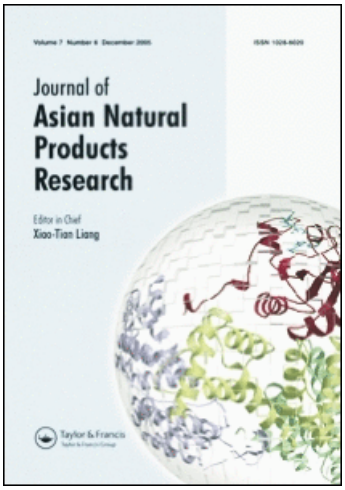


Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

A new compound, brefeldin A formylate, from *Penicillium* sp. strain HLKG-44

Han-Wen Zhang[a]; Hong-Ming Cheng[a]; Hua Fang[b]; Yu-Fen Zhao[acd]; Mei-Juan Fang[c]
[a] Key Laboratory for Chemical Biology of Fujian Province College of Chemistry and Chemical Engineering, Department of Chemistry, Xiamen University, Xiamen, China [b] The Third Institute of Oceanography of the State Oceanic Administration, Xiamen, China [c] Department of Pharmaceutical Science, Medical College, Xiamen University, Xiamen, China [d] The Key Laboratory for Bioorganic Phosphorus Chemistry and Chemical Biology, National Ministry of Education, Department of Chemistry, Tsinghua University, Beijing, China

To cite this Article Zhang, Han-Wen , Cheng, Hong-Ming , Fang, Hua , Zhao, Yu-Fen and Fang, Mei-Juan(2009) 'A new compound, brefeldin A formylate, from *Penicillium* sp. strain HLKG-44', Journal of Asian Natural Products Research, 11: 1, 54 — 57
To link to this Article: DOI: 10.1080/10286020802513970
URL: http://dx.doi.org/10.1080/10286020802513970

Journal of Asian Natural Products Research
Vol. 11, No. 1, January 2009, 54–57

Taylor & Francis
Taylor & Francis Group

A new compound, brefeldin A formylate, from *Penicillium* sp. strain HLKG-44

Han-Wen Zhang[a], Hong-Ming Cheng[a], Hua Fang[c], Yu-Fen Zhao[abd] and Mei-Juan Fang[b]*

[a]*Key Laboratory for Chemical Biology of Fujian Province College of Chemistry and Chemical Engineering, Department of Chemistry, Xiamen University, Xiamen, China;* [b]*Department of Pharmaceutical Science, Medical College, Xiamen University, Xiamen, China;* [c]*The Third Institute of Oceanography of the State Oceanic Administration, Xiamen, China;* [d]*The Key Laboratory for Bioorganic Phosphorus Chemistry and Chemical Biology, National Ministry of Education, Department of Chemistry, Tsinghua University, Beijing, China*

(*Received 16 April 2008; final version received 18 September 2008*)

A novel compound named as brefeldin A formylate (**1**), together with two known compounds, brefeldin A (**2**) and ergosterol (**3**), was isolated from the *Penicillium* sp. strain HLKG-44, which was isolated from polluted environment in Fujian Province. Their structures were identified based on the spectral and X-ray crystallographic analyses. The compound **1**, brefeldin A formylate, exhibited moderate cytotoxic activity against the human lung cancer cell line A549 with IC_{50} value of 18.9 μg/ml by the MTT assay protocol.

Keywords: *Penicillium*; brefeldin A formylate; crystal structure

1. Introduction

Less attention was focused on the fungi that were isolated from an extreme environment, such as a polluted environment, which might provide a good alternative to search for useful natural product [1]. *Penicillium* sp. strain HLKG-44 is an endophytic fungus isolated from a highly contaminated river in southern China. The extracts of the fungus show high cytotoxicity against several human cancer cell lines, such as KB cell (IC_{50}, 0.028 μg/ml) and Raji cell (IC_{50}, 0.035 μg/ml). Little work has been carried out on the fungal genus, *Penicillium*. During initial investigations into the metabolites of this species, we have isolated three compounds, including a new compound, named as brefeldin A formylate (**1**), and two known compounds, brefeldin A (**2**) and ergosterol (**3**). Compound **2** is a macrocyclic lactone fungal metabolite exhibiting a wide range of antifungal, antiviral, antimitotic, and antitumor activities [2]. It has attracted research interest for many years due to its peculiar molecular structure and its bioactivity [3,4]. Here, we find brefeldin A formylate for the first time from *Penicillium* sp. strain HLKG-44. Brefeldin A formylate also shows antitumor activity. In the present paper, we report the isolation and structural elucidation of the new compound **1**, together with two known compounds, brefeldin A and ergosterol. The structure of the compound **1** was established by the spectral and X-ray crystallographic analyses (Figure 1).

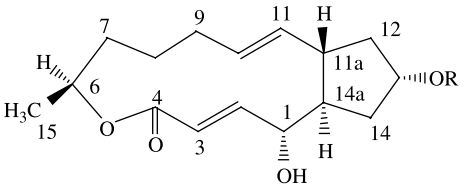
2. Results and discussion

Compound **1** was isolated as colorless needles with mp 128.6–130.3°C and $[\alpha]_D^{28} = +67.5$

*Corresponding author. Email: fangmj@xmu.edu.cn

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020802513970
http://www.informaworld.com



1: R = CHO; **2**: R = H

Figure 1. The structures of compounds **1** and **2**.

(*c* 1.0, $CHCl_3$). Its molecular formula was established as $C_{17}H_{24}O_5$ by ESI-MS at *m/z* 331 $[M + Na]^+$ and elemental analysis. Its IR spectrum showed the absorption bands due to the hydroxyl (3451 cm^{-1}) and the α, β-unsaturated lactone (1711 cm^{-1}) groups. The ^1H NMR spectrum of compound **1** displayed signals for four olefinic methine protons at δ_H 7.34, 5.92, 5.70, and 5.22, three methine protons at δ_H 5.29, 4.86, and 4.13 linked to the oxygen atom (—CH—O—), two methine protons at δ_H 2.41 and 1.93, 10 methylene protons (Table 1), and one methyl at δ_H 1.26.

The ^{13}C NMR (DEPT) spectrum of compound **1** showed 17 signals consisted of 1 × CH_3, 5 × CH_2, 10 × CH groups, including one formyloxy at δ_C 160.9 and one quaternary C-atom at δ_C 166.4, indicating the existence of the ester and the aldehyde functionality. The ^{13}C NMR spectral data of compound **1** and brefeldin A are listed in Table 1.

Comparison of its ^1H and ^{13}C NMR spectral data with those of compound **2** suggested that compound **1** was the C-13 formylate derivative of compound **2**, which was confirmed by the presence of the ^1H and ^{13}C signals at δ_H 8.01 and δ_C 160.9, as well as the downfield shifts of H-13 and C-13 from δ_H 4.34 to δ_H 5.29 and δ_C 72.6 to δ_C 75.3. Moreover, H-16 and H-13 showed HMBC correlations with C-13 and C-16, respectively (Figure 2). Thus, the structure of compound **1** was determined as brefeldin A formylate, identical with the result obtained from the X-ray structure analysis. The X-ray crystallographic structure is shown in Figure 3.

Table 1. ^1H and ^{13}C NMR spectral data of compound **1** and brefeldin A ($CDCl_3$, δ ppm).

	δ_H (ppm)		δ_C (ppm)	
Position	**1**	Brefeldin A	**1**	Brefeldin A
1	4.13 (m, 1H)	4.10 (m, 1H)	75.9	76.0
2	7.34 (dd, J = 16.0, 3.0 Hz, 1H)	7.35 (dd, J = 16.0, 3.0 Hz, 1H)	151.7	151.5
3	5.92 (d, J = 16.0 Hz, 1H)	5.91 (d, J = 16.0 Hz, 1H)	118.0	117.6
4			166.4	166.6
6	4.86 (m, 1H)	4.86 (m, 1H)	72.0	71.8
7	1.73 (m, 1H)	1.74 (m, 1H)	34.3	34.1
	1.53 (m, 1H)	1.53 (m, 1H)		
8	0.94 (m, 1H)	0.95 (m, 1H)	26.8	26.7
	1.88 (m, 1H)	1.87 (m, 1H)		
9	1.85 (m, 1H)	1.84 (m, 1H)	32.0	31.8
	2.03 (m, 1H)	2.02 (m, 1H)		
10	5.72 (m, 1H)	5.70 (m, 1H)	131.3	130.5
11	5.22 (dd, J = 15.0, 9.0 Hz, 1H)	5.27 (dd, J = 15.0, 9.0 Hz, 1H)	135.9	136.5
11a	2.41 (m, 1H)	2.36 (m, 1H)	44.1	44.3
12	2.34 (m, 1H)	2.20 (m, 1H)	40.2	43.2
	1.63 (m, 1H)	1.51 (m, 1H)		
13	5.29 (m, 1H)	4.34 (m, 1H)	75.3	72.6
14	2.26 (m, 1H)	2.08 (m, 1H)	38.7	41.3
	1.89 (m, 1H)	1.81 (m, 1H)		
14a	1.93 (m, 1H)	1.95 (m, 1H)	52.2	52.0
15	1.26 (d, J = 8.0 Hz, 3H)	1.26 (d, J = 8.0 Hz, 3H)	21.0	20.9
16	8.01 (s, 1H)		160.9	

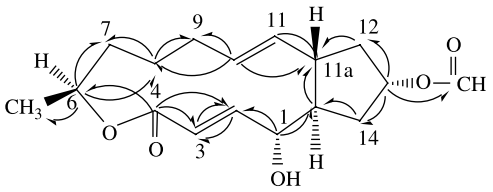


Figure 2. HMBC correlations of compound **1**.

3. Experimental

3.1 General experimental procedures

Melting point was determined on a Yanaco MP-500 micro-melting point apparatus and is uncorrected. Optical rotations were measured using a Perkin-Elmer 341 automatic polarimeter. Infrared spectra were recorded on a Nicolet AVATAR 360 FT-IR spectrophotometer using KBr disks. ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra were obtained with a Bruker AV 400 spectrometer (^{1}H, 400 MHz; ^{13}C, 100 MHz) with TMS as an internal standard. All mass spectra were acquired with a Bruker ESQUIRE-3000 plus ion trap spectrometer equipped with a gas nebulizer probe in the positive ion mode. The X-ray crystallographic study was performed on a Bruker SMART CCD X-ray diffractometer.

3.2 Fungus material

The fungus for production was isolated from a highly contaminated river in southern China. In the present experiment, the fungus strain was cultured on potato dextrose agar medium with gentamicin for 7 days at 25°C. The identification of the strain was performed by Prof. Yao-Jian Huang, the Key Laboratory of National Ministry of Education for Cell Biology and Tumor Cell, Engineering and School of Life Science, Xiamen University.

3.3 Extraction and isolation

The fungus material was extracted with CH_3OH/AcOEt/AcOH (15:75:10), and the mixed extract was concentrated under reduced pressure, to obtain a crude residue (19.2 g), which was subjected to silica gel column chromatography and eluted with a gradient solvent system of petroleum ether and dichloromethane (10:0 → 0:10) and ethyl

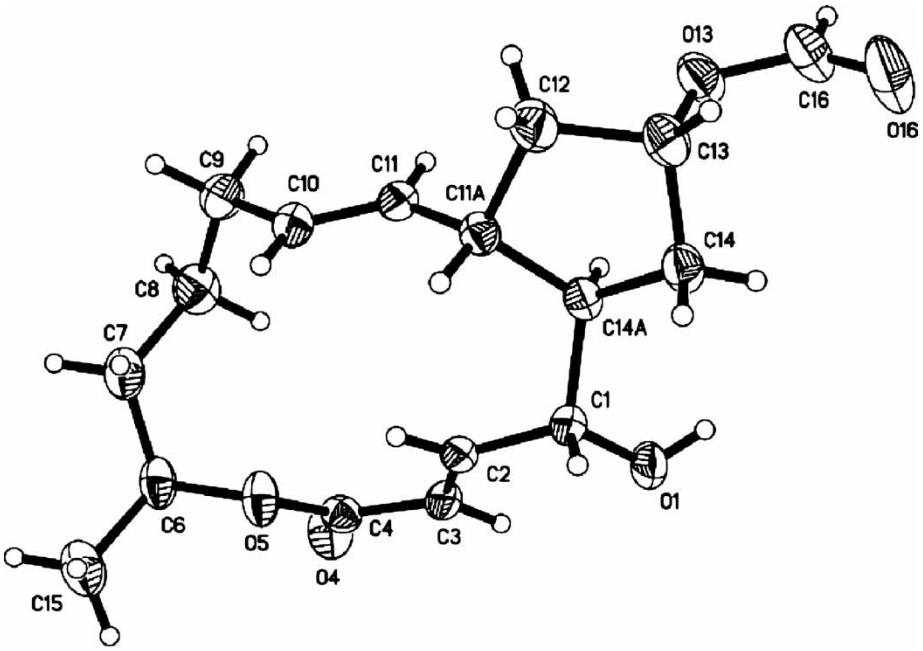


Figure 3. ORTEP drawing of the compound **1**.

acetate and methanol (10:0 → 5:5) to give eight fractions. Fractions 4, 5, and 7 were further subjected to silica gel flash column chromatography to afford compounds **3** (ethyl acetate/dichloromethane = 1:2, 65 mg), **1** (petroleum ether/ethyl acetate = 4:1, 316 mg), and **2** (petroleum ether/ethyl acetate = 1:3, 35 mg), respectively.

3.3.1 Compound *1*

A colorless crystal; $[\alpha]_D^{28} = +67.5$ (*c* 1.0, $CHCl_3$); UV (CH_3OH) λ_{max}: 223, 265 nm; IR ν_{max}^{KBr} (cm^{-1}): 3451, 2930, 1711, 1257, 1178, 1118, 1070; ^1H and ^{13}C NMR spectral data, see Table 1. ESI-MS *m/z*: 331 $[M + Na]^+$; Elemental analysis: Found: C, 66.55%, H, 7.49%; calcd for $C_{17}H_{24}O_5$: C, 66.21%, H, 7.84%.

3.3.2 Compound *2*

A colorless crystal; ^1H and ^{13}C NMR spectral data, see Table 1. The ^1H and ^{13}C NMR spectral data were consistent with the literature [5]; ESI-MS *m/z*: 281 $[M + H]^+$.

3.3.3 Compound *3*

A colorless crystal; ^1H and ^{13}C NMR spectral data were identified with those reported in literature [6,7]; ESI-MS *m/z*: 397 $[M + H]^+$.

3.4 Crystallographic data of compound 1

$C_{17}H_{24}O_5$, $M = 308.36$, monoclinic, space group $P\,2_1$, $a = 9.901$ (4) Å, $b = 5.818$ (2) Å, $c = 14.803$ (6) Å, $\beta = 103.0$ (7)°, $V = 830.8$ (6) Å^3, $Z = 2$, Dc = 1.233 g/cm^3, F (0 0 0) = 332, colorless sheet. A crystal of dimensions 0.28 × 0.31 × 0.37 mm was used for X-ray measurements on a Bruker SMART CCD X-ray area detector diffractometer at room temperature using Mo Kα radiation ($\lambda = 0.7173$ Å) with ϕ and ω scans. The total number of independent reflections measured was 4117, of which 1605 were considered to be observed ($|F|^2 \geq 2\sigma|F|^2$). The crystal structure was solved by the direct methods yielding the positions of all non-hydrogen atoms, and refined with full-matrix least squares procedure based on F^2 using the SHELX-97 program system. The final indices were $R_1 = 0.0418$, $wR_2 = 0.1002$ for 1605 observed reflections, and 199 parameters. Crystallographic data for the structure has been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 684762).

3.5 Testing for cytotoxic activity against A549 cell

Brefeldin A formylate exhibited high-potent cytotoxic activity against the human lung cancer cell line A549 with IC_{50} value of 18.9 μg/ml by the MTT assay protocol, which was adapted from that described by Mosmann [8].

Acknowledgements

The authors would like to thank the financial support from the National Natural Science Foundation of China (No. 20572061), Foundation of Xiamen University (No. 0070Z03134), and the National Natural Science Foundation of China (No. 20732004).

References

[1] M.J. Fang, H. Fang, Y.J. Huang, and Y.F. Zhao, *Tetrahedron Lett.* **46**, 2147 (2005).
[2] S. Budavari, editor, *The Merck Index*, 12th ed. (Merck, Rahway, NJ, 1996), p. 224, and references cited therein.
[3] G.K. Tamura, K. Ando, S. Suzuki, A. Takai'mki, and K. Arhna, *J. Antibiotics* **l21**, 160 (1968).
[4] A. Takatsuki, L. Yamaguchi, G. Tamura, T. Misato, and K. Arima, *J. Antibiotics* **22**, 442 (1969).
[5] G. Robert, S. Dror, and F. Mark, *Magn. Reson. Chem.* **38**, 274 (2000).
[6] A.N. Starratt, *Phytochemistry* **15**, 2002 (1976).
[7] C. Delseth, *Helv. Chim. Acta* **62**, 2037 (1979).
[8] F. Mosmann, *Immunol. Methods* **65**, 55 (1983).