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Reliability of Reactions Used to Locate Assimilated Carbon in Propionic Acid[1]

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Glycerol is fermented by the propionic acid bacteria with accompanying fixation of carbon dioxide.[2] Using carbon dioxide labeled with an isotope of carbon of atomic weight thirteen (C^{13})[3] and atomic weight eleven (C^{11})[4] the fixed carbon dioxide has been shown to occur mainly in the products, propionic and succinic acids. In order to obtain information concerning the chemical reactions involved in this fixation of carbon dioxide, it was essential to locate the position of the fixed carbon within the molecule of the products of fermentation. In the case of succinic acid, the carbon dioxide carbon was shown to be exclusively in the carboxyl groups.[3] Conflicting reports have appeared, however, with respect to the position of the fixed carbon in propionic acid. Carson, *et al.*,[5] degraded propionic acid, which was formed in the fermentation of glycerol and contained fixed radioactive carbon (C^{11}), by the reactions

$$CH_3CH_2C^{11}OONa \xrightarrow[KMnO_4]{\text{alkaline}} NaOOCCOONa + Na_2C^{11}O_3 \qquad (1)$$

$$(CH_3CH_2C^{11}O_2)_2Ba \xrightarrow{350°} (CH_3CH_2)_2C^{11}O + BaC^{11}O_3 \qquad (2)$$

If the fixed carbon was in the carboxyl group, it was supposed the C^{11} would have the distribution indicated above. Actually they found, using reaction 1, that from 70 to 75% of the C^{11} was in the oxalate and 25 to 30% in the carbonate. By reaction 2 there was 88% in the diethyl ketone and 12% in the barium carbonate. Carson, *et al.*, did not place much confidence in reaction 1 but the divergence from a fifty-fifty distribution in reaction 2 was considered conclusive evidence that the fixed carbon dioxide was not exclusively in the carboxyl group but more likely was distributed to every position of the molecule.

Wood, *et al.*,[6] degraded propionic acid containing fixed C^{13} obtained from similar fermentations by the following reactions

$$CH_3CH_2C^{13}OOH \xrightarrow[\text{then } H_2O]{\text{P and Br}} CH_3CHBrC^{13}OOH \qquad (3)$$

$$CH_3CHBrC^{13}OOH \xrightarrow{AgOH} CH_3CHOHC^{13}OOH$$

$$CH_3CHOHC^{13}OOH \xrightarrow{KMnO_4} CH_3CHO + C^{13}O_2$$

If the fixed carbon (C^{13}) was in the carboxyl group, as indicated in reaction 3, the acetaldehyde obtained would contain only the natural complement of C^{13} and the carbon dioxide an amount of C^{13} equivalent to that present in the carbon dioxide fixed during the fermentation. Wood, *et al.*, found this to be the case.

The determination of the causes of the discrepancy between the two investigations seemed desirable, particularly in view of the fundamental importance of the results. If the observations of Carson, *et al.*, are correct, it means that the propionic acid bacteria possess the ability to synthesize a carbon chain completely from 1-carbon compounds. We have, therefore, synthesized propionic acid by the reaction

$$CH_3CH_2MgBr + C^{13}O_2 \longrightarrow CH_3CH_2C^{13}OOMgBr \xrightarrow{H_2O} CH_3CH_2C^{13}OOH \qquad (4)$$

It is apparent that the propionic acid obtained contains the concentrated C^{13} in the carboxyl group. The acid was recovered from the reaction mixture by steam distillation, concentrated and fractionated (b. p. 139°) as described previously.[6]

The results from the degradation of propionic acid synthesized by reaction 4 are shown in Table I. Two values are given for each compound (1) the experimental value as determined with the mass spectrometer, and (2) the calculated value.[7] The calculations are on the basis of the synthesized propionic acid containing C^{13} in the carboxyl carbon equivalent to that of the carbon dioxide used in the synthesis, *i. e.*, 4.92% and the reactions proceeding as pictured in 1 and 2.

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(1) Journal paper No. J-858 of the Iowa Agricultural Experiment Station, Project No. 572. The authors wish to acknowledge grants from the Rockefeller Foundation and Dazian Foundation.

(2) Wood and Werkman, *Biochem. J.*, **30**, 48 (1936); **34**, 129 (1940).

(3) Wood, Werkman, Hemingway and Nier, *J. Biol. Chem.*, **135**, 789 (1940); **139**, 365, 377 (1941).

(4) Carson and Ruben, *Proc. Natl. Acad. Sci.*, **26**, 422 (1940).

(5) Carson, Foster, Ruben and Kamen, *Science*, **92**, 433 (1940).

(6) Wood, Werkman, Hemingway and Nier, *Proc. Soc. Exptl. Biol. Med.*, **46**, 313 (1941).

(7) The previous papers[3] should be consulted for more complete information on methods of calculation and procedures used in determination of the C^{13} compounds.

Table I

Degradation of Propionic Acid Containing Concentrated C^{13} in the Carboxyl Group

	Compound	% C^{13a} Found	% C^{13a} Calcd.
	Synthesized propionate	2.43	2.37[b]
	CO_2 used in synthesis (carboxyl-C)	4.92	
Alkaline permanganate oxidation (1)	Na_2CO_3	2.52	4.92
	NaOOCCOONa	2.28	1.09
Dry distillation of the barium salt (2)	$BaCO_3$	4.83	4.92
	$(CH_3CH_2)_2CO$ (distillate)	1.82	1.86[b]
	$(CH_3CH_2)_2C{=}N{-}NHC_6H_3(NO_2)_2$	1.38	1.44[b]

[a] $\frac{\text{moles of } C^{13}}{\text{moles of } C^{13} + \text{moles of } C^{12}} \times 100$. [b] Calculated from the general equation: $(1.09\,N + X)/M$ = average % C^{13} in molecule. 1.09 = % C^{13} in normal carbon. N = no. of carbons with normal complement of C^{13}. M = total no. of carbons in molecules. X = % C^{13} in carbon containing concentrated C^{13}.

It is apparent by comparison of the experimental and calculated values that alkaline permanganate oxidation, reaction 1, is not a reliable method of obtaining the carboxyl carbon. The heavy carbon of the propionic acid was distributed almost uniformly in the oxalate and sodium carbonate, and each had approximately the same average C^{13} content as the entire molecule of propionic acid (2.43). Apparently the sodium carbonate formed during alkaline permanganate oxidation contains approximately 37% of the carboxyl carbon. The oxidation was performed by heating on a steam-bath for sixteen hours a mixture containing 0.1 g. of propionic acid, 10 ml. of 1 N sodium hydroxide and 35 ml. of one-quarter saturated permanganate solution in a total volume of 90 ml. The excess permanganate was removed with 3% hydrogen peroxide and then the manganese dioxide was filtered off. The carbon dioxide from the carbonate was obtained by acidifying the solution with 15 ml. of 1 M acetic acid. The oxalate was then precipitated from the solution by addition of 30 ml. of 25% calcium acetate and heating on a steam-bath for twelve hours. The oxalate was oxidized to carbon dioxide for the mass spectrometer determination by use of acid permanganate. From 1.35 mmole. of propionic acid there were obtained 0.58 mmole. of oxalate and 0.72 mmole. of carbonate. We have been unable to obtain quantitative conversion to oxalate and carbonate as claimed by McNair.[8]

The dry distillation of barium propionate, reaction 2, was a reliable method for decarboxylation under the condition of our experiments. This is shown by the agreement between the calculated and experimental values of Table I. For example, the barium carbonate which should contain only carboxyl carbon contained 4.83% C^{13} as compared to 4.92, the per cent. in original carbon dioxide used in the synthesis. Likewise the ketone fraction contained the required concentration of C^{13}. The C^{13} of the carboxyl carbon is diluted in this case by C^{12} from the other carbons of the molecule in the oxidation to carbon dioxide preparatory to the C^{13} determination. The dry distillation was carried out at 460° for one hour in a 25-ml. distilling flask submerged in an alloy bath. Dry, oxygen-free nitrogen was passed through the flask at a rate of two liters per hour. The diethyl ketone was collected in 45 ml. of ice-cold water contained in a 22-mm. test-tube. The inlet was drawn to a capillary. At the conclusion, the carbon dioxide was liberated from the residue of distillation with 1 N hydrochloric acid and collected for analysis. An approximate estimate of the diethyl ketone was also obtained by weighing the 2,4-dinitrophenylhydrazone which was prepared from an aliquot part of the distillate. After recrystallization from alcohol, the hydrazone melted at 155° corresponding to the melting point given in the literature for that hydrazone. From 2.95 mmole. of propionate there were obtained 2.83 mmole. of carbonate and 0.95 mmole. of hydrazone (calculated on the basis that the original uncrystallized compound was the hydrazone of diethyl ketone).

In our experiments, similar to those of Ardagh, *et al.*,[9] with calcium acetate, practically no decarboxylation was found at 350° in one hour. At 405° only 26% of the theoretical yield of carbon dioxide and 18% of the diethyl ketone were obtained in one hour. The yield at 460° was 96% of carbon dioxide and 64% of the diethyl ketone. Since Carson, *et al.*,[5] carried out their distillation

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(8) McNair, This Journal, **54**, 3249 (1932).

(9) Ardagh, Barbour, McClellan and McBride, *J. Ind. Eng. Chem.*, **16**, 1133 (1924).

at 350°, it is doubtful whether they actually were dealing with a decarboxylation of propionate.[10]

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Summary

The reliability of the reactions used to locate carbon dioxide fixed in propionic acid by bacterial fermentations has been investigated.

Alkaline permanganate oxidation is not a reliable method of decarboxylating propionic acid. Dry distillation of barium propionate gives reliable results under proper experimental conditions. The results have been obtained using synthetic propionic acid containing the isotope of carbon (C^{13}) as a tracer of carboxyl carbon.

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[10] After completion of this investigation, Dr. Ruben informed us by private communication that they have been unable to duplicate their previous results[5] and have now found radioactive carbon only in the carboxyl group of the propionic acid.

[CONTRIBUTION FROM THE BELL TELEPHONE LABORATORIES]

Macromolecular Properties of Linear Polyesters: Molecular Weight Determinations on ω-OH-Undecanoic Self-polyesters

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Polymers resulting from the self-esterification of ω-OH acids represent isochemical systems whose number average molecular weights can be unambiguously determined by end group titration. In addition they consist of molecules which are strictly linear and structurally well defined.[1] They are therefore model macromolecular assemblies on which to test the Staudinger viscosity relation,[2,3,4] the connection between polymer size and solubility,[5] and the relation between melt viscosity and average molecular weight.[6] The present investigation attempts to explore and compare these relationships for the ω-OH-undecanoic series of polyesters of high purity and known average size. In addition, it was desired to present further experimental evidence for the chain segment theory of macromolecular properties, which has been found to explain satisfactorily the melting point and solid state behavior[7] of these substances.

Methods

Materials.—The pure ω-hydroxyundecanoic acid was prepared and isolated by Mr. W. S. Bishop of these Laboratories, using largely the method of Walker and Lumsden.[8] Equal weights of the acid were weighed into cylindrical Pyrex reaction tubes fitted with ground-glass stoppers containing a gas inlet tube extending to the bottom of the reactant. The reaction vessels had exhaust tubes near the top. All polymers were prepared in an electric furnace comprising a metal block of high heat capacity whose temperature was kept at 200 ± 1° and was automatically recorded throughout the reaction. Uniformity of temperature all around the reacting mass was established by tests with thermocouples at various points. Pure tank hydrogen was passed through a copper reducing furnace and dried over sulfuric acid. It was then passed through the reaction tubes at a nearly constant rate. The control of gas ebullition through the polymer melt is important not only in water removal during the condensation but also as a stirring agent. The gas flow through each reaction tube could be independently checked at any time by a flowmeter by-pass. There was no reflux in the system.

Equivalent Weights.—Solutions of equal weight concentrations within 0.2% were made by dissolving weighed portions of the dried polyesters in reagent chloroform. These corresponded always to about 1 g. of polymer per 100 cc. of solution, and were exceedingly dilute in terms of normality. They were titrated at room temperature with 0.0096 *N* alcoholic sodium hydroxide standardized with Bureau of Standards potassium acid phthalate. No information on the probable degree of dissociation of the polymer acids or their salts in organic solvents is available. A pronounced effect of non-aqueous solvents on the behavior of organic acids has been widely noted.[9,10] However, the end-points with constant phenolphthalein concentrations as indicators were sharp, and it is felt that the equivalent weights (number average molecular weights) thus obtained are at least equal in accuracy to those of any other size determination on macromolecules.

Solution Viscosity.—The solution viscosities were determined on solutions of the polymer in reagent chloroform. It was found of critical importance for use in the Ostwald

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(1) Carothers and Van Natta, THIS JOURNAL, **55**, 4714 (1933).

(2) Staudinger, *Ber.*, **65**, 267 (1932).

(3) Staudinger, "Die hochmolekularen organischen Verbindungen," Julius Springer, Berlin, 1932.

(4) Kraemer and Van Natta, *J. Phys. Chem.*, **36**, 3175 (1932).

(5) Schulz, *Z. physik. Chem.*, **A179**, 321 (1937).

(6) Flory, THIS JOURNAL, **62**, 1057 (1940).

(7) Fuller, Baker and Pape, *ibid.*, **62**, 3275 (1940).

(8) Walker and Lumsden, *J. Chem. Ind.*, **79**, 1193 (1902).

(9) Richardson, *Proc. Roy. Soc.* (London), **B115**, 170 (1934).

(10) Parton and Gibbons, *Trans. Faraday Soc.*, **35**, 542 (1939