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Strepsesquitriol, a Rearranged Zizaane-Type Sesquiterpenoid from the Deep-Sea-Derived Actinomycete *Streptomyces* sp. SCSIO 10355

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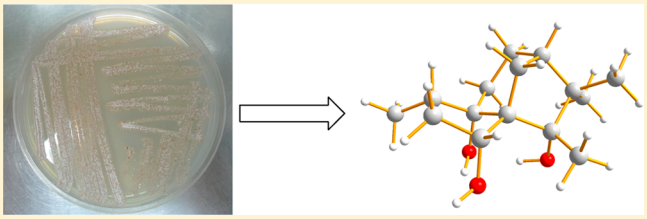
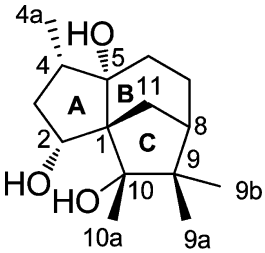
Supporting Information

ABSTRACT: Strepsesquitriol, a new caged sesquiterpene, was isolated from *Streptomyces* sp. SCSIO 10355. Its absolute structure was established as (1*R*,2*R*,4*S*,5*S*,8*S*,10*S*)-4,9,9,10-tetramethyl-2,5,10-trihydroxytricyclo[6.2.1.0^{1,5}]undecane by NMR analysis and a theoretical optical rotation derived from quantum-chemical calculations. It showed moderate inhibitory activity against lipopolysaccharide-induced TNFα production in RAW264.7 macrophages.



The ocean is host to taxonomically unique and phylogenetically diverse populations of marine actinomycetes.[1] In the past decade, they have been clearly demonstrated as a rich source of structurally novel and biologically potent secondary metabolites.[2] These diverse structures and their important bioactivities have elicited the attention of scientists with regard to their total synthesis and biosynthetic origins,[3] which, in turn, encouraged more and more natural product chemists to focus on marine actinomycetes, especially those from deep-sea sediments. In our continuing investigations on novel structures from the deep-sea-derived actinomycetes,[4] a new sesquiterpene, strepsesquitriol (**1**), was isolated from the fermentation broth of the *Streptomyces* sp. SCSIO 10355. Herewith, we report the isolation, structure, and bioactivity of strepsesquitriol, the first representative of a new sesquiterpene structure class.

Strepsesquitriol (**1**) was obtained as a white, amorphous powder. The HRESIMS spectrum of **1** gave an $[M + Na]^+$ adduct ion peak at m/z 277.1776, which suggested the molecular formula $C_{15}H_{26}O_3$, accounting for three degrees of unsaturation. The ^{1}H NMR spectrum exhibited one oxygenated methine at δ_H 3.79 (1H, d, J = 6.3 Hz, H-2) and one doublet and three singlet methyls (δ_H 1.02, 1.03 s, 1.09 s, and 1.48 s, each 3H, Me-4a,9a,9b,10a). These signals were supported by resonances in the ^{13}C NMR spectrum at δ_C 80.1 (CH, C-2), 14.6 (CH_3, C-4a), 23.3 (CH_3, C-10α), 21.8 (CH_3, C-9b), and 30.2 (CH_3, C-9a). Altogether, the ^{1}H, ^{13}C, and DEPT NMR spectra gave 15 signals including four quaternary carbons [two were oxygenated: δ_C 87.3 (C-5) and 85.0 (C-10)], three methines, four methylenes, and four methyls (Table 1). Because all 15 carbons were sp^3 hybridized, **1** was concluded to bear three rings in its structure.



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In the COSY spectrum, two spin systems were observed, for H-2 (δ_H 3.79)/H-3β (δ_H 2.41)/H-4 (δ_H 2.21)/H-4a (δ_H 1.02) and H-6β (δ_H 1.87)/H-7β (δ_H 1.43)/H-7α (δ_H 1.84)/H-8 (δ_H 1.52)/H-11β (δ_H 1.13), which allowed the assembly of two segments: C-2/C-3/C-4/C-4a and C-6/C-7/C-8/C-11 (Figure 1). These two fragments could be connected based on the key HMBC correlations originated from four methyls, one oxymethine (H-2), and two methylenes (H-6 and H-11), which constructed the planar structure as 4,9,9,10-tetramethyl-2,5,10-trihydroxytricyclo[6.2.1.0^{1,5}]undecane (Figure 1). For further confirmation, **1** was dissolved in DMSO-d_6 and was subjected to another HMBC experiment. As shown in Figure 1, the HMBC correlations of 5-OH (δ_H 5.61 s) to C-5/C-4/C-1/C-6, 2-OH (δ_H 4.11, d, J = 9.9 Hz) to C-2/C-1, and 10-OH (δ_H 5.43 s) to C-1/C-10/C-9 established undoubtedly the planar structure of strepsesquitriol.

The assignment of the relative configuration of strepsesquitriol was achieved by exhaustive analysis of the NOESY

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for Strepsesquitriol (1)

	1[a]		1[b]		
position	δ_C	δ_H	δ_C	δ_H	NOESY[c]
1	63.4, C		61.5, C		
2	80.1, CH	3.79, d (6.3)	77.7, CH	3.65, dd (9.8, 6.3)	H-3β s, H-10a s, H-11α s, 2-OH m, H-3α w
3	43.4, CH_2	α: 1.37, dd, (14.7, 6.3)	42.3, CH_2	α: 1.23, dd (14.4, 6.3)	H-3β s, H-4a s, 2-OH m, H-4 w
		β: 2.41, ddd(14.7, 10.9, 6.4)		β: 2.30, ddd (14.4, 10.8, 6.4)	H-3α s, H-2 s, H-4 m, H-11β m
4	38.8, CH	2.21, ddq (10.9, 6.9, 6.4)	36.7, CH	2.11, ddq (10.8, 6.7, 6.4)	H-4a s, H-11β s, H-3β m, H-7β w, H-6β w, H-3α w
4a	14.6, CH_3	1.02, d (7.1)	14.4, CH_3	0.93, d (6.9)	H-4 s, H-3α s, H-6β m, 5-OH w, 2-OH vw
5	87.3, C		84.9, C		
6	31.3, CH_2	α: 1.78, dd (14.2, 7.8)	30.1, CH_2	α: 1.64, dd (13.9, 7.7)	H-6β s, H-9b s, 5-OH m
		β: 1.87, dt (14.2, 7.8)		β: 1.75, dt (13.9, 7.8)	H-6α s, H-4a m, H-7α m, H-7β m, 5-OH w, H-4 vw
7	27.1, CH_2	α: 1.84, m	25.7, CH_2	α: 1.70, m	H-7β s, H-9b s, H-6β m, H-8 m
		β: 1.43, m		β: 1.34, dddd 12.8, 8.1, 6.9, 2.8)	H-7α s, H-11β m, H-6β m, H-8 w
8	46.2, CH	1.52, m	44.0, CH	1.42, m	H-9a s, H-9b s, H-7α m, H-7β w
9	47.4, C		45.9, C		
9a	30.2, CH_3	1.03, s	29.5, CH_3	0.94, s	H-10a s, H-11α s, H-8 s, H-9b s, 10-OH w
9b	21.8, CH_3	1.09, s	21.4, CH_3	1.00, s	H-7α s, H-6α s, H-9a s, H-8 s, 10-OH m, 5-OH vw
10	85.0, C		83.1, C		
10a	23.3, CH_3	1.48, s	22.6, CH_3	1.39, s	H-9a s, H-2 s, 10-OH s, H-11α s, 2-OH m, 5-OH w
11	36.5, CH_2	α: 1.54, m	34.8, CH_2	α: 1.43, ddd (12.8, 4.8, 2.3)	H-11β s, H-9a s, H-2 s, H-10a s
		β: 1.13, d (12.0)		β: 1.02, dd (12.8, 2.9)	H-11α s, H-4 s, H-3β m, H-7β m
2-OH				4.11, d (9.9)	H-3α s, H-10a s, H-2 m, H-4a w
5-OH				5.61, s	H-6α s, H-4a s, H-10a m, H-9b m, H-6β w, H-3α w
10-OH				5.43, s	H-10a s, H-9b s, H-6α m, H-9a w, H-3α w

[a]Data were recorded on a Bruker Avance 500 NMR spectrometer; chemical shifts (δ) are given in parts per million with references to the center peak of CD_3OD with δ 3.30 for ^{1}H and δ 49.0 for ^{13}C. [b]Data were recorded on a Bruker Avance 600 NMR spectrometer; chemical shifts (δ) are given in parts per million with references to the center peak of DMSO-d_6 with δ 2.50 for ^{1}H and δ 39.5 for ^{13}C. [c]NOESY intensities are marked as strong (s), medium (m), weak (w), or very weak (vw).

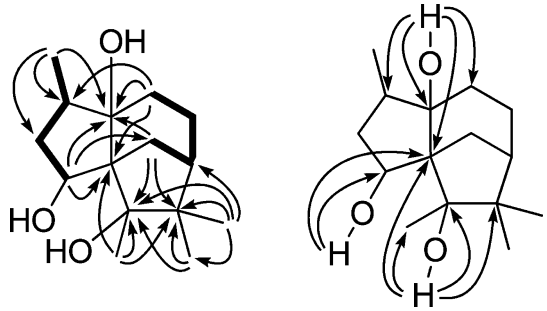

Figure 1. Key COSY (bold) and HMBC (arrow) correlations of strepsesquitriol (**1**) in CD_3OD (left) and DMSO-d_6 (right).

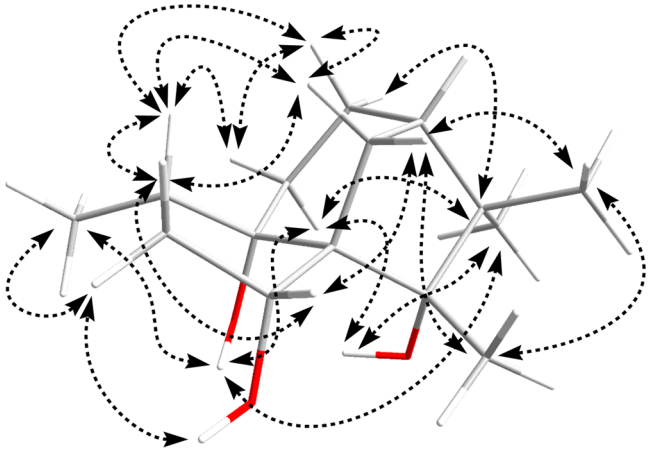

Figure 2. Key NOESY correlations of strepsesquitriol (**1**).

spectrum in DMSO-d_6 (Table 1). As shown in Figure 2, H-3β was correlated to H-2/H-11β/H-4, H-4 to H-6β/H-7β/H-11β, and H-11α to H-2/H-9a/H-10a, while H-3α was correlated to 2-OH/H-4a, H-4a to 5-OH, 5-OH to H-6α/H-9b, and H-9b to H-7α/H-6α/10-OH. This indicated that H-2/H-3β/H-4/H-6β/H-7β/H-11/H-9a/H-10a were cofacial, which was opposite 2-OH/H-3α/H-4a/5-OH/H-6α/H-7α/H-9b/10-OH. As H-6α was correlated to H-9b/10-OH, H-11β was correlated to H-7β, and H-11α was correlated to H-9a/H-10a, ring B was deduced to be in a chair configuration and ring C was in an envelope configuration. In addition, H-11β was correlated to H-4/H-3β, suggesting the envelope configuration of ring A. On the basis of the above evidence, the relative configuration of strepsesquitriol was established to be 4α,9,9,10β-tetramethyl-2α,5α,10α-trihydroxytricyclo[6.2.1.0^{1,5}]undecane.

Quantum-chemical calculations, such as theoretical optical rotation (OR), have been utilized extensively to determine the absolute configurations of organic molecules.[5] Here we performed a theoretical calculation of the OR of **1** using the time-dependent density functional theory (TDDFT) method to establish the absolute configuration of strepsesquitriol.

All of the hydrogen atoms were added and overlaid with key correlations observed in the NOESY spectrum prior to conformational search. The random conformational distribution search performed by SYBYL software using the TRIPOS force field yielded enantiomers **1a** and **1b**, respectively, within a 5 kcal/mol energy difference. The minimum geometries were further optimized by TDDFT at the B3LYP/6-31G(d) level as implemented in the Gaussian 09 program package.[6] Subsequently, the stable conformers (Figure 3) obtained for each enantiomer were submitted to theoretical OR calculations at the B3LYP/6-311++G(2d, p) level. The resulting OR values of

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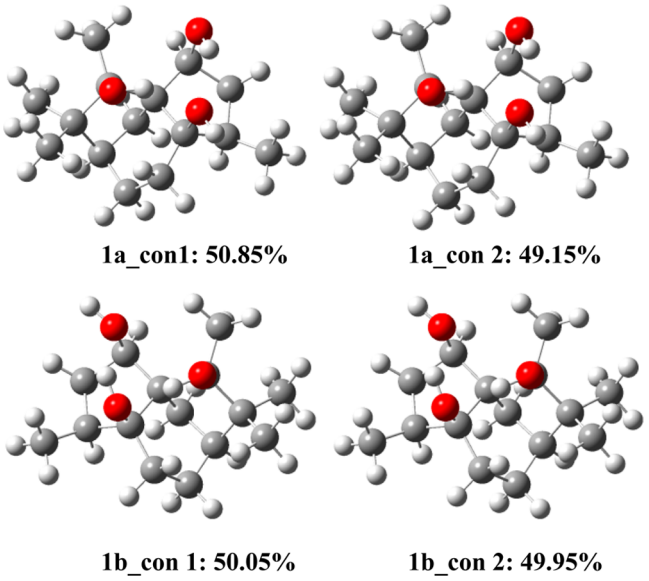

Figure 3. Lowest energy conformers of enantiomers **1a** and **1b** obtained from geometry optimizations by TDDFT at the B3LYP/6-31G(d) level.

1a and **1b** were energetically weighted according to the respective conformational distribution by the Boltzmann statistics (Figure 3).

The OR values of **1a** and its enantiomer **1b** were predicted to be −28.6 and +28.5, respectively. The OR value for compound **1b** was closer to the experimental one (+21.0). Therefore, the absolute configuration of strepsesquitriol (**1**) is proposed to be (1*R*,2*R*,4*S*,5*S*,8*S*,10*S*)-4,9,9,10-tetramethyl-2,5,10-trihydroxytricyclo[6.2.1.0^{1,5}]undecane. This compound is a compact, caged, tricyclic molecule that represents a new sesquiterpenoid carbon skeleton. However, from a biogenetic perspective, it is a rearranged zizaane derivative that may share the same precursor, *epi*-isozizaene (see Scheme S1 in the Supporting Information).[7]

Tumor necrosis factor (TNF)-α is one of the well-recognized inflammatory cytokines. Inhibition of its release is regarded as beneficial in many inflammation-related diseases. Therefore, the ability of strepsesquitriol to inhibit lipopolysaccharide (LPS)-stimulated TNFα production was measured in RAW264.7 macrophages according to the reported protocol.[8] As shown in Figure 4, at the concentration of 100 μM, strepsesquitriol showed moderate activity with 35.4% inhibition ($p < 0.01$), which was comparable to 60.6% inhibition for the positive control, *N*-*p*-tosyl-L-phenylalanine chloromethyl ketone (TPCK).

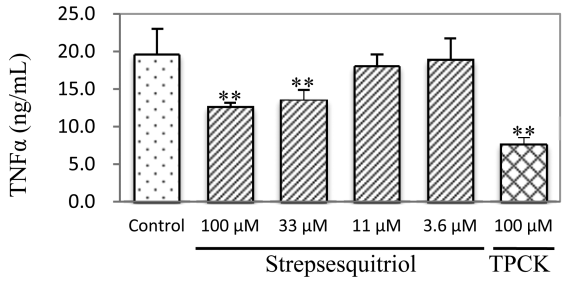

Figure 4. Inhibitory effects of strepsesquitriol (**1**) against LPS-induced TNFα production in RAW264.7 macrophages (n = 3, means ± SD).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with an Anton Paar MCP500 polarimeter. NMR spectra were recorded on a Bruker Avance 500 or 600 MHz spectrometer with TMS as an internal standard. ESIMS was performed on an Agilent LC/MSD Trap XCT spectrometer, and HRESIMS was completed on a Bruker microTOF-QII mass spectrometer. Materials for column chromatography were silica gel, Sephadex LH-20, and ODS. Preparative TLC was conducted with glass precoated silica gel GF254.

Bacterial Isolation. The strain SCSIO 10355 was isolated from a sediment sample (−3412 m, E 87°59.7′, N 9°59.3′) in the Bay of Bengal, Indian Ocean. Genomic DNA of the strain SCSIO 10355 was isolated using standard methods. The 16S rRNA gene was amplified using PCR with the Universal primers FC27 and RC 1492. The almost-complete 16S rRNA sequence (1487 bp) was compared with the 16S rRNA gene sequences available in GenBank using the BLASTIN program. The strain SCSIO 10355 showed the highest 16S rRNA gene sequence similarity of 99.59% to *Streptomyces rubrogriseus* LMG 20318(T). The 16S rRNA sequence of SCSIO 10355 was deposited in GenBank (accession no. KF267718). A voucher strain of this actinomycete (CGMCC 4.7120) is preserved at the China General Microbiological Culture Collection Center.

Cultivation and Extraction. The strain SCSIO 10355 was grown on a medium consisting of 1.5% soluble starch, 0.5% soybean meal, 1.5% peptone, 1.5% glycerol, 0.2% $CaCO_3$, and 3% marine salt (pH 7.4). A large-scale fermentation (15 L) by shake fermentation was performed in 75 Erlenmeyer flasks each containing 200 mL of the medium. After incubating at 28 °C on a rotary shaker (200 rpm) for 10 days, the culture (15 L) was centrifuged to yield the supernatant and a mycelia cake. The supernatant was partitioned with EtOAc and evaporated to give extract A. The mycelia cake was extracted three times with acetone; the latter was evaporated to provide extract B. Both extracts A and B were combined to give a total residue (31.9 g).

Strepsesquitriol Isolation. The total extract (31.9 g) of *Streptomyces* sp. SCSIO 10355 was column chromatographed over ODS using a H_2O−MeOH gradient. The 50% MeOH fraction (0.7 g) was further separated by repeated Sephadex LH-20 chromatography with MeOH and $CHCl_3$−MeOH (1:1). Final purification using preparative TLC using $CHCl_3$−acetone (10:1) gave strepsesquitriol (**1**, 5.4 mg, 0.0017%).

Strepsesquitriol (1): amorphous, white powder; $[\alpha]_D^{20}$ +21.0 (*c* 0.67, MeOH); ^{1}H and ^{13}C NMR data, see Table 1; ESIMS 277.5 [M + Na]$^+$, 531.3 [2M + Na]$^+$; HRESIMS *m*/*z* 277.1776 [M + Na]$^+$ (calcd for $C_{15}H_{26}O_3Na$, 277.1774).

Determination of TNF-α Production Induced by LPS in RAW264.7 Macrophages. RAW264.7, a murine macrophage cell line, was obtained from Shanghai Institute of Cell Biology, Chinese Academy of Sciences, and maintained in media supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μg/mL) in a humidified 5% CO_2 atmosphere at 37 °C. RAW264.7 cells (2 × 10^5 cells/well) were pretreated with different concentrations of compound **1**, TPCK, or vehicle solution for 30 min and then incubated with LPS (100 ng/mL) overnight. Supernatants were collected to determine the TNF-α level by using the mouse TNF-ELISA kit (eBioscience).

ASSOCIATED CONTENT

Supporting Information

The postulated biogenetic pathway, the optimized coordinates of lowest energy conformers **1a** and **1b**, and 1D and 2D NMR spectra in CD_3OD and DMSO-d_6 for compound **1** are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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