Gleditsiosides N−Q, New Triterpenoid Saponins from *Gleditsia sinensis*

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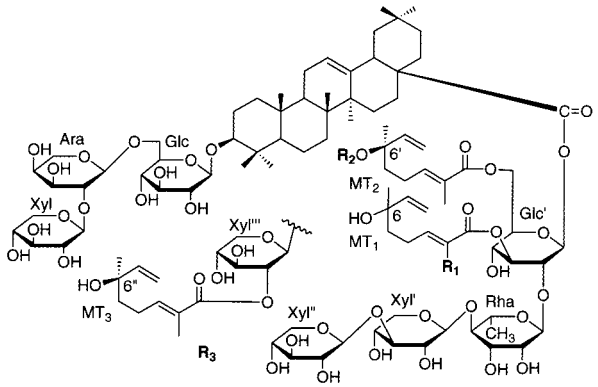
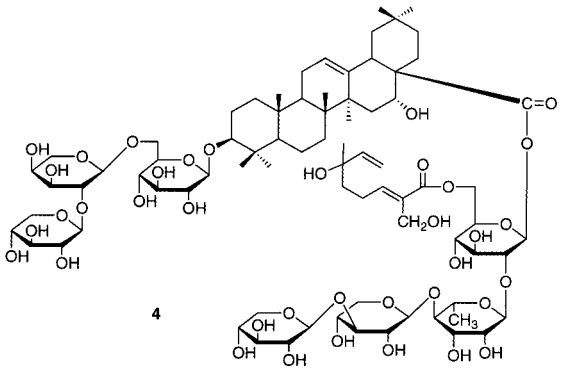
The structures of gleditsiosides N, O, P, and Q (**1**−**4**), isolated from anomalous fruits of *Gleditsia sinensis*, were characterized as novel complex bisdesmosidic triterpenoid glycosides acylated with monoterpenoid units, by means of extensive 1D and 2D NMR studies. The four compounds shared a common structural feature with a trisaccharide [(*β*-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranoside)] affixed to C-3 and a tetrasaccharide [(*β*-D-xylopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester)] attached to C-28. Gleditsioside P (**3**) is the first saponin of this type found to date bearing three monoterpenoid units.

The medicinal plant *Gleditsia sinensis* Lam. (Leguminosae) is a perennial shrub widely distributed throughout the People's Republic of China. Its anomalous fruit called "Zhu Yao Zao", produced by old or injured plants, has long been known in traditional Chinese medicine as a saponin-rich herbal medicine used for treating various diseases.[1] In previous communications,[2−4] we have reported 11 new triterpenoid saponins, named gleditsiosides A−K. Further investigation on the saponin fractions furnished four additional new saponins, designated as gleditsiosides N, O, P, and Q (**1**−**4**). In this contribution, we describe the isolation and structure elucidation of **1**−**4** by extensive NMR studies, including DEPT, DQF−COSY, HETCOR, HOHAHA, HMBC, and ROESY experiments. To the best of our knowledge, gleditsioside P (**3**) is the first saponin containing three monoterpenoid units. Gleditsiosides N (**1**) and O (**2**) represent a new type of compound with two monoterpenoid units acylated at the C-3 and C-6 positions of the glucose moiety, which are directly connected to the C-28 carbonyl group of the aglycon. It is interesting to note that many saponins from the title plant[2−4] and from the fruits of *Gleditsia japonica*[5] have identical oligosaccharide chains, namely, 3-*O*-*β*-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranoside and 28-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester, suggesting a common chemotaxonomic feature for this genus.



1 $R_1 = CH_2OH$ $R_2 = H$
2 $R_1 = CH_3$, 6 = *S* $R_2 = H$
3 $R_1 = CH_3$, 6 = *S* $R_2 = R_3$



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Results and Discussion

Gleditsioside N (**1**) was obtained as a white amorphous solid and analyzed for $C_{88}H_{138}O_{38}$ by its ^{13}C NMR data as well as from the MALDI-TOF MS (positive ion mode) data at *m*/*z* 1825 $[M + Na]^+$ and 1841 $[M + K]^+$. The IR spectrum exhibited characteristic absorptions of a carbonyl group at 1711 cm^{-1} and an α,*β*-unsaturated carbonyl group at 1647 cm^{-1}. The ^1H and ^{13}C NMR spectra displayed resonances due to the seven tertiary methyls and two olefinic carbons and suggested that the aglycon of **1** was based on an olean-12-ene skeleton. After detailed examination of the NMR spectra, the aglycon was identified as oleanolic acid (Table 1). The seven anomeric carbon and seven anomeric proton signals (Tables 2 and 3) and signals for a methyl group (δ_C 18.6; δ_H 1.77, d, J = 6.1 Hz) in the ^{13}C and ^1H NMR spectra suggested that compound **1** contained seven monosaccharides, with one of these being a deoxy sugar. Acidic hydrolysis furnished oleanolic acid identified by co-HPLC analysis with an authentic sample,[2] and the monosaccharide components were detected as glucose, xylose, rhamnose, and arabinose based on GLC analysis. Alkaline hydrolysis afforded a prosapogenin characterized as oleanolic acid 3-*O*-*β*-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranoside by comparison with an authentic sample.[2] The above information showed that **1** was a bisdesmosidic triterpenoid glycoside with glucose, arabinose, and xylose linked to the C-3

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Table 1. ^{13}C NMR Data for the Aglycon Moieties of Compounds **1**−**4** (125 MHz in Pyridine-d_5)

position	1	2	3	4
1	38.9	38.9	39.0	39.0
2	26.9	26.8	26.9	26.8
3	88.6	88.6	88.6	88.8
4	39.6	39.6	39.7	39.6
5	56.0	55.9	56.0	56.0
6	18.7	18.7	18.8	18.7
7	33.3	33.3	33.3	33.5
8	40.0	40.0	40.1	40.1
9	48.1	48.1	48.1	47.2
10	37.1	37.1	37.2	37.1
11	24.1	24.1	24.1	23.9
12	123.1	123.1	123.0	122.7
13	143.9	143.9	144.0	144.3
14	42.3	42.3	42.3	42.2
15	28.5	28.6	28.5	36.2
16	23.5	23.4	23.5	74.1
17	47.3	47.2	47.3	49.3
18	41.9	41.9	42.0	41.5
19	46.3	46.3	46.3	47.4
20	30.8	30.8	30.8	30.8
21	34.0	34.0	34.1	36.0
22	32.5	32.5	32.5	32.0
23	28.3	28.2	28.3	28.3
24	17.1	17.1	17.1	17.1
25	15.8	15.7	15.8	15.8
26	17.6	17.6	17.6	17.6
27	26.1	26.1	26.1	27.2
28	176.5	176.4	176.4	176.0
29	33.2	33.2	33.2	33.2
30	23.9	23.9	23.9	24.7

position of the aglycon, and the other four monosaccharides were attached to the C-28 of the aglycon through an ester bond.

The identification and the full assignments of the proton and carbon signals for the sugar moieties were achieved by a combination of DQF−COSY, HOHAHA, HETCOR, HMBC, and ROESY NMR experiments as described previously.[2,7] Accordingly, the seven monosaccharides were determined to be two glucoses, three xyloses, one arabinose, and one rhamnose, and the assignments of the protons and protonated carbons were established as listed in Tables 2 and 3. All the monosaccharides were determined to be in the pyranose form from their ^{13}C NMR data. The anomeric proton configurations for the sugar moieties were fully defined from their chemical shifts and $^3J_{H1,\ H2}$ and $^1J_{CH}$ coupling constants (Tables 2 and 3), as well as from the NOE relationships of H-1 with H-3 and H-1 with H-5. For the rhamnose moiety, the large $^1J_{CH}$ (173 Hz) and three-bond strong HMBC correlations from the anomeric proton to C-3 and C-5 (the dihedral angles between H-1 and C-3, H-1 and C-5 about 180°) indicated the anomeric proton was equatorial and thus possessed an α configuration.[7] The two oligosaccharide moieties, connected, in turn, to C-3 and C-28 of the aglycon, were confirmed by the significant long-range correlations of H-1 (δ 4.90) of Glc with C-3 (δ 88.6) and H-1 (δ 6.16) of Glc′ with C-28 (δ 176.5). The sequence of two oligosaccharide chains was deduced unequivocally from the HMBC and ROESY information, as illustrated in Figure 1.

The ^{13}C NMR spectrum of **1** showed 88 signals, of which 30 were assigned to the triterpenoid moiety, 38 to the saccharide portion, and the remaining 20 to two monoterpenoid units identified from the extensive NMR data obtained. One monoterpenoid unit MT_2 was characterized as (6′*S*),(2′*E*)-6′-hydroxy-2′,6′-dimethyl-2′,7′-octadienoic acid,[2,3] which was obtained from the alkaline hydrolysate of **1**. The other one, MT_1, was identified as (2*E*)-2-hydroxymethyl-6-hydroxy-6-methyl-2,7-octadienoic acid by comparison of its NMR data with those of MT_2 and from literature data.[3,5,6,8] As observed in the HMBC spectrum, the long-range correlation of H-3 (δ 5.70) of Glc′ with C-1 (δ 167.3) of the monoterpenoid unit MT_1 and H_2-6 (δ 4.59, 4.63) of Glc′ with C-1 (δ 167.8) of the monoterpenoid unit MT_2 established that two monoterpenoid units, MT_1 and MT_2, were attached to C-3 and C-6 of Glc′, respectively. The downfield shifts of H-3 and H_2-6 of Glc′ also indicated they were positions of acylation. On the basis of all the foregoing evidence, gleditsioside N (**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)-[(2*E*)-2-hydroxymethyl-6-hydroxy-6-methyl-2,7-octadienoyl-(1→3) and (6′*S*),(2′*E*)-6′-hydroxy-2′,6′-dimethyl-2′,7′-octadienoyl-(1→6)]-*β*-D-glucopyranosyl ester.

The minor compound **2** displayed an intensive $[M + Na]^+$ peak at *m/z* 1809 and a $[M + K]^+$ peak at *m/z* 1825 in the MALDI-TOF MS, equivalent to 16 mass units fewer than **1**, implying **2** was a derivative of **1** with one less oxygen

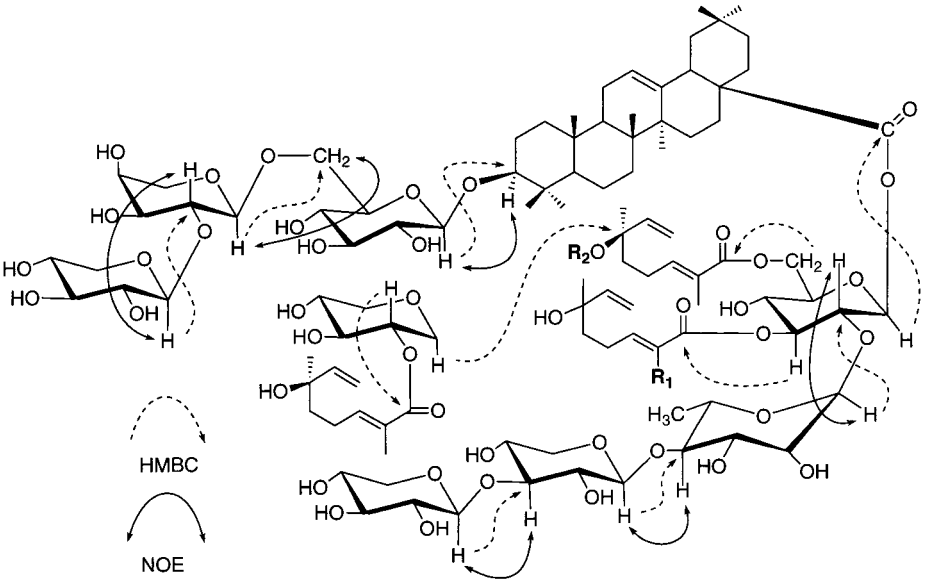

Figure 1. The sequence and linkage position of monosaccharide and monoterpenoid units of compounds **1** and **3** established by HMBC and ROESY experiments.

Table 2. ^{13}C NMR Data for the Sugar Moieties of Compounds **1**−**4** (125 MHz in Pyridine-d_5)[a]

position	1	2	3	4
C_3-Glc				
1	106.7 (158)[b]	106.7	106.7	106.8
2	75.7	75.7	75.7	75.7
3	78.4	78.4	78.4	78.4
4	72.3	72.3	72.3	72.2
5	76.3	76.2	76.3	76.1
6	69.6	69.6	69.6	69.6
Ara				
1	102.4 (164)[b]	102.4	102.5	102.3
2	80.8	80.7	80.7	80.5
3	72.7	72.7	72.7	72.6
4	67.6	67.6	67.6	67.5
5	64.5	64.4	64.5	64.3
Xyl				
1	106.5 (162)[b]	106.3	106.4	106.3
2	75.5[c]	75.5[c]	75.5[c]	75.5[c]
3	77.9	77.9	77.9	77.9
4	70.8[d]	70.8[d]	70.8[d]	70.8[d]
5	67.3	67.3	67.3	67.3
C_{28}-Glc′				
1	94.3 (159)[b]	94.3	94.3	94.6
2	76.0	76.0	76.0	76.6
3	72.3	72.1	72.1	79.0
4	73.5	73.4	73.5	71.5
5	76.5	76.6	76.5	75.9
6	63.5	63.6	63.6	64.4
Rha				
1	101.4 (173)[b]	101.4	101.4	101.4
2	71.5	71.5	71.6	71.8
3	72.5	72.5	72.5	72.5
4	84.9	84.9	84.9	83.5
5	68.5	68.4	68.5	68.4
6	18.6	18.6	18.5	18.7
Xyl′				
1	106.8 (161)[b]	106.8	106.8	106.1
2	75.1[c]	75.1[c]	75.1[c]	75.0[c]
3	87.4	87.3	87.3	87.5
4	68.9	68.9	69.0	68.0
5	67.0	66.9	67.0	66.9
Xyl″				
1	105.9 (160)[b]	105.9	105.9	106.1
2	75.2[c]	75.2[c]	75.2[c]	75.1[c]
3	78.1	78.1	78.1	78.2
4	70.9[d]	70.9[d]	70.9[d]	70.8[d]
5	67.4	67.4	67.4	67.4
Xyl‴				
1			97.4	
2			74.1	
3			78.4	
4			69.6	
5			66.7	

[a] The assignments are based upon DEPT, DQF-COSY, HOHAHA, HETCOR, ROESY, and HMBC experiments. [b] The number in the parentheses is the $^1J_{CH}$ coupling constant (Hz). [c,d] The data with the same label in each column may be interchangeable.

Table 3. ^1H NMR Data for the Sugar Moieties of Compounds **1**−**4** (500 MHz in Pyridine-d_5)[a]

position	1	2	3	4
C_3-Glc				
1	4.90 d (7.9)	4.89 d (7.9)	4.89 d (7.9)	4.89 d (7.5)
2	4.04	4.05	4.04	4.06
3	4.20	4.19	4.21	4.20
4	4.15	4.15	4.14	4.15
5	4.09	4.08	4.07	4.09
6	4.25	4.24	4.26	4.25
	4.68	4.67	4.66	4.67
Ara				
1	5.16 d (5.5)	5.15 d (4.9)	5.14 d (4.9)	5.15 d (5.7)
2	4.52	4.50	4.51	4.53
3	4.38	4.39	4.39	4.40
4	4.37	4.38	4.37	4.39
5	3.75	3.75	3.74	3.75
	4.30	4.29	4.31	4.31
Xyl				
1	4.98 d (6.7)	4.97 d (6.7)	4.97 d (6.7)	4.99 d (7.1)
2	4.04	4.05	4.04	4.05
3	4.06	4.07	4.08	4.07
4	4.15	4.13	4.14	4.15
5	3.59	3.59	3.58	3.57
	4.40	4.39	4.41	4.38
C_{28}-Glc′				
1	6.16 d (7.0)	6.18 d (7.0)	6.16 d (7.0)	6.08 d (8.4)
2	4.41	4.40	4.41	4.37
3	5.70 t (9.2)	5.67 t (9.2)	5.66 t (9.2)	4.22
4	4.20	4.21	4.22	4.10
5	4.42	4.43	4.41	4.07
6	4.59	4.52	4.55	4.78
	4.63	4.63	4.63	4.95
Rha				
1	6.32 s (br)	6.31 s (br)	6.29 s (br)	6.36 s (br)
2	4.79 s (br)	4.78 s (br)	4.76 s (br)	4.80 s (br)
3	4.65	4.65	4.65	4.70
4	4.40	4.39	4.38	4.36
5	4.46	4.45	4.46	4.40
6	1.77 d (6.1)	1.76 d (6.1)	1.76 d (6.1)	1.72 d (5.9)
Xyl′				
1	5.08 d (7.3)	5.07 d (7.1)	5.05 d (7.0)	5.13 d (7.3)
2	4.05	4.05	4.04	4.05
3	4.06	4.07	4.06	4.02
4	4.10	4.09	4.10	4.03
5	3.48	3.50	3.49	3.46
	4.25	4.24	4.25	4.21
Xyl″				
1	5.20 d (7.3)	5.18 d (7.6)	5.17 d (7.9)	5.17 d (7.9)
2	4.05	4.05	4.05	4.05
3	4.10	4.10	4.08	4.11
4	4.15	4.14	4.15	4.15
5	3.68	3.65	3.68	3.65
	4.30	4.29	4.30	4.28
Xyl‴				
1			4.86 d (7.3)	
2			5.89 dd (9.2, 7.3)	
3			4.25	
4			4.20	
5			3.77	
			4.30	

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

atom. The ^{13}C NMR data for the aglycon and sugar parts of **2** bore a close resemblance to those of **1**, but differed in their monoterpenoid moieties. Comparison of the NMR data of the monoterpenoid moieties between **1** and **2** suggested the two compounds differed structurally in the monoterpenoid moiety MT_1 attached to C-3 of the Glc′ unit. As shown in Table 4, the hydroxymethyl group at C-2 (δ_C 56.2, δ_H 4.74) of MT_1 in **1** was replaced by an olefinic methyl group at C-2 (δ_C 12.5, δ_H1.83) in **2**. Therefore, both monoterpenoid moieties in **2** were the same and were characterized as (6*S*),(2*E*)-6-hydroxy-2,6-dimethyl-2,7-octadienoic acid. Accordingly, gleditsioside O (**2**) was defined 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)-[(6*S*),(2*E*)-6-hydroxy-2,6-dimethyl-2,7-octadienoyl-(1→3) and (6′*S*),(2′*E*)-6′-hydroxy-2′,6′-dimethyl-2′,7′-octadienoyl-(1→6)]-*β*-D-glucopyranosyl ester.

Another minor compound, gleditsioside P (**3**), displayed the highest quasimolecular ions at 2107 $[M + Na]^+$ and 2123 $[M + K]^+$ in the MALDI-TOF MS among all the

Table 4. ^{13}C and ^{1}H NMR Data for the Monoterpenoid Moieties of Compounds **1**−**3** (125 and 500 MHz in Pyridine-d_5)[a]

position	**1**	**2**	**3**	position	**1**	**2**	**3**
MT_1				MT_1			
1	167.3	167.4	167.4	3	7.18 t (7.9)	7.06 dd (7.6, 1.2)	7.05 dd (7.6, 1.5)
2	132.9	127.4	127.5	4	2.55, 2.60	2.38, 2.46	2.34, 2.42
3	147.1	144.3	144.3	5	1.73	1.73	1.73
4	24.0	24.1	24.1	7	6.04 dd (17.1, 10.7)	6.08 dd (17.1, 10.7)	6.08 dd (17.4, 10.7)
5	41.8	41.4	41.5	8	5.11 dd (10.7, 1.8);	5.15 dd (10.7, 2.1);	5.15 dd (10.7, 2.1);
6	72.2	72.2[b]	72.2[b]		5.50 dd (17.1, 1.8)	5.53 dd (17.1, 2.1)	5.51 dd (17.4, 2.1)
7	146.5	146.5[c]	146.6	9	4.74	1.83	1.84
8	111.8	111.7	111.7[c]	10	1.41	1.43	1.44
9	56.2	12.5	12.5	MT_2			
10	28.5	28.5[d]	28.5	3	7.13 dd (7.6, 1.2)	7.11 dd (7.6, 1.2)	6.96 dd (7.6, 1.5)
MT_2				4	2.00, 2.50	2.00, 2.45	2.35, 2.46
1	167.8	167.8	167.8	5	1.75	1.75	1.68
2	127.6	127.6	128.1	7	6.10 dd (17.1, 10.7)	6.12 dd (17.1, 10.7)	5.96 dd (17.7, 10.98)
3	143.8	143.9	143.3	8	5.18 dd (10.7, 1.8);	5.18 dd (10.7, 2.1);	5.26 dd (10.98, 1.0);
4	23.9	23.9	23.9		5.56 dd (17.1, 1.8)	5.55 dd (17.1, 2.1)	5.34 dd (17.7, 1.0)
5	41.5	41.5	41.0	9	1.94	1.94	1.95
6	72.2	72.1[b]	79.5	10	1.48	1.48	1.50
7	146.6	146.6[c]	142.9	MT_3			
8	111.7	111.7	115.4	3			7.11 dd (7.6, 1.2)
9	12.5	12.5	12.5	4			2.46, 2.50
10	28.6	28.6[d]	24.1	5			1.70
MT_3				7			6.12 dd (17.4, 10.7)
1			167.3	8			5.17 dd (10.7, 2.1);
2			127.9				5.54 dd (17.4, 2.1)
3			143.4	9			1.94
4			23.6	10			1.48
5			41.7				
6			72.1[b]				
7			146.6				
8			111.7[c]				
9			12.7				
10			28.3				

[a] The assignments are based upon DEPT, DQF-COSY, HOHAHA, HETCOR, ROESY, and HMBC experiments. [b−d] Data with the same letter in each column may be interchangeable.

saponins isolated from the title plant. This, combined with its NMR data, gave a molecular formula of $C_{103}H_{160}O_{43}$. Among the 103 signals observed in the ^{13}C NMR spectrum, 30 were assigned to the aglycon, 43 to the sugar portion, and the remaining 30 to three monoterpenoid units. The IR spectrum of **3** suggested the presence of a carbonyl group (1717 cm^{-1}) and an α,β-unsaturated carbonyl group (1656, 1647 cm^{-1}). The ^{1}H and ^{13}C NMR data for the aglycon portion of compounds **1**−**3** were superimposable, indicating that **3** possessed the same aglycon, oleanolic acid, as **1** and **2**. The ^{1}H and ^{13}C NMR spectra of **1** displayed eight sugar anomeric protons [δ 4.86 d (J = 7.3 Hz), 4.89 d (J = 7.9 Hz), 4.97 d (J = 6.7 Hz), 5.05 d (J = 7.0 Hz), 5.14 d (J = 4.9 Hz), 5.17 d (J = 7.9 Hz), 6.16 d (J = 7.0 Hz), 6.29 (br s)] and carbons (δ 94.3, 97.4, 101.4, 102.5, 105.9, 106.4, 106.7, 106.8), respectively (Tables 2 and 3). The overall structural identification and NMR assignments were accomplished using the same procedure as for compound **1**. Accordingly, the eight monosaccharides were determined to be two β-glucopyranoses, four β-xylopyranoses, one α-L-arabinopyranose, and one α-L-rhamnopyranose. The ^{1}H and ^{13}C NMR data were assigned for **3** as listed in Tables 2 and 3. Detailed comparison of the ^{13}C NMR data with **2** suggested that compound **3** possessed the same trisaccharide unit at C-3 and same tetrasaccharide unit at C-28, including the two monoterpenoid moieties linked to C-3 and C-6 of the glucose (Glc′) moiety. Further confirmation was obtained from HMBC and ROESY NMR experiments, as illustrated in Figure 1. The remaining sugar (Xyl‴) was determined to be linked to C-6 of the monoterpenoid unit MT_2 acylated at C-6 of the glucose (Glc′), as indicated by the HMBC correlation between H-1 (δ 4.86) of Xyl‴ and C-6 (δ 79.5) of MT_2. The structure of the third monoterpenoid unit MT_3 was shown to be the same as MT_2 and to be linked to C-2 of the Xyl‴ unit by an HMBC coupling between H-2 (δ 5.89) of Xyl‴ and C-1 (δ 167.3) of MT_3. Consequently, the structure of **3** 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)-{(6*S*),(2*E*)-6-hydroxy-2,6-dimethyl-2,7-octadienoyl-(1→3) and (6′*S*), (2′*E*)-6′-*O*-[2″-*O*-(6″*S*),(2″*E*)-6″-hydroxy-2″,6″-dimethyl-2″,7″-octadienoyl)-β-D-xylopyranosyl]-2′,6′-dimethyl-2′,7′-octadienoyl-(1→6)}-β-D-glucopyranosyl ester. This is the first triterpenoid saponin containing three monoterpenoid acyl group units reported so far.

Gleditsioside Q (**4**) had the molecular formula $C_{78}H_{124}O_{37}$ as deduced from the $[M + Na]^+$ peak at m/z 1675 and the $[M + K]^+$ peak at m/z 1691 in the MALDI-TOF MS, and from its ^{13}C NMR data. The IR spectrum of **4** exhibited carbonyl group (1709 cm^{-1}) and α,β-unsaturated carbonyl group (1648 cm^{-1}) absorptions. It was apparent from the chemical shifts of C-3 (δ 88.8) and C-28 (δ 176.0) of the aglycon in the ^{13}C NMR spectrum that **4** was also a bisdesmosidic triterpenoid glycoside. The ^{1}H and ^{13}C NMR spectra displayed seven anomeric proton and carbon signals (Tables 2 and 3) and a monoterpenoid unit. Hydrolysis of **4** with 1 M HCl gave the aglycon echinocystic acid, identified by co-HPLC analysis with an authentic sample,[2] and the sugar units were determined by GLC to be D-glucose, D-xylose, L-arabinose, and L-rhamnose. Alkaline hydrolysis of **4** resulted in a prosapogenin characterized as echinocystic acid 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside,[2] indicating that the oligosaccharide unit connected to C-28 was a tetrasaccharide. Extensive NMR data obtained for **4** showed that this compound possessed the same sugar structures at both the C-3 and C-28 positions as did **1**. The monoterpenoid

unit was identified as (2*E*)-2-hydroxymethyl-6-hydroxy-6-methyl-2,7-octadienoic acid by its NMR data and was determined to be linked to C-6 of the Glc′ unit, from the downfield shifts of H_2−6 (δ 4.78, 4.95) and C-6 (δ 64.4) due to an acetylation effect.[2] Hence, compound **4** was elucidated 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)-[(2*E*)-2-hydroxymethyl-6-hydroxy-6-methyl-2,7-octadienoyl-(1→6)]-β-D-glucopyranosyl ester.

Experimental Section

General Experimental Procedures. Instrumentation used for obtaining the physicochemical and spectral data, and the experimental condition for chromatography, are the same as described in our previous paper.[2]

Plant Material. The anomalous fruits "Zhu Ya Zao" of *G. sinensis* were purchased from a market in Nanchang, Jiangxi Province, People's Republic of China, in January 1998, and were identified by Prof. Fan Chuishen (Jiangxi College of Traditional Chinese Medicine). A voucher specimen has been deposited in the Division for Pharmacognostical Biotechnology, School of Pharmaceutical Sciences, Beijing Medical University.

Extraction and Isolation. The powdered anomalous 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 H_2O, and then partitioned successively with $CHCl_3$ (45 g) and *n*-BuOH (480 g). The *n*-BuOH fraction was applied to a column of Diaion HP-20 (2.5 L) and washed in turn with H_2O and 30, 50, 70, and 100% MeOH. The 100% MeOH fraction (100 g) was chromatographed over Si gel eluting with a gradient solvent mixture ($CHCl_3$−MeOH−H_2O) to give four saponin fractions [A (2.5 g), B (5 g), C (25 g), D (60 g)]. Fraction B was chromatographed over an open octadecyl silica (ODS) column eluting with 30−70% MeOH to yield fractions B_1 (0.8 g), B_2 (1.0 g), and B_3 (2.6 g). Fraction B_1 was repeatedly subjected to medium-performance liquid chromatography (MPLC) isolation (30−70% MeOH), following by reversed-phase HPLC purification (45% CH_3CN−0.06% TFA in H_2O) to afford **1** (80 mg). Further MPLC isolation (30−70% MeOH) and HPLC purification (47% CH_3CN−0.06% TFA in H_2O) of fraction B_2 furnished **2** (21 mg) and **3** (16 mg). By the same procedure, fraction A was applied in turn to an ODS column (30−70% MeOH), MPLC isolation (30−70% MeOH), and HPLC purification (70% MeOH), to furnish **4** (60 mg).

Gleditsioside N (1): an amorphous white solid from MeOH; mp 191−192 °C (dec); $[\alpha]^{21}_D$ −20° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3403, 2928, 1711, 1647, 1078 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) aglycon δ 5.47 (1H, br t, H-12), 3.30 (1H, m, H-3), 1.37, 1.34, 1.08, 1.00, 0.98, 0.91, 0.88 (each 3H, s, H_3-23, -27, -26, -24, -30, -25, -29); for other NMR data, see Tables 1−4; MALDI-TOF MS (positive ion mode) *m*/*z* [M + Na]$^+$ 1825, [M + K]$^+$ 1841.

Gleditsioside O (2): an amorphous white solid from MeOH; mp 207−208 °C (dec); $[\alpha]^{21}_D$ −20° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3417, 2932, 1705, 1654, 1077 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) aglycon δ 5.46 (1H, br t, H-12), 3.30 (1H, m, H-3), 1.37, 1.34, 1.08, 1.00, 0.98, 0.91, 0.88 (each 3H, s, H_3-23, -27, -26, -24, -30, -25, -29); for other NMR data, see Tables 1−4; MALDI-TOF MS (positive ion mode) *m*/*z* [M + Na]$^+$ 1809, [M + K]$^+$ 1825.

Gleditsioside P (3): an amorphous white solid from MeOH; mp 175−176 °C (dec); $[\alpha]^{21}_D$ −12° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3425, 2939, 1717, 1656, 1647, 1078 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) aglycon δ 5.45 (1H, br t, H-12), 3.30 (1H, m, H-3), 1.36, 1.34, 1.08, 1.00, 0.97, 0.91, 0.88 (each 3H, s, H_3-23, -27, -26, -24, -30, -25, -29); for other NMR data, see Tables 1−4; MALDI-TOF MS (positive ion mode) *m*/*z* [M + Na]$^+$ 2107, [M + K]$^+$ 2123.

Gleditsioside Q (4): an amorphous white solid from MeOH; mp 210−211 °C (dec); $[\alpha]^{21}_D$ −18° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3415, 2926, 1709, 1648, 1086 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) aglycon δ 5.65 (1H, br t, H-12), 5.22 (1H, br t, H-16), 3.50 (1H, m, H-3), 1.87, 1.33, 1.10, 1.10, 1.00, 0.93, 0.90 (each 3H, s, H_3-27, -23, -26, -30, -24, -25, -29); monoterpenoid acid δ 7.21 (1H, t, J = 7.9 Hz, H-3), 6.08 (1H, dd, J = 17.1, 10.6 Hz, H-7), 5.22 (1H, dd, J = 17.1, 1.95 Hz, H_2-8), 5.14 (1H, dd, J = 10.6, 1.95 Hz, H_2-8), 4.75 (2H, br s, H_2-9), 2.46 (1H, m, H_2-4), 2.33 (1H, m, H_2-4), 1.78 (2H, m, H_2-5), 1.46 (3H, s, H_3-10); ^{13}C NMR (pyridine-d_5, 125 MHz) monoterpenoid acid δ 167.8 (C-1), 146.6 (C-3), 146.5 (C-7), 133.0 (C-2), 111.8 (C-8), 72.2 (C-6), 56.2 (C-9), 41.8 (C-5), 28.6 (C-10), 24.0 (C-4); for other NMR data, see Tables 1−3; MALDI-TOF MS (positive ion mode) *m*/*z* [M + Na]$^+$ 1675, [M + K]$^+$ 1691.

Alkaline Hydrolysis of Gleditsiosides N (1) and Q (4). Compound **1** (30 mg) was refluxed with 2 mL 1 M NaOH at 100 °C for 4 h. On 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 a prosapogenin (10 mg) [oleanolic acid 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside, $[\alpha]^{21}_D$ +26° (*c* 0.10, MeOH)] and a monoterpenoid acid (4.1 mg) [(6*S*),(2*E*)-6-hydroxy-2,6-dimethyl-2,7-octadienoic acid, $[\alpha]^{21}_D$ +12.8° (*c* 1.0, MeOH)]. Compound **4** (30 mg) in 2 mL 0.8 M NaOH was heated at 80 °C for 4 h and then purified in a similar manner to furnish the prosapogenin echinocystic acid 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside [11 mg, $[\alpha]^{21}_D$ +12° (*c* 0.10, MeOH)].

Acidic Hydrolysis of Gleditsioside N (1) and Q (4). Compound **1** (25 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 the dioxane was removed, the solution was extracted with EtOAc (1 mL × 3). The extraction was washed with H_2O and then concentrated to give an aglycon (7 mg) [oleanolic acid, $[\alpha]^{21}_D$ +82° (*c* 0.10, MeOH)]. The monosaccharide portion was neutralized by passing it through an ion-exchange resin (Amberlite MB-3) column, concentrated (dried overnight), and then treated with 1-(trimethylsilyl)imidazole at room temperature for 2 h. After the excess reagent was decomposed with H_2O, the reaction product was extracted with *n*-hexane (1 mL × 2). The TMSi derivatives of the monosaccharides were identified as glucose, xylose, arabinose, and rhamnose by co-GLC analysis with standard monosaccharides. By the same method, compound **4** (25 mg) furnished an aglycon (7.2 mg) [echinocystic acid, $[\alpha]^{21}_D$ + 52° (*c* 0.10, MeOH)], and the monosaccharides were also identified as glucose, xylose, arabinose, and rhamnose.

For the physicochemical properties and the spectral data of the prosapogenins, the aglycons and the monoterpenoid acid, see the literature.[2]

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