New anti-inflammatory guaianes from the Atlantic hydrotherm-derived fungus Graphostroma sp. MCCC 3A00421

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Nine new guaianes (graphostromanes A–I, 1–9) were isolated from the deep-sea-derived fungus Graphostroma sp. MCCC 3A00421, along with four known ones (10–13). The relative configurations were established mainly by detailed analysis of the NMR and HRESIMS data, while the absolute configurations were assigned using the X-ray crystallography and modified Mosher’s method. All isolates were evaluated for their inhibitory effects against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7 macrophages. Graphostromanes F (6) showed remarkable inhibitory effect with an IC50 value of 14.2 μM, which was even stronger than that of aminoguanidine, a positive control with an IC50 value of 23.4 μM.

Guaianes are sesquiterpenoids bearing a bicyclo[5.3.0]-decane skeleton with enormously structural diversities, including nor-guaianes1, guaiane alkaloids2,3, guaiane dimers4,5, etc. They possessed a variety of intriguingly biological activities, such as antioxidant6, antimalarial7,8, antinociceptive9, anti-emetic10, antitumor11–13, anti-inflammatory12,13, and antibacteria14,15. Guaianes occur mainly in terrestrial plants16. Very rarely they were found from terrestrial microorganisms and corals17–19. However, they have never been isolated from marine microbes. In our continuing investigation on the deep-sea-derived Graphostroma sp. MCCC 3A0042120, nine new (1–9) and four known (10–13) guaianes were obtained (Fig. 1). Herein, we report the isolation, structure elucidation, and anti-inflammatory activities of these compounds.

### Results and Discussion

Compound 1 was isolated as a colorless oil. The molecular formula C15H26O2 was established on the basis of the [M + H]+ ionic peak at m/z 239.2044 in its positive HRESIMS, requiring three indices of hydrogen deficiency. The 1H NMR spectroscopic data (Table 1) showed two singlet (δH 1.40 and 1.76) and one doublet (δH 1.08) methyls, one exomethylene (δH 4.83, 4.72), and one oxygenated methine (δH 4.22). These signals were resonance in the 13C NMR data (Table 2) as three methyls (δC 14.4, 19.9, and 29.4), one exocyclic methylene (δC 107.8), one oxygenated methine (δC 77.4). Altogether, the 1H and 13C NMR exhibited 15 carbons attributing to three methyls, five methylenes, five methines, and two nonprotonated carbons (one sp2 at δC 152.7 and one oxygenated sp3 at δC 73.0). Since an olefinic bond accounted for one unsaturation degree, a bicyclic framework was required for 1.

In the COSY spectrum, one spin coupling system from H-1 through H2-2 to H-3/H-4/H-5/H2-6/H-7/H2-8/H2-9, from H-5 to H-1, and from H-4 to H2-15 constructed a long fragment. In the HMBC spectrum, correlations from 14-Me (δH 1.40) to C-1 (δC 53.0), C-9 (δC 37.8), and C-10 (δC 73.0) and from 12-Me to C-7, C-11 (δC 152.7), and C-13 (δC 107.8) established 1 as a guaiane sesquiterpene (Fig. 2).

In the NOESY spectrum, correlations from H-5 (δH 2.66) to H-1/H-4/H-7 (δH 2.59) and from Me-15 to Me-14 and H-3 disclosed that H-1, H-4, H-5, and H-7 were on the same face, whereas H-3, 14-Me, and 15-Me were on the opposite side (Fig. 2). This was unambiguously confirmed by the Cu-Kα X-ray crystallography (Fig. 3). Therefore, 1 was determined to be (1R,3R,4R,5S,7R,10R)-11(13)-en-3,10-dihydroxyguaianoi, and named graphostromane A.
Compound 2 showed the formula molecular of C_{13}H_{20}O as established by the positive HRESIMS at m/z 223.2057 [M + H]^+. The ^1H and ^13C NMR spectra were nearly identical to those of 1 except that a methylene (δ_C 32.1) instead of an oxygenated methine was located at C-3 position. This observation was evidenced by the HMBC correlation of 15-Me (δ_H 0.92) to the methylene unit. On the basis of its key NOESY correlations (Fig. 4) and the similar optical rotation value ([α]_D^28 = 28.5 for 2, while −22.0 for 1), 2 was therefore deduced to be (1R,4S,5S,7R,10R)-11(13)-en-10-hydroxyguaiene, and named graphostromane B.

Compound 3 shared the same molecular formula as that of 1 by the positive HRESIMS at m/z 261.1829 (calc for C_{13}H_{20}O_2Na, 261.1830). Interestingly, it also exhibited almost the same ^1H and ^13C NMR spectra, except for the shielded chemical shift of H-3 (δ_H 1.87) and its peak pattern, indicating C-3 and 1 might be C-3 stereoisomers. The NOESY correlations between H-3/H-1 (δ_H 2.61), H-1/H-9a (δ_H 1.98), H-9a/H-7, and H-9b (δ_H 2.24) to H_{11} (δ_H 1.50) deduced H-1, H-3, and H-7 were in the α orientations, while H_{11}-14 was β-oriented. However, it is difficult to establish the relative configurations of C-4 and C-5 positions by the NOESY correlations because of the overlap signals of H-4 (δ_H 1.67) and H-5 (δ_H 1.69). Therefore, all four possible stereoisomers including a pair of cis-fused C-4 epimers [(1^R^*,3^S^*,4^R^*,5^S^*,7^R^*,10^R^*)-3a and (1^R^*,3^S^*,4^S^*,5^S^*,7^R^*,10^R^*)-3b] and a pair of trans-fused C-4 epimers [(1^R^*,3^S^*,4^R^*,5^R^*,7^R^*,10^R^*)-3c and (1^R^*,3^S^*,4^S^*,5^R^*,7^R^*,10^R^*)-3d] were subjected to the theoretical calculation of the CMR data at mPW1PW91/6-311 + G(2d,p) level using the IEFPCM model in pyridine-d_5 by Gaussian 09. As shown in Fig. 5, 3c displayed the smallest deviation, suggesting the relative configuration of 3 to be 1^R^*,3^S^*,4^R^*,5^R^*,7^R^*,10^R^*. By the modified Mosher’s method, C-3 was determined to be S configuration (Fig. 6). Based on the above evidences, 3 was then established to be (1R,3S,4R,5R,7R,10R)-11(13)-en-3,10-dihydroxyguaiene, and named graphostromane C.

Compound 4 was established the molecular formula C_{13}H_{22}O_2 on the basis of its HRESIMS. The ^1H and ^13C NMR spectra were very similar to those of 3 except that a ketone group (δ_C 200.6) rather than a hydroxy unit was located at C-3. The assumption was corroborated by the HMBC relationships from 15-Me (δ_C 220.6) rather than a hydroxy unit was assigned the molecular formula C_{15}H_{26}O_2 Na on the basis of its HRESIMS. The ^1H and ^13C NMR spectra were nearly identical to those of 1, except for an additional hydroxy unit at C-12. This was evidenced by the HMBC cross-peaks from H_{9a} (δ_H 4.42) to the carbonyl (δ_C 170.7) of the acetyl group. Therefore, 4 was assigned the molecular formula C_{15}H_{26}O_3 by the HRESIMS at m/z 265.1938 [M + Na]^+. Its 1H and 13C NMR spectra were very similar to those of 1 except for an additional hydroxy unit at C-12 (δ_C 64.1). The assumption was evidenced by the HMBC correlations from H-2-13 (δ_H 5.47, 5.08) to C-7 (δ_C 43.7), C-11 (δ_C 157.9), and C-12. Accordingly, 6 was determined to be (1S,2S,4S,5S,7R,10R)-11(13)-en-2,10,12-trihydroxyguaiene, and named graphostromane E.

Compound 7 gave a molecular formula C_{14}H_{22}O_4, from its positive HRESIMS at m/z 337.1979 [M + Na]^+. Its ^1H and ^13C NMR spectra were close to those of (1S,2S,4S,5S,7R,10R)-guaiane-2,10,11,12-tetraol (10)[2], except for an additional acetyl group (δ_H 1.99; δ_C 20.6, 170.7) at C-12. This was evidenced by the HMBC cross-peaks from H_{12} (δ_H 4.42) to the carbonyl (δ_C 170.7) of the acetyl group. The absolute configuration of C-2 was established to be S on the basis of the modified Mosher’s method (Fig. 6), which was further corroborated by the X-ray
single-crystal experiment (Fig. 7). Therefore, 7 was unambiguously determined to be (1S,2S,4S,5S,7R,10R,11R)-12-acetyl-2,10,11-trihydroxyguaiane, and named graphostromane G. Compound 8 had the molecular formula of C_{18}H_{24}O_{4} as established by its HRESIMS. Analysis of the 1D and 2D NMR spectra established the structure of 8 was closely related to 10, except that the hydroxy group was located at C-4 instead of C-2. This was evidenced by the HMBC correlations from 15-Me (δ_H 1.58) to C-3 (δ_C 59.6), C-4 (δ_C 80.9), and C-5 (δ_C 54.5). The NOE cross-peaks from H-2a (δ_H 1.20) to 14-Me (δ_H 1.46) and 15-Me revealed that 4-OH was in α-oriented. Therefore, 8 was identified to be (1R,4R,5R,7R,10R)-4,10,11-trihydroxyguaiane, and named graphostromane H.

Compound 9 was assigned the molecular formula C_{18}H_{22}O_{5} on the basis of the HRESIMS at m/z 277.1783 [M + Na]^+ . Its 1H and 13C NMR spectra were very similar to those of (1R,4S,5S,7R,10R,11S)-guaiane-10,11,12-triol (11) except that a hydroxy group was absent at C-11, while a carboxyl moiety (δ_C 180.2) instead of an oxymethylene was attached to C-11. This was corroborated by the HMBC correlations from 13-Me (δ_H 1.10) to C-7 (δ_C 41.4), C-11 (δ_C 47.0), and C-12 (δ_C 180.2). By the theoretical calculation of CMR spectra, C-11 was assigned as R-configuration (Fig. 8). Therefore, 9 was established to be (1R,4S,5S,7R,10R,11R)-10-hydroxyguaiane-12-ol, and named graphostromane I.

By comparison of the NMR, MS, and OR data with those published in the literature, four known guaianes were identified as (1S,2S,4S,5S,7R,10R)-guaiane-2,10,11-triol (12), (1R,4S,5S,7R,10R,11S)-guaiane-10,11,12-triol (11) except that a hydroxy group was absent at C-11, while a carboxyl moiety (δ_C 180.2) instead of an oxymethylene was attached to C-11. This was corroborated by the HMBC correlations from 13-Me (δ_H 1.10) to C-7 (δ_C 41.4), C-11 (δ_C 47.0), and C-12 (δ_C 180.2). By the theoretical calculation of CMR spectra, C-11 was assigned as R-configuration (Fig. 8). Therefore, 9 was established to be (1R,4S,5S,7R,10R,11R)-10-hydroxyguaiane-12-ol, and named graphostromane I.

All isolates were evaluated for their anti-inflammatory activities against LPS-induced NO production in RAW264.7 macrophages (Table 3). Compound 6 exhibited remarkable anti-inflammatory activity with an IC_{50} value of 14.2 μM, which was stronger than that of the positive control, aminoguanidine, with an IC_{50} of 23.4 μM. In addition, 4, 9, and 13 showed weak anti-inflammatory activities with IC_{50} values of 72.9, 79.1, and 88.2 μM, respectively.

In conclusion, chemical investigation on the deep-sea-derived fungus Graphostroma sp. MCC 3A00421 led to the isolation of 9 new (graphostromanes A–I, 1–9) and 4 known (10–13) guaianes. They are first examples of guaiane sesquiterpenoids reported from the marine-derived fungi. Additionally, 6 showed potent anti-inflammatory activity against LPS-induced NO production in RAW264.7 macrophages, indicating its potential usage for anti-inflammatory drugs.

**Materials and Methods**

**General Experimental Procedures.** An automatic polarimeter Rudolph IV Autopol was used for recording optical rotation data at 25°C. A Xevo G2 Q-TOF mass spectrometer was used for measuring HRESIMS. A Bruker Avance 400 MHz NMR spectrometer was used for measuring 1H, 13C, HSQC, COSY, HMBC, and NOESY spectra. Chemical shifts (δ) were expressed in ppm referring to the solvent peaks, and coupling constants are in Hz. A Bruker D8 Advance X-ray single-crystal diffractometer was used for measuring X-ray data with Cu Kα radiation. Column chromatography (CC) were performed on Sephadex LH-20 (18–110 μm, Pharmacia, Uppsala, Sweden), silica gel (100–200 or 200–300 mesh, Qingdao Marine Chemistry Co. Ltd, Qingdao, China), and ODS.
(50 μm, Daiso, Japan). TLC precoated silica gel plates (GF254, Qingdao Marine Chemistry Co. Ltd, Qingdao, China) were used for TLC detection. All chemical reagents used were analytical grade.

**Table 2.** $^{13}$C NMR spectroscopic data of compounds 1–9. $^{a}$Measured in pyridine-$d_5$ at 100 MHz. $^{b}$Measured in CD$_3$OD at 100 MHz.

**Fungal Identification and Fermentation.** The fungus *Graphostroma* sp. MCCC 3A00421 was isolated from a hydrothermal sulfide deposit in August 2012 from the Atlantic Ocean (W 13.36°, S 15.17°) at a depth of −2721 m. It was identified to be *Graphostroma* genus on the basis of comparison of its ITS1-5.8S-ITS2 rRNA gene sequence (KM190888) with those deposited in GenBank of the NCBI using a BLAST searching tool. The
voucher strain was deposited at the Marine Culture Collection of China (MCCC) with the accession number MCCC 3A00421.

The working strain was cultured on a PDA plate medium under 25 °C for 3 days. Then the fresh mycelia were inoculated into 30 Erlenmeyer flasks (1 L), each containing 120 mL distilled water and 80 g rice, and then statically fermented for 28 days at 25 °C.

Extraction, Isolation, and Purification. After 28 days, the fermented cultures were extracted with EtOAc for three times. The EtOAc solution was evaporated under reduced pressure to get an organic extract, which was then partitioned between MeOH and petroleum ether (PE). The MeOH fraction was evaporated to get the defatted extract (7.0 g), which was separated by column chromatography (CC) over ODS eluting with gradient MeOH-H2O (5→100%) to yield 24 fractions (F1–F24). Fraction F7 (624 mg) was subjected to CC over Sephadex LH-20 (MeOH) and silica gel (CHCl3–MeOH, 100:1–20:1) to yield 39.2 mg. Fraction F16 (462 mg) was subjected to CC over Sephadex LH-20 (MeOH) and silica gel (CHCl3–MeOH, 15:1; EtOAc–MeOH, 200:1–20:1) to yield 8.2 mg. Compounds 6 (18.8 mg) and 13 (24.2 mg) were isolated from fraction F10 (603 mg) by CC over Sephadex LH-20 (MeOH) and silica gel (PE-acetone, 3:1; PE-EtOAc, 3:1→1:1; EtOAc), respectively. Fraction F14 (389 mg) was subjected to CC over silica gel using gradient PE–EtOAc to give seven subfractions (SF14-1→SF14-7). SF14-2 was purified by Prep. TLC (CHCl3–MeOH, 60:40) to get three subfractions (SF14-1→SF14-3). SF9-1 was purified by CC over silica gel using PE-acetone (5:1), followed by Prep. TLC (CHCl3–MeOH, 8:1) to provide 7 (12.6 mg). SF9-3 was subjected to CC over silica gel (CHCl3–MeOH, 15:1) to afford 8 (8.2 mg). Compounds 6 (18.8 mg) and 13 (24.2 mg) were isolated from fraction F10 (603 mg) by CC over Sephadex LH-20 (MeOH) and silica gel (PE-acetone, 3:1; PE-EtOAc, 3:1→1:1; EtOAc), respectively. Fraction F14 (389 mg) was subjected to CC over silica gel using gradient PE–EtOAc to give seven subfractions (SF14-1→SF14-7). SF14-2 was purified by Prep. TLC (PE-acetone, 2:1) to get 1 (14.7 mg). Compound 3 (10.2 mg) was isolated from SF14-5 by Prep. TLC (EtOAc-acetone, 30:1). SF14-6 was separated by CC over Sephadex LH-20 (MeOH) and silica gel (CHCl3–MeOH, 20:1) to yield 11 (39.2 mg). Fraction F16 (462 mg) was subjected to CC on Sephadex LH-20 (MeOH) and silica gel (CHCl3–MeOH, 100:1→1:1; EtOAc–MeOH, 200:1→1:1; and PE-acetone; 5:1) to yield 2 (23.0 mg), 5 (15.5 mg), and 9 (11.6 mg).

\[\text{Graphostromane A (1): colorless oil; } \delta^1H = 22.0 (c 0.58, MeOH); \text{ H and C NMR data, Tables 1 and 2; HRESIMS } m/z = 239.2044 [M-H]^+ \text{ (calcld for C}_{15}\text{H}_{27}\text{O}_2, 239.2011).\]

\[\text{Graphostromane B (2): colorless oil; } \delta^1H = 28.5 (c 2.16, MeOH); \text{ H and C NMR data, Tables 1 and 2; HRESIMS } m/z = 223.2057 [M-H]^+ \text{ (calcld for C}_{15}\text{H}_{26}\text{O}_2, 223.2062).\]

\[\text{Graphostromane C (3): colorless oil; } \delta^1H = 8.5 (c 0.25, MeOH); \text{ H and C NMR data, Tables 1 and 2; HRESIMS } m/z = 261.1829 [M+Na]^+ \text{ (calcld for C}_{15}\text{H}_{26}\text{O}_4\text{Na}, 261.1830).\]

\[\text{Graphostromane D (4): colorless oil; } \delta^1H = 15.4 (c 0.73, MeOH); \text{ H and C NMR data, Tables 1 and 2; HRESIMS } m/z = 237.1851 [M+H]^+ \text{ (calcld for C}_{15}\text{H}_{25}\text{O}_3, 237.1855).\]

\[\text{Graphostromane E (5): colorless oil; } \delta^1H = 8.13 (c 0.39, MeOH); \text{ H and C NMR data, Tables 1 and 2; HRESIMS } m/z = 261.1833 [M+Na]^+ \text{ (calcld for C}_{15}\text{H}_{26}\text{O}_4\text{Na}, 261.1830).\]

\[\text{Graphostromane F (6): colorless oil; } \delta^1H = 3.41 (c 0.44, MeOH); \text{ H and C NMR data, Tables 1 and 2; HRESIMS } m/z = 277.1767 [M+Na]^+ \text{ (calcld for C}_{15}\text{H}_{27}\text{O}_3Na, 277.1780).\]

\[\text{Graphostromane G (7): colorless oil; } \delta^1H = 6.0 (c 0.56, MeOH); \text{ H and C NMR data, Tables 1 and 2; HRESIMS } m/z = 337.1991 [M+Na]^+ \text{ (calcld for C}_{15}\text{H}_{26}\text{O}_4Na, 337.1991).\]

\[\text{Graphostromane H (8): colorless oil; } \delta^1H = 21.0 (c 0.25, MeOH); \text{ H and C NMR data, Tables 1 and 2; HRESIMS } m/z = 295.1885 [M+Na]^+ \text{ (calcld for C}_{15}\text{H}_{26}\text{O}_4Na, 295.1885).\]

\[\text{Graphostromane I (9): colorless oil; } \delta^1H = -10.3 (c 0.12, MeOH); \text{ H and C NMR data, Tables 1 and 2; HRESIMS } m/z = 277.1783 [M+Na]^+ \text{ (calcld for C}_{15}\text{H}_{26}\text{O}_4Na, 277.1780).\]

\[\text{Compound 10: } \text{C NMR (CD}_{3}\text{OD, 100 MHz): } \delta 63.4 (\text{CH}-1), 75.1 (\text{CH}-2), 43.3 (\text{CH}-3), 37.3 (\text{CH}-4), 47.3 (\text{CH}-5), 25.8 (\text{CH}-6), 45.7 (\text{CH}-7), 26.4 (\text{CH}-8), 39.5 (\text{CH}-9), 75.4 (\text{C}-10), 76.6 (\text{C}-11), 69.2 (\text{CH}-12), 19.0 (\text{Me}-13), 29.6 (\text{Me}-14), 16.7 (\text{Me}-15).\]

Preparation of (R)- and (S)-MPA Esters of Compounds 3, 5, and 7. Compound 3 (2.0 mg) was dissolved in CHCl3 (600 µL). Then (R)-MPA (2.5 mg), DCC (2.5 mg), and DMAP (2.5 mg) were added. After stirred 16 h at room temperature, the reactive products were subjected to CC over silica gel (PE-acetone, 3:1) to give the R-MPA ester 3a (1.7 mg). Similarly, the S-MPA ester 3b (1.9 mg) was obtained from (S)-MPA. Analogue treatment of compounds 5 and 7 separately with (R)-MPA and (S)-MPA obtained (R)-MPA esters (5a and 7a) and (S)-MPA esters (5b and 7b), respectively.
Figure 5. Calculated 13C NMR spectroscopic data of four possible stereoisomers of 3 (3a, 3b, 3c, and 3d) at mPW1PW91/6-311+G(2d,p) level in pyridine-\(\text{d}_5\).

Figure 6. \(\Delta\delta_{\text{H}} (\delta_{\text{H} \text{R}} - \delta_{\text{H} \text{S}})\) values of (R)- and (S)-MPA esters of 3, 5, and 7 in CDCl3.

Figure 7. X-ray crystallographic structure of 7.
H-8a), 1.75 (1 H, m, H-8b), 1.67 (1 H, m, H-9a), 1.82 (1 H, m, H-9b), 1.69 (3 H, s, Me-12), 4.61 (1 H, br s, H-13a), 4.65 (1 H, br s, H-13b), 1.22 (3 H, s, Me-14), 0.87 (3 H, d, J = 7.0 Hz, Me-15).

(S)-MPA ester of 5 (5b): 1H NMR (CDCl 3, 400 MHz) δ H  7.31–7.44 (5 H, m, phenyl protons), 4.73 (1 H, s, CH of MPA), 3.41 (3 H, s, OMe of MPA), 2.21 (1 H, m, H-1), 5.15 (1 H, m, H-2), 1.74 (2 H, m, H-3), 2.22 (2 H, m, H-4 and H-5), 1.17 (1 H, m, H-6a), 1.44 (1 H, m, H-6b), 2.10 (1 H, dt, J = 4.1, 11.4 Hz, H-7), 1.74 (2 H, m, H-8b and H-9b), 1.55 (1 H, t, J = 10.1 Hz, H-9a), 1.69 (3 H, s, Me-12), 4.60 (1 H, br s, H-13a), 4.63 (1 H, br s, H-13b), 0.90 (3 H, s, Me-14), 0.91 (3 H, d, J = 7.0 Hz, Me-15).

(R)-MPA ester of 7 (7a): 1H NMR (CDCl 3, 400 MHz) δ H  7.32–7.44 (5 H, m, phenyl protons), 4.74 (1 H, s, CH of MPA), 3.41 (3 H, s, OMe of MPA), 2.31 (1 H, t, J = 6.9 Hz, H-1), 5.17 (1 H, dt, J = 2.9, 7.6 Hz, H-2), 1.45 (1 H, m, H-3a), 1.68 (1 H, m, H-3b), 2.16 (1 H, m, H-4), 2.18 (1 H, m, H-5), 0.91 (1 H, m, H-6a), 1.81 (1 H, m, H-6b), 1.77 (1 H, m, H-7), 1.19 (1 H, m, H-8a), 1.76 (1 H, m, H-8b), 1.59 (1 H, m, H-9a), 1.82 (1 H, m, H-9b), 1.06 (3 H, s, Me-12), 3.97 (1 H, d, J = 11.5 Hz, H-13a), 4.06 (1 H, d, J = 11.5 Hz, H-13b), 1.14 (3 H, s, Me-14), 0.90 (3 H, d, J = 6.5 Hz, Me-15).

(S)-MPA ester of 7 (7b): 1H NMR (CDCl 3, 400 MHz) δ H  7.32–7.44 (5 H, m, phenyl protons), 4.71 (1 H, s, CH of MPA), 3.40 (3 H, s, OMe of MPA), 2.16 (1 H, m, H-1), 5.05 (1 H, dt, J = 2.8, 7.7 Hz, H-2), 1.68 (1 H, m, H-3a), 1.80 (1 H, m, H-3b), 2.23 (1 H, m, H-4), 2.21 (1 H, m, H-5), 0.86 (1 H, m, H-6a), 1.71 (2 H, m, H-6b and H-8b), 1.77 (1 H, m, H-7), 1.15 (1 H, m, H-8a), 1.46 (1 H, m, H-9a), 1.72 (1 H, m, H-9b), 1.05 (3 H, s, Me-12), 3.95 (1 H, d, J = 11.5 Hz, H-13a), 4.04 (1 H, d, J = 11.5 Hz, H-13b), 0.71 (3 H, s, Me-14), 0.96 (3 H, d, J = 6.8 Hz, Me-15).

X-ray Crystal Data of Compounds 1 and 7. Graphostromane A (1) was obtained as colorless crystals. The monoclinic crystals (0.1 × 0.2 × 0.8 mm 3) was recorded on a Bruker D8 Advance X-ray single-crystal diffractometer with Cu Kα radiation. Crystal data of 1: empirical formula C 15H 26O 2, M = 236.36; space group P2 1, unit cell dimensions a = 6.3808 (4) Å, b = 7.7943 (4) Å, c = 13.9771 (8) Å, α = 90.00°, β = 101.329 (6)°.
Colorless crystals of 7 were obtained from MeOH. The monoclinic crystals (0.3 × 0.4 × 0.8 mm) was measured on a Bruker D8 Advance X-ray single-crystal diffractometer with Cu Kα radiation. Crystal data of 7: empirical formula C17H30O5, M = 314.42; space group C2, unit cell dimensions a = 12.0727 (4) Å, b = 14.3161 (3) Å, c = 13.4457 (4) Å, α = γ = 90.00°, β = 114.561 (4)°, Volume = 2317.16 (12) Å³, Z = 9, Dcalcd = 1.173 g/cm³, μ = 0.774 mm⁻¹, F (000) = 828.0; A total of 11239 reflections were collected in the range of 7.228° < 2θ < 123.85°, of which 3337 independent reflections [Rint = 0.0330, Rsigmac = 0.0280] were used for the analysis. The final R indexes [all data] gave R1 = 0.0368, wR2 = 0.1006 and the Flack parameter = −0.07 (3). Crystallographic data of 7 have been deposited in the Cambridge Crystallographic Data Center (deposition number CCDC 1577811).

Theoretical Calculation of CMR Data. The calculated CMR data of 3 and 9 were carried out by Gaussian 0923. Conformational analyses were initially performed using Confab24 with MMFF94 force field for configurations of both compounds. The conformers, which were chosen for CMR calculations with Boltzmann-population over 1%, were firstly optimized at PM6 by semi-empirical theory method to filter some conformers with low Boltzmann-populations. Then, the remaining conformers were optimized at B3LYP/6-31+G(d,p) in gas phase. The CMR calculation was conducted by the Gauge-Including Atomic Orbitals (GIAO) method at mPW1PW91/6-311+G(2d,p) level using the IEFPCM model in pyridine for 3, whereas in MeOH for 9, respectively. Finally, the TMS-corrected 13C NMR chemical shift values were averaged according to Boltzmann distribution for each conformer and fitting to the experimental values by linear regression. The calculated CMR chemical shift values of TMS in pyridine and in MeOH were 187.3194 ppm and 187.3772 ppm, respectively.

Anti-Inflammatory Assay. This experiment was conducted according the reported procedure25.

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Author Contributions
Xian-Wen Yang designed the project; Siwen Niu isolated compounds and determined their structures; Chun-Lan Xie and Jin-Mei Xia conducted the anti-inflammatory experiments; Zhu-Hua Luo and Zongze Shao isolated and identified the fungus; Siwen Niu and Xian-Wen Yang wrote and revised the paper. All authors reviewed the manuscript.

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