

Carbon-13 fractionation in the oxidation of propionate of natural isotopic composition with permanganate and manganate

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Abstract Carbon-13 fractionation in the oxidation of sodium propionate of natural isotopic composition with permanganate in water solution and with manganate in alkaline solution has been investigated and compared with carbon-14 kinetic isotope effects in the oxidation of (3-¹⁴C) – and (2-¹⁴C) propionate with permanganate. The experimental carbon-13 isotope ratios, $R(^{13}\text{C}/^{12}\text{C})_{\text{cumul}}$, corrected for dilution of $R(^{13}\text{C}/^{12}\text{C})_{\alpha}$ by carbon dioxide derived from all external sources and by carbon dioxide derived from (C-1) and from (C-3) of propionate at its complete and partial oxidations, used for calculation of $^{13}\text{C}_{\alpha}$ KIE, provided the carbon-13 isotope effects corresponding to initial oxidation of alpha carbon, which correlate well with (2-¹⁴C) KIE determined previously, $(k_{12}/k_{13} - 1) \times 100 \cong 1/2(k_{12}/k_{14} - 1) \times 100 \cong 1/2(4.6 \pm 0.3)$ at 373 K. The possibility of extension of isotope ratio measurements, using modern double collector mass spectrometers, from geochemical, biomedical, agriculture and environmental research to carbon-13 isotope effects studies in chemical organic reactions is briefly discussed.

Key words carbon-13 • carbon-14 • fractionation • manganate • oxidation • permanganate • propionate

Introduction

Carbonates and oxalates are the final products of oxidation of propionates with permanganate and manganate in alkaline medium [14]. This reaction drew therefore attention of radiochemists as early as in 1942 [11] in view of the possibility of using it to reveal the distribution of radioactivity within different possible positions of propionic acid molecule, a frequent isolation in biomolecular studies or for location of bond cleavage. Unfortunately it appeared soon using 1-, 2- and 3-¹¹C and ¹⁴C labelled propionates that the radiocarbon found in carbonates (or in oxalates) originates from different positions of propionate and only the preferential transformation of a given carbon atom in the propionate into carbonate or oxalate can be established [3, 9, 15].

Subsequent kinetic studies indicated that, in spite of complex distribution of radioactivity between products of oxidation of propionate (P), this reaction as such has a definite simple rate determining step and proceeds according to one mechanism in alkaline medium [17]. Investigation of the carbon-14 KIE in the oxidation of propionate ¹⁴C labelled in the alpha and beta positions indicated that rupture of the C_α-H bond is the rate determining step in the oxidation of P. with permanganate [20–22]. A substantial (k_{12}/k_{14}) KIE of 1.046 ± 0.003 has been found in the oxidation of propionate [²⁻¹⁴C] at 100°C (373 K), and the negligible one of secondary origin in the oxidation of [³⁻¹⁴C] propionate. This radiochemical result

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prompted us to investigate tritium and deuterium KIEs in the oxidation of tritium and deuterium labelled propionates. A very large deuterium kinetic isotope effect in the oxidation of [2-D₂] propionate, CH₃CD₂COONa, has been found. Its large temperature dependence revealed the tunnelling in the hydrogen transfer from the alpha carbon of propionate to the oxidizer especially pronounced in alkaline medium [16, 18, 19, 23]. Both the radiocarbon and hydrogen isotope effects solved the problem of rate determining step in the oxidation of propionate with Mn(VII) and Mn(VI) in alkaline medium. But from experimental point of view both the above approaches require the access to a radioisotope laboratory and quite expensive isotopically labelled compounds.

In this study we attempted to determine the carbon-13 fractionation in the oxidation of propionate of natural isotopic composition with permanganate. Propionic acid has three different positions with three different sets of bonds around each carbon atom. At complete oxidation of P. we obtained the average (¹³C/¹²C) carbon isotope ratio for all three carbons diluted by isotopic carbons from all "external" sources. Carbon dioxide obtained at partial oxidation of propionate should have different (¹³C/¹²C) ratio due to one primary ¹³C KIE caused by the rupture of the C_α-H bond and due to the two secondary ¹³C KIEs caused by the [¹³C] methyl and [¹³C] carboxyl groups. The primary ¹³C KIE will result the carbon dioxide slightly depleted with ¹³C. In the most favourable circumstances the non-homogeneous distribution of ¹³C between Me- and carboxyl groups might be balanced. The (¹³C/¹²C) ratio for CH₃- and -COO[⊖] groups might depend on the method of synthesis of propionate and differ from C-13 equilibrium thermodynamic distribution which depends on the number and types of bonds at the isotopic carbon [3(C-H) + 1(C-C) bond in the Me-group, and the 2(C=O) and 1(C-C) bond in -CO₂[⊖] group]. The magnitude of the secondary ¹³C KIE highly depends on the structure of the transition state (T.S.). If 3(C-H) and 2(C=O) bonds do not change in the T.S. then the S.KIE of ¹³C will be negligible. Weakening of these bonds will increase the S.¹³C KIE. This experiment should show how much the above "man made" [7, 13] and "natural" complications perturb the final ¹³C isotope effect determination since the exact ¹⁴C KIE data are already known (S. denotes: secondary).

Methods and results

The natural abundance of carbon-13 is sufficiently large to notice its variations in the course of chemical reactions using modern isotope ratio mass spectrometers [1, 10] now widely available also in Europe [2, 5, 8, 12]. Nevertheless the mechanisms of organic reactions have been and are investigated using carbon-14 isotope effect methodology [4, 6] applying liquid scintillation counters for precise determinations of the specific activities of the ¹⁴C labelled substrates and products of organic reactions at different degrees of chemical conversion [22]. Having already in hands the ¹⁴C KIE data concerning the oxidation of propionates successively labelled with ¹⁴C in the α- and β-positions with permanganate [20, 21] we decided to explore the possibility of determination of carbon-13

fractionation in the course of oxidation of propionate by measuring the mean carbon isotope ratios, R(¹³C/¹²C), of the cumulative carbon of oxalates and carbonates obtained at complete oxidation of propionate of natural isotopic composition and at partially known oxidations of propionate (at f < 1). The determinations of the radiocarbon in carbonates and oxalates have been replaced in modern ¹⁴C KIE studies by measuring the radioactivity of the easily obtained in high purity crystalline organic derivatives of propionic acid [22]. To simplify the analytical procedure the carbon dioxide derived from all external sources was isolated first and its delta (PDB) determined in the blank experiment reproducing all the analytical steps applied in the actual isotope effect oxidation of propionate with permanganate (0.0319 mmoles of cumulative CO₂ was derived with delta (PDB) equal to -18.33 and R(¹³C/¹²C) equal to 0.01103122). This correction has been applied to carbon dioxide derived from carbonates and oxalates to obtain a correct value of degree of oxidation of propionate listed in column (4) of Table 1.

The δ(PDB) values listed in column (5) of Table 1 are the rough uncorrected values provided by a mass spectrometer. They are smaller (more negative) than δ(PDB) = -34.66 of carbon dioxide obtained at complete oxidation of propionate samples with permanganate and with "CrO₃/H₃PO₄/Oleum" oxidizing solution, and indicate immediately that the heavier propionate molecules containing ¹³C are oxidized with smaller probability than the light P. molecules containing ¹²C. They indicate also that the carbon-hydrogen bonds are weakened in the T.S. Nevertheless these δ(PDB) values are insufficient to draw more detail mechanistic conclusions concerning the interaction of the organic substrate with the inorganic oxidant. Permanganate can attack all three carbon atoms in propionate but the earlier kinetic studies of oxidation of propionate with permanganate at different temperatures resulted a straight line Arrhenius temperature dependence of the second order oxidation rate constants which indicates that the oxidation proceeds according to one mechanism. The subsequent ¹⁴C KIE determinations in the oxidation of propionate labelled with ¹⁴C at C-2 and C-3 positions successively showed that there is a substantial ¹⁴C_α KIE and the very small (secondary) ¹⁴C_β KIE. The above pure kinetic and kinetic isotope effect data have been interpreted as indicating that the initial rate determining oxidation step consists in the effective attack of the oxidizer at the alpha-carbon which results in rupture of the C_α-H bond in the T.S. The subsequent steps are the fast ones and contribute to the actual distribution of ¹⁴C (or ¹³C) between products of the oxidation of propionate. Within this interpretation of kinetics and ¹⁴C KIEs the depletion of ¹³C in product carbon dioxide (listed in column (5)) is caused primarily by ¹³C KIE in the oxidation of the methylene group (or uniquely), augmented by small secondary ¹³C KIE of isotopic carbons located in the neighbouring methyl and carboxyl groups. In this introductory study only the isotopic composition of the total carbon dioxide obtained at complete and at partial oxidations of propionate has been determined. In the first approximation we assume that bonding at C-1 and at C-3 is not weakened (not changed) in the T.S. with respect to the initial state of the substrate and the carbon dioxide derived

Table 1. Carbon-13 isotope effect for oxidation of sodium propionate with permanganate.

Temperature °C	mmoles of substrate		Degree "f" of oxidation	Experimental	R(¹³ C/ ¹² C) α carbon	k ₁₂ /k ₁₃
Exp. No.	Sodium propionate	Oxidizer KMnO ₄	of propionate	δ(PDB) ^a	isotope ratio	
(1)	(2)	(3)	(4)	(5)	(6)	(7)
in 10 cm ³ of water						
95.1°C/N-5	2.518	2.101	0.1438	-40.88	0.01061526 (pf) 0.01084766 (so)	1.0237
95.6/N-14	7.329	4.295	0.1345	-41.64	0.01060409 (pf) 0.01084766 (so)	1.0247
95.6/N-16	0.9035	3.1589	0.3371	-39.43	0.01066222 (pf) 0.01084766 (so)	1.0215 1.0233±0.0013
90.2/N-3	1.5364	3.114	0.2491	-41.23	0.01060494 (pf) 0.01084766 (so)	1.0265
80.35/N-2	1.5822	3.1551	0.2828	-42.04	0.01058011 (pf) 0.01084766 (so)	1.0300
15.3/N-1	2.6554	5.8230	0.02567	-40.73	0.01052556 (pf) 0.01084766 (so)	1.0310
95.05/N-6	CH ₃ CD ₂ COONa	KMnO ₄	0.1823	-42.21	0.0105670 (pf)	1.0269
	3.1361	2.5723			0.0108233 (so)	
in 10 cm ³ of 3M NaOH						
95.6/N-13	2.1196	3.2767	0.1985	-41.40	0.0105555 (pf) 0.0108233 (so)	1.0283

^a) δ(PDB) of carbon dioxide obtained at partial (f<1) oxidation of sodium propionate; (pf) – carbon isotope ratios corresponding to carbon dioxide derived from alpha carbon of propionate; (so) – initial mean carbon isotope ratio in propionate (calculated using mean δ(PDB) equal -34.665). The δ(PDB) values were determined by Europa Scientific 20-20 mass spectrometer with ANCA-TG preparation module operating in the Reactor Center of the J. Stefan Institute of Ljubljana. The detailed description of the experimental procedure used for partial oxidations of propionate and for quantitative extraction of cumulative carbon dioxide from the post reaction mixture in vacuum reactor is given in refs. [16, 18, 19].

from C-1 and C-3 has the same isotopic composition as in the substrate before its partial oxidation. We calculated the carbon isotope ratio R(¹³C/¹²C) of carbon dioxide derived from the methylene carbon at partial oxidations of propionate (f<1) given in column (6) by correcting the measured values of δ(PDB) given in column (5) by initial dilution with the carbon derived from cumulative external sources and by a subsequent twice dilution of the carbon dioxide corresponding to the methylene carbon with carbon dioxide derived from (C-1) and (C-3) positions. The corrected R(¹³C/¹²C) values given in column (6) were used to calculate the ¹³C KIEs given in column (7) with the equation (1)

$$(1) \quad (k_{12}/k_{13}) = \ln[1-f(1+R_{so}) / (1+R_{pf})] / \ln[1-f(R_{pf}/R_{so}) / (1+R_{so}) / (1+R_{pf})]$$

In the course of calculations of R values a relation: δ(‰) = [(R_{sample}/R_{standard}) - 1] × 1000 was applied as usually. The assessed (k₁₂/k₁₃) KIEs are in good agreement with ¹⁴C KIE.

In the first approximation the per cent of ¹⁴C KIE = 4.6±0.3 at 100°C (373 K) is roughly twice as large as the per cent of ¹³C KIE = 2.33±0.13 at 96°C (369 K), (k₁₂/k₁₄ - 1)×100 ≅ 2(k₁₂/k₁₃ - 1)×100. There are no data on ¹⁴C KIE in the oxidation of (2-¹⁴C) (2-D₂) propionates, CH₃¹⁴CD₂COO⁻, with permanganate and manganate.

The ¹⁴C KIE studies provide directly the magnitude of the isotope effect caused by the rupture of the carbon-hydrogen bond at the given labelled position in the molecule. But before starting the time consuming ¹⁴C KIE research requiring the synthesis of the needed ¹⁴C labelled compound preliminary ¹³C KIE study is sufficient to instruct the researcher about the magnitude of the ¹⁴C KIE expected or about the necessity of constructing a fully automatic apparatus to avoid manual operations and any contact of the alkaline medium with carbon dioxide containing air and other external sources of carbon dioxide. The necessity of the detailed chemical degradation of the given organic compound on the isotope effect level to reveal the actual distribution of ¹³C among all isotopically important positions is also a laborious task.

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