Tetrahedron 66 (2010) 5101–5106

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Three new sorbicillin trimers, trisorbicillinones B, C, and D, from a deep ocean sediment derived fungus, *Phialocephala* sp. FL30r

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ARTICLE INFO

Article history:
Received 11 March 2010
Received in revised form 22 April 2010
Accepted 27 April 2010
Available online 24 May 2010

Keywords:
Deep ocean derived fungus
Sorbicillin trimer
Trisorbicillinone
Phialocephala sp

ABSTRACT

Eighteen sorbicillonoids, including three new sorbicillin trimers, trisorbicillinones B (**2**), C (**3**), and D (**4**), were isolated from a deep ocean sediment derived fungus, *Phialocephala* sp. FL30r. Their structures were determined by IR, MS, CD, and NMR spectral data. The cytotoxic activities of the new trisorbicillinones B (**2**), C (**3**), and D (**4**) were tested against both P388 and K562 cell lines.

1. Introduction

Characteristic carbon skeletons defining sorbicillin family of molecules have been found in a wide variety of fungal metabolites since the 1940s.[1] These molecules were classified as either sorbicillinoid (monomer) or, in the case of dimers, bisorbicillinoid natural products. The bisorbicillinoids, which were formed from two molecules of sorbicillinol or other oxidatively activated sorbicillin species either by Diels–Alder cycloaddition or by Michael addition to ketalization sequences, have been shown to occur through similar biosynthetic routes, which lead to different kinds of interesting compounds.[2–5] In addition to their novel chemical scaffolds, sorbicillinoids have also been shown to display various biological activities such as antifungal, antitumor, and antioxidant activities.[2,6] In a previous article,[7] we reported an unprecedented sorbicillin trimer, trisorbicillinone A (**1**). Further examination of the fermentation products of this fungus resulted in three new sorbicillin trimers, trisorbicillinones B–D (**2**–**4**) (Fig. 1). Unlike the previously described molecule trisorbicillinone A (**1**), these new compounds formed at the unusual [4+2] positions. In addition to the trisorbicillinoids, we also isolated seven known bisorbicillinoids: bisorbibutenolide,[8] oxosorbiquinol,[9] dihydrooxosorbiquinol,[9] bisvertinolone,[10] tricodimerol,[11] dihydrotricodimerol,[11] and tetrahydrotricodimerol,[11] and seven monosorbicillinoids: sorbicillin,[12] oxosorbicillin,[13] dihydrosorrentanone,[6] sohirnone B,[8] trichodimerol,[10] and rezishanone D,[8] based on the HPLC-UV analysis from fractions with weak cytotoxitic activity against K562 cell line. In this paper, we report the isolation, structural elucidation, and antitumor activity of these new compounds.

2. Results and discussion

2.1. Structure elucidation

Trisorbicillinone B (**2**) was obtained as yellow powder with a molecular formula $C_{42}H_{48}O_{13}$ assigned by HRESIMS analysis (obsd $[M+Na]^+$ at *m/z*: 783.2969, calcd $[M+Na]^+$: 783.2993). This is the same molecular formula as trisorbicillinone A (**1**). The ^1H and ^{13}C NMR spectra (Table 1) indicated the presence of nine methyls, three of them attached to methines; four sp^3 methines, and ten sp^2 methines. The ^1H NMR spectrum also showed resonances of three extremely downfield exchangeable proton signals of enols at δ 17.71, 16.20, and 14.01 attributed to protons hydrogen-bonded to keto-functionalities, which were similar to trisorbicillinone A. These assignments were further confirmed by corresponding IR absorptions at 3439, 1728, and 1633 cm^{-1} indicating the presence of hydroxyl and enolized β-diketone groups.

Interpretation of the ^1H–^1H COSY and HMBC correlations, together with comparison of the ^1H and ^{13}C NMR data between trisorbicillinones A (**1**)[7] and B (**2**), allowed us to assemble several structure fragments **a**–**e** (Fig. 2). Fragments **a**, **b**, and **c** were sorbyl side chains, fragment **d** was a [2,2,2] octane group and fragment **e**

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0040-4020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2010.04.111

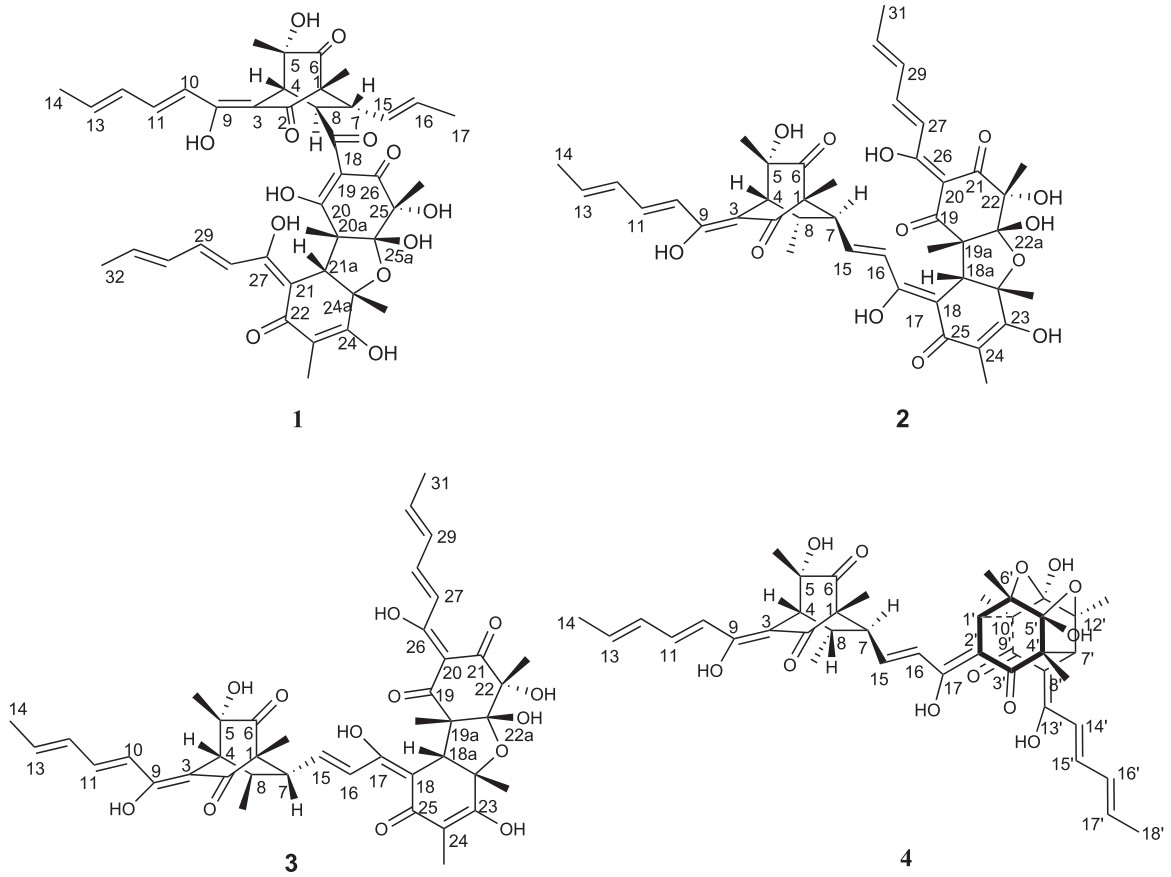

Figure 1. New compounds (**2**–**4**) isolated from *Phialocephala* sp.

was a hydrodibenzofuran moiety. The main differences between **1** and **2** were in fragments **c** and **d**. In trisorbicillinone B (**2**), it were carbons (C-7 and C-8) attached to terminal methyl in the sorbyl side chain (fragment **c**), that composed the [2,2,2] octane group (fragment **d**). While in trisorbicillinone A (**1**), it were carbons (C-7 and C-8) attached to the head carbonyl in sorbyl side chain to composed the [2,2,2] octane group. This attachment was confirmed by HMBC correlations between the proton of CH_3-8 (δ 1.04) to C-4 (δ 47.2), C-8 (δ 32.9), and C-7 (δ 55.9); and by the COSY relationships between CH_3-8 (δ 1.04) and H-8 (δ 2.66). The remaining structure fragments were attached based on key HMBC correlations. Fragment **a** attached to **d** via C-9 based on the HMBC correlations between OH-9 (δ 14.01) and C-10 (δ 117.7), and C-9 (δ 169.1) and C-3 (δ 108.0). Additional HMBC correlations between OH-26 (δ 17.71) and C-27 (δ 121.6), and between C-26 (δ 185.5) and C-20 (δ 107.1) connected fragment **b** to fragment **e** via C-26; and HMBC correlations between H-18a (δ 3.67) and C-17 (δ 168.2) connected fragments **c** and **e** via C-17, thereby completed the plane structure of trisorbicillinone **B**.

The relative configurations of some of the stereocenters in trisorbicillinone B (**2**) were deduced by several key NOESY correlations. The *E*,*E* configurations of the double bonds in the three sorbyl residues were deduced by the large coupling constants (between 10.2 and 14.8) and were further confirmed by NOESY correlations between H-10 and H-12, H-11 and H-13, H-12 and H-14; between H-7 and H-16; and between H-27 and H-29, H-28 and H-30, and H-29 and H-31. Similar to trisorbicillinone A (**1**), H-4 and CH_3-1 were located in the equatorial position of the boat structure of the [2,2,2] octane moiety. NOESY correlations between H-7 and CH_3-8 indicated that they existed in a cis relationship, which was also confirmed by the coupling constant of $J_{7,8}$=6.4 Hz, meaning H-7 was trans to H-8.[7] NOESY correlaitons between H-18a and CH_3-19a, CH_3-23a, and CH_3-22 indicated that they were all on the same side of hydrodibenzofuran moiety. Although there is no direct evidence to confirm the relative configurations at C-5 and OH-22a in trisorbicillinone B (**2**), it is likely that they are the same as trisorbicillinone **A** based on strong similarities between their chemical shifts and also based on the hypothesis of the similar biosynthetic route[6,7] (Fig. 8). Moreover, the OH-22a in all the sorbicillinoids isolated from nature containing bisvertinolone moitif were β, so we assigned the relative configuration of OH-22a as β Figure 3.

Trisorbicillinone C (**3**) (Fig. 4.) was isolated as yellow powder. Analysis of the HRESIMS allowed the assignment of a molecular formula of $C_{42}H_{48}O_{13}$ (obsd $[M+Na]^+$ at *m*/*z*: 783.2975, calcd: $[M+Na]^+$: 783.2993), which the same as trisorbicillinone **B**. The IR spectrum indicated the appearance of hydroxyl groups (3433 cm^{-1}) and carbonyl groups (1730, 1632 cm^{-1}).

Carefully analysis the 1D NMR data (Table 1) indicated that compound **3** was very similar to **2** while the differences between them lay at C-7 and C-8. In trisorbicillinone B (**2**), H-7 and H-8 appeared at 2.14 ppm (1H, dd, 10.2, 6.4) and 2.66 ppm (1H, m) in the ^{1}H NMR spectrum. However, in trisorbicillinone C (**3**), they appeared at 2.88 ppm (1H, ddd, 10.2, 7.4, 2.5) and 3.07 ppm (1H, dqd, 10.2, 7.4, 1.9), respectively. In the ^{13}C NMR, C-7 and C-8 in **2** were at 55.9 ppm and 32.9 ppm while in **3** they were at 51.2 ppm and 30.4 ppm. The coupling constant of $J_{7,8}$=7.4 Hz, meaning H-7 was also trans to H-8.[7] Carefully analysis the HMBC, COSY, and NOESY spectra of compounds **2** and **3**, they had the same plane structure, the same *E* configurations of the double bonds and the same relative configuration of the hydrodibenzofuran moiety and C-1, C-4, and C-5. The difference of NMR data suggested that compounds **2** and **3** were diastereomers with opposite absolute configurations at C-7 and C-8 Figure 5.

Table 1
^{1}H and ^{13}C NMR data of **2** and **3** (600, 150 MHz, $CDCl_3$, TMS, δ ppm)

No	**2**		**3**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	63.3 s		63.0 s	
2	198.4 s		197.4 s	
3	108.0 s		108.5 s	
4	47.2 d	2.96 (1H, d, 1.9)	48.0 d	2.95 (1H, d, 1.9)
5	75.7 s		75.7 s	
6	211.4 s		212.3 s	
7	55.9 d	2.14 (1H, dd, 10.2, 6.4)	51.2 d	2.88 (1H, ddd, 10.2, 7.4, 2.5)
8	32.9 d	2.66 (1H, m)	30.4 d	3.07 (1H, dqd, 10.2, 7.4, 1.9)
9	169.1 s		169.2 s	
10	117.7 d	6.17 (1H, d, 14.8)	117.7 d	6.18 (1H, d, 15.4)
11	142.6 d	7.37 (1H, m)	142.8 d	7.37 (1H, m)
12	139.9 d	6.23 (1H, m)	140.0 d	6.21 (1H, m)
13	131.3 d	6.38 (1H, m)	131.3 d	6.36 (1H, m)
14	19.3 q	1.93 (3H, d, 5.2)	19.2 q	1.92 (3H, d, 5.1)
15	139.1 d	6.47 (1H, dd, 14.7, 10.2)	138.3 d	6.36 (1H, m)
16	124.8 d	6.34 (1H, m)	125.5 d	6.36 (1H, m)
17	168.2 s		167.9 s	
18	100.1 s		99.9 s	
18a	54.2 d	3.67 (1H, s)	54.4 d	3.68 (1H, s)
19	199.8 s		199.8 s	
19a	59.6 s		59.7 s	
20	107.1 s		107.2 s	
21	196.3 s		196.3 s	
22	78.6 s		78.9 s	
22a	104.3 s		104.1 s	
23	163.9 s		164.1 s	
23a	79.5 s		79.7 s	
24	110.0 s		111.1 s	
25	191.7 s		191.7 s	
26	185.5 s		185.7 s	
27	121.6 d	7.34 (1H, dd, 14.8, 10.4)	121.7 d	7.40 (1H, dd, 15.0, 7.8)
28	148.7 d	7.59 (1H,m)	148.6 d	7.58 (1H,m)
29	144.5 d	6.38 (1H, m)	144.4 d	6.36 (1H, m)
30	130.9 d	6.31 (1H, m)	130.9 d	6.29 (1H, m)
31	18.9 q	1.91 (3H, d, 7.2)	18.9 q	1.91 (3H, d, 7.2)
CH_3-1	10.4 q	1.09 (3H, s)	10.9 q	1.14 (3H, s)
CH_3-5	24.7 q	1.22 (3H, s)	24.6 q	1.23 (3H, s)
CH_3-8	19.4 q	1.04 (3H, d, 6.4)	16.8 q	0.92 (3H, d, 7.2)
CH_3-19a	18.6 q	1.45 (3H, s)	18.6 q	1.42 (3H, s)
CH_3-22	23.0 q	1.37 (3H, s)	23.0 q	1.37 (3H, s)
CH_3-23a	25.8 q	1.42 (3H, s)	25.7 q	1.47 (3H, s)
CH_3-24	6.9 q	1.49 (3H, s)	6.9 q	1.48 (3H, s)
OH-9		14.01 (1H, s)		14.25 (1H, s)
OH-17		16.20 (1H, s)		16.11 (1H, s)
OH-26		17.71 (1H, s)		17.70 (1H, s)

For the failure of making crystals, compounds **2** and **3** were identified by the tentatively absolute configurations assignment at C-7 and C-8 using CD analysis. We used a series of the spectra comparisons between trisorbicilliones B (**2**), C (**3**) and a related compared bisorbibutenolide, which was also isolated from this train.[8] The main difference in the CD spectrum between trisorbicillinones B (**2**) and C (**3**) was at 286 nm, which likely results from the change in configuration at C-7. Similar the related compared bisorbibutenolide, compound **3** displayed a positive cotton effect at 286 nm in the CD spectrum, while **2** displayed negative. These comparisons allowed us to presume the absolute configurations of **2** at C-7 and C-8 as 7R, 8S, and compound **3** as 7S, 8R accordingly. The comparison was not rigorous to determine the absolute structures, which still need to further confirm by synthesis or other method because the chromophores of **2** and **3** were complex, and the model compound bisorbibutenolide was not satisfied enough. We also tried to confirm it by using quantum chemical calculation of the CD spectra, but failed.

Trisorbicillinone D (**4**) (Fig. 6) was obtained as a yellow powder. Positive HRESIMS gave the molecular formula $C_{42}H_{48}O_{12}$ (obsd $[M+H]^+$ at m/z: 745.3222, calcd: $[M+H]^+$: 745.3224). The IR spectrum showed the appearance of hydroxyl groups (3447 cm^{-1}) and carbonyl groups (1730,1617 cm^{-1}).

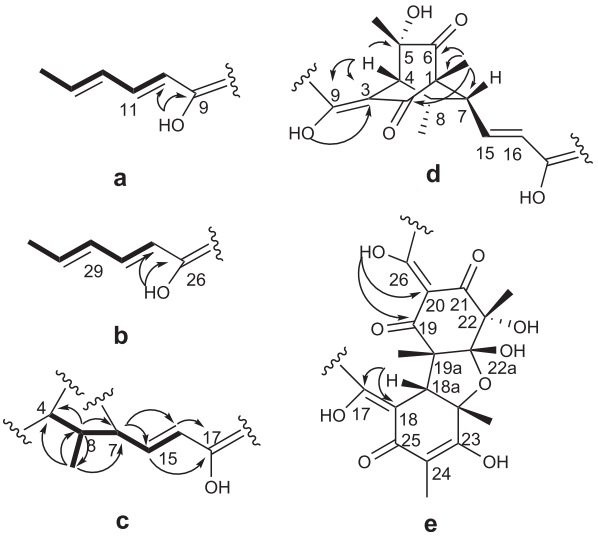

Figure 2. Structure fragments **a**–**e** of trisorbicillinone B (**2**) (^{1}H–^{1}H COSY indicated by bold line and key HMBC correlations by arrows).

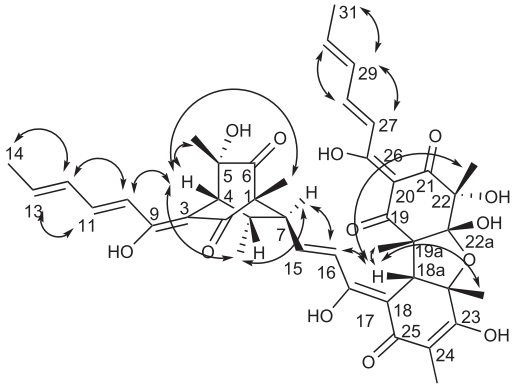

Figure 3. Key NOESY correlations of trisorbicillinone B (**2**).

The NMR data (Table 2) for trisorbicillinone D (**4**) displayed the character signals of sorbicillin trimer family: three extremely downfield exchangeable protons signals of enols (δ: 16.38, 16.10, and 14.20), ten olefinic protons between 6.0 and 7.7 ppm and nine methyl signals at highfield.

Carefully compared the NMR data with trisorbicillinones B (**2**) and C (**3**), they have the very similar data except the data in hydrodibenzofuran moiety. Further comparing and analysis the NMR data with the isolated trichodimerol and dihydrotrichodimerol from this strain result in the containing tricholdimerol moiety (Fig. 6), which also confirmed by the HMBC correlations (Table 2).

The coupling constant of H-7 and H-8 was 7.2 Hz, which suggested that H-7 and H-8 were, trans.[7] The relative configurations were also deduced by the NOESEY spectrum (Fig. 7). Trisorbicillinone D (**4**) has negative Cotton effect at 303 nm, like

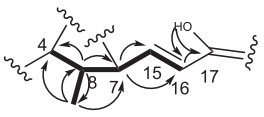

Figure 4. Structure fragment **c** of trisorbicillinone C (**3**) (^{1}H–^{1}H COSY indicated by bold line and key HMBC correlations by arrows).

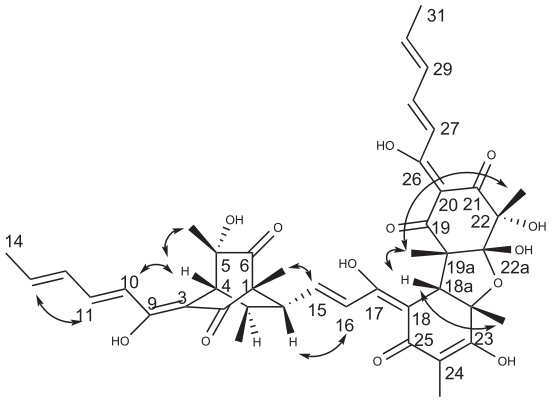

Figure 5. Key NOESY correlations of trisorbicillinone C (**3**).

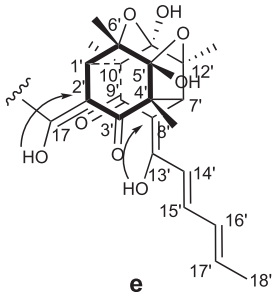

Figure 6. Structure fragment **e** of trisorbicillinone D (**4**) (^{1}H–^{1}H COSY indicated by bold lines and key HMBC correlations by arrows).

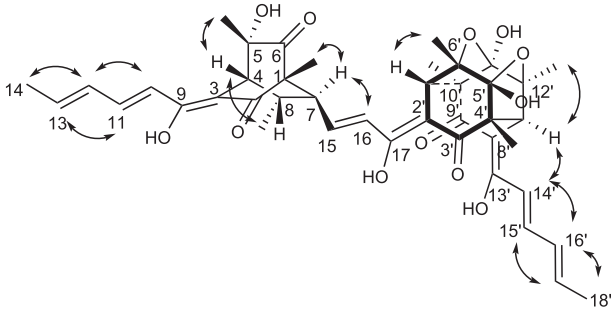

Figure 7. Key NOESY correlations for trisorbicillinone D (**4**).

trisorbicillinone **B** (**2**), suggested the stereochemistry at C-7 and C-8 were 7R, 8S, which also need to further confirm.

The structures of known compounds were established by comparing the NMR data with previously reported data.[6,8–13]

2.2. The proposed biosynthetic pathway of trisorbicillinones B (2), C (3), and D (4)

Sorbicillin monomers and their corresponding dimmers have previously been isolated from different kinds of fungi such as *Trichoderma logibrachiatum*,[10] *Verticillium intertextum*,[14] *Acermonium strictum*,[15] etc. The bisorbicillinoids have been shown to be formed through similar dimmeric reactions, [4+2] cycloadditions or Michael additions.[2] Based on the previous report, we proposed a possible biosynthetic pathways for the formation of trisorbicillinones B (**2**), C (**3**), and D (**4**) in Figure 8.

In the proposed pathway, the chiral center of C-5 in trisorbicillinones B (**2**) and C (**3**) came from sorbicillinol and was not modified during the biosynthesis. Therefore the absolute

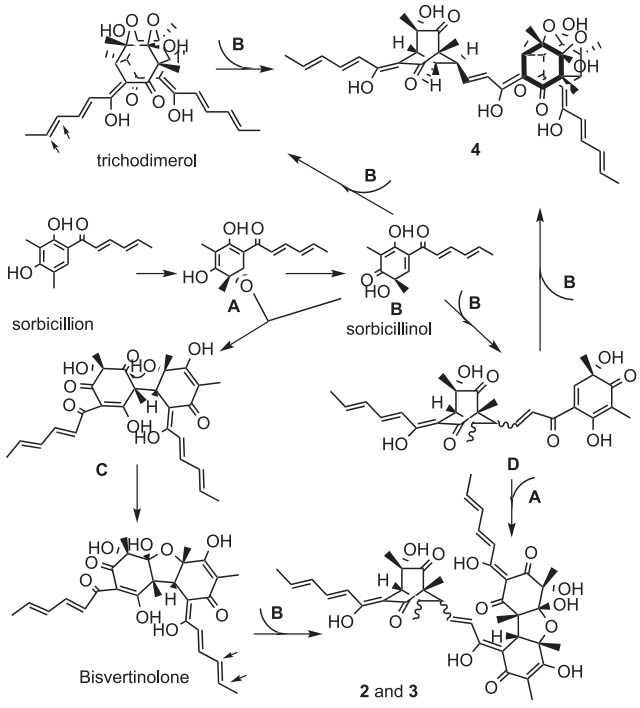

Figure 8. Proposed biosynthetic route of trisorbicillinone B (**2**), C (**3**), and D (**4**).

configuration at C-5 was assigned the same as sorbicillinol. Bisvertinolone was also isolated from this strain but compound **D** in the pathway was totally a supposed compound, which made the route via bisvetinolone priority. So the configuration at C-22a of trisorbicillinones B (**2**) and C (**3**) were the same as bisvertinolone. The differences at C-7 and C-8 between trisorbicillinones B (**2**) and C (**3**) were result in the *exo* and *endo* adduct type during the Diels–Alder cycoladditions, like spirosorbicillinols A and B.[16] Similarly, the biosynthetic route (Fig. 8) to trisorbicillinone D (**4**) was more likely via intermediate trichodimerol than **D**, and the configuration of C-5 in trisorbicillinone **D** was same as sorbicillinol.

This Diels–Alder reaction between a sorbyl chain and a hexacyclic ring of sorbicillin is rare in nature.[7] Only bisorbicillinoid, sorbiquinol,[9] and trisorbicillinone A (**1**) have been showed to arise via this kind of reaction. In the previous reported structures, the changes related to sorbyl side chains including hydrogenation and dimeric reactions, occurred at the double bonds near the hexacyclic rings. This is the first time to report a Diels–Alder reaction, related to sorbyl side chain, occurred at the double bond near methyl group but hexacyclic ring. This kind of reaction increase the distance between the two hexacyclic rings of the monomer sorbicillins. This maybe the reason why the *exo* and *endo* addition products, such as trsorbicillinones B (**2**) and C (**3**), were exist at the same time from the point of organic chemistry.

2.3. Cytotoxic activities

The cytotoxic activities of trisorbicillinones B (**2**), C (**3**), and D (**4**) were preliminarily evaluated using P388 and K562 cell lines by MTT method. All the compounds showed very weak cytotoxic activities against the two cell lines (IC_{50}s: 77.1, 78.3, and 65.7 on P388; and 88.2, 54.3, and 51.2 on K562, respectively).

2.4. General

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

Table 2
^{1}H and ^{13}C NMR, HMBC and ^{1}H–^{1}H COSY data of **4** (600, 150 MHz, $CDCl_3$, TMS, δ ppm)

No	δ_C	δ_H (J in Hz)	HMBC (H→C)	^{1}H–^{1}H COSY
1	62.7 s			
2	197.2 s			
3	108.4 s			
4	47.9 d	2.95 (1H, d, 1.9)	2, 3, 5, 6, 7, 8, 9, CH_3-8	
5	75.7 s			
6	212.0 s			
7	51.1 d	2.83 (1H, dd, 11.0, 10.2)	1, 16	8, 15
8	30.4 d	3.10 (1H, dqd, 10.2, 7.2, 1.9)	3	7, CH_3-8
9	169.3 s			
10	117.6 d	6.13 (1H, d, 14.8)	9	11
11	142.7 d	7.37 (1H, dd, 14.7, 10.8)		10, 12
12	140.1 d	6.30 (1H, m)		11, 13
13	130.8 d	6.25 (1H, m)		12, 14
14	18.9 q	1.93 (3H, d, 6.7)		13
15	142.9 d	6.38 (1H, dd, 14.7, 11.0)		7, 16
16	124.5 d	6.17 (1H, d, 14.7)	17	15
17	173.7 s			
1′	57.4 d	2.84 (1H, s)	17, 2′, 3′, 5′, 6′, 10′, 11′	
2′	102.8 s			
3′	199.0 s			
4′	58.9 s			
5′	102.8 s			
6′	78.6 s			
7′	57.5 d	2.98 (1H, s)	4′, 8′, 9′, 11′, 13′	
8′	102.6 s			
9′	197.3 s			
10′	58.6 s			
11′	104.0 s			
12′	78.7 s			
13′	176.2 s			
14′	118.3 d	6.13 (1H, d, 14.7)		15′
15′	143.3 d	7.29 (1H, dd, 14.4, 10.8)		14′, 16′
16′	130.9 d	6.26 (1H, m)		15′, 17′
17′	140.7 d	6.24 (1H, m)		16′, 18′
18′	18.7 q	1.90 (3H, d, 6.0)		17′
CH_3-1	10.9 q	1.08 (3H, s)	1, 2, 6, 7	
CH_3-5	24.6 q	1.09 (3H, s)	4, 5, 6	
CH_3-8	16.6 q	0.92 (3H, d, 7.2)	4, 7	
CH_3-4′	21.1 q	1.45 (3H, s)	3′, 4′, 7′	
CH_3-6′	21.2 q	1.45 (3H, s)	1′, 5′, 6′	
CH_3-10′	18.5 q	1.42 (3H, s)	9′, 10′, 11′	
CH_3-12′	18.5 q	1.42 (3H, s)	7′, 11′, 12′	
OH-9		14.20 (1H, s)		
OH-17		16.10 (1H, s)	16, 17, 2′	
OH-13′		16.38 (1H, s)	8′, 13′, 14′	

spectrophotometer. ESIMS were measured on a Q-TOF ULTIMA GLOBAL GAA076 LC mass spectrometer. ^{1}H, ^{13}C NMR, and DEPT spectra and 2D-NMR were recorded on a JEOL JNM-ECP 600 spectrometer using TMS as internal standard. Semipreparative HPLC was performed using an ODS column (YMC-Pack ODS-A, 10×250 mm, 5 μm).

2.5. Fungus and culture

The fermentation was carried out as follows. A small spoon full of spores growing on a PDA slant was inoculated into a 250 mL Erlenmeyer flask containing 75 mL sea-water based culture medium (glucose 2%, potato extract 20%, yeast extract 0.2%, peptone 0.3%, NaCl 1%, $MgCl_2 \cdot 6H_2O$ 0.08%, KCl 0.1%) 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 500×500 mL Erlenmeyer flask containing 150 mL of the above culture medium and incubated for 10 days at the same conditions.

2.6. Isolation

Seventy liters of whole broth was filtered through cheese cloth to separate the broth supernatant and mycelia. The former was extracted with ethyl acetate, while the latter was extracted with acetone. The acetone extraction was evaporated under reduced pressure to afford an aqueous solution and then extracted with ethyl acetate. The two ethyl acetate extractions were combined and concentrated in vacuo to give a crude extract (50.0 g). It was subjected to silica gel column chromatography eluted with petroleum ether/acetone/MeOH gradiently and then chromatographied using petroleum ether/ethyl acetate (20:1–1:1) followed by Sephadex LH-20 eluted with chloroform/methanol 1:1 for three times. Further purification was carried about using HPLC on a ODS semi-preparative column (gradient eluted with 80–90% methanol/water containing TFA 3‰) to obtain a complex fraction. And then the fraction was purified by PHPLC twice eluted 90% methanol/water with containing TFA 3‰ to yield 2.5 mg trisorbicillinone B (**2**), 7.5 mg trisorbicillinone C (**3**), 1.3 mg trisorbicillinone D (**4**).

*2.6.1. Trisorbicillinone B (**2**).* Yellow powder; HRESIMS $[M+Na]^+$ m/z: 783.2969, calcd for $C_{42}H_{48}O_{13}Na$, 783.2993; UV (MeOH) λ_{max} nm 228, 365; $[\alpha]_D^{25}$ −46.9 (c 0.23, MeOH); CD (MeOH) λ_{max} nm ($\Delta\epsilon$) 345 (+177.2), 301 (−23.8), 286 (−20.0), 243 (+71.7), 213 (−24.8); IR (KBr) cm^{-1}: 3439, 2927, 1728, 1633, 1604, 1563, 1514, 1445, 1414, 1380, 1348, 1204; t_R=5.91 min (90% MeOH, 0.3% TFA) ^{1}H, ^{13}C NMR, data see Table 1.

*2.6.2. Trisorbicillinone C (**3**).* Yellow powder; HRESIMS $[M+Na]^+$ m/z: 783.2975, calcd for $C_{42}H_{48}O_{13}Na$, 783.2993; UV (MeOH) λ_{max} nm 228, 351; $[\alpha]_D^{25}$ −46.9 (c 0.20, MeOH); CD (MeOH) λ_{max} nm ($\Delta\epsilon$) 354 (+79.0), 312 (−27.3), 286 (+15.6), 267 (+6.0), 242 (+66.7), 211 (−27.5); IR (KBr) cm^{-1}: 3433, 1730, 1632, 1603, 1564, 1446, 1410, 1380, 1348, 1205, 996, 942; t_R=6.38 min (90% MeOH, 0.3% TFA). ^{1}H, ^{13}C NMR, data see Table 1.

*2.6.3. Trisorbicillinone D (**4**).* Yellow powder; HRESIMS $[M+H]^+$ m/z: 745.3222, calcd for $C_{42}H_{49}O_{13}$, 745.3224; UV (MeOH) λ_{max} nm 223, 346; $[\alpha]_D^{25}$ +5.2 (c 0.20, MeOH); CD (MeOH) λ_{max} nm ($\Delta\epsilon$) 343 (+25.4), 303 (−6.2), 241 (+4.1), 209 (+2.8); IR (KBr) cm^{-1}: 3447, 2980, 2937, 1730, 1617, 1557, 1449, 1381, 1298, 1127, 994, 940; t_R=5.93 min (90% MeOH, 0.3% TFA). ^{1}H, ^{13}C NMR, data see Table 2.

2.7. Biological assay

Cytotoxic activity was evaluated by the MTT method. The IC_{50} values were obtained using the Bliss method.

Acknowledgements

This work was funded by the Chinese Ocean Mineral Resource R & D Association (DY105-2-04). We thank Dr. Raymond Andersen and Dr. Wendy Strangman for their advice to the manuscript.

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