Notes

NEW QUINONE ANTIBIOTICS OF THE GRANATICIN TYPE, ISOLATED FROM *STREPTOMYCES LATERITIUS*

I. PRODUCTION, ISOLATION AND PROPERTIES

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During the course of a screening programme for novel antibacterial agents produced by microorganisms, six antibiotics have been isolated, which inhibit the growth of Gram-positive bacteria. The antibiotics were co-produced by *Streptomyces lateritius* ATCC 19913 and found to be quinones of the granaticin series[1~3]. They were designated MM 44325, MM 44326, MM 44785, MM 44786, MM 44787 and MM 44788. One of them, MM 44326, has been shown to be identical to the previously described granaticin B, but the five other metabolites are new members of the series.

This paper describes the production, isolation and purification of the six antibiotics. Some physical and biological properties of the compounds are also reported. Structural studies of these granaticin antibiotics is described in part II of this paper[4].

S. lateritius ATCC 19913 was maintained on agar slopes consisting of yeast extract 0.4%, malt extract 1%, glucose 0.4%, agar 2% in deionised water, pH 7.3. After inoculation, slopes were incubated at 28°C for 1 week before use. A suspension of spores and mycelia in sterile deionised water containing 0.005% Triton X-100 (10 ml) was prepared from an agar slope contained in a Universal bottle, and portions (1 ml) used to inoculate 500-ml Erlenmeyer flasks containing 100 ml of production medium. The production medium consisted of casein hydrolysate 0.25%, beef extract 'Lab-Lemco'

Table 1. Results of TLC on metabolites isolated from *Streptomyces lateritius* ATCC 19913.

Compound	Colour[a]	Rf	
		Solvent A	Solvent B
MM 44785	Yellow	0.38	0.58
MM 44786	Yellow	0.30	0.63
MM 44788	Yellow	0.63	0.82
MM 44325	Red	0.28	0.63
MM 44326	Purple	0.55	0.81
MM 44787	Red	Origin	0.50

Support; Silica gel 60. Solvent system A; $CHCl_3$ - MeOH (9 : 1). Solvent system B; EtOAc - MeOH (1 : 1).

[a] Colour seen on TLC plate. The colour of the compounds varies with pH.

Table 2. UV maxima of metabolites from *Streptomyces lateritius* (log ε in parenthesis).

Compound	MeOH and MeOH - HCl (nm)	MeOH - NaOH (nm)
MM 44326 (granaticin B)	221 (4.55), 282 (3.77), 490 (3.77), 522 (3.84), 564 (3.72)	222 (4.54), 308 (3.65), 588 (3.95), 630 (3.95)
MM 44325 (dihydro-granaticin B)	222 (4.47), 282 (3.80), 488 (3.80), 513 (3.82), 549 (3.66)	224 (4.40), 314 (3.64), 570 (3.87), 610 (3.89)
MM 44785	204 (4.11), 241 (4.21), 270 sh (3.75), 400 (3.86)	232 (3.85), 282 (3.39), 444 (3.47)
MM 44787	206 (4.27), 232 (4.15), 305 (3.62), 474 sh (3.61), 495 (3.62), 525 sh (3.48)	258 (3.95), 536 (3.67), 576 (3.56)
MM 44786	218, 257, 432	226, 299, 568
MM 44788	217, 277, 408	210, 242, 302, 478

Table 3. Antibacterial activity of *Streptomyces lateritius* metabolites.

Organism	MIC (μg/ml)					
	MM 44326 (granaticin B)	MM 44325	MM 44786	MM 44788	MM 44787	MM 44785
Bacillus subtilis ATCC 6633	<0.5	4	64	128	128	64
Corynebacterium xerosis NCTC 9755	16	64	2	32	128	>512
Micrococcus luteus NCTC 8340	8	16	32	64	128	256
Staphylococcus aureus Oxford	1	4	16	64	128	128
S. aureus Russell	2	4	32	64	128	64
S. aureus V573 MR[a]	<0.5	2	16	32	32	32
S. saprophyticus FL1	<0.5	2	16	16	128	64
S. epidermidis 60137	16	32	64	64	256	512
S. epidermidis 54815	0.5	2	32	32	64	32
Streptococcus pyogenes CN10	4	16	4	64	128	128
S. agalactiae 'Hester'	16	32	1	64	256	256
S. sanguis ATCC 10556	16	64	4	64	256	256
S. pneumoniae Pu7	32	64	4	64	256	256
S. faecalis I	128	128	32	128	256	512

[a] Multi-resistant (methicillin, tetracycline, erythromycin and gentamicin resistant).

Tests were carried out by serial dilution in Nutrient broth by microtitre. Inoculum was prepared by dilution of an overnight broth culture to give the equivalent of approx 10^6 cells/ml.

powder 0.1%, soybean flour 1%, distillers solubles 0.2%, corn steep liquor 0.5%, glucose 2%, NaCl 0.5%, K_2HPO_4 0.2% and $CaCO_3$ 1% in deionised water. Inoculated flasks were incubated at 26°C for 72 hours on a rotary shaker. The harvest broth was clarified by centriedfugation.

Fermentation samples were monitored for antibiotic production by bioassay on *Staphylococcus aureus* Oxford using the conventional hole in plate method. Samples generated during purification of the active components were monitored both by bioassay on *S. aureus* Oxford (disc diffusion) and by TLC on silica gel plates in $CHCl_3$ - MeOH (9:1). (The plates were precoated Silica gel 60 F_{254}.)

The antibiotics were isolated using the following procedures. Clarified brew (800 ml) was adjusted to pH 3.0 with 5 N HCl and extracted with $CHCl_3$ (400 ml). The aqueous phase was re-extracted with a further 200-ml of $CHCl_3$. The combined $CHCl_3$ extracts were washed with water adjusted to pH 3.0. The $CHCl_3$ extract was evaporated under reduced pressure to dryness. The solid was dissolved in a minimum of $CHCl_3$ and loaded onto a column (425×40 mm) of Sephadex LH-20 previously equilibrated with the same solvent. MM 44326 and MM 44785 were eluted sequentially from the column in $CHCl_3$. MM 44325, MM 44786 and MM 44788 were then eluted sequentially with $CHCl_3$ - MeOH (9:1) and MM 44787 was eluted with MeOH.

The MM 44326 eluted from the Sephadex column was substantially pure, yielding a purple solid (158 mg). All other components required further purification, and were chromatographed on Silica gel 60 columns. MM 44785 required two further column stages utilising $CHCl_3$ - MeOH (9:1) and (9.5:0.5) to yield 7.5 mg of the yellow antibiotic. MM 44325 similarly required chromatography using $CHCl_3$ - MeOH (9:1), and 50 mg of the red antibiotic were obtained. Antibiotics MM 44786 and MM 44788 were eluted from the Sephadex LH-20 column as a mixture. These components were separated and purified by chromatography using $CHCl_3$ - MeOH (9:1) (MM 44786, 2.9 mg of a yellow solid) followed by $CHCl_3$ - MeOH (8:2) (MM 44788, 1.4 mg of a yellow solid). Finally MM 44787 was purified by sequential elution of a silica gel column with EtOAc - MeOH (1:1), MeOH and finally water. The MM 44787 was eluted with the water to yield 5.0 mg of the red antibiotic.

The antibiotics were characterised by their mobilities on TLC on silica gel (Rf values in Table 1) and by their UV spectra (Table 2). The ratio of the antibiotics produced varied between fermentation runs.

The compounds demonstrated activity against Gram-positive bacteria (Table 3) but were essentially inactive against representative strains of Gram-negative organisms and *Candida albicans*. The antibacterial spectrum of the compounds described in this paper was similar to that of previously reported members of the granaticin series.

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