

K. Okawa, K. Nakajima and T. Tanaka

Department of Chemistry, Faculty of Science, Kwansai Gakuin University, Nishinomiya 662, Japan

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Dedicated to Professor John C. Sheehan on the occasion of his sixty-fifth birthday.

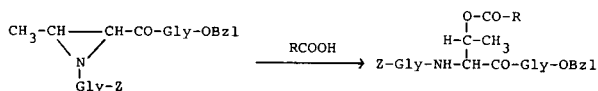
Actinomycin D(C<sub>1</sub>) has been synthesized by a route involving the ester formation between two peptide fragments, (2*S*,3*S*)-1-(2-nitro-3-benzyloxy-4-methylbenzoyl)-3-methyl-2-aziridine-carbonyl-D-valylproline *t*-butyl ester and *N*-benzyloxycarbonylsarcosyl-*N*-methylvaline, via a ring-opening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

*J. Heterocyclic Chem.*, **17**, 1815 (1980).

Sir:

A number of cyclic peptide lactone antibiotics such as the actinomycin group, etamycin, and echinomycin have been reported during the last few years. However, since they possess a 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 as shown in Scheme 1 (1). This paper



reports an application of the ester formation method to the synthesis of the naturally occurring cyclic peptide lactone, actinomycin D(C<sub>1</sub>).

Thus far, the general synthetic method for peptide lactones involved initial formation of the ester bond followed by peptide elongation and cyclization by the formation of the amide bond. A new route is designed in which the linear pentapeptide ester (11) is formed directly by the ring-opening reaction of the aziridine-carboxylic acid containing peptide (8) with dipeptide (10) as shown in 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 the *N*-protected dipeptide.

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

The aziridine segment 8, (2*S*,3*S*)-1-(2-nitro-3-benzyloxy-4-methylbenzoyl)-3-methyl-2-aziridinecarboxyl-D-valylproline *t*-butyl ester, was synthesized as follows.

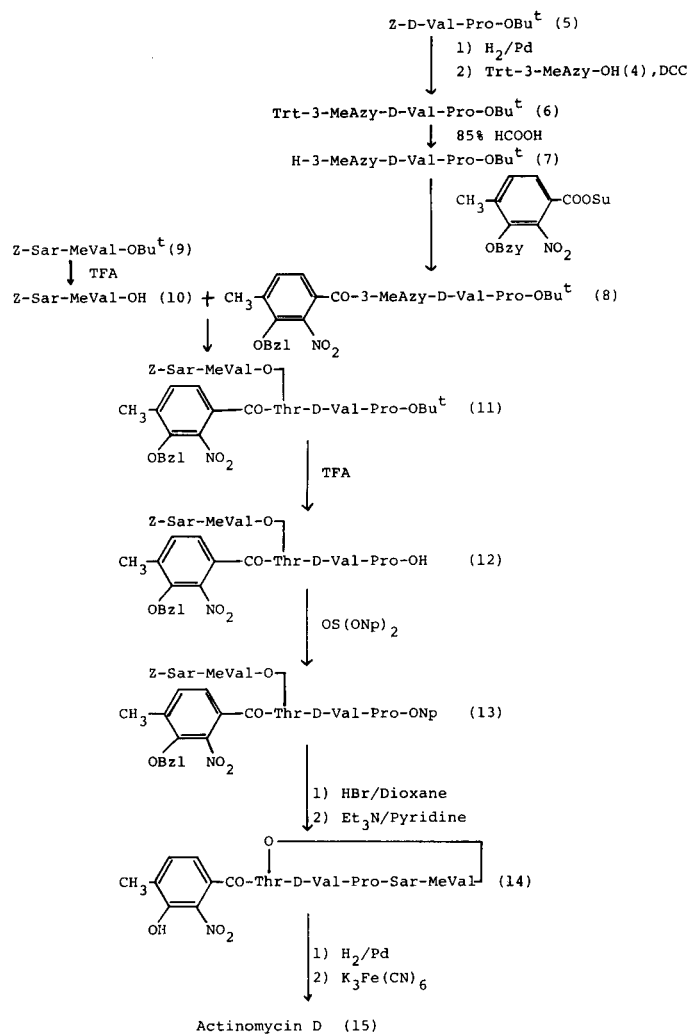


Table  
Physical Properties and Biological Activities of Actinomycin D

Characteristics	Synthetic	Natural Actinomycin (Lit.)	
	Actinomycin D (15)	(a)	(b)
Melting point/(°C)	242-243	241-243	246-247
Optical rotation ( $[\alpha]_D^{23}$ (c))	-316	-323 ± 10	-328 ± 10
Uv absorption	24,900 (443)	24,400 (443)	25,000 (443)
$[\epsilon$ in methanol ( $\lambda$ , nm)]	35,000 (240)	34,100 (240)	34,000 (231)
Ir absorption, (cm <sup>-1</sup> potassium bromide)	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, $\mu$ g./ml.)			
<i>B. Subtilis</i> ATCC-6633	0.78	0.78 (e)	
<i>E. Coli</i> NIHJ	100	—	

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

Coupling of benzyloxycarbonyl-D-valine and proline *t*-butyl ester using dicyclohexylcarbodiimide (DCC) (2) gave the dipeptide (5). Hydrogenation of 5, followed by coupling with 4 using DCC gave the tripeptide (6) in 91.5% yield. Selective removal of the *t*-butyl 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). Benzyloxycarbonyl-sarcosine was coupled with *N*-methylvaline *t*-butyl ester using DCC to give the dipeptide (9). The *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° for 5 hours in the dark. The coupling reaction afforded a mixture of 11 and the hydrolyzed by-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 mmole in 2 l. of pyridine, 60°, 8 hours, and purification on a Sephadex LH-20 column in methanol, gave the cyclic pentapeptide lactone (14) in 21.4% yield. Catalytic hydrogenolysis of 14, 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<sub>1</sub>) (15), which was quantitatively crystallized from ethyl acetate-hexane.

The synthetic actinomycin D(C<sub>1</sub>) was indistinguishable from the natural substance with respect to physical properties and biological activity against *B. Subtilis* as shown in the following Table.

#### REFERENCES AND NOTES

- (1) T. Tanaka, K. Nakajima, T. Maeda, A. Nakamura, N. Hayashi and K. Okawa, *Bull. Chem. Soc. Japan*, **52**, 3579 (1979).
- (2) The abbreviations according to IUPAC-IUB commission, *J. Biol. Chem.*, **247**, 977 (1972), are used. "Azyline" is used as the name of an 2-aziridinecarboxylic acid, "Azy" being its abbreviation. 3-MeAzy: (2*S*,3*S*)-3-methyl-2-aziridinecarboxylic acid.
- (3) J. Meienhofer, *J. Am. Chem. Soc.*, **92**, 3771 (1970).
- (4) J. Meienhofer, *J. Org. Chem.*, **32**, 1143 (1967).
- (5) H. Brockmann and H. Lackner, *Chem. Ber.*, **101**, 1312 (1968).
- (6) H. Brockmann and H. Lackner, *Angew. Chem.*, **66**, 1 (1954).
- (7) Natural actinomycin D was purchased from P-L Biochemicals, Inc., Lot No. 610111.