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Dammarane-Type Triterpenoids from *Gentianella azurea*

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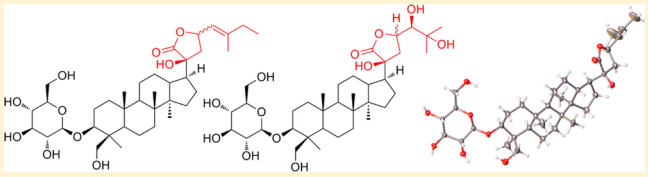
Supporting Information

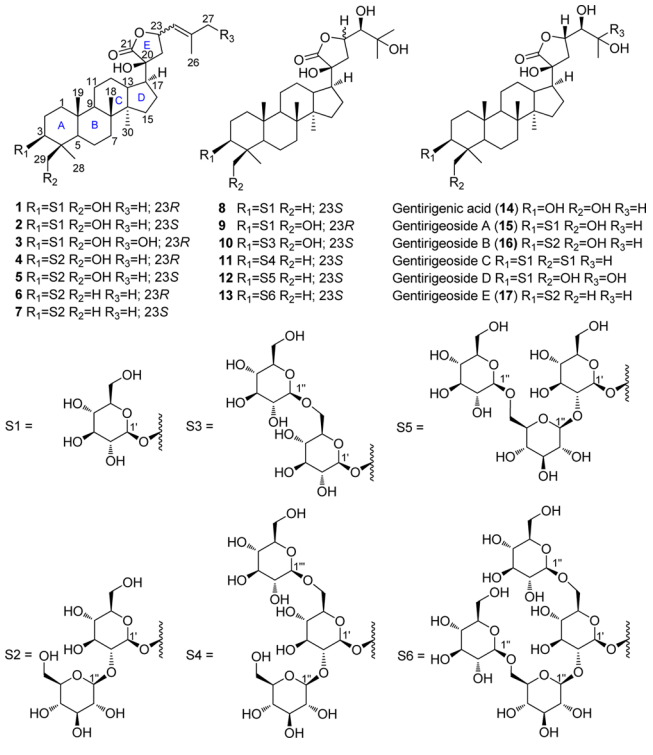
ABSTRACT: Thirteen new dammarane-type triterpenoids (**1**−**13**) and four known analogues, gentirigenic acid (**14**) and the gentirigeosides A, B, and E (**15**−**17**), were isolated from *Gentianella azurea*. Their structures were elucidated by detailed analysis of the NMR, MS, and X-ray crystallographic data. This is the first report of dammarane-type triterpenoids in the *Gentianella* genus. In addition, the known structures of gentirigenic acid (**14**) and the gentirigeosides A, B, and E (**15**−**17**) were revised based on the X-ray diffraction analysis. Gentirigeoside A (**15**) was found to inhibit nitric oxide production in RAW 264.7 macrophages with an IC_{50} value of 6.6 ± 2.1 μM.



Gentianella azurea, a traditional Tibetan medicinal plant, is widely distributed in west China, including Tibet, Sichuan, Yunnan, and Xinjiang Provinces, and has been used to treat inflammation resulting from hepatitis, icterus, and rheumatism.[1] Previous studies on plant constituents from the Gentianaceae family have demonstrated their high anti-inflammatory activities.[2]

Nitric oxide (NO) is a signaling molecule that plays an important role in inflammation and immunity.[3] Several studies have shown that NO plays a crucial role in the modulation of multiform acute and chronic inflammatory disorders.[4] Therefore, inflammatory conditions can be treated by inhibiting NO production.[5]

To identify anti-inflammatory natural products from *G. azurea*, its crude extract was investigated and led to the isolation and identification of 13 new dammarane-type triterpenoids (**1**−**13**) and four known analogues, gentirigenic acid (**14**) and gentirigeosides A, B, and E (**15**−**17**).[6] Their inhibitory activities against lipopolysaccharide-induced NO production in RAW 264.7 macrophages were assessed, and an IC_{50} value of 6.6 ± 2.1 μM was observed for gentirigeoside A (**15**). In addition, these results add to the sparse literature concerning *G. azurea*.[1,7]



RESULTS AND DISCUSSION

Compound **1** was obtained as an amorphous powder. The molecular formula of **1** was determined to be $C_{36}H_{58}O_{10}$ based on the quasi-molecular ion at m/z 673.3917 $[M + Na]^+$ (calcd 673.3922) in the HRESI(+)MS analysis and ^{13}C NMR spectroscopic data. The IR spectrum showed absorption bands for hydroxy (3423 cm^{-1}) and γ-lactone (1764 cm^{-1}) groups. The ^{13}C NMR data (Table 1) exhibited 36 carbon resonances, which were classified by a DEPT experiment into six tertiary methyl, 11 methylene, 12 methine, and seven quaternary carbons. The NMR spectroscopic data (Tables 1 and 2) showed characteristic resonances of carbons from one lactone carbonyl carbon (δ_C 179.8), one trisubstituted double bond (δ_C 138.9 and 125.7), and one hexosyl (δ_C 106.6, 79.2, 79.1, 76.0, 72.2, and 63.3) moiety. These moieties accounted for four indices of hydrogen deficiency, suggesting four additional rings in the structure of **1**; thus, compound **1** was likely a tetracyclic triterpenoid glycoside. Acid hydrolysis of **1** yielded D-glucose, and the coupling constant of the anomeric

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Table 1. ^{13}C NMR Data for Compounds 1−13 in Pyridine-d_5[a] (δ_C in ppm)

no.	1	2	3	4	5	6	7	8	9	10	11	12	13[b]
1	39.4	39.4	39.4	39.3	39.3	39.6	39.7	39.6	39.2	39.2	39.7	39.6	39.7
2	27.3	27.4	27.3	27.1	27.1	27.2	27.2	27.1	27.2	27.2	27.3	27.1	27.3
3	89.2	89.2	89.2	91.0	90.9	89.3	89.3	89.2	89.1	89.2	89.5	89.4	89.6
4	44.9	44.9	44.9	44.2	44.2	40.1	40.1	40.0	44.7	44.6	40.1	40.1	40.1
5	57.0	57.0	56.9	57.0	56.9	56.8	56.7	56.7	56.8	56.7	56.7	56.7	56.8
6	19.1	19.1	19.1	18.8	18.8	18.8	18.8	18.7	19.0	18.9	18.7	18.7	18.8
7	36.2	36.3	36.2	36.0	36.9	36.0	36.1	36.0	36.1	36.0	36.0	36.0	36.0
8	41.0	41.0	41.2	40.9	40.8	41.4	41.0	40.9	40.8	40.7	40.9	40.8	40.9
9	51.3	51.4	51.3	51.2	51.2	51.4	51.4	51.5	51.2	51.1	51.4	51.4	51.4
10	37.1	37.1	37.0	36.9	36.9	37.3	37.3	37.3	36.9	36.9	37.3	37.2	37.3
11	22.3	22.4	22.3	22.3	22.3	22.1	22.1	22.1	22.2	22.1	22.1	22.0	22.1
12	27.6	28.4	27.6	27.6	28.3	27.6	28.4	28.1	27.5	28.3	28.5	28.4	28.5
13	43.6	45.3	43.6	43.5	45.2	43.6	45.3	45.1	43.4	44.9	45.1	45.1	45.1
14	51.0	50.5	51.0	50.9	50.3	51.0	50.5	50.5	50.8	50.3	50.5	50.5	50.5
15	32.0	32.0	32.1	32.0	32.0	32.0	32.1	32.0	31.9	31.8	32.0	32.0	32.0
16	26.2	26.6	26.2	26.1	26.5	26.2	26.6	26.3	25.8	26.1	26.3	26.3	26.3
17	46.1	45.6	46.1	46.1	45.6	46.1	45.6	46.1	46.0	45.9	46.1	46.0	46.1
18	15.8	15.9	15.8	15.7	15.7	15.9	16.0	15.9	15.7	15.6	15.9	15.9	15.9
19	16.5	16.5	16.4	16.6	16.5	16.8	16.6	16.6	16.3	16.3	16.6	16.5	16.6
20	79.3	81.5	79.2	79.3	81.4	79.3	81.5	81.4	78.6	81.3	81.4	81.4	81.4
21	179.8	178.7	179.8	179.8	178.6	179.8	178.7	178.9	179.7	178.8	178.9	178.9	178.9
22	41.2	39.5	41.2	41.1	39.4	41.1	39.4	33.4	35.9	33.3	33.5	33.3	33.5
23	74.5	75.6	74.3	74.4	75.5	74.4	75.6	80.0	78.5	79.8	80.0	79.9	80.0
24	125.7	124.0	123.8	125.7	124.2	125.8	124.2	77.7	78.1	77.5	77.7	77.6	77.7
25	138.9	139.1	143.1	138.8	139.8	138.8	139.8	72.1	72.0	72.0	72.1	72.0	72.1
26	26.0	26.0	14.5	25.9	26.0	25.9	26.0	27.5	27.4	27.9	28.1	28.0	28.5
27	18.5	18.5	67.0	18.5	18.5	18.5	18.5	28.1	28.1	27.3	27.5	27.4	27.3
28	23.7	23.7	23.7	22.9	22.9	28.4	28.4	28.4	23.6	23.5	28.4	28.5	28.1
29	63.6	63.6	63.5	63.7	63.7	17.0	16.9	17.1	63.5	63.4	17.0	17.0	17.1
30	16.8	16.5	16.8	16.8	16.6	16.8	16.7	16.8	16.7	16.4	16.9	16.8	16.9
1′	106.6	106.6	106.6	104.9	104.9	105.5	105.5	107.3	106.5	106.3	105.4	105.3	105.3
2′	76.0	76.0	76.0	82.8	82.7	83.8	83.8	76.1	75.9	75.2	83.6	83.5	83.2
3′	79.1	79.1	79.2	78.6	78.5	78.5	78.5	79.1	79.0	77.4	77.4	78.3	77.4
4′	72.2	72.2	72.3	70.2	70.2	72.0	72.0	72.2	72.1	71.7	71.8	71.8	71.7
5′	79.2	79.1	79.2	78.8	78.7	78.3	78.3	78.7	79.0	78.5	78.3	78.3	78.4
6′	63.3	63.3	63.3	61.9	61.9	63.0	63.0	63.4	63.2	70.7	70.7	63.1	70.4
1″				105.5	105.4	106.5	106.4			105.7	106.4	106.1	106.1
2″				76.1	76.1	77.6	77.5			75.5	77.0	77.0	77.0
3″				79.2	79.2	78.7	78.7			78.6	78.6	78.3	78.5
4″				71.5	71.4	72.0	72.0			71.9	72.1	72.0	72.0
5″				79.0	78.9	78.7	78.7			78.7	78.5	77.3	77.0
6″				63.1	63.0	63.2	63.2			62.8	63.1	70.2	70.6
1‴											105.9	105.6	105.7
2‴											75.4	75.5	75.6
3‴											78.9	78.5	78.6
4‴											72.0	71.8	72.0
5‴											78.8	78.6	78.7
6‴											63.1	63.1	63.1

[a]Chemical shifts (ppm) referenced to pyridine-d_5 (δ_C 135.9) at 125 MHz. [b]Data for Glc-IV of **13**: δ 105.8 (C-1‴), 75.5 (C-2‴), 78.7 (C-3‴), 72.0 (C-4‴), 78.8 (C-5‴), 63.2 (C-6‴).

proton at δ_H 5.07 (d, J = 7.8 Hz) indicated a β-configuration for the sugar unit. The HMBC data (Supporting Information) allowed the connections of the aforementioned moieties to be established, and the structure of **1** was determined to be 3,20,23,29-tetrahydroxydammar-24-en-21-oic acid-21,23-lactone.[8] In addition, the glucose moiety was attached at C-3, as supported by the HMBC correlation of H-1′/C-3. Therefore, the planar structure of **1** was established.

The configuration of the dammarane skeleton was assumed to be consistent with those of previously reported compounds.[8a,9] The large coupling constant of H-3 (δ_H 3.61, dd, J = 11.6, 4.9 Hz) suggested an α-axial orientation. In the ROESY spectrum, the correlation of H_2-29/H_3-19 showed that the hydroxy group was located at C-29. In addition, the correlation of H-24/H_3-27 indicated that they were cofacial. Previously, the configurations of C-20 and C-23 had been determined by comparing either the NMR or the electronic circular dichroism

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Table 2. ^{1}H NMR Data for Compounds 1−7 in Pyridine-d_5[a] (δ_H in ppm, J in Hz)

no.	1	2	3	4	5	6	7
1	1.48 m, 0.72 m	1.52 m, 0.77 m	1.44 m, 0.70 m	1.34 m, 0.64 m	1.40 m, 0.70 m	1.43 m, 0.71 m	1.49 m, 0.77 m
2	2.17 m, 2.00 m	2.20 m, 2.01 m	2.05 m, 1.95 m	2.30 m, 1.87 m	2.33 m, 1.88 m	2.21 m, 1.82 m	2.25 m, 1.86 m
3	3.61 dd (11.6, 4.9)	3.63 dd (11.8, 4.1)	3.62 dd (11.6, 4.7)	3.43 dd (11.7, 4.8)	3.44 dd (11.7, 4.8)	3.29 dd (11.8, 4.5)	3.32 dd (11.8, 4.5)
5	0.85 m	0.88 m	0.84 m	0.76 m	0.77 m	0.66 m	0.69 m
6	1.45 m, 1.21 m	1.49 m, 1.20 m	1.64 m, 1.46 m	1.48 m, 1.25 m	1.49 m, 1.26 m	1.46 m, 1.39 m	1.48 m, 1.21 m
7	1.47 m, 1.22 m	1.51 m, 1.23 m	1.46 m, 1.20 m	1.60 m, 1.08 m	1.54 m, 1.07 m	1.42 m, 1.21 m	1.47 m, 1.21 m
9	1.27 m	1.28 m	1.26 m	1.19 m	1.21 m	1.24 m	1.28 m
11	1.64 m, 1.46 m	1.51 m, 1.28 m	1.45 m, 1.20 m	1.34 m, 1.16 m	1.43 m, 1.21 m	1.43 m, 1.24 m	1.50 m, 1.28 m
12	2.42 m, 1.36 m	2.53 m, 1.49 m	2.44 m, 1.37 m	2.38 m, 1.31 m	2.49 m, 1.43 m	2.41 m, 1.34 m	2.53 m, 1.48 m
13	2.09 m	1.86 m	2.08 m	2.05 m	1.81 m	2.07 m	1.86 m
15	1.64 m, 1.12 m	1.57 m, 1.11 m	1.60 m, 1.08 m	1.39 m, 1.14 m	1.53 m, 1.07 m	1.45 m, 1.19 m	1.58 m, 1.09 m
16	2.02 m, 1.70 m	2.10 m, 1.34 m	2.02 m, 1.70 m	1.99 m, 1.67 m	2.05 m, 1.30 m	1.64 m, 1.10 m	2.08 m, 1.34 m
17	2.55 ddd (10.5, 5.3, 5.3)	2.72 ddd (10.6, 5.6, 5.6)	2.58 ddd (10.6, 5.1, 5.1)	2.52 ddd (10.5, 5.4, 5.4)	2.68 ddd (10.8, 5.8, 5.8)	2.54 ddd (10.6, 5.2, 5.2)	2.72 ddd (10.6, 5.5, 5.5)
18	0.92 s	0.97 s	0.92 s	0.86 s	0.92 s	0.92 s	1.00 s
19	0.71 s	0.77 s	0.71 s	0.57 s	0.63 s	0.75 s	0.81 s
22	2.72 dd (13.2, 7.2)	2.61 dd, (13.2, 5.6)	2.76 dd (13.2, 7.4)	2.71 dd (13.2, 7.2)	2.59 dd (13.3, 5.6)	3.29 dd (12.5, 4.5)	2.61 dd (13.3, 5.6)
	2.30 dd (13.2, 6.2)	2.14 dd (13.2, 9.8)	2.36 dd (13.2, 6.0)	2.28 dd (13.2, 6.3)	2.11 dd (13.3, 9.6)	2.72 dd (12.5, 5.2)	2.13 dd (13.3, 9.9)
23	5.50 ddd (8.9, 7.2, 6.2)	5.72 ddd (9.8, 8.7, 5.6)	5.66 ddd (9.0, 7.4, 6.0)	5.49 ddd (8.5, 7.2, 6.3)	5.70 ddd (9.6, 8.8, 5.6)	5.49 ddd (8.9, 5.2, 4.5)	5.72 ddd (9.9, 8.9, 5.6)
24	5.59 d (8.9)	5.42 d (8.7)	6.26 d (9.0)	5.58d (8.5)	5.40 d (8.8)	5.59 d (8.9)	5.42 d (8.9)
26	1.70 s	1.69 s	4.30 d (12.0), 4.28 d (12.0)	1.62 s	1.68 s	1.70 s	1.69 s
27	1.63 s	1.64 s	1.87 s	1.69 s	1.63 s	1.62 s	1.64 s
28	1.55 s	1.55 s	1.55 s	1.35 s	1.34 s	1.29 s	1.30 s
29	4.37 d (11.1)	4.39 d (11.2)	4.40 d (11.1)	4.28 d (11.4)	4.28 d (9.7)	1.11 s	1.13 s
	3.66 d (11.1)	3.68 d (11.2)	3.67 d (11.1)	3.36 d (11.4)	3.37 d (9.7)		
30	0.89 s	0.92 s	0.88 s	0.85 s	0.86 s	0.89 s	0.89 s
1′	5.07 d (7.8)	5.07 d (7.7)	5.08 d (8.0)	4.88 d (7.7)	4.86 d (7.8)	4.94 d (7.5)	4.95 d (7.5)
2′	4.03 dd (8.5, 7.8)	4.03 dd (8.8, 7.7)	4.05 dd (8.4, 7.8)	4.09 dd (8.4, 7.7)	4.07 dd (8.8, 7.8)	4.25 dd (8.5, 7.5)	4.27 dd (8.8, 7.5)
3′	4.27 dd (9.1, 8.5)	4.27 dd (8.9, 8.8)	4.09 dd (9.1, 8.4)	4.20 dd (8.5, 8.4)	4.17 dd (9.2, 8.8)	4.26 dd (9.0, 8.5)	4.28 dd (8.8, 8.6)
4′	4.21 dd (9.1, 9.1)	4.20 dd (9.2, 8.9)	4.23 dd (9.1, 9.1)	4.39 m	4.36 m	4.35 m	4.37 m
5′	4.06 ddd (9.1, 5.6, 2.5)	4.06 ddd (9.2, 5.6, 2.5)	4.28 ddd (9.1, 5.9, 2.4)	3.73 m	3.71 m	3.94 m	3.95 m
6′	4.64 dd (11.7, 2.4)	4.64 dd (11.6, 2.5)	4.67 dd (11.7, 2.4)	4.38 m	4.38 m	4.48 dd (11.6, 3.8)	4.50 dd (11.5, 3.8)
	4.43 dd (11.7, 5.6)	4.42 dd (11.6, 5.6)	4.45 dd (11.7, 5.9)	4.33 m	4.30 m	4.35 dd (11.6, 2.6)	4.36 dd (11.5, 2.3)
1″				5.57 d (7.3)	5.54 d (7.8)	5.39 d (7.6)	5.40 d (7.7)
2″				4.10 dd (8.5, 7.3)	4.08 dd (8.8, 7.8)	4.13 dd (8.9, 7.9)	4.15 dd (8.7, 7.7)
3″				4.29 dd (8.9, 8.5)	4.27 dd (9.1, 8.8)	4.33 dd (8.9, 8.9)	4.34 dd (8.9, 8.7)
4″				4.17 dd (8.9, 8.7)	4.15 dd (9.2, 9.1)	4.15 dd (9.4, 9.1)	4.16 dd (9.4, 8.9)
5″				3.94 ddd (8.7, 5.9, 2.4)	3.91 m	3.94 ddd (9.1, 4.2, 2.4)	3.95 ddd (9.4, 3.3, 2.5)
6″				4.60 dd (11.8, 2.4)	4.58 (12.1, 2.4)	4.59 dd (11.8, 2.4)	4.59 dd (11.9, 2.5)
				4.58 dd (11.8, 5.9)	4.35 m	4.50 dd (11.8, 3.4)	4.50 dd (11.9, 3.3)

[a]Chemical shifts (ppm) referenced to pyridine-d_5 (δ_H 7.58) at 500 MHz.

(ECD) data.[8] X-ray diffraction analysis of crystals obtained by recrystallization from MeOH not only confirmed the gross structure of **1** but also established its absolute configuration as (3β, 20S, 23R) (Figure 1), which agreed with the ECD results.[8a] Thus, the structure of **1** was determined as (3β,20S,23R)-3,20,23,29-tetrahydroxydammar-24-en-21-oic acid-21,23-lactone 3-O-β-D-glucopyranoside.

Compound **2** was defined as an isomer of **1**, as indicated by HRESI(+)MS analysis and the spectroscopic data. Analysis of the NMR data (Tables 1 and 2) supported this conclusion, as most of the resonances of **2** were nearly superimposable on those of **1**, with the exception of the resonances of ring E. The HMBC spectrum (Supporting Information) established that **2** had the same planar structure as **1**. The (20S, 23S) configuration was proposed by comparing the ^{1}H and ^{13}C NMR data with the data for (3β,20S,23S)-3,20,23-trihydroxydammar-24-en-21-oic acid-21,23-lactone 3-O-[α-L-rhamnopyranosyl-(1→2)]-[β-D-xylopyranosyl(1→3)]-α-L-arabinopyranoside,[8a] and these assignments were confirmed by the ECD spectrum (Supporting Information). Thus, the structure of **2** was established as shown.

The molecular formula of **3** was determined to be $C_{36}H_{58}O_{11}$ from the ^{13}C NMR and HRESI(+)MS data, which showed a molecular ion at m/z 689.3883 $[M + Na]^+$ (calcd 689.3871), 16 amu greater than the molecular weight of **1**. The NMR data for **3** (Tables 1 and 2) were similar to those for **1**, except for an additional hydroxy group at C-27, thus accounting for the difference in molecular weight. These observations were further supported by the HMBC correlations between H_2-27/C-24 and C-25. The ROESY spectrum showed correlations between H-

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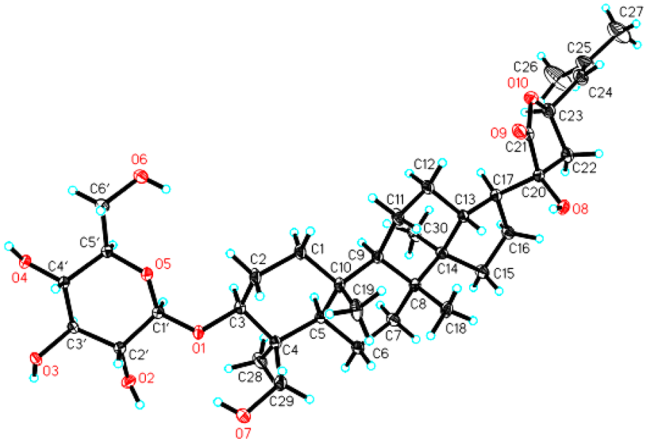

Figure 1. X-ray ORTEP drawing of compound **1**.

24/H_2-27 and H_3-19/H_2-29, which established the relative configuration. The configurations of C-20 and C-23 were established by comparing the NMR and ECD data with the data for **1**. Thus, the structure of **3** was defined as (3*β*,20*S*,23*R*)-3,20,23,26,29-pentahydroxydammar-24-en-21-oic acid-21,23-lactone 3-*O*-*β*-D-glucopyranoside.

Compounds **4** and **5** shared the same molecular formula of $C_{42}H_{68}O_{15}$, as determined by the ^{13}C NMR data and HRESI(+)MS ions at *m*/*z* 835.4452 $[M + Na]^+$ (calcd 835.4450) and 835.4447 $[M + Na]^+$, respectively. The ^1H and ^{13}C NMR data (Tables 1 and 2) of **4** and **5** were similar to the data for **1** and **2**, respectively, except for additional resonances resulting from a hexosyl unit (H-1″ of **4**, δ_H 5.57, d, J = 7.3 Hz; H-1″ of **5**, δ_H 5.54, d, J = 7.8 Hz). Acid hydrolysis of **4** yielded only D-glucose and the aglycone. The sugar moiety of **4** was determined to be *β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl based on the HMBC correlations of H-1″ (δ_H 5.57, d, J = 7.3 Hz)/C-2′ (δ_C 82.8) and H-1′ (δ_H 4.88, d, J = 7.7 Hz)/C-3 (δ_C 91.0). Therefore, the structure of **4** was established as (3*β*,20*S*,23*R*)-3,20,23,29-tetrahydroxydammar-24-en-21-oic acid-21,23-lactone 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside. The structure of **5** was elucidated in a similar manner and named (3*β*,20*S*,23*S*)-3,20,23,29-tetrahydroxydammar-24-en-21-oic acid-21,23-lactone 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside.

Compounds **6** and **7** gave sodiated molecular ions at *m*/*z* $[M + Na]^+$ 819.4513 and 819.4518, respectively, by HRESI(+) MS, which together with the ^{13}C NMR spectroscopic data suggested the same molecular formula of $C_{42}H_{68}O_{14}$ (calcd 819.4501). This formula suggests that compounds **6** and **7** are deoxy derivatives of **4** and **5**, respectively. The NMR data of **6** and **7** (Tables 1 and 2) were similar to the data for **4** and **5**, except that the C-4 hydroxymethyl resonances in **4** and **5** were replaced by methyl resonances in **6** and **7**. The configurations of **4** and **5** were identical to those of **6** and **7**, respectively. Therefore, the structures of compounds **6** and **7** were established as (3*β*,20*S*,23*R*)-3,20,23-trihydroxydammar-24-en-21-oic acid-21,23-lactone 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside and (3*β*,20*S*,23*S*)-3,20,23-trihydroxydammar-24-en-21-oic acid-21,23-lactone 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside, respectively.

Compound **8** exhibited a sodiated molecular ion at *m*/*z* 691.4020 (calcd 691.4028) by HRESI(+)MS analysis, which together with the ^{13}C NMR data suggested a molecular formula of $C_{36}H_{60}O_{11}$. Comparing the NMR data (Tables 1 and 3) with the data for **2**, the resonances of the olefinic bond in **2** were replaced by resonances for one methine and one quaternary carbon (both oxygenated), suggesting that the double bond had been dihydroxylated. In addition, C-29 was deoxygenated. These observations were supported by the HMBC correlations of H_3-26/C-24, C-25, and C-27; H_3-27/C-24, C-25, and C-26; H_3-28/C-3, C-4, and C-5; and H_3-29/C-3, C-4, and C-5. In addition, these observations were consistent with the chemical shifts of these protons and carbons and with the molecular formula. Single-crystal X-ray diffraction analysis permitted definition of the absolute configuration (Figure 2) of **8** as (20*S*, 23*S*, 24*R*). Thus, the structure of **8** was established as (3*β*,20*S*,23*S*,24*R*)-3,20,23,24,25-pentahydroxydammaran-21-oic acid-21,23-lactone 3-*O*-*β*-D-glucopyranoside.

Compound **9** displayed a molecular formula of $C_{36}H_{60}O_{12}$, as deduced from the ^{13}C NMR spectroscopic data and the HRESI(+)MS ion at *m*/*z* 707.3991 $[M + Na]^+$ (calcd 707.3977), which suggested that **9** was a hydroxy analogue of **8**. The hydroxy group was located at C-29 based on the HMBC correlations of H_2-29/C-4 and C-28. The chemical shifts for C-20 (δ_C 78.6), C-22 (δ_C 35.9), C-23 (δ_C 78.5), and C-24 (δ_C 78.1) showed minor differences from the data for **8**, which suggested that **9** had a different C-23 configuration than **8**. Fortunately, a single crystal of **9** was obtained via slow evaporation from a ternary solvent system of H_2O/pyridine/MeOH (2:1:7). The X-ray diffraction of a single crystal of **9** permitted definition of the absolute configuration of **9** as (20*S*, 23*R*, 24*R*) (Figure 3). Therefore, the structure of compound **9** was defined as (3*β*,20*S*,23*R*,24*R*)-3,20,23,24,25,29-hexahydroxydammaran-21-oic acid-21,23-lactone 3-*O*-*β*-D-glucopyranoside.

Compound **10** had a molecular formula of $C_{42}H_{70}O_{17}$, as determined by the ^{13}C NMR and HRESI(+)MS data, which showed an ion at *m*/*z* 869.4508 $[M + Na]^+$ (calcd 869.4505). The NMR resonances for **10** were similar to those for **8**, except for the presence of an additional hexosyl moiety (δ_C 105.7, 78.7, 78.6, 75.5, 71.9, and 62.8) and a C-29 hydroxy substituent. Acid hydrolysis of **10** yielded only D-glucose and the aglycone, and the coupling constants of the anomeric protons at δ_H 5.00 (d, J = 7.8 Hz) and 5.10 (d, J = 7.7 Hz) suggested *β*-configured glucosyl moieties. The sugar chain was determined to be *β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl based on the HMBC correlation of H-1″/C-6′. Thus, the structure of **10** was established as (3*β*,20*S*,23*S*,24*R*)-3,20,23,24,25,29-hexahydroxydammaran-21-oic acid-21,23-lactone 3-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside.

Compound **11** had a molecular formula of $C_{48}H_{80}O_{21}$ based on the ^{13}C NMR and HRESI(+)MS data ($[M + Na]^+$ *m*/*z* 1015.5064, calcd 1015.5084). Comparisons between the NMR data of **11** and **8** showed similar resonances but with two additional *β*-D-glucopyranosyl groups, which was confirmed by 2D NMR spectroscopic data and acid hydrolysis of **11**. In addition, HMBC correlations of H-1″/C-2′ and H-1‴/C-6′ established the linkage of the sugar moieties. Thus, the structure of compound **11** was defined as (3*β*,20*S*,23*S*,24*R*)-3,20,23,24,25-pentahydroxydammaran-21-oic acid-21,23-lactone 3-*O*-[*β*-D-glucopyranosyl-(1→2)]-[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside.

Compound **12** showed the same molecular formula as **11**, as determined by the ^{13}C NMR spectroscopic data and the HRESI(+)MS ion at *m*/*z* 1015.5064 ($[M + Na]^+$, calcd 1015.5084), indicating that they are isomers. D-Glucose and the aglycone were obtained from hydrolysis. Detailed analysis of the NMR data indicated that **12** had the same aglycone as **11**,

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Table 3. ^{1}H NMR Data for Compounds 8−13 in Pyridine-d_5[a] (δ_H in ppm, J in Hz)

no.	8	9	10	11	12	13[b]
1	1.47 m, 0.77 m	1.41 m, 0.66 m	1.58 m, 0.89 m	1.60 m, 0.87 m	1.45 m, 0.74 m	1.60 m, 0.84 m
2	2.27 m, 1.85 m	2.15 m, 1.92 m	2.37 m, 2.04 m	2.50 m, 1.41 m	2.22 m, 1.87 m	2.34 m, 1.94 m
3	3.40 dd (11.8, 4.4)	3.58 dd (11.7, 4.8)	3.59 dd (11.8, 4.8)	3.26 dd (11.7, 4.5)	3.28 dd (11.7, 4.4)	3.25 dd (11.6, 4.6)
5	0.72 m	0.82 m	0.79 m	0.61 m	0.64 m	0.62 m
6	1.43 m, 1.12 m	1.60 m, 1.41 m	1.59 m, 1.41 m	1.38 m, 1.28 m	1.44 m, 1.31 m	1.44 m, 1.34 m
7	1.43 m, 1.12 m	1.44 m, 1.17 m	1.40 m, 1.12 m	1.38 m, 1.10 m	1.41 m, 1.12 m	1.39 m, 1.09 m
9	1.26 m	1.22 m	1.24 m	1.21 m	1.23 m	1.24 m
11	1.46 m, 1.20 m	1.38 m, 1.10 m	1.24 m, 1.16 m	1.49 m, 1.22 m	1.46 m, 1.24 m	1.49 m, 1.25 m
12	2.56 m, 1.48 m	2.38 m, 1.34 m	2.50 m, 1.42 m	2.38 m, 1.92 m	2.55 m, 1.46 m	2.49 m, 1.44 m
13	1.95 m	2.06 m	1.93 m	1.93 m	1.95 m	1.94 m
15	1.48 m, 0.96 m	1.57 m, 1.06 m	1.46 m, 0.96 m	1.48 m, 0.95 m	1.53 m, 0.99 m	1.51 m, 0.99 m
16	2.02 m, 1.66 m	1.97 m, 1.72 m	2.00 m, 1.66 m	2.01 m, 1.65 m	2.02 m, 1.65 m	2.02 m, 1.65 m
17	2.76 m	2.54 m	2.72 m	2.70 m	2.74 m	2.73 m
18	0.84 s	0.90 s	0.84 s	0.85 s	0.86 s	0.84 s
19	0.73 s	0.66 s	0.73 s	0.79 s	0.78 s	0.81 s
22	3.17 dd (13.4, 9.5), 2.78 dd (13.4, 5.9)	3.21 dd (13.5, 5.9), 2.78 dd (13.5, 7.6)	3.13 dd (13.4, 9.5), 2.75 dd (13.4, 6.0)	3.14 dd (13.4, 9.5), 2.77 dd (13.4, 5.9)	3.12 dd (13.4, 9.4), 2.75 dd (13.4, 6.0)	3.13 dd (13.4, 9.4), 2.75 dd (13.4, 5.8)
23	5.67 ddd (9.5, 5.9, 2.6)	5.37 ddd (7.6, 5.9, 3.0)	5.64 ddd (9.5, 6.0, 2.5)	5.65 ddd (9.5, 5.9, 2.5)	5.65 ddd (9.4, 6.0, 2.4)	5.64 ddd (9.4, 5.8, 2.1)
24	4.34 d (2.6)	4.19 d (3.0)	4.32 d (2.5)	4.33 d (2.5)	4.33 d (2.4)	4.30 d (2.1)
26	1.60 s	1.56 s	1.59 s	1.60 s	1.59 s	1.60 s
27	1.56 s	1.56 s	1.55 s	1.55 s	1.55 s	1.55 s
28	1.30 s	1.52 s	1.48 s	1.23 s	1.28 s	1.25 s
29	0.99 s	4.33 d (11.2) 3.63 d (11.2)	4.37 d (10.1) 3.64 d (10.1)	1.11 s	1.13 s	1.15 s
30	0.84 s	0.83 s	0.82 s	0.82 s	0.83 s	0.83 s
1′	4.96 d (7.7)	5.04 d (7.8)	5.00 d (7.8)	4.88 d (7.5)	4.92 d (7.3)	4.87 d (7.5)
2′	4.06 dd (8.3, 7.7)	3.99 dd (8.4, 7.8)	4.05 m	4.16 m	4.25 m	4.19 m
3′	4.03 dd (8.9, 8.3)	4.25 dd (9.1, 8.4)	4.32 m	4.33 m	4.30 m	4.23 m
4′	4.23 dd (9.0, 8.9)	4.17 dd (9.1, 9.0)	4.23 m	4.06 m	4.20 m	4.10 m
5′	4.28 ddd (9.0, 5.5, 2.5)	4.21 ddd (9.0, 5.6, 2.4)	4.22 m	4.23 m	3.91 m	4.03 m
6′	4.63 dd (11.7, 2.5)	4.62 dd (11.8, 2.4)	4.91 dd (11.3, 1.9)	4.85 m	4.53 m	4.78 m
	4.42 dd (11.7, 5.5)	4.39 dd (11.8, 5.6)	4.30 dd (11.3, 5.2)	4.29 m	4.35 m	4.47 m
1″			5.10 d (7.7)	5.33 d (7.7)	5.33 d (7.6)	5.28 d (7.6)
2″			3.95 m	4.05 m	4.07 m	4.22 m
3″			4.18 m	4.23 m	4.23 m	4.24 m
4″			4.08 m	4.23 m	4.16 m	4.32 m
5″			3.93 m	3.93 m	4.03 m	4.03 m
6″			4.51 dd (11.8, 2.7), 4.38 dd (11.8, 5.3)	4.49 m, 4.37 m	4.78 dd (11.3, 2.7), 4.47 dd (11.3, 4.3)	4.81 m, 4.30 m
1‴				5.08 d (7.7)	5.12 d (7.7)	5.11 d (7.7)
2‴				4.06 m	4.06 m	4.05 m
3‴				4.23 m	4.23 m	4.23 m
4‴				4.34 m	4.32 m	4.21 m
5‴				3.93 m	3.91 m	3.93 m
6‴				4.49 m, 4.37 m	4.53 m, 4.35 m	4.51 m, 4.37 m

[a]Chemical shifts (ppm) referenced to pyridine-d_5 (δ_H 7.58) at 500 MHz. [b]Data for Glc-IV of **13**: δ 5.07 d (7.7 Hz, H-1⁗), 4.05 m (H-2⁗), 4.22 m (H-3⁗), 4.21 m (H-4⁗), 3.90 m (H-5⁗), 4.51 m (H-6a⁗), 4.36 m (H-6b⁗).

suggesting that the difference between compounds **11** and **12** is the linkage of the sugar units. The HMBC correlations of H-1‴/C-6″ and H-1″/C-2″ specified the linkages of the sugar units. Therefore, compound **12** was defined as (3*β*,20*S*,23*S*,24*R*)-3,20,23,24,25-pentahydroxydammaran-21-oic acid-21,23-lactone 3-*O*-{[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranosyl-(1→2)}-*β*-D-glucopyranoside.

The molecular formula of **13** was identified as $C_{54}H_{90}O_{26}$ by the ^{13}C NMR and HRESI(+)MS data. The NMR spectra of **13** were similar to those of **12**, except for the presence of resonances resulting from an additional hexosyl moiety. Acid hydrolysis of **13** yielded only D-glucose and the aglycone. The coupling constants of the anomeric protons at δ_H 4.87 (H-1′, d, J = 7.5 Hz), 5.28 (H-1″, d, J = 7.6 Hz), 5.07 (H-1‴, d, J = 7.7 Hz), and 5.11 (H-1⁗, d, J = 7.7 Hz) suggested they are all *β*-configured. The NMR resonance assignments for **13** were confirmed by 2D NMR data analysis. In particular, the HMBC correlations of H-1⁗/C′-6, H-1‴/C″-6, and H-1″/C′-2 specified the linkages of the sugar units. Thus, compound **13** was determined as (3*β*,20*S*,23*S*,24*R*)-3,20,23,24,25-pentahydroxydammaran-21-oic acid-21,23-lactone 3-*O*-{[*β*-D-glucopyr-

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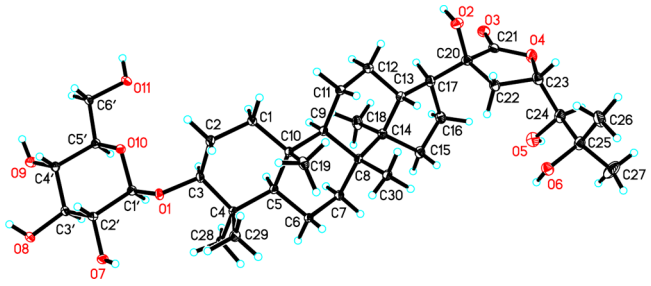

Figure 2. X-ray ORTEP drawing of compound **8**.

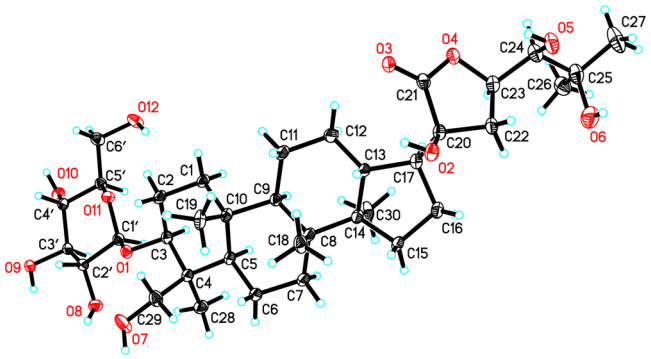

Figure 3. X-ray ORTEP drawing of compound **9**.

anosyl-(1→6)]-*β*-D-glucopyranosyl-(1→2)}-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside.

The IR, NMR, and HRESIMS spectrometric data and the specific rotations for compounds **14**–**17** (Supporting Information) were similar to the data for the known compounds gentirigenic acid and gentirigeosides A, B, and E, respectively. However, the characteristic resonances in the ^{1}H and ^{13}C NMR spectra for ring E were also similar to the resonances of **8**, which was surprising. Thus, we reexamined the chemical structures of these reported compounds[6] and also obtained crystals of compounds **14** (Figure 4) and **15** (Figure 5) from

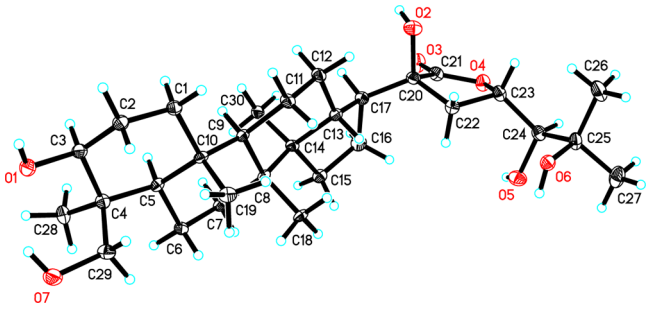

Figure 4. X-ray ORTEP drawing of compound **14**.

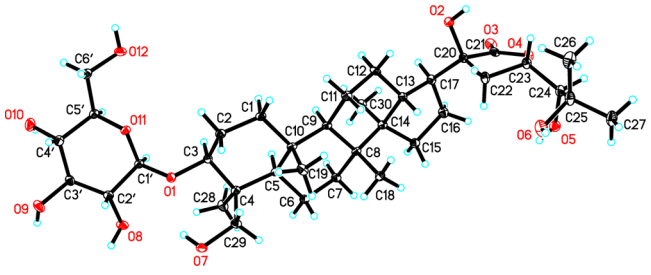

Figure 5. X-ray ORTEP drawing of compound **15**.

MeOH at room temperature. The X-ray diffraction data showed that compounds **14**, **15**, and **8** had identical E-rings. Thus, the structures of gentirigenic acid and gentirigeosides A–E should be revised as (3*β*,20*S*,23*S*,24*R*)-3,20,23,24,25,29-hexahydroxydammaran-21-oic acid-21,23-lactone, (3*β*,20*S*,23*S*,24*R*)-3,20,23,24,25,29-hexahydroxydammaran-21-oic acid-21,23-lactone 3-*O*-*β*-D-glucopyranoside, (3*β*,20*S*,23*S*,24*R*)-3,20,23,24,25,29-hexahydroxydammaran-21-oic acid-21,23-lactone 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside, (3*β*,20*S*,23*S*,24*R*)-3,20,23,24,25,29-hexahydroxydammaran-21-oic acid-21,23-lactone 3-*O*-*β*-D-glucopyranosyl-29-*O*-*β*-D-glucopyranoside, (3*β*,20*S*,23*S*,24*R*)-3,20,23,24,25,26,29-heptahydroxydammaran-21-oic acid-21,23-lactone 3-*O*-*β*-D-glucopyranoside, and (3*β*,20*S*,23*S*,24*R*)-3,20,23,24,25-pentahydroxydammaran-21-oic acid-21,23-lactone 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside, respectively.

Compounds **1**–**17** were examined for anti-inflammatory activity by evaluating the inhibition of LPS-induced NO production in RAW 264.7 macrophages. Only gentirigeoside A (**15**) inhibited NO production in RAW 264.7 macrophages, with an IC_{50} value of 6.6 ± 2.1 *μ*M, while indomethacin, the positive control, showed an IC_{50} value of 1.25 ± 0.52 *μ*M.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a PerkinElmer 341 polarimeter. UV data were acquired on a Shimadzu UV-2550 spectrophotometer. IR spectra were recorded on a PerkinElmer 577 spectrometer using KBr disks. NMR experiments were recorded in pyridine-d_5 on a Bruker AM-500 spectrometer referenced to solvent peaks (δ_H 7.58; δ_C 135.9). HRESIMS analyses were carried out on an Agilent 6224 TOF mass spectrometer with an ESI interface. Semipreparative HPLC was performed on an Agilent 1100 series HPLC system using a YMC-Pack ODS-A column (250 × 10 mm, S-5 *μ*m). Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd.), C_{18} reversed-phase (RP-18) silica gel (20–45 *μ*m; Fuji Silysia Chemical Ltd.), C_8 reversed-phase silica gel (20–45 *μ*m; Fuji Silysia Chemical Ltd.), CHP20P MCI gel (75–150 *μ*m, Mitsubishi Chemical Industries Co., Ltd.), Toyopearl HW-40F (32–63 *μ*m; Tosoh), and Sephadex LH-20 gel (Amersham Biosciences) were used for column chromatography (CC). Precoated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co. Ltd.) were used for TLC. All solvents used for CC were of analytical grade (Shanghai Chemical Reagents Co., Ltd.), and the solvents used for HPLC were of HPLC grade (TEDIA Ltd.).

Plant Material. The aerial parts of *G. azurea* were collected in May 2012 from Yunnan Province, People's Republic of China, and authenticated by Prof. He-Ming Yang. A voucher specimen (No. SIMMPZ323) was deposited at the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences, People's Republic of China.

Extraction and Isolation. Air-dried and pulverized *G. azurea* plant material (2.6 kg) was soaked in 95% ethanol (3 × 30 L; 24 h each time) at room temperature. After removing the solvent under reduced pressure at 40 °C, the combined extracts were concentrated to afford the crude extract (321.1 g), which was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ (131.7 g) fraction was loaded onto a silica gel column and eluted with a gradient of petroleum ether/acetone (20/1, 10/1, 5/1, 3/1, 1/1, and 0/1) to give fractions 1–7. Fraction 7 (4.3 g) was subjected to a column of reversed-phase silica gel eluting with a MeOH/H_2O (50/50 to 100/0) gradient to yield subfractions 7a–7c. Subfraction 7c (67 mg) was purified by semipreparative HPLC with 82% MeOH in H_2O as the mobile phase to yield compounds **1** (16 mg) and **2** (17 mg).

The water-soluble fraction (171.3 g) was subjected to MCI gel column chromatography (MeOH/H_2O, 0/100 to 100/0) to afford fractions A–G. Fraction B was separated using a C_8 column (MeOH/

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H_2O, 10/90 to 50/50) to afford subfractions B1−B5. Subfraction B2 was further separated on a Toyopearl HW-40F column (MeOH/H_2O, 0/100 to 30/70) to yield **17** (89 mg). Compounds **15** (132 mg) and **16** (44 mg) were obtained from subfractions B3 and B4, respectively, using the same method as that used for B1. Fraction C (17.8 g) was further purified through repeated C_8 and Sephadex LH-20 columns to afford compound **14** (12 mg). Fraction D (7.0 g) was purified by chromatography using a C_{18} column eluting with a 30/70 to 80/20 MeOH/H_2O gradient. The major fraction (MeOH/H_2O = 40/60, 3.4 g) was purified by semipreparative HPLC eluting with 58% MeOH in H_2O to yield compounds **10** (12 mg), **11** (57 mg), and **12** (132 mg), and the minor fraction (MeOH/H_2O = 50/50, 300 mg) was purified through repeated LH-20 columns with MeOH to yield compound **13** (22 mg). Fraction F was separated using a C_{18} column eluting with MeOH/H_2O (50/50 to 100/0) to yield three subfractions (F1−F8). Subfraction F1 (5.0 g) was then subjected to a Toyopearl HW-40F column eluting with MeOH/H_2O (0/100 to 50/50) to give subfractions F1A−F1E. Subfraction F1A was purified by semipreparative HPLC with 65% MeCN in H_2O as the mobile phase to yield compounds **8** (15 mg), **9** (9 mg), and **3** (4 mg). Subfraction F1B was purified by semipreparative HPLC with 76% MeOH in H_2O as the mobile phase to yield compounds **4** (21 mg), **5** (48 mg), **6** (14 mg), and **7** (8 mg).

*(3β,20S,23R)-3,20,23,29-Tetrahydroxydammar-24-en-21-oic acid-21,23-lactone 3-O-β-D-glucopyranoside (**1**):* colorless crystals (MeOH); mp 189−191 °C; $[\alpha]_D^{20}$ −8 (*c* 0.1, MeOH); ECD (MeOH) 227 (Δε +1.05), 204 (Δε −3.89); IR (KBr) ν_{max} 3423, 2940, 1764, 1452, 1378, 1200, 1076, 1031 cm^{-1}; ^{13}C NMR, Table 1; ^1H NMR, Table 2; HRESIMS *m/z* 673.3917 $[M + Na]^+$ (calcd for $C_{36}H_{58}O_{10}$, 673.3922).

*(3β,20S,23S)-3,20,23,29-Tetrahydroxydammar-24-en-21-oic acid-21,23-lactone 3-O-β-D-glucopyranoside (**2**):* amorphous powder; $[\alpha]_D^{20}$ +26 (*c* 0.1, MeOH); ECD (MeOH) 232 (Δε −2.09), 202 (Δε +4.12); IR (KBr) ν_{max} 3423, 2938, 1754, 1452, 1379, 1201, 1076, 1032 cm^{-1}; ^{13}C NMR, Table 1; ^1H NMR, Table 2; HRESIMS *m/z* 673.3917 $[M + Na]^+$ (calcd for $C_{36}H_{58}O_{10}$, 673.3922).

*(3β,20S,23R)-3,20,23,26,29-Pentahydroxydammar-24-en-21-oic acid-21,23-lactone 3-O-β-D-glucopyranoside (**3**):* amorphous powder; $[\alpha]_D^{20}$ −6 (*c* 0.1, MeOH); ECD (MeOH) 226 (Δε +2.08), 200 (Δε −9.90); IR (KBr) ν_{max} 3423, 2945, 1765, 1452, 1379, 1211, 1078, 1030 cm^{-1}; ^{13}C NMR, Table 1; ^1H NMR, Table 2; HRESIMS *m/z* 689.3883 $[M + Na]^+$ (calcd for $C_{36}H_{58}O_{11}$, 689.3871).

*(3β,20S,23R)-3,20,23,29-Tetrahydroxydammar-24-en-21-oic acid-21,23-lactone 3-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**4**):* amorphous powder; $[\alpha]_D^{20}$ −4 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3419, 2942, 1765, 1452, 1379, 1200, 1076, 1028 cm^{-1}; ^{13}C NMR, Table 1; ^1H NMR, Table 2; HRESIMS *m/z* 835.4452 $[M + Na]^+$ (calcd for $C_{42}H_{68}O_{15}$, 835.4450).

*(3β,20S,23S)-3,20,23,29-Tetrahydroxydammar-24-en-21-oic acid-21,23-lactone 3-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**5**):* amorphous powder; $[\alpha]_D^{20}$ +22 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3396, 2943, 1761, 1452, 1379, 1200, 1076, 1026 cm^{-1}; ^{13}C NMR, Table 1; ^1H NMR, Table 2; HRESIMS *m/z* 835.4447 $[M + Na]^+$ (calcd for $C_{42}H_{68}O_{15}$, 835.4450).

*(3β,20S,23R)-3,20,23-Trihydroxydammar-24-en-21-oic acid-21,23-lactone 3-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**6**):* amorphous powder; $[\alpha]_D^{20}$ −2 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3423, 2939, 1763, 1452, 1379, 1200, 1076 cm^{-1}; ^{13}C NMR, Table 1; ^1H NMR, Table 2; HRESIMS *m/z* 819.4513 $[M + Na]^+$ (calcd for $C_{42}H_{68}O_{14}$, 819.4501).

*(3β,20S,23S)-3,20,23-Trihydroxydammar-24-en-21-oic acid-21,23-lactone 3-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**7**):* amorphous powder; $[\alpha]_D^{20}$ +26 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3421, 2943, 1761, 1452, 1379, 1201, 1076 cm^{-1}; ^{13}C NMR, Table 1; ^1H NMR, Table 2; HRESIMS *m/z* 819.4518 $[M + Na]^+$ (calcd for $C_{42}H_{68}O_{14}$, 819.4501).

*(3β,20S,23S,24R)-3,20,23,24,25-Pentahydroxydammaran-21-oic acid-21,23-lactone 3-O-β-D-glucopyranoside (**8**):* colorless crystals (MeOH); mp 261−263 °C; $[\alpha]_D^{20}$ +28 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3423, 2945, 1778, 1749, 1639, 1377, 1217, 1076, 1032 cm^{-1}; ^{13}C NMR, Table 1; ^1H NMR, Table 3; HRESIMS *m/z* 691.4020 $[M + Na]^+$ (calcd for $C_{36}H_{60}O_{11}$, 691.4028).

*(3β,20S,23R,24R)-3,20,23,24,25,29-Hexahydroxydammaran-21-oic acid-21,23-lactone 3-O-β-D-glucopyranoside (**9**):* colorless crystals (H_2O/pyridine/MeOH, 2:1:7); mp 256−258 °C; $[\alpha]_D^{20}$ +7 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3419, 2939, 1761, 1595, 1454, 1377, 1076, 1030 cm^{-1}; ^{13}C NMR, Table 1; ^1H NMR, Table 3; HRESIMS *m/z* 707.3991 $[M + Na]^+$ (calcd for $C_{36}H_{60}O_{12}$, 707.3977).

*(3β,20S,23S,24R)-3,20,23,24,25,29-Hexahydroxydammaran-21-oic acid-21,23-lactone 3-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (**10**):* amorphous powder; $[\alpha]_D^{20}$ −1 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3396, 2944, 1761, 1454, 1379, 1205, 1076, 1030 cm^{-1}; ^{13}C NMR, Table 1; ^1H NMR, Table 3; HRESIMS *m/z* 869.4508 $[M + Na]^+$ (calcd for $C_{42}H_{70}O_{17}$, 869.4505).

*(3β,20S,23S,24R)-3,20,23,24,25-Pentahydroxydammaran-21-oic acid-21,23-lactone 3-O-[β-D-glucopyranosyl-(1 → 2)]-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (**11**):* amorphous powder; $[\alpha]_D^{20}$ +2 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3396, 2943, 2779, 1761, 1620, 1354, 1076 cm^{-1}; ^{13}C NMR, Table 1; ^1H NMR, Table 3; HRESIMS *m/z* 1015.5064 $[M + Na]^+$ (calcd for $C_{48}H_{80}O_{21}$, 1015.5084).

*(3β,20S,23S,24R)-3,20,23,24,25-Pentahydroxydammaran-21-oic acid-21,23-lactone 3-O-{[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→2)}-β-D-glucopyranoside (**12**):* amorphous powder; $[\alpha]_D^{20}$ +7 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3406, 2943, 1761, 1641, 1454, 1377, 1207, 1167, 1076, 1028 cm^{-1}; ^1H NMR, Table 3; ^{13}C NMR, Table 1; HRESIMS *m/z* 1015.5064 $[M + Na]^+$ (calcd for $C_{48}H_{80}O_{21}$, 1015.5084).

*(3β,20S,23S,24R)-3,20,23,24,25-Pentahydroxydammaran-21-oic acid-21,23-lactone 3-O-{[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→2)}-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (**13**):* amorphous powder; $[\alpha]_D^{20}$ −25 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3406, 2939, 1761, 1645, 1456, 1377, 1203, 1074 cm^{-1}; ^{13}C NMR, Table 1; ^1H NMR, Table 3; HRESIMS *m/z* 1177.5614 $[M + Na]^+$ (calcd for $C_{54}H_{90}O_{26}$, 1177.5613).

*Gentirigenic acid (**14**):* colorless crystals (MeOH); mp 166−168 °C; $[\alpha]_D^{20}$ +18 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3425, 2929, 1761, 1620, 1466, 1097 cm^{-1}; ^1H NMR and ^{13}C NMR see Supporting Information; HRESIMS *m/z* 1045.7182 $[2 M + H]^+$ (calcd for $C_{60}H_{100}O_{14}$, 1045.7186).

*Gentirigeoside A (**15**):* colorless crystals (MeOH); mp 220−222 °C; $[\alpha]_D^{20}$ +8 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3421, 2933, 1761, 1456, 1379, 1205, 1076, 1028 cm^{-1}; ^1H NMR and ^{13}C NMR see Supporting Information; HRESIMS *m/z* 707.4002 $[M + Na]^+$ (calcd for $C_{36}H_{60}O_{12}$, 707.3977).

*Gentirigeoside B (**16**):* amorphous powder; $[\alpha]_D^{20}$ +16 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3406, 2943, 1763, 1454, 1379, 1203, 1076, 1028 cm^{-1}; ^1H NMR and ^{13}C NMR see Supporting Information; HRESIMS *m/z* 869.4511 $[M + Na]^+$ (calcd for $C_{42}H_{70}O_{17}$, 869.4505).

*Gentirigeoside E (**17**):* amorphous powder; $[\alpha]_D^{20}$ +10 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3419, 2939, 1761, 1595, 1454, 1377, 1076, 1030 cm^{-1}; ^1H NMR and ^{13}C NMR see Supporting Information; HRESIMS *m/z* 853.4574 $[M + Na]^+$ (calcd for $C_{42}H_{70}O_{16}$, 853.4556).

X-ray Diffraction Analysis. The X-ray crystallographic data were obtained on a Bruker APEX-II CCD detector employing graphite-monochromated Cu Kα radiation operated in the $\phi-\omega$ scan mode. The structures were solved by the direct method using SHELXS-97 (Sheldrick 2008) and refined with full-matrix least-squares calculations on F^2 using SHELXL-97 (Sheldrick 2008). All non-hydrogen atoms were refined anisotropically. The hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms.

*Crystallographic data for **1**:* $C_{36}H_{58}O_{10}$, M = 650.82, size 0.25 mm × 0.08 mm × 0.06 mm, monoclinic, space group $P2_1$, a = 15.2138(3) Å, b = 8.0132(2) Å, c = 16.3249(3) Å, α = 90°, β = 116.9640(10)°, γ = 90°, V = 1773.84(7) Å^3, T = 140(2) K, Z = 2, d = 1.219 Mg/m^3, λ(Cu Kα) = 1.541 78 Å, $F(000)$ = 708, reflections collected/unique 13 834/5381 [R(int) = 0.0245], h (−18/18), k (−7/9), l (−19/19), theta range 3.037° to 69.676°, completeness 98.2%, data/restrains/parameters 5381/1/427, final R indices R_1 = 0.0364 and wR_2 = 0.1031 ($I > 2\sigma(I)$), R_1 = 0.0370 and wR_2 = 0.1038 (all data), GOF =

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1.028, largest diff peak and hole, 0.382 and −0.245 e Å^{-3}, absolute structure parameter 0.05(6).

Crystallographic data for ***8****:* $C_{36}H_{60}O_{11}$, M = 668.82, size 0.35 mm × 0.04 mm × 0.02 mm, monoclinic, space group $P2_12_12_1$, a = 7.3172(10) Å, b = 23.8986(3) Å, c = 40.6627(5) Å, α = 90°, β = 90°, γ = 90°, V = 7110.72(16) Å^3, T = 140(2) K, Z = 8, d = 1.296 Mg/m^3, λ(Cu Kα) = 1.541 78 Å, F(000) = 3024, reflections collected/unique 48 526/12 683 [R(int) = 0.0534], h (−8/6), k (−28/28), l (−48/49), theta range 2.144° to 69.650°, completeness 97.5%, data/restrains/parameters 12 683/0/912, final R indices R_1 = 0.0344 and wR_2 = 0.0860 ($I > 2\sigma(I)$), R_1 = 0.0382 and wR_2 = 0.0890 (all data), GOF = 1.052, largest diff peak and hole, 0.189 and −0.173 e Å^{-3}, absolute structure parameter −0.05(6).

Crystallographic data for ***9****:* $C_{36}H_{60}O_{12}$, M = 684.84, size 0.29 mm × 0.16 mm × 0.10 mm, monoclinic, space group $P2_1$, a = 15.2751(3) Å, b = 8.0457(2) Å, c = 15.9079(3) Å, α = 90°, β = 116.3950(10)°, γ = 90°, V = 1751.25(7) Å^3, T = 140(2) K, Z = 2, d = 1.299 Mg/m^3, λ(Cu Kα) = 1.541 78 Å, F(000) = 744, reflections collected/unique 12 092/4840 [R(int) = 0.0333], h (−18/18), k (−7/9), l (−18/19), theta range 3.230° to 69.571°, completeness 99.2%, data/restrains/parameters 4840/1/447, final R indices R_1 = 0.0468 and wR_2 = 0.1362 ($I > 2\sigma(I)$), R_1 = 0.0472 and wR_2 = 0.1368 (all data), GOF = 1.027, largest diff peak and hole, 0.553 and −0.286 e Å^{-3}, absolute structure parameter 0.11(9).

Crystallographic data for ***14****:* $C_{30}H_{50}O_7$, M = 522.80, size 0.26 mm × 0.13 mm × 0.08 mm, orthorhombic, space group $P2_12_12$, a = 11.2093(3) Å, b = 38.8302(9) Å, c = 7.2838(2) Å, α = 90°, β = 90°, γ = 90°, V = 3170.34(14) Å^3, T = 140(2) K, Z = 4, d = 1.267 Mg/m^3, λ(Cu Kα) = 1.541 78 Å, F(000) = 1328, reflections collected/unique 17 922/5668 [R(int) = 0.0468], h (−11/13), k (−46/47), l (−8/6), theta range 2.276° to 69.623°, completeness 96.5%, data/restrains/parameters 5668/12/390, final R indices R_1 = 0.0822 and wR_2 = 0.2311 ($I > 2\sigma(I)$), R_1 = 0.0844 and wR_2 = 0.2343 (all data), GOF = 1.153, largest diff peak and hole, 0.823 and −0.927 e Å^{-3}, absolute structure parameter 0.24(7).

Crystallographic data for ***15****:* $C_{36}H_{60}O_{12}$, M = 684.81, size 0.23 mm × 0.12 mm × 0.08 mm, triclinic, space group $P1$, a = 7.1558(3) Å, b = 12.8203(4) Å, c = 21.0330(7) Å, α = 85.672(2)°, β = 88.636(2)°, γ = 77.863 (2)°, V = 1880.95(12) Å^3, T = 140(2) K, Z = 1, d = 1.333 Mg/m^3, λ(Cu Kα) = 1.541 78 Å, F(000) = 822, reflections collected/unique 23 575/10 540 [R(int) = 0.0442], h (−8/8), k (−15/15), l (−25/25), theta range 3.535° to 69.252°, completeness 95.6%, data/restrains/parameters 10 540/3/966, final R indices R_1 = 0.0477 and wR_2 = 0.1226 ($I > 2\sigma(I)$), R_1 = 0.0479 and wR_2 = 0.1230 (all data), GOF = 1.045, largest diff peak and hole, 0.540 and −0.289 e Å^{-3}, absolute structure parameter 0.05(6).

The crystallographic data for **1** (deposition no. CCDC 970575), **8** (deposition no. CCDC 970577), **9** (deposition no. CCDC 970579), **14** (deposition no. CCDC 970576), and **15** (deposition no. CCDC 970578) have been deposited in the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, by application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0) 1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Acid Hydrolysis of Compounds 1−13. Compound **1** (2 mg) was hydrolyzed in 2 M HCl (3 mL) at 100 °C for 3 h. The reaction mixture was neutralized with 10% Na_2CO_3 and extracted with CH_2Cl_2 (3 × 5 mL). The aqueous layer was desalted (Sephadex LH-20, H_2O) to afford a sugar residue (0.5 mg). The sugar was confirmed to be D-glucose through comparison of the TLC [using *n*-BuOH/EtOAc/pyridine/H_2O (6:1:5:4) as the development solution] with an authentic sample of glucose (R_f 0.42) and by its specific rotation ($[\alpha]_D^{20}$ +43.6 *c* 0.04, H_2O). The constituent sugars of compounds **2**−**13** were identified using the same method.

Anti-inflammatory Bioassay. RAW 264.7 cells were cultured in RPMI medium 1640 supplemented with 10% heat-inactivated FBS with penicillin G (60 units/mL) and streptomycin (100 μg/mL) in a humidified 5% CO_2/95% air atmosphere at 37 °C.[10] The cells were harvested with trypsin-EDTA and diluted and suspended in fresh medium. The suspended cells were seeded in 96-well plates (2 × 10^4 cells/well) and allowed to adhere for 24 h. The stock solution for each test sample was dissolved in DMSO and diluted with RPMI medium 1640 to a final concentration of less than 0.1%. To determine the inhibitory activity of each compound, the cells were co-incubated with fresh medium (200 μL/well) containing 200 ng/mL of LPS and the tested compounds at various concentrations (0.1−50.0 μM) for 24 h. For the positive control group, the cells were co-incubated with indomethacin. The Griess reagent was used to determine NO production by measuring the accumulation of NO_2^- in the culture supernatant. Briefly, 100 μL of the supernatant from the incubate was mixed with an equal volume of Griess reagent (0.2% naphthylenediamide dihydrochloride and 2% sulfanilamide in 5% H_3PO_4) and agitated at room temperature. The absorbance was measured with a microplate reader at 540 nm. Inhibition activity (%) was calculated using the following equation, and the IC_{50} values were calculated using the GraphPad Prism program: Inhibition activity (%) = $(A - B)/(A - C) \times 100$, where A−C is the NO_2^- concentration (μM) [*A*: LPS (+), sample (−); *B*: LPS (+), sample (+); *C*: LPS (−), sample (−)].

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR data for compounds **14**−**17**. Copies of IR and 1D and 2D NMR spectra for compounds **1**−**17**. Copies of the ECD spectra for compounds **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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