Caffeic Acid Phenylethyl Ester (CAPE): Total Synthesis of a Natural Product

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Caffeic acid phenethyl ester (CAPE) is a naturally occurring compound that is found in both plants and propolis in honeybee hives. It has shown to have many pharmaceutical benefits with it being an antimitogenic, anticarcinogenic, antinflammatory and an immune system booster[4]. It is easily synthesized through acylation, esterfication, de-*o*-acylation. CAPE is synthesized at the conclusion of this experiment.
Key Words: Caffeic acid phenethyl ester (CAPE), acylation, esterfication, de-*o*-acylation

INTRODUCTION

Caffeic acid phenethyl ester (CAPE) **[Figure 1**] was first identified in 1987[1]. It was first found in honeybee propolis[1] that has been used since 300 BC as a traditional folk medicine[2,3]. This phenolic compound is known for having a multitude of health benefits including reported antitumoral activities both in vitro and in vivo in addition to its ability to inhibit acetyl-cholinesterase activity[1]. Other biological benefits of CAPE include it being an antioxidant, anti-inflammatory, antiviral, immunostimulatory, antimitogenic, and an anticarcinogenic[3-5].

CAPE has been reported to be able to produce a cytoprotectve enzyme called, heme oxygenase (HO-1) protein that decreases ischemia-reperfusion, balloon angioplasty, and increases its activity in kidney cells and astrocytes[7]. CAPE also suppresses associate polyposis conditions (APC) associated with intestinal carcinogenesis in chemotherapy along with inducing apoptosis in human leukemic HL-60 cells when accompanied by other agents[3]. Other studies show that CAPE has ameliorative effects on changes induced my cigarette smoke[8]. As an inhibitor it blocks mitogenic kinases methyl ethyl ketones (MEK) and AKT as well as oxidized low-density lipoprotein (ox-LDL) that medicates the degradation of the transcription of nuclear factor kappa beta (NF-κB) signal transduction that results in the reduction of human coronary artery endothelial cell and ischemia-reperfusion injury [3-5].

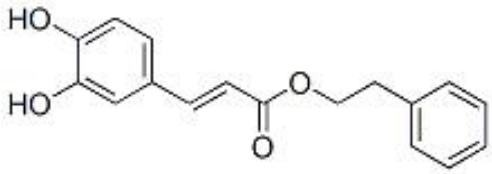

Figure 1. Caffeic Acid Phenethyl Ester

The structure of CAPE allows the electron donating *ortho*-positioned hydroxyl group to lower the bond dissociation of the alcohol group and therefore increases the hydrogen transfer to peroxyl radicals[6]. Its carbon-carbon double bond maximizes its stabilization of the phenolic radical through resonance[6].

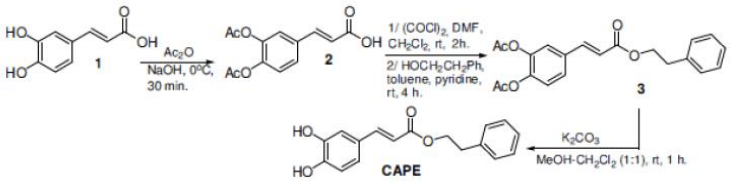

Figure 2. The three-step synthesis of CAPE. **1**, Caffeic acid; **2**, Diacetylcaffeic acid; **3**, Acetylated CAPE.

CAPE can be synthesized in three-step process starting with diacetylation of caffeic acid salt followed by recrystallization to produce diacetylcaffeic acid[9] (**1-2**). A carboxylic chloride synthesis with oxayl chloride is followed by esterification (**2-3)**. The total CAPE synthesis is complete after de-acetylation with potassium carbonate and a dichloromethane-methanol mixture.

METHODS

Diacetylcaffeic Acid Synthesis

Caffeic acid (1 g, 5.5 mmol) was dissolved in NaOH (15 mL, 1.0M). *O*-acetic anyhydride (2 mL) was added to the dark yellow mixture and stirred for 30 minutes in an ice bath. The solid product was isolated using vacuum filtration. It was washed with ice-cold distilled water. Dissolved in boiling ethanol (1 mL, 95%), the crude was recrystallized. Crystals were cooled for 30 minutes at room temperature then for another 10 minutes in an ice bath to isolate the pure product. Product was vacuum filtered and dried overnight. The mass, melting point, and IR spectrometry were recorded.

Acetylated CAPE Synthesis

DCM (5 mL) was added to diacetylcaffeic acid (250 mg, 0.94 mmol) under argon atmosphere. Oxayl chloride (161 μL, 1.88 mmol) and dimethylformamide (2 drops, 100 μL) was slowly added at 0 °C. The mixture was stirred for 2 hours. The mixture was rotary evaporated to remove the excess oxayl chloride.

Esterfication

The residue was dissolved in dry tetrahydrofuran (10 mL). Pyridine (1 mL) was added. The reaction turned bright yellow. 2-phenylethanol (1.04 mmol, 125 μL) was added drop wise to the mixture that turned the reaction white. The reaction stirred for a week at room temperature.

Purification by Flash Column Chromatography

The solvents were removed by rotary evaporation and dissolved in ethyl acetate (20 mL). It was then washed twice with 20 mL of water and then twice with brine (20 mL) and dried in $MgSO_4$. The solvent was dissolved in a minimum amount of dichloromethane, and loaded into a column packed with silica gel (10.0 g). The column was eluted with hexane (20 mL), 10:90 ethyl acetate:hexane (20 mL), and 20:80 ethyl acetate:hexane (20 mL). An extra 50:50 ethyl acetate:hexane (20 mL) was performed to isolate the product from column.

De-O-acetylation

The product dried for a week. The final mass and melting point was determined. In 1:1 MeOH:dichloromethane (5 mL), acetylated CAPE (200 mg, 0.54 mmol) was dissolved. Potassium carbonate (225 mg, 1.63 mmol) was added to the solution and stirred at room temperature for 30 minutes. The reaction was monitored with TLC in 50:50 ethyl acetate:hexane. The residue was dissolved in ethyl acetate (20 mL) and washed twice with water (10 mL), and then twice with brine (10 mL) and dried in $MgSO_4$. There was a noticeable color change from light yellow to white. The solvent was filtered and dried for a week before the final mass and melting point of white, powdery CAPE were taken.

RESULTS

	Mass	% Recovery	Melting Point
diacetylcaffeic acid	1.65 g	11.20%	178° C
Caffeic acid phenethyl ester	0.01g	7.10%	115° C

DISCUSSION

Caffeic acid phenethyl ester is synthesized from caffeic acid. The first part of the reaction was the diacetylation of caffeic acid salt produce diacetylcaffeic acid. Ac_2O acts as an acylating agent. The oxygen electrons of caffeic acid, bond to the carbonyl of acetic anhydride (**1-2**). After the diacetylcaffeic acid product was obtained the melting point was 178° Celsius and 11.2% (1.65 g) was recovered. The IR spectroscopy confirmed the product. A carboxylic chloride synthesis with oxayl chloride proceeds after. Using a catalytic amount (100 μL) of dimethyl formamide (DMF) the reaction undergoes a Vilsmeier-Haak mechanism.

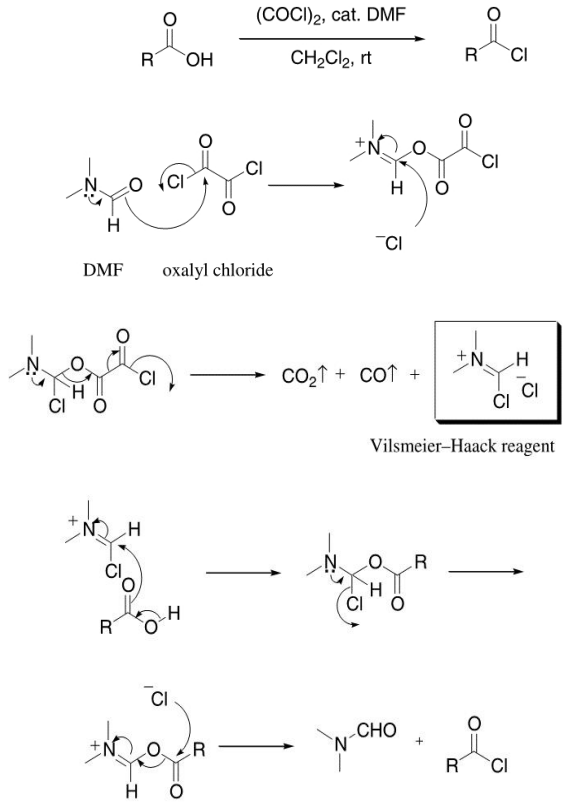

Figure 3. Vilsmeier-Haak Mechanism

The nitrogen electrons in DMF bond to the carbonyl group. Electrons from the C-O double bond attack the carbonyl group of oxayl chloride and the chlorine is kicked off **(Figure 3)**. When the Vilsmeier-Haak reagent is formed it reacts with the diacetylcaffeic acid and substitutes the alcohol group with chlorine (**Figure 3**). This is followed by esterification (**2-3)**. The acid chloride reacts with the alcohol group on the 2-phenylethanol under basic pyridine conditions. The result of the alcholysis is ether with CH_2CH_3Ph attached (**3**). When a TLC was run for this reaction to show that pyridine was present with Rf value are .13 both of pyridine and reaction.

The total CAPE synthesis is complete after de-acetylation by the means of potassium carbonate and a dichloromethane-methanol mixture. While monitoring the TLC plates during column chromatograph all pyridine was gone by the third filter, but the product remained in the column. An additional 50:50 filter was performed in order to flush the product out. After de-*o*-acylation the Rf value was .04 for the product. There was also a noticeable color change to white. Once the product was weighed, the calculated percent recovery was 7.1% and the melting point was 115° C. Possible reason for low percent recovery value was due to product being lost during the multi-step synthesis.

CONCLUSION

The complete synthesis of CAPE was achieved. The final Rf value was 0.4 with a melting point of 115° and 7.1% recovery. The results of final IR, ^{1}H-NMR, and ^{13}C-NMR confirm that CAPE was the product. To increase low percent yield in the future more caution will be taken during purification steps.

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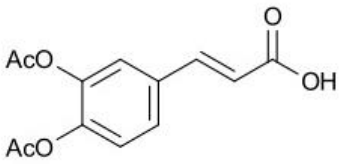
SUPPLEMENTAL INFORMATION

Abbreviations: APC, Associate polyposis conditions; CAPE, Caffeic acid phenethyl ester or 3-(3,4-Dihydroxyphenyl)-2-propenoic Acid 2-Phenylethyl Ester; DMF, dimethylformamide; HL-60, Human promyelocytic leukemia cells; HO-1, heme oxygenase; MEK, Methyl Ethyl Ketone; NF-κB, nuclear factor kappa beta; ox-LDL, oxidized low-density lipoprotein; TLC, Thin-Layer Chromatography;

Diacetylcaffeic Acid



IR (CH_2Cl_2): 3700, 1700, 1500, 1250, 750 cm^{-1}. ^{1}**H-NMR** ($CDCl_3$, 400Hz): δ (ppm) 11 (s, 1H), 7.18-7.45 (aromatic CH, 3H) 6.3 (s, 1H), 6.2 (s, 1H), 2.26 (s, 6H), ^{13}**C-NMR** ($CDCl_3$, 100MHz): δ 171, 169 (2), 145, 143, 142, 132, 126, 124, 123, 116, 20 (2)

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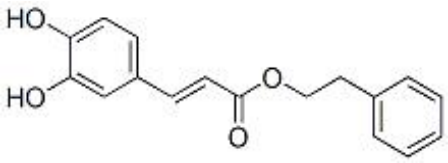
Caffeic Acid Phenethyl Ester



IR (CH_2Cl_2): 3700, 1700, 1550, 1250, 750 cm^{-1}. ^{1}**H-NMR** ($CDCl_3$, 400Hz): 7.5-.7.9 (aromatic CH, 6H), 7.3 (aromatic CH, 1H), 6.85 (aromatic CH, 1H), 6.75 (d, 1H). 6.26 (d, 1H), 5.7 (s, 2H), 4.5 (t, 2H) 2.8 (t, 2H) ^{13}**C-NMR** ($CDCl_3$, 100MHz): δ (ppm) 166, 146, 145, 144, 138 (2), 129, 127.5 (2), 127, 125,123, 118.5, 117.5, 116.5, 65, 34

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