A New Anthracycline Antibiotic Micromonomycin from *Micromonospora* sp.

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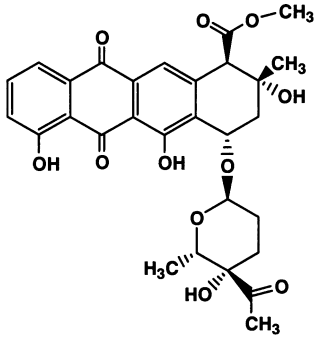
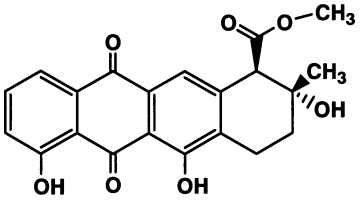
In the course of our continuing search for novel antimicrobial agents,[1~5] we have isolated a novel antibacterial anthracycline, micromonomycin (**1**), from culture *Micromonospora* sp. Micromonomycin was identified as a new anthracycline using high resolution ESI-MS and extensive NMR spectroscopic analyses. In this paper, we describe the isolation and structure elucidation of **1**. The biological activity of **1** against Gram-positive and Gram-negative bacterial strains as well as fungal pathogens is also reported.

The germination and fermentation conditions of this culture were described previously.[5] The fermentation broth (2 liters) was stirred with 100 g of NaCl and 4 liters of acetonitrile (MeCN). The organic layer was separated and dried in vacuum. The extract was absorbed onto the polymeric resin, CG161 (~100 ml) and the NaCl salt was washed out with water (200 ml). The absorbed organic material was eluted with 200 ml 40% aq. MeCN, and 80% aq. MeCN to yield 432 and 73 mg of dried material, respectively, after removing solvent *in vacuo*. The organic material of 80% MeCN fraction was fractionated on an HPLC semi-preparative ODS-A column (YMC, 120 Å, S-7, 20 mm×250 mm). The column was eluted with a three-step gradient of MeCN-H_2O: 5~40% MeCN in 50 minutes, 40~85% gradient in 35 minutes, and then 85% MeCN isocratic for another 15 minutes, with a flow rate of 15 ml/minute. Fractions were collected (13 ml/fraction) by a fraction collector. Pure **1** (~1 mg) and 7-deoxyauramycinone (**2**, ~2 mg) were obtained with two injections of total 73 mg of above 80% MeCN fraction at retention time ~68 and 81 minutes, respectively.

On the basis of analysis of high-resolution ESI-MS data, the molecular formula of **1** was established as $C_{29}H_{30}O_{11}$ ($[M+Na]^+$: Found 577.1700; calcd. 577.1680) indicating 15 degree of unsaturation in the molecule (performed on a PE Sciex QSTAR mass spectrometer, positive ion ESI-HR-MS measurements). The structure of **1** was further elucidated by extensive NMR data analysis. In the downfield region of the ^{13}C NMR spectrum, one carbonyl (C-7′, δ 210.1) and a carboxyl signals (C-14, δ 171.5) were observed and assigned to an acyl and a methyl ester groups, respectively, based on the long range correlations of CH_3-15 (δ 3.87) to C-14, and CH_3-8′ (δ 2.24, s) to C-7′ observed in the HMBC spectrum. The ^{13}C signals of two conjugated-carbonyl (C-5, δ 192.6; C-12, δ 181.3) and twelve aromatic carbons in the downfield region indicated the anthraquinone moiety. This was confirmed by HMBC and ^1H-^1H COSY correlations shown in Figure 1. Among those correlations, both H-1 (δ 7.84, d) and H-11 (δ 7.59, s) having correlations to C-12 (δ 181.3, s) indicated the location of carbonyl C-12 and led to assignment of regio location for H-1 and H-11. No observation of any proton having long-range correlation to the carbonyl C-5 signal in the HMBC spectrum suggested that both C-4 (δ 162.6) and C-6 (δ 161.6) were substituted. Two hydroxyl proton



Micromonomycin (**1**)



7-Deoxyauramycinone (**2**)

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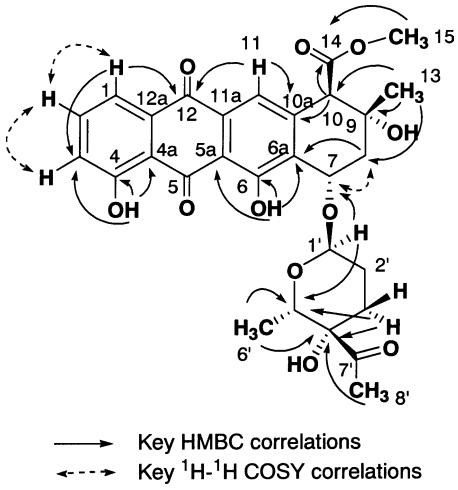
Table 1. NMR spectral data for compound **1** in $CDCl_3$.[a]

C/H no.	^{1}H (δ)	^{13}C (δ)
1	7.84, dd, *J* = 7.5, 1.2	120.2 d
2	7.70, dd, *J* = 7.5, 8.4	137.4 d
3	7.32, dd, *J* = 8.4, 1.2	124.8 d
4		162.6 s
4a		115.8 s
5		192.6 s
5a		114.3 s
6		161.6 s
6a		131.0 s
7	5.30, dd, *J* = 4.4, 3.2	69.2 d
8α	2.51, dd, *J* = 14.6, 3.2	40.8 t
8β	2.03, dd, *J* = 14.6, 4.4	
9		69.7 s
10	3.96, s	57.3 d
10a		142.9 s
11	7.59, s	121.5 d
11a		132.7 s
12		181.3 s
12a		133.5 s
13	1.50 s	29.2 q
14		171.5 s
15	3.87, s	52.5 q
1'	5.44, brs	99.8 d
2'-α	2.13, m	24.5 t
2'-β	1.74, m	
3'-α	1.45, m	27.5 t
3'-β	2.13, m	
4'		78.5 s
5'	4.58, q, *J* = 6.6	66.8 d
6'	1.04, d, *J* = 6.6	14.7 q
7'		210.1 s
8'	2.24, s	24.7 q
4-O*H*	12.02, s	
6-O*H*	12.69, s	
O*H*	3.85, s	
O*H*	4.34, s	

[a] Recorded on a Varian Unity 500 NMR instrument at 500 MHz for ^{1}H and 125 MHz for ^{13}C, using standard Varian pulse sequence programs (VNMR Version 6.1 Software). δ in ppm; *J* in Hz.

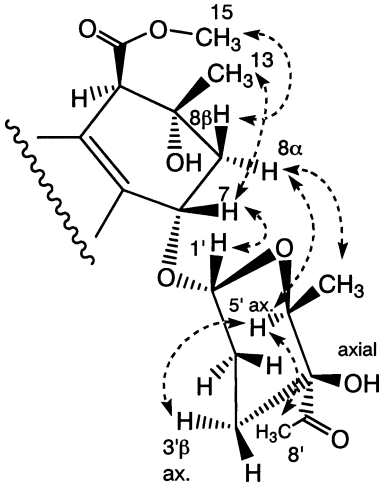
Fig. 1. 2D NMR correlations of **1**.



signals (δ 12.02, s; δ 12.69, s) in the downfield region of the ^{1}H-NMR spectrum revealed the presence of the strong hydrogen bonding, and the HMBC correlations of O*H*-4 and O*H*-6 to their adjacent carbons (Figure 1) confirmed the assignment of the hydroxyl substitutions on C-4 and C-6. This is a typical phenomenon observed in the anthraquinone type of the compounds with β-hydroxyl substitution to the carbonyl functionality of ring B.[6~8] Thus, rings A, B, and C were identified. The connectivity of rings C and D was established based on detailed correlations shown in Figure 1, for instance, the correlation of H-10 (δ 3.96, s) and H-11 to a quaternary aromatic carbon (C-10a, δ 142.9), the correlations of CH_3-13 (δ 1.50, s) to C-8 (δ 40.8), C-9 (δ 69.7), and C-10 (δ 57.3) observed in the HMBC spectrum, and H-7 (δ 5.30, dd) and H_2-8 (δ 2.51, 2.03) observed in the ^{1}H-^{1}H COSY spectrum. Connectivity of C-7 (δ 69.2) and C-6a (δ 131.0) was determined by the observation of the long-range correlation from H-8 (δ 2.51) to C-6a and C-7. Thus, the anthracycline skeleton, which belongs to auramycinone[6~8], was determined.

Remaining 6 carbons consist of one hemi-acetal (H-1′, δ 5.44; C-1′, δ 99.8), two methylenes (C-2′, δ 24.5; C-3′, δ 27.5), one *O*-substituted quaternary carbon (C-4′, δ 78.5), one oxygenated methine carbon (C-5′, δ 66.8), and a methyl group (C-6′, δ 14.7). These functionalities plus the previously identified acyl group could be assembled to an acylated sugar by analyses of the HMBC and ^{1}H-^{1}H COSY data shown in Figure 1. These correlations led to establishment of the 2,3,6-tri-deoxy sugar moiety with an unusual acyl substitution on C-4′ position through C–C

Fig. 2. Key NOE correlations in ring D and glycoside of **1**.



bond. The deoxy-sugar was assigned to the 7-*O* position attachment based on the correlation of H-1′ to C-7 in the HMBC spectrum and the NOE correlation of H-7 and H-1′. Thus, the full structure was elucidated.

The relative stereochemistry of **1** was determined by the analyses of the ^{1}H-^{1}H coupling patterns and the NOE data. In the ring D, NOE correlations between CH_3-15 and CH_3-13, CH_3-15 and H-8β, and CH_3-13 and H-7 established the same (β) orientation of these protons. Hence, 7-*O*-glycoside is substituted on the opposite (α) orientation. This was supported by the observation of NOE correlations of H-8α (δ 2.51) to H-5′ (δ 4.58) and CH_3-6′ (δ 1.04) of glycoside, which showed that they are oriented to the same direction. Thus, the relative stereochemistry of ring D was determined as shown in Figure 2. This configuration is in consistent with that of related known anthracyclines, such as auramycins.[7,8]

The small coupling pattern of H-1′ to H-2′α (δ 2.13) and H-2′β (δ 1.74) established the equatorial orientation of H-1′. NOE correlation between H-3′β (δ 2.13) and H-5′ showed the typical axial-axial space proximity. Acyl substitution on C-4′ (δ 78.5) was determined as β equatorial position as a result of the observation of NOE between H-5′ and CH_3-8′. (δ 2.24). Thus, the structure elucidation of **1** was completed.

Compound **2** was identified as 7-deoxyauramycinone by using extensive 1D and 2D NMR analyses, and by comparing with those of compound **1** and the literature data.[9]

Compound **1** exhibited antibacterial activity against various strains. The MIC values of **1** are listed in Table 2, in comparison with gentamicin as reference standard. Micromonomycin (**1**) showed potent inhibitory activity against *Staphylococcus aureus*, *Streptococcus pneumoniae*, and supersensitive *E. coli*[10] strains with MIC values 1～2, 0.5, and 4 μg/ml, respectively, and also displayed weak antifungal activity against *Saccharomyces cerevisiae* (PM503)[1] and *Candida albicans* (C43).

Table 2. Antimicrobial activity of compound **1**, MIC-48 hours (μg/ml).

Strain[a]	MIC (μg/mL)	
	1	Gentamicin
S. aureus supersensitive (HS999)	1	8
S. aureus (ATCC 29213)	2	0.06
S. pneumoniae (ATCC 49619)	0.5	0.5
E. coli supersensitive (HS294)	4	2
E. coli (ATCC 10536)	>128	0.125
S. cerevisiae supersensitive (PM503)	32	>64
C. albicans (C43)	32	>64
A. fumigatus (ND158)	>128	>64

[a] Incubation for 24 hours for bacteria, 48 hours for fungi

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