JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2004; **47**: 895–901.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.878

Note

Microwave-enhanced, solvent-free synthesis of singly and doubly ^{13}C-labelled *trans*-cinnamic acid at the α- and β-carbon positions

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Summary

^{13}C-labelled *trans*-cinnamic acid (3-phenyl-2-propenoic acid) has been synthesized in one step using benzaldehyde-carbonyl-^{13}C and malonic acid-2-^{13}C in the presence of ammonium acetate under microwave irradiation and solvent-free conditions. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: *trans*-cinnamic acid; 3-phenyl-2-propenoic acid; ^{13}C label; solid-phase reaction; microwave synthesis

Introduction

Trans-cinnamic acid (3-phenyl-2-propenoic acid) and its derivatives are widely used chemicals in a variety of fields. Recently, they have been applied as antibacterial agents for suppression of bacterial growth[1–3] or as a monomer unit to modify the properties of polymer materials.[4,5] *Trans*-cinnamic acid undergoes a [2 + 2] cycloaddition reaction under photoirradiation, and therefore it has a potential application in memory storage materials.[6,7] In connection with these various applications, ^{13}C-labelled derivatives of cinnamic acid can serve as valuable mechanistic probes. For example, one can imagine that the process of polymerization could be monitored by ^{13}C NMR spectroscopy, or that the interaction between *trans*-cinnamic acid and a biological target molecule could be investigated to probe the environment of the macromolecular domain.

A commonly used route to prepare *trans*-cinnamic acid is the condensation of aryl aldehyde and malonic acid, known as the Knoevenagel reaction. The methods used to synthesize the natural abundance *trans*-cinnamic acid and its

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Received 2 June 2004
Revised 16 July 2004
Accepted 19 July 2004

derivatives have been very well documented in the literature.[8–11] Furthermore, microwave-assisted synthesis has been utilized for the synthesis of cinnamic acid,[12] for deuteration of cinnamic acid, and for other related derivatives.[13]

Isotopic ^{14}C labelling of *trans*-cinnamic acid was first developed by Herbert *et al*. in 1968 using the condensation reaction between malonic acid and ^{14}C-labelled benzaldehyde in pyridine.[14] Pichat *et al*. used the same procedure to prepare ^{14}C-labelled *trans*-cinnamic acid that was subsequently reduced by aluminum hydride to generate 3-^{14}C-cinnamyl alcohol.[15] In 1979, this preparation was modified by using pyridine and piperidine as the condensation medium.[16] By using this method, the preparation of labelled compounds of 2-^{14}C, 2-^{13}C and 2,3-^{13}C cinnamic acids were reported in the literature.[17,18]

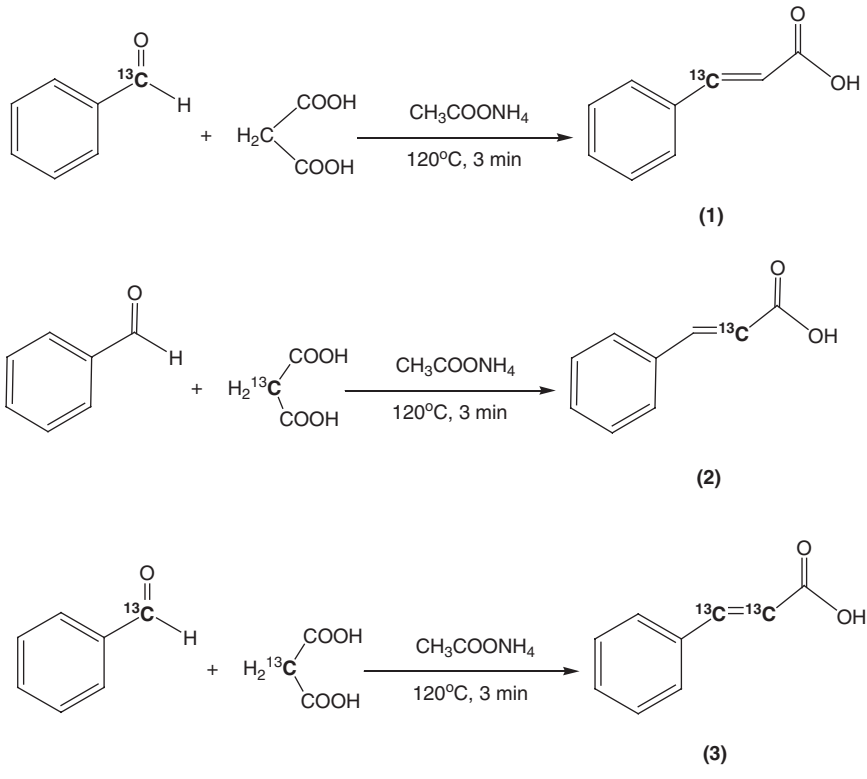
We are investigating [2 + 2] cycloadditions in the solid-state, where the sp^2-hybridized α- and β-carbons photodimerize with a neighboring molecule to create four sp^3-hybridized cyclobutane carbons. An isotopic label at either, or both, of the vinyl positions would provide a sensitive probe of that process. However, few papers address labelling of *trans*-cinnamic acid,[17,18] and no systematic ^{13}C labelling of either or both the α- and β-carbons of *trans*-cinnamic acid has been studied. We report here a rapid, very efficient approach to ^{13}C-labelled cinnamic acid derivatives using inexpensive ammonium acetate as a condensation medium.

Results and discussion

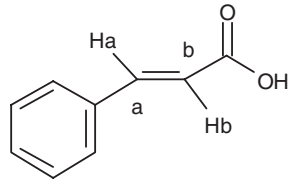
Three different ^{13}C isotopomers of *trans*-cinnamic acid were labelled by the reaction shown in Scheme 1 using the commercially available compounds, benzaldehyde-carbonyl-^{13}C and malonic acid-2-^{13}C (Cambridge Isotope Laboratories, Inc.). The three isotopomers were formed in 67–73% yield when a mixture of benzaldehyde and malonic acid, or their isotopically labelled analogues, and ammonium acetate were combined and subjected to microwave irradiation.

All the reactions were performed in an open Erlenmeyer flask in the absence of any solvent. The irradiations were carried out in microwave 'pulses' of 1 min each at approximately 600 W with 2 min cooling between pulses. This procedure enables one to easily monitor the course of the reaction by TLC. The temperature of the mixture reached a maximum of 110–120°C at the end of each irradiation, and the conversion was completed within a short time (approximately 3–4 min after 3–4 microwave 'pulses'). The glassy reaction mass was subsequently poured into ice water, and the precipitated *trans*-cinnamic acid was isolated by filtration. The melting point was measured to be 132–134°C, indicating that the products were very pure.

The 3-^{13}C-labelled *trans*-cinnamic acids were fully characterized by ^{1}H and ^{13}C NMR spectroscopy in chloroform-*d* (positions indicated in Scheme 2). Figure 1(a) depicts the ^{1}H spectrum of the doubly labelled compound, **3**.



Scheme 1. Synthesis of singly and doubly ^{13}C-labelled *trans*-cinnamic acid



Scheme 2.

Figure 1(b) depicts the ^{13}C spectra also of the doubly labelled compound ($^{13}C\{^1H\}$ in the lower image and ^{13}C without decoupling in the upper image). Assignments were made from spectra of singly labelled compounds. The $^{13}C\{^1H\}$ spectrum of **1** showed a single resonance at 147.6 ppm corresponding to the α-carbon (C_a) position, and the ^{1}H spectrum showed a multiplet, similar to that resolved in Figure 1(a) as a doublet of doublets with equal intensities. The spin–spin coupling observed from the H_a and H_b signals at 7.82 and 6.44 ppm, respectively, was ^1J ($^{13}C_a$–^1H_a) = 156 Hz, ^3J (^1H_a–^1H_b) = 16 Hz and ^2J(^1H_b–$^{13}C_a$) = 1 Hz. In a similar manner, the $^{13}C\{^1H\}$ spectrum of **2** exhibited

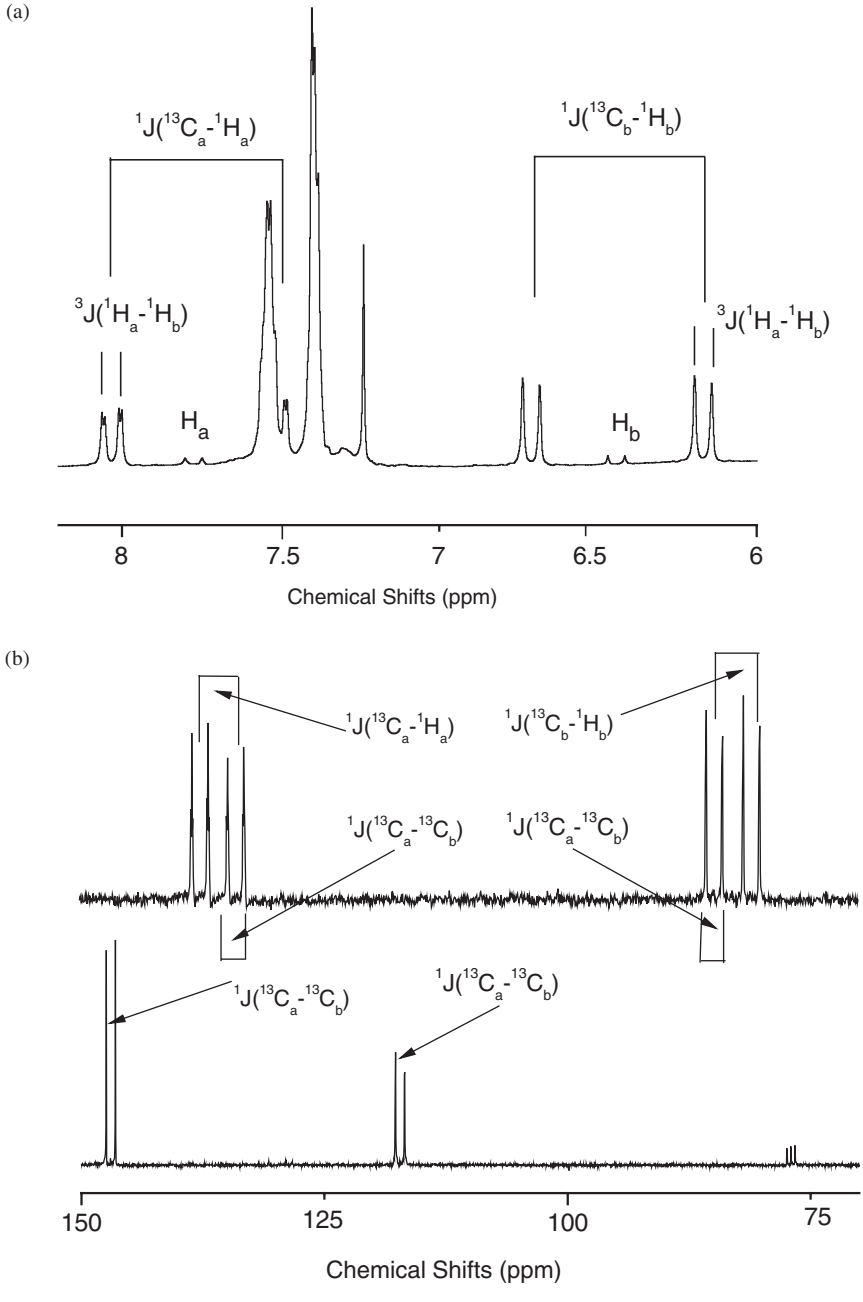


Figure 1. ^{1}H (a) and ^{13}C (b) spectra of *trans*-cinnamic acid doubly ^{13}C-labelled at the α- and β-carbon positions in $CDCl_3$. (b) (upper expanded image) ^{13}C spectrum without proton-decoupling; (b) (lower image) proton-decoupled ^{13}C spectrum

a single peak at 117.9 ppm corresponding to the β-carbon (C_b), and the ^{1}H spectrum produced a similar multiplet pattern with spin–spin couplings of ^1J ($^{13}C_b$–^1H_b) = 163 Hz, ^3J (H_a–H_b) = 16 Hz, and $^2J(^1H_a$–$^{13}C_b) = 3$ Hz. The

spin–spin coupling between C_a, C_b, H_a, and H_b were consistent with those measured for compounds **1** and **2** (Table 1).

The double label allowed us to observe spin–spin coupling between the two carbons of $^1J(^{13}C_a–^{13}C_b) = 70.5$ Hz at each of the carbon resonances, 147.6 and 117.9 ppm. The ^{13}C spectrum of **3** was also recorded with and without proton decoupling. The lower spectrum in Figure 1(b) is the proton-decoupled ^{13}C spectrum, and the upper spectrum was recorded without proton decoupling.

The splitting pattern observed arises from ^1J(C–C) coupling (evident in both ^{13}C spectra). In addition, the experiment without proton decoupling shows the effects of ^1J(C–H) coupling, forming a doublet of doublets, as expected. The same spin–spin coupling constants observed in the proton spectra of $^1J(^{13}C_a–^{13}C_b) = 70.5$, $^1J\,(^{13}C_b–^1H_b) = 163$ Hz, and $^1J\,(^{13}C_a–^1H_a) = 156$ Hz were detected in the ^{13}C spectrum.

Mass spectroscopy was used to confirm the identity of the products. Mass spectra of natural abundance *trans*-cinnamic acid has an intense peak at 148.2 amu. The ^{13}C-labelled compounds **1** and **2** both had spectra that showed an intense peak at 149.2 amu, clearly demonstrating the incorporation of one ^{13}C isotope. Furthermore, compound **3** exhibited an intense peak at 150.2 amu, indicative of two ^{13}C isotopes being incorporated. Smaller peaks were observed at 132.2 and 104.2 amu for compounds **1** and **2**, and at 133.2 and 105.2 amu of compound **3**, arising from the fragment peaks corresponding to the loss of –OH and –COOH groups, respectively, for these three labelled compounds.

In summary, three different vinyllic ^{13}C-labelled compounds were prepared in a one-step reaction. The products were fully characterized by ^1H and ^{13}C NMR and mass spectroscopy in solution. This synthetic route provides an easy and fast method to produce both singly and doubly ^{13}C-labelled *trans*-cinnamic acids that can be used in ^{13}C NMR experiments for biological and polymerization studies.

Experimental

General procedure

Microwave irradiations were carried out using a commercial microwave oven (Sanyo EMF-3400, 1500 W). ^1H and ^{13}C spectra were recorded on a Varian 300 MHz NMR instrument using a 300 Auto SW PFG probe at resonance frequencies of 300.119 MHz (^1H) and 75.472 MHz (^{13}C). Benzaldehyde-carbonyl-^{13}C and malonic acid-2-^{13}C were purchased from Cambridge Isotope Laboratories, Inc. (99% ^{13}C-enriched). 98% ammonium acetate salt was purchased from ACROS. Mass spectrometry was recorded using a VG Analytical Mass Spectrometer.

Table 1. Summary of the ^{1}H and ^{13}C NMR and mass spectrometry data

Compound	^{13}C chemical shifts (ppm)	Coupling constants from ^{13}C spectrum (Hz)	^{1}H chemical shifts (ppm)	Coupling constants from ^{1}H spectra (Hz)	Mass (m/Z)	Label incorporated (%)
1	147.6		6.46, 7.78	$^{1}J(^{13}C_a–^{1}H_a) = 156$ $^{3}J(^{1}H_a–^{1}H_b) = 16$ $^{2}J(^{1}H_b–^{13}C_a) = 1$	149.2	99
2	117.9		6.46, 7.77	$^{1}J(^{13}C_b–^{1}H_b) = 163$ $^{3}J(^{1}H_a–^{1}H_b) = 16$ $^{2}J(^{1}H_a–^{13}C_b) = 3$	149.2	99
3	117.9, 147.6	$^{1}J(^{13}C_a–^{13}C_b) = 70.5$ $^{1}J(^{13}C_b–^{1}H_b) = 163$ $^{1}J(^{13}C_a–^{1}H_b) = 156$	6.50, 7.78	$^{1}J(^{13}C_a–^{1}H_a) = 156$ $^{1}J(^{13}C_b–^{1}H_b) = 163$ $^{3}J(^{1}H_a–^{1}H_b) = 16$ $^{2}J(^{1}H_b–^{13}C_a) = 1$ $^{2}J(^{1}H_a–^{13}C_b) = 3$	150.2	99

A mixture of benzaldehyde (0.50 ml, 4.9 mmol), malonic acid (0.49 g, 4.7 mmol) and ammonium acetate (0.39 g, 5.1 mmol) was added to an Erlenmeyer flask and subjected to microwave irradiation at an energy level of 60% of total power (approximately 600 W, estimated) for approximately 3–4 min. Microwaves were pulsed for units of 1 min, followed by a 2-min cooling period. The temperature of the reaction flask was recorded to be ~120°C. After complete conversion as indicated by TLC, the resulting condensed light-yellow reaction mixture was poured into ice water (25 ml), and the precipitated solid was filtered and dried under vacuum to afford 0.47–0.51 g *trans*-cinnamic acid (yield of 67–73%), melting point 132–134°C.

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