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Saponins and acylated saponins from *Dizygotheca kerchoveana*

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Abstract

Four new triterpenoid saponins were isolated from the leaves and stem of branches of *Dizygotheca kerchoveana* along with seven known ones. The new saponins were respectively characterized as 3-*O*-[β-D-glucopyranosyl-(1 → 3)]-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranosyl echinocystic acid, 3-*O*-[β-D-glucopyranosyl-(1 → 3)]-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranosyl echinocystic acid 28-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester, 3-*O*-[β-D-3-*O*-*trans*-*p*-coumaroyl-glucopyranosyl-(1 → 3)]-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranosyl echinocystic acid 28-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester and 3-*O*-[β-D-3-*O*-*cis*-*p*-coumaroyl-glucopyranosyl-(1 → 3)]-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranosyl echinocystic acid 28-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester. Their structures were elucidated by 1D and 2D NMR experiments, FAB-MS as well as chemical means.

Keywords: *Dizygotheca kerchoveana*; Araliaceae; Triterpenoid saponins

1. Introduction

Dizygotheca species (Araliaceae) are ornamental trees cultivated in public gardens in Egypt. In our previous studies on the family members, we have described the isolation and structures of several numbers of saponins (Melek et al., 2002, 2003a,b, 2004). As a part of our continuing search for bioactive saponins from plants grown in Egypt, we present in this report the isolation and structure elucidation of eleven saponins including four new ones, from the leaves and stem of branches of *Dizygotheca kerchoveana* Hort – Veitch.

2. Results and discussion

The leaves and stem of branches of *D. kerchoveana* with the same saponin content (TLC profile) were mixed and extracted with methanol. The crude saponin mixture obtained from the methanolic extract was subjected to column chromatography on a porous polymer polystyrene resin (Diaion HP-20), silica gel and Sephadex LH-20 followed by HPLC to give eleven saponins (**1**–**4**, **6**–**12**) including four new ones **1**–**4**. The seven known saponins **6**, 3-*O*-α-L-arabinopyranosyl-ursolic acid (Stolyarenko et al., 2000); **7**, 3-*O*-α-L-arabinopyranosyl-echinocystic acid (Grishkovets et al., 2001); calenduloside E **8**, 3-*O*β-D-glucuronopyranosyl-oleanolic acid (Hu et al., 1995); **9**, 3-*O*-α-L-arabinopyranosyl-ursolic acid 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-gentiobiosyl ester (Stolyarenko et al., 2000); ciwujianoside C_3 **10**, 3-*O*-α-L-arabinopyranosyl-oleanolic acid 28-*O*-α-L-rhamnopy-

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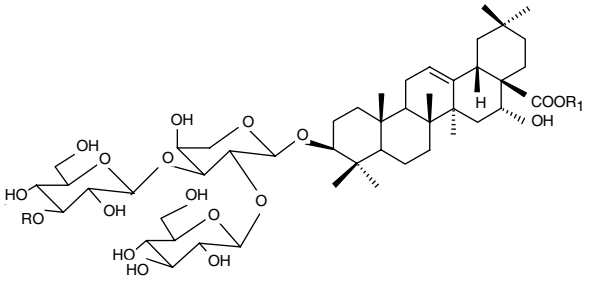
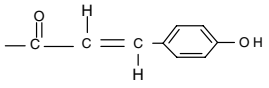
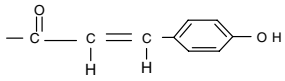
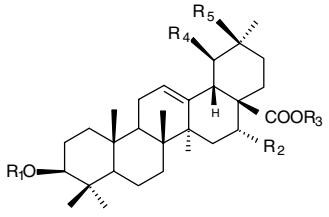
ranosyl-(1 → 4)-β-D-gentiobiosyl ester (Shao et al., 1988); **11**, 3-*O*-α-L-arabinopyranosyl-echinocystic acid 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-gentiobiosyl ester (Melek et al., 2002) and hederasaponin F **12**, 3-*O*-β-sulphate-oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-gentiobiosyl ester (Elias et al., 1991), together with oleanolic acid **5**, were isolated and identified from their NMR data and comparison with the literature values. The NMR data of the new saponins **1**–**4** are presented in Tables 1 and 2.

preparation of their thiazolidine derivatives (Hara et al., 1986). The positive FAB-mass spectrum of **1** displayed the quasi-molecular ion peak $[M + Na]^+$ at *m/z* 951 corresponding to a molecular formula $C_{47}H_{76}O_{18}$ and compatible with a trisaccharide glycoside containing an aglycone of molecular mass 472 and three sugar units, one pentose and two hexoses. The resonances due to seven sp^3 carbons at δ 15.6, 16.8, 17.5, 24.8, 27.3, 28.1, 33.4 and two sp^2 carbons at δ 122.5 and 145.2 in the ^{13}C NMR spectrum of **1** coupled with the information



	R	R_1
1	H	H
2	H	α-L-Rhap-(1→4)-ß-D-Glcp-(1→6)-ß-D-Glcp-
3		α-L-Rhap-(1→4)-ß-D-Glcp-(1→6)-ß-D-Glcp-
4		α-L-Rhap-(1→4)-ß-D-Glcp-(1→6)-ß-D-Glcp-



	R_1	R_2	R_3	R_4	R_5
5	H	H	H	H	CH_3
6	α-L-Arap	H	H	CH_3	H
7	α-L-Arap	OH	H	H	CH_3
8	ß-D-GlcpA	H	H	H	CH_3
9	α-L-Arap	H	α-L-Rhap-(1→4)-ß-D-Glcp-(1→6)-ß-D-Glcp-	CH_3	H
10	α-L-Arap	H	α-L-Rhap-(1→4)-ß-D-Glcp-(1→6)-ß-D-Glcp-	H	CH_3
11	α-L-Arap	OH	α-L-Rhap-(1→4)-ß-D-Glcp-(1→6)-ß-D-Glcp-	H	CH_3
12	SO_3H	H	α-L-Rhap-(1→4)-ß-D-Glcp-(1→6)-ß-D-Glcp-	H	CH_3

Acid hydrolysis of compounds **1** with 2 M hydrochloric acid in dioxane (1:1) yielded the sugar components L-arabinose, and D-glucose, identified by GC analysis after

from the ^1H NMR spectrum (seven methyl proton singlets and a broad triplet vinyl proton at δ 5.63) suggested **1** to be a triterpenoid glycoside with an

Table 1
^{1}H NMR spectral data for compounds **1**–**4** in pyridine-d_5

Aglycone	**1**	**2**	**3**	**4**
3	3.27 (*dd*, 12.0, 4.5)	3.26 (*dd*, 12.0, 4.5)	3.25 (*dd*, 12.0, 4.5)	3.25 (*dd*, 11.5, 4.0)
5	0.81 (*d*, 12.0)	0.80 (*d*, 12.0)	0.80 (*d*, 11.5)	0.81(*d*, 11.5)
12	5.63 (*brt*, 3.5)	5.59 (*brt*, 3.0)	5.59 (*brt*, 3.0)	5.58 (*brt*, 3.0)
16	5.24 (*brs*)	5.27 (*brs*)	5.27 (*brs*)	5.27 (*brs*)
18	3.61 (*dd*, 14.0, 3.5)	3.50 (*dd*, 14.0, 3.5)	3.50 (*dd*, 14.0, 3.5)	3.49 (*dd*, 14.0, 3.5)
23	1.25 (*s*)	1.24 (*s*)	1.23 (*s*)	1.22 (*s*)
24	1.08 (*s*)	1.09 (*s*)	1.10 (*s*)	1.09 (*s*)
25	0.87 (*s*)	0.91 (*s*)	0.90 (*s*)	0.90 (*s*)
26	1.03 (*s*)	1.11 (*s*)	1.11 (*s*)	1.11 (*s*)
27	1.84 (*s*)	1.80 (*s*)	1.81 (*s*)	1.80 (*s*)
29	1.07 (*s*)	0.99 (*s*)	0.99 (*s*)	0.99 (*s*)
30	1.18 (*s*)	1.06 (*s*)	1.06 (*s*)	1.06 (*s*)
3-*O*-sugar				
Ara				
1	4.79 (*d*, 6.7)	4.78 (*d*, 6.7)	4.77 (*d*, 6.7)	4.76 (*d*, 6.7)
2	4.72	4.72	4.68	4.68
3	4.33	4.33	4.29	4.28
4	4.48	4.48	4.50	4.47
5	3.67(*brs*, 11.5)	3.67	3.68	3.66
5‘	4.18	4.18	4.18	4.15
Glc I at Ara C_2				
1	5.49 (*d*, 8.0)	5.49 (*d*, 8.0)	5.43 (*d*, 8.0)	5.40 (*d*, 8.0)
2	4.03	4.03	4.02	4.00
3	4.19	4.18	4.12	4.13
4	4.14	4.13	4.09	4.09
5	3.70 (*m*)	3.70	3.69	3.68
6	4.27	4.27	4.25	4.24
6′	4.35	4.34	4.36	4.35
Glc II at Ara C_3				
1	5.28 (*d*, 8.0)	5.28 (*d*, 8.0)	5.28 (*d*, 8.0)	5.24 (*d*, 8.0)
2	3.99	3.99	4.10	4.05
3	4.20	4.20	5.98	5.93
4	4.18	4.18	4.39	4.32
5	3.93 (*m*)	3.93 (*m*)	3.97 (*m*)	3.93
6	4.31	4.31	4.35	4.33
6′	4.46	4.47	4.45	4.42
28-*O*-sugar				
Glc III				
1		6.22 (*d*, 8.5)	6.22 (*d*, 8.5)	6.22 (*d*, 8.5)
2		4.05	4.05	4.05
3		4.17	4.17	4.17
4		4.22	4.23	4.23
5		4.08	4.08	4.08
6		4.30	4.30	4.30
6′		4.64	4.64	4.63
Glc IV				
1		4.96 (*d*, 8.0)	4.96 (*d*, 8.0)	4.96 (*d*, 8.0)
2		3.91	3.91	3.91
3		4.15	4.13	4.12
4		4.37	4.37	4.37
5		3.66 (*m*)	3.66	3.66 (*m*)
6		4.08	4.08	4.06
6′		4.20	4.20	4.22
Rha				
1		5.82 (*brs*)	5.82 (*d*, 2.0)	5.82 (*d*, 2.0)
2		4.65 (*dd*, 3.0, 1.5)	4.65 (*dd*, 3.0, 2.0)	4.66 (*dd*, 3.0, 2.0)
3		4.52 (*dd*, 9.0, 3.5)	4.52 (*dd*, 9.5, 3.5)	4.52 (*dd*, 9.0, 3.5)
4		4.30	4.30	4.30
5		4.92 (*dq*, 9.0, 6.5)	4.92 (*dq*, 9.0, 6.5)	4.92 (*dq*, 9.0, 6.0)
6		1.69 (*d*, 6.5)	1.69 (*d*, 6.5)	1.69 (*d*, 6.0)

(*continued on next page*)

Table 1 (*continued*)

Aglycone	1	2	3	4
p-Coumaroyl moiety				
2,6			7.48 (*d*, 8.8)	8.07 (*d*, 8.8)
3,5			7.13 (*d*, 8.8)	7.12 (*d*, 8.8)
α			6.53 (*d*, 16.0)	5.96 (*d*, 12.5)
β			7.88 (*d*, 16.0)	6.83 (*d*, 12.5)

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

aglycone of olean-12-ene skeleton. In comparison of the ^{13}C NMR spectral data with those reported for various echinocystic acid glycosides (Nagao et al., 1993), **1** was suggested to be an echinocystic acid derivative with three sugar moieties attached at C-3 (C-3, 89.1 ppm; C-28, 180 ppm). The three sugar anomeric proton signals appeared in the ^{1}H NMR spectrum at δ 4.79 (*d*, $J = 6.7$ Hz), 5.49 (*d*, $J = 8.0$ Hz) and 5.28 (*d*, $J = 8.0$ Hz) were assigned to one L-arabinose and two D-glucose units, respectively. The HOHAHA and NOE difference experiments of **1** allowed the identification of the spin systems of the three monosaccharides. The complementary data from the ^{1}H–^{1}H COSY spectrum defined the sequential assignments of the proton resonances and the intra-residue NOEs was used to discriminate between resonances arising from H-3 and H-4 of glucose unit. The corresponding ^{13}C resonances of the individual monosaccaride, deduced from the HMQC spectrum of **1**, revealed the presence of an inner α-L-arabinopyranose (Ara) substituted at C-2 and C-3 as well as two terminal β-D-glucopyranoses (Glc). The HMBC experiment of **1** was used to verify the branched nature of the trisaccharide chain at C-3 of the triterpene moiety and exhibited cross peaks correlating the resonances due to C-3 (δ 89.1) of the echinocystic acid moiety and Ara H-1 (δ 4.79), Ara C-2 (δ 77.5) and Glc I H-1(δ 5.49), Ara C-3 (δ 83.4) and Glc II H-1 (δ 5.28). The deduced linkages were also in agreement with the observed inter-residue NOEs. Therefore, compound **1** was assigned to the structure of 3-*O*-[β-D-glucopyranosyl-(1 → 3)]-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranosyl echinocystic acid.

Compound **2** exhibited $[M + Na]^{+}$ ion peak at *m/z* 1421 in its FAB-mass spectrum which allowed us to propose a molecular formula $C_{65}H_{106}O_{32}$. Analysis of the ^{1}H and ^{13}C NMR spectra of **2** in comparison with those of **1**, defined the nature of **2** as another echinocystis acid saponin. The presence of six monosaccharide units was indicated from six anomeric proton resonances in the ^{1}H NMR spectrum at δ 4.78 (*d*, $J = 6.7$ Hz), 5.49 (*d*, $J = 8.0$ Hz), 5.28 (*d*, $J = 8.0$ Hz), 6.22 (*d*, $J = 8.5$ Hz), 4.96 (*d*, $J = 8.0$ Hz) and 5.82 (*brs*), attributable to one L-arabinose, four D-glucoses and one L-rhamnose, respectively, after acid hydrolysis. From the assigned ^{13}C NMR resonances due to the echinocystic acid moiety, it was apparent that the six sugars were present in two saccharide chains, one attached to C-3 (δ 89.0) and the other to C-28 (δ 176.0). The unambiguous identification of the individual spin systems associated with the six monosaccharides and the assignments of their NMR resonances were established by the combined use of 1D and 2D NMR techniques (HOHAHA, ^{1}H–^{1}H COSY, NOE, HMQC, HMBC). All monosaccharides were in the pyranose form and the anomeric configurations, as determined from $^{3}J_{H\text{-}1,H\text{-}2}$, were α- for arabinose and β- for glucoses. The anomeric configuration of rhamnose was determined as α- from the δ value of C-3 and C-5 (Agrawal, 1992). The structure of the oligosaccharide chain at C-28 of the echinocystic acid moiety was characterized as 28-*O*-α-L-rhamnopyranosyl (1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl based on the HMBC correlations between resonances of Glc IV C-4 (δ 78.5) and Rha (α-L-rhamnopyranose) H-1 (δ 5.82), Glc III C-6 (δ 69.4) and Glc IV H-1 (δ 4.96), C-28 (δ 176.0) of the triterpene moiety and Glc III H-1 (δ 6.22). The other HMBC correlations were found the same as those observed for 3-*O*-sugar chain of **1**, indicating the presence of identical trisaccharide unit attached to C-3 of the echinocystic acid moiety in **1** and **2**. Saponins with the same triose moiety at C-28 were reported by us from other Araliaceae members (Melek et al., 2004). Based on the above studies, the structure of compound **2** was concluded to be 3-*O*-[β-D-glucopyranosyl-(1 → 3)]-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranosyl echinocystic acid 28-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester.

Compound **3** ($C_{74}H_{112}O_{34}$) exhibited $[M + Na]^{+}$ion peak at *m/z* 1567 in the FAB-mass spectrum and differed from the formula of compound **2** by supplementary 146 uma. Analysis of 1D and 2D NMR of **3**, in comparison with those of **2**, indicated the presence of echinocystic acid moiety and six monosaccharides present in two saccharide units similar in composition and linkages to those of **2**. The UV spectrum of **3** exhibited absorption maxima at 312, 290 (sh) and 288 nm characteristic for cinnamoyl chromophore. The ^{1}H–^{1}H correlations in the ^{1}H–^{1}H COSY spectrum allowed us to identify additional resonances due to *trans*-olefinic protons of the cinammoyl moiety, which appeared as two doublets at

Table 2
^{13}C NMR spectral data for compounds **1**–**4** in pyridine-d_5

Aglycone	1	2	3	4
3	89.1	89.0	89.3	89.3
12	122.5	122.8	122.8	122.8
13	145.2	144.5	144.5	144.5
16	74.8	74.4	74.4	74.4
23	28.1	28.1	28.1	28.0
24	16.8	16.8	16.8	16.8
25	15.6	15.8	15.8	15.8
26	17.5	17.6	17.6	17.6
27	27.3	27.3	27.3	27.2
28	180.0	176.0	176.0	176.0
29	33.4	33.2	33.2	33.2
30	24.8	24.7	24.8	24.8
3-*O*-sugar				
Ara				
1	105.5	105.4	105.4	105.4
2	77.5	77.4	77.4	77.4
3	83.4	83.3	84.1	84.3
4	68.8	68.8	68.6	68.6
5	66.0	65.9	65.9	65.9
Glc I				
1	104.4	104.4	104.5	104.5
2	76.2	76.2	76.1	76.0
3	78.7	78.5	78.4	78.4
4	72.5	72.5	72.6	72.6
5	77.5	77.3	77.3	77.3
6	63.3	63.3	63.4	63.4
Glc II				
1	105.0	105.0	104.9	104.9
2	75.3	75.2	73.4	73.2
3	78.5	78.7	79.2	79.0
4	71.6	71.6	69.5	69.5
5	78.4	78.4	78.4	78.4
6	62.6	62.6	62.1	62.1
28-*O*-sugar				
Glc III				
1		95.8	95.8	95.8
2		74.0	74.0	74.0
3		78.8	78.7	78.7
4		71.0	71.0	71.0
5		78.1	78.1	78.1
6		69.4	69.5	69.5
Glc IV				
1		105.0	105.0	105.0
2		75.3	75.3	75.3
3		76.5	76.5	76.6
4		78.5	78.4	78.5
5		77.2	77.2	77.2
6		61.4	61.4	61.4
Rha				
1		102.8	102.8	102.8
2		72.6	72.6	72.6
3		72.8	72.8	72.8
4		74.0	74.0	74.0
5		70.4	70.4	70.4
6		18.5	18.5	18.5
p-Coumaroyl moiety				
1			126.2	126.6
2,6			130.6	133.8
3,5			116.8	116.7
4			161.4	160.5
α			115.8	115.9
β			145.1	143.6
CO			167.4	166.8

δ 6.53 and 7.88 (1H each, J = 16.0 Hz) and *p*-substituted benzene ring protons at δ 7.13 and 7.48 (2H each, *d*, J = 8.8 Hz). Their associated ^{13}C resonances assigned from the HMQC and HMBC experiments, were at δ 115.8 and 145.1 for the olefinic carbons and δ 126.2 (C-1), 130.6 (C-2, C-6), 116.8 (C-3, C-5), 161.4 (C-4) for the aromatic ring together with a carbonyl ester resonance at δ 167.4. Acid hydrolysis of **3** afforded sugar components identical to those obtained from **2**. Mild alkaline hydrolysis of **3** with 1% KOH yielded *p*-coumaric acid and a deacylated saponin identical to **2**. The above findings indicated that the structural difference between the sugar part of **3** and **2** was confined to the presence of a *trans*-*p*-coumaroyl group in **3**. The location of this group at C-3 of Glc II was suggested by the deshielded value of Glc II H-3 ($\Delta\delta$ + 1.78 ppm) compared to saponin **2**. Furthermore, the downfield δ value of Glc II C-3 resonance at 79.2 ppm (α-effect) and the upfield δ value of both C-2 at 73.4 ppm and C-4 at 69.5 ppm (β-effect), relative to the corresponding values in **2**, can be explained if the hydroxyl at C-3 of Glc II in **3** was esterified. Further supporting information for the site of esterification was derived from the HMBC experiment of **3** which exhibited cross peaks correlating the resonance of the Glc II H-3 (δ 5.98) and the resonance due to Glc II C-2 (δ 73.4), Glc II C-4 (δ 69.4) and carbonyl ester group (δ 167.4) of the *trans*-*p*-coumaroyl moiety. Therefore the structure of **3** was elucidated as 3-*O*-[β-D-3-*O*-*trans*-*p*-coumaroyl-glucopyranosyl-(1 → 3)]-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranosy echinocystic acid 28-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyrsanosyl] ester.

Compound **4** was another echinocystic acid hexasaccharide from NMR analysis. Upon acid hydrolysis, **4** afforded sugar components identical to those obtained from **2** and **3**. The analysis of 1D and 2D NMR of the sugar part of **4** and **3** revealed similarity with regard to the structure of the individual monosaccharides and their linkages. The only structural difference between **4** and **3** was due to the geometry of the double bond between the olefinic carbons of the *p*-coumaroyl moiety, being *cis* in **4** instead of *trans* in **3**. This was evident from the position of the two doublets attributable to the *cis* olefinic protons at δ 5.96 and 6.83 (1H each, J = 12.0 Hz) in the ^{1}H–^{1}H COSY spectrum of **4**. The *p*-substituted benzene ring protons appeared as two doublets at δ7.12 and 8.07 (2H each, *d*, J = 8.8 Hz). Mild alkaline hydrolysis of **4** using 1% KOH afforded *p*-coumaric acid

and deacylated saponin **2**. As expected, the $[M + Na]^+$ ion peak was observed at *m/z* 1567 in the FAB-mass spectrum of **4**. Thus, the structure of **4** was elucidated as 3-*O*-[β-D-3-*O*-*cis*-*p*-coumaroyl-glucopyranosyl-(1 → 3)]-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranosyl echinocystic acid 28-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester.

Saponins contained sugar units esterified with variable organic acids were reported from several plant species. Acylated glycosides of echinocystic acid have been isolated from the roots of *Tragopogon porrifolius* (Warashina et al., 1991).

It is worthnoting that saponins with α-L-arabinopyranosyl as an inner glycosyl unit at C-3 of the triterpene moiety and α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyrano syl as the ester glycosyl unit at C-28, have been reported not only from genera of Araliaceae family but also from genera of other families such as Ranunculaceae (Ansari et al., 1988; Shao et al., 1995), Boraginaceae (Mohammad et al., 1995) Berberidaceae (Chen et al., 1997), Lardizabalaceae (Chandel and Rastogi, 1980) and Podophyllaceae (Chandel and Rastogi, 1980; Agarwal and Rastogi, 1974). This probably indicates similar biosynthetic pathways in these plants.

The saponin mixture of *D. kerchoveana* was found to exhibit a weak molluscicidal activity (LC_{90} = 27.9 ppm). Another species, *D. elegantissima* from Egypt was studied and showed saponins, which possessed similar activity. One of them is a saponin with the same glycosidic chain at C-28 (Abdel-Gawad et al., 1997).

3. Experimental

3.1. General

Optical rotations were measured with Jascodip – 1000 digital polarimeter. MS spectra 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. UV spectra were recorded on Hitachi U-2010 spectrophotometer. HPLC was performed on a Jasco system 800 instruments. GC analysis was carried out on Hitachi G-3000 gas chromatograph.

3.2. Plant material

The leaves and stem of branches of *D. kerchoveana* were collected from a public garden in Cairo, Egypt. Mrs T. Labib, head specialist for plant identfication in El-Orman public garden, Cairo, Egypt, confirmed plant identification. The herbarium voucher specimen (Melek, F. 25) was deposited in the Herbarium of NRC (CAIRC).

3.3. Extraction and isolation

A mixture of dried and powdered leaves and stem of branches of *D. kerchoveana* (1.5 kg) was extracted with $CHCl_3$ twice then extracted with MeOH twice at room temperature. The combined methanolic extract (95.5 g) was concentrated and dropped into a 10-fold amount of $(CH_3)_2CO$ to precipitate the crude saponin mixture (16.5 g). The mixture was dissolved in water and the aqueous solution was passed through a porous polymer gel column (Misubishi Diaion HP-20). The column was then eluted with water, 50%, 75% and 100% MeOH. The 75% MeOH eluate. (650 mg) was chromatographed on a silica gel column eluted with $CHCl_3$–MeOH (65:35) followed by Sephadex LH-20 column eluted with methanol to give **12** (109 mg). The MeOH eluate (5.6 g) was chromatographed on a silica gel column eluted with $CHCl_3$–MeOH–H_2O (80:18:2–61:33:6) to give 17 fractions (A–Q). Fraction A (224 mg) afforded oleanolic acid **5** (26 mg) after silica gel column chromatography eluted with $CHCl_3$:MeOH (98:2). Fraction B (420 mg), C (221 mg), E (223 mg), F (589 mg), I (336 mg), L (193 mg) and P (166 mg) were subjected to silica gel column chromatography eluted with $CHCl_3$–MeOH–H_2O mixs. of increasing polarity. The purification was finally performed over HPLC [ODS, 0.2 × 2.5 cm; solvent, CH_3-CN–H_2O (25:75–55:45) + 0.05 TFA; monitored at 205 nm]. Fraction A gave **5** (18 mg), fraction B afforded **6** (10 mg) and **7** (9 mg), fraction C gave **8** (38 mg), fraction E afforded **1** (18 mg), fraction F gave **9** (22 mg) and **10** (29 mg), fraction I afforded **11**(22 mg), fraction L gave **3** (10 mg) and **4** (16 mg) and fraction P afforded **2** (18 mg).

3.4. Saponin (*1*)

Amorphous powder $[\alpha]_D^{23} + 19.5$ (C = 0.94, MeOH); FAB-MS *m/z*: 951 $[C_{47}H_{76}O_{18} + Na]^+$, 905, 817, 717, 687, 593, 455. ^1H and ^{13}C NMR: see Tables 1 and 2.

3.5. Saponin (*2*)

Amorphous powder $[\alpha]_D^{23} - 13.0$ (C = 0.94, MeOH); FAB-MS *m/z*: 1421 $[C_{65}H_{106}O_{32} + Na]^+$, 1112, 959, 836, 717, 613. ^1H and ^{13}C NMR: see Tables 1 and 2.

3.6. Saponin (*3*)

Amorphous powder $[\alpha]_D^{23} + 4.9$ (C = 0.96, MeOH). UV λ_{max}^{MeOH} nm : 228, 290 (sh), 312. FAB-MS *m/z*: 1567 $[C_{74}H_{112}O_{34} + Na]^+$, 1403, 910, 836, 717, 613. ^1H and ^{13}C NMR: see Tables 1 and 2.

3.7. Saponin (*4*)

Amorphous powder $[\alpha]_D^{23} - 14.9$ (C = 0.84, MeOH). UV λ_{max}^{MeOH} nm : 227 (sh), 290 (sh), and 310. FAB-MS

m/z: 1567 $[C_{74}H_{112}O_{34} + Na]^+$, 1403, 836, 717, 613. ^1H and ^{13}C NMR: see Tables 1 and 2.

3.8. General method for acid hydrolysis

Acid hydrolysis and detection of the aglycones and sugar constituents of **1**–**4** were performed as previously described by Melek et al., 2002, 2003a,b.

3.9. Mild alkaline hydrolysis of *3* and *4*

Each compound (5 mg) was hydrolysed separately with 1% KOH at room temperature for 1 h. the mix was neutralized with dilute HCl and extracted with Et_2O. The Et_2O afforded *p*-coumaric acid identified by silica gel TLC (toluene–ether 1:1, satd. With HOAC 10%). The aq. layer was extracted with *n*-BuOH to give saponin **2**.

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