Effect of 3-Phenylpropan-1-ol, 2-Phenylethanol, and Benzyl Alcohol on *Pseudomonas aeruginosa*

R. M. E. RICHARDS[▲] and R. J. McBRIDE

Abstract □ 3-Phenylpropan-1-ol, 2-phenylethanol, and benzyl alcohol were investigated for their inhibitory action against *Pseudomonas aeruginosa*. 3-Phenylpropan-1-ol was the most effective and benzyl alcohol was the least effective as shown by: (*a*) growth rate studies using subinhibitory concentrations of the alcohols, (*b*) determination of minimum inhibitory concentrations, and (*c*) determination of sterilization times. The three compounds enhance the bactericidal action of benzalkonium chloride against *P. aeruginosa* in the same ranking order. It is suggested that 3-phenylpropan-1-ol may be a suitable preservative for oral suspensions and mixtures.

Keyphrases □ 3-Phenylpropan-1-ol effect on *Pseudomonas aeruginosa*—compared to 2-phenylethanol and benzyl alcohol with/without benzalkonium chloride □ 2-Phenylethanol effect on *Pseudomonas aeruginosa*—compared to 3-phenylpropan-1-ol and benzyl alcohol with/without benzalkonium chloride □ Benzyl alcohol effect on *Pseudomonas aeruginosa*—compared to 3-phenylpropan-1-ol and 2-phenylethanol with/without benzalkonium chloride □ Bactericidal activity—comparison of 3-phenylpropan-1-ol, 2-phenylethanol, and benzyl alcohol with/without benzalkonium chloride □ *Pseudomonas aeruginosa* inhibition—effect of 3-phenylpropan-1-ol, 2-phenylethanol, and benzyl alcohol with/without benzalkonium chloride

As the result of a survey of the contamination of pharmaceutical preparations, Hooper (1) stated that: "... aqueous medicines containing no preservative were very favourable breeding grounds for water-borne Gram-negative bacteria including Pseudomonas/Alcaligenes/Achromobacter groups. Mixtures such as magnesium trisilicate, aluminum hydroxide, ammonia and ipecacuanha, kaolin, magnesium carbonate, magnesium sulphate and magnesium hydroxide were often found to contain enormous numbers of organisms, counts of 10^6 per ml were often commonplace." Thus there is an urgent need for a preservative suitable for inclusion in such preparations.

The aim of this present investigation was to compare 3-phenylpropan-1-ol[1], 2-phenylethanol, and benzyl alcohol, both as single compounds and in combination with benzalkonium chloride, for their effects on *Pseudomonas aeruginosa*.

The initial member of this series of phenyl-substituted aliphatic alcohols is benzyl alcohol, which has been used for its antibacterial and analgesic properties in injections over the past 20 years. Gershenfeld (2) stated that, in a concentration of 1% or more, benzyl alcohol is bacteriostatic to a number of organisms. Its sharp burning taste, however, curtails its usefulness in oral products (3).

2-Phenylethanol is active against Gram-negative organisms (4) and is compatible with many ophthalmic solutions (5). Therefore, it is recommended in USP XVIII (6) and NF XIII (7) for preserving ophthalmic solutions. 2-Phenylethanol also has been shown to enhance the activities of a number of preservatives commonly used in ophthalmic solutions (8–13).

3-Phenylpropan-1-ol, the homolog of 2-phenylethanol having the synonym hydrocinnamyl alcohol, is a liquid with a cinnamon-like odor and is slightly soluble in water. The antibacterial properties of this compound have not been reported previously, but it was thought that it may prove to be a suitable preservative for mixtures and suspensions.

EXPERIMENTAL

Materials—Benzyl alcohol[2], 2-phenylethanol[2], and 3-phenylpropan-1-ol[2] were of laboratory reagent grade, and benzalkonium chloride BP[3] was used. The liquid growth medium was oxoid[4] nutrient broth No. 2 and the incubation temperature was 37°. *P. aeruginosa* NCTC 6750[5] was the test organism and was maintained as stab cultures in oxoid[4] nutrient agar at a temperature of 4°. The inactivating medium was that described by Riegelman *et al.* (14).

Methods—The evaluations used either log phase cultures for growth rate studies or overnight cultures for determinations of

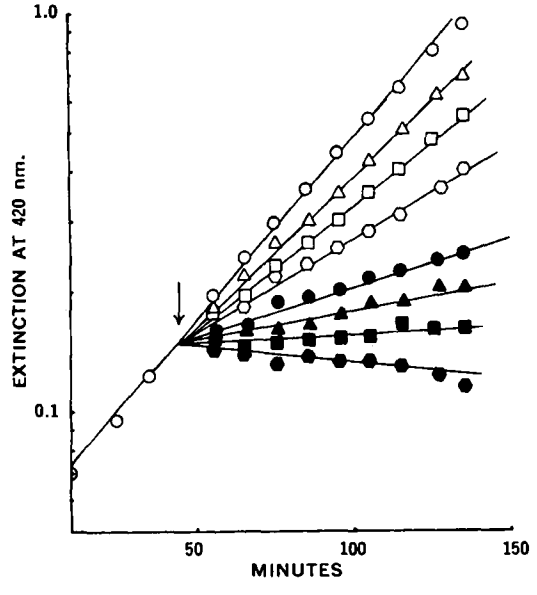

Figure 1—*Effect of various concentrations of phenylethanol on the growth of* P. aeruginosa *NCTC 6750. Sufficient phenylethanol was added at the time indicated by the arrow to give the final concentrations as follows:* ○, *no phenylethanol added;* △, *0.05% phenylethanol;* □, *0.1% phenylethanol;* ○, *0.15% phenylethanol;* ●, *0.2% phenylethanol;* ▲, *0.25% phenylethanol;* ■, *0.3% phenylethanol; and* ●, *0.35% phenylethanol.*

[1] Patent applied for.

[2] British Drug Houses, Poole, Dorset, England.
[3] MaCarthy Ltd., Glasgow, Scotland.
[4] Oxo Ltd., London, England.
[5] National Collection of Type Cultures, Colindale, London, England.

Table I—Minimum Inhibitory Concentrations for 3-Phenylpropan-1-ol, 2-Phenylethanol, and Benzyl Alcohol against *P. aeruginosa* NCTC 6750 in Nutrient Broth at 25°

Alcohol	Minimum Inhibitory Concentration, %—Log Phase Cells	Minimum Inhibitory Concentration, %—1.9×10^7 Overnight Cells/ml.
Benzyl alcohol	0.41	0.42
2-Phenylethanol	0.30	0.34
3-Phenylpropan-1-ol	0.27	0.26

growth inhibition and cell death. Cell numbers were estimated by colony counts as previously described (13).

Effect on Growth Rate—Log phase cultures were prepared by inoculating 100 ml. prewarmed nutrient broth with 1 ml. of an overnight culture, prepared from a stored stab culture of *P. aeruginosa*, and incubating in a shaking incubator[6] at 100 throws a minute. (Each stab culture was used once only.) Growth was followed by measuring the absorbance of samples in a spectrophotometer[7] at 420 nm. When the absorbance reading was 0.3–0.35, 1-ml. samples were transferred to each of the required number of 100-ml. quantities of prewarmed broth in 250-ml. conical flasks. Chemicals were subsequently added to these log phase cultures when the absorbances were approximately 0.1.

Effect of Alcohol Concentration—Varying concentrations of one of the alcohols were added to a number of similar cultures and simultaneously an equivalent volume of water was added to the control culture. (All liquids, as well as the pipets used in making their addition, were prewarmed to 37°.) The growth in each culture was followed by making absorbance measurements over the next 1.5–2.5 hr. The log of the absorbance so obtained for each concentration of alcohol was graphed against the time over which the determinations were made to give a result similar to that in Fig 1. By using the method of least squares, the equation for each line was calculated to obtain the slope which is the growth rate. The growth rate for each concentration, when expressed as a percentage of the growth rate of the control culture, was then plotted against the concentration of alcohol to produce a graph such as Fig. 2. The point where the baseline is crossed indicates the concentration of alcohol that will inhibit log phase cultures of *P. aeruginosa*, and these values are given in Table I.

Comparative Activity of Alcohols—To three log phase cultures, sufficient benzyl alcohol, 2-phenylethanol, and 3-phenylpropan-1-ol were added to give final concentrations of 0.178, 0.2, and 0.223% (w/v), respectively. An equivalent volume of water was added to the control culture. The concentrations used gave equimolar concentrations of the three alcohols. The growth in all flasks was followed as already described; the results are expressed in Fig. 3.

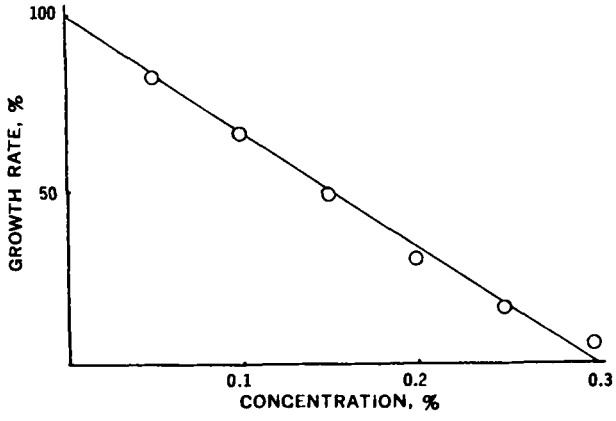

Figure 2—*Correlation between subinhibitory concentrations of phenylethanol and growth rate of* P. aeruginosa *NCTC 6750 in subinhibitory concentrations of phenylethanol expressed as a percentage of the growth rate in the absence of phenylethanol.*

[6] Mickle Engineering Co, Gomshall, Surrey, England.
[7] Unican SP600, Pye Unicam Ltd., Cambridge, England.

Table II—Sterilization Times at 25° for 5.6×10^6 Cells/ml. *P. aeruginosa* NCTC 6750 in Aqueous Solutions of Benzyl Alcohol, 2-Phenylethanol, and 3-Phenylpropan-1-ol Alone and in Combination with Benzalkonium Chloride, 0.01% (w/v)

Antibacterial Concentrations, %	Sterilization Time, min.—Single Substance	Sterilization Time, min.—Combination
Benzalkonium chloride, 0.01	50–60	—
Benzyl alcohol, 0.4	>24 hr.	25–30
2-Phenylethanol, 0.4	>24 hr.	20–25
2-Phenylpropan-1-ol, 0.4	75–90	<5

Alcohol–Benzalkonium Combinations—To eight log phase cultures the following were added: (*a*) water; (*b*) benzalkonium chloride, 0.003%; (*c*) benzyl alcohol, 0.175%; (*d*) 2-phenylethanol, 0.175%; (*e*) 3-phenylpropan-1-ol, 0.175%; (*f*) benzyl alcohol, 0.175%, and benzalkonium chloride, 0.003%; (*g*) 2-phenylethanol, 0.175%, and benzalkonium chloride, 0.003%; and (*h*) 3-phenylpropan-1-ol, 0.175%, and benzalkonium chloride, 0.003%. The growth of each culture was followed as before and the results form Fig. 4.

Effect on Growth Inhibition and Cell Death—*Minimum Inhibitory Concentration*—A series of concentrations of benzyl alcohol, 2-phenylethanol, and 3-phenylpropan-1-ol were prepared in 10-ml. volumes of nutrient broth. Each tube was inoculated with 0.1 ml. overnight culture of *P. aeruginosa* to give a final viable concentration of approximately 1.9×10^7 cells/ml. The tubes were incubated for 5 days and observed for growth daily. The lowest concentration of each alcohol showing no growth is given in Table I.

Sterilization Times—Tubes containing 9.9-ml. volumes of the aqueous solutions under test were equilibrated in a water bath at 25°, after which 0.1 ml. of overnight culture of *P. aeruginosa* was added to give a final inoculum of approximately 5×10^6 cells/ml. At intervals of 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, and 120 min. and 24 hr. after the addition of the inoculum, 0.5-ml. samples were added

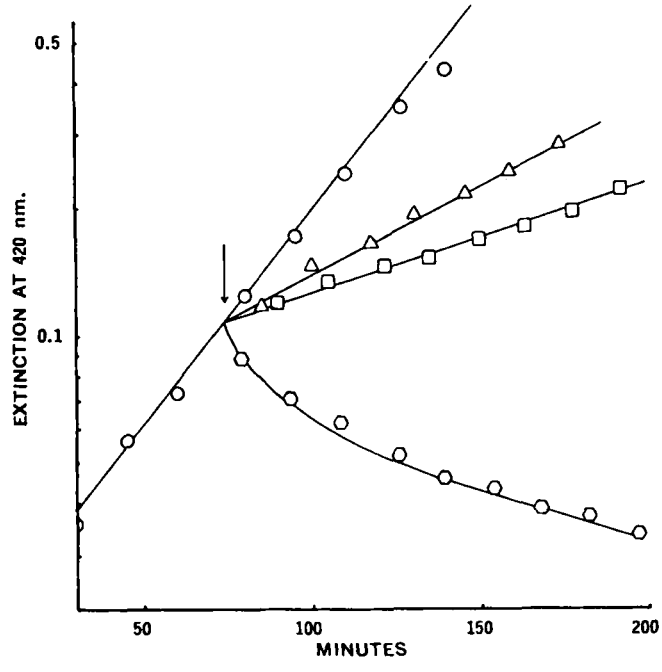

Figure 3—*Comparison of the effect of equimolar concentrations of benzyl alcohol, phenylethanol, and 3-phenylpropan-1-ol on the growth of* P. aeruginosa *NCTC 6750. Sufficient alcohol was added at the time indicated by the arrow to give a final concentration of* 1.6×10^{-3} M. *Key:* ○, *no alcohol;* △, *benzyl alcohol;* □, *phenylethanol; and* ○, *3-phenylpropan-1-ol.*

to 9.5 ml. of inactivating recovery medium and incubated for 3 days. Positive controls were prepared by adding 0.1 ml. of a 10^6 dilution in nutrient broth of an overnight culture of *P. aeruginosa* to tubes of recovery medium separately containing 0.5 ml. of each of the preservative solutions under test; all showed growth. The results are tabulated in Table II.

RESULTS AND DISCUSSION

After the addition of subinhibitory concentrations of either of the three alcohols, the log phase cultures continued to grow exponentially but at a rate dependent on the alcohol concentration. Typical results for phenylethanol are given in Fig. 1. Lang and Rye (15), using a different experimental technique, investigated the growth inhibitory properties of benzyl alcohol and phenylethanol against *Escherichia coli*. These workers found that growth occurring in the presence of benzyl alcohol was exponential, but that the growth rate in the presence of phenylethanol occurred at a gradually decreasing rate throughout the period of incubation. It was not apparent why the response of the *E. coli* to the action of the alcohols was different.

The concentration of each alcohol causing inhibition of growth of log phase cultures was then determined by plotting the growth rate for each concentration of alcohol, as a percentage of the growth rate of the control containing no alcohol, against the alcohol concentration. Figure 2 shows the results for phenylethanol, and the same linear response was obtained with the other two alcohols; these findings demonstrate the effectiveness of these antibacterials under the conditions of the test. Unpublished results[8] with benzalkonium chloride, however, showed a different response pattern to that of the alcohols. When the percentage of the control growth rate was plotted against the concentration of benzalkonium chloride, a straight line was obtained at low concentrations but this became asymptotic at higher concentrations. This lack of linearity shows that the antibacterial action of benzalkonium chloride is inadequate under the conditions of the growth rate experiments using *P. aeruginosa* as the test organism. This technique might prove useful in determining the suitability of a given antibacterial for use as a preservative against a given organism.

The concentrations of the alcohols causing inhibition of log phase cultures of *P. aeruginosa* are given in Table I, together with the minimum inhibitory concentrations for overnight cultures. The closeness of the two sets of values indicates that the alcohols have a uniform activity against *P. aeruginosa* cells in different phases of the growth cycle.

Figure 3 shows that at a concentration of 1.6×10^{-3} *M*, 3-phenylpropan-1-ol is more active against log phase *P. aeruginosa* than 2-phenylethanol, which is more active than benzyl alcohol.

2-Phenylethanol has been shown to enhance the antibacterial effect of benzalkonium chloride on log phase cultures of *P. aeruginosa* (10) and to give shorter sterilization times in ophthalmic preparations (11). Figure 4 shows that all of the alcohols increase the effect of benzalkonium chloride. Again 3-phenylpropan-1-ol is the most effective and benzyl alcohol is the least effective. This result is confirmed by the sterilization times for aqueous solutions of the preservatives alone and in combination with 0.01% benzalkonium chloride against large inocula of *P. aeruginosa* (Table II). 3-Phenylpropan-1-ol sterilized in less than 90 min., but phenylethanol and benzyl alcohol both gave positive samples after 24 hr. No growth was detected in any of the samples from the 3-phenylpropan-1-ol–benzalkonium chloride combination.

SUMMARY AND CONCLUSION

3-Phenylpropan-1-ol is more effective than 2-phenylethanol which, in turn, is more effective than benzyl alcohol in inhibiting the growth of *P. aeruginosa* and in enhancing the activity of benzalkonium chloride against *P. aeruginosa*. The compound 3-phenylpropan-1-ol with its cinnamon-like odor is quite pleasant at a concentration of 0.4% in water and may be suitable, either alone or in combination, for use in the preservation of oral preparations.

[8] R. M. E. Richards and R. J. McBride.

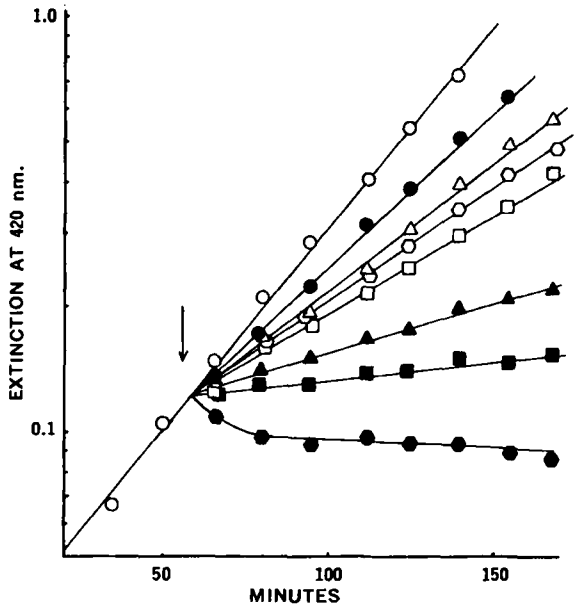

Figure 4—*Effect of 0.175% benzyl alcohol, 0.175% phenylethanol, and 0.175% 3-phenylpropan-1-ol, alone and in combination with 0.003% benzalkonium chloride, on the growth of* P. aeruginosa *NCTC 6750. The substances were added at the time indicated by the arrow. Key:* ○, *water;* ●, *benzalkonium chloride;* △, *benzyl alcohol;* ▲, *benzyl alcohol and benzalkonium chloride;* □, *phenylethanol;* ■, *phenylethanol and benzalkonium chloride;* ○, *3-phenylpropan-1-ol; and* ●, *3-phenylpropan-1-ol and benzalkonium chloride.*

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