BIOSYNTHETIC STUDIES ON OLEANDOMYCIN BY INCORPORATION OF THE CHEMICALLY SYNTHESIZED AGLYCONES

Sir:

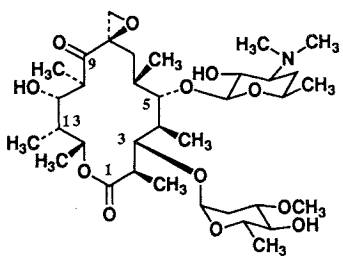
Although a variety of biosynthetic studies have been focused on macrolide antibiotics[1~3], the biosynthetic processes, especially the final steps in the biosynthesis of the 14-membered-ring macrolide antibiotic, oleandomycin (**1**) have not yet been well elucidated. Recently, an Abbott group isolated (8*R*)-8,8a-deoxyoleandolide (**3**) from a fermentation broth of an erythromycin-producing strain, and suggested the aglycone **3** to be a biosynthetic intermediate of oleandomycin[4]. We have chemically synthesized the aglycone **3** and the related aglycones including the intact aglycone, oleandolide (**5**) for chemical simulations of the biosynthesis[5,6] and the total synthesis of oleandomycin (**1**)[7,8]. The syntheses of various aglycones enabled us to study their incorporation by using blocked mutants of an oleandomycin-producing organism, *Streptomyces antibioticus*[9].

Herein, a plausible scheme for the final steps in oleandomycin biosynthesis will be proposed by the incorporation studies.

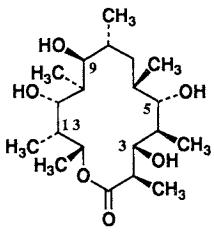
The following aglycones were synthesized by our procedures[5~8] for the incorporation: (8*R*,9*S*)-9-dihydro-8,8a-deoxyoleandolide (**2**)[5], (8*R*)-8,8a-deoxyoleandolide (**3**)[6], (9*R*)-9-dihydro-8,8a-dehydro-8,8a-deoxyoleandolide (**4**)[7,8] and oleandolide (**5**)[7,8]. A new aglycone, (9*R*)-9-dihydro-oleandolide (**6**) was derived from **4** by oxidation with *m*-chloroperbenzoic acid in chloroform: MP 127°C (recrystallization from acetone-hexane); $[\alpha]_D^{23}+8°$ (*c* 0.25, $CHCl_3$): FD-MS 389 $(M+1)^+$; ^1H NMR (270 MHz, CD_3OD): δ 0.90, 1.00, 1.04, 1.07, 1.17 and 1.26 (3H, each d, CH_3), 2.66 (dq, $J=7.2$ and 9.3 Hz, 2-H), 2.82 and 3.00 (each 1H, ABq, $J=3.8$ Hz, 8-CH_2) and 5.48 (dq, $J=1.5$ and 7.2 Hz, 13-H). The configuration at C-8 was confirmed by selective benzylidenation of **6** (*p*-bromobenzaldehyde dimethylacetal and 10-camphorsulfonic acid in CH_2Cl_2 at 0°C for 1.5 hours) to give the corresponding 3,5-*O*-*p*-bromobenzylidene compound: MP 235°C (recrystallization from ethyl acetate-hexane); $[\alpha]_D^{23}$ +8° (*c* 0.5, $CHCl_3$), which was identical with our previously reported compound[7,8].

The blocked mutants were prepared from an oleandomycin-producing strain, *S. antibioticus* SF760 (Meiji Seika Culture Collection) by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine treatment according to the DELIC procedure[10]. Ten mutants, which were isolated from about 1,500 colonies, were lacking in ability to produce any aglycones[11,12], but they could produce oleandomycin (**1**) only when provided with appropriate biosynthetic aglycones.

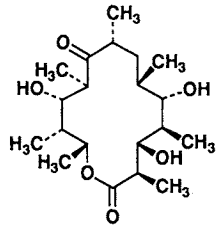
The following medium (30 ml) was used for the



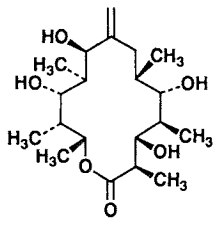
Oleandomycin (**1**)



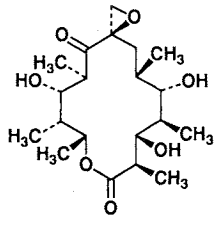
2



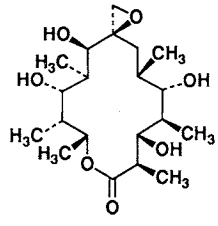
3



4



5



6

Table 1. Incorporation of chemically synthesized aglycones (**2**~**6**) to oleandomycin (**1**) by the non-producing blocked mutant.

Aglycones (500 μg/ml)	**2**	**3**	**4**	**5**	**6**
Production (μg/ml) of **1**	45	72	153	155	0

fermentation which was carried out in the usual way at 28°C for 4 days[9]: Dextrin 2.0%, corn starch 1.0%, soy bean meal 1.5%, soluble vegetable protein (Sungross, Suntory Ltd.) 0.5%, NZ-amine type A 0.5%, NaCl 0.5% and $CaCO_3$ 0.5% (pH 7.2). After 24 hours, to the broth was added 500 μg/ml of each aglycone (**2**~**6**) and then the resulting broth was assayed for oleandomycin (**1**) using *Bacillus subtilis* ATCC 6633 at appropriate time intervals. The oleandomycin (**1**) produced was extracted with $CHCl_3$ from the supernatant of the broths and quantitatively detected by HPLC according to a modified TSUJI's procedure as follows[13]. An ODS stainless column (4.6×250 mm; Yamamura Kagaku YMC A-303) was operated with UV detector (280 nm) at 40°C in a flow rate 1.0 ml/minute of themobile phase (CH_3CN - MeOH - 0.2 M NH_4OAc - H_2O, 30 : 10 : 10 : 25). Oleandomycin (**1**) showed the Rt of 5.9 minutes. At the same time, glucose, starch and NH_3-nitrogen in the aforementioned broth were also determined by the autoanalyzer system (Tenicon Instruments Corp.). No significant metabolic changes (*e.g.* growth, pH and consumption of sugar) were observed with or without the aglycones.

The aglycones (500 μg/ml) were converted into oleandomycin (**1**) by the incorporation as shown in Table 1. The aglycones **4** and **5** were most efficiently incorporated and aglycones **2** and **3** were incorporated to a lesser extent. No oleandomycin (**1**) was detected after feeding with aglycone **6**. The fact that the olefin **4** led to production of oleandomycin (**1**) as efficiently as did the fully elaborated aglycone, oleandolide (**5**), was perhaps remarkable and provides strong presumptive evidence that the unique epoxide of this antibiotic is produced *via* such an intermediate. The somewhat less efficient conversion of the aglycone **3** into oleandomycin (**1**) may reflect the multiple steps required for its conversion into the olefin **4**. The intra or extracellular nature of the final steps in the biosynthesis has not been established and cellular uptake considerations may play a determining factor in the extent of conversion of the possible intermediates into oleandomycin (**1**). Perhaps most surprising was the small but definite production of oleandomycin (**1**) after the feeding of the 9-alcohol (**2**). This opens the possibility that **2** may be a biosynthetic intermediate and in this case a logical biosynthetic pathway would be **2**→**3**→?→**4**→**5**→**1**.

Acknowledgments

We are grateful to the Institute of Microbial Chemistry for the generous support of our program, and also thank Pfizer Taito Co., Ltd. for a generous gift of oleandomycin. Financial support by Ministry of Education, Science and Culture (Grant-in-Aid Scientific Research) and The Asahi Glass Foundation is gratefully acknowledged.

KUNIAKI TATSUTA
HIROKI GUNJI
SHUICHI TAJIMA
TAKASHI ISHIYAMA

Department of Applied Chemistry,
Keio University,
3-14-1 Hiyoshi, Kohoku-ku,
Yokohama 223, Japan

SATOSHI IMAI
SATOSHI OKUYAMA
SHUNZO FUKATSU

Pharmaceutical Research Laboratories,
Meiji Seika Kaisha, Ltd.,
Morooka-cho, Kohoku-ku,
Yokohama 222, Japan

(Received January 10, 1990)

References

1) ŌMURA, S. & Y. TANAKA: Chapter 5. Biochemistry, regulation, and genetics of macrolide production. *In* Macrolide Antibiotics. Chemistry, Biology, and Practice. *Ed.*, S. ŌMURA, pp. 199~229, Academic Press, Inc., 1984
2) YUE, S.; J. S. DUNCAN, Y. YAMAMOTO & C. R. HUTCHINSON: Macrolide biosynthesis. Tylactone formation involves the processive addition of three carbon units. J. Am. Chem. Soc. 109: 1253~1255, 1987
3) CANE, D. E. & C.-C. YANG: Macrolide biosynthesis. 4. Intact incorporation of a chain-elongation intermediate into erythromycin. J. Am. Chem. Soc. 109: 1255~1257, 1987
4) MARTIN, J. R.; R. S. EGAN, A. W. GOLDSTEIN, S. L. MUELLER, E. A. HIRNER & R. S. STANASZEK: 8,8a-Deoxyoleandolide: Elaborated by a blocked mutant of the erythromycin-producing organism

Streptomyces erythreus. J. Antibiotics 27: 570~572, 1974

5) TATSUTA, K.; Y. KOBAYASHI, K. AKIMOTO & M. KINOSHITA: An enantioselective synthesis of a macrolide from the polyketide lactone derived from oleandomycin. Chem. Lett. 1987: 187~190, 1987
6) TATSUTA, K.; Y. KOBAYASHI & M. KINOSHITA: Synthesis of a biosynthetic precursor of oleandomycin, 8,8a-deoxyoleandolide (8-methyloleandolide), from oleandomycin. J. Antibiotics 40: 910~912, 1987
7) TATSUTA, K.; Y. KOBAYASHI, H. GUNJI & H. MASUDA: Synthesis of oleandomycin through the intact aglycone, oleandolide. Tetrahedron Lett. 29: 3975~3978, 1988
8) TATSUTA, K.; T. ISHIYAMA, S. TAJIMA, Y. KOGUCHI & H. GUNJI: The total synthesis of oleandomycin. Tetrahedron Lett. 31: 709~712, 1990
9) SOBIN, B. A.; J. B. ROUTIEN & T. M. LEES (Chas. Pfizer & Co., Inc.): Oleandomycin and hydrochloride. U.S. 2,757,123, July 31, 1956
10) DELIC, V.; D. A. HOPWOOD & E. J. FRIED: Mutagenesis by N-methyl-N'-nitro-N-nitrosoguanidine (NTG) in *Streptomyces coelicolor*. Mutat. Res. 9: 167~182, 1970
11) BALTZ, R. H. & E. T. SENO: Properties of *Streptomyces fradiae* mutants blocked in biosynthesis of the macrolide antibiotic tylosin. Antimicrob. Agents Chemother. 20: 214~225, 1981
12) WEBER, J. M.; C. K. WIERMAN & C. R. HUTCHINSON: Genetic analysis of erythromycin production in *Streptomyces erythreus*. J. Bacteriol. 164: 425~433, 1985
13) TSUJI, K. & J. F. GOETZ: High-performance liquid chromatographic determination of erythromycin. J. Chromatogr. 147: 359~367, 1978