Oleanane-Type Glycosides from *Tremastelma palaestinum* (L.) JANCHEN

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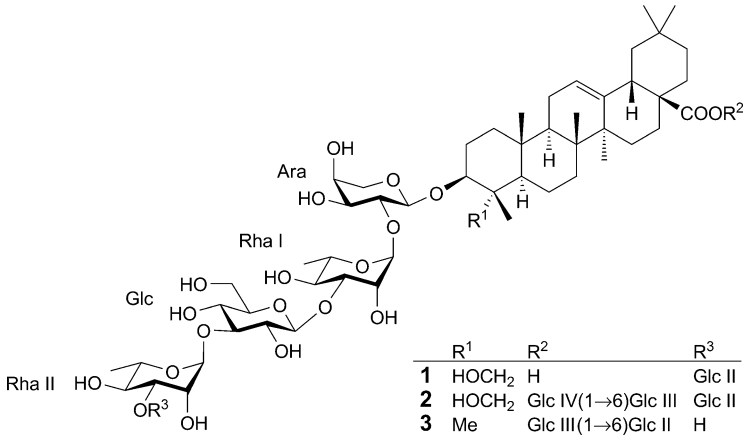
Three new oleanane-type glycosides, **1**–**3**, were isolated from the whole plant of *Tremastelma palaestinum* (L.) JANCHEN, along with eight known triterpene glycosides. The structures of the new compounds were established as 3-*O*-[*β*-D-glucopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl]hederagenin (**1**), 3-*O*-[*β*-D-glucopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl]hederagenin 28-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester (**2**), and 3-*O*-[*α*-L-rhamnopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl]oleanolic acid 28-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester (**3**) by using 1D- and 2D-NMR techniques and mass spectrometry. This is the first report on the phytochemical investigation of a species belonging to *Tremastelma* genus.

Introduction. – The genus *Tremastelma* (Dipsacaceae) is represented by one species in Turkey [1]. *T. palaestinum* (Syn: *Knautia palaestina* L., *Scabiosa brachiata* SM., *Pterocephalus palaestinus* COULT., *Asterocephalus brachiatus* RCHB., *Callistemma brachiatum* Boiss., *Callistemma palaestinum* L.) is an annual plant which mainly grows in West and Northwestern part of Anatolia, some of the Greek islands, Balkan peninsula, and West of Syria [1]. From a phylogenetic point of view [2][3], Dipsacaceae are considered among the most advanced families within the Dicotyledons. Most of the taxa are widespread over the Mediterranean region and the Middle East [3][4]. Although there are no reports of the medicinal uses of genus *Tremastelma*, some of the Dipsacaceae plants have been used for their antidermatosic, anti-eczematous, antipyretic, antihypertensive, and pharyngeal antiseptic properties [5], as well as for the treatment of breast cancer [6], diphtheria [7], hemorrhoids [8], cold, flu, rheumatoid arthritis, and enteritis [9], and also as analgesic and anti-inflammatory agent to treat spermatorrhea, pain, and fractures [10]. This is the first phytochemical report on the genus *Tremastelma*.

Results and Discussion. – The MeOH extract of the air-dried and powdered whole plant *T. palaestinum* was partitioned with hexane, $CHCl_3$, and BuOH. The BuOH extract was fractionated by vacuum liquid chromatography (VLC), followed by column chromatography (CC) to yield compounds **1**–**3** (*Fig.*). The aglycones of the isolated compounds were identified as oleanane-type triterpenes by ^{1}H- and ^{13}C-NMR analysis (*Table 1*) [11][12].



	R^1	R^2	R^3
1	$HOCH_2$	H	Glc II
2	$HOCH_2$	Glc IV(1→6)Glc III	Glc II
3	Me	Glc III(1→6)Glc II	H

Figure. *Structures of compounds* **1**–**3**, *isolated from* Tremastelma palaestinum

The HR-MALDI-TOF mass spectrum of **1** (*m/z* 1243.6096 ($[M+Na]^+$, $C_{59}H_{96}NaO_{26}^+$; calc. 1243.6088) indicated the molecular formula $C_{59}H_{96}O_{26}$. The ESI-MS showed a major ion peak at *m/z* 1243 ($[M+Na]^+$). Significant fragment-ion peaks, in MS/MS analysis, at *m/z* 1081 ($[M+Na-162]^+$), 935 ($[M+Na-162-146]^+$), 773 ($[M+Na-162-146-162]^+$), 627 ($[M+Na-162-146-162-146]^+$), 477 ($[M+Na-162-146-162-146-150]^+$), corresponding to the loss of one pentose unit and four hexose units, were observed.

The ^{1}H-NMR spectrum displayed signals for six tertiary Me groups at δ 0.73, 0.87, 0.91, 0.97, 1.00, and 1.19, for an olefinic H-atom at δ 5.25 (t, $J = 3.5$), one oxygen-bearing CH group at δ 3.65 (dd, $J = 11.5$, 4.2, H–C(3)), and one primary alcohol function at δ 3.36 and 3.61 (H–C(23)) (*Table 1*). These signals, along with the resonances in the ^{13}C-NMR spectrum for the Me groups at δ 13.8, 18.0, 33.8, 24.0, 16.5, and 26.5, and for the two olefinic C-atoms at δ 122.6 and 145.9, suggested that compound **1** possessed hederagenin as aglycone [13]. The downfield shift of C(3) (δ 82.4) of the aglycone evidenced that compound **1** was a monodesmosidic glycoside. The ^{13}C-NMR spectrum showed 59 C-signals, of which 30 were assigned to the aglycone moiety and 29 to a sugar moiety containing of five sugar units. The ^{1}H-NMR spectrum displayed in the sugar region signals corresponding to five anomeric H-atoms at δ 4.52 (d, $J = 3.7$), 4.54 (d, $J = 7.5$), 4.62 (d, $J = 7.5$), 5.21 (d, $J = 1.2$), and 5.22 (d, $J = 1.2$), which were unambiguously correlated by HSQC experiment to the corresponding C-resonances at δ 104.7, 105.2, 104.9, and 101.5 (2 C), respectively. The chemical shifts of all the individual H-atoms of the five sugar units were ascertained from a combination of 1D-TOCSY and DQF-COSY spectral analysis, and the ^{13}C chemical shifts of their respective attached C-atoms were assigned unambiguously on the basis of the HSQC spectrum (*Table 2*). These data evidenced the presence of two β-glucopyranosyl units (δ 4.54 and 4.62), two α-rhamnopyranosyl units (δ 5.21 and 5.22), and one α-arabinopyranosyl unit (δ 4.52). The β-configurations of the glucopyranosyl units were deduced from the J values of the anomeric H-atom ($J = 7.5$). The α-configuration of the

Table 1. *^{1}H- and ^{13}C-NMR Data of the Aglycone Moieties of Compounds* **1**–**3** (recorded in CD_3OD at 600 Mz; δ in ppm, J in Hz)

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	1.64–1.62 (*m*), 0.99–0.97 (*m*)	39.6	1.64–1.62 (*m*), 0.98–0.96 (*m*)	39.8	1.65–1.63 (*m*), 1.01–0.99 (*m*)	39.9
2	1.89–1.87 (*m*), 1.78–1.76 (*m*)	26.6	1.89–1.87 (*m*), 1.77–1.75 (*m*)	26.2	1.87–1.85 (*m*), 1.74–1.72 (*m*)	27.1
3	3.65 (*dd*, *J* = 11.5, 4.2)	82.4	3.65 (*dd*, *J* = 11.5, 4.2)	82.9	3.16 (*dd*, *J* = 11.5, 4.2)	90.5
4	–	43.7	–	43.4	–	40.1
5	1.30–1.29 (*m*)	47.8	1.30–1.29 (*m*)	47.7	0.82–0.80 (*m*)	56.8
6	1.52–1.50 (*m*), 1.40–1.38 (*m*)	18.9	1.51–1.49 (*m*), 1.40–1.38 (*m*)	19.0	1.57–1.55 (*m*), 1.44–1.42 (*m*)	19.2
7	1.66–1.65 (*m*), 1.31–1.29 (*m*)	33.3	1.66–1.65 (*m*), 1.30–1.28 (*m*)	33.1	1.52–1.50 (*m*), 1.44–1.42 (*m*)	33.7
8	–	40.4	–	39.8	–	40.3
9	1.66–1.65 (*m*)	48.8	1.66–1.65 (*m*)	48.4	1.61–1.60 (*m*)	49.0
10	–	37.3	–	37.0	–	37.5
11	1.95–1.94 (*m*, 2 H)	24.5	1.94–1.93 (*m*, 2 H)	24.5	1.92–1.90 (*m*, 2 H)	24.6
12	5.25 (*t*, *J* = 3.5)	122.6	5.27 (*t*, *J* = 3.5)	123.4	5.31 (*t*, *J* = 3.5)	123.3
13	–	145.9	–	144.4	–	145.8
14	–	42.9	–	42.9	–	42.6
15	1.87–1.85 (*m*), 1.07–1.05 (*m*)	28.8	1.80–1.78 (*m*), 1.11–1.09 (*m*)	28.6	1.81–1.79 (*m*), 1.10–1.08 (*m*)	28.6
16	1.96–1.94 (*m*), 1.64–1.62 (*m*)	24.4	2.07–2.05 (*m*), 1.74–1.72 (*m*)	24.0	2.08–2.06 (*m*), 1.74–1.72 (*m*)	23.8
17	–	47.5	–	47.9	–	47.6
18	2.90 (*dd*, *J* = 12.5, 3.5)	42.9	2.91 (*dd*, *J* = 12.5, 3.5)	42.6	2.88 (*dd*, *J* = 12.5, 3.5)	42.5
19	1.71–1.69 (*m*), 1.13–1.11 (*m*)	47.5	1.74–1.72 (*m*), 1.17–1.15 (*m*)	47.1	1.73–1.71 (*m*), 1.18–1.16 (*m*)	47.2
20	–	31.5	–	31.3	–	31.4
21	1.40–1.38 (*m*), 1.19–1.17 (*m*)	35.0	1.43–1.41 (*m*), 1.25–1.23 (*m*)	34.5	1.43–1.41 (*m*), 1.25–1.23 (*m*)	34.8
22	1.76–1.74 (*m*), 1.56–1.54 (*m*)	34.2	1.75–1.73 (*m*), 1.63–1.61 (*m*)	33.3	1.75–1.73 (*m*), 1.63–1.61 (*m*)	33.0
23	3.61 (*d*, *J* = 11.5), 3.36 (*d*, *J* = 11.5)	64.4	3.59 (*d*, *J* = 11.5), 3.36 (*d*, *J* = 11.5)	64.2	1.06 (*s*)	28.2
24	0.73 (*s*)	13.8	0.73 (*s*)	13.8	0.88 (*s*)	17.3
25	1.00 (*s*)	16.5	1.00 (*s*)	16.6	0.97 (*s*)	16.2
26	0.87 (*s*)	18.0	0.82 (*s*)	17.9	0.82 (*s*)	17.8
27	1.19 (*s*)	26.5	1.18 (*s*)	26.3	1.18 (*s*)	26.3
28	–	181.2	–	178.3	–	178.1
29	0.91 (*s*)	33.8	0.94 (*s*)	33.4	0.94 (*s*)	33.6
30	0.97 (*s*)	24.0	0.96 (*s*)	24.2	0.96 (*s*)	24.0

rhamnopyranosyl units was established by the H–C(1)/C(1) J value of 169 Hz, determined by the residual direct correlation observed in the HMBC spectrum, in agreement with that reported for the α-anomer of rhamnopyranose [14]. For the arabinopyranosyl unit, the coupling constant of the anomeric H-atom was reported not to be diagnostic on its own, owing to the high conformational mobility of arabinopyranosides ($^4C_1 \leftrightarrow {}^1C_4$). Evidence supporting an α-L-arabinopyranoside configuration in rapid conformational exchange was obtained from the ROESY experiment which showed correlations between H–C(1) and H–C(2), and between H–C(1) and H–C(3) as expected for $^1C_4$ and $^4C_1$, conformations, respectively. The ROESY correlation between H–C(1) and H–C(3) would not be expected for both $^1C_4$- and $^4C_1$-β-L-arabinopyranosides. A ROESY correlation was also observed between H–C(1) and H–C(5) as expected for an α-L-arabinopyranoside in a $^4C_1$ conformation [15].

An unambiguous determination of the sequence and linkage sites was achieved from the HMBC spectrum, which showed key correlation peaks between H–C(1)(Ara) (δ 4.52) and C(3) (δ 82.4), between H–C(1)(RhaI) (δ 5.22) and C(2)(Ara) (δ 76.6), and between H–C(1)(GlcI) (δ 4.54) and C(3)(RhaI) (δ 82.6), between H–C(1)(RhaII) (δ 5.21) and C(3)(GlcI) (δ 83.3), and between H–C(1)(GlcII) (δ 4.62) and C(3)(RhaII) (δ 82.8). The D-configuration of glucose and the L-configurations of the arabinose and rhamnose units were established by hydrolysis of **1** with 1N HCl, trimethylsilylation, and determination of retention time on a chiral column by GC [16]. On the basis of all these evidences, the structure of **1** was established as 3-*O*-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]hederagenin.

The HR-MALDITOF mass spectrum of **2** (m/z 1567.7151 ($[M+\mathrm{Na}]^+$, $C_{71}H_{116}NaO_{36}^+$; calc. 1567.7144) indicated the molecular formula $C_{71}H_{116}O_{36}$. The ESI-MS showed a major ion peak at m/z 1567 ($[M+\mathrm{Na}]^+$), and significant fragment-ion peaks, in MS/MS analysis, at m/z 1405 ($[M+\mathrm{Na}-162]^+$), 1259 ($[M+\mathrm{Na}-162-146]^+$), 1097 ($[M+\mathrm{Na}-162-146-162]^+$), 951 ($[M+\mathrm{Na}-162-146-162-146]^+$), 801 ($[M+\mathrm{Na}-162-146-162-146-150]^+$), 639 ($[M+\mathrm{Na}-162-146-162-146-150-162]^+$), 477 ($[M+\mathrm{Na}-162-146-162-146-150-162-162]^+$), corresponding to the loss of one pentose unit and six hexose units, were observed.

The ^{1}H- and ^{13}C-NMR chemical shifts of the aglycone moiety of **2** were almost superimposable on those of **1** (*Table 1*), suggesting hederagenin as aglycone.

With respect to the sugar portion, the occurrence of two additional sugar units was observed compared to **1**. The ^{1}H NMR spectrum displayed in the sugar region signals corresponding to seven anomeric H-atoms at δ 4.37 ($d, J=7.5$), 4.52 ($d, J=3.7$), 4.55 (d, $J=7.5$), 4.62 (d, $J=7.5$), 5.21 (d, $J=1.2$), 5.22 (d, $J=1.2$), and 5.38 (d, $J=7.5$) which showed correlations in the HSQC spectrum with the anomeric C-atom signals at δ 104.2, 104.7, 105.2, 105.1, 101.4 (2 C), and 95.3, respectively. A detailed analysis of NMR data revealed the presence of four β-glucopyranosyl units (δ 4.37, 4.55, 4.62, and 5.38), two α-rhamnopyranosyl units (δ 5.21 and 5.22), and one α-arabinopyranosyl unit (δ 4.52). An unambiguous determination of the sequence and linkage sites was obtained from the HMBC spectrum, which allowed us to identify at C(3) the same sugar chain as in **1**, and the occurrence of a further sugar chain at C(28), as indicated by the upfield shift of C(28) at δ 178.3 when compared to compound **1**. Key HMBCs between the resonance of H–C(1)(GlcIII) (δ 5.38) and C(28) (δ 178.3), and between

Table 2. *^{1}H- and ^{13}C-NMR Data of the Sugar Portions of Compounds* **1**–**3** (recorded in CD_3OD at 600 Mz; δ in ppm, J in Hz)

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
	α-L-Ara (at C(3))		α-L-Ara (at C(3))		α-L-Ara (at C(3))	
1	4.52 (*d*, *J*=3.7)	104.7	4.52 (*d*, *J*=3.7)	104.7	4.49 (*d*, *J*=3.7)	105.3
2	3.68 (*dd*, *J*=8.5, 3.7)	76.6	3.66 (*dd*, *J*=8.5, 3.7)	76.6	3.73 (*dd*, *J*=8.5, 3.7)	76.8
3	3.68 (*dd*, *J*=8.5, 3.0)	73.8	3.68 (*dd*, *J*=8.5, 3.0)	73.8	3.72 (*dd*, *J*=8.5, 3.0)	73.5
4	3.81–3.80 (*m*)	69.5	3.81–3.80 (*m*)	70.4	3.81–3.80 (*m*)	68.8
5	3.86 (*dd*, *J*=11.9, 2.0), 3.54 (*dd*, *J*=11.9, 3.0)	65.6	3.87 (*dd*, *J*=11.9, 2.0), 3.54 (*dd*, *J*=11.9, 3.0)	65.9	3.87 (*dd*, *J*=11.9, 2.0), 3.52 (*dd*, *J*=11.9, 3.0)	64.7
	α-L-Rha I (at C(2)(Ara))		α-L-Rha I (at C(2)(Ara))		α-L-Rha I (at C(2)(Ara))	
1	5.22 (*d*, *J*=1.2)	101.5	5.21 (*d*, *J*=1.2)	101.4	5.16 (*d*, *J*=1.2)	101.6
2	4.27 (*dd*, *J*=1.2, 3.2)	70.8	4.26 (*dd*, *J*=1.2, 3.2)	70.7	4.25 (*dd*, *J*=1.2, 3.2)	70.9
3	3.90 (*dd*, *J*=9.7, 3.2)	82.6	3.90 (*dd*, *J*=9.7, 3.2)	82.5	3.88 (*dd*, *J*=9.7, 3.2)	82.7
4	3.59 (*t*, *J*=9.7)	72.5	3.59 (*t*, *J*=9.7)	72.4	3.59 (*t*, *J*=9.7)	72.4
5	3.95–3.94 (*m*)	70.0	3.95–3.94 (*m*)	69.9	3.95–3.94 (*m*)	69.9
6	1.28 (*d*, *J*=6.5)	17.8	1.28 (*d*, *J*=6.5)	18.0	1.26 (*d*, *J*=6.5)	17.9
	β-D-Glc I (at C(3)(RhaI))		β-D-Glc I (at C(3)(RhaI))		β-D-Glc I (at C(3)(RhaI))	
1	4.54 (*d*, *J*=7.5)	105.2	4.55 (*d*, *J*=7.5)	105.2	4.53 (*d*, *J*=7.5)	105.5
2	3.43 (*dd*, *J*=7.5, 9.0)	75.9	3.45 (*dd*, *J*=7.5, 9.0)	75.9	3.42 (*dd*, *J*=7.5, 9.0)	73.8
3	3.57 (*dd*, *J*=9.0, 9.0)	83.3	3.57 (*dd*, *J*=9.0, 9.0)	83.1	3.54 (*dd*, *J*=9.0, 9.0)	83.8
4	3.33 (*dd*, *J*=9.0, 9.0)	71.1	3.33 (*dd*, *J*=9.0, 9.0)	71.2	3.32 (*dd*, *J*=9.0, 9.0)	71.3
5	3.30–3.29 (*m*)	77.9	3.29–3.28 (*m*)	77.8	3.39–3.38 (*m*)	77.8
6	3.88 (*dd*, *J*=3.5, 12.0), 3.72 (*dd*, *J*=4.5, 12.0)	62.4	3.88 (*dd*, *J*=3.5, 12.0), 3.72 (*dd*, *J*=4.5, 12.0)	62.5	3.87 (*dd*, *J*=3.5, 12.0), 3.76 (*dd*, *J*=4.5, 12.0)	62.4
	α-L-Rha II (at C(3)(GlcI))		α-L-Rha II (at C(3)(GlcI))		α-L-Rha II (at C(3)(GlcI))	
1	5.21 (*d*, *J*=1.2)	101.5	5.22 (*d*, *J*=1.2)	101.4	5.20 (*d*, *J*=1.2)	102.1
2	4.00 (*dd*, *J*=1.2, 3.2)	71.9	4.00 (*dd*, *J*=1.2, 3.2)	71.9	3.98 (*dd*, *J*=1.2, 3.2)	69.9
3	3.65 (*dd*, *J*=9.7, 3.2)	82.8	3.65 (*dd*, *J*=9.7, 3.2)	82.9	3.73 (*dd*, *J*=9.7, 3.2)	72.0
4	4.12 (*t*, *J*=9.7)	68.5	4.11 (*t*, *J*=9.7)	68.3	3.43 (*t*, *J*=9.7)	73.8
5	3.99–3.98 (*m*)	72.2	3.99–3.98 (*m*)	71.9	3.99–3.98 (*m*)	72.1
6	1.27 (*d*, *J*=6.5)	17.8	1.28 (*d*, *J*=6.5)	18.0	1.26 (*d*, *J*=6.5)	17.9

Table 2 (cont.)

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
	β-D-Glc II (at C(3)(RhaII))		β-D-Glc II (at C(3)(RhaII)		β-D-Glc II (at C(28))	
1	4.62 (*d*, *J*=7.5)	104.9	4.62 (*d*, *J*=7.5)	105.1	5.38 (*d*, *J*=7.5)	95.4
2	3.23 (*dd*, *J*=7.5, 9.0)	75.9	3.23 (*dd*, *J*=7.5, 9.0)	75.4	3.37 (*dd*, *J*=7.5, 9.0)	73.7
3	3.36 (*dd*, *J*=9.0, 9.0)	77.7	3.45 (*dd*, *J*=9.0, 9.0)	77.8	3.43 (*dd*, *J*=9.0, 9.0)	75.8
4	3.42 (*dd*, *J*=9.0, 9.0)	69.6	3.33 (*dd*, *J*=9.0, 9.0)	71.2	3.43 (*dd*, *J*=9.0, 9.0)	69.6
5	3.41–3.40 (*m*)	77.9	3.41–3.40 (*m*)	77.7	3.54–3.53 (*m*)	77.6
6	3.88 (*dd*, *J*=3.5, 12.0), 3.72 (*dd*, *J*=4.5, 12.0)	62.4	3.88 (*dd*, *J*=3.5, 12.0), 3.72 (*dd*, *J*=4.5, 12.0)	62.5	4.15 (*dd*, *J*=3.5, 12.0), 3.78 (*dd*, *J*=4.5, 12.0)	69.5
			β-D-Glc III (at C(28))		β-D-Glc III (at C(6)(GlcII))	
1			5.38 (*d*, *J*=7.5)	95.3	4.37 (*d*, *J*=7.5)	104.4
2			3.37 (*dd*, *J*=7.5, 9.0)	73.2	3.24 (*dd*, *J*=7.5, 9.0)	74.9
3			3.44 (*dd*, *J*=9.0, 9.0)	76.1	3.45 (*dd*, *J*=9.0, 9.0)	78.1
4			3.40 (*dd*, *J*=9.0, 9.0)	69.6	3.46 (*dd*, *J*=9.0, 9.0)	70.7
5			3.56–3.55 (*m*)	77.4	3.28–3.27 (*m*)	77.8
6			4.15 (*dd*, *J*=3.5, 12.0), 3.80 (*dd*, *J*=4.5, 12.0)	69.5	3.87 (*dd*, *J*=3.5, 12.0), 3.74 (*dd*, *J*=4.5, 12.0)	62.4
			β-D-Glc IV (at C(6)(GlcIII))			
1			4.37 (*d*, *J*=7.5)	104.2		
2			3.24 (*dd*, *J*=7.5, 9.0)	75.4		
3			3.45 (*dd*, *J*=9.0, 9.0)	77.8		
4			3.47 (*dd*, *J*=9.0, 9.0)	70.6		
5			3.28–3.27 (*m*)	77.8		
6			3.88 (*dd*, *J*=3.5, 12.0), 3.72 (*dd*, *J*=4.5, 12.0)	62.5		

the resonance of H–C(1)(GlcIV) (δ 4.37) and C(6)(GlcIII) (δ 69.5), confirmed this hypothesis. On the basis of all these evidences, the structure of **2** was established as 3-*O*-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]hederagenin 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

The HR-MALDI-TOF mass spectrum of **3** (*m/z* 1389.6677 ($[M+Na]^+$, $C_{65}H_{106}NaO_{30}^+$; calc. 1389.6667) provided the molecular formula $C_{65}H_{106}O_{30}$. The ESI-MS exhibited the major ion peak at *m/z* 1389 ($[M+Na]^+$), and significant fragment-ion peaks, in MS/MS, analysis at *m/z* 1243 ($[M+Na-146]^+$), 1081 ($[M+Na-146-162]^+$), 935 ($[M+Na-146-162-146]^+$), 785 ($[M+Na-146-162-146-150]^+$), 623 ($[M+Na-146-162-146-150-162]^+$), 461 ($[M+Na-146-162-146-150-162-162]^+$), corresponding to the loss of one pentose unit and five hexose units were observed.

The ^{1}H-NMR spectrum of **3** displayed signals for seven tertiary Me groups at δ 0.88, 0.94, 0.96, 0.97, 1.06, 1.18, and 0.82, and for a vinyl H-atom at δ 5.31 (*t*, *J* = 3.5). These signals, along with the resonances in the ^{13}C-NMR spectrum for the Me groups at δ 17.8, 17.3, 33.6, 24.0, 16.2, 28.2, and 33.6, and of the two olefinic C-atoms at δ 123.3 and 144.4, evidenced that compound **3** possessed an olean-12-ene skeleton as aglycone [17]. Detailed NMR studies allowed us to identify the aglycone as oleanolic acid [18]. The downfield shifts of C(3) (δ 90.5) and C(28) (δ 178.3) of the aglycone indicated that compound **3** was a bidesmosidic glycoside. The ^{13}C-NMR spectrum showed 65 C-atom signals of which 30 were assigned to the aglycone moiety (*Table 1*) and 35 to a sugar portion. The ^{1}H-NMR spectrum displayed in the sugar region signals corresponding to six anomeric H-atoms at δ 4.37 (*d*, *J* = 7.5), 4.49 (*d*, *J* = 3.7), 4.53 (*d*, *J* = 7.5), 5.16 (*d*, *J* = 1.2), 5.20 (*d*, *J* = 1.2), and 5.38 (*d*, *J* = 7.5).

Complete assignments of the resonances of each sugar unit were achieved by extensive 1D- (^{1}H, ^{13}C, TOCSY) and 2D- (HSQC, HMBC) NMR analyses. These data revealed the presence of three β-glucopyranosyl units (δ 4.37, 4.53, and 5.38), two α-rhamnopyranosyl units (δ 5.16 and 5.20), and one α-arabinopyranosyl unit (δ 4.49). A detailed analysis of the HMBC spectrum indicated that compound **3** differed from **2**, only in the absence of the terminal glucose unit in the sugar chain linked at C(3). Thus, compound **3** was identified as 3-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]oleanolic acid 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

Additionally, eight known oleanane-type glycosides, 3-*O*-α-L-arabinopyranosylhederagenin 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester [19], 3-*O*-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]oleanolic acid 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester [20], 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]oleanolic acid 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester [21], 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]hederagenin 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester [22], 3-*O*-[β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]hederagenin [23], 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]hederagenin [24], 3-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]hederagenin [25], and 3-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-

arabinopyranosyl]hederagenin 28-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester [26], were isolated.

As mentioned above, compounds **1**–**3** have never been reported before. 3-*O*-[*β*-D-Glucopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl]oleanolic acid 28-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester, 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl]oleanolic acid 28-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester, 3-*O*-[*β*-D-glucopyranosyl(1→3)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl]hederagenin, and 3-*O*-[*α*-L-rhamnopyranosyl-(1→3)-*β*-D-glucopyranosyl(1→3)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl]hederagenin are reported for the first time in a species belonging to the Dipsacaceae family. 3-*O*-[*α*-L-Arabinopyranosyl]hederagenin 28-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester and 3-*O*-[*α*-L-rhamnopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl]hederagenin 28-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester have been found in *Cephalaria gigantea* and *Dipsacus asperoides* [27–30], while 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl]hederagenin 28-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester has been previously isolated from the MeOH extract of *Cephalaria pastricensis* leaves [30] and 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl]hederagenin from the roots of *Scabiosa soongorica* [31]. The species cited above belong all to Dipsacaceae family.

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Experimental Part

General. GC Analysis: *ThermoFinnigan Trace* GC apparatus; L-*Chirasil-Val* column (0.32 mm × 25 m). Column chromatography (CC): silica gel *60* (0.063–0.200 μm; *Merck*) and *LiChroprep RP-18* (25–40 μm, *Merck*). TLC: silica gel *60* F_{254} (*Merck*) and *RP-18* F_{254s} (*Merck*) plates. Optical rotations: *JASCO DIP 1000* polarimeter. IR Spectra: *Bruker IFS-48* spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker DRX-600* spectrometer (*BrukerBioSpin*, D-Rheinstetten) equipped with a *Bruker* 5-mm *TCI CryoProbeat 300 K;* all 2D-NMR spectra were acquired in CD_3OD (99.95%, *SigmaAldrich*), and standard pulse sequences and phase cycling were used for DQF-COSY, HSQC, and HMBC spectra; the NMR data were processed using UXNMR software. Exact masses were determined by a *Voyager DE* mass spectrometer. Samples were analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDITOF) mass spectrometry. A mixture of analyte soln. and *α*-cyano-4-hydroxycinnamic acid (*Sigma*) was applied to the metallic sample plate and dried. Mass calibration was performed with the ions from ACTH (fragment 18–39) at 2465.1989 Da and angiotensin III at 931.5154 Da as internal standard. ESI-MS: *ThermoFinnigan LCQ Deca XP Max* ion-trap mass spectrometer, equipped with Xcalibur software.

Plant Material. Tremastelma palaestinum (L.) JANCHEN (whole plant) was collected from Bornova, Izmir, Turkey, in June, 2011. Samples of plant material were identified by Assoc. Prof. Dr. *S. G. Şenol* (Ege University, Faculty of Science, Department of Biology). A voucher specimen (EGE 40858) was deposited with the Ege University Botanical Garden & Herbarium Research and The Application Center, Izmir, Turkey.

Extraction and Isolation. The air-dried and powdered plant material of *T. palaestinum* (whole plant; 400 g) was extracted with MeOH (3 × 3 l) at r.t. for 3 d. After filtration, the solvent was removed by rotary evaporation to give a crude extract (40 g). The residue was dissolved in H_2O (300 ml) and then partitioned successively with hexane (3 × 250 ml), $CHCl_3$ (3 × 250 ml), and BuOH saturated with H_2O (3 × 250 ml). The BuOH extract (5 g) was subjected to vacuum liquid chromatography (VLC) using

reversed-phase (RP) material (*Lichroprep RP-18*, 25–40 μm, 150 g) employing H_2O (1000 ml), H_2O/MeOH (8:2, 1200 ml; 6:4, 2400 ml; 4:6, 3000 ml; 2:8, 1600 ml) and MeOH (600 ml) to give ten main fractions (*A–J*). *Fr. D* (500 mg) was subjected to CC SiO_2 (60 g); $CHCl_3$/MeOH/H_2O 80:20:2, 1000 ml; 70:30:3, 500 ml; 61:32:7, 750 ml) to yield 12 subfractions, and **3** (8 mg), 3-*O*-[α-L-arabinopyranosyl]-hederagenin 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (6 mg), 3-*O*-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]oleanolic acid 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (10 mg), 3-*O*-[β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]hederagenin (10 mg) and 3-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]hederagenin 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (10 mg). *Subfr. 2* (40 mg) was submitted to CC (SiO_2 (15 g); $CHCl_3$/MeOH/H_2O 80:20:2, 500 ml; 70:30:3, 300 ml; 61:32:7, 250 ml) to give **2** (7 mg). *Subfr. 5* (35 mg) was chromatographed on RP material (*Lichroprep RP-18*, 25–40 μm, 10 g), employing MeOH/H_2O 6:4 (500 ml) to yield arabinopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester [20], 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]oleanolic acid 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, (12 mg), and 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]hederagenin-28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (9 mg). *Subfr. 8* (80 mg) was subjected to CC (SiO_2 (15 g); $CHCl_3$/MeOH/H_2O 80:20:2, 200 ml; 70:30:3, 200 ml; 61:32:7, 250 ml) to yield 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-hederagenin (8 mg). *Fr. E* (850 mg) was subjected to CC (SiO_2 (80 g); $CHCl_3$/MeOH/H_2O 80:20:2, 1000 ml; 70:30:3, 500 ml; 61:32:7, 700 ml) to yield 9 subfractions. *Subfr. 3* (80 mg) was submitted to CC (SiO_2 (15 g); $CHCl_3$/MeOH/H_2O 80:20:2, 300 ml; 70:30:3, 300 ml; 61:32:7, 250 ml) to give 3-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]hederagenin (6 mg). *Subfr. 4* (30 mg) was chromatographed on RP material (*Lichroprep RP-18*, 25–40 μm; 10 g), employing MeOH/H_2O (6:4, 500 ml), to yield **1** (8 mg).

Acid Hydrolysis. A soln. (1.0 mg each) of compounds **1**–**3** in 1N HCl (0.25 ml) was stirred at 80° for 4 h. After cooling, the soln. was concentrated by blowing with N_2. The residue was dissolved in 1-(trimethylsilyl)-1*H*-imidazole and pyridine (0.1 ml), and the soln. was stirred at 60° for 5 min. After drying the soln. with a stream of N_2, the residue was partitioned between H_2O and CH_2Cl_2 (1 ml; 1:1 (*v*/*v*)). The CH_2Cl_2 layer was analyzed by GC using an L-*Chirasil-Val* column (0.32 mm × 25 m). Temps. of the injector and detector were 200° both. A temp. gradient system was used for the oven; the initial temp. was maintained at 100° for 1 min and then raised to 180° at the rate of 5°/min [16]. The peaks of D-glucose (14.74 min), L-rhamnose (10.72 min), and L-arabinose (8.91 and 9.81 min) were detected in the hydrolysate of **1**.

Authentic samples treated in the same manner with 1-(trimethylsilyl)-1*H*-imidazole in pyridine were detected at 14.71 (D-glucose), 10.70 (L-rhamnose), and 8.92 and 9.80 min (L-arabinose).

3-O-*[β-D-Glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]hederagenin* (=*(3β)-3-[[β-D-Glucopyranosyl-(1→3)-6-deoxy-α-L-mannopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-6-deoxy-α-L-mannopyranosyl-(1→2)-α-L-arabinopyranosyl]oxy]-23-hydroxyolean-12-en-28-oic Acid*; **1**). Amorphous white solid. $[\alpha]_D^{25} = +19.8$ (*c* = 0.1, MeOH). IR (KBr): 3428 (OH), 2934 (CH), 1680 (C=O), 1658 (C=C). ^{1}H- and ^{13}C-NMR (CD_3OD, 600 MHz): see *Tables 1* and *2*. ESI-MS: 1243 ($[M+Na]^+$). ESI-MS/MS: 1081 ($[M+Na-162]^+$), 935 ($[M+Na-162-146]^+$), 773 ($[M+Na-162-146-162]^+$), 627 ($[M+Na-162-146-162-146]^+$), 477 ($[M+Na-162-146-162-146-150]^+$). HR-MALDITOF-MS: 1243.6096 ($[M+Na]^+$, $C_{59}H_{96}NaO_{26}^+$; calc. 1243.6088).

3-O-*[β-D-Glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]hederagenin 28*-O-*β-D-Glucopyranosyl-(1→6)-β-D-glucopyranosyl Ester* (=*6*-O-*β-D-Glucopyranosyl-1*-O-*[(3β)-3-[[β-D-glucopyranosyl-(1→3)-6-deoxy-α-L-mannopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-6-deoxy-α-L-mannopyranosyl-(1→2)-α-L-arabinopyranosyl]oxy]-23-hydroxy-28-oxoolean-12-en-28-yl]-β-D-glucopyranose*; **2**). Amorphous white solid. $[\alpha]_D^{25} = +22.5$ (*c* = 0.1, MeOH). IR (KBr): 3442 (OH), 2930 (CH), 1670 (C=O), 1655 (C=C). ^{1}H- and ^{13}C-NMR (CD_3OD, 600 MHz): see *Tables 1* and *2*. ESI-MS: 1567 ($[M+Na]^+$). ESI-MS/MS: 1405 ($[M+Na-162]^+$), 1259 ($[M+Na-162-146]^+$), 1097 ($[M+Na-162-146-162]^+$), 951 ($[M+Na-162-146-162-146]^+$), 801 ($[M+Na-162-146-162-146-150]^+$), 639 ($[M+Na-162-146-162-$

146 – 150 – 162]$^+$), 477 ([M + Na – 162 – 146 – 162 – 146 – 150 – 162 – 162]$^+$). HR-MALDITOF-MS: 1567.7151 ([M + Na]$^+$, $C_{65}H_{106}NaO_{30}^+$; calc. 1567.7144).

3-O-*[α*-L-*Rhamnopyranosyl-(1→3)-β*-D-*glucopyranosyl(1→3)-α*-L-*rhamnopyranosyl-(1→2)-α*-L-*arabinopyranosyl]oleanolic Acid 28*-O-*β*-D-*Glucopyranosyl-(1→6)-β*-D-*glucopyranosyl Ester* (=*1*-O-*[(3β)-3-{[6-Deoxy-α*-L-*mannopyranosyl-(1→3)-β*-D-*glucopyranosyl-(1→3)-6-deoxy-α*-L-*mannopyranosyl-(1→2)-α*-L-*arabinopyranosyl]oxy}-28-oxoolean-12-en-28-yl]-6*-O-*β*-D-*glucopyranosyl-β*-D-*glucopyranose*; **3**). Amorphous white solid. $[\alpha]_D^{25} = -10.5$ ($c = 0.1$, MeOH). IR (KBr): 3450 (OH), 2938 (CH), 1680 (C=O), 1660 (C=C). ^{1}H- and ^{13}C-NMR (CD_3OD, 600 MHz): see *Tables 1* and *2*. ESI-MS: 1389 ([M + Na]$^+$). ESI-MS/MS: 1243 ([M + Na – 146]$^+$), 1081 ([M + Na – 146 – 162]$^+$), 935 ([M + Na – 146 – 162 – 146]$^+$), 785 ([M + Na – 146 – 162 – 146 – 150]$^+$), 623 ([M + Na – 146 – 162 – 146 – 150 – 162]$^+$), 461 ([M + Na – 146 – 162 – 146 – 150 – 162 – 162]$^+$). HR-MALDITOF-MS: 1389.6677 ([M + Na]$^+$, $C_{71}H_{116}NaO_{36}^+$; calc. 1389.6667).

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