Studies on 2-Aziridinecarboxylic Acid. IV. Total Synthesis of Actinomycin D (C_1) *via* Ring-opening Reaction of Aziridine

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Actinomycin D (C_1) has been synthesized by a route involving the ester formation between two peptide fragments, (2*S*,3*S*)-1-(2-nitro-3-benzyloxy-4-methylbenzoyl)-3-methyl-2-aziridinecarbonyl-D-valylproline *t*-butyl ester and *N*-benzyloxycarbonylsarcosyl-*N*-methylvaline, *via* a ring-opening reaction of aziridine. Cyclization, followed by reduction and oxidation, gave actinomycin D (C_1). The synthetic actinomycin D (C_1) was indistinguishable from natural substance as regards physical properties and biological activity.

A number of cyclic peptide lactone antibiotics such as actinomycin group, etamycin, and echinomycin have been reported during the last few years. However, since they possess cyclic lactone structure and unusual amino acids, the synthetic approaches were limited.

It was found that the reaction of the peptide containing (2*S*, 3*S*)-3-methyl-2-aziridinecarboxylic acid with *N*-protected amino acids or dipeptides affords *O*-acylthreonine peptides.[1] This paper reports an application of the ester formation method to the synthesis of naturally occurring cyclic peptide lactone, actinomycin D (C_1) (Fig. 1).

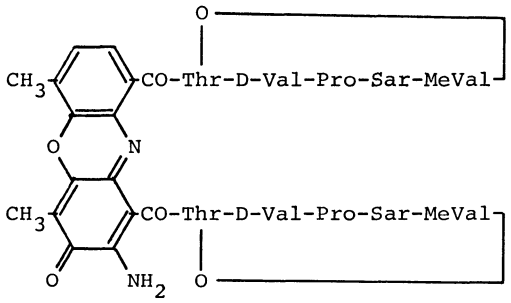
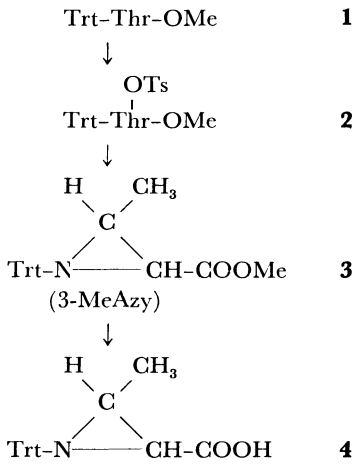


Fig. 1.

So far, the general synthetic method for peptide lactones involved formation of the ester bond (didepsipeptide) at the start, followed by peptide elongation and cyclization by the formation of the amide bond. A new route is designed in which linear pentapeptide ester **11** is directly formed by the ring-opening reaction of aziridinecarboxylic acid-containing peptide **8** with dipeptide **10** (Scheme 2). The method needs no activating reagent for the preparation of *O*-acylthreonine peptide, no racemization taking place during the course of direct introduction of *N*-protected dipeptide.

(2*S*,3*S*)-1-Trityl-3-methyl-2-aziridinecarboxylic acid (**4**) was prepared from threonine by the following route (Scheme 1). Tritylthreonine methyl ester (**1**) was treated with *p*-toluenesulfonyl chloride in a pyridine solution at −10 °C to give *O*-tosyl derivative **2**, which was refluxed in tetrahydrofuran with triethylamine to convert aziridinecarboxylic acid derivative **3**. Saponification of **3** with lithium hydroxide gave the desired amino acid **4**.

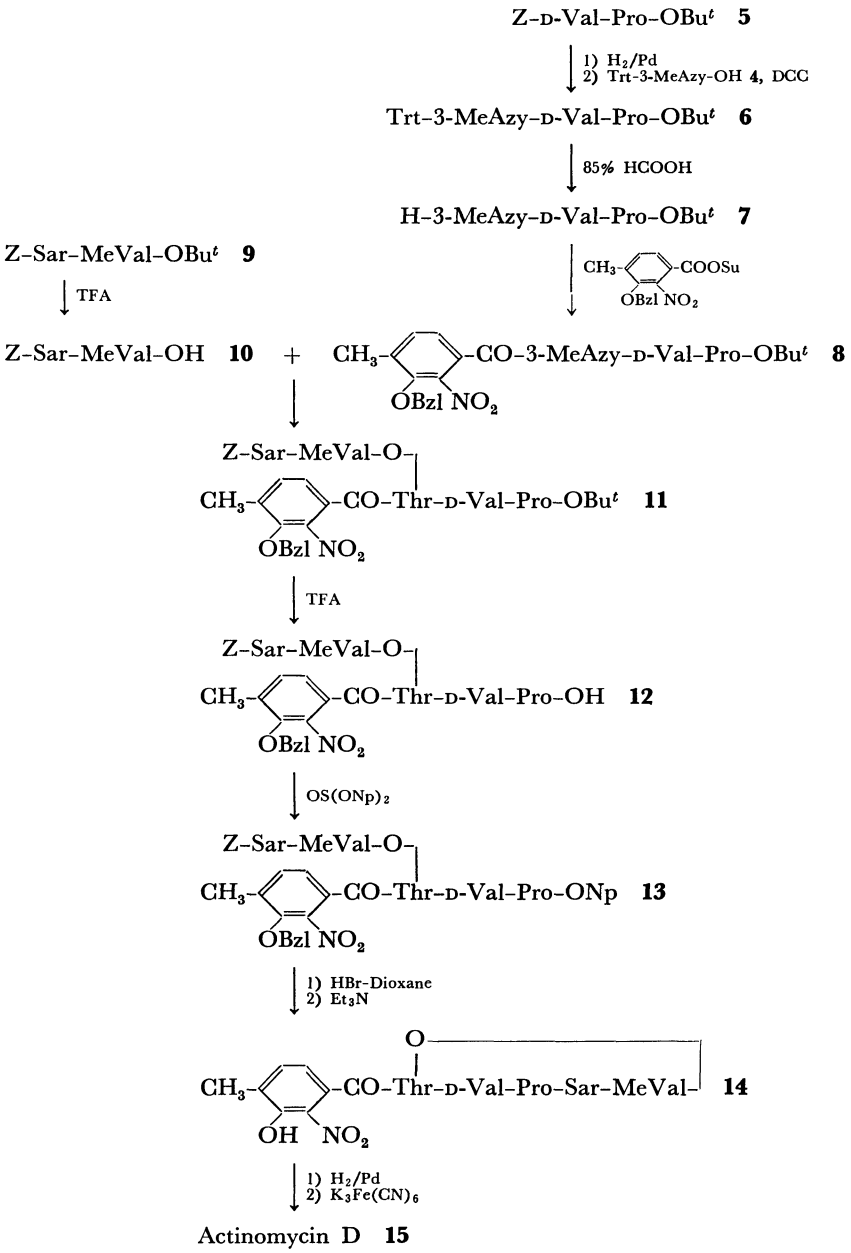
The aziridine segment **8**, (2*S*,3*S*)-1-(2-nitro-3-benzyloxy-4-methylbenzoyl)-3-methyl-2-aziridinecarbonyl-D-valylproline *t*-butyl ester, was synthesized as follows. Coupling of benzyloxycarbonyl-D-valine and proline



Scheme 1.

t-butyl ester using dicyclohexylcarbodiimide (DCC)[2] gave the dipeptide **5**. Hydrogenolysis of **5**, followed by coupling with **4** using DCC gave the tripeptide **6** in 91.5% yield. Selective removal of the trityl group of **6** with 85% formic acid containing a small amount of methanol gave **7** in 94.6% yield, which was then coupled, in the dark, with 2-nitro-3-benzyloxy-4-methylbenzoic acid *N*-hydroxysuccinimide ester. Compound **8** was obtained as colorless amorphous powder in 94.7% yield after purification by silica gel column chromatography using benzene–ethyl acetate (1 : 1 v/v). Benzyloxycarbonylsarcosine was coupled with *N*-methylvaline *t*-butyl ester using DCC to give the dipeptide **9**. *t*-Butyl group of **9** was removed by the action of trifluoroacetic acid to give the dipeptide acid **10** as an acid component.

The formation of the ester bond between *N*-methylvaline and threonine was carried out by heating **8** together with **10** at 110 °C for 5 h, in the dark. The coupling reaction afforded a mixture of **11** and the hydrolyzed product of **8** (threonine derivative), from which **11** was isolated in 45—55% yields by silica gel column chromatography. The *t*-butyl group of **11** was removed by the action of trifluoroacetic acid, the product **12** being treated with bis(*p*-nitrophenyl) sulfite in a pyridine solution to give the *p*-nitrophenyl ester **13**. Deprotection of benzyloxycarbonyl group of **13** with hydrogen bromide in dioxane, followed by neutralization, cyclization [0.98 mmol in 2 l pyridine, 60 °C, 8 h], and purification on Sephadex LH-20 column in methanol, gave cyclic pentapeptide lactone **14** in 21.4% yield. Catalytic hydrogenolysis of **14**,



Scheme 2.

Table 1. Physical properties and biological activities of actinomycin D

Characteristics	Synthetic actinomycin D (**15**)	Natural actinomycin D (Lit.) a)	b)
Melting point/°C	242—243	241—243	246—247
Optical rotation ($[\alpha]_D^{23}$)[c]	−316	−323±10	−328±10
UV absorption	24900 (443)	24400 (443)	25000 (443)
[ε in methanol (λ/nm)]	35000 (240)	34100 (240)	34000 (231)
IR absorption (cm^{-1}, KBr)	1745 (lactone C=O)	1745	1760
	1620—1670 (amide)	1620—1670	1620—1670
	1580 (chromophore)	1580	1585
	1195 (lactone COC)	1195	1200[d]
Antibacterial activity (MIC, μg/ml)			
B. Subtilis ATCC-6633	0.78	0.78[e]	
E. Coli NIHJ	>100	—	

a) Ref. 3. b) Ref. 5. c) In methanol at c 0.21. d) Ref. 6. e) Ref. 7.

followed by oxidation with potassium hexacyanoferrate(III) in the 1:1 mixture of methanol and 0.067 M phosphate buffer, pH 7.1,[3] gave actinomycin D (C_1) (**15**), which was quantitatively crystallized from ethyl acetate–hexane.

The synthetic actinomycin D (C_1) was indistinguishable from the natural substance as regards physical properties and biological activity against *B. Subtilis*. The physical and biological data are summarized in Table 1.

The method using the ring-opening reaction of aziridine is an efficient means for synthesizing peptide lactone compounds.

Experimental

Melting points are uncorrected. Infrared spectra were obtained on a Hitachi EPI-G3 spectrometer, ultraviolet spectra on a Shimadzu UV-200 spectrophotometer. Optical rotations were determined at the D line on a Perkin-Elmer 141 polarimeter. Thin layer chromatography was carried out on silica gel G in the following solvent systems: R_f^1; chloroform–ethyl acetate (1:1), R_f^2; benzene–ethyl acetate (1:1), R_f^3; chloroform–methanol–acetic acid (8:1:1), R_f^4; chloroform–methanol–ethyl acetate (8:1:1), R_f^5; 1-butanol–acetic acid–water (4:1:1).

*Tritylthreonine Methyl Ester (**1**).* To a solution of threonine methyl ester hydrochloride (85.3 g, 0.5 mol) and triethylamine (140 ml, 1 mol) in $CHCl_3$ (280 ml) was added a solution of trityl chloride (139 g, 0.5 mol) in $CHCl_3$ (250 ml) at 0 °C. After being stirred for 24 h at 0 °C the solution was concentrated *in vacuo* and the residue was dissolved in ethyl acetate. The solution was washed with water, 10% citric acid and water. Drying over Na_2SO_4 and concentration gave an oil (186 g) of **1** in a theoretical yield, R_f^1 0.86, $[\alpha]_D^{23}$ +5.4 (*c* 0.94, $CHCl_3$).

*O-Tosyl-N-tritylthreonine Methyl Ester (**2**).* To a solution of **1** (5.72 g, 15.2 mmol) in dry pyridine (45 ml) was added at −10 °C a solution of *p*-toluenesulfonyl chloride (8.71 g, 45.7 mmol) in dry pyridine (50 ml). After being stirred for 3 d at −10 °C the reaction mixture was poured onto ice water (400 ml). The oily product was extracted with ethyl acetate. The extract was further washed with water, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was crystallized from ethyl acetate–ether–hexane, 6.59 g (81.9%), mp 120—122 °C, $[\alpha]_D^{23}$ +87.7 (*c* 0.9, $CHCl_3$). Found: C, 70.21; H, 6.01; N, 2.50; S, 6.25%. Calcd for $C_{31}H_{31}O_5NS$: C, 70.30; H, 5.90; N, 2.64; S, 6.05%.

*(2S, 3S)-1-Trityl-3-methyl-2-aziridinecarboxylic Acid Methyl Ester (**3**).* A solution of **2** (16.5 g, 31.1 mmol) and triethylamine (36.4 ml, 93.3 mmol) in tetrahydrofuran (100 ml) was refluxed at 80 °C for 2 d. After removal of solvent the oily product was dissolved in ethyl acetate. The solution was washed with water, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was crystallized from methanol, 10.1 g (90.4%), mp 109—110 °C, $[\alpha]_D^{23}$ −102.8 (*c* 1.0, $CHCl_3$). Found: C, 80.29; H, 6.39; N, 3.56%. Calcd for $C_{24}H_{23}O_2N$: C, 80.64; H, 6.48; N, 3.92%.

*(2S, 3S)-1-Trityl-3-methyl-2-aziridinecarboxylic Acid (**4**).* To a solution of **3** (6.0 g, 16.8 mmol) in acetonitrile (80 ml) and methanol (20 ml) was added drop by drop at 20 °C a solution of 2 M lithium hydroxide (24 ml, 84 mmol). After being stirred for 24 h at 20 °C the solvent was removed and the residue was dissolved in ethyl acetate. The solution was washed with 10% citric acid and water, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was crystallized from hexane, 5.47 g (94.8%), mp 173—175 °C, $[\alpha]_D^{23}$ −77.3 (*c* 0.9, $CHCl_3$). Found: C, 79.54; H, 6.23; N, 3.80%. Cacld for $C_{22}H_{21}O_2N$: C, 79.73; H, 6.39; N, 4.23%.

*Benzyloxycarbonyl-D-valylproline t-Butyl Ester (**5**).* To a solution of benzyloxycarbonyl-D-valine (16.5 g, 70.5 mmol) and proline *t*-butyl ester (12.0 g, 70.5 mmol) in CH_2Cl_2 (150 ml) was added at −10 °C a solution of dicyclohexylcarbodiimide (DCC, 14.5 g, 70.5 mmol) in CH_2Cl_2 (50 ml). The reaction mixture was stirred at −10 °C for 3 h, and then overnight in a refrigerator. After removal of *N,N'*-dicyclohexylurea by filtration the filtrate was concentrated *in vacuo*. The residue was dissolved in ethyl acetate and the solution was washed with 10% citric acid, 1 M sodium hydrogencarbonate and water, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was crystallized from ethyl acetate–ether–hexane. 24.5 g (89.9%), mp 90—91 °C, $[\alpha]_D^{23}$ −21.4 (*c* 1.0, MeOH). Found: C, 65.74; H, 8.58; N, 7.13%. Calcd for $C_{22}H_{32}O_5N_2$: C, 65.32; H, 7.97; N, 6.93%.

*(2S, 3S)-1-Trityl-3-methyl-2-aziridinecarbonyl-D-valylproline t-Butyl Ester (**6**).* Compound **5** (12.35 g, 30.5 mmol) was hydrogenated over palladium in methanol for 2 h to give N^{α}-free dipeptide. This was coupled with **4** (9.20 g, 28 mmol) in CH_2Cl_2 (150 ml) containing DCC (6.30 g, 30.5 mmol) using the same method as in the preparation of **5** to give **6**, which was crystallized from methanol, 15.3 g (91.5%), mp 93—94.5 °C, $[\alpha]_D^{23}$ −81.5 (*c* 0.82, MeOH). Found: C, 74.29; H, 7.85; N, 6.99%. Calcd for $C_{37}H_{45}O_4N_3$: C, 74.59; H, 7.61; N, 7.05%.

*(2S, 3S)-3-Methyl-2-aziridinecarbonyl-D-valylproline t-Butyl Ester (**7**).* **6** (12.0 g, 20 mmol) was added to an ice-cold solution of 85% formic acid (80 ml) containing metanol (20 ml), and the resulting solution was stirred for 3 h. After removal of solvent the residue was partitioned between ether and water. Sodium hydrogencarbonate was added to the aqueous layer producing alkaline solution, and the isolated oil was extracted with ethyl acetate. After being dried over Na_2SO_4 the solvent was concentrated *in vacuo* to give **7**, which was crystallized from methanol, 6.69 g (94.6%), mp 81—84 °C, $[\alpha]_D^{23}$ −39.0 (*c* 2.0, MeOH). Found: C, 61.42; H, 9.09; N, 11.79%. Calcd for $C_{18}H_{31}O_4N_3$: C, 61.17; H, 8.84; N, 11.89%.

*(2S, 3S)-1-(2-Nitro-3-benzyloxy-4-methylbenzoyl)-3-methyl-2-aziridinecarbonyl-D-valylproline t-Butyl Ester (**8**).* A mixture of **7** (4.8 g, 13.6 mmol) and 2-nitro-3-benzyloxy-4-methylbenzoic acid *N*-hydroxysuccinimide ester[4] (5.23 g, 13.6 mmol) was stirred in $CHCl_3$ (40 ml) in the dark at room temperature for 24 h. After removal of solvent the residue was dissolved in ethyl acetate, and the solution was washed with 10% citric acid and water, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography using benzene–ethyl acetate (1:1 v/v) to give **8** as an amorphous powder, 8.53 g (94.7%), R_f^2 0.65, $[\alpha]_D^{23}$ −81.1 (*c* 1.1, MeOH). Found: C, 63.85; H, 7.13; N, 8.71%. Calcd for $C_{33}H_{42}O_8N_4$: C, 63.65; H, 6.80; N, 9.00%.

*Benzyloxycarbonylsarcosyl-N-methylvaline t-Butyl Ester (**9**).* Benzyloxycarbonylsarcosine (17.6 g, 79 mmol), *N*-methylvaline *t*-butyl ester (14.8 g, 79 mmol), and DCC (19.6 g, 95 mmol) were allowed to react in CH_2Cl_2 (250 ml) using the same method as in the preparation of **5**. After purification of the residue by silica gel column chromatography using chloroform–ethyl acetate (1:1 v/v), **9** was obtained as an oil, 29.8 g (96.0%), R_f^1 0.80, $[\alpha]_D^{23}$ −75.8 (*c* 1.4, MeOH).

*Benzyloxycarbonylsarcosyl-N-methylvaline (**10**).* Trifluoroacetic acid (20 ml) was added to **9** (4.79 g, 12.2 mmol) at

−5 °C, and the reaction mixture was stirred for 3 h at the same temperature. After removal of trifluoroacetic acid *in vacuo* the residual oily product was partitioned between ethyl acetate and sodium hydrogencarbonate solution. Citric acid was added to the aqueous layer producing acidic solution, and the isolated oil was extracted with ethyl acetate. The ethyl acetate layer was washed with water successively, dried over Na_2SO_4, and concentrated *in vacuo* to give **10** as an oil, 3.7 g (90.0%), R_f^4 0.58, $[\alpha]_D^{23}$ −77.7 (*c* 1.07, MeOH).

O-*(Benzyloxycarbonylsarcosyl*-N-*methylvalyl)*-N-*(2-nitro-3-benzyloxy-4-methylbenzoyl)threonyl*-D-*valylproline* t-*Butyl Ester (**11**).* A mixture of **8** (1.87 g, 3 mmol) and **10** (4.0 g, 12 mmol), protected from light, was heated at 110 °C for 5 h in an oil bath. After cooling the reaction mixture was dissolved in ethyl acetate and the solution was washed with 1 M sodium hydrogencarbonate and water, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography using chloroform–ethyl acetate (1:1 v/v) to give **11** as an amorphous powder, 1.54 g (53.6%), R_f^1 0.59, $[\alpha]_D^{23}$ −6.47 (*c* 1.12, MeOH). Found: C, 62.65; H, 7.07; N, 8.23%. Calcd for $C_{50}H_{66}O_{13}N_6$: C, 62.61; H, 6.94; N, 8.76%. Another product possessing a lower R_f value (R_f^1 0.23) was identified as *N*-(2-nitro-3-benzyloxy-4-methylbenzoyl)threonyl-D-valylproline *t*-butyl ester, 769 mg, amorphous powder, $[\alpha]_D^{23}$ −17.4 (*c* 0.9, MeOH). Found: C, 61.20; H, 6.88; N, 8.42%. Calcd for $C_{33}H_{44}O_9N_4$: C, 61.19; H, 6.92; N, 8.74%.

O-*(Benzyloxycarbonylsarcosyl*-N-*methylvalyl)*-N-*(2-nitro-3-benzyloxy-4-methylbenzoyl)threonyl*-D-*valylproline (**12**).* Trifluoroacetic acid (15 ml) was added to **11** (1.30 g, 1.35 mmol) containing anisole (0.5 ml), and the reaction mixture was stirred for 1 h in the dark. After removal of trifluoroacetic acid, absolute ether was added. The solid precipitate was collected by filtration and washed throughly with absolute ether, 1.00 g (82.0%), R_f^3 0.85, mp 118—121 °C, $[\alpha]_D^{23}$ −4.5 (*c* 1.0, MeOH). Found: C, 60.80; H, 6.44; N, 9.39%. Calcd for $C_{46}H_{58}O_{13}N_6$: C, 61.18; H, 6.47; N, 9.31%.

O-*(Benzyloxycarbonylsarcosyl*-N-*methylvalyl)*-N-*(2-nitro-3-benzyloxy-4-methylbenzoyl)threonyl*-D-*valylproline* p-*Nitrophenyl Ester (**13**).* Bis(*p*-nitrophenyl)sulfite (4.4 g, 13.5 mmol) was added to a solution of **12** (1.20 g, 1.32 mmol) in pyridine (20 ml), and the reaction mixture was stirred at room temperature for 20 h in the dark. After removal of solvent the residue was dissolved in ethyl acetate, and the solution was washed with 1 M sodium hydrogencarbonate and water, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by column chromatography on Sephadex LH-20 in ethyl acetate to give **13** as an amorphous powder, 1.09 g (80.8%), R_f^1 0.42, $[\alpha]_D^{23}$ −1.7 (*c* 0.29, DMF). Found: C, 60.28; H, 6.00; N, 9.11%. Calcd for $C_{52}H_{61}O_{15}N_7$: C, 60.98; H, 6.00; N, 9.58%.

(2-Nitro-3-hydroxy-4-methylbenzoyl)threonyl-D-*valylprolylsarcosyl*-N-*methylvaline(threonine hydroxyl) Lactone (**14**).* Hydrogen bromide gas was bubbled at 0 °C, in the dark, into a solution of **13** (1.0 g, 0.98 mmol) in dioxane (10 ml) for 15 min. The reaction mixture was allowed to stand at room temperature for 1 h. After removal of solvent the residue was dissolved in *N*,*N*-dimethylformamide (20 ml) and acetic acid (1 ml). The solution was added drop by drop over a period of 2.5 h to stirred pyridine (2000 ml) containing triethylamine (1 ml) at 60 °C. After being stirred for 5.5 h at the same temperature the pyridine was concentrated *in vacuo* and the residual oil was dissolved in ethyl acetate. The solution was washed with water, 10% citric acid, 1 M sodium hydrogencarbonate and water, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product (568 mg) was purified by column chromatography on Sephadex LH-20 in methanol to give the desired product (159.8 mg, 24.8%), which was crystallized from ethyl acetate–hexane, 137.8 mg (21.4%), R_f^4 0.38, mp 179—181 °C, $[\alpha]_D^{23}$ −21.0 (*c* 0.5, MeOH). IR (KBr) 1730 (ester C=O), 1655 (amide), 1190 cm^{-1} (ester COC). Found: C, 56.45; H, 6.71; N, 12.62%. Calcd for $C_{31}H_{44}O_{10}N_6$: C, 56.35; H, 6.71; N, 12.72%.

*Actinomycin D (C_1) (**15**).* A solution of **14** (49 mg, 0.074 mmol) in methanol (10 ml) was hydrogenated over palladium black, in the dark. After 2 h, the mixture was filtered and the filtrate was added to an equal volume of stirred 0.067 M phosphate buffer pH 7.1 containing potassium hexacyanoferrate(III) (70.8 mg, 0.215 mmol). The mixture was stirred at room temperature for 20 min. It was then partitioned between ethyl acetate and water, the aqueous layer being separated and extracted three times with ethyl acetate. The combined ethyl acetate layer was washed successively with 1 M sodium hydrogencarbonate, 1 M HCl, and saturated NaCl, dried over Na_2SO_4, and concentrated *in vacuo* to afford a red crystalline residue, which was recrystallized from ethyl acetate–hexane to give actinomycin D, 44 mg (95.7%), R_f^5 0.76. Physical and spectral data are given in Table 1. Found: C, 58.59; H, 6.96; N, 13.29%. Calcd for $C_{62}H_{86}O_{16}N_{12}\cdot H_2O$: C, 58.48; H, 6.97; N, 13.20%.

Microbiological Assay. The results of the assay and microorganism employed are given in Table 1. The minimum amount of the compound necessary for the complete inhibition of growth was determined by the Streak method using HI-agar.

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References

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2) The abbreviations according to IUPAC-IUB commission, *J. Biol. Chem.*, **247**, 977 (1972), are used. *Z*: benzyloxycarbonyl, OBu^t: *t*-butyl ester, Trt: trityl. "Azyline" is used as the name of an 2-aziridinecarboxylic acid, "Azy" being its abbreviation. 3-MeAzy: (*2S*, *3S*)-3-methyl-2-aziridinecarboxylic acid.

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7) Natural actinomycin D was purchased from P-L Biochemicals, Inc., Lot No. 610111.