BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 43 897—901 (1970)

The Total Synthesis of Dehexyl-deisovaleryloxy-antimycin A_1*1

Mitsuhiro KINOSHITA and Sumio UMEZAWA

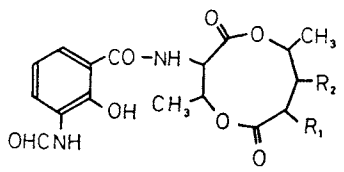
Department of Applied Chemistry, Faculty of Engineering, Keio University, Koganei-shi, Tokyo

(Received September 8, 1969)

A biologically active prototype of antimycin A series has been synthesized. The dilactone moiety was synthesized from a masked L-threonine and γ-hydroxyvalerate and condensed with an *O*-masked derivative of 3-nitrosalicylic acid *N*-hydroxysuccinimide ester. Hydrogenation followed by formylation afforded the anti-fungal substance. This synthesis established a possible general synthetic pathway to the members of antimycin A.

In 1949 F. M. Strong and his co-workers[1] isolated antimycin A and by later works[2] it was revealed that the crystalline antibiotic was a complex of at least four closely related active components. Antimycin A is a potent antibiotic against fungi and, especially, owes its interest to the fact that it is remarkably effective in inhibiting the hydrogen transport systems of aerobic organisms.[3]

The structures of antimycin A_1 (**1b**) and A_3 (**1c**) were elucidated by several research groups[4] on chemical and spectral evidences, and variations in the higher alkyl side chains account for structural differences between the members of the antimycin complex.



	1a	**1b**	**1c**
R_1:	H	n-C_6H_{13}	n-C_4H_9
R_2:	H	i-C_4H_9COO	i-C_4H_9COO

The most striking characteristic of the structure of antimycin A is its dilacton ring linked *via* an amide linkage to 3-formamidosalicylic acid. We were interested in the total synthesis of antimycin A, and attempted, in the first place, to synthesize a prototype in which the structure of dilactone moiety was made somewhat simpler than that in the natural antimycin A. This paper presents the total synthesis of dehexyl-deisovaleryloxy-antimycin A_1 (**1a**) which differs from natural antimycin A

*1 Part XXXIX of "Studies on Antibiotics and Related Substances" by Sumio Umezawa. This paper was read before the 22nd Annual Meeting of the Chemical Society of Japan, Tokyo, April, 1969. (See Preprints for the Meeting, Vol. 3, p. 1874). A part of this work has been briefly communicated: M. Kinoshita and S. Umezawa, This Bulletin, **42**, 854 (1969).

1) B. R. Dunshee, C. Lebens, G. W. Keitt and F. M. Strong, *J. Amer. Chem. Soc.*, **71**, 2436 (1949).

2) H. G. Schneider, G. M. Tenner and F. M. Strong, *Arch. Biochem. Biophys.*, **37**, 147 (1951); J. L. Lockwood, C. Lebens and G. W. Keitt, *Phytopathology*, **49**, 438 (1954).

3) For a general review, see J. S. Rieske, "Antimycin A" in "*Antibiotics*," Vol. I., Mechanism of Action, ed. by D. Gottlieb and P. D. Shaw, Springer-Verlag (1967), p. 542.

4) F. M. Strong, J. P. Dickie, M. F. Loomans. E. E. van Tamelen and R. S. Deway, *J. Amer. Chem. Soc.*, **82**, 1513 (1960); E. E. van Tamelen, J. P. Dickie, M. F. Loomans, R. S. Deway and F. M. Strong, *ibid.*, **83**, 1639 (1961); A. J. Birch, D. W. Cameron, Y. Harada and R. W. Rickard, *J. Chem. Soc.*, **1961**, 889; H. Yonehara and S. Takeuchi, *J. Antibiot.* (Tokyo), *Ser. A*, **11**, 122, 254 (1958); K. Uzu, H. Kato, K. Kumabe and Y. Harada, *ibid.*, *Ser. A*, **14**, 209 (1961).

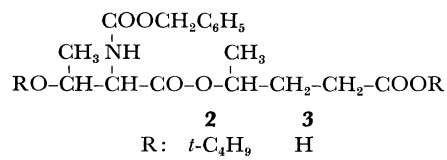
in that the acyloxy and higher alkyl side chains in its dilactone ring are replaced by hydrogen atoms.

As for the amino acid moiety in the dilactone ring, L-threonine was incorporated as it was in the natural antimycin A, because the characteristic antibiotic activity of antimycin A seems to be strictly dependent upon the maintenance of the stereochemical feature of the amino acid moiety.

t-Butyl levulinate was prepared from levulinic acid and isobutene by a modification of the method of Gal and Sletzimyer[5] in a good yield.

Catalytic reduction of *t*-butyl levulinate over Raney Ni W-5 afforded *t*-butyl *γ*-hydroxyvalerate in a 91.8% yield.

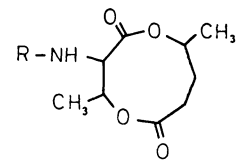
t-Butyl *γ*-hydroxyvalerate was allowed to react with *N*-benzyloxycarbonyl-*O*-*t*-butyl-L-threonine[6] in the presence of *N*,*N*′-dicyclohexylcarbodiimide and pyridine in ether according to the condition in which Hassall *et al.*[7] had obtained the excellent result on the preparation of benzyl *β*-(*N*-benzyloxycarbonyl-*O*-*t*-butyl-DL-seryloxy)propionate. In our case, however, the reaction was accompanied by byproduction of a considerable amount of acyl dicyclohexyl urea. Separation of the condensation product from the by-product was effected on a silica gel column chromatography with a *n*-hexane-diisopropyl ether-ether (2 : 2 : 1) system to afford *t*-butyl *γ*-(*N*-benzyloxycarbonyl-*O*-*t*-butyl-L-threonyloxy) valerate (**2**) in a 45% yield as a syrup.



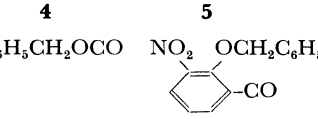
	2	**3**
R:	*t*-C_4H_9	H

Treatment of **2** with trifluoroacetic acid, followed by purification through a silica gel column with *n*-hexane-ethyl acetate-acetic acid (20 : 10 : 1) system gave *γ*-(*N*-benzyloxycarbonyl-L-threonyloxy)-valeric acid (**3**) in a 90% yield. The free linear ester acid **3** was cyclized by high dilution technique in a benzene solution by adding 2 mol of trifluoroacetic anhydride at 75°C during 19 hr.[8]

Treatment of the reaction product with ether gave crystal **A** in a 2.6% yield. The mother-liquor was concentrated and chromatographed on a silica gel column with a benzene-diisopropyl ether-ether (6 : 2 : 1) system to give two fractions. The first fraction gave crystal **B** in a 16.7% yield. On the basis of elemental analysis, infrared spectrum and molecular weight determination by mass spectrum, the crystal **B** was found to be the desired intramolecular cyclization product, namely, 3-(benzyloxycarboxamido)-4,9-dimethyl-1,5-dioxa-2,6-cyclononadione (**4**).



4



	5
R:	NO_2 $OCH_2C_6H_5$ -CO

The second fraction of the chromatography gave a crystalline solid, which, on treatment with ether, was further devided into crystal **C** and syrup **D**. Each of the crystal **A** and **C** and syrup **D** proved to be a diastereomer of dimeric intermolecular cyclization products by the results of elemental analyses, IR-spectra, molecular weight determinations and optical rotatory powers.

When the cyclization reaction was carried out by directly using a freshly prepared crude ester-acid **3** without purification through a silica gel column, the yields of cyclization products markedly increased (about twice).

On NMR spectrum of the compound **4**, the proton signals of the two methyl groups were observed as four doublets having coupling constant $J=7.5$ Hz at τ values in deuteriochloroform 8.53, 8.60, 8.69 and 8.76 (Fig. 1). This indicates that the compound **4** is a mixture of two diastereomers. The presence of the two diastereomers is natural because racemic *t*-butyl *γ*-hydroxyvalerate was used as one of the starting materials, and the diastereomeric rela-

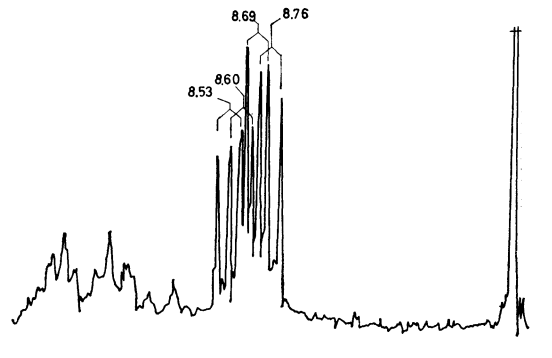


Fig. 1. Partial NMR spectrum of the compound **4** in $CDCl_3$ at 60 MHz.

5) Levulinic anhydride was used instead of levulinic acid in a patent by G. Gal and M. Sletzimyer, Fr. 1384248 (1965); *Chem. Abstr.*, **62**, 16197b (1965).

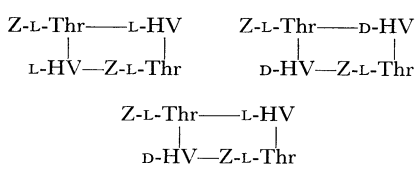
6) E. Schröder, *Ann.*, **670**, 127 (1963).

7) C. H. Hassall, T. G. Martin, J. A. Schofield and J. O. Thomas, *J. Chem. Soc., C*, **1967**, 997.

8) O. Th. Schmidt and W. Staab, *Chem. Ber.*, **81**, 388 (1954).

tionship is concerned with the two possible configurations at C-9 of the dilactone molecule.[9]

The formation of three kinds of cyclic dimers may reasonably explained by the theoretical combinations of *N*-benzyloxycarbonyl-L-threonine (Z-L-Thr), D- and L-γ-hydroxyvaleric acid (D-HV, L-HV) as shown in the schematic diagram.



The benzyloxycarbonyl group of **4** was removed by catalytic hydrogenolysis over palladium black in methanol to give the free amino dilactone, which was directly *N*-acylated with an active ester, *O*-benzyl-3-nitrosalicylic acid *N*-hydroxysuccinimide ester in tetrahydrofuran. A silica gel column chromatography of the reaction product with *n*-hexane-ethyl acetate (5 : 3) system gave 3-(*O*-benzyl-3-nitrosalicyloylamido)-4,9-dimethyl-1,5-dioxa-2,6-cyclononadione (**5**) as crystals in a 72.4% yield.

The compound **5** was then hydrogenolysed over palladium black in methanol to yield *N*-(3-aminosalicyloyl)amino dilactone which was immediately *N*-formylated with 98% formic acid and dicyclohexylcarbodiimide. Column chromatography of the crude product followed by recrystallization afforded dehexyl-deisovaleryloxy-antimycin A_1 (**1a**) which was considered to be a diastereomeric mixture in a 54% yield based on the compound **5**.

The UV spectrum of **1a** showed the characteristic absorption maxima at 226 and 320 mμ which correspond to the *N*-formyl-3-aminosalicyloyl chromophore as a constituent portion of antimycin A.

The IR spectrum of **1a** also was similar to that of antimycin A in respect of the characteristic absorptions assignable to ester carbonyl, formamido and aromatic amido groups.

Bioassay: The synthetic dehexyl-deisovaleryloxy-antimycin A_1 showed strong inhibition against certain fungi. Minimal inhibitory concentrations (mcg/ml) of the synthetic specimen against tested fungi were as follows: *Corticium rosii* 50, *Gloeosporium lacticola* 100, *Glomerella cingulate* 100, *Leptosphaeria sulvinii* 50 by dilution method with Sabouraud medium, 27°C 5 days; *Piricularia oryzae* 0.1, *Trichophyton asteroides*-429 12.5 by agar dilution method with 1% glucose nutrient agar, 27°C 42 hr.

9) A possibility of racemization at C-4 which may occur during the ring closure reaction with trifluoroacetic anhydride might be excluded on the basis of the fact that Taub *et al.* had elucidated in their synthetic studies on zearalenone;[9] D. Taub, N. N. Girotra, R. D. Hofsommer, C. H. Kuo, H. L. Slates, S. Weber and N. L. Wendler, *Tetrahedron*, **24**, 2443 (1968).

Experimental

Melting points were determined on a micro hot stage and were uncorrected. Thin-layer chromatography (TLC) was conducted by the use of silica gel (Daiichi Pure Chemical Co., Inc.). Silica gel column chromatography was carried out by using the silica gel (Kanto Chemical Co., Inc. and Junsei Chemical Co., Inc.) which was activated at 110°C for 1 hr. Infrared spectra were recorded on a Hitachi IPI-2 spectrometer. Ultraviolet spectra were taken in a Hitachi Perkin-Elmer UV-VIS spectrometer 139. NMR spectra was recorded on a Varian A-60D spectrometer (TMS as an internal standard). In general, all concentrations were carried out in a rotary evaporator at reduced pressure below 40°C.

***t*-Butyl Levulinate.** Liquid isobutene (100 ml, *ca.* 1.2 mol) was added to a solution of levulinic acid (20 g, 0.172 mol) in a mixture of dichloromethane (100 ml) and conc. sulfuric acid (1 ml) which was cooled at −30°C in a 500-ml round-bottomed flask. The stoppered flask was allowed to stand at room temperature for 70 hr. The reaction mixture was neutralized with triethylamine (2.52 ml) and evaporated. The residue was taken with ethyl acetate (100 ml) and the solution was washed with water (20 ml × 3) and saturated sodium chloride solution (20 ml) and dried over sodium sulfate. After evaporation of the solvent, the residue was distilled at reduced pressure. The fraction boiling at 49.5—51.0°C/0.8 mmHg was collected: yield 25.8 g (87%); n_D^{20} 1.4232; $\nu_{max}^{CHCl_3}$ 1735 cm^{-1} (C=O and ester). [lit.,[5] bp 72—74°C/2 mmHg].

Found: C, 62.94; H, 9.50%. Calcd for $C_9H_{16}O_3$: C, 62.76; H, 9.36%.

***t*-Butyl γ-Hydroxyvalerate.** *t*-Butyl levulinate (14 g) was hydrogenated with Raney Ni W-5 (1.5 ml) in an initial hydrogen pressure of 75 kg/cm^2 at 66—68°C for 2 hr. After removal of the catalyst, the filtrate was distilled at reduced pressure to afford a colorless liquid: yield 13.27 g (91.8%); bp 70—73°C/1 mmHg; n_D^{20} 1.4291; $\nu_{max}^{liq.}$ 3420 (OH) and 1735 cm^{-1} (ester).

Found: C, 62.26; H, 10.58%. Calcd for $C_9H_{18}O_3$: C, 62.04; H, 10.41%.

***t*-Butyl γ-(*N*-Benzyloxycarbonyl-*O*-*t*-butyl-L-threonyloxy)valerate (2).** A solution of *N*-benzyloxycarbonyl-*O*-*t*-butyl-L-threonine[6] (8.00 g, 25.9 mmol) in dry ether (15 ml) was added dropwise during 40 min to a stirred solution of *t*-butyl γ-hydroxyvalerate (4.50 g, 25.9 mmol), *N,N'*-dicyclohexylcarbodiimide (DCCI) (5.89 g, 28.6 mmol) and dry pyridine (2.2 ml, 25.9 mmol) in dry ether (20 ml) cooled to 0°C. Stirring at 0°C was continued for a further 1 hr. After standing at 0°C for 23 hr, the precipitate of *N,N'*-dicyclohexylurea (3.54 g, 55.2%) was filtered off and the filtrate was treated with acetic acid (2 ml) under stirring at 0°C for 2 hr. An additional urea was removed by filtration and the filtrate was diluted with ether. The solution was then washed with 5% sodium hydrogen carbonate solution, 5% citric acid solution and saturated sodium chloride solution, dried over sodium sulfate, and concentrated to dryness. The oily residue (13.4 g) was chromatographed on a silica gel column (2.2 kg, 8 × 54 cm). Elution with a *n*-hexane-diisopropyl ether-ether (2 : 2 :1) system gave a straw-colored syrup (5.42 g, 45%) as a first fraction which behaved as a homogeneous compound

having R_f-value of 0.66 on TLC using the same solvent system as in the column chromatography: $[\alpha]_D^{19}$ −4.1°C (*c*, 2.0, ethanol); $\nu_{max}^{lig.}$ 3480, 3380 (NH), 1740 (ester and amide I) and 1510 cm^{-1} (amide II).

Found: C, 64.78; H, 8.60; N, 3.22%. Calcd for $C_{25}H_{39}O_7N$: C, 64.49; H, 8.44; N, 3.01%.

The second fraction of the column chromatography gave a syrup (3.05 g) having R_f-value of 0.56 on TLC. A part of the syrup was purified through a silica gel column with the same solvent system to afford *N*-(*N*-benzyloxycarbonyl-*O*-*t*-butyl-L-threonyl)-*N*,*N*'-dicyclohexylurea as a colorless glass.

Found: C, 67.61; H, 8.44; N, 7.80%. Calcd for $C_{29}H_{45}O_5N_3$: C, 67.54; H, 8.80; N, 8.15%.

γ-(*N*-Benzyloxycarbonyl-L-threonyloxy)valeric Acid (3). A mixture of **2** (692 mg) and trifluoroacetic acid (12 m*l*) was kept at room temperature with occasional swirling for 20 min, after which the reaction mixture was evaporated below 20°C. The residual syrup was then dissolved in ether and again evaporated. This procedure was repeated thrice to remove trifluoroacetic acid. The final residue was chromatographed on silica gel (25 g) with a *n*-hexane-ethyl acetate-acetic acid (20 : 10 : 1) system. The eluents containing **3** were evaporated to afford the pure sample of **3** (475 mg, 90%) as a pale-yellow syrup: $[\alpha]_D^{21}$ −16.4°C (*c* 2.0, ethanol); $\nu_{max}^{lig.}$ 3340 (NH and OH), 2700—2500 (carboxyl OH), 1720 (ester, carboxyl and amide I) and 1525 cm^{-1} (amide II).

Found: C, 57.91; H, 6.85; N, 4.08%. Calcd for $C_{17}H_{23}O_7N$: C, 57.78; H, 6.56; N, 3.96%.

Cyclization of 3. a) A mixture of **3** (991 mg, 2.81 mmol) purified by column chromatography, dry benzene (70 m*l*) and trifluoroacetic anhydride (0.39 m*l*, 2.81 mmol) was heated at 75°C for 10 hr, after which additional trifluoroacetic anhydride (0.39 m*l*) was added and again heated at the same temperature for 9 hr. The reaction mixture was evaporated and the resulting syrup was dissolved in absolute ether. On ice-cooling of the solution, there was obtained a crystalline product **A**, which was collected by filtration: mp 206—208°C; yield 24.5 mg (2.6%). Recrystallization of the product **A** from ethyl acetate-petroleum ether (bp 40—60°C) gave colorless needles: mp 208—210°C; $[\alpha]_D^{11}$ −45° (*c* 2.0, chloroform); *m/e* 670; ν_{max}^{KBr} 3340 (NH), 1732 (ester), 1715 (amide I) and 1530 cm^{-1} (amide II).

Found: C, 61.44; H, 6.16; N, 4.41%. Calcd for $(C_{17}H_{21}O_6N)_2$: C, 60.88, H, 6.31; N, 4.18%.

The mother liquor of the crystal **A** was concentrated and chromatographed on silica gel (200 g) with a benzene-diisopropyl ether-ether (6 : 2 : 1) system to afford two fractions having R_f-values, 0.55 and 0.43 on TLC with the same solvent stysem. The first fraction (R_f 0.55) gave a crystalline product **B** (158 mg, 16.7%); mp 99—103°C. Recrystallization of **B** from ethyl acetate-petroleum ether gave a pure sample of 3-benzyloxycarboxamido-4,9-dimethyl-1,5-dioxa-2,6-cyclononadione (**4**): mp 105—107°C; $[\alpha]_D^{21}$ +14° (*c* 2.0, ethanol); $[\alpha]_D^{21}$ +31° (*c* 1.0, chloroform), *m/e* 335; ν_{max}^{KBr} 3320 (NH), 1745 (ester), 1693 (amide I) and 1539 cm^{-1} (amide II); τ^{CDCl_3} 8.53, 8.60, 8.69 and 8.76 (d, C_4- and C_9-CH_3, J=7.5 Hz).

Found: C, 61.21; H, 6.70; N, 4.37%. Calcd for $C_{17}H_{21}O_6N$: C, 60.88; H, 6.31; N, 4.18%.

The second fraction (R_f 0.43) afforded a crystalline product melting at 151—154°C (26.6 mg, 2.8%). The product (71 mg) was washed with ether (4 m*l*) and the resulting crystal (46 mg) was recrystallized from ethyl acetate-petroleum ether to afford crystals **C**: mp 167—169°C; $[\alpha]_D^{13}$ +1.3° (*c* 2.0, chloroform); ν_{max}^{KBr} 3340 (NH), 1732 (ester), 1710 (amide I) and 1525 cm^{-1} (amide II); *m/e* 670.

Found: C, 61.00; H, 6.57; N, 4.23%. Calcd for $(C_{17}H_{21}O_6N)_2$: C, 60.88; H, 6.31; N, 4.18%.

The ether washing was concentrated and worked up with petroleum ether to give additional crystals **C** (5 mg). The filtrate was evaporated to afford a syrup **D** (14 mg): $[\alpha]_D^{18}$ +15° (*c* 1.0, chloroform); $\nu_{max}^{CCl_4}$ 3450 (NH), 1746—1732 (ester and amide I) and 1505 cm^{-1} amide II); *m/e* 670.

Found: N, 4.35%. Calcd for $(C_{17}H_{21}O_6N)_2$: N, 4.18%.

b) Treatment of **2** (162 mg) with trifluoroacetic acid (2.6 m*l*) for 10 min, followed by rapid evaporation below 10°C afforded crude **3**, which was immediately subjected to cyclization by the same procedures as described above. The concentrated reaction mixture afforded the product **A** (3.6 mg, 3.08%, mp 205—208°C), product **B** (**4**) (38 mg, 32.5%, mp 98—101°C, and product **C**+**D** (6.3 mg, 5.38%, mp 151—154°C).

***O*-Benzyl-3-nitrosalicylic Acid *N*-Hydroxysuccinimide Ester.** To a solution of *O*-benzyl-3-nitrosalicylic acid[10] (1.38 g, 5.05 mmol) and *N*-hydroxysuccinimide (0.58 g, 5.05 mmol) in dry tetrahydrofuran (16 m*l*) was added a solution of DCCI (1.04 g, 5.05 mmol) in tetrahydrofuran and the mixture was kept at room temperature overnight. The precipitated *N*,*N*'-dicyclohexylurea (1.08 g, 95.6%) was filtered off and the filtrate was evaporated to dryness. The residue (1.92 g) was recrystallized from ethanol (80 m*l*) to afford pure title compound as pale-yellow needles: yield 1.62 g (87%); mp 140—141°C; ν_{max}^{KBr} 1810, 1780, 1741, 1600, 1540, 1360, 770 and 700 cm^{-1}.

Found: C, 58.56; H, 3.79; N, 7.27%. Calcd for $C_{18}H_{14}O_7N_2$: C, 58.38; H, 3.81; N, 7.57%.

3-(*O*-Benzyl-3-nitrosalicyloylamido)-4,9-dimethyl-1,5-dioxa-2,6-cyclononadione (5). A solution of **4** (crystal **B**) (138 mg, 0.413 mmol) in methanol (6 m*l*) was stirred with palladium black (*ca.* 15 mg) for 50 min under bubbling with hydrogen. The filtered reduction mixture was evaporated to yield the free amino dilactone (85 mg) which showed a single ninhydrin positive spot on TLC with a benzene-diisopropyl ether-ether (6 : 2 : 1) system. The free amino dilactone (83 mg, 0.413 mmol) was dissolved in dry tetrahydrofuran (2 m*l*) and *O*-benzyl-3-nitrosalicylic acid *N*-hydroxysuccinimide ester (153 mg, 0.413 mmol) was added. The mixture was kept for 20 hr at room temperature, after which it was allowed to stand for 27 hr at 36°C in an incubator. The resulting solution was concentrated to afford a yellow crystalline mass which was purified through silica gel (45 g) column. Elution with *n*-hexane-ethyl acetate (5 : 3) system gave the title compound (**5**) as pale-yellow crystals: yield 135 mg (72.4%), mp 186—189°C. Recrystallization from tetrahydrofuran-petroleum ether (bp 60—70°C): mp 188—189.5°C; $[\alpha]_D^{23}$ +20° (*c* 2.0, tetrahydrofuran); ν_{max}^{KBr} 3300 (NH), 1743 (ester), 1645 (amide I) and 1532 cm^{-1} (amide II and NO_2).

Found: C, 60.57; H, 5.48; N, 6.11%. Calcd for

10) F. S. Okumura, M. Masumura and T. Horie, *J. Amer. Chem. Soc.*, **81**, 5215 (1959).

$C_{23}H_{24}O_8N_2$: C, 60.52; H, 5.30; N, 6.14%.

Dehexyl-deisovaleryloxy-antimycin A_1 (1a). A solution of **5** (62.6 mg, 0.137 mmol) in methanol (10 m*l*) was stirred with palladium black (*ca.* 45 mg) under bubbling with hydrogen gas for 40 min. The filtered yellow-green reduction mixture was concentrated to give a yellow oil (48.4 mg, 100%). The product (46.1 mg, 0.137 mmol) was dissolved in tetrahydrofuran (2 m*l*) and to the solution was added DCCI (28.2 mg, 0.137 mmol) and 98% formic acid (6.8 mg) under ice-cooling. After standing for 15 hr in a refrigerator, the reaction mixture was filtered to remove *N,N'*-dicyclohexylurea (23.2 mg, 72.4%). The filtrate was evaporated and the residue was chromatographed on silica gel (10 g) with a *n*-hexane-ethyl acetate (4 : 3) system to afford **1a** (31.7 mg) as crystals. The crystals were again chromatographed on silica gel (7 g) with the same solvent system to give a practically pure of **1a** (26.1 mg, 54%). Recrystallization from ethyl acetate-*n*-hexane: mp 154—156°C; *m/e* 364; $[\alpha]_D^{19}$ +70° (*c* 1.0, chloroform); λ_{max}^{MeOH} 226 (log ε 4.62), 320 mμ (log ε 3.93); $\nu_{max}^{CHCl_3}$ 3435 (NH), 1747 (ester), 1706 (NHCHO), 1647 (ArCONH), 1614 (Ar–H) and 1531 cm^{-1} (ArCONH).

Found: C, 56.29; H, 5.76; N, 7.77%. Calcd for $C_{17}H_{20}O_7N_2$: C, 56.04; H, 5.53; N, 7.69%.

We wish to thank Professor H. Umezawa for his helpful advice, Mr. S. Nakada for the microanalyses and Mr. F. Kishimoto for his technical assistance. We also wish to thank Professor S. Zen of Kitasato University for the measurement of mass spectra, Dr. M. Hamada of Institute of Microbial Chemistry and Mr. S. Fukatsu of Meiji-Seika Co. for their microbiological tests.