THE GERMICIDAL ACTIVITY OF SOME PYRIDINIUM SALTS CONTAINING UNSATURATED HYDROCARBON RADICALS

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The germicidal potency of quaternary ammonium compounds is known to depend mainly on the chemical structure of the cation, the nature of the anion having little if any effect (Glassman, 1948). The relation of structure of the cation to bactericidal activity has been studied extensively insofar as chain length of the hydrophobic or hydrocarbon group is concerned. In compounds with only one long chain aliphatic group the maximum activity is associated almost always with a chain length of 12, 14, or 16 carbon atoms in this radical, the activity decreasing markedly outside this range (Kolloff et al., 1942; Rawlins et al., 1943; Hoogerheide, 1945; Valko and DuBois, 1945; Shelton et al., 1946c, d). No systematic studies, however, have been reported so far on the influence of unsaturation in the hydrocarbon group. In view of the fact that unsaturated fatty acids by themselves possess significant bacteriostatic properties (Kodicek and Worden, 1945; Hirsch, 1947) it was decided to investigate the effect of introducing unsaturated hydrocarbon radicals in quaternary ammonium compounds.

In the present investigation the effect of unsaturation in the hydrocarbon group of aliphatic pyridinium salts was examined by studies on stearyl, oleyl, linoleyl, and linolenyl-pyridinium bromides. Pyridinium salts containing hydrocarbon radicals derived from the mixed fatty acids of commercially available hydnocarpus and shark liver oils were synthesized also and assayed for activity, the former being selected on account of its therapeutic application in the treatment of leprosy and the latter on account of its content of polyethenoid fatty acids which are characteristic of fish oils.

MATERIALS AND METHODS

For the synthesis of the stearyl compound, n-octadecyl alcohol was used as the starting material. The oleyl, linoleyl, and linolenyl derivatives were prepared from the corresponding fatty acids. The oleic acid used was a commercial preparation while linoleic and linolenic acids were obtained by debromination of their bromo derivatives isolated from safflower and linseed oils, respectively. The general steps in the synthesis were reduction of methyl, ethyl, or butyl esters of the fatty acids with sodium and alcohol, conversion of the resulting alcohols to bromides, and condensation of the aliphatic bromides with pyridine. Steryl-pyridinium bromide was purified by crystallization from ethyl alcohol and the unsaturated derivatives obtained as oils by repeated solution in absolute ethyl alcohol and precipitation with dry diethyl ether. Compounds containing mixed hydrocarbon radicals were prepared by the same general methods from the esters of the total fatty acids obtained from hydnocarpus and shark liver oils. Details of synthesis of individual compounds are described elsewhere (Damodaran and SivaRaman, 1951).

The per cent ionizable bromine in the various preparations is shown in table 1.

The critical 10 minute killing dilutions of the compounds were determined using the standard strain 209 P of Micrococcus pyogenes var. aureus. The determinations were carried out at 37 C by a modification of the Food and Drug Administration method. Dilutions of the test substances were treated with the bacterial suspension in the usual manner, and 0.02 ml samples were withdrawn by sterile capillary pipettes after thorough mixing at 5, 10, and 15 minute intervals and transferred to 15 ml subculture media consisting of nutrient broth containing 0.3 per cent agar (Difco). The agar was added to neutralize the effect of bactericide carried over in subculturing (Quiro et al., 1946). Phenol controls were run with each determination as a check on the resistance of the test organism.

RESULTS AND DISCUSSION

The bactericidal concentrations of the compounds are shown in table 2.

The above data clearly indicate that the substitution of the stearyl group in stearyl-pyridinium bromide with unsaturated aliphatic radicals
such as oleyl, linoleyl, and linolenyl progressively increases the germicidal activity of the derivative. Valko and DuBois (1945) have shown that the introduction of an oleyl radical in place of stearyl in stearyl-dimethyl-benzyl-ammonium and stearyl-dimethyl-ethyl-ammonium salts produces a marked increase in bactericidal efficacy. This influence of unsaturation, however, was not noticed in alkyl-trimethyl-ammonium and alkyl-

**SUMMARY**

Stearyl, oleyl, linoleyl, and linolenyl-pyridinium bromides were synthesized and assayed for germicidal activity. It has been shown that in these compounds bactericidal potency increases with the degree of unsaturation in the long chain hydrocarbon radical of the cation, maximal activity being shown by the linolenyl, and minimal activity by the stearyl derivative.

**TABLE 1**

*Analysis of pyridinium salts*

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>FORMULA</th>
<th>IONIZABLE BROMINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stearyl-pyridinium bromide</td>
<td>C₉H₁₈NBr·H₂O</td>
<td>Found 18.47</td>
</tr>
<tr>
<td>Oleyl-pyridinium bromide</td>
<td>C₁₀H₂₁NBr</td>
<td>19.92</td>
</tr>
<tr>
<td>Linoleyl-pyridinium bromide</td>
<td>C₁₀H₂₄NBr</td>
<td>19.85</td>
</tr>
<tr>
<td>Linolenyl-pyridinium bromide</td>
<td>C₁₁H₂₈NBr</td>
<td>19.68</td>
</tr>
<tr>
<td>Mixed pyridinium bromides derived from fatty acids of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hydnocarpus oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed pyridinium bromides derived from fatty acids of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>shark liver oil</td>
<td></td>
<td></td>
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</table>

For practical utilization of these observations pyridinium bromides were prepared from the mixed fatty acids of two naturally occurring fats, hydnocaropus oil and shark liver oil, which contain a high proportion of unsaturated acids. The mixed pyridinium bromides prepared from these sources possess high bactericidal activity.

**REFERENCES**

DAMODARAN, M., AND SIVARAMAN, C. 1951 The preparation of new bactericidal and surface-active quaternary ammonium compounds. Indian patent, 45,118.


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dimethyl-allyl-ammonium compounds, the substitution of stearyl with oleyl radical in the former case producing actually a reduction in activity towards *M. pyogenes* (Valko and DuBois, 1945; Shelton et al., 1946a).

The high bactericidal potency of the pyridinium salts derived from the mixed fatty acids of hydnocarpus and shark liver oils suggest interesting possibilities for their commercial production.
GERMICIDAL ACTIVITY OF PYRIDINIUM SALTS