Communications to the editor

THE ANALYSIS OF A NEW ANTIVIRAL SUBSTANCE S-15-1, STREPTOTHRICIN GROUP ANTIBIOTIC

Sir:

In a previous communication[1] we reported on the isolation, purification, physicochemical and biological properties of a new antivial antibiotic S-15-1 produced by *Streptomyces* S-15-1 isolated from a soil sample. This product was characterized as a new streptothricin group antibiotic. It exhibited *in vitro* activity against Newcastle disease virus, gram-positive and gram-negative bacteria, and some yeasts.

S-15-1 hydrochloride has been hydrolyzed with 6 N HCl at 105°C for 10 hours and 40 hours and the hydrolysate was compared with the hydrolysate of racemomycin by paper chromatography, thin-layer chromatography, and automatic amino acid analysis. Aliquots of hydrolysates were applied to Toyo Roshi No. 51 and No. 51 UH type papers and developed (ascending) with *n*-butanol - acetic acid - water (2 : 1 : 1), and *n*-butanol - pyridine - acetic acid - water - *t*-butanol (15 : 10 : 3 : 12 : 4) solvent systems. The thin-layer chromatograms on Avicel SF plate (Funakoshi Co.) were developed (ascending) with *n*-propanol - pyridine - acetic acid - water (15 : 10 : 3 : 12). Spots detected by ninhydrin reaction are indicated in Figs. 1, 2 and 3. Two spots of S-15-1 hydrolysate (Rf 0.3 and 0.25) in Fig. 1 correspond to *β*-lysine and streptolidine obtained with the hydrolysate of racemomycin, and this agrees with the results reported by TSURUOKA *et al.*[2] Similar agreement is also indicated for *β*-lysine and streptolidine in Figs. 2 and 3. S-15-1 hydrolysates yield an unidentified component (Rf 0.5) not present in racemomycin hydrolysates (Fig. 2). There were no spot corresponding to glycine in the hydrolysate of S-15-1 (Figs. 1 and 2).

When amino sugars in chromatogram were being detected by the ELSON-MORGAN reaction (Fig. 4) a faint spot (Rf 0.5) of amino sugar was detected in S-15-1 hydrolysate. This spot was not detected in racemomycin hydrolysates.

Fig. 1. Paper chromatography of acid hydrolysates of S15-1 and racemomycin.
A : Hydrolysate of racemomycin (for 10 hours)
B : Hydrolysate of S15-1 (for 10 hours)
C : Hydrolysate of racemomycin (for 40 hours)
D : Hydrolysate of S15-1 (for 40 hours)
E : *β*-Lysine HCl
F : Streptolidine HCl
G : Glycine
Solvent system : *n*-Butanol - acetic acid - water (2 : 1 : 1)
Paper : Toyo Roshi No. 51
Detection : Ninhydrin

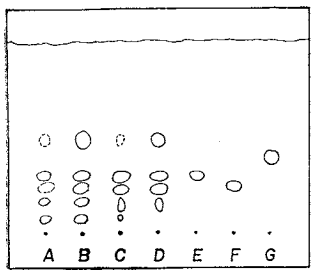


Fig. 2. Paper chromatography of acid hydrolysates of S15-1 and racemomycin.
A : Hydrolysate of racemomycin (for 10 hours)
B : Hydrolysate of S15-1 (for 10 hours)
C : Hydrolysate of racemomycin (for 40 hours)
D : Hydrolysate of S15-1 (for 40 hours)
E : *β*-Lysine HCl
F : Streptolidine HCl
G : Glycine
Solvent system : *n*-Butanol - pyridine - acetic acid - water - *t*-butanol (15 : 10 : 3 : 12 : 4)
Paper : Toyo Roshi No. 51 UH type
Detection : Ninhydrin

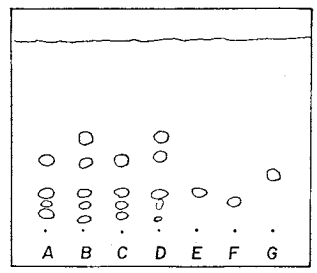


Fig. 3. Thin-layer chromatography of acid hydrolysates of S15-1 and racemomycin.
A : Hydrolysate of racemomycin (for 10 hours)
B : Hydrolysate of S15-1 (for 10 hours)
C : Hydrolysate of racemomycin (for 40 hours)
D : Hydrolysate of S15-1 (for 40 hours)
E : *β*-Lysine HCl
F : Streptolidine HCl
Solvent sysem : *n*-Propanol - pyridine - acetic acid - water (15 : 10 : 3 : 12)
Plate : Avicel SF plate (Funakoshi Co.)
Detection : Ninhydrin

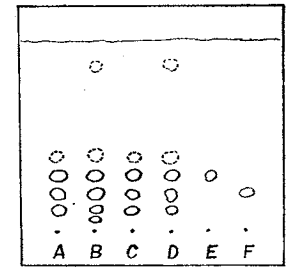


Fig. 4. Paper chromatography of acid hydrolysates of S 15-1 and racemomycin.

A : Hydrolysate of racemomycin (for 10 hours)
B : Hydrolysate of S 15-1 (for 10 hours)
C : Hydrolysate of racemomycin (for 40 hours)
D : Hydrolysate of S 15-1 (for 40 hours)
Solvent system : *n*-Butanol - pyridine - acetic acid - water - *t*-butanol (15 : 10 : 3 : 12 : 4)
Paper : Toyo Roshi No. 51 UH type
Detection : ELSON-MORGAN reaction

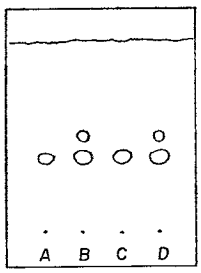
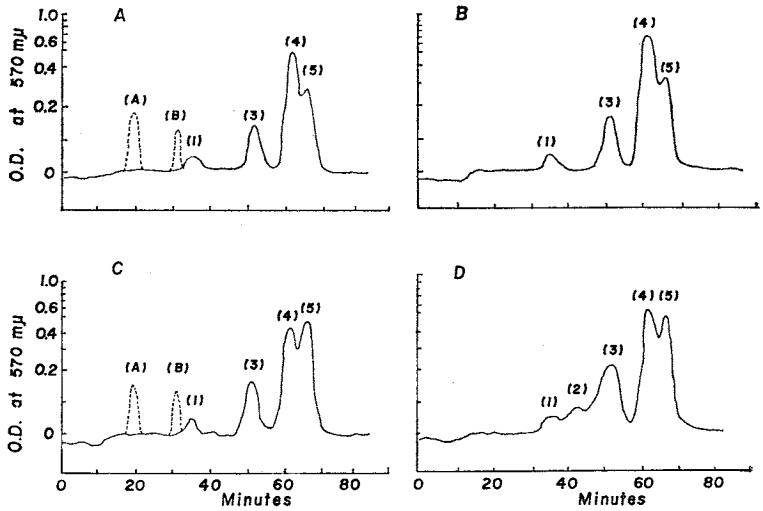


Fig. 5. Amino acid analysis of the hydrolysates of S15-1 and racemomycin.

A : Hydrolysate of racemomycin (for 10 hours)
B : Hydrolysate of racemomycin (for 40 hours)
C : Hydrolysate of S 15-1 (for 10 hours)
D : Hydrolysate of S 15-1 (for 40 hours)
Condition : Column, 2.5×15 cm ; resin, Aminex A-4 ; buffer, 0.7 M Na citrate, pH 5.28 ; temperature, 58℃ ; apparatus, Shibata automatic amino acid analyzer type AA-600.
Peak A : Glycine (authentic), Peak B : D-Glucosamine (authentic), Peak 4 ; Ammonia.



A previously described method[6] was used for amino acid autoanalysis of S-15-1 and racemomycin hydrolysates (Fig. 5). Close agreement was observed with these antibiotics for peaks 1, 2 and 3 corresponding to an amino sugar, β-lysine and streptolidine respectively. Peak 1 had a somewhat slower mobility than reference D-glucosamine. Neither racemomycin nor S-15-1 produced a component which corresponds to glycine (or N-methyl glycine) which was reported in hydrolysates of the new streptothricin group antibiotics E-749C[3], SF-701[4], and LL-AB 664[5]. The 40-hour hydrolysate of S-15-1 contained an identified component (peak 2) not observed in racemomycin hydrolysates.

Although it was not possible to resolve the components sufficiently for quantitative analysis, our data differentiate S-15-1 antibiotic from racemomycin, E-749C, SF-701 and LL-AB 664. It will be of interest to further compare the components in hydrolysates of S-15-1 and racemomycin.

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