STRUCTURE OF A NEW ANTIBACTERIAL ANTIBIOTIC, THIOTETROMYCIN

Sir:

In the course of screening for new substances, a new antibacterial antibiotic, thiotetromycin (**1**)[1], mp 92°C, $[\alpha]_D^{25}$ +124° (*c* 1.0, MeOH), $C_{13}H_{18}O_2S$ (M^+, *m/z* 238.102), was found in the culture broth of *Streptomyces* sp. strain OM-674.

We now report structural analysis of **1** by means of NMR spectroscopy. The following data suggested that **1** possesses two chromophore moieties, a conjugated diene and an α,β-unsaturated thiolactone: UV λ_{max}^{EtOH} 238 (ε 30,100) and 300 nm (4,700) and IR $\nu_{max}^{CHCl_3}$ 1620 cm^{-1} (R–S–C(=O)–C=C). The ^{13}C NMR spectrum of **1** suggested the presence of a carbonyl carbon (δ 198.3), an oxygenated olefinic carbon (δ 179.2), five olefinic carbons (δ 141.3, 140.4, 129.4, 118.1 and 113.8), a quaternary carbon (δ 60.8), two methylenes (δ 33.3 and 16.1) and three methyls (δ 12.5, 12.3 and 8.5). The presence of an enol in **1** was confirmed from the IR absorption at 1780 cm^{-1} in the monoacetate **2**, which was obtained by acetylation of **1** with Ac_2O/pyridine. The disappearance of a broad signal at δ 7.5 in the ^1H NMR spectrum of **1** on addition of D_2O also confirmed the presence of an enol. The ^1H NMR spectrum indicated the presence of one methyl (δ 1.75) and one ethyl group (δ 1.03, 3H, t, J= 7.5 Hz and δ 2.30, 2H, q, J=7.5 Hz) attached to double bond, one ethyl group (δ 0.96, 3H, t, J= 7.2 Hz and δ 2.10, 2H, q, J=7.2 Hz) attached to a quaternary carbon, four olefinic protons (δ 5.10, 5.30, 5.60 and 6.30) and one enolic proton. The detailed proton spin decoupling experiment of **1** revealed the presence of a butadienyl moiety **[I]** containing a methyl group. The validity of the partial structure **[I]** was also confirmed from the $^{13}C\{^1H\}$ long range selective proton decoupling (LSPD) experiment of **1**. The methyl group of C-13 in **[I]** should be located at C-6 from the observation that irradiation of the methyl proton (δ 1.75) collapsed each of the two olefinic carbons at C-5 (δ 129.4, broad singlet) and at C-7 (δ 141.3, multiplet), into a clear doublet ($^3J_{CH}$=4.3 Hz with H-7 for C-5 and $^3J_{CH}$=9.3 Hz with H-5 for C-7, respectively), since the values of ^2J (C-7, H-8) and ^2J (C-5, H-11) are negligibly small. Furthermore, the configuration of the conjugated diene moiety was found to be identical with that of thiolactomycin[2,3], with ethyl groups being replaced by methyl groups in the latter case, from comparison of both NMR spectral data.

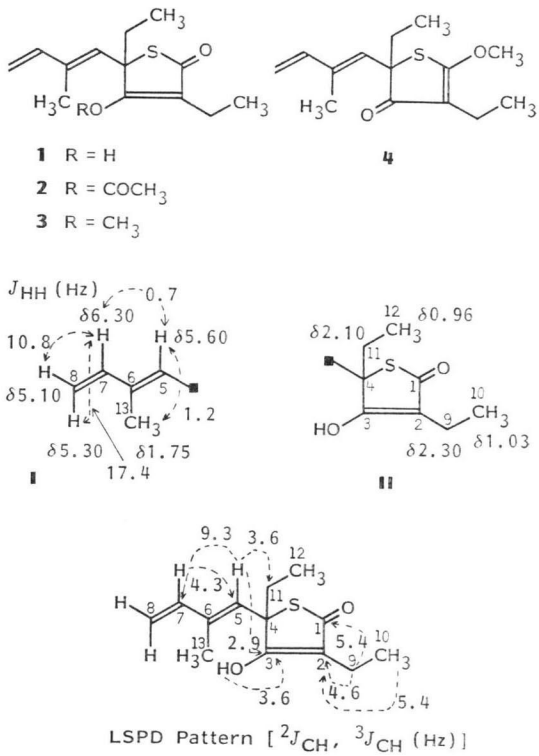

Table 1. ^{13}C NMR data in $CDCl_3$.

Carbon No.	Chemical shift (ppm)/TMS		
	1	**3**	**4**
1	198.3 (s)*	194.0	185.8
2	118.1 (s)	120.0	114.7
3	179.2 (s)	178.2	201.5
4	60.8 (s)	60.2	67.7
5	129.4 (d)	131.0	131.5
6	140.4 (s)	139.1	139.7
7	141.3 (d)	141.5	141.4
8	113.8 (t)	113.5	113.5
9	33.3 (t)	33.6	34.0
10	12.5 (q)	12.4	13.6
11	16.1 (t)	17.4	16.6
12	8.5 (q)	8.6	8.8
13	12.3 (q)	14.4	12.8
3-OCH_3		59.6	
1-OCH_3			59.0

* Multiplicity: s; singlet, d; doublet, t; triplet, q; quartet.

The existence of two ethyl groups, a quaternary carbon and a carbonyl and enolic carbons in the remaining portion $C_5H_{11}O_2S$ and the following LSPD experiment of **1** led us to the 5-membered α,β-unsaturated thiolactone ring [II] as another chromophore. Upon irradiation of the methylenic proton (δ 2.30) at C-9, the carbonyl carbon (δ 198.3, $^3J_{CH}$=5.4 Hz) and the olefinic carbon (δ 118.1, $^2J_{CH}$=4.6 Hz and $^3J_{CH}$=5.4 Hz) collapsed to a singlet and a quartet, respectively. This suggested that one of two ethyl groups must be located at the α-position to the thioester carbonyl. On the other hand, upon irradiation of the olefinic proton at δ 5.60, the methylenic carbon (δ 16.1, broad doublet, $^3J_{CH}$=3.6 Hz) at C-11 and the broad carbon signal (δ 179.2) at C-3 collapsed to a broad singlet and a broad doublet ($^3J_{CH}$=3.6 Hz) coupled with a hydroxyl proton, respectively. Upon the same irradiation under the presence of D_2O, the signal at C-3 appears as a broad triplet coupled with the methylenic protons at C-9 and C-11. This spectral evidence means that the terminal carbon at C-5 in the butadienyl moiety, an ethyl group and an enolic group must be attached to the same quaternary carbon, which is bonded also to a sulfur atom. Further, the observation of a fermentation ion peak at *m/z* 140 ($C_8H_{12}S$) in the mass spectrum of **1** afforded the structural evidence for CH_2=CH-C(CH_3)=CH-C(CH_2-CH_3)-S-. Thus, we can propose the most suitable structure **1** for thiotetromycin. The validity of the structure as a 5-membered thiolactone was also supported by the spectroscopic characterization of two monomethyl ethers, **3** $[\alpha]_D^{27}$ +63.5° (*c* 1.0, $CHCl_3$); UV λ_{max}^{EtOH} 238 nm (ε 16,600); IR $\nu_{max}^{CCl_4}$ 1620 cm^{-1} and ^{13}C NMR δ 194.0 (thioester carbonyl), and **4**: $[\alpha]_D^{27}$ +194.4° (*c* 1.0, $CHCl_3$); UV λ_{max}^{EtOH} 235 (ε 16,100) and 313 nm (6,200); IR $\nu_{max}^{CCl_4}$ 1580 cm^{-1} and ^{13}C NMR δ 201.5 (α,β-unsaturated ketone carbonyl), obtained by treatment of **1** with diazomethane. These spectral data demonstrated that **4** is an tautomeric isomer of **3**. The synthesis of **1** and its related compounds are now in progress.

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References

1) ŌMURA, S.; Y. IWAI, A. NAKAGAWA, R. IWATA, Y. TAKAHASHI, H. SHIMIZU & H. TANAKA: Thiotetromycin, a new antibiotic. Taxonomy, production, isolation, and physicochemical and biological properties. J. Antibiotics 36: 109~114, 1983
2) OISHI, H.; T. NOTO, H. SASAKI, K. SUZUKI, T. HAYASHI, H. OKAZAKI, K. ANDO & M. SAWADA: Thiolactomycin, a new antibiotic. I. Taxonomy of the producing organism, fermentation and biological properties. J. Antibiotics 35: 391~395, 1982
3) SASAKI, H.; H. OISHI, T. HAYASHI, I. MATSUURA, K. ANDO & M. SAWADA: Thiolactomycin, a new antibiotic. II. Structure elucidation. J. Antibiotics 35: 396~400, 1982