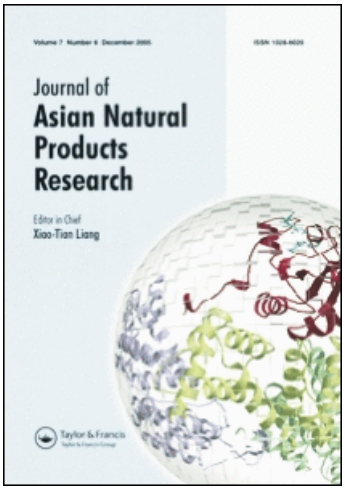

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Cytotoxic macrocyclic trichothecenes from the mycelia of calcarisporium arbuscula preuss

Neng-Jiang Yu[a]; Shun-Xing Guo[a]; Hai-Yan Lu[a]
[a] Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Online publication date: 09 September 2010

To cite this Article Yu, Neng-Jiang , Guo, Shun-Xing and Lu, Hai-Yan(2002) 'Cytotoxic macrocyclic trichothecenes from the mycelia of calcarisporium arbuscula preuss', Journal of Asian Natural Products Research, 4: 3, 179 — 183
To link to this Article: DOI: 10.1080/10286020290011387
URL: http://dx.doi.org/10.1080/10286020290011387

Journal of Asian Natural Products Research, 2002 VOL. 4 (3), pp. 179–183

CYTOTOXIC MACROCYCLIC TRICHOTHECENES FROM THE MYCELIA OF *CALCARISPORIUM ARBUSCULA* PREUSS

NENG-JIANG YU, SHUN-XING GUO* and HAI-YAN LU

Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100094, China

(Received 30 October 2001; Revised 21 December 2001; In final form 2 January 2002)

A new cytotoxic macrocyclic trichothecene calcarisporin B1 (**1**), and two known compounds roridin H (**2**) and roridin J (**3**) were isolated from the cultured mycelia of *Calcarisporium arbuscula* Preuss. The structure of **1** was determined to be 8α-acetoxy roridin H on the basis of spectral data. The cytotoxic activities of **1**–**3** were evaluated *in vitro*.

Keywords: *Calcarisporium arbuscula* Preuss; Macrocyclic trichothecene; Cytotoxic; Calcarisporin B1

INTRODUCTION

Several hundred fungi were isolated from Chinese medicinal plants including *Dendrobium candidum*, *D. noble*, *Ganoderma lucidum*. During the course of an investigation for anticancer agents from these fungi, the crude extract of the cultured mycelia of a fungus identified as *Calcarisporium arbuscula* Preuss showed cytotoxic activities against GLC-82 and HCT-8 cells *in vitro*. Consequently, a large-scale liquid culture collection of *C. arbuscula* has been made from which a new macrocyclic trichothecene calcarisporin B1 (**1**), two known compounds roridin H (**2**) and roridin J (**3**) were isolated. In this report, we give the details of the isolation and structural characterization of compound **1**–**3**, along with the cytotoxic activities *in vitro*.

RESULTS AND DISCUSSION

The dry mycelia of *C. arbuscula* was extracted with MeOH, dispersed in water and partitioned with petroleum ether and CH_2Cl_2. By chromatography on silica gel, a new compound calcarisporin B1 (**1**), two known compounds roridin H (**2**) and roridin J (**3**) were

*Corresponding author. Tel.: +86-10-62899729. Fax: +86-10-62895120. E-mail: sxguo8@hotmail.com

ISSN 1028-6020 print/ISSN 1477-2213 online © 2002 Taylor & Francis Ltd
DOI: 1080/10286020290011387

isolated from the petroleum ether fraction, while a large amount of **1** was isolated from the CH_2Cl_2 fraction. **2** and **3** were isolated from *Calcarisporium* genus for the first time.

Compound **1** was isolated as white amorphous powder, mp $>320°C$. Its molecular formula was determined as $C_{31}H_{38}O_{10}$ by HR-EIMS [*m/z* 570.2452 (calcd. for $C_{31}H_{38}O_{10}$, *m/z* 570.2464)] and TOFMS [*m/z* 593.67 $[M + Na]^+$, 609.63 $[M + K]^+$]. IR spectrum revealed the presence of carbonyl group (1705, 1725 cm^{-1}) and double bond (1650, 1600 cm^{-1}). UV spectrum indicated the presence of conjugated double bonds (210, 246 nm).

^{13}C NMR and DEPT spectra of **1** (see Table I) revealed 31 carbon resonances with 38 directly attached protons. They also indicated the presence of three carbonyl groups (δ 170.8, 166.1, 165.7), four double bonds (δ 155.0, 143.0, 136.3, 134.8, 126.0, 123.9, 118.5, 118.3), an aldehyde acetal group (δ 100.6), and eight oxygenated carbons (δ 81.9, 78.9, 76.5, 73.3, 68.6, 67.1, 65.2, 64.3). The ^1H NMR spectrum (see Table I) exhibited five methyl groups as four singlets (δ 2.27, 1.92, 1.76, 0.83) and a doublet (δ 1.34, $J = 6.0$ Hz), six olefinic protons (δ 7.65, 6.56, 5.95, 5.79, 5.69, 5.63), nine protons directly attached to oxygenated carbons (δ 5.90, 5.52, 5.19, 4.35, 4.39, 4.06, 3.83, 3.75, 3.67) and the characteristic signal of H-13 (δ 3.10, 2.83, AB, $J = 4.0$ Hz) due to the influence of the epoxy ring; Coupling constants showed that the protons δ 7.65 and δ 6.56, 7.65 and δ 5.95, 6.56 and δ 5.79 were coupled to each other, respectively, indicating the presence of 1, 4-disubstituted double bonds. All these evidence suggested **1** to be a macrocyclic trichothecene. Comparison of ^1H, ^{13}C NMR

TABLE I NMR data of compound **1** (400 MHz for ^1HNMR and 100 MHz for ^{13}CNMR in $CDCl_3$)

Position	^1HNMR (*J* in Hz)	^{13}CNMR	COSY correlation	HMBC
2	3.83(1H, d, 5.0)	78.9d	H-3b	C-4,C-5,C-11,C-12
3a	2.48(1H, dd, 8.4, 15.4)	34.6t	H-3b, H-4	C-2,C-5,C-12
3b	2.20(1H, m)			
4	5.90(1H, dd, 8.4, 4.5)	73.3d	H-3a, H-3b	C-12
5		48.9s		
6		42.0s		
7	2.20(2H, m)	26.2t		
8	5.19(1H, d, 4.3)	68.6d	H-7, H-16	C-6,C-9
9		136.3s		
10	5.69(1H, br.d, 5.5)	123.9d	H-11, H-16	C-6,C-8,C-16
11	3.75(1H, d, 5.5)	67.1d	H-10, H-16	C-7,C-9,C-10
12		65.2s		
13	3.10,2.83(2H, AB, 4.0)	47.7t		
14	0.83(3H, s)	7.1q		C-4, C-5, C-6, C-12
15	4.35,4.39(2H, AB, 12.5)	64.3 t		C-5,C-7
16	1.76(3H, s)	20.3q	H-8, H-10, H-11	C-8,C-9,C-10
1′		166.1s		
2′	5.63(1H, s)	118.5d	H-4′a, H-12′	C-4′,C-12′
3′		155.0s		
4′a	2.65(1H, dd, 12.4, 3.3)	47.6t	H-2′, H-5′	C-2′,C-3′,C-5′
4′b	2.20(1H, m)			
5′	5.52(1H, dd, 8.6, 3.3)	100.6d	H-4′	C-4′
6′	4.06(1H, dd, 8.6, 2.6)	81.9d	H-7′, H-8′, H-13′	C-7′,C-8′,C-13′
7′	5.95(1H, dd, 2.6, 15.2)	134.8d	H-6′, H-8′	C-6′,C-9′
8′	7.65(1H, dd, 15.2, 11.3)	126.0d	H-6′, H-7′, H-9′, H-10′	C-6′
9′	6.56(1H, dd, 11.3, 11.3)	143.0d	H-8′, H-10′	C-7′,C-11′
10′	5.79(1H, d, 11.3)	118.3d	H-8′, H-9′	C-8′
11′		165.7s		
12′	2.27(3H, s)	18.1q	H-2′	C-4′
13′	3.67(1H, dq, 8.6, 6.0)	76.5d	H-6′, H-14′	
14′	1.34(3H, d, 6.0)	16.3q	H-13′	C-6′,C-13′
8-Ac	1.92(3H, s)	20.8q		
		170.8s		

Assignments are aided by $^1H-^1H$ COSY and HMBC data. Multiplicity of ^{13}CNMR is given from DEPT observations.

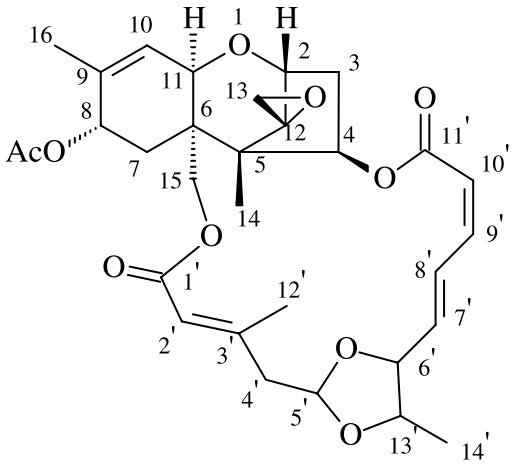

FIGURE 1 Structure of Compound **1**.

spectral data for **1** and **2** suggested that the skeleton of **1** was similar as that of **2**. NMR spectra of **1** showed that the presence of an acetoxy group in the structure according to signals at δ 1.92 (s), 5.19 (d, $J = 4.3$ Hz) and signals at δ 170.8, 20.8. Comparing the signals at δ 68.6d and δ 26.2t of **1** to these at δ 27.6t (C-8) and δ 20.5t (C-7) of **2** suggested that the acetoxy group was at C-8.

The structure of **1** was elucidated by means of 2D NMR experiments (^{1}H–^{1}H COSY, HMQC, HMBC (see Table I)). The signal at δ 68.6 was assigned to C-8 since HMBC showed long-range correlations between the signal at δ 1.76 (H-16) and the signals at δ 68.6, 136.3 (C-9), 123.9 (C-10), respectively. It also indicated that the signal at δ 5.19 (H-8) correlated with the signals at δ 42.0 (C-6) and δ 136.3 (C-9).

According to the literature [1] that $J_{2\beta,\,3\alpha}$, $J_{3\alpha,\,4\beta}$, $J_{7\alpha,\,8\beta}$ were zero due to dihedral angles, the relative stereochemistry of C-8 was thus established as 8β-H since only one hydrogen at C-7 was coupled with H-8 ($J = 4.3$ Hz). Hence, compound **1** was elucidated as 8α-acetoxy roridin H (Fig. 1).

The structures of two known compounds **2** and **3** were identified by spectral comparison with roridin H [2] and roridin J [3,4].

Human tumor cells including BEL-7402, GLC-82 and HCT-8 cell were selected to test the cytotoxic activity of compounds **1**–**3** *in vitro*. It was discovered that the activity against BEL-7402 cell was higher than that against other tumor cells. IC_{50} of compound **1**, **2**, **3** against BEL-7402 was 0.75, 0.50, 0.35 μg/ml, respectively.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were measured on a Fisher–Johns apparatus. Optical rotation was recorded with a Perkin–Elmer 341 polarimeter. IR spectra were measured on a Perkin–Elmer 983G infrared spectrometer as pressed KBr disks. UV spectra were recorded in MeOH using a Philips PYE Unican 8800 spectrophotometer. HR-EIMS spectra were measured on an Autospec-Ultima ETOF mass spectrometer, TOFMS spectra were measured on a BIFLEX III mass spectrometer and EIMS spectra were measured on an AEI MS-50 mass spectrometer at

70 eV. NMR spectra were measured on a Bruker ARX400 spectrometer operating at a basic frequency of 400 MHz, using TMS as the internal standard.

Fungal Material

Fungus *Calcarisporium arbuscula* Preuss (strain BMS-9707) was isolated from *Ganoderma lucidum* in Sichuan province of China in September of 1994 and identified by Zhang Ying. A voucher specimen (No. MP-00202) is deposited in the Department of Mycology, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, China.

Cultivation, Extraction and Isolation

Fungus *C. arbuscula* was cultivated in 500 or 1000 ml rotary shaker on shaking table at 120 rpm for seven days at 25 °C (culture medium: 3% wheat bran, 2% glucose, 0.75‰KH_2PO_4, 1.5‰$MgSO_4$ in water).

About 450 l cultures were collected and filtered into two parts, mycelia and culture filtrate. 2.6 kg of air-dried mycelia were extracted four times with methanol at boiling temperature. The combined extracts were evaporated to give a brown syrup (400 g), which was dispersed in water and partitioned with petroleum ether and CH_2Cl_2 to give two fractions.

The petroleum ether fraction was subjected to silica gel column chromatography, eluting with petroleum ether and increasing proportions of EtOAc. Fractions were combined by monitoring with TLC. Purified by recrystallization in $CHCl_3$–CH_3OH, **1** (250 mg), **2** (430 mg), **3** (65 mg) were obtained. The CH_2Cl_2 fraction was also subjected to silica gel column chromatography, eluting with $CHCl_3$ and increasing proportions of CH_3OH to yield a large amount of **1** (5.5 g).

Calcarisporin B1 (**1**) a white amorphous powder, mp >320°C, $[\alpha]_{20}^{D} + 70.0$ (*c* 0.95, $CHCl_3$). UV $\lambda_{max}^{MeOH}(\log \epsilon)$: 246 (4.23), 210 (4.45) nm. $IR_{max}^{KBr}\,cm^{-1}$: 2970, 1725, 1705, 1650, 1600, 1430, 1370, 1240, 1223, 1188, 1158, 1120, 1072, 998, 970. HREIMS *m/z*: 570.2452 (M^+) (calcd for $C_{31}H_{38}O_{10}$, *m/z* 570.2464). TOFMS *m/z*: 593.67 $[M + Na]^+$, 609.63 $[M + K]^+$. EIMS *m/z* (%): 526, 510, 466, 388 (5), 264 (4), 247 (5), 220 (3), 182 (3), 161 (5), 137 (12), 121 (11), 95 (8), 82 (100). ^{1}HNMR, ^{13}CNMR data, see Table I.

Roridin H(**2**) colorless crystals, mp >320°C. EIMS *m/z*(%): 512 [M^+], 484, 468, 330 (9), 264 (6), 247 (15), 137 (24), 82 (100). ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 7.69 (1H, dd, $J = 15.0$, 11.3 Hz, H-8′), 6.56 (1H, dd, $J = 11.3$, 11.3 Hz, H-9′), 5.95 (1H, dd, $J = 2.4$, 15.0 Hz, H-7′), 5.93 (1H, dd, $J = 8.2$, 4.8 Hz, H-4), 5.79 (1H, d, $J = 11.3$ Hz, H-10′), 5.68 (1H, s, H-2′), 5.53 (1H, dd, $J = 8.4$, 3.2 Hz, H-5′), 5.44 (1H, br.d, $J = 4.2$ Hz, H-10), 4.32 (1H, d, $J = 12.5$ Hz, Ha-15), 4.06 (1H, br.d, $J = 7.0$ Hz, H-6′), 4.04 (1H, d, $J = 12.5$ Hz, Hb-15), 3.84 (1H, d, $J = 5.0$ Hz, H-2), 3.66 (2H, m, H-11,13′), 3.12 (1H, d, $J = 4.0$ Hz, Ha-13), 2.82 (1H, d, $J = 4.0$ Hz, Hb-13), 2.64 (1H, dd, $J = 12.4$, 3.2 Hz, Ha-4′), 2.48 (1H, dd, $J = 8.2$, 15.3 Hz, Ha-3), 2.27 (3H, s, H-12′), 2.25 (1H, m, Hb-4′), 2.16 (1H, ddd, $J = 15.3$, 4.8, 5.0 Hz, Hb-3), 1.90 (4H, m, H-7,8), 1.71 (3H, s, H-16), 1.34 (3H, d, $J = 6.0$ Hz, H-14′), 0.86 (3H, s, H-14); ^{13}CNMR ($CDCl_3$, 100 MHz) δ: 166.3s (C-11′), 166.2s (C-1′), 154.8s (C-3′), 142.8d (C-9′), 140.4s (C-9), 134.8d (C-7′), 126.2d (C-8′), 119.0d (C-2′), 118.8d (C-10′), 118.7d (C-10), 100.9d (C-5′), 81.9d (C-6′), 79.2d (C-2), 76.7d (C-13′), 73.9d (C-4), 67.7d (C-11), 65.5s (C-12), 63.2t (C-15), 49.0s (C-5), 47.9t (C-4′), 47.6t (C-13), 43.2s (C-6), 34.9t (C-3), 27.6t (C-8), 23.3q (C-16), 20.5t (C-7), 18.3q (C-12′), 16.5q (C-14′), 7.3q (C-14). Multiplicity is given from DEPT observations.

Roridin J(**3**) a white amorphous powder, mp 250-253°C. TOFMS *m/z*: $[M + Na]^+$ 551.67. EIMS *m/z* (%): 327 (4), 299 (8), 285 (6), 267 (24), 239 (44), 134 (37), 98 (86), 84 (48).

^{1}HNMR ($CDCl_3$, 400 MHz) δ: 7.65 (1H, dd, $J = 15.0, 11.4$ Hz, H-8′), 6.55 (1H, dd, $J = 11.4, 11.2$ Hz, H-9′), 5.92 (1H, dd, $J = 2.5, 15.0$ Hz, H-7′), 5.89 (1H, m, H-4), 5.79 (1H, d, $J = 11.2$ Hz, H-10′), 5.75 (1H, s, H-2′), 5.44 (1H, br.d, $J = 4.1$ Hz, H-10), 5.24 (1H, d, $J = 7.0$ Hz, H-5′), 4.39 (1H, d, $J = 12.5$ Hz, Ha-15), 4.08 (1H, br.d, $J = 8.7$ Hz, H-6′), 4.01 (1H, d, $J = 12.5$ Hz, Hb-15), 3.84 (1H, d, $J = 4.9$ Hz, H-2), 3.82 (1H, d, $J = 7.0$ Hz, H-4′), 3.64 (1H, m, H-11,13′), 3.12 (1H, d, $J = 4.0$ Hz, Ha-13), 2.81 (1H, d, $J = 4.0$ Hz, Hb-13), 2.47 (1H, dd, $J = 15.3, 8.4$ Hz, Ha-3), 2.31 (1H, br.s, OH-4′), 2.27 (3H, s, H-12′), 2.18 (1H, ddd, $J = 15.3, 4.9, 4.9$ Hz, Hb-3), 1.85-2.02 (4H, m, H-7,8), 1.71 (3H, s, H-16), 1.37 (3H, d, $J = 6.0$ Hz, H-14′), 0.85 (3H, s, H-14). ^{13}CNMR ($CDCl_3$, 100 MHz) δ: 166.2 (C-11′), 165.9 (C-1′), 155.3 (C-3′), 143.1 (C-9′), 140.4 (C-9), 134.5 (C-7′), 126.1 (C-8′), 119.8 (C-2′), 118.8 (C-10′), 118.6 (C-10), 103.3 (C-5′), 82.2 (C-6′), 79.7 (C-4′), 79.1 (C-2), 76.8 (C-13′), 73.8 (C-4), 67.8 (C-11), 65.5 (C-12), 63.4 (C-15), 49.1 (C-5), 47.9 (C-13), 43.2 (C-6), 34.7 (C-3), 27.6 (C-8), 23.3 (C-16), 20.3 (C-7), 15.9 (C-12′), 13.0 (C-14′), 7.4 (C-14).

Cytotoxicity Experiments

Cytotoxicity against human tumor cells was measured in a 5-day MTT test for GLC-82 human lung adenocarcinoma cell line, HCT-8 human ileocecal carcinoma and BEL-7402 human hepatic tumor cells [5,6].

Acknowledgements

We thank Mr Mujiang Lu and Jingqi Pan, Department of Chemistry, Peking University, for NMR measurement; the group of MS laboratory, Institute of Materia Medica, CAMS and PUMC, for HREIMS measurement; Mrs Weihua Hu and Mr Shaoxiang Xiong, Institute of Chemistry, Chinese Academy of Sciences, for EIMS and TOFMS measurement.

References

[1] Hesketh, A.R., Gledhill, L., Marsh, D.C., Bycroft, B.W., Dewick, P.M. and Gilbert, J. (1991), *Phytochemistry* **30**(7), 2237–2243.
[2] Matsumoto, M., Minato, H., Tori, K. and Ueyama, M. (1977), *Tetrahedron Lett.* **47**, 4093–4096.
[3] Jarvis, B.B., Stahly, G.P., Pavanasasivam, G., Midiwo, J.O., Desilva, T. and Holmlund, C.E. (1982), *J. Org. Chem.* **47**, 117–1124.
[4] Jarvis, B.B., Midiwo, J.O. and Mazzola, E.P. (1984), *J. Med. Chem.* **27**, 239–244.
[5] Mosmann, T. (1983), *J. Immunol. Meth.* **65**, 55–63.
[6] Scudiero, D.A., Shoemaker, R.H., Paull, K.D., Monks, A., Currents, M.R., Seniff, D. and Boyd, M.R. (1988), *Cancer Res.* **48**, 4827–4833.