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ONE UNDESCRIBED SESQUITERPENE FROM *Streptomyces* sp.

**Ming-Jen Cheng,[1] Ming-Der Wu,[2] Chih-Ling Chang,[3]
Yuan-Hsiang Yu,[3] Chung-Yi Chen,[4*]
Jih-Jung Chen,[5,6,7*] and Chih-Zen Chang[8*]**

*One previously undescribed sesquiterpene analogue, M2 (**1**), together with five known ones, indole-3-carboxylic acid (**2**), methyl 4-hydroxycinnamate (**3**), ergosterol peroxide (**4**), 6β-hydroxystigmast-4-en-3-one (**5**), as well as betulonic acid (**6**), were isolated from the EtOAc extract of the culture broth of an actinomycete* Streptomyces *sp. M2. The structures of all isolates were elucidated on the basis of extensive analyses of spectroscopic data and comparison with literature data.*

Keywords: *Streptomyces* sp. M2, sesquiterpene.

Actinobacteria, tiny microorganisms residing in the soil, are well-known for producing a diverse range of secondary metabolites showcasing various bioactivities, including antimicrobial and insecticidal properties. These compounds have proven to be crucial in developing new pharmaceuticals [1–3]. Among the Actinobacteria, *Streptomyces* species are particularly notable for their exceptional ability to produce antibiotics and other bioactive compounds. Strain M2, isolated from Chiayi's Wetland, was identified as a *Streptomyces* species based on morphological characteristics and its 16S rDNA sequence analysis. *Streptomyces* encompasses a vast genus consisting of over 800 species, with many strains capable of generating biologically active secondary metabolites that hold significant medical importance. This specific strain thrives in neutral conditions and exhibits growth within a temperature range of 20 to 45°C. In dual culture assays, it demonstrated strong inhibitory activity against various pathogenic fungi, including *Fusarium* sp. LC8, *Neopestalotiopsis* sp. BCRC 35002, and *Colletotrichum gloeosporioides* BCRC 35178. Metabolic products derived from this strain displayed a diverse profile, as revealed by high-performance liquid chromatography (HPLC) fingerprinting analysis. Upon a thorough review of the existing literature, no references were found regarding the chemical and biological activity of the aforementioned Actinobacteria.

The scaled-up fermentation and extensive chromatographic separation of the EtOAc extract resulted in the isolation of one new metabolite, M2 (**1**), together with five known compounds, indole-3-carboxylic acid (**2**) [4], methyl 4-hydroxycinnamate (**3**) [5], ergosterol peroxide (**4**) [6], 6*β*-hydroxystigmast-4-en-3-one (**5**) [7], as well as betulonic acid (**6**) [8]. All of these compounds were found for the first time from the title Actinobacteria strain M2. Herein, we report the structural determination of the new compound.

Compound **1** was obtained as a colorless oil. The molecular formula was determined as $C_{17}H_{24}O_4$ (four degrees of unsaturation) by HR-ESI-MS *m/z* 315.1573 $[M + Na]^+$ (calcd for $C_{17}H_{24}NaO_4$, 315.1572), which was in agreement with the ^1H and ^{13}C NMR data (Table 1). The UV spectrum absorption λ_{max} (MeOH) at 226 nm, and a strong IR absorption at 1702 cm^{-1}, as well as the observation of the featuring carbon resonances [δ_C 150.0 (C-11), 123.7 (C-12), and 172.3 (C-13)] in the ^{13}C NMR spectrum (Table 1), revealed the presence of a conjugate carbonyl functionality in compound **1**.

1) Department of Life Science, Fu Jen Catholic University, 242, New Taipei City, Taiwan, e-mail: chengfirdi@gmail.com; 2) Bioresource Collection and Research Center, Food Industry Research and Development Institute, 300, Hsinchu, Taiwan; 3) Department of Chemistry, Fu Jen Catholic University, 242, New Taipei City, Taiwan; 4) School of Medical and Health Sciences, Fooyin University, Ta-Liao, 83102, Kaohsiung, Taiwan; 5) Department of Pharmacy, School of Pharmaceutical Sciences, National Yang Ming Chiao Tung University, 112, Taipei, Taiwan; 6) Department of Medical Research, China Medical University Hospital, 404, Taichung, Taiwan; 7) Traditional Herbal Medicine Research Center, Taipei Medical University Hospital, 110, Taipei, Taiwan; 8) Department of Neurosurgery, Yuan's General Hospital, 802, Kaohsiung, Taiwan. Published in *Khimiya Prirodnykh Soedinenii*, No. 5, September–October, 2024, pp. 739–741. Original article submitted December 31, 2023.

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TABLE 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data of Compound **1** ($CDCl_3$, δ, ppm, J/Hz)

C atom	δ_H	δ_C[a]	C atom	δ_H	δ_C[a]
1	1.60–1.65 (m)	29.0	10	–	38.0
2	1.51–1.58 (m)	17.8	11	7.13 (dd, J = 16.0, 11.2)	150.0
3	1.42 –1.51 (m)	39.3	12	5.84 (d, J = 16.0)	123.7
4	1.13–1.15 (m)	47.0	13	–	172.3
5	1.96 (dd, J = 10.2, 1.5)	48.5	17	4.80 (s)	110.2
6	1.32–1.36 (m)	25.5	18	4.43 (s)	16.7
7	2.10–2.17 (m); 2.36–2.42 (m)	36.2	19	1.17 (s)	183.8
8		145.9	20	0.89 (s)	15.4
9	2.62 (d, J = 11.2)	61.9			

Assignment was aided by 2D NMR, COSY, HSQC, and HMBC experiments; [a] ^{13}C NMR multiplicities were determined by DEPT experiment.

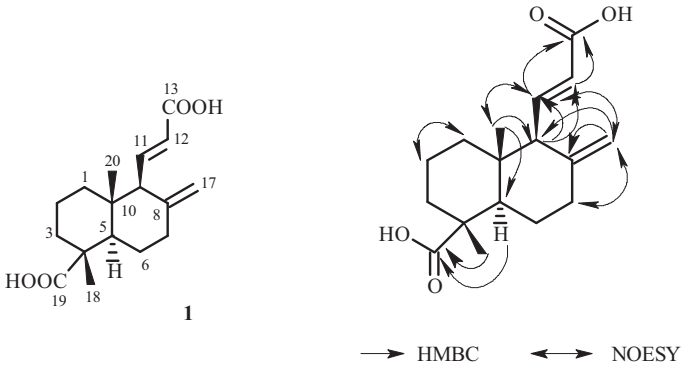

Fig. 1. Structure and key HMBC and NOESY correlations of compound **1**.

Its residual IR spectrum showed absorption bands for CO<u>OH</u> (3400 cm^{-1}), and one vinyl (1650 cm^{-1}) functionalities. The ^{1}H NMR spectrum (Table 1) of compound **2** showed the presence of one terminal double bond at δ_H 4.80/4.43, (1H, s, CH_2-17), one (*E*)-11,12-double bond at δ_H 7.13 (1H, dd, J = 16.0, 11.2 Hz, H-11), 5.84 (1H, d, J = 16.0 Hz, H-12), and two Me moieties [δ_H 1.17 (3H, s, Me-19) and 0.89 (3H, s, Me-20)]. From ^{13}C NMR data, it can be found that compound **1** was composed of 17 carbons. It is speculated that it was originally composed of 20 carbons, but the double bond was cleaved owing to the oxidation of the side chain. The C-20 labdane diterpene becomes a C-17 derivative. The presence of electron-withdrawing groups in proximity to the double-bond position causes a downfield shift in the signal of δ_H 7.13 (H-11), as observed. The HMBC analysis (Fig. 1) indicates a correlation between both H-11 and H-12 with C-13 (δ_C 172.3), which corresponds to a carboxyl group. The ^{13}C NMR of C-19 shows another group of carboxyl groups at δ_C 183.8. The relative configuration of **1** was derived by a NOESY spectrum (Fig. 1), the relative configuration of which was based on a NOESY analyses. NOEs for Me-19/Me-20, and H-5/H-9 indicated that H-5/H-9 were on the same side of the molecular plane, tentatively assumed as α-orientation. The (*E*)-2-carboxyvinyl was β-oriented, which was further confirmed by the NOE H-11/H-20. Consequently, the relative configuration of C-4, 5, 9, and 10 was assigned as *rel*-(*R*,*R*,*S*,*S*) (Fig. 1). Thus, compound **1** was elucidated as M2, which was further confirmed by ^{13}C NMR, COSY, HMBC, NOESY, and HSQC experiments.

Owing to the early development and research applications of *Streptomyces* species, our team has been isolating and collecting actinomycetes resources from various regions and environments in Taiwan over the years. As well as the common *Streptomyces* strains, we have also obtained numerous rare genera, including new species. Guided by the concept of "new species, new compounds," we hope to discover unique compounds from these novel species in the future.

EXPERIMENTAL

General Procedures. TLC: silica gel 60 F_{254} precoated plates (Merck). Column chromatography (CC): silica gel 60 (230–400 mesh, Merck) and Spherical C18 100A Reversed Phase Silica Gel (RP-18) (particle size: 40–63 μm) (LiChroprep). Optical rotation: Jasco P-2000 polarimeter. IR spectra: Perkin-Elmer-2000 FT-IR spectrophotometer. ^{1}H, ^{13}C, and 2D NMR spectra: Varian-VNMRS-600 spectrometers; with Me_4Si, as an internal standard. EI-MS: VG-Biotech Quatro-5022 mass spectrometer. ESI and HR-ESI-MS: Bruker APEX-II mass spectrometer. Reverse-phase HPLC (RP-HPLC): Hitachi model L-2310 pump equipped with a diode-array detector (model L-2455, Hitachi).

Microorganism and Fermentation. The actinomycete, *Streptomyces* sp. M2, was isolated from both the wetland of Houmei in Chiayi and soil samples from agricultural fields in Taiwan, by using HV agar, and was then incubated at 45°C for 7 days. These Actinobacteria were identified byMin Tseng and specimens were deposited at the Bioresource Collection and Research Center, Food Industry Research and Development Institute. The strain was maintained on oatmeal agar and the spores or mycelia suspension were harvested with 20% (v/v) glycerol and stored at –20°C. Liquid culture method was carried out in following six steps: 1. collect spores of the fungal strain; 2. inoculate the spores into separate TSB (Tryptic Soy Broth) and YG (Yeast Extract Glucose) culture media; 3. shake the cultures at 120 rpm for 48–72 h; 4. transfer the fungal broth to TSB and YG agar media for expansion culture; 5. shake the agar cultures at 120 rpm for 48–72 h. 6. The cultures are now ready for further use.

Fermented broth (1 L) was filtered to separate mycelium and culture broth. The culture broth was repeatedly extracted three times with EtOAc. The EtOAc layers were combined and dried to give a fraction soluble in EtOAc (≈ 4.4 g) and was chromatographed over silica gel (70–230 mesh), eluting with *n*-hexane and enriched with EtOAc to produce five fractions (1–5). Fraction 1 (170 mg) was chromatographed over silica gel and eluted with *n*-hexane–EtOAc (10:1→0:1) to afford three fractions (1-1–1-3). Fraction 1-2 (8.7 mg) was purified by preparative TLC (*n*-hexane–acetone, 2:1) to afford compounds **1** (7.9 mg) and **4** (2.8 mg). Fraction 2 (800 mg) was applied to silica gel (230–400 mesh, 24 g) and eluted with hexane–acetone (20:1) to give four fractions (2-1–2-4). Fraction 2-3 (32 mg) was chromatographed on a silica gel column (230–400 mesh, 1 g), eluting with *n*-hexane–acetone (10:1) to give six fractions (2-3-1–2-3-6). Fraction 2-3-1 was further purified by preparative TLC (*n*-hexane–acetone, 10:1) to afford compound **3** (1.4 mg). Fraction 2-3-5 (79 mg) was further purified by preparative TLC (*n*-hexane–acetone, 10:1) to afford compounds **2** (1.3 mg), **5** (2.8 mg), and **6** (2.6 mg).

M2 (1), oil; $[\alpha]_D^{25}$ –1.1° (*c* 0.08, $CHCl_3$). UV (MeOH, λ, nm): 226 (2.18). IR (neat, ν, cm^{-1}): 3400, 1702 (COOH), 1650 (C=C). For ^{1}H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$), see Table 1. EI-MS *m/z* 293 $[M + H]^+$; HR-ESI-MS *m/z* 315.1573 $[M + Na]^+$ (calcd for $C_{17}H_{24}NaO_4$, 315.1572).

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