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

II. STRUCTURE DETERMINATION

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A number of new granaticin type quinone antibiotics have been isolated from *Streptomyces lateritius* ATCC 19913. Spectroscopic evidence is presented which has led to the structure elucidation of three new antibiotics and the chemical relationship between members of the granaticin series has been studied.

Six antibiotics of the granaticin group have been isolated from *Streptomyces lateritius* ATCC 19913 and designated MM 44325, MM 44326, MM 44785, MM 44786, MM 44787 and MM 44788 (Fig. 1). One of them, MM 44326, was found to be identical to the previously described granaticin B[1,2] but the five other metabolites are new members of the series. The production, isolation, purification, physical and biological properties of the six antibiotics are discussed in part I[3]. This paper presents spectroscopic evidence for the structure elucidation of MM 44325, MM 44787 and MM 44785. Although the structures of MM 44786 and MM 44788 have not been elucidated the physical data obtained on these metabolites defines them as previously undescribed members of the series.

The chemical conversion of granaticin B into dihydrogranaticin B, granaticin A and granaticinic acid, and its oxidation to MT 44330 are also described.

The granaticins are a well-documented series of quinone antibiotics and are reported to have antibacterial, antitumour and antiprotozoal activities[4,5]. Their isolation has been reported from several sources including *S. lateritius* (granaticin A, dihydrogranaticin A and its methyl ester)[6], *Streptomyces violaceoruber* (granaticins A and B)[2], *Streptomyces olivaceus* (granaticin A)[7], *Streptomyces litmogenes* (granaticin A)[5], *Streptomyces thermoviolaceus* WR-141 (granaticin A and dihydrogranaticin A)[4] and a thermophilic streptomycete (granaticin A and granaticinic acid)[8].

Structural Studies

All spectroscopic data were obtained on the free acid form of the quinone metabolites, which were all chloroform soluble. The acid forms were prepared by partitioning products from silica gel chromatography between chloroform and dilute hydrochloric acid.

MM 44326 (Granaticin B)

The IR and ^{1}H NMR spectra (see Tables 1 and 2) obtained on this red compound were consistent with those reported[1,2] for the quinone antibiotic granaticin B. Quinones are known to be readily reduced in the mass spectrometer when operating in the fast atom bombardment (FAB) mode and large $(M+2H)^+$ and $(M+3H)^+$ ions are observed in the case of quinones with low reduction potentials[9]. Such is the case with MM 44326 (Table 3), and indeed all the quinone antibiotics derived from this culture. The base peak in the positive ion spectrum was at *m/z* 447 corresponding to the

Fig. 1. Structures of *Streptomyces lateritius* metabolites and related compounds.

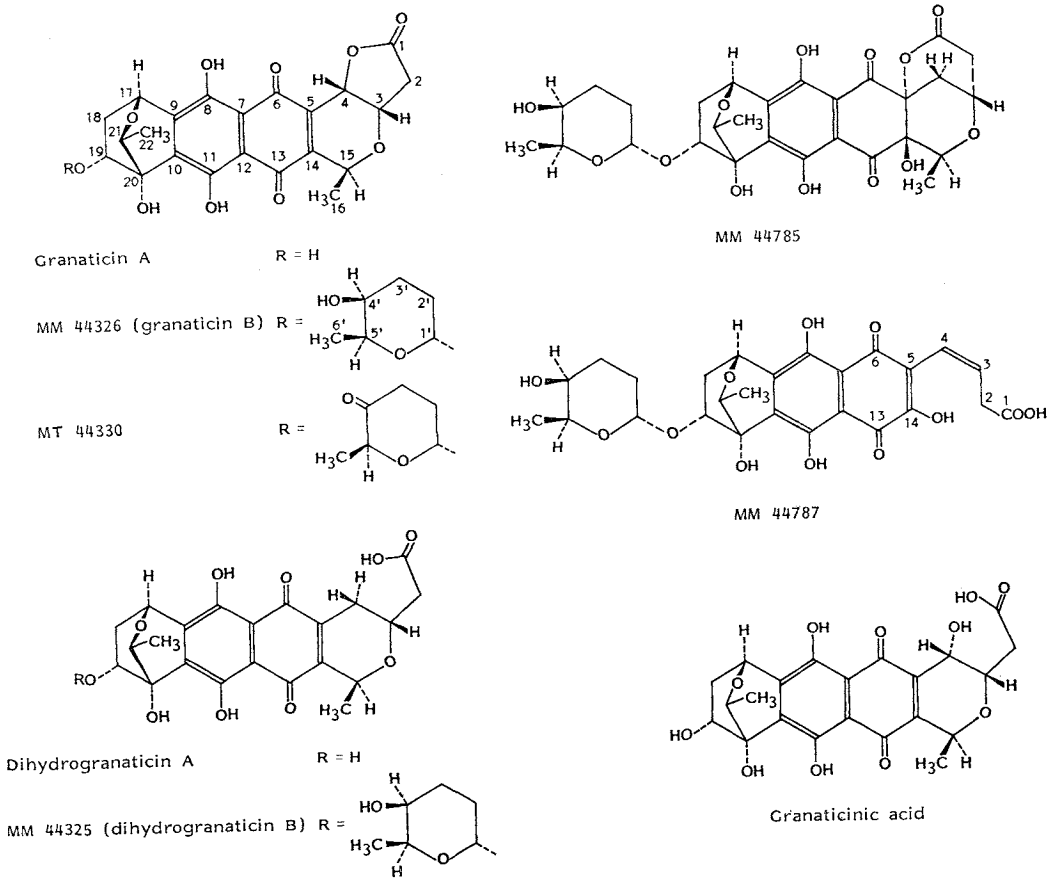

Table 1. IR data (cm^{-1}) on metabolites from *Streptomyces lateritius* and their derivatives.

MM 44326 (granaticin B)	3450, 1795, 1660, 1610, 1565
MM 44325 (dihydrogranaticin B)	3400, 1720, 1600, 1560
MM 44787	3400, 1700, 1640, 1600, 1560
MM 44785	3300, 1760, 1660
Granaticinic acid	3400, 1720, 1600, 1560
MT 44330	3450, 1795, 1720, 1660, 1610, 1565

loss of the L-rhodinose sugar moiety.

Acid hydrolysis of granaticin B afforded granaticin A[1,5] but under basic conditions concomitant γ-lactone cleavage was also observed leading to the formation of granaticinic acid, a previously described[8] antibiotic isolated from *Streptomyces* sp.

The secondary hydroxyl group at position 4′ in the L-rhodinose unit of granaticin B was readily oxidised with pyridinium chlorochromate to give MT 44330. Spectral data was consistent with the proposed structure.

Table 2. ^{1}H NMR data on metabolites from *Streptomyces lateritius* and their derivatives.

Position	δ ($CDCl_3$) (*J* values in parenthesis, Hz)					
	MM 44326 (granaticin B)	MM 44325 (dihydrogranaticin B)	MM 44787	MM 44785	Granaticinic acid	MT 44330
2	3.00, dd (17.8, 5.0), 2.72, d (17.8)	2.74, d (6.3)	4.05~3.95, m	3.00, d (19.6), 2.87, dd (19.6, 5.1)	2.90, d (6.6)	2.99, dd (17.7, 5.1), 2.72, d (17.7)
3	4.76, dd (5.0, 3.0)	4.38, m	6.35, m	4.55, m	4.39, m	4.75, dd (5.1, 2.9)
4	5.23, d (3.0)	2.95, dd (19, 3.3), 2.42, ddd (19, 10.7, 1.6)	5.96, d (11.4)	2.57, ddd (14.6, 4.3, 1.9), 2.31, dd (14.6, 1.4)	4.78, d (2.2)	5.26, d (2.9)
15	5.23, q (6.8)	5.12, q (6.7)	—	4.32, q (6.0)	5.15, q (6.8)	5.23, q (6.8)
16	1.59, d (6.8)	1.62, d (6.7)	—	1.52, d (6.0)	1.61, d (6.7)	1.59, d (6.8)
17	5.35, d (3.0)	5.25, dd (3.3, 2.0)	5.31, br s	5.29, dd (3.5, 1.7)	5.22, m	5.34, d (2.9)
18	2.69, ddd (14.2, 7.7, 3.0), 1.6, obscured	2.69, ddd (14.4, 7.8, 3.3), 1.55, ddd (14.4, 2.0, 1.5)	2.72, ddd (14.3, 8.1, 3.5), 1.53, d (14.3)	2.72, ddd (14.3, 8.3, 3.5), 1.48, partially obscured	2.74, ddd (14.5, 8.1, 3.6), 1.20, ddd (14.5, 6.2, 2.0)	2.75, partially obscured, 1.60, obscured
19	4.06, dd (7.7, 1.3)	4.07, dd (7.8, 1.5)	4.05~3.95, m	4.03, dd (8.3, 2.0)	4.04, dd (8.1, 2.0)	4.16, dd (7.7, 1.3)
21	3.87, q (6.2)	3.87, q (6.3)	3.88, q (6.1)	3.85, q (6.2)	3.80, q (6.2)	3.88, q (6.3)
22	1.05, d (6.2)	1.02, d (6.3)	0.98, d (6.1)	0.86, d (6.2)	1.02, d (6.2)	1.05, d (6.3)
1′	4.83, br s	4.82, br s	4.85, br s	4.84, br s	—	5.03, t (4.8)
2′	1.95~1.2, m	1.95~1.2, m	2.0~1.6, m	2.0~1.2, m	—	2.20, m, 1.82, m
3′	1.95~1.2, m	1.95~1.2, m	2.0~1.6, m	2.0~1.2, m	—	2.40, t (7.0)
4′	3.59, br s	3.59, br s	3.59, br s	3.63, br s	—	—
5′	4.17, q (6.5)	4.23, q (6.7)	4.17, q (6.7)	4.21, q (6.6)	—	4.50, q (6.7)
6′	1.24, d (6.5)	1.24, d (6.7)	1.25, d (6.7)	1.24, d (6.6)	—	1.36, d (6.7)
Aryl O*H*	12.92, s, 12.90, s	13.24, s, 12.80, s	12.93, s, 12.46, s	11.67, br s, 11.35, s	11.12, s, 10.97, s	12.89, s, 12.88, s

Table 3. Molecular ion data from FAB-MS (glycerol matrix).

	Positive ion	Negative ion	Accurate mass	Calcd
MM 44326	560 (M+2H), 561 (M+3H)	558 (M), 559 (M+1)	—	
MM 44325	562 (M+2H), 563 (M+3H)	560 (M), 561 (M+1)	562.2051	$C_{28}H_{34}O_{12}$ (M+2H), 562.2050
MM 44787	—	531 (M−H), 532 (M)	—	
MM 44785	579 (M+3H), 578 (M+2H)	575 (M−H), 576 (M), 577 (M+H)	579.2089	$C_{28}H_{35}O_{13}$ (M+3H), 579.2078
Granaticinic acid	463 (M+H), 464 (M+2H)	462 (M), 463 (M+H)		
MT 44330	—	556 (M), 557 (M+H)		

MM 44325 (Dihydrogranaticin B)

An examination of the FAB-MS (Table 3) indicated a molecular weight of 560, two mass units higher than that found for granaticin B (MM 44326). A comparison of the IR data (Table 1) obtained on these two compounds suggests that in MM 44325 the γ-lactone unit has been replaced by a carboxylic acid group and these features are readily accommodated in the proposed structure, dihydrogranaticin B.

Dihydrogranaticin B is a previously undescribed antibiotic, although dihydrogranaticin A has been isolated by several groups[4,6,7]. Confirmation of the proposed structure for MM 44325 comes from ^1H and ^{13}C NMR studies. In particular, the low-field 4-H at δ 5.23 in the ^1H NMR spectrum of granaticin B (Table 2) has been replaced in MM 44325 by an AB quartet at higher field and furthermore the observed ^1H and ^{13}C chemical shifts (Tables 2 and 4) are in close agreement with those reported[4,7,10] for the related compound dihydrogranaticin A.

Dihydrogranaticin B was prepared synthetically from granaticin B by sodium dithionite reduction followed by aerial oxidation. The lactone cleavage is readily understood in terms of a β-elimination following initial reduction to the hydroquinone system.

MM 44787

Whilst similar in many other respects the ^1H NMR spectrum (Table 2) of MM 44787 featured two striking differences from that of a typical granaticin. One was the absence of signals associated with carbon atoms 15 and 16 of the dihydropyran ring and the second was the appearance of two signals, presumably olefinic, at about 6 ppm. A ^1H-^1H correlated spectroscopy NMR experiment elucidated the relationship between the protons at positions 2, 3 and 4 and, since no unassigned signals remained, position 14 in the proposed structure must bear an oxygen atom in order to comply with a molecular weight of 532.

MM 44785

Whilst the ^1H NMR spectral data (Table 2) on MM 44785 indicated that it was closely related to the granaticins, its yellow colour suggested that the typical red dihydroxynaphthoquinone chromophore had been modified in some way. The carbonyl stretching frequency of 1760 cm^{-1} in the IR spectrum (Table 1) was not consistent with either a γ-lactone or a free carboxylic acid and the presence of a

Table 4. ^{13}C NMR data on MM 44325 and MM 44785.

MM 44325 (dihydrogranaticin B) δ ($CDCl_3$) ppm	Position[a]	MM 44785 δ ($CDCl_3$) ppm	Position
176.2, C	1, 6, 13	199.4, C	6, 13
176.1, C		193.2, C	
175.1, C		167.5, C	
167.6, C	11	149.3, C	
161.9, C	8	137.7, C	
145.0, C	14	135.0, C	
141.8, C	9	112.2, C	
140.1, C	5	111.4, C	
137.8, C	10	96.4, CH	1′
110.4, C	7, 12	83.2, C	5 or 14
110.2, C		78.4, C	20
95.0, CH	1′	76.7, CH	4′ or 5′
78.7, C	20	75.0, C	14 or 5
75.5, CH	4′ or 5′	73.0, CH	21
72.8, CH	21	67.4, CH	3, 15, 19, 5′ or 4′
67.7, CH	5′ or 4′, 15, 19	67.1, CH	
67.4, CH		65.3, CH	
67.0, CH		64.9, CH	
63.0, CH	3	62.1, CH	17
61.8, CH	17	35.8, CH_2	2, 18
40.4, CH_2	2	35.6, CH_2	
35.3, CH_2	18	25.4, CH_2	2′
27.7, CH_2	4	23.6, CH_2	4, 3′
25.4, CH_2	2′	23.5, CH_2	
23.6, CH_2	3′	17.1, CH_3	6′
19.2, CH_3	16	16.5, CH_3	22
17.1, CH_3	6′ or 22	15.6, CH_3	16
16.8, CH_3	22 or 6′		

[a] Assignments made by comparison with published data on dihydrogranaticin A, refs 4, 7 and 10.

δ-lactone was indicated. Since position 4 in the molecule was unoxygenated (from ^1H NMR data) and the dihydropyran ring still intact, the lactone ring must be completed with the C-5 atom. To account for the molecular formula, C-14 must then carry a hydroxyl group. Further evidence for the loss of olefinic character in positions 5 and 14 is given by the ^{13}C NMR spectrum (Table 4). In dihydrogranaticin B the quinone carbonyls C-6 and C-13 appear at approximately 176 ppm but in the spectrum of MM 44785 the carbonyl carbons appear at considerably lower field. This is entirely consistent with the loss of the adjacent C-5 – C-14 double bond and furthermore the signals at 140.1 and 145.0 ppm attributable to these olefinic carbons in the spectrum of dihydrogranaticin B appear at 75.0 and 83.2 ppm in MM 44785, again indicating that C-5 and C-14 are now oxygen-bearing sp^3 carbon atoms.

Presumably, the biosynthetic precursor to MM 44785 is the epoxide of dihydrogranaticin B which is ring-opened by nucleophilic attack of the suitably positioned carboxylic acid group. Our proposed C-5 and C-14 stereochemistry for MM 44785 is based on this assumption.

Experimental

The IR spectra were recorded on a Perkin-Elmer 197 and the UV spectra on a Pye-Unicam SP7-500 spectrometer. The ^1H NMR data was recorded on a Bruker WM 250 instrument operating at

250 MHz and ^{13}C NMR data on a Bruker AM 400 operating at 100 MHz. TMS was used as internal standard. FAB-MS were obtained on a VG ZAB 1F instrument and accurate mass measurements were determined by multi-channel analysis using a 11-250J data system.

Reduction of Granaticin B

A stirred solution of granaticin B (15 mg) in THF (2 ml) was treated with an aqueous solution of sodium dithionite in water until a yellow colour persisted for a few moments. Continued stirring in the presence of air led to the development of a red colour. The reaction mixture was now partitioned between $CHCl_3$ and water. The organic layer was dried ($MgSO_4$) and evaporated to afford a solid (8 mg) which was chromatographed on silica gel. Initial elution with $CHCl_3$ - MeOH (10 : 1) followed by neat MeOH afforded a blue material which was converted into the acidic (red) form by partition between dilute HCl and $CHCl_3$. The organic layer afforded dihydrogranaticin B as a red solid (5 mg). IR, ^1H NMR and TLC characteristics identical to those of authentic material.

Acidic Hydrolysis of Granaticin B

A stirred solution of granaticin B (23 mg) in a mixture of 5 M HCl solution (2 ml) and THF (2 ml) was heated to 50~60°C for 1 hour. The solution was then diluted with $CHCl_3$ and water and the organic layer dried ($MgSO_4$) and evaporated to afford crude product. Chromatography on silica gel, eluting with $CHCl_3$ - MeOH (9 : 1 ~ 1 : 1) gave the product which was partitioned between $CHCl_3$ and dilute HCl solution. The organic layer afforded pure granaticin A (14 mg). IR and ^1H NMR consistent with published data[1,5].

Basic Hydrolysis of Granaticin B

A stirred solution of granaticin B (18 mg) in 10% sodium hydroxide solution (1 ml) was heated to 60°C for 1.5 hours. The cooled solution was acidified with dilute HCl solution, saturated with salt, and extracted with $CHCl_3$. The organic layer was dried ($MgSO_4$) and evaporated to afford crude product (14 mg). Chromatography on silica gel, eluting with $CHCl_3$ - MeOH (4 : 1) gave granaticin A (3 mg). Continued elution with neat MeOH gave granaticinic acid (MT 44329) (6 mg). After conversion to the acidic form (see above) this material gave IR and ^1H NMR data that was consistent with published figures[6].

Oxidation of Granaticin B

A stirred solution of granaticin B (20 mg) in dichloromethane was treated with pyridinium chlorochromate (50 mg) and stirred at room temperature for 2 hours. This reaction mixture was then partitioned between $CHCl_3$ and dilute HCl. The organic layer was dried ($MgSO_4$) and evaporated to afford a crude product which was chromatographed on silica gel. Elution with $CHCl_3$ - MeOH (9 : 1) afforded MT 44330 (3.3 mg) followed by recovered starting material (2 mg).

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