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Enzymatic Synthesis of Gallic Acid Esters

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ABSTRACT

Esters of gallic acid were synthesized by enzymatic means using tannase from *Aspergillus niger*. Alcohols ranging from C_1 through C_{12} and diols from C_3 through C_6 were tested and found to form esters in the presence of the enzyme.

Index Entries: Enzymatic synthesis, of gallic acid esters; synthesis, enzymatic, of gallic acid esters; gallic acid esters, enzymatic synthesis of; esters, enzymatic synthesis of gallic acid.

INTRODUCTION

Antioxidants are compounds used in many industries because they increase the life and temperature range of a variety of different products by prevention of atmospheric oxidative deterioration.

Deterioration involves a complex series of chemical events. These events can cause breakage of chemical bonds, cross-linking, and free radical formation that eventually leads to changes in strength, clarity, electrical properties, surface characteristics, color, taste, and smell.

Atmospheric oxidation is the primary cause of deterioration of fats and oils in foods. Although generally only small amounts of the fats and oils are broken down, their byproducts can render foods unpalatable and unfit for marketing.

One of the antioxidants used in the food industry is propyl gallate. It is used in fats and oils as well as in some beverages. Its fat solubility is rather limited owing to the presence of the propyl group. It is possible that gallic acid esters of higher alcohols might be more useful in fats and oils because of high solubility. It is also possible that such compounds might find their way into other oil-related products outside the food industry.

The synthesis of gallic acid esters requires heat and acid. In addition the product must be purified from the degradation products formed during synthesis. It was my hope that the biological synthesis of propyl gallate could be accomplished with no degradation. It was also my hope that I could synthesize other alcohol esters of potential value.

The enzyme tannase from *Aspergillus niger* is capable of hydrolyzing propyl gallate as well as several other gallic acid esters (*1–6*). Since this enzyme is capable of hydrolysis, it should be possible to synthesize these esters under proper conditions.

Recently there have been several reports on the synthesis of fatty acid esters utilizing lipases (*7–9*). In all these studies the enzyme is immobilized and utilized for synthesis in an organic solvent system.

In the case of gallic acid the problem is rather simplified since the gallic acid and its esters are soluble in the alcohol of the corresponding ester. Thus for these studies the immobilized enzyme was added to a solution of gallic acid dissolved in the appropriate alcohol.

MATERIALS AND METHODS

Tannase from *Aspergillus niger* was covalently coupled to alkylaminosilanized porous silica as previously described (*10*). The product was washed with 0.1*M* Na_2HPO_4 buffer, pH 6.0, and filtered on a Buechner funnel until no more buffer could be extracted. The product was stored as a damp cake at 4°C until used.

The substrate, gallic acid, was dissolved in the appropriate alcohol at a final concentrate of 0.1*M* (170 mg gallic acid to 10 mL alcohol).

To 2 mL of the substrate was added 1.0 g of the immobilized tannase. The reaction was allowed to continue for 18–48 h at room temperature before analysis.

Analysis was by thin layer chromatography (TLC). The TLC was performed using Silica Gel G Chromosorb plates (Eastman Kodak, Rochester, New York). The solvent system was chloroform/methanol 80:20 (v/v) with two drops of acetic acid added per 100 mL of solvent. Visualization was accomplished by exposure to iodine vapor.

RESULTS

The TLC data showed synthesis of esters from alcohols ranging from C_1 through C_{12}. The results are summarized in Table 1.

TABLE 1
Thin Layer Chromatography Results on the Synthesis of Gallic Acid Esters after 18–48 h of Reaction

Alcohol	Results[b]
Methanol	>10% synthesis
Ethanol	20–30% synthesis
n-Propanol	60–70% synthesis
2-Propanol	60–70% synthesis
n-Butanol	85–95% synthesis
n-Amyl alcohol	85–95% synthesis
Isoamyl alcohol	85–95% synthesis
n-Octanol	20–30% synthesis
n-Decanol	20–30% synthesis
n-Laurylalcohol	>10% synthesis[c]

[a]Controls containing both gallic acid and the appropriate alcohol without enzymes showed no indication of ester synthesis.

[b]Percent synthesis were estimates based on the observed loss of gallic acid.

[c]The gallic acid was only slightly soluble in the C_{12} alcohol.

The data in Table 1, although only semiquantitative, indicates that maximum esterification occurs with C_5 alcohols. Based on the quantity of gallic acid remaining at the origin on the TLC plates, it appeared as though synthesis was almost complete.

Since the synthesis of alcohol esters of gallic acid was successful, it was logical to attempt synthesis of esters of several diols under the identical conditions. For these studies, C_3 through C_5 diols were chosen. As in the case of the alcohols, the gallic acid was dissolved in the diol to a final concentration of 0.1*M* (170 mg gallic acid to 10 mL of the appropriate diol).

The reactions were carried out as described for the alcohols. Analysis was also by TLC as previously described.

In the case of several of the diols used in this study, it was possible to synthesize at least three different esters, whereas in the other cases two forms are possible. In all cases the mono-esters were produced. It is unlikely that TLC could separate the different forms of the mono-esters. However, in a few cases there was definitely an additional, more rapidly migrating spot that most likely represents the di-ester.

Once more quantitative analytical procedures are developed, more information on the specific nature of these esters will be determined.

In summary, tannase, an enzyme capable of hydrolysis of a variety of gallic acid esters can, under appropriate conditions, synthesize esters of both a variety of alcohols and diols. The kinetics, optimal conditions for synthesis, and absolute nature of the products remain to be deter-

TABLE 2
Thin Layer Chromatography Results on the Synthesis of Diol Esters of Gallic Acid after 48 h of Reaction

Diol	Results[a]
1,3-Propanediol	50–60% ester synthesis
1,2-Butanediol	70–80% ester synthesis with strong indication of at least two different esters
1,3-Butanediol	70–80% ester synthesis with strong indication of more than one form of ester
1,4-Pentanediol	50–60% ester synthesis.
1,5-Pentanediol	70–80% ester synthesis with indication of more than one form of ester
2,4-Pentanediol	50–60% ester formation

[a]Estimates of percent synthesis were made by comparison of the remaining gallic acid on the TLC compared against known quantities of gallic acid spotted on the plates as controls.

mined. In addition, further studies on the exact specificity of the enzyme for both alcohols and acids yet remains to be determined.

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