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NOTE

A new polysubstituted cyclopentene derivative from *Streptomyces* sp. HS-NF-1046

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Microorganisms are considered as important resources for researchers to prove and develop new natural products and have yielded some of the most important products of the pharmaceutical industry, such as penicillin, erythromycin and avermectin.[1–4] Although research in antibiotics and natural products has declined significantly during the last decade,[5–7] the search for novel bioactive microbial metabolites for potential pharmaceutical applications has been and still is important.[8,9] During the course of searching for novel microbe-derived bioactive secondary metabolites, we investigated the chemical constituents of a strain *Streptomyces* sp. HS-NF-1046. As a result, a new polysubstituted cyclopentene derivative, named hisunic acid (**1**, Figure 1), was isolated from the fermentation broth of this strain. In this paper, we describe the fermentation, isolation, structure elucidation and bioactivity of **1**.

The producing strain HS-NF-1046 was isolated from a soil sample collected from Jilin, Jilin province, China using the standard dilution plate method. The strain was identified as the genus *Streptomyces* because its a 16S rDNA sequence (accession no.: KX118440 in the GenBank) that exhibited a high-sequence similarity of 100% with that of *Streptomyces* sp.YIM8 (accession no.: AF389344).

The strain was grown and maintained on the YMS medium containing malt extract (Becton, Dickinson and Company, Franklin Lake, NJ, USA) 10.0 g, yeast extract (Oxoid, Basingstoke, UK) 4.0 g, glucose (Sinopharm Chemical Reagent, Shanghai, China) 4.0 g, $CoCl_2{\cdot}6H_2O$ (Sinopharm Chemical Reagent) 0.005 g and agar (Becton, Dickinson and Company) 20.0 g in 1.0 l tap water at pH 7.0. The seed medium consisted of glucose 4.0 g, malt extract 10.0 g and yeast extract 4.0 g in 1.0 l tap water, pH 7.0. All of the media were sterilized at 121 °C for 20 min. The producing medium was composed of mannitol (Shangdong Tianli Pharmaceutical, Weifang, China) 20.0 g and soybean powder (Ningbo Beilun Jiangnan Grease, Ningbo, China) 20.0 g in 1.0 l tap water, pH 6.8–7.0 before sterilization. Slant culture was incubated for 5–7 days at 28 °C. Then the slant culture was inoculated on 1 l Erlenmeyer flasks containing 250 ml of the seed medium and incubated at 28 °C for 24 h, shaken at 250 r.p.m. Fermentation was carried out in a 50 l fermentor containing 30 l of producing medium at 28 °C for 7 days, stirred at 200 r.p.m. with an aeration rate of 1000 l of air per hour, tank pressure control at 0.05 MPa.

The final 30 l of broth from 50 l fermentor was filtered to separate mycelial cake and supernatant. The mycelial cake was washed with water (3 l) and subsequently extracted with MeOH (3 l). The supernatant and the wash water were subjected to a Diaion HP-20 resin column eluting with 95% EtOH. The MeOH extract and the EtOH eluents were evaporated under reduced pressure to yield the crude extract and the mixture was chromatographed on a silica gel (Qingdao Haiyang Chemical Group, Qingdao, China; 100–200 mesh) column and successively eluted with a stepwise gradient of $CHCl_3$/MeOH (100:0–50:50, v/v) to give four fractions (Fr.1–Fr.4) based on the TLC profiles. TLC was performed on silica-gel plates ($HSGF_{254}$, Yantai Chemical Industry Research Institute, Yantai, China) with a solvent system of $CHCl_3$/MeOH (9:1) and the developed TLC plates were observed under a UV lamp at 254 nm or by heating after spraying with sulfuric acid-ethanol, 5:95 (v/v). The Fr.3 was subjected to a Sephadex LH-20 (GE Healthcare, Glies, UK) column eluted with $CHCl_3$/MeOH (1:1, v/v) and detected by TLC to give three subfractions (Fr.3-1–Fr.3-3). The Fr.3-3 was further purified by semi-preparative HPLC (Agilent 1100, Zorbax SB-C18, 5 μm, 250 × 9.4 mm inner diameter; 1.5 ml min^{-1}; 220 nm; Agilent, Palo Alto, CA, USA) eluting with CH_3CN/H_2O (50:50, v/v) to give compound **1** (t_R 10.0 min, 15.2 mg). Optical rotation was measured on Perkin-Elmer 341 Polarimeter (Perkin-Elmer, Suzhou, China). The UV spectrum was recorded on a Varian CARY 300 BIO spectrophotometer (Varian, Cary, NC, USA), and IR spectrum in pressed KBr disk was obtained on a Nicolet Magna FT-IR 750 spectrometer (Nicolet, Tokyo, Japan). ^1H and ^{13}C NMR spectra were measured with a Bruker DRX-400 (400 MHz for ^1H and 100 MHz for ^{13}C) spectrometer (Bruker, Rheinstetten, Germany). Chemical shifts are reported in p.p.m. (δ), using residual CH_3OH (δ_H 3.30; δ_C 49.0) as an internal standard, and coupling constants (J) in Hz. ^1H

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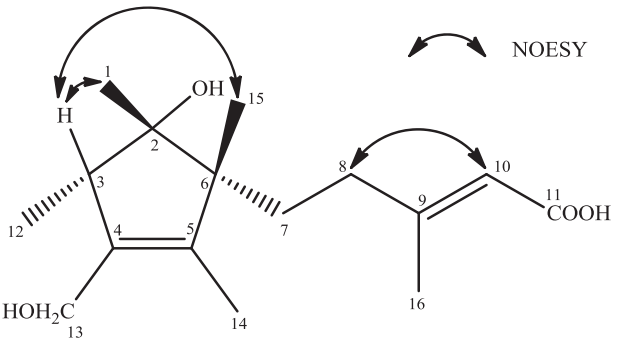

Figure 1 The structure and NOESY correlations of compound **1.**

and ^{13}C NMR assignments were supported by ^{1}H-^{1}H COSY, HMQC and HMBC experiments. The ESIMS and high resolution ESIMS (HRESIMS) spectra were taken on a Q-TOF Micro LC-MS-MS mass spectrometer (Waters, Milford, MA, USA).

Compound **1** was obtained as a white powder with $[\alpha]_D^{25}$ -34.3 (*c* 0.04, EtOH) and UV (EtOH) λ_{max} nm (log ε): 231 (sh, 3.72), 211 (4.04). It had the molecular formula of $C_{16}H_{26}O_4$ determined by HRESIMS at *m/z* 281.1765 $[M\text{-}H]^-$ (calcd as 281.1758 for $C_{16}H_{25}O_4$) indicating four degrees of unsaturation. The IR spectrum displayed absorption bands for hydroxy group (3398 cm^{-1}) and carbonyl group (1689 cm^{-1}). The ^{1}H NMR spectrum (Table 1) showed two olefinic methyls at δ_H 1.66 (d, $J=2.4$ Hz), 2.18 (s), two aliphatic methyl singlets at δ_H 0.97, 1.24, one aliphatic methyl doublet at δ_H 1.07 (d, $J=7.2$ Hz), one olefinic proton at δ_H 5.71 (s), four aliphatic methylene proton signals at δ_H 1.51 (td, $J=13.0$, 3.9 Hz), 1.78 (td, $J=13.0$, 4.6 Hz), 2.27 (td, $J=13.0$, 3.9 Hz), and 2.45 (td, $J=13.0$, 4.6 Hz), one aliphatic methine proton signal at δ_H 2.67 (m) and one hydroxymethyl at δ_H 4.01 (d, $J=11.8$ Hz), 4.20 (d, $J=11.8$ Hz). Sixteen carbon signals, including two sp^3 quaternary carbons (one oxygenated) at δ_C 83.8, δ_C 55.0, three olefinic quaternary carbon signals at δ_C 135.2, 143.2, 162.7, one olefinic methine at δ_C 116.5, five methyl groups at δ_C 10.1, 10.5, 19.1, 19.1 and 21.5, three methylenes (one oxygenated) at δ_C 34.5, 37.5, 56.9, one methine at δ_C 48.7 and one carbonyl carbon at δ_C 170.8, were observed in the ^{13}C NMR and DEPT135 spectra (Table 1).

The structure of compound **1** could be established by the analysis of the ^{1}H-^{1}H COSY, HMQC and HMBC experiments. Interpretation of the ^{1}H-^{1}H COSY NMR data (Figure 2) led to the identification of two isolated proton spin-systems corresponding to the C-3–C-12 and C-7–C-8 subunits of structure **1**. In detail, the HMBC correlations (Figure 2) of the protons and carbon atoms, especially between the H_3-1 (δ_H 1.24) with C-3 (δ_C 48.7), C-2 (δ_C 83.8), C-6 (δ_C 55.0), between H_2-13 with C-3, C-4, C-5 (δ_C 143.2), between H_3-14 (δ_H 1.66) with C-4, C-5, C-6 and between H_3-15 (δ_H 0.97) with C-2, C-6, C-7 (δ_C 34.5), displayed the structure as a five-membered ring with the substituents of four methyl groups, one hydroxyl group, one hydroxymethyl group and a methylene (C-7). The observed HMBC correlations from H_3-16 to C-8, C-9 and C-10 established the connections of C-7–C-10 and C-9–C-16. Taking the molecular formula $C_{16}H_{26}O_4$ of **1** into account, a carboxyl group was situated at C-10. The correlated signal of H-10 with C-11 (δ_C 170.2) in the HMBC spectrum further confirmed the assignment. Thus, the planar structure of **1** was established as shown in Figure 1. The NOESY correlations (Figure 1) between H_2-8 and H-10 revealed the geometry of $\Delta^{9,10}$ was *E*. Furthermore, the correlations of H_3-1/H-3/H_3-15 in the NOESY spectrum indicated these protons were cofacial and arbitrarily assigned as having a β-orientation.

Table 1 ^{1}H and ^{13}C NMR data for **1** (in CD_3OD)

Position	δ_H (J *in Hz*)	δ_C (*p.p.m.*)
1	1.24 (s)	21.5
2		83.8
3	2.67 (m)	48.7
4		135.2
5		143.2
6		55.0
7	1.51 (td, 13.0, 3.9)	34.5
	1.78 (td, 13.0, 4.6)	
8	2.27 (td, 13.0, 3.9)	37.5
	2.45 (td, 13.0, 4.6)	
9		162.7
10	5.71 (s)	116.5
11		170.8
12	1.07 (d, 7.2)	10.1
13	4.01 (d, 11.8)	56.9
	4.20 (d, 11.8)	
14	1.66 (d, 2.4)	10.5
15	0.97 (s)	19.1
16	2.18 (s)	19.1

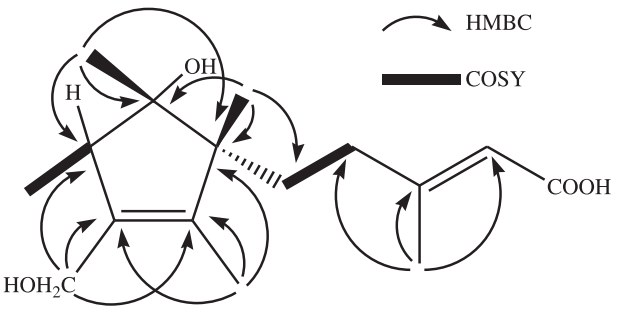

Figure 2 Key HMBC and COSY correlations of compound **1.**

We tested the cytotoxicity of compound **1** against the growth of human erythroleukemia cell line K562 using the sulforhodamine B method.[10] The result showed that compound **1** dose dependently inhibited the growth of K562 cells with IC_{50} value of 59.2 μg ml^{-1}.

The antimicrobial activity of **1** was assessed against the pathogenic microorganism *Candida albicans* with the broth microdilution MIC method recommended by the Clinical and Laboratory Standards Institute Standards[11] using Amphotericin B (Aladdin Industrial Corporation, Shanghai, China) as a positive control. Compound **1** showed weak inhibitory activity against the pathogenic microorganism *C. albicans* (MICs: **1**, 7.5 mg ml^{-1}; Amphotericin B, 0.5 μg ml^{-1}).

A number of natural cyclopentane derivatives have been obtained from microbial resource.[12–14] Most of them displayed weak antitumor or inhibitory activities against two isozymes of 11β-hydroxy-steroid dehydrogenases (11β-HSD1 and 11β-HSD2) except that the C_{11} cyclopentenones isolated from marine organisms showed strong antitumor activities.[15–17] This suggested that the activity of **1** might be improved by means of chemical modification. To the best of our knowledge, compound **1** represent a new kind of natural cyclopentene derivatives of which all carbon atoms of the carbocycle existed substituents and it may provide an important clue for investigation of the biosynthetic mechanism and structure-activity relationships of

this kind of compounds. In addition, studies on the other bioactivities of **1** are currently underway.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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