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Influence of Substituted Phenols on the Growth of Hydrilla

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INTRODUCTION

Previous research has established that leachates of lake sediments have the capability of inhibiting the growth of hydrilla (*Hydrilla verticillata* Royle). Several lakes (including Lake Starvation) in northwest Hillsborough County had sediments derived from cypress (*Taxodium distichum* (L.) Richard), and leachates of these sediments inhibited the growth of hydrilla in the laboratory (5, 8). Sediments from other lakes were studied by Barko and co-workers, and these sediments, typically having high organic content, also appeared to inhibit the growth of hydrilla, but whether the action was the result of a leachate or effect on nutrition or both would not have been distinguished by the experiments described (1, 2).

In addition, Lake Kerr near Ocala, Florida also appeared to be an example of a lake with high-organic substrate in which hydrilla disappeared for a time (7).

More recently, we have been able to identify a hydrilla-inhibiting component of leachates, lake water, and other natural waters (9). The peak was identified using high performance liquid chromatography (HPLC), and an inverse correlation was found between the intensity of the hydrilla inhibitor and the amount of hydrilla in the Withlacoochee River in Pasco County, Florida.

Our interest in allelopathic chemicals, i.e., substances produced by plants that exert an influence on other plants (6, 10, 13) suggested an examination of soil organic acids to measure their influence in comparison with the leachate from Lake Starvation. These substances are known to have allelopathic activity on other plants, and the concentrations were selected to be about 0.4 ppm (as organic carbon), the concentration at which Lake Starvation leachate is active.

MATERIALS AND METHODS

Hoagland's solution (10%) was prepared according to Steward and Elliston (11) and enriched with $KHCO_3$ to provide 5 ppm of inorganic carbon. Eleven substituted phenols, listed in Figure 1, were obtained from Aldrich Chemical Co.[2] Each phenol was added to 3.0 L of the medium to provide a final concentration of 5×10^{-5} *M*. Hydrilla sprigs (about one gram fresh weight) were placed in 500-ml flasks, which were filled with medium (six flasks for control, and six containing the phenol for test). The flasks were closed with rubber stoppers and inverted under lights (84-87 $\mu E/m^{-2}/sec$) programmed for a 12-hour light-dark photoperiod.

After two weeks, the hydrilla sprigs were re-weighed and the changes in weight were recorded and averaged.

Acid dissociation constants (as pK_a values) of substituted phenols were obtained from standard compilations (3, 4).

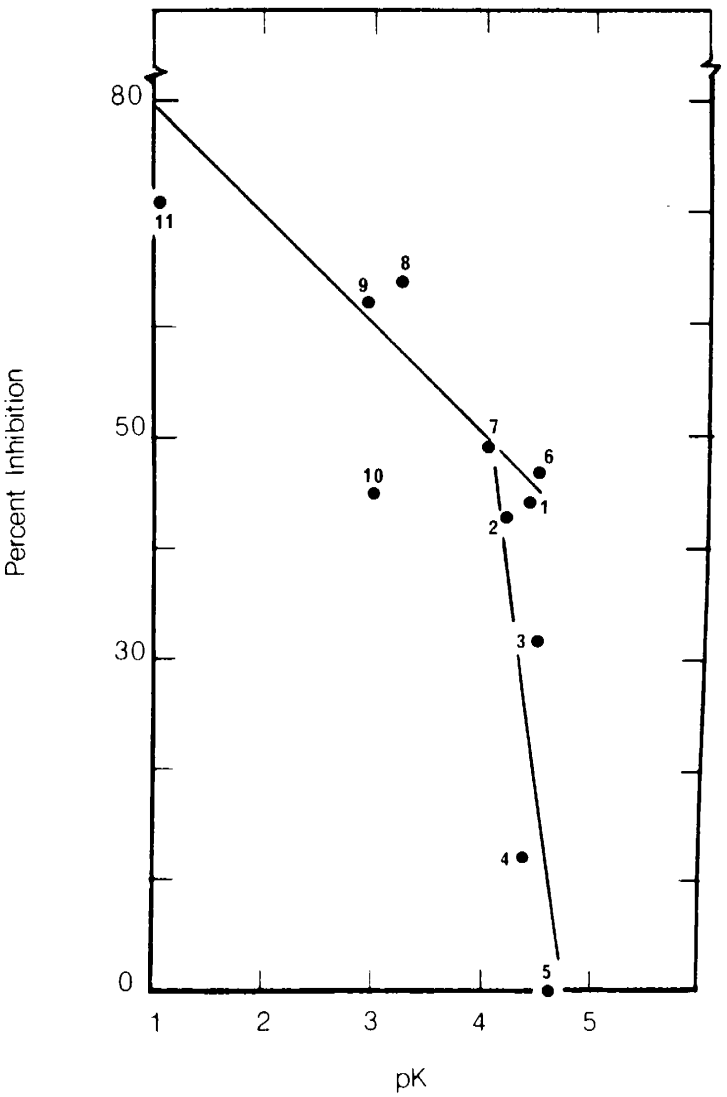

Figure 1. Percent inhibition of freshweight change of hydrilla as a function of pK_a of substituted phenols: (1) *m*-coumaric acid, (2) ferulic acid, (3) vanillic acid, (4) gallic acid, (5) caffeic acid and the following substituted benzoic acids, (6) 3,4-dihydroxy-, (7) 3,5-dihydroxy-, (8) 2,4 dihydroxy-, (9) 2,3-dihydroxy-, (10) 2,5-dihydroxy-, and (11) 2,6-dihydroxy-. Each value represents the mean of five replicates.

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RESULTS

Conditions for the experiments were consistent with those described previously (5), and test results were compared with control experiments for which a minimum of 20% increase in fresh wet weight was observed.

The concentrations of substituted phenols and known allelochemicals used were 5×10^{-5}. This is less than some concentrations used by Sutton (12), but this particular concentration is useful for two reasons. First, it corresponds to about 0.4 ppm as organic carbon, which is the concentration at which significant inhibition of hydrilla growth was observed previously (5). Second, it corresponds to the concentration of allelochemicals that have been isolated from soils, i.e., 49 μm in soil solution (13).

The effectiveness of the added chemical was expressed as percent inhibition, was taken as being equal to $[1-(\Delta FW)_t/(\Delta FW)_c]100$. Here ΔFW refers to mean change in fresh weight for five samples, and the subscripts refer to test and control respectively.

We sought a comparison of our results and those reported (12) for inhibition of hydrilla tuber sprouting. This was unsuccessful for a couple of reasons: the concentrations used by Sutton (12) were significantly greater in most instances, and there is a limited overlap in the compounds investigated. For example, in our study, 5×10^{-5} *M* salicylic acid had no inhibitory effect, but 5×10^{-3} *M* solutions produced a 13% weight loss. The data from Sutton (12) indicate a linear correlation between percent sprouting and log concentration of salicylic acid ($r = 0.966$; $0.05 > P > 0.025$). Thus, we suspect that the inhibition of tuber sprouting as well as inhibition of hydrilla growth could be related to the concentration of inhibitor used, and, given similar concentrations, better agreement might be observed.

We sought a correlation between chelating tendencies and percent inhibition, but found no correlation between the effect of substituents and percent inhibition for a series of closely related substituted benzoic acids (Figure 1, compounds 6-11).

Third, the truly successful correlation was between percent inhibition and the acidity of the substituted phenol. The acidity was expressed as pK_a values (the negative logarithm of the acid dissociation constant). Percent inhibition correlated with pK_a (Figure 1) and two patterns were observed. The majority of the compounds were substituted benzoic acids, and these fall on a single line and have a correlation coefficient, r, of -0.82($N=8$; $0.01>P>0.001$). Five other organic acids fall into a second group, and these did not show a significant correlation between percent inhibition of hydrilla fresh weight change and pK_a.

These results indicate that while organic acids can have several properties that may account for allelopathy, including chelation, redox properties, and acidity (10), in the present instance, acidity appears to be a significant parameter, not only for inhibition of hydrilla fresh weight increase, but for tuber sprouting as well. Percent tuber sprouting was related to the acidity (as pK_a) for some six substituted phenols (caffeic, gallic, *m*-coumaric, salicylic, *p*-hydroxybenzoic, and vanillic acids). Using data from Sutton (12), we found a significant correlation between percent sprouting and pK_a for m*M* concentrations of phenol ($r = -0.964$; $P<0.001$).

Two additional comments should be made. First, the results are inevitably based upon a limited range of acidity for structurally related phenols. For our compounds, the acidity covered a thousand fold range; the acids selected from Sutton (12) covered an acidity range of $10^{1.5}$. Second, at the concentrations used, none of the substances used was as effective as the naturally occurring inhibitor (5).

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