

Two New Triterpene Saponins from *Eryngium campestre*

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Two new triterpene saponins, 3-*O*- β -D-glucopyranosyl-(1 \rightarrow 2)-[α -L-rhamnopyranosyl-(1 \rightarrow 4)]- β -D-glucuronopyranosyl-22-*O*-angeloyl-R1-barrigenol (**1**) and 3-*O*- β -D-glucopyranosyl-(1 \rightarrow 2)-[α -L-rhamnopyranosyl-(1 \rightarrow 4)]- β -D-glucuronopyranosyl-22-*O*- β , β -dimethylacryloyl-A1-barrigenol (**2**), were isolated from the roots of *Eryngium campestre* (Apiaceae). Their structures were established mainly by 2D NMR techniques and mass spectrometry.

Key words *Eryngium campestre*; Apiaceae; triterpene saponin; R1-barrigenol; A1-barrigenol

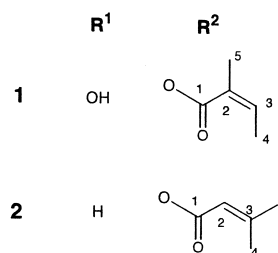
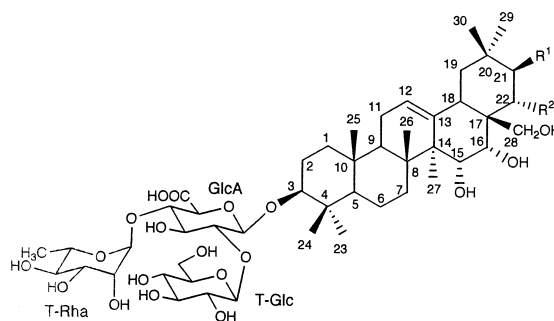
Eryngium genus (Apiaceae) is represented by 317 species, subspecies and varieties.¹⁾ The species *E. campestre* L. grows in most parts of Europe and northern Africa and has been introduced into North America. In the flora of Turkey and East Aegean Islands,²⁾ *Eryngium* genus is represented by 23 species, and *E. campestre* is known in Turkish folk-medicine as "Bogadikeni". Infusions of the aerial and root parts are used as an antitussive, diuretic, appetizer, stimulant and aphrodisiac.³⁾ Previous phytochemical studies on *E. campestre* described the isolation of flavonoids from the aerial parts,^{4,5)} and from the roots, monoterpene glycosides of the cyclohexanone type,⁶⁾ coumarin derivatives,⁷⁾ and small amounts of saponins.⁸⁾ We describe in this paper the isolation and structural elucidation of two new triterpene saponins, **1** and **2**, from the roots of *E. campestre*.

A concentrated *n*-BuOH-soluble fraction of the MeOH extract of the roots of *E. campestre* was subjected to multiple chromatographic steps over silica gel to yield compounds **1** and **2**. Their structures were elucidated mainly by 600 MHz NMR analysis, including 1D and 2D NMR (¹H-¹H COSY, TOCSY, NOESY, HSQC, HMBC) spectroscopy.

Compound **1**, a white amorphous powder, exhibited in the HR-ESI-MS (positive-ion mode) a pseudo-molecular ion peak at *m/z* 1095.5399 [M+Na]⁺ (Calcd 1095.5352), consistent with a molecular formula of C₅₃H₈₄O₂₂Na. Its FAB-MS (negative-ion mode) showed a quasi-molecular ion peak at *m/z* 1071 [M-H]⁻, indicating a molecular weight of 1072. Other fragment ion peaks were observed at *m/z* 925 [(M-H)-146]⁻ and 909 [(M-H)-162]⁻ which revealed the elimination of one terminal 6-desoxyhexosyl and one terminal hexosyl moiety.

The structure of the aglycon of **1** was recognized to be the triterpene R1-barrigenol by ¹H- and ¹³C-NMR analysis (Table 1) using the correlations observed in COSY, HMQC, HSQC and NOESY spectra, and was in full agreement with literature data.⁹⁾ The ¹H-NMR spectrum of **1** showed signals for seven angular methyl groups as singlets, one olefinic proton at δ_{H} 5.54 (1H, brt, *J*=3.0 Hz, H-12), five oxygen bearing methine protons at δ_{H} 3.17 (1H, dd, *J*=10.5, 5.5 Hz, H-3), 4.15 (H-21), 4.39 (1H, d, *J*=3.6 Hz, H-15), 4.55 (1H, d,

J=3.6 Hz, H-16), 6.27 (1H, d, *J*=7.4 Hz, H-22), and one primary alcoholic function at δ_{H} 3.79, 3.83 (2H, d, *J*=12.7 Hz, H-28). In the HMBC spectrum, crosspeaks between δ_{H} 1.91 (3H, s, H-27) and δ_{C} 67.6 (C-15), and between δ_{H} 1.51 (3H, s, H-29), δ_{H} 1.53 (3H, s, H-30) and δ_{C} 71.3 (C-21) allowed the location of two secondary alcoholic functions at C-15 and C-21. The proton at δ_{H} 6.27 (1H, d, *J*=7.4 Hz), showed HMBC connectivities with the C-28 at δ_{C} 64.8, the C-21 at δ_{C} 71.3, the C-16 at δ_{C} 72.8 and the C-18 at δ_{C} 54.0, which revealed its assignment at position 22. The absolute configuration of C-3, C-15, C-16, C-21, and C-22 of the R1-barrigenol is determined from a NOESY experiment and by the multiplicity and coupling constants of carbinol protons. In the NOESY spectrum, correlations between δ_{H} 3.17 (1H, dd, *J*=10.5, 5.5 Hz, H-3) and δ_{H} 0.72 (H-5) and δ_{H} 1.13 (3H, s, H-23), between δ_{H} 4.39 (1H, d, *J*=3.6 Hz, H-15) and δ_{H} 0.98 (3H, s, H-26), and between δ_{H} 4.55 (1H, d, *J*=3.6 Hz, H-16) and δ_{H} 3.79, 3.83 (2H, d, *J*=12.7 Hz, H-28), confirmed the proposed absolute configuration of the R1-barrigenol.⁹⁾ For



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Table 1. ¹H- (600 MHz) and ¹³C- (150 MHz) NMR Data of the Aglycons and Acyl Groups of Compounds **1** and **2** (Pyridine-*d*₅, δ in ppm)

Position	1		2	
	¹³ C	¹ H	¹³ C	¹ H
Agly				
1	38.5	0.72, 1.30	38.5	0.74, 1.32
2	26.0	1.85, 2.18	26.0	1.87, 2.20
3	89.5	3.17 dd (10.5, 5.5)	89.4	3.18 dd (10.5, 5.5)
4	39.1	—	39.2	—
5	55.2	0.72	55.0	0.74
6	18.6	nd	18.8	nd
7	36.5	1.98, 2.10	36.4	2.02, 2.12
8	41.0	—	41.0	—
9	46.8	1.68	46.6	1.68
10	36.6	—	36.6	—
11	23.5	1.78, 1.81	23.5	1.81, 1.87
12	125.5	5.54 brt (3.0)	125.0	5.56 brt (3.0)
13	141.4	—	143.8	—
14	47.6	—	47.0	—
15	67.6	4.39 d (3.6)	66.9	4.28
16	72.8	4.55 d (3.6)	74.3	4.54
17	nd	—	nd	—
18	54.0	3.12 br s	41.3	3.04 br s
19	35.2	2.30, 2.95	46.8	1.38, 2.86 t (13.8)
20	nd	—	31.2	—
21	71.3	4.15	41.3	2.00, 2.76 t (11.9)
22	80.9	6.27 d (7.4)	71.8	6.12 dd (11.9, 5.0)
23	27.6	1.13 s	27.6	1.14 s
24	16.4	1.05 s	16.5	1.06 s
25	15.3	0.81 s	15.4	0.82 s
26	17.5	0.98 s	17.2	1.06 s
27	21.0	1.91 s	21.0	1.87 s
28	64.8	3.79 d (12.7), 3.83 d (12.7)	62.5	3.62 d (10.5), 3.79 d (10.5)
29	28.7	1.51 s	33.2	1.06 s
30	27.6	1.53 s	24.8	1.29 s
Ang				
1	169.2	—		
2	128.8	—		
3	136.6	5.90 qd (7.0, 1.0)		
4	15.5	2.06 br dd (7.0, 1.0)		
5	20.5	2.00 br s		
dMA				
1			167.0	—
2			117.0	5.62 br s
3			157.0	—
4			20.0	2.20 s
5			26.7	1.68 s

nd: not determined. Overlapped proton NMR signals are reported without designated multiplicity. Ang: angelic acid, dMA: β,β-dimethyl-acrylic acid.

C-21 and C-22, the NOESY correlations between δ_H 6.27 (1H, d, *J*=7.4 Hz, H-22) and δ_H 1.53 (3H, s, H-30) and δ_H 3.12 (1H, br s, H-18) revealed a β-axial position of the H-22, and its multiplicity as a doublet and its coupling constant of 7.4 Hz suggested an α-axial position of H-21. Moreover, the deshielded signal of H-22 at δ_H 6.27 and HMBC correlations between δ_H 6.27 (H-22) and δ_C 169.2 (COO) suggested that position 22 was acylated. Two vinylic methyl groups at δ_H 2.00 (3H, br s) and δ_H 2.06 (3H, br dd, *J*=7.0, 1.0 Hz), correlating in the COSY spectrum with a vinylic proton at δ_H 5.90 (1H, qd, *J*=7.0, 1.0 Hz), suggested the presence of an angeloyl residue. This proton at δ_H 5.90, which is not influenced by the carbonyl function, revealed a *Z* double bond, in opposition to a *E* orientation in the tigloyl residue. At this stage, the sugar part of compound **1** needed to be deter-

Table 2. ¹H- (600 MHz) and ¹³C- (150 MHz) NMR Data of the Sugar Moieties of Compounds **1** and **2** (Pyridine-*d*₅, δ in ppm)^{a)}

Position	1		2	
	¹³ C	¹ H	¹³ C	¹ H
GlcA				
1	<i>104.6</i>	<i>4.81 d (7.6)</i>	<i>104.6</i>	<i>4.82 d (7.6)</i>
2	<i>80.5</i>	<i>4.47 t (8.4)</i>	<i>80.5</i>	<i>4.47 t (8.3)</i>
3	76.0	4.28	76.0	4.28
4	<i>79.7</i>	<i>4.62</i>	<i>79.7</i>	<i>4.63</i>
5	78.4	4.45	78.4	4.43
6	176.9	—	176.8	—
T-Glc				
1	<i>104.3</i>	<i>5.43 d (7.4)</i>	<i>104.3</i>	<i>5.44 d (7.4)</i>
2	76.0	4.05 t (8.4)	76.0	4.07 t (7.4)
3	77.4	4.21 t (8.9)	77.4	4.21 t (9.2)
4	71.3	4.09 t (9.3)	71.3	4.10 t (9.4)
5	78.0	3.91	78.0	3.91
6	62.5	4.28, 4.53	62.5	4.29, 4.54
T-Rha				
1	<i>101.6</i>	<i>5.97 br s</i>	<i>101.6</i>	<i>5.99 br s</i>
2	71.4	4.99 br s	71.4	5.00 br s
3	71.5	4.62	71.5	4.62
4	73.4	4.26	73.4	4.26
5	69.7	4.90 dq (9.3, 6.0)	69.7	4.91 dq (9.3, 6.2)
6	18.1	1.62 d (6.0)	18.1	1.62 d (6.2)

a) Chemical shifts of substituted residues are italicized. nd: not determined. Overlapped proton NMR signals are reported without designated multiplicity.

mined. The ¹H-NMR spectrum of **1** displayed signals for three anomeric protons at δ_H 4.81 (1H, d, *J*=7.6 Hz), 5.43 (1H, d, *J*=7.4 Hz), and 5.97 (1H, br s), which gave correlations, in the HSQC spectrum, with anomeric carbon signals at δ_C 104.6, 104.3, and 101.6, respectively. The ring protons of the monosaccharide residues were assigned starting from the readily identifiable anomeric protons by means of COSY, TOCSY, HSQC, HMBC experiments (Table 2). Units of one β-D-glucuronopyranosyl (GlcA), one β-D-glucopyranosyl (Glc), and one α-L-rhamnopyranosyl (Rha) were identified. The relatively large ³*J*_{H-1,H-2} values of the anomeric protons of GlcA and Glc (7.6, 7.4 Hz, respectively) indicated a β anomeric orientation for GlcA and Glc, and the multiplicity of the anomeric proton of Rha (br s) indicated an α anomeric orientation.¹⁰⁾ Correlations observed in the HMBC spectrum between signals at δ_H 4.81 (1H, d, *J*=7.6 Hz, GlcA-1) and δ_C 89.5 (C-3), and in the NOESY spectrum between δ_H 4.81 (GlcA-1) and δ_H 3.17 (1H, dd, *J*=10.5, 5.5 Hz, H-3), confirmed the substitution at the C-3 position of the R-1 barigenol by a 3-*O*-β-D-glucuronopyranose. The assignments of the ¹H- and ¹³C-NMR signals of Glc and Rha attached to GlcA by a (1→2) and a (1→4) linkage, respectively, were deduced from the TOCSY, NOESY, HSQC and HMBC spectra. Cross-peaks in the HMBC spectrum between an anomeric signal at δ_H 5.43 (1H, d, *J*=7.4 Hz) and the deshielded carbon at δ_C 80.5 (GlcA-2), and between another anomeric signal at δ_H 5.97 (1H, br s) and a signal at δ_C 79.7 (GlcA-4), suggested a 2, 4 substitution of this glucuronic acid by one terminal glucose (T-Glc) and one terminal rhamnose (T-Rha), respectively. These substitutions were confirmed by a reverse correlation between δ_H 4.62 (GlcA-4) and δ_C 101.6 (T-Rha-1), and in the NOESY spectrum by a correlation between δ_H 4.47 (1H, t, *J*=8.4 Hz, GlcA-2) and δ_H 5.43 (1H, d, *J*=7.4 Hz, T-Glc-1). The structure of **1** was thus established

as 3-*O*- β -D-glucopyranosyl-(1 \rightarrow 2)-[α -L-rhamnopyranosyl-(1 \rightarrow 4)]- β -D-glucuronopyranosyl-22-*O*-angeloyl-R1-barrigenol, a new natural compound.

Compound **2**, a white amorphous powder, exhibited in the HR-ESI-MS (positive-ion mode) a pseudo-molecular ion peak at m/z 1079.5356 [M+Na]⁺ (Calcd 1079.5403), consistent with a molecular formula of C₅₃H₈₄O₂₁Na. Its FAB-MS (negative-ion mode) displayed a quasi-molecular ion peak [M-H]⁻ at m/z 1055 indicating a molecular weight of 1056, which differs from **1** by 16 amu. Other fragment ion peaks were observed at m/z 909 [(M-H)-146]⁻ and 893 [(M-H)-162]⁻ which revealed the elimination of one terminal 6-desoxyhexosyl and one terminal hexosyl moiety. The signals of the sugar part of **2**, assigned from 1D and 2D NMR spectra, were almost superimposable on those of **1**. The differences between the two molecules were thus located at the aglycon moiety. The ¹H-NMR spectrum of **2** showed signals for seven angular methyl groups as singlets, one olefinic proton at δ_H 5.56 (1H, brt, $J=3.0$ Hz, H-12), four oxygen bearing methine protons at δ_H 3.18 (1H, dd, $J=10.5, 5.5$ Hz, H-3), 4.28 (H-15), 4.54 (H-16), and 6.12 (1H, dd, $J=11.9, 5.0$ Hz, H-22), and one primary alcoholic function at δ_H 3.62, 3.79 (2H, d, $J=10.5$ Hz, H-28). In the HMBC spectrum, crosspeaks between δ_H 1.06 (3H, s, H-29), δ_H 1.29 (3H, s, H-30) and two methylene carbons at δ_C 41.3 and 46.8, revealed the absence of the hydroxyl group at C-21. The deshielded proton at δ_H 6.12 (1H, dd, $J=11.9, 5.0$ Hz), showing HMBC connectivities with the C-16 at δ_C 74.3, can be assigned at position 22, which must be acylated. These values are in accordance with the literature data of A-1 barrigenol as an aglycon.¹¹ In the HMBC spectrum, correlations between two vinylic methyl groups at 1.68 (3H, s) and 2.20 (3H, s) and one *sp*² quaternary carbon at δ_C 157.0 and a *sp*² methine carbon at δ_C 117.0 were observed. These signals belonged to β,β -dimethyl-acrylic acid acylating the C-22 position. This group rarely encountered acylating the aglycon of saponins, was already described esterifying camelliagenin A from *Harpullia cupanioides* (Sapindaceae) but at the C-16 position.¹² On the basis of the above results, the structure of **2** was established as 3-*O*- β -D-glucopyranosyl-(1 \rightarrow 2)-[α -L-rhamnopyranosyl-(1 \rightarrow 4)]- β -D-glucuronopyranosyl-22-*O*- β,β -dimethylacryloyl-A1-barrigenol, a new natural saponin.

R1 and A1-barrigenol are aglycons already isolated from plants belonging to different families, especially from the Apiales order like Apiaceae (genera *Hydrocotyle*,¹³ *Hacquetia*,¹⁴ *Steganotaenia*,¹⁵ *Sanicula*¹⁶) and Pittosporaceae (*Pittosporum*⁹). Therefore, it is not surprising to find them as aglycons of saponins from an *Eryngium* species, but the acylation of C-22 by a dimethylacryloyl unit is rare among triterpene saponins.

Experimental

Optical rotations were taken with a Rudolph Autopol IV Polarimeter using a sodium lamp operating at 589 nm. The 1D and 2D NMR spectra (¹H-¹H COSY, TOCSY, HSQC, and HMBC) were performed using a UNITY-600 spectrometer at an operating frequency of 600 MHz on a Varian INOVA 600 instrument equipped with a SUN 4 L-X computer system (600 MHz for ¹H and 150 MHz for ¹³C spectra). The carbon type (CH₃, CH₂, CH) was determined by DEPT experiments. All chemical shifts (δ) are given in ppm, and the samples were solubilized in pyridine-*d*₅ (δ_C 149.3,

135.8, 123.5). High-resolution ESI mass spectrometry (HR-ESI-MS) (positive-ion mode) was carried out on a Q-TOF 1-micromass spectrometer. FAB-MS (negative-ion mode, glycerol matrix) were obtained on a JEOL SX 102 mass spectrometer. TLC employed precoated silica gel plates 60F₂₅₄ (Merck). For saponins, the TLC solvent system CHCl₃-MeOH-H₂O (61:32:7) was used. The spray reagent for saponins was vanillin (1% in EtOH) and aqueous H₂SO₄ (30%). Isolations were carried out using a medium-pressure liquid chromatography (MPLC) system [Büchi 681 pump Rheodyne injector, Büchi column (46×2.5 cm), LiChroprep RP-18 (25–40 μ m), Büchi 684 fraction collector]. For column chromatography, Si gel 60 (63–200 μ m, Merck) was used.

Plant Material In August 2003, *E. campestre* was collected from Balıkesir-Edremit, Turkey and identified by Pr. Dr. Hayri Duman (Department of Biology, Faculty of Sciences, Gazi University, Ankara, Turkey). A voucher specimen (AEF 22964) was deposited in the herbarium of the Faculty of Pharmacy, Ankara University, Ankara, Turkey.

Extraction and Isolation The dried powdered roots (150 g) were extracted with MeOH under reflux for 24 h. The MeOH extract (17.2 g) was concentrated to dryness, and the residue was dissolved in an H₂O-MeOH mixture (9:1), then partitioned with *n*-butanol (100 ml) saturated with H₂O. The *n*-BuOH layer was concentrated to dryness, and the residue (9.5 g) was separated by column chromatography on Si gel eluted with CH₂Cl₂-MeOH-H₂O mixtures (80:20:2, 750 ml; 70:30:3; 60:40:4; 50:50:5, each 1000 ml), yielding 13 main fractions, A–M. The fraction H (310 mg), eluted with CH₂Cl₂-MeOH-H₂O (60:40:4), was purified by MPLC on a LiChroprep RP-18 column eluted with MeOH-H₂O mixtures (50:50 to MeOH; in steps of 5% MeOH, each 100 ml) to give **1** (11 mg) and **2** (5 mg).

Compound **1**: A white amorphous powder; [α]_D²⁵ -33.0° ($c=0.03$ in MeOH); ¹H-NMR (pyridine-*d*₅, 600 MHz) and ¹³C-NMR (pyridine-*d*₅, 150 MHz), see Tables 1 and 2; Positive HR-ESI-MS m/z 1095.5399 [M+Na]⁺ (Calcd 1095.5352); Negative FAB-MS m/z 1071 [M-H]⁻, 925 [(M-H)-146]⁻, 909 [(M-H)-162]⁻.

Compound **2**: A white amorphous powder; [α]_D²⁵ -33.0° ($c=0.03$ in MeOH); ¹H-NMR (pyridine-*d*₅, 600 MHz) and ¹³C-NMR (pyridine-*d*₅, 150 MHz), see Tables 1 and 2; Positive HR-ESI-MS m/z 1079.5356 [M+Na]⁺ (Calcd 1079.5403); Negative FAB-MS m/z 1055 [M-H]⁻, 909 [(M-H)-146]⁻, 893 [(M-H)-162]⁻.

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