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Research Article

Methyl Caffeate Ether Derivatives as Future Potential Drug

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Abstract: Methyl (E)-3-(3, 4-dihydroxyphenyl) prop-2-enoate were treated with various alkyl halides in presence of K_2CO_3 and acetone to obtain their ether derivatives. Further methyl and ethyl ethers were also obtained using DMS and DES respectively. The structures of these compounds were confirmed by IR, NMR and Mass Spectroscopy. All the compounds give satisfactory elemental analysis. One of them shows promising Antibacterial activity.

Keywords: phytochemicals, Caffeic acid, K_2CO_3 , (3, 4-dipropoxyphenyl) prop-2.

INTRODUCTION

Phenolic phytochemicals are known to exhibit anti-inflammatory, antioxidant, anticarcinogenic, antidiabetic, antiatherosclerosis and immunomodulatory activities in animals^{1, 2}. These are mostly polyphenols known as secondary plant metabolites³, present in plants and trees. Polyphenols are commonly divided into flavonoids and the hydroxyl cinnamic acids. The 3, 4-dihydroxy cinnamic acid derivatives are known as caffeic acid derivatives are widely distributed in the plant kingdom. The most common caffeic acid derivatives are esters of caffeic acid with quinic acid and the caffeic acid phenylethyl ester (CAPE). CAPE is an active component of propolis from honeybee hives and is widely known for its antiviral, anti-inflammatory, and immunomodulatory properties⁴. Caffeic acid

esters also have the ability to alter the redox state and induce apoptosis⁵⁻⁹. Since CAPE, an ester derivative of Caffeic acid or esterification of Caffeic acid with phenyl ethyl groups is a naturally occurring active compound having antimicrobial, anti-inflammatory and antioxidant / anticancer properties, we thought of synthesizing compounds with novel ether, ester and hybrid derivatives of Caffeic acid wherein Caffeic acid would be etherified, esterified and hybridized with various other compounds and to check whether these compounds possess above biological activities.

The objective of this study is to condense two molecules of the same disease domain to produce more potent candidate in the same disease domain or to condense two molecules of different disease domain to produce mixed variety of those disease domain or to have drug candidate with entirely different biological activity.

Synthesis of various derivatives of alkyl caffeate by different methods is reported in the literature¹⁰⁻¹⁴. We are hereby reporting the synthesis of various derivatives of alkyl caffeate by reacting methyl/ethyl (E)-3-(3, 4-dihydroxyphenyl) prop-2-enoate with various alkyl halides in the presence of K₂CO₃ and acetone. Further methyl and ethyl ethers were also obtained using dimethylsulphate and diethylsulphate respectively.

MATERIALS AND METHODS

Materials: Chemicals used were of a laboratory grade. The reactions were monitored by TLC on aluminium-backed silica plate visualized by UV-light.

EXPERIMENTAL

Melting points were determined on a Thomas Hoover capillary melting point apparatus using digital thermometer. IR spectra were recorded on a Shimadzu FTIR Prestige model as KBr pellet. ¹H NMR spectra were recorded on a Varian 400 MHz spectrometer in CDCl₃. Chemical shifts were recorded in parts per million down fields from tetramethyl silane. Mass spectra were recorded on a TOF MS ES mass spectrometer. Elemental analysis was carried out as a percentage on a Thermo finnigan, Flash EA 1112 series, Italy.

RESULTS AND DISCUSSION

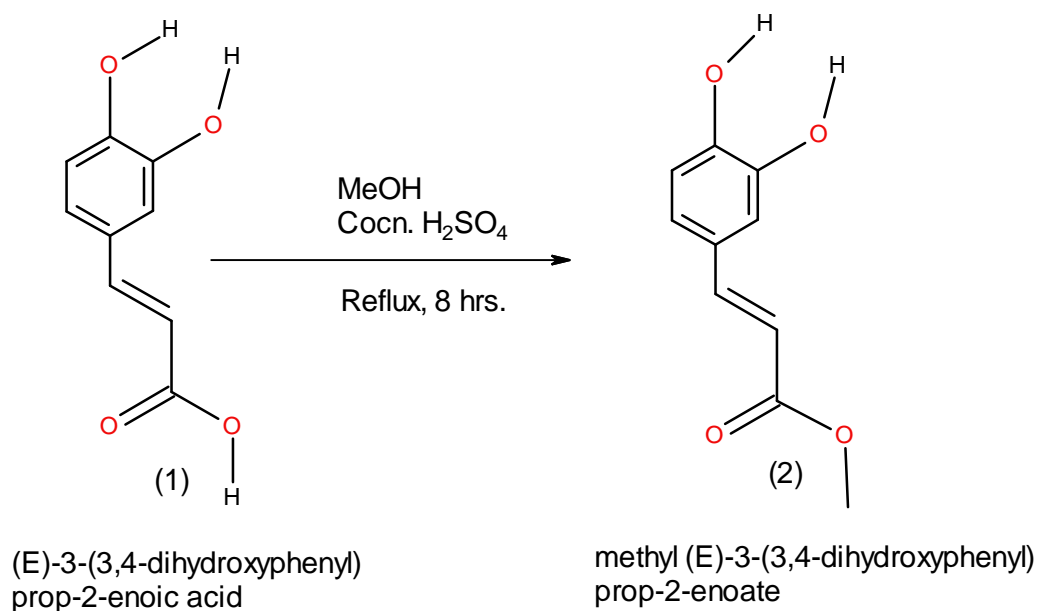
Preparation of methyl caffeate: It was prepared by refluxing 3, 4-dihydroxy cinnamic acid (25 Gms) with methanol (LR grade 400 ml) using sulphuric acid as a catalyst (2 ml) for 8 hrs. The progress of the reaction is monitored by TLC for the completion of reaction.

Work up: The reaction mixture concentrated under reduced pressure to minimum and to that 200 ml of dichloromethane + 200 ml of water were added. The aqueous layer was extracted successively with dichloromethane (3 x 100 ml). The total organic layer was washed with water (200 ml), brine (100 ml) and concentrated to yield methyl caffeate. The yield was 96 %.

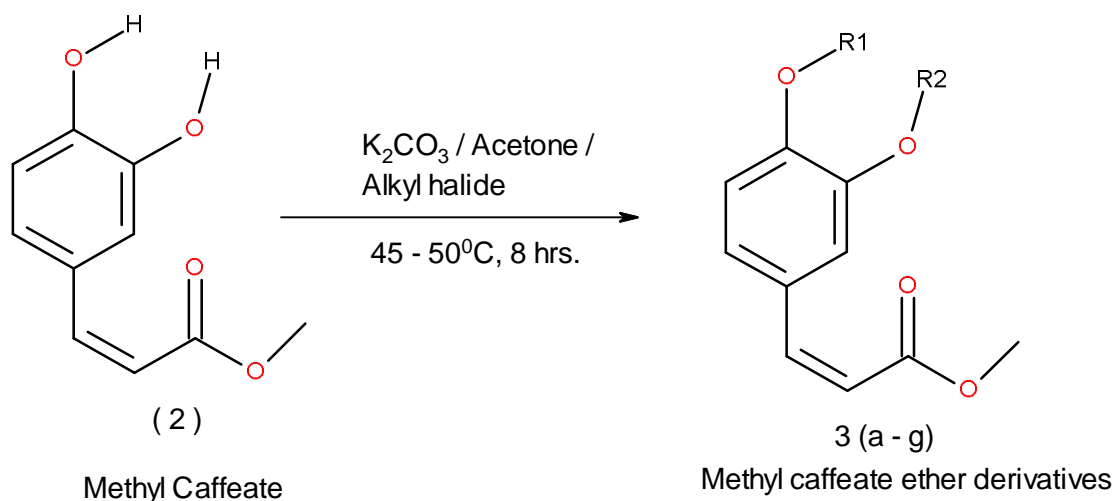
Compound 2: Methyl (E)-3-(3, 4-dihydroxyphenyl) prop-2-enoate

¹H NMR (400 MHz, CDCl₃) δ ppm : 3.8 (s, 3H, -OCH₃ from -COOCH₃ group), 6.22 (d, J = 16.0 Hz, 1H, Trans double bond), 6.7 (brs, 1H, -OH, D₂O exchangeable), 6.8 – 7.4 (m, 3H, ArH), 7.6 (d, J = 16.0 Hz, 1H, Trans double bond); TOF MS ES : 193 (M - H), 217 (M + Na). Molecular formula C₁₀H₁₀O₄; White solid (26.4 gms, 96.0 %). Melting range 158 – 161⁰C; Elemental Analysis, Requires C 61.85 %, H 5.19 %, O 32.96 % Found C 61.89 %, H 5.22 %, O 32.92 %;

The below procedure can be scaled up to get more quantities of methyl caffeate for further diversification.



Reaction Scheme 1



Reaction Scheme 2

Methyl (E)-3-(3,4-dihydroxyphenyl)prop-2-enoate (2) was reacted with various alkyl halides to get the corresponding ethers 3(a - g). Dimethyl ether and diethyl ether derivatives of methyl caffeate were also prepared by treating methyl caffeate with dimethyl sulphate and diethyl sulphate respectively and was further confirmed by coltc.

Synthesis of compounds (3b) and (3e) can be explained as follows: Synthesis was aimed to prepare diether derivatives. However, in above cases TLC shows two major spots. Both were isolated by column chromatography with increase in concentration of ethyl acetate in petroleum ether. The non polar spot was suspected to be di-ether derivative whereas polar spot suspected to be mono-ether derivative which was further confirmed by IR, ¹H NMR / D₂O exchange and mass spectroscopy.

Monoether derivatives shows the signal between δ 5.4 to δ 5.8 in ^1H NMR spectra and in IR spectra a peak was shown at around $3500 - 3550\text{ cm}^{-1}$, due to tertiary $-\text{OH}$ group, which is not seen in the ^1H NMR and IR spectra of the diether derivatives. H of the $-\text{OH}$ group have been replaced by various groups, this has been confirmed by ^1H NMR spectra, where signal have appeared in the region of δ 1 to δ 4, which are in accordance with the respective group. Peak between 1705 cm^{-1} to 1731 cm^{-1} in the IR spectra indicates the presence of ester $>\text{C}=\text{O}$ group.

SYNTHESIS OF 3A, 3B AND 3C USING DIMETHYL SULPHATE / DIETHYL SULPHATE

Synthesis of methyl (E)-3-(3, 4-dimethoxyphenyl) prop-2-enoate (3a): To a stirred solution of [1] (1 eq.) in 30 ml acetone was added K_2CO_3 (2.5 eq.) and stirring continued at 40°C for the next 30 min. For complete formation of K-salt. To this compound Dimethyl sulphate (1.5 eq.) was added and stirring continued at $45 - 50^\circ\text{C}$ for the next 8 hrs. (TLC). The reaction mixture filtered through Buchner funnel; wash the cake with 25 ml acetone. The total organic layer was concentrated to minimum, preadsorbed on silica gel and purified by silica gel (100 - 200 mesh) column chromatography with increase in concentration of ethyl acetate in petroleum ether. The other compounds 3b, 3c were obtained using same method by using Diethyl sulphate instead of Dimethyl sulphate. Compound (3a, 3b, 3c) were also prepared by stirring $\text{CH}_3\text{I} / \text{C}_2\text{H}_5\text{I} - \text{K}_2\text{CO}_3 - \text{Acetone}$ for 24 hrs. And confirmed by co-tlc.

Synthesis of methyl (E)-3-(3, 4-dipropoxyphenyl) prop-2-enoate (3d): To a stirred solution of [1] (1 eq.) in 30 ml acetone was added K_2CO_3 (2.5 eq.) and stirring continued at 40°C for the next 30 min. For complete formation of K-salt. To this compound propyl bromide (2.2 eq.) was added and stirring continued at $45 - 50^\circ\text{C}$ for the next 8 hrs. (TLC). The reaction mixture filtered through Buchner funnel; wash the cake with 25 ml acetone. The total organic layer was concentrated to minimum, preadsorbed on silica gel and purified by silica gel (100 - 200 mesh) column chromatography with increase in concentration of ethyl acetate in petroleum ether. The other compounds were obtained using same method.

Methyl (E)-3-(3, 4-Dimethoxyphenyl) Prop-2-Enoate (3a):

M. Pt. 73°C . Yield - 79 %.

IR (KBr) cm^{-1} : 1705 (α , β -unsaturated $>\text{C}=\text{O}$), 1400 – 1500 (Aromatic), 1380 (C-O).

^1H NMR (CDCl_3 , 400 MHz) δ ppm: 3.78 (s, 3H, Ar x $-\text{COOCH}_3$), 3.92 (s, 6H, 2 x Ar- OCH_3), 6.3 (d, $J = 16.0\text{ Hz}$, 1H, Trans double bond), 6.8 – 7.2 (m, 3H, ArH), 7.4 (d, $J = 16.0\text{ Hz}$, 1H, Trans double bond); TOF MS ES: 245 (M + Na);

Anal. Calcd. For $\text{C}_{12}\text{H}_{14}\text{O}_4$: C 64.85 % H 6.35 % O 28.80 %; Found C 64.89 % H 6.39 % O 28.76 %.

Methyl (E)-3-(4-ethoxy-3-hydroxy-phenyl) prop-2-enoate (3b):

Pure viscous mass. Yield- 74 %

IR (KBr) cm^{-1} : 3516 (sharp peak due to tert. $-\text{OH}$), 1708 (α , β -unsaturated $>\text{C}=\text{O}$), 1400 – 1500 (Aromatic), 1376 (C-O).

^1H NMR(CDCl_3 , 400 MHz) δ ppm- 1.42 (t, $J = 7.0\text{ Hz}$, 3H, $-\text{CH}_3$ from ethyl bromide moiety), 3.8 (s, 3H, Ar x $-\text{COOCH}_3$), 4.14 (q, $J = 7.3, 14.2\text{ Hz}$, 2H, $-\text{OCH}_2$ from $-\text{OCH}_2\text{CH}_3$ moiety), 5.6 (brs, 1H, $-\text{OH}$, D_2O exchangeable), 6.2 (d, $J = 16.0\text{ Hz}$, 1H, Trans double bond), 6.7 – 7.4 (m, 3H, ArH), 7.6 (d, $J = 16.0\text{ Hz}$, 1H, Trans double bond); TOF MS ES: 245 (M + Na);

Anal. Calcd. for $\text{C}_{12}\text{H}_{14}\text{O}_4$: C 64.85 % H 6.35 % O 28.80 %; Found C 64.88 % H 6.38 % O 28.77 %.

Methyl (E)-3-(3, 4-Diethoxyphenyl) Prop-2-Enoate (3c):

Pure viscous mass (72 %);

IR (NaCl) cm^{-1} : 1731 (α , β -unsaturated $>\text{C}=\text{O}$), 1400 – 1500 (Aromatic), 1376 (C-O).

^1H NMR(CDCl_3 , 400 MHz) δ ppm :- 1.42 (t, J = 7.0 Hz, 6H, 2 x $-\text{CH}_3$ from ethyl bromide moiety), 3.76 (s, 3H, Ar x $-\text{COOCH}_3$), 4.14 (q, J = 7.3, 14.2 Hz, 2H, $-\text{OCH}_2$ from $-\text{OCH}_2\text{CH}_3$ moiety), 6.2 (d, J = 16.0 Hz, 1H, Trans double bond), 6.7 – 7.4 (m, 3H, ArH), 7.6 (d, J = 16.0 Hz, 1H, Trans double bond);

TOF MS ES: 273 ($\text{M} + \text{Na}$);

Molecular Formula $\text{C}_{14}\text{H}_{18}\text{O}_4$; Elemental Analysis, Calcd: C 67.18 % H 7.25 % O 25.57 %; Found C 67.22 % H 7.29 % O 25.53 %;

Methyl (E)-3-(3, 4-Dipropoxyphenyl) Prop-2-Enoate (3d):

Pure viscous mass (78 %);

IR (NaCl) cm^{-1} : 1726 (α , β -unsaturated $>\text{C}=\text{O}$), 1400 – 1500 (Aromatic), 1380 (C-O).

^1H NMR(CDCl_3 , 400 MHz) δ ppm :- 1.05 (t, J = 7.0 Hz, 3H, terminal $-\text{CH}_3$ from n-propyl moiety), 1.84 (m, 2H, 1 x $-\text{CH}_2$ from n-propyl moiety), 3.8 (s, 3H, $-\text{OCH}_3$ from $-\text{COOCH}_3$ group), 4.0 (t, J = 6.8 Hz, 4H, 2 x $-\text{OCH}_2$ group); 6.3 (d, J = 16.0 Hz, 1H, Trans double bond), 6.8 – 7.2 (m, 3H, ArH), 7.6 (d, J = 16.0 Hz, 1H, Trans double bond);

TOF MS ES: 301 ($\text{M} + \text{Na}$);

Molecular Formula $\text{C}_{16}\text{H}_{22}\text{O}_4$; Elemental Analysis, Calcd : C 69.04 % H 7.97 % O 22.99 %; Found C 69.07 % H 7.94 % O 22.96 %;

Methyl (E)-3-(4-Butoxy-3-Hydroxy-Phenyl) Prop-2-Enoate (3e):

Pure viscous mass (68 %);

IR (NaCl) cm^{-1} : 3528 (sharp peak due to tert. $-\text{OH}$), 1726 (α , β -unsaturated $>\text{C}=\text{O}$), 1400 – 1500 (Aromatic), 1380 (C-O).

^1H NMR(CDCl_3 , 400 MHz) δ ppm :- 1.2 (t, J = 7.0 Hz, 3H, Terminal $-\text{CH}_3$ from n-butyl bromide moiety), 1.6 (m, 2H, 1 x $-\text{CH}_2$ from n-butyl moiety), 1.8 (m, 2H, $-\text{CH}_2$ from n-butyl moiety); 3.84 (s, 3H, $-\text{OCH}_3$ from $-\text{COOCH}_3$ group), 4.04 (t, J = 6.8 Hz, 2H, 1 x $-\text{OCH}_2$ group), 5.4 (brs, 1H, $-\text{OH}$, D_2O exchangeable), 6.84 (d, J = 16.0 Hz, 1H, Trans double bond), 7.0 – 7.8 (m, 3H, ArH), 7.98 (d, J = 16.0 Hz, 1H, Trans double bond);

TOF MS ES : 273 ($\text{M} + \text{Na}$);

Molecular Formula $\text{C}_{14}\text{H}_{18}\text{O}_4$; Elemental Analysis, Calcd : C 67.18 % H 7.25 % O 25.57 %; Found C 67.21 % H 7.29 % O 25.54 %;

Methyl (E)-3-(3, 4-dibutoxyphenyl) prop-2-enoate (3f):

Pure viscous mass (72 %);

IR (NaCl) cm^{-1} : 1726 (α , β -unsaturated $>\text{C}=\text{O}$), 1400 – 1500 (Aromatic), 1380 (C-O).

^1H NMR(CDCl_3 , 400 MHz) δ ppm :- 1.0 (t, J = 7.0 Hz, 3H, terminal $-\text{CH}_3$ from n-butyl moiety), 1.5 (m, 2H, 1 x $-\text{CH}_2$ from n-butyl moiety), 1.82 (m, 2H, 1 x $-\text{CH}_2$ from n-butyl moiety), 3.8 (s, 3H, $-\text{OCH}_3$ from $-\text{COOCH}_3$ group), 4.0 (t, J = 7.0 Hz, 2H, $-\text{OCH}_2$ from n-butyl moiety), 6.3 (d, J = 16.0 Hz, 1H, Trans double bond), 6.8 – 7.4 (m, 3H, ArH), 7.6 (d, J = 16.0 Hz, 1H, Trans double bond);

TOF MS ES: 329 (M + Na);

Molecular Formula C₁₈H₂₆O₄; Elemental Analysis, Calcd: C 70.56 % H 8.55 % O 20.89 %; Found: C 70.60 % H 8.58 % O 20.86 %;

Methyl (E)-3-(3, 4-diacetonyloxyphenyl) prop-2-enoate (3g):

Pure viscous mass (78 %);

IR (NaCl) cm⁻¹: 1726 (α , β -unsaturated >C=O), 1400 – 1500 (Aromatic), 1380 (C-O).

¹H NMR(CDCl₃, 400 MHz) δ ppm :- 2.3 (s, 6H, 2 x -COCH₃), 3.8 (s, 3H, -OCH₃ from -COOCH₃ group), 4.64 (s, 4H, 2 x -OCH₂ group), 6.3 (d, J = 16.0 Hz, 1H, Trans double bond), 6.6 – 7.2 (m, 3H, ArH), 7.6 (d, J = 16.0 Hz, 1H, Trans double bond);

TOF MS ES: 329 (M + Na);

Molecular Formula C₁₆H₁₈O₆; Elemental Analysis, Calcd : C 62.74 % H 5.92 % O 31.34 %; Found C 62.77 % H 5.95 % O 31.30 %;

Table 1: Ether derivatives of methyl caffeate

Compound No.	R ₁	R ₂
3a	-CH ₃	-CH ₃
3b	-C ₂ H ₅	H
3c	-C ₂ H ₅	-C ₂ H ₅
3d	-CH ₂ CH ₂ CH ₃	-CH ₂ CH ₂ CH ₃
3e	-CH ₂ CH ₂ CH ₂ CH ₃	H
3f	-CH ₂ CH ₂ CH ₂ CH ₃	-CH ₂ CH ₂ CH ₂ CH ₃
3g	-CH ₂ COCH ₃	-CH ₂ COCH ₃

BIOLOGICAL ACTIVITY

Antibacterial Activity using ditch plate method¹⁵: The synthesized molecules were screened for their antibacterial activity at 100 μ g/ml concentration using ditch plate method against Gram positive (*Staphylococcus aureus*, *Corynebacterium diphtheriae*) and Gram negative (*Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*) bacterial species qualitatively. The results of the antibacterial activities are summarized in **Table 2**.

Table- 2: Antibacterial Activity Results

COMPOUND NO.	ACTIVE AGAINST
2	<i>Staphylococcus aureus</i> [Gram positive] <i>Salmonella typhi</i> [Gram negative] <i>Corynebacterium diphtheriae</i> [Gram positive] <i>Escherichia coli</i> [Gram negative]
3g	<i>Staphylococcus aureus</i> [Gram Positive] <i>Escherichia coli</i> [Gram negative]

Theory: Ditch plate method is the method of chosen to test the anti-bacterial activity of compounds. It is a preliminary method to screen the anti-microbial potential of compounds / drugs, which are insoluble or partially soluble in aqueous phase. In this method, the test compound is seeded in an agar plate and the test organisms are streaked across to test the inhibition of the growth as a marker of anti-microbial activity.

Procedure: A ditch (10 mm x 70 mm) is cut into sterile MH agar plate. The test drug / compound is added to 5 ml molten MH agar butt at 40⁰C and this mixture is poured into the ditch and allowed to solidify. The ditch should be made in level with the rest of the agar by pouring the mixture. The different bacterial cultures are streaked perpendicular to the ditch using nichrome wire loop. The plate is then incubated at 37⁰ C for 24 hours. The results are observed as inhibition of bacterial growth on the ditch as well as adjacent to the ditch.

The above results shows that the base molecules, methyl and ethyl caffeate (**2**) have moderate antibacterial activity against both the bacterial cultures. Its derivative viz. **3g** was also active against both the specific bacterial cultures as mentioned in the Table 2. In depth analysis of these compounds through structure activity relationship studies would provide further insight and can be an interesting topic of future studies.

CONCLUSION

The structural diversity and the pronounced biological activities encountered in the caffeic acid ether derivatives suggests that this class of compounds is worthy for further studies that may lead to derivatives by using combinatorial chemistry approach is an alternative strategy to new therapeutic discovery. In other words the generation of diverse coffee acid ether derivatives develops new therapeutic molecules that might result in candidates having better activity.

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