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Antibacterial and Antitumor Macrolides from *Streptomyces* sp. Is9131

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Four compounds, including two novel macrolides, were isolated from an endophyte *Streptomyces* sp. Is9131 of *Maytenus hookeri*. Spectral data indicated that these compounds were dimeric dinactin (**1**), dimeric nonactin (**2**), cyclo-homononactic acid (**3**), and cyclo-nonactic acid (**4**). Bioassay results showed that dimeric dinactin had strong antineoplastic activity and antibacterial activity.

Key words: Macrolide, *Streptomyces* sp. Is9131, Antineoplastic activity, Antibacterial activity

INTRODUCTION

Maytansinoids are compounds having strong cytotoxic and antineoplastic activities (Kupchan *et al.*, 1977; Reider and Roland, 1984) and are products of the bacterium *Actinosynnema pretiosum* (I-Egashide *et al.*, 1977), mosses (Suwanborirux *et al.*, 1990; Sakai *et al.*, 1988), and three closely related plant families, Celastraceae, Rhamnaceae, and Euphorbiaceae (Kupchan *et al.*, 1977; Reider and Roland, 1984). Maytansinoids produced by endophytic microorganisms could be accountable for their occurrence in higher plants. Endophytes occurring in higher plants are important sources of natural products with pharmaceutical potential (Strobel and Long, 1998; Tan and Zou, 2001). The cytotoxic activity of maytansine is under evaluation in new experimental models to discover and develop new drugs (Coghlan, 1996; Liu *et al.*, 1996). Therefore, the isolation of maytansine-producing endophytic microorganisms from plants would be a significant advance in understanding plant-microbe interactions (coevolution) and new drug discovery.

In the course of searching for maytansine-producing endophytic microorganisms from the plant *Maytenus hookeri*, we found that the extract of the culture material of *Streptomyces* sp. Is9131 (an endophyte of *M. hookeri*) exhibited potent activities against several pathogenic strains in diffusion assays (Espinel-Ingroff *et al.*, 1999), and also had antineoplastic activity. Four com- pounds were isolated from the fermentation products of Is9131, and the biological activities of compound **1** were assayed. The present paper describes the isolation and structure identification of these compounds, as well as the antineoplastic and antibacterial activity of dimeric dinactin.

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MATERIALS AND METHODS

General procedure

Optical rotations were measured with a JASCO DIP-370 digital polarimeter in $CHCl_3$ solution. Mass spectra were measured on a VG Auto Spec-3000 spectrometer and Thermo Finnigan LCQ Advantage. NMR spectra were measured on Bruker DRX-500 NMR spectrometers with TMS as internal standard. The reversed-phase (RP) C_{18} silica gel for the column chromatography was obtained from Merck and the Sephadex LH-20 from Amersham Biosciences.

Material

The seeds of *M. hookeri* were collected at Xishuangbanna, Yunnan, People's Republic of China, in April 2003. The plant material was washed in running tap water and was sterilized successively with 75% ethanol

for 1 min and 1.2% sodium hypochlorite for 8 min, then rinsed five times in sterile water and cut into small pieces. These small pieces were incubated at 25°C on YMG media (yeast extract 4.0 g, malt extract 10.0 g, glucose 4.0 g, agar 15.0 g, distilled water 1,000 mL) and cultured until colony or mycelium appeared surrounding the segments. After culturing about one month, a strain named Is9131 appeared, was isolated from the sterilized seed, and identified as *Streptomyces* sp. and deposited in Kunming Institute of Botany, Chinese Academy of Science, Kunming, China. The strain was cultured on YMG plates up to 10 L. After cultivation for two weeks, the cultures were extracted three times by 80% ethyl acetate, 15% methanol and 5% formic acid exhaustively.

Extraction and isolation

The extracts were extracted with chloroform and the chloroform fraction (CH 2.0 g) was run on MPLC over reversed-phase (RP-18) silica gel (125 g) column, eluting with water containing increasing amounts of acetone to produce 5 fractions (CH-1 to CH-5). Fraction CH-5 was subjected to column chromatography over Sephadex LH-20 (20 g), eluted with methanol, and subjected again to MPLC over RP-18 (125 g) eluting with acetone - H_2O (7:3, v/v) containing 0.1% diethylamine to afford compound **1** (8 mg), compound **2** (5 mg), compound **3** (15 mg), and compound **4** (10 mg).

Dimeric dinactin (1)

Colorless oil, $[\alpha]_D^{17}$ –0.0 (*c* 0.01, $CHCl_3$); ESIMS *m/z*: 383 $[M + H]^+$; UV (MeOH) λ_{max} (log ε): 208.0 (2.73); NMR data in Table I.

Dimeric nonactin (2)

Colorless powder, $[\alpha]_D^{25}$ –2.38 (*c* 0.08, $CHCl_3$); ESIMS *m/z*: 369 $[M + H]^+$; UV (MeOH) λ_{max} (log ε): 210.2 (3.82); IR (KBr) ν_{max}: 1727 (s), 1193, 1061 cm^{-1}; NMR data in Table I.

Cyclo-homononactic acid (3)

Colorless oil, ESIMS *m/z*: 199 $[M + H]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.86 (3H, t, *J* = 7.4, H-10), 1.07 (3H, d, *J* = 7.4, H-11), 1.43 (1H, m, H-5), 1.47-1.92 (4H, m, H-7, H-9), 1.62 (1H, m, H-4), 1.92 (1H, m, H-4), 1.99 (1H, m, H-5), 2.48 (1H, m, H-2), 3.82 (1H, m, H-6), 4.05 (1H, m, H-3), 4.87 (1H, m, H-8); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 9.3 (C-10), 12.9 (C-11), 28.3 (C-4), 31.5 (C-5), 39.9(C-7), 41.5 (C-9), 45.1 (C-2), 76.5 (C-6), 79.0 (C-8), 80.0 (C-3), 175.0 (C-1). These data are consistent with previous work (Stadler *et al.*, 2001).

Cyclo-nonactic acid (4)

Colorless oil, ESIMS *m/z*: 185 $[M + 1]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.05 (3H, d, *J* = 7.4, H-10), 1.14 (3H, d, *J* = 7.4, H-9), 1.43 (1H, m, H-5), 1.62-1.89 (2H, m, H-7), 1.62 (1H, m, H-4), 1.92 (1H, m, H-4), 1.99 (1H, m, H-5), 2.48(1H, m, H-2), 3.82 (1H, m, H-6), 4.05 (1H, m, H-3), 4.87 (1H, m, H-8); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 12.9 (C-10), 20.5 (C-9), 26.5 (C-4), 31.5 (C-5), 39.9 (C-7), 45.1 (C-2), 68.8 (C-8), 71.5(C-6), 79.7 (C-3), 178.0 (C-1). These data are consistent with previous work (Stadler *et al.*, 2001).

Biological activity

The antibacterial activity was assessed against gram-positive bacterium, *Staphylococcus aureus*, and gram-negative bacterium, *Mycobacterium tuberculosis*, using the disk diffusion method (Espinel-Ingroff *et al.*, 1999), with Rifampicin as a control. The antitumor assay was done by the National Center for Drug Screening.

RESULTS AND DISCUSSION

Identification of compounds

For compound **1**, the HRESIMS determined the molecular formula to be $C_{21}H_{34}O_6$ (*m/z* 383.2448 $[M + H]^+$, calcd.: 383.2433). The UV (MeOH) spectra showed absorption at λ_{max} (log ε) 208.0 (2.73). The ^{13}C-NMR spectra indicated the preferable symmetry in the structure of **1** as the carbon signals appeared in pairs: two carbonyls at δ 174.3 and 174.6, four oxygen-substituted methines at δ 79.8, 79.7, 76.2 and 76.0, two methines at δ 45.1 and 44.9, four methylenes at δ 31.0, 31.2, 27.9 and 27.8, and two methyls at δ 12.4 and 12.9 (Table I). The NMR data of **1** was very similar to those of dimeric nonactic acid (Wener *et al.*, 1996). But **1** was cyclized to form a dimacrolide, and the substituent at C-8' was ethyl in this compound but methyl in dimeric nonactic acid. The HMBC experiment showed the ^{1}H-^{13}C long-range correlations between the proton at δ 4.89 (H-8) and the carbon at δ 174.6 (C-1'), δ 4.84 (H-8'), and δ 174.3 (C-1), which determined the structure of dimeric dinactin relative to dinactin (Martyn *et al.*, 1997). The NOESY experiment showed ^{1}H-^{1}H correlations between δ 3.99 (H-3) and δ 3.80 (H-6), δ 3.80 (H-6) and δ 4.89 (H-8). Moreover, the signals of H-3 and H-3', H-6 and H-6' were completely superposed. The dimeric nonactic acid was an optically nonactive mixture (Wener *et al.*, 1996), and **1** had no optical activity, indicating that the relative configuration was the same (Fig. 1). Therefore, compound **1** was determined to be dimeric dinactin (Fig. 1). A similar compound (dilactone) was reported by Tomas and coworkers (Tomas *et al.*, 2004), but dilactone was an optical compound. Moreover, the NMR data of **1** were also different from dilactone: the chemical shift of C-6 (C-14) was at δ 72.2 (70.5) in dilactone, but that of C-6 (C-6') was at δ 76.0 (76.2) in dimeric dinactin (**1**). This difference may result in

Table I. NMR Data for Compounds **1** and **2** (in $CDCl_3$)[a]

Position	1			2		
	δ_C	δ(m, *J* in Hz)	HMBC[b]	δ_C	δ_H (m, *J* in Hz)	HMBC[b]
1	174.3	/	/	174.2	/	/
2	44.9	2.48 m	CH_3-2,C-4,C-3,C-1	45.2	2.47 m	CH_3-2, C-4, C-3,C-1
3	79.7	3.99 (ddd)(7.4)	CH_3-2,C-5,C-2,C-1	80.0	4.00 (ddd)(7.3)	CH_3-2, C-1
4	27.9	1.56 m	C-6,C-5, C-3,C-2	28.1	1.58 m	C-5(w), C-2, C-6, C-3
		1.90 m	C-7,C-6, C-3,C-2		1.93 m	C-5, C-2(w)
5	31.0	1.99 m	C-7,C-6 (w), C-4,C-3	31.3	1.98 m	C-4, C-3
		1.43 m	C-7, C-6 (w), C-4, C-3		1.49 m	CH_3-2, C-4, C-7, C-6(w)
6	76.0	3.80 m	C-8,C-5, C-3 (w)	76.3	3.83 m	C-8
7	42.1	1.72 m	CH_3-8, C-8,C-5, C-6	42.1	1.76 m	C-5, C-8, C-6
8	69.0	4.89 m	CH_3-8(w),C-7,C-6,C-1'	69.0	4.96 m	CH_3-8, C-7, C-6, C-1
2-CH_3	12.9	1.05 d(7.4)	C-3, C-2, C-1	12.8	1.06 d(7.2)	C-2, C-3, C-1
8-CH_3	20.1	1.17 d(6.2)	C-8, C-7, C-6(w)	20.4	1.24 d(6.2)	C-5, C-7, C-8
1'	174.6	/	/	174.2	/	/
2'	45.1	2.48 m	CH_3-2', C-4', C-3', C-1'	45.0	2.47 m	CH_3-2', C-4', C-3', C-1'
3'	79.8	3.99 (ddd)(7.4)	CH_3-2',C-2', C-1'	80.0	4.00 (ddd)(7.4)	CH_3-2', C-1'
4'	27.8	1.90 m	C-6', C-5', C-3', C-2'	28.1	1.58 m	C-6', C-5', C-3', C-2'
		1.56 m	C-7',C-6', C-3', C-2'		1.93 m	C-5', C-2'
5'	31.2	1.99 m	C-7', C-6'(w), C-4', C-3'	31.3	1.98 m	C-4', C-3'
		1.43 m	C-7', C-6'(w), C-4', C-3'		1.49 m	CH_3-2', C-4', C-7', C-6' (w)
6'	76.2	3.80 m	C-8', C-5',C-3' (w)	76.3	3.83 m	C-8'
7'	39.6	1.69 m	$\underline{C}H_2CH_3$-8', C-8', C-6', C-5'	42.1	1.76 m	CH_3-8', C-5', C-8', C-6'
8'	73.1	4.84 m	$CH_2\underline{C}H_3$-8', $\underline{C}H_2CH_3$-8' (w), C-7',C-6',C-1	69.0	4.96 m	CH_3-2', C-7', C-6', C-1
2'-CH_3	12.4	1.05 d(7.4)	C-2', C-3', C-1	12.8	1.06 d(7.3)	C-2', C-3', C-1
8'-$\underline{CH_2}CH_3$(8-CH_3)	27.1	1.92 m	C-6'	20.4	1.20 d(7.6)	C-7', C-8'
		1.47 m	$CH_2\underline{C}H_3$-8', C-7', C-6'			
8'-$CH_2\underline{CH_3}$	9.0	0.83 t(7.4)	$\underline{C}H_2CH_3$-8', C-8'			

[a] Run at 500 MHz for ^1H and 125 MHz for ^{13}C.
[b] (w) indicated weak correlations.

a change of configuration, which is supported by previous studies: the NMR value of C-6 gave at δ 77.1~77.4 in nonactic acid (Prikrylova *et al.*, 1994), but at δ 70.6 in methyl nonactate (Tomas *et al.*, 2004).

For compound **2**, the positive HRESIMS determined the molecular formula to be $C_{20}H_{32}O_6$ (*m/z* 369.2274 $[M + H]^+$, calcd.: 369.2277). The UV (MeOH) spectra showed absorption at λ_{max} (log ε) 210.2 (3.82). The IR (KBr) spectra of **2** revealed the presence of the carbonyl group (1727(s) cm^{-1}) and ether group (1193, 1061 cm^{-1}). The ^{13}C-NMR and DEPT experiments (Table I) only showed ten carbon signals: one carbonyl at δ 174.2, three oxygen-substituted methines at δ 80.0, 76.3 and 69.0, one methine at δ 45.2/45.0, two methylenes at δ 31.3, 28.1, and two methyls at δ 20.4 and 12.8, which indicated that **2** had complete symmetry. The NMR data of **2** was very similar to compound **1**. The HMBC experiment showed the ^1H-^{13}C long-range correlations between the proton at δ 4.96 (H-8 or H-8') and the carbon at δ 174.3 (C-1' or C-1'), δ 3.83 (H-6 or H-6') and δ 80.0 (C-3 or C-3'). The NOESY experiment showed NOE between the protons at δ 2.47 (H-2/H-2') and δ 4.00 (H-3/H-3'), and δ 4.00 (H-3/H-3') and δ 3.83 (H-6/H-6'), and δ 3.83 (H-6/H-6') and δ 4.96 (H-8/H-8'). Therefore, compound **2** was determined to be dimeric nonactin (Wener *et al.*, 1996; Martyn *et al.*, 1997) (Fig. 1). The skeleton of compound **2** was mentioned on other publication (Bartlett *et al.*, 1984), but according to the report, the pure compound had not been obtained, and its structure and physical data just were speculated. In our present research, we provided NMR data and its relative structure.

Biological activity

The results from disc diffusion showed that compound **1**

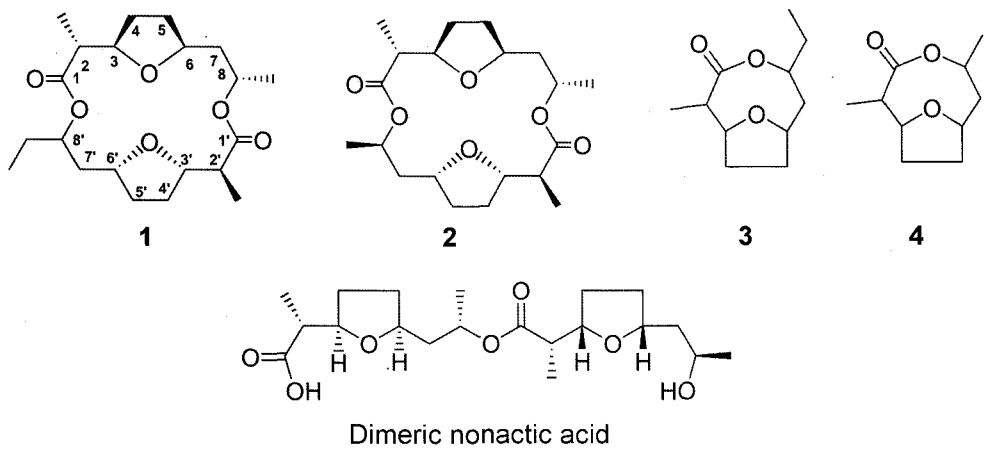

Fig. 1. Structures of compounds **1-4** and dimeric nonatic acid

Table II. Antibacterial activity of dimeric dinactin*

	Dimeric dinactin		Rifampicin	
	μg/disc	Diameter of inhibition zone (cm)	μg/disc	Diameter of inhibition zone (cm)
S. aureus	0.08	0.9	1.5×10^{-3}	0.7
	0.04	0.7	1.35×10^{-3}	—
	0.02	—	1.2×10^{-3}	—
M. tuberculosis	2.0	0.9	7.5×10^{-3}	1.0
	1.0	0.8	6.75×10^{-3}	0.8
	0.5	—	6.0×10^{-3}	—

*Note: diameter of the paper disk was 0.6 cm, "—" : didn't show obvious inhibition activity.

Table III. Effect of dimeric dinactin to the growth of human tumor cell line

Cell lines	IC_{50} (μM)	Sources
HL60	0.26	leukaemia
A549	1.28	lung cancer
SGC7901	1.80	gastric cancer
BEL7402	2.16	liver cancer

exhibited strong antibacterial activities against *S. aureus* and *M. tuberculosis* (Table II).

The inhibitory effect of dimeric dinactin on growth of various human tumor cell lines is shown in Table III. Growth of all the cell lines was inhibited with similar doses, and the IC_{50} was about 1 μM.

The macrotetrolide antibiotics (dinactin) have diverse activities and are formed by a cyclic tetraester structure from four monomeric units of thenonactic acids and their homologues (Martyn *et al.*, 1997). Dimeric nonactic acid did not show antibacterial nor antifungal activity at concentrations < 2 mg/mL; however the dimeric dinactin showed strong antibacterial and antitumor activity, indicating the importance of the lactone ring in these activities.

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