Triterpenoidal Saponins Acylated with Two Monoterpenic Acids from *Gleditsia sinensis*

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The isolation and characterization of three new oleanane-type triterpenoidal saponins, called gleditsiosides E—G, along with two known ones, from the anomalous fruits of *Gleditsia sinensis* LAM. are described. Their structural details were unambiguously determined by using a combination of modern NMR techniques, including distortionless enhancement by polarization transfer (DEPT), double-quantum filtered ^{1}H–^{1}H correlated spectroscopy (DQF-COSY), homonuclear hartmann-hahn (HOHAHA), ^{1}H–^{13}C heteronuclear correlation (HETCOR), heteronuclear multiple-bond connectivity (HMBC) and rotating-frame overhauser enhancement spectroscopy (ROESY) experiments as well as some chemical methods. The five bisdesmosidic triterpenoidal saponins consisting of the same sugar sequence were all acylated with two different or identical monoterpenic acids to the C-2 and C-3 positions of rhamnose moiety.

Key words *Gleditsia sinensis*; Leguminosae; oleanane-type triterpenoidal saponin; gleditsioside E; gleditsioside F; gleditsioside G

"Zhu Ya Zao," an anomalous fruit produced by old or injured plants of *Gleditsia sinensis* LAM. (Leguminosae), has long been known in traditional Chinese medicine as a saponin rich herbal medicine and used for the treatment of apoplexy, as an expectorant and a pesticide.[1)] In our previous communication,[2)] we reported four new triterpenoidal saponins, gleditsiosides A—D, which were acylated with one monoterpenic acid to the C-6 of the glucose directly linked to the C-28 of the aglycone. Further investigation on the saponin fractions led to the isolation of other three new saponins, termed gleditsiosides E—G (**1**, **3**, **4**), and two known ones, gleditsia saponins B (**5**) and C (**2**). We here wish to deal with their structural elucidation by using extensive NMR techniques, including distortionless enhancement by polarization transfer (DEPT), double-quantum filtered ^{1}H–^{1}H correlated spectroscopy (DQF-COSY), homonuclear hartmann-hahn (HOHAHA), ^{1}H–^{13}C heteronuclear correlation (HETCOR), heteronuclear multiple-bond connectivity (HMBC) and rotating-frame overhauser enhancement spectroscopy (ROESY) experiments, as well as by some chemical degradation. All the five saponins consisting of the same sugar sequence were acylated with two monoterpenic acids to the C-2 and C-3 of the rhamnose, which was attached to the C-6 of the glucose connected to the C-28 of the aglycone. This type of saponin, such as gleditsia saponin C, exhibited anti-human immunodeficiency virus (HIV) activity.[3)]

Gleditsioside E (**1**), an amorphous solid, $[\alpha]_D$ −23°, had a molecular formula $C_{94}H_{148}O_{43}$, as determined by ^{13}C, DEPT NMR data and the $[M+Na]^+$ ion at *m/z* 1987 and $[M+K]^+$ ion at *m/z* 2003 in the matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) (positive ion mode).The IR spectrum of **1** showed absorption of the carbonyl group at 1710 cm^{-1} and the α, β-unsaturated carbonyl group at 1646 cm^{-1}. The ^{13}C-NMR spectrum showed 94 signals, of which 30 signals were assigned to the triterpenoid moiety, 44 to the saccharide portion, and the remaining 20 to two monoterpenic units. The sugar portion of **1** contained eight anomeric proton signals at δ 4.88 [d, *J*=7.6 Hz, glucose (Glc)], 4.99 [d, *J*=7.0 Hz, xylose (Xyl)], 5.15 [arabinose (Ara)], 5.15 [xylose″ (Xyl″)], 5.19 [d, *J*=7.0 Hz, xylose′ (Xyl′)], 5.25 [br s, rhamnose′ (Rha′)], 6.05 [d, *J*=7.9 Hz, glucose′ (Glc′)], 6.29 [br s, rhamnose (Rha)] and eight anomeric carbon signals at δ 94.6 (Glc′), 98.2 (Rha′), 101.3 (Rha), 102.1 (Ara), 105.9 (Xyl″), 106.2 (×2, Xyl, Xyl′) and 106.7 (Glc) in the ^{1}H- and ^{13}C-NMR spectra. Detailed NMR analysis identified the aglycone to be echinocystic acid (**8**). It was apparent from the chemical shifts of the C-3 (δ 88.8) and C-28 (δ 175.9) that **1** was a bisdesmosidic glycoside. Alkaline hydrolysis of **1** afforded prosapogenin (**6**) and one monoterpenic acid (**10**). The prosapogenin (**6**) was characterized as echinocystic acid 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside[4)] based on its NMR data, and the monoterpenic acid (**10**) was identified as (6*S*), (2*E*)-6-hydroxy-2,6-dimethyl-2,7-octadienoic acid by comparison of its NMR and optical rotation data to literature values.[5a—d)] Acidic hydrolysis of **1** furnished **8** identified as echinocystic acid,[5b)] and the monosaccharide components were identified as glucose, xylose, rhamnose and arabinose based on the gas-liquid chromatography (GLC) analysis. The above evidence confirmed that **1** was a bisdesmosidic triterpenoidal glycoside with glucose, arabinose and xylose linked to the C-3 position of the aglycone, and the other five monosaccharides were attached to the C-28 of the aglycone through an ester bond.

The structures of the oligosaccharide moieties were determined through DQF-COSY, HOHAHA, HETCOR and HMBC experiments. The HOHAHA experiment allowed the subspectrum of a single monosaccharide unit to be extracted from the crowded overlapped region. Starting from the well resolved anomeric proton signals or the methyl group proton signals for the deoxy sugars, the sequential assignments of all the proton resonances to individual monosaccharides were achieved using DQF-COSY with the aid of two dimensional (2D)-HOHAHA and ROESY experiments. On the basis of the assigned proton signals, a HETCOR experiment then gave the corresponding carbon assignments, and these were further clarified by HMBC experiment. Accordingly, the assignments of the protons and protonated carbons were estab-

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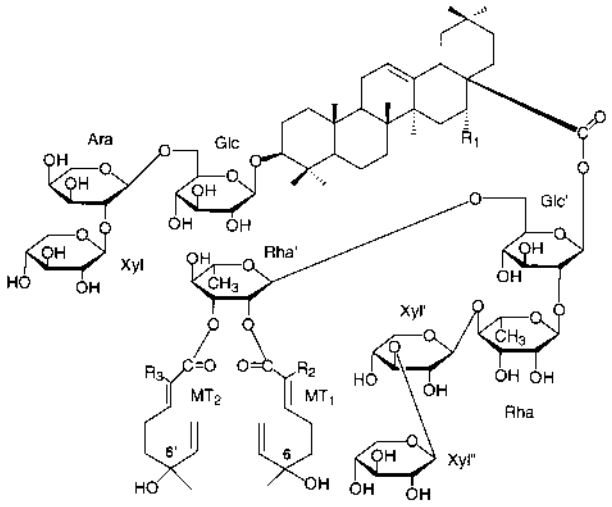

1	R_1 = OH	R_2 = CH_2OH	R_3 = CH_3 (6′ = *S*)
2	R_1 = OH	R_2 = CH_3 (6 = *S*)	R_3 = CH_2OH
3	R_1 = OH	R_2 = CH_3 (6 = *S*)	R_3 = CH_3 (6′ = *S*)
4	R_1 = H	R_2 = CH_3 (6 = *S*)	R_3 = CH_2OH
5	R_1 = OH	R_2 = CH_2OH	R_3 = CH_2OH

Chart 1

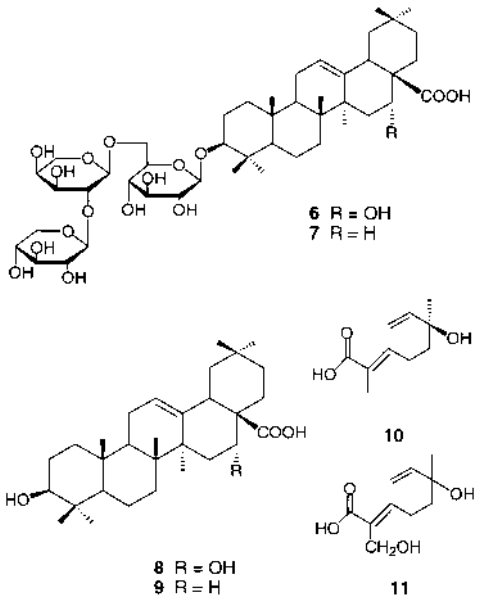

Chart 2

lished (Tables 2, 3), and the eight sugar units were identified as two glucoses, three xyloses, two rhamnoses and one arabinose, and further confirmed by GLC analysis of the acidic hydrolysate. The position of the trisaccharide moiety was unambiguously defined by the HMBC experiment. A significant cross peak due to long-range correlation between C-3 (δ 88.8) of the aglycone and H-1 (δ 4.88) of Glc indicated Glc was linked to the C-3 of the aglycone. A cross peak between H-1 (δ 5.15) of Ara and C-6 (δ 69.5) of Glc, and a cross peak between H-1 (δ 4.99) of Xyl and C-2 (δ 80.3) of Ara demonstrated that Ara was linked to C-6 of Glc and Xyl was attached to C-2 of Ara. Similarly, the sequence of the pentasaccharide chain at C-28 was deduced from the following long-range correlations: H-1 (δ 6.29) of Rha with C-2 (δ 76.7) of Glc′, H-1 (δ 5.19) of Xyl′ with C-4 (δ 83.7) of Rha, H-1 (δ 5.15) of Xyl″ with C-3 (δ 87.4) of Xyl′ and H-1 (δ 5.25) of Rha′ with C-6 (δ 66.8) of Glc′. A long-range correlation between H-1 (δ 6.05) of Glc′ and C-28 (δ 175.9) of the aglycone carbonyl group provided definitive evidence for an ester linkage between the pentasaccharide chain and the aglycone. The two sugar chains of **1** were also deduced from the following key nuclear Overhause effect (NOE) correlations observed in the ROESY spectrum: H-1 (δ 4.99) of Xyl with H-2 (δ 4.51) of Ara, H-1 (δ 5.15) of Ara with H-6 (δ 4.64) of Glc and H-1 (δ 5.15) of Xyl″ with H-3 (δ 4.02) of Xyl′, H-1 (δ 5.19) of Xyl′ with H-4 (δ 4.40) of Rha, H-1 (δ 6.29) of Rha with H-2 (δ 4.28) of Glc′, H-1 (δ 5.25) of Rha′ with H-6 (δ 4.38) of Glc′. The ^{13}C-NMR data for the sugar moieties indicated that all the monosaccharides were in pyranose forms. The anomeric configurations were fully defined from their $^3J_{H1,H2}$ coupling constants and $^1J_{C1,H1}$ coupling constants (see Tables 2, 3) as well as from NOE information. Although the anomeric protons of arabinose and one of the three xyloses (Xyl″) were overlapping, evidence supporting an α-arabinopyranoside and a β-configuration for xylopyranosyl unit (Xyl″) was obtained from the ROESY experiment. The ROESY experiment in **1** showed significant through space interaction of H-1 (δ 5.15) with H-3 (δ 4.40) as well as H-1 (δ 5.15) with H-5 (δ 3.75) for Ara, and H-1 (δ 5.15) with H-3 (δ 4.21) as well as H-1 (δ 5.15) with H-5 (δ 3.61) for Xyl″. Accordingly, the two glucopyranosyl units and three xylopyranosyl units were concluded to have the β-configuration, and the two rhamnopyranosyl units and one arabinopyranosyl unit to have the α-configuration.

The presence of the two monoterpenic units in compound **1** was indicated by various NMR data. One monoterpenic unit was identified as (6*S*), (2*E*)-6-hydroxy-2,6-dimethyl-2,7-octadienoic acid (**10**), which was further confirmed by alkaline hydrolysis described above. The ^{1}H-NMR data (Table 5) of the other one (**11**) were similar to those of **10**, except for exhibition of a hydroxymethyl signal at δ 4.74 instead of the olefinic methyl signal at δ 1.86 in **10**. The stereochemistry of the $\Delta^{2,3}$ double bond was determined as *E* from the chemical shifts H-3 (δ 7.35), since the olefinic proton of the *Z*-isomer appeared at a higher field.[5a,6,7] However, several attempts of alkaline hydrolysis did not afford intact **11**. Consequently, the absolute configuration of C-6 position was not established. Thus, the monoterpenic unit (**11**) was shown to be (2*E*)-2-hydroxylmethyl-6-hydroxy-6-methyl-2,7-octadienoic acid.[5b,5c] The long-range correlations of H-2 of Rha′ (δ 5.91) with C-1 of monoterpenic acid (δ 166.7) (**11**) and H-3 of Rha′ (δ 5.93) with C-1 of monoterpenic acid (δ 167.4) (**10**) established that the monoterpenic acids (**11**) and (**10**) were attached to the C-2 and C-3 of Rha′, respectively. On the basis of the foregoing evidence, gleditsioside E (**1**) 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-hydroxylmethyl-6-hydroxy-6-methyl-2,7-octadienoyl-(1→2) and (6*S*), (2*E*)-6-hydroxy-2,6-dimethyl-2,7-octadienoyl-(1→3)-α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranosyl ester.

Compound **2**, an amorphous solid, $[\alpha]_D$ −21°, had the same molecular formula $C_{94}H_{148}O_{43}$ as **1** deduced from its

^{13}C and DEPT NMR data, and from the $[M+Na]^+$ ion at *m/z* 1987 and the $[M+K]^+$ ion at *m/z* 2003 in the MALDI-TOF MS (positive ion mode). The IR spectrum of **2** displayed characteristic absorption of the carbonyl group at 1711 cm^{-1} and α, β-unsaturated carbonyl group at 1645 cm^{-1}. Alkaline hydrolysis of **2** led to prosapogenin (**6**) and the monoterpenic acid (**10**) as found in **1**. Acidic hydrolysis of **2** allowed the identification of the same aglycone (**8**), and the same sugar components as **1**, that is, glucose, xylose, arabinose and rhamnose based on the GLC analysis. The chemical shifts of protons and carbons for the sugar parts of **2** and **1** were almost superimposable, indicating that they had identical saccharide chains at C-3 and C-28. The two monoterpenic moieties in **2** were indicated as **10** and **11** from NMR data. The key long-range correlations of H-2 of Rha′ (5.92) with C-1 of monoterpenic acid (δ 167.3) (**10**), and H-3 of Rha′ (δ 5.95) with C-1 of monoterpenic acid (δ 167.1) (**11**) showed clearly that the two monoterpenic units (**10**) and (**11**) were respectively linked to the C-2 and C-3 of Rha′, obviously different from compound **1**. Therefore, compound **2** was identified 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)-[(6*S*), (2*E*)-6-hydroxy-2,6-dimethyl-2,7-octadienoyl-(1→2) and (2*E*)-2-hydroxylmethyl-6-hydroxy-6-methyl-2,7-octadienoyl-(1→3)-α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranosyl ester, a triterpenoid saponin called gleditsia saponin C isolated early from *G. japonica*.[5b]

It is very interesting to note that compounds **1** and **2** contained the same aglycone, same sugar sequences, and identi-

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

	1	2	3	4	5	6	7	8	9
1	38.9	39.0	39.0	38.9	39.0	38.9	38.8	39.1	39.4
2	26.7	26.7	26.8	26.8	26.8	26.8	26.8	28.2	28.1
3	88.8	88.9	88.9	88.7	88.9	88.7	88.5	78.1	78.1
4	39.5	39.6	39.6	39.6	39.6	39.6	39.6	39.1	39.0
5	56.0	56.1	56.1	56.0	56.1	55.9	55.9	55.9	55.8
6	18.6	18.8	18.8	18.8	18.8	18.5	18.5	18.9	18.1
7	33.4	33.5	33.5	33.2	33.5	33.5	33.3	33.6	33.2
8	40.0	40.1	40.1	40.0	40.1	39.9	39.8	39.9	39.8
9	47.1	47.2	47.2	48.1	47.2	47.2	48.1	47.3	48.2
10	37.0	37.1	37.1	37.1	37.1	37.1	37.1	37.5	37.4
11	23.8	23.9	23.9	23.9	23.9	23.9	23.9	23.9	23.9
12	122.4	122.6	122.6	122.8	122.6	122.4	122.6	122.5	122.6
13	144.3	144.4	144.4	144.1	144.5	145.1	144.8	145.1	144.8
14	42.1	42.2	42.2	42.3	42.3	42.1	42.1	42.1	42.2
15	36.0	36.3	36.3	28.6	36.3	36.2	28.3	36.2	28.3
16	74.0	74.0	74.0	23.3	74.0	74.8	23.7	74.8	23.8
17	49.4	49.5	49.5	47.3	49.5	48.9	46.7	48.9	46.5
18	41.3	41.5	41.5	41.8	41.5	41.4	41.9	41.5	42.0
19	47.5	47.6	47.6	46.4	47.6	47.2	46.4	47.3	46.7
20	30.7	30.9	30.9	30.8	30.9	31.0	30.7	31.1	31.0
21	35.9	36.1	36.1	34.1	36.1	36.1	34.3	36.1	34.3
22	31.8	32.0	32.0	32.5	32.0	32.9	33.2	32.9	33.3
23	28.3	28.3	28.4	28.3	28.3	28.2	28.2	28.8	28.8
24	17.0	17.1	17.1	17.1	17.1	17.1	17.1	16.6	16.6
25	15.7	15.8	15.8	15.7	15.8	15.6	15.5	15.7	15.6
26	17.4	17.0	17.5	17.5	17.5	17.5	17.4	17.6	17.5
27	27.0	27.1	27.1	26.0	27.1	27.3	26.3	27.3	26.2
28	175.9	175.9	175.9	176.5	176.0	180.0	180.4	179.9	180.2
29	33.1	33.2	33.3	33.2	33.2	33.4	33.4	33.4	33.2
30	24.7	24.8	24.8	23.9	24.8	24.8	23.8	24.8	23.8

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

	1	2	3	4	5	6	7
C_3-Glc							
1	106.7 (158)[b]	106.8	106.8	106.8	106.8	106.8	106.8
2	75.5	75.7	75.7	75.7	75.7	75.7	75.7
3	78.2	78.4	78.2	78.4	78.4	78.4	78.4
4	72.2	72.3	72.3	72.2	72.3	72.2	72.3
5	76.0	76.0	76.0	76.2	76.2	76.1	76.2
6	69.5	69.6	69.6	69.6	69.6	69.6	69.6
Ara							
1	102.1 (163)[b]	102.3	102.3	102.3	102.3	102.3	102.4
2	80.3	80.5	80.5	80.6	80.5	80.5	80.6
3	72.5	72.6	72.6	72.6	72.6	72.6	72.7
4	67.4	67.5	67.5	67.5	67.4	67.5	67.5
5	64.3	64.3	64.3	64.3	64.3	64.2	64.4
Xyl							
1	106.2 (164)[b]	106.3	106.3	106.4	106.4	106.3	106.4
2	75.3[c]	75.4[c]	75.4[c]	75.5[c]	75.5[c]	75.3	75.5
3	77.8	77.9	77.9	77.9	77.9	77.9	77.8
4	70.7[d]	70.8[d]	70.8[d]	70.8[d]	70.8[d]	70.9	70.9
5	67.2	67.3	67.0	67.2	67.3	67.3	67.3
C_{28}-Glc′							
1	94.6 (158)[b]	94.6	94.6	94.6	94.7		
2	76.7	76.7	76.7	76.8	76.8		
3	78.9	79.1	79.1	79.1	79.1		
4	71.4	71.4	71.5	71.4	71.4		
5	77.1	77.3	77.4	77.3	77.2		
6	66.8	66.7	66.8	66.7	66.7		
Rha							
1	101.3 (172)[b]	101.5	101.4	101.5	101.5		
2	71.6	71.7	71.7	71.5	71.7		
3	72.4	72.5	72.5	72.5	72.5		
4	83.7	83.9	83.9	85.1	83.9		
5	68.4	68.4	68.4	68.3	68.4		
6	18.4	18.7	18.7	18.6	18.7		
Xyl′							
1	106.2 (164)[b]	106.3	106.3	106.9	106.4		
2	74.8[c]	75.0[c]	74.9[c]	75.1[c]	75.0[c]		
3	87.4	87.5	87.4	87.3	87.5		
4	68.9	69.1	68.9	69.0	69.1		
5	66.8	66.9	66.9	66.9	66.9		
Xyl″							
1	105.9 (167)[b]	106.1	106.1	105.9	105.1		
2	75.0[c]	75.1[c]	75.1[c]	75.2[c]	75.2[c]		
3	78.0	78.1	78.2	78.1	78.2		
4	70.7[d]	70.9[d]	70.9[d]	70.9[d]	70.9[d]		
5	67.2	67.3	67.3	67.3	67.3		
Rha′							
1	98.2 (171)[b]	98.2	98.3	98.1	98.3		
2	70.9	71.1	71.0	71.1	71.1		
3	73.1	73.4	73.2	73.3	73.4		
4	70.8[d]	70.9[d]	70.9[d]	70.9[d]	70.9[d]		
5	69.7	69.7	69.7	69.7	69.7		
6	18.4	18.7	18.7	18.6	18.7		

a) The assignment was based upon DEPT, 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 interchangeable.

cal monoterpenic moieties **10** and **11**. The only difference was that monoterpenic acids **10** and **11** were respectively appended to C-3 and C-2 of Rha′ in **1**, while the attached positions were just the opposite in **2**. Although the ^{13}C-NMR data was almost the same between the two monoterpenic moieties, significant difference for ^{1}H chemical shifts was observed for H-3 (δ 7.10) of **11** and CH_3-9 (δ 1.98) of **10** in **2** as compared with their counterparts (δ 7.35 and 1.86, respectively) in **1**. Therefore, besides the HMBC experiment, the proton chemical shift differences ($\Delta\delta$ 0.25 for H-3 of **11** and $\Delta\delta$ 0.12 for CH_3-9 of **10**) discussed above can also be used to determine the positions of the monoterpenic moieties acylated to C-2 and C-3 of Rha′.

Gleditsioside F (**3**) gave a $[M+Na]^+$ peak at *m*/*z* 1971 and a $[M+K]^+$ peak at *m*/*z* 1987 in the MALDI-TOF MS (positive ion mode), consistent with a molecular formula of $C_{94}H_{148}O_{42}$, 16 mass units lower than that of **2**. Alkaline hydrolysis of **3** resulted in prosapogenin (**6**) and monoterpenic acid (**10**). Acidic hydrolysis of **3** furnished the aglycone (**8**), and the monosaccharide components were identified as glucose, xylose, arabinose and rhamnose based on the GLC analysis. Comparison of the NMR spectra of **3** with those of **2** concluded that the chemical shifts for the aglycone part and sugar units bore a close resemblance, indicating compounds **3** and **2** had the same aglycone and sugar sequence. The only difference of chemical shifts for monoterpenic moieties was that the hydroxylmethyl group at C-2 (δ_C 56.3, δ_H 4.70) of one monoterpenic moiety in **2** was replaced by a methyl group (δ_C 12.4, δ_H 1.83). Hence, both monoterpenic moieties in **3** were shown to be (6*S*), (2*E*)-6-hydroxy-2,6-dimethyl-2,7-octadienoic acid (**10**). The above evidence led to the elucidation of the structure of gleditsioside F (**3**) 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)-[(6*S*),(2*E*)-6-hydroxy-2,6-dimethyl-2,7-octadienoyl-(1→2) and (6*S*),(2*E*)-6-hydroxy-2,6-dimethyl-2,7-octadienoyl-(1→3)-α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranosyl ester.

Gleditsioside G (**4**), an amorphous solid, $[\alpha]_D$ −10°, gave a $[M+Na]^+$ peak at *m*/*z* 1971 and a $[M+K]^+$ peak at *m*/*z* 1987, appropriate for a molecular formula $C_{94}H_{148}O_{42}$. Acidic hydrolysis of **4** furnished oleanolic acid (**9**) as the aglycone, and the monosaccharide components were identified as glucose, xylose, arabinose and rhamnose by the GLC analysis. Alkaline hydrolysis of **4** afforded prosapogenin (**7**) and monoterpenic acid (**10**). The prosapogenin (**7**) was identified as oleanolic acid 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside[4] based on its NMR data. Detailed NMR spectral comparison of **4** with that of **2** strongly suggested that **4** differed from **2** only in the absence of an α-hydroxyl group at C-16. Accordingly, compound **4** contained the same sugar sequences at C-3 and C-28, and the same monoterpenic acids at C-2 and C-3 of Rha′

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

	1	2	3	4	5	6	7		1	2	3	4	5
C_3-Glc								Rha					
1	4.88 d	4.88 d	4.88 d	4.87 d	4.88 d	4.91 d	4.92 d	1	6.29	6.29	6.30	6.34	6.34
	(7.6)	(7.6)	(7.6)	(7.8)	(7.6)	(7.7)	(7.8)		(br s)	(br s)	(br s)	(br s)	(br s)
2	4.03	4.04	4.03	4.03	4.03	4.05	4.04	2	4.80	4.80	4.80	4.86	4.86
3	4.20	4.21	4.23	4.20	4.23	4.21	4.24	3	4.75	4.73	4.75	4.74	4.74
4	4.15	4.15	4.16	4.14	4.15	4.15	4.12	4	4.40	4.40	4.40	4.38	4.38
5	4.06	4.09	4.08	4.06	4.07	4.09	4.10	5	4.48	4.48	4.50	4.49	4.49
6	4.22	4.23	4.23	4.21	4.22	4.26	4.28	6	1.71 d	1.72 d	1.72 d	1.78 d	1.78 d
	4.64	4.63	4.64	4.64	4.63	4.68	4.67		(6.1)	(6.7)	(6.1)	(5.9)	(5.9)
Ara								Xyl′					
1	5.15[b]	5.16[b]	5.15[b]	5.15 d	5.15[b]	5.16 d	5.17 d	1	5.19 d	5.20 d	5.20 d	5.08 d	5.08 d
				(5.1)		(5.1)	(5.1)		(7.0)	(7.0)	(7.2)	(7.1)	(7.1)
2	4.51	4.50	4.53	4.52	4.52	4.52	4.52	2	4.04	4.04	4.03	4.05	4.05
3	4.40	4.40	4.40	4.39	4.40	4.39	4.38	3	4.02	4.01	4.02	4.03	4.03
4	4.41	4.42	4.41	4.41	4.41	4.40	4.40	4	4.05	4.05	4.05	4.10	4.10
5	3.75	3.74	3.75	3.75	3.75	3.75	3.75	5	3.44	3.45	3.45	3.50	3.50
	4.31	4.31	4.32	4.30	4.30	4.30	4.30		4.20	4.19	4.19	4.22	4.22
Xyl								Xyl″					
1	4.99 d	4.99 d	4.99 d	4.98 d	4.95 d	5.00 d	5.00 d	1	5.15[b]	5.16[b]	5.15[b]	5.19 d	5.15[b]
	(7.0)	(7.0)	(6.9)	(6.8)	(7.0)	(7.0)	(6.6)					(7.6)	
2	4.03	4.04	4.04	4.05	4.04	4.03	4.03	2	4.03	4.04	4.04	4.05	4.04
3	4.05	4.06	4.06	4.05	4.06	4.07	4.08	3	4.21	4.22	4.21	4.23	4.21
4	4.12	4.13	4.12	4.13	4.13	4.15	4.14	4	4.12	4.12	4.12	4.13	4.13
5	3.57	3.57	3.58	3.57	3.59	3.60	3.53	5	3.61	3.69	3.65	3.68	3.69
	4.40	4.35	4.35	4.40	4.40	4.40	4.35		4.28	4.25	4.28	4.30	4.30
C_{28}-Clc′								Rha′					
1	6.05 d	6.09 d	6.07 d	6.08 d	6.07 d			1	5.25	5.30	5.31	5.30	5.25
	(7.9)	(7.8)	(7.8)	(7.3)	(7.4)				(br s)	(br s)	(br s)	(br s)	(br s)
2	4.28	4.25	4.23	4.30	4.28			2	5.91	5.92	5.89	5.90	5.90
3	4.16	4.16	4.16	4.19	4.18			3	5.93	5.95	5.92	5.94	5.93
4	4.31	4.26	4.28	4.28	4.30			4	4.12	4.13	4.12	4.13	4.13
5	3.96	3.98	3.96	3.98	3.98			5	4.39	4.38	4.40	4.38	4.39
6	4.20	4.21	4.20	4.20	4.19			6	1.68 d	1.70 d	1.72 d	1.68 d	1.69 d
	4.38	4.40	4.39	4.39	4.38				(6.1)	(6.7)	(6.1)	(5.9)	(5.3)

a) The assignment was based upon DEPT, DQF-COSY, HOHAHA, HETCOR, ROESY and HMBC experiments. *b*) These pairs of signals were overlapping.

as compound **2**. The proton signals for H-3 (δ 7.10) of **11** and CH_3-9 (δ 1.99) of **10** in **4** were similar to those for H-3 (7.10) of **11** and CH_3-9 (1.98) of **10** in **2**, further confirming the monoterpenic moieties **10** and **11** were respectively linked to C-2 and C-3 of Rha′. Consequently, the structure of gleditsioside F (**4**) 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→2) and (2*E*)-2-hydroxylmethyl-6-hydroxy-6-methyl-2,7-octadienoyl-(1→3)-α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranosyl ester.

Table 4. ^{13}C-NMR Data for the Monoterpenic Moieties (125 and 100 MHz in Pyridine-d_5)[a]

	1	2	3	4	5	10
MT_1						
1	166.7	167.3	167.4	167.3	167.0	170.9
2	132.8	127.7	127.7	127.6	132.8	129.0
3	147.1	144.3	144.2	144.3	147.4	142.4
4	24.1	24.0	24.0	24.0	24.0	23.4
5	41.8	41.5	41.6	41.5	41.8	41.8
6	72.1	72.1	72.1	72.2	72.2	72.2
7	146.4[b]	146.5[b]	146.5[b]	146.5[b]	146.5[b]	146.6
8	111.5[c]	111.7[c]	111.7[c]	111.7[c]	111.7[c]	111.7
9	56.1	12.7	12.7	12.7	56.2	12.8
10	28.4[d]	28.6[d]	28.6[d]	28.6[d]	28.5[d]	28.5
MT_2						
1	167.4	167.1	167.2	167.1	167.2	
2	127.5	132.9	127.8	132.9	132.9	
3	143.6	146.7	143.5	146.7	147.0	
4	23.9	24.1	24.2	24.1	24.2	
5	41.3	41.9	41.6	41.9	41.9	
6	72.1	72.1	72.1	72.2	72.2	
7	146.4[b]	146.4[b]	146.5[b]	146.4[b]	146.5[b]	
8	111.7[c]	111.8[c]	111.8[c]	111.8[c]	111.8[c]	
9	12.5	56.3	12.4	56.3	56.2	
10	28.5[d]	28.5[d]	28.5[d]	28.5[d]	28.6[d]	

a) The assignment was based upon DEPT, DQF-COSY, HOHAHA, HETCOR, ROESY and HMBC experiments. *b*—*d*) The data with the same labels in each column may be interchangeable.

Compound **5** gave a $[M+Na]^+$ peak at *m*/*z* 2003 and a $[M+K]^+$ peak at *m*/*z* 2019 in the MALDI-TOF MS (positive ion mode), 16 mass units higher than that of **2**, implying the presence of an additional oxygen-bearing function in **5**. Alkaline hydrolysis of **5** afforded prosapogenin (**6**). Acidic hydrolysis of **5** resulted in the same aglycone (**8**), and the same monosaccharide components as found in compound **2**. A NMR spectral comparison of **5** with **2** showed that both compounds possessed the same aglycone and sugar-substitution pattern, but differed in the monoterpenic units. After extensive NMR analysis, both of the monoterpenic units were identified as (2*E*)-2-hydroxylmethyl-6-hydroxy-6-methyl-2,7-octadienoic acid (**11**). From the above information, the structure of compound **5** 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-hydroxylmethyl-6-hydroxy-6-methyl-2,7-octadienoyl-(1→2) and (2*E*)-2-hydroxylmethyl-6-hydroxy-6-methyl-2,7-octadienoyl-(1→3)-α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranosyl ester, a triterpenoid saponin termed gleditsia saponin B isolated previously from *G. japonica*.[5b]

Experimental

The dried anomalous fruits (Zhu Ya Zao) of *G. sinensis* were purchased from a market in Nanchang, the capital of Jiangxi province, P. R. China in January 1998, and were identified by Professor Fan Chuishen (Jiangxi College of Traditional Chinese Medicine). Melting points were measured with a Yanaco microscope and are uncorrected. Optical rotations were performed with a JASCO DIP-370 digital polarimeter. IR spectra were carried out on a JASCO 300E FTIR spectrometer. MALDI-TOF MS was conducted using a Perseptive Biosystems Voyager DE-STR mass spectrometer. The ^{1}H- and ^{13}C-NMR were recorded on a JEOL α-500 or a JEOL EX-400 FT-NMR spectrometer in pyridine-d_5 solution and chemical shifts were expressed in δ (ppm) referring to tetramethylsilane (TMS). Diaion HP-20 (Mitsubishi Chemical), silica gel (Silica gel 60, Merck), and octadecyl silica (ODS) (Chromatorex, 100—200 mesh, Fujisylisia) were used for open column chromatography. Preparative medium-performance liquid chromatography (MPLC) and high-performance liquid chromatography (HPLC) was per-

Table 5. ^{1}H-NMR Data for the Monoterpenic Moieties (500 and 400 MHz in Pyridine-d_5)[a]

	1	2	3	4	5	10
MT_1						
3	7.35 t (7.6)	7.20 t (7.6)	7.21 t (7.5)	7.20 t (7.6)	7.34 t (7.6)	7.20 t (7.15)
4	2.65	2.45	2.42	2.43	2.65	2.44, 2.52
5	1.75	1.75	1.80	1.80	1.81	1.77
7	6.06 dd[b] (17.1, 10.8)	6.10 dd[b] (17.1, 10.8)	6.14 dd[b] (17.1, 10.8)	6.11 dd[b] (17.3, 10.5)	6.05 dd[b] (17.1, 10.8)	6.10 dd (17.2, 10.6)
8	5.12 dd (10.8, 1.8); 5.48 dd (17.1, 1.8)[c]	5.16 dd (10.8, 1.8); 5.51 dd (17.1, 1.8)[c]	5.19 dd (10.8, 2.0); 5.56 dd (17.1, 2.0)[c]	5.18 dd (10.5, 1.8); 5.56 dd (17.3, 1.8)[c]	5.08 dd (10.8, 1.8); 5.47 dd (17.1, 1.8)[c]	5.15 dd (10.6, 1.8); 5.53 dd (17.2, 1.8)
9	4.74	1.98	2.00	1.99	4.79	2.04
10	1.42[d]	1.49[d]	1.49[d]	1.48[d]	1.39[d]	1.46
MT_2						
3	7.03 t (7.6)	7.10 t (7.6)	6.96 t (7.5)	7.10 t (7.6)	7.18 t (8.0)	
4	2.45	2.65	2.60	2.65	2.45	
5	1.70	1.70	1.70	1.70	1.70	
7	6.11 dd[b] (17.1, 10.8)	6.04 dd[b] (17.1, 10.8)	6.08 dd[b] (17.2, 10.8)	6.04 dd[b] (17.3, 10.5)	6.09 dd[b] (17.1, 10.8)	
8	5.15 dd (10.8, 1.8); 5.53 dd (17.1, 1.8)[c]	5.11 dd (10.8, 1.8); 5.48 dd (17.1, 1.8)[c]	5.15 dd (10.8, 2.0); 5.52 dd (17.2, 2.0)[c]	5.14 dd (10.5, 1.8); 5.48 dd (17.3, 1.8)[c]	5.13 dd (10.8, 1.8); 5.52 dd (17.1, 1.8)[c]	
9	1.86	4.70	1.83	4.73	4.97	
10	1.45[d]	1.42[d]	1.45[d]	1.42[d]	1.44[d]	

a) The assignment was based upon DEPT, DQF-COSY, HOHAHA, HETCOR, ROESY and HMBC experiments. *b*—*d*) The data with the same labels in each column may be interchangeable.

formed 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.

Extraction and Isolation 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 BuOH (480 g). The BuOH soluble fraction was passed through a column of Diaion HP-20 (2500 ml) and the absorbed materials were eluted with H_2O and containing an amount of MeOH (30, 50, 70, 100%). The 70% MeOH fraction (120 g) was chromatographed over silica 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 B (10 g) was chromatographed over ODS columns to yield B_1 (4.5 g), B_2 (3.5 g) and B_3 (1.0 g). Part of fraction B_1 (2.0 g) was repeatedly subjected to MPLC (30, 50, 70, 90% MeOH) and HPLC (MeOH : H_2O) purification to afford **1** (142 mg), **2** (120 mg), **3** (35 mg) and **5** (30 mg). By the same method, fraction B_3 furnished **4** (80 mg).

Gleditsioside E (**1**): An amorphous solid from MeOH; mp 200—201 °C (dec.); $[\alpha]_D^{21}$ −23° (c=0.10, MeOH); IR ν_{max}^{KBr}: 3409, 2950, 1710, 1646, 1078 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 500 MHz): aglycone δ 5.63 (1H, br s, H-12), 5.19 (1H, br s, H-16), 3.45 (1H, m, H-3), 1.87, 1.34, 1.16, 1.13, 1.01, 0.97, 0.89 (each 3H, s, H_3-27, 23, 30, 26, 24, 29, 25); other NMR data see Tables 1—5; MALDI-TOF MS (positive ion mode) m/z 1987 $[M+Na]^+$, 2003 $[M+K]^+$.

Gleditsia Saponin C (**2**): An amorphous solid from MeOH; mp 197—198 °C (dec.); $[\alpha]_D^{21}$ −21° (c=0.10, MeOH); IR ν_{max}^{KBr}: 3411, 2931, 1711, 1645, 1078 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 500 MHz): aglycone δ 5.63 (1H, br s, H-12), 5.20 (1H, br s, H-16), 3.43 (1H, m, H-3), 1.83, 1.33, 1.16, 1.14, 1.01, 0.99, 0.89 (each 3H, s, H_3-27, 23, 30, 26, 24, 29, 25); other NMR data are given in Tables 1—5; MALDI-TOF MS (positive ion mode) m/z 1987 $[M+Na]^+$, 2003 $[M+K]^+$.

Gleditsioside F (**3**): An amorphous solid from MeOH; mp 195—196 °C (dec.); $[\alpha]_D^{21}$ −20° (c=0.10, MeOH); IR ν_{max}^{KBr}: 3423, 2931, 1710, 1646, 1080 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 500 MHz): aglycone δ 5.63 (1H, br s, H-12), 5.20 (1H, br s, H-16), 3.43 (1H, m, H-3), 1.83, 1.32, 1.15, 1.10, 1.00, 0.96, 0.89 (each 3H, s, H_3-27, 23, 30, 26, 24, 29, 25); other NMR data are shown in Tables 1—5; MALDI-TOF MS (positive ion mode) m/z 1971 $[M+Na]^+$, 1987 $[M+K]^+$.

Gleditsioside G (**4**): An amorphous solid from MeOH; mp 202—203 °C (dec.); $[\alpha]_D^{21}$ −10° (c=0.10, MeOH); IR ν_{max}^{KBr}: 3405, 2933, 1714, 1644, 1080 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 500 MHz): aglycone δ 5.45 (1H, br s, H-12), 3.50 (1H, m, H-3), 1.38, 1.33, 1.11, 1.02, 0.97, 0.88, 0.87 (each 3H, s, H_3-23, 27, 26, 30, 24, 25, 29); other NMR data see Tables 1—5; MALDI-TOF MS (positive ion mode) m/z 1971 $[M+Na]^+$, 1987 $[M+K]^+$.

Gleditsia Saponin B (**5**): An amorphous solid from MeOH; mp 226—227 °C (dec.); $[\alpha]_D^{21}$ −20° (c=0.10, MeOH); IR ν_{max}^{KBr}: 3410, 2930, 1712, 1646, 1079 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 500 MHz): aglycone δ 5.64 (1H, br s, H-12), 5.20 (1H, br s, H-16), 3.50 (1H, m, H-3), 1.87, 1.34, 1.15, 1.14, 1.01, 0.96, 0.88 (each 3H, s, H_3-27, 23, 30, 26, 24, 29, 25); other NMR data are listed in Tables 1—5; MALDI-TOF MS (positive ion mode) m/z 2003 $[M+Na]^+$, 2019 $[M+K]^+$.

Alkaline Hydrolysis of Compounds 1—5 Compound **1** (40 mg) was refluxed with 2 ml 0.8 M NaOH for 4 h. After cooling down, the reaction mixture was neutralized with 1 M HCl and then extracted with BuOH (2 ml×3 times). The organic layers were combined and then evaporated to dryness in vacuum. The residue was subjected to HPLC purification affording prosapogenin **6** (13.5 mg) and monoterpenic acid **10** (3.8 mg). By the same method, **2** and **3** afforded **6** and **10**, respectively; **4** afforded prosapogenin **7** and **10**, and **5** afforded prosapogenin **6**.

Acidic Hydrolysis of Compounds 1—5 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 extraction was washed with water and then concentrated to give the aglycone **8** (10 mg). The monosaccharide portion was neutralized by passing through an 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 water, the reaction product was extracted with hexane (1 ml×2 times). 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**, **3**, **5** afforded **8**, and **4** afforded **9**. GLC analyses showed the monosaccharides of **2**, **3**, **4** and **5** were also to be glucose, xylose, arabinose and rhamnose.

Prosapogenin (**6**): An amorphous solid from MeOH; mp 229—230 °C (dec.); $[\alpha]_D^{21}$ +12° (c=0.10, MeOH); IR ν_{max}^{KBr}: 3423, 2938, 1695, 1047 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 400 MHz): aglycone 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); for other NMR data see Tables 1—3; MALDI-TOF MS (positive ion mode) m/z 921 $[M+Na]^+$.

Prosapogenin (**7**): An amorphous solid from MeOH; mp 235—236 °C (dec.); $[\alpha]_D^{21}$ +26° (c=0.10, MeOH); IR ν_{max}^{KBr}: 3423, 2938, 1695, 1047 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 400 MHz): aglycone 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); other NMR data are listed in Tables 1—3; MALDI-TOF MS (positive ion mode) m/z 905 $[M+Na]^+$, 921 $[M+K]^+$.

Echinocystic Acid (**8**): An amorphous solid from MeOH; mp 280—281 °C; $[\alpha]_D^{21}$ +52° (c=0.10, MeOH); IR ν_{max}^{KBr}: 3435, 2942, 1689, 1032 cm^{-1}; ^{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 495 $[M+Na]^+$.

Oleanolic Acid (**9**): An amorphous solid from MeOH; mp 275—276°C; $[\alpha]_D^{21}$ +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 479 $[M+Na]^+$.

Monoterpenic Acid (**10**): Colorless oil; $[\alpha]_D^{21}$ +12.8° (c=1.0, MeOH); ^{13}C- and ^{1}H-NMR data see Tables 4 and 5; MALDI-TOF MS (positive ion mode) m/z 207 $[M+Na]^+$, 223$[M+K]^+$.

References and Notes

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