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ORIGINAL PAPER

Preparation and Characterization of 4-Methoxy Cinnamoyl Glycerol

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Abstract Glycerol was reacted with 4-methoxy cinnamic acid to prepare the corresponding 4-methoxy cinnamoyl glycerol. The reaction proceeded in toluene under reflux conditions with *p*-toluenesulfonic acid catalyst. Reaction of equimolar amounts of reactants produced the monoester in 20% yield after 2 h. The product was isolated and characterized by FTIR, mass spectrometry, and NMR techniques. Results of mass spectrometry and NMR experiments showed that the ester linkage formed between the carboxylic acid and the primary hydroxyl of glycerol. The ester displayed strong absorbance between 250 and 350 nm and shows potential as a UV filter in formulations where hydrophilic compounds are advantageous.

Keywords Glycerol · 4-methoxy cinnamic acid · Ester

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Introduction

Derivatives of cinnamic acid such as octyl methoxycinnamate (OMC) and 2-ethylhexyl-*p*-methoxycinnamate find use as organic UV filters in sunscreens and cosmetic formulations [1, 2]. These cinnamates and the related esters formed by combining *p*-methoxy cinnamic acid or ferulic acids with long chain acylglycerides exhibit predominantly lipophilic characteristics [3–5]. However, for certain applications it would be advantageous to have a UV filter with more hydrophilic character, e.g., to limit the penetration of a topical formulation into the skin [6–8]. This can be achieved by appropriate selection of the alcohol used to form the ester. For example, reducing the length of the carbon chain or introducing polar groups will increase the hydrophilic properties of the resulting product. Glycerol is a particularly attractive substrate for this reaction due to its small size, three carbons, and three hydroxyl groups. The additional hydroxyl groups introduce significant polarity to the structure and provide sites for further derivatization.

The current supply of glycerol is increasing as biodiesel production expands in response to the demand for renewable fuels which has made the co-product glycerol widely available and relatively inexpensive. Glycerol is also obtained from plant and animal triglycerides during the production of soaps, fatty acids, and fatty esters for nonfuel applications [9].

The monoester of glycerol and 4-methoxy cinnamic acid was prepared directly in one step to produce an organic UV filter with more hydrophilic character than related compounds in the cinnamate class [10]. The development of such new products can provide additional outlets for this surplus glycerol. Applications for these compounds include polymer additives and personal care formulations where

the UV absorbing properties may limit damage from outdoor exposure [11, 12].

Experimental Procedures

Materials

Glycerol, 4-methoxy cinnamic acid, toluene, and diethyl ether were purchased from Sigma-Aldrich Chemicals, (St. Louis, MO, USA). The *p*-toluenesulfonic acid catalyst was purchased from Fisher Scientific Co. (Pittsburgh, PA, USA). BSTFA reagent was purchased from Pierce Chemicals (Rockford, IL, USA). All materials were used as received.

Esterification

Reactions were performed in 250-mL glass vessels fitted with a condenser and Dean Stark trap. Equimolar reactions used 2 g of 4-methoxy cinnamic acid (8 mMol) and 0.73 g glycerol (8 mMol). The reactants were placed into the reactor with 100 mL toluene and 100 mg of *p*-toluenesulfonic acid catalyst (6 mol%, relative to glycerol). The mixture was heated to reflux, 110 °C, on a magnetically stirred hot-plate. Separation and recovery of the monoester from the reactants was achieved by extraction of the reaction mixture with distilled water, 2:1 volume ratio, followed by extraction with diethyl ether, 1:1 volume ratio. The product was obtained as a clear viscous liquid.

HPLC Analysis

Reaction samples were analyzed by HPLC on an Agilent 1100 system equipped with DAD-ELSD-MS detectors. The strong UV absorbance of 4-methoxy cinnamic acid and the glycerol ester products provided a sensitive method of detection. The absorbance of column effluents was monitored at 192, 210, 215, 273, and 288 nm using the diode array detector with UV spectra collected under the peaks by scanning from 190 to 400 nm. The 4-methoxy cinnamic acid and the esters strongly absorb from 250 to 350 nm with maxima near 290 nm. Glycerol does not absorb in this region but was detected by ELSD. The MS was not routinely used for quantitative analysis. Compounds were separated on an Alltech C18AQ column (Grace, Deerfield, IL, USA) measuring 150 mm × 4.6 mm with 5 μm packing. Isocratic methanol at a flow rate of 0.5 mL/min was used to elute compounds. Data were collected and processed via Chemstation software.

Infrared Spectroscopy (IR)

Infrared spectra were obtained with a Thermo Nicolet, Nexus FT-IR 470 spectrometer, using the ZnSe ATR accessory. Samples, 10–20 mg, were dissolved in diethyl ether (2 mL) and a drop was placed onto the ZnSe crystal. The solvent was allowed to evaporate before the spectra were collected. Spectral data were collected over the range 600–4,000 cm^{-1} and processed by Omnilab software. Strong absorptions measured in the 3,200–3,600 cm^{-1} region were observed for hydroxyl groups and near 1,700 cm^{-1} for the ester linkage.

GC–MS

Mass spectra of silylated samples were collected using the Agilent 6890N gas chromatograph equipped with the 5973 mass selective detector operated in EI mode. Separations were achieved on the HP-5 ms column, 30 m × 0.25 mm ID × 0.25 micron film thickness. Helium was used as the carrier gas with a linear velocity of 35 cm/sec. The oven temperature was programmed from 120 to 240 °C at 10 °C/min with an initial 2-min hold and a final ten minute hold. The inlet was heated to 230 °C and set for splitless injections with a one microliter injection volume. The detector source was heated to 230 °C and the detector quadrapole was heated to 150 °C. Data were collected and processed via Chemstation software.

HPLC–MS–MS

Samples were analyzed with a tandem MS system using a methanol/water gradient flowing at 0.2 mL/min. Each solvent contained 1 vol% formic acid with the gradient starting at 50% methanol and increasing to 100% over 50 min with a ten minute hold. Injections were made using a Finnigan Spectra System AS3000 autosampler onto a Beckman Ultrasphere C18 column measuring 4.6 mm × 250 mm. The eluent was monitored at 280 nm by the Finnigan Spectra System UV 6000 LP before entering the MS.

Mass spectrometry was performed using the Finnigan LCQ Duo with electrospray ionization in positive ion mode. The MS parameters were optimized for the acid and the instrument scanned from 100 to 1,000 mass units with the most intense ions fragmented at 27% normalized collision energy. Typical settings were 4.5 kV source voltage, 80 μA source current, 56 V capillary voltage, and 95 °C capillary temperature. All recorded spectra were the average of three microscans.

^{1}D NMR

Spectra were collected at 300.0 K with the Varian Mercury Plus 400 Spectrometer using the 5-mm Mercury probe. Samples were dissolved in CD_3OD and spectra were acquired at 400.15 MHz for ^{1}H NMR and 100.63 MHz for ^{13}C NMR. Chemical shifts (δ) for ^{1}H NMR are reported as ppm from 3.3 ppm for CD_3OD and from 47.9 ppm for CD_3OD for ^{13}C NMR.

^{1}H NMR

7.7 (d, $J = 16.0$ Hz, 1H), 7.5 (d, $J = 8.8$ Hz, 2H), 7.0 (d, $J = 8.9$ Hz, 2H), 6.4 (d, $J = 16.0$, 1H), 4.3 (dd, $J = 4.2$, 11.4, 2H), 4.2 (m , 1H), 3.8 (s, 3H), 3.6 (d, $J = 1.9$, 2H).

^{13}C NMR

168.0 C10, 162.0 C4, 145.3 C8, 130.0 C2, 130.0 C6, 127.1 C1, 114.7 C9, 114.3 C3, 114.3 C5, 70.1 C12, 65.5 C11, 62.9 C13, 54.8 C7.

DEPT Experiments

Spectra were collected at 300.0 K with the Varian Mercury Plus 400 Spectrometer using the 5-mm Mercury probe. Samples were dissolved in CD_3OD and spectra were acquired at 400.15 MHz for ^{1}H NMR and 100.63 MHz for ^{13}C NMR. Samples were scanned 64 times.

HSQC Experiments

Spectra were collected at 300.0 K with the Varian Mercury Plus 400 Spectrometer using the 5-mm Mercury probe. Samples were dissolved in CD_3OD and spectra were acquired at 400.15 MHz for ^{1}H NMR and 100.63 MHz for ^{13}C NMR. Samples were scanned 64 times.

HMBC Experiments

Spectra were collected on the Varian Inova 500 Spectrometer equipped with the 5 mm gradient inverse probe operated at a temperature of 298 K with a frequency of 499.80 Hz and 125.67 Hz for ^{1}H NMR and ^{13}C NMR, respectively. Samples were dissolved in CD_3OD and scanned 16 times.

Results and Discussion

The esterification of glycerol with 4-methoxy cinnamic acid proceeded in toluene at reflux conditions using *p*-toluenesulfonic acid catalyst to form the monoester product (Fig. 1). The disappearance of the acid and the formation of the ester were followed over the course of the reaction by HPLC with UV detection (Fig. 2). While the absorbance spectrum of the 4-methoxy cinnamic acid was unchanged by the esterification with glycerol the polarity of the ester product was significantly increased compared to the free acid. This is indicated by the elution order obtained with the C18 HPLC column. Also, as shown in a related work this esterification reaction can proceed to form the diester at longer reaction times [13]. However, the condensation of glycerol with two molecules of 4-methoxy cinnamic acid to produce such a diester results in a product that is less polar than the free acid. The objective to obtain a more hydrophilic UV filter was satisfied by synthesis of the monoester rather than the diester. This was achieved at shorter reaction times to avoid the sequential reaction of monoester to diester [13].

The 4-methoxy cinnamoyl glycerol product (monoester) was recovered from the reaction mixture by extraction with deionized water followed by extraction of the resulting aqueous phase with diethyl ether. The first extraction separated the product from unreacted 4-methoxy cinnamic acid and the second extraction separated the product from unreacted glycerol. The product was obtained in greater

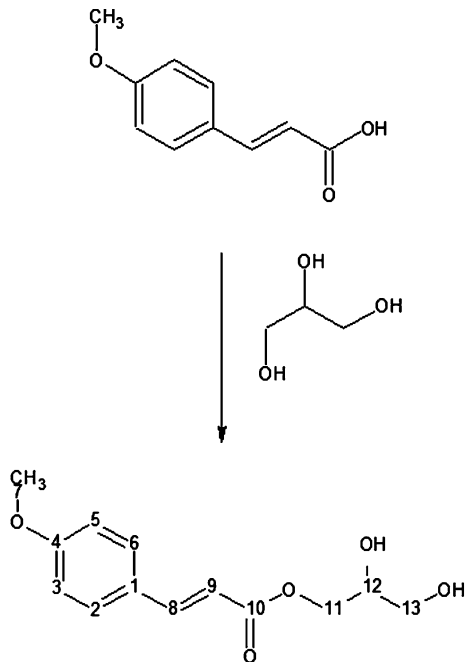

Fig. 1 Esterification of glycerol and 4-methoxy cinnamic acid to form the monoester

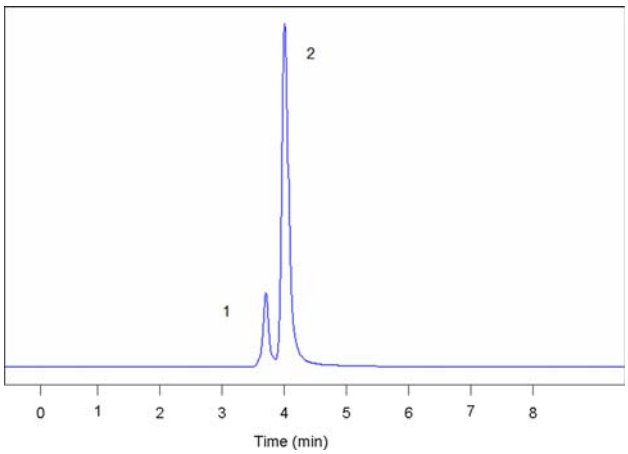

Fig. 2 Chromatogram of an equimolar reaction mixture showing the elution of the product 4-methoxy cinnamoyl glycerol (*1*) before 4-methoxy cinnamic acid (*2*). Separation obtained with methanol mobile phase on a C18 column and detection by UV absorption

than 98% purity after evaporation of the ether. The yield of product was nearly 20% after a two hour reaction with complete selectivity for the monoester.

The 4-methoxy cinnamoyl glycerol product was further characterized by FTIR, LC–MS, GC–MS, and NMR. The infrared spectrum showed the presence of a broad band in the 3,200–3,600 cm^{-1} region typical of the O–H stretching mode of the hydroxyl group. This was attributed to the unreacted hydroxyl groups of the glycerol portion of the molecule following formation of the ester linkage. A signal at 1,701 cm^{-1} indicated the presence of the ester function shifted from the typical 1,740 cm^{-1} location by the double bond conjugated with the aromatic ring structure. A pair of strong sharp bands was observed at 1,172 and 1,116 cm^{-1} consistent with the C–O stretching mode.

Analysis by LC–MS identified the molecular ion of the ester with *m/z* 252.8. The location of the ester linkage on the glycerol moiety was determined from the mass spectrum of the corresponding trimethyl silyl derivative (TMS). The fragmentation pattern of such a derivatized compound provides evidence of the location of the ester linkage. Different ion fragments are generated depending on whether the ester formed at the primary or secondary hydroxyl of glycerol. This approach has been applied to determine the positional isomers for structures such as monoacylglycerides, mono-alkyl glycerides, and in particular feruloyl glycerol [14, 15]. The latter compound is relevant because of the structural similarity between ferulic acid, 4-methoxy cinnamic acid, and the esters these compounds form with glycerol. Mass spectra obtained by GC-MS analysis of the TMS derivative of the isolated product, 4-methoxy cinnamoyl glycerol, are presented in Fig. 3. The molecular ion (M) is observed at *m/z* 396 with the characteristic ion *m/z* 293 (*m/z* M-103) produced by cleavage between carbons 12 and 13. This ion, *m/z* M-103, is observed with 1-substituted glycerols but not 2-substituted glycerols and provides evidence that the ester linkage formed at the primary hydroxyl of glycerol. Other peak assignments are shown for the ions generated by cleavage at the bonds indicated in Fig. 3 and include *m/z* 161, 133, 179, and 191.

The results of DEPT and HMBC experiments provided additional evidence that the ester linkage formed between the primary hydroxyl of glycerol and 4-methoxy cinnamic acid. The DEPT spectra (not shown) displayed all carbons directly bonded to hydrogens. The carbons comprising the glycerol moiety (C11, C12, and C13) were of particular interest and shown to have two protons attached to C11, one proton attached to C12, and two protons attached to C13. Moreover, C11 and C13 displayed different shifts and were therefore not equivalent. If the ester had formed at the C12 hydroxyl group then C11 and C13 would be equivalent. The connectivity across multiple bonds was investigated through HMBC experiments that examined the long-range coupling between carbons and protons. Figure 4 shows results obtained for such an experiment optimized for an 8-Hz coupling. The relevant peaks from

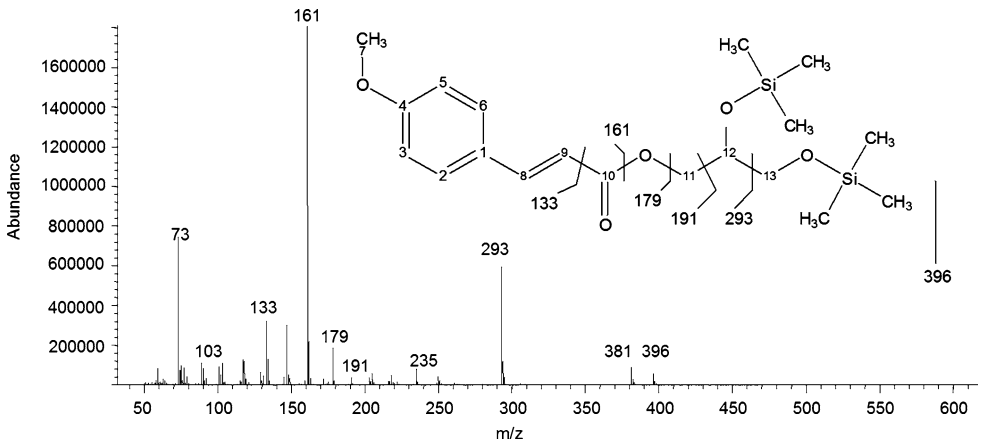

Fig. 3 Mass spectrum of TMS derivatized 4-methoxy cinnamoyl glycerol

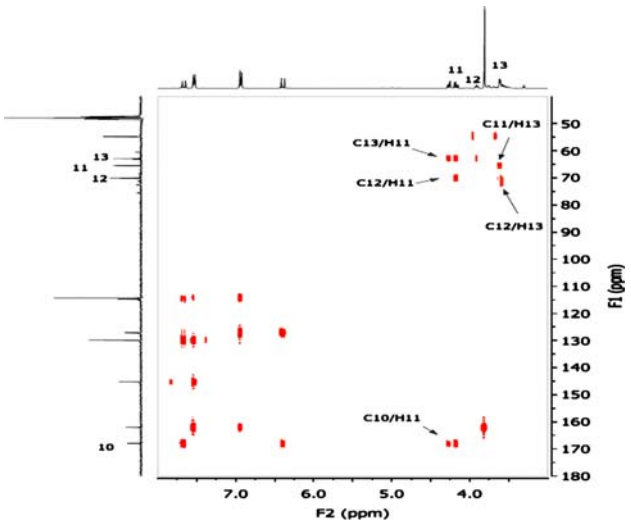

Fig. 4 HMBC spectrum of 4-methoxy cinnamoyl glycerol

the 1D carbon (F1) and proton (F2) spectra are annotated at the sides of the corresponding axes. The cross peaks for C10, C11, C12, and C13 are shown with the assignments of the resonant carbon and proton nuclei, respectively. The long range correlations obtained from this experiment, especially between C10 and H11, show the connectivity through the ester linkage and are consistent with a product formed at the primary hydroxyl site of glycerol.

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