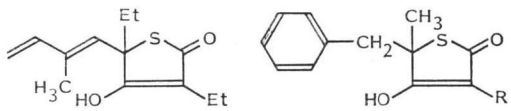
SYNTHESES AND BIOLOGICAL ACTIVITIES OF THIOTETROMYCIN ANALOGS

Sir:

As reported in the previous paper,[1] thiotetromycin (**1**) produced by *Streptomyces* sp. OM-674 contains a unique thiolactonic structure and shows selective activity against *Bacteroides fragilis* as well as inhibiting the proliferative response of T-cells stimulated with concanavalin A. In the course of structure-activity studies for the thiolactonic antibiotics,[1~8] we have synthesized thiotetromycin analogs which possess a methyl and a benzyl group at C-4 of thiolactone. This paper describes the convergent synthesis of thiolactones, **2**~**5** and their biological activities.

Ethyl 2-mercaptopropionate, possessing a methyl group to provide an alkyl residue at C-4 of the thiolactone, was chosen as starting material. The thiol group was protected as a thioether by treatment with β-methoxyethoxymethyl (MEM) chloride and triethylamine at room temperature (86% yield). Metalation of **6** with lithium diisopropylamide (LDA) in tetrahydrofuran at −78°C followed by alkylation with benzyl chloride gave **7** in 94% yield. Ester (**7**) was hydrolyzed with sodium hydroxide in ethanol and water at 50°C to give the corresponding acid, which was immediately treated with thionyl chloride at reflux to afford a 92% yield of the acid chloride (**8**). Reactions of **8** with sodio anions of methyl malonate and methyl acetoacetate afforded undesirable acylation products of the corresponding enols. The lithio anions of ethyl acetate, ethyl phenylacetate, and methyl 1-butyrate generated, instead by treatment with LDA in tetrahydrofuran at −78°C, gave rise to *C*-acylation products as 1:1 mixtures of diastereoisomers (except for **9b**) in 55~72% yield. The SMEM group was cleaved by using mercuric acetate in aqueous acetic acid followed by hydrogen sulfide (61~80% yield). Hydrolysis of the

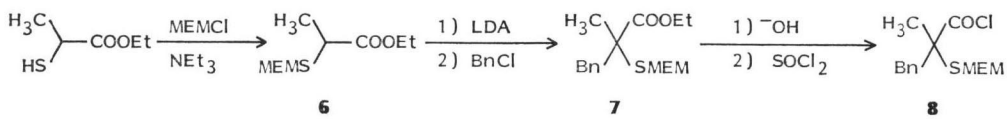
Chart 1.



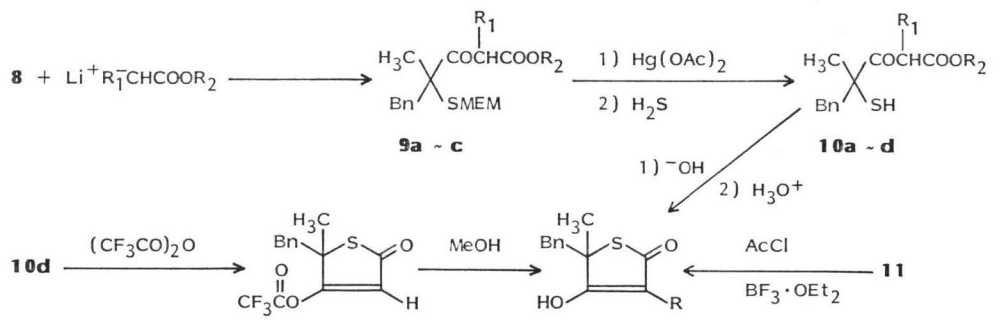
Thiotetromycin (**1**)

2 R = Et
3 R = H
4 R = Ph
5 R = $COCH_3$

Chart 2.



6 **7** **8**



9a ~ c **10a ~ d** **11**

a R_1 = Et R_2 = Me
b R_1 = H R_2 = Et
c R_1 = Ph R_2 = Et
d R_1 = R_2 = H

Bn = $PhCH_2$−

2 R = Et
3 R = H
4 R = Ph
5 R = $COCH_3$

Table 1. Physicochemical properties of thiolactones (**2**~**5**).

Compounds[a]	Mp (°C)[b]	IR (cm^{-1})[c]	NMR (δ, ppm)[e]
2	107~108	1610	0.85 (3H, t, J=7 Hz) 1.70 (3H, s) 2.14 (2H, q, J=7 Hz) 3.16 (2H, s) 7.18 (5H, s)
3	119~120	1610	1.68 (3H, s) 3.18 (2H, s) 5.16 (1H, s) 7.26 (5H, s)
4	128~129	1600	1.78 (3H, s) 3.25 (2H, s) 7.29 (5H, s) 7.1~7.5 (5H, m)
5	163~164	1580[d]	1.59 (3H, s)[f] 2.29 (3H, s) 3.20 (2H, s) 7.11 (5H, s)

[a] Elemental analysis of all compounds are in good agreement with the calculated value.
[b] Melting points are uncorrected.
[c] IR spectra were measured in $CHCl_3$ solution or KBr[d].
[e] Chemical shifts are reported in parts per million relative to internal Me_4Si shift=0 in $CDCl_3$ or $(CD_3)_2CO$[f] solution.

resulting thiols (**10a, c**) with sodium hydroxide, and then acidic workup gave the cyclized thiolactones (**2, 4**) in 80~85% yield. Thiolactonization of the mercapto acid (**10d**) was surprisingly difficult. Standard methods such as simple acid catalysis served only to destroy the substrate, however, the trifluoroacetic anhydride method did work to give rise to the enol trifluoroacetate (**11**) of the thiolactone, which could be removed easily by methanolysis at room temperature to yield a 82% yield of thiolactone (**3**). Introduction of the acyl group at C-2 of the thiolactone was performed by Lewis-acid catalyzed acylation. Treatment of **11** with acetyl chloride in the presence of BF_3-etherate at 50°C followed by methanolysis at room temperature afforded a 46% yield of **5**. The physicochemical properties of the thiolactones are summarized in Table 1.

Inhibitory activities of the synthesized thiolactones against *B. fragilis* and concanavalin A-stimulated T-cells are summarized in Table 2. Most of the thiolactones show weak activities against *B. fragilis*, however, thiolactones (**4**, **5**) possess enhanced inhibitory activities against T-cells. These results suggest that electron-withdrawing groups at C-2 of the thiolactone play an important role in the inhibitory activity against T-cells stimulated with concanavalin A whereas the diene residue of thiotetromycin contributes to the antibiotic activity.

Table 2. Inhibitory activities against *B. fragilis* and concanavalin A stimulated T-cells.

Compounds	MIC (μg/ml)[a]	ID_{50} (μg/ml)[b]
1	6.25	14.5
2	50	155
3	100	34
4	100	9
5	100	9

[a] MIC against *B. fragilis* (GAM-agar, 37°C, 20 hours).
[b] ID_{50} against [^{3}H]thymidine incorporation by concanavalin A-stimulated T-cells.

In order to further clarify the structure-activity relationship, synthetic studies of thiotetromycin analogs designed to functionalize the diene unit are currently in hand.

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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 physico-chemical 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