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STRUCTURE OF THIOTROPOCIN, A NEW SULFUR-CONTAINING 7-MEMBERED ANTIBIOTIC

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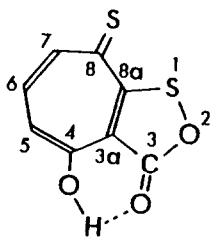
Summary: The structure of a new antibiotic thiotropocin is described.

In the course of our antibiotic screening, a new antibiotic has been isolated as crystals by ethyl acetate extraction and silica gel chromatography from the fermentation broth of Pseudomonas sp. CB-104. The acidic lipophilic antibiotic has broad antibacterial spectra. This paper deals with the structural elucidation of the antibiotic, thiotropocin (1), named from its novel skeleton in combination with tropothione and oxathiole.

The physico-chemical data of 1 are as follows; orange fine needles, mp 222-225°C(dec.), pKa' 5.1, IR(KBr), 1630, 1600 cm^{-1}, UV(MeOH), 216.5 nm(ε 25000), 245(11300), 307(16300), 356(6200), 452(2100), ^{1}H-NMR(400 MHz, $CDCl_3$, Jeol Ltd. GX 400), δ 7.12 ppm(1H, dd, J=0.20,

Chart 1



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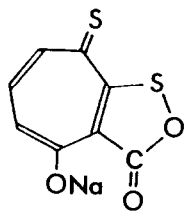

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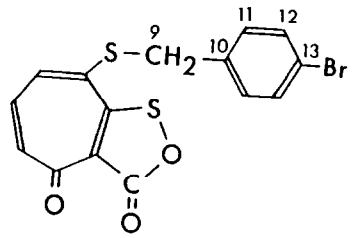

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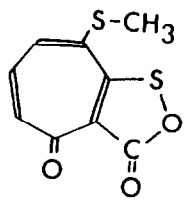

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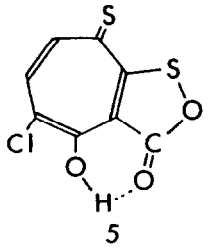

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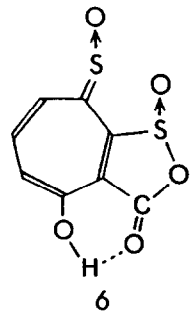


6

9.16 Hz, Ar.H), 7.44 (1H, dd, J=0.20, 12.3 Hz, Ar.H), 7.45(1H, dd, J=9.16, 12.3 Hz, Ar.H) and 16.7(1H, s, exchangeable with D_2O, chelated OH), EI-Mass, m/e 212(M^+), 184(M^+-28(CO)), and 168(M^+-44 (CO_2)), Anal. Calcd. for $C_8H_4O_3S_2$ (212.25), C,45.27; H,1.90; O, 22.61; S, 30.22, Found, C,45.23; H,1.77; S, 30.14 (%). The ^{13}C-NMR spectrum indicated 8 carbon signals derived from aromatic double bonds and carbonyl groups as shown in Table 1.

Table 1. ^{13}C-NMR data (100MHz, DMSO-d_6, Jeol Ltd. GX 400)

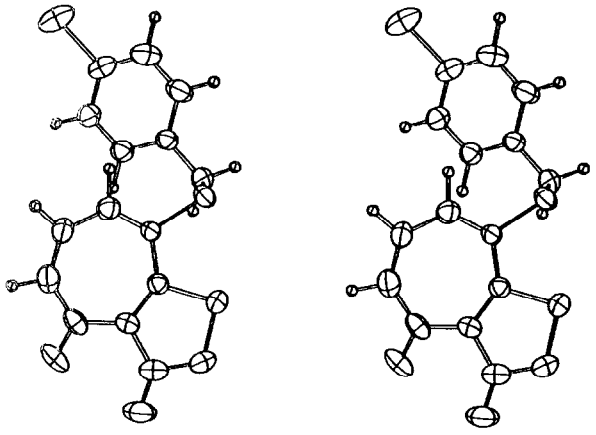
carbon	δ(ppm)	$^1_{\sim}$Jc-H(Hz)	δ(ppm)	$^5_{\sim}$Jc-H(Hz)	δ(ppm)	$^3_{\sim}$Jc-H(Hz)
3	170.60	s	170.31	s	167.91	^{4}J=1.8
3a	120.04	^{3}J=5.5	117.79	s	116.60	^{3}J=5.0
4	150.09	^{3}J=11.6	149.88	^{3}J=12.2, ^{4}J=1.0	o178.41	^{3}J=12.8
5	*133.80	^{1}J=168.8, ^{3}J=11.9	144.58	^{2}J=7.0, ^{3}J=12.5	*142.71	^{1}J=159.9, ^{3}J=9.2
6	137.64	^{1}J=161.7	130.24	^{1}J=170.0, ^{2}J=1.8	132.55	^{1}J=161.1
7	*137.74	^{1}J=161.4, ^{3}J=10.7	136.62	^{1}J=164.8	*142.85	^{1}J=166.9, ^{3}J=11.9
8	182.62	^{3}J=8.6	176.36	^{3}J=9.8	130.45	m
8a	167.65	^{3}J=9.8	166.37	^{3}J=12.2	o177.79	^{3}J=11.0
9					40.98	
10					135.55	
11					•131.52	
12					•131.47	
13					121.13	

o,•,*; Tentative assignments

From these data, $\underset{\sim}{1}$ was assumed to be a sulfur-containing aromatic (especially benzoquinone-typed) compound. However, the spectral data of $\underset{\sim}{1}$ showed curious patterns when compared with those of known compounds, and crystals of $\underset{\sim}{1}$ were not available for an X-ray crystallographic analysis. Therefore, derivatization of $\underset{\sim}{1}$ was carried out to obtain a suitable sample for the analysis. When treated with sodium carbonate in acetone, $\underset{\sim}{1}$ gave monosodium salt $\underset{\sim}{2}$[2] (95% yield). On treatment with p-bromobenzyl bromide in DMF (rt, 4 h), $\underset{\sim}{2}$ afforded a p-bromobenzyl thioether derivative, $\underset{\sim}{3}$[3], mp 152°C(dec.), $C_{15}H_9O_3S_2Br$ (65% yield).

The crystal data of $\underset{\sim}{3}$ are as follows; monoclinic, space group C2/c, a=15.664(3), b=14.557(2), C=12.983(3) Å, β=102.61(2)°, V=2888.9(9) Å^3, Z=8,Dx=1.75 g.cm^{-3}. The intensity measurements were performed for $3° \leq 2\theta \leq 50°$ on a Rigaku automated four-circle diffractometer with MoKα radiation. A total of 1871 reflections with $Fo \geq 3\sigma(Fo)$ was considered as observed. The structure was solved by direct methods using the MULTAN program,[4] and refined[5] to an R of 0.055[6]. A stereoscopic drawing of $\underset{\sim}{3}$ is shown in Fig. 1.

Fig. 1.



Comparing the IR spectrum of 1 with that of 3, the absorption at 1630 cm^{-1} in 1 was shifted to the carbonyl region at 1770 cm^{-1} in 3 as an ordinary absorption of 5-membered lactone. Furthermore, the singlet proton signal observed at δ 16.7 ppm in 1 disappeared in 3. These data show the presence of an intramolecular hydrogen bond between the hydroxyl and lactone carbonyl groups in 1. The structure of 1 was thus determined to be 4-hydroxy-3-oxo-8-thioxo-cyclohepto[c]-2,1-oxathiol-4,5,6,7,8a,3a-triene. To our knowledge, this skeletone is new and the S-O-C(O) moiety is very few in natural sources.

When treated with methyl iodide in DMF, 2 was converted into its methyl thioether, 4[7], mp 145-150°C(dec), $C_9H_6O_3S_2$ (66% yield) (Chart 1). On treatment with N-chlorosuccinimide in DMF (4°C, 15 min → rt, 30 min), 1 gave 5[8], $C_8H_3O_3S_2Cl$ (53% yield). A chloro group of 5 was substituted at the C_5 position from the long range coupling studies as shown in Table 1. When 1 was oxidized with m-chloroperbenzoic acid in methylene dichloride (rt, 2 h), 1 was converted into 6[9], mp 167-171°C (dec), $C_8H_4O_5S_2$ (71% yield). The IR spectrum showed absorptions at 1065, 1085, 1150 and 1165 cm^{-1}, which indicates the presence of -C=S → O and -C-S(O) → O functions[10].

Thiotropocin and its sodium salt showed antibacterial, antifungal and antiprotozoal activities in vitro and especially stronger antimicrobial activities under acidic conditions of the medium than neutral as shown in Table 2.

It is well known that a lot of tropolon analogues have been found as plant or microbial metabolites[11]. Thiotropocin may be the first example that a tropothione derivative has been found as a natural product.

Table 2. Antimicrobial spectra of 1, MIC(μg/ml)

Organism	E.coli	K. pneum.	P. mirab.	Ps. aerug.	St. aureus	B. subtilis
pH 5*	0.1	0.1	0.1	0.1	-	0.1
pH 7	12.5	6.25	1.56	25	6.25	12.5
pH 9	50	25	12.5	100	25	25

*pH of the assay medium (nutrient agar containing diaminopimelic acid).

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REFERENCES and NOTES

1) K. Kintaka, S.Tsubotani, S. Harada, H. Ono and H. Okazaki, J. Antibiotics, submitted for publication

2) All the samples in this report gave satisfactory elemental analyses. 2, a yellowish orange powder, UV, 214 nm (ε 25200), 243 (12700), 302 (18100), 354 (7200), 430 (2600), IR(KBr), 1695, 1635, 1600 cm^{-1}, ^{1}H-NMR(DMSO-d_6), δ 6.73 ppm (1H, dd, J=1.5, 11.0 Hz), 6.83(1H, dd, J=1.5, 8.5 Hz), 7.00(1H, dd, J=8.5, 11.0 Hz). ^{13}C-NMR (DMSO-d_6), δ 121.56, 127.32, 133.26, 135.52, 152.52, 173.19, 177.98, 180.09 ppm.

3) 3, yellowish green plates, EI-Mass, 382, 380 (M^+), 212, UV(CH_3CN), 217 nm (ε 35400), 234 (29000, sh), 280 (17500, sh), 292(19300), 350(4600), IR(KBr), 1770, 1630, 1610 cm^{-1}, ^{1}H-NMR($CDCl_3$), δ 4.10 ppm (2H, s, Ar$\underline{CH_2}$), 6.84 (1H, dd, J=3.0, 8.0 Hz), 6.90 (1H, dd, J=3.0, 8.0 Hz), 7.08 (2H, d, J=8.5 Hz, Ar.$\underline{H}$), 7.14 (1H, t, J=8.0 Hz), 7.50 (2H, d, J=8.5 Hz, Ar.$\underline{H}$).

4) G. Germain, P. Main and M. M. Woolfson, Acta Crystallogr., A27, 368(1971).

5) "The X-ray System". J. M. Stewart, Technical Report TR-446 of the Computer Science Center, University of Maryland, MD, U.S.A.

6) Crystallographic coordinates have been deposited with the Cambridge Crystallographic Data centre.

7) 4, yellowish green plates, EI-Mass, 226(M^+), 198, UV(CH_3CN), 218 nm (ε 24400), 279 (15200, sh), 291 (17200), 364 (4400), IR(KBr), 1760, 1630, 1600 cm^{-1}, ^{1}H-NMR(DMSO-d_6), δ 2.70 ppm (3H, s), 6.90 (1H, dd, J=1.0, 12.0 Hz), 7.22 (1H, dd, J=8.0, 12.0 Hz), 7.59 (1H, dd, J=1.0,8.0 Hz), ^{13}C-NMR (DMSO-d_6), δ 21.05, 116.42, 132.88, 134.19, 140.13, 141.92, 167.88, 176.52, 178.57 ppm

8) 5, orange powder, EI-Mass, 248, 246 (M^+), 218, 202, UV(CH_3CN) 223 nm (ε 18200), 250 (11300), 289 (11800), 304 (14800), 319 (16600), 332 (15000), 354 (7400), 368 (5900), 384 (3900), 468 (3400), IR(KBr), 1650, 1610,1590 cm^{-1}, ^{1}H-NMR (DMSO-d_6), δ 7.59ppm (1H,d, J=10.0Hz), 8.35(1H, d, J=10.0Hz).

9) 6, yellow needles, CI-Mass (iso-C_4H_{10}), 245 (M^++1), 213, UV(CH_3CN), 216 nm (ε 14800), 279 (18100, sh), 288 (19700), 345 (4400), ^{1}H-NMR (CD_3CN), δ 7.35 ppm (1H, dd, J=2.0, 11.5 Hz), 7.54 (1H, dd, J=7.5, 11.5 Hz), 7.75 (1H, dd, J=2.0, 7.5 Hz), 15.2 (1H, s). 6 is an optically active compound ($[\alpha]_D^{25}$-12.2° (CH_3CN, C=0.09)), which will be described elsewhere.

10) J. Straiting, L. Thijs and B. Zwaneburg, Tetrahedron Lett., 65 (1966).

11) F. Pietra, Chem. Rev., 73, 293 (1973).

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