Two New Sesquiterpenes from the Fungus *Stereum* sp.

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Two new sesquiterpenes, stereumin F (**1**) and stereumin G (**2**), together with two known compounds (3*β*,5*α*,22*E*,24*R*)-5,8-epidioxyergosta-6,22-dien-3-ol (**3**) and 4-(2-hydroxyethyl)phenol (**4**), were isolated from the AcOEt extract of the culture broth and the MeOH extract of the mycelium of the fungal strain *Stereum* sp. CCTCC AF 207024. Their structures were established on the basis of spectral analyses.

Introduction. – Mushrooms produce a great diversity of substances that are potentially active in many fields. Strain CCTCC AF 207024 belongs to the Stereaceae family, *Stereum* genus, and separates from wild fruiting body. Previous phytochemical studies on the fungal genus *Stereum* have resulted in the isolation of several interesting new compounds including acetylenic aromatics [1], sesquiterpenes [2], phenolic compounds [3], chromene, and aromatic-aldehyde derivatives [4], some of which showed antimicrobial, phytotoxic, antitumor, and nematicidal activities [5–7].

In our previous study, a series of novel sesquiterpenes was obtained from the strain CCTCC AF 207024 [7]. In the present investigation, four compounds including two new sesquiterpenes were isolated from this fungus. We now report the isolation and structure elucidation of these compounds (*Fig. 1*).

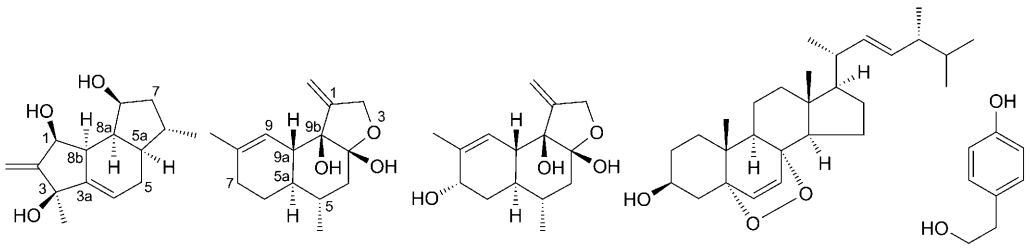

Fig. 1. *Compounds* **1**–**4** *isolated from the fungal strain* Stereum *sp. CCTCC AF 207024 and stereumin A*

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Results and Discussion. – Compound **1** was obtained as colorless powder. The HR-ESI-MS data determined the molecular formula to be $C_{15}H_{22}O_3$ (*m/z* 273.1464 ($[M+Na]^+$)). The DEPT experiment (*Table 1*) showed 15 C-atom signals for two Me, three CH_2, and seven CH groups, and three quaternary C-atoms including 4 sp^2 C-atoms (δ(C) 112.9 and 158.0, and δ(C) 122.0 and 136.8). The HMBC data (*Table 1*) showed ^{1}H,^{13}C-NMR long-range correlations between the exocyclic CH_2=C moiety at δ(H) 5.38 and 5.30 (CH_2=C(2)) and C(3) and C(2), between the Me group at δ(H) 1.81 (Me−C(3)) and C(3), C(2), C(3a), and C(8b), between δ(H) 4.19 (H−C(1)) and C(2), C(3a), and *C*H_2=C(2), between δ(H) 2.52 (H−C(8b)) and C(3a), C(4), and C(1), and between δ(H) 5.42 (H−C(4)) and C(8b), C(5) and *Me*−C(3), to afford subunit **1a** (*Fig. 2*). At the same time, the HMBC data (*Table 1*) showed correlations between the Me−C(6) at δ(H) 0.81 (*d*, *J* = 6.6) and C(8), C(7), and C(6), and between the CH group at δ(H) 0.62–0.58 (H−C(8a)) and C(5), C(6), and C(7), which together with the ^{1}H,^{1}H-COSY cross-peaks H−C(6)/Me−C(6), and H−C(8a)/H−C(5a) provided the fragment **1b** (*Fig. 2*). Fragments **1a** and **1b** were connected according to the correlations between H−C(5a) and C(5), and between H−C(8b) and C(8a). The relative configuration was deduced from a ROESY experiment, establishing the correlations H−C(1)/H−C(8a), H−C(8b), and H−C(8), H−C(8b)/Me−C(3) and H−C(5a), and H−C(5a)/Me−C(6) (*Fig. 2*). The above spectroscopic data established the structure of **1** to be *rel*-(1*R*,3*S*,5a*S*,6*R*,8*R*,8a*R*,8b*R*)-1,2,3,5,5a,6,7,8,8a,8b-decahydro-3,6-dimethyl-2-methylene-*as*-indacene-1,3,8-triol (*Fig. 1*), for which we propose the trivial name stereumin F [7].

Table 1. *1D- and 2D-NMR Data* (500/125 MHz) *of Compound* **1** *in* CD_3OD

	δ(H)	δ(C)	HMBC (H → C)
H−C(1)	4.19 (br. *d*, *J* = 7.8)	81.2 (*d*)	C(2), C(3a), *C*H_2=C(2)
C(2)	–	158.0 (*s*)	–
C(3)	–	80.3 (*s*)	–
C(3a)	–	136.8 (*s*)	–
H−C(4)	5.42 (br. *d*, *J* = 5.4)	122.0 (*d*)	C(8b), C(8a), C(5), *Me*−C(3)
CH_2(5)	2.10–2.05 (*m*, H_α),	30.7 (*t*)	C(3a), C(4), C(8a)
	1.61–1.59 (*m*, H_β)		–
H−C(5a)	1.87–1.83 (*m*)	49.0 (*d*)	C(8), C(7), C(5), C(6)
H−C(6)	1.54–1.52 (*m*)	29.8 (*d*)	–
CH_2(7)	1.78–1.76 (*m*, H_α),	40.1 (*t*)	C(8), C(6)
	1.30–1.24 (*m*, H_β)		C(6)
H−C(8)	4.05–4.00 (*m*)	70.0 (*d*)	C(1) (*w*), C(8b)
H−C(8a)	0.62–0.58 (*m*)	41.6 (*d*)	C(5), C(6), C(7), C(8b)
H−C(8b)	2.52–2.47 (*m*)	49.8 (*d*)	C(3a), C(4), C(1), C(8a)
Me−C(3)	1.81 (*s*)	23.0 (*q*)	C(2), C(3a), C(3), C(8b)
CH_2=C(2)	5.38 (*s*), 5.30 (*s*)	112.9 (*t*)	C(2), C(3); C(2), C(3)
Me−C(6)	0.81 (*d*, *J* = 6.6)	19.0 (*q*)	C(8), C(7) (*w*), C(6)

Compound **2** was obtained as colorless powder. The HR-ESI-MS data determined the molecular formula to be $C_{15}H_{22}O_3$ (*m/z* 273.1461 ($[M+Na]^+$)). The DEPT experiment (*Table 2*) showed 15 C-atom signals for two Me, five CH_2, and four CH

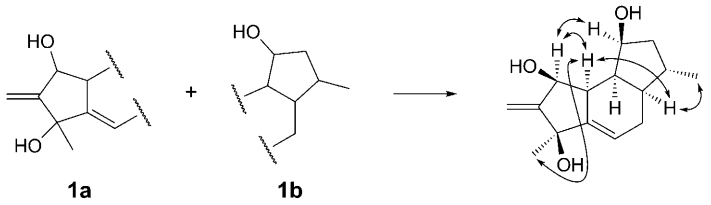

Fig. 2. *The fragments of compound* **1** *and its NOEs*

groups, and four quaternary C-atoms including two C=C bonds (δ(C) 111.7 and 147.5, and δ(C) 120.6 and 135.3). The NMR data of compound **2** (*Table 2*) was very similar to those of stereumin A [7]. The HMBC data (*Table 2*) showed ^{1}H,^{13}C-NMR long-range correlations between the CH=C moiety at δ(H) 5.71 (H–C(9)) and *Me*–C(8), C(7), and C(5a) and between the Me group at δ(H) 1.68 (Me–C(8)) and C(7), C(9) and C(8). The relative configuration was deduced from a ROESY experiment, which showed the correlations H–C(9a)/H–C(5) and Me–C(5)/H–C(5a). These spectroscopic data established the structure of stereumin G (**2**) to be *rel*-(3a*R*,5*S*,5a*R*,9a*R*,9b*S*)-1,2,4,5,5a,6,7,9a-octahydro-5,8-dimethyl-1-methylenenaphtho[2,1-*b*]furan-3a,9b-diol (*Fig. 1*).

Table 2. *1D- and 2D NMR Data* (500/125 MHz) *of Compound* **2** *in* $CDCl_3$

	δ(H)	δ(C)	HMBC (H → C)
C(1)	–	147.5 (*s*)	–
CH_2(2)	4.54 (*d*, *J* = 13.6),	67.0 (*t*)	C(9b), C(3), C(1) (*w*)
	4.16 (*d*, *J* = 13.5)		C(1) (*w*), *C*H_2=C(1)
C(3a)	–	105.8 (*s*)	–
CH_2(4)	1.89–1.96 (*m*),	42.0 (*t*)	C(9b), C(9), C(8), C(5a), C(3)
	0.99–1.09 (*m*)		–
H–C(5)	1.35–1.43 (*m*)	33.7 (*d*)	–
H–C(5a)	1.23–1.26 (*m*)	41.6 (*d*)	C(5), C(5a), *Me*–C(5), C(4)
CH_2(6)	1.89–1.96 (*m*),	26.7 (*t*)	C(9), C(8), C(5a)
	1.10–1.16 (*m*)		C(5a), C(4)
CH_2(7)	2.16–2.20 (*m*),	30.7 (*t*)	–
	1.89–1.96 (*m*)		C(9), C(8), C(5a)
C(8)	–	135.3 (*s*)	–
H–C(9)	5.71 (*s*)	120.6 (*d*)	*Me*–C(8), C(7), C(5a)
H–C(9a)	2.25 (*d*, *J* = 9.8)	46.1 (*d*)	–
C(9b)	–	77.5 (*s*)	–
CH_2=C(1)	5.52 (*s*), 5.02 (*s*)	111.7 (*t*)	C(2), C(1) (*w*); C(2), C(1) (*w*)
Me–C(5)	0.88 (*d*, *J* = 6.5)	19.1 (*q*)	C(5), C(5a), C(4), C(3)
Me–C(8)	1.68 (*s*)	23.8 (*q*)	C(9), C(8), C(7)

Compounds **3** and **4** were determined as (3β,5α,22*E*,24*R*)-5,8-epidioxyergosta-6,22-dien-3-ol (**3**) [8] and 4-(2-hydroxyethyl)phenol (**4**) [9] by comparison with the literature data.

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Experimental Part

General. TLC: precoated silica gel *G* plates (SiO_2; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China). Column chromatography (CC): *Sephadex LH-20* (*Pharmacia*); SiO_2 (200–300 mesh; *Qingdao Marine Chemical Factory*). Optical rotations: *Jasco-DIP-370* digital polarimeter. UV Spectra: *Shimadzu-2401PC* spectrophotometer; λ_{max} log (ε) in nm. NMR Spectra: *Bruker-DRX-500* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI- and ESI-MS: *VG-Auto-Spec-3000* mass spectrometer and *Finnigan LCQ-Advantage*; in *m*/*z* (rel. %).

Fungal Material. The fungus used in this study was collected in Xishuangbanna, Yunnan Province, P. R. China. The mycelium of the basidiomycete was separated from its fruiting body, and the strain was deposited with the *China Center for Type Culture Collection* (number of strain, CCTCC AF 207024). The fungus was grown in a shake culture (150 ml per 250 ml triangular flask) on a PDB medium consisting of glucose (20 g) and potato (200 g, boiled and filtered) (per liter of H_2O), and incubated for 8 d at 120 rpm under 25°. The culture was harvested for further study.

Extraction and Isolation. The culture (10 l) of *Stereum* sp. CCTCC AF 207024 was filtered to separate cell and broth. The culture broth was extracted with AcOEt, and the extract was concentrated. The AcOEt extract (2.2 g) was subjected to CC (SiO_2 *G* (30 g), petroleum ether/acetone 20:1 → 1:1): *Fractions* A_1–A_8. *Fr.* A_1 was repeatedly applied to CC (*Sephadex LH-20* (30 g), acetone): *Frs.* $A_{1.1}$–$A_{1.4}$. *Fr.* $A_{1.2}$ was purified by CC (SiO_2 *G* (20 g), petroleum ether/AcOEt 9:1): *Frs.* $A_{1.2.1}$–$A_{1.2.5}$, *Fr.* $A_{1.2.2}$ was subjected to CC (SiO_2 *G* (4 g), petroleum ether/acetone 12:1) and purified further by CC (*Sephadex LH-20*, acetone): **2** (10 mg). *Fr.* A_2 was applied to CC (SiO_2 *G* (15 g), petroleum ether/acetone 9:1): *Frs.* $A_{2.1}$–$A_{2.5}$. *Fr.* $A_{2.2}$ was purified further by CC (*Sephadex LH-20* (15 g), acetone): **1** (5 mg). *Fr.* A_3 was subjected to CC (SiO_2 *G* (10 g), petroleum ether/AcOEt 9:1 → 7:1) and further purified by CC (SiO_2 *G* (2 g), petroleum ether/acetone 9:1): **4** (6 mg). The mycelium of the strain CCTCC AF 207024 was extracted with MeOH. The crude MeOH extract (1.8 g) was subjected to CC (SiO_2 *G* (30 g), petroleum ether/AcOEt 20:1–10:1): *Frs.* B_1–B_{10}. *Fr.* B_7 was purified further by CC (*Sephadex LH-20* (15 g), acetone): **3** (8 mg).

Stereumin F (= rel-*(1*R,*3*S,*5a*S,*6*R,*8*R,*8a*R,*8b*R*)-1,2,3,5,5a,6,7,8,8a,8b-Decahydro-3,6-dimethyl-2-methylene*-as-*indacene-1,3,8-triol*; **1**): Colorless powder. $[\alpha]_D^{26} = +27.40$ ($c = 0.10$, MeOH). UV (MeOH): 203 (3.45). ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS: 273 ($[M+Na]^+$). HR-ESI-MS: 273.1464 ($[M+Na]^+$, $C_{15}H_{22}NaO_3^+$; calc. 273.1467).

Stereumin G (= rel-*(3a*R,*5*S,*5a*R,*9a*R,*9b*S*)-1,2,4,5,5a,6,7,9a-Octahydro-5,8-dimethyl-1-methylenenaphtho[2,1-*b*]furan-3a,9b-diol*; **2**): Colorless powder. $[\alpha]_D^{26} = +146.00$ ($c = 0.025$, $CHCl_3$). UV (MeOH): 197 (3.75), 204 (3.22). ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS: 273 ($[M+Na]^+$). HR-ESI-MS: 273.1461 ($[M+Na]^+$, $C_{15}H_{22}NaO_3^+$; calc. 273.1467).

*(3β,5α,22*E,*24*R*)-5,8-Epidioxyergosta-6,22-dien-3-ol* (**3**): Colorless needles. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.88 (*s*, Me(18)); 0.81–0.83 (*m*, Me(26), Me(27)); 0.88 (*s*, Me(19)); 0.91 (*d*, *J* = 6.8, Me(28)); 1.01 (*d*, *J* = 6.4, Me(21)); 3.95–3.99 (*m*, H–C(3)); 5.13 (*d*, *J* = 7.6, H–C(22)); 5.27 (*d*, *J* = 7.6, H–C(23)); 6.25 (*d*, *J* = 8.5, H–C(6)); 6.51 (*d*, *J* = 8.4, H–C(7)). ^{13}C-NMR (125 MHz, $CDCl_3$): 13.3 (*q*, C(18)); 17.9 (*q*, C(28)); 18.6 (*q*, C(19)); 20.0 (*q*, C(27)); 20.2 (*q*, C(26)); 21.3 (*q*, C(21)); 21.0 (*t*, C(15)); 23.8 (*t*, C(11)); 29.0 (*t*, C(16)); 30.5 (*t*, C(2)); 33.5 (*d*, C(25)); 35.1 (*t*, C(1)); 37.4 (*s*, C(10)); 37.4 (*t*, C(4)); 39.8 (*t*, C(12)); 40.1 (*d*, C(20)); 43.1 (*d*, C(24)); 45.0 (*s*, C(13)); 51.6 (*d*, C(9)); 52.1 (*d*, C(14)); 56.7 (*d*, C(17)); 66.9 (*d*, C(3)); 79.8 (*s*, C(8)); 82.6 (*s*, C(5)); 131.1 (*d*, C(7)); 132.7 (*d*, C(23)); 135.6 (*d*, C(22)); 136.8 (*d*, C(6)). ESI-MS: 429 ($[M+H]^+$).

4-(2-Hydroxyethyl)phenol (= *4-Hydroxybenzeneethanol*; **4**): Colorless amorphous solid. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.10 (*d*, *J* = 8.2, H–C(3,5)); 6.79 (*d*, *J* = 8.3, H–C(2,6)); 3.84 (*t*, *J* = 6.4, CH_2CH_2OH); 2.82 (*t*, *J* = 6.5, CH_2CH_2OH). ^{13}C-NMR (125 MHz, $CDCl_3$): 154.7 (*s*, C(1)); 130.8 (*s*, C(4)); 130.6 (*d*, C(3,5)); 115.9 (*d*, C(2,6)); 38.7 (*t*, CH_2CH_2OH); 64.2 (*t*, CH_2CH_2OH). ESI-MS: 183 ($[M+HCOO]^-$), 277 ($[2M+H]^+$).

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