Four 6*H*-Dibenzo[*b,d*]pyran-6-one Derivatives Produced by the Endophyte *Cephalosporium acremonium* IFB-E007

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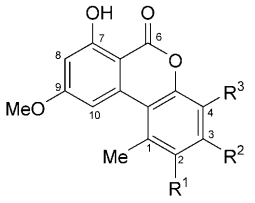
Two novel 6*H*-dibenzo[*b,d*]pyran-6-one derivatives, graphislactone G (**1**) and graphislactone H (**2**), besides graphislactone A (**3**) and alternariol monomethyl ether (**4**) were isolated from the $CHCl_3$/MeOH 1:1 extract of the solid-substrate culture of *Cephalosporium acremonium* IFB-E007, an endophytic fungus in *Trachelospermum jasminoides* (LINDL.) LEM. The structures of compounds **1** and **2** were unambiguously established as 2-chloro-7-hydroxy-3,9-dimethoxy-1-methyl-6*H*-dibenzo[*b,d*]pyran-6-one and 7-hydroxy-3,4,9-trimethoxy-1-methyl-6*H*-dibenzo[*b,d*]pyran-6-one, respectively, by a combination of spectroscopic analyses. Compound **1** was unique in its bearing a Cl-atom. Anticancer tests showed that compounds **1**–**4** had pronounced activities against SW1116 cell with IC_{50} values of 21, 12, 8.5, and 14 μg ml^{-1}, respectively.

Introduction. – Endophytic fungi, residing almost ubiquitously inside the fresh healthy tissue of plants, have been accepted as a big but nearly untapped microbial reservoir that can be expected to provide a wide variety of structurally unique and/or biologically potent natural products [1]. In continuation of our previous chemical and biological investiations of endophyte-generated metabolites [2][3], an endophytic fungal strain, identified as *Cephalosporium acremonium* IFB-E007, isolated from the fresh normal roots of *Trachelospermum jasminoides*, was ascertained to be able to produce bioactive metabolite(s) during its solid-substrate fermentation. The subsequent bioassay-directed fractionation of the $CHCl_3$/MeOH 1:1 extract of the solid-substrate culture of *C. acremonium* IFB-E007 led to the isolation of two novel 6*H*-dibenzo[*b,d*]-pyran-6-one derivatives, graphislactone G (**1**) and graphislactone H (**2**), besides graphislactone A (**3**) and alternariol monomethyl ether (**4**). The 6*H*-dibenzo[*b,d*]pyran-6-one derivatives graphislactone A–F had been isolated from cultured lichen mycobionts of *Graphis prunicola*, *G. cognata* and *G. scripta* [4][5], and the C-skeleton of 6*H*-dibenzo[*b,d*]pyran-6-one derivatives was established to be biosynthesized *via* a common pathway to a single heptaketide [4][6].



1 R^1 = Cl, R^2 = MeO, R^3 = H
2 R^1 = H, R^2 = MeO, R^3 = MeO
3 R^1 = H, R^2 = MeO, R^3 = OH
4 R^1 = H, R^2 = OH, R^3 = H

The structures of the known compounds graphislatone A (**3**) and alternariol monomethyl ether (**4**) were assigned by spectroscopic comparisons with literature data [4][7]. Structure arguments for the new compounds **1** and **2** are detailed below.

Results and Discussion. – Compound **1**, called graphislactone G, was isolated as light purple amorphous powder and had a molecular formula $C_{16}H_{13}ClO_5$ requiring 10 double-bond equivalents, as deduced from the molecular-ion peak at *m/z* 320.0430 (M^+) in the HR-EI-MS. Its IR bands at 3425, 1667, 1628, 1595, and 1580 cm^{-1} suggested the presence of OH group(s), a chelated carbonyl group and a substituted aromatic skeleton. The ^{1}H- and ^{13}C-NMR (*Table*), HMQC, and HMBC and NOED data (*Figure*) of **1** and comparison with graphislactone A (**3**) [4] and alternariol monomethyl ether (**4**) [6] established the structure of **1** as 2-chloro-7-hydroxy-3,9-dimethoxy-1-methyl-6*H*-dibenzo[*b,d*]pyran-6-one.

Table. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**. In $CDCl_3$, δ in ppm, *J* in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
C(1)		136.5 (*s*)		131.7 (*s*)
C(2) or H–C(2)		122.3 (*s*)	6.73 (*s*)	113.0 (*d*)
C(3)		157.2 (*s*)		152.6 (*s*)
H–C(4) or C(4)	6.80 (*s*)	99.7 (*d*)		135.2 (*s*)
C(4a)		156.1 (*s*)		146.0 (*s*)
C(6)		165.8 (*s*)		165.0 (*s*)
C(6a)		100.5 (*s*)		99.2 (*s*)
C(7)		165.9 (*s*)		165.2 (*s*)
H–C(8)	6.56 (*d*, *J* = 2.1)	100.2 (*d*)	6.53 (*d*, *J* = 2.0)	99.1 (*d*)
C(9)		167.2 (*s*)		166.3 (*s*)
H–C(10)	7.19 (*d*, *J* = 2.1)	106.3 (*d*)	7.22 (*d*, *J* = 2.0)	104.8 (*d*)
C(10a)		137.9 (*s*)		138.0 (*s*)
C(10b)		112.9 (*s*)		111.7 (*s*)
Me–C(1)	2.88 (*s*)	22.0 (*q*)	2.78 (*s*)	25.7 (*q*)
MeO–C(3)	3.96 (*s*)	57.3 (*q*)	3.94 (*s*)	56.1 (*q*)
MeO–C(4)	–	–	3.95 (*s*)	61.5 (*q*)
MeO–C(9)	3.92 (*s*)	56.5 (*q*)	3.90 (*s*)	55.6 (*q*)
OH–C(7)	11.78 (br. *s*)		11.93 (br. *s*)	

The ^{1}H-NMR spectrum of **1** exhibited signals for a Me group at δ(H) 2.88 (*s*), two MeO groups at δ(H) 3.92 and 3.96 (each *s*), a pair of *meta*-positioned aromatic protons at δ(H) 6.56 and 7.19 (each *d*, *J* = 2.1), an aromatic proton at δ(H) 6.80 (*s*) and a chelated phenolic OH group at δ(H) 11.78. NOE Difference spectroscopy revealed cross-peaks between the MeO at δ(H) 3.96 and a signal at δ(H) 6.80, between the MeO at δ(H) 3.92 and the 2*d* at δ(H) 6.56 and 7.19, and between the MeC group at δ(H) 2.88 and the signal at δ(H) 7.19. The ^{13}C-NMR spectrum of **1** indicated 16 C resonances, almost all of which were assigned by HMQC and HMBC (*Figure*) experiments and comparison of the δ(C) with those of **3** [4] and **4** [6].

Compound **2**, called graphislactone H, was obtained as yellowish amorphous powder. Its HR-ESI-MS showed a pseudo-molecular-ion peak at *m/z* 317.1023 ($[M+H]^+$) supporting a molecular formula ($C_{17}H_{16}O_6$) requiring 10 double-bond equivalents. The UV and IR data of **2** were nearly identical to those of **1**, suggesting the presence of the

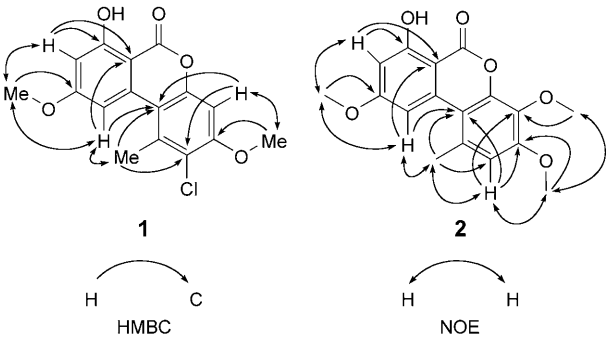


Figure. *Selected HMBC and NOE correlations of* **1** *and* **2**

same molecular skeleton. Further comparison of the ^{1}H- and ^{13}C-NMR spectra of **2** (*Table*) with those of **1** showed that Cl–C(2) and H–C(4) of **1** were replaced by H–C(2) and MeO–C(4) in **2**. This assumption was subsequently confirmed by the HMQC, HMBC, ^{1}H, ^{1}H COSY, and NOE difference data (*Figure*) allowing the exact assignment of all ^{1}H- and ^{13}C-NMR signals. Thus, the structure of **2** was established as 7-hydroxy-3,4,9-trimethoxy-1-methyl-6*H*-dibenzo[*b,d*]pyran- 6-one.

The anticancer tests performed according the reported protocol [8] showed that compounds **1**–**4** had pronounced activities against SW1116 cell with IC_{50} values of 21, 12, 8.5, 14 μg ml^{-1}, respectively. The value of 5-FU co-assayed as positive reference against the tumor cell was 6.0 μg ml^{-1}.

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Experimental Part

General. All chemicals used in the study were of anal. grade. Column chromatography (CC): silica gel (200–300 mesh) from *Qingdao Marine Chemical Factory*, Qingdao, P. R. China. TLC: silica gel GF_{254} from *Qingdas Marine Chemical Factory.* M.p. *XRC-1* apparatus uncorrected. UV Spectra: *Hitachi-UV-3000* spectrometer; λ_{max} in nm. IR Spectra: *Nexus 870* FT-IR; KBr pellets; in cm^{-1}. NMR Spectra: *Bruker-DRX-500* instrument; $CDCl_3$ solns. with $SiMe_4$ as internal standard; chemical shifts δ in ppm and coupling constants J in Hz. HR-EI- and HR-ESI-MS (positive-ion mode): *VG-ZAB-HS* and *Mariner* spectrometer, resp.; in *m/z* (rel. %).

Material. Cephalosporium acremonium IFB-E007 was isolated from the fresh normal roots of *Trachelospermum jasminoides* collected in July, 2002, in the suburb of Nanjing, P. R. China. The identity of the stain was verified by associate Prof. *Yong-chun Song*, and a voucher specimen (IFB-E007) has been deposited in the Institute of Functional Biomolecules, Nanjing University, P. R. China. The culture of *C. acremonium* IFB-007 was obtained according to the process reported in [2].

Extraction and Isolation. The air-dried solid-substrate culture, after roughly milled, was extracted exhaustively with $CHCl_3$/MeOH 1:1. Evaporation of the extract yielded a brown residue (110 g) which was subjected to CC ($CHCl_3$/MeOH mixtures of increasing polarity): 10 combined fractions (TLC monitoring). The combined fractions containing **1**, **2**, **3**, or **4** were resubjected to CC ($CHCl_3$/MeOH gradient). The obtained subfractions were each subjected to gel filtration (*Sephadex LH-20* $CHCl_3$/MeOH 1:1): **1** (12 mg), **2** (15 mg), **3** (18 mg), and **4** (30 mg).

Graphislactone G (=*2-Chloro-7-hydroxy-3,9-dimethoxy-1-methyl-6*H- *dibenzo[*b,d*]pyran-6-one*; **1**): Light purple amorphous powder. M.p. 242–244°. UV (MeOH): 346 (3.95), 334 (3.96), 304 (3.52), 258 (4.58). FT-IR (KBr): 3165.4, 3082.4, 2943.8, 2851.3, 1735.9, 1667.0, 1627.6, 1595.7, 1580.7, 1459.2, 1420.6, 1389.7, 1349.8, 1280.4, 1234.2, 1203.3, 1160.8, 836.4, 823.7, 810.6, 771.0. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS: 322 (40, $[M+2]^+$), 320 (100, $[M]^+$), 285 (4, $[M-Cl]^+$), 277 (21, $[M-CO-Me]^+$). HR-EI-MS: 320.0430 ($C_{16}H_{13}ClO_5^+$; calc. 320.0452).

Graphislactone H (=*7-Hydroxy-3,4,9-trimethoxy-1-methyl-6*H-*dibenzo[*b,d*] pyran-6-one*; **2**): Yellowish amorphous powder. M.p. 165–166°. UV (MeOH): 340 (3.82), 332 (3.80), 298 (3.78), 260 (4.25). FT-IR (KBr): 2964.6, 2928.0, 2846.3, 1736.9, 1664.9, 1626.5, 1602.0, 1571.0, 1460.0, 1440.0, 1401.1, 1349.4, 1248.5, 1239.8, 1210.7, 1138.0, 830.2, 798.4, 780.5. ^{1}H- and ^{13}C-NMR: *Table.* HR-ESI-MS: 317.1023 ($C_{17}H_{17}O_6^+$; calc. 317.1020).

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