Phytochemistry 70 (2009) 128–132

Contents lists available at ScienceDirect

Phytochemistry

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

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

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ARTICLE INFO

Article history:
Received 2 May 2008
Received in revised form 21 October 2008
Available online 10 December 2008

Keywords:
Aspergillus terreus
Marine natural products
Butenolides
Butyrolactone I
Aspernolides

ABSTRACT

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

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 and Ireland, 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 *A. terreus* associated with a soft coral *Sinularia kavarattiensis*.

Terrestrial isolates of *A. 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 *A. 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, *A. 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 the 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 $C_{48}H_{48}O_{14}Na$). The IR spectrum showed the presence of ester/lactone carbonyl at 1731 and 1738 cm^{-1}, phenolic OHs were evident at 3330 cm^{-1} and the presence of an absorption at 1660 cm^{-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, $J = 8.7$ Hz and 6.86, *d*, 2H, $J = 8.7$ Hz revealed the presence of para di-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

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doi:10.1016/j.phytochem.2008.10.017

of 10 aromatic signals for two aromatic rings, two ester carbonyls δ_c 169.3 *s*, and 169.6 *s*, olefenic carbon signals δ_c 137.2 *s* and 128.8 *s*, three sp^3 CH_2 *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. The presence of two aromatic rings accounts for eight while two carbonyls and two olefenic carbons account for another three, which makes a total of eleven degrees of unsaturation. Therefore **1** must posses two aliphatic rings in addition to two aromatic rings.

A detailed comparison of the NMR data of **1** with that of butyrolactone I (**3**) (Rao et al., 2000), confirmed a common hydroxyphenylpyruvate 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 an olefinic proton signal 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 in good agreement with 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 a dihydropyran ring fused through the C3″–C4″ bond of a 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 were evidence of the benzodihydropyranmethylene moiety linked to a lactone ring at C-4. Furthermore HMBC correlation from H-2′(6′) to C-3 established *para* di-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 H_3–5OMe.

Aspernolide B (**2**) (Rf, 0.51), more polar than **1** (Rf, 0.81) was obtained as a light brown syrup $[\alpha]_D$ + 48.27 (c 0.29, MeOH). The IR spectrum showed the presence of – OHs at 3330 cm^{-1}, ester/lactone carbonyls overlapping peaks at 1732 and 1747 cm^{-1} and 1610 and 1519 cm^{-1} for aromatic rings. Although chemical shift variations were present in the ^1H and ^{13}C NMR of **2**, they were similar to those of **1**. Significant variations in the chemical shifts were observed for the ring carrying the 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 ($\Delta\delta$ 4.6 ppm), 124.0 ($\Delta\delta$ 3.7 ppm), 24.2 ($\Delta\delta$ 2.1 ppm), 43.2 ($\Delta\delta$ 10.7 ppm), 28.4 ($\Delta\delta$ 1.8 ppm) and 28.5 ($\Delta\delta$ 1.9 ppm) while C-5″ and C-9″ were considerably shielded to resonate at δ_c 114.6 ($\Delta\delta$ 2.0 ppm) and 70.8 ($\Delta\delta$ 3.4 ppm) 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 a molecular weight of 442 for the compound **2**, which was 18 units more than that of **1**. Based on these observations it was evident that **2** has open chain hydroxylated prenyl chain ortho to a 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 *A. terreus,* it is apparent that the structures of **1** and **2** are an extension of the biosynthesis of **3**, which is derived from p-hydroxyphenylpyruvate. The enzyme-catalysed cyclization or

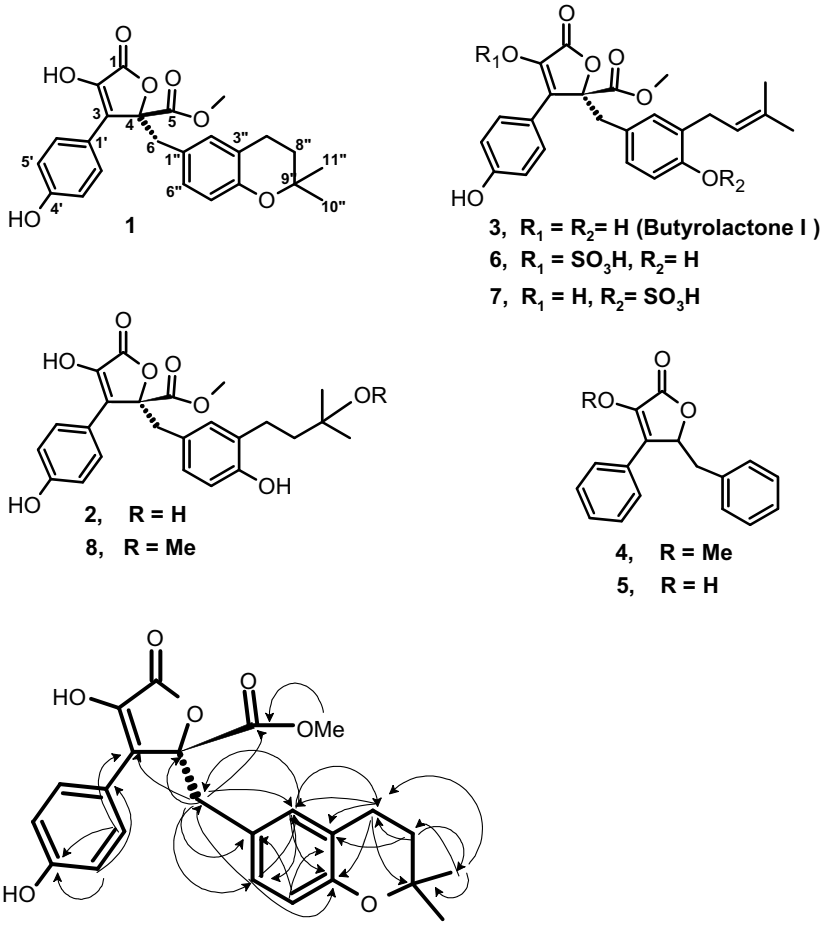

Fig. 1. HMBC correlation for 1.