VOL. 57 NO. 6, JUNE 2004 THE JOURNAL OF ANTIBIOTICS pp. 373 — 378

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

II. Physico-chemical Properties and Structure Elucidation

ABDELGHANI ZITOUNI[a,b], FLORENCE MATHIEU[a], YANNICK COPPEL[c], FREDERIC PONT[d], NASSERDINE SABAOU[b] and AHMED LEBRIHI[a,*]

[a] Ecole Nationale Supérieure d'Agronomie de Toulouse, INPT, Laboratoire de Génie Chimique, UMR 5503 (CNRS/INPT/UPS), 1, avenue de l'Agrobiopôle, B.P. 107, F-31 326 Castanet-Tolosan Cedex, France
[b] Laboratoire de Recherche sur les Produits Bioactifs et la Valorisation de la Biomasse, Ecole Normale Supérieure de Kouba, B.P. 92, 16 050 Vieux-Kouba, Alger, Algérie et Centre de Recherche Scientifique et Technique sur les Régions Arides, Front de l'Oued, B.P. 1682, 07 000 Biskra, Algérie
[c] Laboratoire de Chimie de Coordination, CNRS, 205, route de Narbonne 31 077 Toulouse Cedex 04, France
[d] Service Commun de Spectrométrie de Masse de l'IFR 30-CHU Purpan-BP 3028, 31 024 Toulouse Cedex, France

(Received for publication January 9, 2004)

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 paper[7], 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 ^{13}C NMR spectroscopy analysis. In this study, all the ^1H and ^{13}C 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-^{13}C HMQC and ^1H-^{13}C HMBC

* Corresponding author: Lebrihi@ensat.fr

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

	(1)	(2)
Appearance	Red powder	Red powder
Color in H_2O 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 (*m/z*)		
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_2O	MeOH, Me_2CO, H_2O, *n*-BuOH, EtOH, *1*-PrOH
Slightly soluble	Me_2CO, *n*-BuOH, *1*-PrOH, *2*-PrOH	CH_2Cl_2, $CHCl_3$, EtOAc, *2*-PrOH
Insoluble	CH_2Cl_2, $CHCl_3$ EtOAc, *n*-hexane, toluene	*n*-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

[a]Silica gel TLC (Merck No 5715). (I): EtOAc-MeOH (100:15). (II): *n*-BuOH-CH_3COOH-H_2O (3:1:1). (III): MeOH-CH_2Cl_2 (4:1).

[b]HPLC conditions: Uptisphere C_{18} UP5ODB (250x7.8 mm, i.d.), Mobile phase: isocratic at 63% MeOH in H_2O, Flow rate: 1.5 ml/min, Detection: UV (220 nm).

experiments. The ^1H and ^{13}C 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 ^{13}C 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. ^{1}H and ^{13}C NMR assignments of compound **1** in DMSO-d_6 at 298 K and in 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.
[a]under 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 ^{1}H-^{1}H and ^{1}H-^{13}C experiments and especially the long range ^{1}H-^{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 ^{1}H 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 MS^2 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 ($J_{H1'/H2'}$=1.8 Hz, $J_{H2'/H3'}$=3.2 Hz, $J_{H3'/H4'}$=9.4 Hz, and $J_{H4'/H5'}$=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. ^{1}H and ^{13}C NMR assignments of compound **2** in 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)	73.5
8	2.13 (dd) / 1.93 (dd)	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]	
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.
[a]signals may be transposed.

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

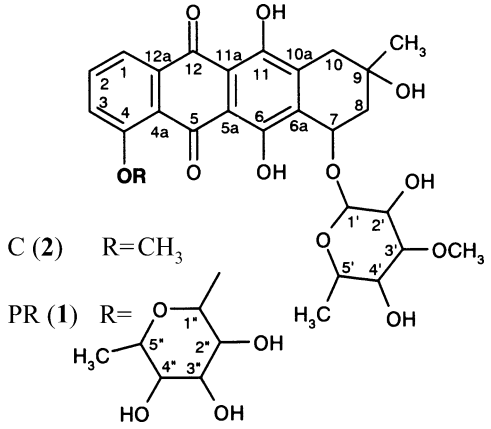
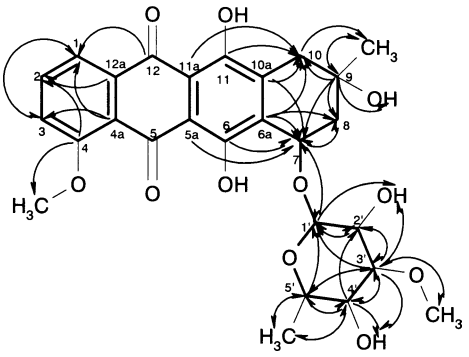


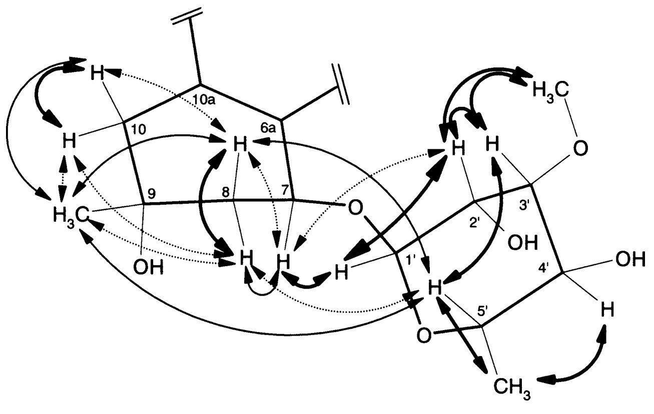
Fig. 2. HMBC correlations of (**2**).



configuration of the aklavinone unit as 7*S**,9*R**. 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: 7*R*,9*S*/α-D or 7*S*,9*R*/α-L). However, the suggested 7*S*,9*R*/α-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 ^{1}H broader lines than for **2** (line broadening was unclear). Due to the low amount available 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 (^{1}H, 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 ^{1}H chemical shifts are slightly shifted between **1** and **2** ($\Delta\delta_H$=0.09 for H1, $\Delta\delta_H$=0.01 for H2 and $\Delta\delta_H$=0.10 for H3). Furthermore, NMR signals of an additional pyranosyl

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



⟷ Strong, ⟷ medium, ⟷ Weak NOE intensities.

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 electronspray 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 source[11] (The Protein Analysis Co., Odense, Denmark).

For NMR analysis, samples were prepared by dissolving 5 mg of compound **2** in 600 μl DMSO-d_6 and 1.5 mg of **1** compound in 600 μl 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 ^{1}H and 100.61 MHz for ^{13}C. All chemical shifts for ^{1}H and ^{13}C are relative to TMS using ^{1}H (residual) or ^{13}C 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.

Acknowledgements

This work was gratefully supported by the "Ministère de l'Enseignement supérieur et de la Recherche Scientifique" (MESRS and ANDRU) of Algeria, and the "Comité d'Evaluation et de Prospective de Coopération Interuniversitaire Franco-Algérienne (CMEP No 02 MDU 564), French Embassy in Algiers.

References

1) Jin, W.; J. Chen, Y. Zhang, H. Li & P. Tao: Isolation and structure determination of mutactimycin A, a new anthracycline antibiotic. Kangshengsu 15: 399～406, 1990
2) Zhang, Y. & W. Jin: Isolation and structure determination of minor components of mutactimycins, a group of new anthracycline antibiotics. Zhongguo Kangshengsu Zazhi 16: 156～164, 1991
3) Li, H.; W. Lu, Y. Zhang, W. Jin, P. Tao, X. Liu, Y. He & Y. Zeng: Isolation and characterization of mutactimycin-producing mutant. Weishengwu Xuebao 32: 353～358, 1992
4) Meda, A.; K. Yazawa, Y. Mikami, M. Ishibashi & J. Kobayashi: The producer and biological activities of SO-075R1, a new mutactimycin group antibiotic. J. Antibiotics 45: 1848～1852, 1992
5) Yan, X.; S. Wang & J. Wu: Isolation and structure determination of XF-1, a new anthracycline antibiotic. J. Antibiotics 46: 787～790, 1993

6) SPEITLING, M.; P. NATTEWAN, K. YAZAWA, Y. MIKAMI, I. GRUN-WOLLNY, H. LAATSCH & U. GRAFE: Demethyl mutactimycines, new anthracycline antibiotics from *Nocardia* and *Streptomyces*. J. Antibiotics 51: 693～698, 1998
7) ZITOUNI, A.; H. BOUDJELLA, F. MATHIEU, N. SABAOU & A. LEBRIHI: New member of mutactimycins, an anthracycline group antibiotic from *Saccharothrix* sp. strain SA 103. I. Taxonomy, fermentation, isolation and biological activities. J. Antibiotics 57: 367～372, 2004
8) BERDY, J.; A. AZALOS & K. L. MCNITT: Microbial Metabolites, Parts 1, 2, 3. Vol. XIII, *In* CRC Handbook of Antibiotics Compounds. CRC Press, Inc., Boca Raton, 1987
9) CHI-KIT WAT: Microbial quinones. *In* CRC Handbook of Microbiology. Vol. V, Microbial Products. *Ed.*, A. I. LASKIN & H. A. LECHEVALIER, pp. 271～361, CRC Press, Inc., Boca Raton, Florida, 1984
10) KASAI, R.; M. OKIHIRA, J. ASAKAWA, K. MIZUTANT & O. TANAKA: ^{13}C NMR study of α- and β-anomeric pairs of d-mannopyranosides and l-rhamnopyranosides. Tetrahedron 35: 1427～1432, 1979
11) WILM, M. & M. MANN: Analytical properties of the nanoelectrospray ion source. Anal. Chem. 68: 1～8, 1996