3Ar-OH), 8.90 (1H, br.s, Ar-OH), 8.61 (1H, br.s, Ar-OH), 6.94 (2H, s, H-2′, 6′), 6.54 (2H, s, H-2, 6), 4.19 (2H, q, J = 7, H-8), 1.25 (3H, t, J = 7, H-9).

1,3,6-Trigalloylglucose (2). Light-yellow amorphous compound, $C_{27}H_{24}O_{18}$, mp 165–167°C (aqueous EtOH). Mass spectrum (ESI-MS), *m/z* 659 [M + Na]$^+$. UV spectrum (EtOH, λ_{max}, nm): 220, 280. ^{1}H NMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 8.80–9.35 (9H, m, 9Ar-H three gallic acid molecules), 6.99, 6.85, 6.78 (2H each, s, H-2, 6), 6.13 (1H, d, J = 7, Glc H-1), 3.5–5.9 (6H, m, H-Glc).

Tetragalloylglucose (3). Light-yellow amorphous compound, $C_{34}H_{28}O_{22}$. Mass spectrum (ESI-MS), *m/z* 811 [M + Na]$^+$. UV spectrum (EtOH, λ_{max}, nm): 220, 280. ^{1}H NMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 8.5–9.4 (12H, m, 12Ar-OH), 6.99, 6.85, 6.82, 6.78 (2H each, s, H-2, 6), 6.12 (1H, d, J = 7, Glc H-1), 3.6–5.9 (6H, m, H-Glc).

Arbutin (Hydroquinone 1-*O*-*β*-D-Glucopyranoside) (4). White needle-like crystals, $C_{12}H_{16}O_7$, mp 197–199°C ($CHCl_3$–EtOH). Mass spectrum (ESI-MS) *m/z* 295 [M + Na]$^+$. UV spectrum (EtOH, λ_{max}, nm): 230, 282. ^{1}H NMR spectrum

(300 MHz, DMSO-d_6, δ, ppm, J/Hz): 9.02 (1H, s, Ar-OH), 6.85 (2H, d, J = 9, H-2, 6), 6.64 (2H, d, J = 9, H-3, 5), 5.25 (d, J = 7, Glc H-1′), 3.30–5.05 (6H, m, H-Glc).

Gallic Acid (5). White crystals, $C_7H_6O_5$, mp 248–250°C (MeOH). UV spectrum (EtOH, λ_{max}, nm): 218, 275. Mass spectrum (70 eV, 200°C, *m/z*, %): 170 (M^+, 100%). ^{1}H NMR spectrum (300 MHz, DMSO-d_6, δ, ppm): 8.80 (3H, br.s, Ar-OH), 7.02 (2H, s, H-2, 6).

Hyperoside (3,5,7,3′,4′-Pentahydroxyflavone 3-*O*-β-D-Galactopyranoside) (6). Light-yellow crystals, $C_{21}H_{20}O_{12}$, mp 230–233°C (aqueous Me_2CO). Mass spectrum (ESI-MS), *m/z* 487 $[M + Na]^+$. UV spectrum (EtOH, λ_{max}, nm): 258, 266 sh, 363. ^{1}H NMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.63 (s, 5-OH), 7.67 (dd, J = 2.5, 9, H-6′), 7.53 (d, J = 2.5, H-2′), 6.81 (d, J = 9, H-5′), 6.42 (d, J = 2.5, H-8), 6.20 (d, J = 2.5, H-6), 5.37 (d, J = 7.5, Gal H-1″), 3.2–4.9 (6H, m, H-Gal).

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