ORGANIC LETTERS

2013
Vol. 15, No. 10
2522–2525

Asperterpenols A and B, New Sesterterpenoids Isolated from a Mangrove Endophytic Fungus *Aspergillus* sp. 085242

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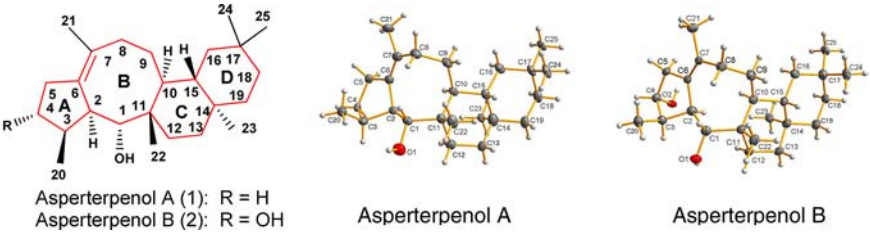
Received April 10, 2013

ABSTRACT



Asperterpenol A (1) and asperterpenol B (2), two novel sesterterpenoids with an unusual 5/8/6/6 tetracyclic ring skeleton, were isolated from a mangrove endophytic fungus *Aspergillus* sp. 085242. The structures were elucidated on the basis of spectroscopic methods and the absolute configurations determined by single-crystal X-ray diffraction analysis. Compounds 1 and 2 inhibit acetylcholinesterase with IC_{50} values of 2.3 and 3.0 μM, respectively.

Globally, Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder, and as global health care improves and the proportion of the elderly increases, the number of AD patients is anticipated to increase dramatically. As of 2010, an estimated 35.6 million people suffer worldwide with dementia. This number will nearly double every 20 years, to an estimated 65.7 million in 2030, and 115.4 million in 2050.[1] Current AD treatment approaches for the disease are primarily symptomatic; the major therapeutic strategy is based on the cholinergic hypothesis which dictates that deterioration of cholinergic neurotransmission in key areas of the CNS underlies AD. Consequently, acetylcholinesterase (AChE) inhibition and other strategies leading to enhanced synaptic acetylcholine concentrations are currently the most well-established approaches to treating AD.[2]

Sesterterpenoids are the smallest and rarest members of the terpenoid family and are known to arise from numerous sources including terrestrial fungi, lichens, higher plants, insects, and various marine organisms. These natural products demonstrate a wide array of interesting pharmacological and ecologically significant characteristics such as cytotoxic activities, enzyme inhibitory functions, antimicrobial effects, and defensive functions.[3,4a]

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10.1021/ol401005j
Published on Web 05/03/2013

In recent years, our research group has been dedicated to investigating novel bioactive compounds from mangrove endophytic fungi collected from the South China Sea.[4] Recently, two novel sesterterpenoids named asperterpenol A (**1**) and asperterpenol B (**2**) were isolated from just such a fungus identified as *Aspergillus* sp. 085242. The structure of **1** was established by spectroscopic methods and its absolute configuration determined by thorough analysis of singl-crystal X-ray diffraction data. Herein we report the isolation and structural elucidation of these compounds as well as the realization that these sesterterpenoids inhibit acetylcholinesterase.

The fungus *Aspergillus* sp. 085242 was fermented on rice solid-substrate medium supplemented with 0.3% sea salt for 28 days at room temperature. The mycelia and rice medium were then extracted with MeOH and solvent removed from the extracts in vacuo to yield 10 g of organic residue. The resulting organic residue was then refined, and its constituents were fractionated by column chromatography (CC) over silica gel eluted using a gradient of petroleum ether/EtOAc from 9:1 to 3:7 to give seven fractions (Fr.1−Fr.7). Further sample refinement was accomplished by subjecting Fr.2 (500 mg) to Sephadex LH-20 CC isocratically eluted with $CHCl_3$/MeOH (1:1) to afford analytically pure **1** (20 mg) and **2** (10 mg), respectively.

Asperterpenol A (**1**)[5] was isolated as colorless crystals (MeOH) and found to have a molecular formula of $C_{25}H_{42}O$ on the basis of HR-EI-MS (M^+ peak at m/z = 358.3233), indicating five degrees of unsaturation. The IR spectrum suggested the presence of an OH group (3403 cm^{-1}). The ^1H and ^{13}CNMR spectra, with the aid of HSQC spectra, revealed the presence of five methyl groups respresented by singlets and one methyl group represented as a doublet, nine methylenes, including two allylic methylenes (δ_H 2.34, 2.20, δ_C 28.8, C-5, and δ_H 2.62, 1.66, δ_C 31.7, C-8), five methines, including an oxygen-bearing methine (δ_H 4.56, δ_C 76.7, C-1) and five quaternary carbons, including two olefinic carbons (δ_C 135.8 and 125.6, C-6 and C-7)(Table 1). The clear presence of one olefin accounts for one degree of unsaturation, thus supporting the identity of **1** as a tetracyclic compound.

The basic carbon skeleton of **1** was established by comprehensive analysis of 2D NMR spectroscopic data, in particular with $^1H-^1H$ COSY and HMBC correlations. Interpretation of the $^1H-^1H$ COSY and HSQC spectra indicated the presence of four substructures, **i**, **ii**, **iii**, and **vi** (bolded lines in Figure 1), and enabled assignment of all protons and corresponding carbons. In the HMBC spectrum, correlations from the secondary methyl signals at δ_H 2.20 and 2.34 (H_2-5) to C-2 (δ_C 51.7) and C-6 established the structure of ring A. HMBC correlations from the methyl signals at δ_H 1.64 (H_3-21) to C-2, C-6, C-7, C-8 and from another methyl signal at δ_H 1.08 (H_3-22) to C-1,

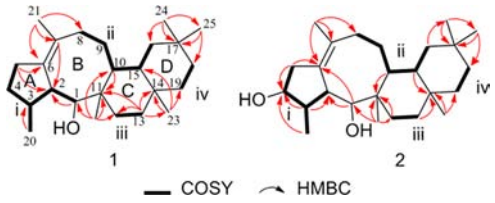

Figure 1. COSY and key HMBC correlations of **1**.

C-10 (δ_C 36.2), C-11 (δ_C 43.1) confirmed the structure of ring B. H_3-22 also showed an HMBC correlation to C-12 (δ_C 25.7) as well as HMBC correlations from the protons of the methylene (δ_H 1.23 and 1.28, H_2-13) to C-11, C-12, C-14 (δ_C 33.5), C-23 (δ_C 16.2), and the HMBC correlation between H_3-23 (δ_H 0.76) and C-14, C-15 (δ_C 40.3) defined one of the six-membered rings (ring C). This methyl signal (H_3-23) also indicated two HMBC correlations to C-18 (δ_C 38.6). Furthermore, the protons of H_3-24 (δ_H 0.87) and H_3-25 (δ_H 0.90) both showed correlations with C-16 (δ_C 38.6), C-17 (δ_C 31.5), C-18, and C-19 (δ_C 37.7) confirming the existence of ring D. Thus, the planar structure of **1** was defined, as shown in Figure 1. The structure of asperterpenol A (**1**) was subsequently confirmed by single-crystal X-ray diffraction experiments using Cu Kα radiation (Figure 2).[6] The absolute configuration of **1** was established as 1*R*,2*S*,3*R*,10*S*,11*S*,14*S*,15*S* through the refinement of Flack's parameter [x = 0.01(2)].[7] Thus,the structure of **1** with a new skeleton was identified and named asperterpenol A.

The molecular formula of asperterpenol B (**2**)[8] was determined to be $C_{25}H_{42}O_2$ on the basis of HR-EI-MS ([M^+] = 374.3181), one oxygen atom greater in size than that of **1**. The close resemblance between the NMR spectra of **1** and **2** indicated that **2** was another sesterterpenoid structurally similar to **1**. The major difference was the replacement of a methylene group in **1** (δ_C 30.2) by a hydroxyl-bearing methine in **2** (δ_C 75.6). The long-range $^1H-^{13}C$ correlations from H-3, H-5, and Me-20 to this methine carbon, and from the corresponding methine proton (δ_H 3.81) to C-2, C-6, and C-20, indicated this methine carbon to be C-4. Analysis of the 2D NMR

(5) Asperterpenol A (**1**): colorless cubic crystals (MeOH); mp 118.4−119.9 °C; $[\alpha]_D^{20}$ = +110 (*c* 0.0020, MeOH); UV (MeOH) λ_{max} 205nm; IR (KBr) 3467, 3403, 2950, 2852, 1627, 1452, 1382, 1029 cm^{-1}; for ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; EIMS 358 $[M]^+$; HREIMS m/z 358.3233 $[M]^+$ (calcd for $C_{25}H_{42}O$, 358.3230).

(6) $C_{25}H_{42}O$, M + 0.75H_2O = 372.10; monoclinic, space group *C*2; *a* = 29.5357(3) Å, *b* = 15.7962(2) Å, *c* = 20.0056(2) Å, α = γ = 90°, β = 96.3130(10)°, V = 9277.05(18) $Å^3$, *Z* = 16, D_x = 1.066 mg/m^3, crystal dimensions: 0.41 × 0.39 × 0.13 mm were used for measurement on an Oxford Gemini S Ultra diffractometer with Cu Kα radiation (λ = 1.54178 Å). The total number of reflections measured was 34830 (R_{int} = 0.0283), $I > 2\sigma(I)$. The final R1 values were 0.0489, wR2 = 0.1309. The crystal structure of compound **1** was solved by direct method SHELXS-97 and expanded using difference Fourier techniques, refined by the program SHLXL-97 and the full-matrix least-squares calculations. Crystallographic data for the structure of asperterpenol A have been deposited with the Cambridge Crystallographic Data Centre (deposition no. CCDC 936220).

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(8) Asperterpenol B (**2**): colorless cubic crystals (MeOH); mp 189.9−190.4 °C; $[\alpha]_D^{20}$ = +70 (*c* 0.0010, MeOH); UV (MeOH) λ_{max} 203nm; IR (KBr) 3313, 2960, 2934, 2913, 2882, 1467, 1451, 1382, 1034 cm^{-1}; for ^1H NMR ($CDCl_3$ and MeOD, 400 MHz) and ^{13}C NMR ($CDCl_3$ and MeOD, 100 MHz) see Table 1; EIMS 374 $[M]^+$; HREIMS m/z 374.3181 $[M]^+$ (calcd for $C_{25}H_{42}O_2$, 374.3179).

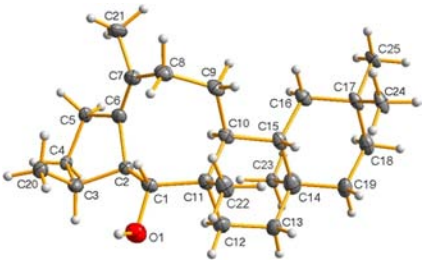

Figure 2. Perspective ORTEP drawing for **1**.

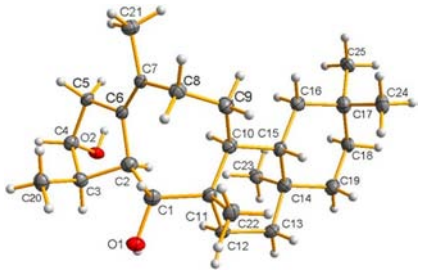

Figure 3. Perspective ORTEP drawing for **2**.

spectra of **2** indicated the presence of an OH with connectivity to C-4. The configuration of **2** was confirmed to be the same as **1** based on single-crystal X-ray diffraction analysis (Figure 3).[9] The absolute configuration of **2** was established to be 1*R*,2*S*,3*R*,4*R*,10*S*,11*S*,14*S*,15*S* through the refinement of Flack's parameter [$x = 0.02$ (3)].[7] From the above findings, the structure of asperterpenol B (**2**) was determined to be 4-hydroxyasperterpenol A.

To the best of our knowledge, the 5/8/6/6 tetracarbocyclic skeleton of asperterpenols A (**1**) and B (**2**) is an unprecedented structure and is presumed to be derived from geranylfarnesyl pyrophosphate through a series of cationic cyclizations, rearrangements and oxidation, as shown in Scheme 1. The initial head-to-tail cyclization of GFPP and removal of the pyrophosphate moiety produces an 11/5 fused ring system, followed by migration of a carbon−carbon σ-bond to generate an 11/6 fused ring system (Scheme 1, structure i). Subsequent cyclization and C−C σ-bond migration of the 11/6 ring system may afford

(9) $C_{25}H_{42}O_2$, $M = 374.59$; monoclinic, space group $P2(1)$; $a = 12.6439(3)$ Å, $b = 6.8702(2)$ Å, $c = 13.2201(4)$ Å, $\alpha = \gamma = 90°$, $\beta = 97.454(2)°$, $V = 1138.67(5)$ Å^3, $Z = 2$, $D_x = 1.093$ mg/m^3, crystal dimensions: 0.37 × 0.31 × 0.25 mm were used for measurement on an Oxford Gemini S Ultra diffractometer with Cu Kα radiation ($\lambda = 1.54178$ Å). The total number of reflections measured was 21175 ($R_{int} = 0.0357$), $I > 2\sigma(I)$. The final R1 values were 0.0595, wR2 = 0.1499. The crystal structure of compound 1 was solved by direct method SHELXS-97 and expanded using difference Fourier techniques, refined by the program SHLXL-97 and the full-matrix least-squares calculations. Crystallographic data for the structure of asperterpenol B has been deposited with the Cambridge Crystallographic Data Centre (deposition no. CCDC 936221).

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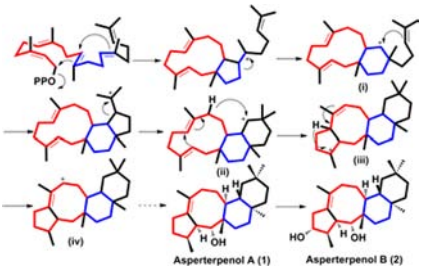
Table 1. Summary of ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data for Terpenoids **1**[a] and **2**[b]

	1		**2**	
no.	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)
1	76.7 d	4.58 (10.4)	76.3, d	4.52, m
2	51.7 d	2.60, m	46.9, d	3.12, br s
3	36.1 d	2.18, m	45.3, d	2.06, m
4a	30.2 t	1.69, m	75.2, d	3.81, d (6.2)
4b		1.35, m		
5a	28.8 t	2.34, m	39.6, t	2.70, dd (17.2, 6.3)
5b		2.20, m		2.13, d (17.2)
6	135.8 s		133.7,s	
7	125.6 s		126.6, s	
8a	31.7 t	2.62, m	31.6, t	2.64, m
8b		1.66, m		1.68, m
9a	29.3 t	1.65, m	29.1, t	1.67, m
9b		1.09, m		1.11, m
10	36.2 d	1.50, m	36.3, d	1.57, m
11	43.1 s		43.1, s	
12a	25.7 t	1.56, m	25.7, t	1.62, m
12b		1.38, m		1.40, m
13a	37.7 t	1.28, m	34.0, t	1.41, m
13b		1.23, m		1.13, m
14	33.5 s		33.6, s	
15	40.3 d	1.26, m	40.5, d	1.30, m
16a	38.6 t	1.27, m	38.5, t	1.32, m
16b		0.83, m		1.28, m
17	31.5 s		31.6, s	
18a	38.6 t	1.31, m	38.6, t	1.15, m
18b		1.16, m		0.82, m
19a	34.1 t	1.39, m	37.6, t	1.27, m
19b		1.12, m		1.25, m
20	14.8 q	0.86, d (6.7)	12.9, q	0.81, d (7.0)
21	23.5 q	1.64, s	23.5, q	1.65, s
22	17.6 q	1.08, s	17.5, q	1.08, s
23	16.2 q	0.76, s	16.2, q	0.79, s
24	26.0 q	0.87, s	26.0, q	0.89, s
25	33.8 q	0.90, s	33.7, q	0.91, s

[a] Measured in $CDCl_3$, [b] Measured in $CDCl_3$ and CD_3OD.

Scheme 1. Plausible Biogenetic Pathway for **1** and **2**



an 11/6/6 fused tricyclic intermediate ii (Scheme 1).[10] H-Migration and carbon cyclization of the tricyclic intermediate is envisioned to form the 5/8/6/6 fused tetracyclic skeleton iii and successive H-migration followed by rearrangement of the double bond affords intermediate iv. Asperterpenol precursor iv can then further oxidize to generate asperterpenol A (**1**) and asperterpenol B (**2**).

In this study, asperterpenols A and B were assayed for in vitro acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibition according to a modified Ellman method.[11] Both compounds strongly inhibited AChE, with IC_{50} values of 2.3 μM for **1** and 3.0 μM for **2**; neither compound inhibited BuChE ($IC_{50} > 100\,\mu$M). Therefore, asperterpenols A and B are moderately potent and apparently selective marine-derived natural inhibitors of AChE (Supporting Information).

Acknowledgment. We are grateful to the National Natural Science Foundation of China (20972197, 41276146, 21102020), the 863 Foundation of China (2011AA09070201), the Science & Technology Plan Project of Guangdong Province of China (2010B030600004, 2011A080403006), Fundamental Research Funds for the Central Universities of China (11lgjc01), and the Guangzhou Pearl River New Star Plan of Science and Technology Program (2012J2200015). We also thank Professor L. Jiang and Dr. J. L. Liu at Sun Yat-sen University for help in analyzing the X-ray crystallographic data.

Supporting Information Available. NMR, MS, and IR spectra and the X-ray crystallographic data of **1** and **2** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org

The authors declare no competing financial interest.

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