NOTES

ANTIBIOTIC Bu-2545, A NEW MEMBER OF THE CELESTICETIN-LINCOMYCIN CLASS

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In the course of screening for new antibiotics active against anaerobic bacteria, a *Streptomyces* strain No. H230-5 that had been isolated from a soil sample collected in England was found to produce a new antibiotic. This agent, designated as Bu-2545, was active against anaerobic organisms as well as aerobic Gram-positive bacteria. Antibiotic Bu-2545 is structurally related to celesticetin[1] and lincomycin[2], having structural units in common with these two antibiotics. This paper describes the producing organism, and the isolation, properties and structure of Bu-2545.

Streptomyces strain No. H230-5 forms white or yellowish white aerial mycelia and straight or flexuous spore chains. The spores are oval or cylindrical in shape and have a smooth surface. It does not produce melanin or a non-melanoid pigment. Strain H230-5 utilizes D-xylose, D-glucose, D-fructose, D-galactose, inositol and D-mannitol, but not L-arabinose, L-rhamnose, raffinose or sucrose. According to the descriptions in BERGEY's Manual (8th ed., 1974), strain H230-5 should be placed in the species group, *rectus flexibilis*, white series, non-chromogenic and smooth spore surface, which includes seven species. Among the seven species, strain H230-5 is considered to be most similar to *Streptomyces aureocirculatus*, a species first reported by KRASILNIKOV and YUAN[3] and further described by SHIRLING and GOTTLIEB[4].

Antibiotic Bu-2545 was produced in 500-ml Erlenmeyer flasks using a medium composed of 2% glycerol, 0.5% beet molasses, 0.5% peptone, 0.5% linseed meal and 0.5% $CaCO_3$. The flasks were shaken on a rotary shaker (250 rpm) at 27°C for 4 days. The antibiotic activity in the fermentation broth was determined by a paper disc-agar plate method using *Bacillus subtilis* PCI 219 as the test organism.

The harvested fermentation broth was filtered and adjusted to pH 7.0. The antibiotic activity in the filtrate was adsorbed on Diaion HP-20 and eluted with 80% acetone. The active eluate was evaporated under reduced pressure, and the resultant aqueous concentrate was extracted with *n*-butanol at pH 10. The active butanol extract was added to acidic water at pH 2.0. The antibiotic agent was then back-extracted into methylene chloride at pH 10. Evaporation of the solvent gave a crude solid of Bu-2545 which was purified by silica gel column chromatography developed with a mixture of benzene - methanol (6: 1). Active fractions were combined, concentrated *in vacuo* and finally lyophilyzed to afford a white powder of Bu-2545 as the free base.

Bu-2545 is a basic substance with a pKa′ of 8.1 in water. It is soluble in lower alcohols, ethyl acetate, methylene chloride and acidic water but insoluble in *n*-hexane and alkaline water. It is optically active: $[\alpha]_D^{24}+140°$ (*c* 0.5, $CHCl_3$). The molecular weight of Bu-2545 (free base) as determined by in-beam EI mass spectroscopy was 378 (M+1: *m/e* 379). The oxalate of Bu-2545 was obtained as colorless crystals (m.p. 201~202°C) which was analyzed as $C_{16}H_{30}N_2O_6S\cdot(COOH)_2$. Calc'd: C 46.16, H 6.88, N 5.98, S 6.88. Found: C 46.30, H 7.23, N 5.98, S 6.33. The UV spectrum of Bu-2545 did not show a maximum between 210 and 360 nm, and the IR spectrum included absorptions of amide bands at 1660 and 1530 cm^{-1}, and an NH and/or OH band at around 3300 cm^{-1}. The NMR spectrum (60 MHz, D_2O) of the sulfate showed signals assignable to >CH–$\underline{CH_3}$ (δ 1.17, 3H, d, J=6), S–CH_3 (δ 2.16, 3H, s), N–CH_3 (δ 2.95, 3H, s) and O–CH_3 (δ 3.40, 3H, s), and indicated the presence of an anomeric proton (δ 5.33, 1H, d, J=5.5).

The analytical and spectroscopic data of Bu-2545 described above suggested that the structure of Bu-2545 should be closely related to that of lincomycin[2], except for the presence of an O–CH_3 group in Bu-2545 and an *n*-propyl group in lincomycin. The mass spectrum of Bu-2545

Table 1. TLC comparison of Bu-2545, lincomycin and celesticetin.

	System I	System II
Bu-2545	0.15	0.38
Lincomycin	0.36	0.55
Celesticetin	0.36	0.40

System I: Silica gel, BuOH - HOAc - H_2O (3 : 1 : 1)
System II: Silica gel, Benzene - MeOH (1 : 1)

showed diagnostic fragment ion peaks at *m/e* 331, 229 and 84 whose assignments are shown in Fig. 1. These fragment ions were also observed in the mass spectra of celesticetins[5]. Thus the structure shown in Fig. 1 was assigned to Bu-2545. The major structural skeleton of the Bu-2545 molecule (non-substituted hygric acid moiety and celestosamine) is the same as that of celesticetin, while Bu-2545 has the same methylthio substituent as lincomycin at the C-1 position. Bu-2545 was differentiated from lincomycin or celesticetin by two TLC systems as shown in Table 1.

Fig. 1. Structure of Bu-2545 and its fragment ions in mass spectrum.

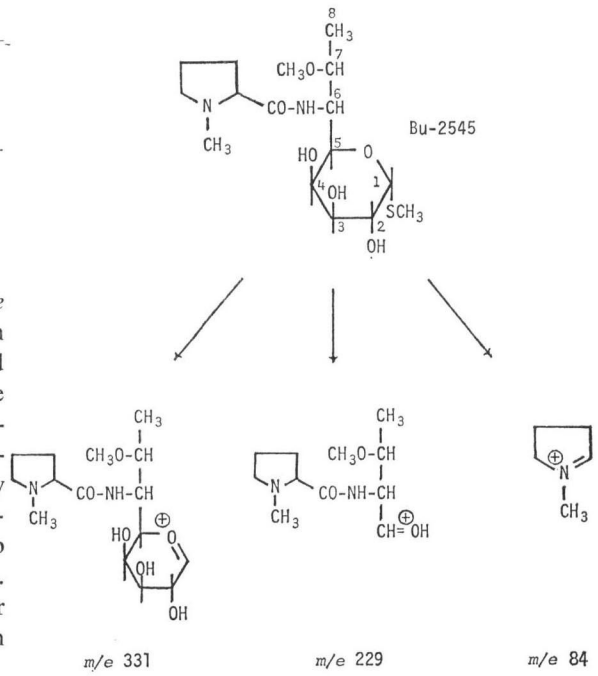

Table 2. Antibacterial activity of Bu-2545.

Test organism		Test medium*	MIC (mcg/ml)	
			Bu-2545	Lincomycin
Anaerobes	*Bacteroides fragilis* A20926	GAM	6.3	1.6
	〃 〃 A20928-1	〃	1.6	0.8
	Fusobacterium varium ATCC 8501	〃	12.5	0.8
	Veillonella parvula ATCC 17745	〃	3.1	0.4
	Clostridium chauvoei A9651	〃	12.5	0.4
	Clostridium perfringens A9635	〃	12.5	0.2
	Propionibacterium acnes A21933	〃	12.5	3.1
	〃 〃 A21953	〃	12.5	3.1
	Peptostreptococcus anaerobius B-43	〃	12.5	3.1
	Peptococcus aerogenes ATCC 14963	〃	12.5	3.1
Aerobes	*Staphylococcus aureus* Smith	NA	6.3	0.4
	〃 〃 A20607**	〃	>100	>100
	Streptococcus pyogenes Dick	GC	12.5	0.05
	Streptococcus pneumoniae Type I	〃	12.5	0.1
	Hemophilus influenzae A9729	〃	25	0.05
	Escherichia coli NIHJ	NA	>100	>100
	Klebsiella pneumoniae D11	〃	>100	>100
	Pseudomonas aeruginosa D15	〃	>100	>100

* GAM: Gifu anaerobe medium (Nissui), NA: Nutrient agar (Eiken), GC: GC medium (Eiken).
** Macrolide-lincomycin resistant strain

The antibacterial activity of Bu-2545 was determined by a two-fold agar dilution method against a variety of aerobic and anaerobic test organisms. As shown in Table 2, Bu-2545 inhibited growth of aerobic Gram-positive bacteria and various anaerobic microorganisms at concentrations generally below 12.5 mcg/ml. The aerobic Gram-negative bacteria tested were not inhibited at 100 mcg/ml. Lincomycin was tested comparatively as a reference agent and showed much greater antimicrobial potency than Bu-2545.

Bu-2545 was tested *in vivo* against experimental infections of *Staphylococcus aureus* Smith and *Clostridium perfringens* A9635. Mice were challenged intraperitoneally with a lethal dose of the pathogens in a 5% suspension of hog gastric mucin. The single intramuscular (im) PD_{50} or protective dose, 50%, found for Bu-2545 was 50 mg/kg for *S. aureus* and 72 mg/kg for *C. perfringens*. Bu-2545 was non-toxic to mice at 400 mg/kg (im).

A number of celesticetin analogs[5~8] have been isolated from the fermentation broth of *S. caelestis* or its mutants, but none has an S-CH_3 substituent at the C-1 position of celestosamine. Although the complete stereochemistry of Bu-2545 has not been elucidated, the structure of Bu-2545 shown in Fig. 1 seems most likely from biosynthetic considerations and also from its optical rotation value (Bu-2545: +140°, lincomycin: +158°[2], celesticetin B: +146°[5]). Further evidence to support the assigned structure of Bu-2545 was recently obtained by C-13 NMR and degradation studies. These findings will be reported in a separate paper[9].

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