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Synthesis and structural analysis of ^{13}C-fatty acids

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Abstract The ^{13}C-labeled fatty acids octanoic-1-^{13}C acid and palmitic-1-^{13}C acid were synthetically prepared from Ba $^{13}CO_3$. The yield of the former was more than 90% and that of the latter was above 85%. MS, IR, ^{1}H-NMR and ^{13}NMR were performed to analyze the structures of the two ^{13}C-fatty acids, compared with their unlabeled fatty acids.

Keywords ^{13}C-fatty acids, Synthesis, Structural analysis
CLC numbers O621.3$^+$5, O623.61, O656.4

1 Introduction

$^{14}CO_2$ breath tests are simple to perform, reliable for diagnosis, and acceptable to patients and staff. So they have been extensively used in the field of Gastroenterology for more than 20 years[1,2]. However, radioactive compounds possess the characteristics of a potential health hazard, which makes $^{14}CO_2$ breath tests unsuitable for infants, children, and pregnant women. With the improvements both in the increased availability and diversity of stable-isotope-labeled compounds and in the analytical instrumentation such as isotope-ratio mass spectrometry(IRMS) and isotope-selective non-dispersive infrared spectrometry(NDIRS) for their quantitative analysis, $^{13}CO_2$ breath tests provide safe and reliable alternations to $^{14}CO_2$ breath tests. In an attempt to perform the CO_2 breath tests in the diagnosis of fat malabsorption[3] and heart diseases[4,5], as well as in the study of gastric emptying rate of solid foods[6], octanoic-1-^{13}C acid and palmitic-1-^{13}C acid were synthesized by different Grignard reagents with high yields. MS, IR, ^{1}H-NMR and ^{13}C-NMR were performed to analyze their structures.

2 Experiments[7]

0.5 g dry magnesium turnings (21 mmol), 40 mL absolute diethyl ether, and crystalline iodine were added to a two-necked reaction flask fitted on a magnetic stirrer. A solution of 18 mmol alkyl halide in 10 mL of anhydrous diethyl ether was gradually added into the flask from a dropping funnel under gently reflux till the reaction was complete. Then the flask was attached to a high-vacuum manifold system. The solution of the Grignard reagent (in excess relative to $Ba^{13}CO_3$) in the flask was frozen with liquid nitrogen and the system was evacuated to 0.66Pa. $^{13}CO_2$ which was generated from $Ba^{13}CO_3$ and concentrated sulfuric acid diffused and condensed rapidly under high vacuum in the two-necked flask. The mixture was thawed and stirred at −20°C and carbonation was performed. The stirring was maintained for suitable time to ensure completion of the reaction. The complex was hydrolyzed with dilute sulfuric acid. Then the product was extracted from the ether solution with dilute alkaline solution. The alkaline solution was acidified again, and the product was extracted by ether.

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3 Structural analyses

3.1 Mass spectrometric analysis (MS)

MS plays an important role in the identification of organic compound. It can determine the correct molecular and fragment ions of compound and provide significant information about its primary structure.

Table 1 and Table 2 show fragmentation patterns of the ^{13}C-labeled and unlabeled fatty acids, respectively. ^{13}C-labeled and unlabeled fatty acids resemble each other in split process. ^{13}C-labeled octanoic acid and palmitic acid, as well as unlabeled octanoic acid and palmitic acid all produce molecular ion peaks, $m/z145$, $m/z257$, $m/z144$, $m/z256$, which prove the correctness of these compounds. The α spilt of carboxylic acid gives rise to two series of fragment ions containing even electron: one is 45, 59, 73, 87, $\cdots$, and the other is 15, 29, 43, 57, 71, $\cdots$, and so on. The former possesses carboxy (As Table 1 and Table 2 listed), while the latter has no carboxy. However, the fragment ions possessing carboxys of ^{13}C-labeled fatty acids contain 1 amu more than those of unlabeled fatty acids. The strong peaks $m/z60$ and $m/z61$ all come from McLafferty rearrangement and are the specific peaks of unlabeled and ^{13}C-labeled carboxylic acids, m/z60 and m/z61 are the basic peaks in the MS of unlabeled and ^{13}C-labeled octanoic acid.

Table 1 Fragmentation pattern for octanoic-1-^{13}C acid

Observed m/z for octanoic acid	Observed m/z for octanoic-1-^{13}C acid	Fragment
60	61	$:\dot{O}H$ $HO\text{-}^{13}C{=}CH_2$
73	74	$CH_2CH_2\,^{13}CO_2H^+$
87	88	$CH_2CH_2CH_2\,^{13}CO_2H^+$
101	102	$CH_2(CH_2)_2CH_2\,^{13}CO_2H^+$
115	116	$CH_2(CH_2)_3CH_2\,^{13}CO_2H^+$
127	128	$CH_3(CH_2)_5CH_2^{13}CO^+$
129	130	$CH_2(CH_2)_4CH_2\,^{13}CO_2H^+$
144	145	$CH_3(CH_2)_5CH_2\,^{13}CO_2H^{+\bullet}$

Table 2 Fragmentation pattern for palmitic-1-^{13}C

Observed m/z for Palmitic acid	Observed m/z for Palmitic-1-^{13}C acid	Fagment
60	61	$:\dot{O}H$ $HO\text{-}^{13}C{=}CH_2$
73	74	$CH_2CH_2\,^{13}CO_2H^+$
87	88	$CH_2CH_2CH_2\,^{13}CO_2H^+$
101	102	$CH_2(CH_2)_2CH_2\,^{13}CO_2H^+$
115	116	$CH_2(CH_2)_3CH_2\,^{13}CO_2H^+$
129	130	$CH_2(CH_2)_4CH_2\,^{13}CO_2H^+$
143	144	$CH_2(CH_2)_5CH_2\,^{13}CO_2H^+$
157	158	$CH_2(CH_2)_6CH_2\,^{13}CO_2H^+$
171	172	$CH_2(CH_2)_7CH_2\,^{13}CO_2H^+$
185	186	$CH_2(CH_2)_8CH_2\,^{13}CO_2H^+$
199	200	$CH_2(CH_2)_9CH_2\,^{13}CO_2H^+$
213	214	$CH_2(CH_2)_{10}CH_2\,^{13}CO_2H^+$
227	228	$CH_2(CH_2)_{11}CH_2\,^{13}CO_2H^+$
256	257	$CH_3(CH_2)_{13}CH_2\,^{13}CO_2H^{+\bullet}$

3.2 Infrared absorption spectrum (IR)

IR mainly provides the information for the analysis of the characteristic functional groups of organic compound.

Table 3 reveals the infrared spectral characteristics of the carbonyl absorptions of the two ^{13}C-labedled and unlabeled fatty acids. The IR absorption bands of ^{13}C-labeled carbonyls are similar to those of unlabeled carbonyls, but the formers move towards the lower fields. ^{13}C-labeled and unlabeled octanoic acid, sodium salts give two IR absorption bands at 1519.7cm-1, 1403.9 cm^{-1} and 1560.2 cm^{-1}, 1446.4 cm^{-1}, respectively. The former comes from the carboxylate ($-^{13}CO_2^-$) of the ^{13}C-labeled octanoic acid, sodium salt, and the latter from the carboxylate($-CO_2^-$) of the unlabeled octanoic acid, sodium salt.

Table 3 Carbonyl absorptions in the ^{13}C-labeled and unlabeled fatty acids

Compounds	$^{13}C{=}O/cm^{-1}$	$C{=}O/cm^{-1}$	Δ (Chemical shift value)/cm^{-1}
Octanoic-1-13C acid,	1519.7	1560.2	1560.2-1519.7=40.5
Sodium salt	1403.9	1446.4	1446.4-1403.9=42.5
Palmitic-1-^{13}C acid	1656.6	1702.9	1702.9-1656.6=46.3

3.3 Nuclear magnetic resonance (NMR)

3.3.1 ^{1}H-NMR

The ^{1}H-NMR can provide the data on the chemical shift of proton, the integral value of peak area, and so on (see Tables 4 and 5). Chemical shift shows the chemical environment of the proton. The integral area is in proportion to the number of the protons that the peak possesses. Hence the ^{1}H-NMR is important to prove the molecular formula and provide useful information on molecular structure.

Table 4 ^{1}H-NMRs of octanoic-1-^{13}C acid, sodium salt and octanoic acid, sodium salt

^{1}H chemical shift/10^{-6}	Compounds	
	$CH_3(CH_2)_4CH_2CH_2^{13}COONa$ a b c d	$CH_3(CH_2)_4CH_2CH_2COONa$ a b c d
a	$\delta 0.92$(3H,q,8-CH_3)	$\delta 0.92$(3H,t,8-CH_3)
b	$\delta 1.33$(8H,s,4~7-CH_2)	$\delta 1.34$(8H,d,4~7-CH_2)
c	$\delta 1.59$(2H,d,3-CH_2)	$\delta 1.60$(2H,q,3-CH_2)
d	$\delta 2.23$(2H,q,2-CH_2)	$\delta 2.23$(2H,t,2-CH_2)

Table 5 ^{1}H-NMRs of palmitic-1-^{13}C acid and palmitic acid

^{1}H chemical shift/10^{-6}	Compounds	
	$CH_3(CH_2)_{12}CH_2CH_2^{13}COOH$ a b c d	$CH_3(CH_2)_{12}CH_2CH_2COOH$ a b c d
a	$\delta 0.89$(3H,q,8-CH_3)	$\delta 0.87$(3H,t,8-CH_3)
b	$\delta 1.28$(8H,s,4~15-CH_2)	$\delta 1.25$(8H,d,4~15-CH_2)
c	$\delta 1.63$(2H,d,3-CH_2)	$\delta 1.60$(2H,q,3-CH_2)
d	$\delta 2.34$(2H,q,2-CH_2)	$\delta 2.24$(2H,t,2-CH_2)

The octanoic-1-^{13}C acid, sodium salt is similar to the octanoic acid, sodium salt in ^{1}H chemical shift value. And so are the palmitic-1-^{13}C acid and the palmitic acid

3.3.2 ^{13}C-NMR

^{13}C-NMR and 1H-NMR are mutually complementary to identify the molecular structure. The ^{13}C-NMR can provide the chemical shift value of carbon atom. From this information, CH_3-, $-CH_2-$ can be differentiated from $-COOH$ or

$-^{13}COOH$ in the same molecule. $-COOH$ and $-^{13}COOH$ will also be distinguished in different molecules according to their peak intensities.

Fig.1, Fig.3, Fig.2, and Fig.4 show that ^{13}C-NMRs(BB+DEPT) of ^{13}C-labeled palmitic acid and octanoic acid, sodium salt are similar to those of unlabeled palmitic acid and octanoic acid, sodium salt, respectively. There are two differences between the ^{13}C-labeled and unlabeled compounds. In the natural abundance samples, two ^{13}C atoms have little chance to couple, so every carbon of the compounds produce single peak. In

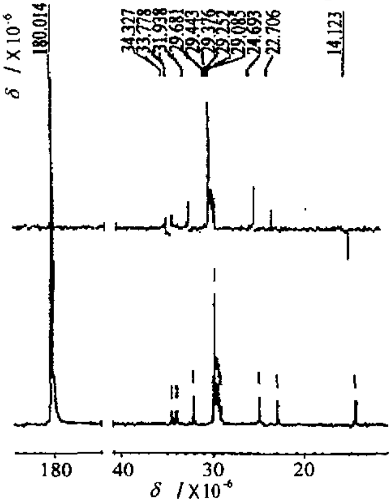

Fig.1 ^{13}C BB+DEPT of palmitic-1-^{13}C acid

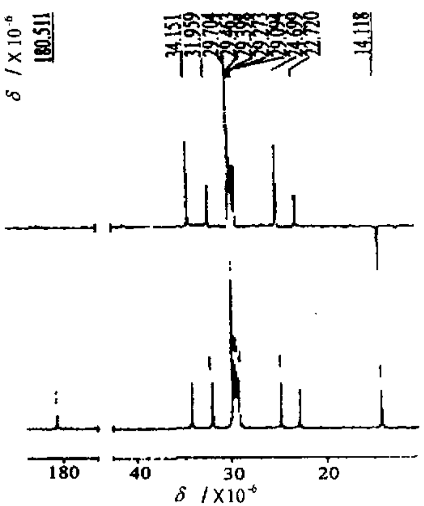

Fig.2 ^{13}C BB+DEPT of palmitic acid

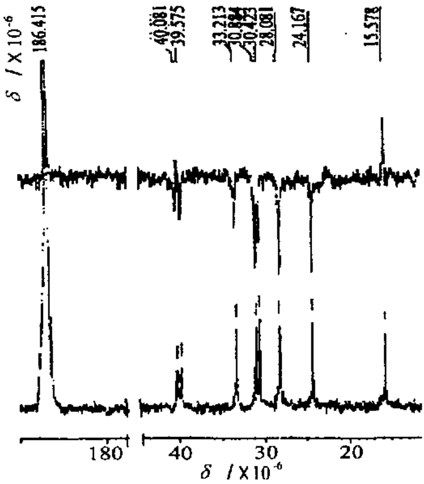

Fig.3 ^{13}C BB+DEPT of octanoic-1-^{13}C acid, sodium salt

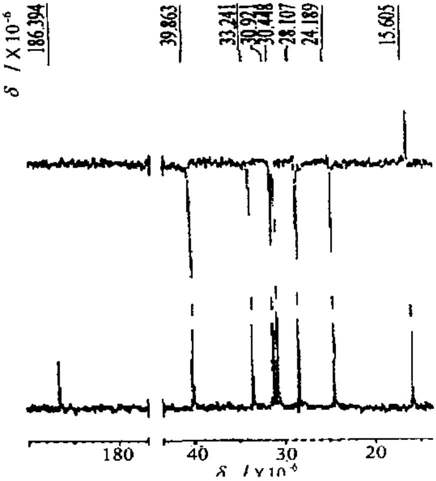

Fig.4 ^{14}C BB+DEPT of octanoic acid, sodium salt

the ^{13}C-labeled compounds, ^{13}C-^{13}C coupling may appear which makes the carbons near ^{13}C give two peaks. On the other hand, the ^{13}C-NMR signal of the compound containing carbons is very weak due to low natural abundance. While the carboxyl containing ^{13}C in the ^{13}C-labeled compound gives strong peak.

4 Discussion

Two step reactions are performed to synthesize the ^{13}C-labeled fatty acids. The first step is Grignard reaction and the second is carbonation. To gain high yield, the conditions minimizing the formation of ketone and carbinol such as anhydrous, a suitable excess of the Grignard reagent, effective mixing, suitable carbonation time and low reaction temperature are required. In this way, we gained ^{13}C-labeled fatty acids in high yields: octanoic-1-^{13}C acid was more than 90% and palmitic-1-^{13}C acid was above 85%. The yield of [1-^{14}C]palmitic acid synthesized by means of the same method was 44.8%[8]. Palmitic-1-^{13}C acid was also prepared through the hydrolysis of cyanide and the yield was 44%[9]. Reducing production cost results from raising the yield of ^{13}C-labeled substrate, which creates favorable conditions for extending the utility of $^{13}CO_2$ breath tests in clinic diagnosis.

Sequential extraction with aqueous alkaline solution and aqueous acid solution removes the impurities from the organic solvent leaving behind the desired product. To further purify palmitic-1-^{13}C acid, recrystallization is needed. TLC are done to identify the purity of the ^{13}C-labeled fatty acid using silica gel G plates developed in two solvent systems and all should show a single spot.

The results of MS, IR, ^{13}C-NMR, ^{1}H-NMR show that the ^{13}C-labeled fatty acids synthesized in our laboratory are correct and pure. MS, IR are not only used in the identification of the molecular structure of the ^{13}C-labeled fatty acid, but in the measurement of $^{13}CO_2/^{12}CO_2$ isotope ratio. With the availability of more ^{13}C-labeled compounds and the improvement of the analytical instrumentation, $^{13}CO_2$ breath tests can be extensively used in clinic diagnosis.

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