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Dammarane Glycosides from the Root of *Machilus yaoshansis*

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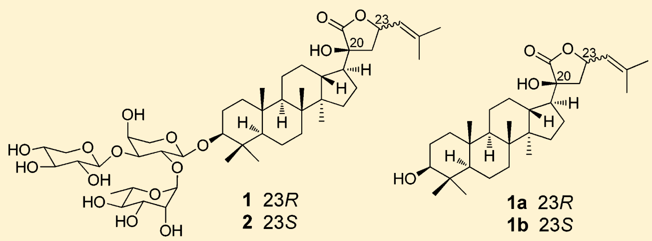
Supporting Information

ABSTRACT: Nine new dammarane triterpene glycosides (**1**−**3** and **8**−**13**) and 12 known analogues have been isolated from an ethanol extract of the roots of *Machilus yaoshansis*. Compounds **1**−**7** have an uncommon 20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone moiety that was previously reported in compounds isolated from *Gynostemma pentaphyllum*. The configurations of the lactone moieties in **1**−**3** were determined by comparison of the experimental ECD spectra of **1**−**3** and the hydrolysates, **1a** and **1b**, with the corresponding calculated ECD spectra. On the basis of NMR and ECD data analysis of **1**−**7**, the previously reported C-20 and C-23 configurations of **4**−**7** and related derivatives from *Gynostemma pentaphyllum* were revised. In addition, the application of NMR data and Cotton effects to the determination of the relative and absolute configurations of the γ-lactone moiety in 3β,20,23-trihydroxydammar-24-en-21-oic acid-21,23-lactone derivatives is discussed.



Species of the genus *Machilus* have long been used for the treatment of edema, abdominal distension, pain, and inflammation in China and Southeast Asia.[1] As part of a program to assess the chemical and biological diversity of *Machilus* species,[2] we focused our study on *Machilus yaoshansis* S. Lee et F. N. Wei, a plant that is widely distributed in the south of China and used as a folk medicine by the ethnic Zhuang in Guangxi Province for the treatment of rheumatism. Our previous studies on the bark[3] and the root[4] of *M. yaoshansis* led to the isolation and characterization of several unusual cucurbitane derivatives and spirolactones with cytotoxic activities. Continuing examination of the root extract has resulted in the characterization of nine new (**1**−**3** and **8**−**13**) and 12 known dammarane glycosides. Herein, we discuss the detailed structural determination of the isolates by extensive spectroscopic analysis, including 1D and 2D NMR and electronic circular dichroism (ECD). Compounds **1**−**7** possess an unusual 20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone moiety that was previously reported in compounds isolated from *Gynostemma pentaphyllum*. The C-20 and C-23 configurations of the 20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone moieties of **1**−**3** were established by comparison of the ECD spectra calculated using the time-dependent density functional theory (TDDFT) with the experimental ECD spectra. On the basis of detailed NMR and ECD data analysis of **1**−**7**, the previously reported C-20 and C-23 configurations of **4**−**7** and related derivatives from *G. pentaphyllum* were revised. In addition, the application of NMR data and Cotton effects to the determination of the relative and absolute configurations of the γ-lactone moiety in 3β,20,23-trihydroxydammar-24-en-21-oic acid-21,23-lactone derivatives is discussed.

■ RESULTS AND DISCUSSION

Compound **1** was obtained as an amorphous powder, and its molecular formula was determined to be $C_{46}H_{74}O_{16}$ by positive-ion HRESIMS data at *m/z* 905.4832 $[M + Na]^+$ (calcd for $C_{46}H_{74}O_{16}Na$, 905.4874), combined with the NMR data (Tables 1 and 3). The IR spectrum suggested the presence of OH (3383 cm^{-1}) and γ-lactone (1760 cm^{-1}) functionalities. The ^1H NMR spectrum showed resonances assignable to seven tertiary methyl groups between δ_H 0.76 and 1.69 and five deshielded methines (anomeric, olefinic, and oxygenated) at δ_H 6.13 (brs, H-1″), 5.59 (d, J = 8.8 Hz, H-24), 5.47 (ddd, J = 8.8, 7.6, 6.0 Hz, H-23), 4.92 (d, J = 5.2 Hz, H-1′), and 5.02 (d, J = 7.2 Hz, H-1‴). The ^1H NMR spectrum also showed partially overlapped signals due to oxymethine and oxymethylene protons between δ_H 3.65 and 4.73. ^{13}C NMR and DEPT spectra showed 46 carbon resonances, of which three were attributed to anomeric carbons [δ_C 105.2 (C-1‴), 104.8 (C-1′), and 102.1 (C-1″)], two were attributed to a trisubstituted

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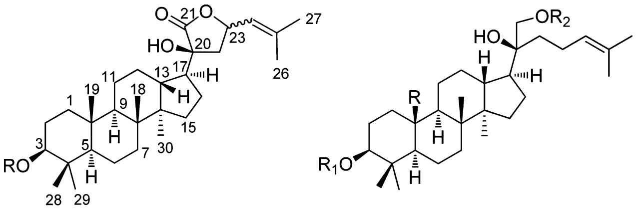
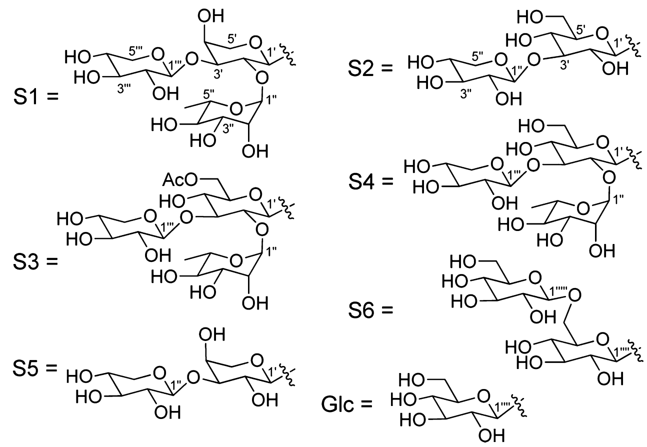


1 R = S1; 23*R*	**8** R = Me, R_1 = S1, R_2 = H
1a R = H; 23*R*	**8a** R = Me, R_1 = R_2 = H
1b R = H; 23*S*	**9** R = Me, R_1 = S1, R_2 = Glc
2 R = S1; 23*S*	**10** R = CHO, R_1 = S5, R_2 = H
3 R = S2; 23*S*	**11** R = CHO, R_1 = S4, R_2 = Glc
4 R = S3; 23*R*	**12** R = Me, R_1 = S3, R_2 = H
5 R = S4; 23*R*	**13** R = Me, R_1 = S4, R_2 = S6
6 R = S3, 23*S*	
7 R = S4, 23*S*	



double bond (δ_C 138.5 and 125.4), and one was attributed to a lactone carbonyl carbon (δ_C 179.4). These spectroscopic data suggested that **1** was a triglycosidic triterpenoid with γ-lactone and olefinic subunits. The sugars obtained by acid hydrolysis of **1** were identified as L-arabinopyranose, D-xylopyranose, and L-rhamnopyranose by GC analysis of the trimethylsilyl-L-cysteine derivatives of the hydrolysate of **1** and the authentic sugars.[5] The structure of **1** was finalized by analysis of the 2D NMR data. The HSQC experiment allowed for the assignments of the proton and protonated carbon resonances in the NMR spectra of **1**. In the $^1H-^1H$ COSY spectrum, the cross-peaks of H_2-1/H_2-2/H-3; H-5/H_2-6/H_2-7; H-9/H_2-11/H_2-12/H-13/H-17/H_2-16/H_2-15; and H_2-22/H-23/H-24 demonstrated the presence of vicinal coupling systems. Coupling constants of the anomeric protons indicated α configurations for the arabinopyranosyl ($J_{1',2'}$ = 5.2 Hz) and rhamnopyranosyl ($J_{1'',2''} \approx 0$ Hz) units and a β configuration for the xylopyranosyl ($J_{1''',2'''}$ = 7.2 Hz) unit.[6] In the HMBC spectrum, two- and three-bond correlations of H_3-18/C-7, C-8, C-9, and C-14; H_3-19/C-1, C-5, C-9, and C-10; H_3-26, H_3-27/C-24 and C-25; H_3-28 and H_3-29/C-3, C-4, and C-5; H_3-30/C-8, C-13, C-14, and C-15; and H_2-22/C-17, C-20, C-21, C-23, and C-24, together with the chemical shifts of these protons and carbons and the molecular formula, revealed that **1** had a 3,20,23-trihydroxydammar-24-en-21-oic acid-21,23-lactone nucleus.[6a,b] In addition, HMBC correlations of H-1′/C-3, H-1″/C-2′, and H-1‴/C-3′ demonstrated that the α-L-arabinopyranosyloxy unit was located at C-3 and that the α-L-rhamnopyranosyloxy and β-D-xylopyranosyloxy units were linked to C-2′ and C-3′ of the arabinopyranosyloxy unit, respectively. Thus, **1** was determined to be 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.

The configuration of the tetracyclic nucleus in **1** was proposed to be identical to that in the natural dammarane derivatives.[6] The chemical shift and splitting pattern of H-3 (δ_H 3.29, dd, J = 12.0 and 4.0 Hz) in the ^1H NMR spectrum indicated that it was axially α-oriented.[6a] The configuration of the γ-lactone in the side chain was established by analyses of the NOESY and ECD data of **1** and its hydrolysates (**1a** and **1b**), in combination with the theoretical ECD spectra based on TDDFT, a powerful tool for the configuration assignment of natural products.[7] In the NOESY spectrum, correlations of H-23/H-17 and H-22a revealed that these protons were cofacial on the γ-lactone ring, while the correlation of H-24/H-22b indicated that these protons were cofacial on the opposite side of the ring.

Acid hydrolysis of **1** with 1 M HCl at 60 °C produced two isomers, **1a** and **1b**. The NOESY spectrum of **1a** showed correlations of H-23/H-17 and H-22a and of H-22b/H-24 and OH-20. In contrast, **1b** did not give the corresponding correlations in its NOESY spectrum (Supporting Information, Figures S20 and S26). This indicated that **1a** had the same configuration as **1**, but **1b** was an epimer of **1a** with the opposite configuration at C-20 or C-23. This was supported by the similarity of the Cotton effects in the ECD spectra of **1** and **1a**, while the ECD spectrum of **1b** displayed Cotton effects opposite those of **1** and **1a** (Figure 1). The ECD spectra of **1a** and the (20*R*,23*S*)-isomer were calculated using TDDFT at the B3LYP/6-311++G(2d,2p) level. The calculated ECD spectrum of **1a** matched the experimental spectra of **1a** and **1**. This indicated the (20*S*,23*R*) configuration for **1** and **1a**. Therefore, compound **1** was determined to be (3β,20*S*,23*R*)-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.

Compound **2** is an isomer of **1**, as indicated by spectroscopic data. Comparison of the NMR data of **2** and **1** indicated that H-22a, H-22b, H-23, and H-24 in **2** were shifted by $\Delta\delta_H$ −0.10, −0.18, +0.24, and −0.18, respectively, while C-20, C-22, C-23, and C-24 were shifted by $\Delta\delta_C$ +2.1, −1.8, +1.1, and −1.4, respectively. This suggested that **2** was the C-23 epimer of **1**, as supported by the ECD spectrum of **2**, which displayed Cotton effects opposite those of **1**. Acid hydrolysis of **2** generated the same compounds as those from **1**, including the same sugars and **1a** and **1b**. ECD calculations of **1b** and the (20*R*,23*R*)-isomer demonstrated that the calculated ECD curve for **1b** was consistent with the experimental ECD spectra of **1b** and **2**. This confirmed that **2** was the C-23 epimer of **1**. Therefore, compound **2** was determined to be (3β,20*S*,23*S*)-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.

Compound **3** exhibited IR and NMR spectroscopic features similar to those of **2**. Comparison of the NMR, HRESIMS, and ECD data of **3** and **2** (Tables 1 and 3 and Experimental Section) indicated that the α-L-arabinopyranosyl and α-L-rhamnopyranosyl moieties in **2** were replaced by a β-D-glucopyranosyl moiety in **3**. This was confirmed by acid hydrolysis of **3** and subsequent GC analysis of the hydrolysate, according to the same protocol as that described for **1**. In the HMBC spectrum of **3**, correlations of H-1′/C-3 and H-1″/C-3′ revealed the linkage of the two sugar units. Thus, compound **3** was assigned as (3β,20*S*,23*S*)-3,20,23-trihydroxydammar-24-en-21-oic acid-21,23-lactone 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranoside.

Table 1. ^{1}H NMR Data for Compounds 1−3, 8, and 9 in Pyridine-d_5 (δ, mult., J in Hz)[a]

no.	1	2	3	8	9[b]
1	1.53 m, 0.81 m	1.56 m, 0.78 m	1.48 m, 0.76 m	1.52 m, 0.82 m	1.51 m, 0.80 m
2	2.05 m, 1.86 qd (13.2, 4.0)	2.08 m, 1.80 m	2.21 m, 1.82 m	2.04 m, 1.85 m	2.04 m, 1.83 m
3	3.29 dd (12.0, 4.0)	3.31 dd (12.0, 4.0)	3.37 dd (12.0, 4.0)	3.28 dd (11.5, 4.0)	3.26 dd (11.5, 4.0)
5	0.73 d (12.0)	0.74 d (11.2)	0.72 d (12.0)	0.74 brd (12.0)	0.72 brd (12.0)
6	1.46 m, 1.36 m	1.48 m, 1.38 m	1.50 m, 1.37 m	1.45 m, 1.36 m	1.44 m, 1.35 m
7	1.50 m, 1.21 m	1.50 m, 1.23 m	1.52 m, 1.22 m	1.52 m, 1.23 m	1.51 m, 1.21 m
9	1.30 m	1.31 m	1.28 m	1.33 m	1.29 m
11	1.46 m, 1.22 m	1.50 m, 1.30 m	1.50 m, 1.29 m	1.49 m, 1.20 m	1.37 m, 1.11 m
12	2.40 m, 1.34 m	2.51 brd (12.0), 1.47 m	2.51 brd (12.0), 1.46 m	2.22 brd (11.0), 1.41 m	2.13 m, 1.30 m
13	2.07 m	1.85 m	1.84 m	2.12 m	2.05 m
15	1.61 m, 1.12 m	1.60 m, 1.10 m	1.58 m, 1.09 m	1.65 m, 1.12 m	1.61 m, 1.08 m
16	2.02 m, 1.69 m	2.05 m, 1.30 m	2.07 m, 1.31 m	2.00, 1.94 m	1.92 m, 1.88 m
17	2.52 td (10.0, 4.8)	2.71 td (10.4, 5.6)	2.71 td (10.5, 4.5)	2.30 m	2.21 m
18	0.91 s	0.99 s	0.98 s	0.95 s	0.92 s
19	0.76 s	0.82 s	0.79 s	0.77 s	0.75 s
21				4.06 d (11.0)	4.38 d (11.0)
				4.00 d (11.0)	4.01 d (11.0)
22	2.70 dd (13.6, 7.2)	2.60 dd (13.2, 5.6)	2.59 dd (13.0, 5.5)	2.08 m	2.09 m
	2.30 dd (13.6, 6.0)	2.12 dd (13.2, 10.0)	2.10 dd (13.0, 9.5)	1.95 m	1.90 m
23	5.47 ddd (8.8, 7.6, 6.0)	5.71 ddd (10.0, 8.8, 5.6)	5.70 ddd (9.5, 9.0, 5.5)	2.49 m, 2.40 m	2.43 m, 2.31 m
24	5.59 d (8.8)	5.41d (8.8)	5.40 d (9.0)	5.33 t (6.0)	5.27 t (7.0)
26	1.62 s	1.67 s	1.67 s	1.66 s	1.64 s
27	1.69 s	1.63 s	1.63 s	1.63 s	1.62 s
28	1.19 s	1.20 s	1.30 s	1.19 s	1.17 s
29	1.11 s	1.14 s	1.00 s	1.11 s	1.10 s
30	0.90 s	0.91 s	0.90 s	0.99 s	0.93 s
1′	4.92 d (5.2)	4.92 d (5.2)	4.91 d (8.0)	4.92 d (5.0)	4.91 d (5.5)
2′	4.67 dd (7.2, 5.2)	4.67 dd (7.2, 5.2)	4.06 dd (8.5, 8.0)	4.66 dd (7.0, 5.0)	4.65 dd (7.0, 5.5)
3′	4.29 dd (7.2, 3.2)	4.31 dd (7.2, 3.2)	4.22 dd (8.5, 8.5)	4.30 dd (7.0, 3.0)	4.30 dd (7.0, 3.0)
4′	4.48 ddd (4.8, 3.2, 2.4)	4.48 ddd (4.8, 3.2, 2.4)	4.11 dd (8.5, 8.5)	4.48 ddd (4.5, 3.0, 2.5)	4.47 ddd (5.0, 3.0, 2.5)
5′a	4.33 dd (11.2, 4.8)	4.33 dd (11.2, 4.8)	3.95 ddd (8.5, 5.5, 2.0)	4.32 dd (12.0, 4.5)	4.33 dd (12.0, 5.0)
5′b	3.81 dd (11.2, 2.4)	3.82 dd (11.2, 2.4)		3.83 d (12.0, 2.5)	3.83 dd (12.0, 2.5)
6′a			4.53 dd (12.5, 2.0)		
6′b			4.33 dd (12.5, 5.5)		
1″	6.13 brs	6.14 brs	5.26 d (8.0)	6.13 brs	6.11 brs
2″	4.73 dd (3.6, 1.6)	4.73 dd (3.2, 1.2)	4.01 dd (8.0, 8.0)	4.73 brs	4.72 brs
3″	4.57 dd (9.6, 3.6)	4.57 dd (9.6, 3.2)	4.14 dd (8.0, 8.0)	4.57 dd (9.0, 3.5)	4.57 dd (9.0, 3.5)
4″	4.27 dd (9.6, 9.6)	4.28 dd (9.6, 9.6)	4.14 ddd (10.0, 8.0, 5.0)	4.27 dd (9.0, 9.0)	4.26 dd (9.0, 8.5)
5″a			4.29 dd (11.0, 5.0)		
5″b	4.59 dq (9.6, 6.0)	4.59 m	3.70 dd (11.0, 10.0)	4.59 m	4.59 m
6″	1.62 d (6.0)	1.62 d (6.0)		1.62 d (6.0)	1.61 d (6.0)
1‴	5.02 d (7.2)	5.02 d (7.6)		5.01 d (7.5)	5.00 d (7.0)
2‴	3.92 dd (8.0, 7.2)	3.93 dd (8.0, 7.6)		3.93 dd (8.0, 7.5)	3.92 dd (8.0, 7.0)
3‴	4.09 dd (8.4, 8.0)	4.08 dd (8.4, 8.0)		4.09 dd (8.0, 8.0)	4.08 dd (8.0, 8.0)
4‴	4.11 ddd (10.8, 8.4, 4.8)	4.11 ddd (10.8, 8.4, 4.8)		4.11 ddd (9.5, 8.0, 4.5)	4.11 ddd (9.5, 8.0, 5.0)
5‴a	4.30 dd (10.8, 4.8)	4.30 dd (10.8, 4.8)		4.30 dd (11.0, 4.5)	4.30 dd (11.0, 5.0)
5‴b	3.65 dd (10.8, 10.8)	3.66 dd (10.8, 10.8)		3.65 dd (11.0, 9.5)	3.66 dd (11.0, 9.5)

[a] ^{1}H NMR data were measured at 400 MHz for **1** and **2** and 500 MHz for **3**, **8**, and **9**. The assignments were based on DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC experiments. [b] Data for Glc-21: δ 5.04 d (7.5 Hz, H-1⁗), 4.08 dd (8.5 and 7.5 Hz, H-2⁗), 4.20 t (8.5 Hz, H-3⁗), 4.22 t (8.5 Hz, H-4⁗), 3.97 m (H-5⁗), 4.55 dd (11.5 and 2.5 Hz, H-6⁗a), and 4.37 dd (11.5 and 4.0 Hz, H-6⁗b).

The IR, NMR, and HRESIMS spectroscopic data for **4**, **5**, **6**, and **7** (Supporting Information, Tables S2 and S3) were identical to those of (3*β*,20*S*,23*S*)-3,20,23-trihydroxydammar-24-en-21-oic acid-21,23-lactone 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-6-*O*-acetylglucopyranoside, (3*β*,20*S*,23*S*)-3,20,23-trihydroxydammar-24-en-21-oic acid-21,23-lactone 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranoside, (3*β*,20*R*,23*R*)-3,20,23-trihydroxydammar-24-en-21-oic acid-21,23-lactone 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-6-*O*-acetylglucopyranoside, and (3*β*,20*R*,23*R*)-3,20,23-trihydroxydammar-24-en-21-oic acid-21,23-lactone 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranoside, respectively, which were isolated from *G. pentaphyllum*.[6b] These compounds, along with five other derivatives[6a,b,8] from *G. pentaphyllum*, represent glycosides with the 3*β*,20,23-trihydroxydammar-24-en-21-oic acid-21,23-lactone aglycone moiety. However, the initial assignment of

Table 2. ^{1}H NMR Data for Compounds **10−13** in Pyridine-d_5 (δ, mult., J in Hz)[a]

no.	10	11[b]	12[c]	13[d]
1	2.63 m, 0.71 m	2.45 m, 0.63 m	1.60 m, 0.96 m	1.38 m, 0.75 m
2	2.16 m, 1.67 m	2.23 m, 1.62 m	2.21 m, 1.87 m	2.23 m, 1.81 m
3	3.38 dd (11.6, 3.6)	3.38 dd (12.0, 4.0)	3.32 dd (12.0, 4.0)	3.34 dd (11.5, 4.0)
5	1.18 m	1.08 brs	0.76 brd (11.0)	0.67 brd (11.5)
6	1.85 m, 1.65 m	1.85 m, 1.60 m	1.47 m, 1.38 m	1.45 m, 1.35 m
7	1.62 m, 1.35 m	1.58 m, 1.31 m	1.52 m, 1.23 m	1.47 m, 1.19 m
9	1.70 m	1.65 m	1.38 m	1.24 m
11	1.72 m, 1.17 m	1.58 m, 1.06 m	1.49 m, 1.20 m	1.35 m, 1.14 m
12	2.17 m, 1.37 m	2.12 m, 1.29 m	2.24 m, 1.42 m	2.26 m, 1.35 m
13	2.05 m	1.97 m	2.13 m	2.03 m
15	1.59 m, 1.17 m	1.56 m, 1.12 m	1.66 m, 1.13 m	1.61 m, 1.08 m
16	2.02 m, 1.92 m	2.03 m, 1.92 m	2.00 m, 1.94 m	1.92 m, 1.81 m
17	2.27 m	2.23 m	2.30 m	2.31 m
18	0.86 s	0.94 s	0.96 s	0.92 s
19	10.03 s	10.25 s	0.79 s	0.73 s
21	4.01 d (10.4)	4.34 d (11.0)	4.06 d (11.0)	4.29 d (11.0)
	3.94 d (10.4)	3.97 d (11.0)	3.99 d (11.0)	4.08 d (11.0)
22	2.05 m, 1.93 m	2.04 m, 1.86 m	2.11 m, 1.96 m	2.01 m, 1.82 m
23	2.45 m, 2.38 m	2.44 m, 2.31 m	2.49 m, 2.39 m	2.49 m, 2.30 m
24	5.32 t (7.2)	5.27 t (7.0)	5.33 t (7.0)	5.27 t (7.0)
26	1.67 s	1.62 s	1.66 s	1.63 s
27	1.61 s	1.61 s	1.62 s	1.65 s
28	1.33 s	1.27 s	1.24 s	1.22 s
29	0.91 s	1.11 s	1.15 s	1.15 s
30	0.98 s	0.83 s	0.98 s	0.92 s
1′	4.75 d (7.6)	4.86 d (7.5)	4.84 d (7.5)	4.88 d (7.5)
2′	4.54 dd (8.8, 7.6)	4.22 d (8.0, 7.5)	4.21 dd (8.0, 7.5)	4.23 dd (8.0, 7.5)
3′	4.18 dd (8.8, 3.6)	4.16 dd (8.0, 8.0)	4.15 dd (9.0, 8.0)	4.17 dd (8.5, 8.0)
4′	4.43 brs	3.98 dd (8.0, 8.0)	3.82 dd (9.0, 9.0)	4.00 dd (8.5, 8.5)
5′a	4.25 dd (11.6, 4.0)	3.89 ddd (80, 5.5, 2.0)	3.97 ddd (9.0, 5.5, 2.0)	3.90 ddd (8.5, 5.5, 2.0)
5′b	3.80 brd (11.6)			
6′a		4.49 dd (12.5, 2.0)	4.82 dd (12.0, 2.0)	4.50 dd (12.0, 2.0)
6′b		4.25 dd (12.6, 5.5)	4.70 dd (12.0, 5.5)	4.26 dd (12.0, 5.5)
1″	5.26 d (7.6)	6.41 brs	6.44 brs	6.44 brs
2″	4.00 dd (8.0, 7.6)	4.79 dd (3.5, 1.5)	4.78 brd (3.5)	4.80 brd (3.5)
3″	4.14 dd (8.4, 8.0)	4.57 dd (9.5, 3.5)	4.58 dd (9.5, 3.5)	4.59 dd (9.0, 3.5)
4″	4.19 dd (10.0, 8.0, 4.8)	4.26 dd (9.5, 9.5)	4.29 dd (9.5, 9.5)	4.29 dd (9.0, 9.0)
5″a	4.34 dd (11.2, 4.8)	4.69 dq (9.5, 6.0)	4.74 dq (9.5, 6.0)	4.75 dq (9.0, 6.0)
5″b	3.70 dd (11.2, 10.0)			
6″		1.64 d (6.0)	1.67 d (6.0)	1.67 d (6.0)
1‴		4.99 d (8.0)	4.98 d (7.5)	4.99 d (8.0)
2‴		3.95 dd (8.0, 8.0)	3.96 dd (8.5, 7.5)	3.96 dd (8.5, 8.0)
3‴		4.07 dd (8.0, 8.0)	4.07 dd (9.0, 8.5)	4.07 dd (8.5, 8.5)
4‴		4.10 ddd (10.0, 8.0, 5.0)	4.11 ddd (10.5, 9.0, 5.0)	4.10 m
5‴a		4.27 dd (11.0, 5.0)	4.27 dd (11.0, 5.0)	4.27 m
5‴b		3.68 dd (11.0, 10.0)	3.68 dd (11.0, 10.5)	3.68 dd (11.0, 10.0)

[a] ^{1}H NMR data were measured at 400 MHz for **10** and 500 MHz for **11−13**. The assignments were based on DEPT, $^{1}H-^{1}H$ COSY, HSQC, and HMBC experiments. [b] Data for Glc-21 of **11**: δ 5.02 d (7.5 Hz, H-1⁗), 4.08 dd (8.0 and 7.5 Hz, H-2⁗), 4.21 dd (8.0 and 9.0 Hz, H-3⁗), 4.23 t (9.0 Hz, H-4⁗), 3.97 m (H-5⁗), 4.55 dd (12.0 and 2.0 Hz, H-6⁗a), and 4.36 dd (12.0 and 5.5 Hz, H-6⁗b). [c] Data for O*Ac* of **12**: δ 2.04 s. [d] Data for inner Glc-21 of **13**: δ 4.95 d (8.0 Hz, H-1⁗), 4.04 t (8.0 Hz, H-2⁗), 4.17 t (8.0 Hz, H-3⁗), 4.08 dd (8.0 and 9.0 Hz, H-4⁗), 4.10 m (H-5⁗), 4.87 brd (12.0 Hz, H-6⁗a), and 4.22 m (H-6⁗b); for terminal Glc-21 of **13**: δ 5.00 d (8.0 Hz, H-1′⁗),4.04 t (8.0 Hz, H-2′⁗), 4.22 dd (8.0 and 9.0 Hz, H-3′⁗), 4.20 dd (8.0 and 9.0 Hz, H-4′⁗), 3.90 m (H-5′⁗), 4.49 dd (12.0 and 2.0 Hz, H-6′⁗a), and 4.35 dd (12.0 and 5.0 Hz, H-6′⁗b).

(20*S*) and (20*R*) configurations for two reported C-20 epimers,[6a] made by comparison of the chemical shifts of C-13 and C-17 in these compounds with those in known ginseng saponins,[9] was ambiguous. This is because the substitution patterns of these epimers differed significantly from those of the reference ginseng saponins, and the configurations of the other reported compounds were determined by comparison of their NMR data with those of the two C-20 epimers. This, together with the configuration assignments of **1−3**, prompted us to re-examine the configurations of the reported compounds by comparing the NMR data of **1−3** with those of **4−7** and the reported compounds.[6a,b,8] Without exception, the data for the aglycone moiety in **1** were consistent with those of the aglycone moieties in **4** and **5** and the (20*S*,23*S*)-aglycone moiety in the reported compounds. In addition, the data for the aglycone moieties in **2** and **3** were similar to those of the aglycone moieties in **6** and **7** and the (20*R*,23*R*)-aglycone moiety in the reported glycosides. This, in combination with the configuration assignments of **1−3** made on the basis of NOESY and ECD data, indicated that **4** and **5** had the same aglycone as **1** and that **6** and **7** had the same aglycone as **2** and **3**. This was proved by the Cotton effects in the ECD spectra of **4−7**, as well as by acid hydrolysis of **4−7**, which generated the same products (**1a** and **1b**) as those from **1−3**. These results also indicated that the configuration of the reported (3*β*,20*S*,23*S*)- and (3*β*,20*R*,23*R*)-3,20,23-trihydroxydammar-24-en-21-oic acid-21,23-lactone derivatives[6b,8] should be revised as (3*β*,20*S*,23*R*) and (3*β*,20*S*,23*S*), respectively. This was further supported by the $[\alpha]^{20}_{D}$ values of **4−7**, **1a**, and **1b**, which were consistent with those of the reported compounds having the same gross structures.[6b,10] In addition, the configurations at C-20 of the (20*R*,23*ξ*)- and (20*S*,23*ξ*)-derivatives[6a,10] did not match the presented structures, and on basis of the above revision, the configurations had to be revised as (20*S*,23*R*) and (20*S*,23*S*), respectively. Therefore, **4**, **5**, **6**, and **7** were defined as (3*β*,20*S*,23*R*)-3,20,23-trihydroxydammar-24-en-21-oic acid-21,23-lactone 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-6-*O*-acetylglucopyranoside, (3*β*,20*S*,23*R*)-3,20,23-trihydroxydammar-24-en-21-oic acid-21,23-lactone 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranoside, (3*β*,20*S*,23*S*)-3,20,23-trihydroxydammar-24-en-21-oic acid-21,23-lactone 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-6-*O*-acetylglucopyranoside, and (3*β*,20*S*,23*S*)-3,20,23-trihydroxydammar-24-en-21-oic acid-21,23-lactone 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranoside, respectively. The sugar

Table 3. ^{13}C NMR Data for Compounds 1−3 and 8−13 in Pyridine-d_5^{a}

no.	1	2	3	8	9^{b}	10	11^{c}	12^{d}	13^{e}
1	39.6	39.8	39.7	39.8	39.8	33.5	33.6	39.7	39.7
2	26.8	26.8	26.7	26.8	26.8	27.7	27.7	26.8	26.9
3	88.3	88.2	89.1	88.3	88.3	87.5	88.1	88.6	88.9
4	39.8	39.6	39.3	39.6	39.5	40.1	40.4	39.7	39.7
5	56.6	56.6	56.4	56.6	56.5	54.6	54.9	56.8	56.6
6	18.4	18.4	18.4	18.5	18.4	17.7	17.6	18.5	18.5
7	35.7	35.7	35.7	35.7	35.8	34.7	34.6	35.7	35.6
8	40.8	40.7	40.7	40.8	40.7	40.4	40.0	40.8	40.7
9	51.2	51.2	51.1	51.2	51.1	52.9	52.8	51.2	51.0
10	37.1	37.1	37.0	37.0	37.0	52.8	52.7	37.1	37.0
11	21.8	21.8	21.8	21.9	21.8	22.4	22.3	21.9	21.8
12	27.3	28.0	27.9	28.1	27.8	27.9	27.9	28.1	27.7
13	43.3	45.0	45.0	41.8	41.7	41.6	41.6	41.8	41.9
14	50.7	50.2	50.2	50.5	50.5	50.3	50.3	50.5	50.3
15	31.7	31.7	31.7	31.8	31.6	32.1	32.0	31.7	31.5
16	25.8	26.2	26.2	24.8	24.7	24.7	24.7	24.8	24.9
17	45.8	45.3	45.3	46.3	46.1	46.1	46.0	46.3	46.5
18	15.6	15.6	15.6	15.8	15.8	16.6	16.0	15.8	15.8
19	16.5	16.6	16.5	16.6	16.6	205.7	205.5	16.6	16.6
20	79.0	81.1	81.1	76.6	76.4	76.4	76.3	76.6	76.3
21	179.4	178.3	178.3	66.8	76.3	66.6	76.2	66.8	77.8
22	40.8	39.0	39.0	36.6	36.6	36.6	36.5	36.6	35.8
23	74.1	75.2	75.3	23.3	23.3	23.2	23.3	23.3	23.1
24	125.4	124.0	124.0	126.2	126.0	126.2	126.0	126.2	126.0
25	138.5	139.4	139.4	130.8	130.8	130.2	130.9	130.8	130.9
26	25.6	25.6	25.6	25.8	25.8	25.8	25.8	25.8	25.8
27	18.1	18.1	18.1	17.7	17.8	17.7	17.8	17.7	17.8
28	27.9	27.9	28.0	28.0	27.9	26.5	26.3	27.8	27.9
29	16.8	16.8	16.8	16.7	16.6	15.9	16.5	16.7	16.7
30	16.6	16.2	16.2	16.8	16.8	17.2	17.2	16.8	16.9
1′	104.8	104.7	106.5	104.8	104.7	107.4	104.9	105.0	104.9
2′	74.7	74.7	74.7	74.7	74.6	71.8	76.9	76.6	77.0
3′	81.5	81.5	87.8	81.6	81.4	83.5	87.6	87.8	88.2
4′	68.3	68.3	69.6	68.3	68.2	69.4	69.9	69.8	69.8
5′	64.8	64.8	78.1	64.8	64.8	67.1	78.2	74.4	78.0
6′			62.7				62.6	64.1	62.6
1″	102.1	102.1	106.3	102.1	102.0	106.9	101.8	101.8	101.8
2″	72.5	72.5	75.2	72.5	72.4	75.3	72.4	72.4	72.4
3″	72.6	72.6	78.2	72.6	72.5	78.2	72.6	72.5	72.5
4″	74.0	73.9	70.9	74.0	73.9	71.1	73.9	73.9	73.9
5″	70.1	70.1	67.4	70.1	70.6	67.2	69.7	69.9	69.7
6″	18.6	18.6		18.6	18.6		18.6	18.6	18.6
1‴	105.2	105.1		105.1	105.0		104.9	104.9	105.0
2‴	74.6	74.6		74.6	74.5		74.8	74.8	74.8
3‴	77.7	77.7		77.7	77.6		78.3	78.3	78.2
4‴	70.7	70.9		70.9	70.9		70.6	70.6	70.6
5‴	67.0	67.0		67.0	67.0		67.3	67.3	67.3

^{a}Data were measured at 100 MHz for **1**, **2**, and **10** and 125 MHz for **3**, **8**, **9**, and **11**−**13**. The assignments were based on DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC experiments. ^{b}Data for Glc-21 of **9**: δ 106.2 (C-1⁗), 75.5 (C-2⁗), 78.7 (C-3⁗), 71.1 (C-4⁗), 78.6 (C-5⁗), 62.8 (C-6⁗). ^{c}Data for Glc-21of **11**: δ 106.2 (C-1⁗), 75.5 (C-2⁗), 78.7 (C-3⁗), 71.1 (C-4⁗), 78.6 (C-5⁗), 62.8 (C-6⁗). ^{d}Data for OAc of **12**: δ 170.6, 20.8. ^{e}Data for inner Glc-21of **13**: δ 106.3 (C-1⁗), 75.4 (C-2⁗), 78.5 (C-3⁗), 71.7 (C-4⁗), 77.0 (C-5⁗), 70.1 (C-6⁗); for terminal Glc-21 of **13**: δ 105.2 (C-1′′′′′), 75.2 (C-2′′′′′), 78.3 (C-3′′′′′), 71.5 (C-4′′′′′), 78.3 (C-5′′′′′), 62.6 (C-6′′′′′).

units in **4**−**7** were confirmed by acid hydrolysis and subsequent GC analysis of the hydrolysates using the protocol described earlier, and their linkages were confirmed by 2D NMR data.

Detailed NMR data analysis of **1**−**7** and the reported compounds[6a,b,8,10] indicated that the readily distinguishable H-23 and H-24 resonances in the ^{1}H NMR spectra should be applicable to the determination of the relative configuration of the γ-lactone moiety in the 3β,20,23-trihydroxydammar-24-en-21-oic acid-21,23-lactone derivatives. In compounds with OH-20 and H-23 in the *trans* orientation [(20*S*,23*R*)- or (20*R*,23*S*)-isomers], the H-24 resonance was deshielded by OH-20. Thus, the chemical shift of H-24 (δ_H 5.60 ± 0.02 for the glycosides in pyridine-d_5; δ_H 5.31 and 5.26 ± 0.01 for the aglycone in acetone-d_6 and $CDCl_3$, respectively) was larger than that of

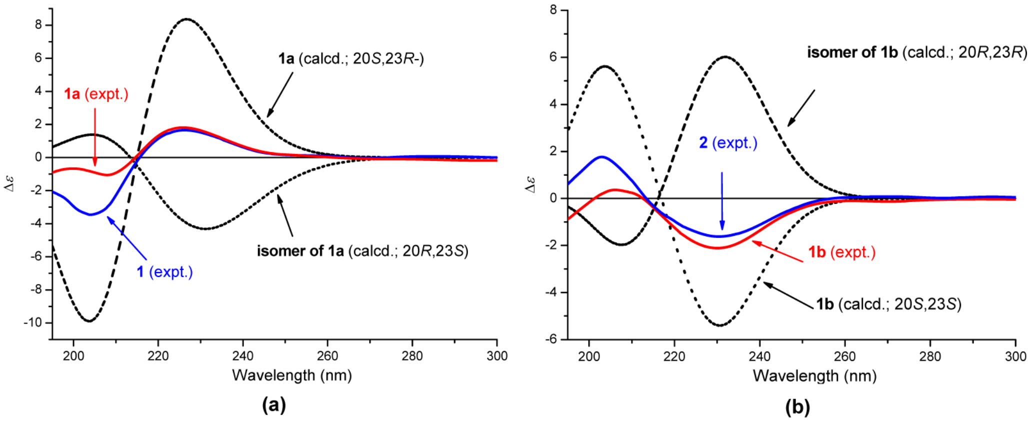


Figure 1. (a) Experimental ECD spectra of **1** and **1a** and the calculated ECD spectra of **1a** and the (20*R*,23*S*)-isomer of **1a**. (b) Experimental ECD spectra of **2** and **1b** and the calculated ECD spectra of **1b** and the (20*R*,23*R*)-isomer of **1b**.

H-23 (δ_H 5.47 ± 0.02 for the glycosides in pyridine-d_5; δ_H 5.23 and 5.12 for the aglycone in acetone-d_6 and $CDCl_3$, respectively). On the other hand, in the compounds with OH-20 and H-23 in the *cis* orientation [(20*S*,23*S*)- or (20*R*,23*R*)-isomers], the H-23 resonance was deshielded by OH-20 and the chemical shift of H-23 (δ_H 5.70 ± 0.02 for the glycosides in pyridine-d_5; δ_H 5.31 and 5.34 for the aglycone in acetone-d_6 and $CDCl_3$, respectively) was larger than that of H-24 (δ_H 5.42 ± 0.02 for the glycosides in pyridine-d_5; δ_H 5.24 and 5.19 for the aglycone in acetone-d_6 and $CDCl_3$, respectively). To eliminate errors, the chemical shift difference between the H-24 and H-23 resonances ($\Delta\delta_H = \delta_{H\text{-}24} - \delta_{H\text{-}23}$) is proposed to be used in determination of the relative configuration of the γ lactone moiety in the 3β,20,23-trihydroxydammar-24-en-21-oic acid 21,23-lactone derivatives. For compounds with OH-20 and H-23 in the *trans* orientation, the $\Delta\delta_H$ value is positive (approximately +0.13 ± 0.02 ppm for the glycosides in pyridine-d_5; +0.08 ± 0.01 and +0.14 ± 0.01 ppm for the aglycone in acetone-d_6 and $CDCl_3$, respectively). Conversely, for the compounds with OH-20 and H-23 in the *cis* orientation, the $\Delta\delta_H$ value is negative (around −0.30 ± 0.02 ppm for the glycosides in pyridine-d_5; −0.07 ± 0.01 and −0.15 ± 0.01 ppm for the aglycone in acetone-d_6 and $CDCl_3$, respectively). In addition, the compounds with OH-20 and H-23 in the *trans* and *cis* orientations showed different chemical shifts for the C-20, C-21, C-22, C-23, C-24, and C-25 resonances in the ^{13}C NMR spectra. In pyridine-d_5, for the glycosides with OH-20 and H-23 in the *trans* orientation, C-20, C-23, and C-25 were shielded by $\Delta\delta_C$ −2.1 ± 0.1, −1.1 ± 0.1, and −1.0 ± 0.1 ppm, respectively, as compared with those for the compounds with OH-20 and H-23 in the *cis* orientation, whereas C-21, C-22, and C-24 were deshielded by $\Delta\delta_C$ +1.1 ± 0.2, +1.6 ± 0.2, and +1.4 ± 0.2 ppm. To eliminate errors, the chemical shift difference between the readily distinguishable C-25 and C-24 resonances ($\Delta\delta_C = \delta_{C\text{-}25} - \delta_{C\text{-}24}$) is suggested to be used in assignment of the relative configuration of the γ-lactone moiety in the 3β,20,23-trihydroxydammar-24-en-21-oic acid-21,23-lactone derivatives. The $\Delta\delta_C$ value is smaller than +14.0 ppm for the glycosides with OH-20 and H-23 in the *trans* orientation (+13.1 ± 0.1 ppm, pyridine-d_5), but for the glycosides with OH-20 and H-23 in the *cis* orientation, the $\Delta\delta_C$ value is larger than +14.0 ppm (+15.6 ± 0.2 ppm, pyridine-d_5). Similar chemical shift differences are observed for the aglycones **1a** and **1b** in $CDCl_3$ ($\Delta\delta_C$: +17.0 ± 0.2 ppm for **1a** and +18.1 ± 0.1 ppm for **1b**) (Supporting Information, Table S1).[10] Furthermore, comparison of the Cotton effects in the experimental ECD spectra of **1**−**7** with those in the calculated ECD spectra of **1a** and the (20*R*,23*S*)-isomer and **1b** and the (20*R*,23*R*)-isomer revealed that the absolute configurations of the γ-lactone moiety in the 3β,20,23-trihydroxydammar-24-en-21-oic acid 21,23-lactone derivatives could be determined by the Cotton effects. For the compounds with OH-20 and H-23 in the *trans* orientation, the (20*S*,23*R*)-isomers showed positive Cotton effects at 227 ± 1 nm and negative effects at 206 ± 2 nm (calculated at 231 and 208 nm), while the (20*R*,23*S*)-isomer showed the reversed Cotton effects at the corresponding wavelengths (calculated at 235 and 208 nm). For the compounds with OH-20 and H-23 in the *cis* orientation, the (20*S*,23*S*)-isomers showed negative Cotton effects at 230 ± 2 nm and positive effects at 204 ± 2 nm (calculated at 235 and 208 nm), while the (20*R*,23*R*)-isomer showed the reversed Cotton effects at the corresponding wavelengths (calculated at 236 and 212 nm). This also demonstrated that the C-23 configuration of the γ-lactone moiety in these compounds is determining the signs of the Cotton effects, while the configuration change at C-20 induces only a variation in the intensity of the Cotton effects.

Comparison of the NMR spectra of compound **8** with those of **1** (Tables 1 and 3) indicated that the only difference between these two compounds was replacement of the oxymethine (C-23) and carbonyl (C-21) units in **1** by methylene and hydroxymethyl groups in **8**, respectively. This suggested that **8** had a 3,20,21-trihydroxydammar-24-ene aglycone moiety,[6a,8a,11] which was further supported by HRESIMS and confirmed by 2D NMR analysis. Particularly, HMBC correlations of H_2-21/C-17, C-20, and C-22; H_2-23/C-20, C-22, C-24, and C-25; and H-1′/C-3, in combination with their chemical shifts, verified the presence of hydroxy groups at C-20 and C-21 in **8**. The *S* configuration at C-20 was proposed from the chemical shifts of C-20 (δ 76.6) and C-17 (δ 46.3).[11a,12] This was confirmed by the *in situ* dimolybdenum ECD method,[13] which was reported for the assignment of the configurations of acyclic 1,2-diols.[4,14] According to the empirical rule proposed by Snatzke,[13,15] the bands around 310 nm (band IV) and 400 nm (band II) in the $Mo_2(AcO)_4$-induced ECD spectrum, which have the same sign as the O−C−C−O torsion angle in the favored conformation, allow for the assignment of the absolute configuration.[13b,15,16] Acid hydrolysis of **8** yielded the aglycone **8a**. In the $Mo_2(AcO)_4$-induced ECD spectrum of **8a** (Supporting Information, Figure S68), positive Cotton effects at 309 and 386 nm supported the

20*S* configuration. Therefore, compound **8** was determined as (3*β*,20*S*)-3,20,21-trihydroxydammar-24-ene 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-xylopyranosyl-(1→3)]-*α*-L-arabinopyranoside.

The spectroscopic data of compound **9** indicated that it was a derivative of **8** with an additional *β*-glucopyranosyl unit. Comparison of the NMR spectra of **9** and **8** demonstrated that the H-21a, H-21b, and C-21 resonances in **9** were deshielded by $\Delta\delta_H$ +0.32, +0.01 and $\Delta\delta_C$ +9.5 ppm, respectively. This suggested that the *β*-glucopyranosyl unit was located at C-21 in **9**, which was confirmed by HMBC correlations of H-1⁗/C-21 and H_2-21/C-1⁗. Thus, compound **9** was determined as (3*β*,20*S*)-3,20,21-trihydroxydammar-24-ene 3-*O*-{[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-xylopyranosyl-(1→3)]-*α*-L-arabinopyranosyl}-21-*O*-*β*-D-glucopyranoside.

Spectroscopic data analysis of compound **10** ($C_{40}H_{66}O_{12}$) indicated that it was another derivative of **8**, with one less rhamnopyranosyl unit and the methyl group replaced by a formyl group (δ_H 10.03 and δ_C 205.7). In the HMBC spectrum, correlations of H-5 and H-9/C-19 and H-19/C-1, C-9, and C-10 revealed that the formyl group was located at C-10 in **10**, while HMBC correlations of H-1′/C-3 and H-1″/C-3′ proved the linkage of the sugar units. Thus, compound **10** was determined as (3*β*,20*S*)-19-oxo-3,20,21-trihydroxydammar-24-ene 3-*O*-*β*-D-xylopyranosyl-(1→3)-*α*-L-arabinopyranoside.

Comparison of the spectroscopic data of compound **11** with those of **9** indicated that the methyl (CH_3-19) and *α*-arabinopyranosyl units in **9** were replaced by a formyl group and a *β*-glucopyranosyl moiety in **11**, respectively. This was confirmed by 2D NMR analysis of **11**, as well as by acid hydrolysis of **11** followed by GC analysis of the hydrolysate using the same protocol as described above. In particular, HMBC correlations of H-5 and H-9/C-19; H-19/C-1, C-9, and C-10; H-1′/C-3; H-1″/C-2′; H-1‴/C-3′; and H-1⁗/C-21 verified the location of the formyl group and the linkage of the sugar units in **11**. Therefore, compound **11** was defined as (3*β*,20*S*)-19-oxo-3,20,21-trihydroxydammar-24-ene 3-*O*-{[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosyl}-21-*O*-*β*-D-glucopyranoside.

Compound **12** had the molecular formula $C_{47}H_{80}O_{16}$ as indicated by HRESIMS and NMR data. Comparison of the NMR data of **12** and **8** suggested that the *α*-arabinopyranosyl group in **8** was replaced by a *β*-D-6-*O*-acetylglucopyranosyl moiety in **12**. The suggestion was confirmed by 2D NMR experiments and GC analysis of the hydrolysate of **12**. Particularly, in the HMBC spectrum of **12**, correlations of H-1′/C-3, H-1″/C-2′, and H-1‴/C-3′ confirmed the linkage of the glycosyl moieties, while a correlation from the H_2-6′ resonance to the acetyl carbonyl carbon (δ_C 170.6) verified the location of the acetyl group at C-6′. Thus, compound **12** was determined as (3*β*,20*S*)-3,20,21-trihydroxydammar-24-ene 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-6-*O*-acetylglucopyranoside.

Compound **13** had the molecular formula $C_{59}H_{100}O_{26}$, as indicated by HRESIMS and NMR data. The NMR spectra of **13** were similar to those of **12**, except for the presence of resonances attributable to two additional *β*-glucopyranosyl units and the absence of the acetyl resonances in **13**. The NMR resonance assignments for **13** were confirmed by 2D NMR data analysis. Particularly, the HMBC correlations of H-1′/C-3, H-1″/C-2′, H-1‴/C-3′, H-1⁗/C-21, and H-1⁗′/C-6⁗ demonstrated the linkage of the sugar units in **13**. Therefore, compound **13** was determined as (3*β*,20*S*)-3,20,21-trihydroxydammar-24-ene 3-*O*-{[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosyl}-21-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside.

The other known compounds were identified by comparison of spectroscopic data with reported data as gylongiposide I, gypenosides XLVIII[11a] and XLIX,[12a] (3*β*,20*S*)-3,20,21-trihydroxydammar-24-ene 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranoside,[12c] (3*β*,20*S*)-3,19,20,21-tetrahydroxydammar-24-ene 3-*O*-{[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-xylopyranosyl-(1→3)]-*α*-L-arabinopyranosyl}-21-*O*-*β*-D-glucopyranoside, (3*β*,20*S*)-3,19,20,21-tetrahydroxydammar-24-ene 3-*O*-{[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosyl}-21-*O*-*β*-D-glucopyranoside, (3*β*,20*S*)-3,20,21-trihydroxydammar-24-ene 3-*O*-{[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosyl}-21-*O*-*β*-D-glucopyranoside, and (3*β*,20*S*)-3,20,21-trihydroxydammar-24-ene 3-*O*-{[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-6-*O*-acetylglucopyranosyl}-21-*O*-*β*-D-glucopyranoside.[11b]

Similar dammarane derivatives from *G. pentaphyllum* have been reported to possess biological activities such as protein tyrosine phosphatase 1B (PTP1B) inhibitory activity[8b,10] and potential apoptotic effect[17] or cytotoxic activity against various cancer cell lines.[18] The isolates from *M. yaoshansis* were tested at 10 *μ*M in preliminary assays to assess their PTP1B inhibitory activity,[19] cytotoxicity against A2780 ovary, HCT-8 colon, Bel-7402 hepatoma, BGC-823 stomach, and A549 lung cancer cell lines,[20] and the TNF-*α* secretion inhibitory activity of mouse peritoneal macrophages.[21] However, all the isolates were found to be inactive in all assays.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a Rudolph Research Autopol III automatic polarimeter. UV and ECD spectra were recorded using a JASCO J-815 spectropolarimeter. IR spectra were recorded using a Nicolet 5700 FT-IR microscope spectrometer (FT-IR microscope transmission). 1D and 2D NMR spectra were obtained at 400, 500, or 600 MHz for ^1H and 100, 125, or 150 MHz for ^{13}C using a Varian 400, 500, or 600 MHz NMR spectrometer in pyridine-d_5, acetone-d_6, or $CDCl_3$, with solvent peaks used as references. ESIMS data were measured using a Q-Trap LC/MS/MS (Turbo Ionspray Source) spectrometer. HRESIMS data were measured using an AccuToFCS JMS-T100CS spectrometer. Column chromatography was performed with HPD-100 macroporous adsorbent resin (Cangzhou Bonchem Co., Ltd., China), silica gel (200−300 mesh, Qingdao Marine Chemical Inc., China), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden). HPLC was performed using a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual *λ* absorbance detector with an Alltima (250 × 10 mm i.d.) preparative C_{18} (5 *μ*m) column. TLC was carried out with glass precoated silica gel GF_{254} plates. Spots were visualized under UV light or by spraying with 7% H_2SO_4 in 95% aqueous EtOH followed by heating.

Plant Material. Roots of *Machilus yaoshansis* were collected at Dayao Mountain, Guangxi, China, in December 2007. The plant was identified by Mr. Guang-Ri Long (Guangxi Forest Administration, Guangxi 545005, China). A voucher specimen (no. 07114) was deposited at the Herbarium of Guangxi Forest Administration, China.

Extraction and Isolation. The air-dried roots of *M. yaoshansis* (10 kg) were powdered and extracted with 95% aqueous EtOH (3 × 15 L) at room temperature (3 × 48 h). The EtOH extract was evaporated under reduced pressure to yield a dark brown residue (1050 g). The residue was suspended in H_2O (5 L) and partitioned with EtOAc (5 × 5 L). The aqueous phase was loaded onto an HPD-100 macroporous adsorbent resin (1500 g, dry weight) column. Successive elution with H_2O, 30% EtOH(aq), 70% EtOH(aq), and 95% EtOH(aq)

(10 L each) and solvent removal yielded four corresponding fractions. The fraction (170 g) eluted by 70% EtOH(aq) was separated over silica gel, with elution using a gradient of increasing MeOH concentration in $CHCl_3$ (2−100%) to give eight fractions (A−H). Fraction C (28 g) was fractionated via RP-MPLC using a preparative C_{18} (5 μm) column, with elution using a gradient of increasing MeOH concentration (0−85%) in H_2O to give fractions C_1−C_7. Fraction C_5 (2.9 g) was further chromatographed over Sephadex LH-20 (MeOH−H_2O, 1:1) to afford fractions $C_{5\text{-}1}$−$C_{5\text{-}4}$. Subsequent separation of $C_{5\text{-}2}$ by RP-HPLC using MeOH−H_2O (85:15) as the mobile phase afforded **1** (36 mg), **9** (15 mg), and **12** (9 mg), and separation of $C_{5\text{-}3}$ gave **2** (16 mg), **3** (33 mg), **8** (17 mg), and **10** (15 mg). Purification of $C_{5\text{-}4}$ by RP HPLC using MeOH−H_2O (83:17) as the mobile phase gave **11** (12 mg) and **13** (35 mg).

*(3β,20S,23R)-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 (**1**):* amorphous powder; $[\alpha]^{20}_{D}$ −14.8 (*c* 0.49, MeOH); ECD (MeOH) 204 (Δε −3.46), 227 (Δε +1.66) nm; IR ν_{max} 3383, 1760, 1647, 1549, 1449, 1377 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) data, see Table 1; ^{13}C NMR (pyridine-d_5, 100 MHz) data, see Table 3; ESIMS *m/z* 905 $[M + Na]^+$; HRESIMS *m/z* 905.4832 $[M + Na]^+$ (calcd for $C_{46}H_{74}O_{16}Na$, 905.4874).

*(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 (**2**):* amorphous powder; $[\alpha]^{20}_{D}$ +7.1 (*c* 0.10, MeOH); ECD (MeOH) 203 (Δε +1.76), 231 (Δε −1.62) nm; IR ν_{max} 3412, 1753, 1666, 1647, 1547, 1448, 1378 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) data, see Table 1; ^{13}C NMR (pyridine-d_5, 100 MHz) data, see Table 3; ESIMS *m/z* 905 $[M + Na]^+$; HRESIMS *m/z* 905.4914 $[M + Na]^+$ (calcd for $C_{46}H_{74}O_{16}Na$, 905.4874).

*(3β,20S,23S)-3,20,23-Trihydroxydammar-24-en-21-oic acid-21,23-lactone 3-O-β-D-xylopyranosyl-(1→3)-β-D-glucopyranoside (**3**):* amorphous powder; $[\alpha]^{20}_{D}$ +13.8 (*c* 0.65, MeOH); ECD (MeOH) 205 (Δε +0.62), 231 (Δε −1.31) nm; IR ν_{max} 3406, 1756, 1642, 1450, 1377 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) data, see Table 1; ^{13}C NMR (pyridine-d_5, 125 MHz) data, see Table 3; ESIMS *m/z* 789 $[M + Na]^+$; HRESIMS *m/z* 789.4394 $[M + Na]^+$ (calcd for $C_{41}H_{66}O_{13}Na$, 789.4401).

*(3β,20S)-3,20,21-Trihydroxydammar-24-ene 3-O-[α-L-rhamnopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside (**8**):* amorphous powder; $[\alpha]^{20}_{D}$ +6.0 (*c* 0.42, MeOH); IR ν_{max} 3401, 1643, 1562, 1451, 1377 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) data, see Table 1; ^{13}C NMR (pyridine-d_5, 125 MHz) data, see Table 3; ESIMS *m/z* 893 $[M + Na]^+$; HRESIMS *m/z* 893.5279 $[M + Na]^+$ (calcd for $C_{46}H_{78}O_{15}Na$, 893.5238).

*(3β,20S)-3,20,21-Trihydroxydammar-24-ene 3-O-{[α-L-rhamnopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranosyl}-21-O-β-D-glucopyranoside (**9**):* amorphous powder; $[\alpha]^{20}_{D}$ −13.6 (*c* 0.80, MeOH) IR ν_{max} 3420, 1642, 1562, 1451, 1375 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) data, see Table 1; ^{13}C NMR (pyridine-d_5, 125 MHz) data, see Table 3; ESIMS *m/z* 1055 $[M + Na]^+$; HRESIMS *m/z* 1055.5735 $[M + Na]^+$ (calcd for $C_{52}H_{88}O_{20}Na$, 1055.5767).

*(3β,20S)-19-Oxo-3,20,21-trihydroxydammar-24-ene 3-O-β-D-xylopyranosyl-(1→3)-α-L-arabinopyranoside (**10**):* amorphous powder; $[\alpha]^{20}_{D}$ +20.9 (*c* 0.85, MeOH); IR ν_{max} 3379, 1700, 1646, 1594, 1443, 1377 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) data, see Table 2; ^{13}C NMR (pyridine-d_5, 100 MHz) data, see Table 3; ESIMS *m/z* 761 $[M + Na]^+$; HRESIMS *m/z* 761.4424 $[M + Na]^+$ (calcd for $C_{40}H_{66}O_{12}Na$, 761.4452).

*(3β,20S)-19-Oxo-3,20,21-trihydroxydammar-24-ene 3-O-{[α-L-rhamnopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl}-21-O-β-D-glucopyranoside (**11**):* amorphous powder; $[\alpha]^{20}_{D}$ +2.2 (*c* 0.38, MeOH); IR ν_{max} 3406, 1702, 1647, 1448, 1378 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) data, see Table 2; ^{13}C NMR (pyridine-d_5, 125 MHz) data, see Table 3; ESIMS *m/z* 1099 $[M + Na]^+$; HRESIMS *m/z* 1099.5642 $[M + Na]^+$ (calcd for $C_{53}H_{88}O_{22}Na$,1099.5665).

*(3β,20S)-3,20,21-Trihydroxydammar-24-ene 3-O-[α-L-rhamnopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→3)]-β-D-6-O-acetylglucopyranoside (**12**):* amorphous powder; $[\alpha]^{20}_{D}$ −5.3 (*c* 0.32, MeOH); IR ν_{max} 3457, 3403, 1725, 1620, 1447, 1388 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) data, see Table 2; ^{13}C NMR (pyridine-d_5, 125 MHz) data, see Table 3; ESIMS *m/z* 965 $[M + Na]^+$; HRESIMS *m/z* 965.5454 $[M + Na]^+$ (calcd for $C_{49}H_{82}O_{17}Na$, 965.5450).

*(3β,20S)-3,20,21-Trihydroxydammar-24-ene 3-O-{[α-L-rhamnopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl}-21-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (**13**):* amorphous powder; $[\alpha]^{20}_{D}$ −9.3 (*c* 0.82, MeOH); IR ν_{max} 3390, 1683, 1646, 1451, 1374 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) data, see Table 2; ^{13}C NMR (pyridine-d_5, 125 MHz) data, see Table 3; ESIMS *m/z* 1223 $[M - H]^-$, 1247 $[M + Na]^+$; HRESIMS *m/z* 1247.6408 $[M + Na]^+$ (calcd for $C_{59}H_{100}O_{26}Na$, 1247.6400).

Acid Hydrolysis of 1−13. Compounds **1**−**13** (5−10 mg) were individually hydrolyzed with 1 N HCl−dioxane (1:1, 3 mL) at 60 °C for 6 h. After dilution with H_2O (5 mL), the reaction mixture was extracted with EtOAc to yield separate EtOAc and H_2O phases. The organic layer was concentrated, and the residue was purified by HPLC using 90% MeOH in H_2O to afford **1a** and **1b** from **1**−**7**, and **8a** from **8**, **9**, **12**, and **13**. **1a**: $[\alpha]^{20}_{D}$ −9.5 (*c* 0.1, MeOH); ECD (MeOH) 208 (Δε −1.06), 226 (Δε +1.82); ^1H NMR (acetone-d_6 and $CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Supporting Information, Table S1; ESIMS *m/z* 495 $[M + Na]^+$. **1b**: $[\alpha]^{20}_{D}$ +41.3 (*c* 0.3, MeOH); ECD (MeOH) 206 (Δε +0.36), 230 (Δε −2.11); ^1H NMR (acetone-d_6 and $CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Supporting Information, Table S1; ESIMS *m/z* 495 $[M + Na]^+$. **8a**: $[\alpha]^{20}_{D}$ +32.1 (*c* 0.3, MeOH); $Mo_2(OAc)_4$-induced ECD (DMSO) 272 (Δε′ −0.26), 309 (Δε′ +0.59), 359 (Δε′ +0.09), 386 (Δε′ +0.11); ^1H NMR ($CDCl_3$, 400 MHz) data, see Supporting Information, Table S2; ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Supporting Information, Table S3; ESIMS *m/z* 483 $[M + Na]^+$, 459 $[M - H]^-$.

The H_2O layer was evaporated under reduced pressure. After addition of H_2O (5 mL), the acidic solution was evaporated again, and this procedure was repeated until a neutral solution was obtained. The neutral solution was evaporated and dried *in vacuo* to furnish a monosaccharide residue. The residue was dissolved in pyridine (0.5 mL), and 2 mg of L-cysteine methyl ester hydrochloride was added. The mixture was maintained at 60 °C for 2 h, evaporated under a stream of N_2, and dried *in vacuo*. Next, 0.2 mL of *N*-trimethylsilylimidazole was added, and the resultant reaction mixture was maintained at 60 °C for 1 h. The mixture was partitioned between *n*-hexane and H_2O (2 mL each), and the *n*-hexane extract was analyzed by GC-MS under the following conditions: capillary column, DB-5 (30 m × 0.25 mm × 0.25 μm); detector, FID; detector temperature, 280 °C; injection temperature, 250 °C; initial temperature, 100 °C for 2 min and subsequent increase to 280 °C at a rate of 10 °C/min; final temperature, 280 °C for 5 min; carrier, N_2 gas. The absolute configurations of the sugars isolated from the hydrolysates of **1**−**13** were determined by comparing the retention times of their trimethylsilyl-L-cysteine derivatives with those of authentic sugars prepared by a similar procedure. The retention times of the trimethylsilyl-L-cysteine derivatives of the sugars were as follows: D-glucose, 19.55 min; D-xylopyranose, 17.65 min; L-rhamnopyranose, 18.38 min; and L-arabinopyranose, 17.79 min.

ECD Calculation. Conformational analyses of **1a** and the (20*R*,23*S*)-isomer were carried out via Monte Carlo searching in the MMFF94 molecular mechanics force field using the SPARTAN 08 software.[22] The two lowest energy conformers for **1a** and the 12 lowest energy conformers for the (20*R*,23*S*)-isomer (Supporting Information, Figure S2), whose relative energies were within 2 kcal/mol, were considered for further DFT calculations. Subsequently, the conformers were reoptimized using DFT at the B3LYP/6-31G(d) level in the gas phase with the Gaussian 09 program.[23] The B3LYP/6-31G(d) harmonic vibrational frequencies were further calculated to confirm their stability. The energies, oscillator strengths, and rotational strengths of the first two electronic excitations of the conformers were calculated using TDDFT methodology at the B3LYP/6-311++G-(2d,2p) level in the gas phase, and the ECD spectra were simulated by the GaussSum 2.25 program (σ = 0.8 eV).[24] To obtain the final spectra of **1a** and the (20*R*,23*S*)-isomer, the simulated spectra of the corresponding lowest energy conformations were averaged according to the Boltzmann distribution theory, in which the relevant Gibbs free energies (G) were adopted.

Conformational analyses in the MMFF94 force field showed three and eight lowest energy conformers for **1b** and the (20*R*,23*R*)-isomer, respectively, whose relative energies were within 2.0 kcal/mol (Supporting Information, Figure S4). The conformers were reoptimized using DFT at the B3LYP/6-31G(d) level in the gas phase. The energies, oscillator strengths, and rotational strengths of the first four electronic excitations for **1b** and the first two electronic excitations for the (20*R*,23*R*)-isomer were calculated using TDDFT methodology at the B3LYP/6-311++G(2d,2p) level in the gas phase, and the ECD spectra were simulated by the GaussSum 2.25 software (σ = 0.6 eV). The final spectra of **1b** and the (20*R*,23*R*)-isomer were obtained by averaging the corresponding spectra according to their relative conformational Gibbs free energies

PTP1B Inhibition Assay. See ref 19.

Cells, Culture Conditions, and Cell Proliferation Assay. See ref 20.

TNF-α Secretion Inhibition Assay. See ref 21.

■ ASSOCIATED CONTENT

Supporting Information

NMR data for compounds **1a** and **1b** (Table S1) and for **4−7** and **8a** (Tables S2 and S3). ECD spectra calculation details of **1a** and the (20*R*,23*S*)-isomer and **1b** and the (20*R*,23*R*)-isomer. Copies of MS, ECD, IR, and NMR spectra of compounds **1−13**. 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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