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Dihydrotrichodimerol and Tetrahydrotrichodimerol, Two New Bisorbicillinoids, from a Marine-derived *Penicillium terrestre*

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Abstract Two new bisorbicillinoids possessing an open-ended cage structure, dihydrotrichodimerol (**1**) and tetrahydrotrichodimerol (**2**), were isolated from a marine-derived *Penicillium terrestre*. Their structures were established by spectroscopic methods. Their cytotoxic activities against P388 and A-549 cell lines were preliminarily evaluated by the MTT method.

Keywords dihydrotrichodimerol, tetrahydrotrichodimerol, bisorbicillinoids, *Penicillium terrestre*, cytotoxicity

Introduction

In previous articles [1, 2] we have reported six new compounds isolated from a marine-derived *Penicillium terrestre*. Further studies led to the isolation of another two new bisorbicillinoids [3] possessing an unusual open-ended cage structure [4], dihydrotrichodimerol (**1**) and tetrahydrotrichodimerol (**2**). This kind of compounds is rare in nature. To the best of our knowledge, only three, trichodimerol [5], demethyltrichodimerol [6] and bisorbibetanone [7], have been previously reported. In this paper, we describe the isolation and structural elucidation of compounds **1**~**3**. In addition, their cytotoxic effects were preliminarily assayed using P388 and A-549 cell lines.

Materials and Methods

General

Melting points were measured using a Yanaco MP-500D micro-melting point apparatus and are uncorrected. Optical rotations were obtained on a JASCO P-1020 digital polarimeter. IR spectra were taken on a NICOLET NEXUS 470 spectrophotometer in KBr discs. UV spectra were recorded on Beckman DU® 640 spectrophotometer. ESI-MS were measured on an Esquir LC mass spectrometer. ^{1}H, ^{13}C NMR and DEPT spectra and 2D-NMR were recorded on a JEOL Eclips-600 spectrometer using TMS as internal standard. Semipreparative HPLC was performed using an ODS column (YMC-Pack ODS-A, 10×250 mm, 5 μm).

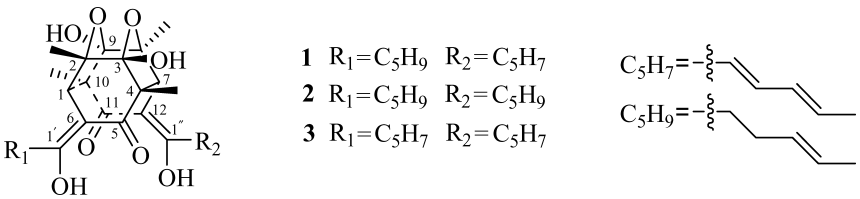

Fig. 1 Structures of compounds **1**~**3**

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† The same new compound (dihydrotrichodimerol) which was isolated independently appears in this issue (pages 615–620).

Fungus and Culture

The producing strain, *Penicillium terrestre*, is preserved in China Center for Type Culture Collection (patent depositary number: CCTCC M 204077). The fermentation procedure was carried out as described previously [2].

Isolation

The active fraction eluted with chloroform–methanol (45 : 1) from a silica gel column [2] yielded compound **3** (300 mg) as a crystalline solid. The mother liquor was then applied to Sephadex LH-20 using chloroform–methanol (1 : 1) as the eluting solvent. The active subfraction was further separated by semipreparative HPLC on an ODS column using methanol–water (80 : 20) as eluting solvent to yield compounds **1** (23 mg) and **2** (30 mg).

Results and Discussion

Physico-chemical Properties

Compound **1**: Yellowish powder; mp 112∼117°C; $[\alpha]_D^{20}$ +60.0° (*c* 0.17, MeOH); HRESIMS *m/z*: 521.2122 $[M+Na]^+$(calcd for $C_{28}H_{34}O_8Na$, 521.2151); UV-vis (MeOH) λ_{max} nm (log ε) 241 (3.87), 308 (4.18), 360 (4.22); CD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 304 (+14.0), 272 (−11.0); IR ν_{max} cm^{-1} (KBr) 3441, 2984, 2940, 1612, 1261, 1127, 992.
Compound **2**: Yellowish powder; mp 40∼50°C; $[\alpha]_D^{20}$ +190.3° (*c* 0.22, MeOH); HRESIMS *m/z*: 523.2292 $[M+Na]^+$ (calcd for $C_{28}H_{36}O_8Na$, 523.2308); UV-vis (MeOH) λ_{max} nm (log ε) 297 (4.27); CD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 319 (+68.0), 277 (−8.0); IR ν_{max} cm^{-1} (KBr) 3428, 2984, 2940, 1595, 1451, 1378, 1261, 1127, 1011, 965, 874.
Compound **3**: Yellowish crystal; mp 235∼238°C; $[\alpha]_D^{20}$ −410.5° (*c* 0.43, MeOH); ESIMS *m/z* (%) 497 $[M+H]^+$ (100); UV-vis (MeOH) λ_{max} nm (log ε) 241 (4.15), 308 (4.36), 363 (4.50); IR ν_{max} cm^{-1} (KBr) 3441, 2983, 2939, 1613, 1547, 1414, 1300, 1264, 1160, 1126, 940.

Structure Determination

Compound **1** was obtained as yellowish amorphous solid resisting crystallization. Its molecular formula was determined to be $C_{28}H_{34}O_8$ by HRESIMS, which was in agreement with its ^1H and ^{13}C NMR data. The absorptions at 3441 and 1612 cm^{-1} in the IR spectrum indicated the presence of an enolized β-diketone [5, 6, 8]. And two enolized β-diketones could be deduced from the carbon signals: four carbonyl-like carbons (δ_C: 206.2, 193.1, 192.7 and 174.5) and two sp^2 quaternary carbons (δ_C: 104.7 and 104.5), and the chemical shifts of two hydroxyls at δ 16.60 and 16.80 in the ^1H NMR spectrum (Table 1).

The ^1H NMR spectrum also displayed the characteristic signals of a sorbyl residue [5, 6, 8] and a 2,3-dihydrosorbyl moiety [8, 9] (Table 1), which could be also deduced from the ^1H-^1H COSY and HMBC spectra. The *E, E*

Table 1 ^1H and ^{13}C NMR data for **1** (600 and 150 MHz, respectively, acetone-d_6, TMS, δ ppm)

No	δ_H (*J* in Hz)	δ_C	No	δ_H (*J* in Hz)	δ_C
1	3.03 (1H, s)	58.6	7	3.14 (1H, s)	58.3
2		79.6	8		79.5
3		105.1[b]	9		105.0[b]
4		58.9	10		60.4
5		193.1	11		201.9
6		104.7[c]	12		104.5[c]
2-CH_3	1.36[a] (3H, s)	21.9	8-CH_3	1.34[a] (3H, s)	21.9
4-CH_3	1.41 (3H, s)	20.1	10-CH_3	1.39 (3H, s)	19.6
1′		192.7	1″		174.5
2′	2.40 (1H, dt, 15.1, 6.9); 2.56 (1H, dt, 15.1, 7.8)	35.2	2″	6.46 (1H, d, 14.7)	120.1
3′	2.25 (1H, m)	29.0	3″	7.26 (1H, dd, 15.1, 11.0)	143.2
4′	5.46 (1H, m)	130.5	4″	6.40 (1H, m)	131.9
5′	5.49 (1H, m)	126.5	5″	6.23 (1H, dq, 15.1, 6.9)	140.1
6′	1.61 (3H, br d, 4.4)	18.0	6″	1.85 (3H, dd, 6.9, 1.4)	18.8
OH	16.60 (s)		OH	16.80 (s)	

1. [a∼c] Assignment of signals bearing the same superscript might be exchanged.
2. In the HMQC spectrum two methyls (δ_H: 1.34 and 1.36) correlated with one carbon signal (δ_C: 21.9), which indicated the signal (δ_C: 21.9) represented two methyls.

Table 2 ^{1}H and ^{13}C NMR data for **2** and **3** (600 and 150 MHz, respectively, acetone-d_6, TMS, δ ppm)

No	**2**		**3**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	3.02 (1H, s)	58.7	3.14 (1H, s)	58.1
2		79.5		79.6
3		105.0		105.2
4		58.7		60.4
5		192.3		201.0
6		104.8		104.5
2-CH_3	1.35[a] (3H, s)	21.9	1.34 (3H, s)	19.7
4-CH_3	1.38[a] (3H, s)	19.9	1.41 (3H, s)	21.8
1′		193.8		176.1
2′	2.41 (1H, dt, 14.9, 7.4); 2.57 (1H, dt, 15.4, 7.7)	35.1	6.45 (1H, d, 14.7)	120.3
3′	2.25 (2H, m)	29.0	7.26 (1H, dd, 14.7, 11.4)	143.3
4′	5.45 (1H, m)	130.4	6.40 (1H, m)	131.9
5′	5.49 (1H, m)	126.5	6.24 (1H, dq, 15.0, 6.6)	140.2
6′	1.61 (3H, br d, 5.0)	18.0	1.86 (3H, d, 6.6)	18.8
OH	16.61 (1H, s)		16.60 (1H, s)	
OH			5.66 (1H, s)	

[a] Assignment of signals bearing the same superscript might be exchanged.

configurations of the two double bonds in the sorbyl moiety were determined on the basis of the magnitude of the coupling constants. The *E* configuration of the double bond in the dihydrosorbyl residue was deduced from the correlation of H-6′ with H-4′ in the NOESY spectrum.

Helped by the HMQC spectrum, twenty-eight carbons could be found in the ^{13}C NMR spectrum. Since there were four carbonyl-like carbons, it was deduced that the carbonyls of the sorbyl and the dihydrosorbyl moieties were a part of two enolized β-diketone systems, respectively. The other carbons could be assigned according to the HMBC correlations (H-1 with C-2, C-3, C-5, C-6, C-9, C-10, C-11, C-1′, CH_3-2 and CH_3-10; H-7 with C-3, C-4, C-5, C-8, C-9, C-11, C-12, C-1″, CH_3-4 and CH_3-8; CH_3-2 with C-1, C-2 and C-3; CH_3-4 with C-3, C-4, C-5 and C-7; CH_3-8 with C-7, C-8 and C-9; CH_3-10 with C-1, C-9, C-10 and C-11). In addition, there were eight oxygen atoms present in **1**, four forming two enolized β-diketones; and there were two monooxygenated carbons (δ_C: 79.6 and 79.5) and two dioxygenated carbons (δ_C: 105.1 and 105.0) in the ^{13}C NMR spectrum, so the remaining four oxygen atoms must comprise two hemiacetals. Thus the plain structure of **1** was established.

In the NOESY spectrum the correlations of H-1 with CH_3-2, CH_3-10 and H-2′, and H-7 with CH_3-4, CH_3-8 and H-2″, showed that H-1 and H-7 were in close proximity to their related functionalities respectively. Its CD spectrum was similar to trichodimerol, so the absolute stereochemistry (Fig. 1) of **1** was deduced.

Compound **2** was also a yellowish amorphous powder. Its molecular formula, $C_{28}H_{36}O_8$, was established on the basis of HRESIMS. Its IR spectrum (3427 and 1595 cm^{-1}) was very similar to those of **1** and trichordimerol [5]. The presence of enolized β-diketone and 2,3-dihydrosorbyl moieties could be deduced from the ^{1}H and ^{13}C NMR spectra (Table 2).

Its ^{13}C NMR spectrum (measured in acetone-d_6) only displayed thirteen singals. But in the HMQC and HMBC spectra, which were measured in $CDCl_3$, a methine and a quaternary carbon appeared at δ_C about 58.0 and 57.8. So the peak (δ 58.7) in the ^{13}C NMR spectrum (measured in acetone-d_6) should represent two carbon signals, a methine and a quaternary carbon. Consequently, fourteen carbon signals could be deduced in the ^{13}C NMR spectrum.

Its molecular formula suggested that **2** was a dimer. The optical activity showed that it must contain a proper axis of symmetry, and not a plane or point of symmetry. Detailed analysis and comparison of its NMR data with those of trichodimerol [5] revealed the differences: the chemical shifts of carbonyl-like carbons in **2** were obviously different from those of **3** owing to the disappearance of the conjugated double bond between C-2′ and C-3′; the sorbyls

Table 3 The activities (IC_{50}, μM) of **1**~**3** on cancer cell lines

	1	**2**	**3**	VP16
P388	2.8	8.8	0.33	0.064
A-549	2.1	4.3	4.7	1.4

in trichodimerol became dihydrosorbyls in **2**. Other data were similar to those of **3**. The above evidence revealed that **2** was the tetrahydrogenated derivative of **3**. The similarity of **1** and **2** in their CD spectra showed **2** possessed the same absolute stereochemistry as **1** (Fig. 1).

Compound **3** was afforded as a yellow crystalline solid. Its molecular formula, $C_{28}H_{32}O_8$, was deduced from ESIMS, together with its ^{1}H NMR and ^{13}C NMR spectra. Fourteen signals were found in the ^{13}C NMR spectrum. Assignment of the ^{1}H NMR and ^{13}C NMR data of **3**, helped by the 2D-NMR experiments (HMQC and HMBC), enabled **3** to be identified as trichodimerol, which had been isolated from a few fungi [5, 6, 10].

Cytotoxicity

The cytotoxic effects (Table 3) of compounds **1**~**3** were preliminarily evaluated by the MTT method [11] in P388 and A-549 cell lines, using VP16 as a positive control. Cell culture and bioassay procedures were the same as article [2]. Bisorbicillinoids had some intriguing biological activities [6, 10, 12]. In our search, they were found to be cytotoxic to cancer cell lines. Detailed studies of their antitumor activities are in progress.

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