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Tetrahedron Letters
Tetrahedron Letters 48 (2007) 5235–5238

Trisorbicillinone A, a novel sorbicillin trimer, from a deep sea fungus, *Phialocephala* sp. FL30r

Dehai Li,[a] Fengping Wang,[b] Xiang Xiao,[b] Yuchun Fang,[a] Tianjiao Zhu,[a] Qianqun Gu[a,*] and Weiming Zhu[a,*]

[a]*Key Laboratory of Marine Drugs, Chinese Ministry of Education, Institute of Marine Drugs and Food, Ocean University of China, Qingdao 266003, PR China*
[b]*Beijing Key Laboratory of Marine Biogenetic Resources, The Third Institute of Oceanography, The State Oceanic Administration, Xiamen, PR China*

Received 6 February 2007; revised 23 May 2007; accepted 24 May 2007
Available online 26 May 2007

Abstract—A novel sorbicillin trimer, trisorbicillinone A (**1**), was isolated from a deep sea fungus, *Phialocephala* sp. FL30r. The structure and relative stereochemistry of **1** were determined based on spectroscopic methods. Trisorbicillinone A (**1**) showed cytotoxicity against P388 and HL60 cells with the IC_{50} value of 9.10 and 3.14 μM, respectively.

Sorbicillinoids and bisorbicillinoids are metabolites that have been found in a variety of fungi and have attracted great attention for their novel dimeric structures produced by two similar biosynthetic pathways and their interesting biological activities such as antifungal, antitumor and antioxidative activities.[1] In our search for novel antitumor compounds from marine-derived microorganisms, a fungal strain FL30r, authenticated as *Phialocephala* sp., was obtained from an underwater sample (depth 5059 m). Its extract exhibited cytotoxicity against the K562 cell line. Investigation of the active constituents of this fungus led to the isolation of a novel sorbicillin trimer, which we named trisorbicillinone A (**1**),[2] together with two new bisorbicillinoids (oxosorbiquinol (**E**) and its dihydro derivative).[2] To date, many bisorbicillinoids natural products have been isolated from a few fungal genera and most of them have unique dimeric structures formed by a postulated Michael addition and Diels–Alder reaction. To the best of our knowledge, trisorbicillinone A (**1**) is the first example with the novel trimeric sorbicillin skeleton. In this Letter, we describe the isolation, structure elucidation and cytotoxicity of **1**.

The producing strain was cultured and the fermentation was carried out as follows. A small spoon of spores growing on PDA slant was inoculated into a 250 mL Erlenmeyer flask containing 75 mL sea-water based culture medium and cultured at 28 °C for 2 days on a rotary shaker at 120 rpm. Then, 10 mL of the resultant seed culture was inoculated into 450 × 500 mL Erlenmeyer flask each containing 150 mL of the above culture medium and incubated for 10 days at the same conditions (Fig. 1).

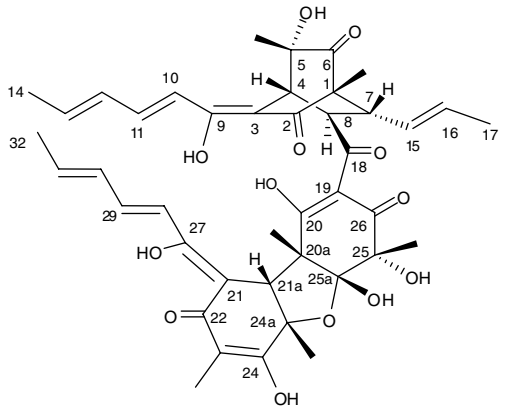

Figure 1. The structure of trisorbicillinone A (**1**).

Keywords: *Phialocephala* sp.; Trisorbicillinone A; Cytotoxicity; Fungus.
* Corresponding authors. Tel.: +86 532 2032065; fax: +86 532 2033054; e-mail addresses: guqianq@ouc.edu.cn; weimingzhu@ouc.edu.cn

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doi:10.1016/j.tetlet.2007.05.134

Seventy liters of the whole broth gave a crude ethyl acetate extract (50.0 g), which was subjected to silica gel column chromatography using petroleum ether–ethyl acetate (20:1–1:1) and followed by Sephadex LH-20 eluted with chloroform–methanol 1:1. Further purification was carried out using HPLC on a ODS semi-preparative column (YMC-Park ODS-A, 10×250 mm, 5 μm, 4 mL/min) gradient eluted with 80–90% of methanol/water containing 0.3% TFA to obtain trisorbicillinone A (**1**) (6.5 mg).

Compound **1** was obtained as brown syrup. The molecular formula was determined as $C_{42}H_{48}O_{13}$ (19 degrees of unsaturation) by HRESIMS (obsd $[M-H]^-$at *m/z*: 759.3043, Calcd: $[M-H]^-$: 759.3017).[2] The IR absorptions at 3439, 1729 and 1620 cm^{-1} indicated the presence of hydroxyl, carbonyl and enolized β-diketone groups. The presence of hydroxyls was confirmed by the existence of three exchangeable downfield protons which have chemical shifts at δ_H 14.15 (s), 16.35 (s) and 17.89 (s) in the ^1H NMR spectrum.[4–6] Analysis of the combined 1D spectral data established that **1** possessed three vinyl methyls (CH_3-14, CH_3-17 and CH_3-32), five disubstituted double bonds (C-10 to C-13, C-15 to C-16 and C-28 to C-31) and two methines (C-7 and C-8), which suggested the presence of three sorbyl side chains (one of the double bonds was substituted).[6] This was in agreement with the COSY and HMBC data and the structure of three fragments (**a**–**c**) was assigned (Fig. 2). The ^{13}C NMR spectrum of **1** revealed forty-two carbon signals (C-2 and C-26 of which were superposed), implying that **1** was a sorbincillin trimer, which was consistent with the molecular formula.

Further analysis and comparison of 1D NMR data with the isolated bisvertinolone (**D**) and oxosorbiquinol (**E**) from this strain resulted in two key moieties, hydrodibenzofuran[4,6–8] and bicyclo [2,2,2] octane.[3,9,10] The

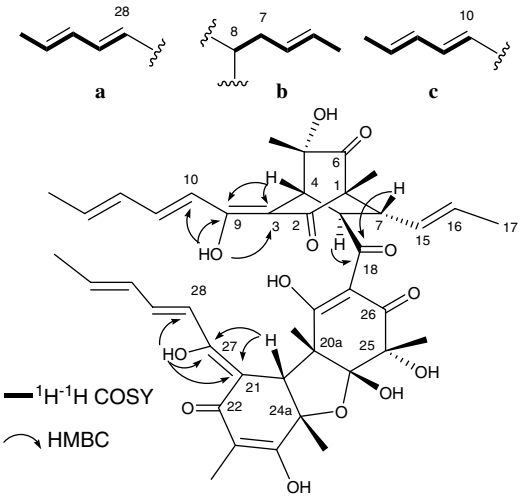

Figure 2. Partial structures **a**–**c** in **1** and its selected HMBC and ^1H–^1H COSY data.

hydrodibenzofuran moiety clearly contained two enolized carbonyls, five sp^2 carbons (two of which were enolic carbons), three sp^3 oxygenated quaternary carbons (one was a hemiacetal carbon), one methine, and four methyls, which were further verified by the HMBC experiment (Table 1). The connectivity of substructure **a** (one of the sorbyl side chains) to C-21 of the hydrodibenzofuran core through C-27 was supported by the key HMBC correlations from OH-27 to C-21, C-27 and C-28, and from H-21a to C-21 and C-27. The above analysis indicated that this moiety had the same structure as bisvertinolone except for the difference of the sorbyl side chain at C-19.

The second key structure unit was the bicyclo [2,2,2] octane[3,9,10] moiety formed by C-1 through C-8. The HMBC correlations from H-7 to C-6 and from H-8 to

Table 1. ^1H (600 MHz) and ^{13}C (125 MHz) NMR data of trisorbicillinone A (**1**) in $CDCl_3$

No	δ_C	δ_H (*J* in Hz)	HMBC (H→C)	Position	δ_C	δ_H (*J* in Hz)	HMBC (H→C)
1	62.9 s			21a	53.7 d	3.81 (s)	20, 20a, 21, 22, 24, 24a,
2	197.4 s						27, CH_3-20a, CH_3-24a
3	107.4 s			23	110.8 s		
4	44.4 d	3.55 (br s)	2, 3, 5, 6, 7, 9	24	163.4 s		
5	76.2 s			24a	79.6 s		
6	210.8 s			25	79.0 s		
7	51.3 d	2.73 (dd, 9.4, 7.6)	6, 8, 15, 18	25a	103.8 s		
8	45.2 d	4.42 (d, 7.2)	3, 4, 7, 15, 18	26	197.4 s		
9	169.3 s			27	170.6 s		
10	118.1 d	6.11 (d, 14.9)	9, 12	28	119.7 d	6.38 (d, 14.9)	27, 30
11	140.1 d	7.32 (m)	9	29	142.8 d	7.30 (m)	
12	131.0 d	6.21 (m)		30	130.9 d	6.30 (dd, 14.3, 12.1)	
13	140.0 d	6.18 (m)		31	138 d	6.15 (m)	32
14	19.0 q	1.87 (d, 6.6)	13	32	18.8 q	1.88 (d, 6.6)	30
15	128.3 d	4.96 (dd, 14.3, 10.9)		CH_3-1	10.0 q	1.09 (s)	1, 2, 6, 7
16	129.8 d	5.30 (m)	17	CH_3-5	24.3 q	1.28 (s)	4, 5, 6
17	17.6 q	1.55 (d, 3.7)	15, 16	CH_3-20a	18.5 q	1.45 (s)	20, 20a, 21a, 25a
18	202.6 s			CH_3-23	7.5 q	1.47 (s)	22, 23, 24
19	110.0 s			CH_3-24a	25.5 q	1.45 (s)	21a, 24a, 24
20	195.8 s			CH_3-25	22.5 q	1.32 (s)	25, 25a, 26
20a	57.9 s			OH-9		14.15 (1H, s)	3, 9, 10
21	99.3 s			OH-20		17.89 (1H, s)	
22	191.1 s			OH-27		16.35 (1H, s)	21, 27, 28

C-3 and C-4 indicated that C-7 and C-8 of substructure **b**, as part of a substituted cyclohexanone ring, were connected to C-1 and C-4, respectively, of the bicyclo [2,2,2] octane moiety. The connectivity of substructure **c** to C-3 via C-9 was confirmed by the key HMBC correlations from OH-9 to C-3, C-9 and C-10, and from H-4 to C-3 and C-9. The NMR data of this moiety were very similar to those of oxosorbiquinol (**E**).[3] Finally, the HMBC correlations from H-7 and H-8 to C-18 and the characteristic chemical shifts of the three carbons of the enolized β-diketone (δ_C 202.6, 195.8 and 110.0) of bisvertinolone[4,6–8] unambiguously suggested that the two main moieties of **1** were connected through C-18 at C-8 and C-19. This completed the planar structure of **1**.

The *E*, *E* configurations of the double bonds in the three sorbyl residues were evident from the large coupling constants (Table 1) and were further confirmed by the correlations of H-14 with H-12, H-13 with H-11, H-12 with H-10, H-15 with H-17, H-28 with H-30, and H-29 with H-31 in the NOESY spectrum (Fig. 3).

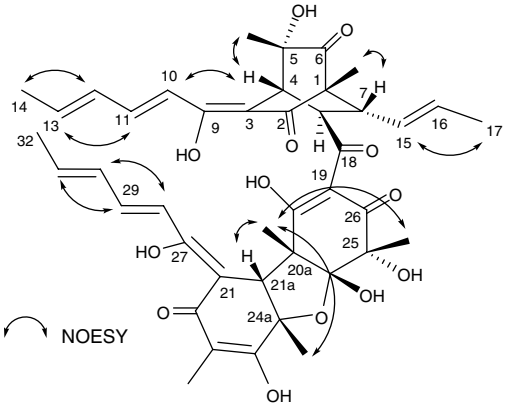

Figure 3. Key NOESY correlations for **1**.

The relative stereochemistry of **1** was established by NOESY experiments (Fig. 3). The H-4 and CH_3-1 were located on the equatorial position of the bicyclo [2,2,2] moiety, which was in the boat form.[11] The cross peaks observed between H-4 and CH_3-5, and between CH_3-1 and H-7 in the NOESY spectrum demonstrated that those groups were on the equatorial bond of the ring. The $J_{7,8} = 7.2$ Hz implied that H-8 was trans to H-7 (the coupling constant over 10 Hz in the cis form).[11] The cross peaks between H-21a and CH_3-20a, between CH_3-20a and CH_3-25, and between CH_3-20a and CH_3-24a suggested that they were on the same side. Therefore, the relative stereochemistry of **1** was constructed as shown in Figure 3.

The configuration of OH-25a in **1** should be identical with bisvertinolone from the point of biogenesis.[6,12] Abe et al.[12] proved that OH-25a was cis to CH_3-20a, based on the elucidation of the biosynthesis of bisvertinolone from oxosorbicillinol and sorbicillinol. So we assigned OH-25a as cis to CH_3-20a in **1**.

Compound **1** was likely biosythesized (Fig. 4) from three molecules of sorbicillinol by [4+2] cycloadditions or Michael addition sequences that are similar to what was proposed for bisvertinolone (**D**)[6,8] and sorbiquinol.[3,9] The two key intermediates, bisvertinolone (**D**) and oxosorbiquinol (**E**), have been isolated in the culture of this strain. Since the configuration at C-6 of sorbicillinol

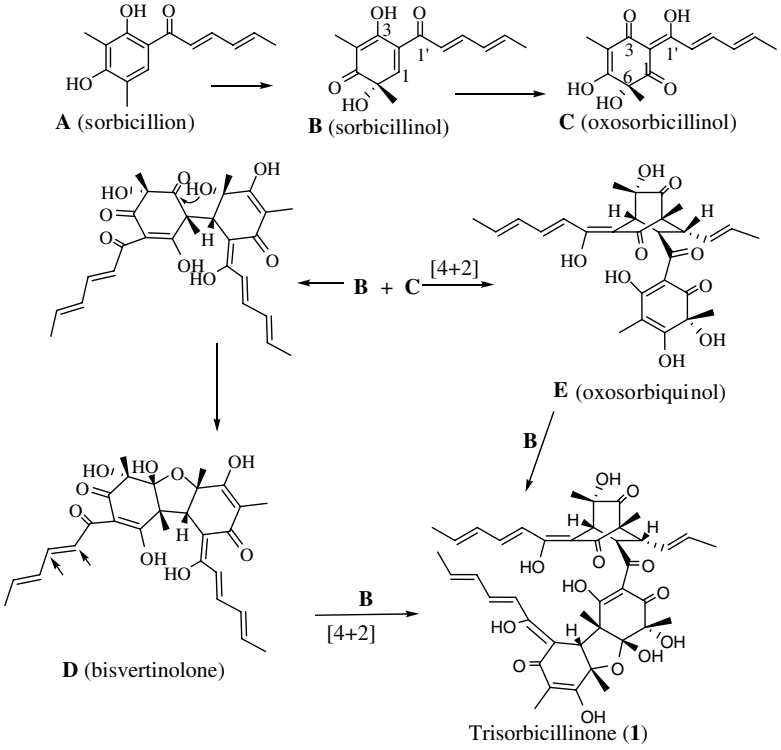

Figure 4. Proposed biogenetic pathway of trisorbicillinone A (**1**).

Table 2. The cytotoxic activities of trisorbicillinone A (**1**)

Cell line	P388	A549	HL60	BEL7402	K562
IC_{50} (μM)	9.10	>100	3.14	60.28	30.21

or oxosorbicillinol did not change in the two reactions,[13] therefore, the configurations at C-5, 24a and C-25 of **1** remain the same. This Diels–Alder reaction between a sorbyl chain and a hexacyclic ring of sorbicillin is rare in nature. Only one bisorbicillinoid, sorbiquinol,[9] has been isolated from a fungus, Trichoderma longibrachiatum, while trisorbicillinone A (**1**) is a unique structure as a sorbicillin trimer.

The cytotoxic activities of trisorbicillinone A (**1**) were preliminarily evaluated using P388, A549, HL60, BEL7402 and K562 cell lines by the MTT method.[14] The result revealed that the cytotoxitiy of **1** was cell type dependent (Table 2).

Acknowledgement

This work was funded by the Chinese Ocean Mineral Resource R&D Association (DY105-2-04).

Supplementary data

^1H, ^{13}C, DEPT, HMQC, HMBC, COSY, NOESY, CD, UV, IR and HRESIMS spectra. This material is available free of charge via the Internet. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.05.134.

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