Tracer Studies with Radioactive Carbon. The Synthesis and Oxidation of Several Three Carbon Acids

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Many three carbon acids, when oxidized with alkaline permanganate, yield one mole each of oxalate and carbonate. Interest in the mechanism of these reactions was aroused when it was discovered[1,2] that with propionate the carbonate is derived mainly from the beta carbon rather than the carboxyl group. In an attempt to learn more about the mechanism, labelled propionate, α- and β-hydroxypropionates have been synthesized and oxidized in the presence of varying concentrations of sodium hydroxide.

Oxidation of Oxalate.—It was found by Wood, et al.,[1] that in 0.1 N sodium hydroxide the oxalate to carbonate ratio was not one, as found by McNair,[3] but considerably less. This low ratio could be due to further oxidation of the oxalate since it is well-known that permanganate in acid solution oxidizes oxalic acid. Accordingly, the oxidation of oxalate by alkaline permanganate at 100° was investigated. 0.05 M sodium oxalate was heated with excess saturated potassium permanganate in varying concentrations of base. After reduction of the excess MnO_4^- by hydrogen peroxide at 0° the manganese dioxide was removed and washed with distilled water. The filtrate and washings were acidified to liberate carbon dioxide, which was swept out with nitrogen and absorbed in 0.3 N sodium hydroxide. The $CO_3^=$ was precipitated as calcium carbonate, filtered, dried and weighed. The data are summarized in Table I.

TABLE I

OXIDATION OF OXALATE BY ALKALINE PERMANGANATE

NaOH concn. (moles/l.)	Time for heating, minutes	% Oxalate oxidized
10^{-4a}	60	19
0.1	80	3
2	60	0 (<0.4)

[a] Buffered solution.

It is apparent from Table I that only at low hydroxide concentrations is oxalate oxidized at an appreciable rate. Thus, in 2 N sodium hydroxide <0.4% of the oxalate was oxidized. This

is indeed interesting since it can be estimated[4] that the standard free energy change for the reaction

$$3C_2O_4^- + 2MnO_4^- + 4OH^- = 6CO_3^- + 2MnO_2 + 2H_2O \quad (1)$$

is ~ -250 kilocalories. The oxidation of oxalate cannot be the cause of its low recovery from the propionate oxidation. We have found, because of the low solubility of sodium oxalate in sodium hydroxide solutions, that an appreciable fraction is included or otherwise carried down with the manganese dioxide precipitate. The oxalate is easily extracted from the precipitate by thoroughly washing with distilled water at 100°. If this precaution is observed, and care is taken to exclude carbon dioxide from all external sources, the carbonate to oxalate ratio for propionate, lactate and β-hydroxypropionate is 1.00 $\pm$ 0.03 for hydroxide concentrations of 2 N and greater.

Synthesis and Oxidation of Propionate.—For the experiments described in this paper the short-lived radioactive isotope of carbon, C^{11} (20.5 minute half life) was used as a tracer. The C^{11} was prepared by bombardment of boron with 8 mev. deuterons. The yield for the nuclear reaction

$$_1D^2 + _5B^{10} \longrightarrow _6C^{11} + _0n^1$$
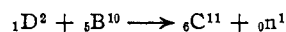
is appreciable at bombarding energies of $\sim$2 mev. Although elementary boron gives the highest yield of C^{11}, boric oxide or boric acid are more satisfactory targets since the newly formed C* is expelled under bombardment almost completely as volatile oxides of carbon.[5,6] Thus the extraction of the radioactivity was achieved with minimum loss of time. The type of target chamber described by Kurie[7] was used in this work. The active gas, (to which $\sim$10 cc. of tank carbon dioxide was added as carrier) from the target chamber was passed into a heated combustion tube containing cupric oxide and oxidized to carbon dioxide. The carbon dioxide was trapped in a glass spiral immersed in liquid air.

(1) Wood, Werkman, Hemingway, Nier and Stuckwisch, THIS JOURNAL, **63**, 2140 (1941).

(2) Nahinsky and Ruben, ibid., **63**, 2275 (1941).

(3) McNair, ibid., **54**, 3249 (1932).

(4) Latimer, "The Oxidation States of the Elements and Their Potentials in Aqueous Solutions," Prentice-Hall, New York. N. Y., 1938.

(5) Ruben, Kamen and Hassid, THIS JOURNAL. **62**, 3443 (1940).

(6) Yost, Ridenour and Shinohara (J. Chem. Phys., **3**, 133 (1935)) found this to be the case to a lesser extent when bombarding at lower energies and intensity than those employed in our experiments.

(7) Kurie, Rev. Sci. Instruments, **10**, 199 (1939).

The propionate* was prepared by treating CH_3CH_2MgBr (in ether) with C^*O_2 for a few minutes at room temperature. The excess Grignard was hydrolyzed with dilute sulfuric acid and the small amount of unreacted C^*O_2 along with the ether was removed by boiling. Propionic acid was rapidly distilled (at low pressure) from this mixture, excess solid silver sulfate being added to prevent the distillation of hydrobromic acid. Trial experiments showed no hydrobromic or sulfuric acid in the distillate and, moreover, the yield of labelled propionic acid to be ~95%.

After purification of the labelled propionic acid and addition of carrier propionic acid, it was treated with a slight excess of potassium permanganate in varying concentrations of sodium hydroxide at 100° for periods ranging from one-half to two hours. The C^{11} content of weighed aliquots was determined with a thin-wall (0.1 mm. Al) Geiger counter. The weighed precipitates (~0.05 g.) were firmly and evenly distributed on a 5 × 7 cm. rectangle of blotting paper and held in position with a thin film of Duco cement and covered with cellophane. These samples were wrapped around the counter so that the most favorable geometrical conditions for detection of the emitted particles were obtained. The oxalate* was precipitated as $CaC_2O_4 \cdot H_2O$, and counted in the same manner. The results are summarized in Table II.

TABLE II

PERMANGANATE OXIDATION OF LABELLED PROPIONATE

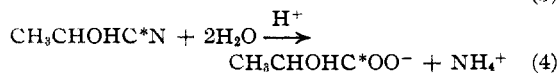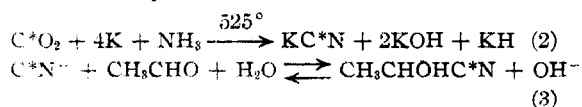| Expt.	Concentration of NaOH, moles/liter	Per cent. of C* in CO₃⁻	C₂O₄⁻	αC-βC rupture αC-COO⁻ rupture
1	10⁻⁴–10⁻⁵ (HCO₃⁻ Buffer)	28.2	71.8	
2	10⁻⁴–10⁻⁵ (HCO₃⁻ Buffer)	27.6 } 28.9	72.4 } 71.1	2.5
3	0.1	30.7	69.3	
4	2	27.8	72.2	
5	2	30.4	69.6	
6	6	16.5	83.5	5.1
7	11	14.2 } 13.1	85.8 } 86.9	6.6
8	11	12.1	87.9	

If the carbonate were derived only from the carboxyl group of the propionic acid, the oxalate, of course, would be devoid of labelled carbon. From the results shown in Table II it is evident that *not only does alpha–beta rupture occur two to six times as often as alpha-carboxyl scission, but that the former process, moreover, is favored significantly by increase of OH^- concentration.*

In addition to the effect of hydroxide ion con-

centration on the splitting ratio, there is a marked effect upon the rate of the propionate oxidation. The oxidation rate is increased by hydroxyl ion, the rate varying approximately linearly with OH^- concentrations above 2 N. This fact should be contrasted with the effect of base upon the oxidation of oxalate by permanganate, where it is found that increasing hydroxyl ion concentration decreases the rate of oxidation so that at 2 N concentration there is no observable oxidation. In one hour permanganate in 2 N sodium hydroxide at 100° oxidizes ~80% of the propionate in a 0.2 M solution. Under the same conditions α- and β-hydroxypropionate are completely oxidized within a few minutes. It was of interest to observe the very large increase in oxidation rate brought about by the presence of a hydroxyl group in either the α or β carbons. If lactate or β-hydroxypropionate are intermediates[8] in the alkaline oxidation of propionate, it follows that the attack upon the ethyl group is the slowest step in the process.

It is desirable to point out that any possible exchange reactions between labelled carbonate ions and any of the possible "active" intermediates, or oxalate, are excluded by the following experiments: (1) Inactive propionate was oxidized in the presence of radioactive carbonate. The oxalate formed during the reaction, as well as the remaining unoxidized propionate, was completely inactive (<0.01% exchanged). (2) Inactive oxalate and radioactive carbonate in 2 N sodium hydroxide, and in contact with freshly precipitated manganese dioxide for one hour at 100° did not exchange (<0.01%).

Synthesis and Oxidation of Lactate.—Radioactive lactic acid was synthesized from $C^{11}O_2$ and CH_3CHO by the method described by Cramer and Kistiakowsky.[9] The reactions are

$$C^*O_2 + 4K + NH_3 \xrightarrow{525°} KC^*N + 2KOH + KH \quad (2)$$

$$C^*N^- + CH_3CHO + H_2O \rightleftharpoons CH_3CHOHC^*N + OH^- \quad (3)$$

$$CH_3CHOHC^*N + 2H_2O \xrightarrow{H^+} CH_3CHOHC^*OO^- + NH_4^+ \quad (4)$$

After addition of carrier lactic acid the labelled acid was purified by treating the cyanohydrin hydrolysate with decolorizing carbon which contained no acid-soluble impurities. Trial experiments showed that <5% of the lactic acid was

(8) The formation of volatile acids (acetic, etc.) other than carbon dioxide during the propionate oxidation could not be detected.

(9) Cramer and Kistiakowsky, *J. Biol. Chem.*, **137**, 549 (1941).

absorbed. The radioactive acid was separated from the volatile impurities by distillation of the clarified solution *in vacuo*. Cyanide was shown to be absent from the high boiling material (any cyanide present would be oxidized by permanganate to carbonate, and thereby not only raise the carbonate/oxalate ratio but also introduce extraneous C^* in the carbonate fraction). Having been made alkaline, the high boiling solution was extracted with several portions of ether, and was freed of dissolved ether by bubbling nitrogen through it for $\sim$ ten minutes. The lactate was oxidized under the conditions described for propionate, only a few minutes being required in this case. A carbonate to oxalate ratio of 1.00 $\pm$ 0.03 was obtained when care was taken to exclude carbonate from external sources and the purification described used.
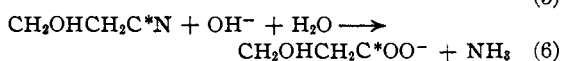
The results are summarized in Table III.

TABLE III
PERMANGANATE OXIDATION OF LABELLED LACTATE

Expt.	NaOH concn. moles/l.	Per cent. of C^* in CO_3^-	$C_2O_4^-$		$\dfrac{\alpha C\text{-}\beta C \text{ rupture}}{\alpha C\text{-}COO^- \text{ rupture}}$
1	2	35.1 }31.9	64.9 }68.1		2.1
2	2	28.8	71.2		
3	12	34.8	65.2		
4	12	37.8 }32.2	62.2 }67.8		2.1
5	12	29.0	71.0		
6	12	27.4	72.6		

It seems, within the experimental limits of error, that hydroxide ion has little or no effect on the $\alpha C\text{-}\beta C$ rupture to $\alpha C\text{-}COO^-$ rupture ratio. A comparison of Tables II and III shows that although the ratios for propionate and lactate in 2 N sodium hydroxide are about the same, the propionate oxidation alone exhibits a marked response to concentrations of OH^- greater than 2 N.

Synthesis and Oxidation of β-Hydroxypropionate.—β-Hydroxypropionate[10] marked with C^{11} in the carboxyl position was prepared from C^*N^- and CH_2ClCH_2OH by the reactions

$$CH_2OHCH_2Cl + C^*N^- \longrightarrow CH_2OHCH_2C^*N + Cl^- \quad (5)$$

$$CH_2OHCH_2C^*N + OH^- + H_2O \longrightarrow$$
$$CH_2OHCH_2C^*OO^- + NH_3 \quad (6)$$

The alkaline solution of KC^*N prepared by Cramer and Kistiakowsky's method[9] (equation 4) was evaporated almost to dryness, and the residue taken up with a small volume of absolute alcohol containing 2-chloroethanol.[11] After being

(10) Jacobs and Heidelberger, THIS JOURNAL, **39**, 1469 (1917).

(11) Eastman Kodak Co. product redistilled, and having a boiling point of 128–129°.

refluxed at 100° for forty minutes, the solution was cooled to 0°, and β-hydroxypropionitrile was added as carrier for the labelled nitrile. The solids separating out on cooling were removed, and alcohol, water and some 2-chloroethanol were distilled from the clear solution *in vacuo*. Next, the remainder of the 2-chloroethanol and the β-hydroxypropionitrile were vacuum distilled off, and a second distillation of this mixture was made to separate the two. Carbonate–bicarbonate buffer solution was added to the alcoholic solution prior to the first distillation in order to prevent distillation of any formate* formed by hydrolysis of cyanide* ion. In the final distillate no HC^*N could be detected using the sensitive Prussian blue test. In order to obtain β-hydroxypropionate*, the pure nitrile* was hydrolyzed in concentrated sodium hydroxide solution for ten minutes at 100°. As in the case of the other acid anions, the carbonate to oxalate ratio obtained on oxidation was found to be 1.00 $\pm$ 0.03 when precautions were taken to exclude carbon dioxide from external sources. The results are given in Table IV.

TABLE IV
OXIDATION OF LABELLED β-HYDROXYPROPIONATE

| Expt.	NaOH concn., moles/l.	Per cent. of C^* in CO_3^-	$C_2O_4^-$		$\dfrac{\alpha C\text{-}\beta C \text{ rupture}}{\beta C\text{-}COO^- \text{ rupture}}$
1	2	28.7 }28.9	71.3 }71.1		2.5
2	2	29.1	70.9		
3	12	30.0 }26.8	70.0 }73.2		2.7
4	12	23.6	76.4		

Discussion

Aside from the fact that the α-β carbon bond is broken about two and one-half times as often as the α-carbon–carboxyl bond, it is surprising that at moderate hydroxide ion concentrations all three acid anions yield the same result. This suggests that under these conditions lactate or β-hydroxypropionate are intermediates in the oxidation of propionate. The finding that at concentrations of OH^- above 2 N, propionate undergoes even greater preferential α-β rupture while lactate and β-hydroxypropionate exhibit no such effect indicates that *in highly alkaline solution the α-β bond in propionate is broken before either of these positions is hydroxylated.*

Although the oxidation of propionate, lactate and β-hydroxypropionate is quite complicated, it may be profitable to formulate a sequence of possible intermediates. The outline suggested below correlates the results obtained thus far, and also

leads to further predictions and suggests future experiments.

TENTATIVE FORMULATION OF PROPIONATE OXIDATION BY ALKALINE PERMANGANATE
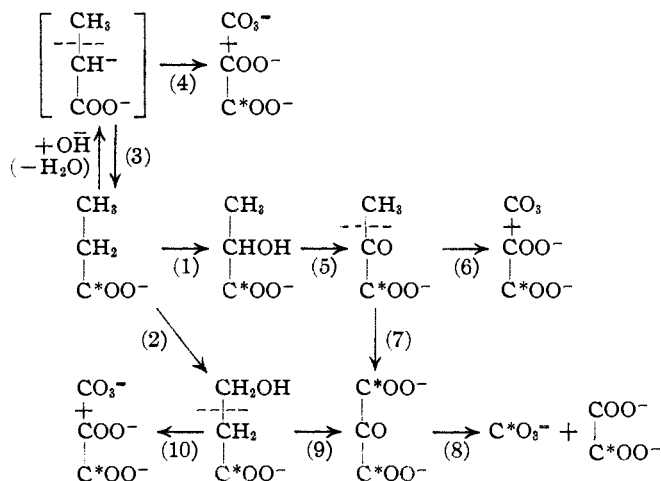
The mesoxalate formed from pyruvate by step (7) is "symmetrical" and therefore the C* content of the carbonate and oxalate formed in (8) will be equal. It is evident from Tables II, III and IV that the rate of (6) is almost equal to the rate of (7), and (10) proceeds at approximately the same rate as (9). In other words *the α-β link is often ruptured before mesoxalate is produced.* While the ratios of (6)/(7) and (9)/(10) are independent of the hydroxide concentration, step (3) is favored over (1) and (2) by increasing alkalinity. The primary step in (3) is the reversible removal of hydrogen ion by OH⁻ resulting in the forma-

tion of $H_3C—\overset{..}{\underset{H}{C}}—COO^-$ which due to the unshared electron pair may be more susceptible to oxidation.

Summary

1. Methods for the rapid synthesis of $CH_3CH_2C^*OOH$, CH_3CHOHC^*OOH, and $CH_2OHCH_2C^*OOH$ using $C^{11}O_2$ are described.

2. Oxidation of these acid anions by alkaline permanganate yields one mole of carbonate and oxalate each.

3. Oxalate is remarkably inert to alkaline permanganate ($2 N$ in sodium hydroxide) even at 100°.

4. For propionate in hydroxide solutions of from 10^{-4} to $2 N$ concentration ~71% of the carbonate is derived from the β-carbon. At higher hydroxyl ion concentration the α-β carbon bond is broken even more preferentially.

5. No exchange between $C^*O_3^=$ and $C_2O_4^=$ could be detected.

6. With lactate and β-hydroxypropionate, ~70% of the carbonate originates from the β-carbon, and this value, within the experimental error, is unchanged by altering the hydroxide ion concentration from 2 to 12 N.

7. The results indicate that with propionate the α-β carbon bond is broken in strongly basic solutions before either α- or β-hydroxypropionate can be formed as an intermediate in the oxidation.

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF HARVARD UNIVERSITY]

Use of Deuterium as a Tracer in the Claisen Rearrangement

BY G. B. KISTIAKOWSKY AND ROBERT L. TICHENOR

In the mechanisms proposed for the Claisen rearrangement[1] the assumption is made that the hydrogen atom displaced by the migrating allyl group moves to the oxygen atom of the resulting phenol. It was thought that definite evidence of the movement of the displaced hydrogen atom could be obtained by using deuterium as a tracer. We have, therefore, carried out the rearrangement of the allyl ethers of 2,4,6-trideutero-phenol (I)

and 4-deutero-2,6-dimethyl-phenol (III). Our results are summarized in Table I.

I(X = D) ⟶ II

III(X = CH₃) ⟶ IV

(1) D. Stanley Tarbell, *Chem. Rev.*, **27**, 495 (1940).