

BIOLOGICAL GLYCOSIDATION OF MACROLIDE AGLYCONES. II  
ISOLATION AND CHARACTERIZATION OF DESOSAMINYL-PLATENOLIDE I

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(Received for publication January 27, 1978)

Biological glycosidation of platenolide I (I), a biosynthetic intermediate of 16-membered macrolide antibiotic platenomycin aglycones, with desosamine by *Streptomyces* producing 14-membered macrolide antibiotics was attempted. *Streptomyces narbonensis* producing narbomycin gave a new product designated as 5-O-desosaminyl-platenolide I (III), and *Streptomyces venezuelae* producing narbomycin and picromycin gave III together with a second new product, 5-O-desosaminyl-14-hydroxyplatenolide I (IV). A nonantibiotic-producing blocked mutant of *Streptomyces platensis* subsp. *malvinus*, a producer of platenomycins, converted III to an antibiologically active compound identified as 3-O-propionyl-5-O-desosaminyl-9-dihydro-18-oxo-platenolide I (V).

In the previous papers dealing with biosynthesis of narbomycin<sup>1)</sup>, picromycin<sup>1)</sup> and platenomycins<sup>2)</sup>, the authors demonstrated that some macrolide aglycones were key substances for the elucidation of biosynthetic pathways of these antibiotics. By adding narbonolide, a 14-membered macrolide aglycone, in the fermentation medium of *Streptomyces platensis* subsp. *malvinus*, a producer of the 16-membered macrolide antibiotic platenomycins, the authors also succeeded in producing some new narbonolide derivatives possessing mycaminose which generally combined to 16-membered macrolide antibiotics<sup>3)</sup>. Recently, LEMAHIEU *et al.* reported the production of 3-O-oleandrosyl-5-O-desosaminyl-erythronolide A oxime by the oleandomycin-producing strain<sup>4)</sup>. These findings show that an aglycone of a macrolide antibiotic is useful not only for the biogenetic studies, but also as a starting material for developing new macrolide antibiotics.

The present paper deals with the glycosidation of platenolide I (I)<sup>1)</sup>, a biosynthetic intermediate of 16-membered macrolide antibiotic platenomycin aglycones, with desosamine by *S. narbonensis* ISP 5016 and *S. venezuelae* MCRL 0376, resulting in the production of 5-O-desosaminyl-platenolide I (III) and/or 5-O-desosaminyl-14-hydroxy-platenolide I (IV). The result suggests that the glycosidation of a macrolide aglycone with a foreign aminosugar is achieved by an appropriate macrolide antibiotic-producing strain. The paper also deals with the biological conversion of the non-antibiotic substance, III to an antibiologically active 3-O-propionyl-5-O-desosaminyl-9-dihydro-18-oxo-platenolide I (V) by a blocked mutant of *S. platensis* subsp. *malvinus*.

#### Materials and Methods

##### Microorganisms and Culture Conditions

*Streptomyces narbonensis* ISP 5016, a producer of narbomycin, and *Streptomyces venezuelae* MCRL 0376, a producer of narbomycin and picromycin as major macrolide antibiotics, were used as desosamine donors. A blocked mutant U 21<sup>5)</sup> of platenomycin-producing *Streptomyces platensis* subsp.

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*malvinus* MCRL 0388 was employed in the bioconversion study. Strain U 21 could not produce platenomycins, but was able to glycosidate **I** with mycaminose<sup>5</sup>).

These organisms were maintained on a BENNET's agar slant at 27°C. Matured spores were inoculated into 500-ml Erlenmeyer flask containing 100 ml of a following seed medium; a medium for strains ISP 5016 and MCRL 0376: glucose 20 g and nutrient broth (Difco) 8.0 g per liter (tap water, pH unadjusted); a medium for strain U 21: glucose 20 g, peptone (Mikuni Kagaku Co., Ltd.) 10 g, beef extract (Mikuni Kagaku Co., Ltd.) 7.5 g, yeast extract (Oriental Kobo Co., Ltd.) 2.5 g and NaCl 3.0 g per liter (tap water, pH was adjusted to 6.5 before autoclaving). After inoculation, the strains were cultivated at 27°C for 40 hours on a rotatory shaker. The vegetative inoculums thus prepared were transformed to 100 ml of a production medium prepared in 500-ml Erlenmeyer flasks (inoculum size : 1.0~2.0 ml) and fermented at 27°C on a rotatory shaker (180 rpm). For fermentation of strains ISP 5016 and MCRL 0376, P-1 medium containing glucose 10 g, glycerol 10 g, Polypeptone (Kyokuto Seiyaku Co., Ltd.) 10 g, yeast extract (Oriental Kobo Co., Ltd.) 1.0 g, NaCl 5.0 g and CaCl<sub>2</sub> 2.0 g per liter (tap water, pH unadjusted) was used, and an appropriate amount of **I** dissolved in ethyl alcohol was added after autoclaving. As the basal medium for the fermentation of strain U 21, P-2 medium was used which contained corn starch 15 g, glucose 5 g, soy bean meal 18 g, gluten feed (Nihon Shokuhin Co., Ltd.) 1.5 g, NaCl 5.0 g and CaCO<sub>3</sub> 2.0 g per liter (tap water, pH was adjusted to 7.0 before autoclaving).

In the present experiments the antibiotic production was assayed by the cylinder cup-plate method using *Bacillus subtilis* PCI 219 as a test organism and picromycin as a reference standard. Antibiotic was expressed in terms of picromycin equivalent.

Detection of 5-O-desosaminyl-platenolide I (III), 5-O-Desosaminyl-14-hydroxy-platenolide I (IV) and 3-O-Propionyl-5-O-desosaminyl-9-dihydro-18-oxo-platenolide I (V)

The harvested broth of strains ISP 5016, MCRL 0376 or U 21 with platenolide **I** was centrifuged and the supernatant was extracted with ethyl acetate at pH 8.5. The extract was concentrated *in vacuo*, and oily residue was chromatographed on a silica gel plate with the solvent system consisted of CHCl<sub>3</sub> - MeOH - AcOH - H<sub>2</sub>O (79: 11: 8: 2) and detected by spraying with 40% H<sub>2</sub>SO<sub>4</sub> followed by heating. R<sub>f</sub> values of the accumulants were 0.41 (coloration blue) for **III**, 0.32 (orange) for **IV**, 0.35 (brownish blue) for **V**, 0.53 (brown) for narbomycin and 0.48 (gray) for picromycin. **III** and **IV** produced in the fermentation beer were measured by the reflective UV spectrophotometer (Hitachi MPF-2A) which was capable to estimate directly the UV absorption of developed compounds on the thin-layer chromatogram. As a standard substance for the quantitative determination, 9-dehydroleucomycin A<sub>8</sub> was employed which showed the UV maximum at 280 nm similar to **III** and **IV**. As the R<sub>f</sub> values of **III**, **IV** and 9-dehydroleucomycin A<sub>8</sub> were fairly close to each other, the estimation of each compound was carried out by the methods reported in the previous paper<sup>6</sup>).

Isolation and Purification of 5-O-Desosaminyl-platenolide I (III) and 5-O-Desosaminyl-14-hydroxy-platenolide I (IV)

The fermentation beer (9.3 liters) of strain MCRL 0376 cultivated about 80 hours in the medium containing **I** (100 mcg/ml) was filtered over Celite 545, and the filtrate was extracted with ethyl acetate (5.0 liters, twice) at pH 8.5. After concentration of the extract, bio-transformed products in ethyl acetate were transferred into diluted hydrochloric acid of pH 4.0. The acidic solution was re-extracted at pH 8.5 with benzene. The benzene layer was concentrated. A crude powder (4.7 g) thus obtained was then dissolved in ethyl acetate and chromatographed on the silica gel column using a solvent system of CHCl<sub>3</sub> - ethyl acetate (9: 1). The fractions of **III** and **IV** monitored by thin-layer chromatography were concentrated respectively, and **III** and **IV** were obtained as a crude powder. Further purification by alumina column chromatography using benzene - ethyl acetate (9: 1) gave **III** (112 mg) and **IV** (86 mg) as a homogeneous substance. These compounds were further recrystallized from benzene - *n*-hexane as colorless needles (**III**: 62 mg, **IV**: 37 mg), respectively.

Conversion of 5-O-Desosaminyl-platenolide I (III) to 5-O-Desosaminyl-14-hydroxy-platenolide I (IV)

After grown for 20 hours at 27°C in the fermentation medium described above, the cells of

strains ISP 5016 and MCRL 0376 were respectively collected by centrifugation (1,200 *g*, 10 minutes) and washed three times with sterilized saline solution. Cells were then suspended in a phosphate buffer (0.05 M, pH 7.0) to give approximately 5.0 mg (dry weight)/ml. **III** as a substrate was then added to the cell suspension to give final concentration of 100 mcg/ml. After incubation for desired time at 27°C the products in the solution was estimated.

#### Isolation and Purification of 3-O-Propionyl-5-O-desosaminyl-9-dihydro-18-oxo-platenolide I (V)

The fermentation beer (9.1 liters) of strain U 21 harvested about 80 hours in a medium added with **III** (200 mcg/ml) was filtered over Celite 545, and the filtrate was treated as described above. Thus, the crude **V** was recovered. **V** was then purified by silica gel column chromatography with CHCl<sub>3</sub> - ethyl acetate (9 : 1) followed by alumina column chromatography with benzene - ethyl alcohol (9 : 1). Thus, **V** was obtained as a thin-layer chromatographically homogeneous powder. Recrystallization from benzene - *n*-hexane gave **V** as colorless needles (320 mg).

### Results and Discussion

#### (A). Fermentations and Accumulation of 5-O-Desosaminyl-platenolide I (**III**) and 5-O-Desosaminyl-14-hydroxy-platenolide I (**IV**)

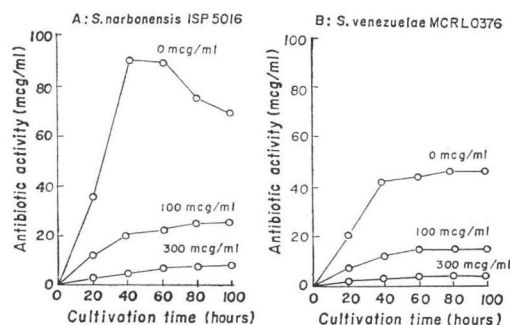
The effects of platenolide I (**I**) on the production of narbomycin and picromycin by strains ISP 5016 and MCRL 0376 were first examined. Figs. 1-A and 1-B show the results. When **I** was added to the fermentation medium, the antibiotic production was depressed, the depression being more marked as the concentrations of **I** became higher. At the levels of 100 mcg/ml of **I**, only 26 mcg/ml (strain ISP 5016) and 15 mcg/ml (strain MCRL 0376) of the antibiotic production were observed. These productivities were respectively corresponded to about 28% and 30% of the fermentation without **I**. From previous experience<sup>3)</sup> the depression of the antibiotic production was suggested due to the accumulation of **I**-related compounds, so that the ethyl acetate extract of the broth was examined by silica gel thin-layer chromatography.

The thin-layer chromatography examined on the sample collected at various cultivation times indicated the production of one or two accumulants. Strain ISP 5016 produced **III** and strain MCRL 0376 gave **III** and **IV**. These compounds could be distinguished from narbomycin and picromycin by R<sub>f</sub> values and colorations. When the thin-layer chromatogram was monitored at 280 nm before coloration by thin-layer reflective UV spectrophotometer, these compounds were able to be detected as the independent chromato-profiles.

Judging from the finding that **III** and **IV** gave the UV absorption maximum at 280 nm similar to **I** and showed the R<sub>f</sub> values lower than **I**, these compounds were speculated as the new products derived from **I** and with more hydrophilic properties. In fact, **III** and **IV** were elucidated as 5-O-desosaminyl-platenolide I and 5-O-desosaminyl-14-hydroxy-platenolide I, respectively, as described below.

Previously the authors experienced that when narbonolide, a 14-membered macrolide aglycone, was added to the medium, platenomycin production by strain MCRL 0388 was depressed and

Fig. 1. The course of antibiotic production of *Streptomyces narbonensis* ISP 5016 and *Streptomyces venezuelae* MCRL 0376 cultured in absence or presence of **I**. Strain ISP 5016 and MCRL 0376 were cultured in the P-1 medium containing 0, 100 and 300 mcg/ml of **I**.



mycaminosyl narbonolide was produced<sup>3</sup>). Therefore, it would be a common phenomenon in macrolide fermentation that the antibiotic production was considerably repressed in the presence of a foreign aglycone, because of the competition between a foreign aglycone exogenously added and an aglycone synthesized *de novo*.

(B). Biological Conversion of 5-O-Desosaminyl-platenolide I (III)  
to 5-O-Desosaminyl-14-hydroxy-platenolide I (IV)

Figs. 2-A and 2-B show the accumulation of III and IV by strains ISP 5016 and MCRL 0376. The maximum production of III was attained at 40 hours cultivation in both strains, when the antibiotic production reached to maximum as well. The accumulation of IV was observed only at the later stage of strain MCRL 0376 fermentation and attained to maximum after 100 hours.

Judging from these findings together with the structure elucidated, it was easily assumed that III and IV were in a precursor (III)—product (IV) relationship. To verify the relative relationships, the conversion experiments were attempted by fermentation and also by washed cell suspension technique using strain MCRL 0376. Fig. 3-A shows the results obtained in the fermentation experiment. III previously added in the medium gradually decreased and IV was accumulated. At 80 hours, 55% of III was converted to IV. Fig. 3-B shows the results by washed cell suspension of strain MCRL 0376. The reaction rapidly proceeded by incubation at 27°C and 40% of III was converted to IV after 5 hours. On the contrary, conversion of III to IV was not observed with strain ISP 5016 either in fermentation or by the washed cell technique.

These results apparently show that IV was converted from III by a picromycin-producing strain, but not by an organism lacking picromycin-producing ability. Similar phenomenon was already resulted in the hydroxylation of narbomycin.<sup>1)</sup>

The authors found that III was easily hydroxylated by strain MCRL 0376. However, direct hydroxylation of platenomycins, demycarosyl platenomycins or leucomycins by this organism was not achieved. Therefore, a methyl function at C-18 position (C<sub>18</sub>-CH<sub>3</sub>) in an aglycone may be a necessary factor in the hydroxylation of 16-membered macrolide antibiotic aglycones by this strain.

Fig. 2. The course of III and IV production by *Streptomyces narbonensis* ISP 5016 and *Streptomyces venezuelae* MCRL 0376 in the medium containing I.

Strain ISP 5016 and MCRL 0376 were cultured in the P-1 medium containing I (100 mcg/ml). The amounts of III and IV accumulated were estimated by thin-layer chromatography using reflective spectrophotometer.

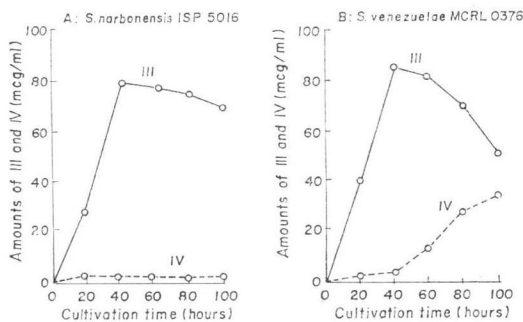
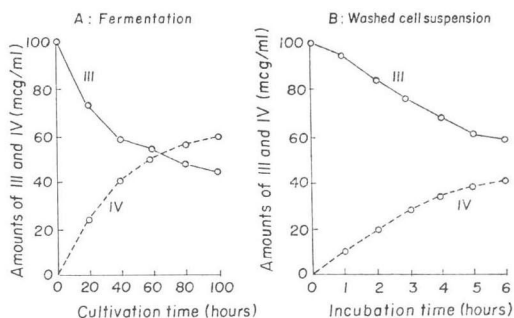


Fig. 3. Biological conversion of III to IV by fermentation or washed cell suspension of *Streptomyces venezuelae* MCRL 0376.

The strain MCRL 0376 was cultured in the P-1 medium, III dissolved in ethyl alcohol was added to the medium at zero time of the fermentation at 100 mcg/ml. The washed cells were prepared by using 20-hour cultured cells in the P-1 medium.



## (C). Biological Conversion of 5-O-Desosaminyl-platenolide I (III)

## to 3-O-Propionyl-5-O-desosaminyl-9-dihydro-18-oxo-platenolide I (V)

ŌMURA *et al.*<sup>8)</sup> and RAKHIT *et al.*<sup>9)</sup> reported that the C<sub>18</sub>-CHO function in the macrolide aglycone was essential for the antibiotic activity. As the compounds possessing a C<sub>18</sub>-CH<sub>3</sub><sup>13)</sup> or C<sub>18</sub>-CH<sub>2</sub>OH<sup>8,10)</sup> function show almost no or very low antibiotic activity, and on that point of view, the biological conversion of III or IV to a C<sub>18</sub>-CHO compound was considered.

As reported previously,<sup>2)</sup> 5-O-mycaminosyl-platenolide I (C<sub>18</sub>-CH<sub>3</sub>) was easily converted to demycarosyl platenomycin (C<sub>18</sub>-CHO) by a platenomycin-producing strain MCRL 0388 or its blocked mutant U 21, so the oxidation of C<sub>18</sub>-CH<sub>3</sub> of III was attempted by these strains. When cultured in the medium containing III, the mutant U 21 could convert III completely after 80 hours. Continuation of fermentation decreased the amount of the product.

The converted products were isolated and their structures were examined. More than five bio-converted products were detected in the fermentation broth, when the ethyl acetate extract of the broth was examined by thin-layer chromatography. Among the products, V elucidated as 3-O-propionyl-5-O-desosaminyl-9-dihydro-18-oxo-platenolide I was dominant. The structure elucidation of minor products were not accomplished, however, the IR, UV and mass spectral analysis showed these compounds were 3-hydroxyl, 3-O-acetyl and/or 9-dihydro derivatives of 16-membered macrolide antibiotic which were similar to platenomycins, but all products contained desosamine in place of mycarosyl-mycaminose. The accumulation of these compounds could easily be speculated by recalling the results of the biogenetic studies of platenomycins.

The biological conversion of IV was also attempted similarly. However, the converted products were not detected in the fermentation beer.

## (D). Physicochemical Properties and Structures of 5-O-Desosaminyl-platenolide I (III),

## 5-O-Desosaminyl-14-hydroxyl-platenolide I (IV) and 3-O-Propionyl-5-O-desosaminyl-9-dihydro-18-oxo-platenolide I (V)

The physicochemical properties of III, IV and V were listed in Table 1. These compounds were soluble in ethyl alcohol, methyl alcohol, ethyl acetate, chloroform and benzene, but hardly soluble in petroleum ether, *n*-hexane and water. They gave negative erythromycin, ferric chloride

Table 1. Physicochemical properties of III, IV and V

|   | III<br>(5-O-Desosaminyl-<br>platenolide I)                | IV<br>(5-O-Desosaminyl-14-<br>hydroxy-platenolide I)      | V<br>(3-O-Propionyl-5-O-<br>desosaminyl-9-dihydro-18<br>oxo-platenolide I) |
|---|---|---|--|
| Appearance  | colorless needles   | colorless needles   | colorless needles  |
| m.p. (°C)   | 169~172   | 120~122   | 106~109  |
| Formula   | C <sub>28</sub> H <sub>47</sub> NO <sub>8</sub>           | C <sub>28</sub> H <sub>47</sub> NO <sub>9</sub>           | C <sub>31</sub> H <sub>51</sub> NO <sub>10</sub>                           |
| Mol. wt. by MS.                                   | 525   | 541   | 597  |
| Elem. anal. %<br>Found (Calcd)                    | C : 64.30 (64.05)<br>H : 9.40 ( 9.02)<br>N : 2.38 ( 2.67) | C : 62.35 (62.16)<br>H : 9.08 ( 8.76)<br>N : 2.32 ( 2.59) | C : 62.50 (62.29)<br>H : 8.71 ( 8.60)<br>N : 2.06 ( 2.34)                  |
| UV λ <sub>max</sub> <sup>EtOH</sup> nm<br>(log ε) | 280 (4.33)  | 280 (4.28)  | 232 (4.37)   |
| IR (KBr, cm <sup>-1</sup> )                       | 3420, 1720, 1680<br>1630, 1595, 1255<br>1160, 1105, 1070  | 3450, 1720, 1685<br>1640, 1595, 1280<br>1165, 1105, 1070  | 3440, 1735, 1630<br>1295, 1160, 1105<br>1070, 1045                         |

and ninhydrin tests.

Structures of these compounds were elucidated as follows: an amino sugar present in **III**, **IV** and **V** was the same and assumed as desosamine by the intense peaks at  $m/e$  158 and 174 in mass spectra and a singlet of six protons of N-dimethyl at about  $\delta$  2.3 in NMR. A doublet ( $J=7.5$ ) at about  $\delta$  4.5 due to an anomeric proton of the sugar suggested the  $\beta$ -glycosidic linkage. The amino sugar was isolated from the acid hydrolysate of each compound and confirmed to be D-desosamine by direct comparison with the authentic sample prepared from narbomycin.

#### (1) The Structure of **III**

The IR spectrum of **III** suggested the presence of hydroxyl ( $3420\text{ cm}^{-1}$ ), lactone and conjugated carbonyl ( $1720$  and  $1680\text{ cm}^{-1}$ ) functions and also double bonds ( $1630$  and  $1595\text{ cm}^{-1}$ ). The UV absorption maximum at 280 nm ( $\log \epsilon$  4.33) suggested the presence of an  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ -dienone chromophore. Besides a molecular ion ( $M^+$ ) at  $m/e$  525, a characteristic fragment peak at  $m/e$  351 was found, which was analysed as  $m/e$  351.2160 ( $C_{20}H_{31}O_5$ ,  $\Delta = -0.9$  mmu) by high resolution mass spectrometry. From the NMR spectrum (Fig. 4-A), the following functions were assigned by comparing with NMR signals shown by **I**<sup>(1)</sup>: a primary methyl ( $\delta$  0.87, 3H, t,  $J=7$ ,  $\text{CH}_3$ -18), three secondary methyls ( $\delta$  1.17, 3H, d,  $J=7$ ,  $\delta$  1.20, 3H, d,  $J=7$  and  $\delta$  1.33, 3H,  $J=7$ ), one  $\text{N}(\text{CH}_3)_2$  ( $\delta$  2.32, 6H, s), one  $\text{OCH}_3$  ( $\delta$  3.60, 3H, s), H-3 ( $\delta$  3.75, 1H, d,  $J=10$ ), H-5 ( $\delta$  4.15, 1H, d,  $J=10$ ), H-1' ( $\delta$  4.51, 1H, d,  $J=7.5$ ), H-15 ( $\delta$  5.20, 1H, m), H-12 and 13 ( $\delta$  6.0~6.2, 2H, m), H-10 ( $\delta$  6.24, 1H, d,  $J=16$ ) and H-11 ( $\delta$  7.20, 1H, bt,  $J=16$ ). Thus, **III** was determined as 5-O-desosaminy-platenolide I.

#### (2) The Structure of **IV**

The structure of **IV** was elucidated in comparison of IR, UV and NMR spectra of **IV** with those of **III**. **IV** showed intense mass ion peaks at  $m/e$  541 ( $M^+$ ), 367, 174 and 158, while the peracetate of **IV** showed peaks at  $m/e$  667 ( $M^+$ ), 451, 216 and 200. A fragment ion peak of **IV** at  $m/e$  367

Fig. 4-A. NMR spectrum of **III** (100 MHz,  $\text{CDCl}_3$ )

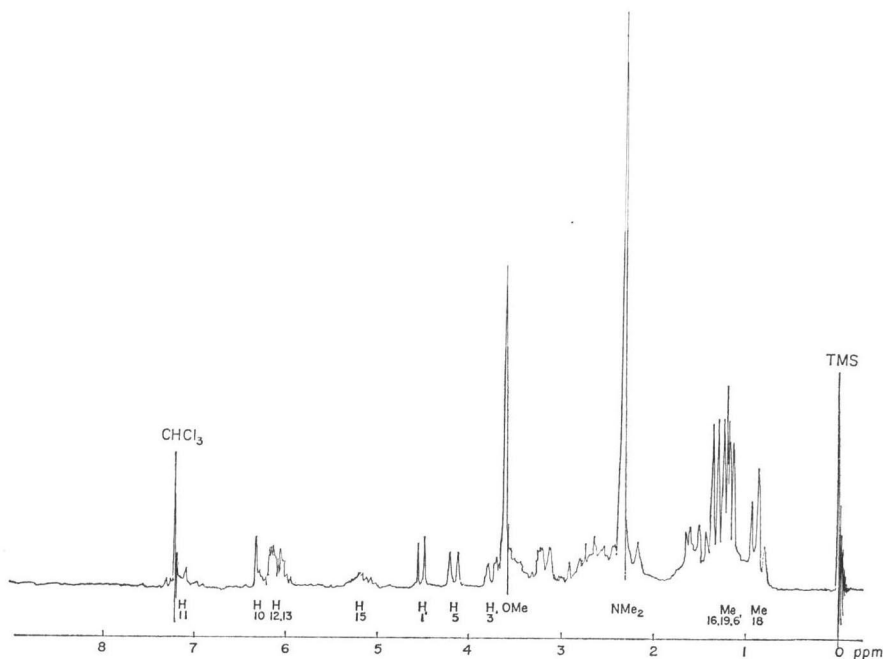
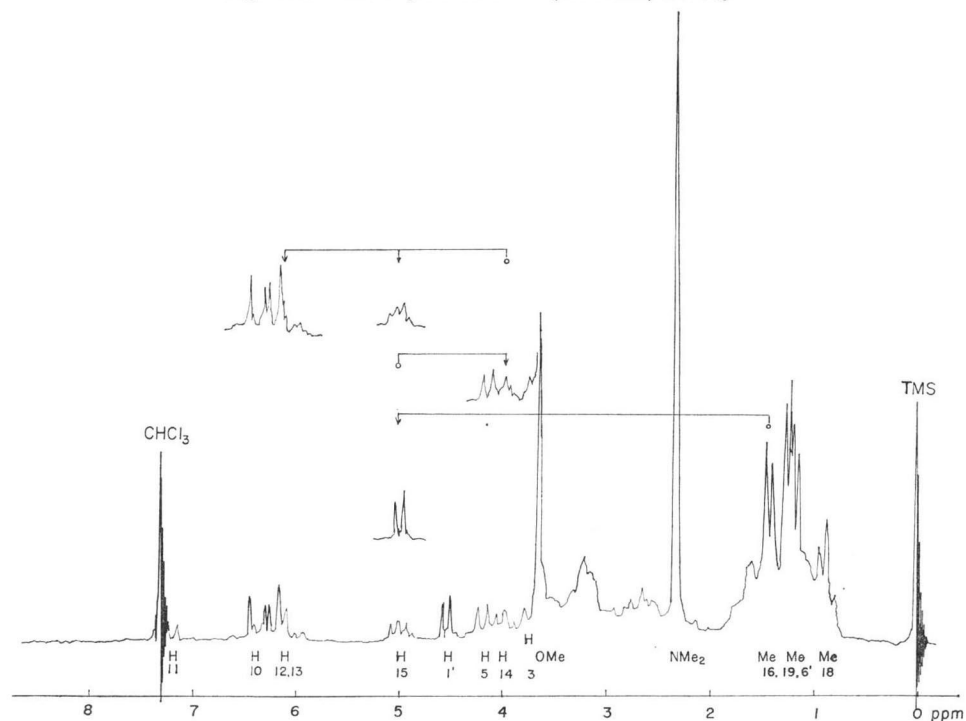
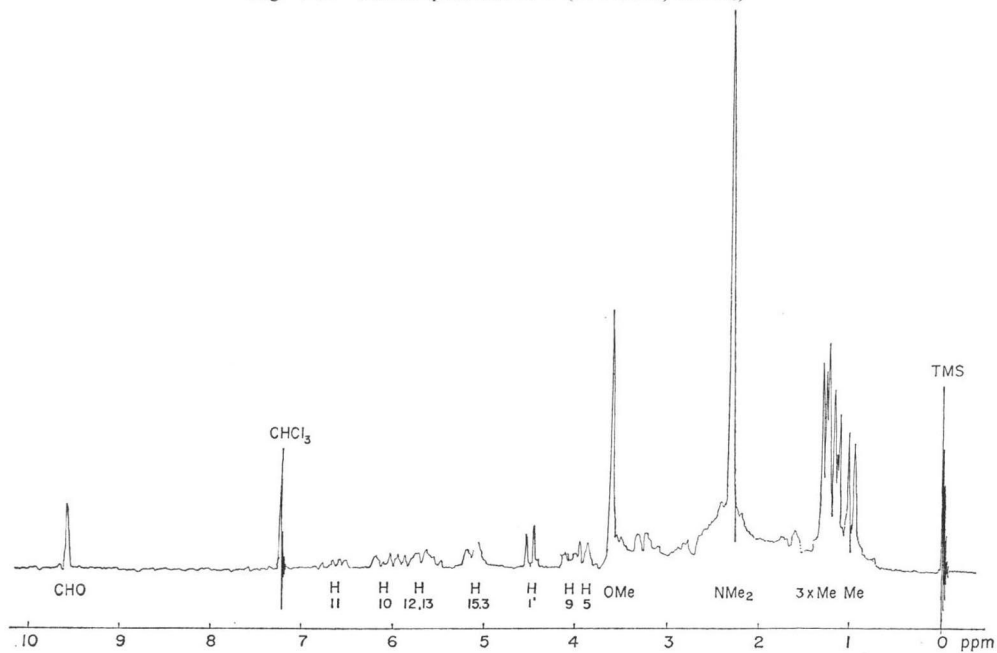


Fig. 4-B. NMR spectrum of IV (100 MHz,  $\text{CDCl}_3$ )Fig. 4-C. NMR spectrum of V (100 MHz,  $\text{CDCl}_3$ )

(367.2128,  $\text{C}_{20}\text{H}_{31}\text{O}_6$ ,  $\Delta=0.9$  mmu) may be comparable to that of **III** at  $m/e$  351. These peaks were derived from an aglycone part. Shifting of the peak at  $m/e$  367 of **IV** to  $m/e$  451 by acetylation showed the presence of two acetylatable units in an aglycone of **IV**. Therefore, an aglycone of **IV** was

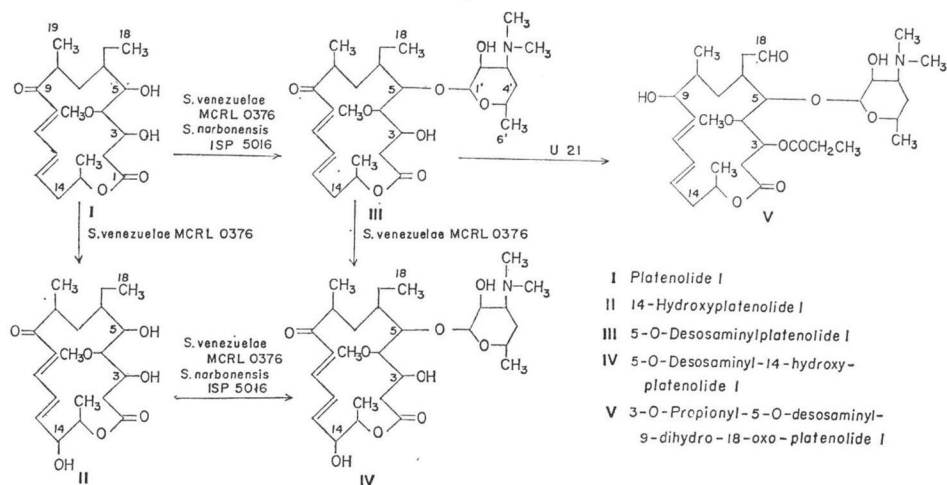
thought to have one more hydroxyl function than **III**. In analogy with **III**, NMR signals of **IV** (Fig. 4-B) were assigned as follows: one primary methyl ( $\delta$  0.88, 3H, t,  $J=7$ ), three secondary methyl ( $\delta$  1.18, 3H, d,  $J=7$ ,  $\delta$  1.21, 3H, d,  $J=7$  and  $\delta$  1.42, 3H, d,  $J=7$ ), one  $N(\text{CH}_3)_2$  ( $\delta$  2.32, 6H, s), one  $\text{OCH}_3$  ( $\delta$  3.62, 3H, s), H-3 ( $\delta$  3.73, 1H, d,  $J=10$ ), H-5 ( $\delta$  4.17, 1H, d,  $J=10$ ), H-1' ( $\delta$  4.54, 1H, d,  $J=7.5$ ), H-15 ( $\delta$  5.00, 1H, m), H-12 and 13 ( $\delta$  5.95~6.42, 2H, m), H-10 ( $\delta$  6.36, 1H, d,  $J=16$ ) and H-11 ( $\delta$  7.24, 1H, bt). Comparing to **III**, a multiplet at  $\delta$  3.96 shown by **IV** was not observed in **III** and the lowest doublet methyl signal ( $\text{CH}_3$ -16) shifted by 0.09 ppm. The multiplicity of H-15 at  $\delta$  5.00 was reduced more than the corresponding signal of **III** and this signal shifted to the upper field by 0.2 ppm. Considering these differences and the alteration of olefinic proton signals, it was assumed that the allylic oxidation took place at the C-14 position in the hydroxylation of **III**. This assumption was resolved by NMR decoupling technique. When a double quartet (H-15) at  $\delta$  5.00 which coupled with a doublet methyl ( $\text{CH}_3$ -16) at  $\delta$  1.42 was irradiated, a multiplet (H-14) at  $\delta$  3.96 was simplified. Furthermore, the irradiation at  $\delta$  3.96 changed the signals at  $\delta$  5.00 and 6.15~6.30 to a quartet (H-15) and the simplified olefinic proton (H-13) signal, respectively. This showed that H-15 and H-13 signals were coupling with a multiplet (H-14) at  $\delta$  3.96. Thus, one additional hydroxyl function was assigned to be present at the C-14 position and the structure of **IV** was concluded to be a 14-oxy derivative of **III**.

### (3) The Structure of V

IR spectrum of **V** showed the presence of hydroxyl ( $3440\text{ cm}^{-1}$ ), lactone ( $1735\text{ cm}^{-1}$ ) and double bond ( $1630\text{ cm}^{-1}$ ) functions. UV absorption maximum at 232 nm ( $\log \epsilon$  4.37) showed the  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ -dienol structure. The mass spectrum showed the molecular ion at  $m/e$  597 and the intense fragment peaks at  $m/e$  423 due to an aglycone.

The aglycone ion ( $m/e$  423.2353,  $\text{C}_{23}\text{H}_{33}\text{O}_7$ ,  $\Delta = -2.7$  mmu) was identical with that of demycarosyl platenomycin A<sub>1</sub>. Comparing the NMR of **V** (Fig. 4-C) with that of demycarosyl platenomycin A<sub>1</sub> previously reported<sup>7)</sup>, many resemblances were noticed. The signals of **V** at  $\delta$  0.99 (3H, d,  $J=7$ ,  $\text{CH}_3$ -19),  $\delta$  1.12~1.30 (9H,  $\text{CH}_3 \times 3$ ),  $\delta$  2.29 (6H, s, N,  $(\text{CH}_3)_2$ ),  $\delta$  3.60 (3H, s,  $\text{OCH}_3$ ), 3.94 (1H, bd,  $J=8$ , H-5),  $\delta$  4.08 (1H, dd,  $J=9$  and 4.5, H-9),  $\delta$  4.50 (1H, d,  $J=7.5$ , H-1'),  $\delta$  5.0~5.25 (m, H-3 and H-15),  $\delta$  5.5~6.2 (3H, m, H-10, 12 and 13),  $\delta$  6.65 (1H, dd,  $J=16$  and 10, H-11) and 9.56

Fig. 5.





(1H, s, -CHO) were assigned as described. These signals seemed almost identical with those of demycarosyl platenomycin A<sub>1</sub> except the chemical shift of N-dimethyl signal ( $\delta$  2.29 for desosamine and  $\delta$  2.55 for mycaminose). Thus, V was decided to be 3-O-propionyl-5-O- $\beta$ -D-desosaminyl-9-dihydro-18-oxo-platenolide I, *i.e.*, 4'-deoxy-demycarosyl-platenomycin A<sub>1</sub>.

The structures and biosynthetic relations of these compounds were shown in Fig. 5. Recently, it was succeeded to isolate a new aglycone, 14-hydroxy-platenolide I (II), in the culture of strain MCRL 0376. The detailed elucidation of this new aglycone will be discussed elsewhere.

(E). Antimicrobial Activities of 5-O-Desosaminyl-platenolide I (III),  
5-O-Desosaminyl-14-hydroxy-platenolide I (IV) and 3-O-Propionyl-  
5-O-desosaminyl-9-dihydro-18-oxo-platenolide I (V)

The antibiotic activities were determined by an agar dilution method. The results are listed in Table 2. III and IV showed no antimicrobial activities as similar as 3-O-propionyl-5-O-mycaminosyl-platenolide I, a C<sub>18</sub>-methyl analog of platenomycin.<sup>11,12)</sup> V showed antimicrobial activities but, contrary to expectation, the activities were considerable less than those of demycarosyl platenomycin.

Recently, the authors succeeded in isolating some new 16-membered macrolide antibiotics named M-4365 A<sub>1</sub> and G<sub>1</sub>.<sup>10)</sup> In spite of these having a methyl function at a C-18 position, they showed fair activities against various organisms. Therefore, the antimicrobial activities of 16-membered macrolide antibiotics were not restricted by the C-18 function but also by an aglycone structure. Among the aglycones of 16-membered macrolide antibiotics, aglycones of antibiotic M-4365, juvenimicin,<sup>13)</sup> rosamicin<sup>14)</sup>, cirramycin<sup>15)</sup> and tylosin<sup>16)</sup> would be more favorable in exhibiting antimicrobial activities.

Table 2. Antimicrobial activities of III, IV, V and related compounds

| Test organisms                               | MIC (mcg/ml) |       |       |       |        |       |
|--|--------------|-------|-------|-------|--------|-------|
|  | I            | III   | IV    | V     | DM-PLM | PLM   |
| <i>Staphylococcus aureus</i> FDA 209 P       | > 100        | > 100 | > 100 | 12.5  | 6.25   | 0.78  |
| <i>Staphylococcus aureus</i> Smith           | > 100        | > 100 | > 100 | 12.5  | 6.25   | 0.78  |
| <i>Streptococcus faecalis</i>                | > 100        | > 100 | > 100 | 12.5  | 12.5   | 1.56  |
| <i>Bacillus subtilis</i> PCI 219             | > 100        | > 100 | > 100 | 6.25  | 3.13   | 0.39  |
| <i>Klebsiella pneumoniae</i>                 | > 100        | > 100 | > 100 | 12.5  | 12.5   | 12.5  |
| <i>Escherichia coli</i> NIHJ                 | > 100        | > 100 | > 100 | > 100 | > 100  | > 100 |
| <i>Escherichia coli</i> K-12                 | > 100        | > 100 | > 100 | > 100 | > 100  | > 100 |
| <i>Pseudomonas aeruginosa</i> A <sub>3</sub> | > 100        | > 100 | > 100 | > 100 | > 100  | > 100 |

I: Platenolide I

III: 5-O-Desosaminyl-platenolide I

IV: 5-O-Desosaminyl-14-hydroxy-platenolide I

V: 3-O-Propionyl-5-O-desosaminyl-9-dihydro-18-oxo-platenolide I

DM-PLM: Demycarosyl platenomycin

PLM: Platenomycins

Acknowledgement

The authors thank Dr. T. OKUDA in this company for his interest and encouragements, and Mr. Y. SEKI as a collaborator of the present experiment.

## References

- 1) MAEZAWA, I.; T. HORI, A. KINUMAKI & M. SUZUKI: Biological conversion of narbonolide to picromycin. *J. Antibiotics* 26: 708~710, 1973
- 2) FURUMAI, T.; K. TAKEDA & M. SUZUKI: Studies on the biosynthesis of basic 16-membered macrolide antibiotics, platenomycins. IV. Biosynthesis of platenomycins. *J. Antibiotics* 28: 789~797, 1975
- 3) MAEZAWA, I.; A. KINUMAKI & M. SUZUKI: Biological glycosidation of macrolide aglycones. I. Isolation and characterization of 5-O-mycaminosyl narbonolide and 9-dihydro-5-O-mycaminosyl narbonolide. *J. Antibiotics* 29: 1203~1208, 1976
- 4) LEMAHIEU, R. A.; H. A. AX, J. F. BLOUNT, M. CARSON, C. W. DESPREAUX, D. L. PRUESS, J. P. SCANNELL, F. WEISS & R. W. KIERSTEAD: A new semisynthetic macrolide antibiotic 3-O-oleandrosyl-5-O-desosaminylerythronolide A oxime. *J. Antibiotics* 29: 728~734, 1976
- 5) FURUMAI, T. & M. SUZUKI: Studies on the biosynthesis of basic 16-membered macrolide antibiotic, platenomycins. I. Selection of and cosynthesis by non-platenomycin-producing mutants. *J. Antibiotics* 28: 770~774, 1975
- 6) MAEZAWA, I.; T. HORI & M. SUZUKI: Accumulation of narbonolide caused by the addition of organic acids. *Agr. Biol. Chem. (Japan)* 38: 91~96, 1974
- 7) KINUMAKI, A.; I. TAKAMORI, Y. SUGAWARA, M. SUZUKI & T. OKUDA: Studies on the macrolide antibiotic YL-704 complex. III. The structures of macrolide antibiotics YL-704 A<sub>1</sub> and B<sub>1</sub>. *J. Antibiotics* 27: 107~116, 1974
- 8) ŌMURA, S.; M. TISHLER, A. NAKAGAWA, Y. HIRONAKA & T. HATA: Relationship of structures and microbiological activities of the 16-membered macrolides. *J. Med. Chem.* 15: 1011~1015, 1972
- 9) RAKHIT, S. & K. SINGH: Structure activity relationship in sixteen membered macrolide antibiotics. *J. Antibiotics* 27: 221~224, 1974
- 10) FURUMAI, T.; I. MAEZAWA, N. MATSUZAWA, S. YANO, T. YAMAGUCHI, K. TAKEDA & T. OKUDA: Macrolide antibiotics M-4365 produced by *Micromonospora*. I. Taxonomy, production, isolation, characterization and properties. *J. Antibiotics* 30: 443~449, 1977
- 11) FURUMAI, T. & M. SUZUKI: Studies on the biosynthesis of 16-membered macrolide antibiotics, platenomycins. III. Production, isolation and structures of platenolide I and II. *J. Antibiotics* 28: 783~788, 1975
- 12) FURUMAI, T. & M. SUZUKI: Studies on the biosynthesis of basic 16-membered macrolide antibiotics, platenomycins. II. Production-isolation and structures of 3-O-propionyl-5-O-mycaminosyl-platenolide I and II, 9-dehydro demycarosyl platenomycin and demycarosyl platenomycin. *J. Antibiotics* 28: 775~782, 1975
- 13) KISHI, T.; S. HARADA, H. YAMANA & A. MIYAKE: Studies on juvenimicin, a new antibiotic. II. Isolation, chemical characterization and structures. *J. Antibiotics* 29: 1171~1181, 1976
- 14) REIMAN, H. & R. S. JARET: Structure of rosamicin, a new macrolide from *Micromonospora rosaria*. *J. Chem. Soc., Chem. Comm.* 1972: 1270, 1972
- 15) TSUKIURA, H.; M. KONISHI, M. SAKA, T. NAITO & H. KAWAGUCHI: Studies on cirramycin A<sub>1</sub>. III. Structure of cirramycin A<sub>1</sub>. *J. Antibiotics* 22: 89~99, 1969
- 16) MORIN, R. B.; M. GORMAN, R. L. HAMILL & P. V. DEMARCO: The structure of tylosin. *Tetrahed. Lett.* 1970: 4737~4740, 1970