Triterpene Saponins from *Gynostemma cardiospermum*

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Six new dammarane glycosides (**1**−**6**) and four known compounds, rutin, kaempferol, quercetin, and linalool 3-*O*-*β*-D-glucopyranoside, were isolated from an ethanol extract of the aerial parts of *Gynostemma cardiospermum.* The structures of **1**−**6** were elucidated by 1D and 2D NMR spectroscopic interpretation as well as by chemical degradation. Triterpene aglycons containing carbonyl groups at both C-21 and C-28, as found in compounds **1**−**5**, are being reported in the family Cucurbitaceae for the first time.

Gynostemma belongs to the family Cucurbitaceae, and these plants are known in mainland China as "xiancao", or "herb of immortality". Plants in this genus are used in folk medicine for lowering cholesterol levels, regulating blood pressure, strengthening the immune system, treating chronic bronchitis and gastritis, and reducing inflammation.[1] Gynostemma species taste sweet and aromatic and can be taken either as a tea or with alcohol. Previous investigations of this genus have shown the occurrence of dammarane-type glycosides called the gypenosides that are structurally related to ginseng saponins.[2] Certain gypenosides have been reported to inhibit the proliferation of Hep-3B and HA22T cells, by affecting calcium and sodium currents in a dose-dependent manner.[3] Currently, there are altogether 14 *Gynostemma* species used in traditional Chinese medicine,[4] and among these, *Gynostemma pentaphyllum* (Thunb.) Makino has been widely investigated for its phytochemical constituents and is sweet-tasting. In contrast, *Gynostemma cardiospermum* Cogn. ex Oliv. has not been subjected to phytochemical investigation before.

In the present study, we have isolated six new triterpene saponins (**1**−**6**) and four known compounds from *G. cardiospermum*. The structure elucidation of **1**−**6** was accomplished mainly on the basis of the interpretation of 2D NMR spectroscopic data, including ^{1}H−^{1}H and ^{1}H−^{13}C chemical shift correlation spectroscopy.

Results and Discussion

The known compounds rutin,[5] quercetin,[5] kaempferol,[6] and linalool 3-*O*-*β*-D-glucopyranoside[7] were identified by comparison of their physical properties and spectroscopic data with those described in the literature.

Compound **1** was obtained as an amorphous powder. The HRESIMS sodiated molecular ion of **1** at *m*/*z* 983.4833 was used to establish its elemental formula as $C_{47}H_{76}O_{20}$ (calcd for $C_{47}H_{76}O_{20}$Na, 983.4828 $[M + Na]^+$). The ^{13}C and DEPT NMR spectra gave 47 signals, of which 17 were assigned to the sugar units and 30 to a triterpene aglycon moiety. The ^{1}H NMR spectrum of **1** showed six signals assignable to aglycon methyl groups at δ 1.02−1.85, of which two were linked to sp^2 carbons (δ 1.70 and 1.61). The ^{1}H NMR spectrum of **1** also showed an olefinic proton at δ 5.44 (m). A 3*β*-hydroxyl substituent was evident from both the chemical shift and the *J* values of the proton ascribable to H-3α (δ 3.49; 1H, dd, J = 11.8, 3.9 Hz). By comparison of its ^{1}H and ^{13}C NMR spectra with those of known dammarane-type saponins,[2] it was evident that the aglycon of **1** has two more carbonyl groups than the common triterpene saponin aglycons. The sites of the two carbonyl groups were deduced from a HMBC experiment. Correlations were observed between H-22 and one carbonyl carbon and between H-29 and the other carbonyl carbon (Figure S1, Supporting Information). Thus, one carbonyl group was located on C-21 and the other was located on C-28. Hydrolysis of **1** yielded a H_2O adduct at C-24 and C-25 of the corresponding aglycon (Scheme S1, Supporting Information). All the ^{1}H and ^{13}C NMR data (Table S1, Supporting Information) were assigned using HSQC, HMBC, ^{1}H−^{1}H COSY, and NOESY experiments. A prominent cross-peak was observed between H-3 and CH_3-29 in the NOESY spectrum (Figure S2, Supporting Information), also indicating that a carbonyl group was located on C-28. The absolute configuration at C-20 was established as *S* by comparing the NMR data with the model compounds.[8] Thus, the aglycon of **1** was elucidated as 3*β*,20*S*-dihydroxydammar-24-en-21,28-dioic acid.

Acid hydrolysis of compound **1** yielded L-arabinose and D-glucose in a ratio of 1:2 by GC analysis of the leucine derivatives of the component monosaccharides compared with the leucine derivatives of the standard sugars. The ^{1}H NMR spectrum, as well as the ^{13}C NMR data, indicated an α-configuration for the arabinosyl unit [δ 5.06 (1H, d, J = 5.5 Hz, H-1 of ara); δ 107.3 (C-1 of ara)], a *β*-configuration for the glucosyl [δ 5.54 (1H, d, J = 7.8 Hz, H-1 of glc); δ 105.8 (C-1 of glc)], and a *β*-configuration for the other glucosyl [δ 6.30 (1H, d, J = 7.8 Hz, H-1 of glc′); δ 94.5 (C-1 of glc′)]. The ^{13}C NMR data allowed the assignment of the pyranose forms of L-arabinose and D-glucose. All ^{1}H and ^{13}C NMR signals of the three sugar units in **1** were assigned using ^{1}H−^{1}H COSY, HMQC, and HMBC spectra. The linkage sites and sequences of the three saccharides and of the aglycon were deduced from a HMBC experiment. Correlations were observed between H-1 of the arabinose and C-3 of the aglycon, H-1 of the glucose and C-3 of the arabinose, and H-1 of the other glucose and C-21 of the aglycon (Figure S1, Supporting Information). Thus, the structure of **1** was elucidated as 3*β*,20*S*-dihydroxydammar-24-en-21,28-dioic acid 3-*O*-[*β*-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl]-21-*O*-*β*-D-glucopyranoside.

Compound **2** was purified as an amorphous powder. It was found to contain five monosaccharide units from the analysis of its ^{1}H and ^{13}C NMR spectra. Comparison of the ^{1}H and ^{13}C NMR spectra of **2** and **1** indicated that they have an identical aglycon moiety. Hydrolysis of **2** yielded L-arabinose, L-rhamnose, and D-glucose in a ratio of 1:2:2. The HRESIMS sodiated molecular ion of **2** at *m*/*z* 1275.5995 suggested a molecular formula of $C_{59}H_{96}O_{28}$ (calcd for

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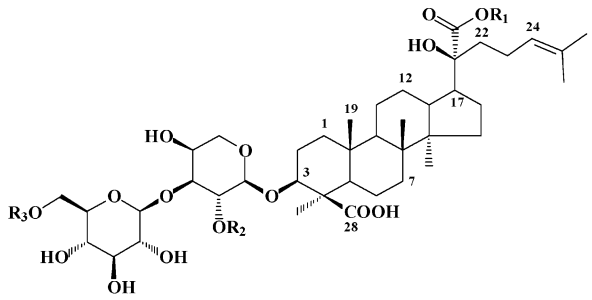
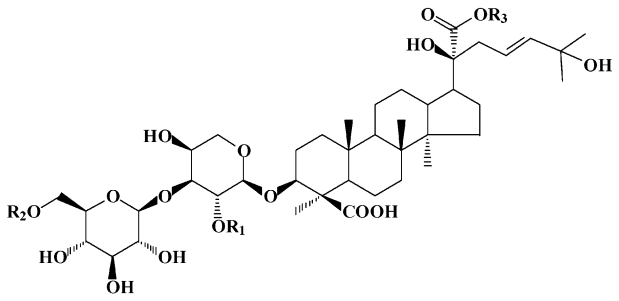


1 R_1 = Glc, R_2 = R_3 = H

2 R_1 = Glc, R_2 = R_3 = Rha

5 R_1 = Glc, R_2 = Rha, R_3 = H



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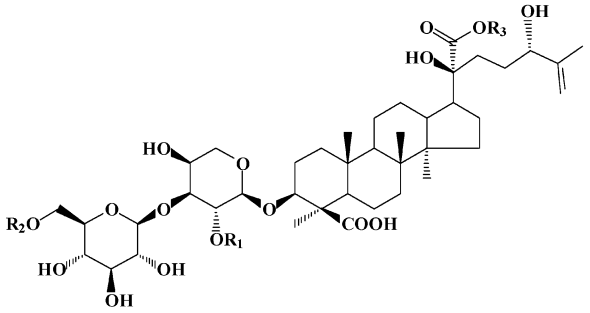

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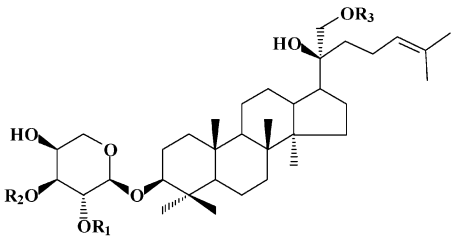

6 R_1 = Rha, R_2 = R_3 = Glc

$C_{59}H_{96}O_{28}Na$, 1275.5986 $[M + Na]^+$). The ^1H NMR spectrum, as well as the ^{13}C NMR data, indicated an α-configuration for the rhamnosyl unit [δ 5.89 (1H, br s, H-1 of rha); δ 101.8 (C-1 of rha)], a β-configuration for the glucosyl uint [δ 5.55 (1H, d, J = 7.7 Hz, H-1 of glc); δ 106.1 (C-1 of glc)], an α-configuration for the other rhamnosyl [δ 5.53 (1H, br s, H-1 of rha′); δ 102.8 (C-1 of rha′)], and a β-configuration for the other glucosyl [δ 6.22 (1H, d, J = 7.3 Hz, H-1 of glc′); δ 94.4 (C-1 of glc′)]. Comparison of the ^{13}C NMR data of **1** and **2**, along with biogenetic considerations, suggested an α-configuration for the arabinosyl unit. The small J value (J < 2 Hz) for H-1 of the arabinosyl unit was due to the strong steric interactions between H-1 of the arabinosyl unit and the carboxylic acid unit at C-28, and H-1 of the arabinosyl unit and C-2 of the rhamnosyl unit. The linkage sites and sequences of the five saccharides and of the aglycon were also determined by a HMBC experiment (Figure S3, Supporting Information). On the basis of the above results, the structure of **2** was elucidated as 3β,-20*S*-dihydroxydammar-24-en-21,28-dioic acid 3-*O*-{[α-L-rhamnopyranosyl(1→2)][α-L-rhamnopyranosyl(1→6)-β-D-glucopyranosyl-(13)]-α-L-arabinopyranosyl}-21-*O*-β-D-glucopyranoside.

The HRESIMS sodiated molecular ion at *m*/*z* 1291.5945 of **3** established its molecular formula as $C_{59}H_{96}O_{29}$ (calcd for $C_{59}H_{96}O_{29}$-Na, 1291.5935 $[M + Na]^+$). The ^{13}C NMR data were very similar to those of **2**, except for the side-chain from the C-22 to C-27 positions. Moreover, it exhibited two methine olefinic carbon resonances instead of signals for one methine olefinic carbon and one quaternary olefinic carbon as in **2**. From the HMBC spectrum (Figure S4, Supporting Information), an oxygen-bearing quaternary carbon signal at 70.3 was correlated with the hydrogen at δ 6.35 (1H, s) and 1.56 (3H, s). According to the literature, it was inferred that the olefinic functional group was between C-23 and C-24, and one hydroxyl functional group is at C-25.[9] Thus, compound **3** was determined as 3β,20*S*,25-trihydroxydammar-23-en-21,28-dioic acid 3-*O*-{[α-L-rhamnopyranosyl(1→2)][α-L-rhamnopyranosyl(1→6)-β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl}-21-*O*-β-D-glucopyranoside.

Compound **4** was isolated as an amorphous powder. The HRESIMS molecular ion of **4** displayed a sodiated molecular ion peak at *m*/*z* 1291.5945, corresponding to an elemental formula of $C_{59}H_{96}O_{29}Na$ (calcd 1291.5935 $[M + Na]^+$). Comparison of the ^1H and ^{13}C NMR spectra of **4** and the known compound 19-oxo-3β,20*S*,21,24*S*-tetrahydroxydammar-25-ene 3-*O*-{[α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-α-L-arabinopyranosyl}-21-*O*-β-D-glucopyranoside[2] indicated that **4** has a 3-hydroxyl-4-methylpent-4-enyl chain moiety at C-20. Moreover, comparison of the ^1H and ^{13}C NMR spectra of **4** and **2** revealed that the only difference was that a 4-methylpent-3-enyl chain moiety at C-20 in **2** is replaced by a 3-hydroxyl-4-methylpent-4-enyl chain moiety in **4**. Thus, the structure of compound **4** was postulated as 3β,20*S*,24*S*-trihydroxydammar-25-ene-21,28-dioic acid 3-*O*-{-[α-L-rhamnopyranosyl(1→2)][α-L-rhamnopyranosyl(1→6)-β-D-glu-copyranosyl(1→3)]-α-L-arabinopyranosyl}-21-*O*-β-D-glucopyranoside. The structure was confirmed by the correlations observed in the HMBC spectrum (Figure S5, Supporting Information).

Compound **5** was obtained as an amorphous powder. The HRESIMS displayed a sodiated molecular ion peak at *m*/*z* 1129.5411, indicating a molecular formula of $C_{53}H_{86}O_{24}$ (calcd for $C_{53}H_{86}O_{24}Na$ 1129.5407 $[M + Na]^+$). Comparison of the ^1H and ^{13}C NMR spectra of **5** and **2** indicated that they had the same aglycon. The only difference between **5** and **2** was that one rhamnosyl unit that linked to C-6 of the glucose in the C-3 saccharide chain in **2** was missing in **5**. Other correlations from the HMBC experiment were the same as those in **2** (Figure S6, Supporting Information). Thus, the structure of compound **5** was assigned as 3β,20*S*-dihydroxydammar-24-en-21,28-dioic acid 3-*O*-{[α-L-rhamnopyranosyl(1→2)][β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl}-21-*O*-β-D-glucopyranoside.

The ^1H and ^{13}C NMR data of **6** were found to be very similar to those of the known compound 3β,20*S*,21-trihydroxydammar-24-ene 3-*O*-{[α-L-rhamnopyranosyl(1→2)][β-D-glucopyranosyl(1→3)]-β-D-glucopyranosyl}-21-*O*-β-D-glucopyranoside,[2] except for the sugar moiety at C-3. The HRESIMS sodiated molecular ion of **6** appeared at *m*/*z* 1085.5876, so compound **6** was determined to have the elemental composition $C_{53}H_{90}O_{21}$ (calcd 1085.5872 $[M + Na]^+$). Comparison of the ^1H and ^{13}C NMR spectra of **6** and the known compound gypenoside XLVIII[10] indicated that they have the same saccharide chain at C-3. Thus, compound **6** was elucidated as 3β,-20*S*,21-trihydroxydammar-24-ene 3-*O*-{[α-L-rhamnopyranosyl-

Table 1. ^{1}H NMR Data of Compounds **1**−**6** in C_5D_5N[a]

position	1	2	3	4	5	6
1	1.70 m, 0.93 m	1.88 m, 1.06 m	1.88 m, 1.08 m	1.86 m, 1.06 m	1.67 m, 0.95 m	1.60 m, 0.90 m
2	2.23 m, 1.54 m	2.22 m, 1.53 m	2.23 m, 1.53 m	2.23 m, 1.51 m	2.21 m, 1.53 m	2.10 m, 1.91 m
3	3.49 dd (11.8, 3.9)	3.41 br d (11.8)	3.41 br d (11.8)	3.38 br d (9.5)	3.41 br d (11.8)	3.35 brd (11.6)
5	1.11 m	1.09 m	1.09 m	1.09 m	1.08 m	0.81 m
6	2.13 m, 2.06 m	2.13 m, 2.04 m	2.13 m, 2.05 m	2.13 m, 2.04 m	2.12 m, 2.03 m	1.61 m, 1.52 m
7	1.61 m, 1.33 m	1.60 m, 1.33 m	1.61 m, 1.34 m	1.61 m, 1.34 m	1.60 m, 1.33 m	1.61 m, 1.31 m
9	1.39 m	1.43 m	1.41 m	1.41 m	1.40 m	1.38 m
11	1.61 m, 1.40 m	1.72 m, 1.42 m	1.72 m, 1.42 m	1.72 m, 1.42 m	1.70 m, 1.40 m	1.72 m, 1.40 m
12	2.12 m, 2.03 m	2.12 m, 2.02 m	2.12 m, 2.04 m	2.12 m, 2.01 m	2.12 m, 2.04 m	2.12 m, 2.01 m
13	2.32 m	2.31 m	2.31 m	2.31 m	2.31 m	2.11 m
15	1.72 m, 1.15 m	1.71 m, 1.17 m	1.72 m, 1.17 m	1.71 m, 1.14 m	1.71 m, 1.18 m	1.70 m, 1.18 m
16	2.96 m, 2.24 m	2.84 m, 2.18 m	2.95 m, 2.18 m	2.85 m, 2.16 m	2.81 m, 2.11 m	1.90 m, 1.43 m
17	2.70 m	2.63 m	2.69 m	2.69 m	2.67 m	2.31 m
18	1.02 s	1.05 s	1.05 s	1.07 s	1.08 s	1.02 s
19	1.11 s	1.13 s	1.13 s	1.12 s	1.12 s	0.86 s
21	-	-	-	-	-	4.43 m, 4.08 m
22	2.47 m, 2.13 m	2.48 m, 2.13 m	3.15 m, 2.91 m	2.21 m, 1.57 m	2.48 m, 2.14 m	2.18 m, 1.99 m
23	2.70 m, 2.41 m	2.71 m, 2.47 m	6.35 s	2.41 m, 2.21 m	2.71 m, 2.47 m	2.53 m, 2.41 m
24	5.44 m	5.50 t (9.0)	6.35 s	4.55 m	5.46 t (9.0)	5.38 t (6.2)
26	1.70 s	1.71 s	1.56 s	5.31 br s, 4.99 br s	1.70 s	1.72 s
27	1.61 s	1.69 s	1.56 s	1.96 s	1.61 s	1.70 s
28						1.30 s
29	1.85 s	1.70 s	1.69 s	1.70 s	1.71 s	1.19 s
30	1.09 s	1.04 s	1.04 s	1.03 s	1.02 s	1.02 s
C-3-Ara						
1	5.06 d (5.5)	5.21 brs	5.20 brs	5.20 brss	5.23 brs	5.18 d (4.9)
2	4.38 m	4.58 m	4.58 m	4.59 m	4.60 m	4.72 m
3	4.38 m	4.36 m	4.27 m	4.29 m	4.37 m	4.40 m
4	4.57 m	4.42 m	4.42 m	4.42 m	4.45 m	4.61 m
5	4.50 m, 4.03 m	4.43 m, 3.97 m	4.43 m, 3.97 m	4.45 m, 3.98 m	4.46 m, 4.02 m	4.33 m, 3.85 m
6						
Rha						
1		5.89 br s	5.88 br s	5.88 br s	5.90 br s	6.20 br s
2		4.58 m	4.58 m	4.58 m	4.59 m	4.65 m
3		4.66 m	4.64 m	4.63 m	4.66 m	4.79 m
4		4.28 m	4.28 m	4.29 m	4.31 m	4.34 m
5		4.52 m	4.52 m	4.52 m	4.52 m	4.65 m
6		1.68 d (5.4)	1.63 d (5.4)	1.67 d (5.4)	1.67 d (5.4)	1.69 d (6.3)
Glc			Glc	Glc	Glc	Glc
1	5.54 d (7.8)	5.55 d (7.7)	5.46 d (7.4)	5.49 d (7.4)	5.54 d (7.3)	4.97 d (7.5)
2	4.15 m	4.12 m	4.19 m	4.19 m	4.14 m	4.02 m
3	4.27 m	4.03 m	4.03 m	4.02 m	4.02 m	4.24 m
4	4.57 m	4.34 m	4.42 m	4.36 m	4.45 m	4.23 m
5	4.02 m	4.17 m	4.15 m	4.16 m	4.24 m	4.06 m
6	4.51 m, 4.46 m	4.62 m, 4.21 m	4.62 m, 4.18 m	4.60 m, 4.19 m	4.48 m, 4.45 m	4.57 m, 4.39 m
Rha′						
1		5.53 br s	5.54 br s	5.54 br s		
2		4.60 m	4.64 m	4.64 m		
3		4.57 m	4.57 m	4.57 m		
4		4.29 m	4.32 m	4.34 m		
5		4.35 m	4.36 m	4.36 m		
6		1.69 d (5.4)	1.68 d (5.4)	1.69 d (5.4)		
C-21-Glc′						
1	6.30 d (7.8)	6.22 d (7.3)	6.20 d (7.7)	6.20 d (7.8)	6.37 d (7.7)	5.14 d (7.7)
2	4.36 m	4.09 m	4.06 m	4.06 m	4.35 m	4.17 m
3	4.41 m	4.27 m	4.27 m	4.28 m	4.40 m	4.29 m
4	4.19 m	4.19 m	4.19 m	4.21 m	4.18 m	4.28 m
5	4.09 m	4.34 m	4.33 m	4.35 m	4.08 m	4.00 m
6	4.67 m, 4.48 m	4.64 m, 4.47 m	4.62 m, 4.45 m	4.63 m, 4.47 m	4.65 m, 4.46 m	4.62 m, 4.45 m

[a] Measured at 500 MHz; referenced to δ 7.58 (C_5D_5N); *J* values (Hz) in parentheses.

(1→2)][β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl}-21-*O*-β-D-glucopyranoside.

Accordingly, as a result of this investigation, the structures of six new compounds (**1**−**6**) from *G. cardiospermum* were identified, and among these, triterpene aglycons containing carbonyl groups at both C-21 and C-28 were reported from a plant in the family Cucurbitaceae for the first time. Sweetness relative to sucrose was evaluated by a human sensory panel.[11] None of the compounds were found to be sweet.

Experimental Section

General Experimental Procedures. Optical rotations were measured in MeOH with a Perkin-Elmer model 341 polarimeter. IR spectra were obtained on a Perkin-Elmer 559B IR instrument. NMR spectra were obtained on a Bruker AMX-500 spectrometer in C_5D_5N solution. Chemical shifts are reported in ppm. ^{1}H NMR chemical shifts were referenced to the center peak of the residual solvent signal (δ 7.58). ^{13}C NMR spectra were referenced to the center peak of the solvent at δ 135.9. HRESIMS were run on a Bruker Atex III spectrometer in MeOH. GC: Shimadzu GC-14BPF; column, 5% OV-225/AW-DMCS-Chromosorb W (80−100 mesh), 3 mm i.d. × 2.5 m; column temperature, 210 °C; injection temperature, 250 °C; carrier gas, N_2 at a flow rate of 25 mL/min; detector, FID.

Plant Material. *Gynostemma cardiospermum* Cogn. ex Oliv. was collected in Chongqing, People's Republic of China, in March 2004. The plant was identified by Mr. Jin-Gui Shen, and a voucher specimen

Table 2. ^{13}C NMR Data of Compounds **1**−**6** in C_5D_5N[a]

carbon	1	2	3	4	5	6
1	40.0	39.9	40.0	40.0	39.9	39.8
2	26.3	26.2	26.2	26.2	26.2	27.0
3	88.9	88.6	88.7	88.7	88.6	88.4
4	50.0	50.0	49.9	50.1	50.0	40.0
5	57.6	57.4	57.4	57.5	57.4	56.8
6	20.7	20.7	20.8	20.8	20.7	18.6
7	36.1	36.1	36.1	36.2	36.1	35.8
8	40.8	40.8	40.8	40.9	40.8	40.9
9	50.9	50.9	50.9	51.0	50.9	51.3
10	37.9	37.9	37.9	38.0	37.8	37.2
11	22.5	22.5	22.4	22.6	22.4	22.0
12	24.3	24.3	24.3	24.3	24.3	24.8
13	41.5	41.5	41.3	41.6	41.5	41.9
14	50.1	50.0	50.5	50.5	50.5	50.7
15	31.0	31.0	31.0	31.0	31.0	31.8
16	27.5	27.4	27.4	27.4	27.4	28.0
17	49.1	49.0	48.4	49.2	49.1	46.4
18	16.2	16.2	16.2	16.3	16.2	16.0
19	14.8	14.7	14.7	14.8	14.7	16.8
20	79.3	79.3	79.7	79.3	79.3	76.5
21	176.2	176.1	175.5	176.1	176.1	76.5
22	40.1	40.1	42.9	30.5	40.1	36.8
23	23.3	23.3	121.5	30.3	23.3	23.5
24	125.5	125.6	144.1	75.8	123.5	126.2
25	131.6	131.5	70.3	150.0	131.6	131.0
26	25.9	25.9	30.7	110.0	25.9	26.2
27	18.0	18.1	30.7	18.5	18.0	17.9
28	177.4	177.7	178.0	178.0	177.8	28.2
29	24.7	24.7	24.7	24.8	24.6	17.0
30	16.7	16.7	16.7	16.8	16.7	16.8
C-3-Ara						
1	107.3	103.9	103.9	103.9	104.0	104.8
2	74.3	75.4	75.3	75.4	75.4	75.0
3	81.1	80.9	81.8	81.5	81.1	82.3
4	68.5	67.2	67.1	67.2	67.3	68.4
5	65.3	62.7	62.7	62.8	62.9	65.0
6						
Rha						
1		101.8	101.7	101.8	101.9	102.1
2		72.8	72.7	72.9	72.8	72.7
3		72.6	72.5	72.6	72.6	72.6
4		74.1	74.1	74.2	74.3	74.1
5		70.2	70.2	70.3	70.2	70.2
6		18.8	18.7	18.8	18.8	18.8
Glc						
1	105.8	106.1	106.0	106.0	105.9	104.9
2	75.6	75.6	75.8	75.6	75.6	75.1
3	78.6	78.6	78.6	78.6	78.7	78.4
4	72.2	74.3	72.3	74.3	72.6	71.6
5	78.6	78.1	78.2	78.4	78.6	78.6
6	62.4	68.1	68.1	68.1	62.4	62.7
Rha′						
1		102.8	102.8	102.8		
2		72.3	72.3	72.1		
3		72.9	72.9	72.9		
4		74.1	74.2	74.2		
5		70.0	69.9	70.0		
6		18.9	18.9	18.9		
C-21-Glc′						
1	94.5	94.4	94.5	94.4	94.5	106.3
2	70.1	70.9	70.9	71.0	70.9	75.6
3	78.7	78.6	78.6	78.6	78.6	78.6
4	72.3	72.3	72.1	72.4	72.3	71.9
5	79.4	78.6	78.7	78.6	79.3	78.6
6	63.4	63.5	63.3	63.2	63.4	63.0

[a] Measured at 125 MHz; referenced to δ 135.9 (C_5D_5N).

(No. 2004005) was deposited at the herbarium of Chinese National Center for Drug Screening, Shanghai, People's Republic of China.

Extraction and Isolation. The dried and powdered aerial parts of *G. cardiospermum* (2.0 kg) were extracted successively with petroleum ether (5 L) and EtOH (3 × 5 L) at room temperature. Removal of EtOH under reduced pressure left a dark residue (50 g). The residue was subjected to silica gel column chromatography, eluted with chloroform−methanol (100:10, 100:20, 100:30, 100:50), to yield four fractions (A−D). Fraction B (10 g) was passed through a Sephadex LH-20 (25−100 μm, Merck, Darmstadt, Germany) column, eluted with methanol to remove flavonoids (2 g), which was further purified on a Sephadex LH-20 column, eluted with methanol−water (50:50), to give rutin (200 mg), kaempferol (50 mg), quercetin (70 mg), and linalool 3-*O*-β-D-glucopyranoside (20 mg). Next, the main fraction was subjected to MCI gel CHP 20P (75−150 μm, Mitsubishi Kasei Industry Co., Ltd., Tokyo, Japan) column chromatography, eluted with water−acetone (9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1), to yield eight subfractions (B-1−8). Subfraction B-7 (4 g) was chromatographed by RP-18 flash column chromatography, eluted with methanol−water (50:50) to afford compound **3** (72 mg), eluted with methanol−water (53:47) to afford compound **4** (110 mg), and eluted with methanol−water (65:35) to give **2** (1100 mg). Fraction C (10 g) was passed over a Sephadex LH-20 column, eluted with methanol to remove flavonoids, and then was subjected to MCI gel CHP 20P column chromatography, eluted with water−acetone (9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1), to yield eight subfractions (C-1−8). Subfraction C-1 (3 g) was further purified by RP-18 flash column chromatography, eluted with methanol−water (70:30), to give compounds **6** (130 mg), **1** (50 mg), and **5** (140 mg).

3β,20*S*-Dihydroxydammar-24-en-21,28-dioic acid 3-*O*-[β-D-glucopyranosyl(1→3)-α-L-arabipyranosyl]-21-*O*-β-D-glucopyranoside (1): amorphous powder; $[\alpha]^{20}_D$ −8.0 (*c* 0.16, MeOH); IR (KBr) ν_{max} 3417, 2933, 1743, 1704, 1637, 1454, 1379 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 983.4833 (calcd for $C_{47}H_{76}O_{20}Na$ $[M + Na]^+$, 983.4828); GC analysis of sugar components, t_R 7.62 and 13.96 min.

3β,20*S*-Dihydroxydammar-24-en-21,28-dioic acid 3-*O*-{[α-L-rhamnopyranosyl(1→2)][α-L-rhamnopyranosyl(1→6)-β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl}-21-*O*-β-D-glucopyranoside (2): amorphous powder; $[\alpha]^{20}_D$ −10.0 (*c* 0.20, MeOH); IR (KBr) ν_{max} 3417, 2933, 1745, 1704, 1637, 1452, 1379, 1070, 983, 584 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 1275.5995 (calcd for $C_{59}H_{96}O_{28}Na$ $[M + Na]^+$, 1275.5986); GC analysis of sugar components, t_R 7.60, 8.86, and 13.94 min.

3β,20*S*,25-Trihydroxydammar-23-en-21,28-dioic acid 3-*O*-{[α-L-rhamnopyranosyl(1→2)][α-L-rhamnopyranosyl(1→6)-β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl}-21-*O*-β-D-glucopyranoside (3): amorphous powder; $[\alpha]^{20}_D$ −4.0 (*c* 0.06, MeOH); IR (KBr) ν_{max} 3417, 2933, 1743, 1702, 1637, 1454, 1378, 1249, 1132, 1072, 983 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 1291.5945 (calcd for $C_{59}H_{96}O_{29}Na$ $[M + Na]^+$, 1291.5935); GC analysis of sugar components, t_R 7.61, 8.85, and 13.95 min.

3β,20*S*,24*S*-Trihydroxydammar-25-ene-21,28-dioic acid 3-*O*-{[α-L-rhamnopyranosyl(1→2)][α-L-rhamnopyranosyl(1→6)-β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl}-21-*O*-β-D-glucopyranoside (4): amorphous powder; $[\alpha]^{20}_D$ −3.0 (*c* 0.17, MeOH); IR (KBr) ν_{max} 3415, 2935, 1735, 1706, 1637, 1450, 1377, 1250, 1132, 1074, 983, 588 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 1291.5945 (calcd for $C_{59}H_{96}O_{29}Na$ $[M + Na]^+$, 1291.5935); GC analysis of sugar components, t_R 7.61, 8.87, and 13.94 min.

3β,20*S*-Dihydroxydammar-24-en-21,28-dioic acid 3-*O*-{[α-L-rhamnopyranosyl(1→2)][β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl}-21-*O*-β-D-glucopyranoside (5): amorphous powder; $[\alpha]^{20}_D$ −2.0 (*c* 0.18, MeOH); IR (KBr) ν_{max} 3406, 2941, 1736, 1637, 1452, 1377, 1076, 982 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 1129.5411 (calcd for $C_{53}H_{86}O_{24}Na$ $[M + Na]^+$, 1129.5407); GC analysis of sugar components, t_R 7.61, 8.85, and 13.96 min.

3β,20*S*,21-Trihydroxydammar-24-ene 3-*O*-{[α-L-rhamnopyranosyl(1→2)][β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl}-21-*O*-β-D-glucopyranoside (6): amorphous powder; $[\alpha]^{20}_D$ −4.0 (*c* 0.12, MeOH); IR (KBr) ν_{max} 3415, 2931, 1716, 1646, 1450, 1376, 1249, 1209, 1074, 983 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 1085.5876 (calcd for $C_{53}H_{90}O_{21}Na$ $[M + Na]^+$, 1085.5872); GC analysis of sugar components, t_R 7.61, 8.88, and 13.94 min.

Acid Hydrolysis of Compounds 1−6. Compounds **1**−**6** (4 mg each) in 10% HCl−dioxane (1:1, 1 mL) were each heated at 80 °C for 4 h in a water bath. The reaction mixtures were neutralized with Ag_2CO_3, filtered, and then extracted with $CHCl_3$ (1 mL × 3). After concentration, each H_2O layer (monosaccharide portion) was examined by TLC with $CHCl_3$−CH_3OH−H_2O (55:45:10) and compared with authentic samples.[12] Compound **1** (15 mg) in 2 N HCl was heated at 50 °C for 4 h in a water bath. The reaction mixture was extracted with $CHCl_3$ (1 mL × 3). The $CHCl_3$ extracts were evaporated to dryness and dissolved in C_5D_5N for a NOESY experiment.

Determination of Sugar Components. The monosaccharide subunits were obtained by hydrochloric acid hydrolysis as described above. The sugar residue was then dissolved in 1 mL of anhydrous pyridine under Ar, 2 mg of L-leucine methyl ester hydrocloride was added, and the mixture was warmed at 60 °C for 1 h. Then 2 mg of $NaBH_4$ was added, and the mixture was stirred for 1 h at ambient temperature. Next, 0.2 mL of trimethylsilylation reagent trimethylchlorosilane (Shengyu Chemical Ltd., Shanghai, People's Republic of China) was added, and warming at 60 °C was continued for another 30 min. The leucine derivatives were subjected to GC analysis to identify the sugars. Column temperature 210 °C; injection temperature 250 °C; carrier gas N_2 at a flow rate of 25 mL/min; derivatives of D-glucose, l-arabinose, and L-rhamnose: 13.95, 7.62, and 8.87 min, respectively.

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Supporting Information Available: Figures of HMBC NMR interrelations of compounds **1**−**6** and NOESY NMR correlations for the hydrolysis product of compound **1**; scheme of acid hydrolysis of compound **1**, and table of ^{1}H NMR and ^{13}C NMR data for the hydrolysis product of compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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