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Sterhirsutins A and B, Two New Heterodimeric Sesquiterpenes with a New Skeleton from the Culture of *Stereum hirsutum* Collected in Tibet Plateau

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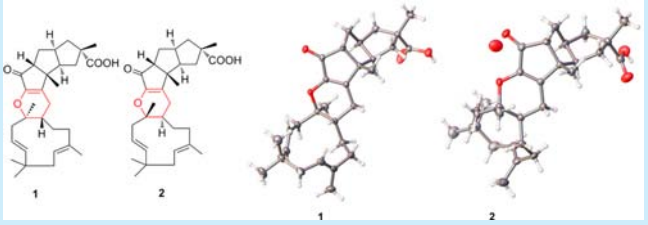
Supporting Information

ABSTRACT: Two new heterodimeric sesquiterpenes, sterhirsutins A (**1**) and B (**2**), and two new sesquiterpenes, hirsutic acids D−E (**3** and **4**), were identified from the culture of *Stereum hirsutum*. The absolute configurations in **1** and **2** were confirmed by single-crystal X-ray diffraction experiments and electronic circular dichroism (ECD) calculations. Compounds **1** and **2** are likely biosynthesized from a hirsutane-type sesquiterpene and α-humulene by a hetero-Diels−Alder cycloaddition. Compounds **1**−**4** showed cytotoxicity against K562 and HCT116 cell lines.



Natural products biosynthesized by Diels−Alder reaction have attracted much attention due to their diverse chemical structures and interesting bioactivities. Several examples of this unique group of natural products include przewalskone with cytotoxicity from the roots of *Salvia przewalskii*,[1] psidial A and (+)-guajadial B from *Psidium guajava*,[2,3] macrocyclic antibiotics nargenicin from *Nocardia argentinensis*[4] and ikarugamycin from *Streptomyces phaeochromogenes*,[5] torreyanic acid with cytotoxicity from the endophytic fungus *Pestalotiopsis microspora*,[6] daphniphyllum alkaloids from *Daphniphyllum macropodum*,[7] cytosporolides A−C with antibacterial activity from *Cytospora* sp.,[8] and chloropupukeananin from the plant endophyte *Pestalotiopsis fici*.[9]

The fungi belonging to the genus of *Stereum* are known to produce bioactive secondary metabolites, including cytotoxic hirsutane-type[10,11] and stereumane-type sesquiterpenes,[12,13] illudalane related sesquiterpenes,[14,15] acetylenic aromatic compounds with phytotoxicity,[16] epidioxysterols with inhibitory activity against *Mycobacterium tuberculosis*,[17] and depsides with 11-hydroxylsteroid dehydrogenase inhibitory activity.[18] In our efforts to search for new bioactive secondary metabolites from fungi collected in the region of Tibet Plateau, a strain of *S. hirsutum* (L515) was isolated from its fruiting body. The strain was grown in a solid-substrate fermentation culture. Chemical investigation on the solid culture of this fungus resulted in the isolation of two unprecedented heterodimeric sesquiterpenes, sterhirsutins A and B (**1** and **2**); two new hirsutane-type sesquiterpenes, hirsutic acids D−E (**3** and **4**); and one known sesquiterpene (**5**) (Figure 1).[11] Details of the structural elucidation and cytotoxicity of **1**−**4**, as well as the plausible biogenesis of **1** and **2**, are reported herein.

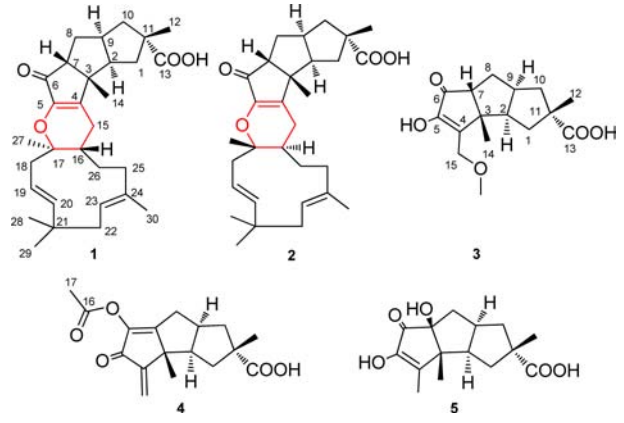


Figure 1. Structures of compounds **1**−**5**.

Sterhirsutin A (**1**) was obtained as a white crystalline and was determined to have a molecular formula of $C_{30}H_{42}O_4$ (10 degrees of unsaturation) on the basis of HRESIMS data at m/z 467.3160 $[M + H]^+$ (calcd 467.3156). The ^1H and APT-^{13}C NMR spectra of **1** (Table S3) showed resonances for six methyl groups [δ_H 0.92 (6H, s), 1.00 (3H, s), 1.20 (3H, s), 1.61 (6H, s); δ_C 19.9, 24.6, 30.6, 21.2, 28.4, and 17.5], eight methylenes,

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four methines, four quaternary carbons with one oxygenated (δ_C 82.1), six olefinic carbons with three protonated [δ_C 121.2 (=CH), 123.6 (=CH), 137.2, 142.8 (=CH), 148.8, 150.8], and two carbonyl carbons (δ_C 181.4, 202.9). The HMBC spectra of **1** revealed the correlations of H_3-12/C-1, C-10, C-11 and C-13, H_3-14/C-2, C-3, C-4 and C-7, H_2-15/C-3, C-4, C-5, and H-7/C-2, C-3, C-4, C-5, C-6, C-8, C-9, and C-14, which in combination with the $^1H-^1H$ COSY correlations of H_2-1-H-2-H-9-H_2-8 (10) established a hirsutane-type sesquiterpene unit (A) (Figure 2).

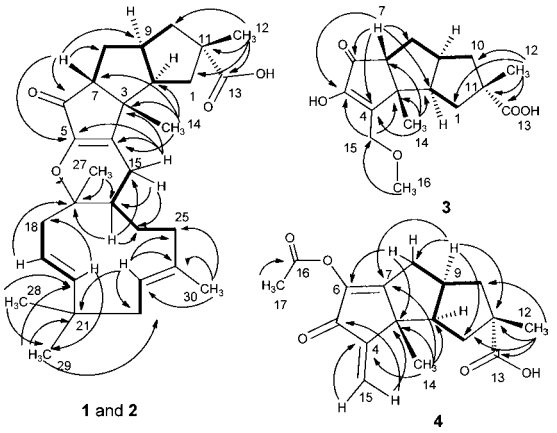

Figure 2. Selected key HMBC and COSY correlations of **1**−**4**.

Furthermore, the HMBC correlations from H_3-27 to C-16, C-17, and C-18, from H_3-28 (29) to C-20, C-21, and C-22, from H_3-30 to C-23, C-24, and C-25, together with the $^1H-^1H$ COSY correlations of H_2-18-H-19-H-20, H_2-22-H-23, and H_2-25-H_2-26-H-16, confirmed the substructure of a humulene unit (B). The presence of the humulene moiety was also supported by the comparison of NMR data with those of (±)-guajadial B.[3] Finally, the HMBC correlations from H_2-15 to C-16, C-17, and C-26, the oxygenated nature of C-5 (δ_C 148.8) and C-17 (δ_C 82.1), and the requirement of unsaturation degree confirmed the linkage between the structural units A and B. Thus, compound **1** was concluded to possess a new chemical skeleton of cyclopenta[5,6]pentaleno[2,1-*b*]cycloundeca[*e*]pyran.

The relative configuration of **1** was determined on the basis of the 1D NOE experiments. NOESY correlations from H-10β (δ_H 1.46) to H-1β (δ_H 1.62) and H_3-12 (δ_H 1.61), from H_3-14 (δ_H 1.20) to H-1β (δ_H 1.62), H-7 (δ_H 2.49), and H-15β [δ_H 2.55 (dd, J = 18.8, 5.4 Hz)], and from H-16 (δ_H 1.94) to H-15β placed these protons on the same face of the 5−5−6−11 ring system. In addition, NOE correlations from H-2 (δ_H 2.77) to H-9 (δ_H 2.41), H-10α (δ_H 2.88), and H-15α [δ_H 1.76 (dd, J = 18.8, 11.3 Hz)] and from H-15α to H_3-27 (δ_H 0.92) demonstrated that H-2, H-9, and H_3-27 were on the opposide side as presented in Figure 3. The coupling constants observed for H_2-15 also confirmed the trans fused ring junction at C-16 and C-17.[19,20] The larger coupling constant of H-19 [δ_H 5.34 (ddd, J = 15.9, 10.7, 2.4 Hz)] and H-20 [δ_H 5.15 (dd, J = 15.9, 1.4 Hz)] and the NOE correlation between H_3-30 (δ_H 1.61) and H-22 (δ_H 2.18) indicated the *E* configuration for two double bonds in the humulene moiety. The relative configuration determined above was further confirmed by X-ray crystallographic analysis using Mo Kα radiation, and the X-ray crystallographic structure of **1** is shown in Figure 4.

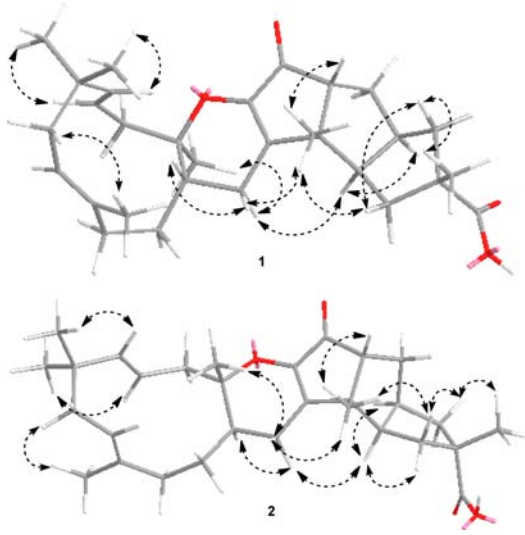

Figure 3. Selected key NOESY correlations of **1** and **2**.

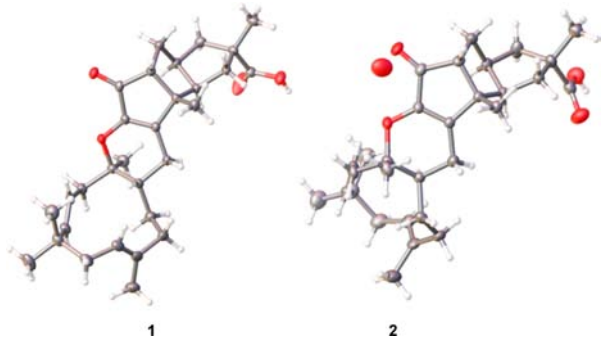

Figure 4. X-ray crystallographic structures of **1** and **2** (the additional oxygen appearing in **2** came from crystal water).

In the CD spectrum of **1**, a positive Cotton effect at ~270 nm (the $\pi-\pi^*$ transition of the α,β-unsaturated cyclopentenone moiety) and a negative Cotton effect at ~310 nm (the n−π^* transition of the α,β-unsaturated cyclopentenone moiety) were observed (Figure 5). To confirm the absolute configuration of **1**, a comparison between the experimental and simulated electronic circular dichroism (ECD) was made. A conformational analysis (Figure S8) was conducted for two isomers (2*R*,3*S*,7*R*,9*S*,11*S*,16*S*,17*R*)-**1a** and (2*S*,3*R*,7*S*,9*R*,11*R*,16*R*,17*S*)-**1b** by the molecular operating environment (MOE) software package using the MMFF94 molecular mechanics force field calculation. Three lowest-energy conformers for each enantiomer were obtained by further reoptimization using TDDFT at the B3LYP/6-31G(d) basis set level. As shown in Figure 5, the calculated ECD curve of **1a** by Boltzmann-weighting of the conformers matched very well with the experimental ECD, indicating that the absolute configuration of **1** was assigned unambiguously. Thus, the absolute configuration of **1** was assigned as 2*R*,3*S*,7*R*,9*S*,11*S*,16*S*,17*R*.

The molecular formula of sterhirsutin B (**2**) was determined to be $C_{30}H_{42}O_4$ by HRESIMS at m/z 467.3151 $[M + H]^+$ (calcd 467.3156). The ^1H, ^{13}C NMR spectra of **2** were quite similar to those of **1** (Table S3), indicating the presence of a hirsutane-type sesquiterpenoid moiety and a humulene moiety. Detailed analysis of its HMBC and $^1H-^1H$ COSY correlations (Figure 2) revealed that compound **2** had the same planar

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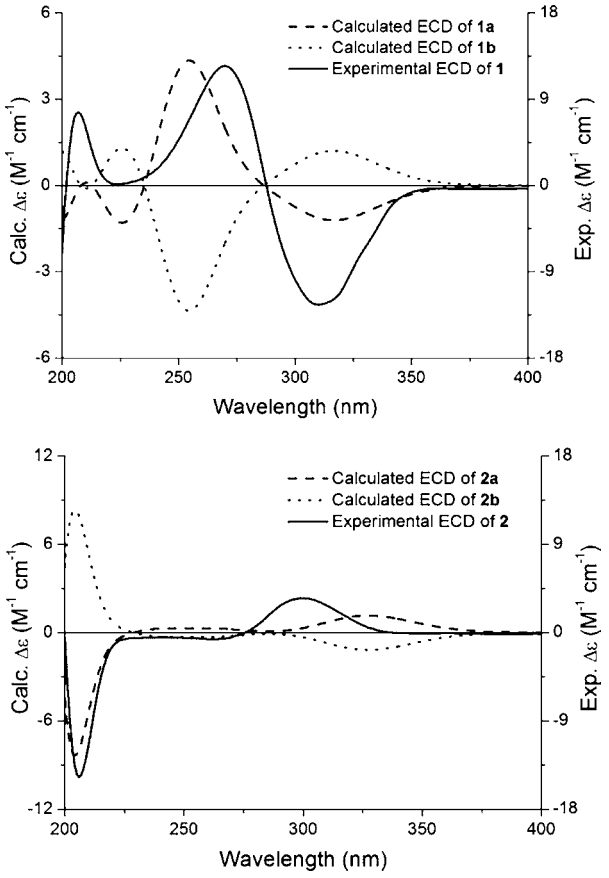


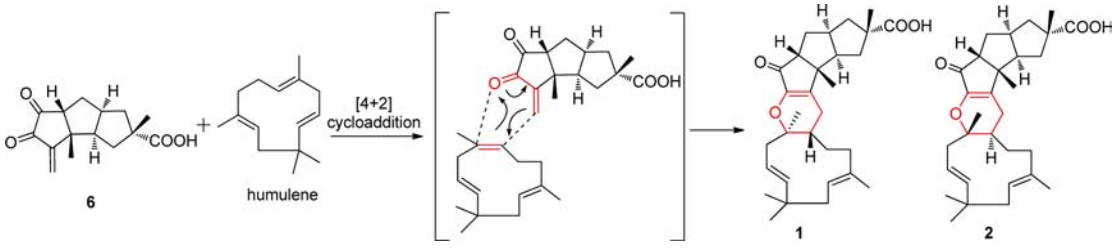
Figure 5. Experimental CD spectrum of **1** and **2** in methanol and the calculated ECD spectra of **1a**, **1b**, **2a**, and **2b**. Structures **1a**, **1b** and **2a**, **2b** represent two possible stereoisomers of **1** and **2**, respectively.

structure as that of **1**. The full assignments of protons and carbons were achieved by the interpretation of 2D NMR. In the 1D NOE experiments, the NOEs from H-10β (δ_H 1.41) to H-1β (δ_H 1.62) and H_3-12 (δ_H 1.57), from H_3-14 (δ_H 1.27) to H-1β, H-7 (δ_H 2.55), and H-15β [δ_H 1.86 (dd, J = 18.5, 11.2 Hz)], from H_3-27 (δ_H 1.04) to H-15β, from H-2 (δ_H 2.82) to H-1α (δ_H 2.58), H-9 (δ_H 2.40), and H-15α [δ_H 2.65 (dd, J = 18.5, 4.9 Hz)], and from H-16 (δ_H 1.94) to H-15α confirmed the same orientation for protons at C-7, C-12, C-14, andC-27 and the opposite orietation for H-2, H-9, and H-16. As described in **1**, the *E* configuration for two double bonds in the humulene moiety was assigned by the NOE from H-22 (δ_H 2.12) to H_3-30 (δ_H 1.57) and the coupling constant analysis. The relative configuration of **2** was further confirmed by X-ray crystallographic analysis (Figure 4). Compounds **1** and **2** are a pair of stereoisomers, with opposite configurations at C-16 and C-17. The CD spectrum of **2** is different from that of **1** and **5**, with a positive Cotton effect at around 300 nm (Figure 5). The absolute configuration of **2** was deduced as 2*R*, 3*S*, 7*R*, 9*S*, 11*S*, 16*R*, and 17*S* by comparison of the experimental and simulated ECD spectra using the procedures described in **1**. The calculated ECD spectrum of **2a** by Boltzmann-weighting of the conformers showed similar Cotton effects with those of the experimental ECD of **2** (Figure 5).

Hirsutic acid D (**3**) possessed a molecular formula of $C_{16}H_{22}O_5$ (six degrees of unsaturaion), as determined by HRESIMS analysis. The NMR data of **3** resembled those of compound **5**, except for the absence of an oxygenated quarternay carbon and one methyl group, and the presence of an oxygenated methylene [δ_H 4.29 (d, J = 12.9 Hz), 4.33 (d, J = 12.9 Hz); δ_C 67.3], an additional methine [δ_H 2.42 (d, J = 10.0 Hz); δ_C 59.4], and one methoxy group [δ_H 3.37 (3H, s); δ_C 59.0] in **3** (Table S4). Analysis of its ^{1}H−^{1}H COSY, HSQC, and HMBC assigned the planar structure of **3** (Figure 2). The relative configuration of **3** was determined to be the same as that of **5** by ROESY spectral analysis (Figure S1). In the CD spectrum of **3**, a positive Cotton effect at about 270 nm and a negative Cotton effect at 324 nm deriving from the α, β-unsaturated cyclopentenone moiety were observed (Figure S18). The absolute configuration of **3** was determined to be 2*R*, 3*S*, 7*R*, 9*S*, and 11*S* by comparing its CD spectrum with that of **1**.

Hirsutic acid E (**4**) had the molecular formula of $C_{17}H_{20}O_5$ as deduced from the HRESIMS at m/z [M + H]$^+$ 305.1382, indicating eight degrees of unsaturaion. The ^{1}H and ^{13}C spectra of **4** exhibited signals due to two methyls [δ_H 1.28 (3H, s), 1.39 (3H, s); δ_C 23.8, 24.7], three methylenes, two methines, two quaternary carbons, one acetyl group [δ_H 2.24 (3H, s); δ_C 20.2, 169.1], four olefinic carbons [δ_C 115.7 (=CH_2), 142.0, 153.1, 174.1], and two carbonyl carbons (δ_C 181.2 and 191.0) (Table S4). The NMR data of **4** showed much similarity with those of chlorostereone,[10] except for the presence of an extra acetyl group. Detailed analysis of its ^{1}H−^{1}H COSY, HMBC, and ROESY spectra further confirmed the hirsutane-type structure of **4** (Figure 2 and Figure S1). The remaining acetyl moiety was finally attached at C-6 to form the enol ester, which was comparable with the chemical shift of olefinic carbon in known compounds 3-hydroxyambrosin damsinate[21] (δ_C 144.4) and arrivacin B[22] (δ_C 145.9) with a similar structural feature. The NOEs from H-1β (δ_H 1.67) to H_3-12 and H_3-14, from H-2 to H-8α (δ_H 2.81) and H-9, and from H-8β (δ_H 2.28) to H_3-14 indicated that H_3-12 and H_3-14 were on the same face, whereas the protons of H-2 and H-9 were on the opposite side. Compound **5** was obained as the major secodary metabolite in the culture of *S. hirsutum*. The absolute configuration in **5** has been assigned as 2*R*, 3*R*, 7*S*, 9*R*, and 11*S* in our early work by X-ray diffraction analysis.[11] Considering the same biosynthetic

Scheme 1. Hypothetical Biogenetic Pathway of 1 and 2



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origin of **3**−**5**, the absolute configuration in **4** was strongly inferred to be 2*R*, 3*S*, 9*S*, and 11*S*.

Although compound **6** was not detected in the culture of *S. hirsutum*, it seems possible that compounds **1** and **2** are synthesized via a hetero-Diels−Alder cycloaddition from the hirsutane-type sesquiterpene **6** and α-humulene (Scheme 1). Natural products biosynthesized by hetero-Diels−Alder cycloaddition with α-humulene include lucidene from *Uvaria lucida*,[19] eupenifeldin from the fungus *Eupenicillium brefeldianum*,[20] epolones A and B from an identified fungus,[23] and pughiinin A from the fungus *Kionochaeta pughii*.[24] Hirsutane-type sesquiterpenes **3**−**5** could be produced through the typical humulane-protoilludane pathway, as often occurs in higher fungi.[25]

To evaluate the cytotoxicity of the sesquiterpenoids from *S. hirsutum*, human colon cancer (HCT116) and human myelogenous leukemia (K562) cell lines were incubated with compounds **1**−**4** for 48 h. The cytotoxicity was determined by the MTT method. As a result (Table S5), compounds **1**−**4** exhibited cytotoxicity against K562 cells with the IC_{50} of 12.97, 16.29, 6.93, 30.52 μg/mL, respectively. For the HCT116 cell line, compounds **1**−**4** showed cytotoxicity with an IC_{50} of 10.74, 16.35, 25.43, and 24.17 μg/mL, repectively.

In conclusion, we isolated and characterized four new cytotoxic sesquiterpenes from the solid culture of *S. hirsutum*. Compounds **1** and **2** possess a new chemical skeleton of cyclopenta[5,6]pentaleno[2,1-*b*]cycloundeca[*e*]pyran. The postulated biosynthetic pathway for **1** and **2** deserves further confirmation by a biomimetic synthesis.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, characterization data, NMR spectra of **1**−**4**, and CD spectra for **3**−**5**; cif files for sterhirsutin A (**1**) and sterhirsutin B (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

‖Q.-Y.Q. and L.B. contributed equally.

Notes

The authors declare no competing financial interest.

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