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Radamycin, a Novel Thiopeptide Produced by *Streptomyces* sp. RSP9

II. Physico-chemical Properties and Structure Determination

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The new cyclic peptide antibiotic, radamycin (**1**) and the known thiopeptide methylsulfomycin I (**2**) have been isolated from the fermentation broth of a *Streptomyces* sp. RSP9. The structure of radamycin was elucidated by NMR, LC-MS and FAB-MS and was established as a thiopeptide with oxazole and thiazole moieties, and several unusual amino acids.

Radamycin is a thiopeptide antibiotic structurally related to a known family of antibiotics whose members include berminamycins[1,2], sulfomycins[3~5], promothiocins[6], and A10255 complex[7]. Members of this family characteristically possess a cyclic peptide core composed mostly of thiazole rings, oxazole rings, several unusual dehydroamino acids and an unique pyridine ring structure.

This report describes the physico-chemical properties and structure determination of a new thiopeptide radamycin (**1**) which was produced by *Streptomyces* sp. RSP9.

Results and Discussion

Physico-chemical Properties

The physico-chemical properties and the behavior of radamycin (**1**) and methylsulfomycin (**2**) on TLC and HPLC are summarized in Table 1.

In the present study two distinct peaks were observed when the extract was analyzed by HPLC-DAD-MS (retention time: 3.50 and 4.09 minutes). One of these compounds with retention time 3.50 was identified as methylsulfomycin I (**2**) by comparison of its NMR and Mass spectra with data given as reference[4,5]. Analysis by HPLC-ESI-MS revealed its molecular mass to be 1258. The molecular formula of **2** was established as $C_{55}H_{54}N_{16}O_{16}S_2$ by HRFAB-MS [$(M+Na)^+$: found *m/z* 1281.3215, calcd for $C_{55}H_{54}N_{16}O_{16}S_2Na$ *m/z* 1281.3242]. The other compound with retention time 4.09 was identified as a new compound, radamycin (**1**). Analysis by HPLC-ESI-MS revealed its molecular mass to be 1105. The molecular formula of **1** was determined as $C_{48}H_{47}N_{15}O_{11}S_3$ from HRFAB-MS [$(M+H)^+$: found *m/z* 1106.2848, calcd for $C_{48}H_{48}N_{15}O_{11}S_3$ *m/z* 1106.2819] and the number of carbons observed in the ^{13}C NMR spectra. The IR absortions at 3400 and 1690 cm^{-1}, suggested the presence of OH/NH and amide carbonyls, respectively.

Structure Elucidation

The ^1H and ^{13}C NMR spectral data of **1** are shown in Table 2. The ^{13}C NMR spectrum demostrated 48 signals which were assigned to six methyls, five methylenes, nine methines and twenty-eight quaternary carbons by DEPT and PFG-HSQC experiments.

Detailed analysis of the ^1H-^1H COSY experiment

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

	1	2
Appearance	Pale yellow powder	Pale yellow powder
Molecular formula	$C_{48}H_{47}N_{15}O_{11}S_3$	$C_{55}H_{54}N_{16}O_{16}S_2$
ESI-MS $(M-H)^-$	1104	1257
FAB-MS $(M+Na)^+$		1281
HRFAB-MS $(M+Na)^+$		1281.3215 (calcd. 1281.3242)
FAB-MS $(M+H)^+$	1106	
HRFAB-MS $(M+H)^+$	1106.2848 (calcd.1106.2819)	
$[\alpha]_D^{25}$	-274.2° (c 0.58, $CHCl_3$)	
UV λ_{max}^{MeOH} nm	250	250
IR υ_{max} (KBr) cm^{-1}	3400, 1690,1550	3380, 1680, 1550, 1490
TLC[a] (Rf value)[b]	0.44 (brown)	0.40 (yellow)
HPLC (Rt, minutes)[c]	2.50	1.90
HPLC-MS (Rt, minutes)[d]	4.09	3.50

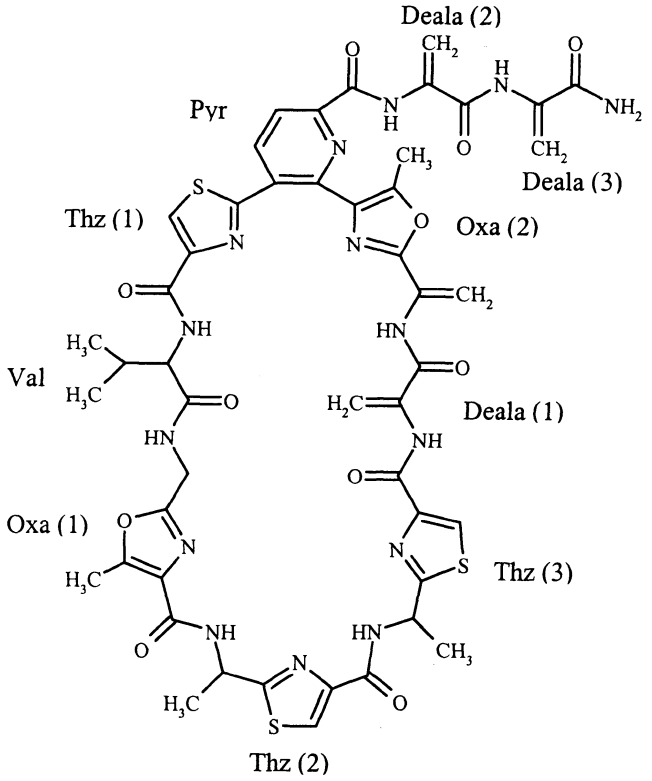
a Silica gel 60 F_{254}, Merck

b Solvent : $CHCl_3$-MeOH (92:8)

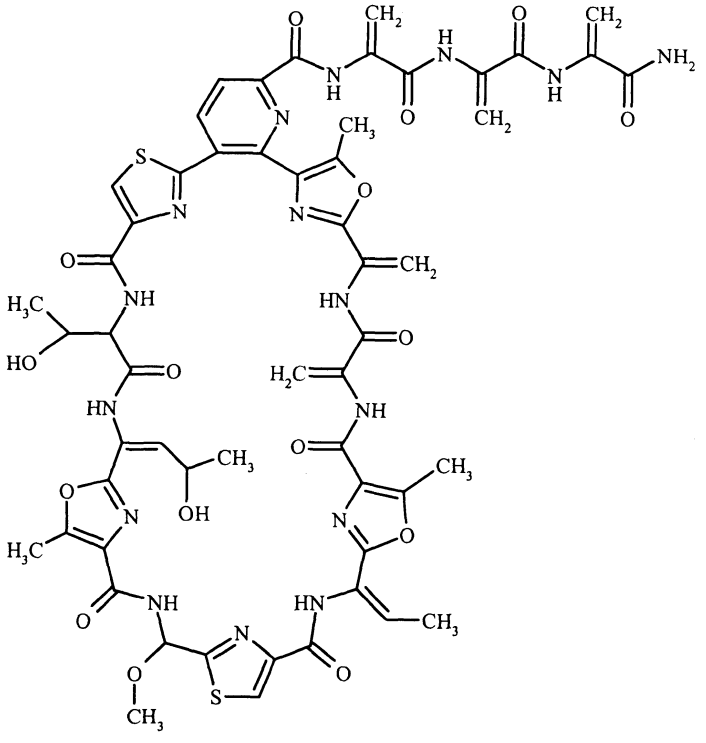
c Resolve C18 radial pack cartridge (10μ); mobile phase: CH_3CN:H_2O (97:3); flow rate: 2 ml/min.; detection: 250 nm

d Symmetry C18 column (5μ); mobile phase: CH_3OH:H_2O+1% HOAc (95:5); flow rate: 0.3 ml/min.; detection: 250 nm

Fig. 1. Structures of radamycin (**1**) and methylsulfomycin (**2**).



Radamycin (**1**)



Methylsulfomycin (**2**)

Table 2. ^{1}H and ^{13}C NMR data of radamycin (**1**).

Position	^{13}C (δ)	^{1}H (δ)	Position	^{13}C (δ)	^{1}H (δ)
Thiazole (1)			Dehydroalanine (1)		
2-C	165.4		NH		8.01 (s)
4-C	150.1		αC	134.7	
5-CH	126.1	8.29 (s)	βCH_2	102.9	6.66 (d, 2.0), 5.01 (s)
CO	160.9		CO	162.1	
Valine			Methyloxazole (2)		
NH		7.72 (d, 9.2)	NH		9.86 (s)
αCH	59.2	4.36 (t, 8.4)	αC	127.8	
βCH	30.6	2.25 (m)	βCH_2	102.7	6.39 (s), 5.66 (s)
γCH_3	19.5	0.95 (d, 6.8)	2-C	154.9	
γCH_3	18.5	0.97 (d, 6.8)	4-C	133.5	
CO	171.3		5-C	152.8	
			$5-CH_3$	13.3	2.91 (s)
Methyloxazole (1)					
NH		6.92 (t, 5.6)	Pyridine		
CH_2	36.6	4.45 (dd, 16.8, 6.0)	2-C	148.6	
		4.30 (dd, 16.8, 6.0)	3-C	130.1	
2-C	157.8		4-CH	141.3	8.11 (d, 8.0)
4-C	128.8		5-CH	120.7	8.28 (d, 8.0)
5-C	154.4		6-C	150.0	
$5-CH_3$	11.8	2.48 (s)	CO	162.2	
CO	161.0				
			Dehydroalanine (2)		
Thiazole (2)			NH		10.63 (s)
NH		8.28 (d, 8.4)	αC	134.3	
αCH	46.5	5.62 (t, 7.2)	βCH_2	103.3	6.82 (d, 2.0), 5.55 (s)
βCH_3	21.7	1.79 (d, 7.0)	CO	162.1	
2-C	171.5				
4-C	149.1		Dehydroalanine (3)		
5-CH	124.1	8.05 (s)	NH		9.01 (s)
CO	160.2		αC	133.1	
			βCH_2	103.5	6.63 (d, 2.0), 5.44 (s)
Thiazole (3)			CO	165.9	
NH		7.68 (d, 8.4)			
αCH	46.5	5.48 (t, 7.2)			
βCH_3	22.4	1.72 (d, 6.8)			
2-C	171.2				
4-C	149.2				
5-CH	125.1	8.15 (s)			
CO	159.9				

revealed the partial structures; $(CH_3)_2$–CH–CH–NH–, CH_3–CH–NH–×2, –CH=CH– and $–CH_2–NH–$. The presence of thiazole and methyl oxazole units were deduced by comparison of the corresponding ^{1}H and ^{13}C chemical shifts with those of methylsulfomycin and other known related thiopeptides. As known in Fig. 2, by PFG-HMBC experiments an aromatic proton signal at 8.29 ppm (Thz(1), 5-H) showed long range couplings to carbons at 165.4 ppm (Thz(1), C-2) and 150.1 ppm (Thz(1), C-4), the aromatic proton signal at 8.28 ppm (Thz(2), 5-H) to carbons at 171.5 ppm (Thz(2), C-2) and 149.1 ppm (Thz(2), C-4), and the aromatic proton signal at 7.68 ppm (Thz(3), 5-H) to carbons at 171.2 ppm (Thz(3), C-2) and 149.2 ppm (Thz(3), C-4). These correlations suggested the presence of three thiazole rings.

A methyl signal at 2.48 ppm (Oxa(1), CH_3-5) showed

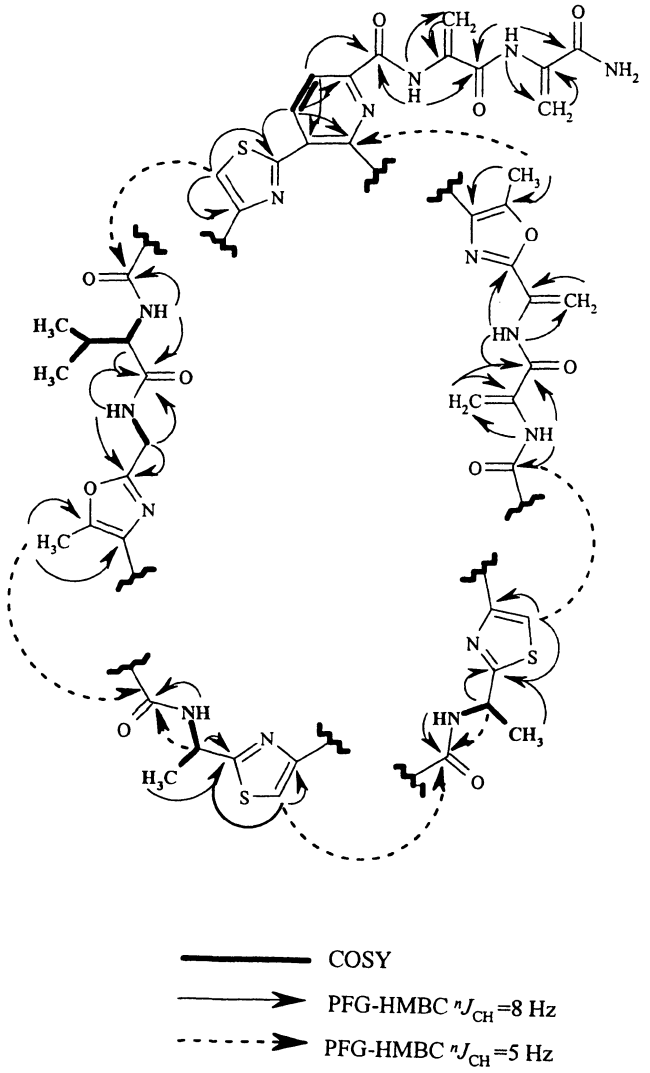
long range correlations to quaternary carbons at 128.8 ppm (Oxa(1), C-4) and 154.4 ppm (Oxa(1), C-5), and another methyl signal at 2.91 ppm (Oxa(2), CH_3-5) to 133.5 ppm (Oxa(2), C-4) and 152.8 ppm (Oxa(2), C-5) indicating the presence of two methyloxazole residues.

Long range connectivities of two adjacent aromatic doublet protons at 8.11 and 8.28 ppm to carbons 130.1, 148.6 and 150.0 ppm revealed the presence of a 2,3,6-trisubstituted pyridine residue. The long range correlation from the aromatic proton at 8.11 ppm (Pyr, 4-H) to a quaternary carbon at 165.4 ppm (Thz(1), C-2) indicated the connection of thiazole(1) and pyridine rings. In addition, a PFG-HMBC experiment optimized for a $^nJ_{CH}$ of 5 Hz revealed a four-bond long range correlation of a methyl signal at 2.91 ppm (Oxa(2), CH_3-5) to C-2 of pyridine at 148.6 ppm and established the linkage of pyridine and oxazole(2) moiety. These results revealed the presence of a thiazole-pyridine-oxazole moiety in the cyclic peptide core as seen in berminamycins[1,2], sulfomycins[3~5], promothiocins[6], and A10255 complex[7].

Eight olefinic protons at 5.01, 5.44, 5.55, 5.66, 6.39, 6,63, 6.66 and 6.82 ppm were assigned as four terminal methylenes by PFG-HSQC experiments in the dehydroalanine residues attached two of them to a carbonyl carbon on pyridine ring, one to a carbonyl carbon on thiazole(3) ring and the last one to C-2 on methyloxazole(2) moiety, as known in Fig. 2 by PFG-HMBC experiments.

The rest of the connectivities of the above partial structures were also established by PFG-HMBC experiments. The partial structures shown in Fig. 2 were further connected by a PFG-HMBC optimized for a $^nJ_{CH}$ of 5 Hz. This experiment revealed the correlation from 5-H of Thz(1) to 160.9 ppm (Thz(1), CO), from CH_3-5 of Oxa(1) and from αCH of Thz(2) to 161.0 ppm (Oxa(1), CO), from 5-H of Thz(2) and from αCH of Thz(3) to 160.2 ppm (Thz(2), CO) and finally, from 5-H of Thz(3) to 159.9 ppm (Thz(3), CO). Thus, the planar structure of radamycin was established as shown in Fig. 1.

Fig. 2. Partial structures of **1** elucidated by the PFG-HMBC experiments and their connectivities.



Experimental

General Procedures

Optical rotation was measured with an Optical Activity AA-10 polarimeter. IR spectrum was recorded on a Perkin Elmer 881 spectrophotometer. HPLC-ESI-MS analysis was performed with a HP 1100 liquid chromatograph equipped with a gradient pump and a mass spectrometer with a nebulizer-assisted electrospray source. NMR spectra were acquired on a Varian Mercury NMR spectrometer (400 MHz for ^1H, 100 MHz for ^{13}C). Chemical shifts are reported in ppm referenced to the $CHCl_3$ peak at 7.26 ppm for ^1H and 77.0 ppm for ^{13}C. FAB-MS and HRFAB-MS were measured with a VG AUTOSPEG spectrometer.

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