NOTES

THE IDENTITY OF YAZUMYCINS A AND C WITH RACEMOMYCINS A AND C

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AKASAKI *et al.*[1] reported that the *Streptomyces* strain IN-183-T, resembling *S. lavendulae* as described by WAKSMAN *et* HENRICI[2], produced a new water-soluble basic antibiotic yazumycin.

In this paper, we wish to report the identification of yazumycins A and C as racemomycins A and C.[3,4]

Purification

The preparation of crude antibiotic from the strain IN-183-T was carried out as previously reported[1]. An aqueous solution of the crude antibiotic (1 g) was chromatographed on a charcoal column (3×16 cm) with water as eluent. Each fraction (10 ml) was examined by spot test, and the ninhydrin-positive fractions were analysed by paper chromatography, using Toyo-Roshi No. 51 UH-type paper with a solvent system of *n*-BuOH - pyridine - HOAc - H_2O - *t*-BuOH (15 : 10 : 3 : 12 : 4). Fractions 17~22 showed spots with Rf values of 0.50, 0.40, 0.38, 0.28 and 0.22 together with minor components, but all they were inactive against the test organisms. Yazumycin A hydrochloride with Rf 0.34 was obtained from fractions 34~42. A second component showing Rf value of 0.36 and a positive SAKAGUCHI test was eluted in fractions 40~43. Yazumycin A was further purified by chromatography on a Sephadex LH-20 column. After lyophilization, pure yazumycin A was obtained as its hydrochloride in a yield of 0.21 g.

A long column (2×150 cm) of Sephadex LH-20, which had been swollen overnight with 10 % aqueous methanol solution, was used for additional separation studies. The crude powder in this experiment was prepared from another culture by the same method, using also charcoal treatment. A solution of crude yazumycin (2 g) in 5 ml of water was applied to the column, and eluted with the same solvent system. The rate of elution was adjusted to 20~40 ml/hour. Fractions of 8 ml were collected. A component which showed an Rf value of nearly zero was found in fractions 22~34.

Yazumycin C hydrochloride with Rf 0.25 was isolated from fractions 35~37, and yazumycin A hydrochloride from tubes 38~41. Fractions 40~43 contained a SAKAGUCHI-positive substance and inorganic salts. The yield of pure yazumycin A hydrochloride was 0.45 g (22.5 % of crude material), and that of yazumycin C hydrochloride was 0.05 g (2.5 %).

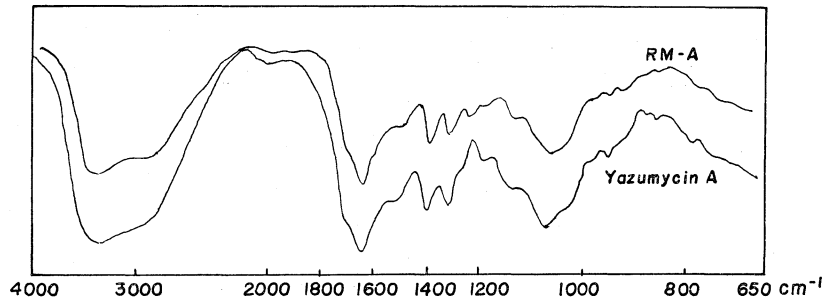
Physical and Chemical Properties of the Yazumycins

Yazumycin A hydrochloride was a colorless powder, m.p. *ca.* 210°C (dec.), $[\alpha]_D^{25}$ −45 ±4° (*c* 1, H_2O). The UV spectrum in water showed no absorption at 220~750 mμ. The IR spectrum of the component is shown in Fig. 1. The hydrochloride is soluble in water, but insoluble in the common organic solvents. Its basic nature was indicated by paper electrophoresis (*ca.* 16 cm, using Toyo-Roshi No. 51 paper, buffer of pyridine - HOAc - H_2O (5 : 0.2 : 95), pH 6.5, 500 V, 3~5 mA, 3 hours). Potentiometric titration of yazumycin A in water indicated a tri-basic substance with pKa′ values 7.10, 8.40 and 10.15 (M.W. 597).

Anal. Found for the hydrochloride: C 35.93, H 6.32, N 17.27, Cl 16.74, H_2O 2.15 %. Calcd. for $C_{19}H_{34}O_8N_8 \cdot 3HCl \cdot H_2O$ (M. W. 629.5): C 36.22, H 6.20, N 17.79, Cl 16.92, H_2O 2.85 %. Found for the reineckate: C 25.15, H 3.97, N 24.08, Cr 10.52, H_2O 2.06 % (M.W. 1462, *c* 1.77 % in THF, by osmometry). Calcd. for $C_{19}H_{34}O_8 \cdot N_8 \cdot 3[Cr(NH_3)_2 \cdot (SCN)_4] \cdot 2H_2O$ (M. W. 1492): C 24.93, H 3.75, N 24.40, Cr 10.46, H_2O 2.41 %.

The reineckate had no definite melting

Fig. 1. The infrared spectrum of yazumycin A hydrochloride and racemomycin A hydrochloride (RM-A) in KBr.

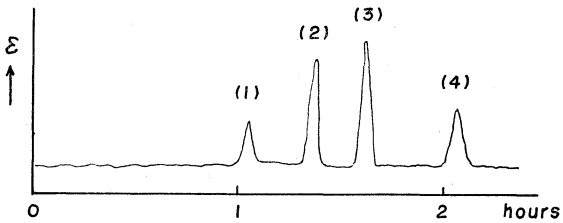


point, and was a rose-colored crystals insoluble in cold water. A molecular weight determination of yazumycin A hydrochloride by Sephadex G-10 chromatography gave a value of approximately 600. The antibiotic gave positive reaction with ninhydrin, PAULY, FEHLING, biuret, and ELSON-MORGAN reagents, but a negative SAKAGUCHI test. The hydrolysate of yazumycin A (6 N HCl, 24 hours, 110°C in a sealed tube) was chromatographed on a paper. Two components, streptolidine (Rf 0.30) and β-lysine (0.36) were detected with ninhydrin, and an aminosugar moiety (0.51) by the ELSON-MORGAN reagent. The three compounds mentioned above were determined quantitatively by an automatic amino acid analysis as shown in Fig. 2.

Fig. 2. Amino acid analysis of the hydrolysates of yazumycins and racemomycins.

Resin : Amberlite CG-120 type III

Column size : 0.9×20 cm. Buffer : 0.35 N sodium citrate (pH 5.28). Column temp. : 50°C. Sample : Hydrolysates of 0.5 mg-antibiotics. Assignment : peak (1) corresponds to aminosugar moiety, (2) to streptolidine, (3) to β-lysine and (4) to ammonia.



	(1)	(2)	(3)	(4)
Yazumycin A	0.09	1.00	1.18	0.54
Racemomycin A	0.08	1.00	1.15	0.49
Yazumycin C	0.06	1.00	1.58	0.63
Racemomycin C	0.10	1.00	1.56	0.46

* The ratios are presented based on the intensity of streptolidine peak (2).

The retention times of the individual components were identified with those of authentic samples obtained from a racemomycin hydrolysate.

The minor component yazumycin C hydrochloride had the following properties : m.p. above 210°C (dec.), $[\alpha]_D^{20}$ −18° (*c* 1, H_2O), molecular weight approximately 750 by gel filtration. Amino acid analysis of the hydrolysate was carried out by the same method.

Therefore the mole ratio of streptolidine, β-lysine and ammonia in yazumycin A was probably 1 : 1 : 1, and that in yazumycin C 1 : 2 : 1 respectively.

Biological Activity of Yazumycin A

Yazumycin A hydrochloride and racemomycin A sulfate were active against Gram-positive and Gram-negative bacteria as shown in Table 1. Most of the bacteria were cultured in brain-heart infusion broth (Difco). Mycobacteria were cultured in bouillon containing glycerin. The acute toxicity of yazumycin A hydrochloride was examined by intravenous injection of mice (ICR strain, female, weighing 20~27 g, 5 animals in each group), the results being presented in Table 2.

No toxicity was observed within a week after administration of a 300 mg/kg dose to mice. In the group administered 500 mg/kg, a delayed toxicity like that of other streptothricins was observed. The mice injected with the antibiotic at 200 and 300 mg/kg lost weight, and the lustre of their skin disappeared. Mice sacrificed after 500 mg/kg injection

Table 1. Antimicrobial activity of yazumycin A hydrochloride and racemomycin-A sulfate

Test organism	MIC (mcg/ml)	
	Yazumycin A	Racemomycin A
Bacillus subtilis PCI 219	4	2~4
Escherichia coli ATCC 11246	2	1~2
Micrococcus luteus ATCC 398	0.25	0.5
Staphylococcus aureus FDA 209P	4~8	4~8
Klebsiella pneumoniae ATCC 10031	4~8	2~4
Pseudomonas aeruginosa NRRL B 1000	32	32
Mycobacterium phlei NCTC 525	1~2	2
Mycobacterium smegmatis ATCC 607	2	4

Table 2. Toxicity of yazumycin A hydrochloride to mice (intravenous injection)

mg/kg	Mortality (dead/treated)							
	0 day	1 day	2 days	3 days	4 days	5 days	6 days	7 days
200	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
300	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
500	0/5	0/5	0/5	1/5	3/5	5/5	—	—

revealed atrophy of the kidney and pancreas.

Discussion

Yazumycin was previously considered to be a new antibiotic because of a positive reaction to the SAKAGUCHI reagent and because of its physico-chemical properties in comparison with the other water-soluble, basic SAKAGUCHI-positive antibiotics such as streptomycin, viomycin *etc.* However, an improved extraction of a culture broth afforded two antibiotics, yazumycins A and C, in order of decreasing Rf value on paper chromatography. Both purified antibiotics were negative to the SAKAGUCHI test, but were quite similar to the racemomycins, and to the streptothricin group antibiotics. The comparison of IR and NMR spectra, amino acid analyses of the hydrolysates, and physico-chemical properties also made it clear that yazumycin A, the major active component, is identical with racemomycin A, and yazumycin C with racemomycin C.

An attempt was made to separate the SAKAGUCHI-positive substance (Rf 0.36) by cellulose chromatography, but failed because of its poor yield. A similar attempt was made to isolate the compound (Rf 0.36) showing a positive SAKAGUCHI test in crude akimycin obtained from the broth filtrate of *S. lavendulae* No. 20-27 strain by ARAI[5]. This substance, which showed a negative ninhydrin test, had no antimicrobial activity.

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