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Triterpenoidal saponins from *Gleditsia sinensis*

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Abstract

Six bisdesmosidic triterpenoidal saponins, gleditsiosides H-K and gleditsia saponins C′ and E′, were isolated from the anomalous fruits of *Gleditsia sinensis*. Their structures were established by a combination of extensive NMR (DEPT, DQF-COSY, HETCOR, HOHAHA, HMBC and ROESY) studies and chemical degradation. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Gleditsia sinensis*; Leguminosae; Fruit; Triterpenoidal saponin; Oleanolic acid; Echinocystic acid; Gleditsioside H; Gleditsioside I; Gleditsioside J; Gleditsioside K; Gleditsia saponin C′; Gleditsia saponin E′

1. Introduction

The medicinal plant *Gleditsia sinensis* Lam. (Leguminosae) is a perennial arbor widely distributed throughout China. Its anomalous fruits called 'Zhu Yao Zao', produced by old or injured plants, have long been known in traditional Chinese medicine as a saponin-rich herbal medicine used for the treatment of apoplexy and as an expectorant and pesticide (Jiangsu New Medical College, 1977). Previous phytochemical studies led to seven new triterpenoidal saponins, named gleditsiosides A–G, together with two known ones (Zhang et al., 1999). All nine bisdesmosidic triterpenoidal glycosides were acylated with one or two monoterpenic acids to the sugar moieties. Further examination of the more polar saponin fractions furnished four new saponins, designated as gleditsiosides H, I, J and K (**1**, **4**–**6**), and two new natural ones (**2**, **3**), called gleditsia saponins C′ and E′, which were obtained previously from the alkaline hydrolysate of gleditsia saponins C and E isolated from the fruits of *Gleditsia japonica*. In this contribution, we describe their isolation and structural elucidation by various NMR spectroscopic techniques (including DEPT, DQF-COSY, HOHAHA, HETCOR, HMBC and ROESY experiments) and some chemical degradation.

2. Results and discussion

Gleditsioside H (**1**), a white amorphous solid, had a molecular formula of $C_{74}H_{120}O_{37}$, as determined by ^{13}C NMR spectroscopic data and the $[M+Na]^+$ ion at m/z 1623 and $[M+K]^+$ ion at m/z 1639 in the MALDI-TOF MS (positive ion mode). The seven tertiary methyl carbon signals at δ 15.8, 17.1, 17.5, 23.8, 26.0, 28.3, 33.2 and the two olefinic carbon signals at δ 122.8, 144.1, coupled with the ^1H information (seven methyl proton singlets at δ 0.86, 0.89, 0.98, 0.99, 1.10,

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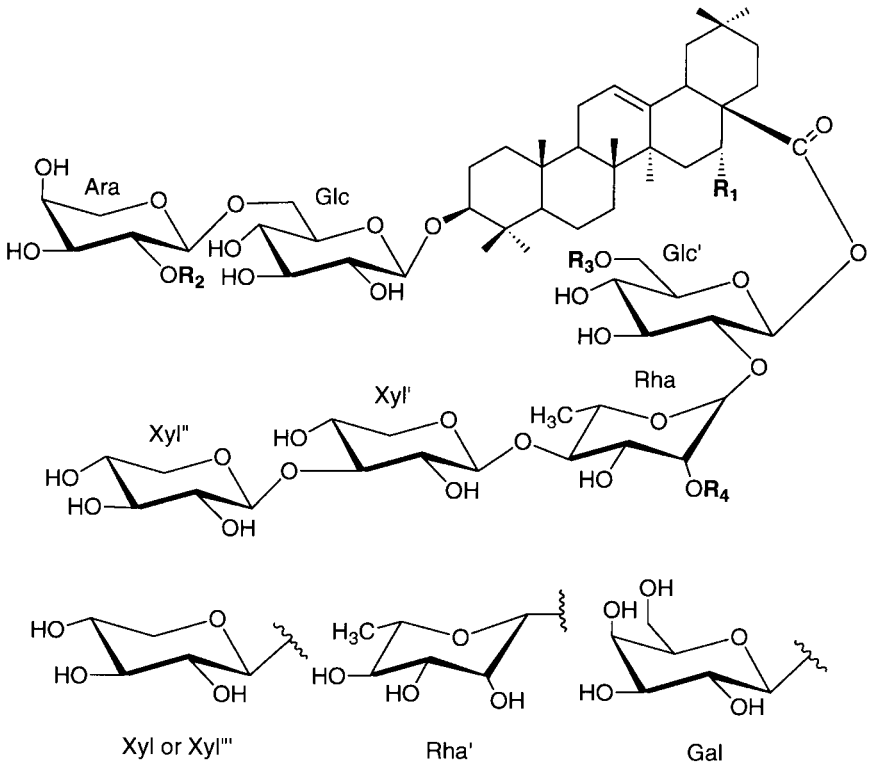
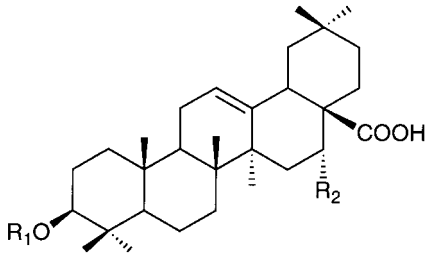


1	R_1 = H	R_2 = Xyl	R_3 = Rha'	R_4 = H
2	R_1 = OH	R_2 = Xyl	R_3 = Rha'	R_4 = H
3	R_1 = OH	R_2 = H	R_3 = Rha'	R_4 = H
4	R_1 = H	R_2 = Xyl	R_3 = H	R_4 = H
5	R_1 = OH	R_2 = Xy	R_3 = H	R_4 = Gal
6	R_1 = OH	R_2 = Xyl	R_3 = H	R_4 = Xyl'"

1.33, 1.37 and a broad triplet vinyl proton at δ 5.40) indicated the aglycone possessed an olean-12-ene skeleton. Detailed NMR studies made it possible to identify that the aglycone was oleanolic acid. The chemical shifts of C-3 (δ 88.7) and C-28 (δ 176.6) of the aglycone showed that compound **1** was a bisdesmosidic triterpenoidal glycoside. Among the 74 carbon signals in the ^{13}C NMR spectrum, 30 signals were assigned to the aglycone (see Table 1); the remaining 44 signals were indicative of the presence of four hexoses and four pentoses, in good agreement with the eight anomeric signals appearing at δ 4.88 d (J = 7.6 Hz), 4.97 d (J = 7.0 Hz), 5.07 d (J = 7.3 Hz), 5.16 d (J = 4.9 Hz), 5.18 d (J = 7.7 Hz), 5.39 (br s), 6.11 d (J = 7.6 Hz), 6.37 (br s) in the ^{1}H NMR spectrum and the eight anomeric carbons observed at δ 94.6, 101.5, 101.8, 102.4, 105.9, 106.4, 106.8 and 106.9 in the ^{13}C NMR spectrum. The two methyl carbon signals at δ 18.6, 18.7 and proton signals at δ 1.59 d (J = 5.8 Hz) and 1.78 d (J = 6.1 Hz) indicated that **1** bore two deoxy sugar moieties. Acidic hydrolysis of **1** furnished oleanolic acid **1b** identified by comparison with the authentic sample (Zhang et al., 1999), and the sugar components were identified as glucose, xylose, arabinose and rhamnose by GLC analysis. Alkaline hydrolysis of **1** afforded prosapogenin **1a** characterized as olenaolic acid 3-*O*-β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranoside based on its spectral data (Zhang et al., 1999). The above evidence indicated that **1** was a bisdesmosidic triterpenoidal glycoside with glucose, arabinose and xylose located at C-3 position of the aglycone, and the other

five monosaccharides were attached to C-28 of the aglycone through an ester bond.



1a R_1 = xyl (1→2)-ara(1→6)-glc R_2 = H

2a R_1 = xyl (1→2)-ara(1→6)-glc R_2 = OH

1b R_1 = H R_2 = H

2b R_1 = H R_2 = OH

The identification and the full assignments of the proton and carbon signals for the sugar moieties were accomplished by a combination of DQF-COSY, HOHAHA, HETCOR, HMBC and ROESY experiments as described previously (Jia, Koike & Nikaido, 1998; Zhang et al., 1999). Accordingly, the eight sugars were determined as two glucoses, three xyloses, two rhamnoses and one arabinose, and the assignments of the protons and protonated carbons were established as shown in Tables 2 and 3. The ^{13}C NMR spectroscopic data for the sugar moieties indicated all the monosaccharides were in pyranose forms. Two glucoses and three xyloses were found to have the β-configuration, and one arabinose to have the α-configuration from the chemical shift and the coupling constant of each of the anomeric protons as well as from the NOE correlations of H-1 with H-3 and H-1 with H-5 of each sugar units. The three-bond strong correlations from the anomeric proton to C-3 and C-5 and the NOE correlation of H-1 with H-2 indicated the two rhamnoses have the α-configuration (Jia et al., 1998).

Table 1
^{13}C NMR spectroscopic data for the aglycone moieties (125 MHz in pyridine-d_5)

	1	2	3	4	5	6
1	38.9	38.9	38.9	38.9	39.0	39.0
2	26.8	26.8	26.8	26.8	26.8	26.8
3	88.7	88.9	89.1	88.6	88.9	88.7
4	39.6	39.6	39.5	39.6	39.6	39.6
5	56.0	56.1	56.0	55.9	56.1	56.0
6	18.8	18.7	18.7	18.7	18.6	18.6
7	33.2	33.5	33.5	33.2	33.5	33.5
8	40.0	40.1	40.1	40.0	40.1	40.1
9	48.1	47.2	47.1	48.1	47.2	47.2
10	37.1	37.1	37.0	37.1	37.1	37.1
11	23.9	23.9	23.8	23.9	23.9	23.9
12	122.8	122.6	122.6	122.8	122.6	122.5
13	144.1	144.4	144.4	144.0	144.4	144.4
14	42.3	42.2	42.2	42.3	42.1	42.1
15	28.7	36.3	36.3	28.5	36.2	36.2
16	23.3	74.0	73.9	23.4	74.1	74.2
17	47.3	49.5	49.5	47.1	49.3	49.2
18	41.9	41.5	41.5	42.1	41.6	41.6
19	46.4	47.5	47.6	46.3	47.4	47.4
20	30.8	30.8	30.8	30.7	30.7	30.7
21	34.1	36.0	36.0	34.0	36.0	35.9
22	32.5	32.0	32.0	32.3	31.9	31.9
23	28.3	28.3	28.3	28.2	28.3	28.3
24	17.1	17.1	17.1	17.1	17.1	17.1
25	15.8	15.8	15.8	15.7	15.8	15.8
26	17.5	17.5	17.5	17.5	17.5	17.5
27	26.0	27.1	27.1	26.1	27.1	27.1
28	176.6	176.0	176.0	176.5	175.9	175.8
29	33.2	33.2	33.3	33.2	33.2	33.2
30	23.8	24.7	24.7	23.7	24.6	24.5

After the protons and carbons of monosaccharides were completely assigned, an unequivocal determination of the sugar sequence and linkage sites was obtained from HMBC and ROESY experiments. The esterified sugar substituent at C-28 was indicated by the following significant C-H correlations obtained in the HMBC spectrum: H-1 (δ 6.11) of Glc′ with C-28 (δ 176.6) of aglycone; H-1 (δ 6.37) of Rha with C-2 (δ 76.5) of Glc′; H-1 (δ 5.07) of Xyl′ with C-4 (δ 85.1) of Rha; H-1 (δ 5.18) of Xyl″ with C-3 (δ 87.4) of Xyl′ and H-1 (δ 5.39) of Rha′ with C-6 (δ 66.5) of Glc′. Further supporting evidence came from NOE experiments as illustrated in Fig. 1. Proceeding in same way, the sequence of the trisaccharide chain at C-3 position was indicated by the following long-range coupling in the HMBC spectrum: H-1 (δ 4.97) of Xyl with C-2 (δ 80.7) of Ara; H-1 (δ 5.16) of Ara with C-6 (δ 69.6) of Glc and deduced from the NOEs observed in the ROESY spectrum (also illustrated in Fig. 1). A key cross-peak between H-1 (δ 4.88) of Glc and C-3 (δ 88.7) of aglycone provided convincing evidence for the trisaccharide moiety attached to C-3 position of aglycone. On the basis of the foregoing evidence, gleditsioside H (**1**) was elucidated as 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)-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-glucopyranosyl ester.

Gleditsia saponin C′ (**2**) exhibited an intense $[M+Na]^+$ peak at m/z 1639 and a $[M+K]^+$ peak at m/z 1655 in the MALDI-TOF MS (positive ion mode), 16 mass units more than that of **1**, suggesting **2** was a derivative of **1** with an additional hydroxyl group, which was confirmed by the proton signal at δ 5.24 (1H, br t), and the protonated carbon signal at δ 74.0, assignable to 16-α-OH of the aglycone. Acidic hydrolysis of **2** furnished echinocystic acid **2b** identified by

Table 2
^{13}C NMR spectroscopic data for the sugar moieties (125 MHz in pyridine-d_5)[a]

	1	2	3	4	5	6
C_3-Glc						
1	106.8	106.8	106.9	106.8	106.8	106.9
2	75.7	75.7	75.6	75.7	75.6	75.7
3	78.4	78.4	78.6	78.4	78.3	78.4
4	72.2	72.2	71.9	72.3	72.4	72.2
5	76.2	76.1	76.7	76.3	76.1	76.2
6	69.6	69.6	70.0	69.6	69.6	69.7
Ara						
1	102.4	102.3	105.4	102.4	102.2	102.3
2	80.7	80.5	72.3	80.8	80.5	80.6
3	72.7	72.5	74.3	72.7	72.4	72.6
4	67.5	67.4	69.1	67.6	67.4	67.5
5	64.3	64.2	66.4	64.4	64.1	64.3
Xyl						
1	106.4	106.3		106.5	106.1	106.4
2	75.5[b]	75.4[b]		75.6[b]	75.3[b]	75.4[b]
3	77.9	77.9		77.9	77.8	77.9
4	70.7[c]	70.7[c]		70.8[c]	70.7[c]	70.7[c]
5	67.3	67.3		67.3	67.2	67.3
C_{28}-Glc′						
1	94.6	94.6	94.6	94.8	94.6	94.8
2	76.5	76.5	76.5	76.4	77.4	76.7
3	79.2	79.1	79.1	79.4	79.0	79.1
4	71.1	71.1	71.1	71.3	71.3	71.2
5	77.6	77.6	77.6	78.9	78.8	78.9
6	66.5	66.7	66.6	62.1	62.1	62.0
Rha						
1	101.5	101.4	101.4	101.3	100.2	100.2
2	71.6	71.7	71.7	71.6	81.4	81.4
3	72.6	72.6	72.5	72.6	72.0	72.5
4	85.1	83.7	83.8	85.1	82.8	82.6
5	68.2	68.4	68.4	68.2	68.1	68.2
6	18.6	18.6	18.6	18.6	18.6	18.6
Xyl′						
1	106.9	106.2	106.2	106.9	105.9	106.1
2	75.1[b]	75.0[b]	75.0[b]	75.1[b]	74.8[b]	75.1[b]
3	87.4	87.5	87.5	87.4	87.3	87.4
4	68.9	69.0	69.0	68.9	69.0	69.0
5	66.9	66.9	66.9	66.9	66.7	66.7
Xyl″						
1	105.9	106.1	106.1	105.9	105.7	105.7
2	75.2[b]	75.1[b]	75.1[b]	75.3[b]	75.1[b]	75.2[b]
3	78.1	78.1	78.2	78.1	78.1	78.2
4	70.8[c]	70.8[c]	70.8	70.9[c]	70.8[c]	70.8[c]
5	67.4	67.3	67.3	67.4	67.3	67.3
					Gal	Xyl‴
Rha′						
1	101.8	101.9	101.9		107.5	107.2
2	72.1	72.1	72.1		73.1	74.9
3	72.7	72.6	72.6		74.6	78.8
4	73.9	73.9	74.0		70.3	69.9
5	69.6	69.7	69.7		77.0	67.3
6	18.7	18.7	18.7		62.2	62.1

[a] The assignments are based upon DEPT, DQF-COSY, HETCOR, HOHAHA, HMBC and ROESY experiments.

[b] The data with same labels in each column may be interchangeable.

[c] The data with same labels in each column may be interchangeable.

comparison with the authentic sample (Zhang et al., 1999), and the monosaccharide components were detected as the same as found in **1**. Alkaline hydrolysis of **2** afforded prosapogenin **2a** determined as echinocystic acid 3-*O*-*β*-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-*β*-D-glucopyranoside by analysis of the spectral data (Zhang et al., 1999). Detailed NMR spectral comparison for compounds **2** and **1** demonstrated that both compounds possessed identical sugar sequences both at C-3 and C-28. Consequently, the structure of compound **2** was concluded to be 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.

Gleditsia saponin E′ (**3**) displayed a $[M+Na]^+$ ion at *m/z* 1507 and a $[M+K]^+$ ion at *m/z* 1523 in the MALDI-TOF MS (positive ion mode), consistent with a molecular formula of $C_{69}H_{112}O_{34}$. The anomeric proton signals appeared at *δ* 4.85 d (*J* = 7.7 Hz), 4.95 d (*J* = 6.6 Hz), 5.15 d (*J* = 7.5 Hz), 5.19 d (*J* = 7.0 Hz), 5.41 (br s), 6.12 d (*J* = 7.9 Hz), 6.38 (br s) in the ^{1}H NMR spectrum and the anomeric carbons observed at *δ* 94.6, 101.4, 101.9. 105.4, 106.1, 106.2, and 106.9 in the ^{13}C NMR spectrum showed that compound **3** contained seven sugar moieties, one sugar moiety fewer than **2**. The NMR spectral resonances due to the aglycone moiety and the sugar unit at C-28 of the aglycone were superimposable with those of **2**, verifying that compound **3** had the common aglycone and the same pentasaccharide chain at C-28 position as did **2**. Thus, the substituent at C-3 hydroxyl group in **3** was a disaccharide moiety, in which the ^{13}C shifts were in good with those of pitheduloside A (Nigam et al., 1997), suggesting the disaccharide sequence, namely, arabinose was the terminal sugar. Therefore, compound **3** was deduced as 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. Although compounds **2** and **3** were obtained earlier from the alkaline hydrolysate of gleditsia saponins C and E, which were isolated from the fruits of *G. japonica* (Konoshima & Sawada, 1980; Konoshima, Umegaki & Sawada, 1981), they were characterized as new natural products for the first time.

Gleditsioside I (**4**) gave a molecular formula of $C_{68}H_{110}O_{33}$ deduced from the $[M+Na]^+$ peak at *m/z* 1477 and the $[M+K]^+$ peak at *m/z* 1493 in the MALDI-TOF MS (positive ion mode), and from the ^{13}C NMR spectral data. Alkaline and acidic hydrolyses of **4** resulted in the same prosapogenin **1a** and the same aglycone **1b** as found in compound **1**. In the ^{1}H and ^{13}C NMR spectra, compound **4** contained seven

Table 3
^{1}H NMR spectroscopic data for the sugar moieties (500 MHz in pyridine-d_5)[a]

	1	2	3	4	5	6
C_3-Glc						
1	4.88 d (7.6)	4.89 d (7.8)	4.85 d (7.7)	4.89 d (7.7)	4.90 d (7.9)	4.90 d (7.7)
2	4.04	4.03	4.00	4.07	4.05	4.06
3	4.19	4.18	4.15	4.20	4.20	4.19
4	4.11	4.12	4.11	4.13	4.14	4.13
5	4.08	4.08	4.09	4.10	4.10	4.09
6	4.25, 4.67	4.25, 4.65	4.27, 4.86	4.24, 4.65	4.25, 4.66	4.24, 4.68
Ara						
1	5.16 d (4.9)	5.16 d (4.9)	4.95 d (6.6)	5.17 d (5.1)	5.16 d (4.9)	5.17 d (4.7)
2	4.50	4.51	4.48	4.52	4.52	4.52
3	4.34	4.36	4.18	4.38	4.38	4.37
4	4.40	4.41	4.32	4.40	4.40	4.41
5	3.75, 4.31	3.75, 4.30	3.75, 4.31	3.75, 4.30	3.75, 4.30	3.76, 4.30
Xyl						
1	4.97 d (7.0)	4.99 d (6.8)		4.98 d (6.7)	4.99 d (7.0)	4.99 d (6.8)
2	4.04	4.05		4.05	4.03	4.04
3	4.06	4.06		4.07	4.08	4.09
4	4.15	4.15		4.15	4.15	4.14
5	3.59, 4.38	3.58, 4.40		3.58, 4.40	3.56, 4.40	3.58, 4.40
C_{28}-Glc′						
1	6.11 d (7.6)	6.12 d (7.8)	6.12 d (7.9)	6.21 d (7.9)	6.12 d (7.6)	6.15 d (7.7)
2	4.34	4.31	4.31	4.38	4.39	4.38
3	4.21	4.20	4.20	4.26	4.20	4.20
4	4.13	4.12	4.12	4.26	4.25	4.25
5	4.03	4.02	4.05	3.97	4.00	4.00
6	4.13, 4.45	4.12, 4.48	4.15, 4.49	4.31, 4.40	4.30, 4.39	4.31, 4.38
Rha						
1	6.37 (br s)	6.36 (br s)	6.38 (br s)	6.46 (br s)	6.54 (br s)	6.51 (br s)
2	4.83	4.81	4.81	4.82	4.85	4.86
3	4.70	4.72	4.71	4.70	4.76	4.77
4	4.38	4.40	4.40	4.40	4.38	4.39
5	4.50	4.50	4.50	4.51	4.48	4.50
6	1.78 d (6.1)	1.72 d (6.0)	1.72 d (5.9)	1.79 d (6.2)	1.65 d (6.1)	1.63 d (6.0)
Xyl′						
1	5.07 d (7.3)	5.14 d (7.3)	5.19 d (7.0)	5.08 d (7.2)	5.10 d (7.6)	5.11 d (7.5)
2	4.07	4.05	4.04	4.07	4.03	4.03
3	4.03	4.01	4.03	4.03	4.01	4.00
4	4.09	4.06	4.05	4.10	4.08	4.09
5	3.50, 4.25	3.48, 4.20	3.47, 4.20	3.50, 4.25	3.49, 4.25	3.47, 4.26
Xyl″						
1	5.18 d (7.7)	5.19 d (7.1)	5.15 d (7.5)	5.19 d (7.5)	5.13 d (7.3)	5.14 d (7.5)
2	4.07	4.06	4.05	4.07	4.03	4.02
3	4.11	4.12	4.13	4.11	4.12	4.11
4	4.15	4.15	4.15	4.15	4.15	4.16
5	3.69, 4.30	3.67, 4.29	3.65, 4.29	3.65, 4.30	3.35, 4.29	3.35, 4.31
Rha′					Gal	Xyl‴
1	5.39 (br s)	5.41 (br s)	5.41 (br s)		5.17 d (7.3)	5.22 d (7.5)
2	4.45	4.45	4.45		4.47	3.99
3	4.41	4.43	4.43		4.00	4.05
4	4.19	4.20	4.21		4.43	4.10
5	4.25	4.26	4.26		4.00	3.37
6	1.59 d (5.8)	1.61 d (6.0)	1.60 d (5.9)		4.31	

[a] The assignments are based upon DEPT, DQF-COSY, HETCOR, HOHAHA, HMBC and ROESY experiments.

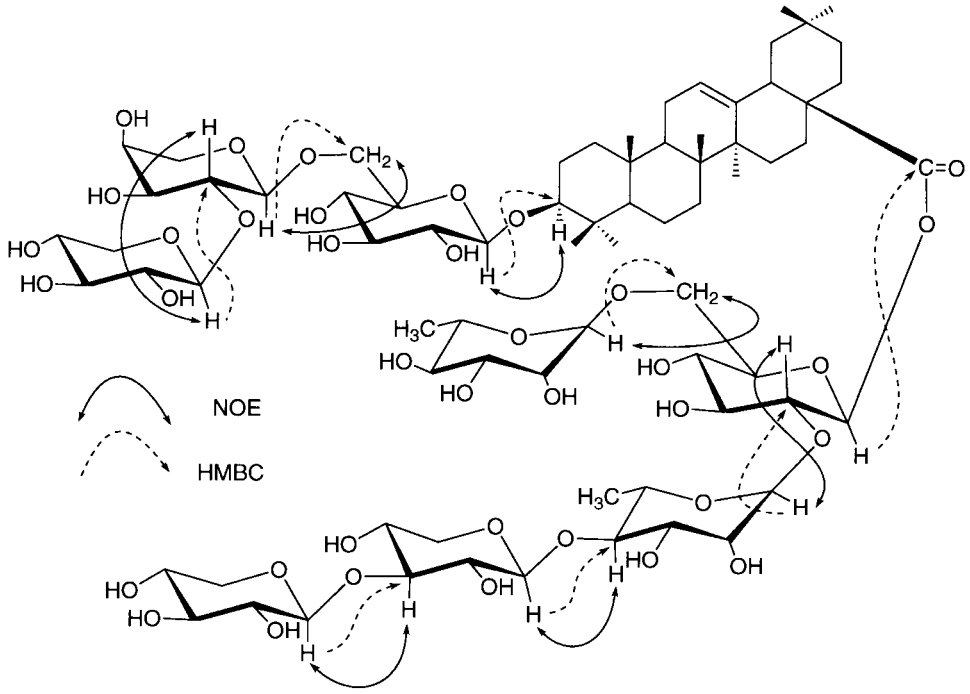


Fig. 1. The sequence and linkage position for the sugar moieties of compound **1** determined by ROESY and HMBC experiments.

anomeric carbon and seven anomeric proton signals (Tables 2 and 3) as well as one methyl signal at δ_C 18.6, δ_H 1.79 d (J = 6.2 Hz)], suggesting the sugar part at C-28 was a tetrasaccharide with only one deoxyhexose moiety, one deoxyhexose fewer than that of **1**. A comparison of the ^{13}C NMR spectroscopic data for the oligosaccharide chain at C-28 of compounds **1** and **4** showed that compound **4** lacked the Rha′ connected to C-6 of Glc′ as found in **1**. Thus, the structure of gleditsioside I (**4**) was determined as 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.

Gleditsioside J (**5**), displaying a $[M+Na]^+$ peak at m/z 1655 and a $[M+K]^+$ peak at m/z 1671 in the MALDI-TOF MS (positive ion mode), yielded prosapogenin **2a** and echinocystic acid **2b** upon alkaline and acidic hydrolyses, respectively, and the monosaccharide components were shown to be glucose, xylose, arabinose, rhamnose and galactose by GLC analysis. The eight sugar anomeric proton and carbon signals (Tables 2 and 3) appearing in the NMR spectra showed that compound **5** possessed a pentasaccharide chain at C-28, one sugar moiety (galactose) more than that of compound **4**. The upfield shift of C-2 (δ 81.4) of Rha indicated the galactose was attached at this position. Consequently, the structure of gleditsioside J (**5**) was established 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)-[*β*-D-galactopyranosyl-(1-2)]-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl ester.

Gleditsioside K (**6**) afforded the same prosapogenin **2a** and echinocystic acid **2b** as for **5** on alkaline and acidic hydrolyses; the monosaccharide components were shown to be glucose, xylose, arabinose and rhamnose based on GLC analysis. The anomeric proton and carbon signals (Tables 2 and 3) observed in its ^1H and ^{13}C NMR spectra indicated compound **6** also contained eight sugar units as compound **5**. From detailed NMR analysis we concluded the galactose in **5** was replaced by a xylose (Xyl‴) in **6**. Accordingly, the structure of gleditsioside K (**6**) was characterized 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)-[β-D-xylopyranosyl-(1-2)]-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl ester.

3. Experimental

3.1. General procedures

Melting points were measured with a Yanaco microscope apparatus and were uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. IR spectra were carried out at a JASCO 300E FTIR spectrometer. MALDI-TOF MS were conducted using a Perseptive Biosystems Voyager DE-STR mass spectrometer. The ^1H and ^{13}C NMR spectra

were recorded on a JEOL α-500 FT-NMR spectrometer in pyridine-d_5 solution and chemical shifts were expressed in δ (ppm) referring to TMS. Diaion HP-20 (Mitsubishi Chemical), silica gel (Silica gel 60, Merck), and ODS (Chromatorex, 100–200 mesh, Fujisylisia) were used for open column chromatography. MPLC and HPLC was performed using an ODS column (SSC-ODS, 40–60 μm, detector: UV 210 nm) and an ODS column (PEGASIL ODS-2, Senshu Pak, 20 mm i.d. × 150 mm, detector: UV 210 nm), respectively. GLC: Shimadzu GC-7A, Column: Silicone OV-17 on Uniport HP (80–100 mesh), 3 mm i.d. × 2.1 m; column temperature, 160°C; carrier gas, N_2, flow rate 30 mL/min.

3.2. Extraction and isolation

The dried fruits (Zhu Ya Zao) of *G. sinensis* were purchased from a market in Nanchang, Jiangxi Province, P.R. China in January 1998, and were identified by Professor Fan Chuishen (Jiangxi College of Traditional Chinese Medicine). The powdered fruits (4.0 kg) of *G. sinensis* were refluxed with 95% EtOH three times for 2 h. The alcoholic extract was concentrated (920 g), suspended in water and then partitioned successively with chloroform (45 g) and *n*-BuOH (480 g). The *n*-BuOH fraction was passed through to a column of Diaion HP-20 (2500 mL) and the absorbed materials were eluted with H_2O and increasing amounts of MeOH (30, 50, 70 and 100%). The 50% MeOH fraction (100 g) was chromatographed over silica gel to give four saponin fractions of A (40 g), B (10 g), C (6 g) and D (10 g). Part of fraction B (5 g) was chromatographed over ODS columns and then repeatedly subjected to MPLC (30, 50, 70 and 90% MeOH) and HPLC (MeOH: H_2O) purification to afford **1** (170 mg), **4** (50 mg). By the same method, fraction C furnished **2** (143 mg), **5**+**6** (98 mg) and fraction D yield **3** (46 mg). Following repeated HPLC [CH_3CN-0.06% TFA (38:62)) purification, **5**+**6** gave **5** (45 mg) and **6** (38 mg).

3.3. Gleditsioside H (*1*)

$C_{74}H_{120}O_{37}$, a white amorphous solid from MeOH, mp 250–251°C (dec.), $[\alpha]_D^{21}$ −12° (MeOH; *c* 0.10). IR ν_{max}^{KBr}: 3400, 2934, 1076 cm^{-1}. MALDI-TOF MS (positive ion mode): *m/z* 1623 $[M+Na]^+$, 1639 $[M+K]^+$. ^1H NMR (pyridine-d_5, 500 MHz): aglycone δ 5.40 (1H, br t, H-12), 3.50 (1H, m, H-3), 1.37, 1.33, 1.10, 0.99, 0.98, 0.89, 0.86 (each 3H, s, H_3-23, -27, -26, -24, -30, -25, -29). Other NMR spectroscopic data see Tables 1–3.

3.4. Gleditsia saponin C′ (*2*)

$C_{74}H_{120}O_{38}$, a white amorphous solid from MeOH, mp 234–235°C (dec.), $[\alpha]_D^{21}$ −18° (MeOH; *c* 0.10). IR ν_{max}^{KBr}: 3406, 2930, 1046 cm^{-1}. MALDI-TOF MS (positive ion mode): *m/z* 1639 $[M+Na]^+$, 1655 $[M+K]^+$. ^1H NMR (pyridine-d_5, 500 MHz): aglycone δ 5.59 (1H, br t, H-12), 5.24 (1H, br t, H-16), 3.48 (1H, m, H-3), 1.86, 1.33, 1.13, 1.12, 1.00, 0.95, 0.90 (each 3H, s, H_3-27, -23, -30, -26, -24, -29, -25). Other NMR spectroscopic data are given in Tables 1–3.

3.5. Gleditsia saponin E′ (*3*)

$C_{69}H_{112}O_{34}$, a white amorphous solid from MeOH, mp 232–233°C (dec.), $[\alpha]_D^{21}$ −33° (MeOH; *c* 0.10). IR ν_{max}^{KBr}: 3414, 2930, 1079 cm^{-1}. MALDI-TOF MS (positive ion mode): *m/z* 1507 $[M+Na]^+$, 1523 $[M+K]^+$. ^1H NMR (pyridine-d_5, 500 MHz): aglycone δ 5.60 (1H, br t, H-12), 5.25 (1H, br t, H-16), 3.30 (1H, m, H-3), 1.87, 1.27, 1.14, 1.13, 1.00, 0.98, 0.89 (each 3H, s, H_3-27, -23, -30, -26, -24, -29, -25). Other NMR spectroscopic data are shown in Tables 1–3.

3.6. Gleditsioside I (*4*)

$C_{68}H_{110}O_{33}$, a white amorphous solid from MeOH, mp 255–256°C (dec.), $[\alpha]_D^{21}$ −17° (MeOH; *c* 0.10). IR ν_{max}^{KBr}: 3413, 2935, 1049 cm^{-1}. MALDI-TOF MS (positive ion mode): *m/z* 1477 $[M+Na]^+$, 1493 $[M+K]^+$. ^1H NMR (pyridine-d_5, 500 MHz): aglycone δ 5.43 (1H, br t, H-12), 3.50 (1H, m, H-3), 1.36, 1.34, 1.09, 0.98, 0.87, 0.86, 0.84 (each 3H, s, H_3-23, -27, -26, -24, -30, -25, -29). Other NMR spectroscopic data are listed in Tables 1–3.

3.7. Gleditsioside J (*5*)

$C_{74}H_{120}O_{39}$, a white amorphous solid from MeOH, mp 256–257°C (dec.), $[\alpha]_D^{21}$ −15° (MeOH; *c* 0.10). IR ν_{max}^{KBr}: 3422, 2926, 1044 cm^{-1}. MALDI-TOF MS (positive ion mode): *m/z* 1655 $[M+Na]^+$, 671 $[M+K]^+$. ^1H NMR (pyridine-d_5, 500 MHz): aglycone δ 5.60 (1H, br t, H-12), 5.22 (1H, br t, H-16), 3.40 (1H, m, H-3), 1.84, 1.30, 1.12, 0.99, 0.94, 0.92, 0.91 (each 3H, s, H_3-27, -23, -30, -26, -24, -29, -25). Other NMR spectroscopic data see Tables 1–3.

3.8. Gleditsioside K (*6*)

$C_{73}H_{118}O_{38}$, a white amorphous solid from MeOH, mp 238–239°C (dec.), $[\alpha]_D^{21}$ −12° (MeOH; *c* 0.10). IR ν_{max}^{KBr}: 3398, 2935, 1077 cm^{-1}. MALDI-TOF MS (positive ion mode): *m/z* 1625 $[M+Na]^+$, 1641 $[M+K]^+$. ^1H NMR (pyridine-d_5, 500 MHz): aglycone δ 5.60 (1H, br t, H-12), 5.25 (1H, br t, H-16), 3.30 (1H, m,

H-3), 1.89, 1.30, 1.13, 1.13, 0.98, 0.92, 0.89 (each 3H, s, H_3-27, -23, -30, -26, -24, -29, -25). Other NMR spectroscopic data see Tables 1–3.

3.9. Alkaline hydrolysis of compounds *1, 2, 4, 5* and *6*

Compound **1** (40 mg) was refluxed with 2 mL 0.8 M NaOH for 4 h. After cooling, the reaction mixture was neutralized with 1 M HCl and then extracted with *n*-BuOH (2 mL × 3). The organic layers were combined and then evaporated to dryness in vacuo. The residue was subjected to HPLC purification affording prosapogenin **1a** (15 mg). By the same method, **2**, **5** and **6** afforded **2a**, and **4** afforded **1a**.

3.10. Acidic hydrolysis of compounds *1, 2, 3, 4, 5* and *6*

Compound **1** (40 mg) was heated in 1 mL 1 M HCl (dioxane-H_2O, 1:1) at 80°C for 2 h in a water bath. After dioxane was removed, the solution was extracted with EtOAc (1 mL × 3). The extract was washed with water and then concentrated to give the aglycone **1b** (10 mg). The aqueous layer was neutralized by passing through an ion-exchange resin (Amberlite MB-3) column, concentrated in vacuo to dryness, and then treated with 1-(trimethylsilyl)imidazole at room temperature for 2 h. After the excess reagent was decomposed with water, the reaction product was extracted with *n*-hexane (1 mL × 2). The TMSi derivatives of the monosaccharides were identified to be glucose, xylose, arabinose and rhamnose by co-GLC analyses with standard monosaccharides. By the same method, **2**, **3**, **5**, **6** afforded **2b,** and **4** afforded **1b**. GLC analyses showed the monosaccharides of **2**, **3**, **4**, **6** were D-glucose, D-xylose, L-arabinose and L-rhamnose, and those of **5** to be D-glucose, D-xylose, D-galactose, L-arabinose and L-rhamnose.

3.11. Compounds *1a, 2a, 1b* and *2b*

For the physicochemical properties and spectral data of compounds **1a**, **2a**, **1b** and **2b** see literature (Zhang et al., 1999).

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