BETA-ALANINE PROTECTION OF YEAST GROWTH AGAINST THE INHIBITORY ACTION OF SEVERAL CHLORINATED ALIPHATIC ACID HERBICIDES

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Chloro-substituted acetic, propionic, and isobutyric acids constitute a class of compounds which in recent years have demonstrated considerable potential use for the control of a number of weedy grasses in several weed-crop situations. The structural similarity of these compounds to propionic and acetic acids makes it seem probable that their phytotoxicity might result, in part, from interference with pantothenic acid synthesis. Such an effect has been demonstrated with the two unchlorinated acids by King and Cheldelin (1) using yeast as a test organism. Their results led to the conclusion that “propionate inhibits growth by competing with β-alanine for attachment within the yeast cell, thereby preventing the coupling of the pantothentic acid moieties.”

In the experiments reported herein, an investigation has been made of the interactions of β-alanine and several chlorinated aliphatic acid herbicides on the growth of yeast using the technique of King and Cheldelin.

Saccharomyces cerevisiae, isolated from the center of a cake of baker’s yeast (Fleischmann’s) was inoculated onto 2% agar nutrient medium containing the same major inorganic salts as used by King and Cheldelin (1) but with 2.5 g yeast extract (Difco) in lieu of their minor elements and vitamin supplements. A liquid subculture was prepared for each experiment in a 125-ml flask containing 30 ml of liquid medium (1) to which was added 100 μg β-alanine in a 10-ml volume of water. The flask was inoculated with yeast cells from an agar slant and incubated for 24 hours at 32°C. The resulting culture was diluted 1:50 with nutrient solution to constitute the experimental inoculum. Growth experiments were carried out under aseptic conditions in 18 x 150-mm Pyrex test tubes. Each tube contained 5 ml of pH 4.8 nutrient, and 1 ml each of β-alanine solution, herbicide solution and diluted liquid subculture of yeast. After 17 hours of incubation at 32°C, turbidimetric growth readings were taken in a Klett-Summerson colorimeter using a blue filter. All experiments were run in triplicate and repeated with three or more cultures.

In the absence of exogenous β-alanine, turbidity readings of 40 to 60 but occasionally as high as 120 Klett-Summerson units were obtained. Addition of 0.2 to 200 μg of β-alanine per tube increased growth to 160 to 180 units. The minimum amount of metabolite required for maximum growth was generally 0.2 μg per tube, and 200 μg appeared to be at least a 10-fold excess for maximum protection against herbi-

cides. These two concentrations of β-alanine were consequently selected for use throughout the present studies.

The herbicidal chemicals, 2 obtained as purified acids, were dissolved in sterile water and the solutions were adjusted to the pH of the medium with sterile NaOH. These solutions were added aseptically to the sterile culture tubes immediately before inoculation. The acids used in these studies, their abbreviated designations, and purities were as follows: 2,3,3′-trichloroisobutyric acid (TCIB), 90% [impurities of 2,3,3′-trichloroisobutyric and 2,3-dichloroisobutyric acids]; 2,3-dichloroisobutyric acid (DCIB), chemically pure; 2,2,3-trichloropropionic acid (TPA), 99+%; 2,2-dichloropropionic acid (dalapon), 99+%; and trichloroacetic acid (TCA), USP.

Evidence was obtained for protection by β-alanine against each of the five herbicides tested. The most definite suggestion of competition between herbicide and metabolite was observed with the chlorinated isobutyric acids. A positive test for a competitive relationship between TCIB and β-alanine (fig 1) was obtained in an analysis of data by the method of Lineweaver and Burk (2). Pantothenic acid was also found to be effective as an antagonist of TCIB.

In comparative studies TCIB was found to be slightly more inhibitory than DCIB. In the presence of 0.2 μg of β-alanine TCIB caused 50% inhibition at 3 to 6 x 10^{-4} M, while ca. 10^{-3} M DCIB was required to produce the same degree of inhibition. At the higher metabolite concentrations (200 μg of β-alanine or 10 μg calcium pantothenate), TCIB at 5 to 8 x 10^{-2} M reduced growth by 50% and at 10^{-2} M generally gave complete inhibition. DCIB, however, at concentrations below 10^{-2} M consistently caused less than 25% inhibition in the presence of the higher concentration of β-alanine. At its high concentrations, therefore, β-alanine more readily overcame the inhibitions caused by DCIB than those produced by TCIB.

Evidence for a protective action by β-alanine against the growth inhibitory actions of unchlorinated isobutyric and n-butyric acids was observed with some cultures; however, results between experiments were

1 The experimental compounds used in these studies were supplied by research personnel of the following companies: American Chemical Paint Company, Ambler, Pa.; American Cyanamid Company, Stamford, Conn.; Dow Chemical Company, Midland, Michigan; Rohm and Haas Company, Philadelphia, Pa.

2 Received August 13, 1957.
too variable to justify quantitative comparisons. The variability appeared to reside in differential requirements of cultures for an exogenous supply of \( \beta \)-alanine. In no instance did the unchlorinated compounds inhibit growth at concentrations as low as those used for the chlorinated isobutyrate.

A recently procured sample of dalapon, 99+\% pure, was compared directly with propionic acid, as shown in figure 2. Although \( \beta \)-alanine offered some protection against this herbicide, the effect was not as pronounced as that observed with the unchlorinated parent acid. Chlorination of propionic acid obviously decreased the ability of the molecule to compete with \( \beta \)-alanine. A 2nd sample of dalapon which was studied had been obtained as the purified acid and allowed to age on the shelf for three years. This sample, which had apparently undergone some decomposition at the time of use, gave a positive competitive inhibition test by the Lineweaver-Burk method and closely approached the inhibitory effectiveness of propionic acid. Although this sample was more inhibitory to growth of yeast than the undeteriorated sample, a similar increase in phytotoxic activity could not be demonstrated against higher plants. Foliar spray application of the deteriorated sample to 8 crop and weed species in the greenhouse showed no evidence that decomposition had in any way altered the herbicidal properties of the sample.

Relatively high concentrations of the trichloropropionic and trichloroacetic acids were required for inhibition of yeast growth. Only slight protection by \( \beta \)-alanine was observed against inhibitor concentrations of \( 10^{-2} \) to \( 10^{-1} \) M. Data are shown in table I for a common concentration of TPA, TCA, and acetic acid. At low \( \beta \)-alanine concentrations the unchlori-

**TABLE I**

<table>
<thead>
<tr>
<th>Beta-alanine Antagonism of Inhibitors of Yeast Growth</th>
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<tr>
<td><strong>INHIBITOR (0.05 M)</strong></td>
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<tr>
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<tr>
<td>2,2,3-Trichloropropionic acid</td>
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<tr>
<td>Trichloroacetic acid</td>
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<tr>
<td>Acetic acid</td>
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</tbody>
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* Amounts in 8 ml of solution.
minated acetic acid was more inhibitory than TCA and was more readily antagonized by \( \beta \)-alanine.

The inability of high concentrations of \( \beta \)-alanine completely to reverse the inhibitions produced by this class of compounds suggest that physical or chemical processes other than the \( \beta \)-alanine - pantothenic acid reaction(s) may be involved in the mechanism of action. Nevertheless, the utilization of \( \beta \)-alanine appears to be the most sensitive metabolic process involved under the experimental conditions employed; the enzymes involved in pantothenic acid synthesis should be investigated as a possible important site of action of these herbicidal compounds. Further investigations on this mechanism of action and its relative importance in species selectivity are in progress and will be the subject of future reports from this laboratory.

A PAPER CHROMATOGRAPHIC SEPARATION OF GIBBERELLIC ACID AND GIBBERELLIN A

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Gibberellin A and gibberellic acid have been separated on buffered partition columns by Stodola et al (1). We now report a paper chromatographic method for separation of these plant growth stimulators.

Takahashi et al (2) reported the use of the upper phase from various mixtures of benzene, acetic acid, and water for paper chromatography of a preparation of gibberellin A using BPB (which we assume to be bromphenol blue) as an indicator. They reported gibberellin A to be immobile in all these mixtures. Phinney et al (3) using four different solvent systems reported that they were unable to separate the gibberellins in any of the four systems.

We have found that the upper phase of a mixture of 10 vol of thiophene-free benzene, 2.5 vol of glacial acetic acid, and 5 vol of deionized water will completely separate gibberellin A and gibberellic acid on a descending chromatogram under conditions which permit sufficient solvent flow.

Samples containing 50 to 200 \( \mu \)g of the gibberellins are applied near one end of a sheet (19\times46 \text{ cm}) of Schleicher and Schuell No. 508 or Munktells, Cramer-Tiselius electrophoretic filter paper. The opposite end of the sheet is serratated to permit solvent to drip off the end more freely, thus allowing a maximum flow. A 10- to 30-minute equilibration of the spotted paper and a descending development of at least 36 hours at 22 to 24\degree \text{ C} are carried out in a glass jar (12\times24 \text{ in}). The jar is fitted with a rack holding a 300-ml stainless steel trough and with a glass lid having a hole for addition of solvent. The inside wall of the jar is lined with a filter paper cylinder, the bottom edge of which is immersed in the lower phase of the solvent mixture in the bottom of the jar. The liner should be allowed to become completely wet before using the jar for chromatography. Once prepared, a jar can be used many times.

After development the chromatogram is removed from the jar and is dried at room temperature. When dry, it is sprayed lightly with a 0.5 % aqueous solution of potassium permanganate. Gibberellin A and gibberellic acid are revealed as yellow spots on a reddish-purple background. The spots are quickly sprayed with more permanganate and the chromatogram is immediately washed in running tap water to remove the unreacted permanganate. The locations of the gibberellins are detected as permanent brown spots on an almost white background. Figure 1 is a photograph of a typical chromatogram treated in the above manner showing the separation of the gibberellins achieved with this solvent system.

Gibberellic acid may also be detected on chromatograms not reacted with permanganate, or sprayed only very lightly with permanganate, by its reaction with sulfuric acid to produce a fluorescence under ultraviolet light. This may be done with concentrated sulfuric acid as reported by Phinney et al (3) or by rapidly dipping the paper in 70 % sulfuric acid and quickly laying it flat on a glass plate. A chromatogram treated with the 70 % sulfuric acid shows yellow-green fluorescent spots instead of the blue fluo-

\textbf{Summary}

Inhibition of yeast growth by chloro-substituted isobutyric, propionic, and acetic acids has been observed. An exogenous supply of \( \beta \)-alanine has been shown to produce varying degrees of protection against these inhibitions.

The results obtained suggest that these herbicides interfere with synthesis of pantothenic acid, presumably by competition with \( \beta \)-alanine.

\textbf{Literature Cited}
