UK-63,052 COMPLEX, NEW QUINOMYCIN ANTIBIOTICS FROM *STREPTOMYCES BRAEGENSIS* SUBSP. *JAPONICUS*; TAXONOMY, FERMENTATION, ISOLATION, CHARACTERISATION AND ANTIMICROBIAL ACTIVITY

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UK-63,052 complex, a new group of quinomycin-like antibiotics comprising UK-63,052 (factor A), UK-63,598 (factor C), UK-65,662 (factor B) and several uncharacterised minor components, is produced by a new subspecies of the genus *Streptomyces* for which the name *Streptomyces braegensis* Dietz subsp. *japonicus*, is proposed. The strain, N617-29, is characterised by a negative melanin reaction, grey aerial mycelium, spiral spore chains and smooth or slightly warty spores. Structure determination has identified UK-63,052, $C_{56}H_{68}N_{10}O_{14}S_2$, UK-63,598, $C_{53}H_{62}N_{10}O_{14}S_2$ and UK-65,662, $C_{55}H_{66}N_{10}O_{14}S_2$ as quinaldic acid substituted quinomycins with unusual bridgehead sulfur substitution as shown in Fig. 3.

In the course of our screening program for new antibiotics, a new actinomycete strain, N617-29, for which the name *Streptomyces braegensis* subsp. *japonicus*, is proposed, was isolated from a soil sample. This strain was found to produce a complex of novel quinomycin-like antibiotics. As well as the taxonomy of the producing strain, this paper describes the production, isolation, physico-chemical properties, antimicrobial activity and the structure determination of the three major components UK-63,052, UK-63,598 and UK-65,662.

Materials and Methods

The actinomycete strain, N617-29, was isolated from a soil sample collected from Kumamoto Castle, Japan. *S. braegensis* NRRL 12567 was obtained from the Northern Regional Center, Peoria, Illinois.

UV spectra were recorded on a Pye Unicam SP8-500 and IR spectra on a Perkin-Elmer 781 spectrophotometer. NMR spectra were measured on a Bruker WM250 spectrometer.

HPLC was performed using a Waters system comprising a 720 System Controller, a WISP 710B and two 6000A pumps. UV detection was by Pye Unicam LC3 detector. Integration was performed by a Spectra Physics 4100 computing integrator.

Bioassays were carried out by the dipped paper disc assay method.

Taxonomy of the Producing Strain N617-29

The morphological properties were observed on inorganic salts - starch agar after 14 days incubation. Examination by scanning electron microscopy showed that the spore chains were composed of 10 to 30 spores, tightly coiled or slightly open with 2 to 7 turns per chain and had a tendency to aggregate into patches or masses, Figs. 1 and 2. The sporophores were monopodially branched and the spores globose (0.8~1.0 μm diameter) or short rods (1.0~1.4×0.8~1.0 μm) with a smooth or slightly warty surface.

The 16 different media given in Table 1 and those in references 1 to 3 were used for cultural and physiological characterisation. Colours were determined by comparison with colour clips from the Colour Harmony Manual (4th Ed., Container Corp. of America, Chicago, 1958). The strain N617-29 had a spore mass which was in the grey-colour series. The strain showed excellent growth at 28~37°C, moderate to good growth at 21°C but no growth at 45°C.[1] Melanin and hydrogen sulfide were not produced and nitrate was not reduced. Gelatin was liquefied and starch hydrolysed. The strain did not grow on LEVINE and SCHOENLEIN's broth[2] and showed only poor growth on JENSEN's broth.[3] Casein, calcium malate and tyrosine digestion were all positive. There was clearing but no coagulation on milk. Strain N617-29 utilised glucose, fructose, inositol, mannitol, raffinose, sucrose or xylose but not arabinose or rhamnose. The methods used for cell wall and sugar analysis were those described by BECKER *et al.*[4] and LECHEVALIER.[5] The whole cell hydrolysates of the strain N617-29 were shown to contain LL-diaminopimelic acid and no diagnostic sugars.

The physiological properties and cell wall analysis, given above, suggest that the strain N617-29 belongs to the genus *Streptomyces*, closely resembling the species *Streptomyces platensis*, *Streptomyces tubercidicus* and *S. braegensis*. It can be readily differentiated from both *S. platensis* and *S. tubercidicus* by the ability to utilise xylose. It can be further differentiated by its greyish-brown substrate mycelium from the red substrate mycelium of *S. platensis*, and by the cream rather than pink soluble pigment of *S. tubercidicus*, in certain media. However, comparisons with *S. braegensis*[6] showed that both cultures

Fig. 1. Scanning electron micrograph of spore chains of strain N617-29 on inorganic salts - starch agar.

Magnification 15,000.

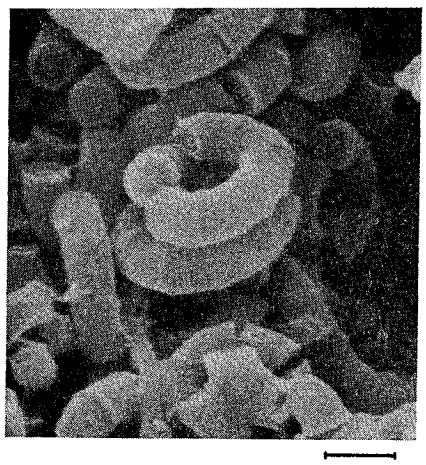


Fig. 2. Scanning electron micrograph of a spore chain of strain N617-29 on inorganic salts - starch agar.

Magnification 20,000.

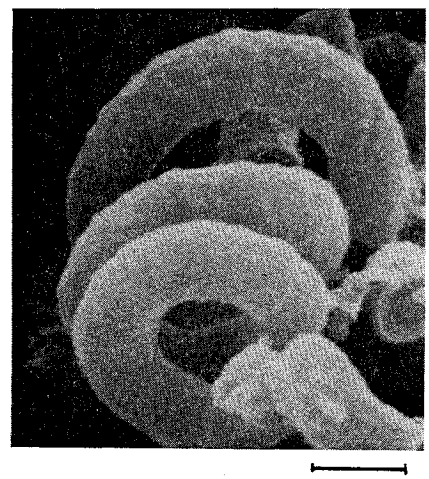


Table 1. Cultural properties of strain N617-29.

Media	ref	Growth	Surface colour[a]	Texture	Aerial mycelium	Reverse colour	Soluble pigment
Yeast extract - malt extract	10	Good	White to pale pink-grey, 4ec	Raised, wrinkled	As surface	Dark brown, 4li, 4nl	Yellowish, 2lc
Oatmeal	10	Moderate	Pink-grey+pale pink edge, 4ec, 4ge, 5ec, ngs5ba, 7ba	Moderately raised smooth to granular	As surface	Greyish-brown, 4ge, 4ig, 4li	Cream, 1.5ca
Inorganic salts - starch	10	Good	White to brownish-grey, 5ec, 5ge, 5ig	Smooth to wrinkled	As surface	Dark brownish-grey, 5li	Cream, 2ca
Glycerol - asparagine	10	Moderate to good	Yellowish+brownish-grey dots, 1ga, 1.5ga, 5ge, 5li	Raised, smooth to slightly wrinkled	Pale greyish-yellowish brown, 3ec	Yellowish+pale grey-yellow-brown, 1.5ga, 3ec	Pale yellowish, 2ea
CZAPEK's - sucrose	11	Moderate to good	Grey to black, ngs3ih, 3ml	Raised, smooth isolated colonies	As surface	Black, ngs3ml, 3po	Yellowish-brown, 3ic
Glucose - asparagine	11	Moderate	Greenish-yellow to pink-grey, 1ic, 3ge, 4li	Moderately raised, smooth or granular or isolated colonies	Pink-grey	Green-grey, grey to greyish yellowish-brown, 1.5ge, 2ge, 3ig	Pale yellow, 2ea
GORDON and SMITH's tyrosine	12	Moderate	Cream, 2ca	Moderately raised, smooth	None	Pale yellowish, 2ea	Yellowish, 2lc
Calcium malate	13	Poor to moderate	Tan to brownish-grey, 3gc, 5ge, 5ig, 5li	Slightly raised, smooth or isolated colonies	Sparse, white	Brownish-grey, 5ge, 5li	Pale yellowish, 2ea

Casein	12	Moderate to good	Yellowish, 2ic	Moderately raised to raised, smooth to wrinkled	None	Yellowish, 2ic	Yellowish-brown, 3pe
BENNETT'S	11	Good	White, pale pink to pale beige, 4ca, 4ec	Raised smooth to slightly wrinkled	As surface	Brown to dark brown, 3lg, 3li, 3nl	Yellowish, 2ga
EMERSON'S	11	Good	Yellowish, 2ic	Raised, wrinkled or isolated colonies	None	Yellowish-brown to yellowish, 3ic, 2ic	Yellowish-brown, 3lc
Nutrient	11	Poor to moderate	Cream, 2ca	Moderately raised or small isolated colonies	None	Cream, 2ca	Pale yellowish, 2ea
Gelatin	14	Moderate to good	Yellowish+pale pink beige dots, 2ic, 4ec	Moderately raised	None	As surface	None
Starch	14	Good	Pink-brownish grey, 6ig	Raised, smooth to slightly wrinkled	White	As surface	Yellowish, 2lc
Potato-carrot[b]	15	Poor to moderate	Light greyish-yellowish-brown to greyish-brown, 3ge, 3ig, 4ig	Thin to slightly raised, smooth or isolated colonies	Greyish-brown	Colourless to greyish-brown, 4ge, 4ig	Cream, 2ca
2% Tap water		Poor	Colourless, pale greyish-yellowish-brown, 3ge, 3ig	Thin, smooth	As surface	As surface	None

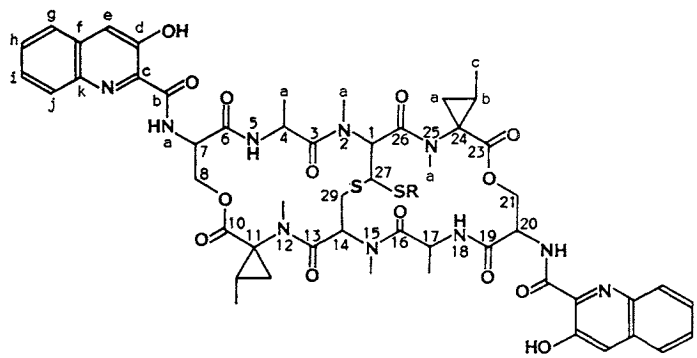
[a] The Colour Harmony Manual, 4th Ed.

[b] Use only 30 g of potatoes, 2.5 g of carrots and 20 g of agar.

Table 2. Comparison of substrate mycelium colours of N617-29 and *Streptomyces braegensis*.

Media	N617-29	*S. braegensis*
Oatmeal	Greyish-brown	Pale grey to grey
Inorganic salts - starch	Greyish-brown	Pale grey to grey
Potato-carrot	Greyish-brown	Pale grey to grey
Tap water	Greyish-brown	Pale grey to grey
Glucose - asparagine	Brownish-grey	Cream to pale pink
BENNETT'S	Brownish-grey	Pale grey to reddish-pink
Starch	Brownish-grey	Pale yellow to reddish

Fig. 3. Structure of UK-63,052 complex.



Component	R
UK-63,052	b c1 c2 d CH-(CH_3)-CH_2-CH_3
UK-65,662	b c1,2 CH-(CH_3)$_2$
UK-63,598	b CH_3

exhibited identical physiological and the majority of the morphological characteristics. The difference in some of the substrate mycelium colours is given in Table 2. In addition the spores of N617-29 are smooth or slightly warty whereas those of *S. braegensis* are smooth.

The similarities between these two strains suggest that N617-29 is a subspecies of *S. braegensis* and the name *S. braegensis* Dietz subsp. *japonicus* subsp. nov. is proposed.

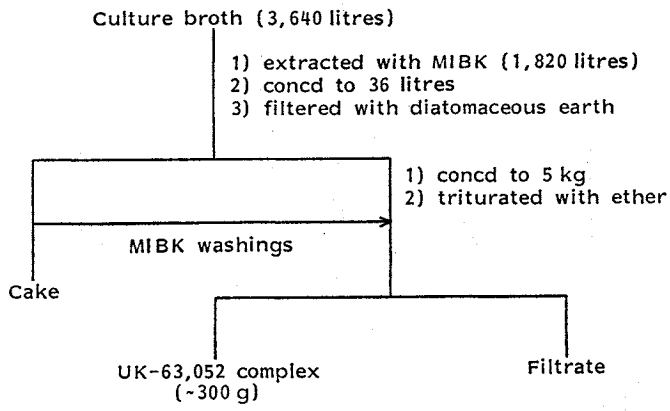
Fermentation

The inoculum was prepared by washing a slant culture of *S. braegensis* subsp. *japonicus*, strain N617-29, maintained on a medium of glucose 4 g, yeast extract 4 g, malt extract 10 g and coconut milk 50 ml and agar 18 g in 1 litre of tap water, pH 7.3, with 10 ml of sterile 0.75% saline.

A seed medium of glucose 10 g, corn starch 5 g, corn steep liquor 5 ml, NZ-Amine YTT 5 g, calcium carbonate 3 g and cobalt chloride 0.002 g in 1 litre of tap water was used. The first stage seed was prepared by inoculation of a 300-ml shake flask containing 80 ml of the seed medium with the inoculum, as prepared above. This was transferred to a second stage seed of 800 ml of the seed medium in a 2.8-litre side arm inoculum flask. Both stages were shaken for 5 days at 28°C and 150 rpm.

The seed, prepared as above, was transferred to 3,640 litres of a production medium containing

Fig. 4. Flow diagram for the isolation of the UK-63,052 complex.

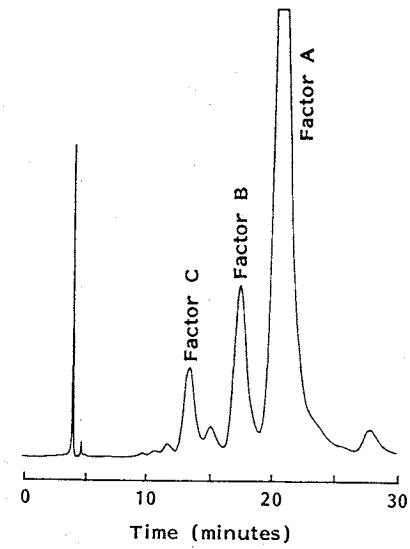


corn flour 10 g, soybean flour 7.5 g, corn steep liquor 5 ml, calcium carbonate 2.5 g, sodium citrate 2 g, magnesium sulfate 0.5 g, cobalt chloride 0.05 g, ferrous sulfate 0.05 g and silicone antifoam 1.1 ml per litre of tap water, pH 7.0~7.2. The fermentation ran for 6 days at 28°C with aeration at 3,640 litres per minute and stirring at 600 rpm. The antibiotic titres, of up to 10 mg/litre, were determined by paper disc assay using *Bacillus subtilis* and *Staphylococcus aureus*, and TLC of the dichloromethane extracts of the whole broth.

Isolation and Purification

The isolation of the UK-63,052 complex is shown in Fig. 4. The fermentation broth (3,640 litres) was extracted with methyl isobutyl ketone (MIBK) (1,820 litres) and concentrated to about 36 litres. This was filtered with the aid of diatomaceous earth, the cake washed with fresh MIBK and the combined filtrates concentrated under vacuum to 4~5 kg of oil. After trituration with ether (2 ml/g of oil), filtering and drying of the resulting solid under vacuum at 50°C, 300 g of UK-63,052 complex was obtained as a yellow solid. The solid was suspended in ethyl acetate and chromatographed on a Jobin-Yvon Chromatospec preparative HPLC system using 1.5 kg of Woelm silica gel 62~200 μm. The fractions were assayed using a dichloromethane - methanol (96 : 4) silica TLC system and 366 nm light for detection, and combined into three bulked fractions containing predominantly the A and B, B and C and C and D factors respectively with an overall recovery of approximately 40%. Each of the three bulked fractions was redissolved in CH_2Cl_2 and decolourised by treatment with activated carbon (1 g/g sample) and the individual factors separated using a Waters Prep LC/system 500 A containing two C_{18} cartridges in series. Initially an isocratic system of

Fig. 5. Typical HPLC trace of enriched UK-63,052 (factor A).



3.9 mm × 30 cm C-18 μBondapak. 0.1% aq AcOH - MeOH (1 : 3) at 1 ml/minute; UV detection at 220 nm.

Table 3. Physico-chemical properties of UK-63,052, UK-65,662 and UK-63,598.

	UK-63,052	UK-65,662	UK-63,598
MP (°C)	252~255	235~241	240~244
$[\alpha]_D$ (c 0.1, $CHCl_3$)	−166.2°	−126.8°	−168.1°
IR ν_{max}^{KBr} cm^{-1}	1730, 1692 (sh), 1662, 1528 (sh), 1516	1730, 1659, 1514	1730, 1690 (sh), 1661, 1512
UV λ_{max} nm (ε):			
MeOH	219 (77,250), 231 (78,300), 300 (9,400), 306 (9,200), 359 (10,150)	219 (76,950), 230 (78,650), 300 (9,500), 306 (9,300), 359 (10,400)	219 (75,050), 231 (76,650), 300 (9,750), 308 (9,600), 359 (10,500)
MeOH+NaOH	223 (65,650), 247 (81,950), 399 (11,200)	223 (68,400), 248 (84,150), 400 (11,400)	223 (67,750), 247 (80,600), 399 (11,500)
Elemental analysis	$C_{56}H_{68}N_{10}O_{14}S_2 \cdot H_2O$	$C_{55}H_{66}N_{10}O_{14}S_2 \cdot H_2O$	$C_{53}H_{62}N_{10}O_{14}S_2 \cdot H_2O$
Theoretical	C 56.64, H 5.94, N 11.79, S 5.39	C 56.29, H 5.84, N 11.94, S 5.46	C 55.58, H 5.63, N 12.23, S 5.59
Found	C 56.34, H 5.88, N 11.52, S 5.21	C 56.33, H 5.84, N 11.81, S 5.50	C 55.37, H 5.59, N 12.18, S 5.63
MW (calcd) (m/z)	1,169	1,155	1,127
TLC[a] (Rf) (solvent)	0.63 (A), 0.39 (B), 0.49 (C), 0.17 (D)	0.61 (A)	0.51 (A)
HPLC[b] (Rt, minutes)	20.20	17.06	13.13

[a] Merck Silica gel 60 pre-coated plates; (A) 35% aq NH_3 - MeOH - $CHCl_3$ (1 : 10 : 89), (B) EtOAc, (C) MeOH - $CHCl_3$ (5 : 95), (D) n-hexane - EtOAc (1 : 3).

[b] 30 cm×4.9 mm C-18 μBondapak, 0.1% aq AcOH - MeOH (1 : 3) at 1 ml/minute. Rt: Retention time.

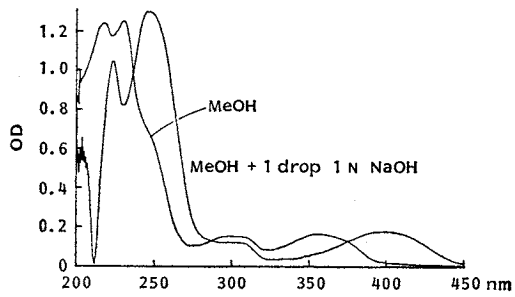
acetonitrile - water (60 : 40) was used for enrichment of the major factors A, B and C followed by rechromatography using a 50 : 50 to 65 : 35 acetonitrile - water gradient system for final purification. A typical HPLC trace of enriched UK-63,052 (factor A), containing factor B 14%, factor C 1% and several minor components, before final purification, is shown in Fig. 5. The required cuts were combined, concentrated to an aqueous slurry and repeatedly extracted into ethyl acetate. After drying over anhydrous Na_2SO_4, decanting and evaporation, the major factors were obtained as off-white or pale yellow solids. Each factor could be recrystallised from a dichloromethane - methanol mixture. Factor A 0.5 g, factor B 7.9 g and factor C 83.2 g were recovered by filtration and dried under vacuum.

Physico-chemical Properties

UK-63,052 complex and its individual factors are readily soluble in pyridine, dimethyl sulfoxide, and halogenated solvents, slightly soluble in methanol and ethyl acetate, and insoluble in water, hexane and ether. UK-63,052 complex and its individual factors exhibit a yellow fluorescence at 366 nm and give positive reactions to vanillin - sulfuric acid, potassium permanganate, *p*-nitrobenzene diazonium tetrafluoroborate and potassium hexacyanoferrate - ferric chloride spray reagents. Each factor appears as an off-white/pale yellow solid.

The physico-chemical properties are summarised in Table 3.

Fig. 6. UV spectrum of UK-63,052.



Structure Determination

An investigation of the antibiotic literature

Fig. 7. IR spectrum of UK-63,052 in KBr.

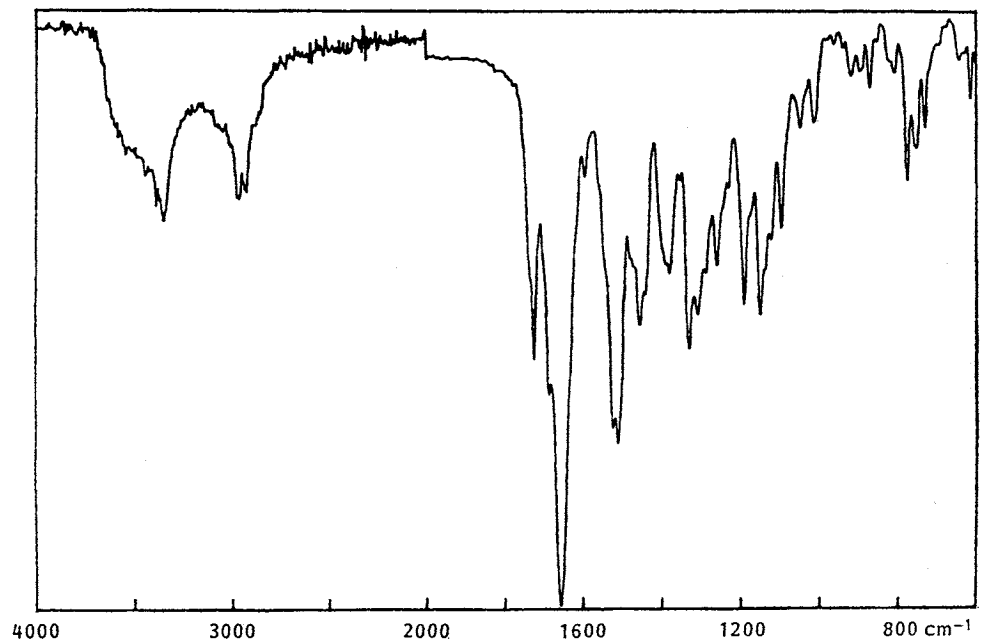
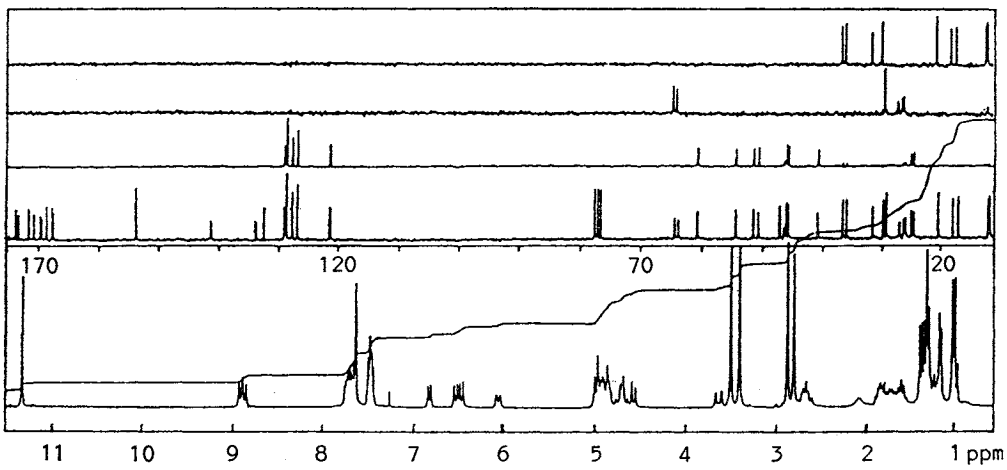


Table 4. ^{13}C NMR (62.9 MHz, $CDCl_3$) chemical shift data for UK-63,052, UK-65,662 and UK-63,598.

Type	UK-63,052	UK-65,662	UK-63,598	Assignment
>C=O	173.62	173.61	173.19	3
	173.18	173.20	172.85	16
	171.56	171.57	171.62	13
	170.64	170.60	170.43	26
	169.60	169.60	169.58	10
	169.37	169.37	169.36	23
	168.55	168.55	168.62	7b, 20b
	168.51	168.52	168.53	
	167.59	167.57	167.70	19
	167.39	167.42	167.36	6
>C=	153.67	153.68	153.68	7d, 20d
	153.67	153.68	153.68	
	141.26	141.26	141.25	
	141.19	141.20	141.20	
	133.71	133.72	133.74	7c, 20c
	133.67	133.69	133.69	
	132.36	132.37	132.38	
	132.36	132.37	132.36	
=CH-	128.96	128.96	128.98	
	128.89	128.89	128.87	
	128.60	128.61	128.62	
	128.60	128.61	128.62	
	127.64	127.63	127.67	
	127.64	127.63	127.61	
	126.73	126.73	126.73	
	126.73	126.73	126.73	
	121.23	121.23	121.23	7e, 20e
	121.13	121.15	121.13	

Type	UK-63,052	UK-65,662	UK-63,598	Assignment
-C-	47.15	47.16	47.18	11, 24
	47.01	47.00	47.00	
-CH-	60.65	60.68	60.48	1
	54.50	54.63	54.33	14
	51.57	51.53	51.60	7, 20
	50.72	50.77	50.65	
	46.37	47.12	51.50	27
	45.95	45.94	46.34	17
	45.65	45.67	45.73	4
	40.74	34.49	—	27b
	24.99	24.98	25.05	24b
	24.59	24.64	24.54	11b
$-CH_2-$	64.30	64.27	64.27	8
	63.72	63.75	63.83	21
	29.25	—	—	27c
	26.97	27.15	26.16	29
	26.23	26.22	26.16	11a
	25.95	25.97	25.97	24a
CH_3-	36.54	36.55	36.52	12a
	35.91	35.95	35.85	25a
	31.48	31.52	31.86	2a
	29.76	29.81	29.71	15a
	20.35	22.78, 22.28	—	27c
	17.76	17.70	17.86	4a
	16.84	16.87	16.59	17a
	—	—	15.22	27b
	11.86	11.85	11.89	24c
	11.73	11.73	11.74	11c
	11.57	—	—	27d

Fig. 8. 250 MHz ^1H and 63 MHz ^{13}C DEPT NMR spectra of UK-63,052 in $CDCl_3$.



revealed that the UV spectrum of UK-63,052 shown in Fig. 6 was identical to that of cinropeptin.[7] It was demonstrated by the Russian workers that the UV spectrum was due to the presence of a 3-hydroxyquinaldic acid residue.[8] However, cinropeptin was shown by the same group to contain only

Fig. 9. 250 MHz ^{1}H NMR spectra of UK-63,052 (top), UK-65,662 (middle) and UK-63,598 (bottom).

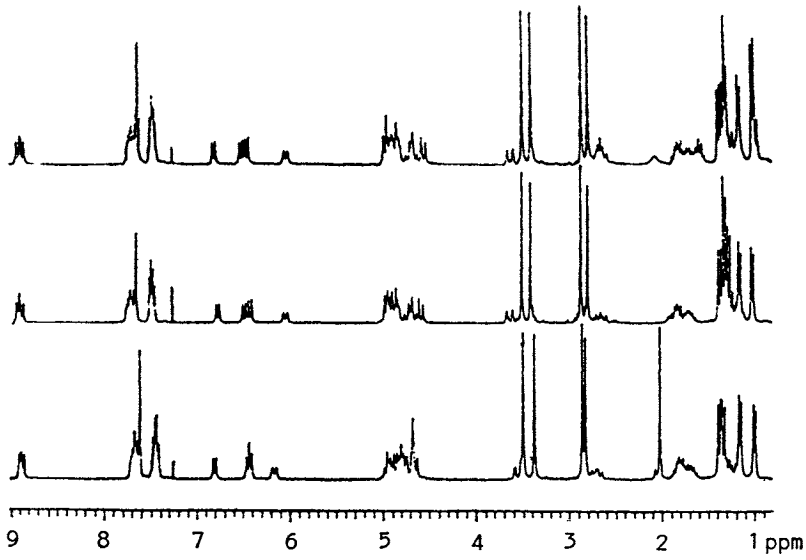


Fig. 10. ^{13}C *J*-resolved spectrum of UK-63,052.

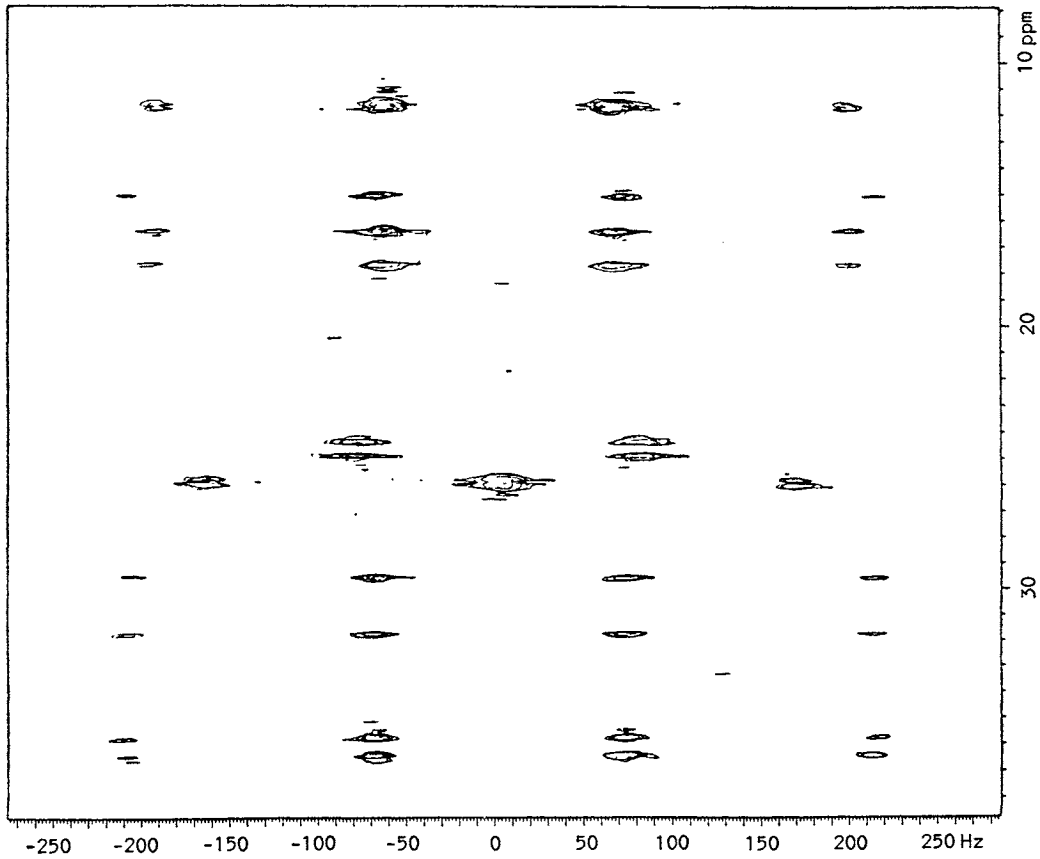


Table 5. Antimicrobial activity of UK-63,052, UK-65,662 and UK-63,598.

Test organism	MIC (μg/ml)		
	UK-63,052	UK-65,662	UK-63,598
Staphylococcus aureus	1.56	<0.20	<0.20
Salmonella choleraesuis	>100	>100	>100
Escherichia coli	>100	>100	50
Pseudomonas aeruginosa	>100	>100	>100
Pasteurella multocida	>100	12.5	1.56
Bordetella bronchiseptica	>100	>100	>100
Moraxella bovis	<0.20	<0.20	<0.20
Erysipelothrix rhusiopathiae	<0.20	<0.20	<0.20

cystine and glycine by amino acid analysis, whereas our studies show that UK-63,052 contains serine and alanine as the only detectable primary amino acids.

According to a recent publication,[9] the 3-hydroxyquinaldic acid group is also present in sandramycin, a non-sulfur containing depsipeptide antibiotic.

The amino acid content of UK-63,052 suggested that it may have a peptolide-type structure as found in the quinomycin family. This similarity was confirmed by comparing the IR spectrum (shown in Fig. 7) with that of quinomycin A. In addition the pale green colouration of the TLC spot (data given in Table 3) after developing with vanillin - sulfuric acid at elevated temperature, was found to be extremely characteristic of the quinomycin family of antibiotics.

The final structures of the three major components, shown in Fig. 3, were obtained by investigation of the ^{1}H and ^{13}C NMR spectra. The DEPT spectra for UK-63,052 (Fig. 8) and ^{13}C peak listing (Table 4) account for all the carbon atoms in the molecule. Comparison of the ^{1}H NMR spectra and ^{13}C peak listings for UK-63,052, UK-65,662 and UK-63,598 are shown in Fig. 9 and Table 4 respectively. In UK-63,052 the $^{1}J_{CH}$ couplings for the two cyclopropyl methine carbon atoms (24b, 11b) are obscured from immediate view by overlap in the high-field region of the proton coupled ^{13}C spectrum. However in the ^{13}C *J*-resolved spectrum, Fig. 10, a coupling of 158 Hz for both carbon atoms is clearly visible, providing final evidence for the presence of the cyclopropyl rings.

Antimicrobial Activity

The MICs of the individual factors of the UK-63,052 complex against a range of microorganisms are shown in Table 5.

Discussion

UK-63,052, UK-65,662 and UK-63,598 are three members of a new antibiotic complex produced by *S. braegensis* subsp. *japonicus*, strain N617-29. The complex belongs to the class of quinomycin antibiotics but has three novel structural characteristics:

1) The quinoxaline rings usually present have been replaced by 3-hydroxy quinaldic acid residues.

2) The usual *N*-methyl valine residue has been replaced by a novel *N*-methylated methylcyclopropane amino acid.

3) The presence of an alkyl substituent on the thioacetal bridge other than the normal methyl substituent.

References

1) Medium No. 196. American Type Culture Collection: Catalogue of strains I. 13th Ed. American Type Culture Collection, Rockville, 1982

2) LEVINE, M. & H. W. SCHOENLEIN: Medium No. 2511. A compilation of Culture Media. Williams & Wilkins Co., Baltimore, 1930
3) JENSEN, H. L.: The genus *Micromonospora Orskov*, a little known group of soil microorganisms. Proc. Linn. Soc. N.S.W. 55: 231~248, 1930
4) BECKER, B.; M. P. LECHEVALIER, R. E. GORDON & H. A. LECHEVALIER: Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole-cell hydrolysates. Appl. Microbiol. 12: 421~423, 1964
5) LECHEVALIER, M. P.: Identification of aerobic actinomycetes of clinical importance. J. Lab. Clin. Med. 71: 934~944, 1968
6) DOLAK, L. A.; F. REUSSER, T. M. CASTLE, B. R. HANNON, A. L. LABORDE & C. K. MARSCHKE (Upjohn): Antibiotic U-64846 prepared from *Streptomyces braegensis* NRRL 12567, active against gram positive bacteria including *Staphylococcus aureus*. U.S. 4,404,190, Sept. 13, 1983
7) FROLOVA, V. I.; M. M. TAIG, A. D. KUZOVKOU & G. C. KATRUKHA: Cinropeptin—a new antibiotic from *Actinoplanes cineraceus*. Antibiotiki 26: 323~327, 1981
8) FROLOVA, V. I.; V. I. SHEYCHENKO, B. V. PROZINOV & A. D. KUSOVKOU: A study of the antibiotic cinropeptin. Structure of the heterocyclic fragment. Antibiotiki 26: 403~407, 1981
9) MATSON, J. A. & J. A. BUSH (Bristol Myers): Antibiotic cyclic depsipeptide with antitumor properties—prepared by fermentation of *Nocardioides* nov. sp. U.S. 621,641, June 18, 1984
10) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterisation of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
11) WAKSMAN, S. A. (*Ed.*): The Actinomycetes. Vol. 2. Classification, Identification and Description of Genera and Species. Williams & Wilkins Co., Baltimore, 1961
12) GORDON, R. E. & M. M. SMITH: Proposed group of characters for the separation of *Streptomyces* and *Nocardia*. J. Bacteriol. 69: 147~150, 1955
13) WAKSMAN, S. A.: Species concept among the actinomycetes with special reference to the genus *Streptomyces*. Bacteriol. Rev. 31: 1~29, 1957
14) GORDON, R. E. & J. M. MIHM: A comparative study of some strains received as *Nocardiae*. J. Bacteriol. 73: 15~27, 1957
15) LECHEVALIER, M. P.: Identification of aerobic actinomycetes of clinical importance. J. Lab. Clin. Med. 71: 934~944, 1968