http://dx.doi.org/10.5935/0103-5053.20150008

J. Braz. Chem. Soc., Vol. 26, No. 3, 545-549, 2015.
Printed in Brazil - ©2015 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Butyrolactones from the Endophytic Fungus *Aspergillus versicolor* and their Anti-Tobacco Mosaic Virus Activity

Min Zhou,[a] Jie Lou,[a] Yin-Ke Li,[b] Yue-De Wang,[a] Kun Zhou,[a] Bing-Kun Ji,[a] Wei Dong,[a] Xue-Mei Gao,[a] Gang Du[a,] and Qiu-Fen Hu[a,*]*

[a]*Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan Minzu University, 650031 Kunming, P. R. China*

[b]*College of Resource and Environment, Yuxi Normal University, 653100 Yuxi, P. R. China*

New butyrolactones aspernolides C and D, along with two known butyrolactones (A and B) were isolated from the culture of the endophytic fungus *Aspergillus versicolor*. Their structures were elucidated on the basis of extensive spectroscopic analysis, including 1D and 2D nuclear magnetic ressonance techniques, and electronic circular dichroism. The butyrolactones aspernolides C and D were tested for their anti-tobacco mosaic virus activity. The results showed that butyrolactones aspernolides C and D exhibited moderate anti-tobacco mosaic virus activity with IC_{50} values of 64.2 and 88.6 μM, respectively.

Keywords: butyrolactones, *Aspergillus versicolor*, anti-tobacco mosaic virus

Introduction

Aspergillus, a genus of filamentous fungi, has been proven to be a prolific source of lifesaving drugs, mycotoxins, and industrial enzymes.[1] Previous phytochemical investigations on the cultures of this genus resulted in the isolation of xanthones, anthraquinones, lactones, polyketides, and tryptophane-derived alkaloids,[2-7] some of them exhibiting several biological activities.[7-10] Here, we report the isolation and structural elucidation of two new butyrolactones, aspernolides C and D (**1** and **2**), along with known butyrolactones **3** and **4**, from an endophytic strain (YNCA1266) of *A. versicolor* (Vuillemin) Tiraboschi isolated from the rhizome of *Paris polyphylla* var. *Yunnanensis*.[11] In addition, the anti-tobacco mosaic virus (anti-TMV) activity of compounds **1** and **2** has been evaluated.

Experimental

General experimental procedures

Optical rotations were measured in a Horiba SEPA-300 polarimeter. Ultraviolet (UV) spectra were obtained using a Shimadzu UV-2401A spectrophotometer. Infrared (IR) spectra were obtained in KBr disc on a Bio-Rad Wininfmred spectrophotometer. Electronic circular dichroism (ECD) spectra were measured on a JASCO J-810 spectropolarimeter. Electrospray ionization mass spectra (ESIMS) and high resolution electrospray ionization mass spectra (HRESIMS) were measured on a VG Auto Spec-3000 MS spectrometer. ^{1}H, ^{13}C and 2D nuclear magnetic ressonance (NMR) spectra were recorded on a Bruker DRX-400 instrument with trimethylsilane (TMS) as internal standard. Column chromatography was performed on silica gel (200-300 mesh), or on silica gel H (10-40 μm, Qingdao Marine Chemical Inc., China), and MCI gel CHP 20P (75-150 μm, Tokyo, Japan). Final purifications utilized an Agilent 1100 high pressure liquid chromatography (HPLC) equipped with ZORBAX-C_{18} (21.2 mm × 250 mm, 7.0 μm) column and diode array detector (DAD). Ningnanmycin (purity > 98%) was provided by the Chengdu Institute of Biology, Chinese Academy of Sciences.

Fungal material

The culture of *Aspergillus versicolor* was isolated from the rhizome of *Paris polyphylla* var. *yunnanensis*, collected from Dali, Yunnan, People's Republic of China, in 2012. The strain was identified by one of authors (Gang Du) based on the analysis of the internal transcribed spacer ITS sequence (Genbank Accession number KJ801852). It was cultivated at room temperature for 7 days on potato dextrose

*e-mail: dugang2006@163.com; huqiufena@163.com

agar at 28 °C. Agar plugs were inoculated into 250 mL Erlenmeyer flasks each containing 100 mL of potato dextrose broth and cultured at 28 °C on a rotary shaker at 180 rpm for five days. Unlike the previous research,[11] large scale fermentation was carried out in 100 Fernbach flasks (500 mL) each containing 300 mL of medium (glucose 5%; peptone 0.15%; yeast 0.5%; KH_2PO_4 0.05%; $MgSO_4$ 0.05% in 1 L of deionized water; pH = 6.5 before autoclaving). Each flask was inoculated with 5.0 mL of cultured broth and incubated at 27 °C for 14 days.

Extraction and isolation

The whole culture broth of *A. versicolor* was extracted four times with ethyl acetate (4 × 10 L) at room temperature and filtered. The crude extract (120 g) was applied to silica gel column chromatography, eluting with a $CHCl_3$-$(CH_3)_2CO$ gradient system (9:1, 8:2, 7:3, 6:4, 5:5). Five fractions were obtained from the silica gel column and individually decolorized on MCI gel CHP 20P to yield fractions A-E. The further separation of fraction B (8:2, 12.5 g) by silica gel column chromatography, eluted with petroleum ether-ethyl acetate (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures B1-B5. Fraction B3 (2.3 g) was subjected to RP-18 column chromatography (MeOH/H_2O 20:80 to 80:40 gradient) to yield four fractions, B3.1-B3.4. Fraction B3.2 (110 mg) was subjected to preparative HPLC (ZORBAX-C_{18}, 7.0 μm, 21.2 mm × 250 mm, flow rate 12 mL min^{-1}, UV detection at λ_{max} = 210, 254, and 280 nm, eluted with CH_3OH/H_2O 65:35) to give 2 (5.8 mg, R_t = 15 min) and 4 (14.1 mg, R_t = 17 min). Fraction B2 (1.25 g) was subjected to RP-18 column chromatography (MeOH/H_2O 20:80 to 60:40 gradient) to provide six fractions, B2.1-B2.6. Fraction B2.2 (98 mg) was subjected to preparative HPLC (CH_3OH/H_2O 70:30) to give 1 (8.5 mg, R_t = 14 min) and 3 (22.3 mg, R_t = 19 min).

Aspernolide C (**1**): White amorphous powder; $[\alpha]_D^{24.8}$ +75.2 (c 0.20, MeOH); UV (MeOH) λ_{max}/nm (log ε): 218 (4.06), 252 (3.63), 297 (0.47); CD (c 0.15 mg mL^{-1}, MeOH) $\Delta\varepsilon_{205}$ +15.8, $\Delta\varepsilon_{232}$ –6.27, $\Delta\varepsilon_{305}$ +3.26; IR (KBr) ν_{max}/cm^{-1} 3450, 3029, 2972, 2928, 1741, 1726, 1610, 1538, 1487, 1439, 1382, 1226, 1147, 1118, 1051, 936, 747; ^1H and ^{13}C NMR (400 and 100 MHz, in $CDCl_3$) see Table 1; ESIMS (positive ion mode) *m/z* 461 $[M + Na]^+$; HRESIMS (positive ion mode) *m/z* 461.1570 $[M + Na]^+$ (calcd. for $C_{25}H_{26}NaO_7$, 461.1576).

Aspernolide D (**2**): White amorphous powder; $[\alpha]_D^{24.6}$ +78.6 (c 0.20, MeOH); UV (MeOH) λ_{max}/nm (log ε): 218 (4.10), 250 (3.58), 295 (3.38); CD (c 0.5 mg mL^{-1}, MeOH) $\Delta\varepsilon_{206}$ +18.6, $\Delta\varepsilon_{234}$ –4.61, $\Delta\varepsilon_{306}$ +4.08; IR (KBr) ν_{max}/cm^{-1} 3460, 3047, 2986, 2915, 1734, 1718, 1609, 1545, 1490, 1432, 1375, 1218, 1163, 1069, 957, 758; ^1H and ^{13}C NMR (400 and 100 MHz, in $CDCl_3$) see Table 1; ESIMS (positive ion mode) *m/z* 463 $[M + Na]^+$; HRESIMS (positive ion mode) *m/z* 463.1376 $[M + Na]^+$ (calcd. for $C_{24}H_{24}NaO_8$, 463.1369).

Anti-TMV assays

TMV (U1 strain) was obtained from the Key Laboratory of Tobacco Chemistry of Yunnan Province, Yunnan Academy of Tobacco Science, P. R. China. The virus was multiplied in *Nicotiana tabacum* cv. K326 and purified as described.[12] The concentration of TMV was determined as 20 mg mL^{-1} with a UV spectrophotometer [virus concentration = $(A_{260}$ × dilution ratio)/$E_{1cm}^{0.1\%,260nm}$, where 260 nm is the ultraviolet wavelength and 1 cm is the optical path length]. The purified virus was kept at –20 °C and diluted to 32 μg mL^{-1} with 0.01 M PBS before use.

Nicotiana glutinosa plants were cultivated in an insect-free greenhouse. *N. glutinosa* was used as a local lesion host. The experiments were conducted when the plants grew to the 5-6 leaf stage. The tested compounds were dissolved in DMSO and diluted with distilled H_2O to the required concentrations. A solution of equal concentration of DMSO was used as a negative control. The commercial antiviral agent ningnanmycin was used as positive control.

For the half-leaf method, the virus was inhibited by mixing with the solution of compound. After 30 min, the mixture was inoculated on the left side of the leaves of *N. glutinosa*, whereas the right side of the leaves was inoculated with the mixture of DMSO solution and the virus as control. The local lesion numbers were recorded 3 or 4 days after inoculation. Three repetitions were conducted for each compound. The inhibition rates were calculated according to the formula:

$$\text{inhibition rate (\%)} = [(C - T)/C] \times 100\% \quad (1)$$

where C is the average number of local lesions of the control and T is the average number of local lesions of the treatment.

Results and Discussion

The whole culture broth of *A. versicolor* was extracted with ethyl acetate. The extract was subjected repeatedly to column chromatography on silica gel, MCI, RP-18 and preparative HPLC to afford the new butyrolactones aspernolides C and D (**1** and **2**), and the known butyrolactones aspernolides A and B (**3** and **4**).[13]

Compound **1** was obtained as a white amorphous powder. Its molecular formula $C_{25}H_{26}O_7$ was determined by positive HRESIMS at *m/z* 461.1570 $[M + Na]^+$, indicating 13 degrees of unsaturation. The IR spectrum showed the absorption bands of hydroxyl and carbonyl groups at 3450 and 1726 cm^{-1}, respectively. The ^1H NMR spectrum (Table 1) displayed signals of a 1,4-disubstituted benzene moiety [δ_H 7.63 (d, 2H, *J* 8.8, Ph-H); 6.91 (d, 2H, *J* 8.8, Ph-H)], a 1,3,4-trisubstituted benzene moiety [δ_H 6.54 (d, 1H, *J* 2.1, Ph-H); 6.52 (d, 1H, *J* 8.2, Ph-H); 6.90 (dd, 1H, *J* 8.2, 2.1, Ph-H)], a prenyl group [δ_H 3.14 (d, 2H, *J* 7.2, CH_2); 5.10 (m, 1H, CH); 1.64 (s, 3H, CH_3); 1.68 (s, 3H, CH_3)], a pair of methylene protons [δ_H 3.48 (d, 1H, *J* 14.7, CH_2); 3.55 (d, 1H, *J* 14.7, CH_2)], and two methoxy protons [δ_H 3.78 (s, 3H, CH_3); 3.97 (s, 3H, CH_3)]. In the ^{13}C NMR and DEPT NMR spectra (Table 1), there were signals for four methyls including two oxygenated ones, two methylenes, eight methines, eleven quaternary carbons (including eight sp^2 ones, one oxygenated sp^3 quaternary carbon, and two carbonyl groups), which were distinguished through analysis of the 2D NMR data. Among them, two carbonyls, two benzene moieties, and two double bonds account for twelve degrees of unsaturation. These data confirmed the molecular formula and also indicated that compound **1** must possess an aliphatic ring in addition to two aromatic rings. Furthermore, the characteristic carbon signals at (δ_C 169.4, 142.9, 127.4, 85.8, 38.6, and 170.3) suggested that **1** should be a butyrolactone.[14] Comparison of the NMR (Table 1) and MS data of **1** with those of butyrolactone I demonstrated that both compounds were similar and had the same basic skeleton.[14,15] The main difference was that the hydroxyl group at C-2 position in butyrolactone I was replaced by a methoxy substituent in **1**. The heteronuclear multiple bond correlation (HMBC) from 2-OMe (δ_H 3.97, s) to C-2 (δ_C 142.9) further confirmed the above inference (Figure 2). Thus, the structure of **1** was established as α-methoxy-β-(4-hydroxybenzyl)-γ-[4-hydroxy-3-(3-methyl-but-2-enyl)-benzyl]-γ-methoxycarbonyl-γ-butyrolactone, and named as aspernolide C.

Compound **2** was also obtained as a white amorphous powder with the molecular formula $C_{24}H_{24}O_8$ determined by its HRESIMS at *m/z* 463.1376 $[M + Na]^+$ (calcd 463.1369). Analyses of the ^1H and ^{13}C NMR spectra (Table 1) revealed that the structure of **2** was very similar to those of butyrolactone I,[14,15] except for the absence of a methyl group and the presence of an oxygenated methylene in **2**. This assignment was further supported by the HMBC from H_2-10'' (δ_H 3.94, s) to C-8'' (δ_C 124.4) and C-11'' (δ_C 15.5), and the rotating-frame overhauser spectroscopy (ROESY) correlation of H_2-10'' (δ_H 3.94, s) with H-8'' (δ_H 5.07, t, *J* = 7.1 Hz). Thus, the structure of compound **2** was determined as α-hydroxy-β-(4-hydroxybenzyl)-γ-[4-hydroxy-3-(2*E*-4-hydroxy-3-methyl-but-2-enyl)-benzyl]-γ-methoxycarbonyl-γ-butyrolactone, and named as aspernolide D.

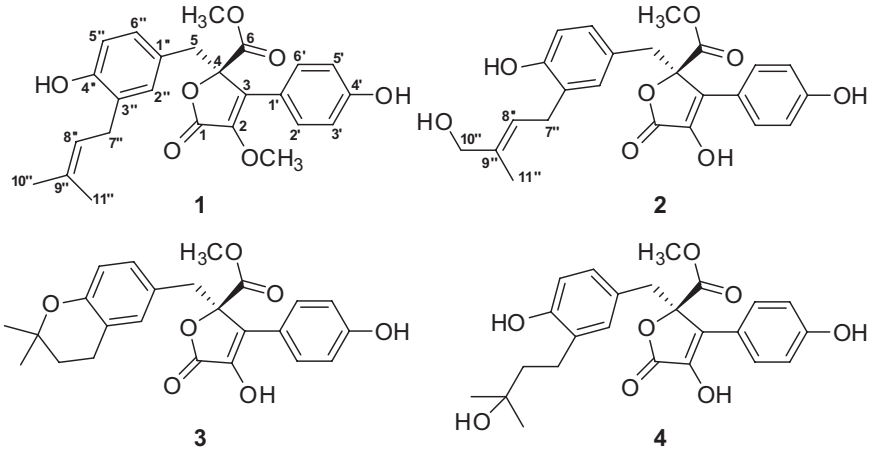

Figure 1. Chemical structures of compounds **1-4** from *A. versicolor*.

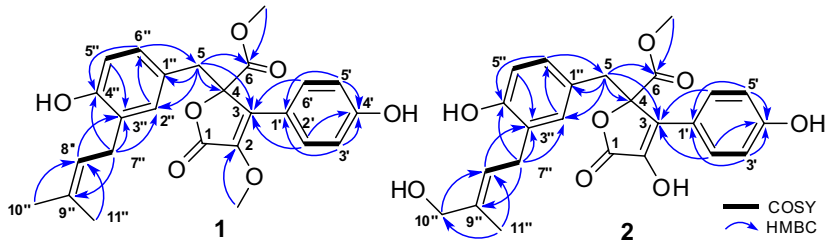

Figure 2. ^1H–^1H COSY and selected HMBC (H → C) of compounds **1** and **2**.

Table 1. ^{1}H and ^{13}C NMR data of compounds **1** and **2** in $CDCl_3$ (δ in ppm, 400 and 100 MHz, respectively)

No.	Compound **1**		Compound **2**	
	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)
1	169.4 s		170.0 s	
2	142.9 s		138.8 s	
3	127.4 s		127.9 s	
4	85.8 s		86.2 s	
5	38.6 t	3.55, 3.48 d (14.7)	38.6 t	3.51, 3.46 d (14.5)
6	170.3 s		170.1 s	
1'	121.8 s		121.7 s	
2',6'	129.3 d	7.63 d (8.8)	129.5 d	7.60 d (8.7)
3',5'	115.8 d	6.91 d (8.8)	116.0 d	6.81 d (8.7)
4'	157.6 s		157.1 s	
1"	124.0 s		124.0 s	
2"	131.6 d	6.54 d (2.1)	131.7 s	6.50 d (1.4)
3"	127.2 s		127.1 s	
4"	153.5 s		153.1 s	
5"	114.4 d	6.52 d (8.2)	114.8 d	6.52 d (8.2)
6"	128.8 d	6.90 dd (2.1, 8.2)	129.0 d	6.58 d (8.2)
7"	28.1 t	3.14 d (7.2)	26.8 t	3.11 d (7.1)
8"	122.1 d	5.10 m	124.4 d	5.07 t (7.1)
9"	132.8 s		133.5 s	
10"	17.5 q	1.64 s	68.0 t	3.94 s
11"	25.5 q	1.68 s	15.5 q	1.66 s
2-OMe	59.5 q	3.97 s		
6-OMe	53.3 q	3.78 s	53.6 q	3.72 s

According to the literature,[14,16] the electronic transitions in the α,β-unsaturated-γ-lactone moiety were responsible for the negative or positive rotatory strength around 210 nm. The experimental ECD spectra of compounds **1** and **2** all showed a positive Cotton effects at 205 and 206 nm, respectively, indicating that compounds **1** and **2** had the same 4*R*-configuration.

Since many butyrolactones have been reported to possess potential anti-virus property,[14,17] compounds **1** and **2** were tested for anti-tobacco mosaic virus (anti-TMV) activity. The anti-TMV activities were tested using the half-leaf method.[17] Ningnanmycin was used as positive control. Compounds **1** and **2** exhibited moderate activity with IC_{50} values of 64.2 and 88.6 μM, respectively (Table 2). The protective effect of **1** and **2** against TMV was also evaluated by pretreating the tobacco leaves with individual compounds for 6 hours before inoculation with TMV.[17] The results (Table 2) showed that at a concentration of 20 μM compounds **1** and **2** showed protective effects to the host plants, with inhibition rates of 18.4% and 15.6%, respectively. The results indicated that pretreatment with these butyrolactones may increase the resistance of the host plant to TMV infection.

Conclusions

Two new butyrolactones, aspernolides C and D (**1** and **2**), along with known butyrolactones aspernolides A and B (**3** and **4**) were isolated from the fermentation products of the endophytic fungus *Aspergillus versicolor*. Their structures were elucidated on the basis of extensive spectroscopic analysis, including 1D and 2D NMR techniques, and ECD. Compounds **1** and **2** exhibited moderate anti-TMV activity with IC_{50} values of 64.2 and 88.6 μM, respectively.

Supplementary Information

Supplementary data is available free of charge at http://jbcs.sbq.org.br.

Acknowledgements

This project was supported financially by the Excellent Scientific and Technological Team of Yunnan High School (2010CI08), the Yunnan Minzu University Green Chemistry and Functional Materials Research for Provincial Innovation Team (2011HC008), and Open Research Fund Program of Key Laboratory of Ethnic Medicine Resource Chemistry (Yunnan Minzu University) (2010XY08).

Table 2. Anti-TMV activity of compounds **1** and **2** on *Nicotiana tabacum* leaf, and protective effect on TMV infection

Compounds	Inhibition rates at 20 μM / %[a]	IC_{50} / μM[a]	Inhibition rates at 20 μM / %[b]
1	22.5 ± 2.8	64.2	18.4 ± 2.5
2	18.6 ± 2.4	88.6	15.6 ± 2.2
Ningnamycin	30.5 ± 2.8	52.4	28.6 ± 3.2

[a] Anti-TMV activity; [b] Protective effect on TMV infection.

References

1. Sanchez, J. F.; Somoza, A. D.; Keller, N. P.; Wang, C. C. C.; *Nat. Prod. Rep.* **2012**, *29*, 351.
2. Wu, Q. X.; Crews, M. S.; Draskovic, M.; Sohn, J.; Johnson, T. A.; Tenney, K.; Valeriote, F. A.; Yao, X. J.; Bjeldanes, L. F.; Crews, P.; *Org. Lett.* **2010**, *12*, 4458.
3. Furtado, N. A. J. C.; Pupo, M. T.; Carvalho, I.; Campo, V. L.; Duarte, M. C. T.; Bastos, J. K.; *J. Braz. Chem. Soc.* **2005**, *16*, 1448.
4. Correa, M. J. C.; Nunes, F. M.; Bitencourt, H. R.; Borges, F. C.; Guilhon, G. M. S. P.; Arruda, M. S. P.; Marinho, A. M. R.; Santos, A. S.; Alves, C. N.; Brasil, D. S. B.; Santos, L. S.; *J. Braz. Chem. Soc.* **2011**, *22*, 1333.
5. Carvalho, M. R.; Barbosa, L. C. d. A.; de Queiroz, J. H.; Howarth, O. W.; *Tetrahedron Lett.* **2001**, *42*, 809.
6. Lin, W. H.; Brauers, G.; Ebel, R.; Wray, V.; Berg, A.; Proksch, S. P.; *J. Nat. Prod.* **2003**, *66*, 57.
7. Zhuang, Y. B.; Teng, X. C.; Wang, Y.; Liu, P. P.; Li, G. Q.; Zhu, W. M.; *Org. Lett.* **2011**, *13*, 1130.
8. Lee, Y. M.; Mansoor, T. A.; Hong, J. K.; Lee, C. O.; Bae, K. S.; Jung, J. H.; *Nat. Prod. Sci.* **2007**, *13*, 90.
9. Leith, J. F.; Andrew, M. P.; Ernest, L.; Robert, J. C.; *J. Nat. Prod.* **2009**, *72*, 666.
10. Dhar, A. K.; Bose, S. K.; *Tetrahedron Lett.* **1969**, *55*, 4871.
11. Zhou, M.; Miao, M. M.; Du, G.; Li, X. N.; Shang, S. Z.; Zhao, W.; Liu, Z. H.; Yang, G. Y.; Che, C. T.; Hu, Q. F.; Gao, X. M.; *Org. Lett.* **2014**, *16*, 5016.
12. Hu, Q. F.; Zhou, B.; Huang, J. M.; Gao, X. M.; Shu, L. D.; Yang, G. Y.; Che, C. T.; *J. Nat. Prod.* **2013**, *76*, 292.
13. Parvatkar, R. R.; D'Souza, C.; Tripathi, A.; Naik, C. G.; *Phytochemistry* **2009**, *70*, 128.
14. Rao, K. V.; Sadhukhan, A. K.; Veerender, M.; Ravikumar, V.; Mohan, E. V. S.; Dhanvantri, S. D.; Sitaramkumar, M.; Babu, J. M.; Vyas, K.; Reddy, G. O.; *Chem. Pharm. Bull.* **2000**, *48*, 559.
15. Kiriyama, N.; Nitta, K.; Sakaguchi, Y.; Taguchi, Y.; Yamamoto, Y.; *Chem. Pharm. Bull.* **1977**, *25*, 2593.
16. Shi, Y. M.; Wang, L. Y.; Zou, X. S.; Li, X. N.; Shang, S. Z.; Gao, Z. H.; Liang, C. Q.; Luo, H. R.; Li, H. L.; Xiao, W. L.; Sun, H. D.; *Tetrahedron* **2014**, *70*, 859.
17. Matsumoto, T.; Hosono-Nishiyama, K.; Yamada, H.; *Planta Med.* **2006**, *72*, 276.

Submitted: October 2, 2014
Published online: January 27, 2015