Total Syntheses of Antimycin A_3 and Its Diastereomer

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Natural antimycin A_3 (**1A**) and its diastereomer (**1B**) were synthesized. By the syntheses, the correlations between configurations of the enantiomeric 2-butyl-4-hydroxy-3-isovaleryloxypentanoic acids present in the dilactone moieties of **1A** and **1B**, and those of natural (+)blastmycinone (+)**7a** and its enantiomer (−)**7a** were confirmed. The absolute configuration of **1B** was also determined.

In the recent papers we reported the total syntheses of dehexyl-deisovaleryloxy-antimycin A_1[1] as a prototype of antimycin A, and a diastereomeric mixture of antimycin A_3.[2] This paper presents the total syntheses of natural antimycin A_3 (**1A**) and its diastereomer (**1B**) (Fig. 1).

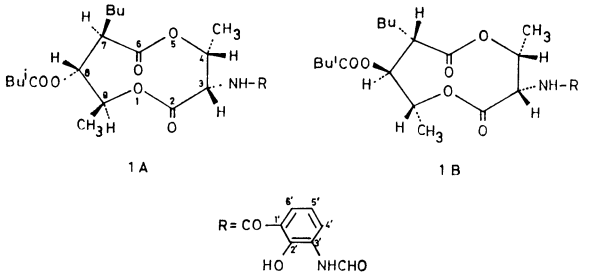
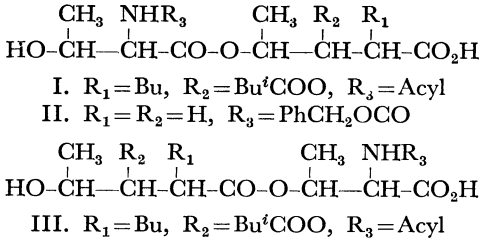


Fig. 1.

The primary structure of antimycin A_3 (blastmycin[3]) which is one of the major components of antimycin A complex was established by van Tamelen, *et al.*,[4] Birch, *et al.*,[5] and Yonehara, *et al.*[6] The absolute configuration for antimycin A_3 was first proposed by Endo and Yonehara[7] in 1967. In their configurational studies, consideration was given to a correlation between the configurations of blastmycin and optically active blastmycinone[6] obtained by saponification of the parent antibiotic and was based on the fact that lithium aluminum hydride reductions of blastmycin and blastmycinone afforded an almostly homogeneous 2-butylpentane-1,3,4-triol,[5] however, the experimental details were not given.

We were interested in the stereochemistry of the unique nine-membered dilactone ring which exists in antimycin A, and in its role playing in biological activity,[8] and attempted to synthesize the natural antimycin A_3 and its diastereomer. After publication of the preliminary report[9] of this paper, we have proposed the revised absolute configuration **1A** of antimycin A_3.[10]

The first important problem in the total synthesis of natural antimycin A_3 was the construction of the nine-membered dilactone ring having the same configuration as that of the natural product. Such a dilactone compound was considered to be obtainable by a lactonization of either type I or III of hydroxyester-acid which is a condensation product of the two kinds of hydroxy acid, *i.e.*, *N*-acyl-L-threonine and 2-butyl-4-hydroxy-3-isovaleryloxypentanoic acid possessing the same configuration as that of the corresponding moiety which exists in the molecule of antimycin A_3.



I. R_1=Bu, R_2=Bu^{t}COO, R_3=Acyl
II. R_1=R_2=H, R_3=$PhCH_2OCO$

III. R_1=Bu, R_2=Bu^{t}COO, R_3=Acyl

It has been known that antimycin A_3 (blastmycin) never afforded the fragments such as type I and III, even in mild saponification condition, whereas it gave blastmycic acid[6] and blastmycinone as the fragmentation products. Therefore, it was impracticable to examine the dilactone formation by the direct use of the naturally occurring hydroxyester-acid fragment or its derivative. In our recent synthetic studies on antimycins,[1,2] it has already been found that the lactonizations of the synthetic diastereomeric mixtures of the hydroxyester-acid, II and I (R_3=$PhCH_2OCO$), by heating with trifluoroacetic anhydride in benzene afforded the corresponding dilactone compounds in 33 and 6% yields, respectively, only this reagent and reaction condition being effective for these lactonizations. In such a lactonization reaction, the hydroxyester-acids of type I have been considered to be more favorable substrates rather than those of type III, because the absence of free terminal carboxyl group

1) M. Kinoshita and S. Umezawa, This Bulletin, **42**, 854 (1969); *ibid.*, **43**, 897 (1970).

2) M. Kinoshita, M. Wada, and S. Umezawa, *J. Antibiot.* (Tokyo), **22**, 580 (1969).

3) K. Watanabe, T. Tanaka, K. Fukuhara, N. Miyairi, H. Yonehara, and H. Umezawa, *J. Antibiot., Ser. A*, **10**, 39 (1957).

4) E. E. van Tamelen, J. P. Dickie, M. E. Loomans, R. S. Dewey, and F. M. Strong, *J. Amer. Chem. Soc.*, **83**, 1639 (1961).

5) A. J. Birch, D. W. Cameron, Y. Harada, and R. W. Rickard, *J. Chem. Soc.*, **1961**, 889.

6) H. Yonehara and S. Takeuchi, *J. Antibiot., Ser. A*, **11**, 254 (1958).

7) T. Endo and H. Yonehara, Abstracts of Papers, 11th Symposium on The Chemistry of Natural Products (Kyoto), P 269, Sept. 1967.

8) J. S. Reeske, A draft of a review on "Antimycin" which will be published in "Antibiotic," new Ed., by Springer-Verlag Publishers.

9) M. Kinoshita, M. Wada, S. Aburaki, and S. Umezawa, *J. Antibiot.* (Tokyo), **24**, 724 (1971).

10) M. Kinoshita, S. Aburaki, and S. Umezawa, *ibid.*, **25**, 373 (1972).

in the *N*-acyl-L-threonine moiety of I will minimize any danger of the racemization which otherwise as in the case of III, may be caused on the α-carbon atom of the *N*-acylated amino acid unit by the activation of its carboxyl group with trifluoroacetic anhydride at higher temperature, even if α-amino group is protected by an urethan type acyl group such as benzyloxycarbonyl.

The next problem was the synthesis of the natural type of racemic diastereomer of 2-butyl-4-hydroxy-3-isovaleryloxypentanoic acid *t*-butyl ester, which was a useful intermediate for the synthesis of the hydroxy-ester-acids of type I. Of the four racemic diastereomers (**6a**—**6d**) which were prepared by the following synthetic route (Chart 1), the major isomer **6a** was the most promising intermediate, because it could be converted to the natural type (±) blastmycinone **7a**.

The starting material 2-benzyloxypropanal **2** was prepared from methyl 2-benzyloxypropanoate by reduction with diisobutylaluminum hydride.[11] The condensation of this compound with *t*-butyl 2-bromohexanoate **3** was effected smoothly by a modified Reformatsky reaction according to Moriwake[12] using magnesium instead of zinc to yield a mixture of four possible racemic diastereomers of *t*-butyl 4-benzyloxy-2-butyl-3-hydroxypentanoate (**4a**, **4b**, **4c**, and **4d**).

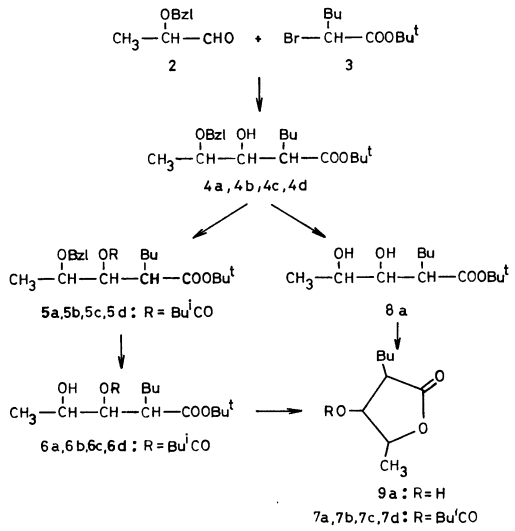


Chart 1.

Preparative isolation of these diastereomers was performed by silica gel column chromatography of the reaction product to give a mixture **4a**+**4b**, **4c**, and **4d** in yields of 43, 12.4, and 6.8% based on **2**, respectively. Repeated column chromatography of fractions containing **4a** and **4b** afforded in a small quantity a homogeneous sample of **4a** and a sample of **4b** contaminated by a trace of **4a**. The samples of **4a** and **4b** showed very similar R_f-values on tlc with several kinds of solvent system, however, their PMR spectra apparently differed in the chemical shifts of *t*-butyl and terminal methyl protons, and this difference was useful for inspection of homogeneity of these diastereomers. Since the preparative separation of **4a** from **4b** by column chromatography was impracticable in this stage, the diastereomeric separation had to be carried over to the next synthetic step.

The mixture **4a**+**4b** obtained above was treated with isovaleric anhydride in pyridine to afford a diastereomeric mixture of *t*-butyl 4-benzyloxy-2-butyl-3-isovaleryloxypentanoate **5a**+**5b**. Column chromatographic separation of the diastereomers was effectively carried out to give **5a** (75%) and **5b** (17%) in homogeneous states, which were identical with the *O*-isovalerylation products derived from the isolated samples of **4a** and **4b**, respectively. These results indicated that the above-mentioned Reformatsky reaction yielded the major diastereomer **4a** with a 56% stereoselectivity.

By the same way other diastereomers, **5c** and **5d** were obtained from **4c** and **4d**, respectively. The four diastereomers, **5a**, **5b**, **5c**, and **5d** were hydrogenolysed over palladium black to afford the corresponding diastereomeric *t*-butyl 2-butyl-4-hydroxy-3-isovaleryloxypentanoates, **6a**, **6b**, **6c**, and **6d**, respectively. In order to determine which diastereomer of the four (**6a**—**6d**) is the objective type of racemic 4-hydroxy-ester, they were converted to the corresponding stable 1,4-lactones, **7a**, **7b**, **7c**, and **7d** by treatment with 5N hydrogen chlorde in dioxane. The resulted structurally homogeneous diastereomeric lactones were readily distinguished by tlc and PMR. From comparison of these lactones with natural antimycin lactone based on tlc, glc, and PMR, it was concluded that the lactone **7a** derived from the major diastereomer **6a** was fortunately the natural type of racemic diastereomer, (±)blastmycinone.

The comparison was first carried out by tlc. The thin-layer chromatogram of natural antimycin lactone obtained from antimycin A complex with a solvent system petroleum ether–diisopropyl ether (7:4) showed a single spot having the same R_f-value as that of **7a**. The comparison by glc was somewhat complicated. The gas chromatogram of **7a** on a polyester succinate column showed a single peak having the same elution time as that of the major peak of natural blastmycinone, which was found in a gas chromatogram of the antimycin lactone. Recently, Yonehara, *et al.*[13] demonstrated by glc that natural blastmycinone is not homogeneous, but it consists of two isomers differing in the structure of the *O*-alkanoyl group. Consequently, our result could confirm that, in the gas chromatogram, the synthetic lactone **7a** shows the same peak with one of the two peaks of so-called blastmycinone and that the peak having longer retention time between the two, corresponds to the blastmycinone possessing isovaleryl group.

The chemical shifts and coupling constants of ring protons, ring methyl and 3-*O*-isovaleryl methyl protons of **7a** were identical with those of natural blastmycinone[14] and antimycin lactone, however, the PMR spectrum in $CDCl_3$ of **7a** showed no doublet at δ 1.15 present in that of the natural lactone. This signal may

11) L. I. Zakharkin, I. M. Khorlina, *Tetrahedron Lett.*, **1962**, 619.
12) T. Moriwake, *J. Org. Chem.*, **31**, 983 (1966).
13) T. Endo and H. Yonehara, *J. Antibiot.* (Tokyo), **23**, 91 (1970).
14) The PMR chart was kindly provided by Prof. H. Yonehara.

be ascribed to the methyl protons of isomeric alkanoyl group in the blastmycinone subcomponent showing the glc peak of shorter retention time. This PMR observation was consisted with the above-mentioned result based on glc. Furthermore, it was confirmed that all the antimycin lactones obtained from antimycin A complex had the same stereochemistry in respect of their lactone rings.

The above stated structural relationship between **7a** and **4a** was also confirmed by an alternative route *via* (±)blastmycinolactol **9a**. Hydrogenolysis of **4a** over palladium black gave *t*-butyl 2-butyl-3,4-dihydroxypentanoate **8a**, which was treated with trifluoroacetic acid. The resulting crystalline hydroxylactone **9a** was *O*-isovalerylated with isovaleric anhydride in pyridine to afford a structurally homogeneous sample of (±)-blastmycinone **7a**. This synthetic route was applied in the syntheses of optically active (+)blastmycinone (+)**7a** and its enantiomer (−)**7a**.

The freshly prepared 4-hydroxyester **6a** was allowed to react with execss amount of *N*-benzyloxycarbonyl-*O*-*t*-butyl-L-threonine in the presence of dicyclohexylcarbodiimide (DCCI) and pyridine at 0°C, to afford a mixture of diastereomeric masked hydroxyester-acids (type I), which was chromatographed on a silica gel column to give optically active homogeneous diastereomers (+)**10A** and (−)**10B** in 29 and 27.7% yields based on **6a**, respectively. It is noteworthy that this step is substantially an optical resolution of the racemic 4-hydroxyester **6a**.

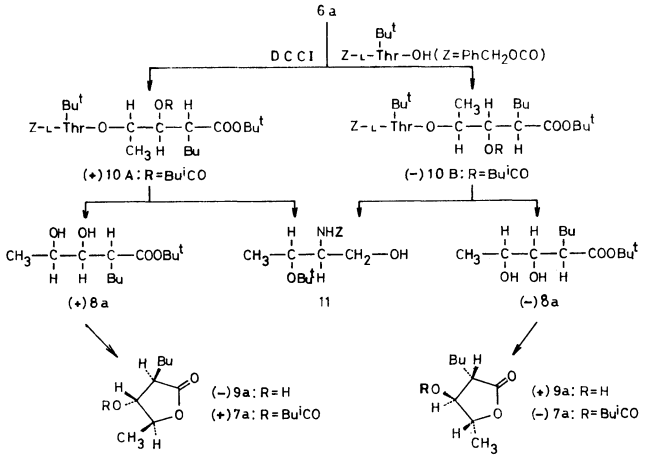


Chart 2.

The configurational assignments of the resolved hydroxyesters present in (+)**10A** and (−)**10B** were undertaken by the following procedure (Chart 2). Selective reduction of (+)**10A** with lithium aluminum hydride at −40°C afforded optically active *t*-butyl (+) 2-butyl-3,4-dihydroxypentanoate (+)**8a** (47.5%) and (−)2-benzyloxycarboxamido-3-*t*-butoxy-1-butanol **11**. De-*t*-butylation of (+)**8a** by the same manner as that of **8a** yielded crystalline (−)blastmycinolactol (−)**9a** with an optical rotation of $[\alpha]_D^{MeOH}$ −18°. Although the rotation of the synthetic specimen was considerably higher than the reported rotation of $[\alpha]_D^{MeOH}$ −5.21° for natural (−)blastmycinolactol[6] obtained from natural (+)blastmycinone, the IR spectrum of (−)**9a** in nujol was very similar to that of natural product.[6] *O*-Isovalerylation of (−)**9a** by the same manner as that of **9a** afforded (+)blastmycinone (+)**7a** with a rotation of $[\alpha]_D^{Chf}$ +10° similar to natural (+)blastmycinone.[6] The PMR spectrum of (+)**7a** was indistinguishable from that of (±)blastmycinone **7a**.

In the same procedure the lithium aluminum hydride reduction of (−)**10B** afforded the enantiomeric dihydroxyester (−)**8a**, from which the corresponding enantiomeric (+)blastmycinolactol (+)**9a** and (−)-blastmycinone (−)**7a** were derived successively.

Recently, we reported in a communication to the editor[10] the absolute configurations of natural (−)-blastmycinolactol and (+)blastmycinone as 2(*R*), 3(*R*), 4(*S*) on the basis of ORD, CD, PMR and through a stereospecific synthesis of the enantiomer (+)**9a** (Chart 2). From these results it was elucidated that (+)**10A** contained an enantiomer of **6a** which had the configuration 2(*R*), 3(*R*), 4(*S*) identical with that of naturally occurring blastmycinone, and that the related racemic diastereomers, **8a**, **6a**, **5a**, and **4a** had a 2,3-*threo*-3,4-*erythro* configuration.

De-*t*-butylation of (+)**10A** with trifluoroacetic acid afforded the desired hydroxyester-acid (type-I, R_3= benzyloxycarbonyl) which was immediately subjected to lactonization. A 0.04M benzene solution of the hydroxyester-acid was heated with about one molar equivalent trifluoroacetic anhydride at 65—70°C for 4 hr. To this mixture additional about one molar trifluoroacetic anhydride was added and was heated at the same temperature for 6 hr. The reaction mixture was immediately evaporated and the residue was chromatographed on a silica gel column to separate fractions containing dilactone compound **12A**. Purification of the crude product by column chromatography afforded **12A** as needles in a 0.8% yield based on (+)**10A**. The compound **12A** was confirmed to be a structurally homogeneous dilactone derivative by elemental analysis, molecular weight determination by mass spectrometry, IR and PMR spectra (Table 1). In this lactonization reaction no other kinds of HBr-ninhydrin positive lactonization product were detected and prolonged heating of the reaction mixture gave rise to a decomposition of **12A** accompanied by rapid formation of some HBr-ninhydrin negative by-products. Attempts to improve the yield of **12A** by use of other kinds of solvent or by changing the quantity of trifluoroacetic anhydride and the reaction temperature were unsuccessful.

On the other hand, the lactonization reaction of the diastereomeric hydroxyester-acid obtained from (−)-

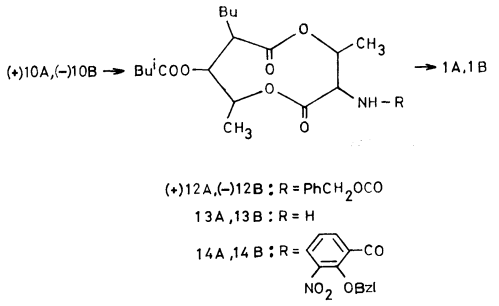


Chart 3.

TABLE 1. PMR-SPECTRA DATA (IN $CDCl_3$). CHEMICAL SHIFTS (δ VALUES) AND COUPLING CONSTANTS (Hz) AT 100 MHz

Compound:	12 A	12 B	14 A	14 B	1 A	Natural antimycin A	1 B
Concentration (*ca.* %)	3	7	3	8	3	3	5
$(C\underline{H}_3)_2CH$	0.96(d)	0.97(d)	0.97(d)	0.97(d)	0.98(d)	0.99(d)	0.99(d)
9-CH_3	1.25(d)[a]	1.30(d)[a]	1.27(d)[a]	1.27(d)[a]	1.29(d)	1.29(d)[a]	1.32(d)[a]
4-CH_3	1.27(d)[a]	1.53(d)[a]	1.09(d)[a]	1.39(d)[a]	1.32(d)	1.32(d)[a]	1.69(d)[a]
H-7	2.46(m)[a]	2.51(m)	2.3—2.6	2.4—2.6	2.54(m)	2.54(m)	2.56(m)
H-9	4.89(dq)[a]	4.75(dq)	4.85(dq)[a]	4.71(dq)	4.96(dq)[a]	4.97(dq)[a]	4.83(dq)
$PhC\underline{H}_2OCO$	5.11(s)	5.11(s)					
H-8	4.8—5.2	5.08(dd)[a]	4.8—5.3	5.16(dd)	5.13(dd)	5.13(dd)[a]	5.14(dd)[a]
H-3	4.8—5.2	4.63(dd)[a]	5.17(dd)	5.11(dd)	5.32(dd)	5.32(dd)[a]	5.13(dd)[a]
$PhC\underline{H}_2Ar$			5.17(s)	5.26(s)			
H-4	5.55(dq)[a]	5.10(dq)[a]	5.55(dq)	4.9—5.4	5.75(dq)	5.75(dq)[a]	5.06(dq)[a]
H-5′			7.35(dd)	7.37(dd)	6.91(dd)	6.90(dd)	6.98(dd)
3-NH	5.48(d)	5.78(d)	8.03(d)	7.9—8.3	7.09(d)	7.10(d)	7.81(d)
H-4′(or H-6′)			7.95(dd)	7.99(dd)	7.25(dd)	7.25(dd)[b]	7.29(dd)
$ArN\underline{H}CHO$					7.97(d)	8.01(d)[b]	7.97(d)
$ArNHC\underline{H}O$					8.50(d)	8.50(d)[b]	8.51(d)
H-6′(or H-4′)			8.25(dd)	8.20(dd)	8.54(dd)	8.54(dd)[b]	8.66(dd)
OH(Ar)					12.55(s)	12.57(s)	12.70(s)
$J_{3,4}$	7.8	6.0	7.6	5.0	7.7	7.7	5.0
J_{4,CH_3}	7.0	6.8	7.0	7.0	7.0	7.0	6.8
$J_{7,8}$		8.8		9.0	*ca.* 9.5	9.5	9.0
$J_{8,9}$		9.8		9.8	*ca.* 10	10.0	9.8
J_{9,CH_3}	6.2	6.2	6.2	6.2	6.2	6.2	6.2
$J_{3,NH}$	*ca.* 8	10.0	7.6	9.0	7.7	7.7	9.0
$J_{4',5'}$ or $J_{5',6'}$			8.0	7.8	8.0	8.0	8.2
$J_{4',6'}$			2.0	2.0	1.6	1.6	1.2

a) Assignment verified by spin decoupling.
b) Splittings were verified by the spectrum of *ca.* 7% solution.

10B was carried out in the condition similar to that described above with exception of the prolonged reaction time (21 hr) to afford a dilactone compound **12B** as needles in a 3.1% yield. Elemental analysis, molecular weight determination, IR and PMR spectra showed **12B** to be a homogeneous diastereomer of **12A**. In this lactonization reaction, no HBr-ninhydrin positive lactones other than **12B** were also detected, however, the formation of two isomeric lactones, (−)**7a** and (−)**7b** as HBr-ninhydrin negative by-products, was found and they were isolated and characterized by tlc, PMR, and optical rotations.

The benzyloxycarbonyl group of **12A** was removed by catalytic hydrogenolysis and the resulting free amino dilactone **13A** was *N*-acylated with *O*-benzyl-3-nitrosalicylic acid *N*-hydroxysuccinimide ester.[1] The product **14A** (65% yield based on **12A**) was then hydrogenolyzed on palladium black and, finally, *N*-formylated with formic acid and DCCI. The final product was isolated by preparative layer chromatography (plc) and purified by recrystallization to afford antimycin A_3 (**1A**) as needles in a 27.3% yield based on **12A**.

The synthetic specimen showed mp 174.0—174.5°C and $[\alpha]_D^{chf}$ +80°, which were identical with the reported values for the natural antimycin A_3.[15] The mass spectrum of **12A** showed a single molecular ion peak at *m/e* 520. The UV absorptions in methanol and IR absorptions in carbon tetrachloride assignable to lactone carbonyl, formamido and aromatic amido groups were also indistinguishable from those observed in the corresponding spectra of an authentic antimycin A complex.[16] The 100 MHz PMR spectrum of *ca.* 3% deuteriochloroform solution of the synthetic specimen measured in a micro cell was very similar to that of a 3% solution of the authentic sample in a standard cell as shown in Table 1.

Inspection of the 100 MHz PMR spectra in deuteriochloroform and deuteriobenzene of the authentic antimycin A complex revealed that all the antimycin components had the same stereochemistry in respect of their dilactone rings. In view of these points, we came to the conclusion that the synthetic antimycin A_3 had the same ring configuration and conformation as those of natural antimycin A. Through this synthesis of antimycin A_3, it has been confirmed that naturally occurring antimycin A_3 retained in its dilactone structure, the same configuration of (+)blastmycinone derived from the parent antibiotic.

De-*N*-benzyloxycarbonylation of (−)**12B** afforded the free amino dilactone **13B** accompanied by (−)-

15) K. Uzu, H. Kato, K. Kumabe, and H. Harada, *J. Antibiot., Ser. A*, **14**, 205 (1961).

16) The sample of antimycin A complex was generously supplied by the Kyowa Hakko Kogyo Co.: mp 139.0—139.5°C; $[\alpha]_D^{24}$ +80° (*c* 0.4, chloroform); molecular ions of the components at *m/e* (relaitve intensities), 562 (1), 548 (12), 534 (7), 520 (16), 506 (2), and 492 (2).

blastmycinone(−)**7a**, which was considered to be resulted by a spontaneous fragmentation (self-saponification) of **13B**. The reaction product was immediately *N*-acylated to give *N*-(*O*-benzyl-3-nitro) salicyloyl derivative **14B** (24%) and (−)**7a** (24%). The low yield of **14B** may be accounted for by the degradation of **13B** which proceeded parallel to the *N*-acylation reaction.

Preparation of **1B** from **14B** was smoothly carried out by the same procedure as that of **1A** from **14A** to afford the antimycin A_3 diastereomer **1B** with $[\alpha]_D^{Chf}$ −5° as a glassy solid in a 83% yield. The UV spectrum of **1B** was identical with that of **1A**, however, the IR spectrum of **1B** in carbon tetrachloride showed two lactone carbonyl bands at 1758 and 1735 cm^{-1}, instead of single band at 1756 cm^{-1} in that of **1A**. The PMR spectrum of *ca.* 5% deuteriochloroform solution of **1B** considerably differed from that of **1A**, especially, in the chemical shifts of ring protons (H-3, H-4), ring methyl protons (4-CH_3) and aromatic amide proton (3-NH) (Table 1).

The PMR spectra data tabulated in Table 1 indicated that such chracteristic differences between the spectra of **1A** and **1B** were also observed between those of other natural and unnatural types of dilactone derivatives, **12A—12B** and **14A—14B**. Therefore, this observation shows that the configurations and conformations of both types of dilactone compounds may be closely reflected on their PMR spectra.

We have already proposed the absolute configuration of antimycin A_3 as **1A**[10] based on the abovementioned configurational correlation between antimycin A_3 and natural (+)blastmycinone whose absolute configuration had independently been determined as (+)**7a**. In a similar manner the absolute configuration of the synthetic diastereomer of antimycin A_3 which was correlated to the enantiomeric (−)blastmycinone (−)**7a** should be determined as **1B**.

On paper chromatography with the solvent system water-ethanol-acetone (7:2:1)[17] which was generally used for detection of antimycin A components, both synthetic specimens of antimycin A_3 and its diastereomer showed, on the bioautogram (test organism, *Piricularia orizae*), the single spot corresponding to that of the natural antimycin A_3 in the antimycin complex.

Table 2. MIC(mcg/ml) of synthetic antimycin A_3(**1A**), natural antimycin A complex and synthetic diastereomer (**1B**)

Organisms	1 A	Natural antimycin A	1 B
Candida albicans 3147	12.5	12.5	>25
Candida krusei	50	50	>25
Trichophyton asteroides 429	50	50	>25
Trichophyton mentagrophytes 833	50	50	25
Piricularia oryzae	0.025	0.025	3.12
Pellicularia filamentosa (Sasaki)	25	25	25

Medium: 1% Glucose nutrient agar, 27°C.

Minimal inhibitory concentrations (MIC) of the synthetic specimens **1A** and **1B** and natural antimycin A complex were summarized in Table 2.

Experimental

Melting points were determined on a micro hot stage and are uncorrected unless otherwise noted. UV spectra were taken on a Hitachi Perkin-Elmer UV-VIS spectrometer 139 and IR spectra on a Hitachi IPI-2 spectrometer. PMR spectra were recorded on Varian A-60D and HA-100D spectrometers using TMS as an internal standard. Optical rotations were measured with a Zeiss Photoelectric Precision Polarimeter. Tlc was carried out on WAKOGEL B-5 (Wako Pure Chemical Industries, Ltd.) and silica gel column chromatography on WAKOGEL C-200 which was activated at 110°C for 1 hr. The following solvent systems were used: (A) petroleum ether–diisopropyl ether (IPE) (3:1), (B) hexane–butanone–acetone (20:1:1), (C) hexane–IPE (10:1), (D) benzene–acetone (20:1), (E) benzene–IPE (7:1), (F) petroleum ether–IPE (7:4), (G) benzene–ethyl actate (3:2), (H) petroleum ether–IPE (2:1), (I) benzene–acetone (10:1), (J) ibid. (15:1), (K) hexane–acetone (3:1), (L) hexane–ethyl acetate (3:1), and (M) ibid. (5:3). In general, all concentrations were carried out at reduced pressure below 40°C.

1) Methyl 2-Benzyloxypropanoate. 2-Benzyloxypropanoic acid[18] was treated with methanol and concd H_2SO_4 in a usual manner to obtain the ester in a 82% yield, bp 96—98°C/4.5 Torr, $n_D^{23.7}$ 1.4942.

Found: C, 68.36; H, 7.42%. Calcd for $C_{11}H_{14}O_3$: C, 68.03; H, 7.27%.

2) 2-Benzyloxypropanal (2). A solution of methyl 2-benzyloxypropanoate (7.0 g, 36.1 mmol) in hexane (131 ml) was cooled to −50°C and diisobutylaluminum hydride[19] (6.25 g, 44.0 mmol) was added under dry nitrogen. The solution was ketp at −50°C for 2.5 hr before saturated sodium bisulfite solution (200 ml) was added. The mixture was allowed to warm to room temperature and the layers were separated. The hexane layer was extracted several times with saturated sodium bisulfite solution (total 1.8 l). The combined aqueous layers were washed three times with 200 ml-portions of ether to remove a small amount of 2-benzyloxypropanol and then basified with 4N NaOH to pH 11 with cooling. The separated aldehyde was extracted with ether. The ether extract was washed with saturated NaCl sloution, dried over Na_2SO_4 and evaporated to give 5.21 g (88%) of **2** as a colorless oil; ν_{max}^{liq} 1735 cm^{-1} (CHO).

2,4-Dinitrophenylhydrazone of 2: Mp 112—113°C (methanol)

Found: C, 56.06; H, 4.92; N, 16.09%. Calcd for $C_{16}H_{14}O_5N_4$: C, 55.81; H, 4.68; N, 16.27%.

3) t-Butyl 2-Bromohexanoate (3). 2-Bromohexanoic acid was treated with isobutene and catalytic amount of concd H_2SO_4 in usual way to afford a fraction of **3** boiling at 63—67°C/2.5 Torr in a 77% yield. Analytical sample was obtained by silica gel column chromatography of the product with hexane: bp 73.0—74.5°C (bath temp.)/6 Torr; n_D^{25} 1.4452.

Found: C, 48.01; H, 7.69; Br, 31.86%. Calcd for $C_{10}H_{19}O_2Br$: C, 47.82; H, 7.63; Br, 31.82%.

4) t-Butyl 4-Benzyloxy-2-butyl-3-hydroxypentanoate (4).

(a) Modified Reformatsky Reaction of 2 and 3: Fresh magnesium shavings (1.35 g, 55.5 mmol) were covered with dry

17) W. C. Liu and F. M. Strong, *J. Amer. Chem. Soc.*, **81**, 4387 (1959).

18) L. Feldmann and H. O. L. Fischer, *Arch. Biochem.*, **14**, 117 (1947).

19) A 25% (W/V) solution of diisobutylaluminum hydride in hexane (Mitsuwa's Pure Chemicals) was used.

ether (22 ml) and they were activated by addition of few drops of methyl iodide. When the activation reaction had started, a solution of **2**[20] (5.7 g, 34.8 mmol) and **3** (9.3 g, 37.0 mmol) in dry ether (32 ml) was added under stirring at such a rate that the mixture refluxed gently. After stirring and refluxing for additional 2.5 hr in a water bath, the reaction mixture was cooled with ice-salt bath and decomposed with a mixture of cold 10% H_2SO_4 (54 ml) and crushed ice (15 g). The ether layer was washed with 5% $NaHCO_3$ and saturated NaCl solution and evaporated. The oily residue was dissolved in hexane (140 ml) and the solution was washed with saturated sodium bisulfite solution to remove unchanged **2**. The hexane layer was washed with saturated NaCl solution, dried and evaporated to afford a viscous oil (11.1 g) containing four diastereomers (**4a**, **4b**, **4c**, and **4d**) of title compound **4**.

(b) Preparative Separation of the Diastereomers of 4: The product (11.1 g) obtained in (a) was chromatographed on a silica gel column (2.20 kg, 8.0×79 cm) with the solvent system A to give four main fractions which were evaporated. The fraction 1 (R_f 0.40 A, 1.36 g) was purified by a silica gel column (270 g) with the solvent system B to afford a homogeneous diastereomer **4c** (1.23 g): bp 105—107°C (bath temp.)/0.001 Torr; R_f 0.40 A; $\nu_{max}^{CCl_4}$ (0.01M) 3500 (OH), 1725 and 1705 cm^{-1} (ester); δ ($CDCl_3$) 1.44 (s, Bu^t), 1.26 (d, 4-CH_3, J_{4,CH_3}=5.8 Hz).

Found: C, 71.35; H, 9.44%. Calcd for $C_{20}H_{32}O_4$: C, 71.39; H, 9.59%.

Additional sample of **4c** (0.23 g) was obtained by chromatographic separation of the fraction 2 (vide infra) and total yield of **4c** amounted to 1.46 g (12.4% based on **2**).

The fraction 2 (R_f 0.40 and 0.34 A, 1.14 g) was separated into two fractions by a silica gel column (230 g) with the solvent system A. The first fraction (R_f 0.40 A) gave **4c** (0.23 g) and the second one (R_f 0.34 A) afforded the product (0.77 g) which was shown to be a mixture of diastereomers **4a** and **4b** by inspection of its PMR spectrum, similar to the following fraction 3.

The fraction 3 (R_f 0.34 A) gave the product (4.33 g) which consisted of major diastereomer **4a** contaminated by minor diastereomer **4b**: δ ($CDCl_3$) 1.41 and 1.44 (s, Bu^t), 1.24 and 1.26 (d, 4-CH_3). Microanalysis of a micro distillated sample agreed with the molecular formula $C_{20}H_{32}O_4$ (Found: C, 71.44; H, 9.61%). The total yield of **4a** accompanied by **4b** thus amounted to 5.10 g (43% based on **2**). The content of **4a** in the combined products may be estimated to be *ca.* 83% on the basis of the result of Exp. 5(a).

The fraction 4 (R_f 0.23 A (main spot), 2.03 g) was purified by a silica gel column (400 g) with the solvent system B to give a homogeneous isomer **4d** (0.80 g, 6.8% based on **2**): bp 120—123°C (bath temp.)/0.005 Torr; R_f 0.23 A; $\nu_{max}^{CCl_4}$ (0.01M) 3570, 3470 (OH), 1735 and 1715 cm^{-1} (ester); δ ($CDCl_3$) 1.24 (d, 4-CH_3, J_{4,CH_3}=6.1 Hz) and 1.43 (s, Bu^t).

Found: C, 71.56; H, 9.48%. Calcd for $C_{20}H_{32}O_4$: C, 71.39; H, 9.59%.

(c) Isolation of the Major Diastereomer 4a: The Reformatsky reaction product (15.0 g) was subjected to silica gel column chromatography (1.5 kg, solvent system A) and the eluted fractions showing R_f 0.34 A were inspected by PMR and the fractions containing homogeneous **4a** were collected and evaporated: yield 1.55 g; bp 108—113°C (bath temp.)/0.001 Torr; R_f 0.34 A; $\nu_{max}^{CCl_4}$ (0.01M) 3590 (OH), 1725 and 1710 cm^{-1} (ester); δ($CDCl_3$) 1.24 (d, 4-CH_3, J_{4,CH_3}=6.0 Hz), 1.41 (s, Bu^t), 2.47 (m, H-2), 3.52 (dq, H-4, $J_{3,4}$=4.7 Hz), and 3.89 (dd, H-3, $J_{2,3}$=6.8 Hz).

Found: C, 71.46; H, 9.65%. Calcd for $C_{20}H_{32}O_4$: C, 71.39; H, 9.59%.

(d) Isolation of 4b: Repeated column chromatography (solvent system A) of several fractions which contained the products showing R_f 0.40 and 0.34 A (main spot) in the experiment 4(c) afforded a small amount of the fraction consisting almostly of the isomer **4b**. Although the PMR spectrum of this sample of **4b** revealed that it was still contaminated by a trace of **4a**, the main PMR signals corresponding to **4b** itself was observed therein as followed; δ ($CDCl_3$) 1.26 (d, 4-CH_3, J_{4,CH_3}=6.3 Hz), 1.43 (s, Bu^t), and 3.40—3.80 (m, overlapped 3-H and 4-H).

5) t-Butyl 4-Benzyloxy-2-butyl-3-isovaleryloxypentanoate (5a and 5b). *(a)*: A sample (1.86 g, 5.53 mmol) of the total mixture of **4a** and **4b** obtained in the experiment 4(b) was dissolved in dry pyridine (24 ml) and to this a solution of isovaleric anhydride (2.06 g, 11.0 mmol) in pyridine (12 ml) was added. After standing this mixture for 24 hr at room temperature, an additional isovaleric anhydride (1.03 g in 2 ml of pyridine) was added and kept for 48 hr in the same temperature. The reaction mixture was partitioned between water and ether and the aqueous layer was extracted with ether. The combined organic layers were washed with aqueous 10% citric acid, 5% $NaHCO_3$ and saturated NaCl solutions, successively. The dried ether solution was evaporated and the residue (2.33 g) was chromatographed on a silica gel column (240 g, 3.4×45 cm) with the solvent system C to afford three fractions which were inspected by tlc (system C). The first fraction (R_f 0.43C) gave the homogeneous isomer **5a** (1.42 g, 61.5%) corresponding to **4a**. Additional **5a** (0.31 g) was obtained by column chromatography (silica gel 60 g, solvent system C) of the second fraction (0.40 g) which consisted of **5a** contaminated by a trace of **5b**. Total yield of **5a** was 1.73 g (75%). This sample (190 mg) was again chromatographed on silica gel (19 g) with the solvent system D and the fraction of **5a** (125 mg) was subjected to micro distillation to afford an analytical sample of **5a**: bp 105—119°C (bath temp.)/0.002 Torr; R_f 0.43C; δ($CDCl_3$) 1.21 (d, 4-CH_3, J_{4,CH_3}=6.4 Hz), 1.38 (s, Bu^t), 2.55 (m, H-2), 3.58 (dq, H-4, $J_{3,4}$=4.0 Hz), and 5.44 (dd, H-3, $J_{2,3}$=8.2 Hz).

Found: C, 71.56; H, 9.69%. Calcd for $C_{25}H_{40}O_5$: C, 71.39; H, 9.59%.

The third fraction (R_f 0.37C) gave the diastereomer **5b** (0.39 g, 16.9%) corresponding to **4b** in a homogeneous state. Rechromatography of this sample using the solvent system C followed by micro distillation afforded an analytical sample of **5b**: bp 155—158°C (bath temp.)/0.04 Torr; R_f 0.37C; δ($CDCl_3$) 1.16 (d, 4-CH_3, J_{4,CH_3}=6.3 Hz), 1.42 (s, Bu^t), 3.73 (dq, H-4, $J_{3,4}$=3.2 Hz), and 5.27 (dd, H-3, $J_{2,3}$=9.1 Hz).

Found: C, 71.59; H, 9.70%. Calcd for $C_{25}H_{40}O_5$: C, 71.39; H, 9.59%.

(b): A sample (1.39 g) of **4a** isolated in the experiment 4(c) was *O*-isovalerylated by the same procedure as mentioned above to give a homogeneous sample of **5a** (1.45 g, 84%).

(c): A sample of **4b** in the experiment 4(d) was also *O*-isovalerylated and purified through a silica gel column to afford a homogeneous sample of **5b** in a 83% yield.

6) t-Butyl 4-Benzyloxy-2-butyl-3-isovaleryloxypentanoate (5c and 5d). *O*-Isovalerylations of **4c** and **4d** were performed by the same procedure as described in the experiment 5 to give the corresponding **5c** and **5d** in good yields, respectively. **5c**: bp 140—142°C (bath temp.)/0.01 Torr; δ($CDCl_3$) 1.18 (d, 4-CH_3, J_{4,CH_3}=6.2 Hz), 1.45 (s, Bu^t), 3.73 (dq,

20) The product **2** obtained in Exp. 2 was thoroughly dried under highly reduced pressure (0.001 Torr) at room temperature before use.

H-4, $J_{3,4}$=5.2 Hz), and 5.34 (dd, H-3, $J_{2,3}$=6.5 Hz).

Found: C, 71.57; H, 9.75%. Calcd for $C_{25}H_{40}O_5$: C, 71.39; H, 9.59%.

5d: bp 126—132°C (bath temp.)/0.002 Torr; $\delta(CDCl_3)$ 1.20 (d, 4-CH_3, $J_{4,CH3}$=6.5 Hz), 1.42 (s, Bu^t), 3.73 (dq, H-4, $J_{3,4}$=4.0 Hz), and 5.20 (dd, H-3, $J_{2,3}$=8.5 Hz).

Found: C, 71.10; H, 9.73%. Calcd for $C_{25}H_{40}O_5$: C, 71.39; H, 5.59%.

*7) t-Butyl 2-Butyl-4-hydroxy-3-isovaleryloxypentanoate (**6a**, **6b**, **6c**, and **6d**).* To a solution of **5a** (2.60 g) in methanol (65 ml) was added freshly prepared palladium black (*ca.* 800 mg) and the mixture was vibrated for 1 hr under bubbling with hydrogen. Completion of the hydrogenolysis was confirmed by tlc (solvent system E). The filtered solution was evaporated to give **6a** (1.98 g, 97%) as a colorless oil: ν_{max}^{liq} 3460 (OH) and 1730 cm^{-1} (ester); $\delta(CDCl_3)$ 1.18 (d, 4-CH_3, J_{4,CH_3}=6.5 Hz), 1.45 (s, Bu^t), 3.89 (dq, H-4, $J_{3,4}$=5.0 Hz), and 5.10 (dd, H-3, $J_{2,3}$=7.8 Hz).

By the same procedure, the diastereomers, **6b**, **6c**, and **6d** were also obtained from the corresponding diastereomers, **5b**, **5c**, and **5d** in a yield of 56,[21] 86,[21] and 96%, respectively. The products, **6a**, **6b**, **6c**, and **6d** were immediately used for the subsequent syntheses without further purifications, because their instability for silica gel chromatography or distillation.

*8) 2-Butyl-4-hydroxy-3-isovaleryloxypentanoic Acid-1, 4-Lactone [(±)Blastmycinone] (**7a**).* *(a)*: A sample (41.2 mg) of **6a** was dissolved in 0.08 ml of 5N HCl in dry dioxane and the solution was allowed to stand for 1 hr at room temperature. The reaction mixture was evaporated to afford **7a** (28.8 mg, 90%) as a colorless oil: bp 125—130°C (bath temp.)/8 Torr; R_f 0.66F; glc (polyester succinate on Shimalite 80—100 mesh, 190°C, He gas 125 ml/min) retention time (min) 7.8; $\nu_{max}^{CCl_4}$ 1782 (1,4-lactone) and 1754 cm^{-1} (ester); $\delta(CDCl_3)$ 1.45 (d, 4-CH_3, J_{4,CH_3}=6.5 Hz), 2.69 (m, H-2), 4.37 (dq, H-4, $J_{3,4}$=4.5 Hz), and 4.95 (dd, H-3, $J_{2,3}$=5.8Hz).

Found: C, 65.40; H, 9.16%. Calcd for $C_{14}H_{24}O_4$: C, 65.59; H, 9.44%.

(b): To a solution of (±)blastmycinolactol **9a** (9.7 mg) in dry pyridine (0.76 ml) was added isovaleric anhydride (0.04 ml). The mixture was kept at room temperature for 40 hr. The reaction mixture was partitioned between water and petroleum ether. The aqueous layer was extracted with petroleum ether and the combined organic layers were washed with aqueous 10% citric acid, 5% $NaHCO_3$ and saturated NaCl solution, successively. The dried solution was evaporated and the residue (14 mg) was purified through a silica gel column (2 g) with the solvent system F to give a sample of **7a** (7 mg, 50%), which was identified to the specimen of **7a** obtained in Exp. 8(a) by tlc, glc, and PMR criteria.

*9) Diastereomers, **7b**, **7c**, and **7d** of (±)Blastmycinone **7a**.* Samples of **6b**, **6c**, and **6d** were treated with 5N HCl in dioxane [Exp. 8(a)] to afford the corresponding diastereomeric lactones, **7b**, **7c**, and **7d**, in a yield of 91, 77, and 86%, respectively. The following data were obtained. **7b**: bp 125—130°C (bath temp.)/2.0 Torr; R_f 0.60F; $\nu_{max}^{CCl_4}$ 1798 (lactone) and 1745 cm^{-1} (ester); $\delta(CDCl_3)$ 1.36 (d, 4-CH_3, J_{4,CH_3}=6.5 Hz), 2.65 (m, H-2), 4.28 (dq, H-4, $J_{3,4}$=5.0 Hz), and 5.24 (dd, H-3, $J_{2,3}$=3.0 Hz).

Found: C, 65.76; H, 9.36%. Calcd for $C_{14}H_{24}O_4$: C, 65.59; H, 9.44%.

7c: bp 114—117°C (bath temp.)/2.0 Torr; R_f 0.48F; $\nu_{max}^{CCl_4}$ 1795 (lactone) and 1755 cm^{-1} (ester); $\delta(CDCl_3)$ 1.40 (d, 4-CH_3, J_{4,CH_3}=6.5 Hz), 2.72 (m, H-2), 4.51 (dq, H-4, $J_{3,4}$=0.7 Hz), and 5.20 (dd, H-3, $J_{2,3}$=6.0 Hz).

Found: C, 65.70; H, 9.41%. Calcd for $C_{14}H_{24}O_4$: C, 65.59; H, 9.44%.

7d: bp 135—139°C (bath temp.)/1.0 Torr; mp 38.5—39.0°C; R_f 0.33F; $\nu_{max}^{CCl_4}$ 1795 (lactone) and 1755 cm^{-1} (ester); $\delta(CDCl_3)$ 1.32 (d, 4-CH_3, $J_{4,CH3}$=6.5 Hz), 2.77 (m, H-2), 4.63 (dq, H-4, $J_{3,4}$=3.4 Hz), and 5.68 (dd, H-3, $J_{2,3}$=5.5 Hz).

Found: C, 65.80; H, 9.39%. Calcd for $C_{14}H_{24}O_4$: C, 65.59; H, 9.44%.

*10) 2-Butyl-3,4-dihydroxypentanoic Acid-1,4-Lactone, [(±)-Blastmycinolactol] (**9a**).* A solution of **4a** (23.5 mg) in methanol (3 ml) was stirred with palladium black for 40 min under bubbling with hydrogen. The reaction mixture was filtered and evaporated to give *t*-butyl 2-butyl-3,4-dihydroxypentanoate **8a** (22 mg) as a crystalline solid. The product **8a** was dissolved in trifluoroacetic acid (0.5 ml) and kept for 15 min at room temperature. The solution was evaporated and the syrupy residue was purified by a silica gel column (3 g) with the solvent system G to afford a crystalline product **9a** (12.7 mg, 89%). The product was recrystallized from ethyl acetate–petroleum ether to give a pure sample of **9a** (9.7 mg): mp 49.5—51.0°C; ν_{max}^{KBr} 3440 (OH) and 1735 cm^{-1} (lactone); $\delta(CDCl_3)$ 1.45 (d, 4-CH_3, J_{4,CH_3}=6.2 Hz), 2.58 (m, H-2), 3.84 (dd, H-3, $J_{2,3}$=8.5 Hz), and 4.25 (dq, H-4, $J_{3,4}$=7.0 Hz).

Found: C, 62.51; H, 9.42%. Calcd for $C_9H_{16}O_3$: C, 62.76; H, 9.36%.

*11) Optically Active Diastereomers [(+) **10A** and (−)**10B**] of t-Butyl 4-(N-Benzyloxycarbonyl-O-t-butyl-L-threonyloxy)-2-butyl-3-isovaleryloxypentanoate Derived from **6a**.* A solution of *N*-benzyloxycarbonyl-*O*-*t*-butyl-L-threonine[22] (1.82 g, 5.88 mmol) in dry ether (4.0 ml) was added dropwise during 20 min to a stirred solution of **6a** (1.94 g, 5.88 mmol), DCCI (1.34 g, 6.52 mmol) and dry pyridine (0.5 ml) in dry ether (6 ml) cooled at 0°C. Stirring at 0°C was continued for an additional hour. After standing at 0°C for 41 hr, to the reaction mixture a solution of DCCI (670 mg) in ether (1.8 ml) and dry pyridine (0.25 ml) was added and then a solution of the threonine derivative (910 mg) in ether (2 ml) was dropped under stirring at 0°C. After stirring at 0°C for 8 hr and standing at 0°C for 41 hr, further additions of DCCI (335 mg), dry pyridine (0.13 ml) and a solution of the threonine derivative (460 mg) in ether (2 ml) were undertaken in the same procedure as in the preceding additions and the reaction mixture was kept at 0°C for 24 hr. The precipitate of *N,N'*-dicyclohexylurea (1.67 g) was filtered off and the filtrate was treated with acetic acid (*ca.* 0.2 ml) under stirring at 0°C for 1 hr. An additional urea was removed and the filtrate was washed with 5% $NaHCO_3$, 10% citric acid and saturated NaCl solutions. The dried solution was evaporated to afford an yellow syrup (5.48 g).

The product (5.48 g) was chromatographed on a silica gel column (1.2 kg, 8×43 cm) with the solvent system H to collect three fractions which were concentrated. The first fraction gave a homogeneous sample of (+)**10A** (R_f 0.57H, 995 mg) and an additional sample of (+)**10A** (115 mg) was obtained by column chromatography of the second fraction (273 mg), whose tlc showed the two spots corresponding to (+)**10A** and (−)**10B**. The combined samples of (+)**10A** (1.11 g) was again chromatographed with the solvent system D to afford a pure sample of (+)**10A** (1.06 g, 29%) as a colorless syrup: $[\alpha]_D^{22}$ +10° (*c* 11.4, chloroform); $\delta(CDCl_3)$

21) In the case of the preparations of **6b** and **6c**, unchanged materials had to be removed through a short silica gel column (solvent system E).

22) E. Schröder, *Ann. Chem.*, **670**, 127 (1963).

1.15 (s, OBu^t), 1.19 (d, 4-CH$_3$, J_{4,CH_3}=6.2 Hz), 1.29 (d, 3'-CH$_3$, J=6.8 Hz), 1.48 (s, COOBu^t), 4.0—4.2 (m, H-2', H-3'), 5.05 (dq, H-4, $J_{3,4}$=3.0 Hz), 5.32 (dd, H-3, $J_{2,3}$=9.0 Hz), and 5.60 (d, 2'-NH, $J_{2',NH}$=9.0 Hz).

Found: C, 65.97; H, 9.10; N, 2.35%. Calcd for $C_{34}H_{55}O_9N$: C, 65.67; H, 8.92; N, 2.25%.

The third fraction afforded a sample of the diastereomer (−)**10B** (R_f 0.50H, 978 mg). To this was added an additional sample of (−)**10B** (102 mg) separated by column chromatography of the second fraction above-mentioned and the combined products (1.08 g) were purified by chromatography (solvent system D) to give a pure sample of (−)**10B** (1.01 g, 27.7%) as a colorless syrup: $[\alpha]_D^{22}$ −6° (*c* 10.1, chloroform); δ(CDCl$_3$) 1.13 (s, OBu^t), 1.19 (d, 4-CH$_3$, J_{4,CH_3}=6.5 Hz), 1.27 (d, 3'-CH$_3$, J_{3',CH_3}=6.8 Hz), 1.48 (s, COOBu^t), 4.1—4.3 (m, H-2', H-3'), 5.02 (dq, H-4, $J_{3,4}$=3.0 Hz), 5.30 (dd, H-3, $J_{2,3}$=8.6 Hz), and 5.53 (d, 2'-NH, $J_{2',NH}$=8.7 Hz).

Found: C, 65.90; H, 8.64; N, 2.29%. Calcd for $C_{34}H_{55}O_9N$: C, 65.67; H, 8.92; N, 2.25%.

*12) t-Butyl (+)(2*R*,3*R*,4*S*)-2-Butyl-3,4-dihydroxypentanoate [(+)**8a**] and Its Enantiomer [(−)**8a**].* A suspension of LiAlH$_4$ (255 mg, 6.73 mmol) in dry tetrahydrofuran (30 ml) was added in one portion to a stirred solution of (+)**10A** (1.05 g, 1.68 mmol) in dry tetrahydrofuran (30 ml) cooled to −45°C in a dry ice-methanol bath. After stirring at −45——40°C for 1 hr, ethyl acetate (4 ml) was added to the reaction mixture. The mixture (pH 9) was gradually allowed to warm to 0°C and acidified to pH 2—3 with 2N HCl aqueous under cooling in an ice-bath. The resulting mixture was extracted with ether and ethereal extract was washed with 5% NaHCO$_3$ and saturated NaCl solution. The dried solution was evaporated to afford a colorless oil (1.09 g). This was chromatographed on a silica gel column (210 g, 4×45 cm) with the solvent system I to collect three fractions. From the first fraction the unchanged (+)**10A** (284 mg) was recovered. The second fraction gave (−)2-benzyloxycarboxamido-3-*t*-butyl-1-butanol (**11**) (302 mg): $[\alpha]_D^{20}$ −8° (*c* 2.19, chloroform).

Found: C, 65.04; H, 8.73; N, 4.70%. Calcd for $C_{16}H_{25}O_4N$: C, 65.06; H, 8.53; N, 4.74%.

The third fraction afforded the title compound (+)**8a** (197 mg, 47.5%): mp 48.0—48.5°C; $[\alpha]_D^{23}$ +16° (*c* 2.35, methanol).

Found: C, 63.56; H, 10.74%. Calcd for $C_{13}H_{26}O_4$: C, 63.38; H, 10.64%.

The another title compound (−)**8a** (116 mg, 44.6%) was obtained from (−)**10B** (655 mg) by the same procedure as described for the preparation of the enantiomer (+)**8a**: mp 48.1—48.7°C; $[\alpha]_D^{21}$ −16° (*c* 1.90, methanol).

Found: C, 63.17; H, 10.44%. Calcd for $C_{13}H_{26}O_4$: C, 63.38; H, 10.64%.

*13) (−)(2*R*,3*R*,4*S*)-2-Butyl-3,4-dihydroxypentanoic Acid-1,4-Lactone [(−)Blastmycinolactol] [(−)**9a**] and Its Enantiomer [(+)**9a**].* By the procedure utilized to obtain **9a** from **8a**, the product (+)**8a** (190 mg) was de-*t*-butylated and lactonized. Purification by chromatography as in the preparation of **9a** followed by recrystallization from ether-petroleum ether afforded (−)**9a** (86.4 mg, 66.5%): mp 50.0—51.0°C; $[\alpha]_D^{22}$ −18° (*c* 1.61, methanol) [lit,[6] mp 49—50°C; $[\alpha]_D^{26}$ −5.27° (*c* 7.8, methanol)].

Found: C, 63.06; H, 9.51%. Calcd for $C_9H_{16}O_3$: C, 62.76; H, 9.36%.

In the same procedure as in the preparation of (−)**9a**, the enantiomer (+)**9a** (80 mg, 70%) was obtained from (−)**8a** (164 mg): mp 50.0—51.0°C; $[\alpha]_D^{23}$ +16° (*c* 1.60, methanol).

Found: C, 62.96; H, 9.56%. Calcd for $C_9H_{16}O_3$: C, 62.76; H, 9.36%.

The PMR spectra (in CDCl$_3$) of (−)**9a** and (+)**9a** were identical with that of (±)blastmycinolactol **9a** and the IR spectra (in nujol) of those were very similar to that[6] of natural product.

*14) (+)(2*R*,3*R*,4*S*)-2-Butyl-4-hydroxy-3-isovaleryloxypentanoic Acid-1,4-Lactone [(+)Blastmycinone] [(+)**7a**] and Its Enantiomer [(−)**7a**].* The synthetic (−)blastmycinolactol (−)**9a** (23.9 mg) was treated with isovaleric anhydride-pyridin. Work up as described in Exp. 8(b) for the preparation of **7a** gave (+)blastmycinone (+)**7a** (15.3 mg, 42.5%) as a colorless oil: $[\alpha]_D^{23}$ +10° (*c* 1.5, chloroform) [lit,[6] $[\alpha]_D^{25}$ +11.5° (*c* 20.8, chloroform)]. This material was indistinguishable from (±)blastmycinone **7a** by PMR, tlc, and glc criteria.

(−)Blastmycinone (−)**7a** (22.6 mg, 46.5%) was obtained from (+)**9a** (32.4 mg) by the same procedure as described in the preparation of (+)**7a**: $[\alpha]_D^{25}$ −10° (*c* 2.26, chloroform). The PMR spectrum of (−)**7a** was identical with that of (+)**7a**.

*15) (+)(3*S*,4*R*,7*R*,8*R*,9*S*)-3-Benzyloxycarboxamido-7-butyl-4,9-dimethyl-1,5-dioxa-8-isovaleryloxycyclononane-2,6-dione [(+)-**12A**].* A mixture of (+)**10A** (2.24 g, 3.6 mmol) and trifluoroacetic acid (27 ml) was kept for 10 min at room temperature and then evaporated below 10°C. The residual syrup was coevaporated with ether repeatedly to remove trifluoroacetic acid. The final residue was dried over NaOH for 2 hr under reduced pressure to afford a syrupy de-*t*-butylated product, hydroxyester-acid. The porduct was immediately dissolved in dry benzene (97 ml) and to this was added trifluoroacetic anhydride (0.54 ml, 3.87 mmol). The mixture was heated at 65—70°C in an oil bath and the reaction course was monitored by tlc (solvent system J). After 2—3 hr a faint spot (R_f 0.77 J, HBr-ninhydrin positive) of (+)**12A** and two HBr-ninhydrin negative spots (R_f 0.60, 0.42J) of by-products appeared on tlc. After an additional hour a new HBr-ninhydrin negative spot (R_f 0.95J) appeared and to the reaction mixture was again added trifluoroacetic anhydride (0.54 ml). The heating was further continued for 6 hr, during which the spot of (+)**12A** became more definite, however, rapid formation of the two by-products (R_f 0.60 and 0.95J) was also observed. The reaction mixture was then immediately cooled and evaporated to dryness below 10°C to afford a syrup (2.20 g). As soon as possible the syrup was chromatographed on a silica gel column (300 g, 4.7×50 cm) with the solvent system J to collect fractions containing (+)**12A** (R_f 0.77J). The combined fractions were evaporated. The residual semicrystalline product (*ca.* 25 mg) was again chromatographed on silica gel (2.3 g) with the solvent system F to afford crystals of (+)**12A** (14.2 mg, 0.8%), whose tlc with solvent system K, J, and F showed a single spot. The crystals (14 mg) was recrystallized from ethyl acetate-petroleum ether to give an analytical sample of (+)**12A** (8.2 mg) as long needles: mp 109.0—109.5°C; $[\alpha]_D^{25}$ +70° (*c* 0.5, chloroform); $\nu_{max}^{CCl_4}$ (0.01M) 3440 (NH) and 1756, 1738 cm^{-1} (ester and amide); molecular ion at *m/e* 491.2473 (calcd, 491.2519).

Found: C, 63.65; H, 7.41; N, 2.61%. Calcd for $C_{26}H_{37}O_8N$: C, 63.52; H, 7.59; N, 2.85%.

*16) (−)(3*S*,4*R*,7*S*,8*S*,9*R*)-3-Benzyloxycarboxamido-7-butyl-4,9-dimethyl-1,5-dioxa-8-isovaleryloxycyclononane-2,6-dione [(−)-**12B**].* By the procedure of the preceding experiment 15, (−)**10B** (1.77 g, 2.84 mmol) was de-*t*-butylated. The product was immediately dissolved in dry benzene (74 ml) and to this was added trifluoroacetic anhydride (0.40 ml, 2.87 mmol). The mixture was heated at 65—70°C. After 30 min a faint spot (R_f 0.60 K, HBr-ninhydrin positive) of

(−)**12B** and a spot (R_f 0.79 K) of blastmycinone were detected on tlc. The heating was continued for 18 hr, during which the two spots became more definite and then trifluoroacetic anhydride (0.40 ml) was again added. After heating for additional 2 hr, the reaction mixture, whose tlc showed the formations of considerable amount of (−)**12B** (R_f 0.60 K) and blastmycinone (R_f 0.77 K) and of additional ninhydrin negative by-product (R_f 0.67 K), was evaporated below 20°C. The resulting syrup was chromatographed on a silica gel column (300 g, 4.5×40 cm) with the solvent system J to collect fractions containing (−)**12B** (R_f 0.77J) and blastmycinone (R_f 0.81J), which were evaporated. The residue (120 mg) was again chromatographed on a silica gel column (12 g, 1.2×25 cm) with the solvent system F to afford the following fractions:

Fraction 1. (−)blastmycinone (−)**7a**; 9.0 mg; $[\alpha]_D^{Chf}$ −9°
Fraction 2. (−)**7a**+(−)**7b** ; 15.2 mg
Fraction 3. (−)**7b** ; 22.3 mg; $[\alpha]_D^{Chf}$ −11°
Fraction 4. (−)**12B** (crystals) ; 43.5 mg (3.1% yield)

The oily products obtained from the fraction 1 and 3 were confirmed to be (−)**7a** and (−)**7b**, respectively, by PMR and optical rotations. The crystals obtained from the fraction 4 showed a single spot on each tlc with the solvent systems, K, J, and F. Recrystallization of the product (43 mg) from petroleum ether gave an analytical sample of (−)**12B** (25.7 mg): mp 87.0—87.5°C; $[\alpha]_D^{25}$ −18° (*c* 1.9, chloroform); $\nu_{max}^{CCl_4}$ (0.01M) 3428 (NH), 1758 and 1738 cm^{-1} (ester and amide); molecular ion at *m/e* 491.2451 (calcd, 491.2519).

Found: C, 63.75; H, 7.57; N, 3.12%. Calcd for $C_{26}H_{37}O_8N$: C, 63.52; H, 7.59; N, 2.85%.

17) Antimycin A_3 (1A). (a) Amino Dilactone (13A): A solution of (+)**12A** (8.1 mg) in methanol (3 ml) was stirred with palladium black for 35 min under bubbling with hydrogen gas. The filtered reduction mixture was evaporated to yield the free amino dilactone **13A** (5.9 mg), whose tlc showed a single sopt detected by ninhydrin and 20% H_2SO_4 reagents.

(b): To a solution of **13A** (5.9 mg) in dry tetrahydrofuran (0.08 ml) was added *O*-benzyl-3-nitrosalicylic acid *N*-hydroxysuccinimide ester (6.2 mg). The mixture was kept for 3 hr at 36°C in an incubator. The reaction mixture (pH 4) was adjusted to pH 6 by addition of triethylamine and again incubated for 25 hr. The resulting solution was evaporated and the residue was chromatographed on silica gel column (0.85 g) with the solvent system L to afford *N*-(*O*-benzyl-3-nitro)salicyloyl derivative **14A** (6.4 mg, 65%) as a yellow glassy solid: $\nu_{max}^{CCl_4}$ (0.01M) 3390 (NH), 1749 (lactone) and 1675, 1603 cm^{-1} (amide).

(c): A solution of **14A** (6.2 mg) in methanol (3 ml) was stirred with palladium black under bubbling with hydrogen gas for 10 min. The filtered solution was evaporated to give a crystalline solid (5 mg). The product was dissolved in tetrahydrofuran (0.06 ml) and to this was added DCCI (4.5 mg) and 98% formic acid (0.8 μl) under ice-cooling. After standing for 4 hr in a refrigerator, the reaction mixture was evaporated and the residue was subjected to plc (one 20×20 cm silica gel plate) with the solvent system M. The strongest fluorescent band which showed the same R_f-value as that of authentic antimycin A complex, was collected and extracted with ether to afford pale orange crystals (4.2 mg). They were recrystallized twice from ether–petroleum ether to give an analytical sample of **1A** (2.3 mg, 27.3% based on (+)**12A**) as colorless needles: mp 174.0—174.5°C (corrected) [lit, 174.5—175°C,[15] 168—169°C,[6] 170.5—171.5°C[17]]; $[\alpha]_D^{24}$ +80° (*c* 0.2, chloroform) [lit, $[\alpha]_D^{26}$ +79.4° (*c* 1, chloroform),[15] $[\alpha]_D^{26}$ +64.3° (*c* 1.0, chloroform)[17]]; λ_{max}^{MeOH} nm (log ε), 225 (4.52) and 320 (3.86); $\nu_{max}^{CCl_4}$ (0.01M) 3420 (NH), 1756 (ester), 1715 (NHCHO), 1644 (ArCONH), 1610 (ArH) and 1531 cm^{-1} (ArCONH); molecular ion at *m/e* 520.2412 (calcd, 520.2421).

Found: C, 59.72; H, 7.15; N, 5.25%. Calcd for $C_{26}H_{36}O_9N_2$: C, 59.99; H, 6.97; N, 5.38%.

18) Diastereomer of 1A (1B). (a): Amino Dilactone (**13B**). By the procedure of Exp. 17(a), (−)**12B** (29 mg) was hydrogenolyzed to afford a reduction product (19.7 mg) whose tlc (solvent L) showed a major ninhydrin positive spot of **13B** and two definite spots of blastmycinone and other ninhydrin positive product.

(b): The reduction product (19.7 mg) was treated with *O*-benzyl-3-nitrosalicylic acid *N*-hydroxysuccinimide ester (22 mg) by the same procedure for **13A**. The concentrated reaction mixture (50 mg) was chromatographed on a silica gel column (5 g) with the solvent system L to collect two fractions. The first fraction gave a colorless oil of (−)**7a** (3.3 mg, *ca.* 24%); $[\alpha]_D^{25}$ −9° (*c* 2.9, chloroform). The PMR spectrum of this ample was identical with that of the specimen of (−)**7a** obtained in Exp. 14. The second fraction afforded *N*-(*O*-benzyl-3-nitro)salicyloyl derivative of **13B** (**14B**) (9.1 mg, *ca.* 24% yield) as a yellow glassy solid: $\nu_{max}^{CCl_4}$ (0.01M) 3410 (NH), 2750 (ester), 1669 and 1602 cm^{-1} (amide).

(c): A sample of **14B** (23.2 mg) was hydrogenolyzed by the procedure of Exp. 17(c) to afford a reduction product whose tlc (solvent system L) showed a single spot by detection with ninhydrin, ethanolic $FeCl_3$ and 20% H_2SO_4 reagents. The product (18 mg) was *N*-formylated with 98% formic acid (2 μl) and DCCI (9.8 mg) in tetrahydrofuran at 0°C. Filtration of urea followed by concentration gave a yellow syrup (21 mg). The syrup was subjected to plc (two 20×20 cm silica gel plates) with the solvent system M to collect the strongest fluorescent bands. The ethereal extract of these bands was evaporated to afford **1B** (15.9 mg, 83% based on **14B**) as a pale yellow syrup. Further purification by plc gave an analytical sample as a colorless glass, whose tlc showed the same R_f-value as that of **1A**: $[\alpha]_D^{21}$ −5° (*c* 1.6, chloroform); λ_{max}^{MeOH} nm (log ε), 225 (4.55) and 320 (3.72); $\nu_{max}^{CCl_4}$ (0.01M) 3420 (NH), 1758 and 1735 (ester), 1714 (NHCHO), 1646 (ArCONH), 1612 (ArH), and 1531 cm^{-1} (ArCONH).

Found: C, 60.29; H, 7.21; N, 5.22%. Calcd for $C_{26}H_{36}O_9N_2$: C, 59.99; H, 6.97; N, 5.38%.

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