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Triterpenoid saponins from *Schefflera arboricola*

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Abstract

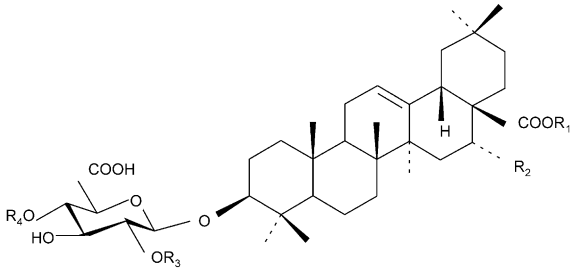
Nine triterpenoid saponins were isolated from the leaves and stems of *Schefflera arboricola*. The saponins were characterised, on the basis of chemical and spectral evidence, as 3-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucuronopyranosyl] oleanolic acid, 3-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucuronopyranosyl] echinocystic acid, 3-*O*-[β-D-apiofuranosyl-(1→4)-β-D-glucuronopyranosyl] oleanolic acid 28-*O*-β-D-glucopyranosyl ester, 3-*O*-α-L-ramnopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→2)-] β-D-glucuronopyranosyl oleanolic acid, 3-*O*-α-L-rhamnopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→2)-] β-D-glucuronopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester, 3-*O*-α-L-rhamnopyranosyl-(1→4)-[β-D-galactopyranosyl-(1→2)-] β-D-glucuronopyranosyl oleanolic acid, 3-*O*-α-L-rhamnopyranosyl-(1→4)-[β-D-galactopyranosyl-(1→2)-] β-D-glucuronopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester, 3-*O*-β-D-apiofuranosyl-(1→4)-[α-L-arabinopyranosyl-(1→2)-] β-D-glucuronopyranosyl oleanolic acid and 3-*O*-β-D-apiofuranosyl-(1→4)-[α-L-arabinopyranosyl-(1→2)-] β-D-glucuronopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester.

Keywords: *Schefflera arboricola*; Araliaceae; Triterpenoid saponin

1. Introduction

Schefflera arboricola (Hayata) Merr. is an ornamental plant which usually grows up to 2–3 m in height. Previous pharmacological studies (Liao, 1986) showed that the ethanolic leaf extract of *S. arboricola* exhibited sedative, hypnotic, analgesic, anticonvulsant and smooth muscle relaxant effects. Previous phytochemical investigation on a mixture of leaves and stems of *S. arboricola* has led to the isolation of the two oleanolic acid glucuronoides cynarasaponin H and olaxoside (Abdel Khalik, 2001).

As a part of our continuing studies on saponins from plant species grown in Egypt (Miyase et al., 1996a; Abdel Khalik et al., 2000, 2001; Melek et al., 2000) we report here the isolation and structure determination of nine new triterpenoidal saponins (**1**–**9**) from *S. arboricola*.



	R_1	R_2	R_3	R_4
1	H	H	H	Rha
2	H	OH	H	Rha
3	Glc	H	H	Api
4	H	H	Ara	Rha
5	Glc	H	Ara	Rha
6	H	H	Gal	Rha
7	Glc	H	Gal	Rha
8	H	H	Ara	Api
9	Glc	H	Ara	Api

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2. Results and discussion

The concentrated methanolic extract of a mixture of leaves and stems of *S. arboricola* was diluted with acetone to precipitate the crude saponins mixture. Chromatography of the mixture using silica gel, Sephadex LH-20 and the polymer gel Mitsubishi Diaion HP-20 followed by HPLC, afforded nine new saponins. Their structures were established on the basis of chemical hydrolysis and NMR data (Tables 1–3).

Saponin **1** had a molecular formula $C_{42}H_{66}O_{13}$ as determined from ^{13}C NMR data and a quasi-molecular ion peak $[M+Na]^+$ at m/z 801 in the positive-ion FAB-mass spectrum. The ^{13}C NMR spectrum of **1** exhibited signals, due to a triterpene moiety, at δ-values similar to those of oleanolic acid 3-*O*-glycosides. Acid hydrolysis of **1** yielded oleanolic acid and the released monosaccharide units were identified as D-glucuronic acid (GlcA) detected by paper chromatography and L-rhamnose (Rha) identified by GC after being converted to its thiazolidine derivative (Hara et al., 1986). The disaccharide nature of **1** was deduced from the presence of two anomeric proton signals at δ 4.97 (*d*, $J=7.6$ Hz) and δ 5.86 (*brs*) in the ^1H NMR spectrum and assigned to β-glucuronic acid and α-rhamnose units, respectively. A ^1H–^1H COSY experiment allowed analysis of their spin systems and assignments of their proton resonances. The assignment of their corresponding carbons, made by a HMQC spectrum, indicated that rhamnose was a terminal unit. The Rha-(1→4)-GlcA structure of the disaccharide moiety at C-3 of the oleanolic acid residue was deduced from the HMBC correlations between C-3 (δ 89.3) and H-1 (δ 4.97) of GlcA unit and between C-4 (δ 80.4) of the GlcA unit and H-1 (δ 5.86) of the Rha unit. The relative stereochemistry of each monosaccharide was determined as β-D-glucuronopyranose and α-L-rhamnopyranose based on the characteristic $J_{H\text{-}1,H\text{-}2}$ coupling constants and ^{13}C NMR data. Therefore, **1** was assigned the structure of 3-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucuronopyranosyl] oleanolic acid. Compound **1** was previously obtained after partial hydrolysis of the saponins olaxoside, previously reported from a number of *Olax* species (Forgacs and Provost, 1981) and narcissiflorinine, isolated from *Anemone*

Table 1
^1H NMR spectral data of saponins **1**–**9** in C_5D_5N

	1	**2**	**3**	**4**	5
Triterpene moiety					
3	3.36 (*dd*, 11.4, 4.5)	3.39 (*dd*, 11.4, 4.5)	3.35 (*dd*, 11.4, 4.5)	3.30 (*dd*, 11.4, 4.4)	3.29 (*dd*, 11.4, 4.5)
12	5.45 (*t*, 3.0)	5.62 (*t*, 3.5)	5.42 (*t*, 3.5)	5.45 (*t*, 3.0)	5.41 (*t*, 3.5)
16(eq)		5.24 (*brs*)			
18	3.28 (*dd*, 13.9, 3.3)	3.61 (*dd*, 14.4, 3.4)	3.19 (*dd*, 14.0, 3.3)	3.27 (*dd*, 14.0, 3.4)	3.18 (*dd*, 14.0, 3.5)
3-0-Sugar GlcA					
1	4.97 (*d*, 7.6)	4.99 (*d*, 7.8)	4.98 (*d*, 7.6)	4.98 (*d*, 7.8)	4.98 (*d*, 7.4)
2	4.06 (*t*, 8.2)	4.08 (*t*, 8.5)	4.08 (*t*, 8.0)	4.18	4.19 (*t*, 8.6)
3	4.25 (*t*, 8.2)	4.26 (*t*, 8.5)		4.31	4.32
4	4.59	4.61	4.58 (*t*, 8.7)	4.63 (*t*, 9.1)	4.63 (*t*, 9.2)
5	4.60	4.62	4.66 (*d*, 9.0)	4.54	4.54
At GlcA C-4 Rha					
1	5.86 (*brs*)	5.88 (*brs*)		5.85 (*brs*)	5.86 (*brs*)
2	4.75 (*brs*)	4.77 (*dd*,2.5,1.5)		4.73 (*brs*)	4.73 (*dd*, 3.1, 1.5)
3	4.56 (*dd*, 9.0, 3.2)	4.57 (*dd*, 9.5, 3.3)		4.52	4.54
4	4.27 (*t*, 9.0)	4.29 (*t*, 9.5)		4.26 (*t*, 9.1)	4.27
5	4.97	4.98		4.94 (*dq*, 9.1, 6.5)	4.95
6	1.67 (*d*, 6.3)	1.69 (*d*, 6.5)		1.65 (*d*, 6.5)	1.65 (*d*, 6.1)
Api					
1			6.09 (*d*, 2.5)		
2			4.77 (*d*, 2.5)		
3					
4			4.81 (*d*, 10.0)		
5			4.16 (*brs*)		
At GlcA C-2 Ara					
1				5.22 (*d*, 6.5)	5.23 (*d*, 6.2)
2				4.55	4.54 (*t*, 6.8)
3				4.19	4.22
4				4.31	4.32
5				3.78 (*d*, 11.7)	3.78 (*dd*, 12.3, 1.8)
5′				4.38 (*dd*, 11.7, 3.3)	4.38

(*continued on next page*)

Table 1 (*continued*)

	1	**2**	**3**	**4**	5
28-0-Glc					
1			6.30 (*d*, 7.8)		6.30 (*d*, 8.0)
2			4.18 (*t*, 8.0)		4.19 (*t*, 8.6)
3			4.26 (*t*, 8.0)		4.26
4			4.33 (*t*, 8.0)		4.34
5			4.01 (*m*)		4.01 (*m*)
6			4.38 (*dd*, 12.4, 3.0)		4.38
6′			4.45 (*dd*, 12.4, 4.3)		4.45 (*dd*, 12.0, 2.7)
	6	**7**	**8**	**9**	
Triterpene moiety					
3	3.28	3.26 (*dd*, 11.5, 4.5)	3.28	3.28 (*dd*, 11.5, 4.4)	
12	5.44 (*t*, 3.0)	5.40 (*t*, 3.0)	5.4 5 (*t*, 3.0)	5.41 (*t*, 3.5)	
18	3.28	3.18 (*dd*, 14.0, 3.4)	3.29	3.18 (*dd*, 14.0, 3.4)	
3-O-Sugar GlcA					
1	4.96 (*d*, 7.4)	4.96 (*d*, 6.8)	4.97 (*d*, 7.6)	4.97 (*d*, 7.4)	
2	4.29	4.28	4.18	4.18	
3	4.32 (*t*, 8.0)	4.32 (*t*, 8.0)	4.32	4.31	
4	4.61 (*t*, 8.6)	4.62 (*t*, 9.1)	4.58	4.58	
5	4.53	4.52	4.58	4.58	
At GlcA-C-4					
Rha					
1	5.85 (*brs*)	5.85 (*brs*)			
2	4.74 (*dd*, 2.5, 1.8)	4.72 (*d*, 2.4)			
3	4.52 (*dd*, 8.0, 3.5)	4.51			
4	4.27 (*t*, 9.2)	4.26 (*t*, 9.0)			
5	4.95	4.95			
6	1.64 (*d*, 6.2)	1.65 (*d*, 6.0)			
Api					
1			6.05 (*d*, 1.2)	6.05 (*d*, 2.2)	
2			4.75 (*brs*)	4.74 (*d*, 2.2)	
3					
4			4.76 (*d*, 10.0)	4.76 (*d*, 10.0)	
5			4.14 (*brs*)	4.14 (*brs*)	
At GlcA-C-2 Ara					
1			5.19	5.19 (*d*, 6.2)	
2			4.55 (*t*, 7.0)	4.53 (*dd*, 8.0, 6.2)	
3			4.19	4.21	
4			4.31	4.31	
5			3.78 (*d*, 12.3)	3.78 (*dd*, 12.3, 1.2)	
5′			4.38 (*dd*, 12.3, 2.4)	4.38	
Gal					
1	5.24 (*d*, 7.6)	5.24 (*d*, 7.8)			
2	4.54	4.54			
3	4.14 (*dd*, 9.2, 3.1)	4.15			
4	4.66 (*d*, 3.1)	4.66 (*d*, 3.1)			
5	4.03 (*dd*, 6.2, 5.5)	4.03			
6	4.38 (*dd*, 11.1, 6.2)	4.38			
6′	4.56	4.57			
28-0-Glc					
1		6.30 (*d*, 7.8)		6.30 (*d*, 8.0)	
2		4.18		4.18	
3		4.26 (*t*, 9.0)		4.26 (*t*, 8.0)	
4		4.32 (*t*, 8.0)		4.34 (*t*,8.6)	
5		4.02		4.01 (*m*)	
6		4.37		4.38	
6′		4.44 (*dd*, 12.4, 2.6)		4.45 (*dd*, 12.3, 2.5)	

Values in parentheses are ^{1}H–^{1}H splittings in cases where these are clearly resolved. GlcA = β-D-glucuronopyranose Gal = β-D-galactopyranose; Ara = α-Larabinopyranose; Api = β-D-apiofuranose; Rha = α-D-rhamnopyranose Glc = β-D-glucopyranose.

Table 2
^{13}C NMR data of the triterpene moieties of saponins **1**–**3** in C_5D_5N

C	1	2	3
1	38.7	38.8	38.7
2	26.6	26.7	26.6
3	89.3	89.2	89.2
4	39.6	39.6	39.5
5	55.9	55.9	55.8
6	18.6	18.6	18.5
7	33.4	33.6	33.2
8	39.8	40.0	40.0
9	48.1	47.2	48.1
10	37.1	37.1	37.0
11	23.8	23.9	23.8
12	122.6	122.5	122.9
13	144.9	145.2	144.2
14	42.3	42.2	42.2
15	28.4	36.3	28.3
16	23.9	74.8	23.5
17	46.8	49.0	47.1
18	42.1	41.6	41.8
19	46.6	47.4	46.3
20	31.0	31.1	30.8
21	34.4	36.2	34.1
22	33.3	32.9	32.6
23	28.3	28.3	28.2
24	17.0	17.0	17.0
25	15.5	15.6	15.5
26	17.5	17.5	17.5
27	26.3	27.3	26.2
28	180.2	180.0	176.4
29	33.3	33.4	33.2
30	23.9	24.8	23.7

narcissiflora (Masood et al., 1981). This is the first reported occurrence of **1** as a natural product.

The molecular formula of saponin **2**, C_{42} H_{66} O_{14}, was assigned by the presence of a quasi-molecular ion peak at *m/z* 817 $[M+Na]^+$ in the positive-ion FAB-mass spectrum and ^{13}C NMR data. Comparison of the ^{13}C NMR spectral data of **2** with those reported for various echinocystic acid glycosides (Nagao et al., 1993), suggested **2** to be an echinocystic acid 3-*O*glycoside. Acid hydrolysis of **2** afforded in addition to echinocystic acid, the sugar components D-glucuronic acid and L-rhamnose. The ^1H and ^{13}C NMR signals due to the sugar units of **2** were almost identical to those of **1**. Therefore, **2** was concluded to be 3-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucuronopyranosyl] echinocystic acid.

The molecular formula of **3**, C_{47} H_{74} $O_{18,}$ was assigned based on the presence of a quasi-molecular ion peak at *m/z* 949 $[M+Na]^+$ in the FAB-mass spectrum. Acid hydrolysis of **3** yielded oleanolic acid and the sugar components D-glucuronic acid, D-glucose (Glc) and D-apiose (Api). The ^1H NMR spectrum of **3** showed the presence of three anomeric proton signals at δ 4.98 (*d*, *J* = 7.4 Hz), 6.09 (*d*, *J* = 2.5 Hz) and 6.30 (*d*, *J* = 7.8 Hz) assignable to D-glucuronic acid, D-apiose and D-glucose units respectively. The bidesmosidic nature of **3** was

Table 3
^{13}C NMR data of the sugar moities of saponins **1**–**9** in C_5D_5N

	1	2	3	4	5	6	7	8	9
Triterpene moiety									
3	89.3	89.2	89.2	89.3	89.3	89.4	89.4	89.3	89.3
28	180.2	180.0	176.4	180.2	176.4	180.2	176.5	180.1	176.4
3-O-Sugar GlcA									
1	107.0	107.0	107.0	105.2	105.1	105.1	105.1	105.2	105.2
2	75.8	75.8	75.4	83.4	83.4	83.8	83.8	82.8	82.9
3	76.4	76.4	76.2	76.0	76.0	76.1	76.1	75.5	75.5
4	80.4	80.4	80.0	79.3	79.3	79.2	79.2	79.2	79.1
5	76.4	76.4	76.2	76.1	76.1	76.1	76.1	75.7	75.9
6	172.6	172.6	172.1	172.4	172.3	172.4	172.4	n.d.	171.9
At GlcA C-4 Rha									
1	102.8	102.8		102.4	102.4	102.4	102.4		
2	72.6	72.6		72.5	72.5	72.5	72.5		
3	72.8	72.8		72.8	72.7	72.7	72.7		
4	74.0	74.0		74.0	74.0	74.0	74.0		
5	70.4	70.4		70.3	70.3	70.3	70.3		
6	18.6	18.6		18.5	18.5	18.5	18.5		
Api									
			110.8					110.6	110.6
			77.8					77.8	77.8
			80.4					80.4	80.4
			75.4					75.7	75.7
			65.5					65.6	65.6
At GlcA C-2 Ara									
1				106.4	106.4			106.4	106.4
2				73.7	73.6			73.7	73.7
3				74.3	74.2			74.2	74.2
4				69.1	69.1			69.1	69.1
5				67.0	66.9			66.9	66.9
Gal									
1						106.9	106.9		
2						74.6	74.6		
3						75.0	75.0		
4						69.7	69.7		
5						77.0	77.0		
6						61.5	61.5		
28-0-Glc									
1			95.8		95.8		95.8		95.8
2			74.2		74.2		74.2		74.2
3			78.9		79.0		79.0		79.0
4			71.3		71.3		71.3		71.3
5			79.3		79.3		79.3		79.3
6			62.3		62.4		62.4		62.4

n.d. = not detected. GlcA = β-D-glucuronopyranose; Rha = α-L-rhamnopyranose; Api = β D-apiofuranose; Gal = β-D-galactopyranose; Ara = α-L-arabinopyranose; Glc = β-D-glucopyranose.

deduced from the δ-values of signals due to C-3 (89.2 ppm) and C-28 (176.4 ppm) of the oleanolic acid moiety. The signals at δ 95.8, 74.2, 78.9, 71.3, 79.3 and 62.3 in the ^{13}C NMR spectrum were typical for a β-D-glucopyranose unit esterifying the oleanolic acid COOH group. The structure of the disaccharide moiety at C-3 position, was established as Api(1→4)-GlcA from the HMBC correlations between apiose H-1 (δ 6.09) and GlcA C-4 (δ 80.0). The anomeric configuration of

glucose was determined to be β from the *J* value of the anomeric proton signal of glucose. The anomeric configuration of apiose was determined to be β by the comparison of the ^{13}C NMR data for **2** with those for methyl α- and β-D-apiofuranosides [^{13}C NMR data for methyl β-D-apiofuranoside: 111.5 (C-1), 77.7 (C-2), 80.3 (C-3), 74.9 (C-4), 65.5 (C-5), 55.5 (1-OCH_3); Kitagawa et al., 1989]. Therefore, **3** was assigned the structure of 3-*O*-[β-D-apiofuranosyl-(1→4)-β-D-glucuronopyranosyl] oleanolic acid 28-*O*-β-D-glucopyranosyl ester.

Saponin **4**, C_{47} H_{74} O_{17}, exhibited $[M+Na]^+$ at *m/z* 933. It afforded oleanolic acid, D-glucuronic acid, L-arabinose (Ara) and L-rhamnose upon acid hydrolysis. The sugar moieties of **4** were characterised by analysis of the NMR data obtained from the combined use of 2D NMR experiments (^1H–^1H COSY, HMQC and HMBC), as β-D-glucuronopyranose (H-1; δ 4.98), α-L-arabinopyranose (H-1; δ 5.22) and α-L-rhamnopyranose (H-1; δ 5.85). The δ-values of signals due to C-28 (180.2 ppm) and C-3 (89.3 ppm) of the triterpene moiety, indicated that **4** was an oleanolic acid 3-*O*-glycoside. The branched nature of the trisaccharide moiety at the C-3 position of the oleanolic acid moiety was revealed from the inter-residue NOEs, in a NOE difference spectrum between H-3 of the oleanolic acid residue and GlcA H-1, between GlcA H-2 and Ara H-1 and between GlcA H-4 and Rha H-1. The HMBC correlations verified the sugar linkages. Therefore the structure of **4** was elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→2)-] β-D-glucuronopyranosyl oleanolic acid.

Saponin **5**, C_{53} H_{84} O_{22}, exhibited $[M+Na]^+$ at *m/z* 1095 in its FAB-mass spectrum, 162 mass units more than that of **4**, suggesting the presence of one more hexose unit. This unit was identified as D-glucose, after being detected by GC analysis of the acid hydrolysate of **5**, together with D-glucuronic acid, L-arabinose and L-rhamnose. The triterpene moiety of **5** was identified as 3, 28-di-*O*-substituted oleanolic acid from NMR data. The assigned NMR signals due to the sugar units of **5** (HOHAHA, ^1H–^1H COSY, HMQC, HMBC spectra) were almost identical to those of **4** except a set of additional signals in **5** due to 28-*O*-β-D-glucopyranose unit as described for saponin **3**. This observation suggested an identical trisaccharide chain at the C-3 position of the oleanolic acid moiety for **4** and **5**. The observed NOEs interaction and the HMBC correlations verified the linkages. Thus the structure of **5** was established as 3-*O*-α-L-rhamnopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→2)-] β-D-glucuronopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester.

Saponin **6** (C_{48} H_{76} O_{18}), on acid hydrolysis, afforded in addition to oleanolic acid, the sugar components D-glucuronic acid, D-galactose (Gal) and L-rhamnose. The sugar moieties of **6** were characterised by analysis of NMR data obtained from the combined use of 2D NMR spectra (HOHAHA, ^1H–^1H COSY, HMQC and HMBC). The data allowed identification of a β-D-glucuronopyranose unit with anomeric proton at δ 4.96 as well as β-D-galactopyranose unit with anomeric proton at δ 5.24 and the H-4 signal was a doublet ($J=3.1$ Hz), which is characteristic for galactose. The remaining unit with the anomeric proton at δ 5.85 was characterised as α-L-rhamnopyranose unit. A β-configuration of the anomeric centre of the galactose moiety was deduced from the $J_{H\text{-}1,H\text{-}2}$ coupling constant. The anomeric configurations of the other sugar units were similar to the corresponding ones in saponin **1**. The branched nature of the trisaccharide moiety at the C-3 position of the oleanolic acid moiety was established from the HMBC correlations between C-3 (δ 89.4) and H-1 (δ 4.96) of GlcA, between C-2 (δ 83.8) of GlcA and H-1 (δ 5.24) of Gal between C-4 (δ 79.2) of GLcA and H-1 (δ 5.85) of Rha. These linkages were also concluded from the observed inter-residue NOEs. Therefore, the structure of **6** was established as 3-*O*-α-L-rhamnopyranosyl-(1→4)-[β-D-galactopyranosyl-(1→2)-] β-D-glucuronopyranosyl oleanolic acid.

Saponin **7** (C_{54} H_{86} O_{23}), on acid hydrolysis yielded oleanolic acid, D-glucuronic acid, D-galactose, D-glucose and L-rhamnose. The 1D and 2D NMR studies of **7** (as described for the previous saponins) indicated that **7** was the 28-glucosyl derivative of **6** and thus, assigned the structure of 3-*O*-α-L-rhamnopyranosyl-(1→4)-[β-D-galactopyranosyl-(1→2)-] β-D-glucuronopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester.

Saponin **8** (C_{46} H_{72} O_{17}), yielded oleanolic acid and the sugar components D-glucuronic acid, L-arabinose and D-apiose upon acid hydrolysis. The trisaccharide nature of **8** was deduced from the presence of three sugar units characterised as β-D-glucuronopyranose (H-1; δ 4.97), α-L-arabinopyranose (H-1; δ 5.19) and β-D-apiofuranose units (H-1; δ 6.05) by NMR studies as described above. The 28-COOH function of oleanolic acid was not esterified as shown from the δ value of C-28 resonance at 180.1 ppm. The attachment of the terminal units α-L-arabinopyranose and β-D-apiofuranose at C-2 and C-4 positions of the inner β-D-glucuronopyranose unit respectively, was established from the inter-residue NOEs and HMBC cross-peaks arising from the anomeric protons to the signals involved in the glycosidic linkage. Thus, the structure of **8** was deduced to be 3-*O*-β-D-apiofuranosyl-(1→4)-[α-L-arabinopyranosyl-(1→2)-] β-D-glucuronopyranosyl oleanolic acid.

Saponin **9,** (C_{52} H_{82} O_{22}), on acid hydrolysis afforded oleanolic acid, D-glucuronic acid, D-apiose, L-arabinose and D-glucose. The tetraglycosidic nature of **9** was established from its NMR data. The comparison of ^1H and ^{13}C NMR data of **9** (1D and 2D spectra) with those of **8** suggested that **9** possessed the same trisaccharide chain at C-3 position of the oleanolic acid moiety, in addition to a β-D-glucopyranose unit esterifying the oleanolic acid COOH group. Sequencing of sugar units

was verified from the observed HMBC correlations and NOEs interactions. Therefore **9** was the 28-glucosyl derivative of **8** and assigned the structure of 3-*O*-β-D-apiofuranosyl-(1→4)-[α-L-arabinopyranosyl-(1→2)-]β-D-glucuronopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester.

Phytochemical studies, carried out by other workers, on several species of *Schefflera*, have resulted in the identification of triterpene saponins of various aglycone types (Sung et al., 1991; Maeda et al., 1994; Zhu et al., 1996). Glucuronides of oleanane-type, bearing a linear sugar sequence, occur frequently in *Schefflera* plants. The newly isolated 2,4 disubstituted glucuronides represent their first occurrence in the genus *Schefflera*. Other glucuronides bearing branched structure with variable linkage sites have been reported from *Aralia* members (Satoh et al., 1994; Hu et al., 1995; Miyase et al., 1996b).

3. Experimental

3.1. General

Optical rotations were measured with Jasco DIP 1000 digital polarimeter. MS were measured on Jeol-JMX-SX 102 mass spectrometer. NMR spectra were obtained with a Jeol GSX-500 FT NMR spectrometer and chemical shifts were given in ppm with TMS as internal standard. GC was performed on a Hitachi G-3000 gas chromatography. Preparative and analytical HPLC were performed on a Jasco model 800 instrument.

3.2. Plant material

Leaves and stems of *S. arboricola* (Hayata) Merr. were collected from El-Orman Public Garden in Giza in June 2001 and identified by Mrs. T. Labib, the senior specialist for plant identification at El-Orman Public Garden. A voucher specimen was deposited at Chemistry of Natural Products Department, NRC.

3.3. Extraction and isolation

Dried powdered mixture of leaves and stems of *S. arboricola* (500 g) was extracted with methanol (2×2 l) at room temperature. The concentrated combined extract was diluted by adding of large excess Me_2CO to precipitate the crude saponins. The crude saponin mixture (4.5 g) was first chromatographed on a silica gel column eluting with $CHCl_3$ containing increasing proportions of MeOH. Four fractions (1–4) were obtained. In order to remove the non-terpenoidal constituents, fr. 1 ($CHCl_3$–MeOH; 85:15; 2.1 g) and fr. 2 ($CHCl_3$–MeOH; 83:17; 1.1 g) were chromatographed separately on Sephadex LH-20 column eluted with a gradient H_2O–MeOH followed by prep. TLC on silica gel developed with $CHCl_3$–MeOH–H_2O–EtOAc (28:35:5:32). The sugars and the rest of phenolic constituents from fr. 3 ($CHCl_3$–MeOH; 80:20; 0.9 g) and fr. 4 ($CHCl_3$–MeOH; 70:30; 0.3 g) were removed by passing through a polymer gel Diaion HP-20 column. Elution was carried out using H_2O then MeOH–H_2O (1:1) and finally MeOH to obtain only saponins. The saponin mixtures obtained from the above processing were repeatedly subjected to HPLC {ODS column 20 mm×25 cm, [CH_3CN–H_2O (30:70) for fr 4; (35:65) for fr 3; (40:60) for fr 2; (45:55) for fr 1]+0.05% TFA, flow rate; 6.5 ml/min, UV; 205 nm} to give in addition to olaxoside and cynarasaponin H, **1** (40 mg), **2** (22mg), **3** (10 mg), **4** (20 mg), **5** (30 mg), **6** (56 mg), **7** (30 mg), **8** (22 mg) and **9** (25 mg).

3.4. Saponin (*1*)

Amorphous powder $[\alpha]_D^{23}$ −8.4° ($c=2.34$, MeOH) FABMS (m/z): 801 $[C_{42}H_{66}O_{13}+Na]^+$, ^1H and ^{13}C NMR: Tables 1–3.

3.5. Saponin (*2*)

Amorphous powder $[\alpha]_D^{23}$ −27.8° ($c=1.10$, MeOH) FABMS (m/z): 817 $[C_{42}H_{66}O_{14}+Na]^+$, ^1H and ^{13}C NMR: Tables 1–3.

3.6. Saponin (*3*)

Amorphous powder $[\alpha]_D^{23}$ −13.1° ($c=1.38$, MeOH) FABMS (m/z): 949 $[C_{47}H_{74}O_{18}+Na]^+$, ^1H and ^{13}C NMR: Tables 1–3.

3.7. Saponin (*4*)

Amorphous powder $[\alpha]_D^{23}$ −4.2° ($c=0.91$, MeOH) FABMS (m/z): 933 $[C_{47}H_{74}O_{17}+Na]^+$, ^1H and ^{13}C NMR: Tables 1 and 3.

3.8. Saponin (*5*)

Amorphous powder $[\alpha]_D^{23}$ −10.2° ($c=2.06$, MeOH) FABMS (m/z): 1095 $[C_{53}H_{84}O_{22}+Na]^+$, ^1H and ^{13}C NMR: Tables 1 and 3.

3.9. Saponin (*6*)

Amorphous powder $[\alpha]_D^{23}$ −10.4° ($c=1.95$, MeOH) FABMS (m/z) 963 $[C_{48}H_{76}O_{18}+Na]^+$, ^1H and ^{13}C NMR: Tables 1 and 3.

3.10. Saponin (*7*)

Amorphous powder $[\alpha]_D^{23}$ −12.1° ($c=1.28$, MeOH) FABMS (m/z) 1125 $[C_{54}H_{86}O_{23}+Na]^+$, ^1H and ^{13}C NMR: Tables 1 and 3.

3.11. Saponin (**8**)

Amorphous powder $[\alpha]_D^{23}$ −3.7° (c = 1.06, MeOH) FABMS (m/z) 919 $[C_{46}H_{72}O_{17}+Na]^+$, ^1H and ^{13}C NMR: Tables 1 and 3.

3.12. Saponin (**9**)

Amorphous powder $[\alpha]_D^{23}$ −13.1° (c = 1.38, MeOH) FABMS (m/z) 1081 $[C_{52}H_{82}O_{22}+Na]^+$, ^1H and ^{13}C NMR: Tables 1 and 3.

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

Each saponin (3 mg) dissolved in dioxane (150 μl) and 2 N HCl (150 μl) was heated at 100 °C for 1 h. The reaction mixture was diluted with H_2O and extracted twice with EtOAc. From the EtOAc layer, the aglycone 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; oleanolic acid (t_R 11.0 min), echinocystic acid (t_R, 7.1 min]. The water layer was passed through an Amberlite IRA-60E column (6×60 mm) and the eluate was concentrated. The residue was examined for sugars by paper chromatography [n-BuOH–HOAc–H_2O (4:1:5)] against standard samples as well as by GC after being converted to their thiazolidine derivatives as described by Hara et al., 1986; conditions: [column Supelco SPB-TM1 (0.25 mm×27 m, column temperature; 215 °C., carrier gas; N_2, retention time D-Glc (22.5 min), L-Glc (21.5 min), D-Api (11.9 min) L-Api (10.9 min), D-Gal(24.7 min), L-Gal (23.1 min), D-Ara (11.0 min), L-Ara (11.9 min), D-Rha (12.7 min), L-Rha (12.9 min). From the new saponins, rhamnose and arabinose were in the L-form, while glucuronic acid, glucose, galactose and apiose were in the D-form.

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