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Cytosporin-related compounds from the marine-derived fungus *Eutypella scoparia*

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ABSTRACT

Chemical investigation of the culture broth of the fungus *Eutypella scoparia* ICB-OBX, isolated from the marine pulmonate mollusc *Onchidium* sp., led to the finding of novel compounds **1** and **2**, structurally related to angiotensin II binding inhibitors cytosporins, along with unrelated known nitrogen metabolites (compounds **3**–**5**). The structure and the relative stereochemistry of the novel metabolites were assigned mainly by a detailed analysis of two-dimensional NMR techniques whereas the absolute stereochemistry was proposed by modified Mosher's method. Compound **2** contains an unusual cyclic carbonate functionality that is rare among natural products.

1. Introduction

Marine microorganisms such as bacteria and fungi inhabit every environment of the sea and are rich sources of pharmacologically active compounds.[1] The interest in marine microorganisms has grown in the last few years due to the possibility of getting abundant biomass by in vitro culturing and consequently large amounts of secondary metabolites. Among marine microorganisms, fungi have shown great potential due to the high structural diversity of the natural products produced.[2] The majority of the chemical data reported in the literature refer to fungi derived from sponges and algae whereas only a few papers deal with the secondary metabolites of mollusc-derived fungi.[2,3] In particular, only the gastropod mollusc, the sea hare *Aplysia kurodai*, has been reported as a source of fungal isolates producing compounds with antitumor and cell adhesion inhibition activities.[4]

In the course of our investigation on marine molluscs[5] we analyzed the pulmonate gastropod *Onchidium* sp., collected along the coast of South China Sea that was found to be characterized by a series of unprecedented polypropionates.[6] Subsequently we also examined the secondary metabolites produced in the culture broth of the fungus *Eutypella scoparia* ICB-OBX, which was isolated from the external part of the mollusc. This study led to two novel molecules **1** and **2**, along with known nitrogen compounds **3**–**5**, previously reported from fungi and other natural sources.[7,8] Compounds **1** and **2** are structurally related to cytosporin A, cytosporin B, and cytosporin C (**6**), isolated from an endophytic strain of the taxonomically related fungus *Cytospora* and showed to be angiotensin II binding inhibitors.[9]

In this paper, the chemical characterization of the new metabolites that we named cytosporin D (**1**) and cytosporin E (**2**) is described.

2. Results and discussion

The EtOAc extract of the culture broth was analyzed by TLC using different eluent systems. The secondary metabolite pattern was found to be dominated by the presence of a main compound along with a series of minor components. The extract was purified by subsequent silica gel column chromatographic steps to give the novel compounds **1** (5.1 mg) and **2** (9.0 mg), along with the main metabolite phenochalasin B (**3**, 28.3 mg), *cyclo*-(D)-Pro-(D)-Leu (**4**, 3.9 mg), and *cyclo*-(D)-Pro-(D)-Phe (**5**, 2.0 mg).

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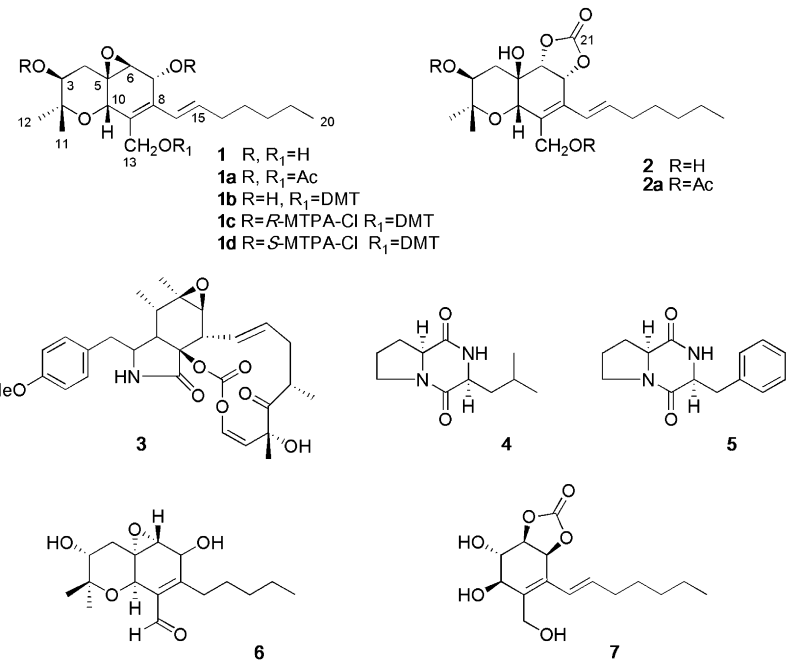
The already known compounds **3**–**5** were identified by comparing their spectral data (^{1}H and ^{13}C NMR, MS, and $[\alpha]_D$) with the literature.[7,8] The new molecules **1** and **2** were characterized as described below.

Cytosporin D (**1**) showed the molecular formula $C_{19}H_{30}O_5$ as deduced by the sodiated-molecular peak at *m*/*z* 361.1988 in the HRESIMS spectrum indicating five degrees of unsaturation. The ^{13}C NMR spectrum disclosed seven sp^3 carbons between δ 57.4 and 77.7 that were assigned to carbons linked to oxygen (one primary, four secondary, and two tertiary), eight sp^3 high-field resonating signals between δ 14.3 and 36.5, and four sp^2 carbons at δ 126.7 (d, C-14), 130.7 (s, C-8), 133.5 (s, C-9), and 135.7 (d, C-15) indicating two double bonds. The remaining unsaturations required by the formula were thus attributed to three rings. The ^{1}H NMR spectrum showed two olefinic signals at δ 6.48 (H-14) and 6.15 (H-15), which were attributed to the protons of a disubstituted double bond, four methine signals due to protons linked to oxygenated carbons at δ 4.65 (H-7), 4.53 (H-10), 3.68 (H-3), and 3.29 (H-6), and an AB system at δ 4.41 (H-13 part A), 4.08 (H-13 part B), assigned to an hydroxymethyl, two methyl singlets at δ 1.30 (H_3-11) and 1.25 (H_3-12), and a methyl triplet at δ 0.95 (H_3-20). A series of multiplet signals due to five additional methylenes completed the spectrum (Table 1). Analysis of ^{1}H–^{1}H COSY spectrum clearly defined some spin systems: the chain C-14/C-20 [δ 6.48 (d, 16, H-14); 6.15 (dt, 7, 16, H-15); 2.23 (td, 7, H_2-16); 1.52–1.43 (m, H_2-17); 1.42–1.33 (m, H_2-18); 1.42–1.33 (m, H_2-19); and 0.95 (t, 7, H_3-20)], an isolated oxygenated AB system [δ 4.41 (d, 12, H-13A) and δ 4.08 (d, 12, H-13B)], two vicinal oxygenated methines (δ 4.65, br s, H-7 and δ 3.29, br s, H-6), and an ABX system constituted by a methylene linked to carbinolic proton [δ 2.32 (t, 12, H-4ax); δ 1.70 (dd, 5, 12, H-4eq); and δ 3.68 (dd, 5, 12, H-3)]. A series of HMBC correlations aided us to link these partial structures. In particular, C-2 had diagnostic correlations with the methyls H_3-11 and H_3-12 and with the protons H-3 and H-10. The carbon C-5 displayed cross-peaks with the protons H-6, H-7, and the methylene H_2-4 whereas C-9 showed correlations with methines H-10, H-7, and H-14, as well as with the isolated hydroxymethyl H_2-13 (Table 1). These data were consistent with a tricyclic structure containing a hexahydrobenzopyrane moiety fused with an oxirane ring as reported in formula **1**. To confirm the suggested gross structure, a sample of compound **1** was acetylated by Ac_2O/DMPA in CH_2Cl_2 leading to the triacetyl derivative **1a**, which was analyzed by ^{1}H NMR. Diagnostic acylation shifts were observed for H-3, H-7, and H_2-13 (see Section 3) in agreement with the proposed structure. Compound **1** exhibits a carbon skeleton characterized by a polyketide moiety and a terpenoid part, reported only previously for cytosporins A–C [e.g., cytosporin C (**6**)], which were isolated some years ago from an endophytic *Cytospora* sp.[9] The full structure characterization of cytosporins A–C has never been published whereas their pharmacological properties in the inhibition of angiotensin II binding have been described.[9,10] The structure proposed for compound **6**[9] displayed strong analogies with compound **1** differing only in the length of the reduced chain at C-8 and in the presence of the aldehyde function at C-9 replacing the hydroxymethyl group. However, the unassigned ^{13}C NMR data reported for **6** match almost completely with those of compound **1**. Our tentative assignment (see Section 3) displayed the main difference for the C-7 carbon value. This anomaly could be justified either by a different orientation of the hydroxy group at C-7 that was not determined for cytosporin C (**6**) or by the influence of the double bond in the side chain of cytosporin D (**1**).



The relative stereochemistry of cytosporin D (**1**) could only partially be defined by NMR analysis. The hydroxyl at C-3 was equatorial as inferred by the multiplicity of the carbinolic proton H-3 (dd, *J*=12.0 and 4.8 Hz) consistent with its axial orientation. A diagnostic NOE effect was observed between H-10 (δ 4.53) and the axial methyl H_3-11 (δ 1.30; 16.4, C-11) supporting the axial orientation of H-10. The other steric effects observed in a series of NOE difference experiments were not useful to establish the orientation of both the epoxide ring at C-5/C-6 and the hydroxyl group at C-7 of cytosporin D (**1**). However, a *cis* relationship between the epoxide ring and H-10 was strongly supported by the comparison of ^{13}C NMR values of compounds **1** and **6** whereas the orientation of 7-OH was suggested by analysis of the spectral data of the co-occurring cytosporin E (**2**).

The molecular formula of compound **2** was $C_{20}H_{30}O_7$ as deduced from the sodiated-molecular peak at *m*/*z* 405.1883 in the HRESIMS spectrum, implying six degrees of unsaturation and an additional

Table 1
NMR data of cytosporin D (**1**) and cytosporin E (**2**) in CD_3OD

C	**1**					**2**				
	δ ^{13}C[a]	m[b]	δ ^{1}H[c]	m (*J*, Hz)	HMBC[d]	δ ^{13}C[a]	m[b]	δ ^{1}H[c]	m (*J*, Hz)	HMBC[d]
2	77.7	s	—		H_3-11, H_3-12, H-10, H-3, H-4eq	77.6	s	—		H_3-11, H_3-12, H-10, H-3, H-4eq
3	74.0	d	3.68	dd (5,12)	H_3-11, H_3-12, H-4eq, H-4ax	71.4	d	4.05	dd (5,12)	H_3-11, H_3-12, H-4eq, H-4ax
4	36.5	t	2.32ax	t (12)		42.3	t	2.30eq	dd (5,12)	
			1.70eq	dd (5,12)				1.90ax	t (12)	
5	57.4	s	—		H-4eq, H-4ax, H-10, H-6, H-7	67.6	s	—		H-4eq, H-4ax, H-10, H-6, H-7
6	62.1	d	3.29	br s	H-4eq, H-4ax, H-10, H-7	81.0	d	4.78	dd (8,2)	H-4eq, H-4ax, H-10, H-7
7	65.2	d	4.65	br s	H-6, H-14	75.3	d	5.58	d (8)	H-6, H-14
8	130.7	s	—		H-14, H-10, H-7, H_2-13	130.1	s	—		H-14, H-15, H-10, H-7, H_2-13
9	133.5	s	—		H-15, H-10, H-7, H_2-13, H-6	135.5	s	—		H-15, H-14, H-10, H-7, H_2-13, H-6
10	67.6	d	4.53	br s	H_2-13, H-4eq	70.3	d	4.25	d (2)	H_2-13, H-4eq
11	16.4	q	1.30	s		16.7	q	1.30	s	
12	27.9	q	1.25	s		28.2	q	1.25	s	
13	59.7	t	4.41A	d (12)		60.1	t	4.45A	d (12)	
			4.08B	d (12)				4.18B	d (12)	
14	126.7	d	6.48	d (16)	H_2-16, H-7	125.5	d	6.53	d (16)	H_2-16, H-7
15	135.7	d	6.15	dt (7,16)	H_2-16, H_2-17	136.8	d	6.08	dt (7,16)	H_2-16, H_2-17
16	34.5	t	2.23	td (7)	H-14, H-15	34.5	t	2.25	td (7)	H-14, H-15
17	30.1	t	1.52–1.43	m	H-15	30.0	t	1.52–1.43	m	H-15
18	32.5	t	1.42–1.33	m		32.5	t	1.42–1.33	m	
19	23.5	t	1.42–1.33	m	H_2-17, H_3-20	23.6	t	1.42–1.33	m	H_2-17, H_3-20
20	14.3	q	0.95	t (7)		14.3	q	0.95	t (7)	
21	—		—		—	156.0	s	—		H-7, H-6

[a] Bruker 300 MHz.
[b] Multiplicity deduced by DEPT.
[c] Bruker 600 MHz.
[d] Significant HMBC correlations (*J*=8 and 6 Hz).

CO_2 unit with respect to **1**. The ^{1}H NMR spectrum of **2** showed strong analogies with that of cytosporin D (**1**) (Table 1) indicating the presence of the same hexahydrobenzopyrane skeleton with an alkyl chain at C-8. In particular, the major differences were observed for H-6 and H-7 protons resonating in compound **2** at δ 4.78 (dd, *J*=8 and 2 Hz) and 5.58 (d, *J*=8 Hz), respectively, whereas in compound **1** these signals resonated at δ 3.29 (br s) and 4.65 (br s) (Table 1). This was consistent with a different substitution pattern in this part of the molecule. The presence of a carbonate moiety in compound **2** was suggested by a strong IR absorption at 1650 cm^{-1} as well as by a diagnostic ^{13}C NMR signal at δ 156.0. The ^{13}C NMR spectrum of **2** showed in addition the expected four sp^2 carbons, seven sp^3 carbons linked to oxygen, and eight sp^3 high-field resonating signals, similar to those of **1**, accounting for 20 carbons as required by mass formula (Table 1).

The ^{1}H–^{1}H COSY spectrum of **2** revealed the presence of the same spin systems as **1** that were connected by HMBC analysis to give the same carbon framework. In particular, diagnostic HMBC correlations between C-21 and both protons H-6 and H-7 led us to link the carbonyl to both oxygen atoms at C-6 and C-7 obtaining a cyclic carbonate moiety, as reported in formula **2**. Such functionality, which is quite rare in nature, has been reported for metabolites of fungi of genera *Phoma*[11] and *Eupenicillium*[12] [e.g., phomoxin C (**7**)[12]].

A sample of compound **2** was submitted to acetylation giving the acetyl derivative **2a**, which was characterized by NMR spectroscopy and mass spectrometry. Diagnostic acylation shifts were observed for H-3 and H_2-13 (see Section 3) in agreement with the proposed structure.

The relative stereochemistry of cytosporin E (**2**) was determined by analysis of both ^{1}H–^{1}H coupling constants and NOE difference experiments. Analogously with **1**, the multiplicity of the carbinolic proton H-3 (δ 4.05, dd, *J*=11.8 and 4.8 Hz) indicated the equatorial orientation of the hydroxyl group at C-3 whereas significant NOE interactions between H-10 (δ 4.25) and the axial methyl H_3-11 (δ 1.30) supported the axial orientation of H-10. The *cis*-junction of the hexahydrobenzopyrane moiety of cytosporin E (**2**) was determined by analyzing a NOESY experiment carried out on the acetyl derivative **2a**, which still retained a free tertiary hydroxyl group (5-OH). A clear cross peak between H-10 (δ 4.25) and 5-OH (δ 5.60) was observed in the spectrum recorded in DMSO-d_6 (see Section 3) thus inferring the same orientation of the angular substituents at C-5 and C-10. Finally, in a NOE difference experiment, the irradiation of H-6 (δ 4.78) caused the enhancement of both H-7 (δ 5.58) and H-4eq (δ 2.30) signals indicating that the carbonate cycle was *trans*-oriented with respect to the angular 5-OH. Accordingly, a strong NOE effect was observed between H-15 and H-7.

Once the relative stereochemistry of cytosporin E (**2**) was established, a possible correlation between the two co-occurring metabolites **1** and **2** was evaluated. From a biogenetic point of view, compound **2** could be considered as derived from compound **1**. In fact, it should be formed by the attack of a carbonate ion at C-6 of the oxirane ring of compound **1** from the less hindered face. The esterification of the carbonate unit by the vicinal hydroxyl at C-7 should give the closure of the ring leading to cytosporin E (**2**). This implies that the relative stereochemistry at C-5, C-6, and C-7 of cytosporin D (**1**) should be that reported with the 5,10 *cis*-junction and the hydroxyl group at C-7 *trans*-oriented with respect to the oxirane ring.

Finally, the absolute stereochemistry of cytosporin D (**1**) was determined by applying the modified Mosher's method.[13] After protection of the primary hydroxyl group at C-13 with DMT (see Section 3), **1b** was reacted with (−)-*R*- and (+)-*S*-MTPA chlorides to obtain *S*-ester (**1c**) and *R*-ester (**1d**), respectively. The $\Delta\delta$ values ($\Delta_S-\Delta_R$) observed for the signals of protons nearby the hydroxyl groups at C-3 and C-7 (see Table 2) indicated the *S* and the *R* configuration, respectively, as reported in the structure. In particular, the *S* configuration at C-3 led to assignment of the absolute stereochemistry at carbons C-5, C-6, and C-10, whereas the observed $\Delta\delta$ values after Mosher esterification at C-7 assigning the *R* absolute stereochemistry at the chiral center, confirmed the suggested *trans* relationship between the oxirane ring and 7-OH. Even though the optical rotation of cytosporin D {$[\alpha]_D$ −3.0 (*c* 0.5, CH_3OH)} is opposite to that of cytosporin E {$[\alpha]_D$ +59.9 (*c* 0.29, CH_3OH)}, the same absolute stereochemistry could be suggested for both compounds on the basis of biogenetical consideration. The structure **6**, closely

Table 2
NMR selective assignments of **1c** (*S*-ester) and **1d** (*R*-ester) in CD_3OD

	1c (*S*-ester)		**1d** (*R*-ester)		$\Delta\delta$
	δ H	m (*J*, Hz)	δ H	m (*J*, Hz)	
H-3	4.48	dd (4.8,12.0)	4.95	dd (4.8, 12.0)	−0.47
H_2-4	2.26	t (12.0)	2.52	t (12.0)	−0.26
	1.91	dd (4.8,12.0)	2.04	dd (4.8, 12.0)	−0.13
H-6	3.39	s	3.53	s	−0.14
H-7	6.27	s	6.18	s	+0.09
H-10	4.48	s	4.61	s	−0.13
H_3-11	0.99	s	1.01	s	−0.022
H_3-12	1.20	s	1.19	s	+0.01
H-14	6.02	d (16.0)	5.89	d (16.0)	+0.13
H-15	5.86	dt (7.0,16.0)	5.66	dt (7.0, 16.0)	+0.20

related to cytosporin D (**1**), is that suggested for cytosporin C, but optical rotation and stereochemical data are not reported in the paper.

Cytosporin D (**1**) and E (**2**) were tested in antimicrobial assays against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. No inhibitory activity was observed for the two molecules.

In conclusion, two new molecules have been isolated from the marine-derived fungus *E. scoparia*. This species, which has been reported from many and diverse environments ranging from soil in Antarctica[14] to tropical forests of Australia[15] and Thailand,[16] was isolated here for the first time from a marine source. However, this is not surprising because other closely related fungi (e.g., *Cytospora*) mostly known in association with woody plants have been also reported from marine invertebrates, in accordance with the suggestion that fungi have the capability of adapting to harsh environments.

3. Experimental

3.1. General experimental procedure

Silica gel chromatography was performed using pre-coated Merck F_{254} plates and Merck Kieselgel 60 powder. Optical rotations were measured on a Jasco DIP 370 digital polarimeter. IR spectra were recorded on a Biorad FTS 155 FT-IR spectrophotometer. NMR experiments were recorded at ICB-NMR Service Centre. 1D and 2D NMR spectra were acquired in CD_3OD (shifts are referenced to the solvent signal) on a Bruker DRX-600 operating at 600 MHz, using an inverse TCI CryoProbe fitted with a gradient along the *Z*-axis. ^{13}C NMR were recorded on a Bruker DPX-300 operating at 300 MHz using a dual probe. High resolution ESIMS were performed on a Micromass Q-TOF Micro™ coupled with a HPLC Waters Alliance 2695. The instrument was calibrated using a PEG mixture from 200 to 1000 MW (resolution specification 5000 FWHM, deviation <5 ppm rms in the presence of a known lock mass).

3.2. Fungal material and fermentation

Isolate ICB-OBX was recovered from the surface of pulmonate *Onchidium* sp. collected in the intertidal zone along the coast of Lingshui County (Hainan province, China), during December 2005. On the basis of ITS sequence data it was identified as *E. scoparia* by the Centraalbureau voor Schimmelcultures (CBS, Utrecht, the Netherlands). The Genbank number for *E. scoparia* is AF 373064.

The fungus *E. scoparia* ICB-OBX was grown on malt extract agar (MEA; malt extract, mycological peptone, agar) at 28 °C. After a week of cultivation the mycelial plugs were cut by a cork borer and inoculated into Erlenmeyer flasks containing 500 mL of malt extract broth (MEB; malt extract 17.0 g, mycological peptone 3.0 g, per liter), prepared with artificial seawater. This fermentation was performed at 28 °C, without shaking for 30 days, in a total volume of 4 L.

3.3. Extraction and isolation

After incubation the mycelium was removed from the culture broth by filtration. Then, the broth (4 L) was extracted with ethyl acetate four times (400 mL×4). The resulting organic extract was evaporated under vacuum to give a brown oil (400 mg) and partitioned by column chromatography on silica gel using stepwise gradient elution from $CHCl_3$ and increasing amounts of CH_3OH to give nine fractions, two of which containing compounds **1**–**5**. The less polar fraction was further purified on silica gel column (*n*-hexane/EtOAc gradient) to obtain *cyclo*-(D)-Pro-(D)-Leu (**4**, 3.9 mg) and *cyclo*-(D)-Pro-(D)-Phe (**5**, 2.0 mg). The more polar fraction was purified on silica gel column (*n*-hexane/EtOAc gradient) to give phenochalasin B (**3**, 28.3 mg), cytosporin D (**1**, 5.1 mg), and cytosporin E (**2**, 9.0 mg), in order of polarity.

3.3.1. Cytosporin D (*1*)

Pale yellow oil; $[\alpha]_D$ −3.0 (*c* 0.5, CH_3OH); IR (liquid film) 3426 (br), 2928, 1792, 1507, 1248 cm^{-1}; HRESIMS: found 361.1988 (361.1991 calculated for $C_{19}H_{30}O_5Na$); ^1H and ^{13}C NMR in CD_3OD see Table 1.

3.3.2. Cytosporin E (*2*)

Pale yellow oil; $[\alpha]_D$ +59.9 (*c* 0.29, CH_3OH); IR (liquid film) 3339 (br), 2936, 1792, 1651, 1462, 1375, 1032 cm^{-1}; HRESIMS: found 405.1883 (405.1889 calculated for $C_{20}H_{30}O_7Na$); ^1H and ^{13}C NMR data in CD_3OD see Table 1.

^1H and ^{13}C NMR data in DMSO-d_6 (δ in ppm, *J* in Hz): 3.78–3.70 (H-3, m), 2.10 (H-4eq, dd, 13, 6), 1.74 (H-4ax, t, 13), 4.70 (H-6, d, 8), 5.52 (H-7, d, 8), 4.08 (H-10, s), 1.13 (H_3-11, s), 1.06 (H_3-12, s), 4.28 (H-13A, dd, 13, 4), 3.92 (H-13B, dd, 13, 6), 6.46 (H-14, d, 16), 5.92–5.84 (H-15, m), 2.18–2.11 (H_2-16, m), 1.44–1.35 (H_2-17, m), 1.34–1.25 (H_2-18, m), 1.34–1.25 (H_2-19, m), 0.88 (H_3-20, t, 7), 4.84 (3-OH, d, 5), 4.70 (13-OH, br t, 5), 5.28 (5-OH, br s); 76.0 (C-2, C), 69.2 (C-3, CH), 41.3 (C-4, CH_2), 67.0 (C-5, C), 79.3 (C-6, CH), 73.5 (C-7, CH), 136.5 (C-8, C), 127.5 (C-9, C), 68.1 (C-10, CH), 16.3 (C-11, CH_3), 28.0 (C-12, CH_3), 58.5 (C-13, CH_2), 125.0 (C-14, CH), 133.0 (C-15, CH), 34.0 (C-16, CH_2), 29.5 (C-17, CH_2), 29.0 (C-18, CH_2), 22.0 (C-19, CH_2), 14.8 (C-20, CH_3), 155.0 (C-21, C).

3.3.3. Cytosporin C (*6*)

Tentative assignment (δ in ppm, $CDCl_3$) of the reported carbon values:[9] 77.2 (C-2), 74.3 (C-3), 35.5 (C-4), 57.8 (C-5), 62.4 (C-6), 55.8 (C-7), 157.7 (C-8), 129.9 (C-9), 68.9 (C-10), 16.0 (C-11), 27.8 (C-12), 189.7 (C-13), 31.6 (C-14), 29.9 (C-15), 29.1 (C-16), 22.3 (C-17), 13.9 (C-18).

3.3.4. Compound *1a*

Cytosporin D (**1**) (1 mg) was dissolved in anhydrous CH_2Cl_2 (0.5 mL) with a catalytic amount of DMAP and 10 μL of acetic anhydride was added to this solution. The reaction was stirred overnight at room temperature. CH_2Cl_2 was removed in vacuo and the residue was worked up by preparative TLC (SiO_2, eluent $CHCl_3/CH_3OH$, 98:2) and the resulting UV band was scraped to afford pure **1a** (0.7 mg). R_f 0.9 ($CHCl_3/CH_3OH$, 98:2); $[\alpha]_D$ 0.0 (*c* 0.13, CH_3OH); IR (liquid film) 2936, 2855, 1738, 1375, 1223, 1037 cm^{-1}; selected ^1H NMR signals (δ in ppm): 6.00 (H-7), 4.85 (H-3), 4.85–4.70 (H_2-13), 2.12 (3H, 13-$COCH_3$), 2.06 (6H, 3-$COCH_3$ and 7-$COCH_3$). ESIMS: *m*/*z* 465 $[M+H]^+$, 405 $[M-60+H]^+$, 345 $[M-2\times60+H]^+$, 285 $[M-3\times60+H]^+$; HRESIMS: found 464.2416 (464.2410 calculated for $C_{25}H_{34}O_8$).

*3.3.5. Compound **1b***

Compound **1** (5.0 mg, 0.015 mM) was stirred in anhydrous CH_2Cl_2 (1 mL), catalytic amount of DMAP, and 5.0 mg of 4,4'-dimethoxytrytil chloride (0.015 mM, DMT) for 2 h at room temperature. CH_2Cl_2 was removed in vacuo and the reaction mixture was purified by chromatography in a Pasteur pipette (SiO_2, $CHCl_3$). R_f 0.5 ($CHCl_3/CH_3OH$, 95:5); $[\alpha]_D$ +12.0 (*c* 0.05, CH_3OH); IR (liquid film) 2922, 2847, 1651, 1516, 1462, 1254, 1032 cm^{-1}; EIMS: *m/z* 640 $[M]^+$; ^1H and ^{13}C NMR data in CD_3OD (δ in ppm, *J* in Hz): 3.66 (H-3, dd, 13, 4), 2.24 (H-4ax, t, 13), 1.64 (H-4eq, dd, 13, 4), 3.26 (H-6, br s), 4.63 (H-7, br s), 4.51 (H-10, br s), 1.29 (H_3-11, s), 1.30 (H_3-12, s), 3.66 (H-13A, d, 11), 3.80 (H-13B, d, 11), 6.06–5.98 (H-14, m), 6.06–5.98 (H-15, m), 2.09–2.00 (H_2-16, m), 1.34–1.38 (H_2-17, m), 1.43–1.34 (H_2-18, m), 1.36–1.25 (H_2-19, m), 0.93 (H_3-20, t, 7), 6.84–7.52 (Ar–H, 13H), 3.80 (OCH_3, s, 6H); 77.9 (C-2, C), 73.9 (C-3, CH), 36.5 (C-4, CH_2), 57.5 (C-5, C), 61.6 (C-6, CH), 65.1 (C-7, CH), 134.4 (C-8, C), 130.0 (C-9, C), 68.6 (C-10, CH), 16.4 (C-11, CH_3), 27.9 (C-12, CH_3), 61.2 (C-13, CH_2), 127.8 (C-14, CH), 135.3 (C-15, CH), 34.3 (C-16, CH_2), 30.7 (C-17, CH_2), 32.4 (C-18, CH_2), 23.0 (C-19, CH_2), 14.4 (C-20, CH_3), 55.7 (OCH_3), 114.0–158.0 (Ar).

*3.3.6. Compound **1c** (S-MTPA-ester)*

Compound **1c** was prepared by treating 2 mg of **1b** with 0.005 mL of *R*-(−)-MTPA chloride in dry CH_2Cl_2 (0.5 mL) with catalytic amount of DMAP under stirring for 16 h at room temperature. The ester was purified by chromatography in a Pasteur pipette (SiO_2, $CHCl_3$). R_f 0.9 ($CHCl_3/CH_3OH$, 98:2); $[\alpha]_D$ +9.8 (*c* 0.06, CH_3OH); IR (liquid film) 2928, 2855, 1752, 1507, 1462, 1246, 1173, 1024, 709 cm^{-1}; selected ^1H NMR values are in Table 2.

*3.3.7. Compound **1d** (R-MTPA-ester)*

Compound **1d** was prepared by treating 2 mg of **1b** with 0.005 mL of *S*-(−)-MTPA chloride in dry CH_2Cl_2 (0.5 mL) with catalytic amount of DMAP under stirring for 16 h at room temperature. The ester was purified by chromatography in a Pasteur pipette (SiO_2, $CHCl_3$). R_f 0.9 ($CHCl_3/CH_3OH$, 98:2); $[\alpha]_D$ −30.5 (*c* 0.01, CH_3OH); IR (liquid film) 2955, 2847, 1749, 1508, 1462, 1252, 1168, 1026, 702 cm^{-1}; selected ^1H NMR values are in Table 2.

*3.3.8. Compound **2a***

Cytosporin E (**2**) (1 mg) was dissolved in anhydrous CH_2Cl_2 (0.5 mL) with catalytic amount of DMAP and 10 μL of acetic anhydride was added to this solution. The reaction was stirred overnight at room temperature. CH_2Cl_2 was removed in vacuo and the residue was worked up by preparative TLC (SiO_2, eluent $CHCl_3/CH_3OH$, 95:5) and the resulting UV band was scraped to afford pure **2a** (0.9 mg). R_f 0.5 ($CHCl_3/CH_3OH$, 95:5); $[\alpha]_D$ +5.0 (*c* 0.03, CH_3OH); IR (liquid film) 2955, 2847, 1749, 1645, 1462, 1352, 1068, 1030, 702 cm^{-1}; selected ^1H NMR signals in CD_3OD (δ in ppm): 5.25 (H-3), 4.95–4.82 (H_2-13), 2.08, 2.10 (6H, 3-$COCH_3$ and 13-$COCH_3$). ESIMS: *m/z* 489 $[M+Na]^+$, 407 $[M-60+H]^+$; HRESIMS: found 489.2110 (489.2101 calculated for $C_{24}H_{34}O_9Na$).

^1H and ^{13}C NMR assignments in DMSO-d_6 (δ in ppm, *J* in Hz): 5.03 (H-3, dd, 12, 6), 2.25 (H-4eq, dd, 12, 6), 1.84 (H-4ax, t, 12), 4.78 (H-6, d, 8), 5.60 (H-7, d, 8), 4.00 (H-10, br s), 1.23 (H_3-11, s), 1.02 (H_3-12, s), 4.82 (H-13A, d, 14), 4.70 (H-13B, d, 14), 6.45 (H-14, d, 16), 6.05–5.97 (H-15, m), 2.21–2.13 (H_2-16, m), 1.44–1.36 (H_2-17, m), 1.24–1.18 (H_2-18, m), 1.32–1.26 (H_2-19, m), 0.88 (H_3-20, t, 7), 5.60 (5-OH, s), 2.03 (3-$COCH_3$, s), 2.01 (13-$COCH_3$, s); 75.0 (C-2, C), 72.3 (C-3, CH), 37.5 (C-4, CH_2), 67.0 (C-5, C), 79.0 (C-6, CH), 74.3 (C-7, CH), 130.0 (C-8, C), 132.0 (C-9, C), 69.2 (C-10, CH), 16.5 (C-11, CH_3), 27.7 (C-12, CH_3), 61.0 (C-13, CH_2), 124.0 (C-14, CH), 135.5 (C-15, CH), 32.9 (C-16, CH_2), 28.2 (C-17, CH_2), 30.2 (C-18, CH_2), 21.9 (C-19, CH_2), 14.1 (C-20, CH_3), 154.2 (C-21, C), 170.0 (3-$COCH_3$- and 13-$COCH_3$, C), 21.5 (3-$COCH_3$ and 13-$COCH_3$, C).

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