FOUR MINOR ANTIBIOTICS FROM MACARBOMYCINS

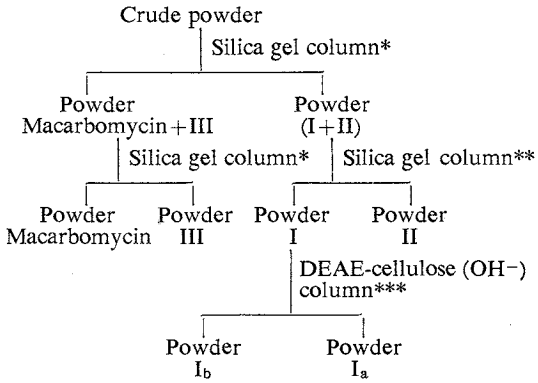
Sir:

Macarbomycin[1], a phosphorus-containing antibiotic, produced by *Streptomyces phaeochromogenes* is a member of the antibiotic group which includes diumycin[2~4], moenomycin[5~10] and prasinomycin[11~18]. It is closely related to diumycin on the basis of their physico-chemical properties, especially, the absence of 6-deoxyglucosamine. During our studies on the improvement of the producing strain and its large scale production, four new minor antibiotics, named macarbomycins I_a, I_b, II and III, were isolated. In this report, the isolation and characterization of these antibiotics are described.

Crude powder (about 50% purity) was obtained from the cultured broth of *Streptomyces phaeochromogenes* by precipitation at pH 3 and DEAE-cellulose column chromatography as described in the previous report[1]. As shown in Fig. 1, the crude powder (4.2 g) was fractionated into macarbomycin (85.8% of weight), macarbomycins I_a (1.07%), I_b (1.03%), II (2.65%) and III (9.42%) by repetitive silica gel column chromatography and by DEAE-cellulose column chromatography. When the activity of macarbomycins isolated was determined by the disc method using a standard macarbomycin preparation designated 1,000 mcg*/mg and *Staphylococcus aureus* 193 as a test organism, the potency of macarbomycin, and macarbomycins I_a, I_b, II and III was found to be 5,470, 3,780, 1,860, 3,170 and 3,460 mcg*/mg, respectively.

As shown in Table 1, all macarbomycins were separated by thin-layer chromatography on silica gel GF_{254} (E. Merck AG) detected with ultraviolet light (2536 Å) and with iodine vapour, and by paper chromatography on Toyo Roshi #51 detecting by bioautography. The physical properties and the elemental analysis of macarbomycins are shown in Tables 2 and 3. Macarbomycin and III show a strong absorption at 257~258 nm in water, while I_a shows only end absorption and I_b and II show weak absorption at 255~259 nm. The phosphorous content of the four macarbomycins I_a, I_b, II and III is slightly less than that of macarbomycin itself. Degradation products of macarbomycins are shown in Table 4. Glycine was found in macarbomycin I_b but not in others. According to

Fig. 1. Isolation of macarbomycins



* Developed with *n*-propanol - 2 N NH_4OH (95 : 5) and (90 : 10) and eluted with (85 : 15)
** Developed with *n*-propanol - 2 N NH_4OH (90 : 10) and eluted with (85 : 15)
*** Developed and eluted with 0.05 N NH_4OH

Table 1. Rf values of macarbomycins on thin-layer chromatography (tlc) and paper chromatography (pc)

	tlc*	tlc**	pc***
Macarbomycin	0.25	0.33	0.34
Macarbomycin I_a	0.20	0.40	0.55
Macarbomycin I_b	0.20	0.34	0.26
Macarbomycin II	0.15	0.25	0.26
Macarbomycin III	0.30	0.29	0.40

* *n*-Propanol - 2 N ammonia (7 : 3)
** *i*-Propanol-water - 0.5 N borate buffer, pH 9.0 (70 : 25 : 5)
*** *n*-Butanol - pyridine - water (4 : 1 : 4)

SLUSARCHYK's review[14], 8036 RP is the only one antibiotic of this group containing glycine and no 6-deoxyglucosamine. The content of glucosamine or 6-deoxyglucosamine was also examined by paper chromatography in the system *n*-butanol - pyridine - water (6:4:3). The glucose content of macarbomycin III was found to be lower than that of the others. By acid hydrolysis (2 N HCl, 100°C, 20 minutes), all macarbomycins gave three kinds of lipids as shown by paper chromatography in the system benzene - chloroform - methanol (8:1:1)[1]. The antimicrobial activity of macarbomycins determined by the agar dilution method is shown in Table 5. Macarbomycin, I_a and II have strong activity against Gram-positive bacteria including resistant strain of *Staphylococcus aureus* FDA 209P, while macarbomycins I_b and

Table 2. Physical properties of macarbomycins

	$[\alpha]_D^{21}$ c 1.0, H_2O	D.P. (°C)	$\lambda_{max}^{H_2O}$ nm	$E_{1cm}^{1\%}$	M.W.	S value
Macarbomycin	+16.0	187~189	257~258	120	32,000	3.4
Macarbomycin I_a	+17.3	193~196	only end abs.		40,000	3.6
Macarbomycin I_b	+12.3	195	257~258	20	35,000	3.8
Macarbomycin II	+10.8	191~193	255~259	12	34,000	3.5
Macarbomycin III	+ 7.2	186~188	257~258	145	48,000	4.0

* Ultracentrifugation in 0.2 M NaCl - 0.02 M sodium phosphate buffer (pH 6.85) by SCHLIEREN method

Table 3. Analysis of macarbomycins*

	C	H	N	P
Macarbomycin	47.30	7.21	4.84	1.93
Macarbomycin I_a	46.39	7.29	4.78	1.45
Macarbomycin I_b	45.43	7.42	5.37	1.63
Macarbomycin II	49.75	7.24	4.70	1.35
Macarbomycin III	51.47	7.40	5.72	1.10

* All macarbomycins analyzed as ammonium salt after drying at 80°C for 3 hours.

III show one-fourth to one-tenth the activity of macarbomycin. Macarbomycins I_a and I_b are more active against Gram-negative bacteria than macarbomycin. All macarbomycins have 8~60 times stronger activity against *Escherichia coli* K-12 ML3966 carrying episomes than the

Table 4. Degradation products of macarbomycins

	Macarbomycin	I_a	I_b	II	III
Glycine*	−	−	±	−	−
Glucosamine (%)**	18	21	17	18	17
Glucose (%)***	22	25	21	22	8
6-Deoxyglucosamine*	−	−	−	−	−
Chromophore	+	−	±	±	+
Lipid	+	+	+	+	+

* Acid hydrolysis (2 N HCl, 100°C, 3 hours)
** ELSON-MORGAN reaction after acid hydrolysis (2 N HCl, 100°C, 3 hours)
*** Orcinol-H_2SO_4 reaction after acid hydrolysis (2 N HCl, 100°C, 3 hours): the corrected values eliminating amounts due to glucosamine

Table 5. Antimicrobial activity of macarbomycins

Test organisms	Minimum inhibitory concentration (mcg*/ml)				
	Macarbomycin	I_a	I_b	II	III
Staphylococcus aureus FDA 209P	0.025	0.0125	0.1	0.0125	0.1
〃 〃 〃 (NB, PC, SM, TC, EM, CP-R)*	0.05	0.05	0.2	0.0125	0.2
Staphylococcus aureus Smith strain	0.025	0.0125	0.1	0.0125	0.1
〃 〃 strain 193	0.025	0.025	0.2	0.0125	0.2
Bacillus subtilis PCI 219	>100	>100	>100	>100	>100
Bacillus cereus ATCC 10702	0.0125	0.025	0.05	<0.006	0.025
Micrococcus flavus M-16	25	12.5	25	25	25
Escherichia coli NIHJ	>100	>100	>100	>100	>100
〃 B	100	50	50	50	25
〃 K-12 W3640	50	25	25	50	25
〃 K-12 ML3996	1.6	0.4	0.8	3.125	3.125
Candida albicans	>100	>100	>100	>100	>100
Saccharomyces cerevisiae	>100	>100	>100	>100	>100

* NB: Novobiocin, PC: Benzylpenicillin, SM: Streptomycin, TC: Tetracycline, EM: Erythromycin, CP: Chloramphenicol, R: resistant

parent strain *E. coli* K-12 W3630.

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