Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 68 (2007) 1261–1266
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

# Triterpenoid saponins with *N*-acetyl sugar from the bark of *Albizia procera*

F.R. Melek [a,*], Toshio Miyase [b], N.S. Ghaly [a], Marian Nabil [a]

[a] *Chemistry of Natural Compounds Department, National Research Center, Dokki 12622, Giza, Egypt*
[b] *School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka 422-8526, Japan*

Received 6 November 2006; received in revised form 18 February 2007
Available online 3 April 2007

**Abstract**

Three (**1**,**2**,**4**) and one known (**3**) triterpenoid saponins were isolated from the bark of *Albizia procera*. The saponins were characterized as 3-*O*-[β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranosyl] echinocystic acid (**1**), 3-*O*-[α-L-arabinopyranosyl-(1 → 2)-β-D-fucopyranosyl-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranosyl] echinocystic acid (**2**) and 3-*O*-[β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranosyl] acacic acid lactone (**4**). Their structures were elucidated by 1D and 2D NMR experiments, FABMS as well as chemical means. Saponins **1** and **3** exhibited cytotoxicity against HEPG2 cell line with $IC_{50}$ 9.13 μg/ml and 10 μg/ml, respectively.


*Keywords:* *Albizia procera*; Leguminosae; Triterpenoid saponin; Echinocystic acid; Acacic acid lactone

## 1. Introduction

*Albizia procera* (Leguminosae) is a tree cultivated in streets and public gardens in Egypt. In folk medicine, the bark is considered useful in pregnancy and stomachache. It is also used as a medicine for water buffalo when given with salt.

As a part of our continuing interest in bioactive saponins from plants grown in Egypt (Miyase et al., 1996; Melek et al., 2002, 2003a,b, 2004a,b), we examined the saponin mixture from the bark of *A. procera* Benth. Previous phytochemical study on saponins from the seeds of this plant led to the isolation of four acylated triterpenoid saponins namely, proceraosides A–D (Yoshikawa et al., 1998). We present in this report the isolation and structure elucidation of four saponins **1**–**4** including three new ones from the bark of *A. procera*.

## 2. Results and discussion

The dried bark of *A. procera* was extracted twice with methanol followed by once with 50% aqueous methanol. The extracts were combined and the crude saponin mixture from the combined extract was subjected to column chromatography on a porous polymer polystyrene resin (Diaion HP-20) and silica gel followed by HPLC to give four saponins (**1**–**4**) including three new ones (**1**, **2**, **4**). The NMR data of the isolates are shown in Tables 1–3.

The positive ion FAB-mass spectrum of **1** exhibited a quasi-molecular ion peak $[M+Na]^+$ at *m*/*z* 962, corresponding to a molecular formula $C_{48}H_{77}NO_{17}$. The $^1H$ NMR spectrum of **1** showed signals due to seven tertiary methyl groups at δ 0.87, 0.98, 1.02, 1.06, 1.18, 1.19 and 1.87. Further feature were signals at δ 5.59 (*br t*, $J = 3.0$ Hz) ascribable to a vinylic proton and at δ 3.42 (*dd*, $J = 12.0$, 4.0 Hz) typical for an axial proton attached to a hydroxylate carbon. The $^{13}C$ NMR spectrum of **1** displayed two signals located at δ 122.5 and 145.1 which confirmed the presence of a double bond and a signal at δ

* Corresponding author.
*E-mail address:* frmelek@link.net (F.R. Melek).

0031-9422/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2007.02.023

Table 1
$^1$H NMR spectral data for compounds **1**–**4** in pyridin-$d_5$

| | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| *Aglycone* | | | | |
| 3 | 3.42(*dd*, 12.0, 4.0) | 3.46(*dd*, 12.5, 4.5) | 3.37(*dd*, 12.0, 5.0) | 3.43(*dd*, 11.5, 4.0) |
| 5 | 0.89 | 0.93(*d*, 12.0) | 0.89 | 0.98 |
| 12 | 5.59(*br t*, 3.0) | 5.58(*br t*, 3.0) | 5.61(*br t*, 2.5) | 5.30(*br t*, 3.0) |
| 16 | 5.23(*br s*) | 5.23(*br s*) | 5.25(*br s*) | 4.52(*brs*) |
| 18 | 3.59 | 3.59(*dd*, 14.0, 3.0) | 3.60(*dd*, 15.0, 3.0) | 2.77(*dd*, 17.0, 5.5) |
| 19 | 1.38 | 1.37 | 1.37 | 1.37 |
| 19′ | 2.80(*t*, 13.0) | 2.79(*t*, 13.0) | 2.81(*t*, 14.0) | 1.82 |
| 21 | 1.38 | 1.37 | 1.37 | 4.23(*d*, 5.0) |
| 21′ | 2.50(*dt*, 13.0, 4.0) | 2.50 | 2.50 | |
| 23 | 1.19(*s*) | 1.22(*s*) | 1.19(*s*) | 1.21(*s*) |
| 24 | 0.98(*s*) | 1.01(*s*) | 0.98(*s*) | 0.99(*s*) |
| 25 | 0.87(*s*) | 0.87(*s*) | 0.86(*s*) | 0.82(*s*) |
| 26 | 1.02(*s*) | 1.03(*s*) | 1.03(*s*) | 0.80(*s*) |
| 27 | 1.87(*s*) | 1.86(*s*) | 1.85(*s*) | 1.38(*s*) |
| 29 | 1.06(*s*) | 1.05(*s*) | 1.07(*s*) | 0.92(*s*) |
| 30 | 1.18(*s*) | 1.19(*s*) | 1.18(*s*) | 1.07(*s*) |
| *3-O-sugar GlcNHAc* | | | | |
| 1 | 5.03(*d*, 8.0) | 5.05(*d*, 8.0) | 5.03(*d*, 7.5) | 5.05(*d*, 8.0) |
| 2 | 4.52(*t*, 8.5) | 4.56(*t*, 8.5) | 4.53(*t*, 9.0) | 4.51(*t*, 9.0) |
| 3 | 4.35(*t*, 8.5) | 4.33(*t*, 8.5) | 4.33(*t*, 9.0) | 4.34(*t*, 9.0) |
| 4 | 4.11 | 4.21(*brt*, 8.5) | 4.13(*dd*, 9.0, 8.0) | 4.11 |
| 5 | 4.03(*m*) | 4.03(*m*) | 4.02(*m*) | 4.02(*m*) |
| 6 | 4.19(*dd*, 11.0, 4.0) | 4.36(*dd*, 11.0, 4.0) | 4.22(*dd*, 11.5, 4.0) | 4.19(*dd*, 10.0, 4.0) |
| 6′ | 4.62(*d*, 11.0) | 4.70(*d*, 11.0) | 4.61(*dd*, 11.5, 2.5) | 4.64(*d*, 10.0) |
| Me(CONH) | 2.14(*s*) | 2.14(*s*) | 2.13(*s*) | 2.15(*s*) |
| NH | 8.80(*d*, 9.0) | 8.80(*d*, 9.0) | 8.79(*d*, 9.0) | 8.80(*d*, 9.0) |
| *Ara* | | | | |
| 1 | 5.13(*d*, 5.0) | | 5.08(*d*, 5.0) | 5.14(*d*, 5.0) |
| 2 | 4.50(*t*, 6.5) | | 4.52(*t*, 6.0) | 4.50(*t*, 6.5) |
| 3 | 4.37(*dd*, 6.5, 4.0) | | 4.33(*dd*, 6.0, 3.5) | 4.36(*dd*, 6.5, 4.0) |
| 4 | 4.37(*brs*) | | 4.33(*brs*) | 4.36(*brs*) |
| 5 | 3.75(*d*, 11.0) | | 3.73(*dd*, 12.0, 2.0) | 3.75(*dd*, 11.5, 2.0) |
| 5′ | 4.28(*dd*, 11.0, 5.0) | | 4.30(*dd*, 1 2.0, 4.0) | 4.28(*dd*, 11.5, 5.0) |
| *Fuc* | | | | |
| 1 | | 4.94(*d*, 7.5) | | |
| 2 | | 4.47(*t*, 8.0) | | |
| 3 | | 4.12(*dd*, 8.0, 3.5) | | |
| 4 | | 3.99(*d*, 3.5) | | |
| 5 | | 3.75(*q*, 6.5) | | |
| 6 | | 1.48(*d*, 6.5) | | |
| *Xyl* | | | | |
| 1 | 4.98(*d*, 7.0) | | | 4.98(*d*, 7.0) |
| 2 | 4.00(*t*, 8.0) | | | 4.00(*t*, 7.5) |
| 3 | 4.05(*t*, 8.0) | | | 4.05(*t*, 8.0) |
| 4 | 4.11 | | | 4.11 |
| 5 | 3.58(*dd*, 12.0, 10.0) | | | 3.59(*dd*, 12.0, 10.0) |
| 5′ | 4.38(*dd*, 12.0, 6.0) | | | 4.38(*dd*, 12.0, 6.0) |
| *Ara* | | | | |
| 1 | | 5.22(*d*, 6.0) | 5.05(*d*, 5.5) | |
| 2 | | 4.50(*t*, 6.5) | 4.47(*dd*, 6.5, 6.0) | |
| 3 | | 4.13(*dd*, 7.0, 3.5) | 4.10(*dd*, 7.5, 3.0) | |
| 4 | | 4.24(*brs*) | 4.25(*brs*) | |
| 5 | | 3.64(*dd*,11.0, 2.5) | 3.67(*dd*, 12.5, 2.0) | |
| 5′ | | 4.48(*dd*,11.0, 3.0) | 4.40(*dd*, 12.5, 3.0) | |

Values in parantheses are $^1$H–$^1$H splitting. GlcNHAc, 2-acetamido-2-deoxy-β-D-glucopyranose; Ara, α-L-arabinopyranose; Fuc, β-D-fucopyranose; Xyl, β-D-xylopyranose.

180.0, suggesting the occurrence of COOH group. The spectrum also contained three signals at $\delta$ 102.4, 104.9 and 106.3 assigned to anomeric carbons of three sugar units. On the basis of the forgoing data, **1** was concluded to be a triterpene triglycoside with a triterpene acid moiety of oleanene skeleton. The downfield position of the axial

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

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| *Aglycone* | | | | |
| 1 | 38.8 | 38.8 | 38.8 | 38.7 |
| 2 | 26.7 | 26.6 | 26.6 | 26.7 |
| 3 | 89.1 | 89.2 | 89.2 | 88.7 |
| 4 | 39.4 | 39.4 | 39.4 | 39.4 |
| 5 | 56.0 | 55.9 | 55.9 | 56.0 |
| 6 | 18.7 | 18.6 | 18.6 | 18.5 |
| 7 | 33.6 | 33.5 | 33.6 | 32.6 |
| 8 | 40.0 | 40.0 | 40.0 | 40.4 |
| 9 | 47.3 | 47.4 | 47.3 | 47.4 |
| 10 | 37.2 | 37.1 | 37.1 | 37.1 |
| 11 | 23.9 | 23.9 | 23.9 | 23.8 |
| 12 | 122.5 | 122.5 | 122.5 | 124.6 |
| 13 | 145.1 | 145.1 | 145.1 | 140.2 |
| 14 | 42.2 | 42.1 | 42.1 | 43.4 |
| 15 | 36.2 | 36.3 | 36.3 | 38.2 |
| 16 | 74.8 | 74.8 | 74.8 | 66.7 |
| 17 | 49.0 | 48.9 | 48.9 | 50.0 |
| 18 | 41.5 | 41.4 | 41.5 | 41.8 |
| 19 | 47.4 | 47.2 | 47.4 | 42.9 |
| 20 | 31.1 | 31.1 | 31.1 | 34.2 |
| 21 | 36.3 | 36.2 | 36.2 | 83.5 |
| 22 | 32.9 | 32.9 | 32.8 | 27.2 |
| 23 | 28.3 | 28.2 | 28.2 | 28.1 |
| 24 | 17.1 | 17.1 | 17.1 | 17.0 |
| 25 | 15.6 | 15.6 | 15.6 | 15.7 |
| 26 | 17.6 | 17.5 | 17.5 | 16.3 |
| 27 | 27.4 | 27.3 | 27.3 | 28.9 |
| 28 | 180.0 | 180.0 | 180.0 | 181.2 |
| 29 | 33.4 | 33.4 | 33. 4 | 28.6 |
| 30 | 24.9 | 24.8 | 24.8 | 24.3 |

Table 3
$^{13}C$ NMR spectral data for the sugar moieties of compounds **1**–**4** in pyridine-$d_5$

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| *C-3-O-sugar GlcNHAC* | | | | |
| 1 | 104.9 | 104.9 | 104.9 | 104.8 |
| 2 | 58.0 | 58.0 | 58.0 | 58.1 |
| 3 | 75.8 | 75.8 | 75.7 | 75.7 |
| 4 | 73.0 | 72.1 | 72.8 | 73.0 |
| 5 | 76.2 | 76.6 | 76.1 | 76.4 |
| 6 | 69.6 | 70.0 | 69.6 | 69.5 |
| C=O | 170.3 | 170.2 | 170.2 | 170.2 |
| Me(CONH) | 23.7 | 23.7 | 23.7 | 23.7 |
| *Ara* | | | | |
| 1 | 102.4 | | 102.5 | 102.4 |
| 2 | 80.5 | | 79.5 | 80.5 |
| 3 | 72.6 | | 72.9 | 72.7 |
| 4 | 67.5 | | 67.7 | 67.5 |
| 5 | 64.3 | | 64.6 | 64.4 |
| *Fuc* | | | | |
| 1 | | 103.5 | | |
| 2 | | 80.4 | | |
| 3 | | 75.5 | | |
| 4 | | 72.5 | | |
| 5 | | 71.4 | | |
| 6 | | 17.1 | | |
| *Xyl* | | | | |
| 1 | 106.3 | | | 106.3 |
| 2 | 75.4 | | | 75.4 |
| 3 | 77.9 | | | 77.9 |
| 4 | 70.9 | | | 70.9 |
| 5 | 67.3 | | | 67.3 |
| *Ara* | | | | |
| 1 | | 105.6 | 105.6 | |
| 2 | | 73.1 | 72.8 | |
| 3 | | 74.2 | 74.3 | |
| 4 | | 68.4 | 68.9 | |
| 5 | | 66.0 | 66.6 | |

group at C-14 (Me-27, $\delta$ 1.87) in the $^1H$ NMR spectrum, implied an additional axial (α) hydroxyl group at C-16. The assignments of the proton and the carbon resonances of the triterpene moiety which were secured by $^1H$–$^1H$ COSY, HMQC and HMBC experiments, allowed identification of this moiety as echinocystic acid. The NMR data due this moiety were in agreement with those reported for similar triterpene saponins (Carpani et al., 1989; Orsini et al., 1991). Glycosylation of the alcoholic function at C-3 was indicated by the downfield position of this carbon resonance ($\delta$ 89.1) with reference to the corresponding signal in echinocystic acid. Acid hydrolysis of **1** afforded the sugar components 2-amino-2-deoxy-D-glucose, L-arabinose and D-xylose.

The sugar portion of **1** contained in the $^1H$ NMR spectrum three anomeric proton signals. Analysis of $^1H$–$^1H$ COSY spectrum allowed the sequential assignments of the proton resonances to the individual monosaccharides as reported in Table 1. The sugar unit with anomeric signal at $\delta$ 5.03 (*d*, $J = 8.0$ Hz) was assigned to a 2-acetamido-2-deoxy-β-glucose unit. The characteristic signals were at $\delta$ 8.80 (*d*, $J = 9.0$ Hz, NHAc) and at $\delta$ 2.14 (*s*, NHCO–$CH_3$) (Ripperger et al., 1981; Carpani et al., 1989; Orsini et al., 1991; Abdel-Kader et al., 2001). The HMBC cross peak between the proton signal at $\delta$ 4.52 (H-2) and the carbonyl signal at $\delta$ 170.3 (NH–CO–$CH_3$) supported the assignment. The sugar units with anomeric proton signals at $\delta$ 4.98 (*d*, $J = 7.0$ Hz) and $\delta$ 5.13 (*d*, $J = 5.0$ Hz) were attributable to a xylose and an arabinose units, respectively. The anomeric centers of the 2-acetamido-2-deoxy glucose and the xylose units were each determined to have a β-configuration based on large $^3J_{H\text{-}1,H\text{-}2}$ values. The α-anomeric configuration of the arabinose unit was deduced from its $^3J_{H\text{-}1,H\text{-}2}$ value (5.0 Hz) expected for an α-arabinose in rapid $^4C_1 \rightarrow {}^1C_4$ conformational exchange. The HMQC experiment of **1** which correlated all the proton resonances with the resonances of their directly attached carbons allowed assignments of the sugar carbons and showed that all the sugars were in the pyranose form. It also permitted the assignments of the interglycosidic linkages by comparison of the observed carbon chemical shifts with those of the corresponding methylpyranosides (Seo et al., 1978). The absence of any $^{13}C$ NMR glycosylation shift for the β-D-xylopyranosyl unit (xyl) suggested that this sugar was a terminal unit. Glycosylation shifts were observed for C-6 ($\delta$ 69.6) of the 2-acetamido-2-deoxy-β-D-glucopyranosyl unit (GlcNHAc) and C-2

($\delta$ 80.5) of the α-L-arabinopyranosyl unit (Ara). The positions of the sugar residues in the trisaccharide chain were unambiguously defined by the HMBC experiment. The cross peak due to a long-range correlation between the echinocystic acid C-3 ($\delta$ 89.1) and GlcNHAc H-1 ($\delta$ 5.03) indicated that this hexose residue was the sugar unit directly linked to the C-3 of the echinocystic acid moiety. The cross peak observed between GlcNHAc C-6 ($\delta$ 69.6) and Ara H-1 ($\delta$ 5.13) established the connectivity between the two sugar units. Similarly, the cross peak between Ara C-2 ($\delta$ 80.5) and xyl H-1 ($\delta$ 4.98) confirmed the terminal position of the xylose unit in the trisaccharide chain. Thus, compound **1** was assigned the structure of 3-*O*-[β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranosyl] echinocystic acid.

The FAB-mass spectrum of **2**, in the positive ion mode exhibited a quasi-molecular ion peak at *m/z* 976 $[M+Na]^+$ corresponding to a molecular formula $C_{49}H_{79}NO_{17}$. The $^1H$ and $^{13}C$ NMR spectra indicated that **2** was also a triterpenoid saponin with the same triterpene moiety as that of **1** (echinocystic acid) but differed in the structure of the sugar part. The presence of three sugar units was deduced from the observed three anomeric proton doublets in the $^1H$ NMR spectrum, at $\delta$ 4.94 ($J = 7.5$ Hz), $\delta$ 5.05 ($J = 8.0$ Hz) and $\delta$ 5.22 ($J = 6.0$ Hz), in addition to a methyl doublet at $\delta$ 1.48 ($J = 6.5$ Hz) which suggested the occurrence of one deoxyhexose unit. From the assigned NMR signals associated with the triterpene moiety and derived from the combined use of $^1H$–$^1H$ COSY, HMQC and HMBC spectra, it was apparent that the three sugars were present in one trisaccharide chain bounded to C-3 of the triterpene moiety. The 1D and 2D NMR studies allowed assignments of the sugar protons and carbons and showed the presence of a terminal α-L-arabinopyranose, a 2-substituted β-D-fucopyranose (Fuc) and a 6-substituted 2-acetamido-2-deoxy-β-D-glucopyranose units. As expected, acid hydrolysis afforded 2-amino-2-deoxy-D-glucose, D-fucose and L-arabinose. The positions of the sugar units in the trisaccharide chain were determined from the HMBC correlations between Ara H-1 ($\delta$ 5.22) and Fuc C-2 ($\delta$ 80.4), Fuc H-1 ($\delta$ 4.94) and GlcNHAc C-6 ($\delta$ 70.0), GlcNHAc H-1 ($\delta$ 5.05) and echinocystic acid C-3 ($\delta$ 89.2). Therefore, **2** was formulated as 3-*O*-[α-L-arabinopyranosyl-(1 → 2)-β-D-fucopyranosyl-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranosyl] echinocystic acid.

Compound **3** showed in its FAB-mass spectrum $[M+Na]^+$ peak at *m/z* 962, consistent with a molecular formula $C_{48}H_{77}NO_{17}$, and identical to that of **1**. The NMR data indicated that **3** was also an echinocystic acid 3-*O*-glycoside identified as 3-*O*-[α-L-arabinopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranosyl] echinocystic acid. Although the structure of **3** was reported before from *A. anthelmintica* (Carpani et al., 1989) we listed here $^1H$ NMR data for the first time and $^{13}C$ NMR data based on 2D NMR ($^1H$–$^1H$ COSY, HMQC, HMBC) analysis.

Compound **4** showed in its FAB-mass spectrum, a quasi-molecular ion peak $[M+Na]^+$ at *m/z* 960, corresponding to a molecular formula $C_{48}H_{75}NO_{17}$. The presence of an absorption band in the IR spectrum of **4** at 1761 $cm^{-1}$ suggested the occurrence of a γ-lactone ring. The $^{13}C$ NMR spectrum of **4** contained signals due to the triterpene moiety very similar to their corresponding in acacic acid lactone 3-*O*-glycosides previously isolated from the bark of *A. lebbek* (Pal et al., 1995) and after alkaline hydrolysis of the stem bark of *A. julibrissin* (Ikeda et al., 1997) as well as from the seeds of *A. prosera* (Yoshikawa et al., 1998). The assignments of the proton and the carbon resonances were established by NMR analysis ($^1H$–$^1H$ COSY, HMQC and HMBC). The presence of three sugar units identified as 6-substituted-2-acetamido-2-deoxy-β-D-glucopyranosyl [H-1: $\delta$ 5.05 (*d*, $J = 8.0$ Hz), C-1: $\delta$ 104.8], 2-substituted-α-L-arabinopyranosyl unit [H-1: $\delta$ 5.14 (*d*, $J = 5.0$ Hz), C-1: $\delta$ 102.4] and a terminal β-D-xylopyranosyl unit [H-1: $\delta$4.98 (*d*, $J = 7.0$ Hz), C-1: $\delta$ 106.3] was deduced from the combined use of 1D and 2D NMR spectra and after acid hydrolysis. The sugar sequence and the sites of attachment were found identical to those of compound **1** after considering the HMBC correlations. Consequently, the structure of **4** was concluded to be 3-*O*-[β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranosyl] acacic acid lactone.

It is worth noting that saponins 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 bark of *A. anthelmintica* (Carpani et al., 1989) and the seeds of *A. lucida* (Orsini et al., 1991) as well as the stems of *A. subdimidiata* (Abdel-Kader et al., 2001) The occurrence of saponins with the same sugar unit, was also reported from the other leguminosae members *Pithecellobium cubense*, *P. arboretum* (Ripperger et al., 1981) and *Tetrapleura tetraptera* (Maillard et al., 1989).

Saponins **1**–**4** were evaluated in vitro for their cytotoxic activity against HEPG2 cell line. Saponins **1** and **3** exhibited cytotoxic effect with $IC_{50}$ 9.13 μg/ml and 10.0 μg/ml, respectively. The rest of compounds were found inactive. The moderately active echinocystic acid saponins **1** and **3** possessed an arabinose unit attached to C-6 of the glucosamine moiety. The inactive saponin **2** was different from **1** and **3** only in the nature of the sugar attached to the glucosamine C-6 moiety being fucose instead of arabinose. The glycosidic parts of **1** and **3** were identical to those of *A. subdimidiata* saponins with oleanolic acid as the triterpene moiety, and showed strong cytotoxicity against A2780 mammalian cell line (Abdel-Kader et al., 2001).

The inactive Saponin **4** contained acacic acid lactone as the triterpene moiety and glycosidic units identical to those of **1** and **3**. The prosapogenins with the same triterpene moiety prepared by alkaline hydrolysis of *Albizia julibrissin* extract were also found inactive against KB cell line (Ikeda et al., 1997). Therefore, it was concluded that the presence of the lactone ring in the triterpene moiety, induced an inhibition of the cytotoxic activity and that an arabinose moiety attached to the position 6 of the glucosamine unit seems to be better than a fucose unit.

## 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 $\delta$-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 the 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 (400 mg) of fraction D (711 mg), eluted with $CHCl_3$–MeOH–$H_2O$ (70:27:3), was subjected to repeated HPLC [ column, ODS, 3 mm × 50 cm; solvent, $CH_3OH$–$H_2O$ (65:35–80:20) + 0.05 TFA; flow rate, 9.5 ml/min; detection, UV 205 nm] to give **1** (72 mg), **2** (13 mg), **3** (23 mg) and **4** (24 mg).

### 3.4. Saponin (*1*)

Amorphous powder $[\alpha]_D^{23} - 0.3$ ($C = 2.20$, MeOH), FAB-MS *m/z*: 962 $[C_{48}H_{77}NO_{17}+Na]^+$, $^1H$ and $^{13}C$ NMR (see Tables 1–3).

### 3.5. Saponin (*2*)

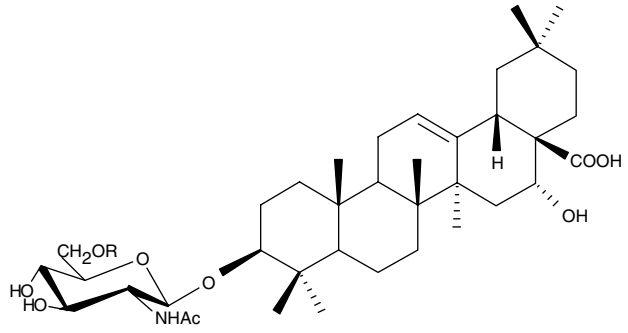
Amorphous powder $[\alpha]_D^{23} + 5.7$ ($C = 1.88$, MeOH), FAB-MS *m/z*: 976 $[C_{49}H_{79}NO_{17}+Na]^+$, $^1H$ and $^{13}C$ NMR (see Tables 1–3).

### 3.6. Saponin (*3*)

Amorphous powder $[\alpha]_D^{23} + 5.0$ ($C = 2.40$, MeOH), FAB-MS *m/z*: 962 $[C_{48}H_{77}NO_{17}+Na]^+$, $^1H$ and $^{13}C$ NMR (see Tables 1–3).

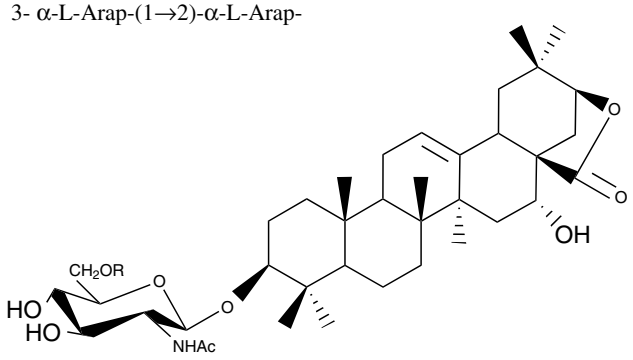
### 3.7. Saponin (*4*)

Amorphous powder $[\alpha]_D^{23} - 9.7$ ($C = 1.55$, MeOH), FAB-MS *m/z*: 960 $[C_{48}H_{75}NO_{17}+Na]^+$, $^1H$ and $^{13}C$ NMR (see Tables 1–3).




R

1- β-D-Xylp-(1→2)-α-L-Arap-
2- α-L-Arap-(1→2)-β-D-Fucp-
3- α-L-Arap-(1→2)-α-L-Arap-




R

4- β-D-Xylp-(1→2)-α-L-Arap-

### 3.8. General method for acid hydrolysis

Each saponin (10 mg) in 9 ml 2 N HCl and 9 ml $CH_3OH$ was heated at 100 °C for 6 h. The mix. was left to cool, diluted with $H_2O$ and extracted with $CHCl_3$. The aq. 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 by GC analysis of their thiazolidine derivatives as previously described (Melek et al., 2002, 2003a,b).

### 3.9. Cytotoxic assay

The procedure for the cytotoxic assay was performed by sulphorhodamine B (SRB) assay as described by Skehan et al. (1990). In this study, the cell line HEPG2 (liver carcinoma cell line) was used.

## References


Abdel-Kader, M., Hoch, J., Berger, J.M., Evans, R., Miller, J.S., Wisse, J.H., Mamber, S.W., Dalton, J.M., Kingston, D.G.I., 2001. Two bioactive saponins from *Albizia subdimidiata* from the Suriname rainforest. J. Nat. Prod. 64, 536–539.

Carpani, G., Orsini, F., Sisti, M., Verotta, L., 1989. Saponins from *Albizia anthelmintica*. Phytochemistry 28, 863–866.

Ikeda, T., Fujiwara, S., Araki, K., Kinjo, J., Nohara, T., Miyoshi, T., 1997. Cytotoxic glycosides from *Albizia julibrissin*. J. Nat. Prod. 60, 102–107.

Maillard, M., Adewunmi, C.O., Hostettman, K., 1989. New triterpenoid *N*-acetyl glycosides with molluscicidal activity from *Tetrapleura tetraptera* TAUB. Helv. Chim. Acta 72, 668–674.

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., 2003a. Triterpenoid saponins from *Meryta lanceolata*. Phytochemistry 62, 557–562.

Melek, F.R., Miyase, T., Abdel-Khalik, S.M., El-Gindi, M.R., 2003b. Triterpenoid saponins from *Schefflera arboricola*. Phytochemistry 63, 401–407.

Melek, F.R., Miyase, T., Ghaly, N.S., Yousif, M.F., 2004a. Further saponins from *Meryta lanceolata*. Phytochemistry 65, 909–914.

Melek, F.R., Miyase, T., Abdel-Khalik, S.M., Mahmoud, I.I., Mina, S.A., 2004b. Saponins and acylated saponins from *Dizygotheca kerchoveana*. Phytochemistry 65, 3089–3095.

Miyase, T., Melek, F.R., El-Gindi, O.D., Abdel-Khalik, S.M., El-Gindi, M.R., Haggag, M.Y., Hilal, S.H., 1996. Saponins from *Fagonia arabica*. Phytochemistry 41, 1175–1179.

Orsini, F., Pelizzoni, F., Verotta, L., 1991. Saponins from *Albizia lucida*. Phytochemistry 30, 4111–4115.

Pal, B.C., Achari, B., Yoshikawa, K., Arihara, S., 1995. Saponins from *Albizia lebbeck*. Phytochemistry 38, 1287–1291.

Ripperger, H., Preiss, A., Schmidt, J., 1981. *O*(3)-(2-acetylamino-2-deoxy-β-D-glucopyranosyl)-oleanolic acid, A novel triterpenoid glycoside from two *Pithecellobium* species. Phytochemistry 20, 2434–2435.

Seo, S., Tomita, Y., Tori, K., Yoshimura, Y., 1978. Determination of the configuration of secondary hydroxyl group in a chiral secondary alcohol using glycosidation shifts in carbon-13 nuclear magnetic resonance spectroscopy. J. Am. Chem. Soc. 100, 3331–3339.

Skehan, P., Storenge, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S., Boyd, M.R., 1990. New colorimetric cytotoxicity assay for anticancer drug screening. J. Natl. Cancer Inst. 82, 1107–1112.

Yoshikawa, K., Satou, Y., Tokunaga, Y., Tanaka, M., Arihara, S., Nigam, S.K., 1998. Four acylated triterpenoid saponins from *Albizia procera*. J. Nat. Prod. 61, 440–445.