reduction of MMC to hydroquinone (**3**) might facilitate a carbonium ion (**4**) formation by elimination of the methoxy group and the aziridine ring opening. This carbonium ion (**4**) might react with 5′-GMP. The product might be oxidized to **1** during work-up or an unknown redox reaction under the reaction conditions.

In conclusion, an alkylation product of a nucleotide by reductively activated MMC was characterized for the first time. This will contribute toward understanding of the molecular basis for the functioning of MMC as a reductively activated alkylating agent.

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Isolation and Characterization of a New 16-Membered Lactone, Protylonolide, from a Mutant of Tylosin-Producing Strain, *Streptomyces fradiae* KA-427[1,2]

A new 16-membered lactone named protylonolide was isolated from the culture of the blocked mutant of *Streptomyces fradiae*, KA-427. Protylonolide was converted to tylosin by both parent strain and several tylosin-non-producing mutants of KA-427. Protylonolide is an important biosynthetic intermediate of tylosin.

Keywords——protylonolide (PTL); tylosin (TYL); 16-membered macrolide antibiotics; blocked mutants; bioconversion and biosynthesis

In the previous paper,[4] the authors reported the biosynthetic relationship between tylosin (TYL) and relomycin (20-dihydro-TYL) using enzyme inhibitor, cerulenin,[5] a specific inhibitor of fatty acid and polyketide biosynthesis. However, investigation on the biosynthetic route of TYL was hampered by the limited amounts of appropriate intermediates. We therefore explored suitable blocked mutants producing the intermediates of TYL, and obtained two mutant strains No. 261 and No. 551 producing 16-membered lactone ring named protylonolide (PTL) by the NTG treatment of the TYL-producing strain of *Sm. fradiae* KA-427.

The mutant strain was cultured in 50-l jar fermentor containing 30 l of the medium (1.0% glucose, 2.0% starch, 0.5% peptone, 0.5% meat extract, 0.3% L-asparagine, and 0.4% $CaCO_3$, pH 7.5) for 3 days at 27°. PTL was extracted with benzene and purified by the preparative silica gel TLC (Kieselgel 60 F_{254}, Merck, developed with $CHCl_3$/MeOH/1.5 N NH_4OH=2:1:1, bottom layer, *Rf* 0.59) to give about 200 mg of PTL as a white powder. PTL was crystallized

1) Bioconversion and biosynthesis of 16-membered macrolide antibiotics. Part XVI. See ref. 3) for Part XV.
2) Abbreviations: PTL, Protylonolide; TYL, Tylosin; NTG, N-methyl-N′-nitro-N-nitrosoguanidine.
3) C. Kitao, H. Hamada, H. Ikeda, and S. Ōmura, *J. Antibiotics*, **32**, 1055 (1979).
4) S. Ōmura, C. Kitao, J. Miyazawa, H. Imai, and H. Takeshima, *J. Antibiotics* **31**, 254 (1978).
5) S. Ōmura, *Bact. Rev.* **40**, 681 (1976).

from a mixed solvent of benzene and cyclohexane to afford prisms [mp 53—56°; $[\alpha]_D^{22}$ −33.6° (c=1.0, $CHCl_3$)]. PTL has a UV maximum at 282 nm (log ε=4.430, MeOH) suggesting the presence of $\alpha,\beta,\gamma,\delta$-unsaturated ketone as a chromophore. The IR spectrum showed significant bands at 3520 cm^{-1} (OH), 2960 and 2880 (CH_3), 2930 (CH_2), 1730 and 1710 (–C(=O)–C(=O)–C(OH)–), 1675 (C=O), 1590 (C=C), 1180 (–O–C(=O)–). The molecular formula of PTL was established by the high resolution mass spectrometry; Observed, M^+ m/z 394.2734; Calcd for $C_{23}H_{38}O_5$, m/z 394.2719. The ^{1}H-NMR spectrum of PTL exhibits characteristic absorptions (δ 6.25, 7.24 and 5.59) corresponding to the dienone system (H_{10}, H_{11} and H_{13}, respectively) and the signals at δ 3.68 and 3.74 which can be assigned to the protons at C-3 and C-5, respectively. Further, the ^{13}C-NMR spectrum shows a total of 23 peaks including six methyl carbons (δ 9.5, 9.6, 11.8, 17.8, 13.0, 16.2) due to C-17, -18, -20, -21, -22 and -23, respectively, ester carbonyl (δ 174.6, C-1) and the ketone carbonyl (δ 203.9, C-9). These data indicates that PTL is derived from the aglycone moiety of TYL, and has the structure as shown in Fig. 1.

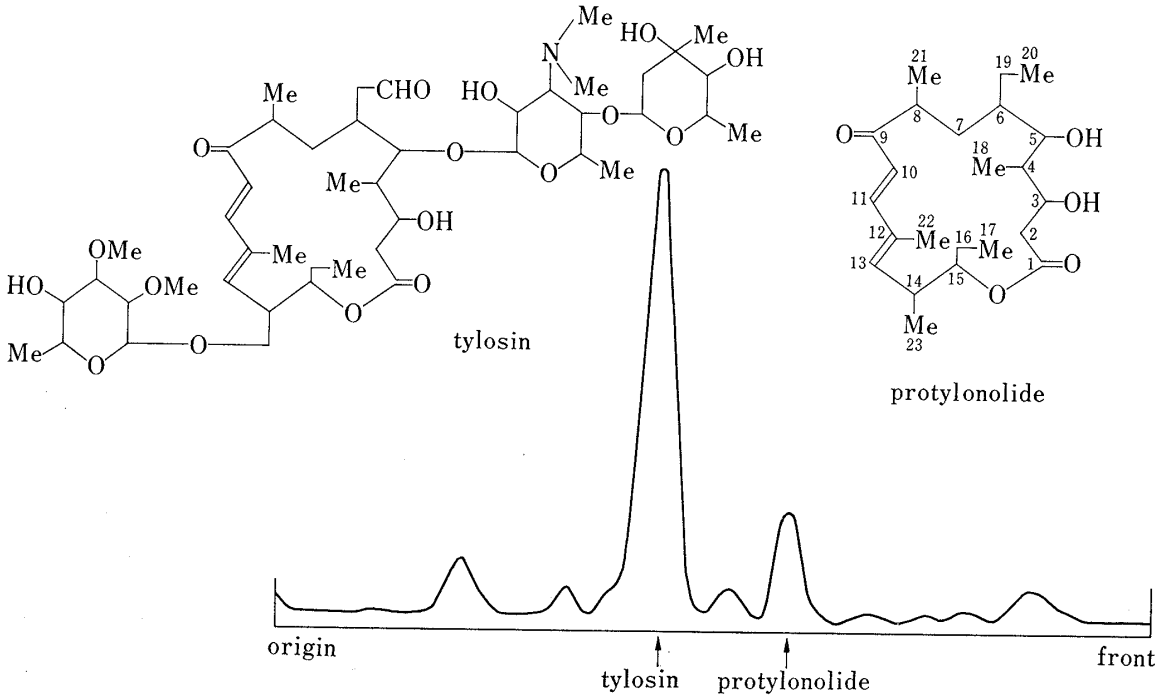

Fig. 1. Bioconversion of Protylonolide by *Sm. fradiae* KA-427 in the Cerulenin-supplemented Culture

Protylonolide was added in the 48 hr culture and incubated for 24 hr. After extraction with benzene, the bioconversion was examined by silica gel TLC scanned at 282 nm.

PTL has no antimicrobial activity. It was converted to TYL by the cultures of the parent strain KA-427 in which 20 μg/ml of cerulenin was added (Fig. 1), and by the TYL-non-producing mutants such as NP-7, NP-10, NP-20 and NP-23 obtained by the same mutation procedure as No. 261. It is therefore suggested that PTL is an important intermediate for the biosynthesis of tylosin.

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The Absolute Configuration of Tsukushinamine-A.[1,2] A New Cage-Type Lupin Alkaloid from *Sophora franchetiana*

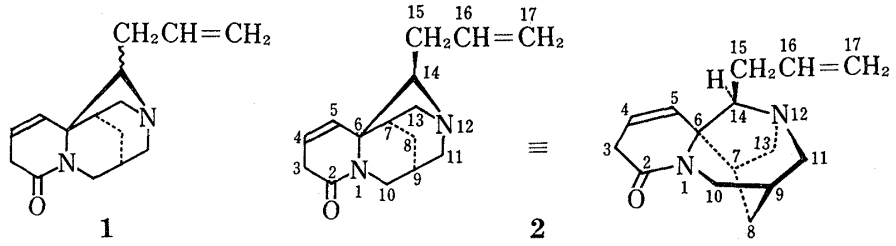
The absolute configuration of tsukushinamine-A, a novel cage-type lupin alkaloid, was determined by the X-ray analysis as **2** (6*R*, 7*R*, 9*S*, 14*R*).

Keywords——*Sophora franchetiana*; Leguminosae; tsukushinamine-A; alkaloid; cage-type lupin alkaloid; lupin alkaloid; X-ray analysis; absolute configuration; biosynthesis; baptifoline

As a result of screening plants belonging to the Leguminosae for lupin alkaloid,[3-6] a novel cage-type lupin alkaloid, tsukushinamine (tsukushinamine-A),[1] was isolated from the fresh epigeal parts of *Sophora franchetiana* as a colourless oil, $[\alpha]_D^{25}$ −72.3° (c=0.56, EtOH), HBr-salt mp 260° (MeOH–acetone), together with (−)-cytisine, (−)-N-formylcytisine, (−)-rhombifoline, (−)-anagyrine, (−)-baptifoline and (±)-ammodendrine. Its structure has been proposed to be shown as **1** from spectroscopic data.[7]

With regard to the final structure determination and the absolute configuration of tsukushinamine-A, we further wish to report that tsukushinamine-A can be drawn as **2** (6*R*, 7*R*, 9*S*, 14*R*) by the X-ray analysis.

Crystals of tsukushinamine-A hydrobromide suitable for an X-ray analysis were grown by the slow evaporation of a methanol/acetone solution. These crystals belonged to the



1 **2**

Chart 1

1) The name of tsukushinamine was altered into tsukushinamine-A as a result of the presence of isomers B and C, and the numbering system of tsukushinamine-skeleton was also changed as **2**.
2) A part of this work was presented at the 22th Symposium on the Chemistry of Natural Products of Japan, Fukuoka, October 26, 1979, "Symposium Papers" p. 525.
3) I. Murakoshi, K. Toriizuka, J. Haginiwa, S. Ohmiya, and H. Otomasu, *Chem. Pharm. Bull.*, **27**, 144 (1979).
4) S. Ohmiya, K. Higashiyama, H. Otomasu, I. Murakoshi, and J. Haginiwa, *Phytochemistry*, **18**, 645 (1979).
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6) I. Murakoshi, K. Toriizuka, J. Haginiwa, S. Ohmiya, and H. Otomasu, *Phytochemistry*, **18**, 699 (1979).
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