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Novel Chromone Derivatives from Marine Fungus *Aspergillus versicolor* Isolated from the Sponge *Xestospongia exigua*

Wen Han LIN[1]*, Hong Zheng FU[1], Jun LI[1], Peter Proksch[2]

[1]National Research Laboratories of Natural and Biomimetic Drugs, Peking University, Beijing 100083
[2]Institut fuer Pharmazeutwasche Biologie, Heinrich-Heine Universitat, Duesseldorf D-40225

Abstract: From the marine sponge *Xestospongia exigua*, fungal isolates of *Aspergillus versicolor* (Vuill)Triab were obtained. Isolation and purification of ethyl acetate extracts from culture filtrates of the fungus led to yield six new chromone derivatives namely aspergione A, aspergione B, aspergione C, aspergione D, aspergione E, aspergione F. The structures of all the new compounds were established on the basis of extensive spectroscopy (UV, MS, ^{1}H and ^{13}C NMR, COSY, HMQC and HMBC) analysis.

Keywords: *Aspergillus versicolor* , chromone derivatives.

In the continuation of our research for the bioactive secondary metabolites from the sponge-associated fungi, the bioassay guiding fractionation led to the isolation of six new secondary metabolites with unusual skeleton based on chromone ring system from the inoculated fungus *Aspergillus versicolor*, that had been isolated from fresh samples of marine sponge *Xestospongia exigua*, collected along coast line of Bali, Indonesia in 1997. The basic skeleton of those compounds possessed an oxohexacyclic chromone. One and two dimensional NMR spectroscopic techniques were employed as main tools for the structural elucidation, and afforded unambiguous confirmation of the signal assignments, as well as the total structures of all novel compounds. While comparing the secondary metabolites of fungus *Aspergillus versicolor* with those of its associated sponge *Xestospongia exigua*, mainly containing the alkaloids of isoquinoline derivatives[1-5], the uncomparable structure patterns indicated that the secondary metabolites isolated from the sponge were not originated from the fungus *Aspergillus versicolor.*

The incubated filtrate and mycelia were collected and extracted with EtOAc exhaustively. The crude extracts were fractionated by VLC on silica gel by employing solvent systems hexane:acetone (2:1), acetone, methanol successively. Before submissionfor separation, all the fractions collected from VLC were undertaken for bio-test by using the assay models of anti-microorganisms (*Escherio coli* HB101, *Candida albicans*, *Staphylococcus aureus*, *E. corl*, *Bacillus subtilwas*), brine shrimp assay, and insecticide test. The bioactive fractions were extensively separated by using flash chromatography on silica gel eluted with hexane-acetone, and semipreparative C-18

HPLC with MeOH/H_2O gradient to afford aspergione A (12.0 mg), aspergione B (1.5 mg), aspergione C (2.5 mg), aspergione D (2.5 mg) , aspergione E (8.0 mg) , aspergione F (3.5 mg), respectively.

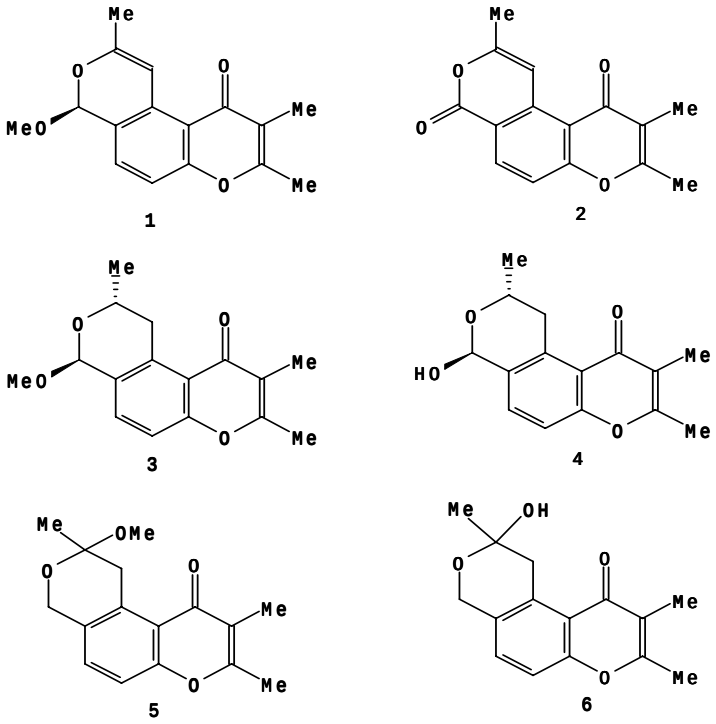
Aspergione A **1** was obtained as colorless amorphous, and showed molecular ion peak [M^+] at *m/z* 272, in association with ^1H and ^{13}C NMR (DEPT) data, compatible to formula $C_{16}H_{16}O_4$ with nine unsaturation. The UV absorption bands at 235, 280, 340 and 390 nm were characteristic of a chromone pattern[4]. The ^1H NMR spectrum exhibited signals of three olefinic methyl singlets at δ 2.08 (s), 2.10 (s) and 2.34 (s), and one methoxyl singlet at δ 3.58 (s), of which two methyl groups were proved to be substituted at C-2 and C-3 due to the HMBC correlation from the methyl protons at δ 2.34 (s) to the unsaturated ketone C-1 (δ 182.68, s) and C-3 (δ 145.49, s), and from the methyl protons at δ 2.10 (s) to C-2 (δ 131.28, s). An AB spin coupling system at δ 6.77 (d, J = 8.0) and 7.63 (d, J = 8.0) was attributable to H-6 and H-7 of aromatic ring, indicating that the positions of C-8 and C-9 of the aromatic ring were substituted by remaining moiety. In HMBC spectrum, an olefinic proton at δ 5.86 (brs) showed long range correlations with C-10 (δ 108.07, s), C-8 (δ 121.67, s) of aromatic ring, and with an olefinic carbon at δ 155.18 (s, C-12) and a methyl carbon δ 20.37 (q), indicating the presence of a propylene moiety attached at C-9. And the acetal proton at δ 6.33 (s) attached to the carbon at δ 94.68 (d), which was observed directly from HMQC spectrum, showed correlations with C-7 (δ 125.16, d), C-9 (δ 138.59, s), C-12 and methoxyl (δ 55.35, q) respectively, suggested that the acetal carbon bearing methoxy was located at C-8 and cyclized to C-12 of the propylene *via* ether bond. The result of spectral analysis mentioned above established the structure as shown in **Figure 1**. The ^1H-^1H COSY, HMQC together with HMBC spectra enable to provide the evidence for assignment of its ^1H and ^{13}C NMR data. It was an unusual structure pattern with oxohexacyclic chromone ring system discovered in marine and terrestrial fungi and bacteria.

Aspergione B **2** had molecular formula $C_{15}H_{12}O_4$, based on pseudo-molecular ion peak at *m/z* 257 (M^++1) in ESI-MS spectrum and its NMR data, and its molecular weight was 16 mu less than that of **1**. The ^1H and ^{13}C NMR spectra showed similar features to those of **1** except for the absence of the proton signal of methoxyl group, and appearance of a carbonyl carbon at δ 166.3 (s) in ^{13}C NMR assigned for unsaturated lactone, two aromatic methyl singlets at δ 2.40 (s) and 2.25 (s) assigned for Me-16 and Me-17 respectively, by directly comparing their ^1H NMR data with that of **1** , an olefinic methyl doublet at δ 2.32 (d, J = 0.8Hz) for Me-15 as well as an olefinic proton δ 6.31 (q, J = 0.8Hz) for H-11. The chemical shifts of proton signals for H-6 (δ 7.02,d,J = 8.0) and H-7 (δ 7.94, d, J = 8.0) shifted more down-field than those of **1** was explained by deshielded effect raised from carbonyl group located at position C-14. Thus, the structure of Aspergione B **2** was identified as shown in **Figure 1**.

Aspergione C **3,** $C_{16}H_{18}O_4$, had molecular ion peak at *m/z* 274 in EI-MS spectrum, 2 mu more than that of **1**. Its UV as well as ^1H and ^{13}C NMR spectral features resembled those of **1**, indicating that it belongs to a chromone analogue of **1**. A methoxyl group at δ 3.62 (s, 3H) and a low field proton δ 5.70 (br) were assigned to acetal group by HMQC and HMBC correlations. **3** differed from **1** in the partial structure of oxohexacyclic ring

where the geminal protons resonated at δ 2.75 (dd, J = 3.8, 17.2, H-11a) and 2.69 (dd, J = 10.6, 17.2, H-11b) coupled with H-12 (δ 4.32, ddq, J = 3.8, 6.2, 10.6), in turn the H-12 displayed a cross peak with the methyl doublet at δ 1.38 (d, J=6.2, Me-15) in the ^{1}H-^{1}H COSY spectrum, referred to saturated carbons C-11 and C-12 of **3** instead of olefinic carbons of **1**. This evidence was also supported by ^{13}C NMR data at δ 36.01 (t) and C-12 (δ 62.88 (d) respectively. The relative configuration at C-12 and C-14 was carried out by coupling constants and NOE experiment. Coupling constant J = 10.6 Hz for vicinal protons H-11a /H-12 agreed with their *trans* axial correlation in a semi chair form in Dreiding model. The NOE correlation between H-12 and MeO confirmed a β-orientation for the methoxyl group annexed to acetal carbon C-14. Thus, **3** was 11,12-dihydroaspgione A.

Figure 1



Aspergione D **4,** $C_{15}H_{16}O_4$, showed UV, ^{1}H and ^{13}C NMR spectral feature identical to those of **3**. It differed from **3** only in the substitution of C-14 where a methoxy in **3** was replaced by a hydroxyl for **4** in the ^{1}H NMR spectrum, and **4** was 14 mu less than **3**. Thus, **4** was corresponded to 11,12-dihydro-14-O-demethyl aspergione A.

Aspergione E **5,** $C_{16}H_{18}O_4$, had the same molecular formula as **3**, and its UV absorptions closely resembled to those of **3**, indicative to a similar structural pattern of chromone ring system. The ^{1}H NMR spectrum exhibited a low field geminal protons

for the oxygen bearing CH_2-14 (δ 5.05,d, J = 16.0; 4.94, d, J = 16.0), and the other geminal protons (δ 3.07, d, J = 17.3; 2.97, d, J = 17.3) assigned for CH_2-11 correlated to a quaternary carbon of C-12 (δ 95.44, s). The remaining methoxyl group (δ 3.35, s) and methyl group (δ 1.58, s) were deduced to substitute at C-12 due to long range correlation of MeO to C-12 in the HMBC spectrum. The HMBC spectrum further provided the correlation of geminal protons CH_2-14 to C-12 (δ 95.44, s) *via* oxygen bridge. Therefore, the structure of **5** was identical to 11, 12-dihydro-12-methoxyl-14-demethoxyl-aspergione A.

Aspergione F **6,** $C_{15}H_{16}O_4$, showed all spectral features (UV, ^1H and ^{13}C NMR) identical to those of **5**, exception of the molecular weight (M^+, *m/z* 260) 14 mu less than that of **5**. Comparing the ^1H and ^{13}C NMR data of **6** with those of **5**, the methoxy group of **5** in C-12 was replaced by hydroxy group. The doublet at δ 6.08 (d, J = 1.3Hz) weakly coupling with H-11 (δ 2.92) was assigned the hydroxy group at C-12. The structure of **6** was therefore identical to 11,12-dihydro-12-hydroxyl-14-demethoxyl aspergione A.

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