ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1975, p. 510-512

Vol. 8, No. 4
Printed in U.S.A.

NOTES

Triethyl-*n*-Hexylammonium Triethyl-*n*-Hexylboride: a New Antimicrobial Showing Activity Against *Candida albicans* and Gram-Positive Bacteria

K. S. ROSENTHAL, D. R. STORM,* AND W. T. FORD

Departments of Biochemistry and Chemistry, School of Chemical Sciences, University of Illinois, Urbana, Illinois 61801*

Received for publication 17 June 1975

The organic salt triethyl-*n*-hexylammonium triethyl-*n*-hexylboride ($N_{2226}B_{2226}$) has biostatic effects against two gram-positive bacteria, *Bacillus subtilis* and *Micrococcus luteus*, and the yeast *Candida albicans*. *Escherichia coli* and chicken embryo fibroblasts grown in tissue culture are more refractory to this compound.

Triethyl-*n*-hexylammonium triethyl-*n*-hexylboride ($N_{2226}B_{2226}$) is one of several tetraalkylammonium tetralkylborides which are liquid salts at room temperature (1). It is miscible in all proportions with most organic solvents but is sparingly soluble in water. The salt can, however, be dispersed into aqueous solutions with gentle physical agitation. $N_{2226}B_{2226}$ is stable to light, heat, and water; however, several days of exposure to air results in some oxidative degradation. The resulting yellow color is thought to be due to trace impurities and does not effect the antimicrobial activity of this compound. The antimicrobial activity of this salt was examined because it is a unique amphipathic molecule which might have a disruptive effect on the structure of biological membranes. The structure of $N_{2226}B_{2226}$ is as follows:

$$(CH_3CH_2)_3\text{—}\overset{+}{N}\text{—}CH_2\text{—}(CH_2)_4\text{—}CH_3$$

$$(CH_3CH_2)_3\text{—}\overset{-}{B}\text{—}CH_2\text{—}(CH_2)_4\text{—}CH_3$$

The anion and cation of this salt pair are isoelectronic with respect to each other, and both ions have a hydrophobic hydrocarbon side chain.

The microorganisms tested with $N_{2226}B_{2226}$ were *Bacillus subtilis*, *Micrococcus luteus*, and *Escherichia coli*, as well as the yeast *Candida albicans*. Varying concentrations of $N_{2226}B_{2226}$ (0.83 μg/ml) were added to 10 ml of nutrient medium (1% meat peptone, 0.5% NaCl, and 0.1% yeast extract, pH 7.0) in 25-ml Erlenmeyer flasks. The flasks were inoculated with 0.1 ml of each microorganism grown to maximum density in nutrient medium. The samples were then incubated at 37 C with shaking, and growth was monitored after 6 and 12 h using a Klett spectrophotometer.

The effects of $N_{2226}B_{2226}$ on the growth of chicken embryo fibroblasts were determined by adding $N_{2226}B_{2226}$ at varying concentrations (0.75 to 750 μg/ml) to freshly plated secondary cultures of chicken embryo fibroblasts. These cells were grown in a medium containing Delbecco modified Eagle medium supplemented with 4% calf serum, 1% chicken serum, and 10% tryptose phosphate broth. Growth was monitored by cell density, and cell morphology was examined using a phase microscope at 200× magnification. Untreated cells grew to confluency in 3 to 4 days (Fig. 1A).

$N_{2226}B_{2226}$ inhibited the growth of the bacteria tested as well as *C. albicans* (Table 1). *B. subtilis* was particularly susceptible, and growth was completely inhibited at 8.3 μg/ml. The minimal inhibitory concentration of $N_{2226}B_{2226}$ (2×10^{-5} M) for *B. subtilis* is 3 to 4 orders of magnitude less than the minimal inhibitory concentrations observed with common nonionic detergents such as Triton X-100 (4). *M. luteus* and *C. albicans* required concentrations of $N_{2226}B_{2226}$ 10-fold higher for inhibition of growth, whereas growth of *E. coli* and chicken embryo fibroblasts was inhibited at 620 and 750 μg/ml, respectively.

Although growth of these bacterial strains was completely inhibited by $N_{2226}B_{2226}$ for a period of 6 to 8 h after inoculation, growth resumed after 12 to 20 h, but at a low level relative to untreated controls. These results

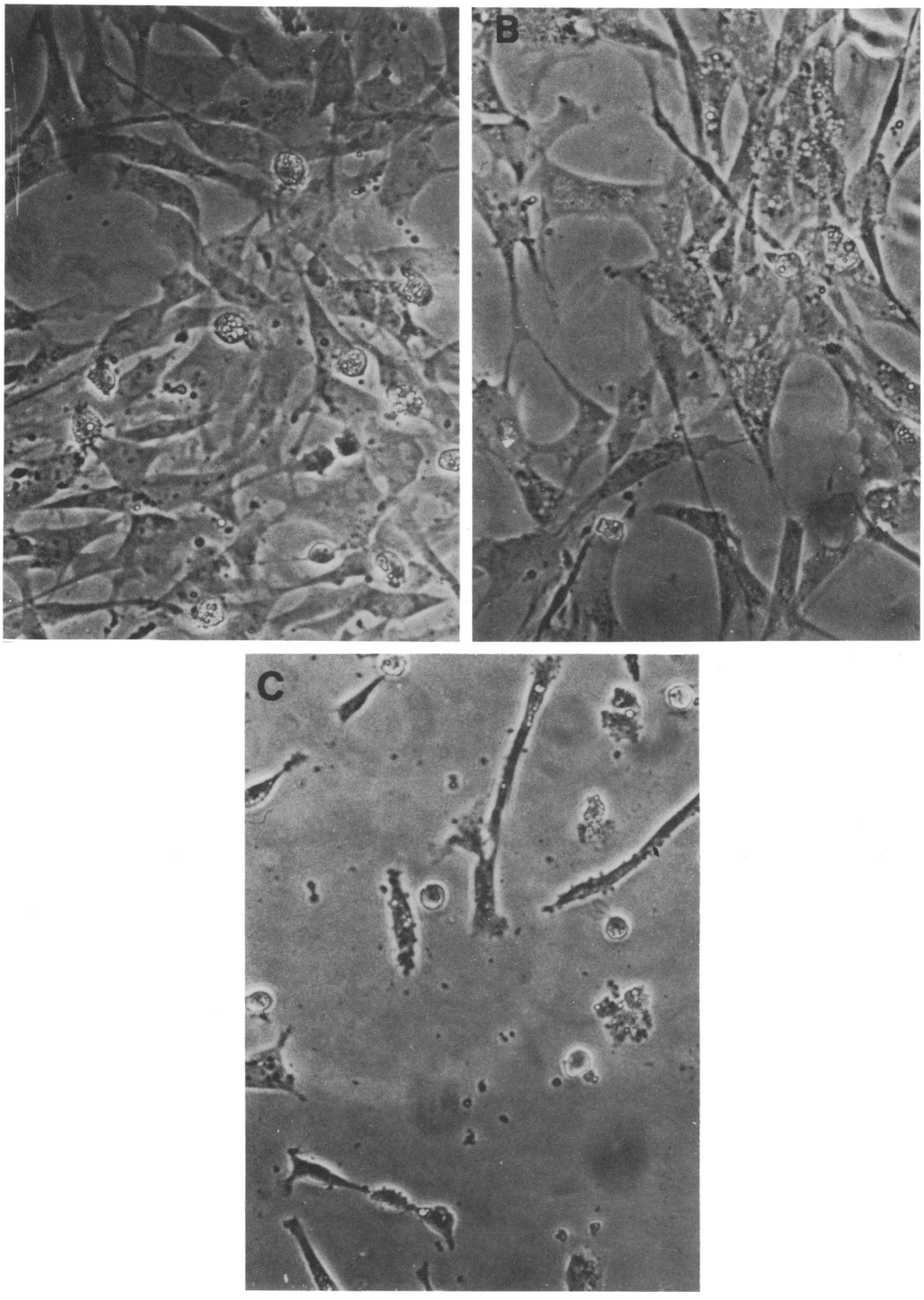

FIG. 1. *Effect of $N_{2226}B_{2226}$ on the growth of chicken embryo fibroblasts. Secondary cultures of chicken embryo fibroblasts were treated at 37 C with N_{2226} B_{2226}* at concentrations of (A) 0, (B) 76, and (C) 760 *μg/ml 3 h after the cells were plated. Pictures were taken 36 h after treatment through a phase microscope at 200× magnification. Cells grown in medium containing N_{2226} B_{2226} at concentrations of 0 and 76 μg/ml ultimately grew to confluency, whereas those grown in 760 μg/ml did not.*

TABLE 1. *Susceptibility of several different cell lines to* N_{2226} B_{2226}

Strain	Growth minimal inhibitory concn (μg/ml)
Bacillus subtilis	8.3
Micrococcus luteus	83
Candida albicans	83
Escherichia coli B	620
E. coli SC9251	620
Chicken embryo fibroblasts	760

suggest that this compound is biostatic rather than biocidal. This resumption of growth was most likely due to slow degradation of $N_{2226}B_{2226}$ in the medium because incubation of $N_{2226}B_{2226}$ in the growth medium for equivalent periods of time in the absence of bacteria reduced the antimicrobial activity of this compound.

The effects of $N_{2226}B_{2226}$ on the growth and morphology of chicken embryo fibroblasts were examined to obtain preliminary data concerning the toxicity of this compound for animal cells. Relatively high concentrations of the salt were required to affect the morphology or growth of these cells (Fig. 1 and Table 1). At a concentration of 760 μg/ml, growth of fibroblasts was completely inhibited (Fig. 1C) and the cells were round, whereas the untreated cells were fibrous and elongated. Although the growth of chicken embryo fibroblasts was slightly retarded by $N_{2226}B_{2226}$ at a concentration of 76 μg/ml (Fig. 1B), the morphology of the treated cells was comparable to the control cells.

It is interesting that gram-positive bacteria were more susceptible $N_{2226}B_{2226}$ than gram-negative bacteria. Gram-negative bacteria are characteristically more resistant to membrane-perturbing agents such as detergents and some peptide anitbiotics (3), and protection of the inner membrane from these agents by the outer membrane is often invoked as an explanation for this phenomenon. The differential susceptibility of these various organisms to $N_{2226}B_{2226}$ may also be due to membrane compositional differences, particularly when comparing the animal cells to bacteria (2). The stability of this compound combined with its unusual chemical characteristics and bacteriostatic action make it a potentially interesting reagent for microbiological research. In addition, its physical properties and biological activity suggest that $N_{2226}B_{2226}$ might be an effective disinfectant.

This work was supported by Public Health Service grant AI 12821 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. Ford, W. T., R. J. Hauri, and D. J. Hart. 1973. Synthesis and properties of molten tetraalkylammonium tetraalkylborides. J. Org. Chem. **38**:3916-3918.
2. Fox, C. F. 1972. Membrane assembly, p. 345-386. *In* C. F. Fox and A. D. Keith (ed.), Membrane molecular biology. Sinauer Associates, Stamford, Conn.
3. Leive, L., 1974. The barrier function of the gram-negative envelope. Ann. N.Y. Acad. Sci. **235**:109-129.
4. Rose, M. J., Jr., S. A. Aron, and B. W. Janicki. 1966. Effect of various nonionic surfactants on growth of *Escherichia coli* J. Bacteriol. **91**:1863-1868.