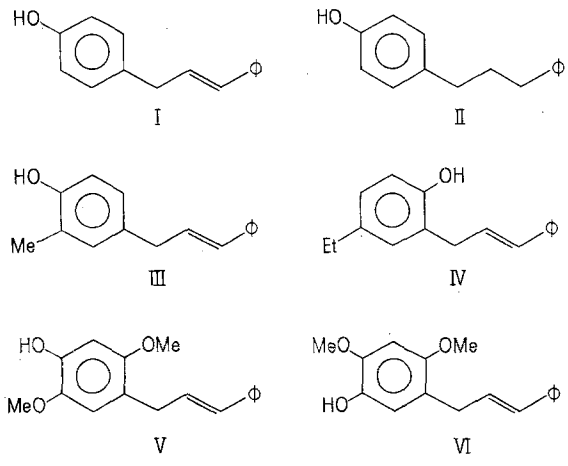
The Antialgal Activity of Obtusastyrene and Related Cinnamylphenols

Timbers of various *Dalbergia* species are resistant to attack by insects, fungi, and the larvae of marine boring organisms[1,2]. Recent chemical studies have established the presence in these species of a wide variety of unusual phenolic and quinonoidal neoflavanoids and cinnamylphenols, including obtusastyrene I, violastyrene V, and isoviolastyrene VI[3–6]. Obtusastyrene and related cinnamylphenols have been shown to inhibit growth of bacteria and fungi[7], to be sporostatic with *Bacillus megaterium*[8], and to be highly toxic to the larvae of marine boring organisms (BULTMAN, 1972, private communication), indicating that these and similar substances are probably the primary protective constituents of these woods. In view of these pronounced microbiocidal properties it was of interest to determine the effects of these phenols on the growth of algae. The observations briefly described in this communication demonstrate that at low concentrations obtusastyrene and related synthetic cinnamylphenols effectively inhibit the growth of *Chlorella pyrenoidosa* and *Scenedesmus obliquus*. The effectiveness of these compounds is dependent on the cinnamyl (or dihydrocinnamyl) substituent, since phenol itself does not appreciably affect the growth of these organisms, even at higher concentrations. The algicidal activities of simple cinnamylphenols of types I-IV appear to compare favorably with those reported for a variety of antibiotics[9–12].



Chlorella pyrenoidosa ATCC 11469 and *Scenedesmus obliquus* ATCC 11457 were grown (5 days) at room temperature in Knoop's solution with Hunter's micronutrients and $KHCO_3$ (100 mg/l) in a Fernback flask (2800 ml)[13] irradiated by fluorescent light of intensity 2.5 mWatts per cm². The filtered algae were reconstituted in a small volume of nutrient solution and centrifuged at $700 \times g$ for 5 min. The top dark-green layer was reconstituted in nutrient solution as inoculum. Algal density in the inoculum was determined by extraction of an aliquot with methanol-chloroform-water (MCW, 6-4-1 v/v for 1 h and spectral analysis of the chlorophyll content[14]. Extraction vessels were sheathed in aluminum foil to prevent photochemical loss of chlorophylls.

The pure cinnamylphenols (I–VI) were synthesized by phenol cinnamyl alcohol condensations in aqueous acid solutions[15,16]. Stock solutions of the cinnamylphenols were prepared in 95% ethanol; 0.5 ml of the appropriate stock solution was added to 100 ml test culture of algae in a 250 ml Erlenmeyer flask to give a concentration of cinnamylphenol of 0.1–120 μg/ml. Cultures were kept at room temperature and irradiated by fluorescent light of intensity 2.5 mWatts per cm². At intervals the algae were collected as previously described and the chlorophyll contents determined by absorbance measurements at 664 nm

Beet slices (*Beta vulgaris*), 2×13 mm, were washed in flowing water for 30 min. 30 disc samples were then bathed in 50 ml solutions of obtusastyrene and dihydroobtusastyrene (30–60 μg/ml). At intervals the betacyanin content of the aqueous solutions were measured by the absorbance at 535 nm.

Obtusastyrene I inhibits the growth of *Chlorella pyrenoidosa*, its inhibitory activity being dependent on the initial algal concentration. Thus, the concentration of obtusastyrene required for complete growth inhibition progressively increases with increasing algal population density. With low algal densities the percentage of viable algal cells recovered after 22 h in the presence of obtusastyrene (30 μg/ml) was reduced to zero (Table).

Effect of inoculum density of *Chlorella pyrenoidosa* ATCC 11469 on the algicidal activity of obtusastyrene[a]

Absorbance (664 nm) of the inoculum	Absorbance at 664 nm after 22 h	Original inoculum (%)
110	65.4	59.5
55	18.2	33.2
11	1.4	13.1
5.5	0.23[b]	4.2
1.1	0.30[b]	—
0.55	0.20[b]	—

[a] Obtusastyrene concentration = 30 μg/ml. Time = 22 hours. Light intensity = 2.5 mwatts/cm². [b] These cultures were colorless.

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The approximate minimal inhibitory concentrations of obtusastyrene, dihydroobtusastyrene II, 2-methyl-4-cinnamylphenol III, and 2-cinnamyl-4-ethylphenol IV, were determined by using a low density algal inoculum, containing 7.6 absorbance units at 664 nm in 100 ml culture solutions. After 44 h the chlorophyll content of recovered algae was measured. Control cultures gave 40 absorbance units. Compounds I, II, III, and IV slightly inhibited growth at concentrations as low as 0.1 µg/ml. As shown in Figure 1, however, complete inhibition of growth occurred with each of these compounds at concentrations of 5–15 µg/ml. The inhibitory effect of these cinnamylphenols was also observed with *Scenedesmus obliquus*. While a control culture of this organism (initial density 5.5 absorbance units in 100 ml) increased to give 143 absorbance units in 110 h, growth in the presence of obtusastyrene at 20–60 µg/ml was completely inhibited, these cultures becoming colorless with the loss of all viable cells.

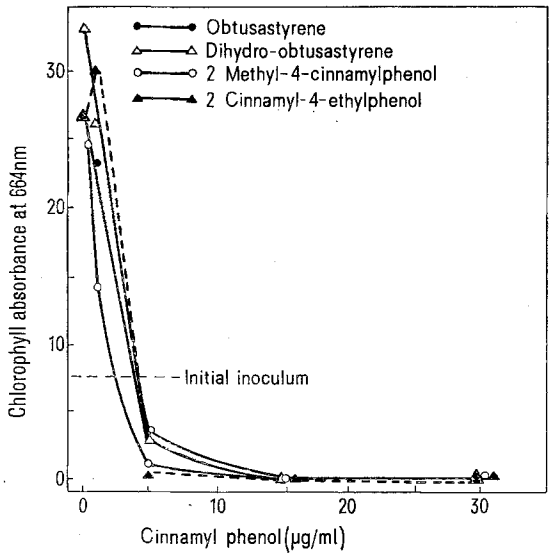

Fig. 1. Chlorophyll absorbance measured in *Chlorella pyrenoidosa* ATCC 11469, after treatments with the cinnamyl-phenols for 44 h. The control culture had chlorophyll absorbance of 40 units after 44 h.

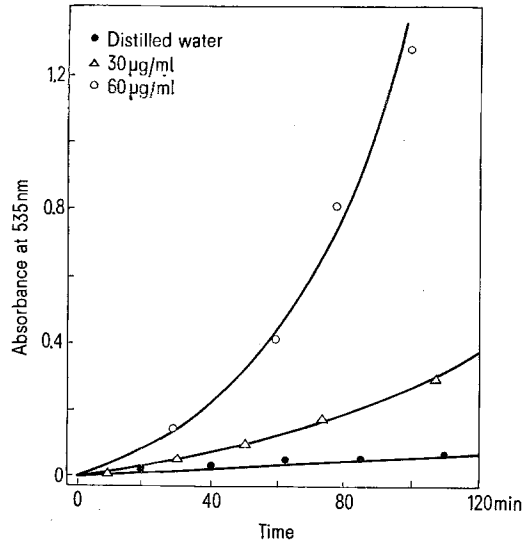

Fig. 2. The rate of betacyanin leakage from 30 discs (5.9 g) of *Beta vulgaris* root into 50 ml solution after treatment with obtusastyrene.

The antialgal properties of the 6 cinnamylphenols I–IV were compared with that of phenol itself as a standard against a dense initial population of *C. pyrenoidosa* (108 absorbance units in 100 ml test medium). Phenol, violastyrene and isoviolastyrene were not inhibitory at concentrations of 100–400 µg/ml. The cinnamylphenol I–IV were not inhibitory at concentrations of 5 µg/ml with this algal density. At concentrations of 15–30 µg/ml, however, I–IV rapidly killed the algal cells. After 20–45 h of exposure they precipitated in brown aggregates, and growth did not occur when these aggregates were washed and transferred to fresh nutriten solutions.

Although the mode of action of the cinnamylphenols on algal cells is uncertain, they do appear to rupture the cellular membranes with subsequent leakage of the cellular constituents. JUDIS[17] has previously suggested that the toxicity of *p*-chloro-*m*-xylenol to *Escherichia coli* is due to a similar lysis of the bacterial cell, and observations on the effects of obtusastyrene on red beet tissue support this interpretation. Thus, when discs of red beet tissue were suspended in aqueous solutions of obtusastyrene, the water-soluble, red betacyanin pigments were rapidly leached into the surrounding medium. As shown in Figure 2, beet discs treated with aqueous obtusastyrene (60 µg/ml) lost 38 % of their total betacyanin pigments to the external solution within 100 min. Significant loss of betacyanin pigment did not occur when the beet tissue was suspended in distilled water.

Zusammenfassung. Der phenolische Inhaltsstoff von *Dalbergia* Arten, Obtusastyrol und verwandte, zimtsäureartige Phenole hemmen das Wachstum der Algen *Chlorella pyrenoidosa* und *Scenedesmus obliquus*.

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The Toxicity of Two Synthetic 3-Substituted Furan Carbamates

Poisoning of livestock through consumption of foliage of various species of plants within the family Myoporaceae is well known in Australia and New Zealand[1]. The toxic compounds involved are furanosesquiterpene essential oils, the best known of which is ngaione (I)[2,3]. Ngaione is of

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