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Echinocystic acid 3,16-*O*-bisglycosides from *Albizia procera*

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ABSTRACT

Three triterpene glycosides and two known ones were isolated from the bark of *Albizia procera* by using chromatographic techniques. The structures of the compounds were determined to be 3-*O*-β-D-xylopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid 16-*O*-β-D-glucopyranoside, 3-*O*-β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid 16-*O*-β-D-glucopyranoside and 3-*O*-α-L-arabinopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid 16-*O*-β-D-glucopyranoside. Their structures were determined by NMR techniques including HOHAHA, ^{1}H-^{1}H COSY, ROE, HMQC and HMBC experiments together with FABMS as well as acid hydrolysis. To the best of our knowledge, the new compounds are considered the first examples of echinocystic acid 3,16-*O*-bisglycosides. In contrast to other cytotoxic echinocystic acid glycosides with *N*-acetyl glucosamine unit, the new glycosides were found inactive when assayed by MTT method for their cytotoxicities against the human tumor cell lines HEPG2, A549, HT29 and MCF7. The results showed the importance of the free hydroxyl group at the aglycone C-16 for exhibiting cytotoxic properties.

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1. Introduction

The genus *Albizia* (Leguminosae) comprises about 150 species distributed in Africa, Asia and Central and South America. In traditional Chinese medicine *Albizia julibrissin* is used for the treatment of insomnia, irritability and wounds (Bown, 1995). *A. lebbeck* was reported to possess antidysenteric, antiseptic and antitubercular activities (Chadha, 1985). The *Albizia* members in Africa are used in folk medicine for the treatment of rheumatism, cough, diarrhea and injuries (Watt and Breyer-Brandwijk, 1962). Phytochemical studies carried out on *Albizia* species led to the isolation of several triterpene glycosides. The root bark of *A. anthelmintica* (Carpani et al., 1989), the seeds of *A. lucida* (Orsini et al., 1991), the stems of *A. subdimidiata* (Abdel-Kader et al., 2001) and the bark of *A. procera* (Melek et al., 2007) afforded a number of *N*-acetyl-D-glucosamine derivatives of oleanolic and echinocystic acids. Three acacic acid lactone glycosides albiziasaponins A–C were obtained from the bark of *A. lebbeck* (Pal et al., 1995). Proceraosides A–D obtained from the seeds of *A. procera* were acacic acid glycosides bearing a common oligosaccharide moiety at C-28 and acylated at C-21 with a monoterpenic carboxylic acid linked to a monoterpene quinovoside (Yoshikawa et al., 1998). Similar antitumor acacic acid glycosides julibrosides with variable monoterpene quinovosyl units at C-21 and oligosaccharide moities at C-3 (trisaccharide substituted by *N*-acetamido unit or tetrasaccharide), were reported from *A. julibrissin* (Ikeda et al., 1995; Ma et al., 1996; Liang et al., 2005; Zheng et al., 2006). Three antitumor acacic acid glycosides grandibracteosides A–C acylated by an *o*-aminobenzoyl unit were isolated from leaves of *A. grandibracteata* (Krief et al., 2005). Cytotoxicity guided fractionation of the extract obtained from the roots of Madagascan plant *A. gummifera*, led to the isolation of three cytotoxic acylated acacic acid glycosides gummiferaosides A–C (Cao et al., 2007). Two recent reports described the isolation of further cytotoxic acylated acacic acid glycosides albizosides A–C from *A. chinensis* (Liu et al., 2009) and coriariosides A and B from *A. coriaria* (Note et al., 2009).

Albizia procera is a tree cultivated in streets and public gardens in Egypt. In folk medicine, the bark is considered useful in pregnancy and stomachache. As a part of our continuous interest in bioactive triterpene glycosides from plants grown in Egypt (Miyase et al., 1996; Melek et al., 2002, 2003a,b, 2004a,b), we reported in our previous communication (Melek et al., 2007) the isolation and structure elucidation of four triterpene glycosides from the bark of *A. procera*. Further investigation on the glycoside-containing fraction led to the isolation of further three new glycosides **1**–**3** and two known ones **6**, **7**. In this contribution, we describe

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the structure determination of **1–3** by NMR analysis using HOHAHA, ^{1}H-^{1}H COSY, ROE, HMQC and HMBC techniques, FAB-mass spectra and acid hydrolysis.

2. Results and discussion

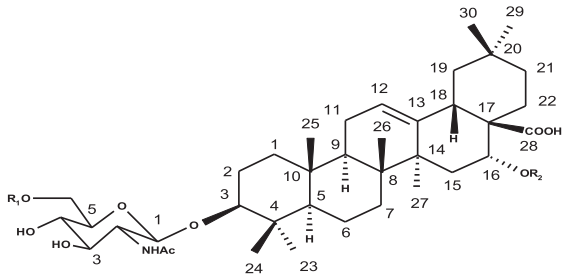
The methanolic extract of the dried bark of *A. procera* was subjected to a combination of HP-20 and silica gel column chromatography followed by HPLC to afford five compounds including three new ones **1–3**.

Compound **1** showed in its positive ion FAB-mass spectrum a quasi-molecular ion peak $[M+Na]^+$ at *m/z* 1154 corresponding to a molecular formula $C_{55}H_{89}NO_{23}$. The ^{1}H NMR spectrum of **1** showed seven angular methyl signals at δ 0.80, 0.84, 0.98, 1.00, 1.10, 1.25 and 1.89 and a typical signal for axial proton attached to a hydroxylate carbon at δ 3.48 (*dd*, *J* = 12.0, 4.0 Hz). Further features were vinylic proton signal at δ 5.50 (*brt*, *J* = 3.0 Hz) and sugar proton signals between δ 3.59 and δ 5.13. In the ^{13}C NMR spectrum of **1**, the signals due to seven sp^3 carbons and two sp^2 carbons at δ 122.5 and 144.9 coupled with the information from ^{1}H NMR spectrum, indicated that **1** was a triterpene glycoside with a triterpene moiety of oleanene skeleton. The deshielded position of the axial methyl group at C-14 (Me-27, δ 1.89) and the presence of a broad singlet proton signal at δ 5.33 (H-16) in the ^{1}H NMR spectrum of **1**, indicated oxygenation at C-16. The assignments of the proton and the carbon signals of the triterpene moiety were established by the combined use of ^{1}H–^{1}H COSY, HMQC and HMBC experiments which allowed identification of the triterpene moiety as echinocystic acid, a common triterpene of triterpene glycosides. The signal at δ 179.5 and the carbon signals of D and E rings suggested the occurrence of a free carboxylic group at C-28. The 3,16 bisdesmosidic nature of **1** was deduced from the presence of two deshielded signals at δ 88.6 and δ 79.9 assigned to echinocystic acid C-3 and C-16, respectively. The assignment of C-16 (δ 79.9) was based on direct correlation of its signal in the HMQC spectrum to that of H-16 (δ 5.33, *brs*) which in turn showed HMBC correlations to the signals of C-17 (δ 48.5) and C-18 (δ 41.7) in the HMBC experiment of **1**. Acid hydrolysis of **1** afforded echinocystic acid detected by TLC and HPLC and the sugar components 2-amino-2-deoxy-D-glucose, D-glucose, D-galactose and D-xylose. The sugar 2-amino-2-deoxy-D-glucose was identified by paper chromatography and optical rotation value while the rest sugars were recognized by GC analysis of their thiazolidine derivatives. The sugar portion of **1** exhibited in the ^{13}C NMR spectrum four anomeric carbon signals at δ 101.7, 103.8, 104.7 and 106.8, a methyl carbon signal at δ 23.8, a carbonyl amide carbon signal at δ 170.1 and a typical amide carbon at δ 58.1. Four corresponding anomeric proton signals were observed in the HMQC spectrum. The structures of the four monosaccharide units were deduced from the use of HOHAHA spectrum of **1** which recognized the spin system of each unit. The ^{1}H–^{1}H COSY and ROE spectra allowed the sequential assignments of the proton resonances to the individual monosaccharides as reported in Table 1. The sugar unit with anomeric signal at δ 5.07 (*d*, *J* = 8.5 Hz) was assigned to a 2-acetamido-2-deoxy-glucose unit (Abdel-Kader et al., 2001; Melek et al., 2007). The sugar with anomeric proton signal at δ 5.10 (*d*, *J* = 7.3 Hz) was due to a galactose unit while the remaining sugars with anomeric signals at δ 5.00 (*d*, *J* = 7.0 Hz) and 5.13 (*d*, *J* = 6.0 Hz) were attributable to glucose and xylose units, respectively. The anomeric centers of the sugar units were all determined to have a β-configuration based on large $^3J_{H1,H2}$ values. The HMQC experiment allowed assignments of the sugar carbons and showed that all the sugars were in the pyranose form. It also allowed the assignments of the interglycosidic linkages by comparison of the observed carbon chemical shifts with those of the corresponding methyl pyranoside (Seo et al., 1978).

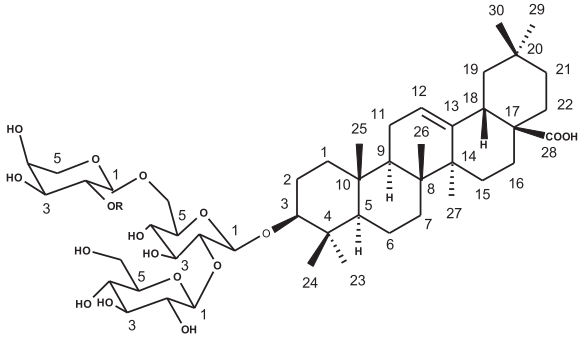
Table 1
^{1}H NMR spectral data for compounds **1–3** in pyridin-d_5.

	1	**2**	**3**
Aglycone			
3	3.48 (*dd*, 12.0, 4.0)	3.36 (*dd*, 13.0, 3.0)	3.36 (*dd*, 13.0, 3.0)
5	0.81	0.81	0.81
12	5.50 (*brt*, 3.0)	5.54	5.54
16	5.33 (*brs*)	5.34 (*brs*)	5.34 (*brs*)
18	3.51	3.54	3.54
19	1.22	1.22	1.22
19′	2.69 (*t*, 13.0)	2.68 (*t*, 13.0)	2.68 (*t*, 13.0)
23	1.25 (*s*)	1.24 (*s*)	1.24 (*s*)
24	1.00 (*s*)	0.99 (*s*)	0.99 (*s*)
25	0.84 (*s*)	0.85 (*s*)	0.85 (*s*)
26	1.10 (*s*)	0.98 (*s*)	0.98 (*s*)
27	1.89 (*s*)	1.89 (*s*)	1.89 (*s*)
29	0.80 (*s*)	0.84 (*s*)	0.84 (*s*)
30	0.98 (*s*)	1.11 (*s*)	1.11 (*s*)
3-O-sugar			
GlcNHAc			
1	5.07 (*d*, 8.5)	5.04 (*d*, 8.0)	5.04 (*d*, 8.0)
2	4.52 (*ddd*, 8.5, 8.5, 8.2)	4.50 (*ddd*, 8.5, 8.2, 8.0)	4.50 (*ddd*, 8.5, 8.2, 8.0)
3	4.33 (*t*, 8.5)	4.36 (*t*, 8.5)	4.36 (*t*, 8.5)
4	4.02	4.11	4.11
5	3.99 (*m*)	4.04 (*m*)	4.04 (*m*)
6	4.33 (*dd*, 11.0, 3.0)	4.19 (*dd*, 11.0, 4.0)	4.19 (*dd*, 11.0, 4.0)
6′	4.72 (*d*, 11.0)	4.61 (*d*, 11.0)	4.61 (*d*, 11.0)
Me(CONH)	2.16 (*s*)	2.16 (*s*)	2.16 (*s*)
NH	8.87 (*d*, 8.2)	8.89 (*d*, 8.2)	8.89 (*d*, 8.2)
Gal			
1	5.10 (*d*, 7.3)		
2	4.52 (*dd*, 7.3, 8.0)		
3	4.19 (*dd*, 8.0, 3.0)		
4	4.55 (*d*, 2.5)		
5	4.00		
6	4.26		
6′	4.37		
Ara			
1		5.13 (*d*, 5.0)	
2		4.50 (*dd*, 5.0, 6.0)	
3		4.36	
4		4.38 (*brs*)	
5		3.75 (*d*, 11.0)	
5′		4.27 (*dd*, 11.0, 3.0)	
Ara′			
1			5.08 (*d*, 5.0)
2			4.52 (*dd*, 5.0, 5.5)
3			4.32
4			4.33 (*brs*)
5			3.72 (*d*, 10.0)
5′			4.26
Xyl			
1	5.13 (*d*, 6.0)	4.99 (*d*, 7.0)	
2	4.07 (*t*, 6.0)	4.00 (*dd*, 7.0, 8.0)	
3	4.01	4.04 (*t*, 8.0)	
4	4.02	4.12	
5	3.59 (*dd*, 12.0, 9.0)	3.60 (*dd*, 11.0)	
5′	4.45 (*dd*, 12.0, 4.5)	4.38 (*dd*, 11.0, 5.0)	
Ara″			
1			5.05 (*d*, 5.5)
2			4.47 (*dd*, 5.5, 6.0)
3			4.10
4			4.23 (*brs*)
5			3.67 (*d*, 11.0)
5′			4.42
16-O-Glc			
1	5.00 (*d*, 7.0)	5.01 (*d*, 7.0)	5.01 (*d*, 7.0)
2	4.04 (*dd*, 7.0, 8.0)	4.06 (*dd*, 7.0, 8.0)	4.06 (*dd*, 7.0, 8.0)
3	4.28 (*t*, 8.0)	4.29 (*t*, 8.0)	4.29 (*t*, 8.0)
4	4.23 (*t*, 9.0)	4.23	4.23
5	4.00 (*m*)	3.99 (*m*)	3.99 (*m*)
6	4.35 (*dd*, 10.0, 3.5)	4.36 (*dd*, 11.0, 2.5)	4.36 (*dd*, 11.0, 2.5)
6′	4.53 (*dd*, 10.0, 2.0)	4.54 (*dd*, 11.0, 2.5)	4.54 (*dd*, 11.0, 2.5)

The absence of any ^{13}C NMR glycosylation shifts for the β-D-xylopyranosyl (Xyl) and β-D-glucopyranosyl (Glc) units, suggested their terminal positions. Glycosylation shifts were observed for C-6 (δ 70.1) of the 2-acetamido-2-deoxy-β-D-glucopyranosyl unit (GlcNHAc) and C-2 (δ 82.1) of the β-D galactopyranosyl unit (Gal). The sequence of the sugar units was unambiguously determined from HMBC experiment. The HMBC correlation between the signals of the echinocystic acid C-3 (δ 88.6) and H-1 (δ 5.07) of GlcNHAc indicated direct attachment of this unit to the triterpene moiety. The correlation between the signal of GlcNHAc C-6 (δ 70.1) with that of Gal H-1 (δ 5.10) established the connectivity between the two sugar units as Gal-(1 → 6)-GlcNHAc and the correlation between the signals of Gal C-2 (δ 82.1) and Xyl H-1 (δ 5.13) confirmed the terminal position of the xylose unit. The correlation between the signals of echinocystic acid C-16 (δ 79.9) and Glc H-1 (δ 5.00) supported by the reverse correlation between the signals of H-16 (δ 5.33) and Glc C-1 (δ 101.7), unambiguously established the glycosylation of the hydroxyl group at C-16 of the echinocystic acid moiety. Accordingly, **1** was assigned as the structure of 3-*O*-β-D-xylopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid 16-*O*-β-D-glucopyranoside.



	R_1	R_2
1	β-D-Xylp-(1→2)-β-D-Galp-	β-D-Glcp-
2	β-D-Xylp-(1→2)-α-L-Arap-	β-D-Glcp-
3	α-L-Arap-(1→2)-α-L-Arap-	β-D-Glcp-
4	β-D-Xylp-(1→2)-α-L-Arap-	H
5	α-L-Arap-(1→2)-α-L-Arap-	H



	R
6	β-D-Xylp-
7	α-L-Arap -

Compound **2** and **3** were obtained as inseparable mixture and gave only one spot on HPTLC but two peaks by HPLC (1:1). Attempts to separate **2** and **3** by preparative HPLC were unsuccessful. Acid hydrolysis of **2** and **3** yielded echinocystic acid and the sugar components 2-amino-2-deoxy-D-glucose, D-glucose, D-xylose and L-arabinose identified as mentioned for **1**. The FAB-mass spectrum of **2** and **3** exhibited a $[M+Na]^+$ at *m*/*z* 1124 corresponding to a molecular formula $C_{54}H_{87}NO_{22}$ and compatible with a tetrasaccharide glycoside containing an aglycone of molecular mass 472 and four sugar units, one 2-acetamido-deoxy hexose, one hexose and two pentoses. The ^{13}C NMR spectrum showed one set of signals due to echinocystic acid very similar to those of **1** but different signals due to the sugar part. Six anomeric carbon signals at δ 101.6, 102.3, 102.5, 104.9, 105.5 and 106.2 correlated in the HMQC spectrum with anomeric proton signals at δ 5.01 (*d*, *J* = 7.0 Hz), 5.08 (*d*, *J* = 5.0 Hz), 5.13 (*d*, *J* = 5.0 Hz), 5.04 (*d*, *J* = 8.0 Hz), 5.05 (*d*, *J* = 5.5 Hz) and 4.99 (*d*, *J* = 7.0 Hz), were observed. The HOHAHA, experiment of **2** and **3** allowed the sub-spectrum of each monosaccharide unit to be extracted from the crowded overlapped sugar region. Starting from the anomeric proton signals, the sequential assignments of the proton resonances to individual monosaccharides were achieved using ^1H–^1H COSY spectrum with the aid of ROE experiment. The sugar with anomeric proton signal at δ 5.04 (*J* = 8.0 Hz) was assigned to a 2-acetamido-2-deoxy-β-glucose and the sugar unit with anomeric proton signal at δ 5.01 was attributable to a β-glucose. The anomeric proton signals at δ 5.05, 5.08 and 5.13 were assigned to three L-arabinoses while the remaining anomeric signal at δ 4.99 was due to β-xylose. The anomeric centers of the sugars 2-acetamido-2-deoxy-glucose, glucose and xylose were each determined to have a β-configuration based on large $^3J_{H\text{-}1,H\text{-}2}$. The α-anomeric configuration of the arabinose was deduced from its $^3J_{H\text{-}1,H\text{-}2}$ values (5.0–5.5 Hz) expected for an α-arabinose in rapid $^4C_1 \leftrightarrow {}^1C_4$ conformational exchange. The HMQC spectrum allowed assignments of the sugar carbons by direct correlation with the assigned proton and showed that all the sugars were in pyranose form. It also showed the presence of 6-substituted 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNHAc), two 2-substituted α-L-arabinopyranose (Ara, Ara′), one terminal α-L-arabinopyranose (Ara″) and one terminal β-D-xylopyranose (Xyl). The sugar pattern of the 3-*O*-sugar chains in **2** and **3** were determined by the HMBC correlations. The cross peak correlated the signals of echinocystic acid H-3 (δ 3.36) and GlcNHAc C-1 (δ 104.9), H-6 and H-6′ of GlcNHAc (δ 4.19, 4.61) and Ara C-1 (δ 102.5) as well as Ara′ C-1 (δ 102.3) indicated that both **2** and **3** contained GlcNHAc unit (one set of NMR signals for **2** and **3**) attached to C-3 of echinocystic acid and substituted at C-2 by Ara unit in **2** and Ara′ unit in **3**. Also, cross peaks correlated the signals of Ara H-2 (δ 4.50) and Xyl C-1 (δ 106.2) as well as Ara′ H-2 (δ 4.52) and Ara″ C-1 (δ 105.5) revealed that the terminal Xyl unit was bound to C-2 of Ara unit in **2** while the terminal Ara″ unit was connected to C-2 of Ara′ in **3**. Accordingly the structure of 3-*O*-sugar chain of **2** and **3** were deduced as Xyl-(1 → 2)-Ara-(1 → 6)-GlcNHAc and Ara"-(1 → 2)-Ara′-(1 → 6)-GlcNHAc, respectively. The attachment of a remaining Glc unit (one set of NMR signals in **2** and **3)** to echinocystic acid C-16 in both **2** and **3**, was established from the cross peak correlated the signals of echinocystic acid H-16 (δ 5.34) and Glc C-1 (δ 101.6) and after considering the tetrasaccharide nature of **2** and **3** from the FABMS data. The close resemblance of sugar data of the 3-*O*-sugar chain in **2** and **3** (Tables 1 and 2) and the corresponding ones in **4** and **5** (Melek et al., 2007), lent further support to the sugar pattern in **2** and **3**. Therefore saponins **2** and **3** were assigned the structures of 3-*O*-β-D- xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid 16-*O*-β-D-glucopyranoside and 3-*O*-α-L-arabinopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid 16-*O*-β-D-glucopyranoside, respectively.

Oligosaccharide chains of triterpene glycosides with oleanane skeleton are usually attached at aglycone C-3 and or C-17 (via C-28). There are few cases in which short saccharide chains of one or two sugar residues only are attached at C-4, C-16, C-20, C-21 and C-22 (Vincken et al., 2007). Inspection of the literature revealed that glycosylation at aglycone C-16 was only observed in

Table 2
^{13}C NMR spectral data for compounds **1**–**3** in pyridine-d_5.

	1	2	3
Aglycone			
1	38.7	38.7	38.7
2	26.7	26.6	26.6
3	88.6	89.1	89.1
4	39.4	39.3	39.3
5	55.9	55.9	55.9
6	18.6	18.6	18.6
7	33.5	33.5	33.5
8	40.0	39.9	39.9
9	47.3	47.2	47.2
10	37.1	37.1	37.1
11	23.8	23.9	23.9
12	122.5	122.5	122.5
13	144.9	144.9	144.9
14	41.3	41.4	41.4
15	30.5	30.4	30.4
16	79.9	79.8	79.8
17	48.5	48.5	48.5
18	41.7	41.7	41.7
19	46.5	46.5	46.5
20	30.9	30.9	30.9
21	36.3	36.3	36.3
22	32.6	32.6	32.6
23	28.3	28.3	28.3
24	17.0	17.0	17.0
25	15.5	15.5	15.5
26	17.4	17.4	17.4
27	27.4	27.4	27.4
28	179.5	179.5	179.5
29	33.4	33.4	33.4
30	24.9	24.9	24.9
C-3-O-sugar			
GlcNHAc			
1	104.7	104.9	104.9
2	58.1	58.0	58.0
3	75.7	75.7	75.7
4	72.6	72.8	72.8
5	75.8	76.1	76.1
6	70.1	69.6	69.6
C=O	170.1	170.1	170.1
Me (CONH)	23.8	23.7	23.7
Gal			
1	103.8		
2	82.1		
3	75.3		
4	69.8		
5	76.8		
6	62.1		
Ara			
1		102.5	
2		80.2	
3		72.9	
4		67.4	
5		64.2	
Ara′			
1			102.3
2			79.4
3			72.9
4			67.7
5			64.6
Xyl			
1	106.8	106.2	
2	76.9	75.2	
3	77.3	77.8	
4	70.9	70.9	
5	67.0	67.2	
Ara″			
1			105.5
2			72.5
3			74.3
4			68.9
5			66.6
16-O-Glc			
1	101.7	101.6	101.6
2	75.8	75.7	75.7
3	79.4	79.4	79.4
4	72.4	72.4	72.4
5	78.1	78.1	78.1
6	63.3	63.3	63.3

a few saiko-saponins isolated from the Labiatae species *Clinopodium micranthum* (Yamamoto et al., 1993) and the Scrophulariaceae plant *Verbascum sinaiticum* (Miyase et al., 1997). Compounds **1**–**3** represent the first occurrence of triterpene 3,16-*O*-bisglycosides in the family Leguminosae. The co-existed metabolites **4** and **5** might represent interesting intermediates in the biosynthesis of glycosides **2** and **3**.

It is worth noting that triterpene glycosides with 2-acetamido-2-deoxy-β-D-glucopyranose as an inner glycosyl unit at C-3 of the triterpene moiety, have been reported before from the other Leguminosae members *Pithecellobium cubense* and *P. arboretum* (Ripperger et al., 1981), *Tetrapleura tetraptera* (Adesina and Reisch, 1985; Maillard et al., 1989) and *Entada phaseoloides* (Okada et al., 1987, 1988a,b).

The known compounds **6** and **7** were identified to be Pitheduloside G and Pitheduloside F from NMR data and comparison with the literature values (Nigam et al., 1997). Detailed ^{1}H NMR data of **6** and **7** are reported here for the first time (Table 3) based on 1D and 2D NMR analyses.

Glycosides **1**, **2** and **3**, **6**, **7** were assayed for their cytotoxic activities against the human tumor cell lines HEPG2, A549, HT29 and MCF7 using MTT method (Mosmann, 1983). In contrast to the cytotoxic glycosides with *N*-acetyl glucosamine unit **4** and **5** (16-*O*-deglucosylated derivatives of **2** and **3**), the glycosides **1**, **2** and **3** showed no cytotoxic effects against the used cell lines. The results showed the importance of the free hydroxyl group at C-16 of echinocystic acid for the cytotoxic activity. On the other hand, the oleanolic acid glycoside **7** showed no cytotoxic effects while the glycoside **6** exhibited only weak activity on HEPG2 cell line (57 μg/ml).

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. HPLC was performed on a Jasco system 800 instrument. GC analysis was carried out on Hitachi G-3000 gas chromatograph.

3.2. Plant material

The bark of *A. procera* (Benth.) was collected from the zoological garden in Giza, Egypt in June 2004. Plant identification was confirmed by Mrs. T. Labib, head specialist for plant identification in El-Orman public garden, Giza, Egypt. The Herbarium voucher specimen was deposited in the Herbarium of NRC (CAIRC).

3.3. Extraction and isolation

Air-dried and powdered bark (750 g) of *A. procera* was extracted twice with MeOH followed by once with 50% aqueous methanol.

The methanolic and aqueous methanolic extracts were combined and the solvent was evaporated to dryness under reduced pressure. The residue (27 g) was dissolved in water and the aqueous solution was passed through a porous polymer gel column (Mitsubishi Diaion HP-20). The column was then eluted with water, 50%, 75%, 85%, and 100% MeOH. The 85% MeOH and MeOH eluates were combined and the material from the combined fraction (5.8 g) was chromatographed on silica gel column eluted with $CHCl_3$–MeOH–H_2O (70:27:3 – 58:35:7) and finally with MeOH to give 11 fractions (A–K). Part (183 mg) of fraction A eluted with $CHCl_3$–MeOH–H_2O (70:27:3) was subjected to repeated HPLC [column ODS, 2 mm × 25 cm; solvent, CH_3CN–H_2O (20:80 – 25:75); flow rate, 6.5 ml/min; detection, UV, 205 nm] to give **1** (14 mg) and mixture of **2** and **3** (26 mg). Part (50 mg) of fraction F was subjected to HPLC [column, capcell pak Ph, 2 mm × 25 cm; solvent, CH_3CN–H_2O (32.5:67.5) + 0.05 TFA; flow rate, 9.5 ml/min; detection, UV, 205 nm] to afford **6** (14 mg) and **7** (10 mg).

Table 3
^{1}H NMR spectral data for compounds **6** and **7** in pyridin-d_5.

	6	7
Aglycone		
3	3.46 (*dd*, 12.0, 4.5)	3.41 (*dd*, 12.0, 4.5)
5	0.85 (*d*, 4.0)	0.86
12	5.44 (*brt*, 3.5)	5.45 (*brt*, 3.5)
16	1.95 (*dt*, 12.0, 3.0)	1.95
16′	2.18	2.18
18	3.27 (*dd*, 14.0, 4.0)	3.28 (*dd*, 14.0, 4.0)
19	1.28	1.27
19′	1.81	1.81
23	1.31 (*s*)	1.33 (*s*)
24	1.11 (*s*)	1.11 (*s*)
25	0.88 (*s*)	0.87 (*s*)
26	1.00 (*s*)	1.00 (*s*)
27	1.32 (*s*)	1.31 (*s*)
29	0.94 (*s*)	0.94 (*s*)
30	1.01 (*s*)	1.02 (*s*)
3-O-sugar		
Inner Glc		
1	4.88 (*d*, 7.0)	4.88 (*d*, 7.0)
2	4.24	4.24
3	4.24	4.23
4	4.10	4.13
5	3.99 (*m*)	3.96 (*m*)
6	4.20	4.21
6′	4.60 (*dd*, 11.5, 2.5)	4.58 (*dd*, 11.5, 2.5)
Inner Ara		
1	5.12 (*d*, 5.0)	5.08 (*d*, 5.0)
2	4.49 (*dd*, 5.0, 6.0)	4.52(*dd*, 5.0, 6.0)
3	4.36	4.33
4	4.31 (*brs*)	4.34 (*brs*)
5	3.75 (*dd*, 11.0, 2.5)	3.73 (*dd*, 11.0, 2.5)
5′	4.30	4.30
Xyl		
1	4.98 (*d*, 7.0)	
2	4.01 (*dd*, 7.0, 8.0)	
3	4.05 (*t*, 8.0)	
4	4.12	
5	3.61 (*dd*, 11.0, 10.0)	
5′	4.39 (*dd*, 11.0, 5.0)	
Terminal Ara		
1		5.04 (*d*, 6.5)
2		4.47
3		4.12
4		4.28 (*brs*)
5		3.71 (*dd*, 12.0, 2.0)
5′		4.41 (*dd*, 12.0, 3.5)
Terminal Glc		
1	5.35 (*d*, 7.5)	5.35 (*d*, 8.0)
2	4.09 (*dd*, 7.5, 8.0)	4.10 (*t*, 8.0)
3	4.22 (*t*, 8.0)	4.22 (*t*, 8.0)
4	4.31	4.33
5	3.92 (*m*)	3.91 (*m*)
6	4.30	4.31
6′	4.46	4.45

3.4. Saponin *1*

Amorphous powder $[\alpha]_D^{23}$ − 15.1 (*c* = 1.79, MeOH), FABMS *m*/*z*: 1154 $[C_{55}H_{89}NO_{23} + Na]^+$, ^{1}H and ^{13}C NMR (see Tables 1 and 2).

3.5. Saponins *2* and *3*

Amorphous powder, FABMS *m*/*z*: 1124 $[C_{54}H_{87}NO_{22} + Na]^+$, ^{1}H and ^{13}C NMR (see Tables 1 and 2).

3.6. Saponin *6*

Amorphous powder $[\alpha]_D^{23}$ + 7.0 (*c* = 1.50, MeOH), ^{1}H NMR (see Table 3).

3.7. Saponin *7*

Amorphous powder $[\alpha]_D^{23}$ + 11.9 (*c* = 1.01, MeOH), ^{1}H NMR (see Table 3).

3.8. General method for acid hydrolysis

A solution of 10 mg of saponin **1** (**2** and **3**) in 9 ml 2 N HCl and 9 ml CH_3OH was heated at 100 °C for 6 h. The mixture was left to cool, diluted with H_2O and extracted with $CHCl_3$. From the $CHCl_3$ layer, the aglycone was detected by TLC using $CHCl_3$–MeOH (10:1) and 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]. The H_2O layer was repeatedly diluted with CH_3OH and evaporated to dryness. 2-Amino-2-deoxy-D-glucose hydrochloride was obtained from the residue by PPC using BuOH–AcOH–H_2O (4:1:5, upper layer) and identified by comparing its PC R_f and $[\alpha]_D$ values with those of authentic sample. The other sugar components were detected from the H_2O Layer by GC analysis after being converted to their thiazolidine derivatives as described by Hara et al. (1986); conditions: [column, Supelco SPB™ −1, 0.25 mm × 27 m; column temperature 215 °C; carrier gas, N_2]. D-glucose, D-galactose and D-xylose were detected from **1** while D-glucose, D-xylose and L-arabinose were detected from **2** and **3**.

3.9. Cytotoxic assay

The procedure for the cytotoxic assay was performed by MTT assay as described by Mosmann (1983). In this study, the cell lines HEPG2, A549, HT29 and MCF7 were used.

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