

THE IDENTITY OF CITROMYCIN
WITH LL-AC541, E-749-C,
AND BY-81

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In the previous paper¹⁾, KUSAKABE *et al.* have reported on the isolation and characterization of citromycin (1483-A), an antibiotic which belongs to the streptothricin group.

The empirical formula, and chromatographic comparisons of citromycin with other streptothricin-like antibiotics showed that citromycin to have the same characteristics as LL-AC541^{2,3)}, E-749-C⁴⁾, and BY-81⁵⁾.

Purification of citromycin was performed by column chromatography first on activated carbon and then on Sephadex LH-20. The product has the following analysis (for the hydrochloride), Found: C 36.29, H 5.71, N 19.45, Cl 12.46, H₂O 3.0%. Calcd. for C₁₇H₂₈O₈N₈·2HCl·H₂O (M.Wt. = 563): C 36.23, H 5.69, N 19.89, Cl 12.61, H₂O 3.2%. M.Wt. was about 550 by Sephadex G-10 column. The reineckate salt recrystallized from water has no definite m.p., and analyzed: *Anal.* Found: C 26.42, H 3.58, N

24.48, Cr 9.56, H₂O 1.33%. Calcd. for C₁₇H₂₈O₈N₈·2[Cr(NH₃)₂·(SCN)₄]·H₂O (M.Wt. 1,126): C 26.91, H 3.73, N 24.87, Cr 9.24, H₂O 1.6%. Molecular weight was obtained as 1,200 (*c* 1.61% in THF by osmometry).

When citromycin hydrochloride was hydrolyzed and the hydrolysate (6 N HCl, 100°C for 10 hours) was applied to a column of Amberlite CG-120 (H⁺ form), and eluted stepwise with 0.1 M acetic acid-pyridine buffer (pH 5.5), glycine (Rf 0.44 on paper), an aminosugar (0.57), and streptolidine (0.34) were identified. These were identical with components of an E-749-C hydrolysate by paper chromatography* and NMR spectra. When citromycin hydrochloride (0.261 g) was hydrolyzed with 3 N sulfuric acid at 120~125°C for 1 hour, and the evolved carbon dioxide was passed through a saturated barium hydroxide solution, precipitate of barium carbonate was obtained (yield: 0.103 g, corresponding to 1.21 mol of carbon dioxide).

When another acid hydrolysate (3 N HCl, 80°C for 1 hour) of citromycin was applied to a column of Sephadex LH-20 and eluted with aqueous 10% methanol, a compound corresponding to streptolidine-sugar Rf 0.33, [α]_D²⁵ -3.6° (*c* 1 in H₂O), was eluted in first fractions. The later fractions gave two unidentifiable spots by paper chromatography (ninhydrin test). Formiminoglycine (Rf 0.45, m. p. 150~152°C) was obtained as a hygroscopic powder from later fractions: NMR δ (from DSS) 7.93

Table 1. NMR spectral and amino acid analytical comparisons of streptothricin-like antibiotics.

		Citromycin	LL-AC 541	E-749-C	BY-81	SF-701 ⁶⁾	LL-AB 664
NMR* δ from DSS in D ₂ O	N-Me (s.)	3.06	3.03	3.04	3.04	2.82	2.83
	Anomeric (d.)	5.58 (J \approx 10 Hz)	5.5	5.52	5.5	3.06	3.01
	Formimino (s.)	7.97	7.91	7.92	7.93	5.56	5.5
Amino acid analysis** (ratio) (6 N HCl, 110°C for 24 hours)	Glycine	0.75	0.74	0.77	0.81	—	0.73
	Streptolidine	1.00	1.00	1.00	1.00	0.64	—
	Ammonia	1.02	1.13	1.08	0.95	1.00	1.00
	Methylamine	0.053	0.05	0.055	0.06	0.35	0.069

* NMR spectra were measured at 60 MHz spectrometer (Hitachi H-60).

** Amino acid analysis⁹⁾ was carried out by an apparatus of Hitachi KLA-3 type using column (Amberlite CG-120 type III, 0.9×20 cm) and buffer (0.35 M sodium citrate, pH 5.28) at 50°C.

* Rf value was obtained by paper (Toyo-Roshi No. 51 UH) with a solvent system of I.

Table 2. Chromatographic comparisons of streptothricin-like antibiotics

Antibiotics	Rf values							
	P.P.C.				T.L.C.			
	Solvent systems				Solvent systems			
	I *	II *	I **	II **	III ***	IV ***	III ****	IV ****
Citromycin _H	0.44	0.53	0.36	0.25	0.52	0.56	0.74	0.35
LL-AC541 _H	0.43	0.53	0.35	0.24	0.52	0.57	0.74	0.35
E-749-C _H	0.44	0.52	0.35	0.24	0.53	0.55	0.73	0.35
BY-81 _H	0.44	0.52	0.35	0.25	0.52	0.56	0.74	0.36
LL-AB664 _S	0.53	0.63	0.45	0.35	0.65	0.65	0.75	0.55
SF-701 _H	0.47	0.55	0.38	0.26	0.45	0.61	0.65	0.33

H : hydrochloride S : sulfate

I : *n*-BuOH-pyridine-acetic acid-water-*t*-BuOH (15:10:3:12:4)

II : *t*-BuOH-acetic acid-water (3:1:1)

III : CHCl₃-methanol-17% aqueous ammonia (upper layer, 2:1:1)

IV : *n*-PrOH-pyridine-acetic acid-water (15:10:3:12)

Detection : Ninhydrin and PAULY tests

* Circular paper using Toyo-Roshi No. 51 UH type

** Ascending method using Toyo-Roshi No. 51

*** Silica-gel thin-layer (Merck Co.)

**** Avicel SF thin-layer (Funakoshi Co.)

1H, 4.23 2H in D₂O. IR_{ν_{max}}^{KBr} (cm⁻¹): 3200, 3100, 1735, 1690, 1622, 1512, 1420, 1408, 1356, 1260, 1240, 1203, 1047, 985, 909, 847, 770, 730. We concluded that complete and partial hydrolysates of citromycin contained streptolidine⁶⁾, N-Me-D-gulosamine²⁾, streptolidyl-N-guan.-N'-methyl-β-D-gulosaminide^{3,7)}, formiminoglycine²⁾, glycine, carbondioxide, and ammonia.

Citromycin contained no β-lysine and could not differentiate from antibiotics of LL-AC541, E-749-C and BY-81. Identifica-

tion was performed by NMR studies, automatic amino acid analysis and chromatographic comparisons.

When antimicrobial activity of citromycin hydrochloride was examined, it was noted that microorganisms such as *Micrococcus luteus*, *Serratia marcescens*, *Corynebacterium spedomicum* and *Diaporthe citri* were more sensitive than previously reported (Table 3). We also found the inhibitory effect against influenza PR-8 strain could not be detected below 100 mcg/ml with citromycin hydrochloride (by shaking culture method¹⁰⁾).

When citromycin was directly compared with streptothricin-like antibiotics, the physical and chemical properties, chromatographic behavior (especially the silica-gel thin-layer chromatogram developed with a solvent system III in Table 2), spot tests (ninhydrin test yellow to purple in water), and automatic amino acid analysis of acid hydrolysate concluded that citromycin, LL-AC541, E-749-C, and BY-81 are identical substances.

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Table 3. Antimicrobial activity of citromycin hydrochloride

Test organisms	MIC (mcg/ml)
<i>Micrococcus luteus</i> IFO-3763	4
<i>Serratia marcescens</i> IFO-3736	4
<i>Corynebacterium spedomicum</i> IFO-3306	8
<i>Pseudomonas aeruginosa</i> IFO-3901	>250
<i>Proteus vulgaris</i> OX-19	16
<i>Klebsiella pneumoniae</i> PCI-602	32
<i>Bacillus subtilis</i> PCI-219	64
<i>Escherichia coli</i> IFO-3806	32
<i>Staphylococcus aureus</i> FDA-209P	64
<i>Xanthomonas citri</i>	64
<i>Xanthomonas oryzae</i>	32
<i>Mycobacterium smegmatis</i> ATCC-607	16
<i>Ophiobolus miyabeanus</i>	256
<i>Diaporthe citri</i>	8
<i>Piricularia oryzae</i>	256
<i>Alternaria kikuchiana</i> NIAS A-14	64
<i>Fusarium oxysporum</i> ATCC-659	128

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