Secondary Metabolites from the Fungus *Chaetomium brasiliense*

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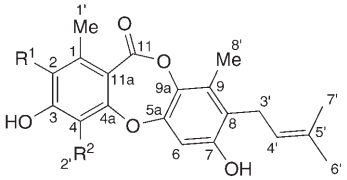
Two new depsidones, mollicellins I and J (**1** and **2**, resp.), and a new chromone, 2-(hydroxymethyl)-6-methylmethyleugenin (**3**), along with six known compounds, **4**–**9**, were isolated from the ethyl acetate extract of a solid-state fermented culture of *Chaetomium brasiliense*. Their structures were elucidated based on spectroscopic analysis. Mollicellins I and H (**5**) exhibited significant growth inhibitory activity against human breast cancer (Bre04), human lung (Lu04), and human neuroma (N04) cell lines with GI_{50} values between 2.5–8.6 μg/ml.

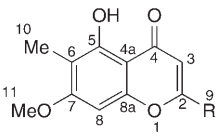
Introduction. – The fungi belonging to the genus *Chaetomium* with *ca.* 100 species are widely distributed in soil, air, plants, and animals [1]. The fungi of *Chaetomium* produce many classes of secondary metabolites with various biological properties such as antifungal, immunomodulatory, cytotoxic, and antitumor effects [2–8]. In this study on *C. brasiliense*, two new depsidones, mollicellins I and J (**1** and **2**, resp.), and a new chromone, 2-(hydroxymethyl)-6-methylmethyleugenin (**3**), were isolated, along with mollicellin D (**4**) [9], mollicellin H (**5**) [9], eugenetin (**6**) [10], *O*-methylsterigmatocystin (**7**) [11], sterigmatocystin (**8**) [12], and chaetocin (**9**) [13]. We herein report the isolation and structure elucidation of compounds **1**–**3**, and cytotoxic activities of compounds **1**, **2**, and **5**.

Results and Discussion. – 1. *Structure Elucidation.* Compound **1** was isolated as a white amorphous powder. Its molecular formula $C_{21}H_{22}O_6$ was established by the quasi-molecular ion peak at *m*/*z* 393.1299 ($[M+Na]^+$) in the HR-ESI-MS. The IR spectrum suggested the presence of OH groups ($\tilde{\nu}_{max}$ 3389 cm^{-1}). The ^{13}C-NMR spectrum showed 21 signals (*Table 1*). The IR band at $\tilde{\nu}_{max}$ 1704 cm^{-1} and the ^{13}C-NMR signal at δ(C) 163.9 (C(11)) indicated the presence of a conjugated ester C=O group. A prenyl group could be concluded from the ^{1}H-NMR signals at δ(H) 1.59 (*s*, Me(6′))[1]), 1.69 (*s*, Me(7′)), 3.20 (*d*, J = 6.7 Hz, CH_2(3′)), and 4.94 (*t*, J = 6.7 Hz, H–C(4′)) (*Table 2*), and the HMBC correlations of Me(6′) and Me(7′) with C(3′) and C(4′), and CH_2(3′) with C(4′) and C(5′). Two benzene moieties were evident from the twelve ^{13}C-NMR signals between δ(C) 105.7 and 163.9, besides those for one ester C=O C-atom and two olefinic C-atoms in the prenyl group. A Me group at δ(H) 2.30 (Me(1′)) was located at C(1) on the basis of the HMBC correlation of Me(1′) with δ(C) 143.1 (C(1)), 115.6 (C(2)), and 112.3 (C(11a)). The HMBC correlations of δ(H) 4.62 (CH_2(2′)) with δ(C) 117.3 (C(4)), 160.5 (C(3)), and 161.7 (C(4a)), and H–C(2) with C(1), C(3), C(4), and

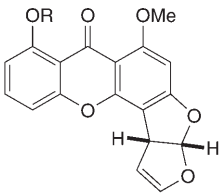
[1]) Arbitrary numbering. For systematic names, see *Exper. Part.*



1 R^1 = H, R^2 = CH_2OH
2 R^1 = Cl, R^2 = CHO
4 R^1 = Cl, R^2 = CH_2OH
5 R^1 = H, R^2 = CHO



3 R = CH_2OH
6 R = Me



7 R = Me
8 R = H

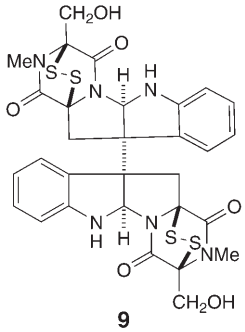


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Table 1. *^{13}C-NMR Data of Compounds* **1**, **2**, **4**, *and* **5**[a])[b])[1])

Position	**1**	**2**	**4**	**5**	Position	**1**	**2**	**4**	**5**
C(1)	143.1	149.5	139.7	151.9	C(11)	163.9	162.7	162.8	162.7
C(2) or H–C(2)	115.6	120.0	119.6	117.4	C(11a)	112.3	114.9	114.8	113.4
C(3)	160.5	161.7	158.0	164.0	Me(1′)	21.1	18.7	17.5	21.8
C(4)	117.3	111.0	115.8	111.9	H–C(2′) or CH_2(2′)	52.3	193.7	56.5	191.7
C(4a)	161.7	160.3	156.2	152.5	CH_2(3′)	25.3	25.0	25.0	25.3
C(5a)	149.0	148.6	149.0	148.6	H–C(4′)	122.6	121.9	122.1	122.4
H–C(6)	105.7	104.4	105.2	105.0	C(5′)	131.2	131.3	131.1	131.4
C(7)	152.3	152.1	151.9	152.6	Me(6′)	25.9	24.9	24.9	25.9
C(8)	124.8	126.0	125.3	125.6	Me(7′)	18.2	17.1	17.1	18.2
C(9)	128.9	129.9	129.3	129.5	Me(8′)	12.7	11.8	11.8	12.8
C(9a)	135.7	135.4	135.8	135.2					

[a]) Assignments based on HSQC and HMBC. [b]) **1** and **5** in (D_6)DMSO, **2** in (D_6)acetone, **4** in $CDCl_3$; 150 MHz.

C(11a) suggested the partial structure **A** (*Fig. 1*). Likewise, the partial structure **B** was determined on the basis of the HMBC and NOESY experiments. The C-atoms at δ(C) 149.0 (C(5a)) and 135.7 (C(9a)) were oxygenated. The remaining C-atom (δ(C) 163.9 (C(11))) could only be adjacent to C(11a). Subunits **A** and **B** could be joined on the basis of strong NOESY correlation between CH_2(2′) and H–C(6), and the degree of unsaturation from the molecular formula.

Compound **2** was obtained as a white amorphous powder. Its molecular formula $C_{21}H_{19}ClO_6$ was deduced from the quasi-molecular ion peaks at *m*/*z* 401.0789 ([*M* –

Table 2. *^{1}H-NMR Data of Compounds* **1**, **2**, **4**, *and* **5**. δ in ppm, J in Hz[a])[b])[1]).

Position	**1**	**2**	**4**	**5**
H–C(2)	6.61 (*s*)	–	–	6.83 (*s*)
H–C(6)	6.76 (*s*)	6.87 (*s*)	6.77 (*s*)	6.80 (*s*)
Me(1′)	2.30 (*s*)	2.55 (*s*)	2.43 (*s*)	2.42 (*s*)
H–C(2′) or CH_2(2′)	4.62 (*s*)	10.63 (*s*)	5.14 (*s*)	10.05 (*s*)
CH_2(3′)	3.20 (*d*, J = 6.7)	3.35 (*d*, J = 6.7)	3.33 (*d*, J = 6.7)	3.23 (*d*, J = 6.7)
H–C(4′)	4.94 (*t*, J = 6.7)	5.02 (*t*, J = 6.7)	5.02 (*t*, J = 6.7)	4.96 (*t*, J = 6.7)
Me(6′)	1.59 (*s*)	1.64 (*s*)	1.63 (*s*)	1.61 (*s*)
Me(7′)	1.69 (*s*)	1.76 (*s*)	1.75 (*s*)	1.71 (*s*)
Me(8′)	2.15 (*s*)	2.27 (*s*)	2.24 (*s*)	2.19 (*s*)

[a]) Assignments based on HSQC and HMBC. [b]) **1** and **5** in (D_6)DMSO, **2** in (D_6)acetone, **4** in $CDCl_3$; 600 MHz.

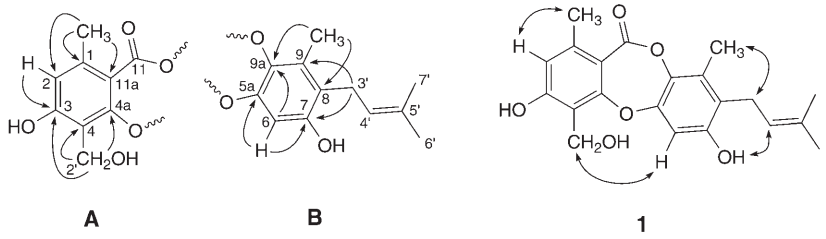

Fig. 1. *Key HMBC correlations* (H → C) *of substructures* **A** *and* **B**, *and NOESY* (↔) *correlations of mollicellin I* (**1**)

H]$^-$) and 403.0775 ([M – H]$^-$) with a relative intensity ratio of *ca.* 3 : 1 in the HR-ESI-MS. The NMR spectra of compound **2** were very similar to those of **1**. However, the CH_2OH group (CH_2(2′))[1]) at C(4) in compound **1** was replaced by a CHO group (δ(H) 10.63, δ(C) 193.7) in **2**, which was supported by the HMBC correlation of H–C(2′) with δ(C) 161.7 (C(3)) and δ(C) 160.3 (C(4a)). Meanwhile, the Cl–C(2) moiety was determined by comparing its NMR data with those of compounds **1**, **4**, and **5**, and the HMBC correlations of Me(1′) with two quaternary olefinic C-atoms (δ(C) 120.0 (C(2)) and 114.9 (C(11a)); *Tables 1* and *2*). The structure of **2** was finally elucidated by HSQC, HMBC, and NOESY experiments (*Fig. 2*).

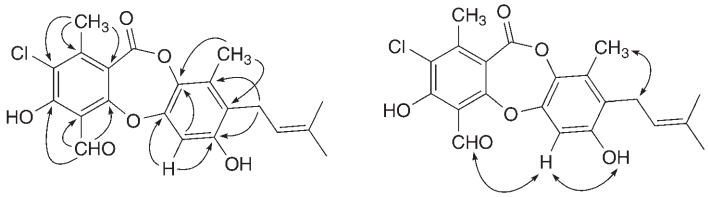

Fig. 2. *Key HMBC* (H → C) *and NOESY* (↔) *correlations of mollicellin J* (**2**)

Compound **3** has a molecular formula of $C_{12}H_{12}O_5$ determined from the HR-ESI-MS quasi-molecular ion peak at *m/z* 259.0568 ($[M+Na]^+$). The IR spectrum of **3** suggested the presence of OH groups ($\tilde{\nu}_{max}$ 3369, 3218 cm^{-1}). The ^{13}C-NMR spectrum showed twelve signals. The UV absorptions at λ_{max} 208 (4.32), 231 (4.12), 257 (4.03), and 293 (3.83) nm, the IR absorption at $\tilde{\nu}_{max}$ 1662 cm^{-1}, and the ^{13}C-NMR signal at δ(C) 182.4 (C(4)) indicated that compound **3** was a benzopyranone derivative, similar to compound **6**. However, C(2) was substituted with a CH_2OH group in compound **3** instead of the Me group in **6**. The bathochromic shift of UV absorptions resulting from the addition of $AlCl_3$ suggested a chelated OH group at C(5). The structure of **3** was elucidated by HMBC and NOESY experiments (*Fig. 3*).

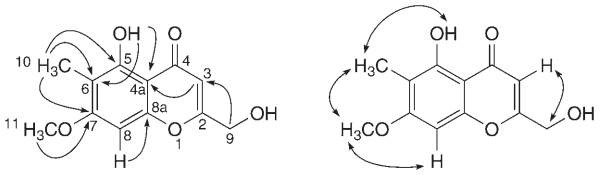

Fig. 3. *Key HMBC* (H → C) *and NOESY* (↔) *correlations of compound* **3**

The structures of the known compounds **4**–**5** were determined by HSQC, HMBC, and NOESY experiments. Compounds **6**–**9** were determined by comparing their physical and spectral data with those reported [10–13].

2. *Biological Studies.* Compounds **1**, **2**, and **5** were evaluated for their cytotoxic properties against human breast cancer (Bre04), and human lung (Lu04) and human neuroma (N04) cell lines (*Table 3*). Compounds **2** and **5** exhibited significant activities. It seems that the HCO group at C(4) is essential for the cytotoxicity.

Table 3. GI_{50} *Values* [μg/ml] *of Compounds* **1**, **2**, *and* **5**

Compounds	Bre04	Lu04	N04
1	> 10.0	> 10.0	> 10.0
2	5.9	8.6	3.8
5	5.1	6.5	2.5
Taxol	0.04	0.03	0.04

Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh, and 10–40 μm; *Qingdao Marine Chemical Inc.*). UV Spectra: *Perkin-Elmer Lambda 35* UV/VIS spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Perkin-Elmer Spectrum One* FT-IR spectrometer, KBr pellets; in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker AM-600* spectrometer; δ in ppm rel. to $SiMe_4$ (=0 ppm), *J* in Hz ESI- and HR-ESI-MS: *BioTOF-Q* mass spectrometer; in *m/z* (rel. %).

Microorganism and Fermentation. C. brasiliense (C. 3.396) was obtained from the Institute of Microbiology, the Chinese Academy of Sciences. It was maintained on potato dextrose agar slant (PDA) at 4° and was stocked in Chengdu Institute of Biology of the Chinese Academy of Sciences. The seed culture medium was comprised of dextrose (20 g/l), yeast extract (1 g/l), KH_2PO_4 (3 g/l), $MgSO_4 \cdot 7\ H_2O$ (1.5 g/l), and potato extract prepared by extracting 200 g of potato with 1 l of boiling H_2O for 20 min. The

pH of the medium was adjusted to 6.0 with 1M NaOH (aq.). The solid culture medium was comprised of rice and 0.1% peptone. The sterilization was carried out at 121° under 15 psi for 30 min.

The fresh mycelium grown on PDA slant at 28° for 3 d was inoculated into 500-ml flasks containing 100 ml of sterilized seed medium. Flasks with inoculated medium were placed in a rotary shaker at 28° and incubated at 180 rpm for 2 d. The seed culture was inoculated into sterilized solid medium for further fermentation at 28° for 25 d.

Extraction and Isolation. The fermented solid medium (4.0 kg) was soaked with AcOEt (8 l × 1, 3 d) at r.t. The solvent was evaporated *in vacuo* to afford a residue (24 g). The residue was separated by CC (silica gel (300 g), ∅ 70 mm × 160 mm; petroleum ether/acetone 10:1, 5:1, 2:1, 1:1, 0:1, each 1000 ml) to yield *Fractions A* (9.5 g), *B* (6.3 g), *C* (3.1 g), *D* (4.5 g), and *E* (1.5 g). The separation of *Fr. B* by CC (silica gel (240 g), ∅ 64 mm × 150 mm; petroleum ether/AcOEt 4:1, 2:1, each 900 ml) yielded *Fr. BA* (2.6 g), *BB* (1.3 g), *BC* (0.8 g), and *BD* (1.0 g). Separation of *Fr. BB* by CC (silica gel (65 g), ∅ 32 mm × 160 mm; petroleum ether/AcOEt 3.5:1, 450 ml) afforded compound **6** (25 mg). *Fr. C* was separated by CC (silica gel (150 g), ∅ 50 mm × 150 mm; petroleum ether/AcOEt 3:1, 2:1, each 800 ml) to afford three subfractions *CA* (550 mg), *CB* (800 mg) and *CC* (620 mg). *Fr. CA* was separated by CC (silica gel column (25 g), ∅ 20 mm × 170 mm; petroleum ether/acetone 6:1, 700 ml) to yield compounds **4** (4 mg), **5** (105 mg), and **3** (8 mg). *Fr. CB* was subjected to silica gel CC (petroleum ether/acetone 4:1, 500 ml) to give **1** (35 mg) and **5** (58 mg). *Fr. CC* was separated by CC (silica gel (75 g), ∅ 30 mm × 200 mm; $CHCl_3$/acetone 10:1, 550 ml) to give **2** (15 mg). *Fr. D* (4.5 g) was separated by CC (silica gel (130 g), ∅ 16 mm × 220 mm; $CHCl_3$/acetone 15:1, 10:1, 5:1, each 1000 ml) to give **9** (310 mg).

Mollicellin I (= *3,7-Dihydroxy-4-(hydroxymethyl)-1,9-dimethyl-8-(3-methylbut-2-enyl)-11*H-*dibenzo[*b,e*][1,4]dioxepin-11-one*; **1**). White amorphous powder. UV (MeOH): 206 (4.79), 269 (4.16). IR (KBr): 3389, 3253, 2920, 1704, 1642, 1608, 1437, 1276. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS (pos.): 393.1299 ($[M+Na]^+$, $C_{21}H_{22}NaO_6^+$; calc. 393.1309).

Mollicellin J (= *2-Chloro-3,7-dihydroxy-1,9-dimethyl-8-(3-methylbut-2-enyl)-11-oxo-11*H-*dibenzo-[*b,e*][1,4]dioxepin-4-carboxaldehyde*; **2**). White amorphous powder. UV (MeOH): 205 (4.59), 267 (3.93). IR (KBr): 3414, 2913, 1712, 1637, 1602, 1558, 1443, 1259, 842. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS (pos.): 401.0789 ($[M-H]^-$, $C_{21}H_{18}{}^{35}ClO_6^-$; calc. 401.0786), 403.0775 ($[M-H]^-$, $C_{21}H_{18}{}^{37}ClO_6^-$).

2-(Hydroxymethyl)-6-methylmethyleugenin (= *5-Hydroxy-2-(hydroxymethyl)-7-methoxy-6-methyl-4*H-*1-benzopyran-4-one*; **3**). White amorphous powder. UV (MeOH): 208 (4.32), 231 (4.12), 257 (4.03), 293 (3.83). UV (MeOH–$AlCl_3$): 211 (4.38), 239 (4.17), 268 (4.08), 312 (3.88). IR (KBr): 3369, 3218, 3086, 1662, 1631, 1577, 1498, 1463, 1328, 1140, 734. ^{1}H-NMR ($CDCl_3$, 600 MHz): 12.67 (*s*, OH); 6.38 (*s*, H–C(8)); 6.32 (*s*, H–C(3)); 4.57 (*s*, CH_2(9)); 3.88 (*s*, Me(11)); 2.09 (*s*, Me(10)). ^{13}C-NMR ($CDCl_3$, 150 MHz): 182.4 (C=O); 167.1 (C(2)); 163.5 (C(7)); 158.6 (C(5)); 156.0 (C(8a)); 109.3 (C(6)); 107.1 (C(3)); 105.5 (C(4a)); 89.3 (C(8)); 61.5 (C(9)); 55.9 (C(11)); 7.2 (C(10)). HR-ESI-MS (pos.): 259.0568 ($[M+Na]^+$, $C_{12}H_{12}NaO_5^+$; calc. 259.0577).

Mollicellin D (= *2-Chloro-3,7-dihydroxy-4-(hydroxymethyl)-1,9-dimethyl-8-(3-methylbut-2-enyl)-11*H-*dibenzo[*b,e*][1,4]dioxepin-11-one*; **4**): White amorphous powder. UV (MeOH): 206 (4.55), 267 (3.91). IR (KBr): 3397, 3265, 2925, 1700, 1640, 1605, 1440, 1270. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS (pos.): 427.0929 ($[M+Na]^+$, $C_{21}H_{20}{}^{35}ClNaO_6^+$; calc. 427.0919), 427.0929 ($[M+Na]^+$, $C_{21}H_{20}{}^{37}ClNaO_6^+$).

Mollicellin H (= *3,7-Dihydroxy-1,9-dimethyl-8-(3-methylbut-2-enyl)-11-oxo-11*H-*dibenzo[*b,e*][1,4]-dioxepin-4-carbaldehyde*, **5**): White amorphous powder. UV (MeOH): 205 (4.76), 269 (3.98). IR (KBr): 3375, 1696, 1661, 1624, 1598, 1568, 1439, 1278. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS (pos.): 391.1169 ($[M+Na]^+$, $C_{21}H_{20}NaO_6^+$; calc. 391.1152).

Cytotoxicity Assay. Cancer cell lines Bre04 (MDA-MB-231), Lu04 (NCI-H460) and N04 (SF-268) were obtained from the *American Type Culture Collection* (ATCC) and cultured according to the supplier's instruction. The cells were seeded into 96-well plates, incubated for 16 h at 37°, and treated with compounds **1**, **2**, and **5** at different concentrations for 48 h. Taxol was used as positive control. The cytotoxic activities were examined by means of colorimetric chemosensitivity assay with SRB (sulforodhamine B). The GI_{50} value (the drug concentration required to inhibit the cell growth by 50%) was used as a parameter for cytotoxicity [14][15].

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