NOTES

TMC-86A, B and TMC-96, New Proteasome Inhibitors from *Streptomyces* sp. TC 1084 and *Saccharothrix* sp. TC 1094

II. Physico-chemical Properties and Structure Determination

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TMC-86A, B and TMC-96, new proteasome inhibitors have been isolated from the fermentation broth of *Streptomyces* sp. TC 1084 and *Saccharothrix* sp. TC 1094, respectively[1]. In the preceding paper, we have reported the taxonomy of producing strains, production, isolation, and biological activities of TMC-86A, B and TMC-96[1]. Here we describe the physico-chemical properties and structure determination of TMC-86A, B and TMC-96.

The physico-chemical properties and NMR spectral data of TMC-86A, B and TMC-96 are summarized in Tables 1 and 2, respectively. The molecular formulas of TMC-86A, B and TMC-96 were determined as $C_{16}H_{26}N_2O_6$, $C_{20}H_{34}N_2O_7$ and $C_{18}H_{32}N_2O_6$, respectively, on the basis of their HRFAB-MS and ^1H and ^{13}C NMR spectral data. Their IR spectra exhibited common signals for the presence of hydroxyl (3300~3400 cm^{-1}), ketone or ester (1720 cm^{-1}), and amide (1640~1650, 1540 cm^{-1}) groups.

The structure of TMC-86A was determined by the analyses of 1D and 2D NMR and MS spectra. The ^{13}C NMR spectrum displayed 16 signals composed of two $-CH_3$, six $-CH_2-$, one $=CH_2$, two $>CH-$, two quaternary carbons, and three carbonyl carbons. The ^1H NMR spectrum showed four D_2O exchangeable protons. In the ^1H-^1H COSY spectrum, four sequential proton networks of $-^8CH_2-OH$, $-NH-^4CH-^5CH_2-$, $-NH-^{2'}CH-^{3'}CH_2-OH$ and $-^{2''}CH_2-^{3''}CH_2-^{4''}CH_3$ were observed (Fig. 1). The presence of the epoxy ring was indicated by the chemical shift of C-1 methylene (δ_C 47.9, δ_H 3.07 and 3.08), and characteristic coupling constants: $^1J_{C\text{-}H}$=180.3 Hz and $^{gem}J_{H\text{-}H}$=5.6 Hz. The connection of these structural fragments was deduced from the observation of the HMBC correlations as shown in Fig. 1. The correlations from C-3 (δ 206.2) to H-4, H-5, H-1 and H-8; and from C-2 (δ 62.9) to H-1 and H-8 (Fig. 1), along with the existence of the epoxy ring, indicated the presence of the epoxy-β-aminoketone moiety. A sequential loss of the assigned fragments was seen in the EI-MS

Table 1. Physico-chemical properties of TMC-86A, B and TMC-96.

	TMC-86A	TMC-86B	TMC-96
Appearance	Colorless oil	White powder	Colorless stickly solid
$[\alpha]_D^{20}$	+10° (*c* 0.27, H_2O)	+30° (*c* 0.41, H_2O)	+25° (*c* 0.45, MeOH)
Molecular formula	$C_{16}H_{26}N_2O_6$	$C_{20}H_{34}N_2O_7$	$C_{18}H_{32}N_2O_6$
EI-MS (*m/z*)	342 $(M)^+$	414 $(M)^+$	372 $(M)^+$
HRFAB-MS (*m/z*)			
Found	343.1863 $(M+H)^+$	415.2430 $(M+H)^+$	373.2343 $(M+H)^+$
Calcd.	343.1869 for $C_{16}H_{27}N_2O_6$	415.2446 for $C_{20}H_{35}N_2O_7$	373.2339 for $C_{18}H_{33}N_2O_6$
UV λ_{max} (MeOH)	End absorption	End absorption	End absorption
IR ν_{max} (KBr) cm^{-1}	3400, 3070, 2960, 2940, 1720, 1650, 1540, 1460, 1380, 1250, 1220, 1050	3400, 3070, 2960, 2940, 1720, 1640, 1540, 1460, 1380, 1250, 1220, 1050	3300, 3070, 2960, 2930, 1720, 1640, 1540, 1465, 1385, 1370, 1215, 1045
Solubility			
soluble in	H_2O, DMSO, MeOH, EtOH	H_2O, DMSO, MeOH, EtOH	DMSO, MeOH, acetone, EtOAc, $CHCl_3$
insoluble in	*n*-hexane	*n*-hexane	H_2O, *n*-hexane

Table 2. ^{1}H and ^{13}C NMR data of TMC-86A, B (in DMSO-d_6) and TMC-96 (in $CDCl_3$).

Position	TMC-86A		TMC-86B		TMC-96	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	47.9 t	3.07 (d, 5.6)	47.9 t	3.06 (d, 5.4)	49.3 t	3.10 (d, 5.0)
		3.08 (d, 5.6)		3.08 (d, 5.4)		3.33 (d, 5.0)
2	62.9 s		62.9 s		62.2 s	
3	206.2 s		206.3 s		207.6 s	
4	50.2 d	4.55 (ddd, 3.2, 7.4, 9.9)	50.2 d	4.55 (ddd, 3.3, 7.4, 9.9)	51.4 d	4.51 (m)
5	36.6 t	2.00 (dd, 9.9, 14.4)		2.00 (dd, 9.9, 14.5)		
		2.42 (dd, 3.2, 14.4)	36.5 t	2.41 (dd, 3.3, 14.5)	38.8 t	1.30 (m)
6	140.6 s		140.6 s			1.61 (m)
7	113.2 t	4.75 (bs)	113.2 t	4.74 (bs)	25.3 d	1.66 (m)
		4.78 (bs)		4.78 (bs)	21.1 q	0.93 (d, 6.3)
8	59.5 t	3.39 (dd, 5.2, 12.5)	59.5 t	3.39 (dd, 5.2, 12.5)	61.5 t	3.75 (dd, 7.0, 12.7)
		4.09 (dd, 6.6, 12.5)		4.07 (dd, 6.5, 12.5)		4.20 (dd, 6.4, 12.7)
9	21.8 q	1.70 (s)	21.9 q	1.70 (s)	23.3 q	0.94 (d, 6.3)
1'	170.3 s		170.3 s		171.5 s	
2'	54.5 d	4.31 (m)	54.5 d	4.30 (m)	56.2 d	4.42 (dd, 2.5, 7.5)
3'	61.6 t	3.46 (m)	61.6 t	3.46 (m)	66.4 d	4.30 (m)
		3.56 (m)		3.54 (m)		
4'					17.7 q	1.15 (d, 6.4)
1"	172.0 s		172.2 s		173.5 s	
2"	37.0 t	2.10 (t, 7.4)	35.2 t	2.10 (t, 7.4)	45.8 t	2.12 (m)
3"	18.5 t	1.49 (tq, 7.4, 7.4)	25.9 t	1.43 (m)	26.3 d	2.10 (m)
4"	13.5 q	0.85 (t, 7.4)	23.5 t	1.23-1.35 (m)	22.5 q	0.96 (m)
5"			43.4 t	1.23-1.35 (m)	22.5 q	0.96 (m)
6"			68.7 s			
7", 8"			29.2 q	1.05 (s)		
4-NH		7.94 (d, 7.4)		7.95 (d, 7.4)		7.13 (d, 7.4)
8-OH		5.05 (dd, 5.2, 6.6)		5.04 (dd, 5.2, 6.5)		2.27 (t-like, ~7)
2'-NH		7.74 (d, 8.2)		7.72 (d, 8.1)		6.46 (d, 7.5)
3'-OH		4.76 (t-like)		4.74 (t, 5.6)		3.70 (d, 3.5)
6"-OH				4.01 (s)		

spectrum (Fig 1).

The ^{1}H and ^{13}C NMR data of TMC-86B corresponded well to those of TMC-86A except for the signals of the fatty acid moiety (C-2" to C-4" in TMC-86A). The *n*-butanoyl group in TMC-86A was replaced by 6-hydrooxy-6-methlheptanoyl group in TMC-86B. Thus the planar structure of TMC-86B was determined to be the 6"-hydroxyl analog of eponemycin[2,3].

The structure determination of TMC-96 was accomplished in the same way as described above by the NMR studies involving ^{1}H-^{1}H COSY, HMQC, HMBC and EI-MS fragmentation.

The configuration of serine in TMC-86A and B, and threonine in TMC-96 was determined to be L by chiral TLC analyses of their acid hydrolysate. The stereochemistry at C-2 and C-4 remains to be determined.

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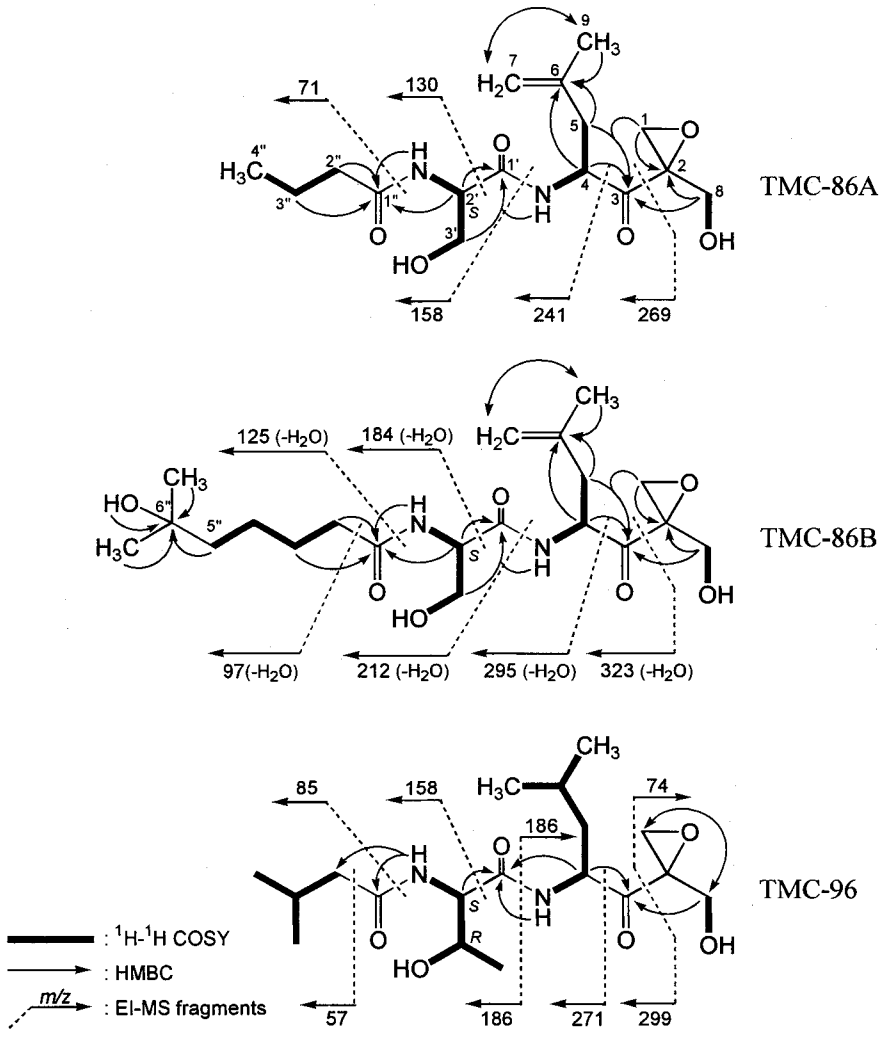
Fig. 1. Structure of TMC-86A, B and TMC-96.



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