Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 65 (2004) 909–914
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Further saponins from *Meryta lanceolata*

F.R. Melek[a,*], Toshio Miyase[b], N.S. Ghaly[a], M.F. Yousif[c]

[a]*Chemistry of Natural Products Department, National Research Center, Dokki, 12622, Cairo, Egypt*
[b]*School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, 422-8526, Japan*
[c]*Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Kasr-El-Aini, 11562, Cairo, Egypt*

Received 30 April 2003; received in revised form 21 November 2003; accepted 16 December 2003

Abstract

Five new oleanane-type saponins along with 11 known ones were isolated from the leaves and stems of *Meryta lanceolata*. The new saponins were characterised by spectroscopic analysis including FAMS, 1 and 2D NMR experiments and the results of hydrolysis as 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl] hederagenin 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester, 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl] oleanolic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]ester, 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl] oleanolic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-6-*O*-acetyl glucopyranosyl-(1→6)-β-D-glucopyranosyl]ester, 3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl] oleanolic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester and 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl] hederagenin, respectively.

Keywords: *Meryta lanceolata*; Araliaceae; Triterpenoid saponin

1. Introduction

In conjunction with a long-term study of bioactive saponins from plants grown in Egypt (Melek et al., 2002, 2003), we reported earlier the structures of five oleanane-type triterpene saponins isolated from the leaves and stems of *Meryta lanceolata* Hort, Araliaceae (Melek et al., 2003). In continuation of this work, another sixteen saponins were isolated from the same plant source, five of these saponins are new natural products. The present paper describes their isolation and structure elucidation

2. Results and discussion

The crude saponin mixture from the methanolic extract of leaves and stems of *M. lanceolata* was passed through a porous-polymer polystyrene resin (Diaion HP-20) column and the methanolic eluate fraction with enriched saponins was subjected to CC using silica gel to give two fractions. Further examination of these fractions using HPLC led to the isolation of additional sixteen saponins (**1**–**16**) including five new ones. The structures of the new saponins (**1**–**5**) were established on the basis of acid hydrolysis and NMR data (Tables 1 and 2).

Saponin **1** showed $[M+Na]^+$ ion peak at m/z 1303 in the FAB-mass spectrum, its molecular formula was then deduced as $C_{60}H_{96}O_{29}$. The ^{1}HNMR spectrum of **1** (C_5D_5N) exhibited signals for anomeric protons of five monosaccharides and a methyl group of one deoxyhexopyranose at δ 5.18 (1H, *d*, $J=7.4$ Hz), 5.40 (1H, *d*, $J=8.0$ Hz), 6.21 (1H, *d*, $J=8.0$ Hz), 4.97 (1H, *d*, $J=8.0$ Hz), 5.81 (brs) and 1.68 (3H, *d*, $J=6.0$ Hz). Acid hydrolysis of **1** with 2M hydrochloric acid in dioxane (1:1) yielded hederagenin and D-glucuronic acid, D-glucose and L-rhamnose as the sugar components. The nature of the monosaccharides and their absolute configuration were established by GC analysis of thiazolidine derivatives for glucose and rhamnose and PC detection for glucuronic acid. The above data, along with five anomeric carbon signals at δ 104.2, 105.9, 95.7, 104.9 and 102.8 indicated that **1** was a hederagenin pentasaccharide. Sequential assignments of the signals of each monosaccharide, including their multiplet patterns and coupling constants, in the ^{1}HNMR spectrum of **1**, were established by the combined use of HOHAHA and

* Corresponding author. Fax: +20-2-5025281.
E-mail address: frmelek@link.net (F.R. Melek).

0031-9422/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2003.12.017

R_1 R_2 R_3

1 ß-D-Glcp-(1 2)-ß-D-GlcpA- OH - ß-D-Glcp-(6 1)-ß-D-Glcp-(4 1)- -L- Rhap

2 ß-D-Glcp-(1 2)-ß-D-GlcpA- H - ß-D-Glcp-(6 1)-ß-D-Glcp-(4 1)- -L- Rhap

3 ß-D-Glcp-(1 2)-ß-D-GlcpA- H -ß-D-Glcp-(6 1)-ß-D-Glcp-(4 1)- -L- Rhap
6
Ac

4 ß-D-Glcp-(1 3)-ß-D-Glcp-(1 3)- -L-Arap- H - ß-D-Glcp-(6 1)-ß-D-Glcp-(4 1)- -L-Rhap

5 ß-D-Glcp-(1 2)- ß-D-GlcpA- OH -H

6 ß-D-Galp-(1 2)-ß-D-GlcpA- OH -H

7 ß-D-Glcp-(1 3)- -L-Arap- H - ß-D-Glcp-(6 1)-ß-D-Glcp-(4 1)- -L- Rhap

8 ß-D-Glcp-(1 2)-ß-D-GlcpA- H -H

9 ß-D-Glcp-(1 2)-ß-D-GlcpA- H -ß-D-Glcp

10 ß-D-Glcp-(1 2)-ß-D-GlcpA- H - ß-D-Glcp-(6 1)-ß-D-Glcp

11 ß-D-Glcp-(1 2)-ß-D-GlcpA- OH - ß-D-Glcp

12 ß-D-Galp-(1 2)-ß-D-GlcpA- OH - ß-D-Glcp

13 HO_3S- H - ß-D-Glcp-(6 1)-ß-D-Glcp-(4 1)- -L- Rhap

14 ß-D-Glcp-(1 2)- -L-Arap- H - ß-D-Glcp-(6 1)-ß-D-Glcp-(4 1)- -L- Rhap

15 -L-Arap- H - ß-D-Glcp-(6 1)-ß-D-Glcp-(4 1)- -L- Rhap

16 -L-Arap- OH - ß-D-Glcp-(6 1)-ß-D-Glcp-(4 1)- -L- Rhap

^1H–^1H COSY experiments. The ^{13}CNMR resonances assigned by an HMQC experiment which correlated the assigned protons and their directly attached carbons, indicated that **1** contained a terminal α-rhamnopyranose (Rha), a terminal β-glucopyranose (Glc I), a 2-substituted glucuronopyranose (GlcA), a 6-substituted β-glucopyranose (GlcII) and a 4-substituted β-glucopyranose (Glc III). In the ^{13}CNMR spectrum, the observed glycosylation shifts in the hederagenin moiety indicated that **1** was a 3, 28 bisdesmoside. The structure of the sugar chain at C-3 was defined from the observed HMBC correlations between: GlcI H-1 (δ 5.40) and GlcA C-2 (δ 83.4), GlA H-1 (δ 5.18) and aglycone C-3 (δ 82.9). The structure of the oligosaccharide chain at C-28 was identified from the HMBC correlations between Glc III C-4 (δ 78.3) and Rha H-1 (δ 5.81), GlcII C-6 (δ 69.3) and Glc III H-1 (δ 4.97). A cross peak correlating GlcII H-1 (δ 6.21) and aglycone C-28 (δ 176.5) implying an ester linkage between the trisaccharide chain and the hederagenin moiety. The sequence and the linkage sites of the sugar moieties were further confirmed by the inter-residue NOEs observed in a NOE difference experiment. Thus the structure of saponin **1** was elucidated as 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl] hederagenin 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

Saponin **2** afforded, upon acid hydrolysis, oleanolic acid and the sugar components D-glucuronic acid, D-glucose and L-rhamnose were released. It exhibited $[M+Na]^+$ ion peak at m/z 1287 $(C_{60}H_{96}O_{28})^+$ and consistent with an oleanolic acid glycoside containing one glucuronic acid one rhamnose and three glucose units. Comparison of the NMR data (1D and 2D NMR) of **2** and those of **1**, indicated that the structures of the two saponins were identical except the replacement of hederagenin in **1** by oleanolic acid in **2**. Thus the structure of **2** was determined as 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl] oleanolic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

Saponin **3** revealed a $[M+Na]^+$ ion peak at m/z 1329 in the FAB-mass spectrum consistent with molecular formula $C_{62}H_{98}O_{29}$ and differed from the formula of saponin **2** by 42 mass units. The spectral features of **3** showed considerable structure similarity to **2**. This is confirmed from the result of acid hydrolysis which gave identical aglycone and sugars. Of the 62 carbons, 30 were assigned to the aglycone part, 30 to the oligo-saccharide moiety and the remaining two to an acetyl group. Spectral analysis (^{1}HNMR, ^{13}CNMR, HOHAHA, ^{1}H-^{1}H COSY, HMQC and HMBC) of **3** revealed similar data to those of **2** except additional signals due to acetyl

Table 1
^{1}HNMR spectral data for compounds **1**–**5** in pyridine-d_5

	1	**2**	**3**	**4**	**5**
Aglycone					
3	4.18	3.29 (*dd*,12.3,4.3)	3.28 (*dd*,12.3,3.7)	3.35 (*dd*, 12.0, 4.0)	4.18 (*dd*, 12.0, 4.4)
12	5.38	5.39 (*brt*,3.0)	5.38 (*br t*, 3.0)	5.42 (*br t*, 3.0)	5.44 (*br t*, 2.5)
18	3.15 (*dd*,13.5,3.1)	3.17 (*dd*,13.5,3.1)	3.16 (*dd*,14.1,3.1)	3.19 (*dd*, 14.0, 3.0)	3.28 (*dd*, 13.5, 3.1)
23	3.71 (*d*,11.1), 4.34	1.28	1.28	1.31	3.73 (*d*, 11.0), 4.35 (*d*, 11.0)
24	1.09	1.11	1.11	1.00	
25	0.94	0.86	0.86	0.90	
26	1.10	1.07	1.06	1.09	
27	1.18	1.25	1.24	1.26	
29	0.86	0.90	0.90	0.90	
30	0.88	0.90	0.92	0.90	
C-3-O-sugar					
	GlcA	*GlcA*	*GlcA*	*Ara*	*Glc A*
1	5.18 (*d*, 7.4)	4.99 (*d*, 7.4)	4.99 (*d*, 7.4)	4.71 (*d*, 6.8)	5.18 (*d*, 6.2)
2	4.27 (*t*, 8.0)	4.32	4.33	4.52	4.27 (*t*, 8.0)
3	4.30	4.37	4.38	4.20	4.28 (*t*, 8.0)
4	4.53	4.55	4.54	4.39	
5	4.47	4.60 (*d*, 8.5)	4.61	3.71 (*d*, 11.7)	
5′				4.18	
	Glc I	*Glc I*	*Glc I*	*Glc I*	*Glc*
1	5.40 (*d*, 8.0)	5.39 (*d*, 8.0)	5.39 (*d*, 8.0)	5.37 (*d*, 8.0)	5.40 (*d*, 8.0)
2	4.12 (*t*, 8.0)	4.11	4.11	4.04	4.12 (*d*, 8.0)
3	4.22	4.24 (*t*, 8.0)	4.24 (*t*, 8.0)	4.20	4.23 (*t*, 8.0)
4	4.29	4.30	4.31	4.09	4.29 (*t*, 8.0)
5	3.92	3.92	3.93	3.91	3.92 (*m*)
6	4.42	4.44	4.44	4.25	4.43 (*dd*, 12.1, 3.5)
6′	4.49 (*d*, 12.0)	4.48	4.48	4.43 (*dd*, 12.0, 2.0)	
				Glc II	
1				5.26 (*d*, 8.0)	
2				4.04	
3				4.22	
4				4.15	
5				4.00	
6				4.26	
6′				4.52	
C-28-O-sugar					
	Glc II	*Glc II*	*Glc II*	*Glc III*	
1	6.21 (*d*, 8.0)	6.21 (*d*, 8.0)	6.20 (*d*, 8.0)	6.21 (*d*, 8.0)	
2	4.10	4.11	4.10	4.11	
3	4.18	4.19 (*t*, 8.0)	4.18 (*t*, 8.0)	4.19	
4	4.27 (*t*, 8.0)	4.28	4.24 (*t*, 8.0)	4.28	
5	4.10	4.10	4.11	4.10	
6	4.31	4.31	4.33	4.31	
6′	4.64	4.65	4.66	4.65	
	Glc III	*Glc III*	*Glc III*	*Glc IV*	
1	4.97 (*d*, 8.0)	4.97 (*d*, 8.0)	4.98 (*d*, 8.0)	4.97 (*d*, 8.0)	
2	3.92	3.92	3.93	3.92	
3	4.12	4.11	4.10	4.13	
4	4.37	4.37	4.07	4.36	
5	3.66 (*m*)	3.66 (*m*)	3.82 (*m*)	3.65 (*m*)	
6	4.07	4.08	4.52 (*dd*, 12.0, 2.5)	4.08	
6′	4.20	4.19 (*d*, 12.0)	4.63	4.20 (*brd*,12.0)	
Ac			1.92		
	Rha	*Rha*	*Rha*	*Rha*	
1	5.81 (*brs*)	5.81 (*brs*)	5.81 (*brs*)	5.81 (*brs*)	
2	4.65 (*d*, 2.5)	4.64	4.60	4.64	
3	4.52 (*dd*, 9.0, 3.5)	4.52 (*dd*, 9.0, 3.0)	4.47	4.52	
4	4.30	4.31	4.29	4.28	
5	4.91 (*dq*, 9.0, 6.0)	4.91 (*dq*, 9.0, 6.0)	4.82 (*dq*, 9.0, 6.0)	4.90 (*dq*, 9.0, 6.0)	
6	1.68 (*d*, 6.0)	1.69 (*d*, 6.0)	1.69 (*d*, 6.0)	1.68 (*d*, 6.0)	

Values in parantheses are ^{1}H–^{1}H splittings in cases where these are clearly resolved. GlcA = β-D-glucuronopyranose; Glc = β-D-glucopyranose; Ara = α-L-arabinopyranose; Rha = α-L-rhamnopyranose.

Table 2
^{13}C NMR spectral data for compounds **1**–**5** in pyridine-d_5

	1	2	3	4	5
Aglycone					
3	82.9	89.4	89.3	88.9	82.8
12	123.0	122.9	123.0	123.0	122.6
13	144.2	144.2	144.1	144.2	144.9
23	65.0	28.2	28.2	28.2	65.0
24	13.4	16.8	16.8	17.0	13.5
25	16.2	15.6	15.6	15.7	16.0
26	17.6	17.6	17.6	17.5	17.4
27	26.1	26.1	26.1	26.1	26.3
28	176.5	176.5	176.5	176.6	180.2
29	33.1	33.2	33.2	33.2	33.3
30	23.7	23.7	23.8	23.8	23.8
Sugar moiety at C-3					
	Glc A	*Glc A*	*Glc A*	*Ara*	*Glc A*
1	104.2	105.4	105.4	107.4	104.3
2	83.4	82.9	82.9	72.0	83.5
3	77.9	77.9	77.8	83.9	77.9
4	73.0	73.2	73.2	69.4	73.0
5	77.4	77.5	77.4	67.1	77.4
6	172.6	172.5	n.d.		n.d.
	Glc I	*Glc I*	*Glc I*	*Glc I*	*Glc*
1	105.9	106.0	106.0	105.7	106.0
2	76.9	77.2	77.1	74.4	76.9
3	78.1	78.0	78.0	88.4	78.1
4	71.6	71.9	71.9	69.8	71.5
5	78.4	78.3	78.1	78.3	78.3
6	62.7	62.9	62.9	62.4	62.8
				Glc II	
1				105.9	
2				75.4	
3				78.2	
4				71.7	
5				78.7	
6				62.6	
28-O-sugar					
	Glc II	*Glc II*	*Glc II*	*Glc III*	
1	95.7	95.7	95.6	95.7	
2	73.9	73.9	73.9	73.9	
3	78.8	78.8	78.8	78.8	
4	71.0	71.0	71.1	71.0	
5	78.1	78.0	78.2	78.1	
6	69.3	69.3	69.5	69.4	
	Glc III	*Glc III*	*Glc III*	*Glc IV*	
1	104.9	104.9	104.8	104.9	
2	75.4	75.4	75.1	75.6	
3	76.6	76.6	76.4	76.6	
4	78.3	78.5	79.3	78.5	
5	77.2	77.1	73.8	77.2	
6	61.4	61.4	63.7	61.4	
Ac			20.6		
C=O			170.6		
	Rha	*Rha*	*Rha*	*Rha*	
1	102.8	102.8	103.0	102.8	
2	72.6	72.6	72.4	72.6	
3	72.8	72.8	72.7	72.8	
4	74.0	74.0	73.9	74.0	
5	70.3	70.4	70.7	70.4	
6	18.5	18.5	18.5	18.5	

group [δ_H 1.92 (s), δ_c 20.6, 170.6] located at the C-6 position of Glc III. This conclusion was deduced from the lower field position of C-6 signal (δ 63.7) in Glc III and higher field position of C-5 signal (δ 73.8) with reference to those of **2** as well as by the HMBC correlation between Glc III H-6 (δ 4.52) and the acetyl carbonyl carbon (δ 170.6). Therefore saponin **3** was considered to be 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl] oleanolic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-6-*O*-acetyl glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

Saponin **4,** molecular formula $C_{65}H_{106}O_{31}$ showed $[M+Na]^+$ ion peak at m/z 1405. Its spectral features indicated that **4** possessed the same aglycone as that of **2** and **3** but differed in terms of the glycosidic structure. The ^{1}HNMR spectrum of **4** showed six anomeric proton signals at δ 4.71 (*d*, $J=6.8$ Hz), 5.37 (*d*, $J=8.0$ Hz), 5.26 (*d*, $J=8.0$ Hz), 6.21 (*d*, $J=8.0$ Hz), 4.97 (*d*, $J=8.0$ Hz), 5.81 (brs) and one methyl doublets at δ 1.68 (*d*, $J=6.0$ Hz). Analysis of 1D and 2D NMR revealed the presence of a terminal β-glucopyranose (Glc II), a terminal α-rhamnopyranose, a 3-substituted α-arabinopyranose (Ara), a 3-substituted β-glucopyranose (Glc I), a 6-substituted β-glucopyranose and a 4-substituted β-glucopyranose. This is confirmed by the result of acid hydrolysis of **4** which yielded oleanolic acid and the sugar components L-arabinose, D-glucose and L-rhamnose. The ^{13}C NMR spectrum of **4** indicated that the sugars were present in two saccharide units, one attached to C-3 of the oleanolic acid moiety and the other at C-28. The structure of the sugar chain at C-3 was defined by the HMBC correlations between aglycone C-3 (δ 88.9) and Ara H-1 (δ 4.71), Ara C-3 (δ 83.9) and Glc I H-1 (δ 5.37), Glc I C-3 (δ 88.4) and GlcII H-1 (δ 5.26). The remaining HMBC correlations were similar to those observed for **1**–**3**, indicating the same trisaccharide chain linked to carboxylic group at C-28. The same conclusion was deduced from the observed NOEs across the glycosidic linkages. Therefore **4** was characterised as 3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl] oleanolic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

Saponin **5** and **6** were obtained as inseperable mixture. Acid hydrolysis of the mixture provided D-glucuronic acid, D-glucose and D-galactose together with an aglycone identified as hederagenin. The FAB-mass spectrum of **5** and **6** showed $[M+Na]^+$ ion peak at m/z 833 compatible with the molecular formula $C_{42}H_{66}O_{15}$ and corresponding to hederagenin containing one uronic acid and one hexose units. The ^{13}C NMR of **5** and **6** showed one set of resonances due to hederagenin moiety except C-3 which appeared as two resonances with close δ values. The monodesmosidic nature of **5** and **6** was demonstrated from the δ value of C-28 at 180 ppm. Analysis of the sugar NMR signals of **5** and **6** and comparison with the corresponding ones in the related and previously

characterized glucuronoids **11**, 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl] hederagenin 28-*O*-β-D-glucopyranosyl ester (Ming-An and Dian-Peng, 2002) and udosaponin F or ilexoside XLIX **12**, 3-*O*-[β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl] hederagenin 28-*O*-β-D-glucopyranosyl ester (Kawai et al., 1989; Amimoto et al., 1993) occurring in the studied plant, allowed us to identify the mixture (1:1 from NMR intensity). Saponin **5** is new natural product 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl] hederagenin and saponin **6** is known isomer udosaponin E, 3-*O*-[β-D-galactopyranosyl-(1→2)-β-D glucuronopyranosyl] hederagenin (Kawai et al., 1989).

The known saponins begoniifolide **7** (Liao et al., 2000), zingibroside R_1 **8** (Yang et al., 1984), chikusetsusaponin V **9** (Kondo et al., 1971), hemsloside G_2 **10** (Kasai et al., 1990), hederasaponin F **13** (Elias et al., 1991), ciwujianoside A_1 **14** (Shao et al., 1989), ciwujianoside C_3 **15** (Shao et al., 1988) and cauloside D **16** (Strigina et. al.,1975; Kizu et al., 1985), were identified by analysis of their spectral data and by comparison with literature values.

3. Experimental

3.1. General

Optical rotations were measured with a Jasco DIP-1000 digital polarimeter. MS were measured on Jeol JMS-SX 102 mass spectrometer. NMR spectra were recorded on Jeol GSX-500 FT NMR spectrometer. Chemical Shifts are given on the δ scale with TMS as internal standard. HPLC was performed on Jasco system 800 instrument. GC analysis was carried out on a Hitachi G-3000 gas chromatograph.

3.2. Plant material

Leaves and stems of *Meryta lanceolata* Hort was collected 60 km west of Alexandria, Egypt in 1998 and identified as mentioned earlier (Melek et al., 2003). The specimen is deposited in the Chemistry of Natural Products Department, NRC.

3.3. Extraction and isolation

The extraction of dried leaves and stems of *M. lanceolata* as well as the isolation using column chromatography and repeated HPLC [acetonitrile–water + 0.05 TFA] were performed as described in our previous publication (Melek et al., 2003). From the more polar saponin fraction (0.5 g), **1** (9 mg), **2** (20 mg), **3** (9 mg), **5** and **6** (7 mg) **8** (12 mg), **9** (20 mg), **10** (7 mg), **11** and **12** (21 mg), **13** (23 mg) were obtained. The less polar fraction (0.5 g) afforded **4** (10 mg), **7** (17 mg), **14** (31 mg), **15** (89 mg) and **16** (21 mg).

3.4. Saponin (*1*)

Amorphous powder $[\alpha]_D^{23} = -18.2^\circ$ ($c = 0.91$, MeOH); FAB-MS *(m/z):* 1303 $[C_{60}H_{96}O_{29} + Na]^+$; ^1H and ^{13}C NMR: see Tables 1 and 2.

3.5. Saponin (*2*)

Amorphous powder, $[\alpha]_D^{23} = -10.5^\circ$ ($c = 2.33$, MeOH); FAB-MS *(m/z):* 1287 $[C_{60}H_{96}O_{28} + Na]^+$, ^1H and ^{13}C NMR: see Tables 1 and 2.

3.6. Saponin (*3*)

Amorphous powder, $[\alpha]_D^{23} = -16.7^\circ$ ($c = 0.88$, MeOH); FAB-MS *(m/z):* 1329 $[C_{62}H_{98}O_{29} + Na]^+$; ^1H and ^{13}C NMR: see Tables 1 and 2.

3.7. Saponin (*4*)

Amorphous powder: $[\alpha]_D^{23} = +3.3^\circ$ ($c = 1.11$, MeOH); FAB-MS *(m/z):* 1405 $[C_{65}H_{106}O_{31} + Na]^+$; ^1H and ^{13}C NMR: see Tables 1 and 2.

3.8. Saponin (*5*)

FAB-MS *(m/z):* 833 $[C_{42}H_{66}O_{15} + Na]^+$; ^1H and ^{13}C NMR: see Tables 1 and 2.

3.9. General method for acid hydrolysis (Hara et al., 1986)

Each saponin (2.0 mg) dissolved in dioxane (100 μl) and 2 M HCl (100 μl) was heated at 100 °C for 1 h. The reaction mixture was diluted with water and extracted twice with EtOAc. The aglycone from the EtOAc layer was detected by HPLC [column YMC R&D ODS; 4.6 mm × 25 cm, solvent; MeOH–H_2O (9:1) + 0.05% TFA; flow rate; 1 ml/min, detection; UV 205 nm], hederagenin (t_R, 7.1 min); oleanolic acid (t_R 11.0 min). The water layer was passed through an amberlite IRA-60E column (6 × 60 mm) and the eluate was concentrated. The residue was compared with standard sugars by PC [*n*-BuOH–HOAc–H_2O (4:1:5)] as well as by preparing and analysing their thiazolidine derivatives as reported earlier (Melek et al., 2003). The results showed that arabinose and rhamnose from the new compounds were in the L-form while glucuronic acid and glucose were in the D-form.

References

Amimoto, K., Yoshikawa, K., Arihara, S., 1993. Triterpenoid saponins of aquifoliaceous plants. XII-Ilexosides XLVI-LI. From the leaves of *Ilex rotunda* Thunb. Chemical and Pharmaceutical Bulletin 41, 77–80.

Elias, R., Diaz Lanza, A.M., Vidal-Ollivier, E., Balansard, G., Faure, R., Babadjamian, A., 1991. Triterpenoid saponins from the leaves of *Hedera helix*. Journal of Natural Products 54, 98–103.

Hara, S., Okabe, H., Mihashi, K., 1986. Separation of aldose enantiomers by gas–liquid chromatography. Chemical and Pharmaceutical Bulletin 34, 1843–1844.

Kasai, R., Tanaka, T., Nie, R., Miyakoshi, M., Zhou, J., Tanaka, O., 1990. Saponins from chinese medicinal plant. *Hemsleya graciliflora*. Chemical and Pharmaceutical Bulletin 38, 1320–1322.

Kawai, H., Nishida, M., Tashiro, Y., Kuroyanagi, M., Ueno, A., Satake, M., 1989. Studies on the structures of udosaponins A, B, C, D, E and F from *Aralia cordata* THUNB. Chemical and Pharmaceutical Bulletin 37, 2318–2321.

Kizu, H., Kilayama, S., Nakatani, F., Tomimori, T., Namba, T., 1985. Studies on Nepalese crude drugs. III. On the saponins of *Hedera nepalensis* K. Chemical and Pharmaceutical Bulletin 33, 3324–3329.

Kondo, N., Marumoto, Y., Shoji, J., 1971. Studies on the constituents of *Panacis japonici* rhizomes. Chemical and Pharmaceutical Bulletin 19, 1103–1107.

Liao, X., Li, B., Ding, L., Pan, Y., Chen, Y., 2000. New triterpenoid saponins from *Anemone begoniifolia*. Yaoxue Xuebao 35, 821–825.

Melek, F.R., Miyase, T., Abdel-Khalik, S.M., Hetta, M.H., Mahmoud, I.I., 2002. Triterpenoid saponins from *Oreopanax guatemalensis*. Phytochemistry 60, 185–195.

Melek, F.R., Miyase, T., Ghaly, N.S., 2003. Triterpenoid saponins from *Meryta lanceolata*. Phytochemistry 62, 557–560.

Ming-An, O., Dian-Peng, L., 2002. Four new triterpenoid saponins from *Ilex godajam*. Journal of Asian Natural Products Research 4, 25–31.

Shao, C., Kasai, R., Xu, J.D., Tanaka, O., 1988. Saponins from leaves of *Acanthopanax senticosus* HARMS. Chemical and Pharmaceutical Bulletin 36, 601–608.

Shao, C., Kasai, R., Xu, J.D., Tanaka, O., 1989. Saponins from leaves of *Acanthopanax senticosus* HARMS. Chemical and Pharmaceutical Bulletin 37, 42–45.

Strigina, L.I., Chetyrina, N.S., Isakov, V.V., Elkin, Yu.N., Dzizenko, A.K., Elyakov, G.B., 1975. Cauloside D, a new triterpenoid glycoside from *Caulophyllum robustum*. Identification of cauloside A. Phytochemistry 14, 1583–1586.

Yang, C., Jiang, Z., Wu, M., Zhou, J., Tanaka, O., 1984. Studies on saponins of rhizomes of *Panax zingiberensis*. Yaoxue Xuebao 19, 232–236.