Dammarane Triterpenoids from the Roots of *Gentiana rigescens*

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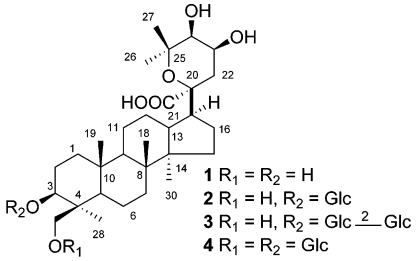
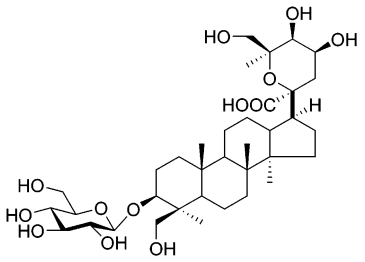
Six new minor dammarane triterpenoids were isolated from the roots of *Gentiana rigescens*. These include one aglycone, gentirigenic acid (**1**), and five glycosides, gentirigeosides A−E (**2**−**6**). Their structures were elucidated through detailed spectroscopic analyses, including 1D and 2D NMR 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 H_2O 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 $C_{30}H_{50}O_7$ on the basis of HRFABMS ([M − H]$^-$, *m*/*z* 521.3476), ^{13}C 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 ^{13}C 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 ^{1}H 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*-tetrahydroxydammarane (neoalsogenin M), an aglycone obtained from acidic hydrolysis of dammarane glycosides of *Neoalsomitra 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 H_2-22 and H-17 with the carboxyl carbon (C-21) at δ 178.7, H_2-29 with C-4 and C-28, and CH_3-28 with C-3, C-4, C-5, and C-29 were observed. At the same time, the ROESY correlation of H-3 with CH_3-28 also indicated that the hydroxyl group was linked to C-29.





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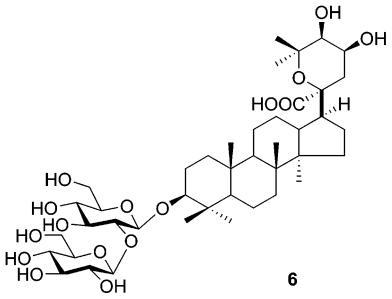

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The coupling constant of H-23 [δ_H 5.65 (ddd, J = 1.7, 8.7, 5.3 Hz)] and H-24 [d_H 4.33 (d, J = 1.7 Hz)] as well as the ROESY correlation (Figure 2) between H-23 and CH_3-27 revealed the equatorial and axial orientations of hydroxyl groups at C-23 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]

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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)	77.5 (CH)	77.4 (CH)	75.7 (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-3β,23β,24β,29-tetrahydroxyldammaran-21-oic acid and was named gentirigenic acid.

Compound **2**, obtained as a white powder, had the molecular formula $C_{36}H_{60}O_{12}$ as deduced from the HRFABMS ([M − H]$^-$, *m*/*z* 683.4004). The FABMS also showed a characteristic fragment ion peak at *m*/*z* 521 [M − 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-3β,23β,24β,-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 [M − H]$^-$, corresponding to a molecular formula of $C_{42}H_{70}O_{17}$. Fragment ion peaks at *m*/*z* 683 [M − H − 162]$^-$ and 521 [M − H − 162 × 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-3β,23β,24β,29-tetrahydroxydammaran-21-oic acid 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside.

Compound **4** had the molecular formula $C_{42}H_{70}O_{17}$, as deduced from the negative HRFABMS ([M − H]$^-$, *m*/*z* 845.4547), which is the same as that of **3**. The characteristic fragment ion peaks at *m*/*z* 683 [M − H − 162]$^-$ and 521 [M − H − 162 × 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-3β,23β,24β,29-tetrahydroxydammaran-21-oic acid 3-*O*-

Table 2. ^{1}H 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	0.73 m	0.72 m	0.79 m	0.78 m	0.76 m
	1.61 m	1.65 m	1.66 m	1.65 m	1.67 m	1.60 m
2	1.16 m	2.16 m	2.04 m	2.04 m	2.16 m	2.21 m
	1.54 m	2.18 m	2.07 m	2.00 m	2.19 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	1.43 m	1.41 m	1.43 m	1.44 m	1.29 m
	1.63 m	1.60 m	1.60 m	1.61 m	1.66 m	1.61 m
7	1.15 m	1.18 m	1.14 m	1.13 m	1.10 m	1.17 m
	1.46 m	1.43 m	1.48 m	1.45 m	1.46 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	1.40 m	1.48 m	1.49 m	1.41 m	1.34 m
	1.32 m	1.28 m	1.22 m	1.24 m	1.24 m	1.24 m
12	1.61 m	1.60 m	1.60 m	1.62 m	1.60 m	1.57 m
	2.56 m	2.51 m	2.53 m	2.52 m	2.54 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	0.99 m	0.99 m	1.00 m	0.98 m	1.06 m
	1.51 m	1.47 m	1.50 m	1.47 m	1.49 m	1.51 m
16	1.51 m	1.57 m	1.55 m	1.57 m	1.51 m	1.54 m
	1.95 m	2.00 m	2.00 m	2.00 m	2.00 m	2.01 m
17	2.73 ddd,5.8,	2.71 ddd,6.0,	2.77 ddd,5.6,	2.75 ddd,5.6,	2.72 ddd,5.7,	2.72 ddd,5.0,
	9.1,10.0	9.8,10.8	8.0,10.9	8.0,10.9	8.2,10.0	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	2.73 dd,6.0,16.5	2.81 dd,5.6,16.6	2.75 dd,5.3,16.9	2.81 dd,5.2,16.8	2.76 dd,5.3,16.6
	3.13 dd,16.0,8.7	3.10 dd,8.8,16.3	3.15 dd,8.7,16.4	3.12 dd,8.0,16.4	3.23 dd,8.6,16.4	3.14 dd,8.7,16.2
23	5.65 ddd,	5.61 ddd,	5.36 ddd,	5.64 ddd,	5.93 ddd,	5.64 ddd,
	1.7,5.3,8.7	1.2,6.0,8.8	1.9,5.6,8.7	1.9,8.0,5.3	2.2,8.6.5.2	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	1.55 s
					4.08 dd,5.5,11.2	
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	3.63 dd,1.6,16.6	3.65 dd,1.7,16.1	4.54 dd,1.8,16.2	3.63 dd,1.5,16.4	1.09 s
	4.86 dd,7.2,16.1	4.38 dd,5.6,16.6	4.24 dd,4.3,16.1	4.43 dd,5.8,16.4	4.42 dd,5.8,16.4	
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
		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
Glu-1″			5.66 d,7.9	4.80 d,7.3		5.37 d,7.6
2″			4.14 m	4.11 m		4.14 dd,8.9,9.6
3″			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	4.42 dd,1.8,11.2		4.58 dd,2.3,11.0
			4.63 dd,6.2,11.2	4.94 dd,6.6,11.2		4.83 dd,6.8,11.0

[a] The assignments were based on DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC experiments.

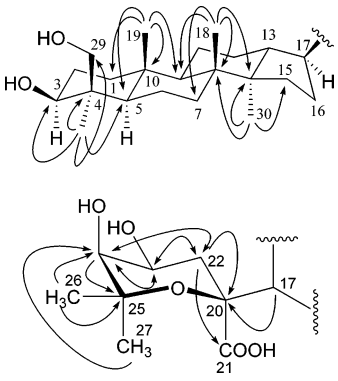

Figure 1. Important HMBC correlations of **1**.

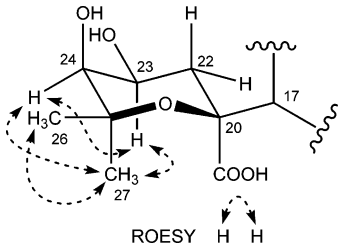

Figure 2. Important ROESY correlations of **1**.

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 $C_{36}H_{60}O_{13}$, as established by the HRFABMS ([M − H]$^-$, *m*/*z* 699.3944). The FABMS displayed a characteristic fragment ion peak at *m*/*z* 537 [M − 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-3β,23β,24β,27,29-pentahydroxydammaran-21-oic acid 3-*O*-β-D-glucopyranoside.

Compound **6** was obtained as a white powder and possessed the molecular formula $C_{42}H_{70}O_{16}$, as determined from its HRFABMS ([M − H]$^-$, *m*/*z* 829.4579) and ^{13}C NMR data. The ^{13}C 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 neoalsogenin M.[7] In the HMBC spectrum of **6**, correlations of the methyl proton at δ_H 1.09 (CH_3-29) with C-4 (δ_C 44.1), C-5 (δ_C 56.7), and C-28 (d_C 27.2) confirmed the structure of **6**. Thus, compound **6** was concluded to be 20*S*,25-epoxy-3β,23β,24β-trihydroxydammaran-21-oic acid 3-*O*-β-D-glucopyranosyl(1→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 *Glorosprium 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 ^{1}H NMR and 125 MHz for ^{13}C 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 μm, 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 H_2O 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 H_2O−MeOH (1:0−0:1) to give seven fractions (B_1−B_7). Fraction B_7 (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 ($CHCl_3$−MeOH−H_2O, 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; $[\alpha]_D^{14}$ +24.7 (*c* 0.093, MeOH); IR ν_{max}^{KBr} cm^{-1} 3432, 2929, 1761, 1629, 1453, 1378, 1075, 1032; ^{1}H NMR data (400 MHz, pyridine-d_5), see Table 1; ^{13}C NMR data (125 MHz, pyridine-d_5), see Table 2; FABMS (negative ion mode) *m*/*z* 521 [M − H]$^-$, 457 [M − H − C_2H_5]; HRFABMS *m*/*z* 521.3476 [M − H]$^-$ (calcd for $C_{30}H_{50}O_7$, 521.3484).

Gentirigeoside A (2): white powder; mp 182−183 °C; $[\alpha]_D^{19}$ +18.9 (*c* 0.27, MeOH); IR ν_{max}^{KBr} cm^{-1} 3418, 2944, 1761, 1632, 1452, 1378, 1202, 1076, 1029; ^{1}H NMR data (500 MHz, pyridine-d_5), see Table 1; ^{13}C 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 $C_{36}H_{60}O_{12}$, 683.4006).

Gentirigeoside B (3): white powder; mp 183−184 °C; $[\alpha]_D^{19}$ +23.5 (*c* 0.29, MeOH); IR ν_{max}^{KBr} cm^{-1} 3418, 2943, 1762, 1634, 1453, 1378, 1201, 1076, 1028; ^{1}H NMR data (500 MHz, pyridine-d_5), see Table 1; ^{13}C 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 × 2]$^-$; HRFABMS *m*/*z* 845.4543 [M − H]$^-$ (calcd for $C_{42}H_{70}O_{17}$, 845.4534).

Gentirigeoside C (4): white powder; mp 185−186 °C; $[\alpha]_D^{18}$ +18.1 (*c* 0.38, MeOH); IR ν_{max}^{KBr} cm^{-1} 3423, 2938, 1761, 1633, 1452, 1378, 1201, 1077, 1030; ^{1}H NMR data (500 MHz, pyridine-d_5), see Table 1; ^{13}C NMR data (125 MHz, pyridine-d_5), see Table 2; FABMS (negative ion mode) *m*/*z* 845 [M − H]$^-$, 683 [M − H − 162 × 2]$^-$, 521 [M − H − 162 × 2]$^-$; HRFABMS *m*/*z* 845.4547 [M − H]$^-$ (calcd for $C_{42}H_{70}O_{17}$, 845.4534).

Gentirigeoside D (5): white powder; mp 182−183 °C; $[\alpha]_D^{20}$ +17.6 (*c* 0.32, MeOH); IR ν_{max}^{KB} cm^{-1} 3424, 2943, 1752, 1631, 1453, 1377, 1074, 1027; ^{1}H NMR data (500 MHz, pyridine-d_5), see Table 1; ^{13}C 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 $C_{36}H_{60}O_{13}$, 699.3955).

Gentirigeoside E (6): white powder; mp 187−188 °C; $[\alpha]_D^{19}$ +11.5 (*c* 0.23, MeOH); IR ν_{max}^{KB} cm^{-1} 3424, 2929, 1761, 1632, 1454, 1377, 1204, 1165, 1076, 1030; ^{1}H NMR data (500 MHz, pyridine-d_5), see Table 1; ^{13}C 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 $C_{42}H_{70}O_{17}$, 829.34585).

Enzymatic Hydrolysis of Compounds 2−6. Solutions of **2**−**6** (each 2 mg) in H_2O (10 mL) were incubated with β-glucosidase (Sigma) at 37 °C for two weeks. The mixture was partitioned between *n*-BuOH and H_2O, 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−H_2O, 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 ($CHCl_3$−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 × 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−H_2O (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−H_2O, 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 sqauld, and *Glorosprium musarum* Cookeet Mass.

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