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NOTE

Antimycin A_{18} produced by an endophytic *Streptomyces albidoflavus* isolated from a mangrove plant

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Mangroves have unique intertidal ecosystems of the tropics, which possess prolific biodiversity of actinomycetes.[1] Recent discoveries[2,3] of new species of microorganisms from the ecosystems encouraged us to explore the bioactive secondary metabolites from endophytic actinomycetes isolated from mangrove plants. As a result, the cultured broth of the strain I07A-01824, an endophytic *Streptomyces albidoflavus*, isolated from the leaf of *Bruguiera gymnorrhiza* collected at Shankou, Guangxi Province, People's Republic of China, was found to show moderate inhibiting activity against *Magnaporth grisea*. By bioassay-guided fractionation, antimycin A_{18} (**1**) was purified by chromatographies. By analyzing the spectroscopic data (including 1D and 2D NMR), its chemical structure was identified to be the first naturally occurring antimycin with an acetoxy group at C-8 (Figure 1).

The strain I07A-01824 identified as *Streptomyces* was isolated from the leaf of *B. gymnorrhiza* collected at Shankou, Guangxi Province, People's Republic of China. A stock culture of the strain I07A-01824 was maintained on yeast and malt extract with glucose (YMG) agar slant consisting of 0.4% yeast extract (Beijing Aoboxing Biotechnology, Beijing, China), 1% malt extract (Beijing Aoboxing Biotechnology), 0.4% glucose and 1.2% agar (pH 7.2). The stock culture was inoculated into 250 ml Erlenmeyer flasks containing 50 ml of seed medium consisting of 0.5% glucose, 0.5% yeast extract, 0.5% peptone, 0.5% beef extract (Beijing Aoboxing Biotechnology), 0.4% corn steep liquor (North China Pharmaceutical Corporation, Shijiazhuang City, China), 2% soluble starch, 1% soybean meal (Beijing Comwin Pharm-Culture, Beijing, China), 0.4% $CaCO_3$ and 0.002% $CoCl_2$ (pH 7.2). The flask culture was incubated on a rotary shaker (180 r.p.m.) at 28 °C for 36 h. The seed culture (50 ml) was transferred into each of 40 5-l Erlenmeyer flasks containing 1 l of the same seed medium. The fermentation was carried out at 28 °C for 72 h on a rotary shaker (180 r.p.m.).

The fermentation broth (40 l) was filtered and the filtrate was extracted with EtOAc (40 l). The extract was dried with Na_2SO_4, and then concentrated under reduced pressure to obtain syrup (20 g). It was then chromatographed on a column of silica gel (120 g, 100–200 mesh, Qingdao Ocean Chemical Group, Qingdao City, China, i.d. 3×40 cm) and developed with stepwise cyclohexane–EtOAc gradient system as below: 19:1 (v/v, 500 ml), 4:1 (500 ml), 2:1 (1000 ml) and 1:1 (500 ml). The fraction of cyclohexane–EtOAc (2:1) showed the strongest fungicidal activity. This fraction (1 g) was further chromatographed on a column of RP-18 silica gel (40 g, 50 μm, Merck, Darmstadt, Germany, i.d. 1×50 cm) and eluted with 75% aqueous MeOH to yield a bioactive semipurified sample (15 mg). This sample was dissolved completely in 1 ml MeOH and was purified by HPLC (detector: SPD-20AVP photodiode array detector (Shimadzu Corporation, Tokyo, Japan); column: YMC-Pack ODS-A (YMC Co. Ltd, Kyoto, Japan), 5 μm, i.d. 10×250 mm; mobile phase: 70% aqueous MeOH; flow rate: 2 ml min^{-1}) to yield **1** (6 mg, R_t 29 min).

Compound **1** was obtained as colorless amorphous solid and is soluble in MeOH, dimethyl sulfoxide, EtOAc and $CHCl_3$, but insoluble in H_2O and *n*-hexane. The other physicochemical properties of **1** are as follows: HR-ESI-MS $(M-1)^-$ *m/z* 477.1872, calcd 477.1878 for $C_{23}H_{29}N_2O_9$; $[\alpha]_D^{25}$ +49° (*c* 0.102, MeOH); λ_{max}^{MeOH} nm (ε) 227

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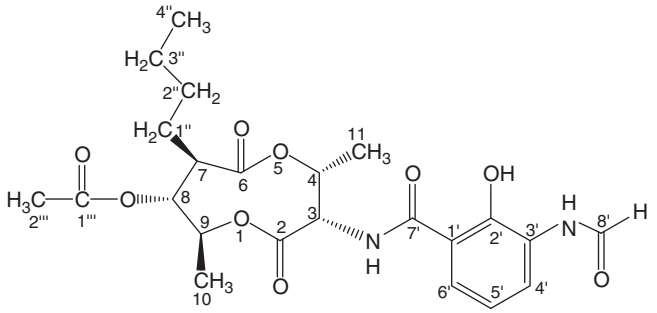

Figure 1 Structure of antimycin A_{18} (**1**).

Table 1 NMR data of antimycin A_{18} (1) in $CDCl_3$

Position	δ_C[a]	δ_H[b] *(mult, J (in Hz))*	*^{1}H-^{1}H COSY*	*HMBC*
2	170.1			
3	53.7	5.29 (dd, 7.2, 7.8 Hz)	7-NH, 3	2, 4, 11
4	71.0	5.73 (dq, 6.6, 7.8 Hz)	3, 11	2, 6, 11
6	172.9			
7	50.1	2.51(dt, 2.7, 10.2, 11.4 Hz)	1″, 8	6, 8, 9
8	75.7	5.07 (dd, 9.6,10.2 Hz)	7, 9	1‴, 7, 9, 10,
9	74.8	4.98 (dq, 6.6, 9.6 Hz)	8, 10	2, 8
10	17.8	1.30 (d, 6.6 Hz)	9	
11	15.0	1.32 (d, 6.6 Hz)	4	3
1′	112.5			
2′	150.6			
2′-OH		12.62 (s)		
3′	127.4			
4′	124.8	8.55 (d, 7.8 Hz)	5′	2′, 6′,
5′	119.0	6.92 (dd, 7.8,7.2 Hz)	4′, 6′	1′, 3′
6′	120.1	7.24 (d, 7.2 Hz)	5′	2′, 4′, 7′
7′	169.4			
7′-NH		7.07 (d, 7.2 Hz)	3	7′,
8′	159.0	8.51 (s)		3′
8′-NH		7.93 (br, s)		4′, 8′
1″	28.2	1.35 (m), 1.70 (m)	2″, 7	3″, 6
2″	29.2	1.15 (m)	1″, 3″	4″
3″	22.4	1.25 (m)	2″, 4‴	1″
4″	13.8	0.88 (t, 6.6 Hz)	3″	2″, 3″
1‴	169.6			
2‴	20.8	2.13 (s)		1‴

[a] ^{13}C-NMR was measured at 150 MHz.
[b] ^{1}H-NMR was measured at 600 MHz.

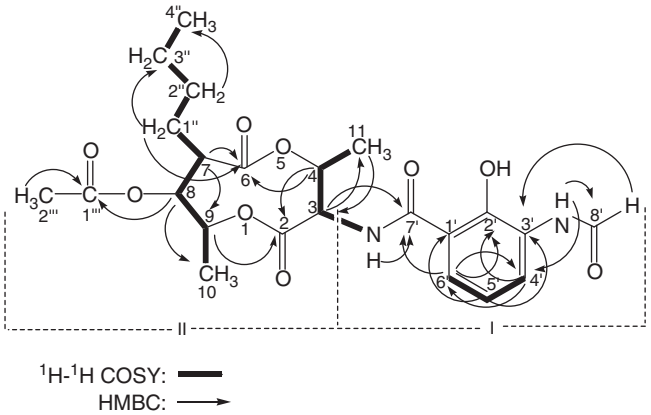

Figure 2 Summary of ^{1}H-^{1}H COSY and selected HMBC correlations of **1**.

(18.230), 319 (3.093); IR υ_{max} (KBr) cm^{-1} 3348, 2929, 1738, 1692, 1635, 1544, 1373, 1234, 1202, 1040, 754. The direct connectivity between protons and carbons was established by the heteronuclear single quantum coherence. The ^{1}H-NMR and ^{13}C-NMR spectral data of **1** are shown in Table 1.

Five carbonyl carbon signals (δ 172.9, 170.1, 169.6, 169.4 and 159) and six olefinic carbon signals (δ 150.6, 127.4, 124.8, 120.1, 112.5 and 119) were readily observed by analysis of ^{13}C-NMR and DEPT of **1**. Further analysis of the six olefinic carbon signals through heteronuclear single quantum coherence together with ^{1}H-^{1}H COSY and HMBC (Figure 2) revealed that the three quaternary carbon signals at δ 112.5 (C-1′), δ 150.6 (C-2′) and δ 127.4 (C-3′), and three tertiary carbon signals at δ 124.8 (C-4′), δ 119 (C-5′) and δ 120.1 (C-6′) formed a 1,2,3-trisubstituted benzene ring, as proton signal at δ 6.92 (1H, dd, J=7.8, 7.2 Hz, 5′-H) was coupled with proton signals at δ 8.55 (1H, d, J=7.8 Hz, 4′-H) and δ 7.24 (1H, d, J=7.2 Hz, 6′-H) in ^{1}H-^{1}H COSY. Both 4′-H and 6′-H were long-range correlated with C-2′, and 5′-H was long-range correlated with C-1′ and C-3′ in HMBC.

The proton signals at δ 12.62, 8.51, 7.93 and 7.07 were also readily observed and assigned to a phenolic hydroxyl proton (2′-OH), formyl proton (8′-H) and two amide protons (3′-NH and 7′-NH), respectively. According to the chemical shift of 150.6 (C-2′), a phenolic hydroxyl was substituted at C-2′. By tracing cross peaks from the two amide protons (3′-NH and 7′-NH) in HMBC, two amide carbonyl carbons of δ 159 (C-8′) and δ 169.4 (C-7′) were assigned. The cross peaks observed in HMBC between 6′-H and C-7′, 8′-H and C-3′, as well as 3′-NH and C-4′, indicated that the side chains of –NHCO$^-$ and –NHCHO were linked to the benzene ring at C-1′ and C-3′, respectively. It led to the unambiguous assignments of NMR data in the substructure I (Figure 2).

As the five carbonyl carbons and one benzene ring in **1** accounted for nine of the ten degrees of unstauration required for the molecular formula, **1** should have another ring. This was confirmed by tracing cross peaks in the ^{1}H-^{1}H COSY and HMBC from the three oxymethine protons at δ 5.73 (1H, dq, J=6.6, 7.8 Hz, 4-H), δ 5.07 (1H, dd, J=9.6, 10.2 Hz, 8-H) and δ 4.98 (1H, dq, J=6.6, 9.6 Hz, 9-H) observed in ^{1}H-NMR. The cross peaks in ^{1}H-^{1}H COSY between the proton at δ 5.29 (1H, dd, J=7.2, 7.8 Hz, 3-H) and 4-H, the proton at δ 2.51 (1H, dt, J=2.7, 10.2, 11.4 Hz, 7-H) and 8-H, 8-H and 9-H, together with the cross peaks in HMBC between 4-H and C-2 (δ 170.1), 4-H and C-6 (δ 172.9), 9-H and C-2, 7-H and C-9 (δ 74.8), 7-H and C-6 established the structure of the nine-membered dilactone ring in **1**. The two methyl proton signals at δ 1.30 (3H, d, J=6.6 Hz, 10-CH_3) and δ 1.32 (3H, d, J=6.6 Hz, 11-CH_3) observed in ^{1}H-NMR were assigned by ^{1}H-^{1}H COSY, and long-range coupling between 3-H and C-11 (δ 15), 11-H and C-3 (δ 53.7), 8-H and C-10 (δ 17.8) in HMBC further confirmed their substitute position in the ring. A butyl side chain linked with the ring at C-7 was identified by the contiguous correlation from 1″-H to 4″-H in ^{1}H-^{1}H COSY and correlations from proton signals at δ 1.35, 1.70 (2H, m, 1″-H) to C-6 and C-3″ (δ 22.4) in HMBC. The final acetoxy group at the C-8 was revealed by the cross peaks between 8-H and C-1‴ (δ 169.6) and between proton signal at δ 2.13 (3H, s, 2‴-H) and C-1‴ in HMBC. These results indicated the presence of substructure II in **1** (Figure 2).

Linkage between the two substructures (I and II) was established by ^{1}H-^{1}H COSY and HMBC. The proton signal at δ 5.29 (1H, dd, J=7.2, 7.8 Hz, 3-H) was coupled with the proton signal at δ 7.07

(1H, d, J=7.2 Hz, 7′-NH) in ^{1}H-^{1}H COSY, meanwhile, the long-range coupling between 3-H and C-7′ (δ 169.4) was observed in HMBC. The data above revealed the linkage of the two substructures through 7′-NH with 3-CH. Thus, the planar structure of **1** was determined.

Hosotani *et al.*[4] has reported the stereochemistry of antimycin A_{13} and Hayashi and Nozaki[5] established the same configuration of the nine-membered dilactones of kitamycins A and B and urauchimycin B with that of antimycin A_{13} by analyzing NOESY. Compound **1** possessed the same configuration of the nine-membered dilactones (Figure 1) with antimycin A_{13} because of the strong agreement between the NMR data of the nine-membered dilactones of **1** and that of the antimycin A_{13}; in addition, the optical rotation of **1** in MeOH is very similar to the urauchimycin B in MeOH.[6] The NOE experiment of **1** further confirmed the result. Irradiation of the proton signal at 3-H (δ 5.29) resulted in an NOE enhancement (+5.58%) in the proton signal at 4-H (δ 5.73); in turn, the irradiation of the 4-H resulted in the enhancement (+8.08%) of the 3-H. Irradiation of the proton signal at the 7-H (δ 2.51) showed no enhancement of the 8-H (δ 5.07), but an enhancement (+4%) of the 9-H (δ 4.98).

As of now, different substituent of the alkyl side chains at C-7 and the oxygen substituent at C-8 in the nine-membered dilactone ring have generated about 30 naturally occurred antibiotics of the antimycin group.[7] Except antimycin A_9 that has an 8-phenylacetyl residue,[8] all the compounds in the antimycin A series from antimycin A_1 to A_{17} possess a C_4 to C_7 aliphatic acyl side chain at C-8.[4,9] Thus, **1** was the first naturally occurring antimycin that has an 8-*O*-acetyl side chain, and we named it antimycin A_{18}.

Hockenbery *et al.*[10] has synthesized a series of 2-methoxy antimycin derivatives, including **1**, as bioactive inhibitors for the Bcl-2 family members in an attempt to treat apoptosis-associated diseases. On the other hand, antimycins have the potential to be developed as fungicide.[9] Acylation of the 8-hydroxy group of antimycins has shown close relationship with their antifungal activities.[7] Owing to the free hydroxyl group at C-8, kitamycins A and B, as well as urauchimycins A and B, showed weak antifungal activities only.[7] Hosotani *et al.*[4] have reported that there are inverse relationships between the antifungal activity and the length of the 7-alkyl and 8-*O*-acyl side chains of antimycins. Using four strains of plant pathogenic fungi: *Colletotrichum lindemuthianum*, *Botrytis cinerea*, *Alternaria solani* and *M. grisea* as test strains, **1** and positive control, blasticidin S (Invitrogen, Carlsbad, CA, USA) were tested in serials dilution assay on a paper (6 mm i.d.) disk with potato dextrose agar medium. The minimum concentration values of **1** to show inhibition zone on plates were 0.01, 0.06, 0.03 and 0.20 μg ml^{-1}, respectively, whereas those of blasticidin S were 0.20, 0.60, 0.12 and 0.01 μg ml^{-1}, respectively. Except *M. grisea*, the respective minimum concentration values of **1** against *C. lindemuthianum*, *B. cinerea* and *A. solani* were 20, 10 and 4 times less than those of blasticidin S, a commercialized fungicide. It indicated that **1**, as a member of antimycins with the shortest *O*-alkylacyl side chain at C-8, has a potential to be developed for plant protection in the field.

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1 Hong, K. *et al.* Actinomycetes for marine drug discovery isolated from mangrove soils and plants in China. *Mar. Drugs* **7,** 24–44 (2009).
2 Thawai, C., Tanasupawat, S. & Kudo, T. *Micromonospora pattaloongensis* sp. nov., isolated from a Thai mangrove forest. *Int. J. Syst. Evol. Microbiol.* **58,** 1516–1521 (2008).
3 Ara, I., Kudo, T., Matsumoto, A., Takahashi, Y. Ōmura, S. *Nonomuraea maheshkhaliensis* sp. nov., a novel actinomycete isolated from mangrove rhizosphere mud. *J. Gen. Appl. Microbiol.* **53,** 159–166 (2007).
4 Hosotani, N., Kumagai, K., Nakagawa, H., Shimatani, T. & Saji, I. Antimycins A_{10}~A_{16}, seven new antimycin antibiotics produced by *Streptomyces* spp. SPA-10191 and SPA-8893. *J. Antibiot.* **58,** 460–467 (2005).
5 Hayashi, K. I. & Nozaki, H. Kitamycins, new antimycin antibiotics produced by *Streptomyces* sp. *J. Antibiot.* **52,** 325–328 (1999).
6 Immamura, N., Nishijima, M., Adachi, K. & Sano, H. Novel antimycin antibiotics, urauchimycins A and B, produced by marine actinomycete. *J. Antibiot.* **46,** 241–246 (1993).
7 Yao, C. B. F., Schiebel, M., Helmke, E, Anke, H. & Laatsch, H. Prefluostatin and new urauchimycin derivatives produced by *Streptomycete* isolates. *Z. Naturforsch. B.* **61,** 320–325 (2006).
8 Shiomi, K. *et al.* A new antibiotic, antimycin A_9, produced by *Streptomyces* sp. K01-0031. *J. Antibiot.* **58,** 74–78 (2005).
9 Chen, G. Y. *et al.* A new fungicide produced by a *Streptomyces* sp. GAAS7310. *J. Antibiot.* **58,** 519–522 (2005).
10 Hockenbery, D. M., Simon, J. A., Tzung, S. P. & (Fred Hutchinson Cancer Research Center) 2-Methoxy antimycin A derivatives and methods of use. U.S. 2005/02398731A1, 27 October 2005.