according to these results from 2.9 in the more dilute solutions to 4.8 in the more concentrated.

In the case of the ethyl chloride reagent, Table II, there is a definite increase in the constant from the more concentrated to the more dilute solutions. The equivalent percentage of the Grignard reagent in the form of diethylmagnesium was very close to 10 for all the solutions studied.

Experimental

Purification of n-Butyl Ether.—Butyl ether was distilled through an efficient fractionating column and the portion boiling between 138–141° collected. This fraction was refluxed over sodium for six hours. The ether was then redistilled and the portion boiling between 139–141° collected and stored over bright sodium wire.

Purification of Nitrogen Gas.—To remove impurities in nitrogen that react with Grignard reagents, the gas was passed through a purification train containing alkaline pyrogallol, water, concentrated sulfuric acid and finally solid sodium hydroxide.

Preparation of Grignard Reagents.—The Grignard reagents were prepared under purified nitrogen from ethyl chloride and n-butyl chloride and magnesium turnings in n-butyl ether without the use of catalysts. The solutions were centrifuged before being transferred to equilibrium tubes.

Solubility of Anhydrous Magnesium Chloride.—Anhydrous magnesium chloride was prepared by the method described by Noller and Raney.[4] The solubility in n-butyl ether was determined by shaking an excess of the powdered material with n-butyl ether until equilibrium was reached. The chloride content of the solution was determined by the Volhard method using 0.01 N solutions of

silver nitrate and potassium thiocyanate. The solubility was found to be approximately 0.0002 mole of magnesium chloride per 1000 g. of solution.

Equilibrium Determination.—In general the procedure for determining the equilibrium composition was similar to that of Noller and Raney.[4] Pyrex glass tubes of about 80 ml. capacity, equipped with stopcocks, were used. Purified nitrogen gas was used in all transfers and special precautions were taken throughout to prevent the solutions from coming in contact with air or moisture.

Anhydrous magnesium chloride was placed in some equilibrium tubes. It soon became evident, however, that this had little or no effect on the rate at which equilibrium was established and it was therefore not continued. Constant shaking was used for a time but as this was apparently unnecessary it was also discontinued.

The time required for equilibrium apparently to become established was about two hundred hours for solutions of n-butylmagnesium chloride and about two hundred fifty hours for ethylmagnesium chloride. Observations were continued for a much longer period, in some cases up to thirteen hundred hours. The solutions were analyzed for active Grignard reagent and for chloride ion. The Volhard method was usually used for the chloride analysis, but this was checked in several cases by gravimetric determinations.

Summary

Determinations have been made of the equilibrium compositions of Grignard reagents prepared from n-butyl chloride and ethyl chloride in n-butyl ether. Concentrations were varied from about 0.6 to 1.9 equivalents per 1000 g. of solution.

IOWA CITY, IOWA   RECEIVED APRIL 6, 1946

Periodate Oxidation of Certain Active Methylene Groups[1,1a]

BY CHARLES F. HUEBNER,[1b] STANLEY R. AMES[1c] AND EDWARD C. BUBL

In applying the periodate oxidation technique to the problem of structural proof of an aldonic acid benzimidazole,[2] it was noted that more periodate was consumed than could be accounted for by the well-known cleavage of 1,2-glycols, ketols and 1,2-diketones.[3] Further study indicated that an activated α-hydrogen was being oxidized. Smith and Duke[4] have reported a reaction of this general nature when using perchlorato-ceric acid in the presence of perchloric acid but data for this reaction are not given for

periodic acid. Oxidation of active α-hydrogens by lead tetraacetate is well known.[5,6]

The present studies give evidence for a new reaction of periodate in aqueous solution, i.e., the oxidation of active α-hydrogens such as those found in malonic acid. The reaction was investigated by both macrodeterminations of periodate consumption and carbon dioxide evolution in the presence of small molar excesses of periodate, and by microdeterminations of carbon dioxide evolution by means of the Warburg respirometer in the presence of large molar excesses of periodate. A number of compounds of related structure were studied, and the probable mechanism of this reaction is indicated.

Malonic acid, on treatment at room temperature with an excess of sodium metaperiodate (6 to 8 molar equivalents (ME)), evolved slightly less than 2 ME of carbon dioxide within two hours (Fig. 1, Curve I). Three ME of periodate were consumed in the reaction during the same time

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. One of us (S. R. A.) was aided in these studies by a grant from the Rockefeller Foundation.

(1a) Presented in part before the Division of Organic Chemistry of the American Chemical Society at the Atlantic City meeting, April 9, 1946.

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(2) C. F. Huebner, R. Lohmar, R. L. Dimler, S. Moore and K. P. Link, J. Biol. Chem., 150, 503 (1945).

(3) E. L. Jackson in R. Adams, "Organic Reactions," Vol. II, John Wiley and Sons, New York, N. Y., 1944, p. 341.

(4) G. F. Smith and F. R. Duke, Ind. Eng. Chem., Anal. Ed., 15, 120 (1943).

(5) O. Dimroth and R. Schweizer, Ber., 65, 1375 (1923).

(6) R. C. Hockett, M. H. Nickerson and W. H. Reeder, III, THIS JOURNAL, 66, 472 (1944).

TABLE I

OXIDATION OF ORGANIC COMPOUNDS USING SODIUM METAPERIODATE AND OTHER OXIDANTS

Compound added	1 hr.	Molar equivalents of oxidant consumed[a]					
		5 hrs.	12 hrs.	1 day	2 days	10 days	20 days
Malonic acid	3.01	3.05	3.07	3.10		3.45	3.58
Malonic acid using periodic acid		1.0		2.3	2.8	3.8	
Malonic acid using lead tetraacetate	0.0	0.5	1.3		1.6		
Tartronic acid	2.05	2.11	2.19	2.28		2.85	2.95
Tartronic acid using periodic acid		1.5		2.2	2.7		
Tartronic acid using lead tetraacetate	1.8	2.3		2.8			
Formic acid				0.07	0.11	0.59	0.85
Mesoxalic acid	1.02 instantaneously						
Monoethyl malonate	1.1	2.0		2.1		2.3	
Malic acid		1.1		3.2		4.0	4.3
Digitoxose		4.0	4.8	5.2	6.1		
Acetoacetic acid	2.5	3.0		3.1			
α-Ethylmalonic acid		0.7	2.2	2.7	2.8	3.0	3.1
α,α'-Diethylmalonic acid				0.0			
Oxalacetic acid				3.9		4.1	
Acetone di-carboxylic acid	4.5	5.1		6.0		6.3	
Citric acid				6.0		6.8 (5 days)	7.1
Lactic acid				0.6	1.2		1.3
Pyruvic acid		0.95		1.00			
Maleic acid				0.0			
Oxalic acid				0.03	0.06	0.21	0.25
1,4-Anhydrosorbitol	2.4	4.4		5.7	6.0		
Ethyl oxomalonate		0.09	0.14		0.37		
Cyanoacetic acid				0.0			
Acetone				0.0			
Acetaldehyde				0.0			
Formaldehyde				0.0			

[a] Unless otherwise noted the oxidant is sodium metaperiodate. Oxidations were carried out with 2 to 3 millimoles of the test substance with 1.2 to 1.6 times the molar equivalent of the oxidant theoretically necessary for complete oxidation. The total volumes were adjusted to 50 ml. and the reactions were run at room temperature (21°).
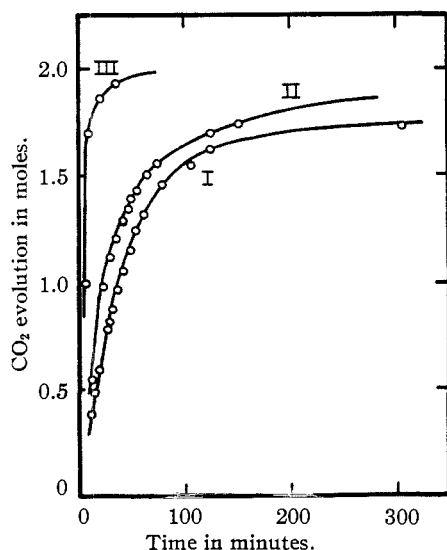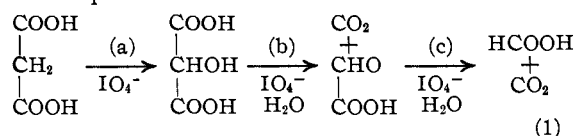

Fig. 1.—Effect of periodate on malonic, tartronic and glyoxalic acids under macroconditions: curve I, 2 millimoles of malonic acid and 4 ME of sodium periodate; curve II, 2 millimoles of tartronic acid and 3 ME of sodium periodate; curve III, 4 millimoles of glyoxalic acid formed *in situ* from 2 millimoles of tartaric acid and 2 ME of sodium periodate. The evolution of carbon dioxide from a total reaction volume of 40 ml. was determined at 22°,

interval (Table I). Under these conditions further utilization of periodate was very slow and in about two weeks approximately 3.6 ME were taken up. The evolution of carbon dioxide falls below the 2 ME level because of a side reaction to be discussed later. After oxidation of · malonic acid for six hours, formic acid was identified as a product by Duclaux constants and as the p-toluidide. The rate of oxidation of formic acid alone as indicated by periodate uptake and carbon dioxide evolution is about the same as both the consumption of the fourth ME of periodate and the rate of evolution of the third ME of carbon dioxide during oxidation of malonic acid. This indicates that the main course of the reaction is probably an oxidation of malonic acid to 2 ME of carbon dioxide and 1 ME of formic acid, the latter being very slowly oxidized to carbon dioxide.

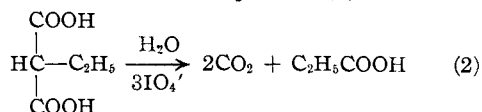The probable course of the reaction is as follows

$$\begin{array}{c} COOH \\ | \\ CH_2 \\ | \\ COOH \end{array} \xrightarrow[IO_4^-]{(a)} \begin{array}{c} COOH \\ | \\ CHOH \\ | \\ COOH \end{array} \xrightarrow[\substack{IO_4^- \\ H_2O}]{(b)} \begin{array}{c} CO_2 \\ + \\ CHO \\ | \\ COOH \end{array} \xrightarrow[\substack{IO_4^- \\ H_2O}]{(c)} \begin{array}{c} HCOOH \\ + \\ CO_2 \end{array}$$

(1)

The over-all result of the action of the first ME of periodate is to replace an active α-hydrogen by a hydroxyl to form tartronic acid (or the corresponding periodate ester). One ME of carbon

dioxide and 1 ME of glyoxalic acid are produced by the action of the second ME of periodate. Reaction (1b) is an example of the oxidation of an α-hydroxy acid such as reported by Fleury[7] for lactic acid. In that case, acetaldehyde and carbon dioxide were formed but the oxidation of lactic acid is slower than that of tartronic acid because the former has one less activating group. Glyoxalic acid, produced in reaction (1b), is then oxidized by the third ME of periodate to yield formic acid and a second ME of carbon dioxide.

If equation (1) is correct, the over-all rate of reaction (1b) + (1c) (the oxidation of tartronic acid) must be as great or greater than the over-all rate of reaction (1a) + (1b) + (1c) and reaction (1c) (the oxidation of glyoxalic acid) must be as rapid or more rapid than reaction (1b) + (1c). Studies of the oxidation rate of each of the several intermediates showed this to be the case (Fig. 1). Reaction (1c) (Curve III) is about twelve times as rapid as reaction (1b) + (1c) (Curve II), while reaction (1b) + (1c) is about 1.4 times as rapid as reaction (1a) + (1b) + (1c) (Curve I), the over-all oxidation of malonate by periodate.[8] The

comparative oxidations of intermediate products were run under comparable conditions (same concentration and same excess of periodate) using both the macro- and micromethods. The glyoxalic acid used in the oxidation studies was prepared *in situ* by prior oxidation of tartaric acid by 1 ME of periodate.

Further evidence for the route of oxidation proposed in equation (1) was furnished by the behavior of α-ethylmalonic acid. Although the oxidation rate was considerably slower than that of malonic acid (Fig. 3, Curve III), 2 ME of carbon dioxide and 1 ME of propionic acid were produced as indicated in equation (2).

$$\begin{array}{c} \text{COOH} \\ | \\ \text{HC—C}_2\text{H}_5 \xrightarrow[3\text{IO}_4{'}]{\text{H}_2\text{O}} 2\text{CO}_2 + \text{C}_2\text{H}_5\text{COOH} \qquad (2) \\ | \\ \text{COOH} \end{array}$$

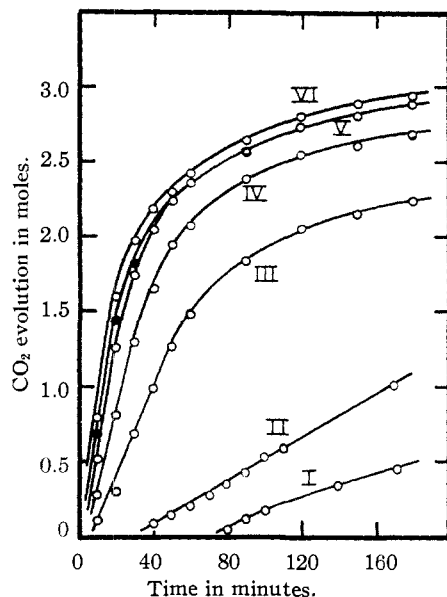Propionic acid was isolated from the oxidation mixture and identified by its Duclaux constants and as the *p*-toluidide.

Fig. 2.—Effect of periodate concentration on the oxidation of malonic acid: curve I, 0.081 M periodic acid containing 1.0 ml. of 0.385 M malonic acid per ml.; curve II, 0.0143 M sodium periodate containing 1.0 ml. of 0.385 M of malonic acid per ml.; curve III, 0.050 M sodium periodate containing 0.50 ml. of 0.834 M malonic acid per ml.; curve IV, same as Curve III, except 0.10 M sodium periodate; curve V, same as curve II, except 0 represents 0.16 M sodium periodate and ● represents 0.33 M sodium periodate; curve VI, same as curve III, except 0.42 M sodium periodate. The test system is described in the text, except for specific details which involve the changes noted.
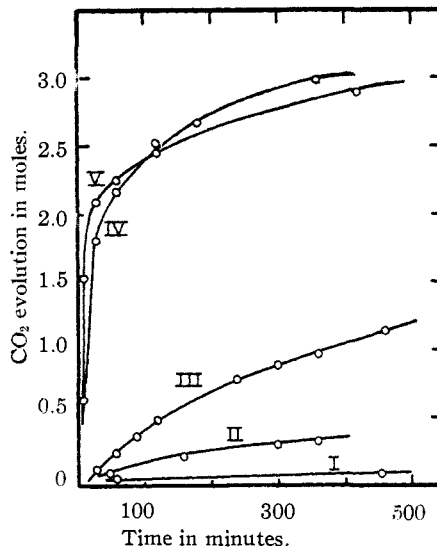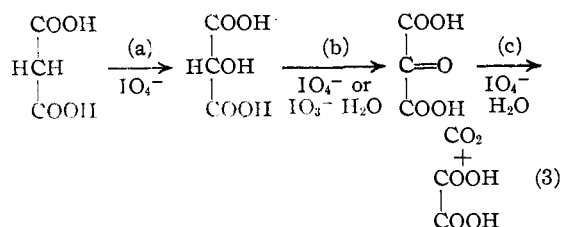
Fig. 3.—Effect of periodate on the oxidation of tartronic, malonic, and α-ethyl malonic acids, and the mono- and diethyl esters of malonic acid: curve I, 0.16 M sodium periodate containing 0.50 ml. of 0.00735 M diethyl malonate; curve II, same as curve I, except containing 0.50 ml. of 0.00854 M half ethyl ester of malonic acid; curve III, same as curve I, except containing 0.50 ml. of 0.00824 M α-ethyl malonic acid; curve IV, same as curve I, except containing 0.50 ml. of 0.00806 M malonic acid: curve V, same as curve I, except containing 0.50 ml. of 0.00869 M tartronic acid. The test system is described in the text, except for specific details which involve the changes noted.

The normal course of oxidation of malonate as indicated in equation (1) is no longer the sole reac-

against time for the three compounds given in Fig. 1, substantially straight lines were obtained. Carbon dioxide evolution was measured and it was assumed that the concentration of substrate was inversely proportional to the gas evolution. The slopes of these lines were compared to obtain a rough estimate of the relative rates of oxidation of the three compounds under consideration.

(7) D. Fleury and M. Boisson, *Compt. rend.*, **204**, 1264 (1937).

(8) When the log of the substrate concentration was plotted

tion if the ME of periodate are three or below. When this occurs, some oxalic acid is formed by a complicating side reaction. In one experiment, in which malonic acid was treated with 3 ME of periodate, a 12% yield of oxalic acid was isolated from the reaction mixture whereas in a similar experiment, using 10 ME of periodate, only a trace (0.1%) of oxalic acid resulted. Oxalic acid is probably produced from malonic acid by the mechanism

$$\begin{array}{ccccc} \text{COOH} & & \text{COOH} & & \text{COOH} \\ | & (a) & | & (b) & | & (c) \\ \text{HCH} & \xrightarrow{IO_4^-} & \text{HCOH} & \xrightarrow{IO_4^- \text{ or}} & \text{C=O} & \xrightarrow{IO_4^-} \\ | & & | & IO_3^- \ H_2O & | & H_2O \\ \text{COOH} & & \text{COOH} & & \text{COOH} \end{array}$$

$$\begin{array}{c} \text{CO}_2 \\ + \\ \text{COOH} \qquad (3) \\ | \\ \text{COOH} \end{array}$$

Tartronic acid, formed by oxidation of malonic acid may be oxidized at a slow rate to mesoxalic acid by either iodate or periodate. It is shown experimentally that iodate is reduced by tartronic acid. With low concentrations of periodate, reaction (1b) presumably becomes slow enough to permit the production of an appreciable amount of mesoxalic acid by the competing reaction (3b). Mesoxalic acid is shown to be instantaneously oxidized to carbon dioxide and oxalic acid. The

latter compound is not appreciably oxidized in twenty-four hours (Table I and Fig. 6, Curve I). Iodate oxidizes tartronic acid but is without effect on malonic or oxalic acids. The amount by which the carbon dioxide production from malonic acid falls below 2 ME (Fig. 1, Curve I) is therefore a measure of the extent of the side reaction which produces oxalic acid.

The evolution of carbon dioxide was measured on a macro scale in the Noller hydrogenation apparatus[9] and on a micro scale in the conventional Warburg constant volume respirometer.[10] Using the latter technique, a concentration of reductant of about 0.0015 M in a total volume of 3.0 ml. theoretically yields a volume of carbon dioxide commensurate with the volume of the reaction vessel. The data included in Fig. 2 show that the rate of oxidation under micro conditions involving these very dilute solutions is a function of the concentration of periodate present and is not dependent upon the molar ratio of periodate and reductant as is the case under macro conditions. With low concentrations of periodate (0.05 M), the rate of oxidation was extremely slow and complete oxidation was not secured even after several days although 33 ME of periodate was present. This dilution effect is to be expected since oxida-
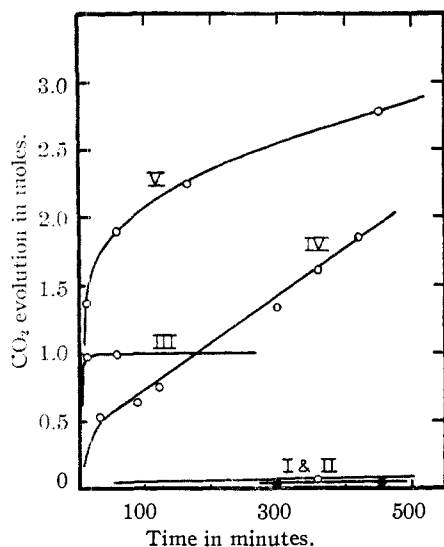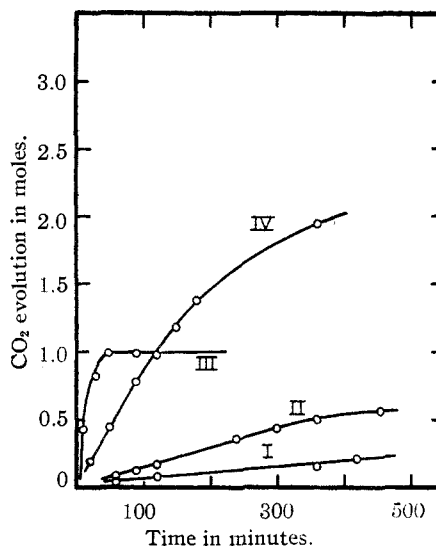

Fig. 4.—Effect of periodate on the oxidation of oxalacetic, acetone dicarboxylic, and acetoacetic acids, ethyl acetoacetate, and acetylacetone: curve I, ●, 0.16 M sodium periodate, containing 0.50 ml. of 0.0122 M acetylacetone; curve II, 0, same as curve I, except containing 0.50 ml. of 0.00942 M ethyl acetoacetate; curve III, same as curve I, except containing 0.50 ml. of 0.0191 M acetoacetic acid; curve IV, same as curve I, except containing 0.50 ml. of 0.0145 M acetone dicarboxylic acid; curve V, same as curve I, except containing 0.50 ml. of 0.00707 M oxalacetic acid. The test system is described in the text, except for specific details which involve the changes noted.

Fig. 5.—Effect of periodate on the oxidation of dl-malic, pyruvic, glycolic and cyanoacetic acids: curve I, 0.16 M sodium periodate containing 0.50 ml. of 0.0115 M cyanoacetic acid; curve II, same as curve I, except containing 0.50 ml. of 0.0169 M glycolic acid; curve III, same as curve I, except containing 0.50 ml. of 0.0143 M pyruvic acid; curve IV, same as curve I, except containing 0.50 ml. of 0.0102 M dl-malic acid. The test system is described in the text, except for specific details which involve the changes noted.

(9) C. R. Noller and M. R. Barusch, Ind. Eng. Chem., Anal. Ed., 14, 907 (1942).

(10) W. W. Umbreit, R. H. Burris and J. F. Stauffer, "Manometric Techniques and Related Methods for the Study of Tissue Metabolism," Burgess Publishing Company, Minneapolis, 1945, p. 1.

tions by periodate are reported to be bimolecular.[11] The concentration of periodate to be used when determining the action of different compounds was chosen in such a manner that, although the maximum possible rate of oxidation was not achieved, the effect of variation in periodate concentration would be minimized. For the micro-determinations, a periodate concentration of 0.17 M or about 115 ME was used.

The use of periodic acid in place of sodium metaperiodate was found to result in a very great decrease in the rate of oxidation (Table I and Fig. 2). While this effect is undoubtedly due to pH differences, the use of buffered solutions was not found to be satisfactory. Considerable variation was obtained at similar pH values using different buffers, and it was possible that such secondary factors might vary too much with the different compounds studied. Sodium metaperiodate without any buffer was therefore employed in the following studies of the effect of structure on the ease of oxidation.

These results introduce a manometric method for the analysis of malonic acid by the use of sodium metaperiodate. Malonic acid is of biological interest since it may occur as a natural cell metabolite.[12] Consideration must be given, however, to possible interfering substances present.

The chemical configuration necessary for this type of oxidation was determined by studying a series of compounds related structurally to malonic acid (Table II). By esterification of one of the carboxyls of malonic acid (monoethyl malonate) the rate of periodate oxidation is greatly decreased and when both carboxyls are esterified (diethyl malonate, ethyl oxomalonate) no reaction takes place. It might be expected that reaction (1a) the hydroxylation of the active methylene group might occur since this type of reaction does occur between diethyl malonate and lead tetraacetate. Accurate periodate determinations in the presence of diethyl malonate (likewise acetylacetone, ethyl acetoacetate and ethyl cyanoacetate) were unsuccessful since the enolic form consumes iodine slowly and results in fading end-points. Hence in such instances other criteria of judging whether oxidations had taken place had to be utilized. In the case of diethyl malonate, sixty per cent. of the compound was isolated unchanged after three days of treatment with periodate. A direct determination of the effect of esterification on periodate consumption was made using ethyl oxomalonate since it does not decolorize iodine. The acid is oxidized instantaneously by periodate but the ester consumes periodate only very slowly (Table I) and this is probably due to a slow hydrolysis under the acid conditions.

Acetoacetic acid, related to malonic acid by the replacement of one carboxyl by a methyl car-

(11) C. C. Price and M. Knell, THIS JOURNAL, **64**, 552 (1942).

(12) B. Vennesland and E. A. Evans, Jr., *J. Biol. Chem.*, **156**, 783 (1944).

TABLE II

PERIODATE OXIDATION OF COMPOUNDS RELATED
STRUCTURALLY TO MALONIC ACID

Compound	Periodate consumption in ME	Carbon dioxide evolution in ME	Other products indicated
α-Ethyl malonic acid	3	2	1 ME propionic acid
Monoethyl malonate	2	1	1 ME ethyl glyoxalate
Diethyl malonate[a]	No reacn.		
Cyanoacetic acid	No reacn.		
Ethyl cyanoacetate[a]	No reacn.		
Malic acid	4	2	2 ME formic acid
Half aldehyde of malonic acid[b]	3	1	2 ME formic acid
Digitoxose	5[c]	0	{ 4 ME formic acid { 1 ME acetaldehyde
Malonyl dialdehyde[d]	3	0	3 ME formic acid
Acetoacetic acid	3	1	{ 1 ME formic acid { 1 ME acetic acid
Ethyl acetoacetate[a]	No reacn.		
Acetylacetone	No reacn.		
Ethyl oxomalonate	No reacn.		
Oxalacetic acid	4	3	1 ME formic acid
Acetone dicarboxylic acid	6	3	2 ME formic acid
Citric acid	7	4	2 ME formic acid

[a] With the concentrations of diethyl malonate used, complete solubility in water was obtained. The same condition applies to the other compounds included in this study which are not easily soluble in water. [b] Presumably obtained by the cleavage of malic acid by one ME of periodate according to the orthodox oxidation of α-hydroxy acids. [c] Nearly 6 ME of periodate were consumed in the oxidation of digitoxose and 4 ME of acid was formed, while a slow evolution of carbon dioxide was noted. Since the theoretical consumption of periodate by digitoxose is 5 ME, it is apparent that oxidation of one of the primary oxidation products is occurring to consume the extra periodate. [d] Presumably obtained by the cleavage of digitoxose by two ME of periodate according to the orthodox oxidation of 1,2,3-triols.

bonyl, is oxidized by periodate, while ethyl acetoacetate resembles diethyl malonate in its non-reactivity. Substitution of methyl carbonyl groups for both carboxyls of malonic acid (acetylacetone) results in a compound which gives no evidence of being oxidized. If the active methylene of acetylacetone were oxidized 2 ME of acetic acid and 1 ME of formic acid would result. The latter compound is slowly oxidized to carbon dioxide (Fig. 6, Curve III), and, since no carbon dioxide was evolved (Fig. 4, Curve I), it was concluded that acetylacetone was probably not oxidized by periodate. However, it can be rapidly oxidized by perchlorato-ceric acid in the presence of perchloric acid.[4]

Replacement of one carboxyl of malonic acid by a nitrile group results in a non-reactive compound. Ethyl cyanoacetate is likewise not susceptible to oxidation by periodate. The compounds formed by replacement of either one or both carboxyls of malonic acid by aldehyde groups (half aldehyde of malonic acid, malonyl dialdehyde) are readily oxidized by periodate presumably by the same mechanism as indicated for malonic acid.

The oxidation of oxalacetic acid can be explained on the basis of hydroxylation of an activated α-methylene carbon followed by oxi-
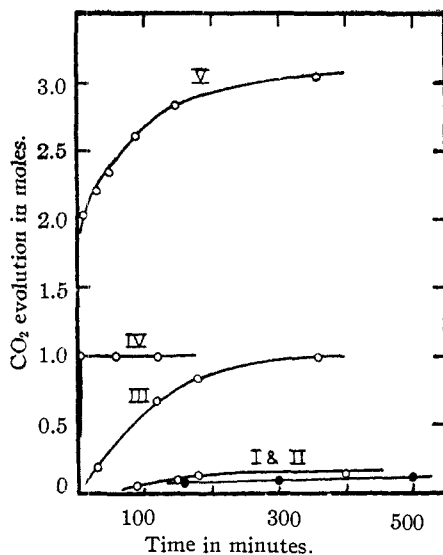
Fig. 6.—Effect of periodate on the oxidation of tartaric, mesoxalic, formic, oxalic and maleic acids: curve I, ●, 0.16 M sodium periodate containing 0.50 ml. of 0.00756 M oxalic acid; curve II, 0, same as curve I, except containing 0.50 ml. of 0.00692 M maleic acid; curve III, same as curve I, except containing 0.50 ml. of 0.00963 M formic acid; curve IV, same as curve I, except containing 0.50 ml. of 0.00822 M mesoxalic acid; curve V, same as curve I, except containing 0.50 ml. of 0.00888 M l-tartaric acid. The text system is described in the text, except for specific details which involve the changes noted.

Fig. 7.—Effect of periodate on the oxidation of citric, gluconic, and lactic acids, glucose and digitoxose: curve I, ●, 0.16 M sodium periodate containing 0.50 ml. of 0.0892 M digitoxose; curve II, 0, same as curve I, except containing 0.50 ml. of 0.0942 M glucose; curve III, same as curve I, except containing 0.50 ml. of 0.0128 M lactic acid; curve IV, same as curve I, except containing 0.50 ml. of 0.0144 M gluconic acid; curve V, same as curve I, except containing 0.50 ml. of 0.00655 M citric acid. The test system is described in the text, except for specific details which involve the changes noted.

dative cleavage of the carbon–carbon bond. Similarly acetone dicarboxylic acid, which contains two activated α-methylene groups is readily oxidized. Citric acid is slowly oxidized by periodate, probably with acetone dicarboxylic acid as an intermediate as proposed by Smith and Duke[4] for perchlorato-ceric acid.

Certain compounds were included in this study since they might occur in conjunction with malonic acid in biological systems. Lactic acid (Fig. 7, Curve III) and glycolic acid (Fig. 5, Curve II) are slowly oxidized to evolve about 1 ME of carbon dioxide. Pyruvic acid (Fig. 5, Curve III) and gluconic acid (Fig. 7, Curve IV) both yield a single ME of carbon dioxide when treated with periodate. Maleic acid gave no evidence of oxidation. Although oxalic acid is reported to be unchanged by periodate,[13] a very slow oxidation does occur. It is 25% oxidized in eighteen days (Table I). Oxalic acid is immediately oxidized by perchlorato-ceric acid.[4]

A number of more complex compounds were oxidized according to the principles developed in this contribution. 1,4-Anhydrosorbitol[14] consumes 6 ME of oxidant while producing 3 ME of acid as indicated in equation (4). The α-hydrogen activated by two aldehyde groups is probably

(13) E. L. Jackson, ref. 3, p. 345.
(14) Adapted from the structure of 3,6-anhydrosorbitol proposed by Hockett, Abstracts of Papers, 100th Meeting, American Chemical Society, Detroit, Division of Organic Chemistry, p. M 22 (1940).
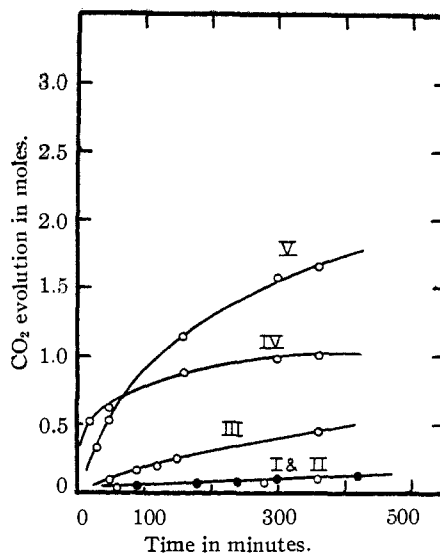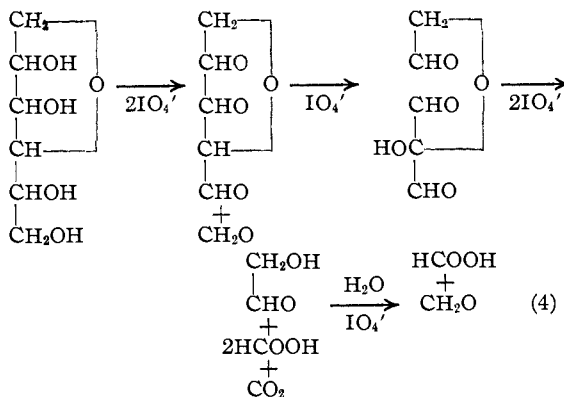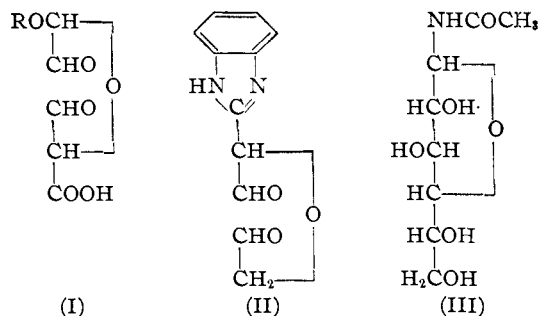
the site of hydroxylation and subsequent oxidation. Other examples of this same type of com-

$$
\begin{array}{ccc}
\underset{\text{CHOH}}{\overset{\text{CH}_2}{|}} & \underset{\text{CHO}}{\overset{\text{CH}_2}{|}} & \underset{\text{CHO}}{\overset{\text{CH}_2}{|}} \\
\underset{\text{CHOH}}{|} \ \text{O} & \underset{\text{CHO}}{|} \ \text{O} & \underset{\text{CHO}}{|} \ \text{O} \\
\underset{\text{CH}}{|} & \underset{\text{CHO}}{|} & \underset{\text{HOC}}{|} \\
\underset{\text{CHOH}}{|} & \underset{\text{CHO}}{|} & \underset{\text{CHO}}{|} \\
\underset{\text{CH}_2\text{OH}}{|} & + & \\
& \text{CH}_2\text{O} & \\
\end{array}
$$

$$\overset{2IO_4'}{\longrightarrow} \quad \overset{IO_4'}{\longrightarrow} \quad \overset{2IO_4'}{\longrightarrow}$$

$$
\begin{array}{c}
\text{CH}_2\text{OH} \\
| \\
\text{CHO} \\
+ \\
\text{2HCOOH} \\
+ \\
\text{CO}_2
\end{array}
\quad \overset{H_2O}{\underset{IO_4'}{\longrightarrow}} \quad
\begin{array}{c}
\text{HCOOH} \\
+ \\
\text{CH}_2\text{O}
\end{array}
\quad (4)
$$

pound are formed as intermediates during the oxidation of bornyl D-glucuronide (I) and anhydro D-xylobenzimidazole (II).[2]
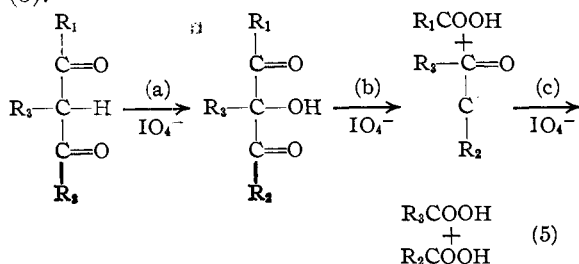
Attention must be directed to this new type of reaction when using periodate as a diagnostic reagent in structure proofs. For example, Niemann[15] recently reported the apparently anomalous behavior of N-acetyl-D-glucofuranosylamine (III) toward periodate. This compound was reported to consume 5 ME of oxidant, a value which is entirely consistent with theory if this new mode of periodate oxidation is considered.

(15) C. Niemann and J. T. Hays, This Journal, 67, 1302 (1945).

ROCH—┐
  |
 CHO |
        O
 CHO |
  |
 CH—┘
  |
 COOH

(benzene/indole ring)
HN   N
  \C/
   |
  CH——
  |
  CHO ——┐
         O
  CHO |
  |
 CH₂—┘

NHCOCH₃
  |
 CH—┐
  |
 HCOH· |
        O
 HOCH |
  |
 HC——┘
  |
 HCOH
  |
 H₂COH

(I)   (II)   (III)

The following generalizations can be made as a result of the study of aqueous solutions of the variously substituted derivatives of malonic acid. The configuration necessary for this new mode of periodate action is a three-carbon system consisting of a free carboxyl or aldehyde, an α-carbon bearing at least one hydrogen, and a β-carbonyl group which may be part of an aldehyde, ketone, carboxyl, or carbalkoxyl or a similar activating structure.

The generalized reaction is indicated in equation (5).

$$R_3-\underset{\underset{R_3}{\overset{\displaystyle C=O}{|}}}{\overset{\overset{\displaystyle R_1}{|}}{\underset{\underset{|}{C=O}}{\overset{|}{C}}}}-H \xrightarrow[IO_4^-]{(a)} R_3-\underset{\underset{R_3}{\overset{\displaystyle C=O}{|}}}{\overset{\overset{\displaystyle R_1}{|}}{\underset{\underset{|}{C=O}}{\overset{|}{C}}}}-OH \xrightarrow[IO_4^-]{(b)} \underset{\underset{R_2}{C}}{\overset{\overset{R_1COOH}{+}}{R_3-C=O}} \xrightarrow[IO_4^-]{(c)}$$

$$\begin{matrix}R_3COOH\\+\\R_2COOH\end{matrix} \quad (5)$$

The general requirements for the reaction are

1. R_1 must be –OH or –H.
2. R_2 and R_3 may be any combination of –H, -alkyl, –OH, or –O alkyl.
3. If R_3 is –alkyl and/or R_2 is –O alkyl, the reaction is slow.
4. If R_1, R_2, or R_3 are H, formic acid will be a product of the reaction and this compound is then slowly oxidized to carbon dioxide, especially if a large excess of periodate is present as in the microanalytical method employed.
5. If R_1 or R_2 are hydroxyl, one of the acids produced is carbonic which decomposes in acid solution to give carbon dioxide.
6. If R_2 is –O alkyl, reaction (5c) does not occur but stops with the intermediate formed in the preceding reaction.
7. The grouping –CO–R_2 may not be replaced by a nitrile and still secure oxidation.

Experimental

With the following exceptions, the compounds used were commercially prepared (c. p. grade). Tartronic acid,[16] acetone dicarboxylic acid,[17] ethyl oxomalonate,[18] acetoacetic

acid[19] and oxalacetic acid[20] were prepared as described in the literature cited. Disodium mesoxalate was prepared by saponification of the diethyl ester. Neutral equivalents for the acids and ash content for the sodium salts were used as the criteria of purity. The solution of acetoacetic acid was standardized according to a manometric procedure.[21] Periodate determinations were performed according to Fleury.[22] In performing long term oxidations, it is essential that a blank containing periodate alone be run simultaneously and that the reaction mixture be kept in the dark. Periodate solutions exposed to light develop an iodine color and possess an ozone-like odor. The determinations of lead tetraacetate were performed according to Hockett.[23]

Measurement of Carbon Dioxide on a Macro Scale.— The requisite amount of 0.48 M sodium metaperiodate was added to the reaction flask of the Noller hydrogenation apparatus.[9] Two to three milliequivalents of the test substance dissolved in the amount of water required to bring the total volume of the two solutions to 40 ml. was added through the sidearm. The mercury level in the gas holder (100-ml. capacity) was adjusted to zero, the sidearm stopcock closed, and stirring begun. At each reading of the gas volume, the mercury was lowered to maintain atmospheric pressure. These readings were reduced to volume of dry gas at standard conditions and corrected for the solubility of carbon dioxide in the aqueous phase.

This solubility was determined experimentally by adding an amount of sodium metaperiodate to the flask equivalent to that used in the oxidations. An amount of sulfuric acid equivalent to the test substance, a known amount of sodium bicarbonate solution, and water were added to make a total volume of 40 ml. Stirring was begun and the final amount of gas evolved was measured. Measurements with increasing amounts of bicarbonate were made and from the experimental gas volumes determined and the corresponding theoretical gas evolutions, a curve was constructed to determine the necessary volume correction for a given evolution of carbon dioxide.

Measurement of Carbon Dioxide on a Micro Scale.—A conventional Warburg constant volume respirometer at 37.0° was used in all experimental and analytical work. The pH was determined in all cases by a Beckman pH meter (glass electrode).

The procedure to determine the volume of carbon dioxide liberated was selected to give a rapid evolution of carbon dioxide with readily oxidized substances under the given experimental conditions. One ml. of 0.50 M sodium metaperiodate was placed in the Warburg flask, and 0.50 ml. of an approximately 0.01 M solution of the compound to be studied was added to the sidearm. Distilled water (1.50 ml.) was added to bring the total volume of the flask contents to 3.0 ml. This gave a molar excess of periodate over the concentration of the compound under study of about 100 fold. The gas phase in the reaction flasks was air. The flasks were equilibrated for ten to fifteen minutes, and the contents of the sidearm were added at zero time. The rate of shaking was 120 complete cycles per minute with a 3-cm. stroke. The final pH of the contents of the reaction flask varied from 3.5 to 4.0, depending upon the compound under consideration. The components of the reaction mixture were added to the flask by means of 1.0-ml. pipets graduated in hundredths. The data were compared for different compounds by dividing the observed total of carbon dioxide evolution in moles by the number of moles of reductant initially present. By adopting this conversion, the number of moles liberated from a mole of compound added can be plotted with relation to the time of oxidation.

Iodate Consumption by Tartronic Acid.—Twenty-five ml. of 0.352 N sodium iodate solution was added to 0.534

(16) B. Bak, Ann., **537**, 286 (1939).

(17) R. Adams, H. M. Chiles and C. F. Rassweiler in H. Gilman and A. H. Blatt, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, New York, N. Y., 1941, p. 10.

(18) H. W. Dox, ibid., p. 266.

(19) Appreciation is expressed to Dr. A. L. Lehninger for a generous sample of acetoacetic acid.

(20) W. W. Umbreit, R. H. Burris and J. F. Stauffer, ref. 10, p. 187.

(21) N. L. Edson, Biochem. J., **29** 2082 (1935).

(22) P. Fleury and J. Lange, J. pharm. chim., **17**, 107 (1933).

(23) R. C. Hockett, M. T. Dienes and H. E. Ramsden, THIS JOURNAL, **65**, 1474 (1943).

g. of tartronic acid and made up to 50 ml. with water. In about thirty minutes iodine appeared in the solution. A 2-ml. aliquot was withdrawn periodically for an iodate determination. Sodium bicarbonate was added to an aliquot and the iodine present was reduced by titrating with 0.1 N arsenite in the presence of starch indicator. Acid and potassium iodide were added and the liberated iodine which is a measure of the remaining iodate was titrated with thiosulfate. The consumption of 0.115 ME of iodate was observed in six hours and 0.432 ME in five days.

Isolation and Characterization of Formic, Oxalic and Propionic Acids.—Five grams of malonic acid and 32 g. of sodium metaperiodate (3.1 ME) were permitted to react in a total volume of 500 ml. aqueous solution for six hours. The mixture was continuously extracted with ether for four days after which an aqueous layer had separated from the ether layer. Evaporation of the ether layer to dryness yielded 270 mg. of oxalic acid dihydrate, m. p. 101°. A mixed melting point with an authentic sample of oxalic acid showed no depression. Calcium chloride was added to 10% of the aqueous fraction described above and 45 mg. of calcium oxalate was obtained which gave 710 mg. as the total yield of oxalic acid dihydrate or 12%. The remainder of the aqueous phase was acidified with phosphoric acid and distilled to a small volume. An aliquot of the distillate was diluted to 0.01 N and the Duclaux constants[24] were 0.110, 0.141 and 0.220. An authentic sample of formic acid give 0.116, 0.149 and 0.227. The remaining aqueous fraction was neutralized with sodium hydroxide and evaporated to dryness. The resultant sodium salt was converted to the p-toluidide, m. p. 55°.

In a similar experiment 2 g. of malonic acid was oxidized by 10 ME of periodate and 2.6 mg. of calcium oxalate was obtained.

Five grams of α-ethyl malonic acid was oxidized as described above with 35 g. of sodium metaperiodate for three days. After ether extraction the ether layer was extracted with sodium hydroxide which in turn was acidified with phosphoric acid and distilled to a small volume. The total amount of volatile acid calculated as propionic acid was 2 g., a 71% yield. Duclaux constants determined on

part of this solution were 0.278, 0.257 and 0.228. An authentic sample of propionic acid gave 0.282, 0.260 and 0.230. The sodium salt of this acid was converted to the p-toluidide, m. p. 122°.

Action of Periodate on Diethyl Malonate.—Five ml. of diethyl malonate was dissolved in a liter of water containing 3.5 ME of sodium metaperiodate. After four days at room temperature the solution was continuously extracted with ether. The ether was concentrated and the residue distilled. Three grams was collected: b. r. 185–190°; N, 1.4139; calcd. 1.4143.

Anal. Calcd. for $C_7H_{12}O_4$: C, 52.5; H, 7.5. Found: C, 52.3; H, 7.4.

Summary

A new mode of oxidation by periodate in aqueous solution is described which consists in the conversion of an activated α-hydrogen to an α-hydroxyl group. The configuration necessary is a three-carbon system consisting of a free carboxyl or aldehyde, and α-carbon bearing at least one hydrogen, and a β-carbonyl group which may be part of an aldehyde, ketone, carboxyl, carbalkoxyl or a similar activating structure. This first reaction is then followed by the well-known oxidative cleavage of α-hydroxy acids and α-hydroxy aldehydes. Using malonic acid as a type compound, intermediates were secured and the mechanism indicated. The influence of structural modification on the oxidation is presented from which generalizations are made as to the specificity of periodate action.

(24) Duclaux constants were determined by a modification of the method of A. I. Virtanen and L. Pulkki, This Journal, **50**, 3138 (1928).

Shape and Size of Pectinic Acid Molecules Deduced from Viscometric Measurements

By H. S. Owens, H. Lotzkar, T. H. Schultz and W. D. Maclay

Until the 1930's chemical evidence had generally been interpreted to indicate that pectin has a small cyclic structure.[2] In 1923 Smolenski, however, offered the opinion that pectin is a high polymer comparable in structure to starch. X-Ray results agreed with that hypothesis, but indicated that comparison with cellulose is more logical. Schneider and co-workers showed that pectin nitrate in acetone solutions has a Staudinger constant between that for cellulose and starch nitrates. Later, Säverborn[3] and Snellman

and Säverborn,[4] on the basis of measurements of flow birefringence, sedimentation and diffusion constants of various pectins and of pectin nitrates, also concluded that pectin has a high molecular weight and is a long chain compound like cellulose.

Shape and size of molecules of high polymers deduced from viscometric measurements of their solutions appear to agree with those calculated from measurements of other types.[5,6] Recent studies[7,8] of the viscosity behavior of pectic materials in electrolytic solutions have made it possible to determine their intrinsic viscosities. It appeared desirable to use these values for cal-

(1) Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) The history of the development of pectin structure is reviewed in detail by K. H. Meyer, "High Polymers," Series 4, Interscience Publishers, Inc., New York, N. Y., 1942, pp. 364–370.

(3) (a) S. Säverborn, Kolloid Z., **90**, 41 (1940). (b) Also "A Contribution to the Knowledge of the Acid Polyuronides," (thesis), Uppsala (1945). The latter publication appeared after the present manuscript was written.

(4) O. Snellman and S. Säverborn, Kolloidchem. Beihefte, **52**, 467 (1941).

(5) M. A. Lauffer, Chem. Rev., **31**, 561 (1942).

(6) R. Simha, J. Chem. Phys., **13**, 188 (1945).

(7) L. Malsch, Biochem. Z., **309**, 283 (1941); C. A., **37**, 4280 (1943).

(8) H. S. Owens, H. Lotzkar, R. C. Merrill and M. Peterson, This Journal, **66**, 1178 (1944).