

of the results shown in Table IV that the difference in behavior between isopropyl iodide and normal propyl iodide is due to a stronger bond between carbon and iodine in the former compound. However, this conclusion is wholly at variance with the commonly accepted view of the effect of substituent alkyl groups on the strength of binding between a carbon atom and a negative group. Experiments in which the ease of ionization of the negative group is measured indicate a weakening of this bond when one goes from primary through secondary to tertiary halides. Apparently the present experiments represent a case in which the simple theory expressed in Eq. 5-7 is inadequate to account for the observations. If the steric hindrance of the extra methyl group on the carbon atom in isopropyl iodide is effective in reducing the number of collisions favorable to exchange, then a temperature dependence of considerable magnitude may be placed upon the reaction velocity by this factor, since with increasing temperature steric effects become less important. If this factor were taken into account, the heat of activation would be found to be smaller than that calculated from Eq. (5); also, a smaller collision diameter would be found.²²

(22) The authors express their indebtedness to Dr. R. T. Arnold for stimulating discussions with him which contributed to the interpretation of these results.

Summary

The rate of exchange of iodine between ethyl, propyl, and isopropyl iodides and sodium iodide in alcohol solution has been studied over a temperature range from 50 to 70°, using ¹²⁸I as a radioactive indicator for the reaction.

The radioactive samples were measured in the form of silver iodide, the precipitate being collected quantitatively in a thin layer on a filter paper which was exposed to a β-ray counter. The reliability of this method of measurement has been studied, and it is shown that measurements are accurate to 1%. Certain improvements in technique over the conventional methods of mixing and separating the reactants are described.

From the specific rate constants measured at three temperatures, an energy of activation has been calculated for each compound. The values are believed to be accurate within 1%. Ethyl and propyl iodides have closely similar energies of activation. However, that of isopropyl iodide appears to be significantly higher, as indicated by a more than 15-fold diminution in rate of exchange at the temperatures studied. A possible explanation of this anomaly is given.

Collision diameters are also calculated.

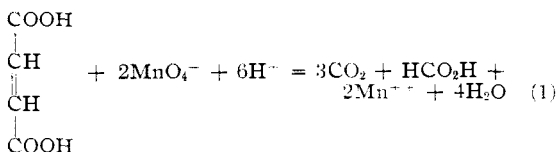
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[CONTRIBUTION FROM THE RADIATION LABORATORY AND THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA]

Tracer Studies with Radioactive Carbon and Hydrogen. The Synthesis and Oxidation of Fumaric Acid

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When fumaric acid is oxidized in 1.5 *N* sulfuric acid by potassium permanganate at 35–50°, carbon dioxide and formic acid are formed.¹ The reaction can be represented quantitatively by the equation



The rate of oxidation of formic acid under these conditions is much slower. The mechanism of reaction (1) is complicated and unknown. Using radioactive carbon as a tracer we have attempted

to determine whether the formic acid originates from the methine or the carboxyl carbons or both. This study is of interest not only for its own sake but also in connection with certain recent bioorganic discoveries made possible by the use of labelled carbon (C¹¹, C¹³, C¹⁴); namely, the synthesis of fumaric and other four carbon dicarboxylic acids from C*O₂ by living systems.^{2,3,4} Indeed, it was the latter that led to the present investigation since it was necessary to have a rapid and convenient method for determining the distribution of C* within succinic and fumaric acids.

(2) Wood, Werkman, Hemingway and Nier, *J. Biol. Chem.*, **135**, 789 (1940); **139**, 377 (1941).

(3) Carson and Ruben, *Proc. Nat. Acad. Sci.*, **26**, 418 (1940).

(4) For a review see Van Niel, Ruben, Carson, Kamen and Foster, *ibid.*, **28**, 8 (1941).

(1) Perdrrix, *Bull. soc. chim.*, [3] **17**, 100 (1897).

Experimental

For the synthesis of labelled fumaric acid, as is the case for most syntheses of labelled carbon compounds, one is forced to begin with $C^{14}O_2$. This limitation is imposed by the method of producing the radioactive carbon. The experiments described below were performed with the short-lived isotope C^{11} (20.5 minute half-life) which is prepared by bombardment of boron (as boric oxide) with 16 m. e. v. deuterons. When boric oxide is the target material it has been found that the C^{11} is expelled for the most part during the bombardment from the boric oxide in the form of carbon monoxide.⁵ This $C^{11}O$, to which 10 cc. of carrier carbon monoxide was added, was oxidized to carbon dioxide (over hot cupric oxide) and collected in a liquid air trap. From this $C^{14}O_2$ $KC^{14}N$ was synthesized by reaction with ammonia and metallic potassium at $\sim 525^\circ$ in a sealed bomb tube as described by Cramer and Kistiakowsky.⁶ The reaction has been represented by the equation



This step required \sim fifteen minutes giving a yield from 40–60% (based on carbon dioxide). After oxidizing the excess potassium with water, the $KC^{14}N$ solution reacted in a sealed tube for fifteen minutes at 150° with excess ethylene dichloride to form succinonitrile. Under these conditions the nitrile yield is $\sim 50\%$ of the theoretical. The nitrile was quantitatively hydrolyzed to succinic acid by treatment with 12 *N* hydrochloric acid for ten minutes at 100° .

The dehydrogenation of succinic to fumaric acid by simple chemical methods is rather difficult so we resorted to a biochemical dehydrogenation using a beef heart preparation⁷ (free of the enzyme fumarase) rich in the enzyme succinic dehydrogenase. Since the enzyme system is readily inactivated by H^+ , CN^- , etc., it was necessary to purify the labelled succinate. This was done by evaporating the solution to dryness. The solid acid was dissolved in phosphate buffer (*pH* 6.9) and shaken with the enzyme preparation in a stream of oxygen at 40° for twenty-five minutes. Proteinaceous matter was precipitated with trichloroacetic acid and centrifuged off. The labelled fumarate was isolated from the filtrate as the mercurous salt.⁸ The mercurous fumarate was treated with excess hydrogen sulfide to obtain the pure acid. It is of interest to note that the fumaric acid with the carboxyl groups labelled with radioactive carbon was ready for oxidation two hours after the cyclotron was turned off.

To the fumaric solution in 1.5 *N* sulfuric acid, potassium permanganate solution was added through a dropping funnel in a stream of nitrogen which carried the evolved carbon dioxide through calcium hydroxide solution. The calcium carbonate was filtered, washed, dried and weighed. By means of a thin wall Geiger counter the C^{11} content of weighed fractions of the calcium carbonate was measured and found to be very radioactive. The formic acid was oxidized to carbonate with alkaline permanganate. (The rate of oxidation of formate by permanganate in alkaline

solution is rapid, in contrast to acid solution where the oxidation is quite slow.) The solution was acidified and the evolved carbon dioxide converted to calcium carbonate and measured for radioactivity as described above. The $CO_2/HCOOH$ ratio from the fumaric oxidation was 3.1/1. The carbonate derived from the formate was inactive. It may therefore be concluded that *the formic acid originates from either of the two methine carbons and not from the carboxyl groups.*

Experiments with Radioactive Hydrogen

It seemed of interest, for reasons to be discussed later, to know whether the hydrogen bonded to the carbon of the formic acid produced in the oxidation is one of the original methine hydrogens from fumaric acid or whether exchange with hydrogen ion takes place during the reaction.

For these experiments we have used radioactive hydrogen,⁹ H^3 , (~ 30 years half life)¹⁰ as a tracer. Since tritium (H^3) emits very low energy beta particles (10–15 k. e. v. upper energy limit)^{9,10,11} it must be counted in gaseous form (hydrogen, water, etc.) inside the Geiger counter. Water vapor, up to a pressure of ~ 0.3 cm. at room temperature, in the presence of an argon-ethanol (10% ethanol) mixture does not seriously impair the performance of the Geiger counter. Hydrogen, although a poor counting gas, can be successfully employed (in the presence of 1.5 cm. of ethanol) at pressures up to ~ 1 atmosphere.

It is first necessary to know if the hydrogen bound to carbon in fumaric and formic acids interchanges with hydrogen ion. We found the exchange to be slow in both acid and neutral solution. The formic acid exchange is easily detectable, $\sim 5\%$ of the hydrogen (bound to carbon) exchanges in one hour in 1.5 *N* sulfuric acid at 80 – 90° . However, no exchange ($< 0.1\%$) of the methine hydrogen of fumaric acid could be detected.

10^{-3} mole of inactive fumaric acid dissolved in 1.5 *N* sulfuric acid solution (having an activity of 9×10^7 counts/min./g. atom of H) was oxidized with potassium permanganate. Sulfate ion was precipitated by the addition of solid barium chloride and the water and formic acid distilled off *in vacuo*. The distillate was divided into two equal portions. The formic acid content of one portion was determined by the alkaline permanganate method described above. The other portion was carefully neutralized with solid sodium bicarbonate and the water distilled off *in vacuo*. The remaining salt containing sodium formate was carefully dried and burned. The resulting water was slightly active, having a specific activity of $2 \pm 1\%$ of the solvent radio-hydrogen water. This result was confirmed by converting the water to hydrogen, which was counted as such. The water was converted quantitatively (to avoid any isotope separation) to hydrogen gas by reaction with fresh magnesium turnings¹² at $\sim 620^\circ$. Since the interchange was small ($\sim 2\%$) and most likely occurred for the most part after the formic acid was formed, we may conclude that *throughout the oxidation of fumaric acid the carbon-hydrogen bond of the product formic acid and all the preceding intermediates was not broken.*

(5) Ruben, Kamen and Hassid, *This Journal*, **62**, 3443 (1940).

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

(7) We are indebted to Mr. Bartley Cardon for this preparation and for advice concerning its use.

(8) Stotz, *J. Biol. Chem.*, **118**, 471 (1937).

(9) Alvarez and Cornog, *Phys. Rev.*, **56**, 613 (1939).

(10) O'Neal and Goldhaber, *ibid.*, **57**, 1086 (1940).

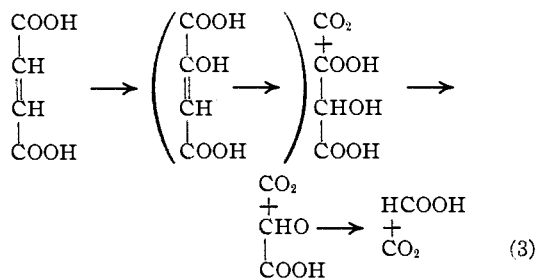
(11) Brown, *ibid.*, **59**, 954 (1941).

(12) We are indebted to Mr. T. H. Norris for valuable assistance in this connection.

This result is of interest in connection with another problem, namely, the proposed use of radioactive hydrogen to label carbon atoms. While C^{11} , C^{13} and C^{14} have been used as tracers, they are available only in limited amounts for divers reasons. For example, production of strong samples of the long-lived radioactive isotope, $^{13}C^{14}$, is difficult and restricted by the very long half-life of C^{14} . On the other hand, hydrogen of very high specific activity is more readily produced and the obvious question arises: why not label carbon (in carbon hydrogen compounds) by firmly bound radio-hydrogen? It is of course essential that exchange of the tritium does not occur while other parts of the molecule are subject to chemical changes. The fumaric oxidation described above indicates that although the molecule is undergoing very profound changes, the hydrogen bound to the carbon has not exchanged. While it is obviously unsound to generalize from a single finding, the possibility of labelling carbon with radio-hydrogen and thus opening new fields of research in chemistry and biochemistry seems very promising. In this Laboratory we are now engaged in further experiments which will furnish additional information on this question.

Discussion

The results obtained with C^* and H^* as tracers lead us to propose the following abbreviated sequence of intermediates in the permanganate oxidation of fumaric acid



We can eliminate $\begin{array}{c} \text{COOH} \\ | \\ \text{CHOH} \\ | \\ \text{CHOH} \\ | \\ \text{COOH} \end{array}$ and $\begin{array}{c} \text{COOH} \\ | \\ \text{CHOH} \\ | \\ \text{CH}_2 \\ | \\ \text{COOH} \end{array}$ as possible

intermediates since they are known^{1,14} to yield 2 moles of formic acid and 2 moles of carbon dioxide. Rupture of the fumaric double bond is

excluded for the same reason. In addition $\begin{array}{c} \text{COOH} \\ | \\ \text{COH} \\ || \\ \text{COH} \\ | \\ \text{COOH} \end{array}$ as an intermediate may be rejected because formic acid produced from it will not contain the original hydrogen from the fumaric acid. The postulation of hydroxymalonic acid is reasonable

(13) Ruben and Kamen, *Phys. Rev.*, **59**, 349 (1941).

(14) Hatcher and West, *Trans. Royal Soc.*, **21**, 269 (1927).

since it is known¹⁵ that malonic acid when oxidized by potassium permanganate under these conditions yields one mole of formic acid and two moles of carbon dioxide. In addition the oxidation of glyoxylic acid gives one mole of formic and 1 mole of carbon dioxide. If the primary process in the oxidation of malonic is the formation of hydroxymalonic, then the above scheme makes definite predictions¹⁶ regarding the mechanism.

If hydroxymalonic is an intermediate in the fumaric oxidation we can eliminate such possibilities as $\begin{array}{c} \text{COOH} \\ | \\ \text{CH}_2 \\ | \\ \text{COOH} \end{array}$, $\begin{array}{c} \text{COOH} \\ | \\ \text{CO} \\ | \\ \text{COOH} \end{array}$, $\begin{array}{c} \text{CH}_2\text{OH} \\ | \\ \text{COOH} \end{array}$ for the same reason the dihydroxyfumaric was rejected. We may also say that $\begin{array}{c} \text{CHO} \\ | \\ \text{COOH} \end{array}$ does not lead to formaldehyde as an intermediate.

The above scheme is admittedly hypothetical but it does lead to certain predictions which can be checked experimentally. Also it strikingly illustrates how sometimes a very simple tracer experiment may yield information usually obtained by laborious kinetic studies.

We wish to thank Dr. M. D. Kamen, Professor E. O. Lawrence, and members of the Radiation Laboratory for their assistance in making these experiments possible.

Summary

1. A rapid method for the synthesis of $\begin{array}{c} C^*\text{OOH} \\ | \\ \text{CH} \\ || \\ \text{CH} \\ | \\ C^*\text{OOH} \end{array}$ starting with $C^{11}O_2$ is described.
2. The formic acid formed by the permanganate oxidation of fumaric acid is derived exclusively from the methine carbons.
3. Using radioactive hydrogen it was found that the exchange of hydrogen bound to carbon in fumaric and formic acids with hydrogen ion is slow in acid and neutral solution.
4. The hydrogen bonded to the methine carbon does not exchange with hydrogen ions during the oxidative formation of formic acid from fumaric acid.
5. The implications of these results are discussed and a tentative mechanism proposed.

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(15) Cartledge and Nichols, *THIS JOURNAL*, **62**, 3057 (1940).

(16) We are studying the malonic acid oxidation using C^* and H^* in order to test these predictions.