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Simple Synthesis of Multiply Labelled Sodium Propionate

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Summary

Sodium [3-^{13}C]- and [3-^{13}CD$_3$]propionate were conveniently synthesized from 2,4,4-trimethyl-2-oxazoline. The numbers and locations of deuterium labels were confirmed by broad-band deuterium and proton-decoupled ^{13}C-nuclear magnetic resonance (^{13}C-{^{1}H}{D}NMR) spectroscopy and fast atom bombardment mass (FAB-MS) spectroscopy.

Key words: [3-^{13}C]propionate, [3-^{13}CD$_3$]propionate, 2,4,4-trimethyl-2-oxazoline, broad-band deuterium and proton-decoupled ^{13}C-NMR, fast atom bombardment MS.

Introduction

Stable-isotope labelled compounds are useful as tracers in studies of biosynthetic pathways and in clinical medicine, and much information can be obtained by utilizing FT-NMR and IR spectroscopy1).

We have reported the synthesis of optically pure ^{13}C-labelled propionates2) for use in a study of the stereochemistry of the chain elongation step in the biosynthesis of macrolides and polyethers. We

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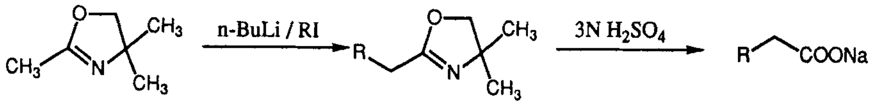
are now interested in the source of protons of the side-chain methyls during the biosynthesis of erythromycin aglycone, and we require propionate, whose methyl protons are labelled. Generally, [3-^{13}C]-propionate is synthesized from [2-^{13}C]iodoethane3), which is more expensive than [^{13}C]iodomethane. In addition, the methyl protons of propionate are variously labelled. We now report a simple and convenient synthesis of sodium [3-^{13}C]- and [3-^{13}CD$_3$]propionates, and confirmation of the numbers and locations of the deuterium labels.

Results and Discussion

As shown in scheme 1, condensation of [^{13}C]iodomethane (1a) or [^{13}CD$_3$]iodomethane (1b) with n-butyllithium-treated 2,4,4-trimethyl-2-oxazoline (2)4) in dry tetrahydrofuran afforded 2-[2'-^{13}C]ethyl-4,4-dimethyl-2-oxazoline (3a) or 2-[2'-^{13}CD$_3$]ethyl-4,4-dimethyl-2-oxazoline (3b), respectively, without dialkylation. Without purification, they were heated under reflux with 3 N sulfuric acid to give sodium [3-^{13}C]-propionate (4a) in 84 % yield from [^{13}C]iodomethane (1a) and sodium [3-^{13}CD$_3$]propionate (4b) in 80 % yield from [^{13}CD$_3$]iodomethane (1b).



(2)

(1a) R=^{13}CH$_3$
(1b) R=^{13}CD$_3$

(3a) R=^{13}CH$_3$
(3b) R=^{13}CD$_3$

(4a) R=^{13}CH$_3$
(4b) R=^{13}CD$_3$

Scheme 1: Synthesis of Labelled Sodium Propionate.

^{13}C-{^{1}H}{D}NMR5) and MS spectroscopy are useful for checking numbers and locations of deuterium labels. The peak (m/z 77 (M^--Na)) of sodium [3-^{13}CD$_3$]propionate (4b) in the FAB-MS spectrum showed the existence of three deuterium atoms (Fig. 1b). The septuplet-coupled and α-shifted (76.3 Hz) methyl signal of sodium [3-^{13}CD$_3$]propionate (4b) in the ^{13}C-{^{1}H}NMR (^{13}C-NMR) spectrum (Fig. 2b) clearly revealed three

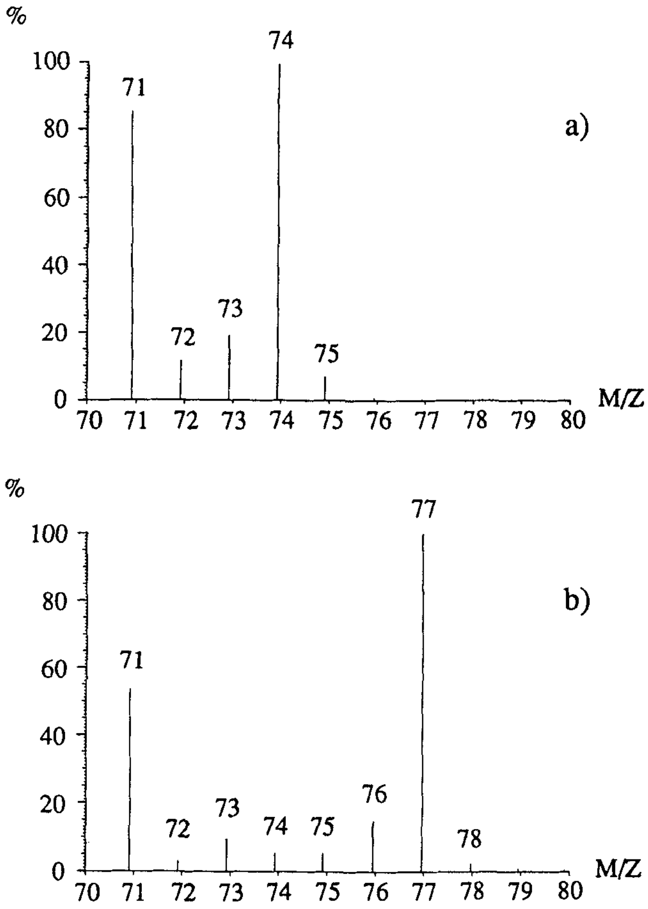


Fig. 1: a) FAB-MS Spectrum of Sodium [3-^{13}C]Propionate (4a), b) FAB-MS Spectrum of Sodium [3-^{13}CD$_3$]Propionate (4b).

deuterium atoms in the methyl group. The ^{13}C-{^{1}H}{D}NMR spectrum (Fig. 2c) showed two singlet signals, indicating the existence of not only sodium [3-^{13}CD$_3$]propionate (4b), but also sodium [3-^{13}CD$_2$H]propionate, although the content of the latter was estimated to be less than 5 %. The latter product could not be readily detected in the ^{13}C-NMR spectrum of 4b (Fig. 2b) owing to the complexity of the α-shifted signal and the lower signal-to-noise ratio.

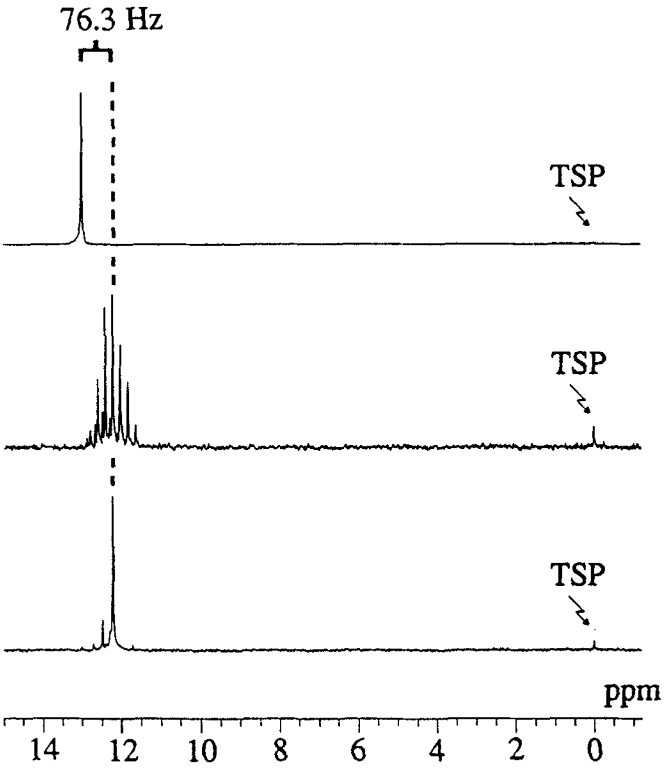


Fig. 2: a)^{13}C-NMR Spectrum of Sodium [3-^{13}C]Propionate (4a), b) ^{13}C-NMR Spectrum of Sodium [3-^{13}CD$_3$]Propionate (4b), c) ^{13}C-{^{1}H}{D}NMR Spectrum of Sodium [3-^{13}CD$_3$]Propionate (4b).

Experimental

Materials

[^{13}C]Iodomethane (99 atm % ^{13}C) was supplied by Cambridge Isotope Laboratories. [^{13}CD$_3$]Iodomethane (99 atom % ^{13}C, 98 atom % D) was supplied by MDS Isotopes.

Instruments

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL GSX-400 (100 MHz) spectrometer in deuterium oxide solution with sodium 3-trimethylsilylpropionate (TSP) as an internal standard. ^{13}C-NMR and ^{13}C-{^{1}H}{D}NMR conditions were: spectral width 24,038.5 Hz; acquisition time 0.682 s; repetition delay 2.5 s; and 30° pulse. FAB-MS spectra were obtained on a Fisons Instrument VG Analytical AutoSpec spectrometer at 8 kV with a DEC VAX-4000 Model 60 data system. Glycerol was used as the sample matrix.

Sodium [3-^{13}C]- and [3-$^{13}CD_3$]Propionates

n-Butyllithium (1.56 M in hexane, 1.03 ml, 1.61 mmol) was added dropwise to a solution of 2,4,4-trimethyl-2-oxazoline ($\underline{2}$) (0.19 ml, 1.49 mmol) in dry tetrahydrofuran (10 ml) at -78 °C under argon, and the solution was stirred for 10 min. To this solution, [^{13}C]iodomethane ($\underline{1a}$) (0.10 ml, 1.60 mmol) was added dropwise, and the whole was stirred for 1 hr at this temperature. The reaction mixture was quenched with sat. ammonium chloride aq. and extracted with ether (20 ml X 3). The combined extracts were washed with brine, dried over magnesium sulfate, and concentrated to approximately 30 ml. This solution, which contained 2-[2'-^{13}C]ethyl-4,4-dimethyl-2-oxazoline ($\underline{3a}$), was mixed with 3 N sulfuric acid (20 ml). The reaction mixture was refluxed for 1 hr, adjusted to pH 10.0 with 1 N sodium hydroxide, and freezed-dried. The residue was taken up in water (50 ml), and the pH was adjusted to 1.0 with 3 N sulfuric acid. The propionate was distilled with water. The distillate was adjusted to pH 10.0 with 1 N sodium hydroxide, and this solution was freezed-dried to give sodium [3-^{13}C]propionate ($\underline{4a}$) (130 mg, 84 %), ^{1}H-NMR (D_2O) 1.06 (dt, 3H, J_{HH}=7.7 Hz, J_{13CH}=127.3 Hz, $^{13}C\underline{H}_3CH_2$), 2.19 (dq, 2H, J_{HH}=7.7 Hz, J_{13CCH}=4.9 Hz, $^{13}CH_3C\underline{H}_2$), ^{13}C-NMR ($D_2O$) 13.0 ($^{13}\underline{C}H_3CH_2$), Negative-Ion FAB-MS m/z 74 (M^--Na, 100 %).

Sodium [3-$^{13}CD_3$]propionate ($\underline{4b}$) (282 mg, 80 %) was synthesized similarly with [$^{13}CD_3$]iodomethane ($\underline{1b}$) (0.22 ml, 3.51 mmol), 2,4,4-trimethyl-2-oxazoline ($\underline{2}$) (0.41 ml, 3.29 mmol) and n-butyllithium (1.60 M in hexane, 2.20 ml, 3.52 mmol), ^{1}H-NMR (D_2O) 2.2 (m, 2H, $^{13}CD_3C\underline{H}_2$), ^{13}C-NMR ($D_2O$) 12.2 (septuplet, J_{13CD}=19.4 Hz, $^{13}\underline{C}D_3CH_2$), ^{13}C-{^{1}H}{D}NMR (D_2O) 12.2 (singlet, $^{13}\underline{C}D_3CH_2$), Negative-Ion FAB-MS m/z 77 (M^--Na, 100 %).

Acknowledgment

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