Mutactimycin PR, a New Anthracycline Antibiotic from *Saccharothrix* sp. SA 103

II. Physico-chemical Properties and Structure Elucidation

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A new antibiotic termed mutactimycin PR (**1**) was isolated along with the known mutactimycin C (**2**) from the fermentation broth of *Saccharothrix* sp. SA 103. The two compounds belong to the anthracycline group. The structure of these antibiotics was elucidated with the aid of NMR and mass spectrometric investigations. The novel compound mutactimycin PR was characterized as 5,12 Naphtacenedione, 7-[(6-deoxy-3-*O*-methyl- α -L-mannopyranosyl)oxy]-4-[(6-deoxy- α -L-mannopyranosyl)oxy]-7,8,9,10-tetrahydro-6,9,11-trihydroxy-9 methyl.

The mutactimycins are a group of anthracycline antibiotics discovered in 1990 by JIN *et al.*¹⁾ At present, this group contains fifteen members^{2~6)} which exhibit anti-Gram-positive bacteria, antiviral and anticancer activities. In the preceding paper⁷⁾, we described the taxonomy and fermentation of *Saccharothrix* sp. SA 103, a producer of mutactimycin C and novel derivative PR, as well as the isolation and biological activities of the two compounds. In this paper, we present the physicochemical properties and the details of the structural elucidation of these antibiotics.

Results and Discussion

Table 1 summarizes the physicochemical properties of the antibiotics (**2**) and (**1**) obtained as red amorphous powder. The UV and IR spectra of **1** were nearly identical to those of **2**. The UV spectra showed absorption maxima in MeOH at 219, 234, 250, 287, 478, 496 and 531 nm. The absorption between 400 and 550 nm was assigned to the

presence of a chromophore conferring the red color of the antibiotics. Many known antibiotics exhibit red color, such as the quinone and the anthraquinone groups, and include a wide number of antibiotics exhibiting different activity spectra.⁸⁾ In our case, the UV maxima values were very similar to those characterizing anthracyclines group.⁹⁾

In the IR spectrum, characteristic absorption bands were observed at 3393, 1611, 1583 and 1048 cm⁻¹, indicating the presence of OH, aromatic structure and methoxy groups. The nano-ESI-IT-MS spectrum of **2** and **1** showed the base peak of the mono-isotopic deprotonated molecule [M-H]⁻ at *m/z* 529 and 661 respectively. These corresponded perfectly with the calculated mono-isotopic mass of 530,1788 and 662,2210 respectively.

For the final characterization of the structures, all these data were combined with ¹H and ¹³C NMR spectroscopy analysis. In this study, all the ¹H and ¹³C NMR signals were assigned on the basis of chemical shifts, spin-spin coupling constants, splitting patterns and signal intensities, and by using ¹H-¹H COSY45, ¹H-¹³C HMQC and ¹H-¹³C HMBC

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Table 1. Physico-chemical properties of (1) and (2).

	(1)	(2)
Appearance	Red powder	Red powder
Color in H ₂ O Solution		
Acid	Yellow	Yellow
Neutral	Red	Red
Basic	Violet-blue	Violet-blue
Molecular formula	C ₃₂ H ₃₈ O ₁₅	C ₂₇ H ₃₀ O ₁₁
Molecular weight	662	530
Nano-ESI-MS (<i>m/z</i>)		
Negative mode	660.8 [M-H] ⁻ , 514.9, 354, 337, 319.1, 291.1	528.8 [M-H] ⁻ , 351, 333, 315, 294.2, 293.1
Positive mode	685.2 [M+Na] ⁺ , 539, 507, 360.9, 342.9	553.1 [M+Na] ⁺ , 375, 356.9, 198.3
UV λ _{max} nm in MeOH	219, 234, 250, 287, 478, 496, 531	219, 234, 250, 287, 478, 496, 531
IR ν _{max} Diamond cell (cm ⁻¹)	3393, 2969, 2932, 2878, 2841, 2709, 2360, 2113, 1611, 1583, 1444, 1408, 1379, 1352, 1285, 1238, 1213, 1133, 1110, 1070, 1048	3393, 2969, 2932, 2878, 2841, 2709, 2360, 2113, 1611, 1583, 1444, 1408, 1379, 1352, 1285, 1238, 1213, 1133, 1110, 1070, 1048
Relative solubility		
Highly soluble	MeOH, EtOH, H ₂ O	MeOH, Me ₂ CO, H ₂ O, <i>n</i> -BuOH, EtOH, <i>l</i> -PrOH
Slightly soluble	Me ₂ CO, <i>n</i> -BuOH, <i>l</i> -PrOH, 2-PrOH	CH ₂ Cl ₂ , CHCl ₃ , EtOAc, 2-PrOH
Insoluble	CH ₂ Cl ₂ , CHCl ₃ , EtOAc, <i>n</i> -hexane, toluene	<i>n</i> -hexane, toluene
TLC (Rf value) ^a		
(I)	0.16	0.44
(II)	0.58	0.64
(III)	0.82	0.80
HPLC (Rt) ^b	31.74 min	35.65 min

^aSilica gel TLC (Merck No 5715). (I): EtOAc-MeOH (100:15). (II): *n*-BuOH-CH₃COOH-H₂O (3:1:1). (III): MeOH-CH₂Cl₂ (4:1).

^bHPLC conditions: Uptisphere C₁₈ UP50DB (250x7.8 mm, i.d.), Mobile phase: isocratic at 63% MeOH in H₂O, Flow rate: 1.5 ml/min, Detection: UV (220 nm).

experiments. The ¹H and ¹³C chemical shifts of **1** and **2** are given in Tables 2 and 3, and the structures are illustrated in the Fig. 1.

For the aglycon unit of **2**, the ¹H NMR spectrum revealed an aromatic ABM spin system pattern (δ_H 7.56, 7.83 and 7.81), an ABX system (δ_H 1.93, 2.13 and 4.86; *J*_{AB}=13.5 Hz, *J*_{AX}=5.5 Hz and *J*_{BX}=6.4 Hz), an AB system (δ_H 2.62 and 2.76, *J*_{AB}=17.8 Hz), two enols protons (δ_H

13.20 and 14.10, 2H, s), a methoxy group (δ_H 3.95, 3H, s), a methyl group (δ_H 1.31, 3H, s) and a hydroxy signal (δ_H 4.78, 1H, s). From the ¹³C data, it was possible to discern two keto-carbonyl groups (δ_C 186.9 and 187.4), 12 *sp*²-hybridized carbons (δ_C 161.5, 157.4, 155.4, 136.8, 136.0, 136.0, 135.7, 120.8, 119.4, 119.1, 111.4 and 111.1), a methoxy group (δ_C 56.2), two *sp*³-hybridized carbons bearing an electronegative heteroatom (δ_C 68.2 and 73.5),

Table 2. ^1H and ^{13}C NMR assignments of compound **1** in $\text{DMSO}-d_6$ at 298 K and in $\text{DMF}-d_7$ at 278 K.

Position	DMSO		DMF	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1	8.00 (d)	121.0	7.96 (d)	120.6
2	7.84 (br dd)	nd	7.81 (dd)	135.3
3	7.66 (br)	nd	7.68 (d)	124.1
4		nd		157.9
4a		nd		122.6
7	4.89 (m)	73.2	4.92 (m)	73.7
8	2.10 (dd) / 1.92 (dd)	43.3	2.16 (dd) / 1.98 (dd)	43.6
9		67.8		67.7
10	2.83 (d) / 2.61 (d)	38.3	2.84 (d) / 2.65 (d)	38.2
12a		nd		136.0
6-OH	nd		nd	
9-OH	4.70 (br)		nd	
9-Me	1.30 (s)	29.7	1.32 (s)	29.2
11-OH	nd		nd	
1'	5.16 (br s)	104.4	5.22 (br s)	104.7
2'	3.83 (m)	67.0	3.92 (m)	67.1
3'	3.00 (dd)	81.5	3.04 (dd)	81.7
4'	3.33 ^a	71.0	3.44 (dd)	72.0
5'	3.64 (dq)	70.2	3.70 ^a	69.8
2'-OH	4.88		nd	
3'-OMe	3.22 (s)	56.9	3.17 (s)	56.6
4'-OH	4.90		nd	
5'-Me	1.21 (d)	18.7	1.17 (d)	18.0
1''	5.69 (br s)	99.1	5.73 (br s)	99.4
2''	4.00 (m)	71.0	4.09 (m)	71.3
3''	4.04 (dd)	70.9	4.10 (dd)	71.3
4''	3.35 ^a	72.4	3.42 (dd)	72.2
5''	3.52 (dq)	70.9	3.54 ^a	70.8
2''-OH	5.15 (br)		nd	
3''-OH	4.89 (br)		nd	
4''-OH	4.97		nd	
5''-Me	1.09 (d)	18.7	1.05 (d)	17.9

Only detected signals are shown. See Fig. 1 for numbering of H and C atoms.

^aunder the residual HOD signal.

nd: not detected.

two sp^3 -hybridized carbons (δ_{C} 42.8 and 37.4) and a methyl group (δ_{C} 56.2). The 2D ^1H - ^1H and ^1H - ^{13}C experiments and especially the long range ^1H - ^{13}C couplings observed in the HMBC spectrum (Fig. 2) permitted the combination of all determined structural elements. Thus, the structure of the aglycon unit of compound **2** was established as an aklavinone unit. This stable structure was obtained on the fragmentation pattern of **2** as a major fragment at m/z 351.

In addition, the ^1H and ^{13}C NMR spectra displayed characteristic signals of a pyranosyl hexose. This moiety corresponded to the loss of the mass fragment of m/z 178 observed on MS² pattern. In the COSY spectrum, correlations were observed between the five sugar protons H1' (δ_{H} 5.12), H2' (δ_{H} 3.87), H3' (δ_{H} 3.02), H4' (δ_{H} 3.32)

and H5' (δ_{H} 3.63). ROE correlations between H1' and H2', absence of ROE correlations between H1' and H3'/H5' protons and chemical shift of C5'⁽¹⁰⁾ identified this sugar as the α -anomer. The observation of coupling constants ($J_{\text{H1'}/\text{H2'}}=1.8$ Hz, $J_{\text{H2'}/\text{H3'}}=3.2$ Hz, $J_{\text{H3'}/\text{H4'}}=9.4$ Hz, and $J_{\text{H4'}/\text{H5'}}=9.4$ Hz) strongly suggested that the sugar was a mannopyranose. Additional COSY and HMBC correlations enabled to identify the sugar part of **2** as a 6-deoxy-3-*O*-methyl- α -mannopyranoside moiety.

The linkage between the aklavinone unit and the sugar moiety was determined by HMBC correlations between H1'/C7 and H7/C1' and a ROE between H1' and H7. ROESY correlations (Fig. 3) detected between the protons of the aglycon part allowed us to fix the relative

Table 3. ^1H and ^{13}C NMR assignments of compound **2** in $\text{DMSO}-d_6$ at 298 K.

Position	δ_{H}	δ_{C}
1	7.81 (m)	119.4
2	7.83 (m)	136.0
3	7.56 (m)	119.1
4		161.5
4a		120.8
5		186.9
5a		111.1
6		157.4
6a		136.0
7	4.86 (dd) 2.13 (dd) / 1.93 (dd)	73.5
8		42.8
9		68.2
10	2.76 (d) / 2.62 (d)	37.4
10a		136.8
11		155.4
11a		111.4
12		187.4
12a		135.7
4-OMe	3.95 (s)	56.2
6-OH	14.13 (s) ^a	
9-OH	4.78 (s)	
9-Me	1.31 (s)	28.1
11-OH	13.19 (s) ^a	
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1'	5.12 (d)	104.2
2'	3.87 (m)	67.1
3'	3.02 (dd)	81.2
4'	3.32 (ddd)	71.7
5'	3.63 (dq)	69.8
2'-OH	4.82 (d)	
3'-OMe	3.23 (s)	56.1
4'-OH	4.91 (d)	
5'-Me	1.21 (d)	17.0

See Fig. 1 for numbering of H and C atoms.

^asignals may be transposed.

configuration of the aklavinone unit as $7S^*,9R^*$. ROE contacts were also observed between the aglycon and the sugar parts (between H7 and H1', between H7 and H2', between H8a,b and H5' and between Me-9 and H5'). However these ROE correlations could not permit clear determination of the relative configuration between these two sections (two possibilities: $7R,9S/\alpha\text{-D}$ or $7S,9R/\alpha\text{-L}$). However, the suggested $7S,9R/\alpha\text{-L}$ -configuration is consistent with the Mutactimycin family, and thus the structure of **2** is consistent with Mutactimycin C, previously obtained from the mutant 113 of *Streptomyces* sp.¹⁾

NMR data for **1** in DMSO, and DMF or Methanol (not showed), exhibited much ^1H broader lines than for **2** (line broadening was unclear). Due to the low amount available

Fig. 1. Structures of mutactimycins PR (**1**) and C (**2**).

Fig. 2. HMBC correlations of (**2**).

of **1** (<2 mg) and broad lines, a complete set of ^{13}C NMR data could not be obtained for **1**. NMR data in DMSO at 298K (^1H , COSY45 and HMQC spectra) showed that **1** and **2** are closely related. The structure of **1** was thus established through comparison with mass spectrometry and NMR data obtained for **2**. In mass spectrometry, **1** exhibited the same fragmentation pattern as **2**, but an additional fragment mass of m/z 179 suggested the presence of another sugar moiety. In NMR analysis, the major differences between **1** and **2** were localized on the aromatic cycle of the aklavinone unit bearing the three aromatic protons H1, H2 and H3. The NMR signals of the methoxy group OMe-4 were not observed for **1**, and H1, H2 and H3 ^1H chemical shifts are slightly shifted between **1** and **2** ($\Delta\delta_{\text{H}}=0.09$ for H1, $\Delta\delta_{\text{H}}=0.01$ for H2 and $\Delta\delta_{\text{H}}=0.10$ for H3). Furthermore, NMR signals of an additional pyranosyl

Fig. 3. Selected ROE correlations for (**2**) (mixing time 200 ms).

Note that the relative stereochemistries of the aklavinone and sugar units are arbitrary (the 7*S*, 9*R*/ α -*L* configuration is depicted).

hexose were detected and could be assigned with COSY and HMQC spectra. The observation of the coupling constants ($J_{H1''/H2''} < 1.5$ Hz, $J_{H2''/H3''} < 1.5$ Hz, $J_{H3''/H4''} = 9.3$ Hz, and $J_{H4''/H5''} = 9.1$ Hz), correlations between H2'', H3'', and H4'' and hydroxyl protons and correlations between H5'' and methyl protons enabled to identify the second sugar residue as a 6-deoxy-mannopyranoside moiety. The configuration of the glycosidic linkage, was determined to be α , on the basis of the chemical shift of C5'' (δ_C 70.9).

It was not possible to detect a HMBC correlation between H1'' and C4 even with a 60 h HMBC experiment (in DMF or in DMSO). However a ROE correlation between H3 and H1'' was observed with a 1D GROESY experiment (mixing time 300 ms) in DMF at 278 K, thus confirming the attachment of the *O*-glycosidic bond to C4. The limited supply of **1** prevented determination of its relative or absolute stereochemistry. Therefore **1** was characterized as 5,12 Naphthacene-dione, -[(6-deoxy-3-*O*-methyl-- α -*L*-mannopyranosyl)oxy]-4-[(6-deoxy- α -*L*-mannopyranosyl)oxy]-7,8,9,10-tetrahydro-6,9,11-trihydroxy-9methyl Mutactimycin PR represents a newly described antibiotic of the anthracycline class. The two sugar moieties of this new compound make PR unique member of mutactimycin group.

Experimental

Analytical Studies

The spectroscopic studies are made with the pure antibiotics. UV-vis absorption spectra are recorded on a Perkin Elmer (lambda 20) spectrophotometer and Infrared spectra on a Perkin Elmer FT-IR 1760x spectrometer. Mass spectroscopic studies were performed on Finnigan/MAT LCQ and TSQ 700 instruments. MS and MS/MS spectra were obtained by electrospray ionization (ESI) in negative and positive mode. Nano-ESI-IT-MS (Electrospray Ionization, Ion Trap Mass Spectrometry) analysis was performed with a commercial nanospray ESI source¹¹⁾ (The Protein Analysis Co., Odense, Denmark).

For NMR analysis, samples were prepared by dissolving 5 mg of compound **2** in 600 μ l DMSO-*d*₆ and 1.5 mg of **1** compound in 600 μ l DMSO-*d*₆ or DMF-*d*₇.

All spectra were recorded on a Bruker AMX400 spectrometer equipped with a 5 mm triple resonance inverse probe operating at 400.13 MHz for ¹H and 100.61 MHz for ¹³C. All chemical shifts for ¹H and ¹³C are relative to TMS using ¹H (residual) or ¹³C chemical shifts of the solvent as a secondary standard. The temperature was set at 298 K for all samples in DMSO and at 278 K in DMF.

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