Antimicrobial Activity of Sodium n-Alkylsalicylates

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The activities are reported of sodium salts of several n-alkylsalicylic acids against Staphylococcus aureus, Pseudomonas aeruginosa, Mycobacterium phlei, and Candida albicans. The acids had alkyl substituents of 1 to 18 carbon atoms in the 3, 4, or 5 positions relative to the carboxyl group of salicylic acid. Generally, the antimicrobial properties were typical of anionic surface-active agents although the salicylates are more potent than most of these, particularly against S. aureus.

Salicylic acids, substituted with alkyl groups of 1 to 18 carbon atoms in the 3, 4, or 5 positions relative to the carboxyl group, have been prepared for their similarity to anacardic acid to evaluate the effect of the position and size of the alkyl substituents on the antimicrobial activity of sodium salicylate.

Anacardic acid is a mixture of salicylic acids substituted in the 6 position with side chains of 15 carbon atoms and different degrees of unsaturation. The average constellation of the mixture is represented by 6-pentadecadienylsalicylic acid (1, 16). The high in vitro antibacterial activity of cashew nut shell liquid (CNSL) has been attributed to its anacardic acid content (11, 12).

The sodium salts of anacardic acid are anionic surface-active agents (5–7). Eichbaum (11) reported that the monosodium salt is more potent as a bactericide, particularly against Staphylococcus aureus, that it possesses a wider spectrum of antibacterial activity than that generally exhibited by anionic surface-active agents, and that this activity is largely independent of pH over the range of pH 5 to 9. Biswas and Roy (5, 6) suggested that disodium anacardate could be a "useful bactericidal surfactant."

The present work reports the activities of homologous and isomeric sodium alkylsalicylates.

MATERIALS AND METHODS

Compounds. The preparation of all compounds has been reported (D. Buckley and J. Thomas, J. Med. Chem., in press).

Organisms. Bacteria were grown on Oxoid nutrient broth no. 2 (Oxoid CM67). Agar slopes were prepared from nutrient broth solidified with Oxoid agar no. 3. Candida albicans was grown on Sabouraud liquid medium (Oxoid CM147, pH 5.6). S. aureus (NCTC6571) and C. albicans were supplied by Commonwealth Serum Laboratories, Melbourne; Pseudomonas aeruginosa and Mycobacterium phlei were supplied by the Microbiology department, Sydney University. All bacteria were grown at 37°C, and C. albicans, at 27°C.

Drug solutions. Stock solutions containing sodium alkylsalicylates (0.5 to 1.0%) were prepared in ethanol (20%, v/v) from equivalent amounts of free acid and sodium hydroxide solution. Precipitation of hydrates occurred on standing from solutions of salts having eight or more carbon atoms in the substituent; this was delayed by storage at 37°C. Stock solutions were heated in a water bath (80°C, 10 min) to redissolve precipitate and allowed to cool to 37°C when the required dilutions were made at 37°C with ethanol (20%, v/v). For experiments with S. aureus and P. aeruginosa, solutions were sterilized (121°C, 10 min) in ampoules (20 ml) and were equilibrated to 37°C before use. The procedure delayed precipitation sufficiently to permit reproducible dosing of the cultures. The sodium alkylsalicylate solutions were shown to be stable under these conditions.

Ethanol concentration in the test systems were <2% (except 5% for M. phlei), and alcohol controls did not exhibit any antibacterial effect.

Antimicrobial assays: C. albicans and M. phlei. Minimum inhibitory concentrations were determined by the method of Taylor and D’Arcy (20).

S. aureus and P. aeruginosa. A turbidometric method, adapted from that of Brown (8) was used. Measurements were carried out in "nephelo" flasks prepared by attaching optically matched side-arm tubes (1.0 cm inner diameter, 10 cm long, approximately 8 ml) to the lower sides of 250-ml Erlenmeyer flasks. The side arm fitted into a 1-cm holder of a Spectronic 20 spectrophotometer. Absorbance was measured at 540 nm. Growth of the cultures was followed by absorbance measurements at 15 to 20-min intervals. When exponential growth was established [optical density (OD) 0.4 equivalent to approximately 200 × 106 cells of S. aureus per ml], drug solution was added and incubation and absorbance measurements were continued for at least 2 hr. The concentration of compound which reduced the growth rate to zero was determined. Some typical results are shown in Fig. 1.
RESULTS

The relative potencies of the sodium n-alkylsalicylates against S. aureus, M. phlei, and C. albicans are reported in Table 1. None of the compounds had significant activity against P. aeruginosa. Cultures of S. aureus inhibited by 3-n- and 5-n-dodecyl homologues recovered from the inhibition after approximately 140 min of exposure to the compounds (Fig. 1) and resumed rapid growth rate. A similar but slower recovery was observed with the 4-n-dodecyl homologue. In the presence of lower homologues, a delayed recovery occurred during prolonged incubation (18 hr). Generally, the time of onset and the rate of recovery of the cultures from the effects of alkylsalicylates appeared to be related to the potency of the compound as well as the dose. That is, the larger the substituent, the more potent the compound and also the more rapid the recovery up to the dodecyl (C_{12}) homologue.

Electron microscopy showed that high concentrations of alkylsalicylates (in excess of inhibitory concentrations) caused a loss of electron-dense material from the cytoplasm of cells of S. aureus. This corresponded with a fall in absorbance of the cultures with time. The activity of the compounds against S. aureus was inhibited by the addition of horse serum. For example, the action of sodium 3-n-octyl- and 5-n-dodecyl salicylate against S. aureus was completely inhibited by the addition of 2.5 and 0.5% serum, respectively.

DISCUSSION

The pattern of increasing potency with increase in size of the substituent until a cut-off point is reached is typical of that seen with homologous series of surface-active agents and other compounds (14, 18, 19, 21). Comparison of the activity of sodium salicylate and the alkylsalicylates against P. aeruginosa and S. aureus indicates that the alkyl substitution results in specificity against gram-positive organisms and an increased potency against this group of organisms. The selective action against gram-positive organisms and the low order of activity against C. albicans is typical of anionic surface-active agents. However, the alkylsalicylates differ from most anionic surface-active agents in possessing a relatively high order of activity against S. aureus and in this respect appear to be similar to sodium anacardate (2, 11, 21).

The position of the substituent in the aromatic nucleus of salicylic acid was important. Generally, substitution in the 3 position resulted in higher activity than the same substituent in the 4 and 5 positions. Where studied, the relative activity of the isomers seemed to depend both on the number of carbon atoms in the substituent and the nature of the test organism. In related groups of compounds (e.g., alkylphenols), positional isomers have a similar pattern of activity (19).

It was evident that viable cells of S. aureus may persist for long periods in solutions of the alkylsalicylates, but the lytic effect indicates that the compounds are bactericidal to some cells, if not cultures, of this organism.

Previous workers have shown that microorganisms may persist in various systems of antimicrobial agents and that, after an initial bactericidal effect, multiplication may occur (3, 4, 9, 10, 13, 17). In the case of the n-alkylsalicylates, it is possible that cell exudates were either providing nutrients to promote multiplication of survivors (3, 4, 15) or inactivating the compounds (11). Alternatively, the recovery effect may be explained in terms of the adsorption of the compound to organic matter present in the system. Thus, in any mixture containing alkylsalicylate, the higher the intrinsic activity of the drug, the greater would be the proportion of compound removed from solution by adsorption onto cells and cell fragments. The different rates of recovery of S. aureus, in the presence of initially inhibitory concentrations of the various homologues, would seem to reflect the extents to which the compounds are
adsorbed from solution. It may be envisaged that a dynamic situation exists during turbidometric assays in which more resistant cells in the cultures continue multiplication in the presence of the drug, although at an extremely slow rate. As a consequence of this multiplication, more cells are produced which can adsorb the drug. Ultimately the concentration of free unbound drug would be reduced to an ineffective level. This would then be reflected by rapid multiplication of the cells. The greater the activity (both surface activity and antimicrobial activity) of a particular homologue, the more rapid would be the onset of recovery of growth rate. It should also be noted that the compound will be adsorbed to high-molecular-weight material other than intact cells.

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**TABLE 1. Antimicrobial activities of monosodium n-alkylsalicylates**

| Substituent | Position | Staphylococcus aureus | Mycobacterium phlei | Candida albicans |
|-------------|----------|------------------------|---------------------|-----------------|
|             |          | Inhibitory dilution     | MIC<sup>b</sup>      | MID             | MIC<sup>c</sup> |
|             |          | (%)                    | (%)                 | (%)             | (%)             |
| H           | 3        | >1-110                 | 1-1,200             | 521             | 1-300           | 108.4           |
| CH<sub>3</sub> | 4        | >1-500                 | 1-1,300             | 479             | 1-1,000         | 57.5            |
| CH<sub>4</sub> | 5        | >1-500                 | 1-1,200             | 479             | 1-900           | 63.9            |
|             | 6        | >1-500                 | 1-1,300             | 479             | 1-504           | 114.2           |
| C<sub>8</sub>H<sub>15</sub> | 3        | >1-500                 | 1-1,100             | 470             | 1-270           | 212.8           |
| C<sub>8</sub>H<sub>15</sub> | 3        | >1-500                 | 1-1,300             | 409             | 1-1,044         | 51              |
| C<sub>12</sub>H<sub>15</sub> | 3       | >1-6,385               | 1-17,000            | 24.5            | 1-9,800         | 4.25            |
| C<sub>12</sub>H<sub>17</sub> | 3       | >1-500                 | 1-1,300             | 409             | 1-1,032         | 51.5            |
| C<sub>15</sub>H<sub>27</sub> | 4       | >1-5,500               | 1-1,300             | 409             | 1-1,044         | 51              |
| C<sub>15</sub>H<sub>27</sub> | 4       | >1-5,500               | 1-1,300             | 409             | 1-1,044         | 51              |

<sup>a</sup> MID, minimum inhibitory dilution; MIC, minimum inhibitory concentration; >, no activity at stated concentration.

<sup>b</sup> Values expressed × 10<sup>-4</sup> M.

<sup>c</sup> Values expressed × 10<sup>-4</sup> M.
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