Antimicrobial Properties of Natural Phenols and Related Compounds

I. Obtusastyrene

L. JURD, A. D. KING, JR., K. MIHARA, AND W. L. STANLEY

Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Albany, California 94710

Received for publication 25 November 1970

Obtusastyrene (4-cinnamylphenol) displays effective antimicrobial activity in vitro against a variety of gram-positive bacteria, yeasts, and molds. The activity of obtusastyrene is not appreciably affected by pH, and its minimal inhibitory concentrations, 12 to 25 μg/ml for bacteria and 12 to 100 μg/ml for fungi, compare favorably with those of a number of synthetic, phenolic antimicrobial agents.

Because of its unusually high resistance to attack by fungi, insects, and termites, the heartwood of Dalbergia siso is a source of valuable timber in India (1). The chemical basis for the durability of this wood has not been determined unequivocally, although in 1962 it was suggested (8) that the presence of phenolic 4-phenylcoumarins, e.g., dalbergin (I), may account for its particular antibiotic properties. Almost simultaneously it was reported (3) that aqueous alcoholic extracts of a related Brazilian species, D. nigra (jacarandá), showed considerable antibiotic activity against gram-positive and gram-negative bacteria and against some acid-fast organisms and fungi. These extracts yielded a crystalline quinone, subsequently (2) identified as 4-methoxydalbergione (II), which at low concentrations (20 to 100 μg/ml) inhibited the growth of Bacillus anthracis and Candida albicans.

As a result of more recent chemical investigations a wide variety of novel, natural phenols have now been isolated and identified from Dalbergia and related Machaerium species. These include phenolic neoflavonoids (2, 5) of different structural types, namely, dalbergiones, e.g., II, and the corresponding quinol, III, dihydrobenzofurans, e.g., IV, and 4-phenylcoumarins similar to dalbergin, I. Cinnamylphenols, which are structurally isomeric with the neoflavonoids of structural type III, co-occur with these neoflavonoids in Dalbergia species. Nine natural cinnamylphenols have been detected in these woods, namely, obtusastyrene (4-cinnamylphenol), V, and partially methylated derivatives of 6-cinnamylresorcinol, VIa, 6-cinnamylpyrogallol, VIIa, and 6-cinnamyl-3-methoxyquinol, VIIIa. With the exception of the early report (3) on the activity of 4-methoxydalbergione (II), the antimicrobial properties of natural and derived compounds of types III to VIIIa do not appear to have been described. In this initial investigation, it has been observed that the cinnamylphenols, particularly obtusastyrene (V) are highly effective and promising antimicrobial agents.
against a wide variety of gram-positive bacteria, yeasts, and molds.

MATERIALS AND METHODS

Obtusastyrene (V) and related cinnamylphenols were synthesized by the facile condensation of cinnamyl alcohol with the appropriate parent phenol in dilute aqueous acetic or citric acid solution (6, 7); e.g.,

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\begin{align*}
\text{HO} \quad & + \quad \text{HOCH}_2-\text{CH}=\text{CH} \quad \rightarrow \\
& \quad \underset{\text{H}^+}{\text{V}}
\end{align*}
\]

oxidation of the cinnamyl alcohol-methoxyquinol condensation products gave pure 4-methoxydalbergione, II, identical with the natural product, and the isomeric cinnamylquinone, IX (6).

\[
\begin{align*}
\text{MeO} \quad & + \quad \text{HOCH}_2-\text{CH} \quad \rightarrow \\
& \quad \underset{\text{H}^+, [O]}{\text{II} \quad + \quad \text{MeO}}
\end{align*}
\]

The synthetic dihydrobenzofuran (X), which is isomeric with natural products of type IV, was prepared by acid condensation of methoxyquinol with anethole epoxide. For comparative purposes the synthetic quinone, XI, and the natural neoflavonoid, brazilin XII, were also included in this study. Brazilin does not occur in Dalbergia species, but it has been reported to have some antimicrobial activity.

All compounds were initially tested at a concentration of 500 µg/ml (w/v). Plates were prepared by adding a measured amount of the candidate compound (in an appropriate solvent, i.e., acetone, ethanol, or water) to 10 ml of sterilized medium, mixing thoroughly, pouring into plastic petri dishes (60 by 15 mm) and allowing the gel to set. The plates were then inoculated with the test organisms. In the case of bacteria and yeasts, the inoculation was done by the replica plating technique of Lederberg and Lederberg (9), applying nine bacteria or seven yeasts on each plate. In the case of molds, drops of homogenized culture were placed on the surface of the plates, applying three or four molds per plate. The media used were: plate count agar (Difco; pH 7.0) for bacteria and potato dextrose agar (Difco; pH 5.6) for yeasts and moulds. Control plates were also prepared containing the media plus the same solvent used for the candidate agents and inoculated with the same organisms. The plates were incubated at 28°C for 1 to 5 days and evaluated by comparison with the controls.

RESULTS AND DISCUSSION

The above compounds were screened initially at concentrations of 500 µg/ml for activity against 27 representative microorganisms. Obtusastyrene (V), 6-cinnamylresorcinol (VIa), and 6-cinnamylpyrogallol (VIIa) completely inhibited the growth of the gram-positive bacteria and the growth of all species of fungi tested. Growth of *Alcaligenes faecalis* and of *Escherichia coli* was also inhibited, but other gram-negative bacteria (*Pseudomonas aeruginosa, Salmonella typhimurium,* and *Serratia marcescens*) were not appreciably affected. 6-Cinnamyl-3-methoxyquinol (VIIIa) inhibited growth of gram-positive bacteria and of some yeasts, but it was ineffective against molds.

In contrast to the earlier report, 4-methoxy-dalbergione (II) and the isomeric cinnamylquinone (IX) proved to be generally inactive in this replica plating bioassay. These quinones and the synthetic dihydro analogue XI inhibited growth of *C. tropicalis* and a limited number of other yeasts but were otherwise ineffective against bacteria and fungi. The reason for the discrepancy between these observations and those reported earlier for II are not presently known, and more extensive screening of these quinones by other methods is being planned.

In contrast to the pronounced inhibitory effect of these cinnamyl derivatives, both phenol and resorcinol were inactive against bacteria, yeasts, and most molds at 500 µg/ml. Brazilin (XIII) inhibited the growth of *B. cereus, Streptococcus lactis,* and three species of yeasts, but it (and the...
The approximate minimal inhibitory concentrations of obtusastyrine and 6-cinnamyl resorcinol at different pH values were measured. The activity of obtusastyrine was not appreciably influenced by a change of pH, and, in the pH range of 4 to 7, complete inhibition of growth of gram-positive bacteria (Bacillus, Sarcina, Staphylococcus, and Streptococcus species) and of gram-negative bacteria (A. faecalis and E. coli) occurred with concentrations of obtusastyrine as low as 12 to 25 µg/ml and 25 to 50 µg/ml, respectively. At pH 3 to 6, the growth of 10 yeasts and molds was inhibited by 12 to 50 µg of obtusastyrine per ml, although with Aspergillus species higher concentrations of obtusastyrine (50 to 100 µg/ml) were required for complete inhibition. The activity of obtusastyrine is decreased by increasing hydroxylation of the aromatic nucleus. Thus, 6-cinnamyl resorcinol, Vla (and 6-cinnamyl pyrogallol), is generally about two to four times less active than obtusastyrine against these microorganisms.

On the basis of this investigation, it would appear that obtusastyrine is a potent, natural antimicrobial agent which may prove to be useful in a variety of applications. Thus, for example, a number of synthetic phenols, such as o-phenylphenol, heptyl-4-hydroxybenzoate, and propyl-4-hydroxybenzoate, are currently used or have been suggested as preservatives for some foods. In the course of this investigation, the inhibitory activities of these compounds were determined under the same conditions used for obtusastyrine (bacteria, pH 7.0; fungi, pH 5.6). As shown in Table 1, obtusastyrine is more effective against a wider variety of microorganisms and, in most cases, at significantly lower concentrations than any of these synthetic preservatives. Further studies on the antimicrobial action of obtusastyrine and structural variants are in progress.

### Table 1. Minimal inhibitory concentrations (µg/ml) of antimicrobials against bacteria and fungi

| Microorganisms | Obtusastyrine | O-phenyl phenol | Heptyl paraben | Propyl paraben | Potassium sorbate |
|----------------|---------------|----------------|----------------|----------------|-----------------|
| Bacillus cereus 2006 | 25 | 100 | 12 | 400 | —a (800)b |
| Sarcina lutea | 25 | 200 | 12 | 400 | — (800) |
| Staphylococcus aureus SG8A | 25 | 200 | 12 | 400 | — (800) |
| Streptococcus lactis | 25 | 100-200 | 12 | 400 | — (800) |
| Alcaligenes faecalis B170 | 50 | 100 | — | 400 | — (800) |
| Escherichia coli ML30 | 50 | 100-200 | — | 400 | — (800) |
| Pseudomonas aeruginosa 111 | — | — | — | — | — (800) |
| Salmonella typhimurium Tml | — | 200 | — | — | — (800) |
| Serratia marcescens | — | — | — | — | — (800) |
| Zygosaccharomyces japonicus C124 | 12 | 100-200 | 12-25 | 200 | 800 |
| Candida tropicalis C147 | 12 | 100 | 12-25 | 150 | 200-400 |
| Pichia chodatii C238 | 25 | 100 | — | 400 | 800 |
| Hansenula anomala | 50 | 200 | — | 400 | 800 |
| Saccharomyces cerevisiae Y44 | 25 | 100-200 | 100 | 200 | 800 |
| Torula utilis NRRL Y660 | 50 | 200 | 25 | 200 | 800 |
| Aspergillus flavus NRRL 3145 | 100 | 100 | — | 200 | — |
| A. niger A-7705 | 50 | 100 | — | 200 | — |
| Penicillium chrysogenum 52 | 50 | 50 | — | 200 | — |
| Rhizopus senti NRRL 2868 | 6 | 100 | 25 | 200 | 200 |
| Botrytis cinerea NRRL 3492 | 25-50 | 12-25 | 50-100 | 100 | 200 |
| Byssoschlamys fulva NRRL 3493 | 25 | 50 | — | 200 | — |
| Alternaria sp. | 25 | 50 | 50-100 | 100 | 400 |

*a* Inactive at 500 µg/ml.  
*b* Potassium sorbate was tested in amounts as high as 800 µg/ml.

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**Dihydrobenzofuran, X** was generally ineffective against a wide variety of organisms.

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