K. Okawa, K. Nakajima and T. Tanaka

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

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

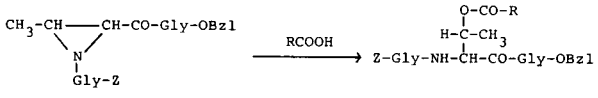
Actinomycin D(C_1) has been synthesized by a route involving the ester formation between two peptide fragments, (2*S*,3*S*)-1-(2-nitro-3-benzyloxy-4-methylbenzoyl)-3-methyl-2-aziridinecarbonyl-**D**-valylproline *t*-butyl ester and *N*-benzyloxycarbonylsarcosyl-*N*-methylvaline, *via* a ring-opening 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 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, etamaycin, 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



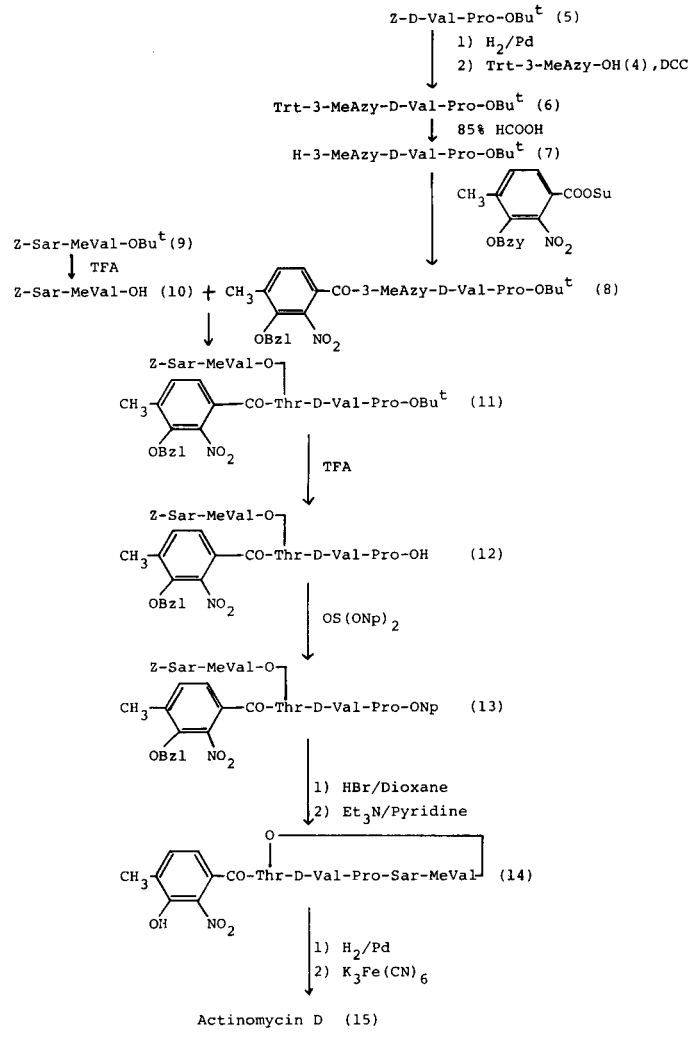
Scheme 1

reports an application of the ester formation method to the synthesis of the naturally occurring cyclic peptide lactone, actinomycin D(C_1).

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 aziridinecarboxylic 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-aziridinecarbonyl-**D**-valylproline *t*-butyl ester, was synthesized as follows.



Scheme 2

0022-152X/80/081815-02$02.25

Table

Physical Properties and Biological Activities of Actinomycin D

Characteristics	Synthetic Actinomycin D (**15**)	Natural Actinomycin (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	24,900 (443)	24,400 (443)	25,000 (443)
[ε in methanol (λ, nm)]	35,000 (240)	34,100 (240)	34,000 (231)
Ir absorption, (cm^{-1} 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, μ g./ml.)			
B. Subtilis ATCC-6633	0.78	0.78 (e)	
E. Coli NIHJ	100	–	

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

Coupline 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 tirtyl 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, *p*H 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 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.