THE IDENTITY OF S15-1-A AND B WITH RACEMOMYCINS A AND C

TAKESHI KAWAMURA*, TSUTOMU KIMURA**, KUNIHIKO TAGO**, TERUHIKO BEPPU and KEI ARIMA

Laboratory of Microbiology, Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo, Japan

(Received for publication May 12, 1976)

In the previous papers[1,2], we have reported that *Streptomyces purpeofuscus* produced a new streptothricin group antibiotic S15-1. S15-1-A, a major component, and S15-1-B and C, minor components of antibiotic S15-1, which were purified by cellulose column chromatography and Sephadex LH-20 column chromatography with a solvent system of 0.05 M NaCl solution, showed the same characteristics of S15-1-A and B as racemomycins A and C. This paper deals with the results of purification and identification of S15-1-A and B.

Purification

The preparation of crude antibiotic S15-1 was carried out as previously reported.[1] Each component of crude antibiotic S15-1 (2.0 g) was isolated by column chromatography, using a cellulose column (7.5×135 cm) with a solvent system of *n*-PrOH - pyridine - AcOH - H_2O (15: 10: 3: 12).

Each fraction (16 ml) was examined by spot test and bioassay, and the fractions which were active against *Bacillus subtilis* PCI 219 were analysed by thin-layer chromatography using Avicel-SF plate (Funakoshi Co.) with a solvent system of *n*-PrOH - pyridine - AcOH - H_2O (15: 10: 3: 12). Fractions 487~532 showed 3 spots with two components (Rf 0.40 and 0.35) which were inactive against *B. subtilis* PCI 219 and S15-1-A′ (Rf 0.32). S15-1-A with Rf 0.30 was obtained from fractions 632~682, S15-1-B (Rf 0.25) from fractions 792~817 and S15-1-C (Rf 0.20) from fractions 912~942. Then these separated components were purified by Sephadex LH-20 column (2×145 cm) with a solvent system of 10% MeOH solution.

S15-1-A was further purified by Sephadex LH-20 column chromatography with another solvent system, that is: The solution (10 ml) of S15-1-A hydrochloride (190 mg) was applied to Sephadex LH-20 column (2×145 cm), and eluted with a solvent system of 0.05 M NaCl solution. Each fraction (5 ml) was detected by the bioassay and thin-layer chromatography above mentioned. S15-1-A was able to separate a ninhydrin-positive component (fractions 16~20) (Rf 0.30) and a SAKAGUCHI-positive component (fractions 23 and 24) (Rf 0.30). The SAKAGUCHI-positive component was inactive against *B. subtilis* PCI 219 and negative for the ninhydrin test. S15-1-A hydrochloride and sodium chloride were separated from each other by Sephadex G-10 column chromatography. This method was applied to the purification of the B and C components.

Further purifications of the S15-1-A′ component were carried out by cellulose and Sephadex LH-20 column chromatography. The purified S15-1-A′ component was one spot (Rf 0.30) on thin-layer chromatography.

Physico-chemical Properties

S15-1-A hydrochloride was a colorless hygroscopic powder, m.p. *ca.* 205°C (dec.) and $[\alpha]_D^{20}$ −46.1° (*c* 1, H_2O). The UV spectrum showed no absorption at 220~400 nm. The IR spectrum was a much closer resemblance to that of racemomycin A.[3] The hydrochloride was soluble in water and slightly soluble in methanol, but insoluble in common organic solvents.

Analytical found for the hydrochloride: C 35.60, H 6.30, N 17.20, Cl 16.70, H_2O 2.60%,

Calculated for $C_{19}H_{34}O_8N_8 \cdot 3HCl \cdot H_2O$: C 36.22, H 6.20, N 17.79, Cl 16.92, H_2O 2.85%.

The hydrochloride gave positive reaction with ninhydrin and ELSON-MORGAN reagents, but a doubtful biuret reaction and a negative SAKAGUCHI reaction. Proton[4] and carbon-13* NMR spectra of S15-1-A and A′ were similar to those

Present address:

* Asahi Chemical Industry Co., Ltd. 2–5, 3-chome, Kasumigaseki, Chiyoda-ku, Tokyo, Japan.

** Asahi Chemical Industry Co., Ltd. 2–1, Samejima, Fuji, Shizuoka, Japan.

* The carbon-13 NMR spectra in D_2O were observed on a Nihondenshi JNM-PS-100 spectrometer operating at 25 MHz in the pulsed FOURIER transformed model. Benzene was used as reference.

Fig. 1. ^{13}C-NMR spectrum of S15-1-A.

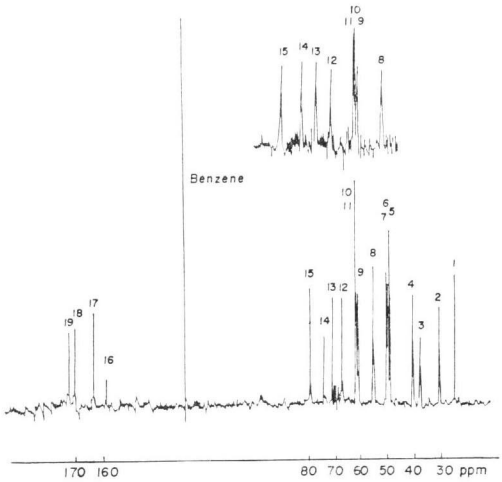

Fig. 2. ^{13}C-NMR spectrum of S15-1-B.

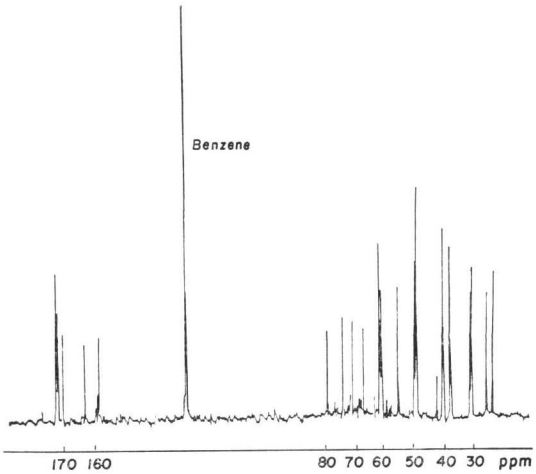

Table 1. Chemical shifts and partial assignments of carbon resonances of S15-1-A, A′ and racemomycin A in D_2O.

	S15-1-A and A′		Racemomycin A	
β-Lysine α CH_2	49.4		49.4	
β CH	37.4		37.4	
γ CH_2	30.1		30.1	
δ CH_2	24.0		24.0	
ϵ CH_2	40.1		40.1	
Streptolidine	50.0	61.8	50.0	61.8
and	50.4	67.6	50.4	67.6
D-gulosamine	55.5	71.1	55.5	71.1
	61.4	74.6	61.4	74.6
	61.7	79.9	61.7	79.9
Carbamoyl and	158.8		158.8	
guanidino	163.7		163.7	
Carbonyls	171.0		171.0	
	173.0		173.0	

1) Chemical shifts are reported in ppm downfield from TMS.
2) The resonances of streptolidine D-gulosamine, carbamoyl, guanidino and carbonyls have not yet been satisfactorily assigned.

Table 2. Chemical shifts of the ^{13}C-NMR spectrum of S15-1-B.

	S15-1-B	
β-Lysine, streptolidine	23.8	55.5
and D-gulosamine	25.2	61.3
	30.0	61.8
	30.4	67.6
	37.8	71.0
	40.0	74.6
	49.5	79.8
	50.1	
Carbamoyl, guanidino	158.8	
and carbonyls	163.6	
	171.0	
	172.7	
	173.2	

Chemical shifts are reported in ppm downfield from TMS.

from racemomycin A, and the chemical shifts of S15-1-A and A′, and racemomycin A on carbon-13 NMR were also similar (Fig. 1, and Table 1). The ^{13}C spectra of S15-1-A, A′ and racemomycin A show the presence of 19 carbons, including 2 carbonyls, 1 guanidino and 1 carbamoyl-groups. The absorptions of β-lysine except carbonyl were assigned by using off-resonance decoupling experiments and chemical shift data of β-lysine prepared from racemomycin A.

The hydrolysates of S15-1-A hydrochloride (6 N HCl, 20 hours, 110°C, sealed tube) were chromatographed on a thin-layer plate (Avicel-SF plate) with a solvent system of *n*-PrOH - pyridine - AcOH - H_2O (15: 10: 3: 12). Two components, streptolidine (Rf 0.25) and β-lysine (Rf 0.30) were detected with ninhydrin, and an amino sugar (Rf 0.45) by ELSON-MORGAN reagent. The 100 MHz proton spectra of streptolidine and

D-gulosamine agree well with the data of BORDERS *et al.*[5] and TANIYAMA *et al.*[6]

In the proton NMR spectrum of S15-1-B the protons (δ 1.5~1.9, and δ 2.5~2.7) of the methylene groups were twice those of S15-1-A, and other protons overlapped on δ 2.7~5.2 and resembled those of S15-1-A. Chemical shifts of S15-1-B on ^{13}C NMR spectrum showed a close resemblance to those of S15-1-A at 35~165 ppm, and three new peaks appeared at 23~35 ppm (2) and 171~174 ppm (1) (Fig. 2 and Table 2). The hydrolysates of S15-1-B hydrochloride (3 N HCl, 10 hours, 100°C, sealed tube) were purified and two spots, streptolidine and one component (Rf 0.20), were detected with the ninhydrin test. The proton NMR spectrum of the component (Rf 0.20) was similar to that of the β-lysine dipeptide of racemomycin C. These results showed that S15-1-B is identical with racemomycin C.[7]

Discussion

Antibiotic S15-1-A was previously considered to be a new streptothricin group antibiotic because of a positive reaction with the SAKAGUCHI reagent in spite of various purification methods having been investigated. However, it was found that the SAKAGUCHI-positive compound was separated from S15-1-A component by Sephadex LH-20 column chromatography with a solvent system of 0.05 M NaCl solution. This result suggested that some quantities of salts in the sample used by TANIYAMA *et al.*[8] was highly effective for separation of SAKAGUCHI-positive compound. The physico-chemical properties, IR and NMR spectra, and the analyses of the hydrolysates made it clear that S15-1-A was identical with racemomycin A, that is streptothricin F[9]. And also S15-1-B was identical with racemomycin C (streptothricin E). An attempt was made to separate S15-1-C by the above purification but failed because of its poor yield. But it was suggested that S15-1-C may be identical with streptothricin D.

Acknowledgement

The authors wish to express their sincere thanks to Prof. H. TANIYAMA, Nagasaki University, for his kind supply of racemomycin A sulfate.

References

1) ARIMA, K.; T. KAWAMURA & T. BEPPU: A new antiviral substance S15-1, streptothricin group antibiotic. J. Antibiotics 25: 387~392, 1972
2) KAWAMURA, T.; K. TAGO, T. BEPPU & K. ARIMA: Antiviral antibiotic S15-1, taxonomy of the producing strain and study of conditions for production of the antibiotic. J. Antibiotics 29: 242~247, 1976
3) TANIYAMA, H.; Y. SAWADA & T. KITAGAWA: The indentity of yazumycins A and C with racemomycins A and C. J. Antibiotics 24: 390~392, 1971
4) TANIYAMA, H.; Y. SAWADA & T. KITAGAWA: Characterization of racemomycins. Chem. Pharm. Bull. 19: 1627~1634, 1971
5) BORDERS, D. B.; K. J. SAX, J. E. LANCASTER, W. K. HAUSMANN, L. A. MITSCHER, E. R. WETZEL & E. L. PATTERSON: Structures of LL-AC 541 and LL-AB 664, new streptothricin-type antibiotics. Tetrahedron 26: 3123~3133, 1970
6) TANIYAMA, H.; Y. SAWADA & T. KITAGAWA: Structure of 476-B, new streptothricin group antibiotic. The 14th symposium on the chemistry of natural products (Fukuoka), "Abstr. paper" p. 55, 1970
7) TANIYAMA, H.; Y. SAWADA, K. MIYAZEKI & F. MIYOSHI: Studies on the β-lysine peptide. III. Synthesis of β-(L-β-lysyl)-L-β-lysine. Chem. Pharm. Bull. 20: 601~604, 1972
8) TANIYAMA, H.; Y. SAWADA & T. KITAGAWA: Chromatography of racemomycins on dextran gel. J. Chromatogr. 56: 360~362, 1971
9) KHOKHLOV, A. S. & K. I. SHUTOVA: Chemical structure of streptothricins. J. Antibiotics 25: 501~508, 1972