NOTES

Sch 40832: A Novel Thiostrepton from *Micromonospora carbonacea*†

MOHINDAR S. PUAR*, T. M. CHAN, V. HEGDE, M. PATEL, P. BARTNER, K. J. NG and B. N. PRAMANIK

Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, N.J. 07033 U.S.A.

R. D. MACFARLANE

College of Science, Texas A & M University, College Station Texas 77873 U.S.A.

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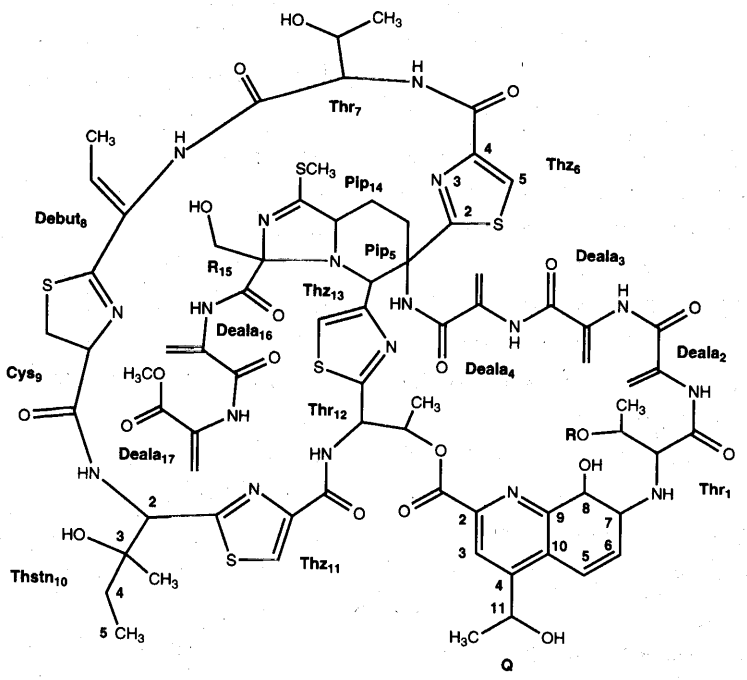
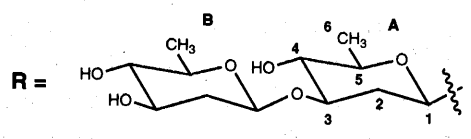
In the course of screening for novel pharmacologically active microbial products from fermentations of soil microorganisms, the structure of a thiostrepton-type antibiotic, Sch 18640 was reported[2]. Several other studies related to thiostrepton-type molecule have also been reported[3~8]. Presently, we report our studies of a first diasaccharide containing sulfur-rich peptide antibiotic, Sch 40832.

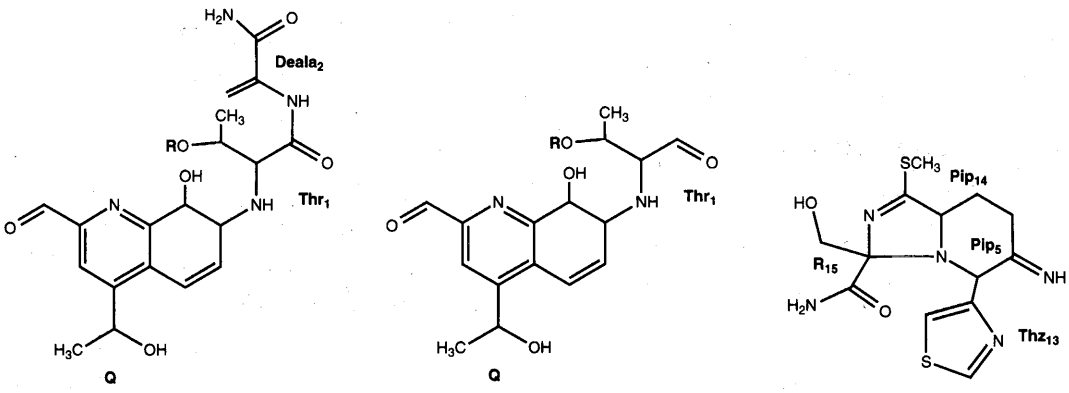
Sch 40832 (**1**) is a minor component of the antibiotic complex (also referred to as 13~384 complex) produced by *Micromonosporia carbonecea* var *africana* (ATCC 39149). Taxonomy, fermentation, isolation and characterization have been reported[9]. Solvent extraction of the fermentation broth followed by chromatography afforded two everninomicins (Sch 27899 and 27900), chloramphenicol, Sch 40832, a diketopiperazine and other minor components. The structure elucidation[10] of Sch 27899 and 27900 and *in vitro* activity[11] of the former have recently been reported. The *in vitro* activity of Sch 40832 was determined using a disc-diffusion agar plate assay against gram positive bacteria following established





Sch 40832

† See ref. 1.



procedures. Results indicated that Sch 40832 had potent activity in the range of 0.1 ~ 1.0 mcg/ml.

Sch 40832 was purified on silica gel, followed by HPLC on Waters Associates reverse phase C18 silica gel column (r.t. = 33.5 minutes, MeOH : 0.01 N NaH_2PO_4, 70 : 30).

Sch 40832, a colorless solid, gave satisfactory and reproducible chemical analysis: %C = 50.17, H = 5.37, N = 12.51 and S = 8.41 for a molecular weight of 2022.21 consistent with the composition of $C_{84}H_{104}N_{18}O_{26}S_5 \cdot H_2CO_3 \cdot H_2O$ which suggested 42 degrees of unsaturation (30 double bonds and 12 rings); $[\alpha]_D^{26} = -163.1°$ (0.9% in $CHCl_3$); IR ($CDCl_3$) 3400, 3310 (OH, NH), 1730 (ester), 1670, 1660 (amide), 1642, 1530, 1500, 1480 (C=N, amide II, C=C), 1210 (C–O), 1052 (C–O–C) cm^{-1}, UV λ_{max} (C 2 mg/100 ml CH_3OH) 234 nm (ε 68,320), 295 nm (ε 16,960) and 307 nm (ε 11,630). The amino acid analysis of Sch 40832 showed the presence of one cysteine, four threonines, and one lysine (from pip) residues.

The molecular weight of Sch 40832 was established as 1940 based on FAB-MS, Cs^+-LSI-MS, PD-MS and Ion-Spray-MS data. The positive ion Cs^+ liquid secondry ion mass spectrum of 1 in thioglycerol-DMSO with 0.1% TFA displayed an intense molecular ion at *m/z* 1941 $(M+H)^+$. Peak-matching Cs^+-LI-MS experiment using CsI as a reference provided an accurate mass of 1941.6219 (1941.6205 calculated for $C_{84}H_{105}N_{18}O_{26}S_5$) and the isotope pattern of the molecular ion region of 1 also matched with the computer generated isotope profile for this composition.

MS/MS experiments of *m/z* 1941 $(M+H)^+$ using ion-spray ionization method generated a limited number of daughter ions at *m/z* 1811, 1680, 1608, 1465, and 1209. The intense ions at *m/z* 1811 and 1680 corresponded to the successive loss of sugar units from the parent ion. FAB-MS showed prominent ions at *m/z* 1941.6 $(M+H)^+$, 1912 $(M+H-C_2H_5)^+$, 1897.5 $(M+H-COCH_3)^+$, 1745 $(M+H-C_8H_9N_2O_4)^+$, 1681.5 $(M+H-C_{12}H_{20}O_6)^+$, and 1665 $(M+H-C_{12}H_{20}O_7)^+$. The data provided information about the side chain and the disaccharide moiety. Additional FAB MS studies provided critical and important fragment ions at *m/z* 663, 580, and 388 (shown above).

In the proton NMR spectrum[12], all directly coupled protons were assigned on the basis of the analysis of 2D DQCOSY and LR COSY. The Hartman-Hahn (HOHAHA) experiment helped to delineate the entire proton spin system of the disaccharide moiety.

^{13}C NMR data[12], edited on the basis of an APT experiment, were analyzed by comparison with the data of thiostrepton and Sch 18640[2]. The assignments of protonated carbons were confirmed by a 2D (^1H-^{13}C) heteronuclear correlation experiment and are presented in Table 1.

The presence of a methyl triplet at δ 0.99, coupled to a methylene residue with non-equivalent proton resonances at δ 1.55 and 1.68, was assigned to thiostreptine (Thstn 10) 5-CH_3. The lack of further correlation confirmed that the C_2H_5 moiety was connected to the quarternary carbon at position 3 and there was no OH function at C_4 as in Sch 18640. The glycosidic bond was established at C_3 of Thr1 on the basis of chemical shifts[13], which also led to the assignment of 1,3 linkage between the sugars, tentatively assigned as β-D chromose A and B[14,15].

Lack of one thiazole unit (compared to Sch 18640 and thiostrepton) led to the consideration of a modified unit (R_{15}). In addition, several long range interactions were observed in the ROESY spectra, *e.g.*, between δ 1.50 (CH_3 debut8) and 4.88 (CH cys9), δ 5.40 (CH thstn 10) and 8.13 (CH thz 11), δ 5.63 (CH thr 12) and 7.83 (CH thz 13), δ 5.22 (CH Q 11) and 7.29 (CH Q 3) and 6.78 (CH Q5), and between δ 6.25 [CH Q6 and 4.41 (CH

Table 1. ^{13}C and ^{1}H NMR spectral data of Sch 40832 (δ in ppm, J in Hz).

Deala(2)	CO	161.1 s	
	2	133.4 s	
	3	100.2 t	5.07 (bs), 6.23 (d, 2.0)
		—	9.27 (bs, NH)
Deala(3)	CO	163.0 s	
	2	132.9 s	
	3	102.1 t	5.55 (t, 2,0, 1,5),
			6.58 (d, 2.0)
		—	8.57 (bs, NH)
Deala(4)	CO	168.9 s	
	2	138.1 s	
	3	103.4 t	5.20 (bs), 5.66E (d, 2.0)
		—	8.28 (bs, NH)
Thz(6)	CO	161.8 s	
	2	169.2 s	
	4	146.7 s	
	5	124.3 d	7.90 (s)
Thr(7)	CO	166.6 s	
	2	55.6 d	4.35 (dd, 8.0, 2.5)
	3	66.5 d	1.12 (m)
	CH_3	19.2 q	0.96 (d, 6.5)
		—	6.42 (d, 8.0, NH)
		—	4.22 (s, OH)
Debut(8)	2	128.4 s	
	3	132.7 d	6.10 (q, 6.5)
	CH_3	15.0 q	1.50 (d, 6.5)
		—	8.44 (bs, NH)
Cys(9)	CO	171.7 s	
	2	170.7 s	
	4	78.9 d	4.88 (dd, 12.0, 9.0)
	5	34.8 t	3.17 (t, 12.0, 12.0)
			3.52 (t, 12.0, 9.0)
Thstn(10)	2	54.6 d	5.40 (d, 9.2)
	3	75.1 s	
	4	30.7 t	1.55 (m), 1.68 (m)
	CH_3	7.6 q	0.99 (t, 6.5)
	$3CH_3$	23.6 q	1.12 (s)
		—	7.56 (d, 9.2, NH)
Thz(11)	CO	162.1 s	
	2	167.2 s	
	4	149.7 s	
	5	125.1 d	8.13 (s)
Thr(12)	2	55.4 d	5.63 (d, 10.0)
	3	72.0 d	6.05 (q, 6.5)
	CH_3	18.9 q	1.28 (d, 6.5)
		—	7.90 (d, 10.0, NH)
Thz(13)	2	169.0 s	
	4	152.9 s	
	5	122.3 d	7.38 (s)
Q	CO	170.5 s	
	2	143.5 s	
	3	122.7 d	7.29 (s)
	4	153.6 s	
	5	122.8 d	6.78 (d, 10.0)
	6	130.1 d	6.25 (d, 10.0, 6.0)
	7	59.5 d	3.51 (dd, 6.0, 2.0)
	8	67.9 d	4.41 (bd, 8.0, 2.0)
	9	154.6 s	
	10	127.6 s	
	11	64.3 d	5.22 (q, 6.5)
	CH_3	22.5 q	1.23 (d, 6.5)
	8OH		7.10 (d, 8.0)
	11OH		5.13 (s)
Thr(1)	CO	164.0 s	
	2	66.4 d	2.87 (d, 8.5)
	3	70.7 d	3.54 (m)
	CH_3	17.4 q	1.16 (d, 6.5)
		—	3.14 (bs, NH)
Pip	2	71.6 d	4.04 (m)
	3	23.8 t	2.08 (m)
	4	33.8 t	2.08 (m), 3.87 (m)
	5	59.3 s	
	6	63.0 d	4.51 (s)
		—	10.22 (bs, NH)
R(15)	CO	162.3 s	
	2	92.3 s	
	3	64.0 t	4.01 (dd, 12.5, 2.0)
			4.07 (dd, 12.5, 2.0)
	4	178.0 s	
	SCH_3	12.1 q	2.45 (s)
	3OH	—	2.37
Deala(16)	CO	160.3 s	
	2	134.2 s	
	3	103.5 t	5.72 (bs), 5.76 (bs)
		—	7.72 (bs, NH)
Deala(17)	CO	165.7 s	
	2	131.4 s	
	3	110.8 t	6.00 (bs), 6.66 (bs)
		—	8.66 (bs, NH)
	OCH_3	52.4 q	3.84 (s)

Q8)]. HMBC data supported several connectivities, *e.g.*, Debut8-Cys9-Thstnl0-Thzl1-Thr12-Q(-Thz13), Thz13-Pip5, Thz6-Thr7, Deala2-3-4, Deala16-17, and Thrl(δ 3.54) to β-D chromose A (δ 97.6). The lack of a hydroxyl group at C_3 (Thstn10), the presence of a modified thiazoline ring (R_{15}), and the presence of a diasaccharide moiety attached to Thr1 makes for a unique structure **1** for Sch 40832.

Acknowledgments

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12) Proton magnetic resonance data of a solution in $CDCl_3$ and $CDCl_3$-CD_3OD were obtained at 100～600 MHz. Highfield, 500 and 600 MHz spectra were obtained at Yale University and Mellon Institute, respectively. ^{13}C NMR spectra of a solution in $CDCl_3$, $CDCl_3$-CD_3OD and $CDCl_3$-CD_3OH were obtained at 25.2～126.7 MHz. ROESY experiment was carried out at 500 MHz utilizing GE-500 NMR Instrument. The chemical shifts are referenced to internal TMS. All spectral data are available upon request.
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15) ^{1}H NMR ($CDCl_3$, *J* in Hz): Sugar A, 1 (4.31, dd, 9.5, 1.5), 2 (1.60, 2.08), 3 (3.40, ddd, 10.0, 9.0, 4.0), 4 (3.02, t, 9.0, 9.0), 5 (3.13, dq, 9.0, 6.5), and 6 (1.23, d); Ring B, 1′ (4.47, dd, 9.5, 1.5), 2′ (1.60, 2.17), 3′ (3.56, ddd, 10.0, 9.0, 4.0), 4′ (3.05, t, 9.0, 9.0), 5′ (3.29, dq, 9.0, 6.5), and 6′ (1.29, d).
^{13}C NMR ($CDCl_3$): Sugar A, 1 (97.6), 2 (37.2), 3 (80.8), 4 (74.8), 5 (72.2), and 6 (17.0); Ring B, 1′ (99.1), 2′ (38.2), 3′ (75.1), 4′ (76.5), 5′ (72.0), and 6′ (18.4).