Communications to the Editor

31-HOMORIFAMYCIN W, A NOVEL METABOLITE FROM *Amycolatopsis mediterranei*

Sir:

Rifamycins and streptovaricins have potent antibacterial activities. They contain an aliphatic ansa chain and naphthalene system as constituents. Several natural rifamycins have been found as the metabolites of *Amycolatopsis mediterranei* originally described by SENSI and coworkers[1]. Rifamycin W was isolated from a mutant strain of *A. mediterranei*, being reported as the missing-linkage product in the biosynthetic pathway leading to the naphthalene ansamycins[2,3]. In the course of our chemical screening program, *A. mediterranei* var. *kanglensis* 1747-64 was found to produce a novel antibiotic, 31-homorifamycin W (**1**) which is belonging to rifamycin family. In this communication, we wish to describe the fermentation, isolation and structural elucidation of **1**.

Strain 1747-64 was cultured in 80-liter jar fermentor containing 40 liters of a production medium consisting of glucose 5%, yeast meal 1.2%, peanut meal 0.5%, peptone 0.5%, soy bean oil 3% and $CaCO_3$ 0.1% (pH 6.5). Fermentation was carried out at 28°C for 86 hours, agitated at 250 rpm and aerated at 10 liters per minute. The broth filtrate (26.5 liters) was adjusted to pH 3 with 6N HCl and extracted twice with 9 liters of ethyl acetate. The organic layers were combined and concentrated to dryness under reduced pressure and the residue was chromatographed on a silica gel column (4 × 50 cm, chloroform - methanol, 5 : 1). The eluate was further purified over Sephadex LH-20 column (2.5 × 100 cm, methanol). The desired fractions were collected, evaporated and applied on preparative TLC (silica gel, chloroform - methanol - acetic acid, 100 : 10 : 1). The yellow band (Rf value of 0.57) on TLC plates were extracted with methanol. The extract was concentrated and rechromatographed on LH-20 column with methanol to give 30 mg brown powder of **1**.

Physico-chemical properties of **1** are summarized in Table 1. **1** was obtained as brown powder, which is soluble in methanol, acetone and ethyl acetate, slightly soluble in chloroform and insoluble in H_2O and hexane, respectively. The molecular formula was determined as $C_{36}H_{47}NO_{11}$ by HRFAB-MS ($[M+H]^+$; calcd: 670.3227, found: 670.3257). The UV spectrum suggested to possess a chromophore very close to that of rifamycin W[2,3]. The IR absorption band at 1690 and 1625 cm^{-1} were attributable to the amide carbonyl and the quinone carbonyl group linked in the intramolecular H-bond, and the band at 1495 cm^{-1} indicated that the chromophoric system is in the quinone form[4].

The structure of **1** was ascertained by detailed analysis of ^1H, ^{13}C and 2D NMR spectra. The ^1H NMR spectrum of **1** is very similar to those of rifamycins[2,3]. ^1H and ^{13}C NMR spectral data were shown in Table 2. ^1H-^1H COSY experiments of **1** established the following connectivities of the carbons: 34-CH_3–26-CH–25-CH–, 33-CH_3–24-CH–23-CH–, 32-CH_3–22-CH–, 31a-CH_3–31-CH_2–, =17-CH–18-CH=19-CH–20-CH–21-CH– and 35-CH–

Fig. 1. Structure of 31-homorifamycin W (**1**).

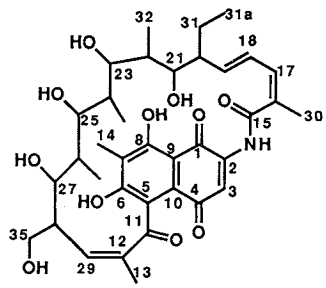


Table 1. Physico-chemical properties of 31-homorifamycin W.

Appearance	Brown powder
Molecular formula	$C_{36}H_{47}NO_{11}$
FAB-MS (*m/z*, positive)	670 $(M+H)^+$, 692 $(M+Na)^+$
(*m/z*, negative)	668 $(M-H)^-$
HRFAB-MS $[(M+H)^+]$	
Calcd for $C_{36}H_{48}NO_{11}$:	670.3227
Found:	670.3257
UV λ_{max}^{MeOH} nm (ε)	233 (34,100), 274 (sh, 20,000), 326 (8,700)
IR ν_{max} (KBr) cm^{-1}	3450, 2950, 2900, 1690, 1625, 1495
TLC (Rf value)[a]	0.60 ($CHCl_3$ - MeOH, 4 : 1)
	0.39 (EtOAc - MeOH, 3 : 1)
Solubility	Soluble in EtOAc, MeOH
	Slightly soluble in $CHCl_3$
	Insoluble in hexane, H_2O

[a] Silica gel TLC (Merck Art. No. 5715).

Table 2. 400 MHz ^{1}H NMR and 100 MHz ^{13}C NMR data of 31-homorifamycin W in CD_3OD (ppm downfield from internal TMS)[a].

No.	δ_H (multi., J in Hz)	δ_C	No.	δ_H (multi., J in Hz)	δ_C
1		182.3 (s)	19	6.04 (dd, 16.1, 7.3)	140.9 (d)
2		142.7 (s)	20	2.24 (m)	45.9 (d)
3	7.52 (s)	117.6 (d)	21	4.09 (br d, 9.1)	73.9 (d)
4		187.3 (s)	22	1.86 (m)	34.5 (d)
5		127.1 (s)[c]	23	3.43 (m)	79.1 (d)
6		164.2 (s)	24	1.77 (m)	37.9 (d)
7		118.7 (s)	25	3.98 (br d, 9.9)	71.2 (d)
8		166.5 (s)	26	1.38 (m)	44.0 (d)
9		106.7 (s)[c]	27	4.36 (br s)	69.3 (d)
10		129.7 (s)	28	2.61 (m)	49.4 (d)
11		201.5 (s)	29	6.41 (d, 9.1)	140.0 (d)
12		140.8 (s)	30	2.09 (s)	20.2 (q)
13	2.06 (s)	12.7 (q)	31	1.24 (m), 1.53 (m)	26.1 (q)
14	2.12 (s)	8.5 (q)	31a	0.88 (t, 7.0)	11.6 (q)
NH	8.92 (br s)[b]		32	1.04 (d, 7.0)	11.7 (q)
15		172.0 (s)	33	0.71 (d, 7.0)	9.0 (q)
16		132.0 (s)	34	0.41 (d, 7.0)	11.1 (q)
17	6.28 (d, 11.0)	135.3 (d)	35	3.56 (m), 3.46 (m)	64.7 (t)
18	6.52 (dd, 16.1, 11.0)	127.8 (d)			

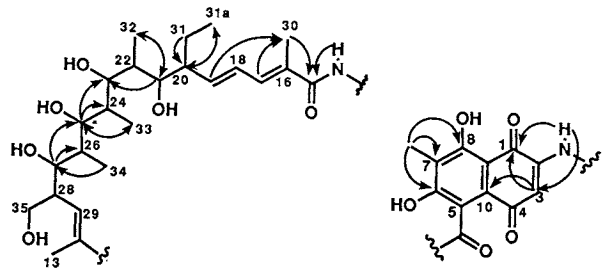
DEPT experiment allowed distinction of carbon multiplicities.

[a] s: Singlet, d: doublet, t: triplet, q: quartet, m: multiplet.

[b] In acetone-d_6.

[c] These signal assignments are interchangeable.

Fig. 2. Partial structures of **1** elucidated by ^{1}H-^{1}H COSY, HMBC and ^{1}H-^{13}C long range COSY experiments.



⟶ : Long range hetero nuclear correlations.

28-CH(27-CH)–29-CH=. These fragments were connected to form a partial structure in Fig. 2 based on the analysis of the ^{1}H-^{13}C long-range couplings obtained by HMBC and ^{1}H-^{13}C long-range COSY experiments. An amide linkage was revealed to be located between C-2 and C-15 carbonyl group by ^{1}H-^{13}C long-range COSY spectrum of **1** acquired in aceton-d_6. The UV and IR spectra suggested that **1** had a chelated naphthoquinone chromophore as shown in Fig. 2, which was confirmed by comparison of the ^{13}C NMR spectral data of **1** with those of rifamycin W[2,3]. Furthermore, in the ^{1}H-^{13}C long-range COSY spectrum of **1** (in acetone-d_6), correlations between 14-CH_3–C-7, 14-CH_3–C-6 and 14-CH_3–C-8 were observed as shown Fig. 2. Thus, the carbon signals at δ 118.7, δ 164.2 and δ 166.5 were assigned to C-7, C-6 and C-8, respectively. Therefore, this indicates the presence of a naphthoquinone chromophore as recognized in **1**.

In conclusion, it was revealed that **1** possesses an ethyl group in place of C-31 methyl branch of rifamycin W, *i.e.* the structure of **1** was determined to be 31-homorifamycin W.

Naphthalene ansamycins are strongly active against Gram-positive and mycobacteria. **1** was tested in some different bioassays. In the standard

antibacterial, antifungal and antiviral tests, each performed with a number of different organisms, **1** exhibited no significant activity.

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