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Aspergilloxide, A Novel Sesterterpene Epoxide from a Marine-Derived Fungus of the Genus *Aspergillus*

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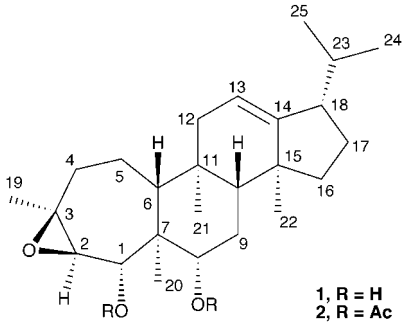
ABSTRACT



A new sesterterpene epoxide-diol, aspergilloxide (1), was isolated from the extract of a cultured marine-derived fungus (strain CNM-713) identified as an undescribed member of the genus *Aspergillus.* The structure of 1 was determined by interpretation of NMR data and by chemical methods. The absolute stereochemistry of aspergilloxide was assigned by application of the modified Mosher method. The carbon skeleton of 1 represents a new addition to the architectural diversity of the sesterterpenoid (C_{25}) class of secondary metabolites.

Although the sesterterpenes (C_{25}) are the rarest of the terpenoid classes of secondary metabolites, their sources are widespread,[1] having been isolated from terrestrial fungi, lichens, higher plants, insects, and various marine organisms,[2] especially sponges. Examples of polycyclic sesterterpenes from terrestrial fungi include variecolol,[3] YW3699,[4] citreohybridone,[5] and aleurodical.[6] In two recent papers, we reported the isolation of several polycyclic sesterterpenoids from marine fungi. These include the cytotoxic, halogen-containing neomangicols[7] and the related mangicols,[8] both rearranged tetracarbocyclic sesterterpenes isolated from a marine-derived *Fusarium* species. Recently, a tricarbocyclic ophiobolin-derivative, halorosellinic acid, was reported from the marine fungus *Halorosellinia oceanica*.[9] As a continuation of our interest in the discovery of new secondary metabolites from marine fungi, we report here the isolation and structure elucidation of a new sesterterpenoid, aspergilloxide (**1**),[10] produced in culture by a marine-derived fungus identified as a member of the taxonomically complex fungal genus *Aspergillus.* The carbon framework of as-

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(10) Compound **1**: amorphous white powder $[\alpha]_D$ −29° (*c* 0.650, CH_2Cl_2); UV λ_{max} ($CHCl_3$) 227 (log ϵ 5.4) nm; IR (film, NaCl) ν_{max} 3236, 2942, 2872, 1637, 1454, 1378 cm^{-1}; $C_{25}H_{40}O_3$ by HRMALDI $[M + Na]^+$ *m/z* 411.2857, calc 411.2870; for ^1H and ^{13}C NMR data see Supporting Information.

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pergilloxide (**1**) has not been described among the sesterterpenoids and is composed of a regular (head to tail isoprenoid) terpenoid tetracarbocyclic carbon skeleton.

Aspergillus sp. (strain CNM-713) was provided by E. B. Gareth Jones during an expedition to the Bahamas Islands in 1989. The strain was identified by its morphological features by both Jones and Microbial ID, Newark, DE. The fungal strain was cultivated without shaking in replicate 2.8-L Fernbach flasks (5 × 1 L) at 25 °C in a seawater-based marine nutrient medium consisting of 2 g of yeast extract, 2 g of bactopeptone, and 4 g of mannitol in 1 L of seawater. After 25 days, the cultures were combined, and the mycelium and broth were extracted together with 7.5 L of ethyl acetate. After removal of the solvent under vacuum, the crude extract was subjected to C-18 silica flash chromatography using gradient elution (100% H_2O to 100% MeOH), followed by size exclusion chromatography (Sephadex LH-20) using isooctane/toluene/MeOH (3:1:1) as the eluent. Final purification was achieved by normal phase HPLC (silica gel, 60 Å) with isooctane/EtOAc (6:4), to yield 20.3 mg of aspergilloxide (**1**) as an amorphous white powder (yield 4.0 mg/L).

Aspergilloxide (**1**) analyzed for $C_{25}H_{40}O_3$ by HRMALDI mass spectrometry ($[M + Na]^+$ *m/z* 411.2857; calc 411.2870), a formula indicating six degrees of unsaturation. The ^{13}C NMR spectrum of **1** showed signals for 25 carbons, while DEPT spectral data indicated the presence of six methyl groups, six methylene carbons, seven methine carbons, two olefinic carbons (one quaternary), and four quaternary sp^3 carbons. Three of the methine carbons were observed at δ 84.2, 76.9, and 67.4, and one of the quaternary carbons was observed at δ 60.4. Since aspergilloxide contains three oxygen atoms and four carbons bearing oxygen, one oxygen-containing ring or an acyclic ether must be present. The assignment of these carbons was accomplished by ^1H NMR data, which showed three methine protons at δ 3.78 (dd, 4.5, 11.4), δ 3.51 (d, 6.0), and δ 2.80 (d, 6.0) indicative of protons attached to carbons bearing hydroxyl and epoxide functionalities. Two broad, D_2O exchangeable hydroxyl protons were observed at δ 4.68 and δ 3.97, indicating the presence of two hydroxyl groups. The ^1H NMR spectrum also showed one olefinic proton at δ 5.12 (br s), two methyl singlets at δ 1.38 (s) and δ 1.12 (s), a complex of sharp bands between δ 0.88−0.93 that integrated for four methyl groups, and a broad band of protons between δ 2.10 and 0.85 that integrated for a total of 16 protons.

Acetylation of aspergilloxide (Ac_2O/pyr/RT) cleanly produced the expected diacetate derivative **2**. Considering the six degrees of unsaturation present in this molecule, the presence of two olefinic carbons, and the presence of two hydroxyl groups, aspergilloxide must be composed of five rings, one of which must be oxygen-containing. Analysis of chemical shift data showed that this ring was a trisubstituted epoxide [^1H δ 2.80 (d, 6.0), ^{13}C δ 60.4, δ 67.4]. Thus, aspergilloxide was defined as a tetracarbocyclic epoxy-diol generally conforming to the spectral characteristics expected of a C_{25} sesterterpene.

A combination of 1D and 2D NMR spectral data allowed numerous structural components of **1** to be defined. COSY and 1D TOCSY proton correlations allowed connections between C-1 and C-2, between C-12 and C-14, and between C-8 and C-10. These connections were confirmed using data obtained from HMBC NMR data. HMBC correlations from the methyl groups C-19 and C-20 led to the confident assignment of all of the oxygenated carbons in close proximity. An HMBC correlation between H-13 and C-18, in conjunction with previous COSY and TOCSY data, established the relationship of carbons 12−18 along the perimeter of the C and D rings.

To further establish the structural features of **1**, exhaustive NMR measurements were conducted on the diacetate **2** in C_6D_6. In this solvent, the six methyl signals (four singlets for C-19, -20, -21, -22, and two doublets for C-24 and -25) were readily resolved. HMBC experiments conclusively placed the methyl groups at strategic locations on the tetracarbocyclic ring structure of aspergilloxide. Correlations from the C-19 protons to C-2, C-3, and C-4 placed the epoxide methyl at C-3. Correlations from the C-20 methyl protons to C-1, C-6, C-7, and C-8 allowed this methyl group to be placed at C-7. Likewise, correlations from the C-21 methyl protons to C-6, C-10, and C-12 and correlations from the C-22 methyl protons to C-10, C-14, C-15, and C-16 allowed these methyl groups to be placed at C-11 and C-15, respectively. Last, three-bond correlations from the two isopropyl methyl group protons (C-24 and C-25) to the opposite carbons C-25 and C-24 and correlations of both to C-18 allowed the isopropyl group to be confidently assigned at C-18 on ring D. The sites of the A/B and C/D ring junctures (C-6/C-7 and C-14/C-15) were defined on the basis of three bond HMBC correlations between C-14 and H_3-22, between C-6 and H_3-20, and between C-10 and H_3-21. Other correlations confirmed these assignments and resulted in the complete planar assignment for aspergilloxide.

The relative configurations of the stereocenters in aspergilloxide (**1**) were assigned on the basis of 2D NOESY experiments for **1** and 1D NOE measurements for the diacetate derivative **2**. The 2D NOESY NMR experiment for **1** (Figure 1) showed correlations between H-2 and H_3-20 and H_3-19. Also, a 1D NOE experiment with **2** showed enhancements of H_3-20 and H_3-22 when H_3-21 was irradiated. These data allowed the methyl groups C-19, C-20, and C-21 to be placed on the same face of the polycyclic ring system and the epoxide to be placed on the opposite face. NOESY measurements for **1** showed a strong correlation

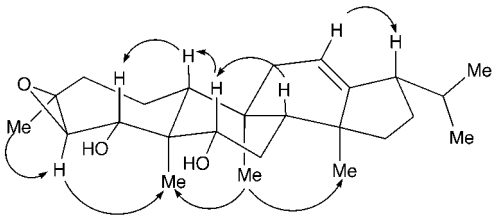

Figure 1. Key NOE correlations for aspergilloxide (**1**).

between H-1 and H-8. In addition, correlations were observed between H-1 and H-6 and between H-8 and H-10. These observations allowed the protons of the ring fusion to be placed on the same face of the molecule as H-1 and H-8, thus establishing the C-6/C-7 and C-10/C-11 ring fusions as *trans*. This assignment is in good agreement with the ^{13}C NMR chemical shifts of the methyl carbons positioned at these junctions (δ 10.0 and δ 18.3).

Once the relative stereochemistry of **1** had been established, a preferred conformation was considered in which the isopropyl group is in the (down) pseudoequatorial position. Supporting this assignment was an NOE enhancement observed between H-13 and H-18. Analysis of molecular models, however, indicates that NOE effects would be observed from H-18 in either the pseudoaxial or pseudoequatorial positions. Intuitively, the isopropyl group was assigned to be pseudoequatorial (down) on the same face of the molecule as C-20, C-21, and C-22, but the alternative pseudoaxial isopropyl group cannot be discounted.

The absolute stereochemistry of compound **1** was established by application of the modified Mosher method.[11,12] *R*- and *S*-Mosher esters of the alcohol at C-8 were prepared.[13] Subsequent NMR analysis of the $\Delta\delta$ values for the two MTPA esters gave clean evidence to assign the absolute stereochemistry at C-8 as *S* (Figure 2). This information, together with the NOESY data, implied that C-4, C-11, C-15, C-8, C-7, C-3, and C-1 are *S* and C-10, C-6, and C-2 are *R*.

Compound **1** showed little in vitro cytotoxicity toward HCT-116 human colon carcinoma, but its acetate derivative showed weak inhibitory activity at 61 μM. Aspergilloxide

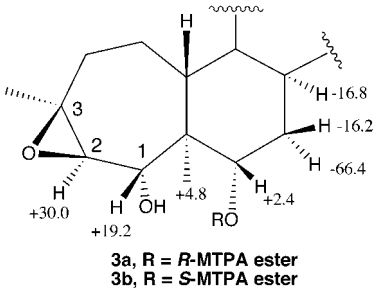

Figure 2. $\Delta\delta$ values ($\delta_S - \delta_R$) in Hz of the two MTPA esters derived from aspergilloxide (**1**).

(**1**) has a novel tetracyclic skeleton that has yet to be defined in the sesterterpenoid class of secondary metabolites. The isopropyl-substituted D ring of aspergilloxide is similar to that observed in stellatic acid, a tricyclic sesterterpenoid isolated from the terrestrial fungus *Aspergillus stellatus*.[14] Analysis of the terpenoid character of **1** shows that it is likely derived from a typical cyclization of a "folded" all-*trans*-geranylfarnesyl diphosphate precursor. We suggest the semisystematic name "asperane" to define this new carbon skeleton.

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Supporting Information Available: ^1H and ^{13}C NMR assignments for compounds **1** and **2** in $CDCl_3$ and C_6D_6. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) **Typical Procedure.** Aspergilloxide (**1**) (3.6 mg, 9.27 μmol), (*S*)-MTPA-Cl (24.4 mg, 92.70 μmol), pyridine (70 μL), and 4-(dimethylamino)-pyridine (5.4 mg, 45 μmol) in CH_2Cl_2 (0.5 mL) were stirred at room temperature for 24 h. The solvent was removed under N_2, and the residue was purified by silica gel chromatography using isooctane/EtOAc (1:1) to give the (*R*)-MTPA ester **3a** (1.6 mg). The same experimental procedure was followed for the production of the corresponding (S)-MTPA ester **3b**.

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