103°. *Anal.* Calcd. for $C_8H_6O_5N_3Br$: C, 31.60; H, 1.99; N, 13.82; Br, 26.28. Found: C, 31.45; H, 1.97; N, 13.98; Br, 26.65.

2-Amino-2′,4′-dinitroacetanilide (14)——Ammonia gas was bubbled into a stirred solution of 10 g of **13** in a mixture of 100 ml of ethyl acetate and 100 ml of methylene chloride over a period of 8 hr at 25°. The resulting solution was filtered and evaporated. The residue was recrystallized from ethanol to give 4.7 g (60%) of **14** as plates, mp 151.5—152°. IR ν_{max}^{Nujol} cm^{-1}: 3407, 3330, 3160, 3085, 1702, 1613, and 1598. UV $\lambda_{max}^{CH_3OH}$ $m\mu$ (ε): 260 (11,100) and 330 (12,200). *Anal.* Calcd. for $C_8H_8O_5N_4$: C, 40.00; H, 3.36; N, 23.33. Found: C, 40.21; H, 3.26; N, 23.22.

2-(2,4-Dinitroanilino)acetamide (15). A. From 14——A solution of 1.0 g of **14** in 5 ml of dimethyl sulfoxide was stirred at room temperature for 7.5 hr. The rearranged acetamide **15** began to separate as orange crystals about 50 min after the beginning of the reaction. TLC analysis (ethyl acetate) showed that the reaction was nearly complete in 6 hr. The precipitate formed was collected by filtration and washed with ethanol. The crude **15** was suspended in 30 ml of ethanol and refluxed. Cooling and filtration afforded 0.70 g (70%) of **15** as yellow needles, mp 224—225°. Recrystallization from ethanol gave analytically pure **15**, mp 224—225°. IR ν_{max}^{Nujol} cm^{-1}: 3460, 3333, 3165, 3100, 3080, 1702, and 1614. UV $\lambda_{max}^{CH_3OH}$ $m\mu$ (ε): 259 (9,700) and 343 (17,800); NMR (DMSO-d_6) δ: 4.13 (2H, d, J=5 Hz, CH_2), 6.95—8.84 (3H, ABX type pattern, J_{ortho}=9.5 Hz, J_{meta}=3 Hz, aromatic), 7.38 and 7.66 (2H, D_2O exchangeable, $CONH_2$) and 9.06 (1H, t, J=5 Hz, D_2O exchangeable, NH). *Anal.* Calcd. for $C_8H_8O_5N_4$: C, 40.00; H, 3.36; N, 23.33. Found: C, 39.83; H, 3.31; N, 23.05.

B. From N-(2,4-Dinitrophenyl)glycine (16)[9]——A solution of 1.3 g of ethyl chloroformate in 3 ml of tetrahydrofuran was added with stirring to an ice-cooled mixture of 2.41 g of **16** and 1.21 g of triethylamine in 20 ml of tetrahydrofuran. After 5 min, the resulting mixture was added with vigorous stirring to 20 ml of ice-cooled 28% aq. ammonia and to this was added 500 ml of water. The precipitate obtained by filtration was subjected to chromatography on 50 g of silica gel eluting with chloroform and then with ethyl acetate. The first fraction gave 0.51 g of ethyl N-(2,4-dinitrophenyl)glycinate, mp 140—141°. An analytical sample was recrystallized from ethanol, mp 143—144° (lit.[13] mp 142—144°). IR ν_{max}^{Nujol} cm^{-1}: 3328, 3095, 1743, and 1622. NMR (acetone-d_6): 1.29 (3H, t, J=7 Hz, CH_2CH_3), 4.24 (2H, q, J=7 Hz, CH_2Me), 4.43 (2H, d, J=5 Hz, CH_2CO), 7.08—8.93 (3H, ABX type pattern, J_{ortho}=9.5 Hz, J_{meta}=2.7 Hz, aromatic) and 8.9 (1H, D_2O exchangeable, NH). *Anal.* Calcd. for $C_{10}H_{11}O_6N_5$: C, 44.61; H, 4.12; N, 15.61. Found: C, 44.41; H, 4.19; N, 15.37.

The second fraction gave 0.10 g of 2,4-dinitroaniline and the third, 0.25 g of **15**, mp 223—224°, identical with the sample obtained in A by mixture melting point, IR and TLC.

13) S. Passeron and G.A. Brieux, *Bull. Soc. Chim. France*, **1963** 35.

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Studies on Chromophore Groups of Streptothricin Group Antibiotics by Optical Rotatory Dispersion and Circular Dichroism

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In the course of searching for antibiotics, streptothricin group antibiotics such as racemomycins,[2] yazumycins,[3] containing β-lysine in their molecule, and new-type antibiotics such

1) Location: *1-14 Bunkyo-machi, Nagasaki, 852, Japan.*
2) H. Taniyama, Y. Sawada, and T. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1627 (1971).
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as SF-701,[4] LL-AC541,[5] LL-AB664,[6] E-749-C,[7] BY-81, BD-12[8] and citromycin,[9] containing sarcosine[4] or formiminoglysine,[5-9] had been found by many laboratories. Characteri-

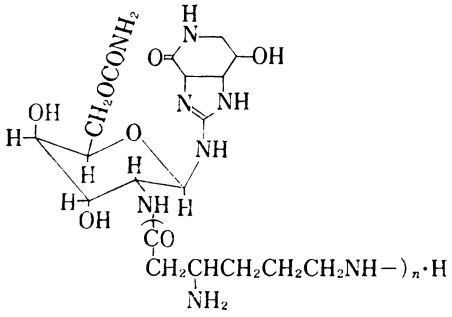
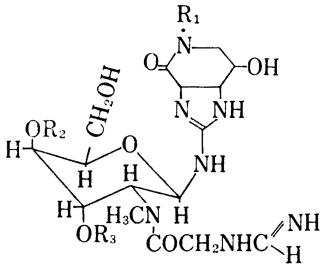


Chart 1[10]



LL-AC541
R_1 : −H
R_2, R_3 : −H, −$CONH_2$
LL-AB 664
R_1 : −CH_3
R_2, R_3 : −H, −$CONH_2$

Chart 2[11]

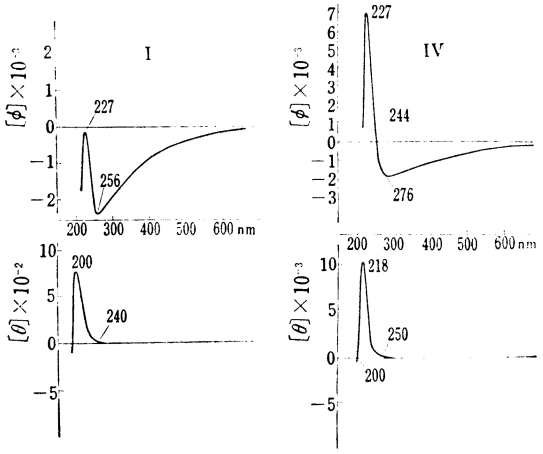


Fig. 1a

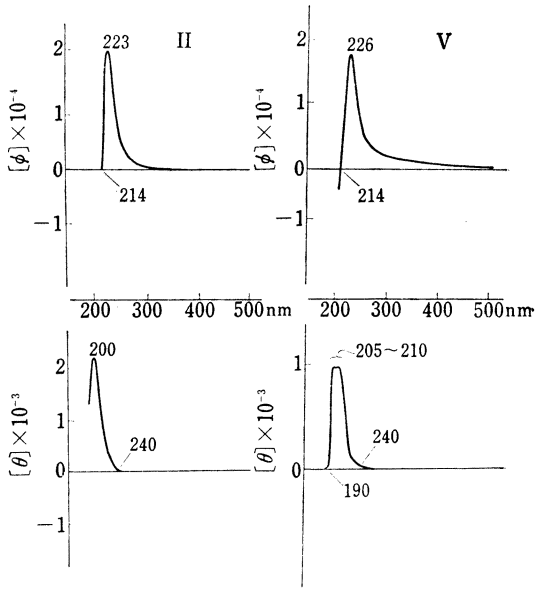


Fig. 1b

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5) V. Zbinovsky, W.K. Hausmann, E.R. Wetzel, D.B. Borders, and E.L. Patterson, *Appl. Microbiol.*, **16**, 614 (1968).
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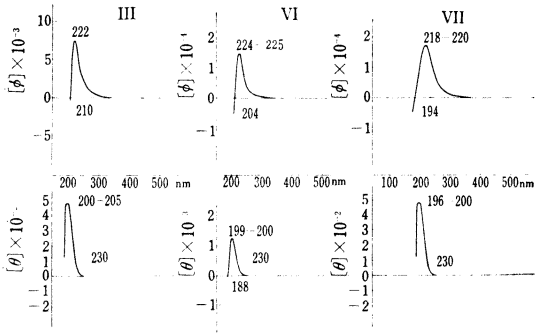

Fig. 1c

zation or identification of these antibiotics were generally performed by paper chromatogtaphy and automatic amino acid analyses of acid hydrolysate as well as the physical and chemical properties. However, it was difficult to clear the properties of an antibiotic isolated because of a water-soluble basic character.

The tentative structure for streptothricin-streptolin was proposed by van Tamelen,*et al.*[10] (Chart 1) and structures of antibiotics LL-AC541 and LL-AB664 has been reported by Borders, *et al.*[11] (Chart 2). However, the elucidative approach for chromophore groups of streptothricins has not been published.

Streptothricins has a water-soluble basic character. The ultraviolet (UV) spectrum of then in aqueous solution showed no maximum absorption from 220 nm to 340 nm. However, from the measurement of optical rotatory dispersion (ORD), antibiotics racemomycin A(I), SF-701 (IV) and E-749-C has a fitst extremum at 227 nm in positive field through negative field. On the other hand, in compounds racemomycinic A acid (II), SF-701 acid derivative (V),[12] E-749-C acid derivative, N-guan.-streptolidyl-β-D-gulosaminide (III) and N-guan.-streptolidyl-N′-methyl-β-D-gulosaminide (VI), they have also a positive extremum at 222—226 nm in positive field. In Fig. 1 and in Table I, examples of ORD and circular dichroism (CD) data in aqueous solution were shown.

In CD curves, the positive Cotton effect at 199—218 nm supported the pattern of positive extremum on their ORD curves. Therefore, these optical active absorption bands at approximately 200 nm were ascribed to the >C=N- group in a guanidino residue in the structures proposed.[10,11]

Table I

Samples	ORD (in H_2O) Cotton effect Ist extremum nm ([θ]), concentration, cell length	ORD (in H_2O) Background rotation	CD (in H_2O) λ 200nm region ([θ]), concentr. cell length	CD (in H_2O) λ270nm region ([θ])
IV·HCl	227—228 (6900°)0.1%, 10,0.35mm	negative	217—218 (10100°)0.1% 0.35mm	not clear
E-749-C·HCl	227—228 (10000°) 0.1% 1mm	negative	216—218 (10000°) 0.1% 0.35mm	not clear
I·H_2SO_4	226—228 (200°) 0.1%, 10,1mm	negative	199—201(790°) 0.03, 0.1% 0.35mm	270 (1.8°) 0.1%,100 mm
V·HCl	226 (17600°) 0.1% 10,1,0.35mm	positive	205—215 (1200°) 0.1% 0.35mm	not clear
II·H_2SO_4	222—223(22600°) 0.1% 10,1mm	positive	199—201 (2300°) 0.03%, 0.35mm	not clear
VI·HCl	224—225 (14700°) 0.1% 10,1mm	positive	199—200 (1200°) 0.03, 0.1% 0.35mm	270 (1.7°) 0.1%, 100mm
III·HCl	222(6700°) 0.085% 5mm	positive	200—205 (500°) 0.085% 5mm	not clear
VII·HCl	218—220 (17500°) 0.1% 1mm	positive	196—200 (500°) 0.1% 0.35mm	not clear

12) It was presented at the 14th symposium on the chemistry of natural products (Fukuoka), "Abstr. paper," 1970, p. 55.

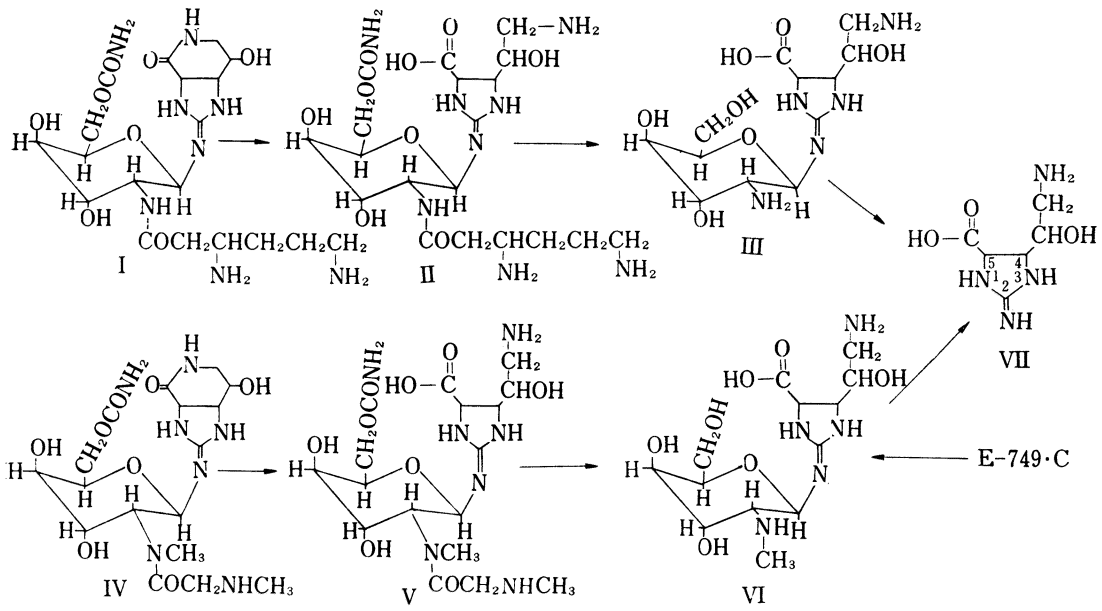

Chart 3. Acid hydrolysates of Streptothricins

Contrary to a back ground rotation of the ORD cruves at long wave region in the lactam system and the open ring system, it was presumed that in lactam system there is an optical active absorption band which gives negative Cotton effect at shorter wavelength region. The ORD difference of streptothricin and streptothricinic acid has been already noted[13] for the conformational discussion of a biologically inactive substance. In this point, the second extremum on ORD curve was most important to elucidate the lactam conformation of streptothricins. Despite of a maximum at 220—235 nm in general, six-membered lactam systems, the lactam system in streptothricins seemed to be show a maximum at below 200 nm. This influences may be considered to be either by a decreasing of the bond-order of -C(=O)-N(H)- in the cyclic conformation, by the solvolysis in water, or by a distorted lactam ring of the cyclic guanidino groups.

Considering the amplitude of optical active absorption band at 200 mμ for both the lactam system and the open ring system, the latter has a larger amplitude than that of the former, suggesting that the positions 4 and 5 in streptolidine moiety are largely affecting to a chromophoric group, and the evidence is also supported by the molecular models. That is, a feature of the NMR spectra of streptolidine moiety was the change in the J_{4-5} coupling constant from 5.0 Hz at δ 5.0 for the open-chain form with the dihedral angle of the protons near 120° to about 15 Hz at δ 6.06 for the lactam form with a dihedral angle near 180°. These, higher coupling constants than predicted by Karplus[14] was attributed to the influence of the guanidino group as noted for the antibiotics LL-AC541 and LL-AB664.[11]

On the contribution ratios of an anomeric position and positions 4 and 5 in streptolidine moiety through lone pair electons on nitrogen atoms of the cyclic guanidino residue, it was suggested that the ORD and CD curves of streptolidine are similar to those of substances having an open ring system. Therefore, the controbution ratio of an anomeric position to a chromophore group seemed to be little.

13) H. Taniyama, Y. Sawada, and T. Kitagawa, *J. Antibiotics*, **24**, 662 (1971).
14) M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870, (1963).

The assigned attachment of N-glycoside bond to the exocyclic nitrogen of streptolidine moiety is based on analogy to the structure of the corresponding glycoside from streptothricin, and a possible fixiation of –C=N- double bond, which could not be hydrogenated on platinum dioxide, on guanidino group is based on the aspects of ORD and CD curves, and of p*K*a′ value.[15]

Another active absorption band at 270 nm, probably ascribed to a guanidino group, was under investigation because of their small amplitude.

Consequently application of ORD and CD for streptothricin group antibiotics or the relative antibiotics was conventionally distinguished from those of other water-soluble basic antibiotics.

Experimental

NMR spectra were determined at 60 MHz spectrometer (Hitachi H-60) in D_2O with DSS as an internal standard. Melting points determined by automatic micro-apparatus are uncorrected. *Rf* values were obtained by paper chromatography of Toyo-Roshi No. 51 UH type using a solvent system of *n*-BuOH–pyridine–HOAc–H_2O–*t*-BuOH (15: 10: 3: 12: 4). Detection: ninhydrin and Pauly tests. Amino acid analysis was carried out by the method described in the previous paper.[2] ORD and CD curves were obtained by JASCO ORD/UV-5 spectrometer attached with J-5 or J-10 attachments in purified water at 28—30°. Compounds I, II and VII were obtained by the same method[2,13] respectively.

N-guan.-streptolidyl-β-D-gulosaminide (III)——Racemomycin A hydrochloride was hydrolysed with 6N HCl, at 90—100° for 12 hr, and applied to the column of Sephadex LH-20 (2×300 cm) using water as eluent. Fractionation: 8 g/Fr. Fractions No. 60—65 gave a spot of streptolidine-sugar. After lyophylization, white and hygroscopic powder was obtained. Then it was converted to the hydrochloride salt. Yield: 1 g→*ca.* 200 mg, mp above 220° with decomp., $[\alpha]_D^{29}=-2.5°$ ($c=1.37$ in H_2O), p*K*a′ values: 2.25, 6.23, 8.77, and 10.80, *Rf* 0.32, IR: 1720 cm^{-1} (COOH), *Anal.* Found: C, 29.98; H, 5.93; N, 14.40; Cl, 22.55%. Calcd. for $C_{12}H_{23}O_7N_5\cdot 3HCl\cdot H_2O$; C, 30.22; H, 5.87; N, 14.68; Cl, 22.35%.

On an Antibiotic SF-701 (IV)——The crude powder containing SF-701 hydrochloride was purified repeatedly by a column chromatography of Sephadex LH-20 (2×140 cm). It was developed with 10% aqueous methanolic solution. Fractionation: 8 g/Fr. Fractions 36—38 afforded the antibiotic hydrochloride. White plate, mp 215—217°, $[\alpha]_D^{20}=-65°$ ($c=1$ in H_2O), p*K*a′ values: 7.2, 9.3, *Rf* 0.46, IR cm^{-1} in KBr: 1720 (COOH), 1710 ($OCONH_2$), NMR δ: 2.82, 3.06 due to N-Me, amino acid analysis; streptolidine: sarcosine: ammonia: methylamine (1.00: 0.64: 0.35: 0.04). Empirical formula was corrected in this paper. *Anal.* Found: C, 35.71; H, 6.12; N, 17.28; Cl, 12.68; H_2O, 6.0%. Calcd. for $C_{17}H_{29}O_8N_7\cdot 2HCl\cdot 2H_2O$ (M.W.=568): C, 35.91; H, 6.16; N, 17.25; Cl, 12.50; H_2O, 6.7%. M.W.=500 (by Sephadex G-10), 570 (by titration), reineckate salt: mp above 165° with decomp., *Anal.* Found: C, 26.62; H, 4.15; N, 23.28; H_2O, 3.0%. Calcd. for $C_{17}H_{29}O_8N_7\cdot 2[Cr(NH_3)_2(SCN)_4]\cdot 2H_2O$ (M.W.=1131): C, 26.52; H, 3.98; N, 23.52; H_2O, 3.18%.

SF-701 Acid (V)——IV·HCl was dissolved in a hundred-fold of 3N HCl and allowed to stand at room temperature for a day. The solution was dried to a white powder below 50° *in vacuo*, mp above 220° with decomp., *Rf* 0.28 $[\alpha]_D^{20}=+1.3°$ ($c=1$ in H_2O), p*K*a′: 2.70, 6.4, 9.0 and 10.50, IR: 1720 cm^{-1} (COOH), NMR δ: 2.82, 3.06 (2×N-Me), amino acid anal.; streptolidine: sarcosine: ammonia: methylamine=1.00: 0.57: 0.4: 0.02. *Anal.* Found: C, 31.56; H, 6.42; N, 15.18; Cl, 16.37%. Calcd. for $C_{17}H_{31}O_9N_7\cdot 3HCl\cdot 3H_2O$: C, 31.85; H, 6.25; N, 15.30; Cl, 16.63%.

N-guan.-streptolidyl-N′-methyl-β-D-gulosaminide (VI)——IV·HCl was hydrolysed with 3N HCl at 100° for 5 hr. The hydrolysate was applied to the column of Sephadex LH-20 (2×300 cm) using water as eluent. Fractionation: 8 g/Fr. Fractions No. 66—70 gave the corresponding compound. Yield: 1 g→*ca.* 200 mg, mp above 210° with decomp., *Rf* 0.32, $[\alpha]_D^{20}=-7.5°$ ($c=1$ in H_2O), p*K*a′ values: 2.70, 6.45, 8.78, and 10.50. IR: 1720 cm^{-1} (COOH), NMR: 2.8 (N-Me). *Anal.* Found: C, 32.02; H, 6.28; N, 14.25; Cl, 21.61; H_2O, 3.50%. Calcd. for $C_{13}H_{25}O_7N_5\cdot 3HCl\cdot H_2O$: C, 31.80; H, 6.10; N, 14.27; Cl, 21.71; H_2O, 3.66%. Found: C, 32.73; H, 6.19; N, 14.53. Calcd. for $C_{13}H_{25}O_7N_5\cdot 3HCl$: C, 33.01; H, 5.94; N, 14.80%.

VI from E-749-C——Antibiotic E-749-C hydrochloride, 200 mg, was hydrolysed with 3N HCl at 80—90° for 5 hr. The concentrated products of the reactant was applied to the column of Sephadex LH-20 (2×300 cm) and eluted with water. Fractionation: 8 g/Fr. Fractions No. 62—66 gave a spot of VI. $[\alpha]_D^{29}=-3.0\pm 0.2°$ ($c=4.05$ in H_2O). *Anal.* Found: C, 31.76; H, 6.08; N, 13.96%.

Acknowledgements The authors wish to thank the Meiji Seika Co. for generous supply of the antibiotic SF-701, Dr. J. Shoji, Shionogi Research Laboratory, for generous supply of antibiotic E-749-C, and also we thank Dr. M. Suzuki, Tanabe Seiyaku Microbial Chemistry Research, and the peoples of JASCO Research Center, for their ardent ORD and CD measurements.

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