

**Aspernolides A and B, butenolides from a marine-derived
fungus *Aspergillus terreus***

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Abstract

Two new aromatic butenolides, aspernolides A (**1**) and B (**2**) along with the known metabolites, butyrolactone I (**3**), terrein and physcion were isolated from the fermentation broth of a soft coral derived fungus *Aspergillus terreus*. The structures of these new metabolites were assigned on the basis of detailed spectroscopic analysis. The absolute stereochemistry of **1** and **2** was established by their preparation from the known butyrolactone I (**3**). Biogenetically **1** and **2** must be derived from **3**, a well known specific inhibitor of cyclin dependent kinase (cdk) from *A. terreus*. When tested, aspernolide A (**1**) exhibited mild cytotoxicity against cancer cell lines.

Keywords: *Aspergillus terreus*, Marine natural products, Butenolides, Butyrolactone I, Aspernolides.

1. Introduction

Marine-derived microbes, fungi in particular have long been recognized as potential source of structurally novel and biologically potent metabolites (Faulkner, 2000; Bugni et al., 2003; Saleem et al., 2007). Fungi belonging to *Aspergillus* genera are one of the major contributors to the secondary metabolites of fungal origin. *Aspergillus terreus* is a ubiquitous fungus in our environment and although *Aspergillus* sp. are normally considered terrestrial species, the genus is tolerant to high salt concentrations. In recent years, we sought to draw marine microbial diversity into the arena of drug discovery. The present investigation is an outcome of such a study on the fungus *Aspergillus terreus* associated with a soft coral *Sinularia kavarattiensis*.

Terrestrial isolates of *Aspergillus terreus* are well known for the production of butenolides. Cytotoxic butyrolactones I-IV, biogenetically derived from tyrosine (Rao et al., 2000; Kiriyama et al., 1977; Nitta et al., 1983) and non prenylated-decarboxylated butenolides, xenofuranones A (**4**) and B (**5**) (Morishima et al., 1994), biogenetically produced from phenyl alanine are known to be derived from *A. terreus*. Xenofuranones A (**4**) and B (**5**) are also known as metabolites of bacterium *Xenorhabdus szentitmaii* (Brachmann et al., 2006). There is a recent report on the identification of **3** and its sulfated derivatives (**6** and **7**) (Niu et al., 2008) from a strain *A. terreus* (HKI0499).

This report focuses on the isolation and structure elucidation of new butenolides, aspernolides A (**1**) and B (**2**) and other known metabolites from culture medium of fungus *Aspergillus terreus*. Although, **1** has been described in the literature (Kiriyama et al., 1977) as a reaction product in the structure elucidation of **3**, neither its NMR data is reported nor it is known to be a natural product. Moreover this is the first report describing the isolation of **3** and other related butenolides from marine-derived fungus.

2. Results and Discussion

Fungus, *Aspergillus terreus* was isolated as an epiphyte from a soft coral *Sinularia kavarattiensis* collected from the coast of Mandapam, Tamil Nadu, India. This fungus was grown on potato dextrose broth prepared in seawater. New secondary metabolites, aspernolides A (**1**) and B (**2**) were identified from the chloroform and ethyl acetate extracts of culture broth respectively. These butenolides along with their plausible biogenetic precursor **3** and the known metabolites physcion and terrein were purified using repeated silica gel and Sephadex LH-20 gel filtration chromatography.

Aspernolide A (**1**) was obtained as white sticky solid. The molecular formula $C_{24}H_{24}O_7$ of **1** was determined by HRESITOFMS which showed pseudomolecular ion peaks $[M+Na]^+$ at 447.1433

(calcd. 447.1420 for $C_{24}H_{24}O_7Na$) and $[2M+Na]^+$ at 871.2959 (calcd. 871.2942 for $C_{48}H_{48}O_{14}Na$). IR spectrum showed presence of ester/lactone carbonyl 1731cm^{-1} and 1738cm^{-1} , phenolic OHs were evident at 3330cm^{-1} and presence of absorption at 1660cm^{-1} was suggestive of aromaticity in the molecule.

The ^1H NMR signals of the A_2B_2 system at δ_{H} 7.56, d, 2H, $J = 8.7\text{Hz}$ and 6.86, d, 2H, $J = 8.7\text{Hz}$ revealed the presence of para substituted benzene moiety. Two aromatic signals 6.53, s, 1H and 6.47, s, 2H (two doublets merged into a singlet) were indicative of the presence of additional unsymmetrical trisubstituted benzene ring in the molecule. Its ^{13}C NMR showed the presence of 10 aromatic signals for two aromatic rings, two ester carbonyls δ_{C} 169.3 s, and 169.6 s, olefinic carbon signals δ_{C} 137.2 s and 128.8 s, three $\text{sp}^3\text{CH}_2\text{s}$ δ_{C} 22.1 t, 32.5 t and 38.6 t, a carbomethoxy δ_{C} 53.4 q and two oxygenated quaternaries δ_{C} 86.1 s and 74.2 s. The molecular formula $C_{24}H_{24}O_7$ requires 13 degrees of unsaturation. Presence of two aromatic rings accounts for eight while two carbonyls and two olefinic carbons accounts for another three, which makes total of eleven degrees of unsaturation. Therefore **1** must possess two aliphatic rings in addition to two aromatic rings.

A detail comparison of NMR data of **1** with that of butyrolactone I (**3**) (Rao et al., 2000), confirmed a common hydroxyphenyl pyruvate dimer type of network in the molecule. The significant difference observed in the NMR spectra of **1** as compared to that of **3** was the absence of olefinic proton signals δ_{H} 5.0, t, 1H, two olefinic carbon signals δ_{C} 121.0 d and 130.7 s and the presence of three methylenes and two oxygenated sp^3 quaternaries compared to two methylenes and one oxygenated sp^3 quaternary in **3** in both ^{13}C and DEPT NMR spectra. This data was indicative of the presence of a dihydropyran ring fused to a trisubstituted benzene ring in place of the open prenyl chain present in **3**. HMBC was well in agreement of the structure **1** (**Fig 1**). Key HMBC correlations from H-2'' to C-3'' and C-7'', from H-7'' to C-2'', C-3'' and C-4'' and from H-8'' to C-9'' and C-10''(11'') established dihydropyran ring fused through C3''-C4'' bond of benzene ring. HMBC correlations from H-2'' and H-6'' to C-6 and from H-6 to C-1'', C-6'', C-2'', C-4, C-5 and C-3 was evident of the benzodihydropyranmethylene moiety linked to lactone ring at C-4. Furthermore HMBC correlation from H-2'(6') to C-3 established p-substituted phenolic moiety at C-3. Out of two carbonyls, C-5 and C-1, δ_{C} 169.6 was assigned to C-5 on the basis of its HMBC correlation to the protons H-6 and H₃-5OMe.

Aspernolide B (**2**) (Rf, 0.51), more polar than **1** (Rf, 0.81) was obtained as light brown syrup $[\alpha]_{\text{D}} + 48.27$ (c 0.29, MeOH). The IR spectra showed presence of – OHs at 3330cm^{-1} , ester/lactone carbonyls overlapping peaks at 1732 and 1747cm^{-1} and 1610 and 1519cm^{-1} for aromatic rings. Although chemical shift variations were present in the ^1H and ^{13}C NMR of **2**, they were similar to

that of **1**. Significant variations in the chemical shifts were observed for ring carrying iso-pentyl chain wherein C-1'', C-3'', C-7'', C-8'', C-10'' and C-11'' were considerably deshielded to resonate at δ_c 128.1($\Delta\delta$ 4.6ppm), 124.0($\Delta\delta$ 3.7ppm), 24.2($\Delta\delta$ 2.1ppm), 43.2($\Delta\delta$ 10.7ppm), 28.4($\Delta\delta$ 1.8ppm) and 28.5($\Delta\delta$ 1.9ppm) while C-5'' and C-9'' were considerably shielded to resonate at δ_c 114.6 ($\Delta\delta$ 2.0ppm) and 70.8($\Delta\delta$ 3.4ppm) as compared to **1**, suggesting a change on the aromatic ring carrying iso-pentyl chain. Compound **2** was well distinguished from ESI-MS spectrum which showed pseudomolecular ions $[M+H]^+$ at 443.1699 (calcd. 443.1706 for $C_{24}H_{27}O_8$) and $[M+Na]^+$ at 465.1516 (calcd. 465.1525 for $C_{24}H_{26}O_8Na$) suggesting molecular weight of 442 for the compound **2**, which was 18 units more than **1**. Based on these observations it was evident that **2** has open chain hydroxylated prenyl chain ortho to phenolic $-OH(C-4'')$

Based on the reported feeding experiments for establishing the biosynthesis of xenofuranones A (**4**) and B (**5**) together with compound **3** (Brachmann et al., 2006; Nitta et al., 1983) and isolation of **1** and **2** from *Aspergillus terreus*, it is apparent that the structures of **1** and **2** are extension of the biosynthesis of **3**, which is derived from p-hydroxy phenylpyruvate. The enzyme catalysed cyclization or addition of water across the double bond of the prenyl chain of **3** results in the formation of **1** and **2** respectively (**scheme-1**). The last step in the biogenetic scheme was mimicked using mild acid catalysis to confirm the structure of **1**.

On heating with 1% aqueous sulphuric acid, **3** was converted to **1** as well as small amount of **2** (**scheme-2**). Only 50% conversion was observed. When the same reaction was carried out using 2% conc. HCl in methanol, complete conversion of **3** was observed as indicated by TLC (90:10, $CHCl_3/MeOH$). On chromatographic separation, 75% of **1** was obtained along with minor amounts of **2** and a new product **8**. Compound **8** displayed an extra methoxy signal δ_H 3.15, s, δ_C 49.0 q in NMR spectra compared with **2**. In the rest of the NMR spectra it was seen that C-7'', C-8'', C-10'' and C-11'' were shielded and observed at δ_c 23.2($\Delta\delta$ 1.0), 40.0($\Delta\delta$ 3.2), 24.0 ($\Delta\delta$ 4.4), and 24.4($\Delta\delta$ 4.1) while only methoxylated quaternary carbon C-9'' was deshielded to 75.4($\Delta\delta$ 4.6) compared to **2**. Pseudomolecular ion peaks $[M+H]^+$ at 457.1851 and $[M+Na]^+$ at 479.1669 observed in ESI-MS indicated molecular weight of 456 for the compound. On the basis of these observations, the structure was assigned as shown in **8**. Compounds **2** and **8** are formed by Markovnikoff's addition of water and methanol respectively, across the double bond of the prenyl chain.

During the structure elucidation of **3**, compound **1** has been reported as the product of its reaction with ethanolic HCl (Kiryama et al., 1977). In the present investigation, the same reaction in methanolic HCl yielded besides **1**, two additional products **2** and **8**. Co-metabolite **3** used in the above reaction was determined to be 4R configured by comparison of specific rotation data $[\alpha]_D +$

84.32 with the previously reported result (Kiryama et al., 1977). The absolute configurations of **1** and **2**, therefore could be deduced to be also 4*R* based on the biosynthetic grounds and similarity of the specific rotations $[\alpha]_D +88.73$ for **1** and $+48.27$ for **2**. Further confirmation of the absolute stereochemistry of **1** and **2** results from the fact that, the natural **1** and **2** and those obtained as products of acid catalyzed reaction had identical spectral data and specific rotations $[\alpha]_D +88.73$ for **1** and $+48.27$ for **2**. Therefore, we conclude that the compounds **1** and **2** also have 4*R*-configuration.

Butyrolactone I (**3**), a metabolite of *Aspergillus terreus* var. *africans* IFO 8355 discovered in 1977, seems to be a common metabolite of this fungus (Kiryama et al., 1977; Rao et al., 2000; Niu et al., 2008; Schimmel et al., 1999). To our best knowledge, this is the first report of **3** and its derivatives from marine-derived fungus. Aspernolides B (**2**) and C (**3**) were unstable and were converted to **1** on long standing. Aspernolide C (**1**) when tested against five cell lines displayed weak cytotoxicity against H460, ACHN, Calu, Panc1 and HCT116 cell lines ($IC_{50} >88, >103, >147, >130, >121\mu\text{M}$ respectively)

3. Conclusion

Aspergillus terreus is known to be a producer of cholesterol lowering agent lovastatin (mevinoline) (Alberts et al., 1980) and many other important secondary metabolites such as terreineol (Macedo et al., 2004), terreulactone A (Kim et al., 2002), terrein, terreic acid and aspulvinones. The list of metabolites also includes butyrolactone I (**3**), specific inhibitor of cdk1/cyclin B and cdk2/cyclin A (Fischer et al., 2000) as potent as roscovitine, a drug currently undergoing phase IIb clinical evaluation (www.cyclacel.com). Compound **3** exhibits antiproliferative activity against colon and pancreatic carcinoma, human lung cancer (Nishio et al., 1996) and prostatic cancer (Suzuki et al., 1999). Computer aided molecular modeling using automated docking methods and molecular dynamics simulations studies (Brana et al., 2004) have proven the importance of alkenyl (prenyl) side chain in the molecule and explains why these butenolides lacking alkenyl side chain do not maintain antitumor activity. After considering the potential of **3** as a cdk inhibitor it can be suggested that these marine natural products **1** and **2** or synthetically modified natural products such as **8**, which retains alkenyl side chain could be tested in future to explore as potential cdk inhibitors.

4. Experimental

4.1. General experimental procedures

Sephadex LH-20 (Pharmacia) and silica gel (60-120 mesh)[Qualigens] were used for column chromatography. Culture media Czapek agar and potato dextrose broth were procured from Himedia Ltd., Mumbai. Solvents of laboratory reagent grade used for column chromatography were

purchased from local supplier and were distilled prior to use. Petroleum ether of boiling range 60-80°C was used for column chromatography. Precoated kieselgel 60 F₂₅₄ TLC plates were used for analytical TLC. Mixture of methanol and chloroform (10:90, v/v) was used as mobile phase for TLC analysis. Compounds were visualized as intense rose colour spots on spraying with methanolic sulphuric acid (95:5, v/v) followed by heating at 120°C. UV and IR spectra were recorded on Shimadzu UV-2401 PC and Shimadzu FTIR-8201 PC spectrometers. Optical rotations were measured on optical polarimeter ADP220 (Bellingham + Stanley Ltd). NMR (¹H, ¹³C, DEPT, HSQC and HMBC) data were obtained on Bruker Avance 300 and Bruker Avance 500 spectrometer with TMS as internal standard. EI-MS and HRESITOFMS were recorded on Shimadzu 2010 and QSTARXL MS/MS, Applied Biosystems, Switzerland.

4.2. Fungal isolation, identification and cultivation

Soft coral *Sinularia kavarattiensis* was collected by scuba diving at a depth of 8-10m from the coast of Mandapam, Tamil Nadu, India in May 2004. After washing the soft coral with sterile sea water, fungus *Aspergillus terreus* was isolated as an epiphyte using Czapek agar containing (g/l) NaNO₃ (2.0), MgSO₄.7H₂O (5.0) FeSO₄.7H₂O (0.1), KH₂PO₄ (1.0), KCl (0.5), agar (3.0) sucrose (30.0) at pH 5.5 prepared in seawater supplemented with penicillin benzyl sodium salt (0.02) to avoid any bacterial growth. After 6-7 days sand brown, velvety, reverse the medium deep dirty brown colonies were observed. The strain was identified as *Aspergillus terreus* from the morphological features of conidiophores by Dr. Sanjay K. Singh, mycologist, Agharkar Research Institute, Pune, India. A voucher specimen of the fungus is deposited at National Institute of Oceanography, Dona Paula, Goa, India. Stock cultures of the fungus, maintained at -20°C preserved with 20% glycerol was used to inoculate 500 ml of seed medium in Erlenmeyer flask (4 L) containing 24 g/l of potato dextrose broth in seawater. It was then cultured at 27±2°C on a rotary shaker at 200 rpm. The flask was incubated for 72 hr and used as a first stage inoculum. The same medium (1 L) was made in 10 Erlenmeyer flasks (4 L) and inoculated with 5% of first stage inoculum. The flasks were incubated for 21 days at 27±2°C on a rotary shaker at 200 rpm for 10 hr/day.

4.3. Extraction and isolation of metabolites

21 days old fermentation broth (10 L) was separated from fungal mat and concentrated to a volume of 1 L under reduced pressure. The broth was extracted first with chloroform (200 ml X 4) followed by ethyl acetate (200 ml X 4). Chloroform and ethyl acetate layers were separately concentrated under reduced pressure to yield chloroform extract (470 mg) and ethyl acetate extract (430 mg). The

ethyl acetate extract was chromatographed over Sephadex LH-20 using MeOH/CHCl₃ (1:1) to yield pure crystalline compound, terrein (92 mg) (Dunn et al., 1975) and a fraction containing an intense rose colour spot. This fraction was flash chromatographed over silica gel using gradient elution of MeOH/CHCl₃ (5:95 - 20:80) to yield **2** (7.2 mg; Rf, 0.51). The chloroform extract was repeatedly chromatographed over Sephadex LH-20 using MeOH/CHCl₃ (1:1) and 100% MeOH, which yielded a pure yellow coloured compound, physcion (17 mg) (Bachmann et al., 1979). Other fractions giving two prominent rose colour spots on TLC was further purified over silica gel column using gradient elution of MeOH/CHCl₃ (0:100- 20:80) to afford **3** (32.0 mg; Rf, 0.64) and another nearly pure compound which was fine purified over Sephadex LH-20 using MeOH/CHCl₃/Pet. ether (40 :40 :20) to yield **1** (26.3 mg; Rf, 0.81)

4.3.1. Aspernolide A (**1**)

White sticky solid; $[\alpha]_D^{28} +88.73$ (c 0.58, CHCl₃); UV (MeOH) λ_{max} nm : 303, 240; IR (NaCl) ν_{max} cm⁻¹ 3340, 3024, 2970, 2935, 1737, 1732, 1610, 1519, 1498, 1436, 1386, 1261, 1182, 1122, 1068, 1037, 948, 839, 754; For NMR data see Table 1; HRESITOFMS $[M+Na]^+$ m/z 447.1433 (calcd. 447.1420 for C₂₄H₂₄O₇Na), $[2M+Na]^+$ m/z 871.2959 (calcd. 871.2942 for C₄₈H₄₈O₁₄Na); EIMS m/z (%): M⁺ 424(2.7), 380(37.8), 348(35.1), 320(6.8), 293(13.5), 265(8.1), 237(10.8), 218(5.4), 205(6.8) 189(6.8), 175(100), 157(8.1), 145(10.8), 131(16.2), 119(13.5), 107(5.4), 91(24.3), 77(3.5), 69(12.1), 44(37.8).

4.3.2. Aspernolide B (**2**)

Light brown syrup; $[\alpha]_D^{28} +48.27$ (c 0.29, MeOH); UV (MeOH) λ_{max} nm : 303, 240; IR (NaCl) ν_{max} cm⁻¹ 3380, 3024, 2975, 2933, 1745, 1610, 1519, 1442, 1386, 1182, 1070, 1037, 838, 762; For ¹H and ¹³C NMR spectroscopic data see Table 2; HRESITOFMS: $[M+H]^+$ m/z 443.1699 (calcd. 443.1706 for C₂₄H₂₇O₈), $[M+Na]^+$ m/z 465.1516 (calcd. 465.1525 for C₂₄H₂₆O₈Na); EIMS m/z (%): $[M-CO_2]^+$ 398(13), 380(70), 348(100), 333(18), 320(15), 293(40), 205(26), 249(10), 237(23), 218(17), 205(25), 188(16), 175(77), 145(13), 131(41), 119(23), 107(15), 91(35), 77(17), 69(20), 59(33), 43(23), 41(11).

4.3.3. Butyrolactone I (**3**)

White powder; $[\alpha]_D^{28.8} + 84.32$ (c 0.617, MeOH); and $[\alpha]_D^{28.8} + 87.56$ (c 0.617, CHCl₃); ¹H NMR and ¹³C NMR data were in agreement with the literature (Rao et al., 2000); HRESITOFMS: $[M+H]^+$ m/z 424.1516(calcd. 424.1522 for C₂₄H₂₅O₇).

4.3.4. Aspernolide C (**8**)

Light brown syrup; ^1H and ^{13}C NMR spectroscopic data see Table 2; HRESITOFMS: $[\text{M}+\text{H}]^+$ m/z 457.1851 (calcd. 457.1862 for $\text{C}_{25}\text{H}_{29}\text{O}_8$) and $[\text{M}+\text{Na}]^+$ m/z 479.1669 (calcd. 479.1682 for $\text{C}_{25}\text{H}_{28}\text{O}_8\text{Na}$).

4.4. Conversion of butyrolactone I (**3**) to aspernolides A (**1**), B (**2**) and C (**8**)

Butyrolactone I (**3**), (76.9 mg) was dissolved in MeOH (10 ml) containing conc. HCl (0.2 ml). Mixture was stirred at r.t. approximately for 2 hr. or till complete conversion of **3** as indicated from TLC. The solvent was removed under vacuum and resulting residue was separated on flash Si-gel column using gradient elution of MeOH/ CHCl_3 (0:100 – 20:80) to yield in the order of increasing polarity **1** (58.6 mg, 75 %), **8** (8.8 mg, 10.4%) and **2** (6.9 mg, 8.3%).

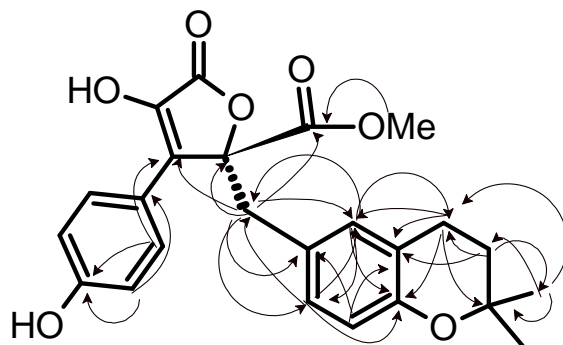
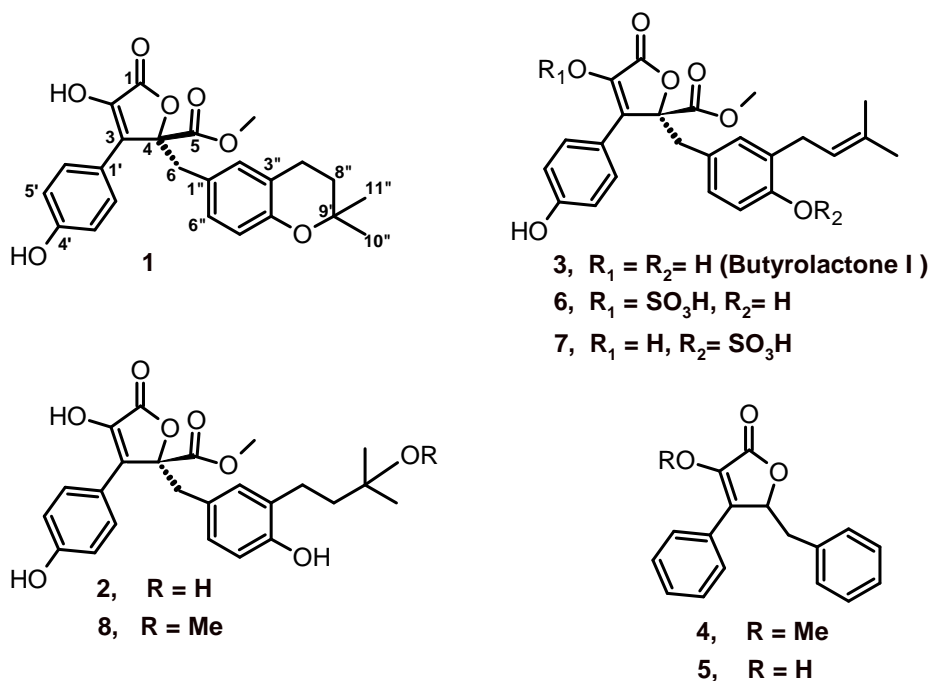
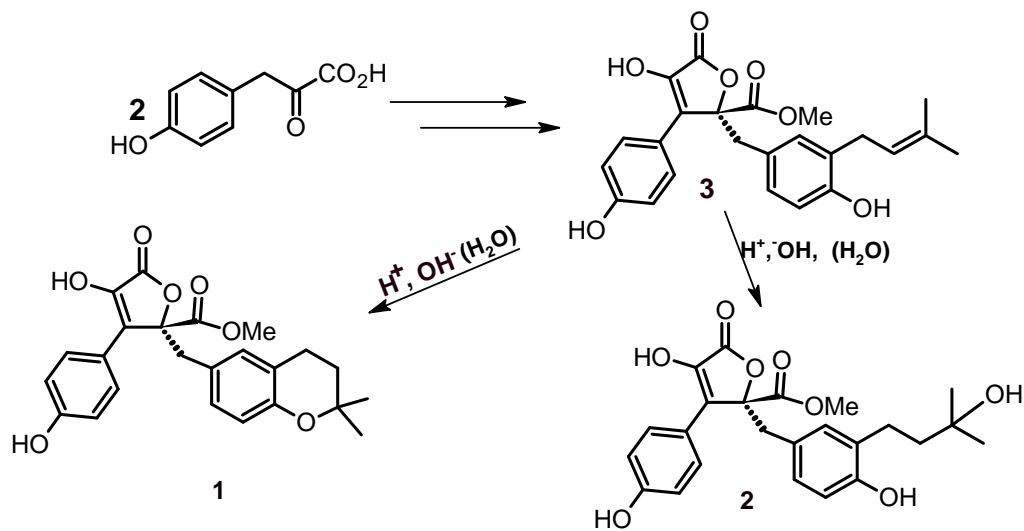


Fig 1. HMBC correlation for **1**

Scheme 1. Biogenic pathway of **1** and **2**



Scheme 2. Conversion of butyrolactone I (**3**) to aspernolide A (**1**)

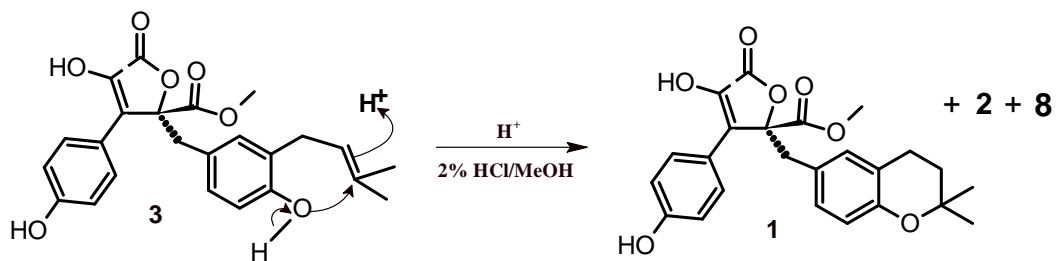


Table 1. NMR spectroscopic data of aspernolide A (**1**) (500 MHz, CDCl₃)

Carbon No	δ_C , mult.	δ_H , mult., J (Hz)	HMBC
1	169.3 s		
2	137.2 s		
3	128.8 s		
4	86.1 s		
5	169.6 s		
6	38.6 t	3.39, d, (15.0) 3.59, d, (15.0)	C4, C1'', C6'', C3, C2'', C5
1'	122.2 s		
2'(6')	129.5 d	7.56, d, (8.7)	C4', C3
3'(5')	115.9 d	6.86, d, (8.7)	C1', C4'
4'	156.4 s		
1''	123.5 s		
2''	131.4 d	6.53, s	C7'', C6, C6'', C4''
3''	120.3 s		
4''	152.9 s		
5''	116.6 d	6.47, s	C1'', C3''
6''	129.0 d	6.47, s	C6, C2'', C4''
7''	22.1 t	2.53m	C8'', C9'', C2'', C3'', C4''
8''	32.5 t	1.66, t, (6.5)	C3'', C7'', C9'', C10''(11'')
9''	74.2 s		
10''(11'')	26.6 q	1.21, s	C7'', C8'', C9''
5-OMe	53.4 q	3.72, s	C5

Table 2. ^1H and ^{13}C NMR spectroscopic data of compounds **2** and **3** (300 MHz, CDCl_3 + 2drops CD_3OD)

Carbon No.	Aspernolide B (2)		Aspernolide C (3)	
	δ_{C} , mult	δ_{H} , mult., J(Hz)	δ_{C} , mult	δ_{H} , mult., J(Hz)
1	169.2 s		169.3 s	
2	137.9 s		137.9 s	
3	128.3 s		128.2 s	
4	85.6 s		85.7 s	
5	170.1 s		170.1 s	
6	38.3 t	3.46, s	38.4 t	3.43, s
1'	121.6 s		121.8 s	
2'(6')	129.1 d	7.55, d, (8.7)	129.2 d	7.54, d, (8.7)
3'(5')	115.6 d	6.87, d, (8.7)	115.0 d	6.85, d, (8.7)
4'	157.5 s		157.5 s	
1''	128.1 s		128.1 s	
2''	131.7 d	6.41, d (1.8)	131.8 d	6.40, d (1.8)
3''	124.0 s		124.0 s	
4''	153.2 s		153.4 s	
5''	114.6 d	6.52, d, (8.7)	114.4 d	6.49, d, (8.1)
6''	128.6 d	6.55, dd, (8.7, 1.8)	128.9 d	6.54, dd, (8.1, 1.8)
7''	24.2 t	2.37, m	23.2 t	2.35, m
8''	43.2 t	1.53, t, (7.8)	40.0 t	1.54, t, (7.8)
9''	70.8 s		75.4 s	
10''	28.4 q	1.20, s	24.0 q	1.12, s
11''	28.5 q	1.19, s	24.4 q	1.12, s
12''	---		49.0 q	3.15, s
5-OMe	53.5 q	3.76, s	53.3 q	3.74, s

Acknowledgement

We sincerely acknowledge the Ministry of Earth Sciences (MoES), New Delhi for funding the project. Authors RRP and CDs are grateful to University Grant Commission (UGC), New Delhi and MoES, for research fellowship and project assistantship. We are grateful to Dr. (Mrs.) Solimabi Wahidulla, senior scientist, National Institute of Oceanography and Prof S. K. Paknikar, retd. Prof., Dept of Chemistry, Goa University for critically reviewing the manuscript. We also thank Dr. P.D. Mishra, Dr. Asha Almeida and Dr.Pari Koteppa, Nicholas Piramal Research Center, Mumbai for carrying out biological screening and 500 MHz NMR facility.

References

- Alberts, A. W., Chen, J., Kuron, G., Hunt, V., Huff, J., Hoffman, C., Rothrock, J., Lopez, M., Joshua, H., Harris, E., Patchett, A., Monaghan, R., Currie, S., Stapley, E., Albers-Schonberg, G., Hensens, O., Hirshfield, J., Hoogsteen, K., Liesch, J., Springer, J., 1980. Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. *Proc. Natl. Acad. Sci. USA.* 77, 3957-3961.
- Bachmann, M., Luethy, J., Schlatter, C., 1979. Toxicity and mutagenicity of molds of the *Aspergillus glaucus* group. Identification of physcion and three related anthraquinones as main toxic constituents from *Aspergillus chevalieri*. *J. Agric. Food Chem.* 27(6), 1342-1347.
- Brachmann, A. O., Forst, S., Furgani, G. M., Fodor, A., Bode, H. B., 2006. Xenofuranones A and B: phenylpyruvate dimers from *Xenorhabdus szentirmaii*. *J. Nat. Prod.* 69, 1830-1832.
- Brana, M. F., Garcia, M. L., Lopez, B., Pascual-Teresa, B., Ramos, A., Pozuelo, J. M., Dominguez, M. T., 2004. Synthesis and biological evaluation of analogues of butyrolactone I and molecular model of its interaction with CDK2. *Org. Biomol. Chem.* 2, 1864-1871.
- Bugni, T. S., Ireland, C. M., 2003. Marine-derived fungi: a chemically and biologically diverse group of microorganisms. *Nat. Prod. Rep.* 21, 143-163.
- Dunn, A.W., Ian, D. E., Johnstone, A.W., 1975. Terrein and other metabolites of *Phoma* species. *Phytochem.* 14, 2081-2082.
- Faulkner, D. J., 2000. Marine natural products. *Nat. Prod. Rep.* 17, 7-55.
- Fischer, P. M., Lane, D. P., 2000. Inhibitors of cyclin-dependent kinases as anti-cancer therapeutics. *Current Medicinal Chemistry.* 7, 1213-1245.
- Kim, W., Cho, K., Lee, C., Yoo, I. D., 2002. Terreulactone A, a novel meroterpenoid with anti-acetylcholinesterase activity from *Aspergillus terreus*. *Tetrahedron Lett.* 43(17), 3197-3198.

- Kiriyama, N., Nitta, K., Sakaguchi, Y., Tagushi, Y., Yamamoto, Y., 1977. Studies on the metabolic products of *Aspergillus terreus*. III. Metabolites of the strain IFO 8835. Chem. Pharm. Bull. (Tokyo). 25(10), 2593-2601.
- Macedo, Jr. C. F., Porto, A. L. M., Marsaioli, A. J., 2004. Terreinol—a novel metabolite from *Aspergillus terreus*: structure and ¹³C labeling. Tetrahedron Lett. 45(1), 53-55.
- Morishima, H., Fujita, K., Nakano, M., Atsumi, S., Ookubo, M., Kitagawa, M., Matsumoto, H., Okunyama, A., Okabe, T., Suda, H., Nishimura, S., 1994. Jpn. Kokai Tokkyo Koho.
- Nishio, K., Ishida, A., Arioka, H., Kurokawa, H., Fukuoka, K., Nomoto, T., Fukumoto, H., Yokote, H., Saijo, N., 1996. Antitumor effects of butyrolactone I, a selective cdc2 kinase inhibitor, on human lung cancer cell lines. Anticancer Res. 16, 3387-3395.
- Nitta, K., Fujita, N., Yoshimura, T., Arai, K., Yamamoto, U., 1983. Metabolic products of *Aspergillus terreus*. IX. Biosynthesis of butyrolactone derivatives isolated from strain IFO 8835 and 4100. Chem. Pharm. Bull. (Tokyo). 31(5), 1528-1533.
- Niu, X., Dahse, H., Menzel, K., Lozach, O., Walther, G., Meijer, L., Grabley, S., Sattler, I., 2008. Butyrolactone I derivatives from *Aspergillus terreus* carrying an unusual sulfate moiety. J. Nat. Prod. 71, 689-692.
- Rao, K. V., Sadhukhan, A. K., Veerender, M., Mohan, E. V. S., Dhanvantri, S. D., Sitaramkumar, S., Babu, M. J., Vyas, K., Reddy, O. G., 2000. Butyrolactones from *Aspergillus terreus*. Chem. Pharm. Bull. (Tokyo). 48(4), 559-562.
- Saleem, M., Ali, M. S., Hussain, S., Jabbar, A., Ashraf, M., Lee, Y. S., 2007. Marine natural products of fungal origin. Nat. Prod. Rep. 24, 1142-1152.
- Schimmel, T. G., Parsons, S. J., 1999. High purity, high yield procedure for butyrolactone I production from *Aspergillus terreus*. Biotechnology Techniques. 13, 379-384.
- Suzuki, M., Hosaka, Y., Matsushima, H., Goto, T., Kitamura, T., Kawabe, K., 1999. Butyrolactone I induces cyclin B1 and causes G2/M arrest and skipping of mitosis in human prostate cell lines. Cancer Lett. 138, 121-130.

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