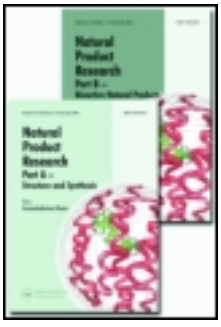

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A new antitumour ansamitocin from *Actinosynnema pretiosum*

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A new compound of ansamitocin was isolated from the extracts of fermentation medium of mutant strain HGF052 derived from *Actinosynnema pretiosum* ssp. *aurantium* ATCC 31565, and identified as *N*-demethyl-desepoxy-9-methoxy-maytansinol (**1**) on the basis of extensive spectroscopic methods. Bioassay results showed that compound **1** had cytotoxic activity against HL-60 and BEL-7402 cell lines.

Keywords: *Actinosynnema pretiosum* ssp. *auranticum* ATCC 31565; ansamitocins; spectroscopic methods; cytotoxic activity

1. Introduction

The maytansinoids are characterised by 19-member macrocyclic lactams attached to a chlorinated benzene ring chromophore and are members of the ansamycins group of natural products of actinomycetes (Higashide et al., 1977), mosses (Sakai et al., 1988; Suwanborirux, Chang, Spjut, & Cassady, 1990) and three related plant families: Celastraceae, Rhamnaceae and Euphorbiaceae (Kupchan et al., 1972, 1977). They have extraordinary cytotoxic and antineoplastic activities (Kupchan et al., 1972, 1977). Recently, a series of glycosides of ansamitosins were isolated from *Actinosynnema pretiosum* that displayed strong biological activities (Lu, Bai, & Shen, 2004; Ma, Zhao, & Shen, 2007).

In our continued search for novel metabolites related to ansamitocins, we investigated the metabolites of a mutant strain HGF052 derived from *A. pretiosum* ssp. *aurantium* ATCC 31565 (Spiteller et al., 2003). By fermentation and isolation of the mutant strain HGF052, we obtained a new metabolite, namely *N*-demethyl-desepoxy-9-methoxy-maytansinol (**1**), together with a known compound *N*-demethyl-desepoxy-maytansinol (**2**) (Figure 1). This article describes the isolation and structural elucidation of the two compounds and their biological activities.

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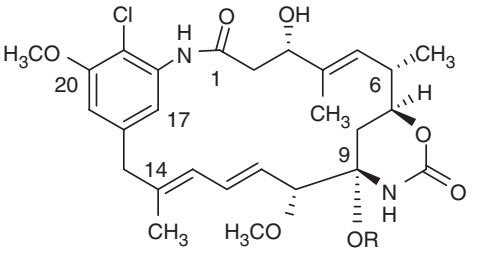

1R=CH_3
2R=H

Figure 1. Structure of compounds **1** and **2**.

2. Results and discussion

2.1. *Structure determination of compounds 1 and 2*

Compound **1** was obtained as a white amorphous power. $[\alpha]_D^{22} = -72.2$ (*c* 0.09, MeOH). IR (KBr) cm^{-1}: 3427, 2933, 1702, 1592. UV (MeOH) λ_{max} (log ε): 220.2 (4.65), 243.2 (4.52), 281.6 (3.49), 345.4 (2.19). Its molecular formula was determined to be $C_{28}H_{37}ClN_2O_7$ by ESI-MS (*m*/*z* 571 $[M + Na]^+$), which was further confirmed by HR-FAB-MS (*m*/*z* 571.2224 $[M + Na]^+$, Calcd 571.2245). The ansamitocin moiety was readily recognised by analysing the NMR data (^{1}H- and ^{13}C-NMR, HSQC, HMBC and COSY) and comparing with literature data (Higashide et al., 1977; Kupchan et al., 1972, Spiteller et al., 2003). The NMR data of **1** (Table 1) were very similar to those of *N*-demethyl-desepoxy-maytansinol (Spiteller et al., 2003). However, an additional –OCH_3 (δ_H 3.42, s; δ_C 52.0) of **1** was observed. In the HMBC spectrum of **1**, –OCH_3 (δ_H 3.42, s) correlating with C-atoms at δ_C 84.3(9) indicated –OCH_3 attached at C-9. Moreover, $J_{H\text{-}5,\text{-}6}$ and $J_{H\text{-}10,\text{-}11}$ were consistent with those assigned in *N*-demethyl-desepoxy-maytansinol (Spiteller et al., 2003), indicating that compound **1** had the same relative configuration as ansamitocins. Therefore, compound **1** was identified as *N*-demethyl-desepoxy-9-methoxy-maytansinol.

Compound **2** was obtained as white amorphous power. $[\alpha]_D^{22} = -95.7$ (*c* 0.55, MeOH). IR (KBr) cm^{-1}: 3510, 2930, 1712, 1592. UV (MeOH) λ_{max}(log ε): 221.2 (4.65), 243.4 (4.54), 282.0 (3.46). ESI-MS *m*/*z*: 557(100) $[M(^{35}Cl) + Na]^+$, 559(33) $[M(^{37}Cl) + Na]^+$. HR-FAB-MS *m*/*z*: 535.2204 $[M + H]^+$, Calcd for $C_{27}H_{35}ClN_2O_7$ 535.2211. By careful comparison of its physical and spectroscopic data with the corresponding literature data (Spiteller et al., 2003), compound **2** was identified as *N*-demethyl-desepoxy-maytansinol.

2.2. *Biological activity*

Investigation of the inhibitory effects of compound **1** on the growth of various tumour cell lines was performed by the National Center for Drug Screening, and the antitumour activity of compound **2** was tested by the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences. Growth of all cell lines evaluated were inhibited at different dose levels: the IC_{50} of compound **1** against HL-60 was 0.12 μM and against

Table 1. NMR data of compound **1** (in CD_3COCD_3; δ in ppm, J in Hz).

No.	δ_C	δ_H	HMBC
1	Not visible	–	–
2	Not visible	Not visible	–
3	72.0	4.20 d (5.0)	C-5, C-4, Me-4
4	138.1	–	–
5	124.7	5.45 d (9.5)	C-3, C-4, C-6, C-7, Me-4
6	38.1	2.54 m	C-4, C-5, C-7, C-8, Me-6
7	78.7	3.93 m	–
8	36.5	1.87 m	–
		1.33 m	C-6, C-7, MeO-9, C-10
9	84.3	–	–
10	90.7	3.69 d (9.3)	C-8, MeO-10, C-9, C-11, C-12
11	127.3	5.58 dd (15.1, 9.3)	–
12	134.0	6.69 dd (15.1, 11.1)	C-10, C-13, C-14
13	127.6	6.16 d (11.1)	C-12, C-14, Me-14, C-15
14	139.2	–	–
15	46.3	3.51 d (14.0)	C-13, C-14, Me-14, C-17, C-21
		3.26 m	C-13, C-14, Me-14, C-17, C-21
16	141.0	–	–
17	113.3	Not visible	–
18	136.8 or not visible	–	–
19	136.8 or not visible	–	–
20	155.9	–	–
21	109.3	6.84 br.s	–
CONH	152.8	–	–
Me-4	14.7	1.56 br.s	C-3, C-5
Me-6	16.6	1.08 d (6.5)	C-5, C-6, C-7
Me-14	17.8	1.72 s	C-13, C-14, C-15
MeO-9	52.0	3.42 s	C-9
MeO-10	56.4	3.29 s	C-10
MeO-20	56.6	3.92 s	C-20

BEL-7402 was 16.05 μM, and the IC_{50} of compound **2** against HL-60 and SMMC-7721 was 0.625 μM and 9.339 μM, respectively.

3. Experimental

3.1. *Spectroscopic measurements and chromatography*

Optical rotations were measured on a Jasco DIP-370 polarimeter. NMR spectra were measured in CD_3COCD_3 and recorded on a Bruker AM-400 spectrometer with TMS as the internal standard. ESI-MS and HR-FAB-MS were performed on Finnigan LCQ-Advantage and VG Auto-Spec-3000 mass spectrometers, respectively. The UV spectrum was measured on a Shimadzu UV-2401PC, $\lambda_{max}(\log \varepsilon)$ in nm. The IR

spectrum was measured on a Bio-Rad FTS-135 spectrometer. Column chromatography (CC) was performed on silica gel (200–300 mesh, 10–40 μm; Qingdao Marine Chemical Factory, China), on Sephadex LH-20 (Amersham Pharmacia, Sweden), on reverse-phase C_{18} (RP-18) silica gel (40–70 μm, Merck, Germany). TLC was performed on silica gel GF_{254} (10–40 μm, Qingdao). All solvents were distilled before use.

3.2. *Microbial material*

The Δ*asm19* strain HGF052 derived from *A. pretiosum* ssp. *aurantium* ATCC 31565 was obtained as a gift from Dr Bai LQ, which was conserved in 20% glycerol and deposited at the Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, P.R. China.

3.3. *Extraction and isolation*

The Δ*asm19* strain HGF052 was cultured on YMG solid medium (10 L) (yeast extract (0.4%), malt extract (1%), glucose (0.4%) and agar (1.5%), pH = 7.2) for 10 days at 28°C. The culture was extracted three times with EtOAc : MeOH : AcOH (80 : 15 : 5, v/v/v), exhaustively, to give an extract (10.134 g). The extract was subjected to MPLC over RP-18 silica gel (40–75 μm, 160 g) eluted with H_2O, 50% MeOH, 75% MeOH and 100% MeOH (3 L each) and yielded four fractions.

The 75% MeOH fraction (935 mg) was subjected to CC, eluted with a petroleum ether : ethyl acetate gradient system (from 1 : 5 to 1 : 1) to provide four fractions: Fra1–4. Fra3 (83 mg) was subjected to CC (silica gel; petroleum ether : acetone, from 1 : 6 to 1 : 1) to afford Fra3-1, and Fra3-1 was further purified by CC over Sephadex LH-20 eluted with acetone to provide **1** (8 mg). Fra4 (778 mg) was subjected to CC (silica gel; petroleum ether : ethyl acetate, from 1 : 6 to 1 : 1) to afford Fra4-1, and Fra4-1 was further purified by CC over Sephadex LH-20 eluted with acetone to provide **2** (162 mg).

3.4. *Test for cytotoxicity* in vitro

Compound **1** was tested for *in vitro* cytotoxicity against HL-60 and BEL-7402 cell lines in the National Center for Drug Screening (Shanghai, P.R. China), and compound **2** was tested against HL-60 and SMMC-7721 cell lines in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

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