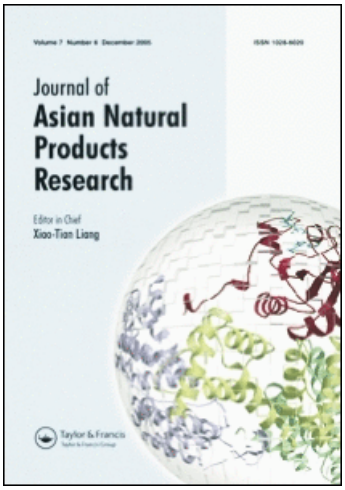


Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Two new metabolites from the mangrove endophytic fungus no. 2106

San-Yong Wang[a]; Zhong-Liang Xu[b]; Zhi-Gang She[a]; Hui Wang[b]; Chun-Rong Li[c]; Yong-Cheng Lin[a]
[a] School of Chemistry and Chemical Engineering, Zhongshan University, Guangzhou, China [b] School of Chemistry and Environment, South China Normal University, Guangzhou, China [c] Guangdong Food Industry Institute, Guangzhou, China

To cite this Article Wang, San-Yong , Xu, Zhong-Liang , She, Zhi-Gang , Wang, Hui , Li, Chun-Rong and Lin, Yong-Cheng(2008) 'Two new metabolites from the mangrove endophytic fungus no. 2106', Journal of Asian Natural Products Research, 10: 7, 622 — 626
To link to this Article: DOI: 10.1080/10286020802133423
URL: http://dx.doi.org/10.1080/10286020802133423

Journal of Asian Natural Products Research
Vol. 10, No. 7, July 2008, 622–626

Two new metabolites from the mangrove endophytic fungus no. 2106

San-Yong Wang[a], Zhong-Liang Xu[b], Zhi-Gang She[a]*, Hui Wang[b], Chun-Rong Li[c] and Yong-Cheng Lin[a]*

[a]*School of Chemistry and Chemical Engineering, Zhongshan University, Guangzhou, China;* [b]*School of Chemistry and Environment, South China Normal University, Guangzhou, China;* [c]*Guangdong Food Industry Institute, Guangzhou, China*

(*Received 21 November 2006; final version received 26 April 2007*)

Two new metabolites, named no. 2106 A (**1**) and *cyclo*-(*N*-MeVal-*N*-MeAla) (**2**), have been produced by the endophytic fungus no. 2106 isolated from the seeds of the mangrove *Avicennia marina* in Hong Kong. The structures were elucidated by 2D NMR, HR-MS, and X-ray diffraction analyses.

Keywords: *Avicennia marina*; marine fungi; metabolites; no. 2106 A; *cyclo*-(*N*-MeVal-*N*-MeAla)

1. Introduction

A large variety of new bioactive compounds have recently been isolated from the marine fungi, especially the mangrove fungi [1,2]. It was reported that most described marine fungi could be found from mangroves [2]. In our search for secondary metabolites of mangrove endophytic fungi from the South China Sea, many cytotoxic and/or novel compounds have been isolated [3,4]. We report here the isolation and structural elucidation of the metabolites from the fungus (strain no. 2106), which was an unidentified endophytic fungus separated from the seeds of *Avicennia marina* (Forsk.) Vierh from the mangroves of Hong Kong. Two new compounds, no. 2106 A (**1**) and *cyclo*-(*N*-MeVal-*N*-MeAla) (**2**), together with four known compounds mannitol (**3**), ergosterol (**4**), cerevisterol (**5**), and 3β-hydroxy-5α,8α-epidioxyergosta-6,22-diene (**6**), were isolated from the fermentation broth of this fungus. The structure of **1** is quite rare. So far, only two appreciably analogous compounds, buergerinin F and G, isolated from the roots of *Scrophularia buergeriana* Miq., have been reported [5].

2. Results and discussion

Compound **1** was obtained as colorless crystals, $[\alpha]_D^{25} + 36.9$ ($CHCl_3$, *c* 0.22). The molecular formula was established as $C_8H_{10}O_4$ by HR-MS at *m/z* 170.0577 $[M]^+$, along with ^1H and ^{13}C NMR spectral data (Table 1). In the ^{13}C NMR spectrum of **1**, there was no signals from δ 107 to 176, indicating no unsaturated bond in **1**. The ^1H NMR spectrum of **1** showed the presence of a methyl group linked to a quaternary carbon (δ 1.52, 3H, s), and two methine groups linked to oxygen (δ 4.90, 1H, dt, $J = 7.8$, 1.8 Hz; 5.11, 1H, dd, $J = 7.8$, 8.0 Hz). The ^{13}C NMR and DEPT spectra of **1** showed the existence of two methylene carbons, a carboxyl carbon at δ 177.2 (s), and an acetal carbon at δ 106.6 (s). The carboxyl carbon at δ 177.2 (s), together with the IR absorption band at 1770 cm^{-1}, suggested the existence of a γ-lactone moiety in **1**. In view of the degree

*Corresponding authors. Email: cesshzhg@mail.sysu.edu.cn; ceslyc@mail.sysu.edu.cn

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020802133423
http://www.informaworld.com

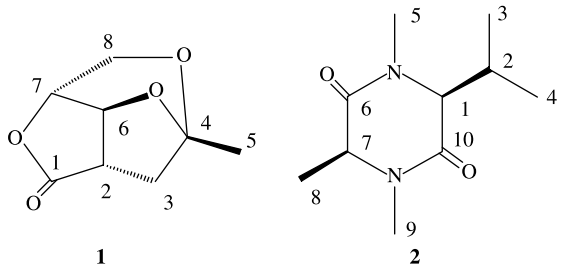
Table 1. NMR spectral data of **1** ($CDCl_3$) (δ in ppm, J in Hz).

No.	^{1}H	^{13}C	^{1}H–^{1}H COSY	HMBC (H → C)
1		177.2 (C)		
2	3.15 (ddd, J = 11.0, 7.8, 1.5)	41.3 (CH)	H-3a, 3b, 6	C-1, 3, 4, 7
3	2.08 (dd, J = 11.0, 13.0, H-10a)	44.3 (CH_2)	H-2, 3b	C-1, 2, 4, 5, 6
	2.30 (dd, J = 13.0, 1.5, H-10b)		H-2, 3a	
4		106.6 (C)		
5	1.52 (s)	22.7 (CH_3)		C-3, 4
6	5.11 (dd, J = 7.8, 8.0)	77.6 (CH)	H-2, 7	C-1, 3, 4, 7, 8
7	4.90 (dt, J = 7.9, 1.8)	79.9 (CH)	H-6, 8b	C-1, 2, 6, 8
8	3.65 (d, J = 12.4, H-3a)	61.6 (CH_2)	H-8b	C-4, 6, 7
	3.91 (dd, J = 12.4, 1.8, H-3b)		H-7, 8a	

of unsaturation, three rings were required. The ^{1}H–^{1}H COSY spectrum of **1** revealed the presence of a contiguous sequence of H-3/H-2/H-6/H-7/H-8. The HMBC spectral data established the overall structure of **1** (Figure 2; Table 1), especially the multiple correlations between H-2 and C-1, C-3, C-4, and C-7. The correlations from H-5 to C-3 and C-4 located the position of methyl group.

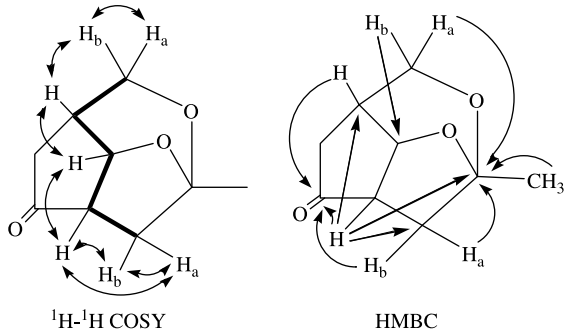
The chemical structure (Figure 1) and chirality of **1** was finally confirmed by X-ray diffraction analysis that showed the relative configurations of **1** to be 2*S**,4*S**,6*S**,7*R** (Figure 3).

Compound **2** was obtained as colorless prismatic crystals, $[\alpha]_D^{25} + 67.3$ ($CHCl_3$, *c* 0.45). The molecular formula was established as $C_{10}H_{18}O_2N_2$ by HR-MS at *m/z* 198.1360 $[M]^+$ and the overall NMR spectroscopic interpretation (Table 2). The ^{13}C NMR and DEPT spectra of **2** contained signals for 10 carbons, including two carbonyl groups



1 **2**

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



^{1}H-^{1}H COSY HMBC

Figure 2. Key HMBC and ^{1}H–^{1}H COSY correlations of **1**.

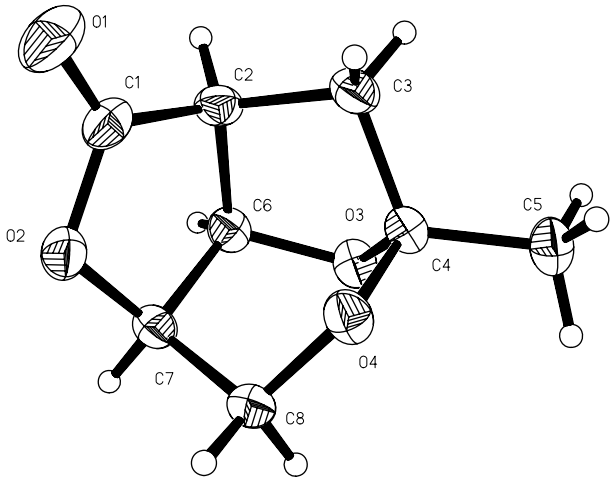


Figure 3. Crystal structure of **1**.

(δ 167.1 and 164.5), five methyl groups (δ 18.7, 18.8, 19.8, 31.8, and 34.5), and three tertiary carbons (δ 32.2, 57.7, and 68.1), respectively. In the ^{1}H NMR spectrum, there were two signals of nitrogen methyl groups at δ 2.97 (s, 3H) and 2.92 (s, 3H).

The ^{1}H–^{1}H COSY and HMBC experiments of **2** provided full information for the elucidation of the structure (Figure 4; Table 2). Interpretation of these spectral data defined the amino acids in the cyclic peptide as *N*-methylalanine and *N*-methyl valine and their linkages. In the HMBC, the key correlations between H-1 and C-2, C-3, C-4, C-5, C-6, and C-10; between H-7 and C-6, C-8, C-9, and C-10; and the correlations from the proton of *N*-methyl-5 to C-1 and C-6, and from the proton of *N*-methyl-9 to C-10 and C-7, assembled the overall structure of **2**.

The chemical structure and the configuration of **2** was finally confirmed by X-ray diffraction analysis that showed the relative configurations to be 1*R**,7*R** (Figure 5). The *N*-methyl cyclic dipeptide structure is uncommon in nature.

In addition, the structures of compounds **3–6** were elucidated by comparison of their spectral data with those in the literature [6].

3. Experimental

3.1 General experimental procedures

Melting points were determined on Stuart Scientific apparatus and are uncorrected.

Table 2. NMR spectral data of **2** ($CDCl_3$) (δ in ppm, J in Hz).

No.	^{1}H	^{13}C	^{1}H–^{1}H COSY	HMBC (H → C)
1	3.70 (d, $J = 4.8$)	68.1 (CH)	H-2	C-2, 3, 4, 5, 6, 10
2	2.16 (dq, $J = 7.0, 4.8$)	32.2 (CH)	H-1, 3, 4	C-1, 3, 4, 10
3	1.10 (d, $J = 7.0$)	19.8 (CH_3)	H-2	C-1, 2, 4
4	0.97 (d, $J = 7.0$)	18.7 (CH_3)	H-2	C-1, 2, 3
5	2.97 (s)	34.5 (CH_3)		C-1, 6
6		167.1 (C)		
7	3.89 (q, $J = 7.2$)	57.7 (CH)	H-8	C-6, 8, 9, 10
8	1.53 (d, $J = 7.2$)	18.8 (CH_3)	H-7	C-6, 7
9	2.92 (s)	31.8 (CH_3)		C-7, 10
10		164.5 (C)		

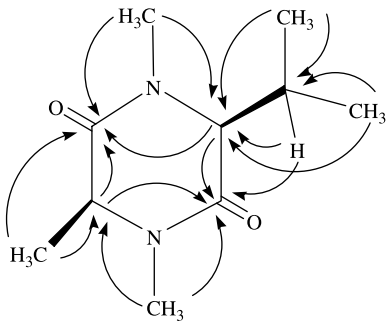


Figure 4. Key HMBC correlations of **2**.

Infrared spectra were measured on Nicolet Nexus 670 Fourier-transform IR spectrometer, ^{1}H and ^{13}C NMR spectra on Inova 400NB NMR or 300NB NMR spectrometer, mass spectra on a VG-ZAB mass spectrometer, optical rotations on Horiba high-sensitivity polarimeter SEPA-300, and X-ray data on Bruker SMART 1000 CCD system diffractometer.

3.2 Fungal strain

The endophytic fungus (strain no. 2106) was separated from the seeds of *A. marina* (Forsk.) Vierh from the mangroves of Hong Kong and unidentified. It was stored at the School of Chemistry and Chemical Engineering, Zhongshan University, Guangzhou, China.

3.3 Culturing

Starter cultures (from Professors E.B.G. Jones and L.L.P. Vrijmoed) were maintained on potato dextrose agar. Plugs of agar supporting mycelial growth were cut and transferred aseptically to an Erlenmeyer flask (250 ml) containing 100 ml liquid medium [1% glucose, 0.5% peptone, 0.5% yeast extract, and 20% seawater (pH 7.5)] that was sterilized at 110°C for 20 min. The flask was then incubated at 28°C on a rotary shaker (rpm = 120) for 5–7 days. The mycelium was subsequently aseptically transferred to Erlenmeyer flasks (500 ml) containing 200 ml of the same liquid medium. The flasks were then incubated at 28°C on a rotary shaker (rpm = 120) for 20 days.

3.4 Extraction and isolation

The cultures (170 l) were filtered through cheesecloth. The filtrate was concentrated to 3.5 l below 50°C and extracted five times by shaking with an equal volume of ethyl acetate. The combined extracts were chro-

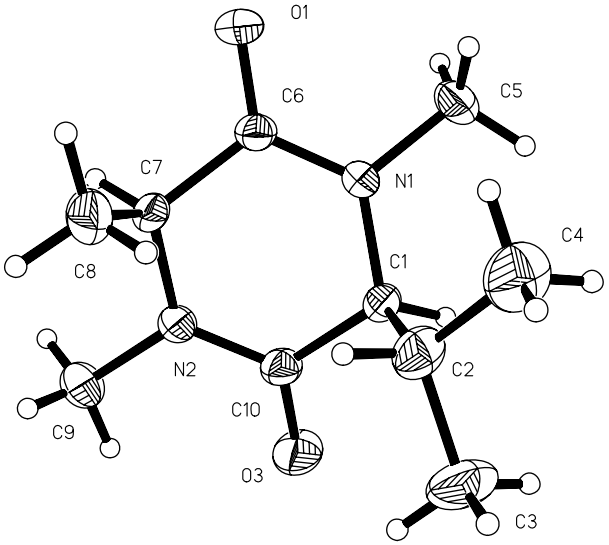


Figure 5. Crystal structure of **2**.

matographed on silica gel column using a gradient elution from petroleum ether (boiling point 60–90°C) to ethyl acetate. The strain no. 2106 A (**1**) (83 mg) was obtained from the 15% EtOAc–petroleum ether fraction, and *cyclo*-(*N*-MeVal-*N*-MeAla) (**2**) (112 mg) from the 30% EtOAc–petroleum ether fraction. The known compounds mannitol (**3**) (1800 mg), ergosterol (**4**) (700 mg), cerevisterol (**5**) (580 mg), and 3β-hydroxy-5α,8α-epidioxyergosta-6,22-diene (**6**) (390 mg) were obtained from the 40–75% fraction.

3.4.1 Compound *1*

A colorless prismatic crystal from EtOAc, mp 178–180°C; $[\alpha]_D^{25} + 36.9$ ($CHCl_3$, *c* 0.22); IR (KBr) ν_{max} (cm^{-1}): 1770, 1165, 1041; ^1H ($CDCl_3$), ^{13}C ($CDCl_3$) and 2D-NMR spectral data: see Table 1. EI-MS *m/z*: 170 $[M]^+$, 140, 97, 43; HR-MS: *m/z*: 170.0577 $[M]^+$ (calcd for $C_8H_{10}O_4$, 170.0574).

3.4.2 Compound *2*

A colorless prismatic crystal from EtOAc, mp 136.3–138.9°C; $[\alpha]_D^{25} + 67.3$ ($CHCl_3$, *c* 0.45); IR (KBr) ν_{max} (cm^{-1}): 3288, 1660, 1484, 1048; ^1H ($CDCl_3$), ^{13}C ($CDCl_3$), and 2D-NMR spectral data: see Table 2. EI-MS *m/z*: 198 $[M]^+$; HR-MS: *m/z*: 198.1360 $[M]^+$ (calcd for $C_{10}H_{18}O_2N_2$, 198.1363).

3.5 X-ray crystallographic analysis of 1

Crystal system, space group monoclinic, $P2_12_12_1$; unit cell dimensions $a = 7.2745(11)$ Å, $b = 9.7015(15)$ Å, $c = 10.8735(17)$ Å, volume = 767.4(2) Å[3], $Z = 4$, $D_{calcd} = 1.473\ mg/m^3$, $m = 0.119\ mm^{-1}$, $F000 = 360$. All single-crystal data were collected using the hemisphere technique on a Bruker SMART 1000 CCD system diffractometer with graphite-monochromated Mo Kα radiation $\lambda = 0.7l073$ at 293(2)K. The structures were solved by direct methods. The final value of *R* was 0.0304, $wR_2 = 0.0756$ $[I > 2\sigma(I)]$. The CIF file of X-ray data of compound **1** was deposited in the CCDC (deposit number: 627541).

3.6 X-ray crystallographic analysis of 2

The conditions and methods of the experiment were the same as those used for **1**, crystal system, space group monoclinic, $P2_12_12_1$; unit cell dimensions $a = 8.9479(12)$ Å, $b = 10.9181(15)$ Å, $c = 11.1593(15)$ Å, volume = 1090.2(3) $Å^3$, $Z = 4$, $D_{calcd} = 1.208\ mg/m^3$, $m = 0.085\ mm^{-1}$, $F000 = 432$. The final value of *R* was 0.0552, $wR_2 = 0.1730$ $[I > 2\sigma(I)]$. The CIF file of X-ray data of compound **2** was deposited in the CCDC (deposit number: 627542).

Acknowledgements

This work was supported by the 863-Foundation of China (2006AA09Z422), the National Natural Science Foundation of China (20572136), and the Guangdong Provincial Public Laboratory of Food Industry, China.

References

[1] Y.C. Lin, W.X. Wu, S. Feng, G. Jiang, S. Zhou, L.L.P. Vrijmoed, and E.B.G. Jones, *Tetrahedron Lett.* **42**, 449 (2001).
[2] Y.C. Lin, W.X. Wu, S. Feng, G. Jiang, J. Lou, S. Zhou, L.L.P. Vrijmoed, and E.B.G. Jones, *J. Org. Chem.* **66**, 6252 (2001).
[3] Y.C. Lin, W.X. Wu, Z. Deng, J. Wang, S. Zhou, L.L.P. Vrijmoed, and E.B.G. Jones, *Phytochemistry* **59**, 469 (2002).
[4] Y.C. Lin, Z. Shao, G. Jiang, S. Zhou, J. Cai, L.L.P. Vrijmoed, and E.B.G. Jones, *Tetrahedron* **56**, 9607 (2000).
[5] S.J. Lin, S.H. Jiang, Y.M. Li, J.F. Zeng, and D.Y. Zhu, *Tetrahedron Lett.* **41**, 1069 (2000).
[6] F. Zhu, X.H. Liu, L. Wen, Y.C. Lin, S.N. Zhou, and L.L.P. Vrijmoed, *Acta Sci. Nat. Univ. Sunyatseni* **44**, 66 (2005).