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SYNTHESIS OF (2,3-$^{13}C_2$) ERUCIC ACID

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SUMMARY

The synthesis of (2,3-$^{13}C_2$) erucic acid, for use in metabolic studies, is reported. The synthesis employs a repeated 3 step reaction sequence using ^{13}C labeled triethylphosphonoacetate to extend $C_{18:1}$, oleyl alcohol, by 4 carbons. The starting alcohol is first oxidized with PCC and the resulting aldehyde is condensed with triethyl(1-^{13}C)phosphonoacetate. The product, an α,ß unsaturated ester, is reduced with $(Ph_3PCuH)_6$ to ethyl eicosenoate. Reduction with LAH gives eicosenoyl alcohol. A repetition of the reaction sequence using triethyl(2-^{13}C) phosphonoacetate results in ethyl(2,3-$^{13}C_2$)erucate. The (2,3-$^{13}C_2$) erucic acid, obtained by hydrolysis with alcoholic KOH, gave ^{13}C NMR signals of δ 24.6 and 34.1 ppm for C-3 and C-2 respectively with a J= 34.9 Hz.The overall yield was 5.5%.

Key words: (2,3-$^{13}C_2$)erucic acid, erucic acid, fatty acid synthesis, ^{13}C NMR

INTRODUCTION

As part of an investigation of the catabolism of very long chain fatty acids in germinating rape seed, we have developed a synthetic sequence for selective placement of ^{13}C in positions C-1 through C-4 by chain extension. It has been applied to the synthesis of erucic acid, $C_{22:1}$, the major storage acid found in the triglycerides of rape seed (1). Traditionally chain extension has been achieved by the addition of 1 carbon units using cyanide, diazomethane or Grignard chemistry, or by 2 carbon additions employing acetylene and Wittig chemistry. The synthetic sequence described in Scheme I allows for selective

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placement of such carbons using ^{13}C labeled triethylphosphonoacetate in a Horner-Emmons reaction (2). This type of condensation has been used for two carbon homologation of aldehydes or esters to α,ß-unsaturated esters and to introduce isotopic labels into the conjugated side chains of retinal (3,4), and coniferyl alcohol(5). It has also been combined with subsequent reduction of the conjugated unsaturation, using catalytic hydrogenation, to construct part of the skeleton of (S)-Leucine-^{13}C (6) and (6' - ^{13}C)all-rac -α-tocopherol (7). We have coupled the use of this condensation reaction with reduction of the α,ß unsaturated product by $(Ph_3PCuH)_6$ in a repeated 3 step reaction sequence extending oleyl alcohol by 4 carbons to give erucic acid. This copper hydride reagent shows no reactivity with the isolated double bond present in the parent oleyl chain (8).

The use of the (2,3 -$^{13}C_2$) label produces a unique ^{13}C NMR spectrum, with the C-2 (34.1 ppm) and the C-3 (24.6 ppm) signals being split having a coupling constant of J = 34.9 Hz. This labeling pattern has a distinct advantage over the typical ^{13}C-1 labels in that the protonated carbons exhibit signal enhancement due to NOE and to shortened spin lattice relaxation times. Metabolism of the compound via normal ß oxidation, a two carbon cleavage, would result in shifts of both of these signals and the loss of coupling.

EXPERIMENTAL

Materials and methods. ^{1}H and ^{13}C spectra were recorded on a Chemagnetics A-200 spectrometer in $CDCl_3$, using trimethylsilane as an internal standard. ^{1}H NMR and ^{13}C NMR signals were assigned by comparison to those from unlabeled compounds. For ^{13}C NMR, only resonances arising from the ^{13}C labels are given. Capillary gas chromatography mass spectroscopic analysis (GC-MS) of methyl (2,3-$^{13}C_2$)erucate was performed by Karl V. Wood, Mass Spectroscopic Center, Department of Chemistry, Purdue University, W. Lafayette, IN, USA.

All reagents, including (1-^{13}C) and (2-^{13}C) triethylphosphonoacetate,(99 atom%^{13}C), were purchased from Aldrich Chemical (Milwaukee, WI, USA) and used without further purification. All solvents were distilled prior to use.

Oleylaldehyde (2). Oleyl alcohol (1) (0.636 g, 2.37 mmol) in 3.0 ml dry distilled methylene chloride was added to pyridinium chlorochromate (PCC) (0.931 g, 4.32 mmol) in 8 ml methylene chloride and allowed to react under reflux. As the reaction progresses it darkens from bright orange to brown-black. The reaction was followed by TLC and had gone to completion in 1.5 hours. In addition to the formation of aldehyde 2, a side product, identified as the oleyl oleate, was also isolated. Compound 2 was purified by flash chromatography in 77% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 9.50 (1H, t, H-1), 5.24 (2H, dt, J_t = 5Hz, H-9,10), 2.34 (2H, m , H-2), 1.96 (4H, m, H-8,11) 1.56 (2H, m, H-3), 1.24 (10H, m, H-4-7,12-17), 0.88 (3H, m, H-18).

Ethyl (1-^{13}C)2,11-eicosadienoate (3). Aldehyde 2 (0.49 g, 1.84 mmol) in 4 ml of diethyl ether was added to a precooled mixture of NaH (0.11 g, 60% NaH oil emulsion, 2.76 mmol) and triethyl(1-^{13}C)phosphonoacetate (0.539 g, 2.39 mmol) in 24 ml of ether maintained under nitrogen. The reaction was stirred at 0° C for 30 minutes. The organic layer was removed and the residual oil washed with water. Following diethyl ether extraction of the water washes, the organics were combined and washed with saturated $NaHCO_3$. After removal of the solvent, the residue was flash chromatographed resulting in a 70% yield of 3. The purified product showed no aldehyde peaks in either the ^{1}H NMR or the ^{13}C NMR. ^{1}H NMR (200 MH_z, $CDCl_3$) δ_H 7.0 (1H, dt, J = 7 Hz, 16Hz, H-3), 5.8 (1H, d, J = 16Hz, H-2), 5.37 (2H, t, J= 5 Hz, H-11,12), 4.2 (2H, q, J = 7 Hz, H-1'), 2.2 (2H, m, H-4), 2.0 (4H, m, 10, H-10,13), 1.24 (25H, m, H-2' ,5-9,14-19), 0.9 (3H, m, H-20), ^{13}C NMR (50 MHz, $CDCl_3$) δ_C 166.7 (C-1).

Ethyl (1-^{13}C)11-eicosenoate (4). The α,ß unsaturated ester 3 (0.39 g, 1.16 mmol) was added to $(Ph_3PCuH)_6$ (0.76 g, 0.388 mmol) in 28 ml of deoxygenated benzene containing 48 ul of H_2O under an N_2 atmosphere. The reaction was stirred for 30 minutes and then opened to the air. Stirring was continued for 30 minutes, during which time the reaction changed from a bright brick red (the hydride) to a muddy brown. $MgSO_4$ was added directly to the reaction and the solids were filtered away using a sintered glass funnel. Removal of the benzene solvent and filtration through silica resulted in a crude yield of 98 %. ^{13}C NMR

of the product, 4, shows complete reduction of the conjugated unsaturation. ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 5.33 (2H, m, H-11,12), 4.12 (2H, q, J = 2.4 Hz, H-1'), 2.31 (2H, q, J = 2.4 Hz, H-2), 2.0 (4H, m, H-10,13), 1.61 (2H, m, H-3), 1.26 (27H, m, H-2',4-9,14-19), 0.9 (3H, m, H-20), ^{13}C NMR (50 MHz, $CDCl_3$) δ_C 173.9 (C-1).

(1-^{13}C)11-eiconenoyl alcohol (5). The ester 4 (0.385 g, 1.14 mmol) in 10 ml of diethyl ether was added dropwise to a refluxing solution of LAH (0.095 g, 2.5 mmol) in 10 ml of diethyl ether. The reaction was allowed to reflux and was monitored by TLC. After approximately 2 hours the reaction was cooled on ice. Following the addition of 10 ml of 1:1, diethyl ether : ethanol, the reaction mixture was acidified with 10 ml of 1M H_2SO_4. The product was extracted into diethyl ether, dried over $MgSO_4$ and purified by flash chromatography. Purified 5, based on ester 3, was isolated in 87 % yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 5.29 (2H, m, H-11,12), 3.59 (2H, dm, J_d = 42.9 Hz, H-1), 2.00 (4H, m, H-10,13), 1.29 (34H, m, H-2-9,14-19), 0.86 (3H, m, H-20), ^{13}C NMR (50 MHz, $CDCl_3$) δ_C 63.1 (C-1).

(1-^{13}C)11-eicosenoyl aldehyde (6), ethyl (2,3-^{13}C)13,2-docosadienoate (7), ethyl (2,3-^{13}C)erucate (8). Steps e,f and g were performed according to the protocol for steps a,b and c, with triethyl(2-^{13}C)phosphonacetate being used in step f. The NMR assignments are shown below.

[1-^{13}C]11-eiconenoyl aldehyde (6).

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 9.75 (1H, d, J = 54 Hz, H-1), 5.35 (2H, m, H-11,12), 2.42 (2H, m, H-2), 2.06 (4H, m, H-10,13), 1.65 (2H, m, H-3), 1.24 (24H, m, H-4-9, 14-19), 0.86 (3H, m, H-20), ^{13}C NMR (50 MHz, $CDCl_3$) δ_C 202.9 (C-1).

ethyl [2,3-^{13}C]2,13-docosadienoate (7).

^{1}H NMR (200 MHz, $CDCl_3$) δ_H 6.93 (1H, dm, J = 44.4 Hz, H-3), 5.8 (1H, dd, J = 4.9 Hz, 48.6 Hz, H-2), 5.37 (2H, m, H-13,14 overlaped by one of the

H-2 doublets.) 4.16 (2H, m, H-1'), 2.16 (2H, m, H-4), 2.0 (4H, m, H-12,15), 1.26 (29H, m, H-5-7,16-21,2'), 0.86 (3H, m, H-20), ^{13}C NMR (50 MHz, $CDCl_3$) δ_C 149.5 (d, J = 69.6Hz, C-3), 121.1 (d, J = 69.6Hz, C-2).

ethyl [2,3-^{13}C]erucate (8).

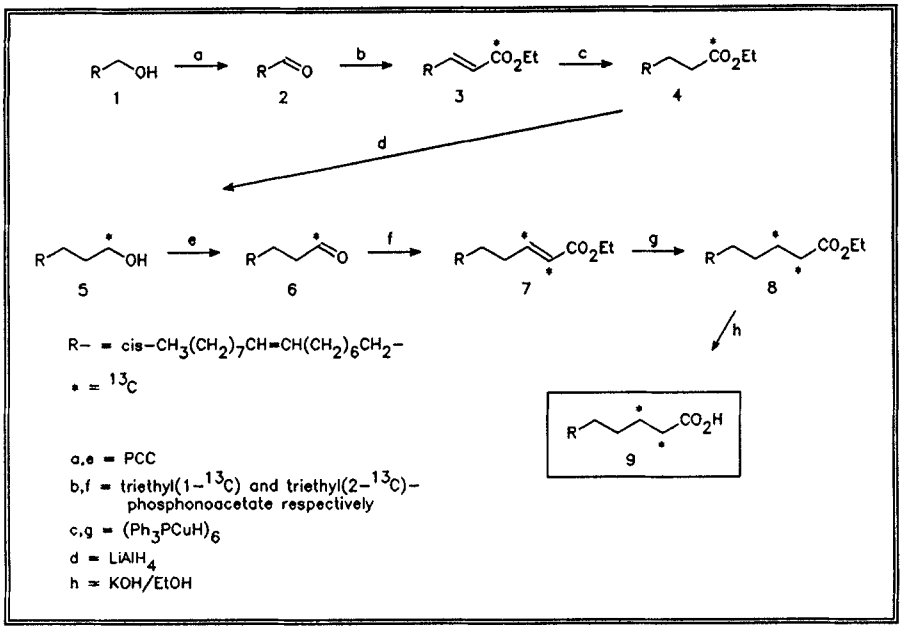
^1H NMR (200 MHz, $CDCl_3$) δ_H 5.35 (2H, m, H-13,14), 4.06 (2H, q, J = 2.45 Hz, C-1'), The unlabeled ester has peaks at 2.31 and 1.58 for H-2 and H-3 respectively. These are coupled, estimated J=36 Hz for both signals, resulting in overlap with the 2.00 H-12,15 and 1.29 CH envelope. Remaining assignments as in 7 . ^{13}C NMR (50 MHz, $CDCl_3$) δ_C 34.2 (d, J = 34.4Hz, C-3), 25.4 (d, J = 34.4Hz, C-2).

(2,3-^{13}C)13-erucic acid (9).

Step g, the conversion of the saturated ester 8 to the free acid was performed in alcoholic KOH (60 mmol KOH : 10 ml EtOH : 1 ml H_2O : 1 mmol ester 8). After refluxing under N_2 for 2.5 hours, 30 ml of H_2O was added and the reaction mixture was neutralized with 20 ml of 6M HCl. Compound 9, the free acid, was extracted into diethyl ether, dried over $MgSO_4$ and, following removal of the ether, chromatographed on reverse phase TLC plates using acetonitrile: H_2O: glacial acetic, 90 : 9 : 1. Characteristic R_f values for eicosenoic and erucic acids were 0.15 and 0.08 respectively, allowing the clean separation of erucic acid from very small amounts of eicosenoic acid present in the final reaction mixture. ^1H NMR (200 MHz, $CDCl_3$) δ_H 5.35 (2H, m, H-13,14) The unlabeled acid has peaks at 2.35 and 1.60 for H-2 and H-3 respectively. These are coupled with an estimated J=35 Hz for both signals, resulting in overlap with the 2.00 H-12,15 and the 1.29 CH envelope. 0.90 (3H, t, H-22) ^{13}C NMR (50 MHz, $CDCl_3$) δ_C 34.1 (d, J = 34.9Hz, C-3), 24.6 (d, J = 34.9Hz, C-2). GC-MS (methyl (2,3-^{13}C)13-erucate) Isotopic purity was 96.9 atom%.

RESULTS AND DISCUSSION

Scheme I. Synthesis of (2,3-$^{13}C_2$) Erucic Acid



The reaction sequence is shown in Scheme 1, and the ^{13}C chemical shifts of the labeled positions are listed in Table 1 and in the experimental section.

Table 1. Chemical Shifts of Labeled Carbons (ppm)

Cpd	C-1	C-2	C-3
3	166.7		
4	173.9		
5	63.1		
6	202.9		
7		121.1	149.5(J=69.6Hz)
8		24.6	34.1(J=34.4Hz)

The sequence was started with oleyl alcohol, providing the double bond at C-13 in the final product. None of the reactions described interfered with this functional group. The oxidation of the alcohol with PCC is uneventful, with the exception of the formation of a

small amount of oleyl oleate as a side product. The condensation reaction was performed using a 1.3: 1.5: 1 molar ratio of triethyl phosphonoacetate : NaH: aldehyde. In our hands, the 0.3 molar excess of triethyl phosphonoacetate was sufficient to drive the reaction and no residual aldehyde was observed at the end of the reaction time. The copper hydride reducing agent, (triphenylphosphine)copper hydride, hexamer, was used to selectively reduce the α,ß unsaturated ester without affecting the C-11 double bond. The reaction was done with an excess of the hydride, a 1:3 molar ratio of the hydride to unsaturated ester being sufficient to give complete reduction. The workup described left some residual triphenylphosphine by product as a contaminant, but this did not interfere with the LAH reduction of the ester and was easily removed during the purification of the resulting alcohol. The next three steps, e, f and g were performed as described for steps a,b and c, respectively, with comparable yields. With the placement of the second ^{13}C, the spectra show the expected splitting patterns in both the ^{1}H NMR and the ^{13}C NMR. The final hydrolysis, step h, gave the most difficulty, as well as dissappointingly low yields of never more than 50 %. This is presumed to be due to solubility problems, resulting from increasing chain length, which are yet unresolved. The hydrolysis products were chromatographed on C_{18} reverse phase TLC plates resulting in an overall yield of purified (2,3-$^{13}C_2$) erucic acid of 5.5%. The GC-MS analysis shows the erucic acid in this sample to be 96.9 atom % the dual labeled material. The ^{13}C NMR displays a pair of coupled peaks, at 34.1 and 24.6 ppm for C-3 and C-2 respectively, having a J=34.9 Hz. The peak position and coupling of the isotopic labels of this erucic acid will be used to investigate its catabolism.

The synthesis of (2,3-$^{13}C_2$) erucic acid shown in Scheme I is centered around a Horner-Emmons condensation reaction for placement of the isotopic labels, and a Stryker reduction of the α,ß unsaturated ester product. This two reaction sequence provides an efficient and repetitive means of homologizing a fatty acid.

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