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New Dithiolopyrrolone Antibiotics from *Saccharothrix* sp. SA 233

II. Physicochemical Properties and Structure Elucidation

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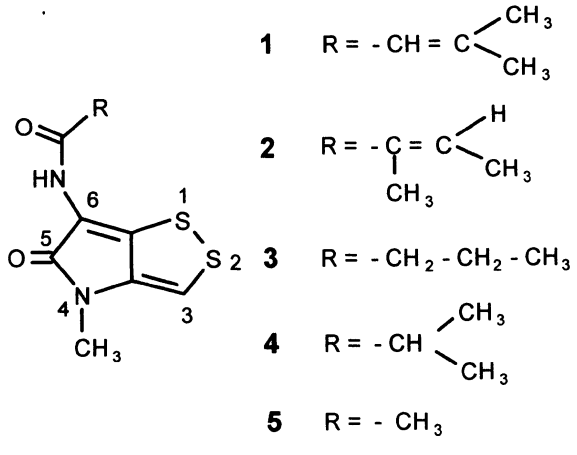
Three new natural dithiopyrrolone antibiotics, 3-methyl-2-butenoylpyrrothine (**1**), tigloylpyrrothine (**2**), and *n*-butyropyrrothine (**3**) were isolated along with the known *iso*-butyropyrrothine (**4**) and thiolutin (**5**) from the fermentation broth of *Saccharothrix* sp. SA 233. The structures of the novel compounds were established on the basis on their spectral data.

During the course of a screening for new antibiotic agents from rare microorganisms present in the soil of the palm groves of Southern Algeria[1,2], several antibiotics (**1**~**5**) were obtained from the fermentation broth of *Saccharothrix* sp. SA 233. The taxonomy of the producing strain, fermentation, isolation, and biological activities of compounds **1**~**5** are described in the preceding paper[3]. We report here the physico-chemical properties and the structural elucidation of novel natural compounds **1**~**3**, together with the identification of **4** and **5**.

Fig. 1. Dithiolopyrrolone antibiotics from *Saccharothrix* sp. SA 233.



Results and Discussion

Physico-chemical properties of novel compounds **1**~**3** are summarized in Table 1. The antibiotics **1**~**5** were obtained as bright yellow to orange yellow amorphous powders. Spectral features common to the five products included: (i) typical IR absorption bands at 3300~3100,

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Table 1. Physico-chemical properties of compounds **1**~**3**.

	1	**2**	**3**
Appearance	Yellow orange powder	Yellow orange powder	Yellow powder
Molecular formula	$C_{11}H_{12}N_2O_2S_2$	$C_{11}H_{12}N_2O_2S_2$	$C_{10}H_{12}N_2O_2S_2$
Molecular weight	268	268	256
EI-MS (*m/z*)	268 (M^+), 186, 83, 55	268 (M^+), 186, 83, 55	256 (M^+), 186, 43
HR-MS			
Found:	268.03430	268.03400	256.03417
Calcd:	268.03402	268.03402	256.03402
UV λ_{max} nm (log ε) in MeOH	302 (3.87), 402 (3.97)	302 (3.85), 402 (3.96)	308 (3.70), 389 (3.92)
IR ν_{max} in KBr (cm^{-1})	3270, 1680, 1655, 1635, 1600, 1520, 1225	3220, 1670, 1650, 1600, 1500, 1210	3280, 1680, 1650, 1600, 1540, 1225
Solubility			
Soluble	MeOH, CH_2Cl_2, $CHCl_3$, DMSO	MeOH, CH_2Cl_2, $CHCl_3$, DMSO	MeOH, CH_2Cl_2, $CHCl_3$, DMSO
Slightly soluble	Me_2CO, H_2O, EtOAc, CH_3CN	Me_2CO, H_2O, EtOAc, CH_3CN	Me_2CO, H_2O, EtOAc, CH_3CN
Insoluble	*n*-hexane	*n*-hexane	*n*-hexane
Color reaction			
Positive	Bromocresol green/ bromophenol blue/ $KMNO_4$ reagent	Bromocresol green/ bromophenol blue/ $KMNO_4$ reagent	Bromocresol green/ bromophenol blue/ $KMNO_4$ reagent
Negative	Ninhydrine, $FeCl_3$, Anisaldehyde-H_2SO_4, Millon, Tollens-Zaffaroni reagents	Ninhydrine, $FeCl_3$, Anisaldehyde-H_2SO_4, Millon, Tollens-Zaffaroni reagents	Ninhydrine, $FeCl_3$, Anisaldehyde-H_2SO_4, Millon, Tollens-Zaffaroni reagents
TLC (Rf value)[a]			
(I)	0.59	0.59	0.59
(II)	0.63	0.63	0.63
(III)	0.65	0.65	0.65
HPLC (Rt)[b]	11.0 min	10.0 min	7.1 min

[a]Silica gel TLC (Merck No 5715). (I): EtOAc- MeOH (100:15). (II): *n*-BuOH-CH_3COOH-H_2O (3:1:1).

(III): EtOH-H_2O (1:2).

[b]HPLC conditions: Uptishere C_{18} (300x7.8 mm, i.d.), Mobile phase: MeOH-H_2O (50:50), Flow rate: 2 ml/min, Detection: UV-220 nm.

~1670, and ~1650 cm^{-1}, accounting for two different amide groups, (ii) strong UV absorptions at 300~310 and 385~405 nm, (iii) the presence of a prominent fragment ion at *m/z* 186 corresponding to the empirical formula $C_6H_6N_2OS_2$ in EI-MS, (iv) the appearance on the ^{1}H-NMR spectra (Table 2) of two singlets at 7.30~6.60 and 3.40~3.20 ppm typical for one isolated olefinic proton and one N–CH_3 group included in a amide function,

Table 2. ^{1}H (300 MHz) NMR data of compounds **1**~**4** in comparison with thiolutin (**5**) (δ [ppm], multiplicity, J [Hz]).

Position	**1** ($CDCl_3$)	**2** ($CDCl_3$)	**3** ($CDCl_3$)	**4** ($CDCl_3$)	**5** (DMSO-d_6)
H-3	6.62 (1H, s)	6.63 (1H, s)	6.65 (1H, s)	6.64 (1H, s)	7.30 (1H, s)
N(4)-CH_3	3.37 (3H s)	3.37 (3H s)	3.37 (3H s)	3.37 (3H s)	3.20 (3H s)
C(6)-NH	7.43 (1H, br. s)	7.69 (1H, br. s)	7.45 (1H, br. s)	7.46 (1H, br. s)	9.95 (1H, br. s)
Amido moiety	5.71 (1H, m)	6.65 (1H, m)	2.33 (2H, t, 7)	2.53 (1H, spt, 7)	2.50 (3H, s)
	2.25 (3H, d, 1)	1.92 (3H, br. s)	1.74 (2H, sxt, 7)	1.23 (6H, d, 7)	
	1.90 (3H, d, 1)	1.83(3H, d, 8)	1.00 (3H, t, 7)		

Table 3. ^{13}C (75 MHz) NMR data of compounds **1**~**3** and **5** (δ [ppm], multiplicity).

Position	**1** ($CDCl_3$)	**2** ($CDCl_3$)	**3** ($CDCl_3$)	**5** (DMSO-d_6)
3	108.5 (d)	108.9 (d)	108.8 (d)	112.1 (d)
3a	131.9 (s)	132.5 (s)	132.2 (s)	133.6 (s)
N(4)-CH_3	29.7 (q)	29.7 (q)	29.7 (q)	27.8 (q)
5	167.0 (s)	167.1 (s)	167.8 (s)	167.3 (s)
6	114.7 (s)	114.9 (s)	115.2 (s)	115.9 (s)
6a	136.9 (s)	136.2 (s)	136.4 (s)	137.1 (s)
Amido moiety	164.3 (s)	165.7 (s)	170.8 (s)	170.0 (s)
	155.7 (s)	133.7 (d)	38.2 (t)	23.5 (q)
	116.8 (d)	129.0 (s)	19.4 (t)	
	27.5 (q)	14.1 (q)	13.6 (q)	
	20.3 (q)	11.8 (q)		

respectively. These data unambiguously characterized compounds **1**~**5** as *N*-acyl derivatives of 6-amino-4-methyl-1,2-dithiolo[4,3-*b*]pyrrol-5[4*H*]-one[4~8], differing only from each other by the amido moiety.

The molecular formula of 3-methyl-2-butenoylpyrrothine (**1**) was established as $C_{11}H_{12}N_2O_2S_2$ by HR-MS [found *m/z* 268.03430 (M^+), calcd. 268.03402]. Observation of a 1H olefinic multiplet at δ 5.71 ppm coupled with two 3H methyl doublets (J=1 Hz) at δ 2.25 and 1.90 ppm in the ^{1}H-NMR spectrum was consistent with the presence of a 3-methyl-2-butenoyl side chain. The ^{13}C-NMR spectrum (Table 3) confirmed the presence of 11 carbons in **1**. It also provided confirmation of the structure of the amido moiety, with five typical signals observed at δ 20.3 (q), 27.5 (q), 116.8 (d), 155.7 (s), and 164.3 (s) ppm, the latter characterizing a conjugated amide carbonyl group. The six other ^{13}C-NMR resonances were assigned to the 4-methyl-1,2-dithiolo[4,3-*b*]pyrrol-5[4*H*]-one basic core.

The empirical formula of tigloylpyrrothine (**2**) was also established as $C_{11}H_{12}N_2O_2S_2$ by HR-MS [found *m/z* 268.03400 (M^+), calcd. 268.03402]. The UV and MS spectra of **2** were merely identical to those of 3-methyl-2-butenoylpyrrothine (**1**). Nevertheless significant differences were noticed in the ^{1}H-NMR spectrum, where the characteristic signals of a 2-methyl-2-butenoyl side chain appeared as a 3H doublet (J=8 Hz) at δ 1.83 ppm, a 3H broad singlet at δ 1.92 ppm, and a 1H multiplet at δ 6.65 ppm. The strongly deshielded position of this latter signal gave evidence for the (*E*) configuration of the amido moiety. In agreement with this statement, signals associated with a tigloyl unit were observed at δ 11.8 (q), 14.1 (q), 129.0 (s), 133.7 (d), and 165.7 (s) ppm in the ^{13}C-NMR spectrum, together with the six signals of the methyldithiolopyrrolone skeleton.

n-Butyropyrrothine (**3**) had a molecular formula of $C_{10}H_{12}N_2O_2S_2$, determined by HR-MS [found *m/z* 256.03417 (M^+), calcd. 256.03402]. The ^{1}H-NMR spectrum displayed a 2H triplet (J=7 Hz) at δ 2.33 ppm, a 2H sextet (J=7 Hz) at δ 1.74 ppm, and a 3H triplet (J=7 Hz) at δ 1.00 ppm accounting for a *n*-butyryl amide side chain, whose resonances appeared at δ 13.6 (q), 19.4 (t), 38.2 (t), and 170.8 (s) ppm in ^{13}C-NMR. It should be mentioned that *n*-butyropyrrothine (**3**) was previously synthesized by condensation of butyric anhydride with 6-amino-4-methyl-1,2-dithiolo[4,3-*b*]pyrrol-5[4*H*]-one obtained by hydrolysis of thiolutin (**5**)[7]. This compound is obtained here for the first time from a natural source.

Finally, the two further antibiotics isolated from *Saccharothrix* sp. SA 233 were identified as *iso*-butyropyrrothine (**4**), previously obtained from *Streptomyces pimprina* broth[9], and thiolutin (**5**) produced by various *Streptomyces* strains[6~11]. The ^{1}H-NMR data of **4** and the ^{13}C-NMR data of **5**, which were not previously described, are summarized in Tables 2 and 3, respectively.

Experimental

General Experimental Procedures

IR spectra (ν_{max} in cm^{-1}) were obtained on a Nicolet 510 FT-IR instrument. UV spectra (λ_{max} in nm) were determined in spectroscopic grade MeOH on a Beckman DU 640B spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 300 MHz and 75 MHz respectively, using a Bruker AC-300 spectrometer. When necessary, the signals were unambiguously assigned by 2D NMR techniques: ^{1}H-^{1}H COSY, ^{13}C-^{1}H HETCOR, and ^{13}C-^{1}H COLOC. These experiments were performed using standard Bruker microprograms. Electron impact mass spectra were recorded at 70 eV with a Nermag R-10-10C spectrometer. High resolution mass spectra were obtained on a Micromass ZAB2-SEQ spectrometer.

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