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Four Novel Hydropyranoindeno- Derivatives from Marine Fungus *Aspergillus versicolor*

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Abstract: From the cultured filtrates of fungus *Aspergillus versicolor*, isolated from marine sponge *Xestospongia exigua*, four novel secondary metabolites, namely aspergillone **1**, aspergillodiol **2**, aspergillol **3** and 12-acetyl-aspergillol **4**, have been isolated by column chromatographic separation. The structures of all the new compounds are established on the basis of extensive 2D NMR spectroscopy in conjugation with MS, UV spectral analysis. The basic structure pattern of those compounds possessed an hydroindenoisopyran nucleus.

Keywords: *Aspergillus versicolor*, aspergillone, aspergillodiol, aspergillol, 12-acetyl-aspergillol.

In the previous report on bioactive secondary metabolites from the sponge-associated fungus *Aspergillus versicolor* (Vuill) Tirab, the bioassay guiding fractionation led to isolation of six new compounds with unusual skeleton based on chromone ring system from the inculated fungus which was isolated from fresh samples of marine sponge *Xestospongia exigua*, collected along coast line of Bali, Indonesia in 1997[1]. In the continuation of our chemical investigation on the marine fungus, four compounds with new skeleton, named aspergillone **1**, aspergillodiol **2**, aspergillol **3**, 12-acetyl-aspergillol **4** have been isolated. The basic structure pattern of those compounds possessed an unique hydroindenoisopyran nucleus. One and two dimensional homo- and hetero-nuclear correlation spectroscopy were employed as main tools for the structural elucidation.

Compound **1** was obtained as colorless amorphous. Its molecular ion [M^+] is at *m/z* 342 in EI-MS spectrum, in association with ^1H and ^{13}C NMR(DEPT) data, was compatible to the formula $C_{21}H_{26}O_4$, with nine unsaturation. The UV absorption at 220, 263 and 350 nm suggested the presence of α, β-unsaturated ketone and/or aldehyde carbonyls and benzene ring. The gross structure of **1** and all of the ^1H and ^{13}C NMR chemical data were determined by an extensive 2D NMR spectra (^1H-^1H COSY, HMQC and HMBC). In the ^{13}C NMR (DEPT, and BB decoupling) spectrum signals at δ147.06(s), 116.51(s), 161.50(s), 116.97(d), 134.17(d) and 133.39(s) indicated a four-substituted aromatic ring. ^1H-^1H COSY spectrum showed an AB coupling system of aromatic protons at δ 6.79 (d, J = 8.4 Hz, H-7) and δ 7.30 (d, J = 8.4 Hz, H-8). The HMBC spectrum showed that a phenolic proton δ 11.22 (s, 6-OH)

Table 1 The ^{13}C NMR Data Comparison of compound **1** to **4** (in $CDCl_3$)

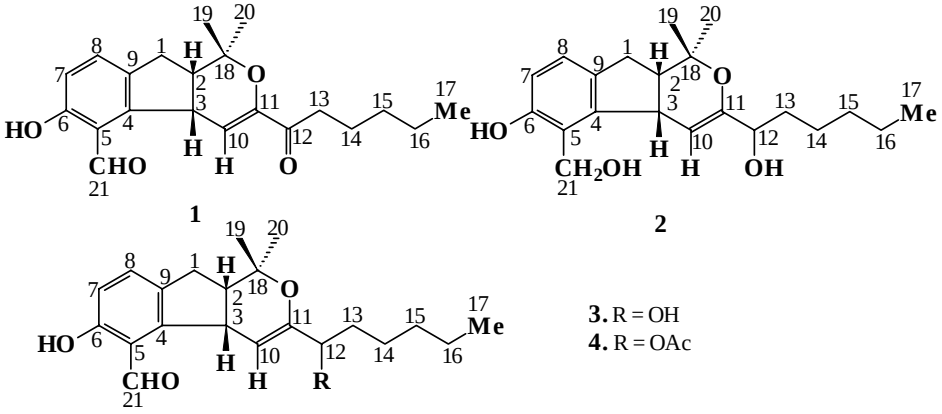
C-atom	**1**	**2**	**3**	**4**
1	31.45(t)	31.70, t	31.65, t	31.81, t
2	48.46(d)	48.68, d	48.96, d	48.96, d
3	38.24(d)	38.52, d	37.72, d	37.71, d
4	147.06(s)	142.50, s	149.62, s	149.33, s
5	116.51(s)	120.00, s	116.49, s	116.25, s
6	161.50(s)	155.00, s	161.23, s	161.25, s
7	116.97(d)	115.13, d	116.19, d	116.26, d
8	134.17(d)	125.19, d	134.14, d	134.18, d
9	133.39(s)	134.00, s	133.32, s	133.52, s
10	106.97(d)	95.67, d	95.86, d	97.27, d
11	148.57(s)	153.00, s	153.44, s	150.25, s
12	197.92(s)	72.57, d	72.52, d	73.97, d
13	37.93(t)	34.95, t	34.96, t	31.16, t
14	23.46(t)	24.75, t	24.18, t	24.75, t
15	31.22(t)	31.70, t	31.21, t	31.16, t
16	22.47(t)	22.58, t	22.57, t	22.51, t
17	13.94(q)	14.00, q	13.96, q	13.98, q
18	76.15(s)	75.08, s	75.07, s	75.28, s
19	27.06(q)	27.06, q	27.09, q	27.10, q
20	24.62(q)	24.75, q	24.75, q	24.84, q
21	194.04(d)	61.03, t	194.49, d	194.46, d
Ac				170.10, s 21.25, q

correlated with δ161.50 (s, C-6) and δ116.97 (d, C-7), and an aldehyde proton δ10.22 (s, H-21) correlated to C-6 and δ116.51 (s, C-5). It means OH located at C-6 and CHO annexed to C-5 of the aromatic ring respectively. The ^1H-^1H COSY spectrum showed the proton at δ2.66 (ddd, J = 1.73, 2.3, 7.5Hz, H-2) correlated to the geminal protons δ 2.80 (dd, J = 2.3,10.6Hz) and δ2.78(d, J = 10.6Hz) for CH_2-1, and in turn H-2 correlated to its vicinal proton δ4.28 (dd, J = 2.8, 7.5 Hz, H-3) which was coupling to the vinyl proton δ5.69 (dd, J = 1.7, 2.8 Hz, H-10), as observed around the hydroindenoisopyran ring. The HMQC spectrum enabled to assign the chemical shifts of all protonated carbons. The HMBC showed the correlations of C-9 to H-1, H-3, H-7 as well as C-4 to H-1, H-3, H-8, indicating the presence of a hydropyranoindeno nucleus. The two methyl singlets at δ1.39 (s) and 1.45 (s) showed correlation to each other and further to δ76.15 (s, C-18) and δ48.46 (d, C-2), suggesting both methyls attached to the oxygenated quaternary carbon C-18. Although the relationship of vinyl carbon C-11 and quaternary carbon C-18 could not be clarified by HMBC correlation, the last one element out of nine unsaturation in association with calculation of molecular weight agreed with C-11 linking to C-18 *via* oxygen atom to form a oxohexacyclic ring. The typical MS fragment *m/z* 99, 71, 57, 43, 29 indicated the existence of a *n*-hexanoyl group, which was further supported by the proton signals of saturated *n*- pentyl at δ0.86 (t, 3H, J = 6.7 Hz,

H-17), 1.28 (m, 2H, H-16), 1.54, 1.30 (m, 2H, H-15), 1.55 (m, 2H, H-14) and 2.57 (ddd, 2H, J = 1.7,7.8,8.4 Hz, H-13) and their correlation in ^{1}H-^{1}H COSY spectrum. The hexanoyl group was proposed to annex to C-11 (δ148.57, s), and was confirmed by the evidence of long range correlations of vinyl proton δ5.69 (dd, J = 1.7, 2.8 Hz, H-10) with C-11 and carbonyl carbon C-12 (δ197.92, s) in HMBC spectrum, in association with H-13 (δ2.57, ddd, J = 1.7, 7.8, 8.4 Hz) showed weakly correlation with H-10 in COSY spectrum. The relative configuration of two chiral centers C-2 and C-3 were clarified according to the coupling constant of H-2/H-3 ($J_{H-2/H-3}$ = 7.5 Hz) as well as NOE correlation between H-2 and H-3 to afford a *cis* orientation for both protons. The Dreiding structure model also supported that *cis* configuration is more stable than *trans* configuration. Consequently, the entire structure of **1** is identified as shown in **Figure 1**.

Compound **2** had molecular formula $C_{21}H_{30}O_4$, on the basis of pseudo-molecular ion at *m/z* 347 (M^+ + 1) in ESI-MS spectrum in associated with ^{13}C NMR spectra (DEPT, BB decoupling). The ^{1}H and ^{13}C NMR spectra showed similar features to those of **1** except for the absence of the aldehyde and keto carbons which were replaced by an oxygenated methylene δ 4.98 (br, 2H, H-21) and an oxygenated methine δ3.82 (dd, J = 5.9, 6.8 Hz, H-12). Those evidences indicated that **2** is a dihydrogenized product of **1,** named aspergillodiol .

Figure 1 The chemical structures of new compounds **1-4**



Compound **3** had a molecular ion peak at *m/z* 344 in its EI-MS spectrum, compatible to the formula $C_{21}H_{28}O_4$, with eight unsaturation, and the molecular weight was 2 mu more than that of **1**. Its UV, ^{1}H and ^{13}C NMR spectral features were closely identical to those of **1**, except for the keto signal of **1** at C-12 to be replaced by an oxygenated methine due to the chemical shifts at δ3.85 (dd, J = 6.3, 6.6 Hz, H-12) and δ72.52 (d, C-12). The entire structure is identified as 12-hydroxyaspergillone, namely aspergillol.

Compound **4** had a pseudo molecular ion peak at *m/z* 387 (M^++1) in ESI-MS spectrum, compatible to formula $C_{23}H_{30}O_5$. Its UV, ^{1}H and ^{13}C NMR spectral data closely resembled those of **3**, but the molecular weight of **4** was 42 mu more than that of **3**, implying that **4** had an additional acetyl group than **3**. This was also evidenced by

the signals of acetyl group at δ170.10 (s), 21.25 (q), and 2.02 (s, 3H) in ^{1}H and ^{13}C NMR spectra. The acetyl was considered to be attached to C-12 since the chemical shift of H-12 was down field shifted to δ5.02 (dd, J = 6.6, 6.8 Hz) compared to that of **3** (δ3.85, dd, J = 6.3, 6.6 Hz, H-12), while the ^{13}C NMR data resonated at δ 73.97 (d, C-12) in **4** and at δ72.52 (d, C-12) in **3**. The structure of **4** was identical to 12-acetyl-aspergillol.

Acknowledgment

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References and notes

1. W. H. Lin, H. Fu, J. Li, P. Proksch, *Chinese Chemical Letters*., to be in press.
2. **1**, ^{1}H NMR ($CDCl_3$, δ ppm): 2.80 (dd, J = 2.3, 10.6 Hz, H-1a), 2.78 (d, J = 10.6 Hz, H-1b); 2.66 (ddd, J = 1.7, 2.3, 7.5 Hz, H-2); 4.28 (dd, J = 2.8, 7.5 Hz, H-3); 6.79 (d, J = 8.4 Hz, H = 7); 7.30 (d, J = 8.4 Hz, H-8); 5.69 (dd, J = 1.7, 2.8 Hz, H-10); 2.57 (ddd, J = 1.7, 7.8, 8.4 Hz, H-13); 1.55 (m, H-14); 1.5,1.30 (m, H-15); 1.28 (m, H-16); 0.86 (t, J = 6.7 Hz, H-17); 1.45 (s, H-19); 1.39 (s, H-20); 10.22 (s, H-21); 11.22 (s, OH-6). **2,** ^{1}H NMR ($CDCl_3$, δppm): 2.80 (dd, J = 11.2,15.2 Hz, H-1a), 2.72 (dd, J = 8.2, 15.2 Hz, H-1b); 2.48 (ddd, J = 11.2,8.2,7.7 Hz, H-2); 3.70 (dd, J = 2.2, 7.7 Hz, H-3); 6.65 (d, J = 8.0 Hz, H-7); 6.95 (d, J = 8.0 Hz, H-8); 4.50 (d, J = 2.2 Hz, H-10); 3.82 (dd, J = 5.9, 6.8 Hz, H-12); 1.54 (m, H-13); 1.50 (m, H-14); 1.52, 1.21 (m, H-15); 1.22 (m, H-16); 0.82 (t, J = 7.0 Hz, H-17); 1.37 (s, H-19); 1.36 (s, H-20); 4.98 (br, H-21). **3,** ^{1}H NMR ($CDCl_3$, δ ppm): 2.81 (dd, J = 11.4,14.8 Hz, H-1a), 2.75 (dd, J = 7.0, 14.8 Hz, H-1b); 2.62 (ddd, J = 1.0, 7.0, 7.5, 11.4 Hz, H-2); 4.17 (dd, J = 2.1, 7.5 Hz, H-3); 6.75 (d, J = 8.4 Hz, H-7); 7.29 (d, J = 8.4 Hz, H-8); 4.57 (dd, J = 1.0, 2.1 Hz, H-10); 3.82 (dd, J = 5.9, 6.8 Hz, H-12); 1.54 (m, H-13); 1.50 (m, H-14); 1.52, 1.21 (m, H-15); 1.22 (m, H-16); 0.82 (t, J = 7.0 Hz, H-17); 1.37 (s, H-19); 1.36 (s, H-20); 10.18 (s, H-21), 11.2 (s, OH-6). **4,** ^{1}H NMR ($CDCl_3$, δppm): 2.80 (dd, J = 11.1,14.8 Hz, H-1a), 2.73 (dd, J= 7.9, 14.8 Hz, H-1b); 2.58 (ddd, J = 7.3, 7.9, 11.1 Hz, H-2); 4.13 (dd, J = 2.0, 7.3 Hz, H-3); 6.75 (d, J = 8.3 Hz, H-7); 7.29 (d, J = 8.3 Hz, H-8); 4.54 (brd, J = 2.0 Hz, H-10); 5.02 (dd, J = 6.6, 6.8 Hz, H-12); 1.66 (m, H-13); 1.57 (m, H-14); 1.52, 1.23 (m, H-15); 1.22 (m, H-16); 0.82 (t, J = 6.7 Hz, H-17); 1.36 (s, H-19); 1.35 (s, H-20); 10.16 (br, H-21), 11.19 (s, OH-6), 2.02 (s, Ac).

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