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Antimicrobial Properties of Natural Phenols and Related Compounds

I. Obtusastyrene

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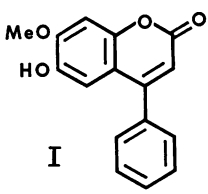
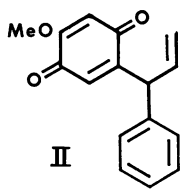
Obtusastyrene (4-cinnamylphenol) displays effective antimicrobial activity in vitro against a variety of gram-positive bacteria, yeasts, and molds. The activity of obtusastyrene is not appreciably affected by *p*H, and its minimal inhibitory concentrations, 12 to 25 μg/ml for bacteria and 12 to 100 μg/ml 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 sisoo* 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,



I



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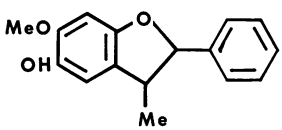
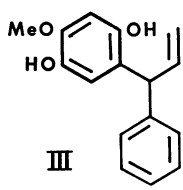


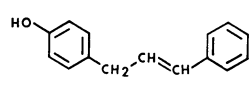
IV



III

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 neoflavanoids (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. Clinnamylphenols, which



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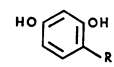
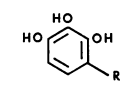
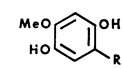


VI a, R=$-CH_2CH=CHC_6H_5$
b, R=H



VII a, R=$-CH_2CH=CH-C_6H_5$
b, R=H



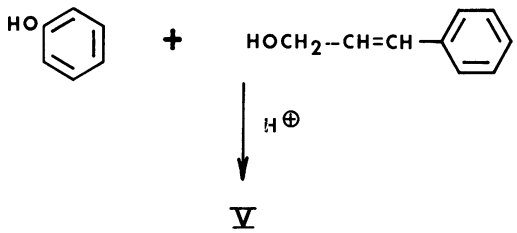
VIII a, R=$-CH_2CH=CH-C_6H_5$
b, R=H

are structurally isomeric with the neoflavanoids of structural type III, co-occur with these neoflavanoids 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.

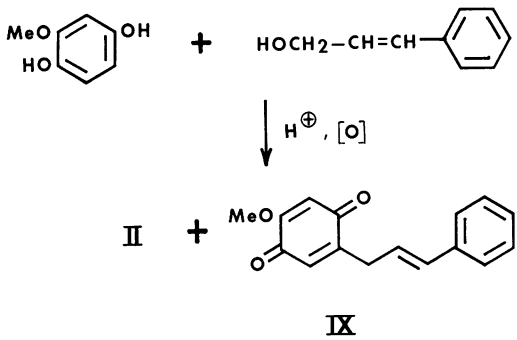
MATERIALS AND METHODS

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.,



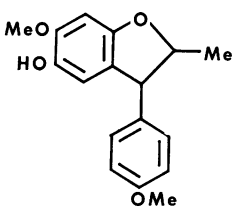
V

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

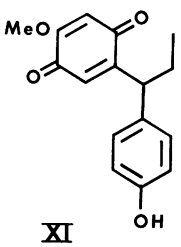


IX

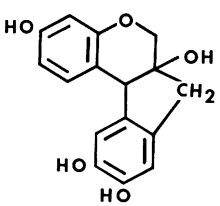
The synthetic dihydrobenzofuran (X), which is



X



XI



XII

isometric 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 neoflavanoid, 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; *p*H 7.0) for bacteria and potato dextrose agar (Difco; *p*H 5.6) for yeasts and molds. 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-methoxydalbergione (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

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

Microorganisms	Obtusastyrene	*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 chodati 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
Byssochlamys 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.

dihydrobenzofuran, X) was generally ineffective against a wide variety of organisms.

The approximate minimal inhibitory concentrations of obtusastyrene and 6-cinnamyl resorcinol at different *p*H values were measured. The activity of obtusastyrene was not appreciably influenced by a change of *p*H, and, in the *p*H 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 obtusastyrene as low as 12 to 25 μg/ml and 25 to 50 μg/ml, respectively. At *p*H 3 to 6, the growth of 10 yeasts and molds was inhibited by 12 to 50 μg of obtusastyrene per ml, although with *Aspergillus* species higher concentrations of obtusastyrene (50 to 100 μg/ml) were required for complete inhibition. The activity of obtusastyrene is decreased by increasing hydroxylation of the aromatic nucleus. Thus, 6-cinnamyl resorcinol, VIa (and 6-cinnamyl pyrogallol), is generally about two to four times less active than obtusastyrene against these microorganisms.

On the basis of this investigation, it would appear that obtusastyrene 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 obtusastyrene (bacteria, *p*H 7.0; fungi, *p*H 5.6). As shown in Table 1, obtusastyrene 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 obtusastyrene and structural variants are in progress.

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