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Note

An improved procedure for the synthesis of labelled fatty acids utilizing diethyl malonate

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Summary

An improved procedure for the preparation and purification of labeled fatty acids by malonic ester synthesis has been developed. This method uses the different hydrolysis rates of the monoalkylmalonic ester intermediate and its dialkylmalonic ester side product. A convenient GC–MS monitoring technique allows performing this 3-step procedure without isolation of intermediates and gives a good yield. Copyright © 2006 John Wiley & Sons, Ltd.

Key Words: carbon-13; hydrogen-2; malonic ester synthesis; fatty acids

Introduction

Diethyl malonate is a valuable synthetic equivalent of the synthons $^{-}CH_2CO_2H$ or $^{2-}CHCO_2H$, and is widely used for the preparation of a variety of carboxylic acids labeled with ^{2}H and ^{13}C isotopes in different positions of the aliphatic chain.[1] When treated with one equivalent of base, such as NaH or C_2H_5ONa, diethyl malonate is converted to a mono metal enolate. This mesomeric anion undergoes an S_N2 reaction with an alkyl halide or alkylsulfonate to give a monoalkylmalonic ester. Alkaline hydrolysis of this malonic ester, followed by acidic decarboxylation, produces a carboxylic acid with the extension of the aliphatic chain by two carbon units. When 2 equivalents of diethyl malonate enolate react with 1 mol of terminal dihaloalkane, dicarboxylic acids with 4 more carbons than dihaloalkane can be prepared.

A reaction starting with an accurate stoichiometry of substrates and the correct combination of base and solvent usually produces a good yield

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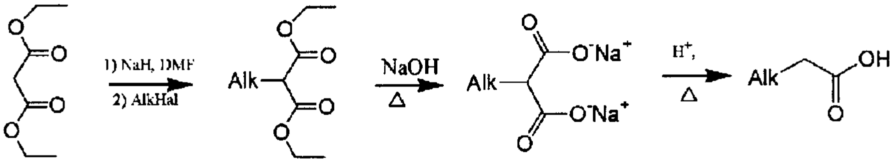
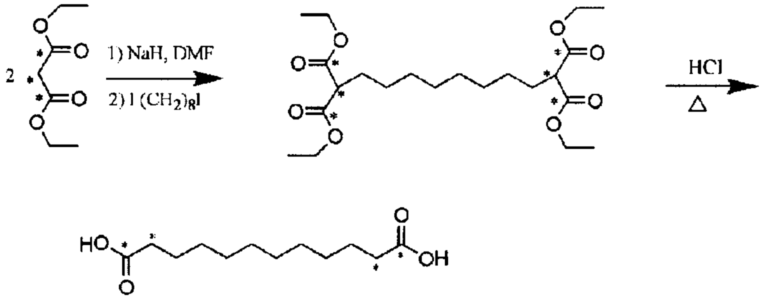
(80–90%) after distillation of the monoalkylmalonic ester.[2] When applied to the synthesis of carboxylic acids in large quantities, this is an excellent technique. However, because of the high cost of labeled compounds, particularly ^{13}C-labeled diethylmalonate or alkylhalides, purification of the alkylmalonic ester by distillation is not cost-effective. Therefore, column chromatography is the most common separation method.[3] Column chromatography purification of the alkylmalonic ester is not only time consuming and relatively costly, it is also associated with the uncertainties of thin-layer chromatography (TLC) monitoring of diethylmalonate and alkyl substituted (both mono- and di-) malonic esters.

Attempts to limit dialkylation and avoid the use of column chromatography to purify alkylmalonic ester have been described.[4,5] The use of excess diethylmalonate minimizes the formation of dialkylated side products.[2] The unreacted substrate can be partially eliminated by a water extraction of free malonic acid after the hydrolysis of the diethyl ester. However, the high cost of carbon-labeled diethylmalonate prohibits such an approach when it is applied to the synthesis of [1-^{13}C]-, [2-^{13}C]- and [1,2-$^{13}C_2$]-carboxylic acids. Even when the cost of substrates is not prohibitive, the complete removal of malonic acid by extraction is not possible.



AlkHal = [1-^{13}C]$C_6H_{13}Br$, C^2H_3I,
$C_6H_5CH_2CH_2Br$, $C_{20}H_{23}Br$

Scheme 1. Synthesis of ^{13}C and ^{2}H labeled short-chain, medium-chain, long-chain fatty acids and aromatic fatty acids



Scheme 2. Synthesis of [1,2,11,12-$^{13}C_4$]dodecanedioic acid

As part of our study on the regulation of both peroxisomal and mitochondrial fatty acid oxidation[6,7] we developed an easy and convenient method for the synthesis of ^{13}C or ^{2}H-labeled short, medium, and long chain fatty acids. We also describe a reliable gas chromatography–mass spectrometry (GC–MS) technique for the monitoring of substrates, reaction intermediates, and products that makes it possible to conduct a 3-step reaction without isolation of intermediates and in good yield (Schemes 1 and 2).

Results and discussion

It is well known that the rate of saponification of malonate esters depends upon the structure of these compounds.[1] Particularly, the hydrolysis of diethyl monoalkylmalonates is much easier than that of dialkylmalonates because of the steric effects of the dialkyl groups. We took advantage of this phenomenon when attempting to prepare a small scale amount of monoalkylmalonic acids, such as hexylmalonic acid and found that overnight refluxing with excess of 2 M KOH solution in water resulted in their complete hydrolysis. Surprisingly, the dialkyl side product of these reactions was not hydrolyzed at all under these conditions. Our test run using unlabeled substrates revealed that the saponification of diethyl 2, 2-dihexylmalonate, a potential dialkyl side product, under these conditions required more than three days. We found that the ethyl ether extraction of the reaction mixture resulted in the complete transfer of the dialkylmalonate ester in the organic phase. After this step, the water solution contained monoalkylmalonate disodium salt. A GC–MS assay of trimethylsilyl (TMS) derivative of the acidified water solution demonstrated the presence of trace amounts (less than 1%) of malonic acid that presumably derived from the unreacted diethylmalonate.

The decarboxylation of the malonic acid, the next step in this synthesis, was performed by refluxing the reaction mixture with acid for 36 h until no starting material was detected by GC–MS of the TMS derivative. These conditions decarboxylated unreacted malonic acid to acetic acid, which could be washed out with water or vacuum-evaporated. Because of their high boiling points and hydrophobic properties, long-chain and medium-chain fatty acids could be purified easily by this technique. Using this procedure, [3-^{13}C]octanoic acid was isolated without distillation with 99% purity. The scope of this method was tested through the synthesis of short and long chain fatty acids: [3,3,3-$^{2}H_3$]propionic, [1-^{13}C]docosanoic, 4-phenyl[1,2-$^{13}C_2$]butyric, and [1,2, 11,12-$^{13}C_4$]dodecanedioic acids. Based on the data of these preparations, we concluded that increasing the length of aliphatic chain decreases the probability of the formation of dialkyl substituted side products. On the other hand, it is difficult to monitor the hydrolysis of the diethyl ester of long chain eicosylmalonate and the complete decarboxylation of eicosylmalonate

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by GC–MS because of the unstability of di-TMS derivative at 320°C, the GC column temperature necessary for the elution of these compounds. It was found that under these conditions, di-TMS of eicosylmalonate decarboxylates to TMS of docosanate.

Experimental

Materials

Unlabeled reagents, including solvents were purchased from Aldrich. Diethyl-[1,2-$^{13}C_2$]malonate, diethyl-[1,2,3-$^{13}C_3$]malonate [1-^{13}C]hexylbromide, and [$^2H_3$]methyliodide were supplied by ISOTEC. *N*,*N*-dimethylformamide (DMF) was dried immediately before the reaction.

Analysis of products

Chemical purity of all compounds was analyzed by GC–MS both as the ethyl ester and the TMS derivatives using a HP-5 column. The structures and isotopic purities were verified by both GC–MS as well as by nuclear magnetic resonance (NMR) spectroscopy.

GC–MS analyses were carried out on an Agilent 5973 mass spectrometer, equipped with a Model 6890 gas chromatograph, auto sampler, and HP-5MS 5% phenyl methyl siloxane fused silica capillary column (60 m, 25 mm id, 0.25 μm film thickness). The injector temperature was set at 270°C and the transfer line at 305°C. The GC temperature program was as follows: start at 80°C, hold for 1 min, increase by 6°C/min to 200°C, increase by 25°C/min to 310°C. The ion source was set at 200°C and the quadruple at 106°C. Under ammonia positive chemical ionization, $m/z+1$ and $m/z+18$ ions were monitored.

All proton magnetic resonance (^{1}H NMR) were recorded on a Varian Gemini spectrometer operating at 300 MHz. Residual protons of $CDCl_3$ ($\delta = 7.24$) was used as an internal reference. Chemical shifts were reported in parts per million (ppm). Carbon magnetic resonance (^{13}C NMR) spectra were recorded on the same instrument at 75 MHz. These spectra are reported in ppm δ scale relative to $CDCl_3$. Each time NMR spectra of synthetic labeled compound was compared with the spectra of unlabeled compound. This comparison and analysis of ^{13}C and ^{2}H coupling was used for the elucidation of the structure labeled carboxylic acid.

[3-^{13}C]Octanoic acid. Sodium hydride 60% dispersion in mineral oil (0.5 g, 12.5 mmol) was suspended in 10 ml of DMF under nitrogen gas. Diethyl malonate (2.00 g, 12.48 mmol) in 3 ml DMF was added slowly over 15 min. The reaction mixture was heated at 55°C until no more hydrogen gas was released, and the reaction mixture became clear. After cooling the reaction

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mixture to 40°C, freshly distilled [1-^{13}C]hexylbromide (1.98 g, 12.4 mmol) in 3 ml DMF was added slowly over 20 min. The reaction mixture was stirred for another 1 h at 60°C. GC–MS analysis of the reaction mixture showed one major peak of diethyl [1-^{13}C]hexylmalonate and two minor peaks, corresponding to diethyl di-[1,1′-$^{13}C_2$]hexylmalonate, and unreacted diethylmalonate. The reaction mixture diluted with 15 ml of water was extracted with ethyl ether. After evaporation of solvent, the residue was suspended in 10 ml of 2.8 M KOH solution in water. The reaction mixture was refluxed for 3 h until the hydrolysis of diethyl hexylmalonate was completed, based on GC–MS analysis. The reaction mixture was diluted with 10 ml of water and ethanol formed from hydrolysis was completely removed by azeotropic distillation. This prevents back esterification with ethanol during acidic decarboxylation of hexylmalonate. The GC–MS assay of the reaction mixture showed that under these conditions diethyl dihexylmalonate was not hydrolyzed. The unhydrolyzed side product was extracted from the alkaline solution with ethyl acetate. The water phase was made acidic and hexylmalonate was extracted with diethyl ether. The crude hexylmalonate was then suspended in 5 ml of dioxane + 10 ml of 12 N HCl and refluxed for 24 h. The cooled solution was extracted with diethyl ether. The combined organic extracts were dried with anhydrous sodium sulfate, filtered, and the solvent completely evaporated under reduced pressure. GC–MS and NMR analysis of the residue showed 99% chemical purity and 98% isotopic enrichment of the product. The yield of [3-^{13}C]octanoic acid was 63% based on [1-^{13}C]hexylbromide.

[3,3,3-$^{2}H_3$]Propionic acid. This compound was prepared from [3,3,3-$^{2}H_3$]methyl iodide and unlabeled diethylmalonate using the same procedure as was used for the synthesis of [3-^{13}C]octanoic acid. The chemical purity, isotope enrichment and yield of the product were 99, 98 and 47%.

[1-^{13}C]Docosanoic acid. This compound was prepared from diethyl [1,3-$^{13}C_2$] malonate and 1-bromoeicosane in the same manner as [3-^{13}C]octanoic acid. The product was recrystallized from hexane. The chemical purity, isotope enrichment and yield of the product were 98, 99 and 69%.

4-*Phenyl[1,2-$^{13}C_2$]butyric acid.* This compound was prepared from diethyl [1,2,3-$^{13}C_3$]malonate and 2-bromoethylbenzene in the same manner as [3-^{13}C]octanoic acid. GC–MS monitoring of the orientation experiment with unlabeled diethylmalonate revealed that during the nucleophilic alkylation reaction, part of the 2-bromoethylbenzene underwent a partial elimination to styrene. This is why in the subsequent synthesis, a slight excess of 2-bromoethylbenzene (1:1.15) was used for the alkylation of diethyl [1,2,3-$^{13}C_3$]malonate. 4-Phenyl[1,2-$^{13}C_2$]butyric acid was recrystallized from

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hexane. The chemical purity, isotope enrichment and yield of the product were 98, 99 and 54%.

[1,2,11,12-$^{13}C_4$]Dodecanedioic acid. This compound was prepared from 2 equivalent of diethyl [1,2,3-$^{13}C_3$]malonate and 1 equivalent of 1,8-diiodooctane in the same manner as [3-^{13}C]octanoic acid. The product was recrystallized from hexane/ether. The chemical purity, isotope enrichment and yield of the product were 98, 99 and 59%.

Conclusion

A convenient and efficient three-step procedure for the preparation of ^{13}C and ^{2}H-labeled carboxylic acids, which avoids the isolation of intermediates, has been developed utilizing malonic ester methodology. The reaction sequence can be easily monitored by GC–MS analysis. The wide availability of GC–MS instrumentation allows this procedure to be applicable in most research labs for the synthesis of labeled (and unlabeled) fatty acids and dicarboxylic acids.

Acknowledgements

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References

1. Baretz BH, Lollo CP, Tanaka K. *J Label Compd Radiopharm* 1978; **15**: 360–379.
2. Cope AC, Holmes HL, House HO. The alkylation of esters and nitriles. *Organic Reactions*. Wiley: New York, 1957; 107–331.
3. Padmakumar R, Gantla S, Banerjee R. *Anal Biochem* 1993; **214**: 318–320.
4. Allevi P, Longo A, Anastasia M. *J Label Compd Radiopharm* 1999; **42**: 1085–1091.
5. Brandstrom A, Junggren U. *Tetrahedron Lett* 1972; **6**: 473–474.
6. Kasumov T, Adams JE, Bian F, David F, Thomas KR, Jobbins KA, Minkler PE, Hoppel CL, Brunengraber H. *Biochem J* 2005; **389**: 397–401.
7. Bian F, Kasumov T, Thomas KR, Jobbins KA, David F, Minkler PE, Hoppel CL, Brunengraber H. *J Biol Chem* 2005; **280**: 9265–9271.

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