Notes

SO-75R1, A NEW MUTACTIMYCIN DERIVATIVE PRODUCED BY *Nocardia brasiliensis*

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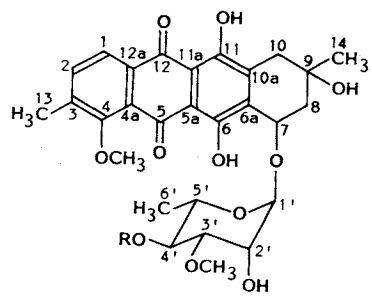
In the course of studies on biologically active substances from pathogenic microorganisms, a new antibiotic SO-75R1 was isolated from the mycelium of *Nocardia brasiliensis* IFM 075. This paper describes the fermentation, isolation and structural elucidation of SO-75R1.

The seed broth was prepared by inoculating mycelial elements of the producing strain (IFM 075) grown on Sabouraud dextrose agar (Difco) into 10 ml brain heart infusion broth with 2% glucose in 50-ml Erlenmeyer shake flasks. The culture was incubated on a rotary shaker at 250 rpm for 96 hours. Ten percent inoculum was transferred to a 2.0-liter fermenter containing 1.0 liter of the production medium composed of meat extract 0.5%, peptone 0.5%, glucose 2.0% and supplemented with antifoam 0.05%. The pH was adjusted to 7.4. The jar fermenter was stirred at 500 rpm with aeration at 1.0 liter/minute at 30°C for 4 days. After 4 days of incubation, one and one half volume of methanol was added to the culture broth and further incubated for 3 hours to kill the *Nocardia* and to extract the active substance from the mycelia. Then the broth was filtered and evaporated under reduced pressure to the original quantity and extracted with a half volume of ethyl acetate. The extract was concentrated *in vacuo* and the crude residue was then subjected to silica gel chromatography using $CHCl_3$ as an eluting agent. The combined active fraction was purified by preparative TLC (silica gel, E. Merck) of the developing solvent of ethyl acetate and followed by a mixture of $CHCl_3$ - acetone (1 : 1). SO-75R1 was further purified using an HPLC column (Capsel Pak-C18, SG-120) using CH_3CN-H_2O (3 : 2) as the eluent.

SO-75R1 (**1**, Fig. 1) was obtained as orange crystals, mp 121~122°C; $[\alpha]_D^{23}$ +216° (*c* 1.0, $CHCl_3$). SO-75R1 (**1**) was a neutral substance, dissolved in methanol, ethyl acetate, chloroform, and other organic solvents, and insoluble in water. The molecular formula of SO-75R1 (**1**) was determined as $C_{29}H_{34}O_{11}$ by the HRFAB-MS data (*m/z* 559.2177, $M^+ + H$, Δ 0.4 mmu). The IR absorption bands (ν_{max} (KBr) cm^{-1} 3450, 2930, 1615, 1575, 1405, 1235, 1135, and 1050) suggested the presence of hydroxyl and conjugated carbonyl groups. The UV absorption spectrum (λ_{max} nm (ε) in MeOH: 234 (23,000), 256 (31,000), 292 (10,000), 470 (11,000), 490 (12,000), and 520 (8,400)) of **1** was reminiscent of the 1,4,5-trihydroxyanthraquinone chromophore commonly present in daunomycin and related antibiotics[1]. The two carbonyl signals observed in the ^{13}C NMR of **1** at δ_C 187.0 and 186.5 also indicated the presence of the anthraquinone group and the two sharp ^1H singlets resonating in the fairly low-field (δ_H 14.0 and 13.4) were assignable to two hydroxyl group attached on the aromatic ring, viz. two phenols, which were coincident with the observation that UV absorption at 490 nm was shifted to 550 nm on addition of alkali. The ^1H NMR

Fig. 1. Structures of SO-75R1 and mutactimycin A.



1 R=Me
2 R=H

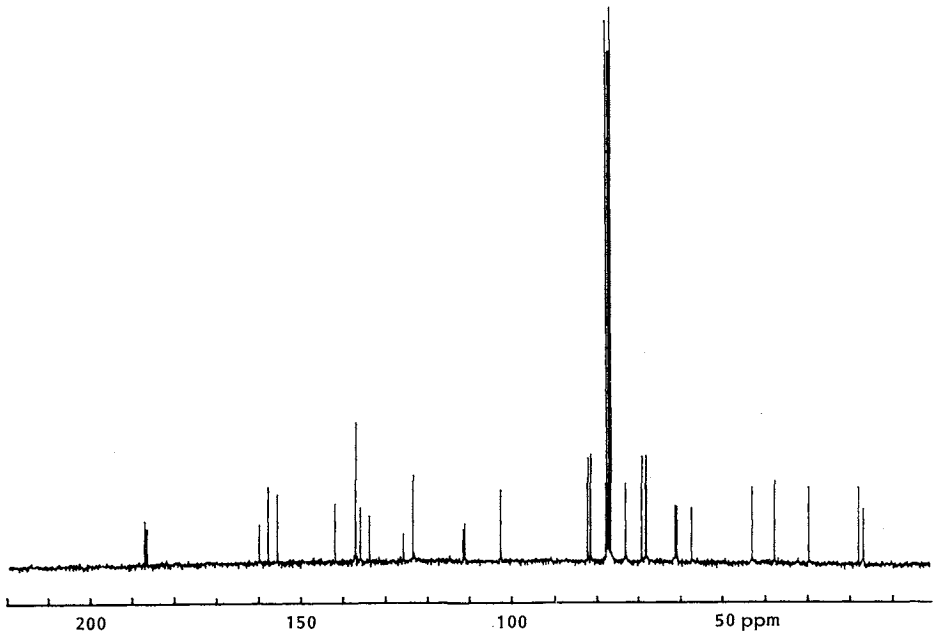
Table 1. ^{1}H and ^{13}C NMR data of SO-75R1 (**1**).

Position	^{1}H	J=Hz	^{13}C	HMBC correlations (^{1}H)
1	8.05 d	8.0	123.2 d	
2	7.63 d	8.0	136.9 d	13-H_3
3			141.7 s	1-H, 13-H_3
4			159.7 s	2-H, 13-H_3, 4-OMe
4-OMe	3.90 s		61.1 q	
4a			125.5 s	1-H
5			187.0 s	
5a			111.0 s	6-OH
6			157.7 s	6-OH
6-OH	14.0 s			
6a			135.8 s	6-OH, 7-H, 8-H_a, 8-H_b, 10-H_a, 10-H_b
7	5.12 t	6.0	72.9 d	8-H_b, 1′-H
8	(a) 2.28 dd	13.7, 6.0	43.0 t	
	(b) 2.09 dd	13.7, 6.0		
9			69.1 s	7-H, 8-H_a, 8-H_b, 10-H_a, 10-H_b, 14-H_3
10	(a) 2.97 d	18.1	37.7 t	8-H_a, 8-H_b
	(b) 2.84 d	18.1		
10a			136.9 s	7-H, 10-H_a, 10-H_b, 11-OH
11			155.5 s	11-OH
11-OH	13.4 s			
11a			111.3 s	11-OH
12			186.5 s	1-H
12a			133.6 s	2-H
13	(3H) 2.45 s		16.7 q	
14	(3H) 1.48 s		29.6 q	8-H_a
1′	5.41 d	1.6	102.5 d	7-H
2′	4.05 dd	3.0, 1.6	67.9 d	1′-H
3′	3.33 dd	9.3, 3.0	81.3 d	1′-H, 2′-H, 3′-OMe
3′-OMe	3.41 s		57.3 q	
4′	3.13 t	9.3	81.9 d	4′-OMe, 6′-H_3
4′-OMe	3.54 s		60.8 q	
5′	3.78 dq	9.3, 6.0	68.2 q	4′-H, 6′-H_3
6′	1.36 d	6.0	17.8 q	

spectrum of **1** showed an AB quartet signal in the aromatic region (δ_H 8.05 and 7.63, J=8.0 Hz), one of which (δ_H 7.63) was coupled with a singlet methyl signal resonating at δ_H 2.45 in the ^{1}H-^{1}H COSY spectrum, thus suggesting the presence of the partial structure (–CH=CH–C(CH_3)=) in the anthraquinone nucleus. Interpretation of the ^{1}H-^{1}H COSY spectrum suggested that SO-75R1 (**1**) contained one 6-deoxyhexose unit and the coupling constants of the sugar ring protons were consistent with those of an *O*-methylrhamnose derivative. The ^{1}H NMR spectrum of **1** revealed another AB quartet (δ_H 2.97 and 2.84, J=18.1 Hz), an ABX system (δ_H 5.12, 2.28, and 2.09), three methoxy (δ_H 3.90, 3.54, and 3.41), and one tertiary methyl (δ_H 1.48) signals in the sp^3 region. The ^{1}H and ^{13}C NMR spectral data of SO-75R1 (**1**) were further examined extensively by using several two-dimensional techniques (^{1}H-^{1}H COSY, HSQC[2], HMBC[3], and NOESY) and assignments of the ^{1}H and ^{13}C signals as well as the long-range ^{1}H-^{13}C connectivities observed in the HMBC spectrum were presented in Table 1. As a result, the anthraquinone nucleus, sp^3 carbon fragments, and the 6-deoxyhexose unit described above were able to be connected to give rise to an anthracycline structure, which proved to be very similar to that of mutactimycin A (**2**), an antiviral anthracycline antibiotic recently isolated from a mutant strain of *Streptomyces* sp[4]. The ^{1}H and ^{13}C NMR chemical shifts as well as coupling constants of mutactimycin A (**2**) corresponded very well to those of SO-75R1 (**1**, Fig. 2). The structural difference was found in the 6-deoxysugar unit, namely the hydroxyl group at C-4′ in **2** is replaced by a methoxy group in **1**. The structure of SO-75R1 was, therefore, concluded to be **1**.

SO-75R1 exhibits antimicrobial activities against most Gram-positive bacteria with MIC values from

Fig. 2. ^{13}C NMR spectrum of SO-75R1 (in $CDCl_3$).



0.2 to 3.1 μg/ml, but not against Gram-negative bacteria and fungi. IC_{50} for L1210 cultured cells was 7.4 μg/ml. Detailed biological activities of SO-75R1 including antiviral effects and its taxonomical studies on IFM 075 strain, will be reported elsewhere.

Acknowledgment

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