Four New Triterpenoidal Saponins Acylated with One Monoterpenic Acid from *Gleditsia sinensis*

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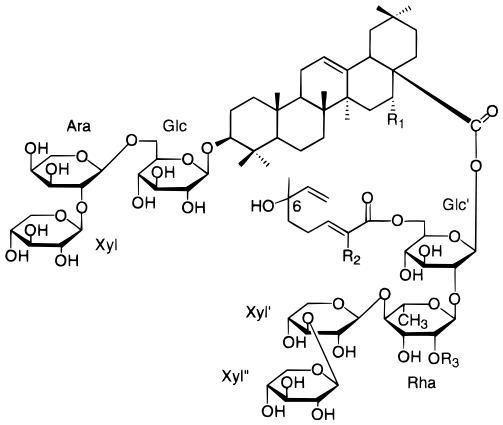
Four new oleanane-type triterpenoidal glycosides, named gleditsiosides A−D (**1**−**4**), were isolated from the anomalous fruits of *Gleditsia sinensis*. Using modern NMR techniques, including DQF−COSY, HETCOR, HOHAHA, HMBC, and ROESY experiments and MS analysis as well as chemical methods, their structures were 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→6)]-*β*-D-glucopyranosyl ester (**1**); 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-hydroxylmethyl-6-hydroxy-6-methyl-2,7-octadienoyl-(1→6)]-*β*-D-glucopyranosyl ester (**2**); 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)-[(2*E*)-2-hydroxylmethyl-6-hydroxy-6-methyl-2,7-octadienoyl-(1→6)]-*β*-D-glucopyranosyl ester (**3**); and 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)-[(6*S*,2*E*)-6-hydroxy-2,6-dimethyl-2,7-octadienoyl-(1→6)]-*β*-D-glucopyranosyl ester (**4**).

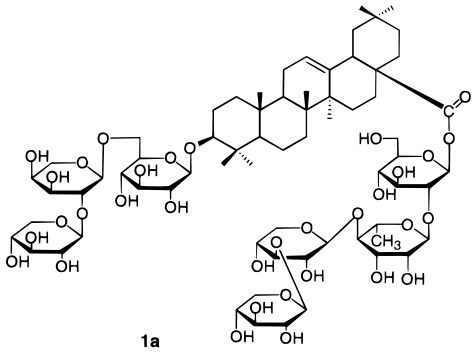
Gleditsia sinensis Lam. (Leguminosae) is widely distributed throughout China. Its different parts—called "Zao Jia", "Zhu Ya Zao", "Zao Jia Zi", "Zao Jia Ye", "Zao Jiao Ci", and "Zao Jia Gen Pi" from the normal fruits, anomalous fruits (a small fruit), seeds, leaves, thorns, and radix cortexes, respectively—possess different curative effects according to the theory of traditional Chinese medicine. "Zhu Ya Zao", the anomalous fruit produced by old or injured plants, has long been known in China as a saponin-rich herbal medicine and used for the treatment of apoplexy, as an expectorant, and for a pesticide.[1] However, until now there has been no report on the chemical constituents making up this medicine, though some saponins bearing two monoterpenic acids were isolated from the fruits of *G. japonica* Miquel.[2−4] To shed light on the chemical entity responsible for its medicinal actions, we conducted a detailed investigation of the saponins occurring as a very complex mixture from this source. In this paper, we describe the isolation and structure elucidation of four new triterpenoidal saponins, named gleditsiosides A, B, C, and D (**1**−**4**), by using modern NMR techniques, including DQF−COSY, HETCOR, HOHAHA, HMBC, and ROESY experiments and MS analysis as well as some chemical degradation. All four compounds were acylated with one monoterpenic acid to the C-6 of the glucose directly linked to C-28 of the aglycon.



1	R_1 = H	R_2 = CH_3 (6 = *S*)	R_3 = H
2	R_1 = H	R_2 = CH_2OH	R_3 = H
3	R_1 = OH	R_2 = CH_2OH	R_3 = Gal
4	R_1 = OH	R_2 = CH_3 (6 = *S*)	R_3 = Gal

Results and Discussion

Gleditsioside A (**1**), an amorphous solid, had a molecular formula of $C_{78}H_{124}O_{35}$ deduced from the $[M - H]^-$ ion at *m*/*z* 1619.7960 in the negative HRFABMS, $[M + Na]^+$ ion at *m*/*z* 1643, and $[M + K]^+$ ion at 1659 in the MALDI-TOF MS (positive ion mode) as well as from its NMR data. The



1a

IR spectrum showed a carbonyl group (1697 cm^{-1}) and a α,*β*-unsaturated carbonyl group (1645 cm^{-1}) absorption.

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Table 1. ^{13}C NMR Data for the Aglycon Moieties (125 and 100 MHz in Pyridine-d_5)

	1	2	3	4	1a	1b	2b	1d	2d
2	26.8	26.8	26.8	26.8	26.8	26.8	26.8	28.1	28.2
3	88.6	88.6	88.8	88.8	88.6	88.5	88.7	78.1	78.1
4	39.6	39.6	39.6	39.6	39.6	39.6	39.6	39.0	39.1
5	55.9	55.9	56.1	56.1	55.9	55.9	55.9	55.8	55.9
6	18.7	18.7	18.8	18.7	18.7	18.5	18.5	18.8	18.9
7	33.3	33.3	33.5	33.6	33.3	33.2	33.5	33.2	33.6
8	40.0	40.0	40.2	40.1	40.0	39.8	39.9	39.8	40.0
9	48.1	48.1	47.2	47.2	48.1	48.1	47.2	48.1	47.3
10	37.1	37.1	37.2	37.1	37.1	37.1	37.1	37.4	37.5
11	23.8	23.9	23.9	23.9	23.9	23.9	23.9	23.9	23.9
12	122.9	122.9	122.7	122.7	122.8	122.6	122.4	122.6	122.5
13	143.9	143.9	143.8	143.5	144.0	144.8	145.1	144.8	145.1
14	42.3	42.3	42.1	42.2	42.3	42.1	42.1	42.2	42.1
15	28.4	28.5	36.2	36.2	28.5	28.3	36.2	28.3	36.2
16	23.4	23.4	74.1	74.1	23.4	23.7	74.8	23.8	74.8
17	47.2	47.2	49.4	49.3	47.1	46.7	48.9	46.7	48.9
18	41.9	41.9	41.5	41.6	42.0	41.9	41.4	42.0	41.5
19	46.3	46.3	47.3	47.4	46.3	46.4	47.2	46.5	47.3
20	30.7	30.7	30.8	30.8	30.7	30.7	31.0	31.0	31.1
21	34.0	34.0	36.0	36.0	34.0	34.3	36.1	34.3	36.1
22	32.5	32.5	31.9	32.0	32.3	33.3	32.9	33.3	32.9
23	28.2	28.2	28.3	28.3	28.2	28.2	28.2	28.8	28.8
24	17.1	17.1	17.1	17.1	17.1	17.1	17.1	16.6	16.6
25	15.7	15.7	15.8	15.8	15.7	15.5	15.6	15.6	15.7
26	17.6	17.6	17.6	17.6	17.5	17.4	17.5	17.5	17.6
27	26.1	26.1	27.1	27.1	26.1	26.3	27.3	26.2	27.3
28	176.5	176.6	175.9	175.9	176.5	180.4	180.0	180.2	179.9
29	33.2	33.2	33.2	33.2	33.2	33.3	33.4	33.3	33.4
30	23.9	23.9	24.7	24.6	23.7	23.8	24.8	23.8	24.8

After an extensive 2D NMR study, the aglycon was identified as oleanolic acid (Table 1). The chemical shifts of C-3 (δ 88.6) and C-28 (δ 176.5) indicated that **1** was a bisdesmosidic glycoside. The ^{1}H and ^{13}C NMR of **1** exhibited seven sugar anomeric protons at δ 4.83 (d, J = 7.9 Hz), 4.93 (d, J = 7.0 Hz), 5.03 (d, J = 7.0 Hz), 5.09 (d, J = 5.5 Hz), 5.12 (d, J = 7.4 Hz), 6.09 (d, J = 8.6 Hz), and 6.34 (br s) and carbons at δ 94.6, 101.5, 102.4, 105.9, 106.4, 106.7, and 106.8, respectively (Tables 2 and 3). The ^{13}C NMR spectrum of **1** showed 78 carbon signals, from which 30 were assigned to the aglycon part, 38 to the oligosaccharide moiety, and the remaining 10 to a monoterpenic acid. Alkaline hydrolysis of **1** afforded prosapogenins **1a** and **1b**, and the monoterpenic acid **1c**. Compounds **1a** and **1b** were identified 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 and oleanolic acid 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside,[5] respectively, based upon their NMR data (Tables 1, 2, and 3). The monoterpenic acid **1c** was characterized as (6*S*,2*E*)-6-hydroxy-2,6-dimethyl-2,7-octadienoic acid by comparison with the published physicochemical and spectral data.[6] Acid hydrolysis of **1** furnished **1d**, identified as oleanolic acid, and the monosaccharide components were identified as glucose, xylose, rhamnose, and arabinose based on GLC analysis. From the above evidence, it was concluded that **1** was bisdesmosidic triterpenoid glycoside with glucose, arabinose, and xylose linked to the C-3 position of the aglycon, and the other four monosaccharides were connected to C-28 of the aglycon through an ester bond.

The identity of the monosaccharides and the sequence of the oligosaccharide chain were determined by a combination of DQF−COSY, HOHAHA, DEPT, HETCOR, HMBC, and ROESY experiments. The individual spin systems can be discerned from the subspectra corresponding to the anomeric protons or methyl groups (for deoxy sugars) in the HOHAHA experiment. Starting from the anomeric proton of each sugar unit, all the hydrogens within each spin system were assigned using DQF−COSY with the aid of 2D HOHAHA and ROESY spectra. Information from COSY, HOHAHA, and ROESY furnished most of the

Table 2. ^{13}C NMR Data for the Sugar Moieties (125 and 100 MHz in Pyridine-d_5)

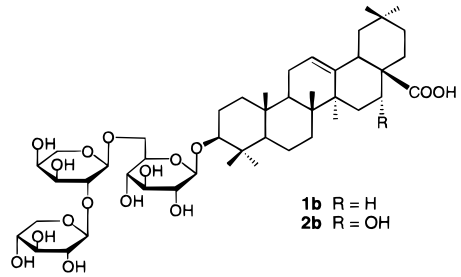
	1	2	3	4	1a		1	2	3	4	1a
C_3-Glc						Rha					
1	106.7 (157)[b]	106.7	106.7	106.8	106.7	1	101.5 (175)[b]	101.5	100.4	100.4	101.3
2	75.7	75.7	75.7	75.7	75.7	2	71.6	71.6	81.2	81.3	71.6
3	78.4	78.4	78.4	78.4	78.4	3	72.5	72.5	72.1	72.1	72.5
4	72.1	72.3	72.2	72.3	72.3	4	84.9	85.0	83.2	83.0	85.1
5	76.2	76.2	76.1	76.1	76.2	5	68.3	68.3	68.2	68.2	68.2
6	69.6	69.6	69.6	69.6	69.6	6	18.4	18.6	18.0	18.6	18.6
Ara						Xyl′					
1	102.4 (163)[b]	102.4	102.3	102.3	102.4	1	106.8 (160)[b]	106.8	105.9	106.1	106.8
2	80.7	80.7	80.4	80.6	80.7	2	75.1[c]	75.1[c]	75.0[c]	75.1[c]	75.1[c]
3	72.7	72.7	72.6	72.6	72.7	3	87.4	87.4	87.2	87.4	87.4
4	67.5	67.5	67.5	67.5	67.5	4	68.9	68.9	69.0	69.0	68.9
5	64.4	64.4	64.4	64.3	64.4	5	66.9	66.9	66.7	66.8	66.9
Xyl						Xyl″					
1	106.4 (163)[b]	106.4	106.2	106.4	106.4	1	105.9 (161)[b]	105.9	105.8	105.8	105.9
2	75.3[c]	75.5[c]	75.3[c]	75.5[c]	75.3[c]	2	75.2[c]	75.2[c]	75.2[c]	75.2[c]	75.2[c]
3	77.9	77.9	77.8	77.9	77.9	3	78.1	78.1	78.0	78.2	78.1
4	70.9[d]	70.9[d]	70.9[d]	70.9[d]	70.9[d]	4	70.8[d]	70.8[d]	70.8[d]	70.8[d]	70.8[d]
5	67.3	67.3	67.2	67.3	67.3	5	67.4	67.4	67.2	67.3	67.4
C_{28}-Glc′						Gal					
1	94.6 (168)[b]	94.6	94.5	94.5	94.8	1			107.6	107.6	
2	76.8	76.6	77.2	77.2	76.4	2			73.7	73.3	
3	79.0	79.0	78.7	78.8	79.3	3			74.8	74.9	
4	71.3	71.5	71.5	71.3	71.3	4			70.2	70.2	
5	75.8	75.8	75.8	75.9	78.9	5			77.0	77.1	
6	64.3	64.4	64.3	64.3	62.1	6			62.2	62.2	

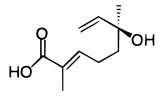
[a] The assignment was based upon DQF-COSY, HOHAHA, HETCOR, ROESY, and HMBC experiments. [b] The number in the parentheses is the $^1J_{C1,H1}$ coupling constant (Hz). [c,d] The data with the same labels in each column may be interchanged.

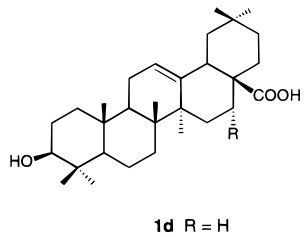
Table 3. ^{1}H NMR Data for the Sugar Moieties (500 and 400 MHz in Pyridine-d_5)[a]

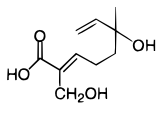
	1	2	3	4	1a		1	2	3	4	1a
C_3-Glc						Rha					
1	4.83 d	4.88 d	4.89 d	4.90 d	4.89 d	1	6.34	6.38	6.45	6.49	6.40
	(7.9)	(7.6)	(7.9)	(7.8)	(7.6)		(br. s)	(br. s)	(br. s)	(br. s)	(br. s)
2	4.06	4.06	4.05	4.08	4.01	2	4.80	4.82	4.82	4.85	4.82
3	4.18	4.20	4.16	4.15	4.21	3	4.69	4.66	4.75	4.70	4.70
4	4.12	4.13	4.12	4.13	4.12	4	4.35	4.37	4.36	4.25	4.36
5	4.07	4.08	4.06	4.08	4.10	5	4.40	4.47	4.30	4.38	4.52
6	4.23	4.25	4.22	4.25	4.25	6	1.72 d	1.77 d	1.64 d	1.63	1.76 d
	4.65	4.80	4.61	4.65	4.68		(6.1)	(6.1)	(6.1)	(6.0)	(6.0)
Ara						Xyl′					
1	5.09 d	5.15 d	5.12 d	5.16 d	5.16 d	1	5.03 d	5.08 d	5.06 d	5.11 d	5.08 d
	(5.5)	(5.2)	(4.9)	(5.6)	(5.2)		(7.0)	(7.3)	(7.3)	(7.1)	(6.8)
2	4.50	4.53	4.50	4.55	4.52	2	4.05	4.06	4.03	4.03	4.01
3	4.36	4.37	4.35	4.38	4.37	3	4.01	4.03	3.96	3.96	4.03
4	4.34	4.30	4.32	4.32	4.39	4	4.07	4.10	4.00	4.00	4.12
5	3.73	3.74	3.69	3.75	3.75	5	3.45	3.52	3.34	3.35	3.50
	4.25	4.30	4.28	4.30	4.31		4.21	4.23	4.07	4.10	4.25
Xyl						Xyl″					
1	4.93 d	4.92 d	4.97 d	4.98 d	4.98 d	1	5.12 d	5.19 d	5.09 d	5.13 d	5.18 d
	(7.0)	(10.7)	(7.3)	(7.0)	(6.8)		(7.4)	(7.6)	(7.6)	(8.3)	(8.8)
2	4.02	4.04	4.03	4.03	4.01	2	4.05	4.06	4.03	4.03	4.01
3	4.05	4.06	4.05	4.06	4.06	3	4.10	4.10	4.10	4.09	4.12
4	4.13	4.15	4.12	4.15	4.15	4	4.13	4.15	4.15	4.15	4.15
5	3.57	3.56	3.56	3.55	3.58	5	3.65	3.67	3.61	3.65	3.69
	4.35	4.41	4.36	4.43	4.40		4.26	4.31	4.27	4.30	4.28
C_{28}-Glc′						Gal					
1	6.09 d	6.13 d	5.93 d	6.09 d	6.20 d	1			5.15 d	5.18 d	
	(8.6)	(8.5)	(7.6)	(8.0)	(8.0)				(7.6)	(7.8)	
2	4.37	4.37	4.25	4.27	4.40	2			4.45	4.51	
3	4.24	4.24	4.18	4.20	4.28	3			4.00	3.99	
4	4.10	4.09	4.08	4.09	4.32	4			4.41	4.47	
5	4.06	4.06	4.10	4.10	3.97	5			3.94	3.95	
6	4.71	4.73	4.75	4.75	4.30	6			4.31	4.35	
	4.88	4.96	4.90	4.90	4.40						

[a] The assignment was based upon DQF−COSY, HOHAHA, HETCOR, ROESY, and HMBC experiments.









assignments. On the basis of the assigned proton signals, a HETCOR experiment then gave the corresponding carbon assignments, and these were further confirmed by an HMBC experiment. After the assignments of the protons and protonated carbons were established (Tables 2 and 3), the seven sugar units were identified as two glucoses, three xyloses, one rhamnose, and one arabinose and further confirmed by GLC analysis of the acid hydrolysate. The linkage of the sugar units at C-3 was established from the following HMBC correlations: H-1 (δ 5.09) of Ara with C-6 (δ 69.6) of Glc; H-1 (δ 4.93) of Xyl with C-2 (δ 80.7) of Ara. The attachment of the trisaccharide moiety to C-3 of the aglycon was confirmed by the long-range correlation between H-1 (δ 4.83) of Glc and C-3 (δ 88.6) of the aglycon. The sequence of the sugar chain at C-28 was deduced from the following HMBC correlations: H-1(δ 6.34) of Rha with C-2 (δ 76.8) of Glc′; H-1(δ 5.03) of Xyl′ with C-4 (δ 84.9) of Rha; H-1 (δ 5.12) of Xyl″ with C-3 (δ 87.4) of Xyl′, while the attachment of the tetrasaccharide chain to C-28 of the aglycon was based on a correlation of H-1 (δ 6.09) of Glc′ with the C-28 (δ 176.5) of the aglycon. The attachment of the monoterpenic acid **1c** to C-6 of Glc′ was established by a long-range correlation of H_2-6 (δ 4.71, 4.88) of Glc′ with C-1 (δ 168.0) of the monoterpenic acid from the HMBC spectrum of **1**. In addition, the downfield shifts of the two H_2-6 also indicated it was the acylated position. The two sugar chains of **1** were also deduced from the following NOE correlations observed in the ROESY spectrum: H-1 (δ 4.93) of Xyl with H-2 (δ 4.50) of Ara, H-1 (δ 5.09) of Ara with H-6 (δ 4.65) of Glc, H-1 (δ 5.12) of Xyl″ with H-3 (δ 4.01) of Xyl′, H-1 (δ 5.03) of Xyl′ with H-4 (δ 4.35) of Rha, and H-1 (δ 6.34) of Rha with H-2 (δ 4.37) of Glc′. The major fragmentation peaks at *m*/*z* 617 [aglycon + Glc]$^-$, 749 [617 + Ara]$^-$, 881 [749 + Xyl]$^-$, 1189 [881 + Glc′ + Rham]$^-$, 1355 [M − Xyl′ − Xyl″]$^-$, 1453 [M − monoterpene]$^-$, and 1487 [M − Xyl″]$^-$ in the negative FABMS of **1** were also in agreement with the structure deduced above.

All the monosaccharides were determined to be in the pyranose form from their ^{13}C NMR data. The anomeric configurations for the sugar moieties were fully defined from their $^3J_{H1,\ H2}$ coupling constants and $^1J_{C1,H1}$ coupling constants as well as from NOE information. Accordingly, the glucoses and the xyloses were established to be in the β configuration, while the arabinose and the rhamnose were in the α configuration. The foregoing evidence led to the elucidation of the structure of gleditsioside A (**1**) 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→6)]-β-D-glucopyranosyl ester.

Gleditsioside B (**2**) gave $[M + Na]^+$ ion at *m*/*z* 1659 and $[M + K]^+$ ion at 1675 in the MALDI-TOF MS (positive ion mode), 16 mass units higher than that of **1**, implying the presence of an additional oxygen-bearing function in **2**. Detailed analysis of the ^{13}C NMR spectrum of **2** indicated the chemical shifts for the aglycon part and sugar moieties of **2** bore a close resemblance to those of **1**, suggesting that both compounds had a common aglycon and sugar-substitution pattern. Alkaline hydrolysis of **2** under the same conditions as that of **1** afforded **1a** and **1b** as shown by co-HPLC analysis. Hydrolysis of **2** with 1M HCl gave **1d**, and the sugar units determined by GLC also were D-glucose, D-xylose, L-arabinose, and L-rhamnose. The ^1H and ^{13}C NMR data obtained for the monoterpenic acid in **2** were different from those of **1**, in that the methyl group at C-2 (δ_C 12.5, δ_H 1.88) was replaced by a hydroxylmethyl group (δ_C 56.2, δ_H 4.74). Thus, the monoterpenic acid **2c** in **2** was shown to be (2*E*)-2-hydroxylmethyl-6-hydroxy-6-methyl-2,7-octadienoic acid.[3] Unfortunately, attempted alkaline hydrolysis of **2** did not afford intact **2c**, giving only **2b**. Consequently, the absolute configuration of **2c** was not established. The proton and carbon assignments were made by a combination of DQF−COSY, HOHAHA, DEPT, HETCOR, and ROESY experiments. From the above evidence, the structure of **2** 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→6)]-β-D-glucopyranosyl ester.

Gleditsioside C (**3**) had a molecular formula of $C_{84}H_{134}O_{42}$ determined from the $[M - H]^-$ ion at *m*/*z* 1813.8357 in the negative HRFABMS, an $[M + Na]^+$ ion at *m*/*z* 1837, and an $[M + K]^+$ ion at 1853 in the MALDI-TOF MS (positive ion mode), and from the NMR data. The IR of **3** featured absorption of the carbonyl group (1696 cm^{-1}) and the α,β-unsaturated carbonyl group (1646 cm^{-1}). It was apparent from the chemical shifts of C-3 (δ 88.8) and C-28 (δ 175.9) of the aglycon in the ^{13}C NMR that **3** was also a bisdesmosidic glycoside. The ^1H and ^{13}C NMR spectra of **3** displayed eight anomeric proton signals at δ 6.45 (br s, Rha), 5.93 (d, *J* = 7.6 Hz, Glc′), 5.15 (d, *J* = 7.6 Hz, Gal), 5.12 (d, *J* = 4.9 Hz, Ara), 5.09 (d, *J* = 7.6 Hz, Xyl″), 5.06 (d, *J* = 7.3 Hz, Xyl′), 4.97 (d, *J* = 7.3 Hz, Xyl), and 4.89 (d, *J* = 7.9 Hz, Glc) and carbon signals at δ 100.4 (Rha), 94.5 (Glc′), 107.6 (Gal), 102.3 (Ara), 105.8 (Xyl″), 105.9 (Xyl′), 106.2 (Xyl), and 106.7 (Glc), respectively (Tables 2 and 3). Acid hydrolysis of **2** afforded **2d**, identified as echinocystic acid,[3] and the monosaccharide components were identified as glucose, xylose, arabinose, rhamnose, and galactose based on the GLC analysis. Alkaline hydrolysis of **3** resulted in the prosapogenin **2b**, characterized as echinocystic acid 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside,[5] indicating that the remaining five sugars were connected to C-28 of the aglycon. The linkage for sugar units at C-28 was established based upon the long-range correlation of H-1 (δ 5.93) of Glc′ with C-28 (δ 175.9) of the aglycon, appeared in the HMBC spectrum of **3**. The existence of the monoterpenic acid in compound **3** was indicated from various NMR data and identified as (2*E*)-2-hydroxylmethyl-6-hydroxy-6-methyl-2,7-octadienoic acid **2c**. The linkage position of the monoterpenic acid was deduced from a correlation between H-6 (δ 4.75, 4.90) of Glc′ and C-1 (δ 167.3) of the monoterpenic acid.

The overall structure assignment was accomplished using the same protocol as in compound **1**. The whole sugar sequence of **3** was deduced from the HMBC information, from the ROESY experiment, and from the analysis of the FABMS as depicted for compound **1**. All the monosaccharides were determined to be in the pyranose form from their ^{13}C NMR data. The anomeric configurations for each sugar were evident from their $^3J_{H1,H2}$ coupling constants (Tables 2 and 3) as well as from the ROESY information. On the basis of above evidence, the structure of gleditsioside C (**3**) 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)-[β-D-galactopyranosyl-(1→2)]-α-L-rhamnopyranosyl-(1→2)-[(2*E*)-2-hydroxylmethyl-6-hydroxy-6-methyl-2,7-octadienoyl-(1→6)]-β-D-glucopyranosyl ester.

Gleditsioside D (**4**) had $[M + Na]^+$ ion at *m*/*z* 1821 and $[M + K]^+$ ion at 1837 in the MALDI-TOF MS (positive ion mode), 16 mass units lower than that of **3**. Detailed comparison of the NMR data of **4** with those of **3** indicated both compounds shared a common aglycon and a common sugar-substitution pattern. The NMR data of the monoterpenic acid in **4** were almost superimposable on those of the monoterpenic moiety in **1**. Alkaline hydrolysis of **4**, under the same condition as that of **1**, furnished **2b** and **1c** as detected by co-HPLC. Acid hydrolysis of **4** afford **2d**, and the sugar units determined by GLC were D-glucose, D-xylose, L-arabinose, L-rhamnose, and D-galactose. Therefore, the structure of **4** was established 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)-[β-D-galactopyranosyl-(1→2)]-α-L-rhamnopyranosyl-(1→2)-[(6*S*,2*E*)-6-hydroxy-2,6-dimethyl-2,7-octadienoyl-(1→6)]-β-D-glucopyranosyl ester.

Experimental Section

General Experimental Procedures. Melting points were measured with a Yanaco microscope apparatus and are uncorrected. Optical rotations were performed with a JASCO DIP-370 digital polarimeter. IR spectra were carried out on a JASCO D-300 FTIR spectrometer. FABMS and MALDI-TOF MS were conducted using JEOL JMS-700 and Perseptive Biosystems Voyager DE-STR mass spectrometers, respectively. The ^1H and ^{13}C NMR measurements were carried out at 500 MHz (JEOL α-500) or 400 MHz (JEOL EX-400), using an standard JEOL sequences for 1D and 2D NMR measurement in pyridine-d_5 solution, and chemical shifts were expressed in δ (ppm) with reference to TMS. ROESY spectra were recorded with the mixing time set at 250 ms. The spin locking time for the HOHAHA experiment was 120 ms. The HMBC experiment was run with the delay time set at 100 ms. Diaion HP-20 (Mitsubishi Chemical), Si gel (Si gel 60, Merck), and ODS (Chromatorex, 100−200 mesh, Fujisylisia) were used for open column chromatography. Preparative MPLC and HPLC were 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.

Plant Material. The anomalous fruits (Zhu Ya Zao) of *G. sinensis* Lam. were purchased from the market of Nanchang, the capital of Jiangxi province, People's Republic of China, in January 1998, and were identified by professor Fan Chuishen (Jiangxi College of Traditional Chinese Medicine). A voucher specimen was deposited in the Division for Pharmacognostical

Biotechnology, School of Pharmaceutical Sciences, Beijing Medical University, Beijing, China.

Extraction and Isolation. The powdered anomalous fruits (Zhu Ya Zao, 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-soluble fraction was applied to a column of Diaion HP-20 (2500 mL) and washed with H_2O and 30, 50, 70, and 100% MeOH. The 70% MeOH fraction (120 g) was chromatographed over Si gel and ODS columns to give four saponin fractions of A (5 g), B (22 g), C (2.4 g), and D (60 g). Part of fraction D (12 g) was chromatographed over ODS columns to yield D_1 (8.0 g), D_2 (1.5 g), and D_3 (1.1 g). Fraction D_3 was repeatedly subjected to MPLC and HPLC purification to afford **1** (180 mg) and **2** (112 mg). By the same method, fraction C furnished **3** (120 mg) and **4** (65 mg).

Gleditsioside A (1): an amorphous solid from MeOH; mp 205−206 °C (dec); $[\alpha]^{21}_D$ −11° (*c* 0.10, MeOH); IR ν_{max}(KBr) 3405, 2933, 1697, 1645, 1078 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) aglycon δ 5.44 (1H, br t, H-12), 3.50 (1H, m, H-3), 1.39, 1.35, 1.04, 0.98, 0.97, 0.89, 0.87 (each 3H, s, H_3-23, -27, -26, -24, -30, -25, -29); monoterpenic acid δ 7.02 (1H, t, J = 7.9 Hz, H-3), 6.09 (1H, dd, J = 17.4, 10.7 Hz, H-7), 5.52 (1H, dd, J = 17.4, 1.8 Hz, H_2-8), 5.17 (1H, dd, J = 10.7, 1.8 Hz, H_2-8), 2.45 (1H, m, H_2-4), 2.35 (1H, m, H_2-4), 1.88 (3H, s, H_3-9), 1.76 (2H, m, H_2-5), 1.46 (3H, s, H_3-10); ^{13}C NMR (pyridine-d_5, 125 MHz) monoterpenic acid δ 168.0 (C-1), 146.6 (C-7), 143.5 (C-3), 127.7 (C-2), 111.7 (C-8), 71.9 (C-6), 41.5 (C-5), 28.6 (C-10), 24.0 (C-4), 12.5 (C-9); other NMR data see Tables 1, 2, and 3; negative FABMS *m/z* 455 [aglycon − H]$^-$, 617 [aglycon + Glc]$^-$, 749 [617 + Ara]$^-$, 881 [749 + Xyl]$^-$, 1189 [881 + Glc′ + Rham]$^-$, 1355 [M − Xyl′ − Xyl″]$^-$, 1453 [M − monoterpene]$^-$, 1487 [M − Xyl″]$^-$, and 1619 [M − H]$^-$; negative HRFABMS *m/z* 1619.7960 [M − H]$^-$ (calcd for 1619.7821); MALDI-TOF MS (positive ion mode) *m/z* [M + Na]$^+$ 1643, [M + K]$^+$ 1659.

Gleditsioside B (2): an amorphous solid from MeOH; mp 203−204 °C (dec); $[\alpha]^{21}_D$ −10.0° (*c* 0.10, MeOH); IR ν_{max}(KBr) 3420, 2930, 1709, 1646, 1079 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) aglycon δ 5.47 (1H, br t, H-12), 3.51, (1H, m, H-3), 1.37, 1.34, 1.08, 0.99, 0.97, 0.90, 0.87 (each 3H, s, H_3-23, -27, -26, -24, -30, -25, -29); monoterpenic acid δ 7.24 (1H, t, J = 7.9 Hz, H-3), 6.08 (1H, dd, J = 17.4, 10.8 Hz, H-7), 5.52 (1H, dd, J = 17.4, 1.8 Hz, H_2-8), 5.14 (1H, dd, J = 10.7, 1.8 Hz, H_2-8), 4.74 (2H, br s, H_2-9), 2.72 (1H, m, H_2-4), 2.60 (1H, m, H_2-4), 1.74 (2H, m, H_2-5), 1.45 (3H, s, H_3-10); ^{13}C NMR (pyridine-d_5, 125 MHz) monoterpenic acid δ 167.7 (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); other NMR data are shown in Tables 1, 2, and 3; MALDI-TOF MS (positive ion mode) *m/z* [M + Na]$^+$ 1659, [M + K]$^+$ 1675.

Gleditsioside C (3): an amorphous solid from MeOH; mp 211−212 °C (dec); $[\alpha]^{21}_D$ −15° (*c* 0.10, MeOH); IR ν_{max}(KBr) 3412, 2925, 1696, 1646, 1078 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) aglycon δ 5.57 (1H, br t, H-12), 5.05 (1H, br t, H-16), 3.44 (1H, m, H-3), 1.75, 1.27, 1.06, 1.05, 0.98, 0.95, 0.92 (each 3H, s, H_3-27, -23, -26, -30, -24, -25, -29); monoterpenic acid δ 7.15 (1H, t, J = 7.9 Hz, H-3), 6.05 (1H, dd J = 17.4, 10.7 Hz, H-7), 5.45 (1H, dd, J = 17.4, 1.8 Hz, H_2-8), 5.11 (1H, dd, J = 10.7, 1.8 Hz, H_2-8), 4.64 (2H, br s, H_2-9), 2.66 (1H, m, H_2-4), 2.55 (1H, m, H_2-4), 1.77 (2H, m, H_2-5), 1.42 (3H, s, H_3-10); ^{13}C NMR (pyridine-d_5, 125 MHz) monoterpenic acid δ 167.3 (C-1), 146.4 (C-3), 145.9 (C-7), 132.3 (C-2), 111.4 (C-8), 72.1 (C-6), 55.8 (C-9), 41.9 (C-5), 28.3 (C-10), 24.0 (C-4); other NMR data are given in Tables 1, 2, and 3; negative FABMS *m/z* 765 [aglycon + Glc + Ara]$^-$, 897 [765 + Xyl]$^-$, 1205 [897 + Glc′ + Rham]$^-$, 1337 [1205 + Xyl′]$^-$, 1469 [1337 + Xyl″]$^-$,1499 [1337 + Gal]$^-$, 1631 [M − monoterpene]$^-$, and 1813 [M − H]$^-$; negative HRFABMS *m/z* 1813.8357 [M − H]$^-$ (calcd for 1813.8221); MALDI-TOF MS (positive ion mode) *m/z* [M + Na]$^+$ 1837, [M + K]$^+$ 1853.

Gleditsioside D (4): an amorphous solid from MeOH; mp 215−216 °C (dec); $[\alpha]^{21}_D$ −19.0° (*c* 0.10, MeOH); IR ν_{max}(KBr) 3405, 2928, 1708, 1646, 1080 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) aglycon δ 5.64 (1H, br t, H-12), 5.01 (1H, br t, H-16), 3.55 (1H, m, H-3), 1.86, 1.32, 1.11, 1.08, 1.01, 0.93, 0.93 (each 3H, s, H_3-27, -23, -26, -30, -24, -25, -29); monoterpenic acid δ 7.04 (1H, t, J = 7.6 Hz, H-3), 6.10 (1H, dd, J = 17.6, 10.8 Hz, H-7), 5.64 (1H, dd, J = 17.6, 1.95 Hz, H_2-8), 5.19 (1H, dd, J = 10.8, 1.95 Hz, H_2-8), 2.45 (1H, m, H_2-4), 2.32 (1H, m, H_2-4), 1.88 (3H, s, H_3-9), 1.80 (2H, m, H_2-5), 1.48 (3H, s, H_3-10); ^{13}C NMR (pyridine-d_5, 125 MHz) monoterpenic acid δ 168.0 (C-1), 146.6 (C-7), 143.5 (C-3), 127.7 (C-2), 111.7 (C-8), 72.2 (C-6), 41.6 (C-5), 28.6 (C-10), 24.0 (C-4), 12.5 (C-9); other NMR data are listed in Tables 1, 2, and 3; MALDI-TOF MS (positive ion mode) *m/z* [M + Na]$^+$ 1821, [M + K]$^+$ 1837.

Alkaline Hydrolysis of Gleditsiosides A (1), B (2), C (3), and D (4). Gleditsioside A (**1**, 40 mg) was refluxed with 2 mL 0.8 M NaOH at 80 °C for 4 h. After cooling, the reaction mixture was neutralized with 1M HCl and then extracted with *n*-BuOH (2 mL × 3 times). The organic layers were combined and then evaporated to dryness in a vacuum. The residue was subjected to HPLC purification affording prosapogenin **1a** (30 mg) and **1c** (4 mg). By the same method, **2** (10 mg) afforded **1a** (7 mg), **3** afforded **2b**, and **4** (10 mg) afforded **2b** (3.7 mg) and **1c** (0.5 mg). Compounds **1** and **2** in 2 mL 1 M NaOH were heated at 100 °C for 4 h, and then isolation and purification as above depicted furnished **1b**.

Acid Hydrolysis of Gleditsiosides A (1), B (2), C (3), and D (4). Compound **1** (40 mg) was heated in 1 mL 1M HCl (dioxane−H_2O, 1:1) at 80 °C for 2 h in a H_2O bath. After 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 amorphous powder (**1d**, 10 mg). The monosaccharide portion was neutralized by passing 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 analyses with standard monosaccharides. By the same method, **2** (10 mg) afforded **1d** (2.4 mg); **3** (40 mg) afforded **2d** (9 mg), and **4** (10 mg) afforded **2d** (2.0 mg). GLC analyses showed the monosaccharides of **2** to be glucose, xylose, arabinose, and rhamnose; those of **3** and **4** were glucose, xylose, arabinose, rhamnose, and galactose.

Prosapogenin 1a: an amorphous solid from MeOH; mp 255−256 °C (dec); $[\alpha]^{21}_D$ −32.0° (*c* 0.10, MeOH); IR ν_{max}(KBr) 3482, 2934, 1744, 1077 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) aglycon δ 5.47 (1H, br s, H-12), 3.51 (1H, m, H-3), 1.37, 1.34, 1.08, 0.99, 0.97, 0.90, 0.87 (each 3H, s, H_3-23, -27, -26, -24, -30, -25, -29); other NMR data see Tables 1, 2 and 3; MALDI-TOF MS (positive ion mode) *m/z* [M + Na]$^+$ 1477, [M + K]$^+$ 1493.

Prosapogenin 1b: an amorphous solid from MeOH; mp 235−236 °C (dec); $[\alpha]^{21}_D$ +26° (*c* 0.10, MeOH); IR ν_{max}(KBr) 3423, 2938, 1695, 1047 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) aglycon δ 5.44 (1H, br s, H-12), 3.53 (1H, m, H-3), 1.35, 1.34, 1.01, 1.00, 0.99, 0.95, 0.86 (each 3H, s, H_3-23, -27, -30, -24, -26, -29, -25); Glc δ 4.92 (1H, d, J = 7.8 Hz, H-1), 4.04 (H-2), 4.24 (H-3), 4.12 (H-4), 4.10 (H-5), 4.28 (H_2-6), 4.67 (H_2-6); Ara δ 5.17 (1H, d, J = 5.1 Hz, H-1), 4.52 (H-2), 4.38 (H-3), 4.40 (H-4), 3.75 (H_2-5), 4.30 (H_2-5); Xyl δ 5.00 (1H, d, J = 6.6 Hz, H-1), 4.03 (H-2), 4.08 (H-3), 4.14 (H-4), 3.53 (H_2-5), 4.43 (H_2-5); ^{13}C NMR (pyridine-d_5, 100 MHz) Glc δ 106.8 (C-1), 75.7 (C-2), 78.4 (C-3), 72.3 (C-4), 76.2 (C-5), 69.6 (C-6); Ara δ 102.4 (C-1), 80.6 (C-2), 72.7 (C-3), 67.5 (C-4), 64.4 (C-5); Xyl δ 106.4 (C-1), 75.5 (C-2), 77.8 (C-3), 70.9 (C-4), 67.3 (C-5); MALDI-TOF MS (positive ion mode) *m/z* [M + Na]$^+$ 905, [M + K]$^+$ 921.

Prosapogenin 2b: an amorphous solid from MeOH; mp 229−230 °C (dec); $[\alpha]^{21}_D$ +12° (*c* 0.90, MeOH); IR ν_{max}(KBr) 3423, 2938, 1695, 1047 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) aglycon δ 5.60 (1H, br s, H-12), 5.26 (1H, br s, H-16), 3.50 (1H, m, H-3), 1.89, 1.33, 1.19, 1.06, 1.03, 0.99, 0.89 (each 3H, s, H_3-27, -23, -30, -29, -26, -24, -25); Glc δ 4.91 (1H, d, J = 7.7 Hz, H-1), 4.05 (H-2), 4.21 (H-3), 4.15 (H-4), 4.09 (H-5), 4.26 (H_2-6), 4.68 (H_2-6); Ara δ 5.16 (1H, d, J = 5.1 Hz, H-1), 4.52 (H-2), 4.39 (H-3), 4.40 (H-4), 3.75 (H_2-5), 4.30 (H_2-5); Xyl δ 5.00 (1H,

d, J = 7.0 Hz, H-1), 4.03 (H-2), 4.07 (H-3), 4.17 (H-4), 3.60 (H_2-5), 4.40 (H_2-5); ^{13}C NMR (pyridine-d_5, 100 MHz) Glc δ 106.8 (C-1), 75.7 (C-2), 78.4 (C-3), 72.2 (C-4), 76.1 (C-5), 69.6 (C-6); Ara δ 102.3 (C-1), 80.5 (C-2), 72.6 (C-3), 67.5 (C-4), 64.2 (C-5); Xyl δ 106.3 (C-1), 75.3 (C-2), 77.9 (C-3), 70.9 (C-4), 67.3 (C-5); MALDI-TOF MS (positive ion mode) m/z $[M + Na]^+$ 921.

Monoterpenic acid 1c: colorless oil; $[\alpha]^{21}_D$ +12.8° (*c* 1.0, MeOH); ^{1}H NMR (pyridine-d_5, 400 MHz) δ 7.20 (1H, t, J = 7.15 Hz, H-3), 6.10 (1H, dd, J = 17.2, 10.6 Hz, H-7), 5.53 (1H, dd, J = 17.2, 1.8 Hz, H_2-8), 5.15 (1H, dd, J = 10.6, 1.8 Hz, H_2-8), 2.52 (1H, m, H_2-4), 2.44 (1H, m, H_2-4), 2.04 (3H, s, H_3-9), 1.77 (2H, m, H_2-5), 1.46 (3H, s, H_3-10); ^{13}C NMR (pyridine-d_5, 100 MHz) δ 170.9 (C-1), 146.6 (C-7), 142.4 (C-3), 129.0 (C-2), 111.7 (C-8), 72.2 (C-6), 41.8 (C-5), 28.5 (C-10), 23.4 (C-4), 12.8 (C-9); MALDI-TOF MS (positive ion mode) m/z $[M + Na]^+$ 207, $[M + K]^+$ 223.

Oleanolic acid (1d): an amorphous solid from MeOH; mp 275−276 °C; $[\alpha]^{21}_D$ +82° (*c* 0.10, MeOH); IR ν_{max}(KBr) 3456, 2940, 1696, 1036 cm^{-1}; ^{1}H NMR (pyridine-d_5, 400 MHz) δ 5.51 (1H, br s, H-12), 3.47 (1H, m, H-3), 1.29, 1.25, 1.03, 1.03, 1.02, 0.96, 0.91 (each 3H, s, H_3-23, -27, -30, -24, -26, -29, -25); ^{13}C NMR data are shown in Table 1; MALDI-TOF MS (positive ion mode) m/z $[M + Na]^+$ 479.

Echinocystic acid (2d): an amorphous solid from MeOH; mp 280−281 °C; $[\alpha]^{21}_D$ +52° (*c* 0.10, MeOH); IR ν_{max}(KBr) 3435, 2942, 1689, 1032; ^{1}H NMR (pyridine-d_5, 400 MHz) δ 5.67 (1H, br s, H-12), 5.26 (1H, br s, H-16), 3.40 (1H, m, H-3), 1.88, 1.24, 1.20, 1.08, 1.07, 1.04, 0.95 (each 3H, s, H_3-27, -23, -30, -29, -26, -24, -25); ^{13}C NMR data are given in Table 1; MALDI-TOF MS (positive ion mode) m/z $[M + Na]^+$ 495.

References and Notes

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