MACARBOMYCIN, A NEW ANTIBIOTIC CONTAINING PHOSPHORUS

Sir:

During screening studies of antibiotics active against *Staphylococcus aureus* 193 (a resistant strain to tetracyclines), a water-soluble and acidic antibiotic was obtained in 1964. It was extracted mainly from the solid portion including mycelia of a cultured broth of a streptomyces, designated as No. C 715-7. The strain was isolated from a soil sample collected in Geneva, Switzerland, and classified as *Streptomyces phaeochromogenes*. The antibiotic, named macarbomycin because of its macromolecular nature, yielded sugars in the acid hydrolysate. Macarbomycin contains phosphorus and is related to moenomycin[1] and prasinomycin[2], but can be differentiated from these antibiotics by paper and thin-layer chromatography and also by the fact that the acid hydrolysate of macarbomycin does not contain 6-deoxyglucosamine. Added to animal feed, macarbomycin exhibited an excellent effect on growth promotion. In the present paper, the production, isolation, physico-chemical and biological properties are described. The mechanism of action was previously reported by J. SUZUKI *et al.*[3] of the Institute of Microbial Chemistry, who observed that macarbomycin inhibits cell wall synthesis, especially the process of polymerization of N-acetylglucosamine and muramic acid pentapeptide.

The macarbomycin-producing strain (C 715-7) was inoculated to a medium consisting of 2 % starch, 2.5 % soybean meal, 0.05 % $MgSO_4 \cdot 7H_2O$, 0.1 % K_2HPO_4, 0.3 % NaCl, 0.0007 % $CuSO_4 \cdot 5H_2O$, 0.0001 % $FeSO_4 \cdot 7H_2O$, 0.0008 % $MnCl_2 \cdot 4H_2O$, 0.0002 % $ZnSO_4 \cdot 7H_2O$ and 0.02 % silicone oil (pH 7), and shake-cultured at 28°C for 4~8 days. The activity of macarbomycin was determined by the disc or cylinder plate method using *Staphylococcus aureus* 193. A standard macarbomycin preparation was designated 1,000 mcg*/mg, and the potency of the purest macarbomycin thus far obtained was 5,000 mcg*/mg.

The cultured broth was filtered, and the filtrate adjusted to pH 3. The precipitate which formed was filtered. The precipitate was extracted with 70 % aqueous acetone. The extract was concentrated and lyophilized to obtain a crude powder. In addition, the solids containing mycelia were extracted with 70~80 % aqueous acetone or methanol, and the extract concentrated to remove the solvent. The residual aqueous solution was adjusted to pH 3, the precipitate dissolved in water at pH 7 and lyophilized to yield a crude powder. It was found that, all the activity in the cultured broth could be recovered with the mycelial cake by acidification of the cultured broth to pH 3 before filtration. The crude powders from the filtrate and mycelial cake were dissolved in water at pH 7 and adsorbed on a column of DEAE-cellulose (OH cycle, produced by Brown Co., Ltd.). The column was treated successively with 0.5 M HCOOH, 0.5 M $HCOONH_4$, water and 0.1 M NH_4OH, and macarbomycin was eluted with 0.2~0.4 M NH_4OH. On lyophilization a crude powder of 200~500 mcg*/mg was obtained. The powder was dissolved in 0.05 M Tris-HCl buffer (pH 8.0) and poured on a column of Ecteola cellulose (OH cycle) pretreated with the same buffer. Elution was done by raising the concentration of NaCl from 0 to 0.3 M NaCl in 0.05 M Tris-HCl buffer (pH 8.0). Macarbomycin appeared mainly in the fractions of 0.04~0.25 M NaCl. The active fractions were combined and macarbomycin was adsorbed on a column of DEAE cellulose. The column was washed with water and the activity eluted with 0.2~0.4 M NH_4OH. The active eluate was concentrated and lyophilized, yielding a powder of 1 mg*/mg. Repetition of the procedure described above gave a powder containing 2~3.5 mg*/mg of macarbomycin. This powder was purified by column chromatography using silica gel. It was successively developed with *n*-propanol - 2 N ammonia (95 : 5), (90 : 10), (85 : 15), and macarbomycin was eluted with *n*-propanol - 2 N ammonia (80 : 20). About eighty per cent of the activity was obtained at the peak and its lyophilization gave a white ammonium salt of macarbomycin having activity of 5 mg*/mg.

Macarbomycin ammonium salt does not show a definite melting point but decomposes at 190~195°C with foaming. It is easily soluble in water, soluble in methanol and dimethylformamide, slightly soluble in ethanol, scarcely soluble in acetone, *n*-butanol, ethyl acetate and benzene, and insoluble in *n*-hexane. Macarbomycin gives a purple color with ferric chloride, violet when heated with 0.2 % ninhydrin in 5 % pyridine acetone solution, dark brown in TOLLENS and green in anthrone, and no color in the SAKAGUCHI, BENEDICT, biuret, and ELSON-MORGAN reactions. Macarbomycin ammonium salt exhibits dextrorotation, $[a]_D^{25}$ +15° (*c* 1, MeOH), and shows maxima at 258~259 mμ in aqueous and 0.1 N NaOH aqueous solution ($E_{1cm}^{1\%}$ 120), and at 246~247 mμ in 0.1 N HCl aqueous solution ($E_{1cm}^{1\%}$ 70). When pelleted with potassium bromide there are strong absorption bands in the infrared region at 3300~3400 cm^{-1} attributable to OH and NH, 1715~1730 cm^{-1} to carbonyl groups and a broad band at 1030~1100 cm^{-1} to the sugars in the molecule. Potentiometric titration in water shows an equivalent weight of 845± 10 with pKa' value of 4.36. Analytical ultracentrifugation[4] in 0.05 M phosphate buffer (pH 7.0) gave 23,200±700 as the molecular weight and in methanol[5] it gave 1,800±65. Macarbomycin ammonium salt dried at 80°C for 3 hours gave analyses of C 47.35, H 7.25, N 4.90, P 2.12. The analytical results suggest a tentative empirical formula in the following range: $C_{68\sim79}$-$H_{123\sim144}N_{6\sim7}O_{41\sim48}P$ (m. w. 1712~1991; Calc'd: C 47.70~47.65, H 7.24~7.29, N 4.90~4.92, P 1.81~1.55).

On thin-layer chromatography of silica gel GF_{254} (E. Merck AG, Darmstadt, Germany), macarbomycin can be differentiated from moenomycins and prasinomycins by a solvent system of *n*-propanol - 2 N aqueous ammonia (70 : 30 by volume) using ultraviolet light at 253.6 mμ, spraying with chlorosulfonic acid - acetic acid (1 : 2) or exposing to iodine vapour for detection. Macarbomycin gave one spot at Rf 0.25, moenomycin gave two main spots at Rf 0.25 and 0.33 with two minor spots at Rf 0.41 and 0.47, and prasinomycin gave three main spots at Rf 0.25, 0.33 and 0.41 with a minor spot at Rf 0.47. The spot of macarbomycin was detected at the position corresponding to moenomycin B_1 and prasinomycin A, but showed strong UV-absorption, whereas those of moenomycin B_1 and prasinomycin A exhibited weak or no absorption.

Table 1. Antimicrobial activity of macarbomycin

Organisms	MID mcg/ml
Staphylococcus aureus FDA 209P	0.05~0.2
〃 (ST·SM-R)	0.2 ~0.4
〃 (Amphomycin-R)	0.2
〃 (NB·PC·SM·TC·EM·CM-R)	0.2
〃 (BM-R)	0.4
Staphylococcus aureus Smith strain	0.05~0.2
〃 (Actinomycin-R)	0.2
Staphylococcus aureus strain 193	0.05~0.2
Bacillus subtilis PCI 219	400
〃 *megaterium*	1,000
〃 *sphericus*	1,000
〃 *anthracis*	0.025
〃 *cereus* ATCC 10702	0.025
〃 *mycoides* strain "O"	0.05
Micrococcus flavus M-16	12.5
Sarcina lutea PCI 1001	1,000
Escherichia coli NIHJ	500
〃 B	50
Salmonella paratyphi A 1015	500
〃 *typhosa*	500
Shigella boydii 1-65	1,000
〃 *sonnei* 11,37148	500
Mycobacterium 607	25
〃 (KM-R)	25
〃 (SM-R)	50
〃 *phlei*	12.5
Candida albicans	1,000
Aspergillus niger	1,000
Saccharomyces cerevisiae	1,000
Xanthomonas oryzae	1,000

Acid hydrolysis of macarbomycin (2 N HCl, 100°C for 3 hours) gave glucose and glucosamine, but not 2-amino-2,6-dideoxy-D-glucose, which was obtained from hydrolysis of both moenomycin and prasinomycin under the same conditions. Acid hydrolysis of macarbomycin (6 N HCl, 100°C for 20 minutes) gave 48 % reducing sugar and 20~23 % amino sugar. Another acid hydrolysis

of macarbomycin gave three lipids corresponding to moenocen, moenocinol, and isomoenocinol which had been isolated from moenomycin[6,7] and prasinomycin[8], and also a chromophore, 2-aminocyclopentandione-1, 3 which was found in moenomycin[9].

Macarbomycin in aqueous solution (50~100 mcg*/ml, at 60°C for 1 hour) is stable at pH 4~10, but unstable in acidic solution (pH less than 3) and in an alkaline solution stronger than 0.1 N NaOH.

The antimicrobial activity of macarbomycin determined by the agar dilution method is shown in Table 1.

The LD_{50} of macarbomycin injected into mice intravenously was 750 mg/kg. Intraperitoneal administration of 5 and 1.25 mg*/kg to mice infected with *Staphylococcus aureus*, Smith strain, gave 100 and 50 % protection, respectively. Subcutaneous injection of 50 and 25 mg*/kg gave 50 and 25 % protection. Oral administration of 200 mg*/kg gave 25 % protection.

Macarbomycin gave good results** in weight gain and feed conversion with both broilers and swine, especially when it was administrated to broilers in feed at a level of 30 g*/ton, and to swine at a level of 2 g*/ton. Its effect on growth promotion was excellent with statistical significance at 5 % level.

As shown by the results described above, macarbomycin has strong antibacterial activity *in vitro* and *in vivo*, shows an excellent effect as feed additive, and is clearly different from moenomycin and prasinomycin. When we had finished the studies described above, the paper of E. MEYERS, *et al.*[10] of the Squibb Institute for Medical Research was published reporting on the diumycins which also do not contain 6-deoxy-D-glucosamine. Although there are some differences between macarbomycin and diumycin A, their identity or difference is not certain and will have to determine by direct comparison.

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