## Lactonamycin, a New Antimicrobial Antibiotic Produced by *Streptomyces rishiriensis*

Sir:

In the course of our screening from soil microorganisms for new antibiotics, we have isolated a new antibiotic, lactonamycin (**1**) (Fig. 1) from a culture broth of *Streptomyces rishiriensis* MJ773-88K4[1,2] which was isolated from a soil sample collected at Yokohama city, Kanagawa prefecture, Japan. **1** shows antimicrobial activities against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA). In this communication, we report the production, isolation, physico-chemical properties and biological activities of **1**.

A slant culture of lactonamycin producing organism was inoculated into three 500-ml Erlenmeyer flasks each containing 110 ml of a producing medium consisting of glycerol 2%, dextrin 2%, Bacto-soytone (Difco) 1%, yeast extract 0.3%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.2%, adjusted to pH 7.4 before sterilization. The fermentation was carried out at 27°C for 6 days. The fermentation broth was centrifuged, and the supernatant (300 ml) was extracted with ethyl acetate (300 ml) at pH 2.0. The ethyl acetate layer was concentrated under reduced pressure to *ca.* 10 ml, which was washed with saturated aq. $NaHCO_3$ (10 ml × 2), and **1** was transfered to 10 ml of water. The water layer was adjusted to pH 2.0 with 1 N

Fig. 1. Structure of lactonamycin.

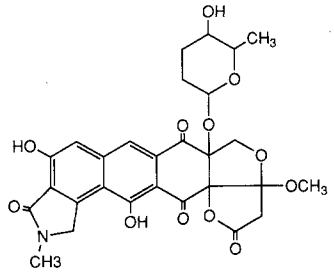



Fig. 2. $^1H$ NMR spectrum of lactonamycin in $CDCl_3$ (500 MHz).

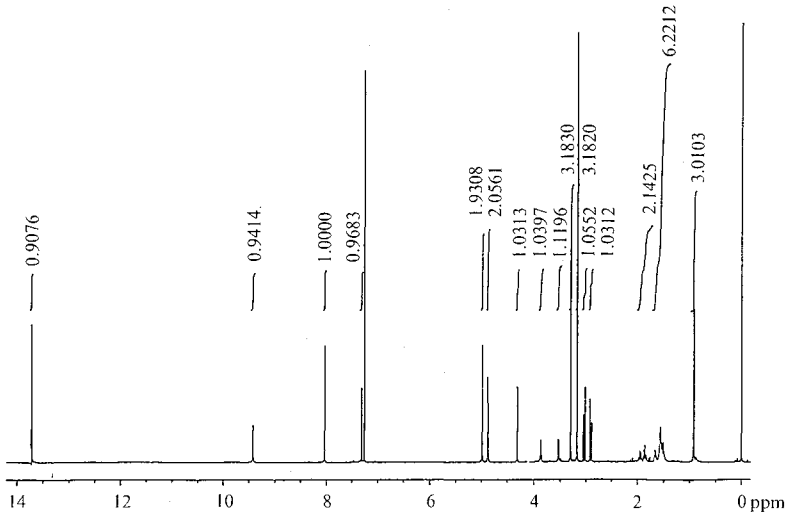
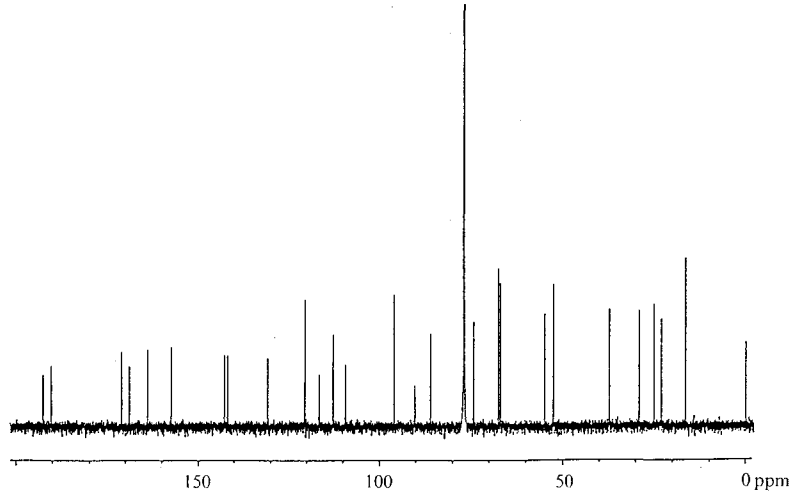



Fig. 3. $^{13}C$ NMR spectrum of lactonamycin in $CDCl_3$ (125 MHz).



HCl, then extracted with ethyl acetate (10 ml). The organic layer was washed with water (10 ml), dried over anhydrous sodium sulfate, and concentrated *in vacuo* to yield a dark brown residue (9.5 mg). The crude material was subjected to preparative HPLC (mobile phase: $CH_3CN$-0.1% aq. AcOH=40:60, flow rate: 10 ml/minute) using an ODS column (SHISEIDO, Capcell Pak UG120 Å i.d. 20 mm × 250 mm). Active fractions against *Bacillus stearothermophilus* were collected and concentrated to give **1** (1.5 mg).

**1** is yellowish powder with melting point of 168~171°C, optically active with $[\alpha]_D^{27}$ +34° (*c* 0.27, MeOH). The moleculer formula was determined to be $C_{28}H_{27}O_9N$ by HR-MS (FAB, positive) (Found: *m/z* 570.1613 $(M+H)^+$. Calcd: *m/z* 570.1612). UV spectrum has an absorption maxima (ε) at 228 (11300), 257 (13200), 300 (20000), 395 (6700) and 412 nm (7500) in MeOH, at 232 (11600), 250 (12900), 287 (12200), 323 (11300), 367 (10700), 398 (sh.) and 448 nm (sh.) in 90% MeOH-0.1 N NaOH. The IR spectrum (KBr) showed absorbtion bands at 3410, 2950, 1810, 1690, 1640sh, 1620 and 1250 $cm^{-1}$. The $^1H$ NMR (500 MHz) and $^{13}C$ NMR (125 MHz) spectra in $CDCl_3$ of **1** are shown in Figs. 2 and 3, respectively. The structure of **1** was determined by NMR spectral analyses including decoupled-HMBC and NOE experiments. Structural elucidation study of **1** will be reported in another paper.

Antimicrobial activity of **1** was potent against Gram-positive bacteria including MRSA (MIC 0.2~0.78 μg/ml)[3]. Single intraperitoneal injection of 50 mg/kg of **1** did not cause death in female ICR mice (4-weeks old).

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## References

1) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. International Journal of Systematic Bacteriology 22: 344~345, 1972
2) WILLIAMS, S. T.; M. GOODFELLOW & G. ALDERSON: Genus *Streptomyces* Waksman and Henrici 339$^{AL}$, 1943. *In* BERGY's manual of systematic bacteriology, Vol. 4. (*Ed.* S. T. WILLIAMS, M. E. SHARPE & J. G. HOLT), pp. 2452~2492, The Williams & Wilkins Co., Baltimore, 1989
3) GOTO, S. *et al.*: MICs were according to the standard method of Japan Society of Chemotherapy. Chemotherapy (Tokyo) 29: 76~79, 1981 (in Japanese)