Notes

Methylsulfomycin I, a New Cyclic Peptide Antibiotic from a *Streptomyces* sp. HIL Y-9420704[†]

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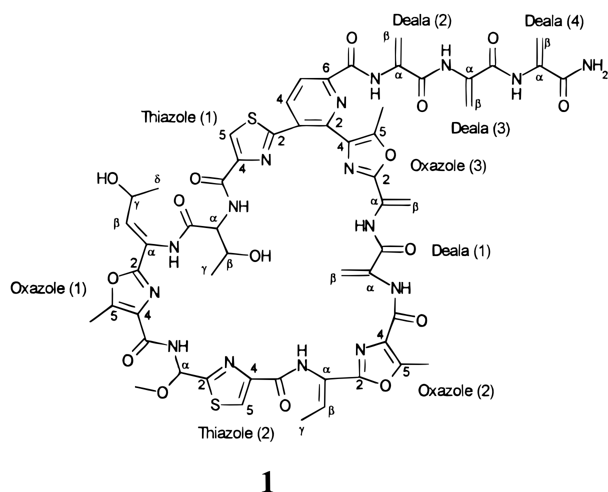
Methylsulfomycin I (**1**) is a new cyclic peptide antibiotic isolated from the fermentation broth of a *Streptomyces* sp. HIL Y-9420704. Its structure was elucidated by NMR and GC−MS. The in vitro activity (MIC) against a wide range of Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-, and teicoplanin-resistant strains, is described.

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are known to be predominant in several infectious conditions such as wounds and burns. Vancomycin and teicoplanin, belonging to the glycopeptide class, are the only two antibiotics clinically used for the treatment of MRSA infections.[1] However, recent emergence of vancomycin- and teicoplanin-resistant strains[2] have made these infections menacing and fatal. A program directed toward an intensive search for a structurally different class of compounds active against these resistant strains resulted in the isolation of methylsulfomycin I (**1**) from the fermentation broth of a *Streptomyces* sp. HIL Y-9420704.[3,4] Herein we report the production, isolation, structure elucidation, and biological properties of **1**.



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Results and Discussion

The crude antibiotic was recovered from the culture filtrate by extraction with EtOAc at the broth pH and from the mycelium by MeOH extraction. The combined crude material was purified by HPLC over reversed-phase Si gel (RP_{18}) using H_2O−MeOH mixtures for elution followed by repeated semipreparative HPLC (32:68 H_2O−MeOH as the mobile phase). The production as well as the purification of methylsulfomycin I (**1**) was monitored both by testing its bioactivity against *Staphylococcus aureus* 3066 and *Enterococcus faecium* R-2 and by HPLC.

The molecular formula of methylsulfomycin I (**1**) was determined to be $C_{55}H_{54}N_{16}O_{16}S_2$ by HRFABMS [$(M + H)^+$: found *m/z* 1259.3428, calcd for $C_{55}H_{55}N_{16}O_{16}S_2$ *m/z* 1259.3423]. The IR spectrum of **1** showed bands at 3380 and 1680 cm^{-1}, indicating the presence of OH/NH and amide carbonyls, respectively. Acid hydrolysis (6N HCl, 115 °C, 20 h) of **1** followed by GC−MS of the hydrolysate (as *N*-TFA/*O*-Me derivatives) indicated the presence of threonine along with several unidentified residues.

Pure methylsulfomycin (**1**), in solution, was found to decompose into a biologically inactive material practically insoluble in most of the commonly used organic solvents. A time bound study of its ^1H NMR in $CDCl_3$, CD_3OD, and DMSO-d_6 revealed that, in a completely degassed solution, a stability of up to 2, 10, and 72 h, respectively, was achieved, thus making DMSO-d_6 the solvent of choice for 2D NMR studies.

Table 1 summarizes the ^1H and ^{13}C NMR spectral data of methylsulfomycin I (**1**). The ^{13}C NMR and DEPT 135 spectra of **1** displayed the presence of 6 × CH_3 , 5 × CH_2, 10 × CH (6 × =CH and 4 × XCH), 33 × C (23 × =C and 10 × CO) and 1 × OCH_3, accounting for 41 protons. The remaining 13 protons were exchanged with D_2O in the ^1H NMR spectrum of **1** in DMSO-d_6 and were thus assigned to NH and/or OH protons. The spectral properties of **1** (NMR and GC−MS) were very similar to those reported for cyclic thiopeptides thioxamycin;[5,6] berninamycin A;[7−9] sulfomycin I;[7] and A 10255 B, G, and J,[10] which are composed of unusual amino acids masked at their carboxyl group by thiazole and/or oxazole units and several dehydroamino acids.

The analysis of DQF H−H COSY and HRFABMS indicated that **1** was different from sulfomycin I[7] in having an additional methyl group (δ_C 11.43; δ_H 2.36). The location of this methyl group was found to be at the C-5 position of oxazole (3) by the absence of C-5 methine and the presence of a quaternary carbon at δ 153.80 in the ^{13}C NMR

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Table 1. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) Spectral Data of Methylsulfomycin I (**1**) in DMSO-d_6

position	δ_C	δ_H	position	δ_C	δ_H
thiazole (1)			deala (1)		
C-2	164.88 (s)		NH		9.28 (brs)
C-4	152.48 (s)		α-C	134.03 (s)	
CH-5	126.71 (d)	8.40 (s)	β-CH$_2$	105.83 (t)	6.34 (brs), 5.78 (brs)
C=O	160.88 (s)		CO	163.00 (s)	
threonine			oxazole (3)		
α-CH	58.44 (d)	4.40 (dd, 8.5, 4.8 Hz)	NH		9.95 (brs)
β-CH	66.42 (d)	4.17 (dd, 6.1, 4.8 Hz)	α-C	134.36 (s)	
γ-CH$_3$	22.51 (q)	1.12 (d, 6.1 Hz)	β-CH$_2$	111.97 (t)	5.66 (brs), 5.62 (brs)
OH		5.26 (d, 6.1 Hz)	C-2	156.27 (s)	
NH		7.77 (d, 8.5 Hz)	C-4	129.50 (s)	
C=O	169.89 (s)		C-5	153.80 (s)	
oxazole (1)			CH$_3$	11.43 (q)	2.36 (s)
NH		9.54 (s)	pyridine		
α-C	121.29 (s)		C-2	149.54 (s)	
β-CH	136.90 (d)	6.29 (d, 7.3 Hz)	C-3	124.09 (s)	
γ-CH	62.28 (d)	4.54 (m)	CH-4	141.02 (d)	8.51 (d, 7.3 Hz)
δ-CH$_3$	22.88 (q)	1.19 (d, 6.1 Hz)	CH-5	121.10 (d)	8.27 (d, 7.3 Hz)
OH		4.96 (d, 3.6 Hz)	C-6	156.09 (s)	
C-2	156.20 (s)		C=O	161.50 (s)	
C-4	128.73 (s)		deala (2)		
C-5	153.70 (s)		NH		10.53 (brs)
CH$_3$	10.96 (q)	2.55 (s)	α-C	134.03 (s)	
C=O	161.50 (s)		β-CH$_2$	106.30 (t)	6.54 (brs), 5.97 (brs)
thiazole (2)			C=O	163.00 (s)	
NH		8.58 (d, 8.5 Hz)	deala (3)		
α-CH	78.31 (d)	6.37 (d, 8.5 Hz)	NH		10.10 (brs)
OCH$_3$	55.74 (q)	3.39 (s)	α-C	136.83 (s)	
C-2	168.72 (s)		β-CH$_2$	110.50 (t)	5.75 (brs), 5.71 (brs)
C-4	149.03 (s)		C=O	162.21 (s)	
CH-5	122.37 (d)	8.41 (s)	deala (4)		
C=O	159.24 (s)		NH		9.11 (brs)
oxazole (2)			α-C	134.60 (s)	
NH		9.75 (brs)	β-CH$_2$	104.39 (t)	6.12 (brs), 5.66 (brs)
α-C	123.59 (s)		C=O	165.03 (s)	
β-CH	129.68 (d)	6.53 (q, 7.3 Hz)	NH$_2$		7.94 (brs), 7.54 (brs)
γ-CH$_3$	13.57 (q)	1.75 (d, 7.3 Hz)			
C-2	156.90 (s)				
C-4	129.11 (s)				
C-5	153.40 (s)				
CH$_3$	11.32 (q)	2.51 (s)			
C=O	159.97 (s)				

spectrum of **1**. This was further substantiated by $^2J_{CH}$ and $^3J_{CH}$ correlations observed for the methyl protons with C-4 and C-5.

The geometry of the trisubstituted double bonds in oxazole (1) and oxazole (2) were found to have *Z* configuration by the analysis of 2D NOESY spectrum of **1**. Thus, NOE interactions were observed between the NH at δ 9.54 (brs) and the methine proton (γ-CH) at δ 4.54 (m) in the case of oxazole (1) and between the NH at δ 9.75 (brs) and the methyl protons (γ-CH$_3$) at 1.75 (d, 7.3 Hz) in the case of oxazole (2).

The structure of methylsulfomycin I was thus established as **1**. The relative instability of **1** in solution, which was in sharp contrast to sulfomycin I, might be due to its sensitivity to oxygen. A similar behavior was reported earlier in the case of thioxamycin.[5,6]

Methylsulfomycin I (**1**) exhibited good in vitro activity against a wide range of Gram-positive bacteria, including vancomycin- and teicoplanin-resistant organisms. Compound **1** was found to be more potent than vancomycin against vancomycin- and teicoplanin-sensitive and -resistant organisms. The MIC values against several *Staphylococcus* and *Entercoccus* species are in the range of 0.06–0.125 μg/mL. Against teicoplanin-sensitive (*S. haemo.* 712 and *S. epi.* 825) and -resistant (*S. haemo.* 712 and *S. epi.* 823) strains, **1** was found to be about 6.2 and 12.4 times, respectively, more active than vancomycin (0.78 and 1.56 μg/mL, respectively). Further, **1** was greater than 800 times more active than vancomycin (>100 μg/mL) against vancomycin- and teicoplanin-resistant (*E. faecium* R-1, *E. faecium* R-2, *E. faecium* P-1, and *E. equinus*). The results of the in vivo activity of **1** against *Streptococcus pyogenes* A 77 and *S. aureus* SG 571 at 25 mg/kg in mice were not conclusive as a result of precipitation in aqueous solvents under the experimental conditions.

Experimental Section

General Experimental Procedures. Melting point is uncorrected. HPLC analysis was carried out on a 5μ ODS–Hypersil [4 mm × (30 + 250 mm)] column using H_2O–MeOH (35:65) as the mobile phase at a flow rate of 1 mL/min and detection at 210 nm. UV spectrum was recorded on a JASCO V 550 spectrophotometer. IR spectrum was recorded on a Perkin–Elmer 782 spectrophotometer. Optical rotation was measured on a Rudolph AP III 589 polarimeter. MS were obtained on VG B10-Q (ESI) spectrometer. HRFABMS was recorded on a VG-ZAB SEQ spectrometer using MeOH–NBA as the matrix and PEG 1540 as the internal reference. NMR spectra were recorded in DMSO-d_6 on a Bruker ACP 300 spectrometer using concentrations of 10 and 20 mg/mL at 295 K. The carbon multiplicities were determined by DEPT 135 spectrum. The protonated carbon resonances were assigned by the analysis of a HMQC experiment, and the quaternary carbons were assigned by the interpretation of HMBC experiments optimized for $^nJ_{CH}$ values of 5 and 12.5 Hz. Protons were assigned by the analysis of phase-sensitive double quantum filtered ^{1}H–^{1}H COSY, NOESY, and HMBC spectra.

Organism and Fermentation. The microorganism, culture number HIL Y-9420704, was isolated from a soil sample collected in Greece and identified as *Streptomyces* species. The producing organism was deposited at the German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany, with the accession number DSM 11008. A loopful of mature slant culture of Y-9420704 was inoculated into Erlenmeyer flasks (500-mL capacity) containing 80 mL of seed medium consisting of glucose 1.5%, soyabean meal 1.5%, corn steep liquor 0.5%, and $CaCO_3$ 0.2%, pH 7.0, before autoclaving. The flasks were cultivated on a rotary shaker for 72 h at 240 rpm at 29 °C (± 1 °C) to give seed culture. The seed culture (3%) was inoculated into a 150-L fermenter containing 90 L of production medium consisting of soluble starch 2.5%, glucose 1.0%, yeast extract 0.2%, soyabean meal 1.0%, $CaCO_3$ 0.1%, NaCl 0.5%, and $MgSO_4 \cdot 7H_2O$ 0.1%, pH 7.0, before autoclaving. Desmophen (45 mL) was added as an antifoam. The aeration and agitation of the fermentation were maintained at 100 $Lmin^{-1}$ and 120 rpm, respectively. The fermentation was carried out at 29 °C for 72 h. The production of the antibiotic was monitored by bioactivity of the culture filtrate against *S. aureus* 3066 and *E. faecium* R-2 and by HPLC.

Isolation and Purification of 1. The culture broth (100 L) was harvested and separated from the mycelium (7.7 kg) by centrifugation. Methylsulfomycin I was found to be present in the culture filtrate as well as in the mycelium. The culture filtrate (92 L) was extracted with EtOAc (2 × 45 L) at broth pH, and the combined EtOAc extracts were concentrated under reduced pressure to obtain crude methylsulfomycin I (25.7 g). The mycelium (7.7 kg) was extracted with MeOH (3 × 30 L). The combined MeOH extracts were concentrated under reduced pressure and lyophilized to get more crude material (30.5 g). This was combined with the crude material obtained from the culture filtrate. The combined concentrate was subjected to HPLC on reversed-phase Si gel (50−70 μm) packed in two (32 mm × 250 mm and 62 mm × 300 mm) stainless steel columns connected in series. The column was developed in H_2O and then eluted with H_2O−MeOH mixtures, where the MeOH concentration was increased in steps of 10% up to 50% and then in steps of 2% up to 60%. A flow rate of 100 mL/min was maintained at a pressure of 150−175 bar. The eluates were collected in fractions of 1 L, and the presence of methylsulfomycin I was monitored by checking each fraction for its bioactivity against *S. aureus* 3066 and *E. faecium* R-2 as well as by HPLC. Methylsulfomycin I eluted in 50−58% MeOH in H_2O system, which was then concentrated under reduced pressure and lyophilized to obtain semi-pure methylsulfomycin I (1.1 g).

The above semi-pure methylsulfomycin I was further purified by two successive preparative HPLC runs on a [16 mm × (30 + 120) mm] 5μ ODS−Hypersil column using MeOH−H_2O (68:32) as the mobile phase at a flow rate of 9 mL/min and detection at 210 nm. The fractions were monitored by HPLC and the pure bioactive fractions pooled. Concentration of the pooled fractions under reduced pressure followed by lyophilization gave methylsulfomycin I (110 mg).

Methylsulfomycin I (1): white powder; mp > 250 °C (dec); $[\alpha]^{25}_{D}$ −57.14° (*c* 0.07, MeOH); soluble in $CHCl_3$, MeOH, CH_3-CN, DMSO; HPLC room temperature, 11.6 min; HRFABMS *m/z* $(M + H)^+$ 1259.3428 (calcd for $C_{55}H_{55}N_{16}O_{16}S_2$, 1259.3423); UV (MeOH) λ_{max} 250, 320 nm (no acid/alkali shift); IR (KBr) ν_{max} 3380, 1670 (broad), 1635, 1505, 1200, 890 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1.

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