NOTE

A WATER-SOLUBLE BASIC ANTIBIOTIC E-749-C IDENTICAL WITH LL-AC541[1,2]

JUN'ICHI SHOJI, SHUICHI KOZUKI, MITSUO EBATA and HIDEO OTSUKA

Shionogi Research Loboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka

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In the previous communication[3] we have reported on the isolation of polyetherin A from a strain E-749 which was identified as *Streptomyces hygroscopicus*. This strain simultaneously produces a water-soluble basic antibiotic, named E-749-C, active against Gram positive and Gram negative bacteria.

The antibiotic produced in the culture filtrate was adsorbed on an IRC-50 (Na^+) column and eluted with 0.5 N HCl. Adsorption on active charcoal at neutral pH and elution with acidic aqueous methanol followed by lyophilization gave crude powders of the antibiotic. Purification was performed by preparation of crystalline reineckate or helianthate. The purified reineckate was treated with pyridine hydrochloride to obtain the hydrochloride salt as a colorless amorphous powder.

Reineckate: no definite m. p., *Anal.* Found: C 24.36, H 4.71, N 22.26, Cr 8.75, H_2O 10.91 %.

Helianthate: m. p.* 220~230°C (dec.), *Anal.* Found: C 46.39, H 5.98, N 16.94, S 5.63, H_2O 5.77 %.

Hydrochloride: m. p. 210~240°C (dec.), *Anal.* Found: C 36.53, 37.20; H 6.50, 5.70; N 18.28, 19.15; Cl 12.40, 13.10 %.

Physico-chemical properties of the hydrochloride were as follows:

Rf values of paper chromatography
ca. 0.11 (*n*-butanol – acetic acid – water, 4 : 1 : 2).
0.45 ~ 0.55 (*n*-propanol – pyridine – acetic acid – water, 15 : 10 : 3 : 12).

$[\alpha]_D^{22}$ −58.5±1.0° (*c*, 1.091, water).

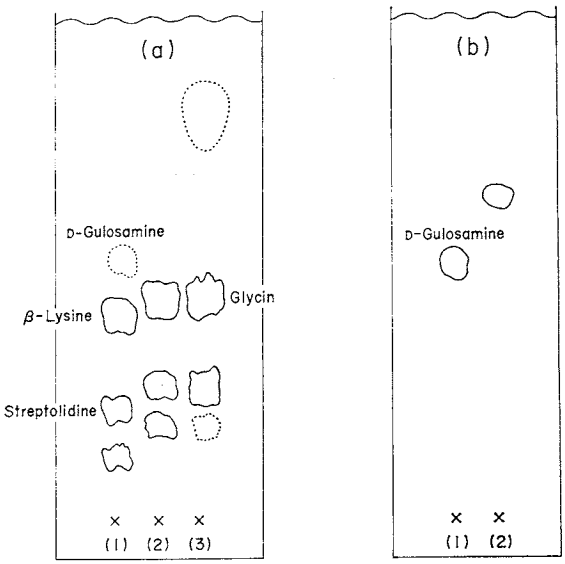
No ultraviolet absorption at 220~750 mμ.

I. R. (KBr tablet): 3300 (broad), 1713, 1663~1645, 1600 (shoulder), 1490, 1390, 1307, 1188, 1072, 1042, 948, 930 cm^{-1}.

Minimum M. W. *ca.* 614 (amino acid analysis).

The hydrochloride was soluble in water, methanol, dimethylformamide, dimethylsulfoxide, but insoluble or only slightly soluble in other common organic solvents. Basic nature was indicated by paper electrophoresis. The antibiotic showed weakly positive ninhydrin reaction (yellow to purple) and PAULY reaction (yellowish green), and decolorized potassium permanganate and bromine solutions. FEHLING, TOLLENS, BENEDICT, anthrone, ELSON-

Fig. 1. Paperchromatogram of the hydrolysates of E-749-C and streptothricin



(a) Detected by ninhydrin reaction
(b) Detected by ELSON-MORGAN reaction
(1) Hydrolysate of streptothricin (for 10 hours)
(2) Hydrolysate of E-749-C (for 10 hours)
(3) Hydrolysate of E-749-C (for 40 hours)

* Melting points were measured by micro-melting point apparatus and were uncorrected.

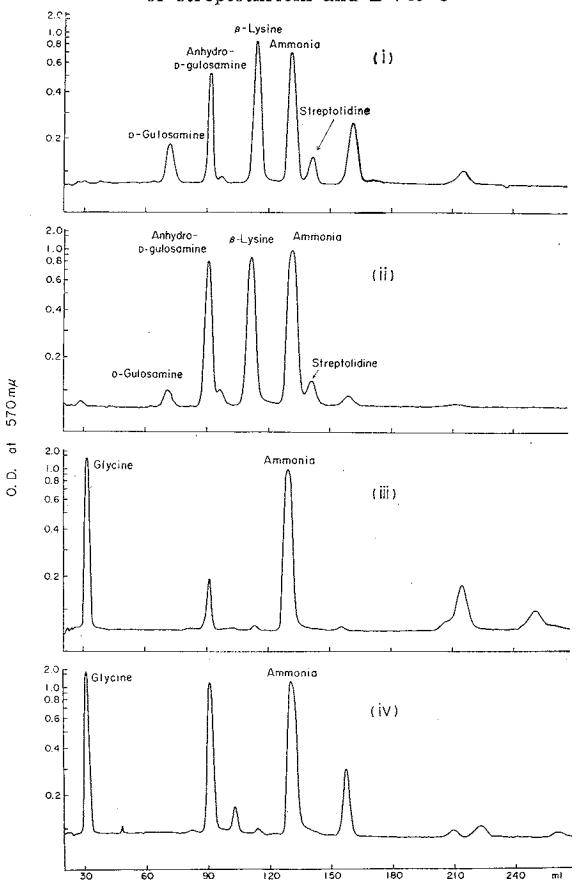
MORGAN, EHRLICH and SAKAGUCHI reactions were substantially negative.

E-749-C hydrochloride was hydrolized with 6 N HCl at 100°C for 10 hours and also 40 hours. The hydrolysates were compared with the hydrolysate of streptothricin sulfate* by paper chromatography and automatic amino acid analysis. The paper chromatogram carried out on Toyo Roshi No. 51 with *n*-butanol - acetic acid - water (3 : 1 : 1) by continuous descending development for 18 hours, and visualized by ninhydrin and ELSON-MORGAN reactions is shown in Fig. 1. The spots assigned to D-gulosamine, β-lysine and streptolidine with the hydrolysate of streptothricin were confirmed by reference to those shown by TSURUOKA *et al.*[4] The following results were obtained with the hydrolysates of E-749-C, the presence of glycine was confirmed by comparison with authentic sample; a slower moving spot below glycine gave the same characteristic greenish gray coloration with ninhydrin as did streptolidine, but had a different mobility and an ELSON-MORGAN positive substance which moved faster than D-gulosamine and was practically ninhydrin negative.

The conditions of the amino acid autoanalysis cited in Fig. 2 were employed so as to bear relation with the quantitative analytical method of streptothricin hydrolysates determined by EGOROV *et al.*[5] It was evident that there were no peaks corresponding to D-gulosamine, β-lysine and streptolidine in the hydrolysates of E-749-C. In another analyses run with our routine analytical conditions with hydrolysates by 6 N HCl, 105°C, 24 hours and 48 hours, *ca.* 1.63 μ moles of glycine and *ca.* 2.22 μmoles of ammonia were found per 1 mg of E-749-C hydrochloride.

BORDERS *et al.*[1] reported that glycine, stereptolidine, N-methyl-α-D-gulosamine, N-*guan*-streptolidyl-β-D-gulosaminide, ammonia, carbon dioxide and formic acid were found from an acid hydrolysate of the antibiotic LL-AC541. This antibiotic** was also examined by us in the same manner as described above in order to compare with our E-749-C. Both intact antibiotics gave the same Rf values in paper chromatograms. Furthermore, completely identical peaks were observed in automatic amino acid analysis with the hydrolysates

Fig. 2. Amino acid analyses of the hydrolysates of streptothricin and E-749-C



i) Hydrolysate of streptothricin (for 10 hours)
ii) Hydrolysate of streptothricin (for 48 hours)
iii) Hydrolysate of E-749-C (for 10 hours)
iv) Hydrolysate of E-749-C (for 40 hours)

Conditions : column, 0.9×50 cm ; resin, Hitachi spherical resin No. 3105 ; buffer, 0.7 M Na citrate, pH 5.28; temperature, 50°C ; rate, 30 ml/hour ; apparatus, Hitachi automatic amino acid analyzer.

* Samples of streptothricin and racemomycin A were used and gave the same results.

** A preparation of antibiotic LL-AC541 was kindly supplied by Dr. E. L. PATTERSON after our experiments with E-749-C were finished.

of both antibiotics. In the light of these data, therefore, it may be concluded that E-749-C and LL-AC541 are identical substances.

However, streptolidine itself could not be found at any rate in our analysis.

Recently, some other new members related to streptothricin group of antibiotics have been reported. LL-AB664[6] was reported to contain glycine and N-methylstreptolidine as a part of the constituents. Antibiotics BD-12 and BY-81[7] which exhibited a cross resistance with streptothricin were considered to be chromatographically identical with LL-AB664 and LL-AC541, respectively. An antibiotic SF-701[4] was also reported to have sarcosin in the constituents. It will be very interesting problem to compare directly the degradative products of these antibiotics.

Acknowledgement

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Addendum

After the submission of the present paper, streptolidine (roseonine) was kindly supplied from prof. H. TANIYAMA, Nagasaki University. In analysis with the conditions described in Fig. 2, this preparation gave a peak whose position coincided with the peak which was formerly assigned to anhydro-D-gulosamine in the figure.

In this respect, the authors amend their former assignment for the peaks of the streptothricin hydrolysate which was based on the literature[5].

According to the amended view point, the peak of streptolidine was obviously observed in the hydrolysate of E-749-C (Fig. 2), and it would be considered that the difference of the mobilities of streptolidine in Fig. 1, was caused by they moved as their sulfate and hydrochloride.