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A NEW TEN-MEMBERED LACTONE FROM *Tubercularia* sp. TF5, AN ENDOPHYTIC FUNGUS OF *Taxus mairei*

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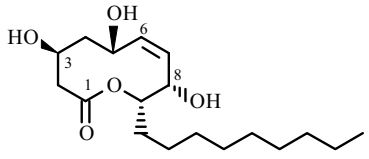
*One new ten-membered lactone (**1**) named (Z)-4,6,9-trihydroxy-10-nonyl-3,4,5,6,9,10-hexahydrooxecin-2-one along with 5-methoxycarbonylmellein (**2**) and cytochalasin D (**3**) were isolated from the culture of the endophytic fungus strain* Tubercularia *sp. TF5, originally separated from the inner bark of* Taxus mairei *obtained in Fujian Province, Southeast China. The structures of compounds **1–3** were elucidated by spectroscopic methods. The antimicrobial and cytotoxic activities of **1** were analyzed but it showed no significant activities.*

Key words: ten-membered lactone, *Tubercularia* sp. TF5, (*Z*)-4,6,9-trihydroxy-10-nonyl-3,4,5,6,9,10-hexahydrooxecin-2-one.

The fungal strain *Tubercularia* sp. TF5 was isolated from the inner bark of *Taxus mairei*. Previous studies have suggested this strain has the potential to produce the anticancer compound taxol [1], and a series of compounds has been isolated [2]. In this article, one new ten-membered macrolide with two known ones, 5-methyoxycarbonylmellein (**2**) [3, 4] and cytochalasin D (**3**) [5]), were isolated and identified from the fermentation extracts of *T.* sp. TF5, and the antibacterial and cytotoxic activities of **1** were discussed.

Compound **1** was obtained as a colorless powder. The molecular formula of **1** was determined to be $C_{18}H_{32}O_5$ by analysis of NMR spectroscopic data and ESI-MS measurement of the quasi-molecular ion at *m/z* 679.3 $[2M + Na]^+$. Inspection of the ^1H and ^{13}C NMR, HMQC, HMBC, and COSY data (Table 1) gave the structure of **1** as (*Z*)-4,6,9-trihydroxy-10-nonyl-3,4,5,6,9,10-hexahydrooxecin-2-one.



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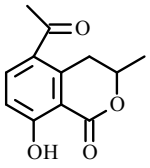

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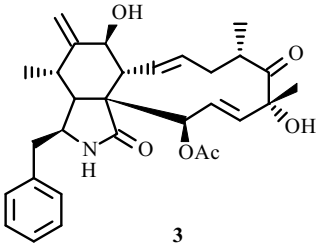

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TABLE 1. ^{1}H NMR (500 MHz), ^{13}C NMR (DEPT) (125 MHz), HMBC and COSY Spectral Data of **1** (CD_3OD, δ, ppm, multiplicity, J/Hz)

C atom	δ_H	δ_C	HMBC (C/H)	COSY (H/H)
1		177.4 s		
2	2.46 (dd, J = 10.0, 15.0)			
	2.18 (dd, J = 10.0, 15.0)	33.3 t	C-1, C-2, C-4	H-3
3	3.65 (br.d, J = 10.0)	76.8 d		H-4
4	2.40 (dd, J = 11.0, 15.0)	27.6 t	C-5, C-3	H-3
	1.64 (m)			
5	4.45 (br.s)	74.2 d		H-6
6	5.71 (d, J = 15.0)	131.3 d	C-4, C-7	H-5, H-7
7	5.81 (d, J = 15.0)	130.3 d	C-6, C-8	H-6, H-8
8	4.29 (br.s)	73.1 d		H-9
9	4.97 (m)	79.6 d	C-1, C-8, C-10, C-11	H-8, H-10
10	1.83 (m)			
	1.68 (m)	30.7 t	C-9, C-11, C-12	H-9, H-11
11	1.29–1.35 (m, 2H)	27.2 t	C-12, C-13	H-10
12		30.5 t*		
13		30.6 t*		
14	1.29–1.35 (m, 8H)	30.4 t*	C-10, C-11, C-18	
15		33.1 t*		
16	1.29–1.35 (m, 4H)	23.7 t*	C-14, C-15, C-18	H-18
17		30.7 t*		
18	0.91 (t, J = 6.3, 7, 3H)	14.4 q	C-16, C-17	H-17, H-18

*Signals overlapped.

The ^{1}H NMR (CD_3OD) showed a methyl triplet at δ_H 0.91 (t, J = 6.3 Hz), three nonequivalent methylenes [δ_H 2.46 (dd, J = 10.0, 15.0 Hz) and 2.18 (dd, J = 10.0, 15.0 Hz)], [δ_H 2.40 (dd, J = 11.0, 15.0 Hz) and 1.64 (m)], and [δ_H 1.83 (m) and 1.68 (m)], four oxy protons [at δ_H 3.65; 4.45; 4.29 and 4.97], and two olefinic protons [at δ_H 5.71 and 5.81] (Table 1). The $J_{H\text{-}6/H\text{-}7}$ value of 15.0 Hz revealed a *trans*-configuration of the olefinic protons. The ^{13}C NMR (CD_3OD) showed 18 signals: one methyl, ten methylenes, six methines, and one quaternary carbon (Table 1). The ^{1}H–^{1}H COSY spectra of **1** demonstrated the connectivity from C-2 to C-12. The ^{1}H–^{13}C long-range correlations from H-9 to C-1 (δ 177.4) and the relatively lower-field resonance of H-9 (at δ 4.97) suggested that **1** was a 10-membered macrolide. The downshift of C-3 (δ 76.8) and C-8 (δ 73.1) revealed the presence of hydroxyl groups. The ^{1}H and ^{13}C NMR (DEPT) spectra showed that there are many overlapped proton signals at δ 1.29–1.83 and eight methylene signals and a broad three-proton triplet at δ 0.91, which suggested the presence of an *n*-octane unit. The methylene protons at δ 1.83 and 1.68 (H-10) showed long-range correlations with C-9, C-11, and C-12, which suggested that the octane unit should be at C-9. The relative configuration of **1** was revealed by the NOEs observed between H-3 and H-5, H-6, and H-8, and H-8 and H-9. Thus compound **1** was elucidated to be (*Z*)-4,6,9-trihydroxy-10-nonyl-3,4,5,6,9,10-hexahydrooxecin-2-one.

EXPERIMENTAL

General Experimental Procedures. Column chromatography (CC): silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, P. R. China), silica gel GF254 (Merck), RP-18 (Merck), and Sephadex LH-20 (Amersham Biosciences). TLC: precoated silica gel GF254 plates (0.20–0.25 mm, Qingdao Marine Chemical Factory). ^{1}H and ^{13}C NMR spectra: Bruker DRX-500 spectrometer, at 500/125 MHz, rep., in CD_3OD; δ in ppm rel. to Me_4Si, J in Hz. ESI-MS: Thermo-Finnigan Advantage LCQ mass spectrometer, in *m/z*.

Microbial Material. The fungus strain TF5 was isolated from *Taxus mairei* obtained in Fujian Province, Southeast China [1].

Extraction and Isolation. The fungal strain TF5 was cultured on PDA media (5 L) for 21 days. The cultures was extracted three times with equal volumes of EtOAc–MeOH–AcOH 80:15:5 (v/v/v) at room temperature. The organic solutions were collected by filtration and removed under vacuum at 40°C to yield a brown syrup. The brown syrup was partitioned between water and EtOAc (1:1) until the EtOAc layer was colorless. The combined organic layer was concentrated by evaporation to obtain the EtOAc extract (3.26 g).

The extract was subjected to MPLC (80 g RP-18; 50%, 70%, and 100% MeOH in water, 1 L for each gradient) to afford three fractions: Fr.1–3.

Fraction 2 (510 mg) was subjected to MPLC (45 g RP-18; MeOH–H_2O 7:3) to afford two subfractions (Fr. 2.1 and Fr. 2.2). Fraction 2.1 (324 mg) and Fr. 2.2 (114 mg) were subjected to Sephadex LH-20 (in MeOH) to afford Fr. 2.1.1, Fr. 2.1.2, Fr. 2.2.1, and Fr. 2.2.2. Fraction 2.1.1 (63 mg) was subjected to silica gel column chromatography, eluting with $CHCl_3$–MeOH (250:1) to afford **3** (11 mg). Fraction 2.1.2 (45 mg) added to Fr. 2.2.2 (31 mg) was subjected to silica gel column chromatography, eluting with $CHCl_3$–MeOH (30:1) to afford **1** (7 mg). Then Fr. 2.2.2 (24 mg) was purified by purification on silica gel column, eluting with petroleum ether–EtOAc (30:1) to afford **2** (5 mg).

Bioassay Procedures. The cytotoxicities against HeLa cell line were measured 72 h post-treatment by the MTT method [6]. The inhibiting rate of **1** at 10 μg/mL and 20 μg/mL against HeLa lines was 27.6% and 40.7%, respectively. The results revealed that **1** displayed weak activities against HeLa cells.

The antibacterial activity of **1** was tested at a concentration of 50 μg/mL against bacteria (*Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*) and yeasts (*Saccharomyces cerevisiae* and *Candida albicaus*) using a similar MIC method with 96-well microplates [7]. The results showed no inhibitory activities.

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