CHEMICAL STUDIES ON ACTINOMYCIN S. II

CHEMICAL STRUCTURES OF ACTINOMYCIN S_2 AND S_3

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A previous paper[1] reported the separation of actinomycin S, which is produced by a streptomyces strain 1048 A, taxonomically related to *Streptomyces flaveolus*. Actinomycin S was separated into two major components, S_2 and S_3, and two minor components, S_0 and S_1 by column chromatography on alumina and silicic acid. The present paper deals with the identification of the S_2- and S_3-components of actinomycin S. The S_0- and S_1-components were separated from components S_{2+3} by column chromatography on acidic alumina (Nakarai Chemicals Ltd.). Components S_2 and S_3 were separated from each other as follows: The mixture was dissolved in very small amount of benzene and applied to a column of alumina equilibrated with benzene. The column was developed with ethyl acetate. The two clearly separated bands thus obtained were eluted with ethyl acetate and evaporated to dryness *in vacuo*. Actinomycin S_2 was recrystallized from a mixture of ethanol and methanol(3 : 1), and S_3 from ethyl acetate.

Table 1. R_D* values of circular paper chromatography of actinomycin S_2, S_3 and D

Solvent system	Actinomycin			
	Found			Reported[5]
	D	S_2	S_3	X_2(A_V or B_V)
1	standard 1.00	1.00	1.59	—
2	standard 1.00	1.00	1.83	1.80
2	standard 1.00	1.00	1.23	—

Solvent system:
1: Isoamyl acetate : 5 % sodium naphthalene sulfonate (1 : 1)
2: Dibutyl ether : ethyl acetate : 2 % β-naphthalene sulfonic acid (3 : 1 : 4)
3: Ethyl acetate : 2 % β-naphthalene sulfonic acid : dibutyl ether (2 : 1 : 1)
* R_D=Rf of unknown/Rf of actinomycin D
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Purified samples of actinomycin S_2 and S_3 were subjected to circular paper chromatography by the method of VINING[2]. As shown in Table 1, actinomycin S_2 had an R_D value similar to actinomycin D, which was kindly supplied by Merck, Sharp and Dohme Research Laboratories. The R_D value of actinomycin S_3 was quite different from that of actinomycin D and corresponded to that of actinomycin X_2 (A_V or B_V) reported by VINING[2,3].

Various physical and chemical properties of actinomycin S_2 and S_3 are shown in Table 2. The ultraviolet absorption spectra of the two compounds are quite similar. The infrared absorption spectra of actinomycin S_2 and S_3 shown in Fig. 1, differed only in the region of

Fig. 1. Infrared absorption spectra of actinomycin S_2, S_3 and D (KBr)
1: Actinomycin S_3
2: Actinomycin S_2
3: Actinomycin D

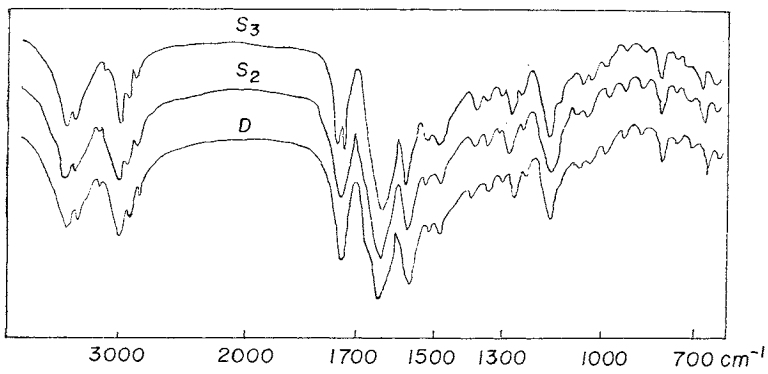

Table 2. Physical and chemical properties of actinomycin S_2 and S_3

Property		Actinomycin			
		S_2	S_3	Reported	
				D (C_1, D_{IV})[4]	X_2[5] (A_V, B_V)
Crystalline form and Color		prism red	fine needle orange red	prism red	fine needle red
Melting point (decomposition)		242~243°C	245~246°C	241°C (235.5~236.5)	244~246°C
Specific rotation		−289±10 ($CHCl_3$)	−320±10 ($CHCl_3$)	−262 ($CHCl_3$)	−341±10 (CH_3OH)
Absorption maximum in mμ		445~446 240~242	445~446 240	445 240	446 —
log ε	445 mμ 240 mμ	4.44 4.53	4.41 4.51	4.43 4.49	4.4 —
Molecular formula				$C_{62}H_{86}N_{12}O_{16}$	$C_{62}H_{84}N_{12}O_{17}$
		Found	Found	Calculated	Calculated
Elemental analysis	C	59.20	57.20	59.33	58.68
	H	6.90	6.65	6.86	6.62
	N	13.47	13.21	13.40	13.25

the carbonyl band, and no differences were detected between the spectra of actinomycin S_2 and D.

Amino acid analyses of acid hydrolyzates of actinomycin S_2, S_3 and of their products of oxidation with hydrogen peroxide[4] were performed in an amino acid autoanalyzer (Hitachi KLA-III model). N-Methylvaline was determined by the conventional method of paper chromatography[6]. An amino acid was isolated from the acid hydrolyzates of actinomycin S_3 by column chromatography on ion exchange resin (Dowex 50×8). This was shown by elementary analysis and comparison of its infrared absorption spectrum with that of authentic material to be identical with γ-oxoproline, which was described by KUHN and OSSWALD[7].

Table 3. Amino acid analyses of acid hydrolyzates of actinomycin S_2, S_3 and oxidation products

Hydrolyzate	Amino acid						
	Asp*	Oxopro	Thr**	Pro	Val	Sar	N-Meval
Actinomycin S_2			1.10	1.98	2.00	2.11	2.01
Actinomycin S_3		0.97	1.21	0.99	2.04	1.99	2.05
Actinomycin S_2 peptide A***			0.88	1.00	1.00	1.04	0.99
Actinomycin S_2 peptide B***			0.72	0.98	1.00	1.01	1.02
Actinomycin S_3 peptide A	0.04		0.89	0.92	1.00	0.98	1.01
Actinomycin S_3 peptide B	0.62		0.93	0.09	1.00	1.00	1.02

Asp: aspartic acid Oxopro: γ-oxoproline Thr: threonine
Pro: proline Val: valine Sar: sarcosine
N-Meval: N-methylvaline

Tne values are expressed as moles of amino acid per mole of actinomycin S_2 and S_3 or of valine.

* Aspartic acid is considered to be the oxidation product of γ-oxoproline formed during the vigorous degradation process with hydrogen peroxide.

** Threonine is known to be partly destroyed under conditions of vigorous acid hydrolysis.

*** These peptides are oxidation products by a method similar to that of BULLOCK and JOHNSON[4].

Peptide A is soluble in organic solvents, and peptide B is soluble in water.

The presence of γ-oxoproline in actinomycin X_2 was suggested by BROCKMANN and GRÖNE[5]. As shown in Table 3, actinomycin S_2 contained two molecules of proline, and actinomycin S_3 contained one molecule of proline and one molecule of γ-oxoproline.

Although the chromophores of all members of the actinomycin group are thought to be identical[8], the chromophore of actino-

mycin S_3 was isolated by treatment of the specimen with barium hydroxide[4,9,10] to obtain actinomycinol. The elemental analysis and ultraviolet and infrared absorption spectra of the isolated actinomycinol were the same as the values described in the literatures[4,9,10].

Although the final elucidation of the structure of actinomycin S_2 must await further investigation, it seems to be identical with actinomycin D. Furthermore the antibacterial and antitumor activities of actinomycin S_2 are identical with those of actinomycin D (unpublished). The chromatographic pattern of actinomycin S_3 and the presence in it of γ-oxoproline suggest that it is similar to actinomycin X_2. A similar result was obtained by KURYŁOWICZ by comparison with an authentic specimen by paper chromatography (personal communication).

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