A FUROSTANOL SAPONIN AND PHYTOECDYSTEROID FROM ROOTS OF *Helleborus orientalis*

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A furostanol saponin mixture and a known phytoecdysteroid were isolated from the roots of Helleborus orientalis *Lam. Their structures were established as 26-[(*β*-D-glucopyranosyl)oxy]-22*α*-hidroxyfurosta-5,25(27)-dien-1*β*,3*β*,11*α*-triol (**1a**), 26-[(*β*-D-glucopyranosyl)oxy]-22*α*-methoxyfurosta-5,25(27)-dien-1*β*,3*β*,11*α*-triol (**1b**), and 20-hydroxy-*β*-ecdyson-3-O-*β*-D-glycoside (**2**). Acid hydrolysis of **1a,b** gave (1*β*,3*β*,11*α*,22*α*)-22,26-dimethoxyfurosta-5,25(27)-dien-1,3,11-triol (aglycone **1**) and of **2** gave 20-hydroxy-*β*-ecdyson (aglycone **2**). Their structures were elucidated by spectral analysis.*

Key words: *Helleborus orientalis*, furostanol saponin, phytoecdysteroid.

Helleborus extracts exhibit high biological activity with antialgic, myorelaxant, antirheumatic, and blood vessel regulating actions [1]. Previously isolated classes of constituents, furostanol saponin [2–5], phytoecdysteroids [6–9], and bufadienolides [10], were characterized from *Helleborus* species.

This study describes the isolation and the exact structure determination of two glycosides, a furostanol saponin **1a,b** and a phytoecdysteroid **2**, from *Helleborus orientalis* Lam roots.

Compounds **1a,b** was obtained as a mixture of the C-22 hydroxyl and C-22 methoxyl forms. It gave two spots at TLC due to the existence of 22-hydroxy and 22-methoxy derivatives of the glycoside. Acid hydrolysis of the mixture gave aglycone **1**. The ^{1}HMR spectral data of aglycone **1** contained signals of olefinic protons at 5.62, 4.78, and 4.83 ppm, two methoxyl protons at 3.21 and 3.63 ppm, and methyl groups at 0.96 (21-CH_3), 0.79 (18-CH_3), and 1.15 ppm (19-CH_3). Furthermore, the presence of a semiketal carbon signal at 109.4 ppm suggested the furostanol nature of the aglycone **1**. The EI-MS spectrum showed an ion peak at m/z: 491 $[M+H]^+$ for $C_{29}O_6H_{46}$. The above ^{1}H NMR spectral data and a comparison of the ^{13}C NMR signals (Table 1) and of – 85.92 of aglycone **1** with those described in the literature [1, 2] showed the structure of the aglycone1 to be (1*β*,3*β*,11*α*,22*α*)-22,26-dimethoxyfurosta-5,25(27)-dien-1,3,11-triol.

The furostanol glycosidic nature of compounds **1a,b** was evidenced by the furostan steroidal structure (916 and 833 cm^{-1}) in the IR spectra. The ^{1}H NMR spectral data of the mixture of compounds **1a,b** contained a signal for one anomeric proton at 4.24 ppm (d, J = 7.3 Hz, H-1′). The ^{13}C NMR spectral data (Table 1) contained an anomeric carbon signal at 104.0 ppm. Furthermore, the presence of a semiketal carbon signal at 110.4 and 110.7 ppm suggested the furostanol nature of compounds **1a,b**. The peak intensities of these semiketal carbon signals were 0.775 and 0.742, respectively. The ratio of these components (**1a** and **1b**) in the mixture of was 1:1. The negative FAB-MS showed an ion peak at *m/z*: 571.4 $[M\text{-}OCH_3\text{-}2H_2O]^-$, suggesting the molecular formula as $C_{34}O_{11}H_{54}$. The structure of **1a** was established as 26-[(*β*-D-glucopyranosyl)oxy]-22*α*-hydroxyfurosta-5,25(27)-dien-1*β*,3*β*,11*α*-triol and **1b** as 26-[(*β*-D-glucopyranosyl)oxy]-22*α*-methoxyfurosta-5,25(27)-dien-1*β*,3*β*,11*α*-triol. In this paper, the NMR data of **1a** and **1b** are reported for the first time. Compounds **1a,b** are presumed to exist as C-22 hydroxyl and C-22 methoxyl derivatives. It is thought that furostanol saponin exists in the form of just **1**a in the roots of Helleborus orientalis Lam and that compound **1b** may be artifacts of the extraction and isolation procedure. Such interconversion between hydroxyl and methoxyl groups at C-22 furostanol glycosides has already been reported in the literature [1, 4].

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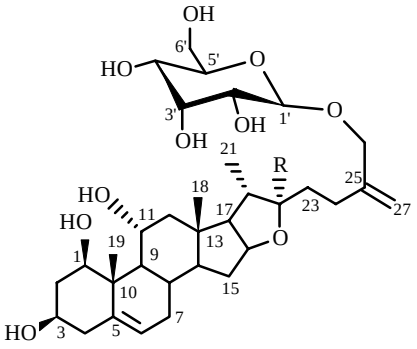
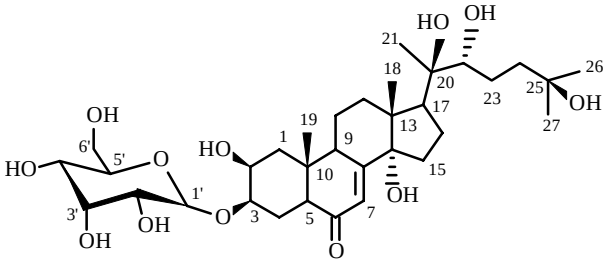
TABLE 1. Chemical Shifts of ^{13}C Atom of Glycosides and of Aglycone (δ, ppm)

C atom	**1a**	**1b**	Aglycone **1**	C atom	**1a**	**1b**	Aglycone **1**
1	78.6	78.6	75.1	18	17.2	17.2	17.0
2	35.5	35.5	35.1	19	16.3	16.3	14.5
3	75.5	75.5	66.2	20	42.1	42.1	41.6
4	40.9	40.9	40.6	21	13.9	13.9	13.3
5	147.2	147.2	143.4	22	**110.4**	**110.7**	109.4
6	126.2	126.2	125.9	23	28.4	28.4	28.5
7	33.3	33.3	32.7	24	37.9	37.9	39.7
8	31.8	31.8	31.0	25	140.5	140.5	138.5
9	50.4	50.4	52.1	26	66.1	66.1	63.0
10	47.8	47.8	42.5	27	112.1	112.1	113.0
11	75.9	75.9	67.7	$-OCH_3$	-	**50.5**	49.4 and 51.5
12	32.7	32.7	32.0	Glc-1′	104.0	104.0	
13	40.7	40.7	40.2	2′	71.7	71.7	
14	58.1	58.1	57.0	3′	75.0	75.0	
15	33.2	33.2	35.0	4′	68.0	68.5	
16	81.4	81.4	81.1	5′	72.1	72.1	
17	63.6	63.6	62.0	6′	62.8	62.8	



1a, b

1a: R = OH; **1b:** R = OCH_3



2

The ^1H NMR spectral data of compound **2** contained signals for one anomeric proton at 4.72 ppm (d, J = 6.7 Hz, H′). The ^{13}C NMR spectral data contained one anomeric carbon signal at 105.5 ppm. The negative FAB-MS showed an ion peak at m/z: 623.3[M-H_2O-H]$^-$. ^1H NMR spectral data and comparison of the ^{13}C NMR signals of **2** with those described in the literature [6, 7] showed the structure of **2** to be 20-hydroxy-*β* ecdyson-3-*O*-*β*-D glycoside.

EXPERIMENTAL

The plant material from *Helleborus orientalis* Lam was collected in Bursa Uludag. A plant specimen was deposited under registration number BULU 26118 in the Herbarium of Uludag University (Bursa, Turkey).

Dried and powdered roots of the plant (1000 g) were extracted with 80% MeOH (4×5000 mL) at room temperature for about 15 days. The mixture was filtered. The combined extracts were evaporated to dryness under vacuum at 40°C. The residue (25 g) was extracted with hexane and partitioned between *n*-BuOH and H_2O phases. The *n*-butanol portion (16 g) was column chromatographed (Silica gel 60, 0.063–0.200 mm) with $CHCl_3$–MeOH (10:1) and three different $CHCl_3$–MeOH–H_2O solvent systems [(65:20:10); (65:25:10); (65:30:10)]. The fractions were separated using preparative TLC [$CHCl_3$–MeOH-H_2O, (65:32:10)]. Preparative TLC was achieved using 20×20 cm glass plates coated with 0.5 mm layers of silicagel (Merck Kieselgel 60 $PF_{254+366}$). Glycosides **1a,b** (450 mg) and glycoside **2** (180 mg) were isolated from the root samples.

Glycosides **1a,b** (90 mg) and glycoside **2** (60 mg) were dissolved in 10 mL MeOH and 10 mL 2M HCI added to this. After refluxing the mixture for 4 hours, H_2O was added and the aglycones were extracted with $CHCl_3$ and purified on a silica

gel column using $CHCl_3$–MeOH (9:1). After acid hydrolysis of glycosides **1a,b** and glycoside **2**, aglycone **1** and aglycone **2** were obtained. After that, sugar analysis was performed using TLC by comparison with authentic samples.

Glycosides **1a,b** (120 mg) were acetylated with pyridine–Ac_2O and purified by preparative TLC using $CHCl_3$–MeOH (97:3).

IR spectra were recorded on a Bruker IFS-48 FTIR spectrometer (KBr disks). The ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker 200 MHz spectrometer with TMS as an internal standard. Optical rotations were measured on a Schmidt+Haensch Polartonic E polarimeter. EI-MS, CI-MS spectra were recorded on a Kratos MS-80, and negative ion FAB-MS spectra were recorded on a Kratos Concept-II H MS instrument using Cs ion (6KV) in glycerin.

Compounds (1a,b) (amorphous): $[\alpha]_D^{20}$ –67.41° (*c* 0.6, MeOH); IR (ν_{max}^{KBr}, cm^{-1}): 1076 and 3382 (OH), 1558 (=CH-),1652(=CH_2), 916,833 (furostanol nature). ^1H NMR (δ, pyridine-d_5, J/Hz): 0.94 (s, 18-CH_3), 1.26 (d, J = 6.7, 21-CH_3), 1.42 (s, 19-CH_3), 3.24 (22-OCH_3), 4.42 (q, H-16) 4.24 (d, J = 7.3, anomeric proton), 5.61 (d, J = 4.9, H-6). The ^{13}C NMR spectral data of acetylation product of **1a,b**: δ = 169.40–176.0 (7×acetyl C=O) and 13.9–21.2 (7×acetyl CH_3). FAB-MS (*m/z*): 571.4 $[M\text{-}OCH_3\text{-}2H_2O]^-$.

Aglycone 1 (amorphous): $[\alpha]_D^{20}$ –85.92° (*c* 0.34, $CHCl_3$); ($[\alpha]_D^{18}$ –86.9°) [lit. 2]. ^1H NMR (δ, $CDCl_3$, J/Hz): 0.79 (s, 18-CH_3), 0.96 (d, J = 6, 21-CH_3), 1.15 (s, 19-CH_3), 3.21 (s, 22-OCH_3), 3.63 (s, 26-OCH_3), 4.39 (q, H-16), 4.78 (d, J = 6.6, H-27a), 4.83 (d, H-27b), 5.62 (d, J = 5.2, H-6). EI-MS (*m/z*): 491$[M+H]^+$, 477, 459, 426, 409, 391, 345, 211,84.

Compound 2. IR (ν_{max}^{KBr}, cm^{-1}): 1087 and 3404 (OH), 1770 (C=O), 1577(C=C). ^1H NMR (δ, pyridine-d_5, J/Hz): 0.86 (s, 18-CH_3), 1.10 (s, 19-CH_3), 1.25 (s, 26-CH_3), 1.27 (s, 27-CH_3), 1.43 (s, 21-CH_3), 4.72 (d, J = 6.7, anomeric proton), 5.63 (d, J = 5.1, H-7). ^{13}C NMR (δ, pyridine-d_5): 140.4 (=C, C-8), 120.5 (=CH-, C-7), 167.0 (C=O, C-6), 105.5 (anomeric carbon), 84.0–62.7 (3C-O-, 7CH-O-), 62.2 (1CH_2-O-, C-6′), 58.0–32.0 (2 quaternary C-, 3 methine C, 8 methylene C), 29.9, 16.8, 16.3, 15.0, 13.8 (5-methyl groups). EIS-MS (*m/z*): 647.3 $[M+Na\text{-}H_2O]^+$, 503.2 [20-hydroxyecdysone+Na]$^+$; FAB-MS (*m/z*): 623.3$[M\text{-}H_2O\text{-}H]^-$.

Aglycone 2. $C_{27}O_7H_{44}$. ^{13}C NMR (δ, $CDCl_3$): 138.6 (=C , C-8), 125.8 (=CH-, C-7), 168.0 (C=O, C-6), 84–66.5 (3C-O-, 3CH-O-), 61.4–26.9 (2 quaternary C-, 3 methine C, 8 methylene C), 24.7, 16.7 14.7, 14.4, 13.3 (5 methyl groups). CI-MS (*m/z*): 444 $[M\text{-}2H_2O]^+$, 426,391 (100), 331, 279, 58.

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