# The Synthesis of Carboxylic Acids with Carboxyl Carbons of Precisely Known Stable Isotopic Composition

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A procedure is described which allows the preparation of carboxylic acids in which the carbon isotopic composition of the carboxyl carbon atom is known to within better than  $0.002\%^{-13}$ C (95% confidence limits). In this procedure, a Grignard reagent is first reacted with CO<sub>2</sub> of known isotopic composition. After hydrolysis of the salt of the carboxylic acid, the unreacted CO<sub>2</sub> is recovered and the isotopic composition determined. The isotopic composition of the carboxyl carbon is then calculated by difference. A differential error analysis in which all relevant uncertainties are considered is presented. Approximate kinetic isotope effect values for carboxylation of a Grignard reagent at -78 C can be determined from the observed isotopic fractionations, with five observations yielding  ${}^{12}k/{}^{13}k = 1.059$  ( $\sigma = 0.009$ ).

# **INTRODUCTION**

It is well known that isotope effects in chemical reactions can lead to significant isotopic fractionations in the products. For example, it has been shown that the carboxyl carbon is enriched in  $^{13}$ C during biosynthesis of amino acids<sup>(1)</sup> and acetic acid.<sup>(2,3)</sup> With the goal of eventually determining the extent to which the isotopic distribution pattern in acetate is retained during biosynthesis of alkyl chains, we are working to develop methods for the determination of the isotopic distributions within alkanoic acids.

Because there exist no purely instrumental methods for the determination of the isotopic composition of each carbon position in alkanoic acids, a chemical degradation scheme must be applied in order to produce CO<sub>2</sub> from each position of interest. Any kinetic isotope effects incurred during the degradation must be carefully investigated and taken into account if accurate results are to be obtained. Determination of isotopic fractionations occurring in the degradative reactions will be greatly facilitated if standard acids with accurately known initial isotopic compositions can be prepared. Useful analyses of natural isotopic abundance variations typically involve an uncertainty of  $\pm 0.0005\%^{13}$ C and the synthetic procedure must be consistent with this requirement.

Carboxylic acids labelled with  ${}^{14}C$  in the carboxyl group have previously been prepared by the addition of  ${}^{14}C$  to Grignard reagents.<sup>(4)</sup> This procedure has been adapted to the present requirements in the following way: First an excess of CO<sub>2</sub> with known isotopic composition is reacted with a Grignard reagent to produce (after hydrolysis of the intermediate) the corresponding alkanoic acid. Second, the unreacted  $CO_2$  is recovered and its isotopic composition is determined. In this case, the isotopic composition of the synthesized carboxyl group can be determined by difference, and the validity of the calculation can be checked by obtaining a mass balance via titration of the acid.

# **EXPERIMENTAL**

The synthesis can be divided into two major procedures: (1) manipulation of the gas-phase reagent,  $CO_2$ ; (2) manipulation of the condensed-phase reagents, Grignard reagent and fatty acid.

# 1. Treatment and manipulation of CO<sub>2</sub>

All measurements of  $CO_2$  were made manometrically in a vacuum apparatus (Fig. 1) of known volume, so that a pressure-volume measurement could be converted into a mass measurement. Both reactant and recovered  $CO_2$  were rigorously purified by sublimation at  $-117^{\circ}C$  (ethanol slush) and, subsequently, at  $-129^{\circ}C$  (*n*-pentane slush). Isotopically representative aliquots of purified  $CO_2$  were transferred in breakseal tubes to the inlet system of an isotope-ratio mass spectrometer like that described by MCKINNEY et al.<sup>(5)</sup>

Carbon dioxide of known isotopic composition (0.05 moles) was prepared for reaction with the Grignard reagent by condensing the  $CO_2$  in a 1000-ml reaction vessel (Fig. 2) cooled in liquid nitrogen  $(-196^{\circ}C)$ .

After the reaction between  $CO_2$  and the Grignard reagent and the hydrolysis of the magnesium salt of the fatty acid were accomplished, the unreacted  $CO_2$ 



FIG. 1. Vacuum apparatus for gas manipulation: A, B. traps; C, 1-l. ballast volume; D, E, sampling ports; F, manometer; TC1, TC2, thermocouple pressure gauges.

was recovered by condensing the  $CO_2$  from the reaction vessel into trap A, cooled in liquid nitrogen (Fig. 2). Trap B and the open-ended condenser were cooled in an ethanol slush to prevent large amounts of solvent from reaching trap A.

#### 2. Manipulation of condensed phase reagents

The alkyl Grignard reagent was produced under anhydrous conditions in flask E by adding the alkyl bromide dropwise to a mixture of refluxing diethyl ether and magnesium turnings.

$$RBr \xrightarrow{Mg}_{dry \text{ ether}} RMgBr.$$
(1)

After refluxing for 1 hr, the Grignard reagent was slowly added through the sidearm to the 1000-ml reaction vessel (D, Fig. 2) containing the reagent  $CO_2$ . The reaction vessel was cooled in a dry-ice-acetone bath ( $-78^{\circ}C$ ) during the course of the reaction.

$$RMgBr + CO_2 \rightarrow RCO_2MgBr.$$
 (2)

After complete addition of the Grignard reagent, an aqueous solution of sulfuric acid (0.1 mole) was added to the reaction vessel in order to hydrolyze the magnesium salt of the fatty acid. This procedure leads to the separation of two phases in the reaction vessel. with the product acid in the ether phase. The dry-ice-acetone cooling bath was left in place during the hydrolysis reaction and during the first stage of  $CO_2$  recovery. The reaction mixture was then allowed to thaw and was thoroughly degassed in order to complete the  $CO_2$  recovery.

The fatty acid was extracted from the ether phase and separated from side reaction products by one of two procedures, depending on the chain length of the acid. Acids containing less than 12 carbon atoms were recovered by washing the ether phase with a saturated aqueous solution of Na<sub>2</sub>CO<sub>3</sub> (100 ml), acidification of the resulting aqueous phase, and ether extraction of the product. Larger acids were recovered using a scaled-up McCARTHY-DUTHIE procedure<sup>(6)</sup> in which the product was recovered by formic acid extraction of a base-washed silica gel on which the product had been adsorbed. The acids containing less than 12 carbon atoms were quantified by titration with standard base. The larger acids were quantified by weighing the purified and dried product of the McCARTHY-DUTHIE separation.

# **RESULTS AND DISCUSSION**

Calculation of isotopic composition of the carboxyl carbon

A mass-balance equation for <sup>13</sup>C introduced to and recovered from the synthetic procedure can be written as follows:

$$P_i N_i = P_a N_a + P_r N_r + P_l N_l, \tag{3}$$

where the *P* terms represent atom per cent <sup>13</sup>C, the *N* terms represent molar amounts, and the subscripts *i*, *a*, *r*, and *l* refer to input CO<sub>2</sub>, carbon incorporated in the carboxyl group of the synthesized acid, recovered (unreacted) CO<sub>2</sub>, and lost CO<sub>2</sub>, respectively. In work near natural isotopic abundance, the atomic percentages differ only very slightly, but can be determined with very high precision  $(\pm 0.0002\%^{13}\text{C})$  by differential comparison to an arbitrary isotopic standard. The results of such measurements are usually expressed in terms of  $\delta^{13}C_{\text{PDB}}$ , where  $\delta^{13}C_{\text{PDB}} = [(R_x/R_{\text{PDB}}) - 1] 10^3$ ,  $R = {}^{13}\text{C}/{}^{12}\text{C}$ , PDB indicates the Pee Dee Belemnite standard (a typical fossil carbonate, see ref. 7), and *x* designates the sample. The units for  $\delta^{13}\text{C}$  are parts per thousand (°<sub>oo</sub>) and are termed "per mil".

Although all high-precision isotope abundance measurement systems are designed for the determination of  $\delta$ , accurate mass balance calculations such as equation (3) must be based on fractional isotopic abundances, not isotope ratios. In the present work, we have first directly determined  $\delta^{13}C_{PDB}$  values by differential isotope ratio mass spectrometry; then calculated atom per cent abundances by adopting  $R_{PDB} = 0.0112372$ ;<sup>(7)</sup> and, for convenience, finally recast these results in terms of  $\Delta_x$ . This variable, un-



FIG. 2. Apparatus for carbonation of Grignard reagent: A, B, traps; C, reflux condenser with external jacket for  $-117^{\circ}$ C cooling bath; D, 1-l. reaction vessel; E, 250-ml flask for preparation of Grignard reagent; F, sidearm for addition of Grignard reagent; G, dropping funnel; H, N<sub>2</sub> purge gas input; I, reflux condenser; J, drying tube (CaCl<sub>2</sub>).

like  $\delta$ , can be directly employed in accurate mass balance calculations. The definition of this quantity is given by  $\Delta_x = [(P_x/P_{PDB}) - 1] \ 10^3$ , and absolute  $^{13}C$  abundances can be calculated by adopting  $P_{PDB} = 1.11123$  atom  $^{\circ}_{0}$   $^{13}C$ . Like  $\delta$ ,  $\Delta$  has units of parts per thousand ( $^{\circ}_{00}$ ).

Important objectives in the present work are to calculate the isotopic composition of the carboxyl carbon atom and to obtain an accurate value for the uncertainty which must be assigned to that result. Recasting equation (3) in terms of  $\Delta$  values, and recalling that  $N_i = N_a + N_r + N_l$ , an expression meeting the first of these objectives is readily obtained.

$$\Delta_a = \frac{\Delta_i N_i - \Delta_r N_r - \Delta_l N_l}{N_a}.$$
 (4)

Determination of  $\Delta_a$  requires that all of the terms on the right-hand side of equation (4) be known. Since  $N_i$ ,  $N_r$ , and  $N_a$  are experimentally determined,  $N_i$  can be calculated. However,  $\Delta_i$  is a parameter that cannot be measured and its significance must be carefully considered. On the one hand, CO<sub>2</sub> might be lost during the recovery procedure. This loss should involve only small isotope effects, and it is reasonable to assign  $\Delta_l = \Delta_r$ . On the other hand, an apparent loss of CO<sub>2</sub> can occur if any side reactions consume some of the carbonated Grignard reagent. The major side reactions contributing to  $N_i$  are

$$RCO_2MgBr + RMgBr \rightarrow R_2CO + MgBr_2 + MgO$$
(5)

$$R_2CO + RMgBr \rightarrow R_3COMgBr$$
 (6)

$$R_3COMgBr + H_2O \rightarrow R_3COH + HOMgBr.$$
 (7)

Acting in competition with the main synthetic reaction, these side reactions could cause appreciable isotopic fractionation, making the estimation of  $\Delta_l$ nearly impossible. An assessment of the relative importance of these loss mechanisms leads to the conclusion that losses during recovery account for nearly all of  $N_l$  and that, therefore,  $\Delta_l$  can be satisfactorily approximated by  $\Delta_r$ . This judgement is based on an analysis of the side products, which were found to contain little or no oxygenated material provided the Grignard reagent had been added in small portions and the reaction temperature had been maintained at  $-78^{\circ}$ C, conditions which are known to limit the side reactions noted above.<sup>(8)</sup>

#### The uncertainty in the isotopic composition

To consider how the uncertainties in the various experimentally measured quantities contribute to the uncertainty in  $\Delta_a$ , it is useful to recast equation (4) in terms of relative molar quantities.

$$\Delta_a = X_i \Delta_i - X_r \Delta_r - X_l \Delta_l, \qquad (8)$$

where  $X_i = N_i/N_a$ , etc. Performing an analysis of the propogation of errors, and further simplifying the

result by defining  $D_a = \Delta_a - \Delta_i$ ,  $D_r = \Delta_r - \Delta_i$ ,  $D_l = \Delta_l - \Delta_l$ , and, consequently,  $D_i = 0$ , yields

$$\sigma_{\Delta_a}^2 = X_i^2 \sigma_{\Delta_i}^2 + X_r^2 \sigma_{\Delta_r}^2 + X_l^2 \sigma_{\Delta_l}^2 + D_r^2 \sigma_{x_i}^2 + D_l^2 \sigma_{x_i}^2, \quad (9)$$

where  $\sigma^2$  terms represent the variances in the parameters indicated by the subscripts. With the exception of  $\sigma_{\Delta_i}$ , all of the terms in equation (9) are experimentally determinable. Given that  $\Delta_i$ , has been taken as an estimate of  $\Delta_i$ ,  $\sigma_{\Delta_i}$  can be viewed as a measure of the adequacy of this approximation, and might, therefore, be assigned some value substantially greater than  $\sigma_{\Delta_i}$  or  $\sigma_{\Delta_i}$ . Even if  $\sigma_{\Delta_i}$  is taken to be an order of magnitude greater than  $\sigma_{\Delta_i}$ , and losses of CO<sub>2</sub> amount to 5%, application of equation (9) shows that the  $X_i^2 \sigma_{\Delta_i}^2$  term will typically account for less than 15% of the total variance. Therefore, perfectly accurate assignment of  $\sigma_{\Delta_i}$  is not critical, and  $\sigma_{\Delta_i}$  is adopted as the best estimate of  $\sigma_{\Delta_i}$ .

## Results of typical syntheses

The results of a number of syntheses are summarized in Table 1. Use of the data given in the first five columns allows calculation of  $\Delta_a$  [using equation 4] and  $\sigma_{\Delta_a}$  [using equation 9]. Considering the typical values shown in Table 1, it can be seen that approximately half of the total variance in  $\Delta_a$  is due to the first term in equation (9). Therefore, the value of  $X_i$  should be reduced to the greatest possible extent, a requirement which is equivalent to obtaining the highest possible yield. The carbon isotopic composition of each carboxyl group is known in each case to within better than  $0.002^{\circ}_{0}$  <sup>13</sup>C, a result hitherto unprecedented in the synthesis of labelled compounds.

### Kinetic isotope effects

The synthetic procedures outlined here incorporate few of the strict controls of reaction conditions which are required for precise determinations of kinetic isotope effects. Nevertheless, the data do allow an approximate calculation of the kinetic isotope effect, a process which seems worthwhile for a number of reasons. First, measurements of heavy-atom kinetic isotope effects are still rather rare, particularly under extreme temperature conditions such as those prevailing in the present work. Second, the carbonation of a Grignard reagent forms a particularly interesting example of carbon-carbon bond formation. Finally, the routine calculation of the approximate kinetic isotope effect provides a useful internal check on the consistency and reliability of the synthetic procedure itself.

The approximate kinetic isotope effects shown in Table 1 were calculated using equation (10):<sup>(9)</sup>

$$\binom{1^{3}k}{1^{2}k} - 1 = \frac{\log(R_{f}/R_{i})}{\log[(1-f)(1+R_{i})/(1+R_{f})]}, \quad (10)$$

where f, the fractional degree of reaction completion,

TABLE 1. Summary of acid syntheses

Product acid	N <sub>i</sub> * (moles)	N,* (moles)	N <sub>a</sub> † (moles)	$\Delta_{i\pm}^+$ $(^{lpha}_{ m oo})$	Δ,‡ (° <sub>00</sub> )	$\Delta_a$ ; (° <sub>00</sub> )	Approx. kinetic isotope effect $({}^{12}k/{}^{13}k, -78^{\circ}C)$
n-octanoic	0.0630	0.0080	0.0536	$-34.95 \pm 0.28$	$+86.75 \pm 0.57$	$-57.63 \pm 1.0$	1.068
n-octanoic	0.0527	0.0269	0.0249	$-34.50 \pm 0.16$	$-1.13 \pm 0.28$	$-71.71 \pm 1.1$	1.057
n-nonanoic	0.0612	0.0156	0.0447	$-34.53 \pm 0.60$	+46.60 + 0.11	$-64.56 \pm 1.8$	1.066
<i>n</i> -tridecanoic <i>n</i> -heptadecanoic§	0.0678 0.00402	0.0225 0.00288	0.0424 0.00116	$-36.98 \pm 0.37$ $-35.53 \pm 0.14$	$+14.38 \pm 0.41$ $-21.66 \pm 0.25$	$-68.30 \pm 1.2$ -69.86 ± 1.6	1.057 1.045

\*  $\sigma_{N_i} = \sigma_{N_r} = 8 \times 10^{-5}$  mole. †  $\sigma_{N_a} = 1.0 \pm 10^{-4}$  mole.

 $\ddagger$  Indicated uncertainty =  $\pm 2\sigma$ , isotopic standard = PDB (see text).

§ For this sample only,  $\sigma_{N_i} = \sigma_{N_r} = 8 \times 10^{-6}$  mole.

is taken here as  $N_a/N_i$ , and  $R_i$  and  $R_f$  indicate the  $^{13}C/^{12}C$  ratios in the CO<sub>2</sub> at the initiation and completion of each experiment. The absolute R values have been calculated from the definition of  $\Delta^{1\,3}C_{PDB}$ assuming  $P_p = 1.11123^{\circ/13}_{\circ 0} \text{ C.}^{(7)}$  The calculated effect refers to the carbonation of a Grignard reagent at −78°°C.

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