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NOTE

Albaflavenol B, a new sesquiterpene isolated from the terrestrial actinomycete, *Streptomyces* sp.

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Secondary metabolites from plant-associated actinomycetes are increasingly attracting attention, with the recent identification of new secondary metabolites, juniperolide A,[1] lorneic acids,[2] leopolic acid[3] and oleaceran.[4] As part of our ongoing campaign to focus natural products discovery on this source, we have isolated and characterized a new sequiterpene, alblaflavenol B (**1**).

The strain (Lv-4-26), identified as belonging to the genus *Streptomyces*, was isolated from the soil collected from the root zone of the plant *Phyllostachys viridiglaucescens*. The strain was later cultivated in M medium consisting of (starch 1%, yeast extract 0.4% and peptone 0.2%) for 8 days at 30 °C in five 5 liters Erlenmeyer flask containing 1.4 liters of medium each. The whole broth was then later extracted with ethyl acetate (6 l) to give a crude extract of 110.5 mg. The EtOAc extract was then sequentially fractionated between 20 ml of hexane, CH_2Cl_2 and MeOH to afford 40.6, 55.7 and 5.5 mg fractions, respectively. The CH_2Cl_2 fraction was subsequently purified by semi-preparative reverse-phase HPLC to yield the sesquiterpene, **1** ($t_R = 14.5$ min; 0.7 mg). The structure of albalfavenol B was elucidated through detailed spectroscopic analysis, while the absolute configuration was assigned by comparison to its known derivatives, albaflavenol (**2a/2b**) and albaflavenone (**3**).

HRESI (+)MS analysis of **1** (Table 1) revealed a pseudomolecular ion ($[M+Na]^+$) indicative of a molecular formula ($C_{15}H_{24}O_2$) requiring four double bond equivalents. The NMR (DMSO-d_6) data (Table 2) (Supplementary Figures S1–S4) revealed two sp^2 carbon resonances (δ_C 137.1 and 150.4) requiring that **1** be tricyclic. Analysis of the COSY correlations led to the generation of the first isolated spin system, which began with a secondary methyl H_3-12 (δ_H 0.89) linked to a methine H-2 (δ_H 1.68) and then to a methylene H_2-3 (δ_H 2.13, 1.21) and terminating at the oxymethine H-4 (δ_H 4.56). The second spin system consisted of two methylenes H_2-9 and H_2-10 extending futher to the methine H-8 (δ_H 1.77) and terminating at the methylene H_2-11 (δ_H 1.44, 1.32). The key HMBCs from H_3-12 to C-1 and H-4 to C-1, C-4 and C-5 led to the generation of a substituted cyclopentane ring (C-1 to C-5). A second set of HMBCs revealed a second

Table 1 Physico-chemical properties of 1

	1
Appearance	Colorless oil
HR-ESI-MS (*m*/z)	
Found	259.1677 $[M+Na]^+$
Calcd	259.1669 ($C_{15}H_{24}O_2Na$)
$[\alpha]_D$ (MeOH)	(+) 24°
UV λ_{max} nm (log ε) (MeOH)	220 (4.40)

Table 2 NMR (500 MHz, DMSO-*d6*) data for albaflavenol B (1)

Pos	$\delta_{H,}$ *mult (J in Hz)*	δ_C[a]	*COSY*	*HMBC*
1		52.5		
2	1.68, m	35.6	3, 12	1, 12
3a	2.13, m	42.9	2, 4	1, 2, 4, 5
3b	1.21, m			
4	4.56, t (*7.8*)	70.2	3	1, 5, 6
5		150.4		
6		137.1		
7		40.4		
8	1.77, m	46.9	9a/b, 11a/b	1, 6
9a	1.73, m	24.4	8, 10a	
9b	1.58, m		8, 10a	
10a	1.42, m	29.6	9a	1
10b	1.23, m			
11a	1.44, m	36.8	8	1
11b	1.32, m			8
12	0.89, d (*6.8*)	14.1	2	1, 2, 3
13	3.94, dd (*13.5*, *11.6*)	57.6		5, 6, 7
14	0.99, s	29.3		6, 7, 8, 15
15	1.05, s	24.9		6, 7, 8, 14

[a] ^{13}C NMR resonances obtained from 2D HSQC and HMBC experiments.

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cyclopentane unit fused at position C-1. Finally, the tertiary methyls H_3-14 (δ_H 0.99) and H_3-15 (δ_H 1.05) showed correlations to C-6, C-7 and C-8, while the methylene H_2-13 (3.94) showed correlations to C-5, C-6 and C-7, leading to the construction of the cyclohexene ring fused alongside the two cyclopentane ring system (Figure 1). The overall planar structure was analogous to albaflavenone (**3**),[5] a sesquiterpene ketone with the two differences being the reduction of the ketone into a secondary alcohol and the presence of a hydroxyl methylene as opposed to a olefinic methyl (Figure 2). Albaflavenols[6] (**2a**/**2b**) were isolated as a mixture of epimeric alcohols in a biosynthetic-related study, where the absolute configuration of the hydroxyls was assigned as 4*R* and 4*S* based on the use of Hyperchem 7.5 for predictions. We observed a coupling of 7.8 Hz for H-4 as a triplet in our experimental data, which was in agreement with the 4*R* configuration (Figure 2). The remaining absolute stereochemistry is drawn as that for albaflavenone based on a comparison of the optical rotation and on biosynthetic grounds. Recently, as part of a characterization of the silent sesquiterpenoid biosynthetic pathway in *Streptomyces avermitilis* led to the discovery of 4β,5β-epoxy-2-*epi*-zizaan-6β-ol (**4**) (Figure 2), which was most likely to be formed by oxidation of (4*S*)-albaflavenol.[7] In summary, we have isolated a new derivative of albaflavenone, albaflavenol B (**1**) the first of the albaflavenols to be isolated as a pure substance rather than an epimeric mixture of alcohols. Owing to low yields and very low reproducibility of the metabolite from the strain, we failed to obtain a biological profile of this metabolite.

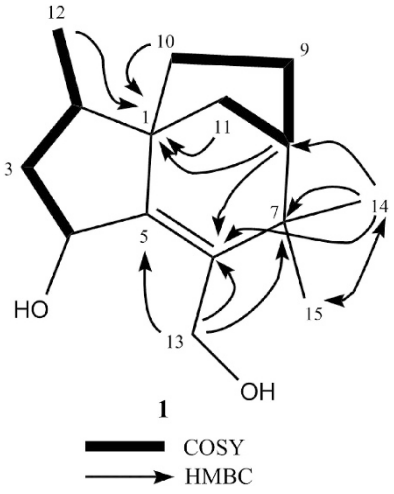

Figure 1 Key 2D NMR correlations for **1**.

EXPERIMENTAL PROCEDURE

NMR spectra were obtained on a Bruker Ascend 500 MHz spectrometer equipped with a cryoprobe system (Bruker Biospin GmbH, Waldbronn, Germany), in the solvents indicated and referenced to residual ^{1}H signals in deuterated solvents. ESI-MS were acquired using an Agilent 1100 Series separations module equipped with an Agilent 1100 Series LC/MSD mass detector in both positive and negative ion modes under the following conditions: Zorbax C_8 column, 150 × 4.6 mm, eluting with 0.4 ml min^{-1} 95% H_2O/MeCN to 5% H_2O/MeCN (with isocratic 0.01% trifluoroacetic acid (TFA)) over 22 min, then held for 5 min. HRMS was carried out using an UltiMate 3000 rapid separation liquid chromatography system (Dionex RSLC, Crawford Scientific, Lanarkshire, UK) coupled to an UHR-TOF mass spectrometer (Bruker Daltonik MaXis, Bremen, Germany) operating in the positive ESI mode.

Sampling was performed in the Nikitsky Botanical Garden in the south part of Crimea (Ukraine). The soil was collected (1 g) from the root zone of the plant *P. viridiglaucescens* and resuspended in sterile water (10 ml). Serial dilutions of the soil suspension were prepared in sterile water and inoculated onto the oatmeal agar (oatmeal—40 g l^{-1}, agar—15 g l^{-1}, pH 7.5). The plates were then later incubated for 20 days at 28 °C. Individual colonies were transferred onto new oatmeal agar plates for further analysis and maintenance. Based on 16s rDNA sequence analysis, strain Lv4-26 was identified to belong to the genus *Streptomyces*. The strain *Streptomyces* sp., Lv-4-26 is deposited in the microorganism collection of Ivan Franko Lviv National University.

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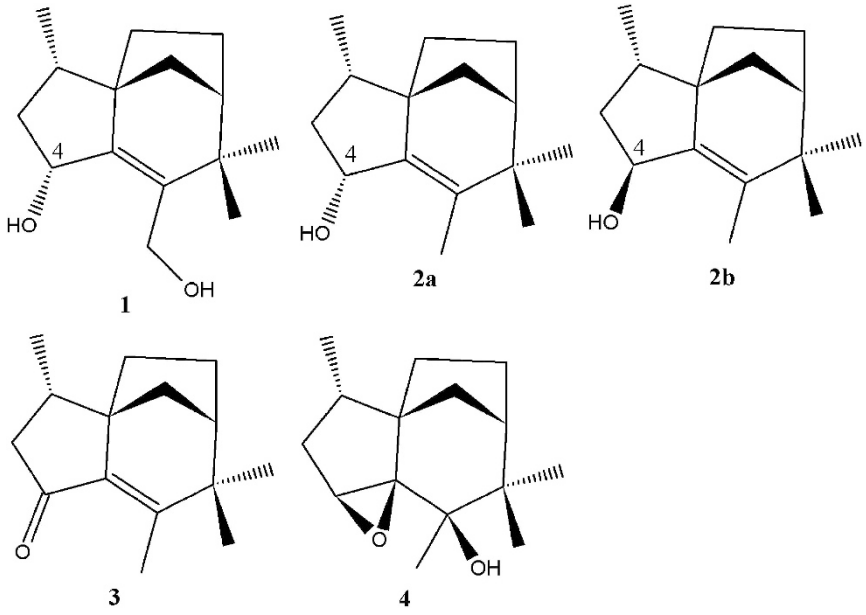

Figure 2 Structures of compounds **1**–**4**.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)