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SHORT COMMUNICATION

Some properties of bronopol, a new antimicrobial agent active against *Pseudomonas aeruginosa*

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MARKED antimicrobial activity was noted in a series of aliphatic halogeno-nitro compounds (unpublished observations of Clark, Croshaw, Leggetter & Spooner). Of these, 2-bromo-2-nitropropane-1,3-diol (bronopol) has been further investigated because it is more stable in aqueous media than other members of the series.

This preliminary communication describes some properties of bronopol.

EXPERIMENTAL AND RESULTS

Physical properties. Bronopol is a colourless, odourless, crystalline solid m.p. 121°. It is slightly hygroscopic, readily soluble in water, lower alcohols and glycols but only slightly soluble in oils; the distribution coefficient water: chloroform is 14·7: 1 at 22–24°. The pH of an aqueous solution varies between 5·1 and 5·5 according to the concentration and falls slowly on storage, the rate of fall increasing with increased temperature.

Antimicrobial activity. The inhibitory activity of bronopol (Table 1) was determined *in vitro* by serial dilution in agar and surface-inoculation using a multi-point inoculator (Hale & Inkley, 1964). The inoculum was 0·01 ml of 18 hr broth cultures of the test bacteria or yeasts, or 0·01 ml of spore suspensions prepared from 7 day cultures of the fungi. The minimum inhibitory concentrations were noted after 24 hr at 37° for the bacteria, 48 hr at 26° for the yeasts and 120 hr at 26° for the fungi.

Bronopol is more active against bacteria than against fungi or yeasts; all bacteria tested, including *Pseudomonas aeruginosa*, were inhibited at 12·5–50 μg/ml. A comparison of the bacteriostatic activity of bronopol and other antibacterial agents against some strains of *Ps. aeruginosa* is shown in Table 2. This inhibitory activity falls two- to eight-times when the pH increases from pH 5·3 to pH 7 or 8. The inhibitory activity is decreased three to four-times in the presence of 75% ox serum or 0·1% cysteine hydrochloride, whilst 75% oxalated horse blood reduces the activity at least thirty-two-fold. Tween 80 1%, suramin 1%, Lubrol W 1% or lecithin 0·1% had no effect on activity.

The bactericidal activity of bronopol was determined using bacterial suspensions prepared by washing off the organisms from an 18 hr agar slope culture and standardising by opacity to approximately 1×10^6 organisms per ml. 1 ml of such a suspension was added to 9 ml of aqueous solution of the compound at 22° and plate counts were made on samples after 15, 30 and 60 min intervals. Bactericidal activity is greater

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against Gram-negative bacteria than against Gram-positive cocci (Table 3), and it shows little variation over a pH range of 5–8. The addition of 50% ox serum has little effect on the bactericidal activity and solutions containing 1·6 mg/ml of bronopol in 50% blood gave at least a 90% reduction in the number of viable cells of *Ps. aeruginosa* (10S) and *Salmonella typhosa* (TY2) in 1 hr.

Twelve daily passages *in vitro* in liquid medium in the presence of bronopol of *Ps. aeruginosa* and *Staphylococcus aureus* have not increased the resistance of these organisms to the compound.

TABLE 1. THE INHIBITORY ACTIVITY OF BRONOPOL IN AGAR*

Organisms	No of strains tested	MIC μg/ml†
Gram-positive bacteria		
Staphyloccus aureus‡	30	12.5–50
Streptococcus pyogenes	2	25
Corynebacterium pyogenes	4	25–50
Bacillus subtilis	1	25
Gram-negative bacteria		
Pseudomonas aeruginosa (see Table 2)	22	25–50
Proteus vulgaris	17	12·5–25
P. rettgeri	1	25
P. morganii	1	25
P. inconstans	1	25
P. mirabilis	3	25–50
Escherichia coli	13	12·5–50
Aerobacter aerogenes	1	25
Salmonella typhosa	2	25
Salm. typhimurium	3	25
Salm. gallinarum	2	25
Salm. enteritidis	2	25
Salm. ser. *dublin*	1	25
Salm. ser. *heidelberg*	2	25
Shigella sonnei	2	25
Klebsiella pneumoniae	2	25
Fungi and yeasts		
Trichophyton mentagrophytes	1	200
T. rubrum	1	100–200
T. tonsurans	1	100
Microsporum canis	1	100–200
Cladosporium herbarum	1	400
Penicillium roqueforti	1	400
Candida albicans	3	400–>400

* "Oxoid" blood agar base (CM 55) for the bacteria (with the addition of 2·5% glucose and 10% ox serum for *Str.* and *Corynebacterium pyogenes*) and "Oxoid" Sabouraud dextrose agar (CM 41) for the fungi and yeasts.
† MIC after 24 hr at 37° for bacteria, 48 hr at 26° for yeasts and 120 hr at 26° for fungi.
‡ Benzylpenicillin-resistant and -sensitive organisms tested.

TABLE 2. THE BACTERIOSTATIC ACTIVITY OF BRONOPOL AND OTHER ANTIBACTERIAL AGENTS AGAINST 22 STRAINS OF *Pseudomonas aeruginosa*

Compound	No of strains with MIC* (μg/ml)			
	25	50	100	>100
Bronopol	14	8		
Chlorhexidine digluconate B.P.	0	0	2	20
Hexachlorophane B.P.	0	9	13	
Cetrimide B.P.	0	0	0	22
Domiphen bromide B.P.	0	0	0	22
Phenoxyethanol B.P.C.	0	0	0	22
Dequalinium chloride B.P.	0	0	0	22

* Serial dilution in agar with surface inoculation incubated for 24 hr at 37°.

Using *Ps. aeruginosa* as test organism, preparations of the compound were assayed by agar diffusion at pH 5·3 (the zones of inhibition were greater at this pH). There is a marked loss of microbiological activity in the presence of cetomacrogol B.P.C., pure propylene glycol or polyethylene glycol 300 although 20% aqueous dilutions of the two glycols appear to have no effect on the compound. Unbuffered aqueous solutions are relatively stable at temperatures up to 50° but solutions buffered at pH > 5 are less stable. Concentrated solutions buffered at pH > 5 turn brown when exposed to heat or light; this discolouration, which can be prevented by the addition of sodium metabisulphite, is not always associated with loss of microbiological activity.

TABLE 3. THE BACTERICIDAL ACTIVITY OF BRONOPOL IN AQUEOUS SOLUTION AGAINST THREE SPECIES OF BACTERIA

Concentration of bronopol mg/ml	% reduction* in number of viable organisms at time in min (Temp. 22°)								
	Ps. aeruginosa (10S)			*Salm. typhosa* (TY2)			*Staph. aureus* (NCTC 8452)		
	15	30	60	15	30	60	15	30	60
1·6	99·9	>99·9	>99·9	99·9	>99·9	>99·9	90	92	99
0·8	99	99·9	99·9	99	99·9	99·9	30	51	80
0·4	40	98	99·9	50	99	99·9	<10	<10	40

* Compared with a water control tube with initial inoculum level of approx. 10^6 organisms per ml.

Toxicity. In aqueous solution the acute LD50 of bronopol (based on small numbers of animals and estimated graphically) to mice is 350 mg/kg orally and 20 mg/kg intraperitoneally; to rats the figure is 400 mg/kg orally and 200 mg/kg subcutaneously.

Male and female albino rats (5–6 weeks old) were fed concentrations of 100 and 1,000 p.p.m. in the diet for twelve weeks, so that the average daily doses ingested were roughly equivalent to one-fortieth and one-quarter of the LD50 respectively; control rats were fed plain diet. Both dose levels were tolerated by the animals without any effect on growth, food consumption, blood picture, liver and kidney weights or histopathology of major organs.

Concentrations of 0·5 and 2·0% in an emulsion base and in solution were tested in rabbits for irritation to skin and to the mucous membrane of the eye. The 2·0% concentration was irritant to skin and to the eye after one application while there was no observable difference in the local reaction between the 0·5% concentration and the vehicle alone after application on four successive days.

Guinea-pigs were injected intradermally with 0·05% aqueous solution on alternate days for a total of ten doses (one of 0·1 ml followed by nine of 0·05 ml). This was followed two weeks later by injection of a challenging dose of 0·05 ml; no evidence of skin sensitisation was observed.

DISCUSSION

Unlike many antibacterial agents, bronopol has activity against *Pseudomonas aeruginosa* as well as against other Gram-negative and Gram-positive bacteria and to a lesser extent against fungi.

Its wide spectrum of activity makes it suitable as a preservative, and its relatively low toxicity on chronic oral administration to rats suggests that it may be safe for use in the preservation of oral medicaments.

The absence of local irritancy to the skin and mucous membranes of experimental animals at a concentration of 0·5%, together with the fact that lower concentrations (0·1 or 0·2%) are bacteriostatic and bactericidal, also suggests that bronopol could be used as a topical antibacterial agent. It shows optimum antibacterial activity and optimum stability in aqueous vehicles at a slightly acid pH; such properties are desirable in a topical skin formulation.

Reference

Hale, L. J. & Inkley, G. W. (1964). *Lab. Pract.*, in the press.

The paper was presented by MR. GROVES.