Two New Meroterpenes from the Mangrove Endophytic Fungus *Aspergillus* sp. 085241B

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Two new meroterpenes, 'acetoxydehydroaustin B' (**1**) and '1,2-dihydro-acetoxydehydroaustin B' (**2**) were isolated in the form of a mixed crystal from the mangrove endophytic fungus *Aspergillus* sp. 085241B. Their structures and absolute configurations were determined by extensive analysis of their spectra and X-ray diffraction data. In a preliminary bioassay, the mixed crystal did not exhibit activities against cancer cell lines of MDA-MB-435, SKBR3, HepG2, HEP3B, PC-3, and A549, as well as against α-glucosidase and tyrosinase.

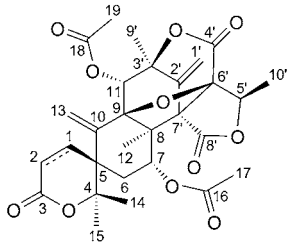
Introduction. – Austin was first isolated as a novel polyisoprenoid mycotoxin from *Aspergillus ustus* in 1976 [1]. Subsequently, some new austin analogs were continually found, including austinol [2] and (−)-dehydroaustinol [3] from *Emericella nidulans var. dentata*, (+)-dehydroaustinol, dehydroaustin, acetoxydehydroaustin, and neoaustin from *Penicillium* sp. MG-11 [4], and preaustinoid A, preaustinoid B, preaustinoid A2, and austinoneol from the *Penicillium* sp. [5]. The structural features of austin compounds are to have a complex cyclic system, contain a spirolactone ring, and many O-atoms. These features and their insecticidal activities have attracted a lot of attention.

Our chemical investigation on the mangrove endophytic fungus *Aspergillus* sp. 085241B collected from the Shankou Mangrove National Nature Reserve, Guangxi Province, P. R. China, led to the isolation of two new meroterpenes, 'acetoxydehydroaustin B'[2]) (**1**), which is the enantiomer of the acetoxydehydroaustin, and '1,2-dihydro-acetoxydehydroaustin B'[2]) (**2**). Herein, we reported their structure elucidation by extensive spectroscopic and crystal analysis, and their bioactivity study.

Results and Discussion. – Compounds **1** and **2** were isolated as an optically active mixed crystal. The UV spectrum showed absorptions at λ_{max} (log ε) 210 (3.7) and 236 (3.2) nm. The IR spectrum exhibited a group of strong absorptions around 1700 cm^{-1}, suggesting the presence of C=O groups. The ^{1}H-NMR data showed that the ratio of the

[1]) These two authors contributed equally to this work.

[2]) Arbitary atom numbering; for sytematic names, see *Exper. Part.*



1[1]) C(1)=C(2)
2[1]) C(1)–C(2)

two compounds in mixture was 2 : 3. In the ^{13}C-NMR spectrum, there were 58 C-atoms signals. The HMQC spectrum allowed to distinguish the 1D-NMR data of **1** and **2** (*Table 1*).

Table 1. *^{1}H- and ^{13}C-NMR Data* (400 and 100 MHz, resp.; (D_6)DMSO) *of Compounds* **1** *and* **2**. Attributions established by HMQC, COSY, and HMBC. δ in ppm, J in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1) or CH_2(1)	6.90 (*d*, $J = 9.9$)	151.1	2.27 (*m*)	29.3
H–C(2) or CH_2(2)	6.00 (*d*, $J = 9.9$)	115.8	2.39, 2.55 (2*m*)	25.4
C(3)		162.6		170.1
C(4)		85.8		87.6
C(5)		45.1		42.4
CH_2(6)	1.91 (*s*)	31.2	1.94 (*s*)	35.3
H–C(7)	5.25 (*m*)	67.1	5.19 (*m*)	67.7
C(8)		54.9		56.0
C(9)		91.1		92.2
C(10)		138.9		137.4
H–C(11)	5.50 (*s*)	73.6	5.46 (*s*)	73.8
Me(12)	1.36 (*s*)	12.0	1.32 (*s*)	11.7
CH_2(13)	5.88 (*d*, $J = 1.9$), 5.67 (*d*, $J = 1.2$)	124.5	5.61 (*d*, $J = 0.4$), 5.56 (*d*, $J = 1.7$)	122.7
Me(14)	1.36 (*s*)	26.4	1.34 (*s*)	23.2
Me(15)	1.40 (*s*)	25.2	1.34 (*s*)	23.2
C(16)		169.8		169.7
Me(17)	1.97 (*s*)	20.3	1.96 (*s*)	20.4
C(18)		169.0		169.3
Me(19)	2.09 (*s*)	20.4	2.06 (*s*)	20.5
CH_2(1′)	5.96 (*s*), 5.82 (*s*)	116.1	5.96 (*s*), 5.82 (*s*)	116.0
C(2′)		136.0		136.0
C(3′)		82.2		82.3
C(4′)		168.5		168.6
H–C(5′)	5.18 (*s*)	75.1	5.20 (*s*)	75.3
C(6′)		83.9		84.4
C(7′)		60.8		60.7
C(8′)		166.5		166.6
Me(9′)	1.51 (*s*)	18.8	1.51 (*s*)	19.0
Me(10′)	1.49 (*s*)	12.7	1.48 (*s*)	12.8

'Acetoxydehydroaustin B'[2]) (**1**) gave a molecular-ion peak in the HR-EI-MS at *m*/*z* 556.1941 (M^+), which corresponded to the molecular formula $C_{29}H_{32}O_{11}$ with 14 degrees of unsaturation. The peak at *m*/*z* 438 in the EI-MS indicated the loss of two AcO groups. The ^{1}H-NMR spectrum of **1** displayed seven Me groups at δ(H) 1.36 (*s*, Me(12), Me(14)), 1.40 (*s*, Me(15)), 1.49 (*s*, Me(10′)), 1.51 (*s*, Me(9′)), 1.97 (*s*, Me(17)), and 2.09 (*s*, Me(19)), two exocyclic olefin moieties at δ(H) 5.67 (*d*, $J = 1.2$ Hz) and 5.88 (*d*, $J = 1.2$ Hz, CH_2(13)) and δ(H) 5.82 and 5.96 (2*s*, CH_2(1′)), one aliphatic CH_2 group at δ(H) 1.91 (*s*, CH_2(6)), two *cis*-positioned olefinic H-atoms at 6.90 (*d*, $J = 9.9$ Hz, H–C(1)), 6.00 (*d*, $J = 9.9$ Hz, H–C(2)), and three oxymethine H-atoms at δ(H) 5.50 (*s*, H–C(7)), 5.18 (*s*, H–C(5′)), and 5.25 (*m*, H–C(7)). Combined with the ^{13}C-NMR data, the presence of five ester C=O groups at δ(C) 162.6 (C(3)), 166.5 (C(8′)), 168.5 (C(4′)), 169.0 (C(18)), and 169.8 (C(16)) and two olefin C-atoms at δ(C) 136.0 (C(2′)) and 138.9 (C(10)) was established, beside fourteen quaternary C-atoms. Six degrees of unsaturation remained in the structure, implying that compound **1** was a hexacyclic compound. The COSY cross-peaks H–C(1)/H–C(2), H–C(7)/CH_2(6), and H–C(5′)/Me(10′) confirmed the corresponding adjacent positions. These resulting partial structures and the HMBC data (*Fig. 1*) allowed to establish the hexacyclic structure of **1** accordingly. The ^{1}H,^{13}C-correlations Me(14)/C(4) and C(15), and Me(15)/C(4) and C(14) placed Me(14) and Me(15) at C(4). The correlations H–C(1)/C(2) and C(5) and H–C(2)/C(1), C(3), C(4), and C(5) established ring *A*, present as a lactone ring. The correlations Me(17)/C(16) and Me(19)/C(18) determined the two AcO groups. The correlations H–C(7)/C(6), C(8), and C(16) confirmed that the group Me(17)C(16)OO was at C(7), and, combined with the correlations CH_2(13)/C(10), C(5), and C(9), and CH_2(6)/C(5) and C(4), elucidated the *B* moiety, present as a six-membered ring, and established that the spiro C-atom between rings *A* and *B* was C(5). Similarly, the correlations H–C(11)/C(3′), C(9), and C(18), and Me(9′)/C(3′), C(2′), and C(11) placed the group Me(19)C(18)OO at C(11) and Me(9′) at C(3′), respectively. Moreover, the correlations CH_2(1′)/C(2′), C(3′), and C(7′), and Me(12)/C(7), C(8), C(9), and C(7′) established that the corresponding six-membered ring (*C*) was fused to ring *B* and that Me(12) was attached to C(8). The position of Me(10′) at C(5′) and the five-membered lactone ring *D* were determined based on the correlations H–C(5′)/C(10′), C(6′), and C(8′). Finally, the connectivities of the O-atom to C(9) and C(6′),

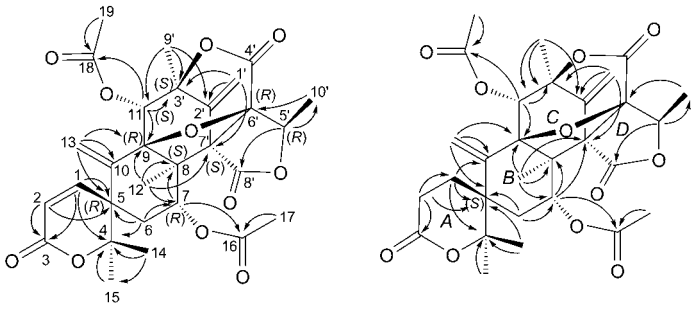


Fig. 1. *Key HMBC features of compounds* **1** *and* **2**[2])

and of the group OC(4′)=O to C(3′) and C(6′) were established by the values of their ^{13}C-NMR chemical shifts. Thus, the structure of compound **1** was determined, which possessed the same planar structure as the acetoxydehydroaustin [4]. However, the colorless crystal of **1** offered the convenience to solve its absolute configuration (*Fig. 2*). It was found that compound **1** was the enantiomer of acetoxydehydroaustin, and was named 'acetoxydehydroaustin B'.

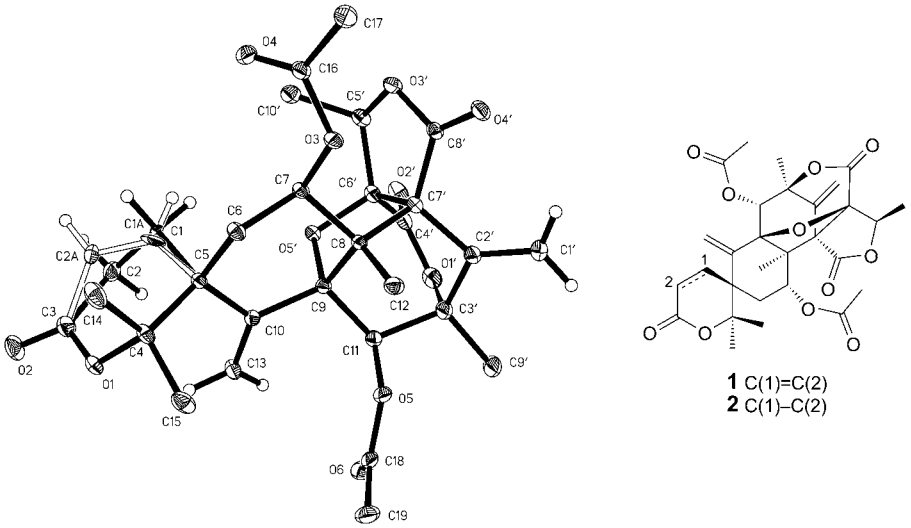


Fig. 2. *X-Ray crystal structure and chemical structure of compounds* **1** *and* **2**[2])

Compound **2** had the molecular formula $C_{29}H_{34}O_{11}$, based on its HR-EI-MS (*m*/*z* 558.2094 (M^+)), *i.e.*, two H-atoms more than **1**. The NMR data of **2** similar to those of **1** offered an efficient way to establish its structure. Detailed 2D-NMR analysis (*Fig. 1*) and the crystal-structure elucidation of **2** (*Fig. 2*) suggested that the differences between **1** and **2** were mainly in the bond between C(1) and C(2). Thus the configuration of C(5) of **2** was (*S*) ((*R*) in **1**), due to the single bond between C(1) and C(2), and **2** was named '1,2-dihydro-acetoxydehydroaustin B'.

In the bioactivity assay of the mixed crystal, it was found that **1** and **2** were almost inactive ($IC_{50} > 50$ μM) against cancer cell lines of MDA-MB-435, SKBR3, HepG2, HEP3B, PC-3, and A549, as well as against α-glucosidase and tyrosinase (*Table 2*). However, some austin compounds have reportedly shown strong insecticidal activities [4][6]; therefore, a systematic study of the bioactivities of austin compounds and their biosynthetic pathway may lead to new significant results in the future.

Table 2. IC_{50} *Values of the Mixture* **1/2** *in the Bioactivity Assay*

	MDA-MB-435	SKBR3	Hep3B	HepG2	PC-3	A549	α-Glucosidase	Tyrosinase
IC_{50}	> 100	56.73	> 100	45.24	> 100	63.2	> 100	> 100

This work was supported by the *National Natural Science Foundation of China* (20972197), the *China's Marine Commonwealth Research Project* 201005022-2, the *Science & Technology Plan Project of Guangdong Province of China* (2010B030600004), and the *Fundamental Research Funds for the Central Universities* (101gpy11). We would like to thank *Syngenta Ltd.* for awarding a Ph.D. studentship to *Y.-X. S.* The single-crystal analysis by Dr. *Long Jiang* is highly appreciated.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Haiyang Chemical Group Co.*). TLC: silica gel GF_{254} (SiO_2; HG/T2354-92, *Qingdao Haiyang Chemical Group Co.*). M.p.: *SFW-X-4* apparatus. Optical rotation: *Polaptronic-HNQW5* apparatus (*Schmidt-Haensch*). UV Spectra: *Shimadzu-UV-2501-PC* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet-5DX-FTIR* spectrometer; in cm^{-1}. CD Spectra: *Jasco-810* CD spectrometer; λ ($\Delta\varepsilon$) [M^{-1} cm^{-1}] in nm. ^{1}H- and ^{13}C-NMR: *Bruker-Avance-400* spectrometer; 400 (^{1}H) and 100 MHz (^{13}C). MS: *VG-ZAB* mass spectrometer; in *m/z* (rel. %).

Fermentation of the Fungal Strain. The strain of fungus 085241B was identified as an *Aspergillus* sp. (Genebank accession no. JF312217) based on its complete ITS1-5.8S-ITS2 gene sequences. It was deposited with the Department of Applied Chemistry, Sun Yat-sen University, Guangzhou, P. R. China. Starter cultures were maintained on potato dextrose agar. Plugs of agar-supporting mycelia growth were cut and transferred aseptically into GYT medium (glucose 1%, yeast extract 0.1%, peptone 0.2%, crude sea salt 0.2%) in 500-ml *Erlenmeyer* flasks containing 250 ml of GYT and incubated as seed at 28°/120 rpm for 5–7 d. Large-scale fermentation of 100 l was performed in multiple 1000 ml *Erlenmeyer* flasks containing 500 ml of GYT; each flask was inoculated with 1 ml of seed and incubated at 28° for 30 d under static conditions.

Extraction and Isolation. The AcOEt extract of the smashed dry mycelia (284 g) was subjected to CC (SiO_2, gradient of petroleum ether → AcOEt → MeOH). The fraction eluted with AcOEt/petroleum ether 6:4 was subjected to extensive CC (SiO_2) to afford the mixture **1**/**2** (30 mg) with AcOEt/petroleum ether 7:3. Their similar properties made the further separation difficult.

'Acetoxydehydroaustin B' (=*(3*R,*3'*R,*3a*R,*6*S,*7*R,*7a*R,*11*R,*11a*S,*11b*S*)-7,11-Bis(acetyloxy)-6,7,11,11a-tetrahydro-2',2',3,6,11a-pentamethyl-8,12-bis(methylene)spiro[4*H,*8*H-*3a,7a-epoxy-1*H-*6,11b-methano-3*H-*furo[3,4-*e*][3]benzoxocin-9(10*H*),3'(6'*H*)-[2*H*]-pyran]-1,4,6'-trione*; **1**) *and '1,2-Dihydro-acetoxydehydroaustin B'* (=*(3*R,*3'*S,*3a*R,*6*S,*7*R,*7a*R,*11*R,*11a*S,*11b*S*)-7,11-Bis(acetyloxy)hexahydro-2',2',3,6,11a-pentamethyl-8,12-bis(methylene)spiro[4*H,*8*H-*3a,7a-epoxy-1*H-*6,11b-methano-3*H-*furo[3,4-*e*][3]benzoxocin-9(10*H*),3'(6'*H*)-[2*H*]pyran]-1,4,6'-trione*; **2**): Colorless square crystals. M.p. >300°. $[\alpha]_D^{25} = +49 \pm 2$ ($c = 0.047$, MeOH). CD (MeOH): 236 (70.2). UV (MeOH): 210 (3.7), 236 (3.2). IR (KBr): 3004, 2983, 2946, 1780–1723, 1215, 1047. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 440 (72), 176 (74), 174 (100). Compound **1**: HR-EI-MS: 556.1941 ($C_{29}H_{32}O_{11}^+$, M^+; calc. 556.1939). Compound **2**: HR-EI-MS: 558.2094 ($C_{29}H_{34}O_{11}^+$, M^+; calc. 558.2096).

X-Ray Crystallography. The X-ray diffraction data for the crystal was collected with an *Oxford-Diffraction-Xcalibur-Nova* diffractometer with CuK_α radiation (*Fig. 2* and *Table 3*). The structures were solved by direct methods with SHELXTLV5.0 (*Siemens Industrial Automation Inc.* Madison, WI) and refined by using full-matrix least-squares difference *Fourier* techniques. All non-H-atoms were refined with anisotropic displacement parameters, and all H-atoms were placed with the relative isotropic parameters. Absorption corrections were applied with the *Siemens* area detector absorption (SADABS) program.

Cytotoxicity Assays. Cytotoxicity of the crystal against cancer cell lines was tested by using the MTT (=2-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay. The EPI (*epirubicin*) was used as positive control. The activities on α-glucosidase and tyrosinase were operated according to the slightly modified method reported in the literature [7][8].

Table 3. *X-Ray Crystal and Refinement Data of the Mixture* **1/2**[a])

Solvent for crystallization	acetone/MeOH *ca.* 4 : 1
Empirical formula	$C_{29}H_{33.3}O_{11}$
M_r	557.86
Crystal size	$0.43 \times 0.40 \times 0.32$ mm
Unit-cell dimensions:	
a [Å]	8.92780(10)
b [Å]	12.42850(10)
c [Å]	12.11670(10)
α [°]	90.00
β [°]	98.83(10)
γ [°]	90.00
V [Å^3]	1328.52(2)
Wavelength [Å]	1.54178
Crystal system	Monoclinic
Space group	$P2(1)$
Z	2
Temp. [K]	150(2)
Calc. density [Mg/m^3]	1.395
Absorption coefficient [mm^{-1}]	0.899
θ-Range [°] for data collection	3.69 – 71.50
F (000)	591
Refinement method	Full-matrix least-squares on F^2
Data, restraints, parameters	5129, 1, 387
Reflections collected/unique	26005/5129 (R(int) = 0.0307)
Goodness-of-fit on F^2	1.038
Final R indices ($I > 2\sigma(I)$)	$R_1 = 0.0342$, $wR_2 = 0.0883$
R Indices (all data)	$R_1 = 0.0358$, $wR_2 = 0.0897$
Absolute structure parameter	0.01(11)
Largest diff. peak and hole	0.201, −0.165 e Å^{-3}
Limiting indices	$-10 \le h \le 10$, $-15 \le k \le 15$, $-14 \le l \le 14$

[a]) CCDC-806951 contains the supplementary crystallographic data for this article. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/data_request/cif.

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Received March 15, 2011