

Acetylation of Cinnamic Acid and Evaluation of Antioxidant Activity of the Resultant Derivative

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Abstract: *Background:* Cinnamic acid is a white crystalline substance, chemically known as 2(E)-3-phenylprop-2-enoic acid or benzopropenoic acid. It is a mono-carboxylic acid naturally occurring in plants with low toxicity and serves as a flavoring agent in the food, pharmaceutical and perfumery industries. *Objectives:* The rising incidence of deleterious and harmful effects of free radicals in the human body leading to wide ranging disease conditions such as diabetes, cardiovascular ailments, cancers, hay-fever amongst many others necessitated this present study. *Method:* The acid was subjected to the acetylation reaction using acetic anhydride in acetic acid. In addition, the acid and the resultant derivative were screened for antioxidant activity using the DPPH (2, 2-diphenyl-1-picrylhydrazyl hydrate) test. The comparison of the observed activities was also done. *Results:* The acetylation of the acid led to a derivative which has been identified to be cinnamyl acetate using the IR spectral technique. Both the cinnamic acid and acetyl derivative demonstrated remarkably significant antioxidant activity of IC_{50} at 0.18 and 0.16 $\mu\text{g/mL}$ respectively which compare favorably with 0.12 $\mu\text{g/mL}$ elicited by Vitamin C (a standard antioxidant drug). *Conclusion:* The obtained results indicate that acetylation slightly enhances the antioxidant activity of cinnamic acid.

Keywords: Cinnamic Acid, Acetylation, Cinnamyl Acetate, Derivative, Antioxidant Activity

1. Introduction

In the search for novel pharmacologically active compounds, research efforts are being focussed on some lead chemicals/compounds. One of such is cinnamic acid and its derivatives which are chemical compounds with high potentials for development into drug templates. Some of these compounds especially the ones containing phenolic hydroxyl group are well-known for their several health benefits due to inherent strong free radical scavenging properties. Hence, these compounds are being studied as potential antioxidants due to the multi-functional activities they exhibit. Furthermore, previous studies have shown that this class of compounds possess anti-microbial, anti-inflammatory, anti-cancer, anti-oxidative and cardiovascular protective properties. A derivative namely, p-coumaric acid (4-hydroxyl-trans-cinnamic acid) has been found to exhibit antioxidant activity involving direct scavenging of ROS

(Reactive Oxidative Species) by minimizing the oxidation of low-density lipoproteins [1-3]. In addition, ethyl cinnamate and cinnamyl alcohol have been found to possess antioxidant activity using the rapid bench-top DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) test [4]. In this present study, the acid was acetylated and the resultant derivative screened for antioxidant activity (IC_{50}) using the DPPH test. Comparison of the activities given by the acid and the derivative was done with a view to determining if any improvements would be observed.

2. Experimental

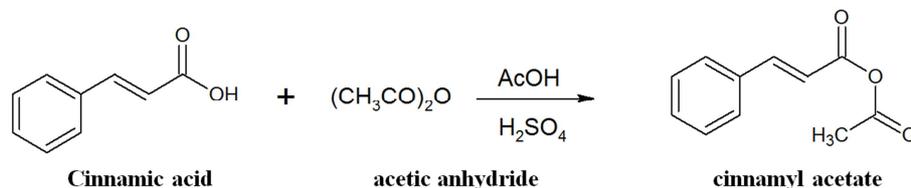
2.1. Reagents/Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) and cinnamic acid were purchased from Sigma Aldrich Chemicals, Germany while acetic acid, acetic anhydride, di-

ethyl ether, methanol and sulphuric acid were obtained as AnaLAR Grade Chemicals from the British Drug House Chemicals Limited, Poole, England.

2.2. Acetylation of Cinnamic Acid

0.4 g of cinnamic acid was dissolved in a beaker containing 10 mL of acetic anhydride and 10 mL of acetic acid. The solution was heated for 20 minutes and allowed to



2.3. Determination of Melting Point

Cinnamic acid and the synthesized derivative (0.05 g) were separately filled to a quarter of the length of a micro-capillary tube and the melting point determined [7] using an Electro-thermal Melting Point apparatus (Electro-thermal Engineering Limited, England).

2.4. Determination of Optical Rotation and Refractive Index

Each sample (0.05 g) was dissolved in 10 mL of methanol. The tube of the Polarimeter (ADP-220, Bellingham Stanley, England) was filled with distilled water and the machine subsequently zeroed. The tube was refilled with 5 mL of sample and the optical rotation and was measured at the wavelength (λ) of sodium line (589.3 nm) at 20.5°C. Similarly, the refractive index of sample was obtained on a refractometer (WAY-15, Abbe, England) at the wavelength (λ) of sodium line (589.3 nm) at 20.5°C [8].

2.5. Antioxidant Activity

2.5.1. Spectrophotometric Determination of Antioxidant Activity Using DPPH Reagent

Substances which are capable of donating electrons or hydrogen atoms can convert the purple-colored DPPH radical (2,2-diphenyl-1-picryl hydrazyl hydrate) to its yellow-colored non-radical form; 1,1-diphenyl-2-picryl hydrazine [10, 11]. This reaction can be monitored by spectrophotometry.

2.5.2. Preparation of Calibration Curve for DPPH Reagent

DPPH (4 mg) was weighed and dissolved in methanol (100 mL) to produce the stock solution (0.004%w/v). Serial dilutions of the stock solution were then carried out to obtain the following concentrations; 0.0004, 0.0008, 0.0012, 0.0016, 0.0020, 0.0024, 0.0028, 0.0032 and 0.0036%w/v. The absorbance of each of the sample was taken at λ_m 512 nm using the Ultra-Violet Spectrophotometer (Jenway 6405, USA). This machine was zeroed after an absorbance had been taken with a solution of methanol without DPPH which served as the blank.

cool. 5 mL of concentrated H_2SO_4 was added as catalyst. Further heating was done for few minutes and it was covered with an aluminum foil and kept in the refrigerator at -4°C. After two weeks, crystals were formed in the beaker 5 mL of warm di-ethyl ether was added and the mixture gently heated again for few minutes. The crystals dissolved on warming, but formed back after so many hours. They were then filtered and allowed to dry [5, 6]. The crystals were then weighed.

2.5.3. Determination of the Antioxidant Activity of Cinnamic Acid, Derivative and Vitamin C

2 mg each of sample was dissolved in 50 mL of methanol. Serial dilutions were carried out to obtain the following concentrations; 0.0004 mgmL⁻¹, 0.0008 mgmL⁻¹, 0.02012 mgmL⁻¹, 0.0016 mgmL⁻¹ and 0.0020 mgmL⁻¹ using methanol. 5 mL of each concentration was incubated with 5 mL of 0.004%w/v methanolic DPPH solution for optimal analytical accuracy. After an incubation period of 30 minutes in the dark at room temperature (25 ± 2 °C), observation was made for a change in the color of the mixture from purple to yellow. The absorbance of each of the samples was then taken at λ_m 512 nm. The Radical Scavenging Activity (RSA %) or Percentage Inhibition (PI %) of free radical DPPH was thus calculated:

$$RSA\%(PI\%) = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

A_{blank} is the absorbance of the control reaction (DPPH solution without the test sample and A_{sample} is the absorbance of DPPH incubated with the sample. Cinnamic acid /derivative/ vitamin C concentration providing 50% inhibition (IC₅₀) was calculated from a graph of inhibition percentage against the concentration of the cinnamic acid /derivative/ vitamin C [12, 13]. Vitamin C was used as a standard antioxidant drug.

2.5.4. Infra-red Spectroscopy of Sample

Each sample (0.2 g) was analyzed for IR characteristics using the FTIR84005 Spectrophotometer (Shimadzu, Japan).

3. Results

Cinnamic acid: C₉H₈O₂; mol. wt. (148 g/mol); white crystalline solid; m.pt. (134-136°C); $[n]_D^{20}$ (1.516); $[\alpha]_D^{20}$ (0°); FTIR (cm⁻¹): 1576 (Ar-C=C), 1627 (acyclic-C=C), 1682 (C=O) and 2923 (-OH).

Cinnamyl acetate: C₁₁H₁₀O₃; mol. wt. (190 g/mol); yellow crystals; m.pt. (169-171°C); $[n]_D^{20}$ (1.552); $[\alpha]_D^{20}$ (0°); FTIR (cm⁻¹): 771 (alkyl substitution), 1071 (C-O-C, ether linkage), 1577 (Ar-C=C), 1629 (acyclic-C=C) and 1683 (C=O).

Table 1. Absorbance of samples incubated with DPPH at different concentrations at λ_{max} 512 nm (Blank absorbance of 0.004% w/v methanolic DPPH reagent: 0.803).

Sample	0.02mg/mL	0.04mg/mL	0.06mg/mL	0.08mg/mL	0.1mg/mL
Cinnamic acid	0.325	0.310	0.307	0.300	0.154
Vitamin C	0.068	0.067	0.065	0.063	0.061
Derivative	0.311	0.298	0.294	0.293	0.289

Key: DPPH=(2,2-diphenyl-1-picrylhydrazylhydrate)

Table 2. Radical scavenging activity (RSA) /percentage inhibition (PI) of samples at different concentrations and the computed IC_{50} values.

Sample	0.02mg/mL	0.04mg/mL	0.06mg/mL	0.08mg/mL	0.1mg/mL	$IC_{50}\mu\text{g/mL}$
Cinnamic acid	59.35	61.39	61.77	62.64	80.82	0.18
VitaminC	91.53	91.96	91.91	92.15	93.40	0.12
Derivative	61.27	62.89	63.89	63.51	64.01	0.16

Keys: IC_{50} = Concentration at which 50% of DPPH is scavenged or inhibited RSA % (PI)=Radical Scavenging Activity (Percentage Inhibition)

4. Discussion

The cinnamic acid used in this study had been put through some monographic determinations in a previous research [4] where its identity, purity, integrity, assay and suitability were established. Its IR spectrum shows diagnostic stretchings at 1576, 1627, 1682 and 2923 cm^{-1} which indicate the characteristic Ar-C=C, acyclic C=C, α , β -unsaturated C=O and -OH groups respectively. The -OH peak of the acid absorbed particularly lower than the expected (>3000) at 2923 cm^{-1} because of inherent intra-molecular hydrogen bonds. Cinnamyl acetate was synthesized as yellow crystals with a balsamic flavor. The compound was found to be soluble in methanol, petroleum ether, acetone, chloroform and ethanol. However, it was insoluble in water conferring a lyphophilic character on the acetyl derivative. Its refractive index was found to be 1.552 (190 g/mol) compared with the parent compound (acid) at 1.516 (148 g/mol). The higher the molecular mass the greater the refractive index [8, 9]. IR peaks of the acetyl product at 771, 1071, 1577, 1629 and 1683 cm^{-1} are characteristic of alkyl substitution (found in COCH_3 substitution in the acetyl derivative), ether linkage --C-O-C, aromatic-C=C (which was observed to have absorbed slightly higher than that seen in the cinnamic acid), acyclic-C=C and C=O stretchings (both observed to have slightly absorbed higher than that seen in the cinnamic acid) respectively. The ether linkage is particularly diagnostic indicating that the hydrogen atom in the -OH group had been substituted with an acetyl group. Both the acid and its acetyl derivative showed optical rotation of 0° indicating that the compounds are optically inactive. Consequently, neither compounds can rotate plane of polarized light in any directions. Hence, the compounds cannot demonstrate either dextro-rotation or laevo-rotation [8, 9]. The acetyl derivative has also been found to be of immense benefit to the pharmaceutical, winery and perfumery industries. It is pertinent to mention that open chain or acyclic carboxylic acids such as cinnamic acid are much difficult to acetylate compared with the ringed or cyclic or aromatic counterparts. Consequently, the acetylation reaction had to be left for 14

days in a refrigerator for the reaction to go into completion.

A calibration curve was prepared for the DPPH (2,2-Diphenyl-1-picryl hydrazyl hydrate) reagent with the aim of confirming its purity and suitability for use in the antioxidant determinations. The Beer-Lambert's Law is the basis of all absorption spectrophotometry [9]. The reduction of the DPPH radical was determined by taking its absorption at a wavelength of λ_m 512 nm. It was observed that the absorbance of DPPH decreased as the concentration of added free radical scavenger (cinnamic acid/ derivative/vitamin C) increased which suggested that the DPPH reagent was being reduced. The tables show radical scavenging activity (RSA %) or percentage inhibition (PI %) and the computed IC_{50} values of cinnamic acid/derivative/vitamin C. The RSA % is an indicator of the antioxidant activity of cinnamic acid/derivative/vitamin C. Interestingly, both the cinnamic acid and acetyl derivative demonstrated significant antioxidant activity (IC_{50}) of 0.18 and 0.16 $\mu\text{g mL}^{-1}$ respectively. These values compare remarkably well with the antioxidant activity given by vitamin (a standard antioxidant drug) at 0.12 $\mu\text{g mL}^{-1}$. However, it can be inferred that the acetyl derivative was slightly more active than the acid. Hence, acetylation slightly enhances the antioxidant activity of the acid. This observation was not surprising because the solubility profile of the synthesized derivative showed that it was soluble inorganic solvents whereas it was insoluble in water implying that it had some lyphophilic characteristics. This feature most probably can account for its slightly higher activity compared with that demonstrated by the acid. Furthermore, this lyphophilic feature favors the acetyl derivative to reach the active sites (allosteric sites) faster than the acid in the process of effecting anti-oxidation. It is also germane to mention that synthesized ethyl cinnamate was observed to be more antioxidant than the acid in a earlier study by this same the same author and others in a early study [4]. Aside from the DPPH assay, other methods for determining the antioxidant activity of compounds include the hydrogen peroxide, nitricoxide, conjugated diene, superoxide, phosphomolybdenum, peroxyxynitrile and xanthine oxidase assay methods amongst many others [14, 15].

5. Conclusion

This present study shows that the cinnamic acid and cinnamyl acetate elicited significant antioxidant activities. However, the activity demonstrated by the acetyl derivative was slightly better. Hence, acetylation enhances the antioxidant activity of the acid.

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