NOTES

BOSEIMYCIN—A NEW STREPTOTHRICIN-LIKE ANTIBIOTIC

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During an antibiotic screening programme, a new biologically active complex was isolated from an unidentified *Streptomyces*, strain Ac_6 569[1], which was obtained from a garden soil in Calcutta, India. This antibiotic complex was found to contain two water-soluble active fractions of which the major one was designated as boseimycin. This commuication describes the isolation, purification and characterization of boseimycin. It was found to have a close relationship to the streptothricin group of antibiotics but was differentiated from them on the basis of its physico-chemical and microbiological properties.

Production and Isolation

The medium used for antibiotic production was composed of : 0.6 % soya peptone, 0.2 % yeast extract, 0.4 % KCl, 0.5 % $(NH_4)_2SO_4$, 0.04 % KH_2PO_4, 0.05 % $CaCO_3$ and 2 % glucose. The pH was adjusted to 7.2 before sterilisation. The fermentation was carried out under agitation and the maximum antibiotic titre was reached at the end of 72 hours of incubation.

Boseimycin was isolated following either of two method. (1) The fermented broth was decanted off the solid mycelia. The culture filtrate was adjusted to pH 2 with hydrochloric acid and stirred with Darco G 60 (0.5 % w/v). The clarified filtrate was treated again with Darco G60 (2.5 % w/v) after bringing the pH to 6.8 and filtered. The carbon cake was washed twice with aqueous methanol (60 % v/v), and the active principles were eluted with a mixture of methanol and 0.01 N HCl (6 : 4, v/v). (2) The harvested broth was passed through a column of Amberlite IRC-50 in the Na^+ cycle. The column was thoroughly washed with deionized water and the antibiotic complex was eluted from the column by 0.1 N HCl.

The active eluates were neutralized over Amberlite IRA-400 in the OH^- cycle and were concentrated to a small volume under reduced pressure at 35°C. Boseimycin complex was precipitated as the hydrochloride in acetone after the pH was adjusted to 6.8.

A separation of boseimycin from the antibiotic complex was achieved over a column of Amberlite IRC-50 (Na^+), 0.01 N ammonium hydroxide being used as the eluting agent. Further purification of boseimycin was achieved over a column of Darco G60 and Celite 545 (1 : 1) with 1 % aqueous acetone as the developing agent. Active fractions were concentrated and lyophilised yielding boseimycin hydrochloride as a white powder.

The homogeneity of boseimycin was esta-

Table 1. Descending paper chromatography of boseimycin (development: 18 hours)

Solvent systems	Boseimycin (Rf)
1. *n*-Propanol, pyridine, acetic acid, water (15 : 10 : 3 : 12)	0.14
2. Pyridine, acetic acid, water (50 : 35 : 15)	0.25
3. *n*-Butanol saturated with water containing 0.25 % *p*-toluene sulphonic acid	0.02
4. *n*-Butanol saturated with water cantaining 2 % *p*-toluene sulphonic acid	0.08
5. *n*-Butanol, acetic acid, water (2 : 1 : 1)	0.07

Table 2. A comparative study by thin-layer chromatography (Silica gel G, 3-hour run)

Solvent systems	Rf values		
	Boseimycin	Streptothricin	Yazumycin
1. *n*-Propanol, pyridine, acetic acid, water (15 : 10 : 3 : 12)	0.75	0.72	0.64
2. Pyridine, acetic acid, water (50 : 35 : 15)	0.68	0.85	0.90
3. *n*-Butanol, acetic acid, water (2 : 1 : 1)	0.09	0.12	0.20

Fig. 1. Infrared absorption spectrum of boseimycin hydrochloride in 1.5 % KCl pellet.

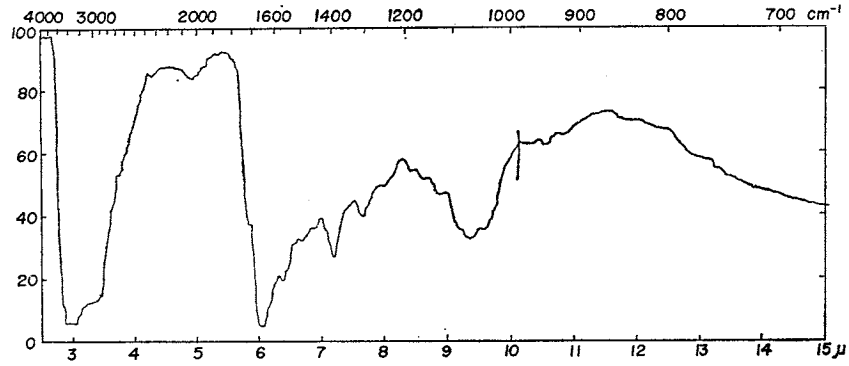
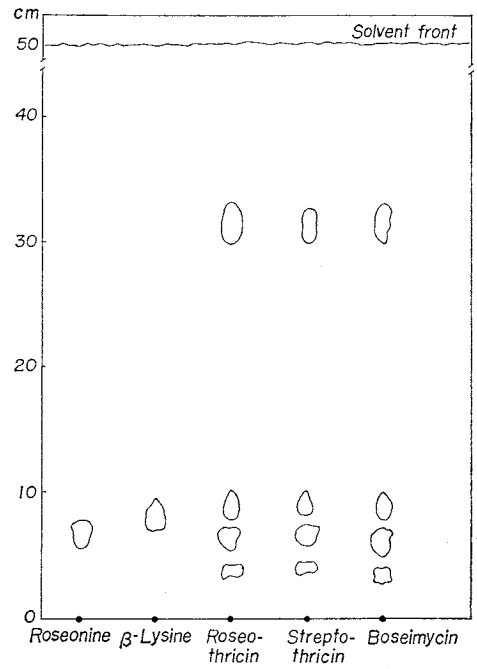


Fig. 2. Descending paper chromatography of acid hydrolysates (5.7 N HCl at 110°C for 12 hours) of streptothricin, roseothricin A and boseimycin compared with streptolidine and β-lysine in Whatman No. 1.
Solvent systems: *n*-butanol, acetic acid and water (3 : 1 : 1)
Detection: ninhydrin



blished by counter-current distribution in a solvent system of *n*-butanol and water (1 : 1) containing 2 % *p*-toluenesulphonic acid as the carrier and also by paper chromatography, thin-layer chromatography (Tables 1 and 2) and paper electrophoretic studies. In paper electrophoresis, boseimycin hydrochloride moves 2.4 cm in pH 3.9 (0.1 M acetate buffer) and 3.8 cm in pH 7.2 (0.1 M phosphate buffer) towards the cathode in a 2 hour run. Consistent microanalytical results of different derivatives of boseimycin established the purity of the product. Further purification did not either change the analytical results or the biological activity.

Table 3. Antimicrobial spectrum of boseimycin (Serial dilution method)

Test organisms	Minimal inhibitory concentration (μg/ml)
Staphylococcus aureus	0.03
Bacillus subtilis PCI 219	0.1
Escherichia coli K12	1.0
Diplococcus pneumoniae	10.0
Salmonella typhimurium	3.0
Proteus vulgaris	3.0
Klebsiella pneumoniae	3.0
Pseudomonas aeruginosa	30.0
Candida albicans ATCC 10257	0.3
Cryptococcus neoformans	0.3
Trycophyton mentagrophytes	10.0
Epidermophyton floccusum	3.0
Microsporum gypseum	>30.0
Nocardia asteroides	>30.0
Aspergillus fumigatus	>30.0

Properties

Boseimycin hydrochloride is readily soluble in water, partially in methanol, almost insoluble in ethanol, acetone, diethyl ether, butanol, chloroform, petroleum ether and benzene. It is stable at room temperature for more than three months in the dry powdery state. The antibiotic is fairly stable at acidic pH but very little activity is retained when it is treated with alkali at pH 10 for 6 hours at room temperature. An aqueous solution of the antibiotic loses 60 % of its antimicrobial activity when it is

Table 4. Comparison of boseimycin with other known strepthricin-like antibiotics

Antibiotics	Color reactions					Acid hydrolysed products			Remarks
	SAKAGUCHI	Ninhydrin	FEHLING	MOLISCH	Biuret	β-Lysine	Streptolidine	Other amino acids	
Boseimycin[1]	+	+	−	+	+	+	+		
Complex A 4788[3]	−	+	+	+	+	+	+		
BD-12[4]	−	+	?		?	−	−		Two unidentified products
BY-81[4]	−	+			−	−	−		Two unidentified products
Citromycin[5]	−	− (in water) + (in pyridine)	−		−	+			
E-749C	−	+ (weak)	−			−	+	glycine	
Geomycin	+ (weak)	+	+	+	+ (weak)	+	+	gly, ala, asp, glu, threo, ser	
LL AB 664[8]							N-methyl	glycine	
LL AC 541[9]	−	−	+	−	+	−	+	glycine	
Mycothricin[10]	−	+	−	−	+	+	+	serine	
Pleocidin[11]									Differs in biological activity
Racemomycin-O[12]	−	+	−		−	+	+		
Roseothricin A[13]	−	+	+	−	+	+	+		
SF-701[14]	−	+	+	−	−	−	+	N-methyl gly	
Streptolin[15]	−	+			+	+	+		
Streptothricin[16]	−	+	+	−	+	+	+		
Viomycin[17]	+	+	+	−	+	+	−	Ser, α,β diamino propionic acid	
Yazumycin[18]	+	+	+		+ (weak)				

(+) Positive (−) Negative (?) Doubtful

placed on a boiling water bath for three minutes.

Boseimycin is optically inactive (*c* 1, water). It shows only end absorption in the ultraviolet spectrum. The IR absorption spectrum in 1.5 % KCl is presented in Fig. 1. It has characteristic absorption at the following frequencies: 3320, 3260, 3100, 2960, 2080, 1713, 1653, 1560, 1456, 1395, 1310 and 975 cm^{-1}. The bands at 1713 and 1653 cm^{-1} are probably attributable to the presence of carbonyl and guanidino groups in the antibiotic molecule. The antibiotic is positive to MOLISCH, SAKAGUCHI (strong), ninhydrin, ELSON-MORGAN, anthrone, biuret and EHRLICH (weak) tests, but negative to FEHLING, BENEDICT, maltol, TOLLENS, PAULY, SCHIFF, ferric chloride, neutral potassium permanganate and MILLON tests.

Boseimycin is a tetraacidic base with pK_a' 7.50, 8.35, 9.85 and >11.0. An equivalent weight 146 was obtained when boseimycin hydrochloride was titrated with perchloric acid in glacial acetic acid[2]. The molecular weight was calculated as 584 and the corresponding hydrochloride as 730. Elemental analysis and molecular weight determination indicate the molecular formula for bosei-

mycin hydrochloride as $C_{24}H_{46}N_9O_8 \cdot 4HCl$, m.p. 216~218°C (decomp.).

Calcd. for $C_{24}H_{46}N_9O_8 \cdot 4HCl$:
C 39.23, H 6.81, N 17.20, Cl 19.40.
Found:
C 39.24, H 7.25, N 18.28, Cl 19.81.

Calcd. for $C_{24}H_{46}N_9O_8 \cdot 2H_2SO_4$:
C 36.73, H 6.37, N 16.10, S 8.32.
Found:
C 36.23, H 6.91, N 16.48, S 8.04.
m.p. 255~260°C (decomp.)

Calcd. for boseimycin picrate, $C_{24}H_{46}N_9O_8 \cdot 4C_6H_3N_3O_7$:
C 38.30, H 3.86, N 19.35.
Found:
C 38.88, H 4.62, N 19.47.
m.p. 174~176°C (decomp.)

Paper chromatographic patterns of acid hydrolysates of boseimycin and the known streptothricins are shown in Fig. 2. The hydrolysate of boseimycin corresponds to the spots of β-lysine and roseonine (streptolidine), two main constituents of streptothricins.

Antimicrobial activities of boseimycin are summarised in Table 3. The antibiotic is mainly active against gram-positive and gram-negative bacteria and highly active against *Candida albicans* (MIC 0.3 μg/ml). Fungi are in general less sensitive to boseimycin than bacteria. It does not exhibit any cross-resistance relationship with streptomycin and streptothricin (unpublished data). The rate of formation of resistance *in vitro* against *Staphylococcus aureus* and *Escherichia coli* with boseimycin is much slower than that of streptomycin and streptothricin. This work will be reported elsewhere in detail.

The toxicity of boseimycin expressed as LD_{50} was 63 mg/kg (intravenously) in mice. However, in keeping with the similarity to streptothricin, delayed toxic symptoms began to appear after 3 days at 69 mg/kg, 4 days at 63 to 40 mg/kg, 5 days at 33 mg/kg, 6 days at 28 mg/kg and 10 days at 24 mg/kg.

Discussion

Boseimycin is a water-soluble, basic antibiotic. Its characteristic IR and UV spectra, the presence of β-lysine and streptolidine in acid hydrolyzed products and its delayed toxicity, identify boseimycin as a streptothricin-streptolin group of antibiotic. As a rule, the antibiotics of this group have been obtained as water-soluble substances with the characteristics in common that they produce amorphous hydrochlorides and sulphates, show end adsorption in UV region and in general they are highly toxic. They have been further characterised by qualitative color reactions, being negative to maltol and SAKAGUCHI but both boseimycin and yazumycin are positive to SAKAGUCHI. Yazumycin contains sulfur in the molecule and is further differentiated from boseimycin in thin-layer chromatographic study on silica gel plates (Table 2). A comparative study of boseimycin with other known streptothricin-like antibiotics was carried out (Table 4). Lack in optical rotation in boseimycin molecule is an unique property for which no explanation has yet been obtained.

Although, the acid hydrolyzed products of boseimycin correspond with those of several streptothricin type of antibiotics, particularly of roseothricin A, racemomycin-O, streptothricin, streptolin, a comparison of their physico-chemical as well as biological properties establish boseimycin as a new addition to streptothricin group of antibiotic.

Summary

Boseimycin was isolated from an unidentified strain of *Streptomyces* and identified as a streptothricin-like antibiotic. It was isolated by adsorption on Darco-G60 or by column chromatography on Amberlite IRC-50 (Na^+) and purified over a column of Darco G60 and Celite 545. It is active against gram-positive and gram-negative bacteria and also against fungi and yeast.

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