Thiocoraline, a Novel Depsipeptide with Antitumor Activity Produced by a Marine *Micromonospora*

II. Physico-chemical Properties and Structure Determination

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Thiocoraline (**1**) is a new antitumor antibiotic isolated from the mycelium of *Micromonospora* sp. L-13-ACM2-092. Its structure was elucidated to be a novel cyclic thiodepsipeptide on the basis of spectroscopic methods.

In the course of screening for new antitumor compounds, thiocoraline (**1**) was isolated from the mycelium of *Micromonospora* sp. L-13-ACM2-092 (Fig. 1) by bioassay-guided fractionation. The taxonomy, fermentation, isolation and biological activities are the subject of a preceeding paper[1]. We will report herein the physico-chemical properties and structural elucidation of thiocoraline.

Structural Elucidation of Thiocoraline

Thiocoraline (**1**) was obtained as a pale yellow crystalline powder. The physico-chemical properties of **1** are summarized in Table 1. It is soluble in ethyl acetate, chloroform, dichloromethane and dimethyl sulfoxide, slightly soluble in methanol and insoluble in water.

The molecular formula of thiocoraline **1** was established as $C_{48}H_{56}N_{10}O_{12}S_6$ from the HRFAB-MS [*m/z* 1157.2521 $(M+H)^+$, calcd. 1157.2481] and NMR spectral analyses. The UV spectrum of **1** suggested the existence of a 3-hydroxyquinaldic acid chromophore[2,3] (218, 230, 298 and 360 nm in CH_3OH). The IR spectrum of **1** showed the presence of amide group (1650 and 1515 cm^{-1}).

The ^1H and ^{13}C NMR spectral data of **1** are shown in Table 2. Since the molecular formula has twice the

Fig. 1. Structure of thiocoraline.

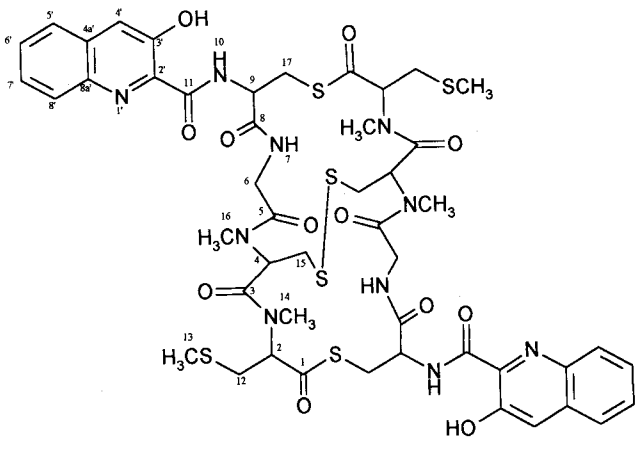

Table 1. Physico-chemical properties of thiocoraline.

Appearance	Pale yellow crystalline powder
Molecular formula	$C_{48}H_{56}N_{10}O_{12}S_6$
HRFAB-MS $(M+H)^+$)	1157.2521 (Calcd; 1157.2481)
MP (°C)	266~266.5
$[\alpha]_D^{25}$	−190.9° (*c* 1.1, $CHCl_3$)
UV λ_{max}^{MeOH} nm (ε)	218 (76,500), 230 (76,700), 298 (9,400), 360 (8,900)
IR ν_{max} (KBr) cm^{-1}	3370, 1650, 1515, 1095
TLC[a] Rf value[b]	0.5
Rf value[c]	0.78
HPLC (Rt, minutes)[d]	2.1

[a] Silica gel 60 F_{254}, Merck.
[b] Solvent I: $CHCl_3$-EtOAc-AcOH (5:10:0.15).
[c] Solvent II: $CHCl_3$-MeOH (95:5).
[d] C18 radial pack cartridge (10 μ); mobile phase: MeOH-H_2O-CF_3COOH (90:10:0.1); flow rate: 2 ml/minute; detection: 360 nm.

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Table 2. Heteronuclear NMR spectral data for thiocoraline in $CDCl_3$.

Position C/H No.	^1H shifts[a]	^{13}C shifts[a,c]	Long range coupled
1		199.6 s	2.85, 3.50, 3.70, 5.80
2	5.80 m, 1H (J=4.3, 11.7)[b]	60.8 d	2.85, 3.05, 3.22
3		170.0 s	2.85, 3.05, 3.50, 5.80, 6.43
4	6.43 m, 1H (J=5.6, 9.0)	56.3 d	2.85, 3.00, 3.50
5		168.2 s	3.00, 3.70, 4.56, 6.43, 6.82
6	3.70 m, 1H (J=2.9, 16.6)	40.3 t	
	4.56 m, 1H (J=7.5, 16.8)		
7-NH	6.82 m, 1H (J=3.1, 7.3)		
8		169.2 s	3.50, 3.70, 4.56, 6.82
9	4.89 m, 1H (J=4.9, 8.8)	53.9 d	3.50, 3.70, 8.80
10-NH	8.80 d, 1H (J=6.1)		
11		169.2 s	4.89, 8.80
12	2.85 m, 1H (J=11.7, 14.4)	32.0 t	2.13, 5.80
	3.22 m, 1H (J=4.3, 14.1)		
13	2.13 s, 3H	15.1 q	2.85, 3.22
14	3.05 s, 3H	30.7 q	
15	2.85 m, 1H (J=5.6, 14.4)	41.2 t	6.43
	3.50 m, 1H (J=9.2, 13.9)		
16	3.00 s, 3H	30.5 q	
17	3.50 m, 1H (J=9.2, 13.9)	30.2 t	8.80
	3.70 m, 1H (J=4.9, 14.1)		
2′		133.6 s	7.58, 8.80
3′		153.5 s	7.58
3′-OH	11.26 s, 1H		
4′	7.58 s, 1H	120.8 d	7.73
4a′		132.1 s	7.48, 7.73
5′	7.73 m, 1H	128.9 d	
6′	7.48 m, 1H (J=5.8)	127.7 d	
7′	7.48 m, 1H (J=5.8)	128.7 d	
8′	7.66 m, 1H, (J=4.3, 5.1)	126.6 d	
8a′		141.2 s	7.48, 7.58, 7.66

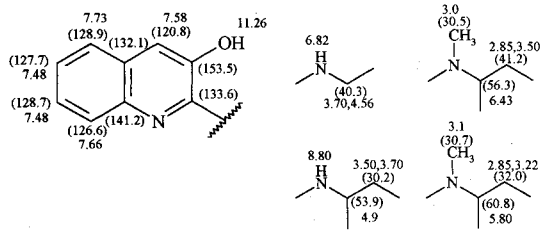
[a] Chemical shifts (δ value in ppm).
[b] Coupling constants in J=Hz.
[c] s: singlet, d: doublet, t: triplet, q: quartet.

number of the carbon and proton atoms observed in the NMR spectra, thiocoraline had to be symmetrical dimer. The ^{13}C NMR spectrum and the DEPT experiment revealed the presence of twenty-four carbons as five carbonyl carbons, four quaternary carbons, eight methine carbons, four methylene and three methyl carbons signals. In addition, the ^1H NMR spectrum, ^1H-^1H COSY[4] and ^1H-^{13}C HMQC[5] spectra established the partial structures shown in Fig. 2.

The sp^2 hybridized carbons (C-2′~C-8a′) and the related five aromatic protons (δ 7.48, δ 7.48, δ 7.58, δ 7.66, δ 7.73) with a proton of –OH (δ 11.26) revealed the presence of 3-hydroxyquinaldic acid[3,6] as an aromatic chromophore.

The N-methyl carbons (C-14 and C-16) showed connectivities to methine protons 2-H and 4-H respectively which are part of isolated methine-methylene (–CH–CH_2–) spin systems. The ^1H shifts (δ 2.85 and δ 3.50) of the N-methylcysteine β-protons are characteristic of

Fig. 2. Partial structures and chemical shifts δ_H (δ_C) in $CDCl_3$ of thiocoraline.



a methylene joined to a sulfur which are part of a disulfide cross-link proposed to attach the two chemically equivalent halves on the basis of analogy to Triostin A[7]. The 7-NH proton was coupled to a methylene carbon (C-6, δ 40.3) which did not show any other correlation while the 10-NH proton was linked to a methine-methylene group.

Fig. 3. ^{1}H-^{13}C long range coupling of thiocoraline chromophore, 3-hydroxyquinaldate, as detected by HMBC.

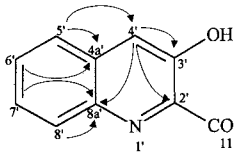
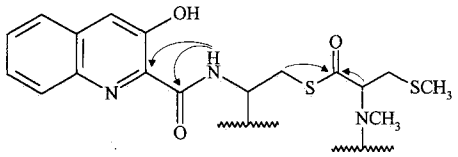


Fig. 4. Chromophore attachment and dimerization site.



The position of each partial structure and the full assignments of ^{13}C and ^{1}H NMR spectra were deduced from the ^{1}H-detected multiple-bond heteronuclear multiple quantum coherence (HMBC)[8] spectrum, as summarized in Fig. 3~6.

The long range couplings from 7′-H (δ 7.48) to C-8a′ (δ 141.2), from 6′-H (δ 7.48) to C-4a′ (δ 132.1), from 8′-H (δ 7.66) to C-8a′, from 5′-H (δ 7.73) to C-4a′ and C-4′ (δ 120.8) and from 4′-H (δ 7.58) to C-3′ (δ 153.5), C-2′ (δ 133.6) and C-8a′ supported the structure of the aromatic chromophore (Fig. 3). The next couplings from 10-NH (δ 8.80) to C-11 (δ 169.2) and C-2′ (δ 133.6) and from the cysteine β-protons (17-H at δ 3.50 and δ 3.70) and the *N*-*S*-dimethylcysteine α-proton (2-H at δ 5.80) to the carbonyl C-1 (δ 199.6) proved the attachment of the aromatic chromophore and the sites of dimerization (Fig. 4).

The amino acids, cysteine, glycine, *N*-methylcysteine and *N*-*S*-dimethylcysteine were readily identified by their respective spin systems as shown in Fig. 5. Next, it was necessary to establish unequivocally the amino acid sequence (Fig. 6). The amino acid sequence was determined by the following long range couplings from the *N*-*S*-dimethylcysteine α-proton (2-H, δ 5.80) and *N*-methyl protons (14-H, δ 3.05) to the *N*-methyl-cysteine carbonyl (C-3) at δ 170.0, establishing the connection between these two amino acids. The attachment of *N*-methylcysteine to glycine was determined by correlations between the α-proton (4-H, δ 6.43) and *N*-methyl protons (16-H, δ 3.0) of *N*-methylcysteine and between the α-proton (4-H) and the glycine carbonyl (C-5, δ 168.2). Furthermore, the glycine geminal protons (6-H, δ 3.70 and 4.56) and the glycine amide proton (7-H, δ 6.82) were coupled to the cysteine carbonyl (C-8). Finally the cysteine α-protons (9-H, δ 4.89) was correlated with the carbonyl (C-11) of aromatic chromophore.

Fig. 5. ^{1}H-^{13}C long range couplings detected in the amino acids of thiocoraline by HMBC.

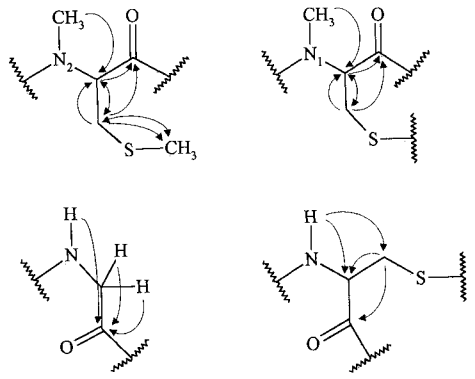
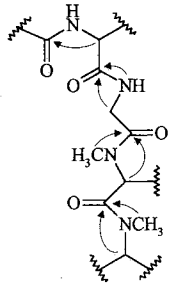


Fig. 6. Amino acid sequence.



Experimental

General Procedures

Optical rotation was measured with an Optical Activity AA-10 polarimeter.

Melting point was determined with a Mettler FP 81 mp apparatus and was uncorrected.

IR and UV spectra were recorded on a Perkin Elmer 881 spectrophotometer and a Perkin Elmer Lambda 15 double beam spectrometer, respectively.

NMR spectra were acquired on a Varian Unity 500 NMR spectrometer (500 MHz for ^{1}H, 125 MHz for ^{13}C). Chemical shifts are reported in ppm referenced to the $CHCl_3$ peak at 7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C. The HMBC and HMQC experiments were carried out using an inverse resonance probe.

FAB-MS spectra were carried out with VG ZAB-SE (LRFAB), 70-VSE (HRFAB) and 70-SE-4F (MS/MS FAB) spectrometers.

Acknowledgments

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References

1) ROMERO, F.; F. ESPLIEGO, J. P. BAZ, T. G. DE QUESADA, D. GRÁVALOS, F. DE LA CALLE & J. L. FERNÁNDEZ-PUENTES: Thiocoraline, a new depsipeptide with antitumor activity produced by a marine *Micromonospora*. I. Taxonomy, fermentation, isolation and biological activities. J. Antibiotics 50: 734~737, 1997
2) MATSON, J. A. & J. A. BUSH: Sandramycin, a novel antitumor antibiotic produced by a *Nocardioides* sp. Production, isolation, characterization and biological properties. J. Antibiotics 42: 1763~1767, 1989
3) KONISHI, M.; H. OHKUMA, F. SAKAI, T. TSUNO, H. KOSHIYAMA, T. NAITO & H. KAWAGUCHI: BBM-928, a new antitumor antibiotic complex. III. Structure determination of BBM-928 A, B and C. J. Antibiotics 34: 148~159, 1981
4) BAX, A. & R. FREEMAN: Investigation of complex networks of spin-spin coupling by two-dimensional NMR. J. Magn. Reson. 44: 542~561, 1981
5) BAX, A. & S. SUBRAMANIAN: Sensitivity-enhanced two-dimensional heteronuclear shift correlation NMR spectroscopy. J. Magn. Reson. 67: 565~569, 1986
6) MATSON, J. A.; K. L. COLSON, G. N. BELOFSKY & B. B. BLEIBERG: Sandramycin, a novel antitumor antibiotic produced by a *Nocardioides* sp. II. Structure determination. J. Antibiotics 46: 162~166, 1993
7) OTSUKA, H. & J. SHOJI: Structure confirmation of triostin A by ^{1}H and ^{13}C magnetic resonance. J. Antibiotics 29: 107~110, 1976
8) BAX, A. & M. F. SUMMERS: ^{1}H and ^{13}C assignments from sensitivity enhanced detection of heteronuclear multiple-bond connectivity by 2D multiple quantum NMR. J. Am. Chem. Soc. 108: 2093~2094, 1986