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Stereumins H−J, Stereumane-Type Sesquiterpenes from the Fungus *Stereum* sp.

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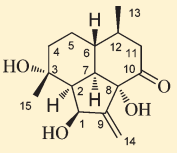
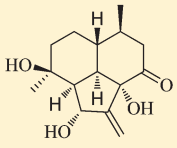
Supporting Information

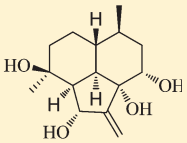
ABSTRACT: Stereumins H (**1**), I (**2**), and J (**3**), three sesquiterpenes possessing a novel stereumane-type backbone, were isolated from an extract of culture broth of the Basidiomycete *Stereum* sp. CCTCC AF 207024. The complete structural assignments including the absolute configurations are reported by using X-ray studies and density functional theory at the B3LYP/aug-cc-pVDZ//B3LYP/6-31G(d) level.



Stereumin H (**1**)

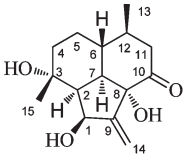


I (**2**)

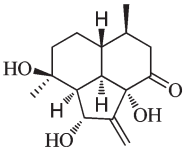


J (**3**)

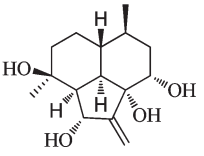
Basidiomycetes produce various classes of primary and secondary metabolites and are a rich source of structurally diverse sesquiterpenes.[1] *Stereum* is a genus of the Stereaceae family (subsequently classified in the Corticiaceae family) of Basidiomycete, and there are a number of species of *Stereum* throughout the world. In investigations of chemical constituents of the genus *Stereum*, several types of sesquiterpenoids, including hirsutanes, sterpuranes, cadinanes, and avicoronanes, have been reported.[2−6] In our previous work,[7,8] seven cadinane-type compounds (stereumins A−G) were obtained from the culture broth of Basidiomycete *Stereum* sp. CCTCC AF 207024, a fungus with nematicidal activity against *Panagrellus redivivus*. Since then, several additional minor stereumane-type sesquiterpenoids, stereumins H (**1**), I (**2**), and J (**3**), have been found.



Stereumin H (**1**)



I (**2**)



J (**3**)

Stereumin H (**1**) had the molecular formula $C_{15}H_{22}O_4$, as deduced from HRESIMS (*m*/*z* 289.1419 $[M + Na]^+$), with 5 degrees of unsaturation. In accordance with the molecular formula, 15 carbon resonances were resolved in the ^{13}C NMR spectrum (Table 1) and were further classified by DEPT experiment into two methyls, four methylenes (one olefinic one), five methines (one oxygenated), and four quaternary carbons (one oxygenated, one carbonyl, and one olefinic one). In addition, one tertiary methyl (δ_H 1.46, s) and one secondary methyl (δ_H 0.99, 3H, d, J = 6.6 Hz) were distinguished by analysis of the ^1H NMR data (Table 1).

The $^1H-^1H$ COSY, HSQC, and HMBC spectra (Figure 1) revealed the structure of compound **1**. The $^1H-^1H$ COSY spectrum of **1** showed the connectivities of three partial structures, **a** (C-1−C-2−C-7−C-6), **b** (C-4−C-5), and **c** (C-13−C-12−C-11), as indicated in Figure 1. The terminal olefinic protons at δ_H 5.11 and 5.51 were assigned to C-14 by the HMBC correlations from H-14 to C-9 (δ_C 159.5), C-1 (δ_C 71.7), and C-8 (δ_C 84.3), and the oxygenated methine H-1 (δ_H 4.74) was correlated with C-7 (δ_C 54.9), C-8 (δ_C 84.3), and C-14 (δ_C 114.5). The key methine proton H-7 (δ_H 2.18), which was among three rings, was correlated with C-5 (δ_C 23.7), C-12 (δ_C 37.4), C-6 (δ_C 42.0), C-2 (δ_C 52.8), C-1 (δ_C 71.7), C-8 (δ_C 84.3), and C-9 (δ_C 159.5). On the basis of HMBC correlations from H-15 to C-5 (δ_C 23.7)(w), C-4 (δ_C 37.1), and C-2 (δ_C 52.8), the methyl was placed at C-3 (δ_C 70.7), and the correlations of H-4a and H-5b with C-3 (δ_C 70.7) indicated that the partial structure **b** was connected to C-3. The other methyl was placed at C-12 (δ_C 37.4) on the basis of HMBC correlations from H-13 to C-12 (δ_C 37.4), C-6 (δ_C 42.0), C-11 (δ_C 47.1), and C-10 (δ_C 210.7), and the HMBC correlations of H-11 with C-13 (δ_C 18.8), C-12 (δ_C 37.4), C-6 (δ_C 42.0), and C10 (δ_C 210.7) revealed that the partial structure **c** was connected to the carbonyl group at C-10. The planar structure of **1** was therefore assigned as indicated.

The relative configuration of **1** was established unambiguously by X-ray diffraction (Figure 2), which was consistent with that of **1**, as shown by the ROESY spectrum: correlations of H-2, H-3, and H-12 and between H-6 and H-13. Thus, the structure of stereumin H was elucidated to be **1**. We assigned the skeleton to the stereumane-type. Its absolute configuration was assigned using density functional theory (DFT) at the B3LYP/aug-cc-pVDZ//B3LYP/6-31G(d) level, which has been widely used.[9−16] The computed optical rotation (OR) value for (1*S*,2*S*,3*R*,6*R*,7*R*,8*S*,12*S*) was −60.7 in the gas phase or −58.4 in methanol. As the relative configuration was established using the X-ray experiment and the

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Table 1. ^{1}H and ^{13}C NMR Data for Stereumins H (1), I (2), and J (3)

	1[a]		2[a]		3[b]	
position	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)
1	4.74 (d, J = 7.3 Hz, 1H)	71.7 (d)	4.31 (d, J = 8.6 Hz, 1H)	74.0 (d)	4.58 (d, J = 7.5 Hz, 1H)	73.2 (d)
2	2.46 (t(dd), J = 5.5 Hz, 1H)	52.8 (d)	2.30 (m, 1H)	56.9 (d)	2.34 (t(dd), J = 5.9 Hz, 1H)	50.9 (d)
3		70.7 (s)		70.5 (s)		71.8 s
4A	1.93 (dt, J = 4.4, 9.1 Hz, 1H)	37.1 (t)	1.68 (m, 1H)	35.7 (t)	1.84 (dd, J = 3.2, 10.8 Hz, 1H)	38.2 (t)
4B	1.69 (m, 1H)		1.43 (m, 1H)		1.61 (m, 1H)	
5A	1.79 (m, 1H)	23.7 (t)	1.61 (m, 1H)	23.8 (t)	1.61 (m, 1H)	25.1 (t)
5B	1.41 (dt, J = 3.7, 12.7 Hz, 1H)		1.25 (m, 1H)		1.29 (dt, J = 4.0, 11.6 Hz, 1H)	
6	1.43 (m, 1H)	42.0 (d)	0.81 (m, 1H)	42.3 (d)	0.95 (m, 1H)	43.1 (d)
7	2.18 (dd, J = 5.5, 11.5 Hz, 1H)	54.9 (d)	2.15 (m, 1H)	52.4 (d)	1.95 (d, J = 5.9, 11.9 Hz, 1H)	49.8 (d)
8		84.3 (s)		84.0 (s)		80.0 (s)
9		159.5 (s)		155.2 (s)		160.0 (s)
10		210.7 (s)		209.6 (s)	4.07 (m, 1H)	70.1 (d)
11A	2.53 (dd, J = 3.7, 15.2 Hz, 1H)	47.1 (t)	2.44 (dd, J = 3.6, 15.6 Hz, 1H)	47.0 (t)	1.84 (dd, J = 3.2, 10.8 Hz, 1H)	40.6 (t)
11B	2.28 (t (dd), J = 15.2 Hz, 1H)		2.10 (m, 1H)		1.39 (m, 1H)	
12	1.69 (m, 1H)	37.4 (d)	1.53 (m, 1H)	36.3 (d)	1.58 (m, 1H)	30.3 (d)
13	0.99 (d, J = 6.6 Hz, 3H)	18.8 (q)	0.95 (d, J = 6.5 Hz, 3H)	18.8 (q)	0.86 (d, J = 6.6 Hz, 3H)	19.1 (q)
14A	5.51 (s, 1H)	114.5 (t)	5.48 (s, 1H)	115.5 (t)	5.32 (s, 1H)	112.9 (t)
14B	5.11 (s, 1H)		5.06 (d, (s, J = 1.9 Hz, 1H)		5.39 (s, 1H)	
15	1.46 (s, 3H)	28.7 (q)	1.40 (s, 3H)	29.3 (q)	1.38 (s, 3H)	29.1 (q)

[a] Data were recorded in $CDCl_3$ on Bruker DRX-500 or 600 MHz spectrometers. [b] Data were recorded in CD_3OD on a Bruker AMD-400 MHz.

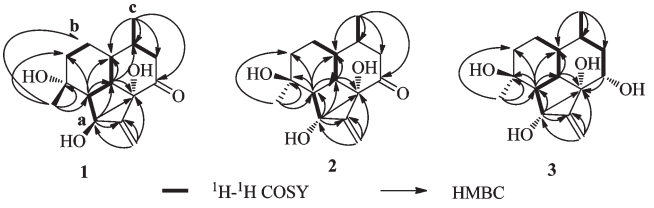

Figure 1. Key HMBC and $^{1}H-^{1}H$ COSY correlations of stereumins H (1), I (2), and J (3).

recorded OR was +67.2, the absolute configuration was assigned as (1*R*,2*R*,3*S*,6*S*,7*S*,8*R*,12*R*).

Stereumin I (2) had the molecular formula $C_{15}H_{22}O_4$, as determined by the HRESIMS ion at m/z 289.1420 $[M + Na]^+$, with 5 degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra of **2** closely resembled those of stereumin H (**1**) except that the chemial shifts of several carbons were different: C-1 at δ_C 74.0, C-2 at δ_C 56.9, C-7 at δ_C 52.4, and H-6 at δ_H 0.81 in **2** instead of at δ_C 71.7, 52.8, and 54.9 and δ_H 1.43 in **1**. The $^{1}H-^{1}H$ COSY, HSQC, and HMBC spectra revealed the structural details of **2** (Figure 1). The ROESY experiment showed that the differences were at the C-1 stereocenter, where there was opposite configuration. The relative configuration of **2** was deduced from the ROESY experiment, which showed correlations between H-6 and H-1, H-13, between H-2 and H-7, H-15, and between H-7 and H-12. Stereumin I (**2**) and stereumin H (**1**) were diastereoisomers, and the relative configuration of **2** was as shown. Its relative configuration was supported by the ^{13}C NMR and computation obtained at the B3LYP/aug-cc-pVDZ//B3LYP/6-31G(d) level.[14,17−24] Most of the chemical shift errors between the computed ^{13}C NMR and recorded one were less than 6 ppm, except for C-7 (6.9 ppm) and C-14 (7.2 ppm), which were less than the maximum control of 8.0 ppm. The absolute configuration

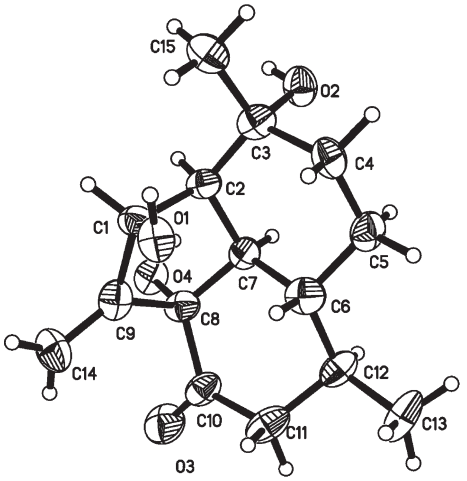

Figure 2. X-ray structure of compound **1**.

was assigned as (1*R*,2*S*,3*R*,6*R*,7*R*,8*S*,12*R*) on the basis of comparing the recorded OR (+57.3) and the computed OR magnitude (+70) in the gas phase for (1*R*,2*S*,3*R*,6*R*,7*R*,8*S*,12*R*)-**2** using the methods above.

Stereumin J (**3**) had the molecular formula $C_{15}H_{22}O_4$ by the HRESIMS ion at m/z 291.1566 $[M + Na]^+$, with 4 degrees of unsaturation. The NMR data of **3** were very similar to those of **2** (Table 1). The main differences were the chemical shifts of C-10 at δ_C 209.6, C-11 at δ_C 47.0, and C-12 at δ_C 36.3 for **2**, contrasting those at δ_C 70.1, 40.6, and 30.3 for **3**, respectively. Considering the molecular weights of **3** and **2**, the structure of **3** had two more hydrogen atoms than **2** and the C-10 carbonyl in **2** was reduced to OH in **3**. In the HMBC spectrum (Figure 1), the oxygenated methine proton H-10 (δ_H 4.07) was correlated

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with C-9 (δ_C 160.0), C-8 (δ_C 80.0), C-7 (δ_C 49.8), C-11 (δ_C 40.6), and C-12 (δ_C 30.3). Furthermore, most correlations supporting the structure of **2** were also observed in the 2D NMR spectroscopic data (Figure 1) of **3**. Thus, the structure of **3** was determined to be as shown.

No inhibitory activity was observed for compounds **1**−**3** against the nematode *Panagrellus redivivus* at 100 μg/mL or against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* at 50 μg/mL using the disk diffusion method, or against cell lines HL-60, SMMC-7721, and A-549 at 40 μM in the MTT assays.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a Jasco DIP-370 digital polarimeter. Melting point was measured using an uncorrected XT-4 Digital Display micromelting point apparatus. IR spectra were measured on a Paragon 1000 PC spectrometer. UV spectra were measured on a Shimadzu UV-2401PC spectrophotometer, λ_{max} (log ε) in nm. NMR spectra were obtained with Bruker AM-400, Bruker DRX-500, and Bruker Avance III-600 NMR spectrometers with TMS as an internal standard. ESIMS and HRESIMS were recorded on a VG Auto-Spec-3000 mass spectrometer. Column chromatography (CC) was performed on silica gel G (200−300 mesh, Qingdao Marine Chemical Factory, China) and Sephadex LH-20 (Amersham Pharmacia, Sweden).

Fungal Material. The voucher specimen of the basidiomycete *Stereum* sp. was deposited in the China Center for Type Culture Collection (strain no. CCTCC AF 207024). The strain was identified on the basis of the ITS sequence and partial 28S rDNA (GenBank accession no. EF067346 and EF600046).[7]

Extraction and Isolation. Fermentation of *Stereum* sp. CCTCC AF 207024 (50 L) was on PDB (potato dextrose broth) medium for 15 days at 25 °C. It was then concentrated under reduced pressure and extracted with EtOAc to obtain the crude extract (10.8 g). The EtOAc extract was subjected to silica gel G CC (300 g) eluted with petroleum ether/acetone with increasing polarity from 5% to 80% to yield fractions A_1−A_{13}. Fraction A_5 (1.06 g) was applied to Sephadex LH-20 CC (50 g) eluted with MeOH to obtain fractions $A_{5\text{-}1}$−$A_{5\text{-}5}$. Fraction $A_{5\text{-}5}$ (62 mg) was applied to Sephadex LH-20 CC (30 g) eluted with acetone to obtain fractions $A_{5\text{-}5\text{-}1}$−$A_{5\text{-}5\text{-}4}$. Fraction $A_{5\text{-}5\text{-}2}$ (11 mg) was purified by silica gel H CC (2 g, petroleum ether/EtOAc, 9:1) to obtain compound **1** (8 mg). Fraction A_7 (254 mg) was applied to silica gel H CC (20 g, petroleum ether/EtOAc, 9:1 to 4:1) to furnish fractions $A_{7\text{-}1}$−$A_{7\text{-}8}$. Fraction $A_{7\text{-}6}$ (15 mg) was purified further by Sephadex LH-20 CC (30 g) three times, eluted with acetone, to yield compound **2** (6 mg). Fraction A_8 (763 mg) was subjected to silica gel H CC (50 g, petroleum ether/acetone, 20:1 to 1:1) to afford fractions $A_{8\text{-}1}$−$A_{8\text{-}13}$. Fraction $A_{8\text{-}6}$ (20 mg) was purified repeatedly by Sephadex LH-20 CC (39 g) eluted with acetone to yield compound **3** (7 mg).

Stereumin H (***1***)*:*. colorless needles; mp 173−175 °C; $[\alpha]_D^{22}$ +67.1 (*c* 0.60, MeOH); UV (MeOH) λ_{max} (log ε) 216 (3.28), 279 (2.58) nm; IR (KBr) ν_{max} 3423, 2926, 1705 (s), 1640, 1376, 1110 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 289 $[M + Na]^+$, 555 $[2M + Na]^+$; HRESIMS *m*/*z* 289.1419 $[M + Na]^+$, calcd for $C_{15}H_{22}O_4Na$, 289.1415.

X-ray measurements were made on a SMART CCD area detector with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). **1** (MF = $C_{15}H_{22}O_4$, M_r = 266.15): crystal dimensions 0.31 × 0.18 × 0.09 mm, orthorhombic, space group triclinic, *P*1, *a* = 8.583(2) Å, *b* = 9.549(3) Å, *c* = 11.107(3) Å, *V* = 774.5(4) Å^3, *Z* = 2, ρ_{calcd} = 1.217 Mg m^{-3}, μ = 0.090 mm^{-1}, *T* = 293(2) K, $2\theta_{max}$ = 28.46°, 5574 measured reflections, 5312 independent reflections (R_{int} = 0.0253), 372 parameters refined, *R* = 0.0875 (for 5312 reflections with $I > 2.00\,\sigma(I)$), R_w = 0.1952, max./min. residual peaks in the final difference map 0.299/−272 e Å^{-3}. Crystallographic data for the stereumin H reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC-754868, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Stereumin I (***2***)*:*. colorless powder; $[\alpha]_D^{22}$ +53.7 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 216 (2.48), 253 (1.99) nm; IR (KBr) ν_{max} 3422, 2927, 1708 (s), 1378, 1087 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 289 $[M + Na]^+$, 555 $[2M + Na]^+$; HRESIMS *m*/*z* 289.1420 $[M + Na]^+$, calcd for $C_{15}H_{22}O_4Na$, 289.1415.

Stereumin J (***3***)*:*. colorless powder; $[\alpha]_D^{22}$ +14.3 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 216 (2.92) nm; IR (KBr) ν_{max} 3425, 2925, 1706, 1371, 1010 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 291 $[M + Na]^+$, 559 $[2M + Na]^+$; HRESIMS *m*/*z* 291.1566 $[M + Na]^+$, calcd for $C_{15}H_{24}O_4Na$, 291.1572.

Computational Methods. Conformational searches were performed using the MMFF94 force field. The geometries with relative energy from 0 to 8 kcal/mol were selected for further optimizations at the B3LYP/6-31G(d) level. These conformers having 0−2.5 kcal/mol energy were used in OR computations at the B3LYP/aug-cc-PVDZ level in the gas phase or in methanol using the PCM model. ^{13}C NMR computations were carried out at the B3LYP/aug-cc-pVDZ//B3LYP/6-31G(d) level.

ASSOCIATED CONTENT

Supporting Information. 1D and 2D NMR (COSY, HSQC, HMBC, ROESY) spectra for compounds **1**−**3** and CIF files of X-ray crystallographic data of compound **1** are available free of charge via the Internet at http://pubs.acs.org.

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