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Chemical composition of white currant seed extract

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Abstract: From the seeds of white currant (*Ribes rubrum*, cv. White Champagne), a new sesquiterpenoid glycoside **1** was isolated, along with two known compounds: dihydrophaseic acid 3'-*O*-*β*-D-glucopyranoside (**2**), and 3-carboxymethyl-indole-1-*N*-*β*-D-glucopyranoside (**3**). The structure of the new compound was identified as dihydrophaseic acid 3'-*O*-*β*-gentiobioside, based on extensive NMR and MS spectral studies.

Keywords: white currant; seeds; sesquiterpenoid glucoside; dihydrophaseic acid derivative; indole derivative; 2D NMR.

INTRODUCTION

The genus *Ribes* is native to the Northern Hemisphere, and several species have been cultivated as a food crop since the early 1500s in Europe, and were taken to North America with the colonists in the 1700s. This genus contains about 150 species, grouped into categories including red and white currants, black currants, ornamental currants, golden currants, and gooseberries.

Despite the low content of dominant berry phenolics such as ellagic acid, anthocyanins and other flavonoids, it was shown that white currants are effective in preventing cancer initiation and progression in the Min mouse.[1] It was also found that currant seed oils are rich in both *α*-linolenic acid and *γ*-linolenic acid, which makes them specific among plant oils.[2]

This paper is a continuation of investigations on the chemical composition of edible berries, in pursuit of sources of potentially pharmacologically active compounds.[3–5] Herein, the isolation and structure elucidation of a new dihydrophaseic acid glucoside, along with two known compounds, from the seeds of white currant (*Ribes rubrum*, cv. White Champagne) are described.

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EXPERIMENTAL

General experimental procedures

The NMR spectra were acquired on a Bruker Avance DRX 500 MHz instrument with a 5 mm inverse detection probe using standard pulse sequences, in acetone-d_6/D_2O (1:1) as a solvent, at 289 K. The spectra were referenced to the residual solvent signal (δ_H 2.05, δ_C 29.92 for acetone-d_6); the chemical shifts are given in δ (ppm), and coupling constants are reported in Hz.

High resolution mass spectral (HR-MS) data (negative ion mode) were generated with an Agilent time-of-flight mass spectrometer equipped with an electrospray ionization source (EPI) (Agilent 6210 system) and Agilent 1200 Series high pressure liquid chromatograph (HPLC). Samples were diluted with water/acetonitrile (1:1) containing 0.1 % formic acid and introduced *via* the HPLC, without a column, using the same solvent system.

Semi-preparative HPLC was performed on an Agilent 1100 system using a Zorbax XDB-C18 column (5 μm, 250 mm×9.4 mm).

Plant material

White currant (*Ribes rubrum*, cv. White Champagne) was grown in the vicinity of Belgrade (experimental orchard of the nursery "Omega", located in Mislođin, Municipality of Obrenovac). Berries at the optimum technological maturity were harvested at the end of June 2009. The seeds from the berries were manually separated from the pulp and dried using filter paper.

Isolation

The samples of whole, dried seeds (20 g) were macerated in 50 mL of 50 % MeOH. The mixtures were sonicated in an ultrasonic bath for 12 h. The extracts were filtered through filter paper, evaporated at reduced pressure at 45 °C, which afforded 500 mg of dry material. The extract was diluted with 1 mL of 0.2 % formic acid/acetonitrile (1:1), and filtered through a 0.45 mm cellulose filter (Millipore).

The separation of the extract was realized using a semi-preparative HPLC method. Mobile phase A was 0.2 % formic acid in water and mobile phase B was acetonitrile. The injection volume was 50 μL, and elution at 4.5 mL min^{-1} with a solvent gradient program (0–20 min, 5–16 % B, 20–28 min, 16–40 % B, 28–30 min, 40–99 % B, 30–35 min, 99 % B, 35–36 min, 99–5 % B). The chromatographic procedure was repeated twenty times, yielding 3 mg of compound **1** (t_R = 11.1 min), 3 mg of compound **2** (t_R = 12.9 min), and 2 mg of compound **3** (t_R = 20.8 min).

Dihydrophaseic acid 3'-O-β-gentiobioside ***(1)***. Colorless film. ^{1}H-NMR (acetone-d_6/ /D_2O (1:1), 500 MHz) and ^{13}C-NMR (acetone-d_6/D_2O (1:1), 125 MHz). HR-ESI–MS, *m/z*, 606.2514 (calcd. for $C_{27}H_{42}O_{15}$: 606.2524, error: –1.6 ppm).

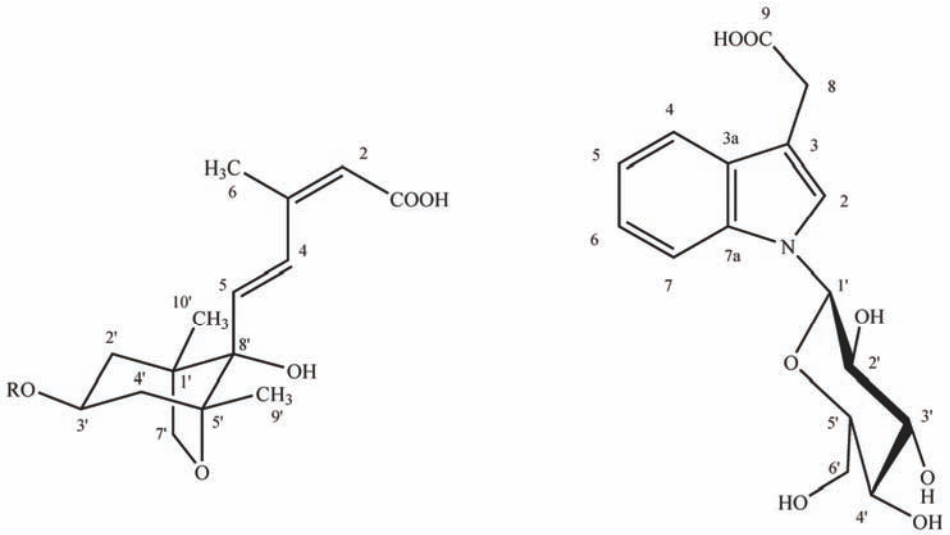
Dihydrophaseic acid 3'-O-β-*D-glucopyranoside* ***(2)***. Colorless film. ^{1}H-NMR (acetone- -d_6/D_2O (1:1), 500 MHz) and ^{13}C-NMR (acetone-d_6/D_2O (1:1), 125 MHz). HR-ESI–MS, *m/z*, 444.1993 (calcd. for $C_{21}H_{32}O_{10}$: 444.1995, error: –0.5 ppm).

3-Carboxymethylindole 1-N-β-*D-glucopyranoside* ***(3)***. Colorless film. ^{1}H-NMR (acetone-d_6/D_2O (1:1), 500 MHz) and ^{13}C-NMR (acetone-d_6/D_2O (1:1), 125 MHz). HR-ESI–MS, *m/z*, 337.1161 (calcd. for $C_{16}H_{19}NO_7$: 337.1162, error: –0.3 ppm).

RESULTS AND DISCUSSION

The whole, dried seeds of white currant were extracted with 50 % MeOH. The resulting extract was repeatedly subjected to semi-preparative HPLC to yield

three pure compounds. Their structures (Fig. 1) were elucidated by 1D and 2D NMR techniques (^{1}H-, ^{13}C-NMR, COSY, NOESY, HSQC, and HMBC), HR--ESI–MS, and by comparison with literature data. The spectroscopic data for compounds **1** and **2** are given in Table I and for compound **3** in Table II.



1 R = β-D-Glc(1→6)-β-D-Glc

2 R = β-D-Glc

3

Fig. 1. Structues of the isolated compounds.

Compound **1** was isolated as a colorless oil and its molecular formula was deduced as $C_{27}H_{42}O_{15}$ based on its negative ESI mass spectrum, which showed $[M–H]^-$ at *m/z* 605.2441. The ^{1}H-NMR spectrum of **1** showed the presence of three methyl singlets at δ 0.84, 1.04, and 1.99 (H-10', 9', and 6, respectively), signals of three methylene groups from the aglycone ring at δ 1.62 (1H, *br d*, H-2'$_{ax}$), 1.89 (1H, *dd*, H-2'$_{eq}$), 1.65 (1H, *br d*, H-4'$_{ax}$), 2.04 (1H, *dd*, H-4'$_{eq}$) and 3.63 ppm (2H, *m*, H-7'), two olefinic methine doublets at δ 6.32 (H-5) and 7.90 ppm (H-4), an olefinic methine singlet at δ 5.69 ppm (H-2), and an oxymethine signal at δ 4.05 ppm (*tdd*, H-3'). The two double bond functionalities of aglycone were also confirmed by the ^{13}C-NMR resonances at δ 119.1, 129.8, 133.7 and 148.0 ppm. A complete assignment of the ^{1}H- and ^{13}C-NMR spectra of the aglycone moiety (Table I) was based on 2D NMR experiments (COSY, NOESY, HSQC and HMBC). A comparison of the NMR data with those from the literature, indicated that compound **1** belongs to dihydrophaseic acid glucoside.[6] The values of vicinal ($^3J_{H,H}$) couplings of H-3' (δ 4.05 ppm, *tdd*, *J* = 10.5, 6.9 and 6.4 Hz), corresponded to two axial and two equatorial neighbors, revealing the axial

position of this proton.[7] Furthermore, the NOESY correlation of protons 3' and 7' was indicative they were on the same side of cyclohexane ring. Similarly, the NOESY correlation of H-2'$_{ax}$ and H-5 suggested that the acyclic moiety of aglycone was attached to the 8' axial position of a bicyclic ring. The configurations of the double bonds (2*Z*, 4*E*) were confirmed by the NOE of H_3-6 with olefinic H-2, and the absence of NOE between olefinic H-4 and H-5. According to the NOESY correlation of H_3-6 with H-5, the 3-*s*-*trans* conformation is preferred.

TABLE I. ^{1}H-NMR (acetone-d_6/D_2O (1:1), 500 MHz) and ^{13}C-NMR (acetone-d_6/D_2O (1:1), 125 MHz) data of compounds **1** and **2**; assignments were based on COSY, NOESY, HSQC and HMBC experiments. Overlapped proton NMR signals (ovl) are reported without designated multiplicity; n.d. – not determined

Position	Compound					
	1			**2**		
	^{1}H-NMR	*J* / Hz	^{13}C	^{1}H	*J* / Hz	^{13}C-NMR
1	–	–	n.d.	–	–	n.d.
2	5.69 s	–	119.1	5.69 *s*	–	119.1
3	–	–	148.0	–	–	148.3
4	7.90 d	16.0	129.8	7.90 *d*	16.0	129.8
5	6.32	d 16.0	133.7	6.32 *d*	16.0	133.7
6	1.99 *s*	–	20.6	2.00 *s*	–	20.6
1'	–	–	48.0	–	–	47.8
2'$_{ax}$	1.62 *br d*	13.2	41.7	1.62 *br d*	13.5	41.5
2'$_{eq}$	1.89 *dd*	13.2; 6.9	–	1.83 *dd*	13.5; 6.9	–
3'	4.05 *tdd*	10.5; 6.9; 6.4	72.3	4.08 *tdd*	10.5; 6.9; 6.4	71.3
4'$_{ax}$	1.65 *br* d	13.2	41.9	1.65 *br d*	13.5	41.7
4'$_{eq}$	2.04 *dd*	13.2; 6.4	–	2.01 *dd*	13.5; 6.4	–
5'	–	–	85.2	-	–	85.3
7'	3.63 *ovl*	–	74.9	3.60 *ovl*	–	75.1
8'	–	–	81.1	–	–	81.1
9'	1.04 *s*	–	19.5	1.04 *s*	–	19.4
10'	0.84 *s*	–	16.0	0.83 *s*	–	16.0
1''	4.19 *d*	7.8	101.9	4.18 *d*	7.8	101.3
2''	2.90 *ovl*	–	73.1	2.89 *ovl*	–	73.2
3''	3.12 *ovl*	–	76.5	3.12 *ovl*	–	76.5
4''	3.02 *ovl*	–	70.1	3.02 *ovl*	–	70.1
5''	3.33 *ovl*	–	75.6	3.09 *ovl*	–	76.5
6''	3.96 *ovl*	–	68.4	3.42 *ovl*	–	61.0
	3.56 *ovl*	–	–	3.66 *ovl*	–	–
1'''	4.26 *d*	7.8	n.d.	–	–	–
2'''	2.96 *ovl*	–	73.5	–	–	–
3'''	3.13 *ovl*	–	76.6	–	–	–
4'''	3.04 *ovl*	–	70.0	–	–	–
5'''	3.05 *ovl*	–	76.8	–	–	–
6'''	3.67 *ovl*	–	61.0	–	–	–
	3.43 *ovl*	–	–	–	–	–

TABLE II. ^{1}H-NMR (acetone-d_6/D_2O (1:1), 500 MHz) and ^{13}C-NMR (acetone-d_6/D_2O (1:1), 125 MHz) data of compound **3**

Position	^{1}H-NMR	*J* / Hz	^{13}C-NMR
2	7.34 *s*	–	124.1
3	–	–	110.1
3a	–	–	128.0
4	7.50 *d*	8.0	118.8
5	7.02 *t*	8.0	118.9
6	7.12 *t*	8.0	121.1
7	7.50 *d*	8.0	110.3
7a	–	–	136.3
8	3.55 *s*	–	31.9
1’	5.39 *d*	9.1	84.2
2’	3.70 *ovl*	–	71.8
3’	3.45 *ovl*	–	79.1
4’	3.25 *ovl*	–	69.8
5’	3.42 *ovl*	–	77.5
6’	3.44 *ovl*	–	60.8
	3.66 *ovl*	–	–

The ^{1}H-NMR spectrum of **1** also indicated the presence of two anomeric proton doublets at δ 4.19 (H-1’’) and 4.26 (H-1’’’), suggesting the presence of two sugar moieties. The application of the COSY, NOESY, HSQC, and HMBC spectra enabled the assignment of the remaining ^{1}H- and ^{13}C-NMR sugar resonances, the chemical shifts of which corresponded to glucose. The values of the vicinal couplings of the anomeric protons were consistent with two β-glucopyranosyl units. The occurrence of strong H-1/H-5 and H-1/H-3 NOE cross-peaks indicated a $^4C_1$ conformation for both β-glucopyranosyl units. The interglycosidic linkage was confirmed by NOE cross-peaks of H-1’’’ with both diastereotopic H-6’’. In the preferred conformation of the sugar subunit, the anomeric proton 1’’ is close in space to H-4’$_{ax}$ of the aglycone moiety, as shown by the corresponding cross peak in the NOESY spectrum. In this way, it was concluded that the sugar unit was connected to the 3’-position of the aglycone moiety. Based on the above spectral data, the structure of **1** was unambiguously established as dihydrophaseic acid 3’-*O*-β-gentiobioside.

Compound **2** was isolated as a colorless oil, and its molecular formula was deduced as $C_{21}H_{32}O_{10}$ based on its negative ESI mass spectrum, showing $[M–H]^–$ at *m/z* 443.1920. The NMR data of this compound were very similar to those of compound **1**, containing the signals for only one β-glucopyranosyl unit. Thus, compound **2** was identified as dihydrophaseic acid 3’-*O*-β-D-glucopyranoside, which was previously isolated from the aerial parts of *Juniperus phoenicea*.[6]

Compound **3** was isolated as a colorless oil, and its molecular formula was deduced as $C_{16}H_{19}NO_7$ based on its negative ESI mass spectrum, which showed $[M–H]^–$ at *m/z* 336.1088, and $[2M–H]^–$ at *m/z* 637.2256. The ^{1}H- and ^{13}C-NMR

chemical shifts corresponded to 3-carboxymethylindole 1-*N*-β-D-glucopyranoside, a nitrogen-containing astringent indole already isolated from red currants.[8]

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И З В О Д

ХЕМИЈСКИ САСТАВ ЕКСТРАКТА СЕМЕНКИ БЕЛЕ РИБИЗЛЕ

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Из семена беле рибизле (*Ribes rubrum*, cv. бела шампањска) изолован је нови сесквитерпенски глукозид (**1**), заједно са два позната једињења: 3'-*O*-β-D-глукопиранозидом дихидрофазеинске киселине (**2**) и 3-карбоксиметилиндол-1-*N*-β-D-глукопиранозидом (**3**). На основу детаљних NMR и MS студија, структура новог једињења је одређена као 3'-*O*-β-генциобиозид дихидрофазеинске киселине.

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