

Dammarane Triterpenoids from the Roots of *Gentiana rigescens*

Min Xu,^{†,‡} Dong Wang,[†] Ying-Jun Zhang,^{†,*} and Chong-Ren Yang^{†,§}

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China, and Graduate School of the Chinese Academy of Sciences, Beijing 100039, People's Republic of China

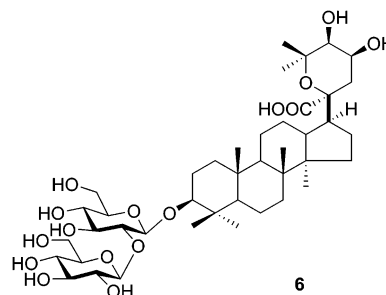
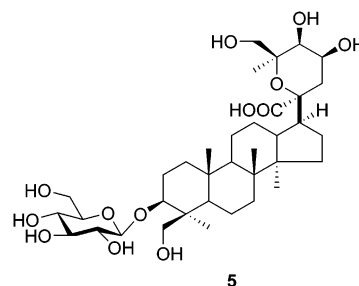
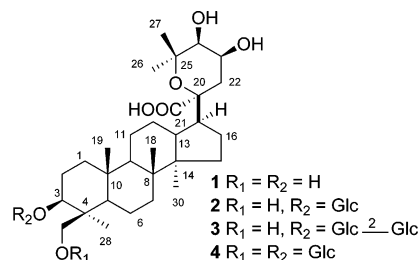
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Six new minor dammarane triterpenoids were isolated from the roots of *Gentiana rigescens*. These include one aglycone, gentirigenic acid (**1**), and five glycosides, gentirigeosides A–E (**2–6**). Their structures were elucidated through detailed spectroscopic analyses, including 1D and 2D NMR techniques, and enzymatic hydrolysis. Antifungal testing of these compounds showed that the glycosides gentirigeosides A (**2**), C (**4**), and E (**6**) had antifungal activity against the plant pathogen *Glomerella cingulata*.

Gentiana rigescens Fr. ex Hemsl. (Gentianaceae) is a plant endemic to the southwest of China. As a traditional Chinese medicine (TCM), its root has been commonly used for the treatment of inflammation, hepatitis, rheumatism, and cholecystitis.¹ We recently reported several iridoidal glucosides as major constituents of the roots.² Further investigation of the same extract has resulted in the isolation of six new dammarane triterpenoids, including an aglycone (**1**) and five glycosides (**2–6**). This paper presents the isolation and structure elucidation of these compounds using detailed spectroscopic analyses, including 2D NMR techniques, and enzymatic hydrolysis. In addition, the *in vitro* antifungal activities of these dammarane triterpenoids on several plant pathogens are also described.

The methanolic extract of air-dried roots of *G. rigescens* was suspended into H₂O and partitioned successively with EtOAc and *n*-BuOH. The *n*-BuOH layer was subjected to column chromatography over Diaion HP20SS, MCI-gel CHP-20P, Chromatorex ODS, Rp-8, and silica gel to afford compounds **1–6**.

Compound **1** was obtained as a white powder. Its molecular formula was assigned as C₃₀H₅₀O₇ on the basis of HRFABMS ([M – H][–], *m/z* 521.3476), ¹³C NMR, and DEPT data, indicating six degrees of unsaturation. The IR spectrum of **1** indicated the existence of hydroxyl (3432 cm^{–1}) and carboxyl groups (1761 cm^{–1}). The ¹³C NMR spectrum (Table 1) gave 30 signals comprising six methyls, 10 methylenes (including one oxygen-bearing carbon at δ_C 64.5), seven methines [three of them bearing an oxygen atom (δ_C 77.6, 79.7, and 80.4)], six quaternary carbons (including two oxygen-bearing ones at δ_C 71.8 and 81.1), and a carboxyl (δ_C 178.7), assignable to a triterpenoid compound. The ¹H NMR spectrum (Table 2) showed six singlet methyl signals, suggesting that all of them were linked to quaternary carbons. The large coupling constant of H-3 (δ_H 3.30, dd, *J* = 5.7, 11.8 Hz) suggested a 3β-hydroxyl substituent. Comparison of the NMR data with those of 20*S*,25-epoxy-3,12β,23*S*,24*R*-tetrahydrodammarane (neosalsogenin M), an aglycone obtained from acidic hydrolysis of dammarane glycosides of *Neosalsomitra interegrifolium* (Cucurbitaceae),³ revealed that **1** had a 20*S*,25-epoxy dammarane skeleton similar to that of neoalsogenin M. However, instead of the methyl groups at C-21 and C-29 as in neoalsogenin M, compound **1** had carboxyl (δ_C 178.7) and hydroxymethylene (δ_C 64.5) groups in the molecule. In addition, obvious differences at C-12 (δ_C 22.1 and 70.6 for **1** and neoalsogenin M, respectively) and C-13 (δ_C 44.8 and 49.5 for **1** and neoalsogenin M, respectively) were also



observed, indicating that compound **1** had no hydroxyl group attached at C-12, relative to neoalsogenin M. The locations of the carboxyl and hydroxymethylene groups at the C-20 and C-4 positions were further confirmed by the HMBC spectrum (Figure 1), in which correlations of both H₂-22 and H-17 with the carboxyl carbon (C-21) at δ 178.7, H₂-29 with C-4 and C-28, and CH₃-28 with C-3, C-4, C-5, and C-29 were observed. At the same time, the ROESY correlation of H-3 with CH₃-28 also indicated that the hydroxyl group was linked to C-29.

The coupling constant of H-23 [δ_H 5.65 (ddd, *J* = 1.7, 8.7, 5.3 Hz)] and H-24 [δ_H 4.33 (d, *J* = 1.7 Hz)] as well as the ROESY correlation (Figure 2) between H-23 and CH₃-27 revealed the equatorial and axial orientations of hydroxyl groups at C-23 and C-24, respectively; that is, both the C-23 and C-24 hydroxyl groups were β-oriented. The absolute configuration at C-20 was established as *S* by comparing the NMR data with model compounds.^{3–5}

* Author to whom correspondence should be addressed. Tel: +86-871-522-3235. Fax: +86-871-515-0124. E-mail: zhangyj@mail.kib.ac.cn.

† Co-corresponding author. E-mail: cryang@mail.kib.ac.cn.

‡ Kunming Institute of Botany.

§ Graduate School of the Chinese Academy of Sciences.

Table 1. ^{13}C NMR Data of Compounds **1**–**6** (pyridine- d_5 , 100 MHz, δ in ppm)^a

position	1	2	3	4	5	6
1	39.4 (CH ₂)	39.6 (CH ₂)	39.2 (CH ₂)	39.5 (CH ₂)	40.7 (CH ₂)	39.4 (CH ₂)
2	28.1 (CH ₂)	27.0 (CH ₂)	26.9 (CH ₂)	28.5 (CH ₂)	27.1 (CH ₂)	26.9 (CH ₂)
3	80.4 (CH)	89.0 (CH)	90.8 (CH)	83.8 (CH)	89.2 (CH)	89.1 (CH)
4	43.4 (C)	44.6 (C)	43.9 (C)	42.9 (C)	44.6 (C)	43.9 (C)
5	56.9 (CH)	56.0 (CH)	56.7 (CH)	57.2 (CH)	56.7 (CH)	56.7 (CH)
6	19.1 (CH ₂)	18.8 (CH ₂)	18.6 (CH ₂)	18.8 (CH ₂)	18.9 (CH ₂)	18.8 (CH ₂)
7	36.2 (CH ₂)	36.0 (CH ₂)	35.8 (CH ₂)	35.9 (CH ₂)	36.9 (CH ₂)	36.7 (CH ₂)
8	40.7 (C)	39.3 (C)	40.6 (C)	40.6 (C)	40.1 (C)	40.6 (C)
9	51.4 (CH)	51.2 (CH)	51.0 (CH)	51.2 (CH)	51.2 (CH)	51.2 (CH)
10	37.3 (C)	36.8 (C)	36.7 (C)	37.2 (C)	37.4 (C)	36.3 (C)
11	30.0 (CH ₂)	28.1 (CH ₂)	28.1 (CH ₂)	28.5 (CH ₂)	28.8 (CH ₂)	32.2 (CH ₂)
12	22.1 (CH ₂)	22.1 (CH ₂)	22.1 (CH ₂)	21.9 (CH ₂)	22.2 (CH ₂)	22.1 (CH ₂)
13	44.8 (CH)	44.8 (CH)	44.8 (CH)	44.7 (CH)	44.9 (CH)	44.7 (CH)
14	50.2 (C)	50.2 (C)	50.2 (C)	50.0 (C)	50.3 (C)	50.5 (C)
15	31.7 (CH ₂)	31.7 (CH ₂)	31.8 (CH ₂)	31.6 (CH ₂)	31.8 (CH ₂)	32.2 (CH ₂)
16	26.0 (CH ₂)	26.0 (CH ₂)	26.0 (CH ₂)	25.9 (CH ₂)	26.1 (CH ₂)	26.0 (CH ₂)
17	45.7 (CH)	45.8 (CH)	45.8 (CH)	45.7 (CH)	45.9 (CH)	45.8 (CH)
18	15.6 (CH ₃)	15.5 (CH ₃)	15.5 (CH ₃)	15.5 (CH ₃)	15.6 (CH ₃)	15.7 (CH ₃)
19	16.9 (CH ₃)	16.2 (CH ₃)	16.4 (CH ₃)	16.7 (CH ₃)	16.3 (CH ₃)	16.7 (CH ₃)
20	81.1 (C)	81.1 (C)	81.3 (C)	81.0 (C)	81.4 (C)	81.0 (C)
21	178.7 (C)	178.6 (C)	178.7 (C)	179.2 (C)	178.8 (C)	178.6 (C)
22	33.2 (CH ₂)	33.2 (CH ₂)	33.2 (CH ₂)	33.1 (CH ₂)	33.2 (CH ₂)	33.9 (CH ₂)
23	77.6 (CH)	77.4 (CH)	77.4 (CH)	77.4 (CH)	77.5 (CH)	77.5 (CH)
24	79.7 (CH)	79.6 (CH)	79.7 (CH)	79.7 (CH)	80.2 (CH)	79.6 (CH)
25	71.8 (CH)	72.0 (CH)	71.9 (CH)	72.7 (CH)	72.1 (CH)	71.9 (CH)
26	27.8 (CH ₃)	27.7 (CH ₃)	27.8 (CH ₃)	27.8 (CH ₃)	23.4 (CH ₃)	28.1 (CH ₃)
27	27.2 (CH ₃)	27.2 (CH ₃)	27.2 (CH ₃)	27.2 (CH ₃)	69.0 (CH ₂)	27.7 (CH ₃)
28	22.2 (CH ₃)	22.1 (CH ₃)	22.7 (CH ₃)	23.4 (CH ₃)	21.8 (CH ₃)	27.2 (CH ₃)
29	64.5 (CH ₂)	63.4 (CH ₂)	63.5 (CH ₂)	71.8 (CH ₂)	63.4 (CH ₂)	16.3 (CH ₃)
30	16.3 (CH ₃)	18.0 (CH ₃)	16.3 (CH ₃)	16.3 (CH ₃)	16.3 (CH ₃)	16.5 (CH ₃)
Glc-1'		106.3 (CH)	104.6 (CH)	102.3 (CH)	106.3 (CH)	105.1 (CH)
2'		75.7 (CH)	82.4 (CH)	72.3 (CH)	74.6 (CH)	83.4 (CH)
3'		78.8 (CH)	78.7 (CH)	78.9 (CH)	78.8 (CH)	78.4 (CH)
4'		71.9 (CH)	71.3 (CH)	70.0 (CH)	72.1 (CH)	71.8 (CH)
5'		78.7 (CH)	78.4 (CH)	77.4 (CH)	78.8 (CH)	78.1 (CH)
6'		63.1 (CH)	61.7 (CH ₂)	61.9 (CH ₂)	63.1 (CH ₂)	62.9 (CH ₂)
Glc-1''			105.1 (CH)	105.4 (CH)		106.1 (CH)
2''			75.9 (CH)	76.1 (CH)		77.1 (CH)
3''			79.0 (CH)	78.5 (CH)		78.6 (CH)
4''			70.1 (CH)	70.9 (CH)		71.8 (CH)
5''			78.4 (CH)	78.3 (CH)		78.1 (CH)
6''			62.8 (CH ₂)	62.4 (CH ₂)		62.9 (CH ₂)

^a The assignments were based on DEPT, ^1H – ^1H COSY, HMQC, and HMBC experiments.

Therefore, the structure of **1** was determined to be 20*S*,25-epoxy-3 β ,23 β ,24 β ,29-tetrahydroxydammaran-21-oic acid and was named gentirigenic acid.

Compound **2**, obtained as a white powder, had the molecular formula $\text{C}_{36}\text{H}_{60}\text{O}_{12}$ as deduced from the HRFABMS ($[\text{M} - \text{H}]^-$, m/z 683.4004). The FABMS also showed a characteristic fragment ion peak at m/z 521 $[\text{M} - \text{H} - 162]^-$, suggesting the existence of a hexosyl unit in the molecule. The ^1H and ^{13}C NMR data (Tables 1 and 2) of **2** were closely related to those of **1**, except for a set of additional signals arising from a β -glucopyranosyl moiety [anomeric C at δ_{C} 106.3, anomeric H at δ_{H} 5.02 (d, $J = 7.8$ Hz)]. Enzymatic hydrolysis of **2** with β -glucosidase afforded D-glucose and compound **1**, indicating that compound **2** was a glucoside of **1**. In the HMBC spectrum of **2**, the anomeric proton at δ_{H} 5.02 (H-1') was correlated with C-3 of the aglycone, indicating that the glucopyranosyl unit was linked to C-3 of **2**. In addition, it was noted that the chemical shift of C-3 was changed by +8.6 ppm, relative to **1**. Thus, compound **2** was assigned to be 20*S*,25-epoxy-3 β ,23 β ,24 β ,29-tetrahydroxydammaran-21-oic acid 3-*O*- β -D-glucopyranoside and was named gentirigeoside A.

Compound **3** was isolated as a white powder. Its HRFABMS displayed a quasi-molecular ion peak at m/z 845.4543 $[\text{M} - \text{H}]^-$, corresponding to a molecular formula of $\text{C}_{42}\text{H}_{70}\text{O}_{17}$. Fragment ion peaks at m/z 683 $[\text{M} - \text{H} - 162]^-$ and 521 $[\text{M} - \text{H} - 162 \times 2]^-$ observed in the negative FABMS indicated the existence of two hexosyl units in the molecule. Enzymatic hydrolysis of **3** with β -glucosidase yielded **1** and D-glucose. The ^1H and ^{13}C NMR data

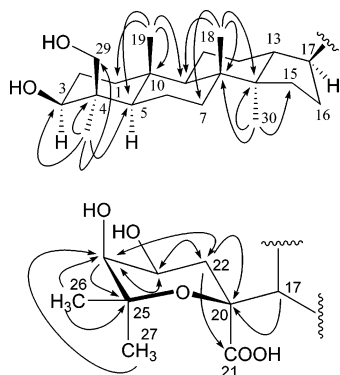
(Tables 1 and 2) of **3** were very similar to those of **2**, except for the appearance of one more β -D-glucopyranosyl unit. Location of the additional glucosyl unit on the C-2' position of the inner glucosyl unit was determined by the long-range correlations between the additional anomeric proton at δ_{H} 5.66 (H-1'') and C-2' (δ_{C} 82.4) of the inner glucosyl unit. Moreover, HMBC correlation of H-1' (δ_{H} 4.88) of the inner glucosyl unit with C-3 (δ_{C} 90.8) of the aglycone was also observed. Therefore, the structure of gentirigeoside B (**3**) was elucidated as 20*S*,25-epoxy-3 β ,23 β ,24 β ,29-tetrahydroxydammaran-21-oic acid 3-*O*- β -D-glucopyranosyl(1 \rightarrow 2)- β -D-glucopyranoside.

Compound **4** had the molecular formula $\text{C}_{42}\text{H}_{70}\text{O}_{17}$, as deduced from the negative HRFABMS ($[\text{M} - \text{H}]^-$, m/z 845.4547), which is the same as that of **3**. The characteristic fragment ion peaks at m/z 683 $[\text{M} - \text{H} - 162]^-$ and 521 $[\text{M} - \text{H} - 162 \times 2]^-$ were also observed in the FABMS of **4**. Enzymatic hydrolysis with β -glucosidase yielded D-glucose and **1**. The NMR data (Tables 1 and 2) of **4** were very similar to those of **3**, except for the obvious differences at C-29 (δ_{C} 71.8) and C-2' (δ_{C} 72.3), suggesting that the two β -D-glucopyranosyl units were located respectively at the C-3 and C-29 positions in **4**. The sites of the two sugar units were further confirmed by the HMBC experiment, in which cross-peaks of one anomeric proton at δ_{H} 4.80 (H-1') with C-3 (δ_{C} 83.8) and another anomeric proton at δ_{H} 4.66 (H-1'') with C-29 were observed. Accordingly, the structure of **4** was characterized as 20*S*,25-epoxy-3 β ,23 β ,24 β ,29-tetrahydroxydammaran-21-oic acid 3-*O*-

Table 2. ^1H NMR Data of Compounds **1–6** (pyridine- d_5 , 500 MHz, δ in ppm, J in Hz)^a

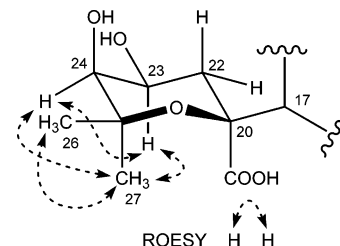
no.	1	2	3	4	5	6
1	0.90 m 1.61 m	0.73 m 1.65 m	0.72 m 1.66 m	0.79 m 1.65 m	0.78 m 1.67 m	0.76 m 1.60 m
2	1.16 m 1.54 m	2.16 m 2.18 m	2.04 m 2.07 m	2.04 m 2.00 m	2.16 m 2.19 m	2.21 m 2.24 m
3	3.30 dd,5.7,11.8	3.48 dd,5.0,11.5	3.47 dd,5.3,11.5	3.49 dd,5.5, 11.9	3.52 dd,5.7,11.8	3.27 dd,5.2,11.7
5	0.89 m	0.81 m	0.80 m	0.99 m	0.88 m	0.65 dd,2.5,11.5
6	1.44 m 1.63 m	1.43 m 1.60 m	1.41 m 1.60 m	1.43 m 1.61 m	1.44 m 1.66 m	1.29 m 1.61 m
7	1.15 m 1.46 m	1.18 m 1.43 m	1.14 m 1.48 m	1.13 m 1.45 m	1.10 m 1.46 m	1.17 m 1.43 m
9	1.29 m	1.29 m	1.21 m	1.21 m	1.29 m	1.24 m
11	1.25 m 1.32 m	1.40 m 1.28 m	1.48 m 1.22 m	1.49 m 1.24 m	1.41 m 1.24 m	1.34 m 1.24 m
12	1.61 m 2.56 m	1.60 m 2.51 m	1.60 m 2.53 m	1.62 m 2.52 m	1.60 m 2.54 m	1.57 m 2.55 m
13	2.00 dd,8.7,10.0	1.98 dd,8.6,10.8	1.97 dd,8.8,10.9	1.90 m	1.94 dd,9.1,10.0	1.96 m
15	0.98 m 1.51 m	0.99 m 1.47 m	0.99 m 1.50 m	1.00 m 1.47 m	0.98 m 1.49 m	1.06 m 1.51 m
16	1.51 m 1.95 m	1.57 m 2.00 m	1.55 m 2.00 m	1.57 m 2.00 m	1.51 m 2.00 m	1.54 m 2.01 m
17	2.73 ddd,5.8, 9.1,10.0	2.71 ddd,6.0, 9.8,10.8	2.77 ddd,5.6, 8.0,10.9	2.75 ddd,5.6, 8.0,10.9	2.72 ddd,5.7, 8.2,10.0	2.72 ddd,5.0, 8.6,10.9
18	0.89 s	0.84 s	0.82 s	0.89 s	0.89 s	0.84 s
19	0.82 s	0.73 s	0.78 s	0.76 s	0.72 s	0.76 s
22	2.75 dd,16.0,5.3 3.13 dd,16.0,8.7	2.73 dd,6.0,16.5 3.10 dd,8.8,16.3	2.81 dd,5.6,16.6 3.15 dd,8.7,16.4	2.75 dd,5.3,16.9 3.12 dd,8.0,16.4	2.81 dd,5.2,16.8 3.23 dd,8.6,16.4	2.76 dd,5.3,16.6 3.14 dd,8.7,16.2
23	5.65 ddd, 1.7,5.3,8.7	5.61 ddd, 1.2,6.0,8.8	5.36 ddd, 1.9,5.6,8.7	5.64 ddd, 1.9,8.0,5.3	5.93 ddd, 2.2,8.6,5.2	5.64 ddd, 1.7,8.7,5.3
24	4.33 d,1.7	4.31 d,1.2	4.17 d,1.9	4.18 d,1.9	4.98 d,2.2	4.36 d,1.7
26	1.55 s	1.57 s	1.54 s	1.54 s	1.70 s	1.62 s
27	1.50 s	1.51 s	1.59 s	1.57 s	4.28 dd,1.2,11.2 4.08 dd,5.5,11.2	1.55 s
28	1.61 s	1.53 s	1.33 s	1.48 s	1.57 s	1.26 s
29	3.69 dd,1.1,16.1 4.86 dd,7.2,16.1	3.63 dd,1.6,16.6 4.38 dd,5.6,16.6	3.65 dd,1.7,16.1 4.24 dd,4.3,16.1	4.54 dd,1.8,16.2 4.43 dd,5.8,16.4	3.63 dd,1.5,16.4 4.42 dd,5.8,16.4	1.09 s
30	0.85 s	0.83 s	0.86 s	0.93 s	0.80 s	0.85 s
Glu-1'		5.02 d,7.8	4.88 d,7.8	4.96 d,7.5	4.99 d,7.7	4.92 d,7.5
2'		3.96 dd,8.2,9.1	4.18 m	4.35 m	4.01 m	4.25 dd,8.6,9.7
3'		4.02 m	4.00 m	4.63 m	4.08 m	3.92 m
4'		4.15 m	4.16 m	4.40 m	4.21 m	4.25 m
5'		4.25 m	3.79 m	4.26 m	4.26 m	3.92 m
6'		4.36 dd,2.1,11.8	4.40 dd,1.6,11.8	4.27 dd,1.5,12.0	4.39 dd,2.1,11.6	4.58 dd,2.3,11.0
Glu-1''		4.59 dd,6.3,11.8	4.63 dd,6.2,11.8	4.67 dd,6.5,12.0	4.63 dd,6.3,11.6	4.83 dd,6.8,11.0
2''			5.66 d,7.9	4.80 d,7.3		5.37 d,7.6
3''			4.14 m	4.11 m		4.14 dd,8.9,9.6
4''			4.15 m	4.08 m		4.12 m
4''			4.60 m	4.57 m		4.54 m
5''			4.29 m	4.47 m		4.42 m
6''			4.40 dd,1.6,11.2 4.63 dd,6.2,11.2	4.42 dd,1.8,11.2 4.94 dd,6.6,11.2		4.58 dd,2.3,11.0 4.83 dd,6.8,11.0

^a The assignments were based on DEPT, ^1H - ^1H COSY, HMQC, and HMBC experiments.

**Figure 1.** Important HMBC correlations of **1**.

β -D-glucopyranosyl-29-O- β -D-glucopyranoside and was named gentirigeoside C.

Compound **5** was obtained as a white powder and had the molecular formula $\text{C}_{36}\text{H}_{60}\text{O}_{13}$, as established by the HRFABMS ($[\text{M} - \text{H}]^-$, m/z 699.3944). The FABMS displayed a characteristic

**Figure 2.** Important ROESY correlations of **1**.

fragment ion peak at m/z 537 $[\text{M} - \text{H} - 162]^-$, indicating the existence of a hexosyl unit in the molecule. Comparison of the NMR data of **5** with those of **2** revealed that the only difference was that a methyl group at C-27 in **2** was replaced by a hydroxymethylene (δ_{C} 69.0) in **5**. The HMBC correlations of the hydroxymethylene protons [δ_{H} 4.28 (dd, $J = 1.2, 9.4$ Hz) and 4.08 (dd, $J = 1.5, 11.2$ Hz)] with C-24 (δ_{C} 80.2) and the ROESY correlation between H-23 [δ_{H} 5.93 (ddd, $J = 2.2, 8.6, 5.2$ Hz)] and CH_3 -26 [δ_{H} 1.70 (s)] also assigned the hydroxymethylene to be at C-27. Therefore, gentirigeoside D (**5**) was determined to be

20*S*,25-epoxy-3 β ,23 β ,24 β ,27,29-pentahydroxydammaran-21-oic acid 3-*O*- β -D-glucopyranoside.

Compound **6** was obtained as a white powder and possessed the molecular formula C₄₂H₇₀O₁₆, as determined from its HRFABMS ([M - H]⁻, *m/z* 829.4579) and ¹³C NMR data. The ¹³C NMR data (Table 1) of **6** were very similar to those of **3**, except for the signals due to C-29 (δ_C 16.3) and C-28 (δ_C 27.2) of the aglycone, indicating that there was no hydroxyl group attached to C-29 in **6**, as in neosolsogenin M.⁷ In the HMBC spectrum of **6**, correlations of the methyl proton at δ_H 1.09 (CH₃-29) with C-4 (δ_C 44.1), C-5 (δ_C 56.7), and C-28 (δ_C 27.2) confirmed the structure of **6**. Thus, compound **6** was concluded to be 20*S*,25-epoxy-3 β ,23 β ,24 β -trihydroxydammaran-21-oic acid 3-*O*- β -D-glucopyranosyl(1 \rightarrow 2)- β -D-glucopyranoside and was named gentirigeoside E.

In this work, six new dammarane triterpenoids were isolated as minor constituents from the roots of *G. rigescens*. All six compounds were evaluated for their antifungal activity against plant pathogens *Peronophythora litchi*, *Glomerella cingulata*, and *Glorosporium musarum*, using the disc diffusion method.⁶ Carbendazim was used as a positive control. At 1 mg/mL concentrations, the new dammarane glycosides **2**, **4**, and **6** showed antifungal activities on *G. cingulata* with inhibitory zones of 2, 0.8, and 1.4 cm, respectively, but demonstrated no activity against the other two fungi. Compounds **1**, **3**, and **5** had no antifungal activity on any of the fungi tested, whereas the positive control, carbendazim, showed antifungal activities against *P. litchi*, *G. cingulata*, and *G. musarum* with inhibitory zones of 1.1, 7.0, and 0.4 cm, respectively.

To the best of our knowledge, dammarane triterpenoids are rare in the genus *Gentiana*. The above research results suggested that the genus *Gentiana* has obvious molecular diversity, which could be a good resource for the discovery of new bioactive natural products.

Experimental Section

General Experimental Procedures. Melting points were obtained on an XRC-1 apparatus and are uncorrected. Optical rotations were measured with a HORIBA SEPA-300 high-sensitive polarimeter. IR (KBr) spectra were measured on a Bio-Rad FTS-135 spectrophotometer. NMR spectra were measured in pyridine-*d*₅ solution and recorded on a Bruker DRX-500 instrument (500 MHz for ¹H NMR and 125 MHz for ¹³C NMR) at 25 °C, using TMS as an internal standard. Mass spectra were recorded on a VG Auto Spec-3000 mass spectrometer using glycerol as matrix. Column chromatography was performed on Diaion HP20SS (Mitsubishi Chemical Co.), MCI-gel CHP-20P (75–150 μ m, Mitsubishi Chemical Co.), and Chromatorex ODS (100–200 mesh, Fuji Silysia Chemical Co. Ltd). Precoated silica gel plates (Qingdao Haiyang Chemical Co.) were used for TLC. Detection was done by spraying the plates with 10% sulfuric acid, followed by heating.

Plant Material. The roots of *Gentiana rigescens* were collected in Chuxiong, Yunnan Province of China, during July 2004. The sample was identified by Prof. Chong-Ren Yang. A voucher specimen (KIB 0552250) has been deposited at the herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The dried roots of *G. rigescens* (9.2 kg) were extracted with methanol. After removal of the solvent by evaporation, the residue (1290 g) was suspended in H₂O and partitioned sequentially with EtOAc and *n*-BuOH. The *n*-BuOH fraction (430 g) was subjected to a column of Diaion HP20SS and eluted with H₂O–MeOH (1:0–0:1) to give seven fractions (B₁–B₇). Fraction B₇ (12.9 g) was repeatedly chromatographed over MCI-gel CHP-20P (20–100% MeOH), Chromatorex ODS (30–85% MeOH), Rp-8 (40–70% MeOH), and silica gel (CHCl₃–MeOH–H₂O, 9:1:0.1–7:3:0.5) to yield compounds **1** (8 mg), **2** (17 mg), **3** (39 mg), **4** (17 mg), **5** (12 mg), and **6** (21 mg).

Gentirigenic acid (1): white powder; mp 160–161 °C; [α]_D¹⁴ +24.7 (*c* 0.093, MeOH); IR ν_{\max}^{KBr} cm⁻¹ 3432, 2929, 1761, 1629, 1453, 1378, 1075, 1032; ¹H NMR data (400 MHz, pyridine-*d*₅), see Table 1; ¹³C NMR data (125 MHz, pyridine-*d*₅), see Table 2; FABMS (negative ion mode) *m/z* 521 [M - H]⁻, 457 [M - H - C₂H₅]; HRFABMS *m/z* 521.3476 [M - H]⁻ (calcd for C₃₀H₅₀O₇, 521.3484).

Gentirigeoside A (2): white powder; mp 182–183 °C; [α]_D¹⁹ +18.9 (*c* 0.27, MeOH); IR ν_{\max}^{KBr} cm⁻¹ 3418, 2944, 1761, 1632, 1452, 1378, 1202, 1076, 1029; ¹H NMR data (500 MHz, pyridine-*d*₅), see Table 1; ¹³C NMR data (125 MHz, pyridine-*d*₅), see Table 2; FABMS (negative ion mode) *m/z* 683 [M - H]⁻, 521 [M - H - 162]⁻; HRFABMS *m/z* 683.4004 [M - H]⁻ (calcd for C₃₆H₆₀O₁₂, 683.4006).

Gentirigeoside B (3): white powder; mp 183–184 °C; [α]_D¹⁹ +23.5 (*c* 0.29, MeOH); IR ν_{\max}^{KBr} cm⁻¹ 3418, 2943, 1762, 1634, 1453, 1378, 1201, 1076, 1028; ¹H NMR data (500 MHz, pyridine-*d*₅), see Table 1; ¹³C NMR data (125 MHz, pyridine-*d*₅), see Table 2; FABMS (negative ion mode) *m/z* 845 [M - H]⁻, 683 [M - H - 162]⁻, 521 [M - H - 162 \times 2]⁻; HRFABMS *m/z* 845.4543 [M - H]⁻ (calcd for C₄₂H₇₀O₁₇, 845.4534).

Gentirigeoside C (4): white powder; mp 185–186 °C; [α]_D¹⁸ +18.1 (*c* 0.38, MeOH); IR ν_{\max}^{KBr} cm⁻¹ 3423, 2938, 1761, 1633, 1452, 1378, 1201, 1077, 1030; ¹H NMR data (500 MHz, pyridine-*d*₅), see Table 1; ¹³C NMR data (125 MHz, pyridine-*d*₅), see Table 2; FABMS (negative ion mode) *m/z* 845 [M - H]⁻, 683 [M - H - 162 \times 2]⁻, 521 [M - H - 162 \times 2]⁻; HRFABMS *m/z* 845.4547 [M - H]⁻ (calcd for C₄₂H₇₀O₁₇, 845.4534).

Gentirigeoside D (5): white powder; mp 182–183 °C; [α]_D²⁰ +17.6 (*c* 0.32, MeOH); IR ν_{\max}^{KBr} cm⁻¹ 3424, 2943, 1752, 1631, 1453, 1377, 1074, 1027; ¹H NMR data (500 MHz, pyridine-*d*₅), see Table 1; ¹³C NMR data (125 MHz, pyridine-*d*₅), see Table 2; FABMS (negative ion mode) *m/z* 699 [M - H]⁻, 537 [M - H - 162]⁻; HRFABMS: *m/z* 699.3944 [M - H]⁻ (calcd for C₃₆H₆₀O₁₃, 699.3955).

Gentirigeoside E (6): white powder; mp 187–188 °C; [α]_D¹⁹ +11.5 (*c* 0.23, MeOH); IR ν_{\max}^{KBr} cm⁻¹ 3424, 2929, 1761, 1632, 1454, 1377, 1204, 1165, 1076, 1030; ¹H NMR data (500 MHz, pyridine-*d*₅), see Table 1; ¹³C NMR data (125 MHz, pyridine-*d*₅), see Table 2; FABMS (negative ion mode) *m/z* 829 [M - H]⁻, 667 [M - H - 162]⁻; HRFABMS *m/z* 829.4579 [M - H]⁻ (calcd for C₄₂H₇₀O₁₇, 829.34585).

Enzymatic Hydrolysis of Compounds 2–6. Solutions of **2–6** (each 2 mg) in H₂O (10 mL) were incubated with β -glucosidase (Sigma) at 37 °C for two weeks. The mixture was partitioned between *n*-BuOH and H₂O, and both parts were concentrated to dryness. TLC analysis indicated the presence of glucose in the water layer for all of the compounds **2–6** (2-propanol–MeOH–H₂O, 25:1:2, *R*_f 0.6). The *n*-BuOH extract from each of the compounds (**2–4**) was identified to be aglycone **1**, by direct TLC comparison with the authentic sample (CHCl₃–MeOH, 9:1, *R*_f 0.43).

Antifungal Activity. The antifungal bioassay was performed as described previously.⁶ Briefly, the assay was executed using 100 \times 15 mm Petri plates containing 10 mL of potato dextrose agar. The sterile blank paper disk (1.0 cm in diameter) was dipped into the solution of test sample (1 mg) in MeOH–H₂O (1:1, 1 mL) and then placed in a Petri plate at a distance of 2 cm away from the center of the plate. Each plate was incubated at 28 °C for 72 h until mycelia had grown and enveloped the disk containing the control (MeOH–H₂O, 1:1) and had produced a zone of inhibition around disks exhibiting antifungal activity. Carbendazim was used as a positive control. The fungal species used included plant pathogens *Peronophythora litchi* Chen ex Ko et al., *Glomerella cingulata* (Stonem) Schr. et squald, and *Glorosporium musarum* Cooke et Mass.

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References and Notes

- Editorial Board of Chinese Pharmacopoeia. *Chinese Pharmacopoeia*; Chemistry and Industry Press: Beijing, 2005; Vol. 1, pp 64–65.
- Xu, M.; Wang, D.; Zhang, Y. Z.; Yang, C. R. *Acta Bot. Yunnanica* **2006**, *28*, 669–672.
- Fujita, S.; Kasai, R.; Qhtani, K.; Yamasaki, K.; Hua, C.-M.; Lin, N.-R.; Tanaka, O. *Phytochemistry* **1995**, *39*, 591–602.
- Yin, F.; Zhang, Y. N.; Yang, Z. Y.; Cheng, Q. Q.; Hu, L. H. *J. Nat. Prod.* **2006**, *69*, 1394–1398.
- Yin, F.; Hu, L. H. *Helv. Chim. Acta* **2005**, *88*, 1126–1134.
- Ye, X.-Y.; Ng, T.-B. *Biochem. Biophys. Res. Commun.* **2000**, *273*, 1111–1115.