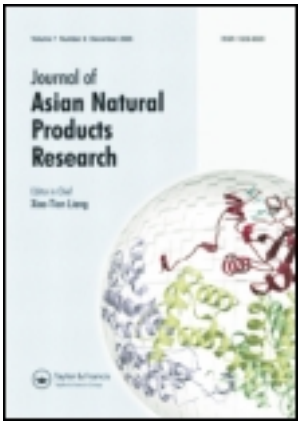

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Three triterpenoid saponins acylated with monoterpenic acid from *Gymnocladus chinensis*

Qi Wen[ab], Dan Yuan[b], Ke-Hui Xie[a], Tian-Zhi Cai[a] and Hong-Zheng Fu[a]*

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A new triterpenoid saponin acylated with monoterpenic acid, together with two known triterpenoid saponins, has been isolated from the fruit of *Gymnocladus chinensis* Baill. Their structures were elucidated as 2β,23-dihydroxy-3-*O*-α-L-rhamnopyranosyl-21-*O*-{(6*S*)-2-*trans*-2,6-dimethyl-6-*O*-[3-*O*-(β-D-glucopyranosyl)-4-*O*-((6*S*)-2-trans-2,6-dimethyl-6-hydroxy-2,7-octadienoyl)-β-L-arabinopyranosyl]-2,7-octadienoyl}-acacic acid 28-*O*-β-D-xylopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-glucopyranosyl ester (**1**), gymnocladus saponin E (**2**), and gymnocladus saponin F_2 (**3**).

Keywords: *Gymnocladus chinensis*; triterpenoid saponins; eight to nine sugar moieties

1. Introduction

Gymnocladus chinensis Baill.(Leguminosae) is a plant widely distributed in southern China, and the dried fruit of this plant is used as an expectorant in traditional Chinese medicine [1]. Previous phytochemical studies showed 3 monoterpene glycosides [1,2], 6 flavones, 1 coumarin [2], 11 saponins [3–7], and 1 peptide [8] were obtained from the fruits of this plant. Some of the saponins from *G. chinensis* showed anti-HIV and anticancer effects [9–10]. In the search for novel bioactive constituents from natural source, we investigated the chemical constituents of *G. chinensis*.

In our recent research, three compounds have been isolated, among which compound **1** was a novel compound (Figures 1 and 2). The structures of these compounds were determined by spectroscopic and chemical analysis.

2. Results and discussion

Compound **1** was obtained as a hygroscopic white powder with $[\alpha]_D^{25} - 6.7$ ($c = 0.97$, MeOH). The molecular formula of $C_{95}H_{150}O_{45}$ was established by TOF-MS at *m/z* 2033 $[M + Na]^+$ and QFT-MS at *m/z* 2033.9368 $[M + Na]^+$. The IR spectrum of **1** showed absorption bands at 1749 and 1694 cm^{-1} ascribable to carbonyl and olefin moieties, and two broad-bands at 3418 and 1079 cm^{-1}, suggestive of an oligoglycoside structure.

The ^1H and ^{13}C NMR spectra of **1** (Tables 1–3), combined with various 2D NMR experiments (including DEPT, $^1H-^1H$ COSY, HMQC, HMBC, 2D-TOCSY, HSQC-TOCSY, and NOESY), showed signals assignable to six tertiary methyl groups at δ_H 0.75, 0.98, 1.00, 1.09, 1.55, and 1.58 (Me-29, Me-30, Me-24, Me-26, Me-25, and Me-27, each 3H, s), one oxygenated methylenes at δ_H 3.48

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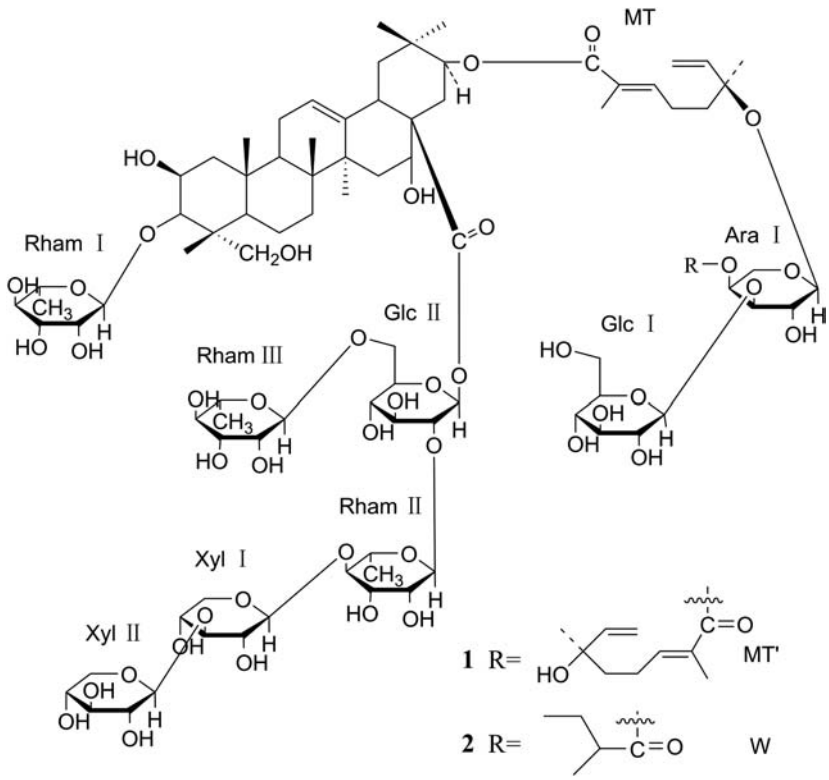

Figure 1. The structures of compounds **1** and **2**.

(1H, H_a-23), 3.95 (1H, H_b-23), four oxygenated methines at δ_H 6.20 (1H, dd, $J = 6.0$, 10.8 Hz, H-21), 4.18 (1H, H-3), 4.54 (1H, H-2), and 5.05 (1H, br s, H-16), an olefinic group at δ_H 5.42 (1H, br s, H-12), as well as a carbonyl group at δ_C 174.5 (C-28). Thus, the aglycone of **1** was concluded to be 2β,23-dihydroxy-acacic acid [3].

In addition, the data of NMR spectra revealed signals for four methyl singlets at δ_H 1.16 (3H, s, Me-10 of MT′), 1.28 (3H, s, Me-10 of MT[1]), 1.61 (3H, s, Me-9 of MT′), and 1.65 (3H, s, Me-9 of MT), eight olefinic protons at δ_H 4.92 (1H, dd, $J = 1.9$, 17.4 Hz, H_b-8 of MT′), 5.04 (1H, d, $J = 10.8$ Hz, H_b-8 of MT), 5.18 (1H, d, $J = 17.5$ Hz, H_a-8 of MT), 5.28 (1H, dd, $J = 1.9$, 10.8 Hz, H_a-8 of MT′), 5.82 (1H, dd, $J = 10.8$, 17.4 Hz, H-7 of MT′), 6.71 (1H, dd, $J = 10.8$, 17.5 Hz, H-7 of MT), 6.85 (1H, t, $J = 6.9$ Hz, H-3 of MT), and 6.92 (1H, t, $J = 6.9$ Hz, H-3 of MT′), as well as two carbonyl groups at δ_C 167.5 (C-1 of MT) and 167.9 (C-1 of MT′). All the results indicated that there were two monoterpene moieties in the structure of **1**.

Acid hydrolysis of **1** yielded D-glucose, D-xylose, L-arabinose, and L-rhamnose (2:2:1:3) as the component sugars, as identified by the GC analysis. By analysis of 1D and 2D NMR spectra of **1**, the data showed the presence of eight glycosyl moieties at δ_H 4.59 (1H, d, $J = 7.5$ Hz, H-1 of Ara I), 4.94 (1H, d, $J = 6.2$ Hz, H-1 of Xyl I), 4.97 (1H, d, $J = 8.0$ Hz, H-1 of Xyl II), 5.10 (1H, d, $J = 7.7$ Hz, H-1 of Glc I), 5.14 (1H, br s, H-1 of Rham III), 5.54 (1H, br s, H-1 of Rham I), 5.85 (1H, *d*, $J = 7.8$ Hz, H-1 of Glc II), and 6.04 (1H, br s, H-1 of Rham II).

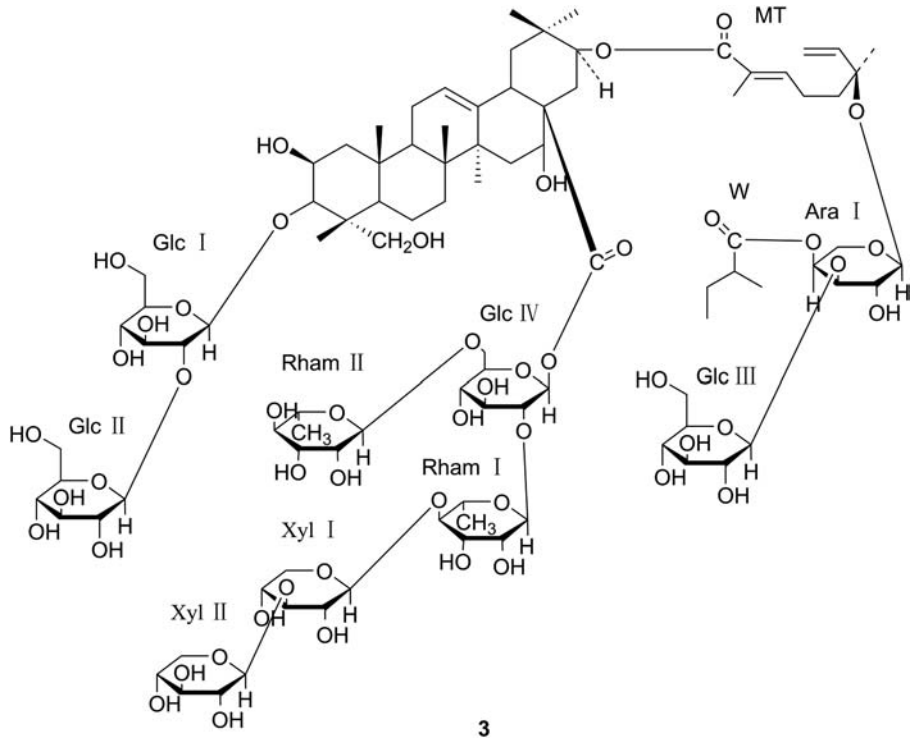

Figure 2. The structure of compound **3**.

Starting from the anomeric proton of each sugar unit, all the protons within each spin system were assigned by HSQC-TOCSY and $^1H-^1H$ COSY experiments, whereas the proton–carbon correlations were assigned by HMQC and further confirmed by HMBC experiments (see Figure 3). The linkage of the sugar units at C-3 of the aglycone was established from the HMBC correlation of H-1 of Rham I at δ_H 5.54 with C-3 at δ_C 81.5.

Similarly, the sugar chain at C-21 was established from the following HMBC correlations: H-21 at δ_H 6.20 with C-1 of MT at δ_C 167.5, H-1 of Ara I at δ_H 4.59 with C-6 of MT at δ_C 79.7, H-1 of Glc I at δ_H 5.10 with C-3 of Ara I at δ_C 81.4, and H-4 of Ara I at δ_H 4.30 with C-1 of MT′ at δ_C 167.9.

The sequence of the sugar unit at C-28 was indicated in the same way: H-1 of Glc II at δ_H 5.85 with C-28 at δ_C 174.5, H-1 of Rham III at δ_H 5.14 with C-6 of Glc II at δ_C 66.7, H-1 of Rham II at δ_H 6.04 with C-2 of Glc II at δ_C 78.7, H-1 of Xyl I at δ_H 4.94 with C-4 of Rham II at δ_C 84.1, and H-1 of Xyl II at δ_H 4.97 with C-3 of Xyl I at δ_C 87.5 (Figure 3). The same conclusion with regard to the sugar sequence was also drawn from the 2D-TOCSY and NOESY experiments.

Considering the above evidences, the structure of compound **1** was elucidated as 2β,23-dihydroxy-3-*O*-α-L-rhamnopyranosyl-21-*O*-{(6*S*)-2-*trans*-2,6-dimethyl-6-*O*-[3-*O*-(β-D-glucopyranosyl)-4-*O*-((6*S*)-2-*trans*-2,6-dimethyl-6-hydroxy-2,7-octadienoyl)-β-L-arabinopyranosyl]-2,7-octadienoyl}-acacic acid 28-*O*-β-D-xylopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-glucopyranosyl ester.

Compound **2** was obtained as a hygroscopic white powder. The molecular formula of $C_{90}H_{144}O_{44}$ was established by

Table 1. ^{1}H and ^{13}C NMR (pyridine-d_5) spectral data of the aglycone moieties of compounds **1**–**3**.

	1		2		3	
No.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	44.6	1.08, 2.54	44.8	1.33, 2.33	44.2	1.25, 2.34
2	70.9	4.54	71.1	4.77	70.3	4.78
3	81.5	4.18	81.3	4.22	83.4	4.20
4	42.7	–	43.0	–	42.8	–
5	47.3	1.65	47.6	1.92	47.7	1.86
6	18.4	1.75	18.3	1.90	18.4	1.87
7	32.9	1.58, 1.65	33.2	1.74, 1.89	33.4	1.74, 1.87
8	40.0	–	40.3	–	40.3	–
9	47.3	1.57	47.6	1.74	48.5	1.72
10	36.9	–	37.2	–	37.1	–
11	23.8	1.90, 2.20	24.1	2.10, 2.25	24.2	2.04, 2.23
12	123.6	5.42 (1H, br s)	123.9	5.65 (1H, br s)	123.9	5.63 (1H, br s)
13	143.2	–	143.4	–	143.4	–
14	42.0	–	42.3	–	42.4	–
15	35.7	1.90, 2.00	36.0	2.00, 2.25	36.0	2.04, 2.25
16	73.2	5.05 (1H, br s)	73.5	5.51 (1H, br s)	73.5	5.22 (1H, br s)
17	51.6	–	51.8	–	51.9	–
18	40.6	3.46	40.9	3.53	41.0	3.52
19	47.7	1.22, 2.72 (1H, t, 13.7)	48.0	1.39, 2.95 (1H, t, 13.7)	48.1	1.44, 2.93 (1H, t, 13.8)
20	35.1	–	35.4	–	35.5	–
21	76.7	6.20 (1H, dd, 6.0, 10.8)	77.0	6.19 (1H, dd, 6.0, 10.8)	77.2	6.18 (1H, dd, 6.0, 10.8)
22	36.3	1.88 (1H, m), 2.53 (1H, m)	36.5	2.28 (1H, m), 2.76 (1H, m)	36.6	2.27 (1H, m), 2.74 (1H, m)
23	64.9	3.48, 3.95	66.9	3.70, 4.37	65.8	3.74, 4.35
24	14.6	1.00 (3H, s)	14.9	1.22 (3H, s)	15.0	1.41 (3H, s)
25	17.3	1.55 (3H, s)	17.6	1.60 (3H, s)	17.5	1.58 (3H, s)
26	17.4	1.09 (3H, s)	17.7	1.20 (3H, s)	17.6	1.17 (3H, s)
27	26.9	1.58 (3H, s)	27.2	1.77 (3H, s)	27.3	1.77 (3H, s)
28	174.5	–	174.8	–	174.7	–
29	28.3	0.75 (3H, s)	29.2	0.99 (3H, s)	29.3	0.97 (3H, s)
30	18.9	0.98 (3H, s)	19.2	1.22 (3H, s)	19.3	1.21 (3H, s)

Notes: The ^{1}H NMR spectra of **1** and **2** were measured at 500 MHz and **3** at 600 MHz. The ^{13}C NMR spectra of **1** and **2** were measured at 125 MHz and **3** at 150 MHz. Assignments were made on the basis of ^{1}H–^{1}H COSY, HMQC, HMBC, 2D-TOCSY, NOESY, and HSQC-TOCSY. Overlapped signals are reported without designated multiplicities.

TOF-MS at m/z 1951 $[M + Na]^+$ and QFT-MS at m/z 1951.8953 $[M + Na]^+$. The IR spectrum of **2** showed absorption bands at 1709 and 1650 cm^{-1} ascribable to carbonyl and olefin moieties, and two broadbands at 3467 and 1078 cm^{-1}, suggestive of an oligoglycoside structure. Acid hydrolysis of **2** yielded D-glucose, D-xylose, L-arabinose, and L-rhamnose (2:2:1:3) as sugar residues on the basis of GC. The ^{1}H and ^{13}C NMR chemical shifts of **2** were similar to those of **1** except for the signals of the 2-methylbutyroyl group. It was observed that two methyl groups at δ_H 0.82 (3H, t, $J = 7.5$ Hz, Me-4 of W^2) and 1.07 (3H, d, $J = 7.2$ Hz, Me-5 of W), as well as one carbonyl carbon at δ_C 176.7 (C-1 of W), all indicating one 2-methylbutyroyl group in the structure of **2**. Thus, the structure of compound **2** was elucidated as 2β,23-dihydroxy-3-*O*-α-L-rhamnopyranosyl-21-*O*-{(6*S*)-2-*trans*-2,6-dimethyl-6-*O*-[3-*O*-(β-D-glucopyranosyl)-4-*O*-(2-methylbutyroyl)-β-L-arabinopyranosyl]-2,

Table 2. ^{1}H and ^{13}C NMR (pyridine-d_5) spectral data of the monoterpene acid moieties and 2-methylbutyroyl groups of compounds **1**–**3**.

	1		2		3	
No.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
MT						
1	167.5	–	167.8	–	167.7	–
2	128.4	–	128.6	–	128.6	–
3	143.5	6.85 (1H, t, 6.9)	142.2	6.95 (1H, t, 7.0)	142.1	6.93 (1H, t, 7.3)
4	23.3	2.33	23.6	2.42	23.7	2.42
5	40.0	1.62	40.4	1.79	40.5	1.79
6	79.7	–	80.0	–	80.1	–
7	141.9	6.71 (1H, dd, 10.8, 17.5)	143.8	6.22 (1H, dd, 10.7, 17.4)	143.8	6.23 (1H, dd, 10.8, 17.4)
8	115.0	5.04 (1H, d, 10.8), 5.18 (1H, d, 17.5)	115.2	5.27 (1H, d, 10.7), 5.39 (1H, d, 17.4)	115.2	5.26 (1H, d, 10.8), 5.42 (1H, d, 17.4)
9	12.4	1.65 (3H, s)	12.7	1.88 (3H, s)	12.8	1.86 (3H, s)
10	23.5	1.28 (3H, s)	23.7	1.51 (3H, s)	23.8	1.50 (3H, s)
MT′						
1	167.9	–				
2	127.7	–				
3	143.7	6.92 (1H, t, 6.9)				
4	23.7	2.12				
5	40.9	1.57				
6	71.8	–				
7	146.3	5.82 (1H, dd, 10.8, 17.4)				
8	111.4	4.92 (1H, dd, 1.9, 17.4), 5.28 (1H, dd, 1.9, 10.8)				
9	12.2	1.61 (3H, s)				
10	28.9	1.16 (3H, s)				
2-*Methylbutyroyl group*						
1			176.7	–	176.7	–
2			41.3	2.42	41.4	2.41
3			27.0	1.38, 1.65	27.1	1.46, 1.68
4			11.6	0.82 (3H, t, 7.5)	11.7	0.83 (3H, t, 7.5)
5			16.6	1.07 (3H, d, 7.2)	16.7	1.05 (3H, d, 7.2)

Notes: The ^{1}H NMR spectra of **1** and **2** were measured at 500 MHz and **3** at 600 MHz. The ^{13}C NMR spectra of **1** and **2** were measured at 125 MHz and **3** at 150 MHz. Assignments were made on the basis of ^{1}H–^{1}H COSY, HMQC, HMBC, 2D-TOCSY, NOESY, and HSQC-TOCSY. Overlapped signals are reported without designated multiplicities.

Table 3. ^{1}H and ^{13}C NMR (pyridine-d_5) spectral data of the sugar moieties of compounds **1**–**3**.

	1		2		3	
No.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
3-Sugars						
	Rham I		Rham I		Glc I	
1	103.9	5.54 (br s)	104.2	5.75 (br s)	103.2	5.07 (d, 7.2)
2	72.5	4.47	72.8	4.69	83.1	4.18
3	72.3	4.38	72.5	4.58	76.4	4.23
4	73.7	4.09	74.0	4.29	71.0	4.29
5	70.1	3.94	70.4	4.19	77.9	4.07
6	18.5	1.38 (d, 6.5)	18.6	1.62 (d, 6.2)	62.3	4.21, 4.49
					Glc II	
1					105.5	5.37 (d, 7.2)
2					75.2	4.01
3					78.4	3.94
4					71.3	4.03
5					78.1	4.17
6					62.5	4.40, 4.68
21-Sugars						
	Ara I		Ara I		Ara I	
1	100.0	4.59 (d, 7.5)	99.8	4.77 (d, 7.6)	99.8	4.77 (d, 7.8)
2	71.3	4.28	71.8	4.39	71.7	4.20
3	81.4	4.17	81.3	4.29	81.2	4.31
4	71.8	4.30	71.6	4.21	71.4	4.03
5	63.9	3.73, 4.08	64.3	3.67, 4.09	64.4	3.67, 4.08
	Glc I		Glc I		Glc III	
1	106.2	5.10 (d, 7.7)	106.2	5.26 (d, 7.6)	106.2	5.27 (d, 7.6)
2	75.3	3.98	75.7	3.95	75.8	4.00
3	78.4	3.69	78.4	4.26	77.9	4.23
4	71.3	3.84	71.7	4.02	72.1	4.04
5	78.0	3.74	78.2	3.93	78.3	4.26
6	62.7	4.08, 4.28	63.1	4.18, 4.60	63.3	4.21, 4.52
28-Sugars						
	Glc II		Glc II		Glc IV	
1	94.7	5.85 (d, 7.8)	95.0	6.06 (d, 7.6)	95.2	6.04 (d, 7.8)
2	78.7	4.00	79.0	4.21	79.3	4.21
3	77.7	3.81	77.9	4.05	78.2	3.99
4	71.2	3.86	71.5	3.94	71.7	4.01
5	76.6	3.74	76.9	4.22	76.6	4.16
6	66.7	4.08, 4.26	66.9	3.49, 4.20	66.9	3.46, 4.35
	Rham II		Rham I		Rham I	
1	101.3	6.04 (br s)	101.6	6.12 (br s)	101.5	6.30 (br s)
2	71.7	4.60	71.8	4.82	72.0	4.49
3	72.3	4.49	72.5	4.69	72.5	4.70
4	84.1	4.16	84.4	4.39	84.3	4.37
5	68.2	4.26	68.4	4.47	68.5	4.47
6	18.5	1.49 (d, 6.0)	18.7	1.71 (d, 6.2)	18.7	1.70 (d, 6.2)
	Xyl I		Xyl I		Xyl I	
1	106.3	4.94 (d, 6.2)	106.5	5.16 (d, 7.6)	106.4	5.15 (d, 7.2)

Table 3 – *continued*

	1		2		3	
No.	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
2	74.9	3.90	75.1	4.04	74.0	4.39
3	87.5	3.81	87.7	4.06	88.0	4.03
4	68.6	4.00	68.9	4.03	68.9	4.04
5	67.2	3.80, 4.26	67.5	3.52, 4.14	67.5	3.98, 4.47
	Xyl II		Xyl II		Xyl II	
1	105.9	4.97 (d, 8.0)	106.1	5.18 (d, 7.6)	106.1	5.12 (d, 7.8)
2	75.0	3.79	75.2	4.02	75.1	4.09
3	78.1	3.69	78.3	4.04	78.2	4.01
4	70.6	3.93	70.9	4.11	70.7	4.13
5	67.1	3.45, 4.09	67.4	3.49, 4.29	67.3	3.65, 4.27
	Rham III		Rham III		Rham II	
1	101.8	5.14 (br s)	102.1	5.36 (br s)	102.1	5.36 (br s)
2	72.2	4.23	72.1	4.60	72.0	5.74
3	72.4	4.29	72.7	4.37	72.6	4.40
4	73.7	4.06	74.0	4.29	73.9	4.18
5	69.4	4.38	69.7	4.58	69.7	4.21
6	18.5	1.38 (d, 6.0)	18.7	1.62 (d, 6.5)	18.8	1.59 (d, 6.4)

Notes: The ^{1}H NMR spectra of **1** and **2** were measured at 500 MHz and **3** at 600 MHz. The ^{13}C NMR spectra of **1** and **2** were measured at 125 MHz and **3** at 150 MHz. Assignments were made on the basis of ^{1}H–^{1}H COSY, HMQC, HMBC, 2D-TOCSY, NOESY, and HSQC-TOCSY. Overlapped signals are reported without designated multiplicities.

7-octadienoyl}-acacic acid 28-*O*-β-D-xylopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-glucopyranosyl ester. By comparing with the literature [5], **2** was identified as gymnocladus saponin E. In the literature, the relative configuration of the sugar (Ara I) located at C-21 was established as α-L-arabinopyranosyl. However, with the aid of COSY, TOCSY, HMQC and HMBC spectrum, Ara I was identified as *β-L*-arabinopyranosyl. Revise is hereby given.

Compound **3** was obtained as a hygroscopic white powder. The molecular formula of $C_{96}H_{154}O_{50}$ was established by TOF-MS at *m/z* 2129 $[M + Na]^+$ and QFT-MS at *m/z* 2129.9424 $[M + Na]^+$. The IR spectrum of **3** showed absorption bands at 1735 and 1644 cm^{-1} ascribable to carbonyl and olefin moieties, and two broadbands at 3420 and 1079 cm^{-1}, suggestive of an oligoglycoside structure. Acid hydrolysis of **3** yielded D-glucose, D-xylose, L-arabinose, and L-rhamnose (4:2:1:2) as sugar residues, on the basis of GC. By comparing NMR data of **3** with those of **2** indicated that **3** differed from **2** only by the presence of the sugar units on the C-3 sugar chain. The units at C-3 were established from the following HMBC correlations: H-1 of Glc I at δ_H 5.07 (1H, d, $J = 7.2$ Hz) with C-3 at δ_C 83.4 and H-1 of Glc II at δ_H 5.37 (1H, d, $J = 7.2$ Hz) with C-2 of Glc I at δ_C 83.1. Thus, with the aid of ^{1}H and ^{13}C NMR spectral data (Tables 1–3), **3** was established as 2β,23-dihydroxy-3-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]-21-*O*-{(6*S*)-2-*trans*-2,6-dimethyl-6-*O*-[3-*O*-(β-D-glucopyranosyl)-4-*O*-(2-methylbutyroyl)-β-L-arabinopyranosyl]-2,7-octadienoyl}-acacic acid 28-*O*-β-D-xylopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-

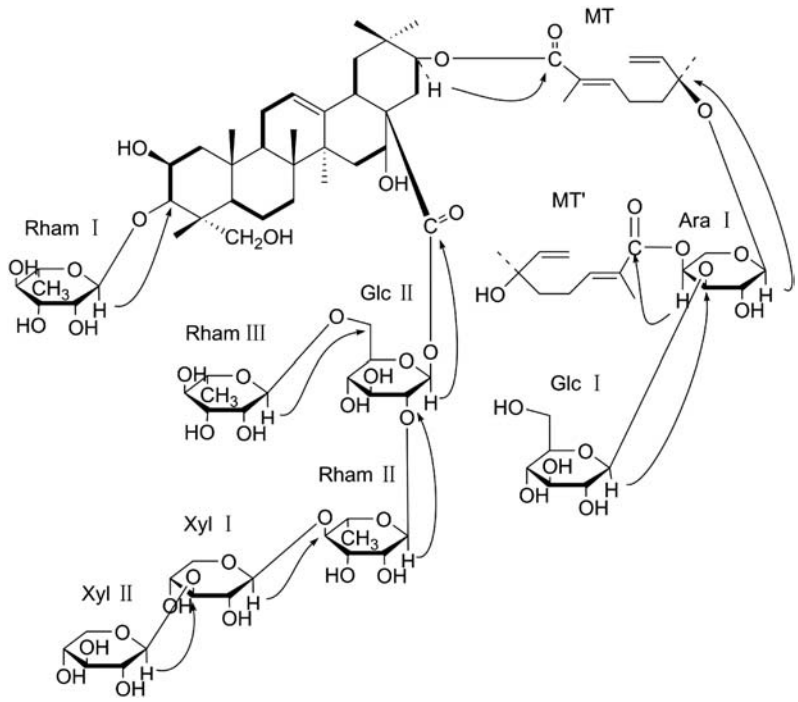

Figure 3. The key HMBC (→) and $^1H-^1H$ COSY (—) correlations of compound **1**.

[α-L-rhamnopyranosyl-(1 → 6)]-β-D-glucopyranosyl ester. By comparing with the literature [5], **3** was identified as gymnocladus saponin F_2. In the literature, the relative configuration of the sugar (Ara I) located at C-21 was established as α-L-arabinopyranosyl. However, with the aid of COSY, TOCSY, HMQC and HMBC spectrum, Ara I was identificated as *β-L*-arabinopyranosyl. Revise is hereby given.

3. Experimental

3.1 General experimental procedures

The IR spectra were recorded on a FT-IR instrument (Magna-IR750, Nicolet, WI, USA), KBr disks. The 1D and 2D NMR spectra were recorded on the Bruker-500 (Avance DRX-500, Bruker, Fällanden, Switzerland) and the Varian-600 (Unity-Inova 600, Varian, CA, USA) instruments. The TOF-MS data were obtained on a quadrupole time-of-flight instrument (Q-Tof Ultima Global, Waters, Manchester, UK). QFT-MS were carried out on a 9.4 T Q-FT-ICR MS instrument (Bruker, MA, USA). GC-MS was performed on the FINNIGAN TRACE DSQ instrument (Thermo Finnigan, San Jose, CA, USA). Optical rotations were taken on a PerkinElmer 241 automatic digital polarimeter (PerkinElmer, Waltham, MA, USA). Chromatography was performed on macroporous resin D_{101} (Nankai University Chemical Factory, Tianjin, China), silica gel (SiO_2, 10–40 μm, 200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), Sephadex LH-20 (Pharmacia, NJ, USA), Amberlite MB-3 ion-exchange resin (Organo, Tokyo, Japan), RP-C_{18} silica gel (100–200 mesh, Pharmacia), and reversed-phase HPLC (Waters 2487, MA, USA). HPLC grade MeCN was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Deionized H_2O was purified by Milli-Q system (Bedford, MA, USA). MeOH, $CHCl_3$, and *n*-BuOH for purification were

of analytical grade from Beijing Reagent Company (Beijing, China).

3.2 Plant material

The dried fruits of *G. chinensis* were purchased from Anhui Province, China, which is the major cultivation area. They were identified by Senior Pharmacist Yu Qiu at Chengdu Laimei Pharmaceutical Co., Ltd, Chendu, China. A voucher specimen (No. 080207) has been deposited at State Key Laboratory of Natural and Biomimetic Drugs, Peking University.

3.3 Extraction and isolation

The air-dried and powdered fruits (8.5 kg) were extracted consecutively with 75% MeOH (*v*/*v*). The MeOH extract (1120 g) was concentrated under vacuum and the residue was suspended in water, and then extracted successively with EtOAc and *n*-BuOH. The *n*-BuOH-soluble portion was concentrated under reduced pressure, and the viscous concentrate was passed through a D_{101} macroporous resin column and successively eluted with 30, 50, 70, and 90% MeOH . The 70% MeOH eluate portion (833 g) was subjected to silica gel column (SiO_2, eluted with a stepwise gradient mixture of $CHCl_3$/MeOH/H_2O, 30:10:1 → 10:5:1 → 6:4:1 and finally with MeOH) to afford three fractions A, B, and C. Fraction B (122 g) was subjected to column chromatography on RP-C_{18} silica gel eluted with MeOH/H_2O (7:3) to yield a crude saponin fraction. Further purification of the fraction by the combination of preparative and semi-preparative reverse-phase HPLC successively yielded compound **1** (32 mg): a hygroscopic white powder, $C_{95}H_{150}O_{45}$, $[\alpha]_D^{25} - 6.7$ ($c = 0.97$, MeOH); IR (KBr) ν_{max} (cm^{-1}): 1749, 1694, 3418, 1079; ^1H and ^{13}C NMR spectral data, see Tables 1–3; TOF-MS: *m*/*z* 2033 $[M + Na]^+$ and QFT-MS at *m*/*z* 2033.9368 $[M + Na]^+$ (calcd for $C_{95}H_{150}O_{45}Na$, 2033.9349), compound **2** (250 mg), and compound **3** (128 mg).

3.4 Acid hydrolysis and identification of sugars

A solution of **1** (10 mg) in 4 N HCl (dioxane/H_2O, 1:1, 1000 ml) was heated at 80°C for 10 h. After dioxane was removed, the solution was extracted with EtOAc to obtain the aglycone. The aqueous layer was neutralized by passing through Amberlite MB-3 ion-exchange resin column and concentrated under reduced pressure to dryness to afford a residue of the sugar fraction. The residue was dissolved in pyridine (0.1 ml), to which 0.08 M L-cysteine methyl ester hydrochloride in pyridine (0.15 ml) was added. The mixture was kept at 60°C for 1.5 h. After the reaction mixture was dried *in vacuo*, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 ml) for 2 h. The mixture was partitioned between *n*-hexane and H_2O (0.3 ml), and the *n*-hexane extract was analyzed by GC-MS under the following conditions: capillary column, DB-1MS (30 m × 0.25 mm × 0.25 μm); column temperature, 260°C; injection temperature, 90°C; carrier gas, He. Then, **2** and **3** (each 10 mg) were carried in the same method [11]. In the acid hydrolyzate of **1**–**3**, L-rhamnose, D-glucose, L-arabinose, and D-xylose were confirmed by comparison with the retention times of the L-rhamnose, D-glucose, L-arabinose, and D-xylose derivatives prepared in a similar way, which showed retention times of 18.41, 19.62, 17.64, and 18.40 min, respectively.

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Notes

1. MT was the abbreviation of the monoterpene acid moiety.
2. W was the abbreviation of the 2-methylbutyroyl group

References

[1] T. Konoshima, T. Sawada, and T. Kimura, *Chem. Pharm. Bull.* **32**, 2617 (1984).
[2] L. Han and H.Z. Fu, *Chin. Herb. Med.* **1**, 66 (2009).
[3] T. Konoshima, T. Sawada, and T. Kimura, *Chem. Pharm. Bull.* **32**, 4833 (1984).
[4] T. Konoshima, T. Sawada, and T. Kimura, *Chem. Pharm. Bull.* **33**, 4732 (1985).
[5] T. Konoshima, T. Sawada, and T. Kimura, *Chem. Pharm. Bull.* **35**, 46 (1987).
[6] T. Konoshima, T. Sawada, and T. Kimura, *Chem. Pharm. Bull.* **35**, 1982 (1987).
[7] K. Wang and H.Z. Fu, *J. Chin. Pharm. Sci.* **18**, 141 (2009).
[8] J.H. Wong and T.B. Ng, *Peptides* **24**, 963 (2003).
[9] T. Konoshima, T. Sawada, and T. Kimura, *J. Natl. Prod.* **58**, 1372 (1995).
[10] Y.X. Ma, H.Z. Fu, M. Li, W. Sun, B. Xu, and J.R. Cui, *Anti-Cancer Drugs* **18**, 41 (2007).
[11] H.Z. Fu, K. Koike, W. Li, T. Nikaido, L.W. Han, and D.A. Guo, *J. Natl. Prod.* **68**, 754 (2005).