BISDESMOSIDIC SAPONINS FROM FRUITS OF *GLEDITSIA CASPICA* DESF.

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ABSTRACT

The isolation and characterization of three oleanane type triterpenoid saponins Gleditsia saponins C' and E', and Gleditsioside I from the methanol extract of fruits of *Gleditsia caspica* Desf. are described. Their structures were determined by 1D and 2D (H1-H1 COSY, HMQC, HMBC) NMR analysis and by acid and alkaline hydrolysis.

Keywords: *Gleditsia caspica*, Triterpenoid saponins, Gleditsiosides, Gleditsia saponins.

RESUMEN

En este trabajo se describe el aislamiento y la caracterización de tres saponinas triterpénicas tipo oleanano, Saponinas Gleditsia C y E y Gleditsiosido I del extracto metanólico de los frutos de *Gleditsia caspita* Desf. Sus estructuras se determinaron por el análisis de RMN de una y dos dimensiones (H^1-H^1COSY, HMQC, HMBC) y por hidrólisis ácida y alcalina.

Palabras clave: *Gleditsia caspica*, saponinas triterpenoides, Gleditsiosidos, saponinas Gleditsia

INTRODUCTION

Genus *Gleditsia* (Family Fabaceae) comprises 14 species of deciduous trees (Huxley *et al.*, 1992). *Gleditsia caspica* (Caspian Locust), a tree that grows up to 12 meters, is grown in public gardens in Egypt mainly for ornamental purposes due to its graceful habit, elegant form and delicate fern-like foliage.

Gleditsia species have been widely used in folk medicine in Egypt-Asia. The anomalous fruits (fruits without seeds) produced by old or injured plants of *Gleditsia sinensis* Lam., have long been used in traditional Chinese medicine. The thorns of *Gleditsia sinensis* have been used for the treatment of carbuncle, scabies and suppurative skin diseases. While the mature pods and anomalous fruits are mainly used for trea-

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ting apoplexy, headache, productive cough and asthma. Also, dried *Gleditsia japonica* Miq. fruits have long been known in oriental medicine as diuretic and expectorant (Jiangsu New Medical College, 1977).

Saponins, the main constituents of *Gleditsia* fruits, were previously reported from fruits of different *Gleditsia* species. Triacanthosides A_1, G and C together with Gleditschosides A, B, C, D and E were isolated from *Gleditsia triacanthos*. Triacanthosides G and C were 3, 28-*O*-bisdesmosides while the rest compounds were all 3-*O*-monodesmosides (Badalbaeva *et al.*, 1972a,b, 1973a,b). Gleditsia saponins B, C, D_2, G, and I , isolated from the fruits of *Gleditsia japonica*, were characterized as echinocystic acid 3,28-O-bisglycosides acylated with two monoterpene carboxylic acids (Konoshima and Sawada, 1982a,b).

Fourteen bisdesmosidic triterpenoidal saponins were isolated from the fruits of *Gleditsia sinensis* (Zhang *et al.*, 1999a,b,c,d). Gleditsiosides A, B, C, D, Q acylated with one monoterpenic unit; Gleditsiosides E, F, G, N, O acylated with two units. Gleditsiosides H, I, J, K lacked the presence of any monoterpenic unit. Gleditsioside P was the only saponin that contained three monoterpenic units. The bisdesmosides Gleditsia saponin C' and Glediststides A and B were isolated from *Gleditsia delavayi* Franch. (Teng *et al.*, 2002a,b).

Crude extracts, partition fractions and purified compounds from *Gleditsia* members exhibited variable biological activities. Saponins isolated from *Gleditsia japonica* showed antiulcerogenic and anti-inflammatory activities (Yamahara *et al.,* 1975). The aqueous extract of *G. sinensis* thorns was suggested to be used in the managing of mast cell-dependent anaphylaxis (Shin and Kim, 2000). The 70% ethanolic extract of the anomalous fruits of *G. sinensis* possessed anti-allergic and anti-inflammatory activities (Dai *et al.*, 2002). The *n*-butanolic fraction from the anomalous fruits of *Gleditsia sinensis* LAM. was reported as a possible clinically effective agent in alleviating the nasal symptoms of allergic rhinitis, proba-bly by inhibiting both histamine release from mast cells and nasal vascular permea-bility (Fu *et al.*, 2003). Also, the saponin pool from anomalous fruits of *Gleditsia sinensis* was suggested to be a candidate novel therapeutic agent for rheumatoid arthritis (Hou *et al.*, 2006).

Gleditsia saponin C, isolated from fruits of *G. japonica*, was reported to be the first example of triterpene saponins to demonstrate significant anti-HIV activity (Konoshima *et al.*, 1995). 2β-carboxyl-3β-hydroxyl-norlupA (1)-20 (29)-en-28-oic acid, a lupane acid isolated from stings of *G. sinensis,* also showed strong anti-HIV activity (Li *et al.*, 2007).

The ethanolic extract of stings of *G. sinensis* exhibited antitumour activity on mice bearing uterine cervical carcinoma (U14) (Long *et al.*, 2006). The methanolic extract of the fruits of *G. triacanthos* exerted moderate oncostatic activity against sarcoma 180 and Ehrlich carcinoma at the total dose 350 mg/kg b.wt. per mouse (Sokoloff *et al.,* 1964). Triacanthine from the leaves of *G. triacanthos* was found highly toxic (LD_{50} 35 mg/kg) and of questionable oncostatic activity. *Gleditsia sinensis* fruit extract (GSE) exhibited cytotoxic activity in vitro against breast cancer (Campbell *et al.*, 2002) and nasopharyngeal carcinoma (Chen and Zhao, 1996). Also GSE could be potentially used as a chemotherapeutic drug to treat patients with acute and chronic myelogenous leukemia (Chow *et al.*, 2003a). Later investigations suggested that GSE could be potentially used as an angiogenic inhibitor in both solid tumour and leukaemia therapy (Chow *et al.*, 2003b), as well as a novel anticancer agent for esophageal squamous cell carcinoma (ESCC). The means of MTS_{50} of GSE for the ESCC cell lines and non-tumor NIH 3T3 cells were 21 and 163 μg/ml, respectively (Tang *et al.*, 2007).

Thirteen saponins were isolated from the anomalous fruits of *Gleditsia sinensis* on the basis of bioassay-guided fractionation. These saponins were tested for their cytotoxicities against six tumor cell lines (HL-60, MCF-7, Bel-7402, BGC-823, HeLa and KB cells) by the MTT method. The tumor cell lines showed different drug sensitivities with HL-60 being the most sensitive. The structure activity relationships of these saponins in cytotoxicity and induction of apoptosis were studied (Zhong *et al.*, 2004). In conclusion, the monoterpene units at the C-28 oligosaccharide chain was crucial for the cytotoxicity. The α-hydroxyl group at the C-16 position increased the cytotoxicity.

As a part of our continuous interest in bioactive saponins from plants growing in Egypt (Melek *et al.*, 2002, 2003a,b, 2004a,b, 2007) we describe here the isolation and structure elucidation of three saponins from *Gleditsia caspica* fruits.

MATERIALS AND METHODS

Plant material

Fruits of *Gleditsia Caspica* Desf. were collected from El-Orman public garden, Giza, Egypt in November 2006. Plant identification was confirmed by Mrs. T. Labib, head specialist for plant identification in El-Orman public garden, Giza, Egypt. A voucher specimen was deposited in the Herbarium of National Research Centre (CAIRC), Cairo, Egypt. Voucher Number 221.

Instrumentation

The NMR spectra were measured on a nuclear magnetic resonance spectrometer Jeol GSX-500 FT NMR operating at 399.65 MHz for ^{1}H NMR and 100.40 MHz for ^{13}C NMR. The spectra were run in pyridine-d_5 and chemical shifts were given on the δ-scale (ppm) with tetramethylsilane (TMS) as an internal standard. HPLC analyses were performed on a JASCO model 800 instrument. GC analysis were carried out on Hitachi G-3000 gas chromatograph.

Extraction and Isolation

The air-dried fruits of the *G. caspica* (1.25kg) were defatted with *n*-hexane (3 x 3 L) at room temperature with occasional stirring for 3 days. The plant material was then extracted with chloroform twice (2 x 3 L) followed by methanol until exhaustion. The combined methanolic extract was evaporated under vacuum to dryness. The residue (66 g) was dissolved in the least amount of methanol and the solution was diluted with tenfold amount of acetone to precipitate 12 g of crude saponin mixture. The mixture was dissolved in water (0.002%) and the aqueous solution was passed through a column chromatography packed with 500gm Diaion HP-20 polymer gel (Mitsubishi). After washing the column with distilled water for several times, elution was carried out with 25%, 50%, 60%, 75% methanol-water mixture and finally with 100% MeOH. The collected fractions were examined by silica gel TLC (Merck) using as solvent systems $CHCl_3$: MeOH: H_2O (60:30:5) and *n*-BuOH: EtOH: NH_4OH (7:2:5) and visualized by spraying with 20% sulphuric acid in methanol followed by heating at 110 °C. Based on TLC analysis, similar fractions were then combined. Fractions eluted with 75% and 100% methanol-water mixture were found similar and contained saponin constituents. The combined fraction (3 g) was applied on column chromatography packed with 120 g PSQ 100B silica gel (Fuji silysia, Nagoya, Japan) and eluted with $CHCl_3$:MeOH :H_2O solvents with increasing polarity (70:27:3-58:35:7). A total of 50 fractions, 50 mL each, were collected. Similar fractions were combined after TLC analysis to yield 18 subfractions (A-R). The subfractions D, E and K were subjected to repeated HPLC (column, TSK gel ODS-80TS, 55 mm x 60 cm; solvent, 30-45% CH_3CN (15 h) linear gradient; flow rate, 45 mL/min; detection, UV 205

nm). Part of Fraction E (100 mg) yielded compound **1** (18 mg). Part of Fraction K (281 mg) and Fraction D (88 mg) furnished compound **2** (21 mg) and compound **3** (20 mg), respectively.

General method for acid hydrolysis

Each saponin (3 mg) was dissolved in 3 mL methanol and 3 mL 2 M HCl and heated at 100°C for 1.5 h in a water bath. The methanol was then evaporated and the residue was dissolved in distilled water. The mixture was extracted with $CHCl_3$ (3 x 7mL). The chloroform layer was washed until acid free and evaporated to dryness. The obtained triterpene was identified by TLC against reference sample using solvent system $CHCl_3$: MeOH (10:1). The aqueous layer was neutralized with repeated addition of methanol and evaporation. The residue was dissolved in methanol and the sugar components were detected by GC analysis of their thiazolidine derivatives as previously described (Melek *et al.*, 2003a,b).

Alkaline hydrolysis

Each saponin (6 mg) was dissolved in 2 mL 1M NaOH and heated under reflux for 4 h at 100°C. The reaction mixture was neutralized with 1M HCl, then extracted with *n*-butanol (3 x 5 ml). The remaining aqueous layer was evaporated to dryness and the residue was dissolved in methanol. Sugars were detected in the methanolic solution as shown before. The prosapogenin in the *n*-butanol layer was subjected to acid hydrolysis using the same method applied for the intact saponin and the sugars in the hydrolysate were examined.

Compound 1

Amorphous powder (18 mg), $[\alpha]_D^{23}$ -39.0 (c= 0.99, MeOH). ^{13}C NMR: Tables 1 and 2, ^{1}H NMR (pyridine-d_5): aglycone δ 0.9, 0.98, 1.0, 1.12, 1.14, 1.27, 1.81 (Each 3H, *s*), 2.77 (1H, *t*, *J*=13.7 Hz, H-19), 3.32 (1H, *dd*, *J*=11.7, 4.4 Hz, H-3), 5.22 (1H, br *s*, H-16), 5.59 (1H, br *t*, *J*=3.0 Hz, H-12). Sugar units δ 1.61 (3H, *d*, *J*=6.2 Hz, Rha' H-6), 1.71 (3H, *d*, *J*=5.9 Hz, Rha H-6), 3.44 (1H, *t*, *J*=10.2 Hz, Xyl' H-5), 3.64 (1H, *t*, *J*=9.8 Hz, Xyl" H-5), 3.75 (1H, *d*, *J*=10.2 Hz, Ara H-5), 4.68 (1H, *dd*, *J*=8.8, 3.2 Hz, Rha H-3), 4.77 (1H, *dd*, *J*=3, 1.8 Hz, Rha H-2), 4.84 (1H, *d*, *J*=7.4 Hz, Glc H-1), 4.94 (1H, *d*, *J*=6.6 Hz, Ara H-1), 5.14 (1H, *d*, *J*=7.3 Hz, Xyl" H-1), 5.17 (1H, *d*, *J*=6.8 Hz, Xyl' H-1), 5.38 (1H, *d*, *J*=1.2 Hz, Rha' H-1), 6.11 (1H, *d*, *J*=7.8 Hz, Glc' H-1), 6.32 (1H, *d*, *J*=1.2 Hz, Rha H-1).

Compound 2

Amorphous powder (21mg), $[\alpha]_D^{23}$ -24.0 (c= 1.05, MeOH). ^{13}C NMR: Tables 1 and 2, ^{1}H NMR (pyridine-d_5): aglycone δ 0.85, 0.86, 0.88, 0.97, 1.08, 1.33, 1.35 (Each 3H, *s*), 3.10 (1H, *dd*, *J*=13.7, 3.9 Hz, H-18), 3.52 (1H, *dd*, *J*=11.2, 4.2 Hz, H-3), 5.42 (1H, br *t*, *J*=2.9 Hz, H-12). Sugar units δ 1.77 (3H, *d*, *J*=6.1 Hz, Rha H-6), 3.49 (1H, *t*, *J*=10.2 Hz, Xyl' H-5), 3.59 (1H, *t*, *J*=10.4 Hz, Xyl H-5), 3.66 (1H, *t*, *J*=10Hz, Xyl" H-5), 3.75 (1H, *dd*, *J*=11.2, 2.2 Hz, Ara H-5), 4.49 (1H, *t*, *J*=4.9 Hz, Ara H-2), 4.64 (1H, *d*, *J*=9.5 Hz, Glc H-6), 4.68 (1H, *dd*, *J*=8.8, 3.4 Hz, Rha H-3), 4.79 (1H, *dd*, *J*=3.1, 1.6 Hz, Rha H-2), 4.88 (1H, *d*, *J*=7.5 Hz, Glc H-1), 4.98 (1H, *d*, *J*=6.8 Hz, Xyl H-1), 5.06 (1H, *d*, *J*=7.0 Hz, Xyl' H-1), 5.15 (1H, *d*, *J*=5.2 Hz, Ara H-1), 5.17 (1H, *d*, *J*=7.3 Hz, Xyl" H-1), 6.19 (1H, *d*, *J*=7.8 Hz, Glc' H-1), 6.40 (1H, *d*, *J*=1.5 Hz, Rha H-1).

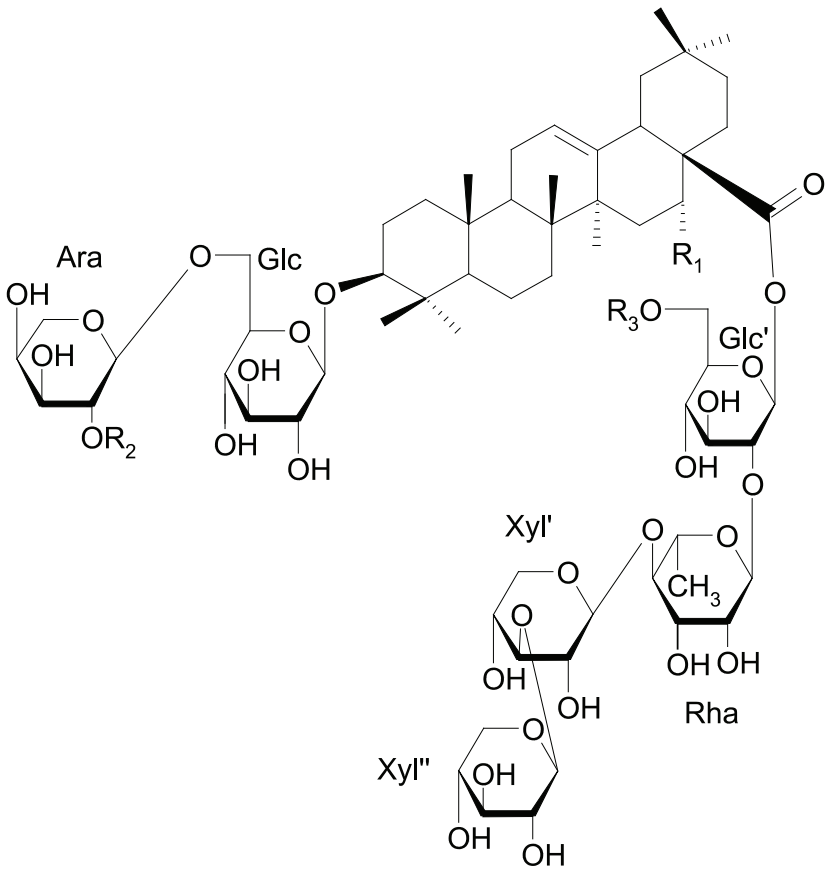
Compound 3

Amorphous powder (20 mg), $[\alpha]_D^{23}$ -42.0 (c= 0.78, MeOH). ^{13}C NMR: Tables 1 and 2. ^{1}H NMR (pyridine-d_5): aglycone δ 0.91, 0.94, 1.0, 1.12,1.12, 1.32, 1.85 (Each 3H, *s*), 2.73 (1H, *t*, *J*=13.7 Hz, H-19), 3.49 (1H, *dd*, *J*=11.7, 4.4 Hz, H-3), 5.21 (1H, br *s*, H-16), 5.58 (1H, br *t*, *J*=3.1 Hz, H-12). Sugar units δ 1.61 (3H, *d*, *J*=5.9 Hz, Rha' H-6), 1.71 (3H, *d*, *J*=5.9 Hz, Rha H-6), 3.44 (1H, *t*, *J*=10.3 Hz, Xyl' H-5), 3.60 (1H, *t*, *J*=10.7 Hz, Xyl H-5), 3.75 (1H, *d*, *J*=11.2 Hz, Ara

H-5), 4.51 (1H, *t*, *J*= 5.5, Ara H-2), 4.64 (1H, *dd*, *J*=11.2, 3.4 Hz, Glc H-6'), 4.68 (1H, *dd*, *J*=9.0, 2.9 Hz, Rha H-3), δ 4.77 (1H, *m*, Rha H-2), 4.88 (1H, *d*, *J*=7.6 Hz, Glc H-1), 4.99 (1H, *d*, *J*=6.8 Hz, Xyl H-1), 5.13 (1H, *d*, *J*=6.8 Hz, Xyl' H-1), 5.14 (1H, *d*, *J*=4.1 Hz, Ara H-1), 5.17 (1H, *d*, *J*=6.8 Hz, Xyl" H-1), 5.38 (1H, br *s*, Rha' H-1), 6.11 (1H, *d*, *J*=7.8 Hz, Glc' H-1), 6.32 (1H, br *s*, Rha H-1).

RESULTS AND DISCUSSION

Phytochemical investigation on the fruits of *G. caspica* resulted in the isolation of three triterpenoidal saponins **1-3** belonging to the oleanane series. Isolation and purification of saponins were achieved by column chromatography followed by HPLC. Characterization of the isolates in general was established by total and partial hydrolysis, spectral analysis (1D and 2D NMR) as well



1	R_1=OH	R_2=H	R_3=Rha'
2	R_1=H	R_2=Xyl	R_3=H
3	R_1=OH	R_2=Xyl	R_3=Rha'

as comparison of their spectral data with those reported in the literature for *Gleditsia* saponins.

The ^{1}H NMR spectrum of saponin **1** showed the existence of seven tertiary methyl groups characterized by the singlets at δ 0.9, 0.98, 1.0, 1.12, 1.14, 1.27 and 1.81. Their corresponding carbon signals were displayed in the HMQC spectrum at δ 15.9, 33.3, 17.1, 17.6, 24.8, 28.3 and 27.2, respectively. Compound **1** showed a distorted triplet of an olefinic proton at δ 5.59 in its ^{1}H NMR spectrum and signals of one double bond at δ 122.7 and 144.5 in its ^{13}C NMR spectrum, indicating the presence of a triterpenoid moiety of the olean-12-ene type. The presence of a downfield methyl group attached to C-14 (Me-27, δ 1.81) and one proton broad singlet at δ 5.22 in the ^{1}H NMR spectrum suggested an axial (α) hydroxyl group at C-16 of the triterpene moiety. Seven anomeric proton signals were also observed in the ^{1}H NMR spectrum at δ 4.84 [*d*, *J* = 7.4 Hz, glucose (Glc)], 4.94 [*d*, *J* = 6.6 Hz, arabinose (Ara)], 5.14 [*d*, *J* = 7.3 Hz, xylose (Xyl")], 5.17 [*d*, *J* = 6.8 Hz, xylose (Xyl')], 5.38 [*d*, *J* =1.2 Hz, rhamnose (Rha')] , 6.11 [*d*, *J* = 7.8 Hz, glucose (Glc')] and 6.32 [*d*, *J* = 1.2 Hz, rhamnose (Rha)] together with two methyl signals at δ 1.61 (*d*, *J* = 6.2 Hz) and 1.71 (*d*, *J* = 5.9 Hz) for two deoxy hexose units. The corresponding anomeric carbon signals assigned by direct correlation from HMQC spectrum were at δ 106.9, 105.3, 106.0, 106.3, 101.9, 94.7, 101.4, respectively. The ^{13}C NMR data of compound **1** (Tables 1 and 2) showed the presence of sixty nine carbon atoms in the molecule, thirty carbons were assigned to the triterpene moiety. The remaining thirty nine carbons were in agreement with the presence of seven monosaccharide moieties corresponding to two hexoses, three pentoses and two deoxy hexoses. Acid hydrolysis of **1** with 2 M hydrochloric acid in methanol-water mixture (1:1) afforded an aglycone that was identified as echinocystic acid (TLC against authentic sample) along with the sugar components identified by GC analysis of their thiazolidine derivatives (Melek *et al.*, 2002, 2003a,b) as D-glucose, L-arabinose, D-xylose and L-rhamnose. The coupling constant values of the anomeric proton signals and the ^{13}C NMR spectral

Table (1): ^{13}C NMR data of the aglycone part of compounds 1-3 in pyridine-d_5 (ppm)

	1	2	3		1	2	3
C				C			
1	39.0	38.9	39.1	**18**	41.6	42.1	41.6
2	26.8	26.9	26.9	**19**	47.7	46.3	47.6
3	89.2	88.6	88.9	**20**	30.9	30.8	30.8
4	39.6	39.6	39.6	**21**	36.0	34.1	36.1
5	56.1	56.0	56.2	**22**	31.9	32.4	31.9
6	18.8	18.8	18.7	**23**	28.3	28.3	28.4
7	33.6	33.3 a	33.6	**24**	17.1	17.1	17.1
8	40.2	40.1	40.2	**25**	15.9	15.8	15.9
9	47.2	48.1	47.3	**26**	17.6	17.6	17.6
10	37.1	37.2	37.2	**27**	27.2	26.1	27.2
11	23.9	24.0	23.9	**28**	176.1	176.5	176.0
12	122.7	122.9	122.6	**29**	33.3	33.2 [a]	33.3
13	144.5	144.1	144.5	**30**	24.8	23.8	24.8
14	42.3	42.3	42.3				
15	36.4	28.5	36.4				
16	74.0	23.5	74.0				
17	49.6	47.2	49.6				

Data with the same labels are interchangeable.

Table (2): ^{13}C NMR data of the sugar moieties of compounds 1-3 in pyridine-d_5 (ppm)

	1	2	3		1	2	3
C-3 Glc				**Rha**			
1	106.9	106.8	106.8	**1**	101.4	101.3	101.4
2	75.7	75.8	75.7	**2**	71.8	71.7	71.8
3	78.7	78.5	78.4	**3**	72.5	72.5	72.7 [c]
4	72.1	72.3	72.3	**4**	83.9	85.1	83.8
5	76.7	76.3	76.2	**5**	68.4	68.2	68.4
6	70.0	69.6	69.7	**6**	18.7[a]	18.6	18.7
Ara				***Xyl'***			
1	105.3	102.5	102.3	**1**	106.3	106.9	106.3
2	72.3	80.7	80.5	**2**	75.0	75.1	75.0
3	74.3	72.7	72.5	**3**	87.5	87.4	87.5
4	69.1	67.6	67.5	**4**	69.0	69.0	69.1
5	66.3	64.5	64.3	**5**	66.9	67.0	66.9
Xyl				***Xyl''***			
1		106.4	106.3	**1**	106.0	105.9	106.1
2		75.5	75.4	**2**	75.1	75.2	75.1
3		77.9	77.9	**3**	78.1	78.1	78.1
4		70.8	70.9	**4**	70.9	70.9	70.9
5		67.3[a]	67.4 [a]	**5**	67.4	67.4 [a]	67.3 [a]
C-28 Glc'				***Rha'***			
1	94.7	94.8	94.7	**1**	101.9		102.0
2	76.7	76.5	76.6	**2**	72.2		72.2
3	79.1	79.4	79.2	**3**	72.7		72.6 [c]
4	71.2	71.4	71.2	**4**	74.0		74.0
5	77.6	78.8	77.7	**5**	69.7		69.7
6	66.8	62.3	66.9	**6**	18.7[a]		18.7

Data with the same labels are interchangeable.

data (Table 2) indicated the *β*-D-pyranosyl configuration for glucoses and xyloses. The coupling constant values of the anomeric proton signals and the ^{13}C NMR data indicated α-L-pyranosyl configuration for arabinoses and rhamnoses. The anomeric carbon signal at δ 94.7 and the carbonyl signal of the triterpene moiety at δ 176.1 revealed the presence of an ester glycoside linkage. The simultaneous presence of 3-*O*-glycosidic linkage of **1** was clearly observed by attendant downfield position of C-3 signal at δ 89.2. Thus, compound **1** was a 3, 28 bisdesmoside. Alkaline hydrolysis of **1** with 1 M NaOH yielded a prosapogenin and the sugar components D-glucose, D-xylose and L-rhamnose were detected in the hydrolysate. Acid hydrolysis of the obtained prosapogenin furnished D-glucose and L-arabinose. The fore mentioned information together with the observed sugar arrangement in saponins of Gleditsia species (Zhang *et al.*, 1999d) allowed us to conclude that the 3-*O*-sugar chain comprised one D-glucose and one L-arabinose units while the pentasaccharide moiety at C-28 contained one D-glucose, two D-xyloses and two L-rhamnose residues. The assignments of the carbon resonances due to the sugar units of **1**, were established based on the combined use of ^{1}H-^{1}H COSY, HMQC and HMBC spectra (Table 2). The site of attachment of the sugar units was determined using the glycosylation rule (Seo *et al.*, 1978; Tori *et al.*, 1977). In the 3-*O*-sugar chain the downfield ^{13}C chemical shift of glucose C-6 at δ 70.0 and the upfield shift of C-5 at δ 76.7 were attributed to glycosylation shift when compared to methyl glucose, thus disclosed that C-6 of an inner *β*-D-gluco-

pyranose (Glc) unit is attached to C-1 of a terminal *a*-L-arabinopyranose (Ara) unit. In the 28-sugar chain, the downfield ^{13}C shift of C-2 at δ 76.7 and C-6 methylene at δ 66.8 of *β*-D-glucopyranose compared to methyl glucose were in agreement with 2, 6 disubstituted *β*-D-glucopyranose (Glc') unit. Similarly, the observed downfield ^{13}C chemical shift of *a*-L-rhamnopyranose C-4 at δ 83.9 and *β*-D-xylopyranosyl C-3 at δ 87.5 with respect to methyl rhamnose and methyl xylose, were in accordance with the presence of 4-substituted *a*-L-rhamnopyranose (Rha) and 3-substituted *β*-D-xylopyranose (Xyl') units. The remaining ^{13}C chemical shift values revealed two terminal *a*-L-rhamnopyranose (Rha') and *β*-D-xylopyranose (Xyl") units, thus disclosed the branched nature of the pentasaccharide moiety attached to the aglycone C-28. The positions of the sugar moieties linked in ester form, were identified based on ^{1}H and ^{13}C NMR spectral data which matched well with those reported for the 28-*O*-β-D-xylopyranosyl-(1→ 3)-β-D-xylopyranosyl-(1→ 4)-α-L-rhamnopyranosyl- (1→ 2)-[α-L-rhamnopyranosyl-(1→ 6)]-β- D-glucopyranosyl ester of echinocystic acid (Konoshima *et al.*, 1981; Konoshima, 1982a,b; Zhang *et al.*, 1999c,d). The sugar pattern of **1** was confirmed by the HMBC experiment. The observed HMBC correlations at 3-O-glycosidic chain were between the resonances of aglycone C-3 (δ 89.2) and Glc H-1 (δ 4.84) and between Glc C-6 (δ 70.0) and Ara H-1 (δ 4.94). The HMBC correlations at 28-*O*-sugar chain were between the resonances of aglycone carbonyl (δ 176.1) and Glc' H-1 (δ 6.11), Glc' C-2 (δ 76.7) and Rha H-1 (δ 6.32), Glc' C-6 (δ 66.8) and Rha' H-1 (δ 5.38), Rha C-4 (δ 83.9) and Xyl' H-1 (δ 5.17) and between Xyl' C-3 (δ 87.5) and Xyl" H-1 (δ 5.14).The above spectral evidences led to conclude that the structure of saponin **1** is 3-O-α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranosyl echinocystic acid 28-O-β- D-xylopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4) -α-L -rhamnopyranosyl-(1→ 2)-[α-L-rhamnopyranosyl- (1→ 6)]-β-D-glucopyranosyl ester. Compound **1** is identical to Gleditsia saponin E' previously isolated from *G. sinensis* (Zhang *et al.*, 1999d) and also obtained after partial alkaline hydrolysis of Gleditsia saponin E isolated from *G. japonica* (Konoshima *et al.*, 1981).

Compound **2** is another triterpenoidal glycoside from the characteristic ^{1}H and ^{13}C NMR signals as observed in compound **1**. In the ^{13}C NMR spectrum of **2** the signals due to the triterpene moiety were in good agreement with those of 28-glycosyl ester of 3-*O*-glycosyl oleanolic acid. Consequently, **2** was considered as a bisdesmoside. Compound **2** exhibited in its ^{1}H NMR spectrum seven anomeric proton signals at δ 4.88 (*d*, *J* = 7.5 Hz, *β*-Glc), 4.98 (*d*, *J* = 6.8 Hz, *β*-Xyl), 5.06 (*d*, *J* = 7.0 Hz, *β*-Xyl'), 5.15 (*d*, *J* = 5.2 Hz, *L*-Ara), 5.17 (*d*, *J* = 7.3 Hz, *β*-Xyl"), 6.19 (*d*, *J* = 7.8 Hz, *β*-Glc'), 6.40 (*d*, *J* = 1.5 Hz, *L*-Rha) and a methyl doublet at δ 1.77 (*d*, *J* = 6.1 Hz) for a deoxy hexose unit. The corresponding seven anomeric carbon signals appearing in the HMQC spectrum at δ 106.8, 106.4, 106.9, 102.5, 105.9, 94.8 and 101.3 made it clear that **2** contained seven sugar moieties. Acid hydrolysis of **2** furnished oleanolic acid (TLC analysis) and sugar component identical to those detected after acid hydrolysis of **1**. Similarly, alkaline hydrolysis of **2** afforded a prosapogenin and the same sugars obtained after alkaline hydrolysis of **1**. Acid hydrolysis of the obtained prosapogenin yielded the sugars D-glucose, L-arabinose as in the case of **1** in addition to D-xylose. The ^{13}C NMR spectrum of **2** showed signals corresponding to sixty eight carbons, thirty were assigned to oleanolic acid and the remaining thirty eight were due to two hexoses, four pentoses and one deoxy hexose unit. Analysis of NMR data deduced from 1D and 2D NMR spectra (^{1}H-^{1}H COSY, HMQC, HMBC) with reference to methyl pyranosides revealed the presence of two terminal *β*-D-xylopyranoses, a 6-substituted *β*-D-glucopyranose,

a 2-substituted *a*-L-arabinopyranose, a 2-substituted *β*-D-glucopyranose, a 4-substituted α-L-rhamnopyranose and a 3-substituted *β*-D-xylopyranose units. Considering the above information and the ^{13}C NMR assignments in Tables 1 and 2, the structure of **2** is similar to that of **1** except that the C-2 of the terminal α-L-arabinopyranose unit in **1** is substituted with a terminal *β*-D-xylopyranose unit in the 3-*O*-glycosyl chain of **2**. Furthermore, the terminal *a*-L- rhamnopyranose linked to C-6 of the ester *β*-D-glucopyranose unit in **1** is absent in **2**. The HMBC correlation between the resonances of Ara C-2 (δ 80.7) and Xyl H-1 (δ 4.98) in 3-*O*-sugar chain of **2** confirmed the connectivity between the two sugar units. The other HMBC correlations were found the same as those observed in the HMBC spectrum of **1** except the absence of inter-residue connectivity for C-6 of the ester linked *β*-D-glucopyranose. Therefore, saponin **2** was assigned the structure of 3-*O*-β -D- xylopyranosyl- (1→ 2) -α-L-arabinopyranosyl- (1 → 6)-β-D-glucopyranosyl oleanolic acid 28-*O*- β-D- xylopyranosyl-(1→ 3)- β- D-xylopyranosyl- (1 → 4) -α -L- rhamnopyranosyl- (1→ 2) -β-D-glucopyranosyl ester. Saponin **2** is identical to gleditsioside I previously isolated from *Gleditsia sinensis* (Zhang *et al.*, 1999d).

Saponin **3** was another bisdesmosidic triterpenoidal saponin from NMR analysis. Its ^{13}C NMR spectrum (Tables 1 and 2) exhibited signals due to 3, 28-disubstituted echinocystic acid moiety as well as eight anomeric carbon signals at δ 94.7, 101.4, 102.0, 102.3, 106.1, 106.3, 106.3 and 106.8. Acid hydrolysis of **3** afforded sugar components identical to those obtained after acid hydrolysis of **1** and **2** in addition to echinocystic acid. Alkaline hydrolysis of **3** and acid hydrolysis of the obtained prosapogenin furnished the same sugars obtained in case of saponin **2**. In the ^{13}C NMR spectrum of **3**, forty four carbon signals were assignable to two hexoses, four pentoses and two deoxy hexoses. Comparison of the ^{13}C NMR data of **3** (1D and 2D NMR analysis) with those of **2** (Table 2) indicated that **3** exhibited the same sugar structure of **2** with additional *a*-L- rhamnopyranose unit substituted the ester-linked *β*-D-glucopyranose at C-6 position. The structure of the triose moiety at aglycone C-3 was confirmed by the observed HMBC correlations in the HMBC experiment of **3** which were found the same at those observed for 3-*O*-sugar of **2**. The HMBC experiment of **3** was also used to verify the branched structure of the pentasaccharide unit at C-28 by displaying the same cross peaks as those observed in the HMBC experiment of **1**. Thus, saponin **3** was formulated as 3-*O*-β-D-xylopyranosyl-(1→ 2)-α-L-arabinopyranosyl -(1 → 6)-β -D-glucopyranosyl echinocystic acid 28-*O*-β-D-xylopyranosyl-(1→ 3)-β-D-xylopyranosyl-(1→ 4) -α-L-rhamnopyranosyl-(1 → 2) -[α-L-rhamnopyranosyl- (1 → 6)]-β-D-glucopyranosyl ester. Saponin **3** is identical to Gleditsia saponin C' isolated previously from *Gleditsia sinensis* (Zhang *et al.*, 1999d) and from *Gleditsia delavayi* (Teng *et al.*, 2002a). It was also obtained after partial alkaline hydrolysis of Gleditsia saponin C isolated from *Gleditsia japonica* (Konoshima *et al.*, 1980).

A Saponin containing fraction was found to exhibit potent central and peripheral analgesic activities as well as moderate acute and chronic anti-inflammatory activities. The acute and chronic toxicities were also measured. The detailed studies will be the subject of another report.

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