

THIOLACTOMYCIN, A NEW ANTIBIOTIC

II. STRUCTURE ELUCIDATION

HIROSHI SASAKI, HIDEO OISHI, TOSHIAKI HAYASHI, IKUTOSHI MATSUURA,
KUNIO ANDO and MIKIO SAWADA

Research Laboratories, Chugai Pharmaceutical Co., Ltd.
Takada, Toshima-ku, Tokyo 171, Japan

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Thiolactomycin, a new antibiotic produced by a soil isolate, *Nocardia* sp. No. 2-200, is shown to have the structure (4*S*)-(2*E*,5*E*)-2,4,6-trimethyl-3-hydroxy-2,5,7-octatriene-4-thiolide on the basis of spectroscopic and X-ray crystallographic analyses. This compound containing an unusual thiolactone moiety is reported here as a representative of a new type of antibiotic.

In our preceding paper,¹⁾ we described the taxonomy of the producing organism, and the fermentation, isolation procedures and biological characteristics of a new antibiotic, thiolactomycin. The antibiotic has been detected with the use of the β -lactam antibiotic-sensitive *Pseudomonas aeruginosa* M-57740. The producing organism was isolated from soil samples and has been designated *Nocardia* sp. No. 2-200. It has a wide antibacterial spectrum and is especially effective against *Salmonella*, *Serratia* and *Bacteroides*. Its acute toxicity is weak.

This paper describes the structure of this antibiotic as determined by IR, UV, NMR and mass spectroscopy.

Physico-chemical Properties

Thiolactomycin is a colorless crystalline substance which melts at 120°C. It is soluble in alkaline water, methanol, acetone, ethyl acetate and chloroform, slightly soluble in benzene and neutral water, and insoluble in acidic water and *n*-hexane. Its optical rotation is $[\alpha]_D^{20} +176^\circ$ (*c* 1.0, MeOH). Elemental analysis indicated the following composition:

Calcd. for C ₁₁ H ₁₄ O ₂ S:	C 62.86, H 6.67, S 15.23
Found	C 62.98, H 6.69, S 15.09

Observation of the parent peak at *m/z* 210 in the mass spectrogram and of eleven carbon signals in the ¹³C NMR spectrum supported the proposed formula C₁₁H₁₄O₂S.

Its color reactions are positive with potassium permanganate, iodine and nitroprusside tests and negative with ninhydrin and MOLISH tests. The ultra violet absorption spectrum is shown in Fig. 1 with two absorption peaks, λ_{max} at 238 nm (ϵ 29,800) and 295 nm (ϵ 4,600) in methanol. This antibiotic gave the R_f value of 0.3 (benzene - acetone, 3:1) on a precoated Merck 60F₂₅₄ silica gel plate. The infrared absorption maxima (in KBr) were: 3400 (ν_{OH}),

Fig. 1. UV spectrum of thiolactomycin (in MeOH).

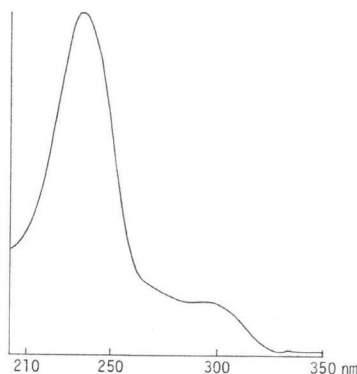


Fig. 2. IR spectrum of thiolactomycin (KBr).

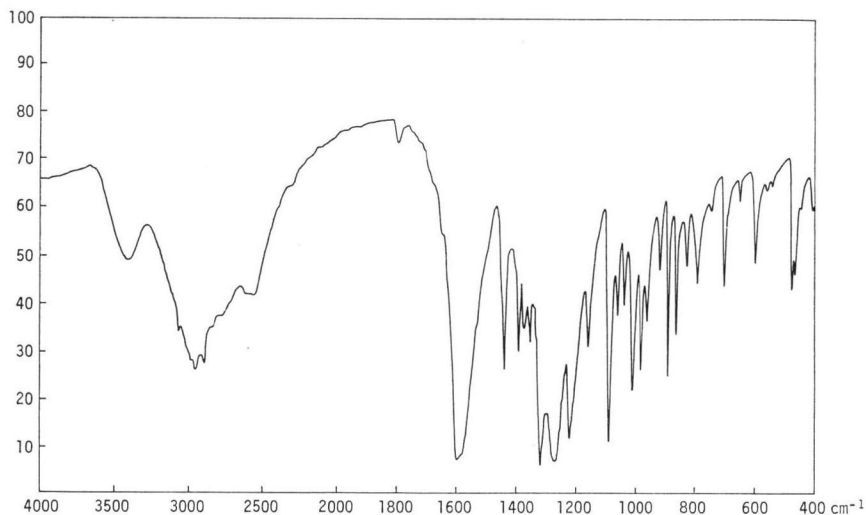
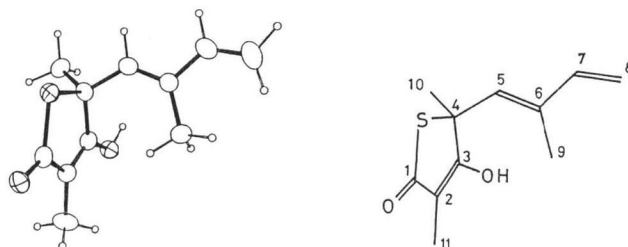


Fig. 3. ORTEP drawing of thiolactomycin molecule (left) and structural formula with numberings of atoms (right).



1605 ($\nu_{C=O}$) and 1090 cm^{-1} (ν_{C-O}) (Fig. 2).

Structure and Spectroscopic Study

The molecular structure of thiolactomycin, determined by X-ray diffraction method,* is shown in Fig. 3. This compound is composed of a five-membered thiolactone ring and a planar zigzag butadienyl chain, and its chemical name is (4*S*)-(2*E*,5*E*)-2,4,6-trimethyl-3-hydroxy-2,5,7-octatriene-4-thiolide.

The proton and C-13 nuclear magnetic resonance spectra of thiolactomycin were measured with a JEOL FX-100 spectrometer in CDCl_3 solution. The PMR of the antibiotic indicated the following characteristic signals as shown in Fig. 4: nine methyl protons at δ 1.73 (3H, s), 1.78 (3H, s) and 1.87 (3H, d, $J=1$ Hz); four olefinic protons at δ 5.07 (1H, d, $J=11$ Hz), 5.21 (1H, d, $J=17$ Hz), 5.62 (1H, s) and 6.30 (1H, d, d, $J=11, 17$ Hz); a proton at δ 8.00 (1H, br.). Irradiation of the signals at δ 5.07 and 5.21 changed the quartet-like signal at δ 6.30 into doublets ($J=17$ Hz and $J=11$ Hz, respectively). The fact that three olefinic protons are coupled with each other indicated the existence of $-\text{CH}=\text{CH}_2$. In addition, the appearance of 4 olefinic protons in low field suggested that the antibiotic had a butadienyl chain. The PMR protons are assigned as summarized in Table 1.

The ^{13}C NMR indicated that the antibiotic had 11 carbon signals composed of 3 methyl signals, 6 olefinic carbon signals, one carbonyl signal and one singlet signal at 55.7 ppm. Among 6 olefinic car-

* The data of X-ray crystallography will be reported elsewhere.

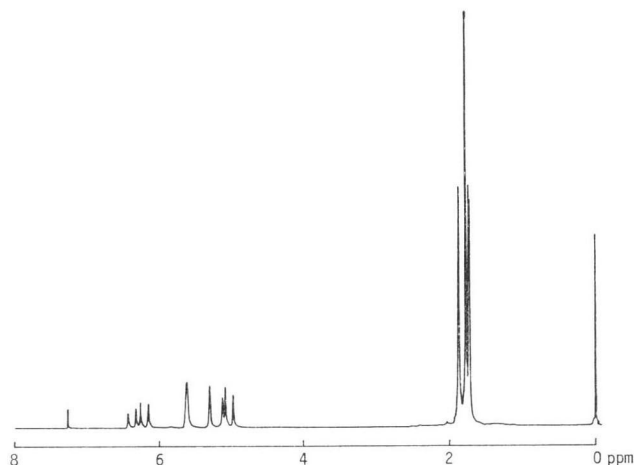
Fig. 4. ^1H NMR spectrum of thiolactomycin (in CDCl_3).

Table 1. PMR spectrum of thiolactomycin.

Chemical shift (ppm)	Number of protons	Shape	Assignment
8.00	1	br	C(3)-OH
6.30	1	d,d	$\begin{array}{c} \text{H} \\ \diagdown \\ \text{C}(8)=\text{C}(7) \\ \diagup \\ \text{H} \end{array} \begin{array}{c} \text{H} \\ \diagup \\ \text{C}(8)=\text{C}(7) \\ \diagdown \\ \text{R} \end{array}$ at C(7), $J_{cis}=11$, $J_{trans}=17$ Hz
5.62	1	s	$\begin{array}{c} \text{H} \\ \diagdown \\ \text{C}(6)=\text{C}(5) \\ \diagup \\ \text{H} \end{array}$ at C(5)
5.21	1	d	$\begin{array}{c} \text{H} \\ \diagdown \\ \text{C}(8)=\text{C}(7) \\ \diagup \\ \text{H} \end{array} \begin{array}{c} \text{H} \\ \diagup \\ \text{C}(8)=\text{C}(7) \\ \diagdown \\ \text{R} \end{array}$ (<i>trans</i>) at C(8), $J=17$ Hz
5.07	1	d	$\begin{array}{c} \text{H} \\ \diagdown \\ \text{C}(8)=\text{C}(7) \\ \diagup \\ \text{H} \end{array} \begin{array}{c} \text{H} \\ \diagup \\ \text{C}(8)=\text{C}(7) \\ \diagdown \\ \text{R} \end{array}$ (<i>cis</i>) at C(8), $J=11$ Hz
1.87	3	s	C(4)-Me
1.78	3	s	C(2)-Me
1.73	3	d	C(6)-Me, $J=1$ Hz

Solvent; CDCl_3 , TMS as internal standard.

Shape; s: singlet, d: doublet, br: broad, d,d: double doublet.

Atomic numberings are in the parentheses.

bons, three carbon signals appeared as singlet, two as doublet and one as triplet. The singlet signal at 55.7 ppm fairly high field and one triplet signal at 113.8 ppm were easily assigned to C4 and C8 carbons, respectively. According to off-resonance studies the triplet carbon should be attached to both the δ 5.21 and the δ 5.07 protons, one of doublet carbons to the δ 6.30 proton and the other to the δ 5.62 proton. The selective proton decoupled ^{13}C NMR spectrum irradiating at δ 5.62 (1H, s) and 6.30 ppm (1H, d, d) revealed conclusively that the signals at 129.3 and 140.7 ppm should be assigned to C5 and C7, respectively. The methyl quartet signals at 7.7, 12.1 and 29.5 ppm were further collapsed to a singlet, a multiplet and a doublet by H-1 Gated decoupling with NOE, and were assigned to C11, C9 and C10, respectively. The two singlet signals at 140.0 and 110.3 ppm were collapsed to a quartet and a multiplet by the same method as the methyl signals, and were identified with C2 and C6. The ^{13}C NMR carbon assignments of thiolactomycin are summarized in Table 2.

Table 2. ^{13}C Chemical shift of thiolactomycin.

Carbon number	Chemical shift	Shape
1	197.7	s
2	140.0	s
3	180.7	s
4	55.7	s
5	129.3	d
6	110.3	s
7	140.7	d
8	113.8	t
9	12.1	q
10	29.5	q
11	7.7	q

Solvent; CDCl_3 , TMS as internal standard.

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

compared with homocysteine thiolactone hydrochloride (1700 cm^{-1}). This result indicated that the antibiotic had a double bond and a hydroxyl function in the 5-membered thiolactone ring, and revealed a strong mesomeric effect. The UV absorption of 238 nm ($\epsilon\ 29,800$) supported the mesomeric effect.

Discussion

Studies of the new antibiotic thiolactomycin by both spectroscopy and X-ray diffractometry have identified it as (4*S*)-(2*E*,5*E*)-2,4,6-trimethyl-3-hydroxy-2,5,7-octatriene-4-thiolide. This compound contains an unusual thiolactone moiety in its molecule. In relation to other natural products, cysteine and homocysteine thiolactones have been known and investigated for thiolation of proteins²⁾ and as anticirrhotic agents.³⁾ However, antimicrobial activity has not been found in thiolactone compounds except thiolactomycin. An antibiotic containing the novel thiolactone moiety is reported for the first time in natural products.

This antibiotic was detected by the use of a β -lactam antibiotic-sensitive mutant but differs from β -lactam antibiotics in containing a unique thiolactone moiety in its molecule. It is interesting that the antibiotic with its 5-membered thiolactone ring structurally resembles penicillin which also has a 5-membered ring containing sulfur.

Biological properties of the antibiotic *in vitro*⁴⁾ and *in vivo*⁵⁾ will be described in detail in following papers.

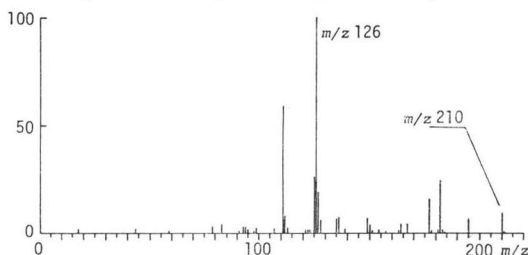
Acknowledgment

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Fig. 5. Mass spectrum of thiolactomycin.



The spectroscopic data supported the structure. The base peak at $m/z\ 126$ in the mass spectrogram was formed by fission of the S-CO and C3-C4 bonds in the thiolactone ring (Fig. 5). In the IR spectrum the carbonyl absorption at 1605 cm^{-1} of the antibiotic was extremely low

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