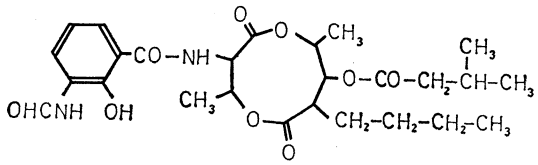
THE TOTAL SYNTHESIS OF A DIASTEREOMERIC MIXTURE OF ANTIMYCIN A_3 (BLASTMYCIN)*

Sir:

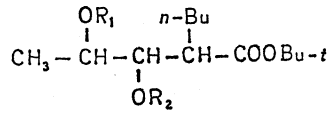
Antimycin A is an antifungal antibiotic produced by a number of *Streptomyces* spp. and of special interest because it inhibits the hydrogen transport systems of aerobic organisms[1]. Antimycin A is a complex of closely related antibiotics, A_3 being one of them. The structures of antimycin A_1 and A_3 (**1**) were elucidated by several research groups[2] from chemical and spectral evidence, and variations in the higher alkyl side chains account for structural differences between the members of the antimycin complex.

The most striking characteristic of the structure of antimycin A is its dilactone ring linked via an amide linkage to 3-formamidosalicylic acid. Recently, we[3] reported the total synthesis of dehexyl-deisovaleryloxyantimycin A_1 which differs from natural antimycin A in that the acyloxy and higher alkyl side chains are replaced by hydrogen atoms. In the present paper, we wish to report the total synthesis of a diastereomeric mixture of antimycin A_3 (**1**).



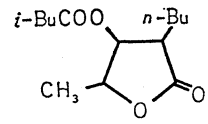
α-Benzyloxypropionaldehyde prepared from methyl α-benzyloxypropionate by reduction with diisobutylaluminum hydride[4] was condensed with *t*-butyl α-bromocaproate by the modified REFORMATSKY reaction[5] to give *t*-butyl γ-benzyloxy-α-*n*-butyl-β-hydroxyvalerate (**2**); b. p. 130°C (bath temp.)/0.0005 mmHg. Anal. Found: C 71.69, H 9.79. Calcd for $C_{20}H_{32}O_4$: C 71.39, H 9.59. The product was shown to be a mixture of four diastereomers (**2a**, **2b**, **2c** and **2d**) by unidimensional multiple chromatography using a solvent system *n*-hexane – butanone – acetone (20 : 1 : 1), two passes[6]. Column chromatography over silica gel employing a *n*-hexane – butanone – acetone (20 : 1 : 1) system separated this mixture into three fractions, a mixture of the two major components (**2a**+**2b**), a single component **2b** and a mixture of the two minor components (**2c**+**2d**), in a total yield 66.1 %.

The single component **2b** was treated with trifluoroacetic acid to remove the *t*-butyl group, hydrogenated over palladium black to remove the benzyl group and, finally, esterified with isovaleryl chloride, to afford DL-blastmycinone (**3**); b. p. 116~123°C (bath temp.)/18 mmHg, $\nu_{max}^{CCl_4}$ 1782 (γ-lactone), 1754 cm^{-1} (ester), nmr peaks: τ^{CDCl_3} 5.05 (d, d, H-β, $J_{\alpha,\beta}$=5.8 Hz), 5.63 (q, d, H-γ, $J_{\beta,\gamma}$=4.5 Hz), 7.31 (d, d, d, H-α), and 8.55 (d, C-CH_3, J=6.5). Recently, YONEHARA *et al.*[7] demon-

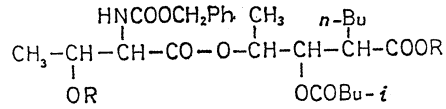


2 R_1 = $PhCH_2$, R_2 = H

4 R_1 = H, R_2 = isovaleryl

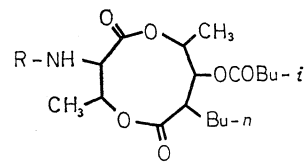


3

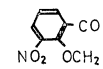


5 R = *t*-Bu

6 R = H



7 R = $PhCH_2OCO$

8 R = 

* A portion of this paper was presented at the Post-Congress Symposium, on Chemistry, Biosynthesis and Transformation of Antibiotics, 6th International Congress of Chemotherapy Aug. 16, 1969, Tokyo.

strated by gas chromatography that the natural blastmycinone is not homogeneous, but it consists of two isomers differing in the structure of the alkanoyl group. Gas chromatography confirmes that our synthetic antimycin lactone has the same retention time as one of the two peaks of natural blastmycinone so that the peak of longer retention time corresponds to the blastmycinone having the isovaleryl group.

The main fraction above (**2a**+**2b**), which contained about 50 %* of the component **2b**, was used for the subsequent synthesis. O-Acylation with isovaleryl chloride in the presence of pyridine was followed by hydrogenolysis over palladium black to give *t*-butyl α-*n*-butyl-γ-hydroxy-β-isovaleryloxy-valerate (**4**) in a 71 % yield ; b. p. 116~120°C (bath temp.)/0.0005 mmHg. Anal. Found : C 65.42 ; H 10.37. Calcd for $C_{18}H_{34}O_5$: C 65.78 ; H 10.88.

The γ-hydroxy ester was esterified with N-benzyloxycarbonyl-O-*t*-butyl-L-threonine[8] in the presence of dicyclohexylcarbodiimide and pyridine to afford *t*-butyl γ-(N-benzyloxycarbonyl-O-*t*-butyl-L-threonyloxy)-α-*n*-butyl-β-(isovaleryloxy)-valerate (**5**) as a syrup in a 65 % yield. Anal. Found : C 66.01 ; H 9.39 ; N 2.38. Calcd for $C_{34}H_{55}O_9N$: C 65.67 ; H 8.92 ; N 2.25.

De-*t*-butylation of the ester (**5**) with trifluoroacetic acid gave a free acid (**6**), which was cyclized with trifluoroacetic anhydride in benzene (concentration of **6** in benzene : 4×10^{-2}M) at 75°C for 16 hours. The reaction product was chromatographed on a silica gel column with benzene - acetone (15 : 1) to afford an intramolecular cyclization product, 3-benzyloxycarboxyamido-7-*n*-butyl-4, 9-dimethyl-8-isovaleryloxy-1,5-dioxa-2, 6-cyclononanedione (**7**) as a pale yellow syrup in a 9 % yield ; $[\alpha]_D^{18}$ +12° (*c* 3, chloroform), m/e 491, $\nu_{max}^{CCl_4}$ 3440 (NH), 1738 (ester and amide), 1504 cm^{-1} (amide). Anal. Found : C 63.79 ; H 7.25 ; N 2.51. Calcd for $C_{26}H_{37}O_8N$: C 63.52 ; H 7.59 ; N 2.85.

The benzyloxycarbonyl group of **7** was removed by hydrogenolysis and the resulting free amino dilactone was N-acylated with O-benzyl-3-nitrosalicylic acid N-hydroxysuccinimide ester[3] to yield 3-(O-benzyl-3'-nitrosalicyloylamido)-7-*n*-butyl-4, 9-dimethyl-8-isovaleryloxy-1, 5-dioxa-2, 6-cyclononanedione (**8**) as a syrup in a 26 % yield ; $[\alpha]_D^{27}$ +2° (*c* 1.2, chloroform), $\nu_{max}^{CCl_4}$ 3390, 1749, 1675, 1603, 1535 and 1513 cm^{-1}. Anal. Found : C 62.93 ; H 6.88. Calcd. for $C_{32}H_{40}O_{10}N_2$: C 62.73 ; H 6.58. The product (**8**) was hydrogenated again and N-formylated with 98 % formic acid in the presence of dicyclohexylcarbodiimide. Purification of the product by preparative thin-layer chromatography with *n*-hexane - ethyl acetate (5 : 3) afforded a diastereomeric mixture of antimycin A_3 (**1**) as a syrup in a 61.5 % yield ; $[\alpha]_D^{25}$ +26.5° (*c* 1, chloroform), λ_{max}^{MeOH} 224 (log ε 4.49) and 320 mμ (log ε 3.93), $\nu_{max}^{CHCl_3}$ 3420, 1747, 1704, 1645, 1613 and 1528 cm^{-1}. Anal. Found : C 59.78 ; H 6.62 ; N 5.19. Calcd for $C_{26}H_{36}O_9N_2$: C 59.99 ; H 6.97 ; N 5.38.

On paper chromatography with a solvent system[9] water - ethanol - acetone (7 : 2 : 1), the synthetic specimen showed on the bioautogram two spots not completely separated and the smaller one corresponding to that of the natural antimycin A_3.

Minimal inhibitory concentrations (mcg/ml) of the synthetic specimen (mixture of isomers) against tested fungi, as compared to those (in parenthesis) of natural antimycin A complex, were as follows : *Corticium rolsii* 50 (100), *Gloeosporium lacticola* 100 (>100), *Glomerella cingulata* >100 (>100), *Leptosphaeria sulvinii* 0.39 (0.0125) by dilution method with SABOURAUD medium, 27°C 5 days ; *Penicillium chrysogenum* Q 176 12.5, *Piricularia oryzae* <0.012 (0.005), *Trichophyton asteroides* 429 6.25 by agar dilution method with 1 % glucose nutrient agar, 27°C 42 hours.

The authors wish to acknowledge with gratitude helpful discussions with Professor HIROSHI YONEHARA, who supplied a sample of antimycin lactone, and the nmr spectrum of blastmycinone and the helpful advice of Professor HAMAO UMEZAWA.

* The content of the major isomer (**2b**) was calculated from the peak area of DL-blastmycinone in the gas chromatogram of the isomeric mixture of the γ-valerolactone derivatives derived from the γ-hydroxyester (**4**).

Mitsuhiro Kinoshita
Masao Wada
Sumio Umezawa

Department of Applied Chemistry,
Keio University, Koganei-shi, Tokyo

(Received August 25, 1969)

References

1) Rieske, J. S. : Antimycin A in "Antibiotics Vol. I, Mechanism of Action", edited by D. Gottlieb & P. D. Shaw, pp. 542～584, Springer 1967
2) Strong, F. M.; J. P. Dickle, M. E. Loomans, E. E. van Tamelen & R. S. Dewey : The chemistry of antimycin A. IX. Structure of the antimycins. J. Am. Chem. Soc. 82 : 1513～1514, 1960
van Tamelen, E. E.; J. P. Dickle, M. E. Loomans, R. S. Dewey & F. M. Strong : The chemistry of antimycin A. X. Structure of the antimycins. J. Am. Chem. Soc. 83 : 1639～1646, 1961
Birch, A. J.; D. W. Cameron, Y. Harada & R. W. Rickard : The structure of the antimycin A complex. J. Chem. Soc. 1961, 889～895, 1961
Yonehara, H. & S. Takeuchi : Studies on the chemical structure of blastmycin. J. Antibiotics, Ser. A 11 : 254～263, 1958
3) Kinoshita, M. & S. Umezawa : The total synthesis of dehexyl-deisovaleryloxy-antimycin A_1. Bull. Chem. Soc. Japan 42 : 854, 1969
4) Zakharkin, L. I. & I. M. Khorlina : Reduction of esters of carboxylic acids into aldehydes with diisobutylaluminum hydride. Tetrahedron Letters 1962-14: 619～620, 1962
5) Moriwake, T. : The Reformatsky reaction. I. Condensation of ketones and *t*-butyl bromoacetate by magnesium. J. Org. Chem. 31 : 983～985, 1966
6) Thoma, J. A. : Application and theory of unidimensional multiple chromatography. Anal. Chem. 35 : 214～224, 1963
7) Endo, T. & H. Yonehara : Abstracts of Papars, 11th Symposium on the Chemistry of Natural Products, p. 269 Sept. 1967, (Kyoto)
8) Schröder, E. : Über Peptidsynthesen. XV. Neue O-*tert*-Butyl-Hydroxyaminosäure-Derivate und ihre Verwendung zur Synthese von Glukagon-Teilsequenzen. Ann. 670 : 127～136, 1963
9) Liu, Wen-chih & F. M. Strong : Separation and properties of antimycin A subcomponents. J. Am. Chem. Soc. 81 : 4387～4390, 1959