Homo- and Heptanor-sterols and Tremulane Sesquiterpenes from Cultures of *Phellinus igniarius*

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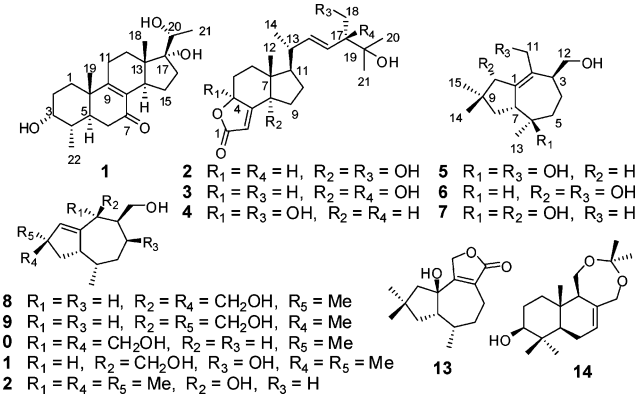
Four steroids, a homopregnene (**1**) and three heptanorergosterane derivatives (**2**−**4**), nine tremulane sesquiterpenes (**5**−**13**), and 18 known compounds have been isolated from cultures of the fungus *Phellinus igniarius*. Their structures and absolute configurations were elucidated by spectroscopic data analysis. In preliminary in vitro assays, at 10^{-5} M, compounds **8**, **9**, **13**, and 3β-hydroxy-11,12-*O*-isopropyldrimene (**14**) showed significant vascular-relaxing activities against phenylephrine-induced vasoconstriction with relaxing rates of 35.7%, 45.4%, 46.6%, and 32.1%, respectively, as compared with the blank control.

Phellinus igniarius (DC. Ex Fr.) Que'l (Polyporaceae) is a fungus that preferably hosts on stems of aspen, robur, and birch. Its fruit body is used to treat fester, abdominalgia, and bloody gonorrhea in traditional Chinese medicine.[1] Our previous investigation on the fruit body of this fungus resulted in isolation of more than 20 metabolites including antioxidant and/or cytotoxic phelligridins A−J and phelligridimer A.[2] As part of a program to study the chemical diversity of this medicinal fungus and its biological effects, we have now carried out an investigation of cultures of this fungus. The constituents of the fruit body were quite different from those of the cultures. This paper describes the isolation, structure elucidation, and preliminary in vitro bioassays of 13 new metabolites (**1**−**13**) and 18 known compounds from the cultures. Compound **1** is an unusual 4-methyl homopregnane derivative,[3] and **2**−**4** are incisterols possessing a highly degraded 1−5,10,19-heptanorergosterane skeleton that are related to eight compounds obtained from the marine sponges *Dictyonella incise*[4] and *Homaxinella* sp.[5] and from the fungi *Lactarius volemus*[6] and *Agrocybe chaxingu*.[7] Compounds **5**−**13** are tremulanes, a class of sesquiterpenoids that were first isolated from cultures of *Phellinus tremulae*[8] and recently from cultures of the basidiomycete *Conocybe siliginea*.[9]



Results and Discussion

Compound **1**, $C_{22}H_{34}O_4$ by HRESIMS, showed the presence of OH (3461, 3343 cm^{-1}) and carbonyl (1737, 1701, 1650 cm^{-1}) groups in its IR spectrum. The ^1H NMR spectrum of **1** displayed resonances attributable to two tertiary methyl [δ_H 0.60 (H_3-18) and 1.14 (H_3-19)], two secondary methyl [δ_H 0.87 (J = 6.6 Hz, H_3-22) and 1.14 (J = 6.0 Hz, H_3-21)], and two oxymethine [δ_H 3.66 (brd, J = 3.0 Hz, H-3) and 3.71 (q, J = 6.0 Hz, H-20)] groups and resonances due to partially overlapped methylenes and methines between δ_H 0.95 and 2.80. The ^{13}C NMR spectrum of **1** gave 22 carbon resonances that were attributed to four sp^3 methyl, seven sp^3 methylene, five sp^3 methine (two oxygen-bearing, δ_C 71.2 and 73.0), and six quaternary carbons including a sp^2 carbonyl (δ_C 202.0), a conjugated tetrasubstituted double bond (δ_C 169.4 and 134.2), and an oxygen-bearing sp^3 carbon (δ_C 84.9). The gHSQC spectroscopic data analysis of **1** furnished assignments of the proton-bearing carbon and corresponding proton resonances in the NMR spectra (Table 1). In the $^1H-^1H$ gCOSY spectrum of **1**, homonuclear coupling correlations for H_2-1/H_2-2/H-3/H-4/H-5/H_2-6 (in which H-4 correlated with H_3-22), H_2-11/H_2-12, H-14/H_2-15/H_2-16, and H-20/H_3-21 indicated structural units containing vicinal coupling protons in **1** (Supporting Information, Figure S1). In the HMBC spectrum of **1**, long-range heteronuclear correlations for H_3-18/C-12, C-13, C-14, and C-17, H_3-19/C-1, C-5, C-9, and C-10, H_3-21/C-17 and C-20, H_3-22/C-3, C-4, and C-5, H_2-6/C-7, and H_2-11 and H-14/C-8 and C-9 (Supporting Information, Figure S1), in combination with the shifts of these protons and carbons, established the planar structure of 3,17,20-trihydroxy-4-methylpregn-8-en-7-one for **1**.

The absolute configuration of **1** was elucidated by analysis of coupling constants and the NOE difference experiment, combined with the CD data. In the ^1H NMR spectrum, the shift and the half-width of the resonance for H-3 ($W_{1/2} \approx 5$ Hz) suggested the equatorial orientation of H-3.[10,11] In the NOE difference spectrum, H-3, H-5, and H-6α were enhanced when H_3-22 was irradiated and irradiation of H-6β gave an enhancement of H_3-19 (Supporting Information, Figure S2). These enhancements combined with coupling constants of H-5 with H-4, H-6α, and H-6β ($J_{4,5}$ = 9.6 Hz, $J_{5,6\alpha}$ = 4.2 Hz, and $J_{5,6\beta}$ = 13.8 Hz) supported the equatorial orientation of the methyl at C-4 and *trans*-fusion of the A/B rings in **1**. This was supported by the absence of enhancement of H_3-19 upon irradiation of H_3-22. Enhancement of H-14 due to irradiation of H-15α and enhancement of H_3-18 upon irradiation of H-15β indicated *trans*-fusion of the C/D rings. Irradiation of H_3-18 enhanced H_3-19 and H-20 and suggested identical orientation of the methyl groups at C-10 and C-13 and the hydroxyethyl unit at C-17. Molecular modeling indicated that the cyclohexenone moiety of the ring system possessed a twisted half-chair conformation for the lowest energy conformational isomer of **1** (Supporting Information, Figure S3), of which the torsion angle for the enone unit is 175.8°, suggesting that the CD octant rule is applicable for determining the absolute configuration of the cyclohexenone

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Table 1. NMR Spectroscopic Data of Compounds **1**−**4**[a]

	1		**2**		**3**		**4**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	α: 1.69 m β: 1.55 m	30.9		176.5		176.5		173.6
2	α:1.70 m β: 1.77 m	30.1	5.71 d (1.2)	111.5	5.71 d (1.2)	111.5	5.62 brs	113.0
3	3.66 brd (3.0)	71.2		176.1		176.1		173.2
4	1.63 ddq (9.6, 2.4, 6.6)	36.8	5.16 brt (8.4)	82.8	5.16 brt (9.0)	82.8		107.5
5	2.04 ddd (13.8, 9.6, 4.2)	42.6	α: 2.28 m β: 1.43 m	31.4	α: 2.30 m β: 1.43 m	31.4	α: 2.18 dt (13.5, 4.0) β: 1.75 dt (4.0, 13.5)	36.2
6	α 2.27 dd (16.2, 4.2) β: 2.18 dd (16.2, 13.8)	39.8	α: 2.01 m β: 1.64 m	29.9	α: 2.00 m β: 1.64 m	29.9	α: 1.90 m β: 1.55 m	36.5
7		202.0		51.4		51.4		49.9
8		134.2		83.9		84.0	2.60 dd (10.5, 7.0)	51.7
9		169.4	α: 1.63 m β: 1.94 m	30.8	α: 1.62 m β: 1.93 m	30.8	α: 1.65 m β: 1.56 m	22.3
10		40.5	α: 1.92 m β: 1.43 m	28.8	α: 1.92 m β: 1.43 m	29.0	α: 1.89 m β: 1.47 m	30.0
11	α: 2.31 m β: 2.50 dd (19.8, 7.8)	26.1	2.08 m	50.6	2.07 m	50.8	1.47 m	56.5
12	α: 1.84 m β: 1.55 m	29.1	0.64 s	16.4	0.65 s	16.4	0.60 s	12.1
13		47.1	2.11 m	41.2	2.11 m	41.2	2.10 m	41.6
14	2.78 m	44.8	1.00 d (6.6)	21.2	1.00 d (6.6)	21.2	1.03 d (6.5)	21.1
15	α: 2.40 m β: 1.37 m	25.2	5.38 dd (15.0, 8.4)	141.1	5.50 dd (15.0, 9.0)	136.3	5.36 dd (18.0, 10.2)	141.0
16	α: 1.70 m β: 2.05 m	39.1	5.24 dd (15.0, 8.4)	127.5	5.61 d (15.0)	133.5	5.22 dd (18.0, 11.4)	127.6
17		84.9	2.11 m	56.5		77.9	2.11 m	56.5
18	0.60 s	14.3	a: 3.78 dd (10.8, 6.0) b: 3.53 dd (10.8, 7.2)	64.2	1.20 s	25.3	a: 3.78 dd (12.6, 7.2) b: 3.52 dd (12.6, 8.4)	64.1
19	1.14 s	17.7		73.6		75.7		73.6
20	3.71 q (6.0)	73.0	1.10 s	26.0	1.11 s	23.0	1.09 s	26.0
21	1.14 d (6.0)	18.8	1.12 s	29.7	1.12 s	25.2	1.12 s	29.7
22	0.87 d (6.6)	16.0						

[a] Data (δ) were measured in MeOH-d_4 at 600 MHz for protons and at 125 MHz for carbons. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, ^{1}H−^{1}H COSY, gHSQC, and HMBC experiments.

moiety.[12] In the CD spectrum of **1**, a positive Cotton effect at 330 nm for a n−π* transition and a negative Cotton effect at 258 nm for a π−π* transition suggested the 5*S*,10*S* configuration for **1** (Supporting Information, Figure S3). On the basis of the empirical rule of Snatzke's method (the dimolybdenum method),[13] in the $Mo_2(AcO)_4$-induced CD spectrum of **1**, positive Cotton effects at 302 (band IV) and 402 nm (band II) and a negative Cotton effect at 342 nm (band III) supported the 20*R* configuration for **1** (Supporting Information, Figure S4). Therefore, **1** was determined to be 3*R*,4*S*,5*S*,17*R*,20*R*-3,17,20-trihydroxy-4-methylpregn-8-en-7-one.

Compound **2** showed IR absorption bands for OH, double-bond, and carbonyl groups. It had the molecular formula $C_{21}H_{32}O_5$ indicated by the (+)-HRESIMS. The ^{1}H NMR spectrum of **2** displayed resonances ascribed to three tertiary and a secondary methyl, a *trans*-disubstituted and a trisubstituted double bond, an oxymethine, and an oxymethylene. It also showed partially overlapped signals due to aliphatic protons of four methylenes and three methines (Table 1). The ^{13}C NMR spectrum of **2** displayed 21 carbon resonances corresponding to the above units and four quaternary carbons. The proton-bearing carbon and the corresponding proton resonances in the NMR spectra were readily assigned (Table 1) by analysis of the gHSQC spectroscopic data. Detailed analysis of the COSY and HMBC spectroscopic data (Supporting Information, Figure S1) concluded that **2** was an incisterol derivative possessing the unusual 1−5,10,19-heptanorergostane skeleton.[4−7]

The configuration of **2** was assigned by the NOE difference and CD spectroscopic data. In the NOE difference spectrum of **2**, irradiation of H_3-12 gave enhancements of H-2, H-5β, H-6β, H-10β, and H-13 (Supporting Information, Figure S2), indicating that these protons were oriented on the same side of the ring moiety. In addition, enhancements of H-5α and *H*O-8 by irradiation of H-4 indicated they were on the opposite face of the molecule. In the CD spectrum, a positive Cotton effect at 235 nm for a π−π* transition of the α,β-unsaturated γ-lactone moiety (Supporting Information, Figure S5)[5,14] and the chemical shift value of H_3-14[15] supported the 4*S*,13*S* configuration for **2**. On the basis of the sector rule of the CD spectroscopy for the in situ complexation between 1,3-diol and $Mo_2(OAc)_4$,[13b,16] in the $Mo_2(OAc)_4$-induced CD spectrum, a positive Cotton effect at 403 nm supported the 17*R* configuration for **2** (Supporting Information, Figure S6). Therefore, the structure of **2** was determined to be as shown, and it was named phellinignincisterol A.

The IR, (+)-HRESIMS, and NMR data of compound **3** indicated that it was an isomer of **2**. Comparing the NMR data between **3** and **2**, resonances for the methine and hydroxymethylene units of the side chain of **2** were replaced by those for a quaternary carbon (C-17) and a methyl group (CH_3-18) of **3**, respectively. In addition, the resonance of H-16 was changed from the double doublet of **2** to a doublet in **3**. Meanwhile, the resonances for H-15 and H-16 and C-16 and C-19 of **3** were deshielded significantly compared with those of **2**, and C-15 of **3** was shielded significantly. These data implied that the OH group at C-18 of **2** was at C-17 in **3**, which was confirmed by 2D NMR and CD data analysis of **3** (Supporting Information, Figures S1, S2, and S7). The position of the OH was supported by HMBC correlations from H_3-18, H_3-20, and H_3-21 to C-17 and C-19 combined with the shifts of these protons and carbons. Similarity of the CD data between **2** and **3** indicated that they had the same configuration for the lactone ring moiety. In the $Mo_2(OAc)_4$-induced CD spectrum, a positive Cotton effect at 298 nm (band IV) for the in situ complexation with $Mo_2(OAc)_4$[13] (Supporting Information, Figure S8) suggested the 17*R*-configuration for **3**. Therefore, the structure of **3** (phellinignincisterol B) was determined to be as shown.

The spectroscopic data of compound **4** indicated that it was another isomeric analogue of **2**. Comparison of the NMR data between **4** and **2** suggested that they had the same side chain and different ring moieties, although several resonances for the quaternary carbons in the ^{13}C NMR spectrum of **4** were not observed due to a limitation of sample amount available (Supporting Information). In order to fix the undetected quaternary carbons and to finalize the structure, 2D NMR spectra were measured for **4**. The gHSQC spectroscopic data analysis of **4** resulted in assignments of the resonances for the proton-bearing carbons and their corresponding protons. In addition to cross-peaks and correlations (Supporting Information, Figure S1) confirming the side chain moiety in **4**, the $^1H-^1H$ COSY spectrum displayed cross-peaks for H_2-5/H_2-6 and H-8/H_2-9/H_2-10/H-11, and the HMBC spectrum exhibited correlations from H-2 to C-1 and two undetected resonances (C-3 and C-4), from H-8 to C-4, C-7, C-9, C-12, and an undetected resonance (C-2), and from H_3-12 to C-6, C-7, and C-11. These data revealed that **4** differed from **2** in the position exchange of H-4 and HO-8. The relative configuration of **4** was supported by the NOE difference experiment showing enhancements of H-2, H-5β, H-10β, and H-13 upon irradiation of H_3-12 and enhancements of H-6α, H-9α, H-10α, and H-11 by irradiation of H-8 (Supporting Information, Figure S2). Compound **4** showed a positive $\pi-\pi^*$ Cotton effect at 237 nm and a positive Cotton effect for the in situ complexation with $Mo_2(OAc)_4$ at 418 nm[13b,16] (Supporting Information, Figures S9 and S10) and hence was proposed to have the same configurations at C-4 and C-17 as those of **2**. Compound **4** was named phellinignincisterol C.

Compound **5** ($C_{15}H_{26}O_3$) showed IR absorptions for OH and double-bond groups. The ^1H NMR spectrum had resonances (Table 2) attributed to three tertiary methyls, an olefinic methine, and two oxymethylenes respectively attached to a quaternary and methine carbons, as well as partially overlapped resonances due to four aliphatic methylenes. The ^{13}C NMR spectrum of **5** showed 15 carbon resonances (Table 3) corresponding to the above units and four quaternary (two sp^2 and an oxygen-bearing sp^3) carbons. The gHSQC spectrum of **5** provided assignments for the proton-bearing carbon and corresponding proton resonances in the NMR spectra (Tables 2 and 3). The gCOSY spectrum of **5** showed cross-peaks for H_2-12/H-3/H_2-4/H_2-5, H-7/H_2-8, H-10a/H-10b, and H-11a/H-11b (Supporting Information, Figure S1) that established fragments divided by the quaternary carbons in **5**. In the HMBC spectrum, correlations for H_2-11/C-1, C-2, and C-3, H_2-12/C-2, C-3, and C-4, H_3-13/C-5, C-6, and C-7, and H-7/C-1, C-2, C-5, and C-6 (Supporting Information, Figure S1) demonstrated the seven-membered ring moiety in **5**. HMBC correlations from both H_3-14 and H_3-15 to C-8, C-9, and C-10 and from H_2-10 to C-1, C-2, C-7, C-8, C-9, C-14, and C-15 revealed the five-membered-ring moiety that fused at C-1 and C-7 with the above ring. This concluded the planar structure of tremulene-6,11,12-triol for **5**.

In the NOE difference spectrum of **5**, irradiation of H-7 enhanced H-8a, H-10b, H_2-12, and H_3-14 and indicated that these protons were oriented on the same side of the ring system, while enhancement of H_3-13 upon irradiation of H-3 indicated that they were on another side. The CD spectrum of **5** displayed a positive Cotton effect at 203 nm, which was in agreement with that of tremulenediol A,[8] supporting the 3*S*-configuration for **5** on the basis of the olefin octant rule[8,17] (Supporting Information, Figure S11). Therefore, **5** was determined to be (+)-(3*S*,6*R*,7*R*)-tremulene-6,11,12-triol.

Compound **6** gave spectroscopic data very similar to those of **5** (Tables 2 and 3 and Experimental Section), suggesting that it was an isomer of **5**. Detailed comparison of the ^1H NMR data between **6** and **5** indicated that the OH group, at C-6 in **5**, was at C-10 in **6**. The 2D NMR data analysis confirmed that the OH was at C-10 in **6**. In the NOE difference spectrum of **6**, irradiation of H-7 gave enhancements of H-6, H-10, H_2-12, and H_3-14, indicating identical

Table 2. ^1H NMR Spectroscopic Data of Compounds **5**–**13**[a]

position	5	6	7	8	9	10	11	12	13
2				2.86 dt (2.8, 6.8)	2.83 dt (1.0, 7.0)	2.13 dt (7.5, 7.0)	2.73 m		
3	2.50 dddd (8.0, 7.2, 4.0, 3.6)	2.61 m	2.20 m	1.59 m	1.59 m	1.34 m	1.86 m	1.21 m	
4a	1.85 m	1.82 m	1.82 m	1.65 m	1.63 m	1.73 m	4.02 brd (7.5)	1.73 m	2.33 m
4b	1.65 m	1.78 m	1.63 m	1.24 m	1.26 m	1.37 m		1.33 m	2.29 m
5a	1.91 m	1.94 m	1.88 m	1.30 m	1.34 m	1.32 m	1.69 m	1.64 m	1.51 m
5b	1.65 m	1.62 m	1.63 m		1.28 m		1.53 dd (14.0, 9.0)	1.19 m	1.48 m
6		1.80 m		1.91 m	1.95 m	1.92 m	2.38 m	2.30 m	2.03 m
7	3.02 brdd (10.8, 7.8)	3.03 brdd (11.5, 8.0)	2.85 brdd (9.0, 8.0)	3.02 m	3.02 m	2.86 m	3.09 m	3.12 m	2.42 m
8a	1.57 ddd (13.2, 7.8, 2.4)	1.73 dd (11.5, 11.5)	1.80 dd (12.5, 9.0)	1.83 dd (12.4, 6.8)	1.48 dd (12.0, 11.0)	1.76 dd (12.5, 7.5)	1.67 dd (11.5, 7.0)	1.54 dd (12.0, 6.6)	1.60 dd (12.8, 7.6)
8b	1.50 dd (13.2, 10.8)	1.36 dd (11.5, 8.0)	1.42 dd (12.5, 8.0)	1.21 dd (12.4, 11.2)	1.43 dd (12.0, 7.0)	1.16 dd (12.5, 10.0)	1.39 t (11.5, 11.0)	1.29 dd (12.0, 12.0)	1.49 dd (12.8, 12.8)
10a	2.24 dd (15.0, 2.4)	4.01 s	3.90 brs	5.21 d (2.0)	5.30 brs	5.17 d (2.5)	5.33 brs	5.23 d (2.4)	1.78 brd (14.0)
10b	1.91 brd (15.0)								1.72 d (14.0)
11a	3.98 d (11.4)	4.15 d (11.5)	1.79 d (2.0)	3.65 d (6.8)	3.68 dd (11.5, 7.0)	3.74 dd (11.0, 6.0)	3.86 dd (11.0, 8.0)	1.45 s	4.81 dt (16.8, 2.0)
11b	3.86 d (11.4)	4.07 d (11.5)		3.65 d (6.8)	3.64 dd (11.5, 7.0)	3.66 dd (11.0, 7.5)	3.72 dd (11.0, 5.0)		4.74 dt (16.8, 2.0)
12a	3.69 dd (10.8, 8.0)	3.70 d (9.0)	3.71 dd (10.5, 10.0)	3.47 dd (10.4, 6.8)	3.47 dd (11.0, 6.5)	3.58 dd (11.0, 5.0)	3.80 dd (11.0, 6.0)	3.74 dd (10.8, 5.4)	
12b	3.62 dd (10.8, 7.2)	3.70 d (9.0)	3.50 dd (10.5, 5.5)	3.40 dd (10.4, 6.0)	3.41 dd (11.0, 6.5)	3.48 dd (11.0, 5.0)	3.64 dd (11.0, 7.5)	3.56 dd (10.8, 3.0)	
13	1.02 s	0.90 d (6.5)	1.08 s	0.83 d (7.2)	0.84 d (7.0)	0.82 d (7.0)	0.89 d (7.5)	0.78 d (7.2)	0.93 d (7.2)
14	0.81 s	0.82 s	0.73 s	3.27 s	0.94 s	1.00 s	1.01 s	0.93 s	1.14 s
15a	1.04 s	1.03 s	0.98 s	0.98 s	3.32 d (10.5)	3.30 d (10.5)	1.05 s	1.00 s	1.00 s
15b					3.28 d (10.5)	3.27 d (10.5)			

[a] Data (δ) were measured in MeOH-d_4 for **8** and **13** at 400 MHz, for **6**, **7**, and **9**–**11** at 500 MHz, and for **5** and **12** at 600 MHz. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on $^1H-^1H$ COSY, gHSQC, and HMBC experiments.

Table 3. ^{13}C NMR Spectroscopic Data of Compounds **5**−**13**[a]

position	5	6	7	8	9	10	11	12	13
1	144.1	140.2	141.2	151.3	150.0	151.5	146.4	153.5	82.6
2	134.7	147.0	137.9	47.7	47.9	44.2	46.0	76.0	167.4
3	44.3	46.0	48.9	50.0	49.8	47.9	52.9	53.2	124.9
4	25.2	21.9	23.8	30.7	30.7	32.2	71.7	32.1	23.5
5	42.2	33.0	41.7	31.8	31.9	30.3	39.9	31.2	27.8
6	73.3	32.5	73.5	37.2	37.1	34.5	28.5	35.5	32.5
7	53.4	46.8	52.3	50.0	49.0	53.6	50.3	49.9	57.7
8	44.0	42.8	40.5	39.0	38.5	37.4	44.6	42.7	40.7
9	36.9	41.9	41.1	48.4	49.0	49.9	42.6	42.7	36.8
10	48.0	81.3	82.2	135.4	135.3	130.7	139.6	136.6	55.7
11	66.2	65.8	23.8	60.8	60.9	65.1	63.0	29.3	72.1
12	61.5	61.5	60.4	66.7	66.8	65.9	63.0	65.7	177.7
13	19.8	13.0	21.0	19.9	20.1	20.2	18.1	20.5	23.6
14	27.1	26.3	26.3	69.9	21.9	24.8	27.1	27.5	30.3
15	28.9	23.0	22.9	24.6	71.7	70.3	29.8	29.8	31.0

[a] Data (δ) were measured in MeOH-d_4 for **8** and **13** at 100 MHz, for **6**, **7**, and **11** at 125 MHz, and for **5, 9**, **10**, and **12** at 150 MHz. The assignments were based on DEPT, $^1H-^1H$ COSY, gHSQC, and HMBC experiments.

orientation of these protons in the molecule. The CD spectrum displayed a positive Cotton effect at 198 nm and a negative Cotton effect at 211 nm. According to the reverse octant rule for allylic alcohols (oxygen-substituted olefins),[17] the CD data supported the 3*S*,7*S* configuration (Supporting Information, Figure S12). Therefore, **6** was deduced to be (+)-(3*S*,6*S*,7*S*,10*S*)-tremulene-10,11,12-triol.

Spectroscopic data of compound **7** indicated that it was another isomer of **5**. However, as compared with **5**, the NMR data of **7** indicated replacement of the resonances for H_2-11 and C-11 of **5** by those attributable to an allylic methyl in **7** [δ_H 1.79 (d, $J = 2.0$ Hz) and δ_C 23.8]. In addition, the resonances for H_2-10 and C-10 of **5** were replaced by those due to an allylic oxymethine [δ_H 3.90 (brs) and δ_C 82.2] in **7**. This indicated that the OH, at C-11 in **5**, was at C-10 in **7**, and this was confirmed by 2D NMR data analysis. In the NOE difference spectrum of **7**, H-10, H_2-12, and H_3-14 were enhanced by irradiation of H-7, demonstrating an identical orientation of these protons. The CD spectrum of **7** gave a negative Cotton effect for an allylic alcohol $\pi-\pi^*$ transition at 208 nm. Application of the inverse octant rule[17] suggested the 3*S*,7*R* configuration for **7** (Supporting Information, Figure S13). Therefore, **7** was deduced to be (+)-(3*S*,6*R*,7*R*,10*S*)-tremulene-6,10,12-triol.

Compound **8** was also an isomer of **5**. However, the NMR spectra of **8** displayed resonances for a trisubstituted double bond and only one tertiary methyl group, suggesting that it differed from **5** in location of the double bond and the presence of an OH at C-14 or C-15. On the basis of assignments of the proton-bearing carbon and corresponding proton resonances (Table 2) in the NMR spectra by gHSQC, gCOSY cross-peaks, and HMBC correlations (Supporting Information, Figure S1), a planar structure of tremul-1(10)-ene-11,12,14-triol or tremul-1(10)-ene-11,12,15-triol for **8** was indicated. In the NOE difference spectrum of **8**, irradiation of H-7 enhanced H-6, H_2-11, and H_2-14, and H_2-11 was enhanced by irradiation of H_2-12. The enhancements indicated that the three hydroxymethylenes and H-6 and H-7 had the same orientation in the molecule and defined the relative configuration of **8**. In the CD spectrum of **8**, a negative Cotton effect due to the $\pi-\pi^*$ olefinic transition at 197 nm[17] supported the 7*S*,9*R* configuration for **8** (Supporting Information, Figure S14). Therefore, **8** was determined to be (−)-(2*S*,3*S*,6*S*,7*S*,9*R*)-tremul-1(10)-ene-11,12,14-triol.

Compound **9** exhibited spectroscopic features very similar to those of **8**. Detailed comparison of the NMR data indicated that **9** was the 9-epimer of **8**, which was confirmed by 2D NMR, NOE difference, and CD data analysis. In particular, in the NOE difference spectrum of **9**, enhancements of H-6, H_2-11, and H_3-14 upon irradiation of H-7 proved the same orientation of these protons, while a positive Cotton effect at 209 nm[17] in the CD spectrum supported the 7*S*,9*S* configuration for **9** (Supporting Information, Figure S15). Therefore, **9** was elucidated to be (−)-(2*S*,3*S*,6*S*,7*S*,9*S*)-tremul-1(10)-ene-11,12,15-triol.

The spectroscopic data of compound **10** indicated that it was the 2-epimer of **8**. This was supported by enhancements of H-2, H-6, and H_2-14 upon irradiation of H-7 in the NOE difference spectrum of **10** and a negative Cotton effect at 197 nm[17] in the CD spectrum of **10** (Supporting Information, Figure S16). Therefore, **10** was assigned to be (−)-(2*R*,3*S*,6*S*,7*S*,9*R*)-tremul-1(10)-ene-11,12,14-triol.

Compound **11** had spectroscopic data similar to those of **8**. However, the NMR data indicated that the OH was at C-4 or C-5 in **11**. The location of the OH group was finalized by 2D NMR data analysis and placed the OH group at C-4 in **11**. In the NOE difference spectrum of **11**, irradiation of H-4 enhanced H-2 and H-3, while irradiation of H-7 enhanced H-6, H-8a, H_2-11, H_2-12, and H_3-14. These data indicated that H-2, H-3, and H-4, opposite H-6, H-7, H-8a, H_2-11, H_2-12, and H_3-14, were oriented on the same side of the ring system. The CD spectrum showed a positive Cotton effect at 211 nm,[17] indicating the 2*S*,7*S* configuration (Supporting Information, Figure S17). The absolute configuration was further analyzed by the CD spectra of in situ formed complexes of **11** with $Rh_2(OCOCF_3)_4$[18] and $Mo_2(OAc)_4$,[13b,16] respectively. On the basis of the bulkiness rule of the CD spectroscopy for in situ complexation of secondary alcohols with $Rh_2(OCOCF_3)_4$ and the sector rule for in situ complexation between 1,3-diol and $Mo_2(OAc)_4$ (Supporting Information, Figures S18 and S19), both positive Cotton effects at 350 nm (the E band) in the $Rh_2(OCOCF_3)_4$-induced CD spectrum and at 409 nm in the $Mo_2(OAc)_4$-induced CD spectrum supported the 4*S* configuration for **11**. Thus, **11** was determined to be (−)-(2*S*,3*S*,4*S*,6*S*,7*S*)-tremul-1(10)-ene- 4,11,12-triol.

Compound **12** ($C_{15}H_{26}O_2$) had one less oxygen atom than **11**. Comparison of the NMR data between **12** and **11** (Tables 2 and 3) indicated that **12** was tremul-1(10)-ene-2,12-diol, which was confirmed by 2D NMR data analysis. HMBC correlations of C-2 with H-10, H_3-11, and H_2-12, together with their chemical shifts, proved the location of the two OH groups in **12**. In the NOE difference spectrum, irradiation of H-7 enhanced H-6, H-8a, and H_3-14, while irradiation of H_3-11 enhanced H-3 and H-10. The enhancement defined the configuration of **12**, in which H-6, H-7, H-8a, and H_3-14, opposite H-3 and H_3-11, were oriented on the same side of the ring system. In the CD spectrum, a positive Cotton effect at 221 nm for the allylic alcohol $\pi-\pi^*$ transition[17] supported the 2*S*,7*S* configuration (Supporting Information, Figure S20). Thus, **12** was determined to be (+)-(2*S*,3*R*,6*S*,7*S*)-tremul-1(10)-ene-2,12-diol.

Compound **13**, $C_{15}H_{22}O_3$, showed IR absorptions for OH (3391 cm^{-1}) and carbonyl (1723 cm^{-1}) groups. The NMR data (Tables 2 and 3) indicated the presence of two tertiary and a secondary methyl, five methylene (one oxygenated), two methine, and three sp^2 (a carbonyl) and two sp^3 (one oxygen-bearing) quaternary carbons. These data suggested that compound **13** was a hydroxylated analogue of tremulenolide A.[8] HMBC correlations (Supporting Information, Figure S1) demonstrated a planar structure of 1-hydroxytremul-2-ene-12(11)-lactone for **13**. In the NOE difference spectrum of **13**, H-6, H-8a, H-10a, and H_3-14 were enhanced by irradiation of H-7, while H_2-5, H-10b, and H_2-11 were enhanced by irradiation of H_3-15. The enhancements indicated that H-6 and H-7 and the OH group at C-1 had the same orientation in **13**. In the CD spectrum, a negative Cotton effect at 242 nm for the $\pi-\pi^*$ transition of allylic alcohols[11,17] supported the 1*R*,7*S* configuration for **13** (Supporting Information, Figure S21). Accordingly, **13** was determined to be (+)-(1*R*,6*S*,7*S*)-tremul-2-ene-12(11)-lactone.

The known compounds were identified by comparison of spectroscopic data with those reported in the literature as 24*R*-ergosta-4,6,8(14),22-tetraen-3-one,[19] stigmasta-7,22-diene-3β,5α,6α-triol,[20] 5α,8α-epidioxyergosta-6,22-diene-3β-ol,[21] 3β-hydroxy-

11,12-*O*-isopropyldrimene (**14**),[22] 3β,11,12-trihydroxydrimene,[22,23] 11,12,13α-trihydroxydrimene,[24] cyclo(L-Pro-L-Val),[25] cyclo(L-Leu-D-Pro), cyclo(L-Leu-L-Pro),[25,26] cyclo(ILe-Pro),[26] cyclo(Ala-Pro),[27] cyclo(Ala-Phe),[27,28] cyclo(4-OH-Pro-Phe),[26a,28] cyclo(L-Phe-D-Pro),[26b,28,29] cyclo(6-HyP-Phe),[30] cycle(Gln-Pro),[31] cyclo(Gly-Leu),[32] and cyclo(Phe-Ser).[33]

In preliminary in vitro assays, at 10^{-5} M, compounds **8**, **9**, **13**, and **14** showed significant vascular-relaxing activities against phenylephrine (PE)-induced vasoconstriction with relaxation rates of 35.7%, 45.4%, 46.6%, and 32.1% [the positive control, verapamile, exhibited 83.3% relaxation], respectively. The isolates were also assessed for their activities against several human cancer cell lines,[2a] the release of β-glucuronidase in rat polymorphonuclear leukocytes (PMNs) induced by platelet-activating factor (PAF),[34] neuroprotective activity against glutamate-induced neurotoxicity in cultures of PC12 cells,[35] the antioxidant activity in Fe^{2+}-cystine-induced rat liver microsomal lipid peroxidation,[2d] and the inhibitory activity against HIV-1 replication[36] and protein tyrosine phosphatase 1B (PTP1B),[2d] but were inactive at concentrations of 10^{-5} M.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Rudolph Research Autopol III automatic polarimeter. UV spectra were measured on a Cary 300 spectrometer. CD spectra were recorded on a JASCO J-815 CD spectrometer. IR spectra were recorded on a Nicolet 5700 FT-IR microscope instrument (FT-IR microscope transmission). 1D- and 2D-NMR spectra were obtained at 400, 500, or 600 MHz for ^{1}H and 100, 125, or 150 MHz for ^{13}C, respectively, on INOVA 400 or 500 MHz or SYS 600 MHz spectrometers, in CD_3OD or acetone-d_6, with solvent peaks used as references. ESIMS data were measured with a Q-Trap LC/MS/MS (Turbo Ionspray Source) spectrometer. HRESIMS data were measured using an Agilent Technologies 6520 Accurate Mass Q-ToF LC/MS spectrometer. Column chromatography (CC) was performed with silica gel (200−300 mesh, Qingdao Marine Chemical Inc. Qingdao, PR China) and Pharmadex LH-20 (Amersham Biosciences, Inc., Shanghai, PR China). Preparative TLC separation was performed with high-performance silica gel preparative TLC plates ($HSGF_{254}$, glass precoated, Yantai Jiangyou Silica Gel Development Co., Ltd., Yantai, PR China). HPLC separation was performed on an instrument consisting of an Agilent 1100 controller, an Agilent 1200 G1310A Isopump, and an Agilent 1100 G1314A VWD with a Prevail (250 × 10 mm i.d.) preparative column packed with C18 (5 μM). TLC was carried out on precoated silica gel GF_{254} plates. Spots were visualized under UV light or by spraying with 7% H_2SO_4 in 95% EtOH followed by heating. Unless otherwise noted, all chemicals were obtained from commercially available sources and were used without further purification.

Fungus and Cultural Conditions. The fungus *Phellinus igniarius* (CGMCC 5.95) was purchased from the China General Microbiological Culture Collection Center (CGMCC) and was stored in slants of PDA (potato extracts 200 g, glucose 20 g, distilled water 1 L, pH 6.0−6.3; the media were autoclaved at 121 °C for 30 min) at 4 °C at the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, China. The cultivation was carried out on a shaker at 26 °C and 180 rpm for 12 days in malt medium (malt extract 10.0 g, glucose 4.0 g, L-phenylalanine 1.0 g, and yeast extraction 4.0 g, in 1 L of tap water). The pH was adjusted to 6.6 before autoclaving.

Extraction and Isolation. The cultures (110 L) were applied to a HDP100 macroporous adsorbent resin column. Elution of the column with H_2O and 95% EtOH (5 L each) yielded two corresponding residues after removing solvents. The 95% EtOH residue (103 g) was suspended in H_2O (850 mL) and then partitioned with EtOAc (4 × 850 mL). The EtOAc extract was evaporated under reduced pressure to yield 6.9 g of residue, which was subjected to silica gel CC eluting with a petroleum−Me_2CO gradient (100:0−0:100) to produce eight fractions (A−H) on the basis of TLC analysis. Fraction B was further fractionated via silica gel CC, eluting with petroleum−Me_2CO (100:5−100:20), to yield B_1 and B_2. Fraction B_2 was subjected to reversed-phase preparative HPLC using a mobile phase of MeOH−H_2O (75:25) to afford **12** (3.6 mg). Fraction D was separated by normal silica gel CC, eluting with a gradient of increasing Me_2CO in petroleum (100:15−100:30), to afford D_1−D_5. Fraction D_1 was repeatedly fractionated by preparative RP-HPLC, using a mobile phase of MeOH−H_2O (65:35) or $CNCH_3$−H_2O (45:55), to obtain **13** (4.9 mg). Fraction E was chromatographed on Sephadex LH-20 (petrol−$CHCl_3$−MeOH, 4:6:1) to afford E_1−E_3. Fraction E_2 was subjected to silica gel CC eluting with petrol−Me_2CO (100:8) to yield **14** (23.2 mg). Fraction F was chromatographed on Sephadex LH-20 (petrol−$CHCl_3$−MeOH, 4:6:1) to afford F_1−F_4. Fraction F_2 was purified by silica gel CC eluting with petrol−Me_2CO (10:1) to yield **8** (15.2 mg). Fraction H was isolated by preparative RP-HPLC using MeOH−H_2O (30:70) to afford H_1−H_5. Fraction H_1 was separated by preparative RP-HPLC by using MeOH−H_2O (22:78) to afford **7** (1.6 mg). Fraction H_2 was subjected to CC over Sephadex LH-20, eluting with petrol−$CHCl_3$−MeOH (4:6:1), to give $H_{2\text{-}1}$−$H_{2\text{-}4}$. Fraction $H_{2\text{-}3}$ was fractionated using silica gel CC [petrol−Me_2CO (100:2−100:20)] to give $H_{2\text{-}3\text{-}1}$−$H_{2\text{-}3\text{-}4}$. Fraction $H_{2\text{-}3\text{-}2}$ was purified by repeated RP-HPLC with a MeOH−H_2O (65:35) or CH_3CN−H_2O (55:45) to yield **5** (3.2 mg), **9** (3.1 mg), and **10** (2.2 mg). RP-HPLC of $H_{2\text{-}3\text{-}4}$ [MeOH−H_2O (65:35)] yielded $H_{2\text{-}3\text{-}4\text{-}1}$−$H_{2\text{-}3\text{-}4\text{-}5}$. Fractions $H_{2\text{-}3\text{-}4\text{-}3}$ and $H_{2\text{-}3\text{-}4\text{-}5}$ were separately purified by RP-HPLC [CH_3CN−H_2O (48:52) and/or CH_3CN−H_2O (44:56)] to yield **3** (1.1 mg) and **1** (0.6 mg), respectively. Fraction $H_{2\text{-}3\text{-}4\text{-}4}$ [RP-HPLC using CH_3CN−H_2O (47:53)] yielded **2** (1.4 mg) and **4** (0.4 mg). CC of H_5 over Sephadex LH-20 [petrol−$CHCl_3$−MeOH (5:5:1)] afforded $H_{5\text{-}1}$−$H_{5\text{-}3}$. Fraction $H_{5\text{-}3}$ was purified with silica gel CC (petrol−Me_2CO, 100:10) and then Sephadex LH-20 (petrol−$CHCl_3$−MeOH, 4:6:1) CC and reversed-phase preparative HPLC (MeOH−H_2O, 65:35 and 56:44) afforded **6** (5.8 mg) and **11** (17.0 mg).

(−)-(3*R*,4*S*,5*S*,17*R*,20*R*)-3,17,20-Trihydroxy-4-methylpregn-8-en-7-one (1): colorless gum, $[\alpha]_D^{20}$ −0.7 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.81) nm, 223 (3.52, sh) nm; CD (MeOH) 214 ($\Delta\varepsilon$ +0.74), 258 ($\Delta\varepsilon$ −0.85), 330 ($\Delta\varepsilon$ +0.30); [$Mo_2(OAc)_4$]-induced CD (DMSO) 302 ($\Delta\varepsilon$ +0.15), 322 ($\Delta\varepsilon$−1.22), 404 ($\Delta\varepsilon$ +0.20); IR ν_{max} 3461, 3343, 2963, 2934, 2875, 2566, 2482, 1650, 1587, 1374, 1107, 1015 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table 1; (+)-ESIMS *m*/*z* 363 [M + H]$^+$, 385 [M + Na]$^+$; HRESIMS *m*/*z* 363.2535 [M + H]$^+$ (calcd for $C_{22}H_{35}O_4$, 363.2530), 385.2358 [M + Na]$^+$ (calcd for $C_{22}H_{34}O_4Na$, 385.2349).

Phellinigncisterol A (2): colorless gum, $[\alpha]_D^{20}$ +5.8 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.59), 215 (3.50, sh) nm; CD (MeOH) 235 ($\Delta\varepsilon$ +2.96); [$Mo_2(OAc)_4$]-induced CD (DMSO) 403 ($\Delta\varepsilon$ +0.17); IR ν_{max} 3368, 2955, 2974, 2902, 1724, 1650, 1364, 1173, 1034, 860 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data, see Table 1; (+)-ESIMS *m*/*z* 365 [M + H]$^+$, 387 [M + Na]$^+$, 403 [M + K]$^+$; (−)-ESIMS *m*/*z* 363 [M − H]$^-$, 727 [2 M − H]$^-$; (+)-HRESIMS *m*/*z* 387.2145 [M + Na]$^+$ (calcd for $C_{21}H_{32}O_5Na$, 387.2142).

Phellinigncisterol B (3): colorless gum, $[\alpha]_D^{20}$ +4.8 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.18) nm, 221 (2.99, sh) nm; CD (MeOH) 235 ($\Delta\varepsilon$ +3.40); [$Mo_2(OAc)_4$]-induced CD (DMSO) 298 ($\Delta\varepsilon$ +5.66); IR ν_{max} 3557, 3399, 2962, 2946, 2899, 2870, 1724, 1710, 1642, 1607, 1367, 1175, 936, 866 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data, see Table 1; (+)-ESIMS *m*/*z* 365 [M + H]$^+$, 387 [M + Na]$^+$, 403 [M + K]$^+$; (−)-ESIMS *m*/*z* 363 [M − H]$^-$; HRESIMS *m*/*z* 387.2159 [M + Na]$^+$ (calcd for $C_{21}H_{32}O_5Na$, 387.2142).

Phellinigncisterol C (4): colorless gum, $[\alpha]_D^{20}$ +9.5 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.30) nm, 216 (3.39) nm; CD (MeOH) 237 ($\Delta\varepsilon$ +9.24); [$Mo_2(OAc)_4$]-induced CD (DMSO) 418 ($\Delta\varepsilon$ +0.83); IR ν_{max} 3344, 2955, 2968, 2884, 1741, 1664, 1593, 1380, 1178 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data, see Table 1; (+)-ESIMS *m*/*z* 365 [M + H]$^+$, 387 [M + Na]$^+$, 403 [M + K]$^+$; (−)-ESIMS *m*/*z* 363 [M − H]$^-$, 727 [2 M − H]$^-$; (+)-HRESIMS *m*/*z* 387.2142 [M + Na]$^+$ (calcd for $C_{21}H_{32}O_5Na$, 387.2142).

(+)-(3*S*,6*R*,7*R*)-Tremulene-6,11,12-triol (5): white, amorphous solid, $[\alpha]_D^{20}$ +68.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 209 (3.89) nm; CD (MeOH) 203 ($\Delta\varepsilon$ +1.52); IR ν_{max} 3501, 3367, 3271, 2951, 2867, 2599, 2493, 2445, 1659, 1627, 1463, 1408, 1100, 1013 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data, see Tables 2 and 3; (+)-ESIMS *m*/*z* 277 [M + Na]$^+$, 509 [2M + H]$^+$, 531 [2M + Na]$^+$; (+)-HRESIMS *m*/*z* 277.1767 [M + Na]$^+$ (calcd for $C_{15}H_{26}O_3Na$, 277.1780).

(+)-(3*S*,6*S*,7*S*,10*S*)-Tremulene-10,11,12-triol (6): white, amorphous solid, $[\alpha]_D^{20}$ +38.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.97) nm; CD (MeOH) 198 ($\Delta\varepsilon$ +6.22), 219 ($\Delta\varepsilon$ −2.40); IR ν_{max} 3390, 3261,

2951, 2925, 2851, 2515, 2457, 1455, 1362, 1281, 1024, 991, 956 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Tables 2 and 3; (+)-ESIMS *m/z* 255 $[M + H]^+$, 277 $[M + Na]^+$, 531 $[2 M + Na]^+$; (−)-ESIMS *m/z* 507 $[2 M - H]^-$; (+)-HRESIMS *m/z* 277.1773 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_3Na$, 277.1780).

(+)-(3*S*,6*R*,7*R*,10*S*)-Tremulene-6,10,12-triol (7): white, amorphous solid, $[\alpha]_D^{20}$ +55.8 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.97) nm; CD (MeOH) 208 ($\Delta\varepsilon$ −1.09); IR ν_{max} 3331, 2959, 2932, 2876, 1599, 1374, 1036, 1006 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Tables 2 and 3; (+)-ESIMS *m/z* 277 $[M + Na]^+$; (+)-HRESIMS *m/z* 277.1767 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_3Na$, 277.1780).

(−)-(2*S*,3*S*,6*S*,7*S*,9*R*)-Tremul-1(10)-ene-11,12,14-triol (8): white, amorphous solid, $[\alpha]_D^{20}$ −51.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.82) nm; CD (MeOH) 197 ($\Delta\varepsilon$ −8.38); IR ν_{max} 3325, 3243, 3045, 2947, 2914, 2896, 2850, 1639, 1452, 1037, 1017, 925, 851 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data, see Tables 2 and 3; (+)-ESIMS *m/z* 255 $[M + H]^+$, 277 $[M + Na]^+$, 509 $[2 M + H]^+$; (−)-ESIMS *m/z* 507 $[2 M - H]^-$; (+)-HRESIMS *m/z* 277.1788 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_3Na$, 277.1780).

(−)-(2*S*,3*S*,6*S*,7*S*,9*S*)-Tremul-1(10)-ene-11,12,15-triol (9): white, amorphous solid, $[\alpha]_D^{20}$ −5.6 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.08) nm; CD (MeOH) 209 ($\Delta\varepsilon$ +5.63); IR ν_{max} 3332, 2955, 2916, 2871, 1707, 1450, 1377, 1037 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data, see Tables 2 and 3; (+)-ESIMS *m/z* 255 $[M + H]^+$, 277 $[M + Na]^+$, 509 $[2 M + H]^+$; (−)-ESIMS *m/z* 507 $[2 M - H]^-$; (+)-HRESIMS *m/z* 277.1759 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_3Na$, 277.1780).

(−)-(2*R*,3*S*,6*S*,7*S*,9*R*)-Tremul-1(10)-ene-11,12,14-triol (10): white, amorphous solid, $[\alpha]_D^{20}$ −58.1 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.91) nm; CD (MeOH) 197 ($\Delta\varepsilon$ −4.04); IR ν_{max} 3312, 2951, 2916, 2870, 1452, 1376, 1065, 1028 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data, see Tables 2 and 3; (+)-ESIMS *m/z* 255 $[M + H]^+$, 277 $[M + Na]^+$, 509 $[2 M + H]^+$; (−)-ESIMS *m/z* 507 $[2 M - H]^-$; (+)-HRESIMS *m/z* 277.1780 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_3Na$, 277.1780).

(−)-(2*S*,3*S*,4*S*,6*S*,7*S*)-Tremul-1(10)-ene-4,11,12-triol (11): colorless needles (MeOH); mp 109−110 °C; $[\alpha]_D^{20}$ −64.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.92) nm; CD (MeOH) 211 ($\Delta\varepsilon$ +0.24); $Rh_2(OCOCF_3)_4$-induced CD ($CHCl_3$) 350 ($\Delta\varepsilon$ +17.1); $Mo_2(OAc)_4$-induced CD (DMSO) 409 ($\Delta\varepsilon$ +0.07); IR ν_{max} 3332, 3223, 2948, 2865, 1450, 1350, 1061, 1038, 712 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Tables 2 and 3; (+)-ESIMS *m/z* 255 $[M + H]^+$, 277 $[M + Na]^+$, 509 $[2 M + H]^+$, 531 $[2 M + Na]^+$; (−)-ESIMS *m/z* 253 $[M - H]^-$, 507 $[2 M - H]^-$; (+)-HRESIMS *m/z* 277.1779 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_3Na$, 277.1780).

(+)-(2S,3*R*,6*S*,7*S*)-Tremul-1(10)-ene-2,12-diol (12): colorless oil, $[\alpha]_D^{20}$ +14.0 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.78) nm; CD (MeOH) 203 ($\Delta\varepsilon$ −0.24), 221 ($\Delta\varepsilon$ +0.31); IR ν_{max} 3353, 2952, 2928, 2863, 1454, 1373, 1274, 1076, 1032, 914, 852 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data, see Tables 2 and 3; (+)-ESIMS *m/z* 261 $[M + Na]^+$, 499 $[2 M + Na]^+$; (−)-ESIMS *m/z* 475 $[2 M - H]^-$; (+)-HRESIMS *m/z* 261.1837 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_2Na$, 261.1831).

(+)-(1*R*,6*S*,7*S*)-Tremul-2-ene-12(11)-lactone (13): white, amorphous solid, $[\alpha]_D^{20}$ +5.0 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.94) nm; CD (MeOH) 206 ($\Delta\varepsilon$ +45.11), 242 ($\Delta\varepsilon$ −11.71); IR ν_{max} 3391, 2952, 2926, 2859, 1723, 1664, 1439, 1049, 1026, 776 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data, see Tables 2 and 3; (+)-ESIMS *m/z* 251 $[M + H]^+$, 273 $[M + Na]^+$, 289 $[M + Cl]^+$, 523 $[2 M + Na]^+$; (−)-ESIMS *m/z* 249 $[M - H]^-$; (+)-HRESIMS *m/z* 273.1447 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_3Na$, 273.1467).

Vasodilating Activity Assays.[37,38] Thoracic aortas of Langendorff-perfused rat were isolated from Sprague−Dawley (SD) rats weighing 200 ± 20 g (Charles River Laboratories, Beijing, China). Isotonic tension of thoracic aortic rings precontracted by PE was recorded to characterize vasorelaxing action of compounds. The relaxation rates were calculated by comparing with the blank control, and verapamile was used as the positive control.

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Supporting Information Available: Figure S1, main $^1H-^1H$ COSY and HMBC correlations of **1**−**13**. Figure S2, main 1D NOESY correlations of **1**, **2**, and **4**−**13**. Figures S3−S21, CD spectra of **1**−**13** together with diagrams for conformations and CD rules applied. Figures S22−S147c, IR, MS, ^1H and ^{13}C NMR, $^1H-^1H$ COSY, HMQC, HMBC, and NOE spectra of compounds **1**−**13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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