NOTES

Components of the GE2270 Complex Produced by *Planobispora rosea* ATCC 53773

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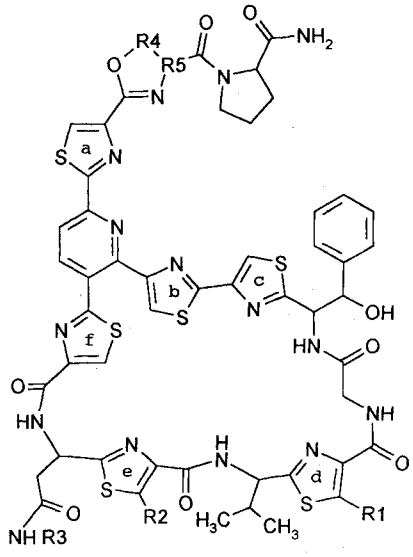
Antibiotic GE2270A inhibits bacterial protein biosynthesis by acting on EF-Tu[1~4]. The antibiotic has a thiazolyl peptide structure[5] which has been recently revised[6,7]. Other structurally related antibiotics are normally co-produced with GE2270A by *Planobispora rosea* ATCC 53773. We describe the isolation and characterization of GE2270 factors E, D1, D2, C1, C2a, C2b, B1, B2 and T.

Fig. 1. Structures of the GE 2270 factors.



GE 2270 factors	R1	R2	R3	R4-R5
E	CH_2OH	CH_3	H	CH_2-CH
D1	H	CH_3	H	CH_2-CH
D2	CH_2OH	CH_3	CH_3	CH_2-CH
C1	H	CH_3	CH_3	CH_2-CH
C2a	CH_2-OMe	CH_2OH	CH_3	CH_2-CH
C2b	CH_2-OMe	H	CH_3	CH_2-CH
B1	CH_2-OMe	CH_3	H	CH_2-CH
B2	CH_3	CH_3	CH_3	CH_2-CH
A	CH_2-OMe	CH_3	CH_3	CH_2-CH
T	CH_2-OMe	CH_3	CH_3	CH=C

Crude preparations from fermentations of *Planobispora rosea* ATCC 53773 were obtained (Scheme 1) which were enriched in the factors co-produced with GE2270A. The individual factors were then isolated by preparative HPLC on a 25×250 mm column (Hibar, E. Merck) packed with Lichrosorb C18 (7 μm) and eluted at a flow rate of 24 ml/minute with phase A and B in the ratio 72:28. Phase A and B were acetonitrile-tetrahydrofuran-40 mM $HCOONH_4$ in the ratio 10:10:80 and 40:40:20, respectively. Factor T was purified by eluting with acetonitrile-water 60:40. The purified antibiotics precipitated from the elutes after elimination of the acetonitrile under vacuum. GE2270 factors were obtained as white powders showing HPLC retention times and molecular weights reported in Table 1. Factor T showed UV spectra with $\lambda_{max}^{0.1\,N\,HCl}$ 250, 280 (sh), 345 (sh); $\lambda_{max}^{0.1\,N\,KOH}$ 240 (sh), 290 (sh), 345 (sh); $\lambda_{max}^{Phosphate\ buffer\ pH\ 7.38}$ 250 (sh), 310 (sh), 345 (sh); λ_{max}^{MeOH} 240 (sh), 275 (sh), 290 (sh), 311, 335 (sh). The other factors showed identical UV spectra in MeOH, in 0.1 N HCl and in 0.1 N NaOH with λ_{max} 245~250 (sh), 310.

The characteristic IR absorptions were common for all factors: IR (Nujol) cm^{-1} 3600~3100 (νNH, νOH); 1681,1650 (νC=O, amide I); 1582 (aromatic νC=C); 1555 (heterocyclic νC=C and νC=N); 1511 (δNH, amide II); 1243, 1209 (aromatic δCH); 1090, 1074 (νC–O and

Scheme 1. Crude preparation of GE 2270 factors.

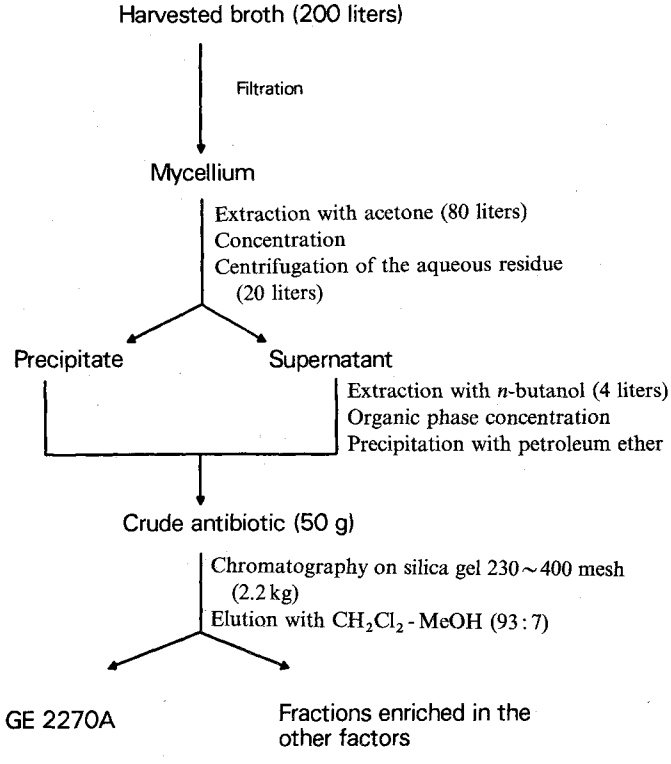


Table 1. HPLC and FAB-MS characteristics.

GE 2270 factors	HPLC[a]	FAB-MS (MH^+)[b]
Factor E	0.52	1262
Factor D1	0.56	1232
Factor D2	0.59	1276
Factor C1	0.65	1246
Factor C2a	0.76	1306
Factor C2b	0.79	1276
Factor B1	0.85	1276
Factor B2	0.97	1260
Factor A	1.00	1290
Factor T	1.19	1288

[a] Retention time relative to factor A (Factor A eluted after 16.6 minutes).
HPLC: Column: Bakerbond C8 (5 μm) 4.6 × 250 mm; Flow-rate: 1.8 ml/minute.
Phase A: CH_3CN-THF-$HCOONH_4$ 40 mM (4:4:2).
Phase B: CH_3CN-THF-$HCOONH_4$ 40 mM (1:1:8).
Elution: Linear gradient from 20% to 30% of phase A in 20 minutes.
Detection: UV 254 nm.

[b] The FAB-MS studies were conducted under the following experimental conditions: Positive ion FAB spectra were obtained on a Kratos MS-50 double focusing mass spectrometer; A saddle field atom gun was used with Xe gas 6 Kv voltage and 1 mA current. In the FAB-MS experiments the sample was mixed with thioglycerol matrix which contained 0.1 M acetic acid. The main peak corresponds to the lowest isotope of the protonated molecular ion.

νC–O–C); 870 (heterocyclic γCH); 763, 703 (aromatic γCH).

The structures of the individual factors were determined by 2D NMR spectroscopy and by comparison with the assignment of GE2270A[5,7]. Most of the proton signals of GE2270 A and of the minor factors are coincident. It indicates that the building blocks of the thiazolyl peptide backbone and the sequence is conserved in all factors. The coincidence of the signals of the aromatic protons of pyridine and of the thiazole rings a, b, c and f for GE2270A and for all other factors shows that the chromophore is identical in all factors which is consistent with the similarity of their UV spectra. The complete ^1H attributions for the factors C1, C2a, C2b and D2 are reported in Table 2.

The assignments of the substituents on thiazoles d and e were made on the basis of the attribution achieved for factor A. The signal around 2.6 ppm which corresponds to the methyl group on thiazole ring e was observed in factor A and in factors E, D1, D2, C1, B1, B2 and T. When both thiazole rings d and e have a methyl substituent, as in factor B2, a second distinct signal at 2.72 ppm is present. The signals of the CH_2OCH_3 substituent at thiazole ring d (singlets around 5.0 and 3.4 ppm) are present in factors A, C2a, C2b, B1, and T. Factor C2a has a CH_2OH group at position 5 of thiazol ring e, reflected by a triplet at 5.72 ppm (OH) and a doublet at 4.88 ppm (CH_2), whereas factor C2b has a proton at this position corresponding to a singlet at 8.07 ppm. Thiazole ring d of factor E is substituted with a CH_2–OH group in position 5 indicated by two doublets of doublets at 4.98 and 5.00 ppm and a triplet at 5.95 ppm. The thiazole ring d of two minor factors, D1 and C1, is unsubstituted in position 5 which is indicated by an additional singlet at 8.29 or 8.27 ppm, respectively. To determine if a substituent is connected to ring d or e a ROESY spectrum was acquired. In this spectrum the amide proton of the valine moiety experiences a cross relaxation with the substituent on position 5 of thiazole ring e. This is also true if the substituent is only a proton like in the case of factor C2b. Additional evidence of these structural assignments was obtained from hydrolysis studies. The substituted thiazole amino acids characteristic of the GE2270 factors were identified by GC-MS analysis of the acid hydrolysates of the complex[8] and of the single factors.

The asparagine moiety of factors A, D2, C1, C2a, C2b, B2, and T has an NH–CH_3 group, identified by a doublet around 2.5 ppm (CH_3) and a quartet around 7.4 ppm (NH); factors E, D1, and B1 are unsubstituted at the NH_2, as indicated by the additional singlets of the $CONH_2$ group (factor E: 6.62, 6.98; factor D1: 6.53, 7.00; factor B1: 6.65, 6.99).

Factor T is characterized by the oxidation of the oxazoline ring. The signals of the oxazoline CH_2–CH fragment are missing and a singlet at 8.66 ppm is present which is consistent with a proton in position 5 of an oxazole ring. The structures of the factors are reported in Fig. 1.

The factors are characterized by the substituents at position 5 of thiazole rings d and e, and on NH_2 of asparagine. In the biosynthesis of thiazolyl peptide antibiotics[9], the thiazoles derive from one molecule of cysteine and the carboxy group of the adjacent amino acid[10] leading to the formation of unsubstituted rings. The types of substituents found in the GE2270 factors suggest that *P. rosea* modifies the GE2270 backbone by introducing a variable number of methylene units. Under most fermentation conditions tested, the strain produces preponderantly GE2270 factor A and small quantities of the other factors. However, in some media and fermentation conditions there is significant production of factors E, D1 and D2. Since factor A has the substituents with the highest number of methylene units and factor D1 represents the lowest extent of methylation, it appears that the methylation efficiency influences the relative production of the factors in the complex.

All the isolated GE2270 minor factors are active against Gram-positive bacteria. The MICs of the factors, reported in Table 3, show that the individual factors generally have antibacterial activities equivalent to or slightly less than that of factor A.

Table 2 ^{1}H chemical shifts of factors A, C1, C2a, C2b and D2[a].

		A[b]	C1[c]	C2a[c]	C2b[c]	D2[b]
PheSer	NH	8.96	8.94	8.99	8.94	8.98
	α	5.27	5.27	5.26	5.26	5.26
	β	5.04	5.04	5.02	5.03	5.02
	β-OH	5.99	5.98	6.04	5.98	6.00
	2, 6	7.29	7.28	7.28	7.28	7.29
	3, 5	7.32	7.31	7.31	7.31	7.31
	4	7.24	7.23	7.23	7.23	7.24
Gly	NH	8.42	8.50	8.43	8.43	8.36
	α	4.31/3.82	4.25/3.88	4.30/3.84	4.30/3.82	4.30/3.80
Val	NH	8.68	8.72	8.67	8.67	8.69
	α	5.21	5.25	5.20	5.25	5.18
	β	2.19	2.21	2.19	2.20	2.18
	γ	0.90	0.90	0.89	0.90	0.90
	γ′	0.86	0.87	0.85	0.87	0.86
Thia-d	5	5.00 (CH_2)	8.27 (H)	4.99 (CH_2)	4.99 (CH_2)	5.00 (CH_2)
		3.40 (CH_3)	—	3.39 (CH_3)	3.39 (CH_3)	5.96 (OH)
Asn	NH	8.72	8.73	8.69	8.74	8.71
	α	5.32	5.36	5.31	5.38	5.30
	β	2.73/1.39	2.73/1.54	2.71/1.35	2.74/1.42	2.73/1.35
	δ-NH	7.37	7.44	7.36	7.37	7.37
	ε-CH_3	2.49	2.47	2.48	2.46	2.49
Thia-e	5	2.61 (CH_3)	2.61 (CH_3)	4.88 (CH_2)	8.07 (H)	2.60 (CH_3)
	5	—	—	5.72 (OH)	—	—
Thia-f	5	8.58	8.59	8.59	8.60	8.58
Pyr	4	8.39	8.41	8.43	8.42	8.40
	5	8.28	8.28	8.28	8.28	8.27
Thia-a	5	8.51	8.53	8.53	8.53	8.52
Thia-b	5	8.28	8.29	8.28	8.29	8.28
Thia-c	5	7.37	7.37	7.38	7.37	7.36
Oxa	α	5.24	5.24	5.24	5.23	5.23
	β	4.83/4.57	4.82/4.57	4.81/4.57	4.81/4.57	4.82/4.57
Pro	α	4.28	4.27	4.26	4.26	4.27
	β	2.15/1.90	2.16/1.95	2.15/1.96	2.14/1.94	2.14/1.94
	γ	1.97	1.99/1.91	1.97/1.91	1.95/1.87	1.96/1.86
	δ	4.01/3.83	4.00/3.84	3.99/3.84	3.99/3.83	3.99/3.83
	NH_2	7.32/6.91	7.32/6.90	7.32/6.90	7.32/6.90	7.35/6.93

[a] All NMR experiments were performed on a Bruker AMX600 spectrometer.
[b] in DMSO-d_6 at 310 K.
[c] in DMSO-d_6 at 313 K.

Table 3. MIC (μg/ml) of GE 2270 factors.

Strain	A	B1	B2	C1	C2b	D1	D2	E	T
Staphylococcus aureus L165	1	1	1	1	1	2	1	2	0.06
S. epidermidis L147	0.25	1	0.25	0.25	1	0.5	0.5	2	0.06
S. haemoliticus L602	2	4	2	1	2	8	4	>128	0.06
Streptococcus pyogenes L49	0.25	0.25	1	0.25	1	0.25	0.5	0.5	>128
S. pneumoniae L44	0.13	0.13	0.5	0.13	0.25	0.13	0.13	0.25	0.5
Enterococcus faecalis L149	0.25	0.5	1	0.25	1	0.5	1	1	0.03
Propionibacterium acnes L1014	0.008	0.016	0.016	0.008	0.008	0.016	0.016	0.03	0.016
Escherichia coli L47	>128	>128	>128	>128	>128	>128	>128	>128	>128
Pseudomonas aeruginosa ATCC 10145	>128	>128	>128	>128	>128	>128	>128	>128	>128
Proteus vulgaris ATCC 881	>128	>128	>128	>128	>128	>128	>128	>128	>128

Minimal inhibitory concentrations (MIC) were determined by microbroth dilution methodology. Inocula were $10^4 \sim 10^5$ CFU/ml. All microorganism were cultured at 37°C. MICs were read at 18 and 24 hours, except for *P. acnes* (48 hours). *P. acnes* was incubated anaerobically. Media used were: Oxoid Iso-Sensitest broth (staphylococci, *E. faecalis*, *E. coli*, *P. vulgaris*, *P. aeruginosa*); Difco Todd-Hewitt broth (streptococci); Difco Wilkins-Chalgren broth for *P. acnes*.

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