Anal. Found: C, 79.45; H, 10.76; methoxyl, 9.61.

A tail fraction of 1.2 g., $n^{20}D$ 1.5176, arbitrarily cut from the main, likewise analyzed properly for $C_{21}H_{34}O_2$ but showed $\lambda_{max.}$ 238 mμ, $E^{1\%}_{1\ cm.}$ 673.

α-Vitamin A Methyl Ether by Dehydration of 4,5-*trans*-XIX.—A 15.0-g. sample of pure 4,5-*trans*-XIX as obtained from the reduction of XVIII was dehydrated with iodine by the procedure described above for the dehydration of VI. Removal of the benzene under vacuum left 14.2 g. of dark amber-colored liquid; $\lambda_{max.}$ 311 mμ, $E^{1\%}_{1\ cm.}$ 926. The extinction corresponds to an α-vitamin A methyl ether content of 48.8%.

Alumina chromatography and subsequent distillation as described above under the dehydration of VI, yielded 4.5 g. of α-vitamin A methyl ether identical in all respects with the specimen obtained from VI.

The results were essentially the same with samples of 4,5-*trans*-XIX obtained by the other two routes.

Dehydration of 4,5-*trans*-XIX was also readily accomplished by allowing a solution of 10.0 g. of the carbinol in 300 ml. of glacial acetic acid to stand at room temperature for three hours. Working up with water and petroleum ether and concentrating under vacuum yielded 10.0 g. of crude product of $\lambda_{max.}$ 311 mμ, $E^{1\%}_{1\ cm.}$ 806.

Stereoisomerization of the Tetraene Glycol X.—A specimen of X was prepared by the lithium aluminum hydride reduction of the corresponding 1,2-dehydro compound,[24] as described by Attenburrow, *et al.*[5] The product, after two recrystallizations from cyclohexane, melted at 126–134° and showed $\lambda_{max.}$ 307.5 mμ, ϵ 52,400. Attenburrow, *et al.*, report m.p. 122–127°, $\lambda_{max.}$ 307 mμ, ϵ 58,000. These data, especially the wide melting ranges, leave no doubt that the reduction product in each case was a mixture of stereoisomers.

A solution of 2.4 g. of the glycol and 25 mg. of iodine in 625 ml. of benzene was stirred under nitrogen at room temperature in moderate artificial light. The shift of $\lambda_{max.}$ from 307.5 to 309.5 mμ was completed during the first half-hour but stirring was allowed to continue an additional half-hour to assure equilibrium. The solution then was washed with sodium thiosulfate solution, dried with anhydrous potassium carbonate, and concentrated under vacuum to a sirup. This was taken up in 5 ml. of warm benzene, diluted to 30 ml. with cyclohexane, and stored overnight under nitrogen at 0°. A portion of the glycol which precipitated was then recrystallized from benzene–cyclohexane. The all-*trans* product consisted of rather large canary-yellow crystals as compared to the almost white mono-*cis* isomer; m.p. 124–125°[25]; $\lambda_{max.}$ 309.5 mμ, ϵ 59,800.

Anal. Calcd. for $C_{20}H_{32}O_2$: C, 78.89; H, 10.60. Found: C, 78.45; H, 10.51.

Biological Assays.—The assays and liver extractions were performed by the Biochemistry Division of this Institute and the Food Research Laboratories, Inc., Long Island City, New York.

Acknowledgment.—The author is indebted to Mr. Joseph Grodsky for the microanalyses and to Mr. Walter Gall for technical assistance.

(24) The author is indebted to Dr. B. A. Hems, Glaxo Laboratories, Ltd., Greenford, Middlesex, England, for a generous supply of the intermediate necessary for the synthesis of the dehydro compound.

(25) Partial polymerization or some other reaction appears to occur during the heating for about one-third of the melting point sample did not fuse.

RARITAN, NEW JERSEY

[CONTRIBUTION FROM THE DIVISION OF APPLIED BIOLOGY, NATIONAL RESEARCH LABORATORIES]

Production of Formic Acid During Oxidation of Carbohydrates with Lead Tetraacetate[1,2]

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The rate of oxidation of formic acid to carbon dioxide by lead tetraacetate in acetic acid was found to be markedly increased in the presence of potassium acetate. The accelerated reaction was used to study production of formic acid during the lead tetraacetate oxidation of methyl glycopyranosides. By measuring evolved carbon dioxide with the Warburg respirometer the course of the reactions was conveniently followed on the micro scale. In glacial acetic acid, the glycosides consumed the expected two moles of oxidant but, with the hexose series, much less than the theoretical one mole of formic acid was found. Evidence is presented suggesting that the formic acid not accounted for was present as the 6-O-formyl ester. In aqueous acetic acid, the extent of ester formation was reduced and over-oxidation of the glycosides also was observed.

In theory, formic acid is produced during the oxidation of many carbohydrates with lead tetraacetate, *e.g.*, when the compounds contain hydroxyl groups on three adjacent carbon atoms.[3] Little is known of this aspect of the oxidations, however, because of the lack of a convenient method of analysis. Moreover, the stoichiometry of the oxidations is obscured when the formic acid is not determined since the latter itself consumes lead tetraacetate.[4] Grosheintz[5] has noted that when oxidations are conducted in aqueous acetic acid,[6] formic acid is quantitatively oxidized to carbon dioxide and consumes an extra mole of oxidant. Although this finding suggests an index of formic acid production in carbohydrate oxidations with lead tetraacetate, it has been used only to a limited extent, *e.g.*, in the degradative assay of radioactive sugars.[7]

An examination of Grosheintz' reaction in this Laboratory showed that the rate of evolution of carbon dioxide was accelerated markedly by the addition of potassium or sodium acetate (Fig. 1) (see also reference 8). The reaction was carried out in the Warburg respirometer,[9] and the evolved gas measured manometrically. An accelerated oxidation also was evident in glacial acetic acid though more potassium acetate was required to attain the same rate. Thus at a concentration of 1.5 moles of acetate per mole of oxidant in 90% acetic acid and of 3 moles per mole in glacial acetic acid, the reaction was complete in 10 to 15 minutes. These rates

(1) Presented in part before the Division of Carbohydrate Chemistry, 124th Meeting, American Chemical Society, Chicago, Ill., 1953.

(2) Issued as N.R.C. No. 3401.

(3) R. Criegee, *Ann.*, **495**, 211 (1932).

(4) R. C. Hockett, M. T. Dienes, H. G. Fletcher and H. E. Ramsden, THIS JOURNAL, **66**, 467 (1944).

(5) J. M. Grosheintz, *ibid.*, **61**, 3379 (1939).

(6) E. Baer, J. M. Grosheintz and H. O. L. Fischer, *ibid.*, **61**, 2607 (1939).

(7) S. Abraham, *ibid.*, **72**, 4050 (1950).

(8) A. S. Perlin, *Anal. Chem.*, **26**, 1053 (1954).

(9) O. Warburg, "Uber der Stoffwechsel der Tumoren," Berlin, Springer, 1926.

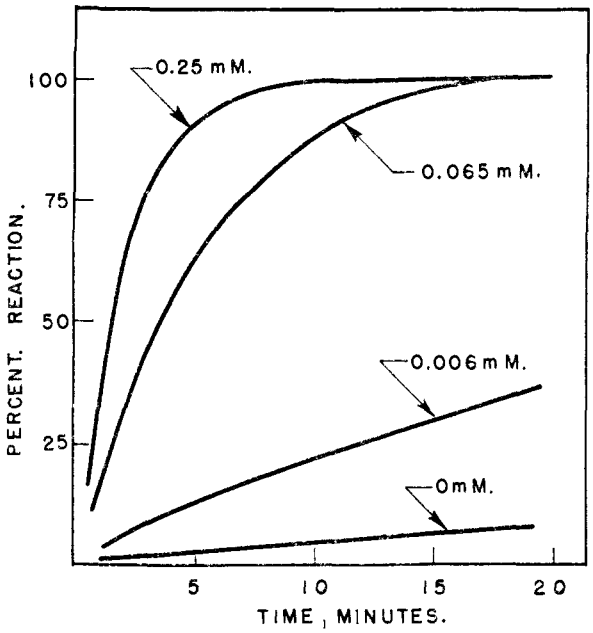


Fig. 1.—Effect of potassium acetate on the rate of oxidation of formic acid with lead tetraacetate as measured by the rate of evolution of carbon dioxide; solvent, 90% acetic acid; temperature, 27°.

were very rapid when compared with the rates of oxidation of many carbohydrates (see below), and the accelerated reaction therefore appeared well-suited to an investigation of formic acid production in these oxidations. Used in conjunction with the Warburg respirometer, as in periodate oxidations,[10] the reaction offered a convenient measure of oxidations on the micro-scale.

The accelerated reaction was studied using methyl glycosides as model compounds since they have already been examined in detail under the more usual conditions of oxidation.[11] It was found that potassium acetate catalyzed not only the oxidation of the formic acid but of the glycosides themselves (Table I). The oxidations were carried out in glacial acetic acid both with and without added potassium acetate and, at the times indicated, the formic acid yields (carbon dioxide pressure) were noted and total lead tetraacetate consumptions were determined by titration. The oxidant consumed by the glycosides therefore could be determined by correcting for the amount consumed in the side-oxidation of formic acid. Except for the pronounced increases in rate, the course of the oxidations in the presence of potassium acetate appeared to differ little from those in its absence. Thus methyl mannoside, containing a 2,3-*cis*-diol, was oxidized more rapidly than methyl galactoside, containing a 3,4-*cis*-diol, both rates being much greater than for methyl glucoside, containing only *trans*-diols, in agreement with the results of Hockett and McClenahan.[11] However, the latter investigators were unable to correct for lead tetraacetate consumed by the formic acid and consequently did not determine whether or not over-oxidation occurred. The present results therefore extend their findings by showing that the oxidations in glacial acetic acid are stoichiometric and involve no over-oxidations.

The rates of lead tetraacetate consumption (Table I) were not paralleled by those of formic acid production (Fig. 2), particularly during later stages of the reactions; eventually acid production from methyl glucoside exceeded that of the other two glycosides. In part, this observed lack of agreement was to be expected from the work of Hockett and McClenahan.[11] For example, to explain the very rapid oxidation of methyl α-D-mannopyranoside (I), and other hexopyranosides containing a 2,3-*cis*-diol, they suggested an initial rapid attack chiefly at the *cis*-diol to yield a dialdehyde. The latter could exist as a cyclic hemiacetal II, the glycol of which, being easily oxidized, then rapidly consumed the second mole. However, oxidation of II could lead to formation of a formyl ester III which would be stable in glacial acetic acid,[12] and the yield of free formic acid should accordingly be small. The observed production of only one-third of the theoretical acid (Fig. 2, curve 1) when two moles of oxidant had been consumed (Table I) is therefore in agreement with this interpretation.

TABLE I

CONSUMPTION OF LEAD TETRAACETATE BY GLYCOSIDES IN THE PRESENCE AND ABSENCE OF POTASSIUM ACETATE[a]

Time, hours	Moles/mole Mannoside	Moles/mole Galactoside	Moles/mole Glucoside
	Without potassium acetate		
2	0.65	0.75	0.22
3	1.11	0.93	..
7	1.72	1.07	0.72
23	2.02	1.20	..
	With potassium acetate		
0.25	0.89	1.18	0.50
1.5	1.87	1.58	1.10
4	2.02	2.07	1.42
21.5	2.07	2.06	2.06

[a] Corrected for oxidant consumed by formic acid.

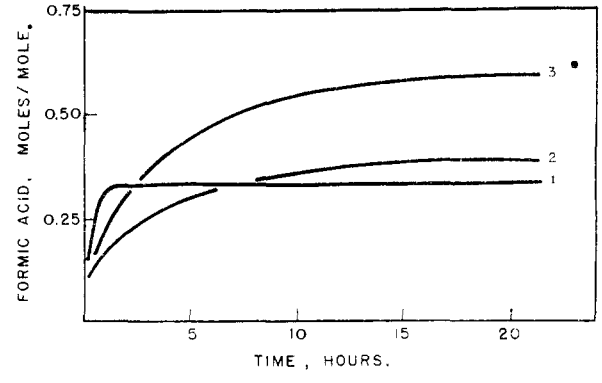


Fig. 2.—Rates of formic acid production (CO_2 evolution) during oxidation of the methyl α-D-pyranosides of mannose (1), galactose (2) and glucose (3) with lead tetraacetate in glacial acetic acid.

During oxidations of hexopyranosides having a 3,4-*cis*-diol, as in methyl α-D-galactopyranoside

(10) A. S. Perlin, THIS JOURNAL, **76**, 4101 (1954).

(11) R. C. Hockett and W. S. McClenahan, *ibid.*, **61**, 1667 (1939).

(12) Ethyl formate was not oxidized by lead tetraacetate in glacial acetic acid during a reaction period of 8 hours.

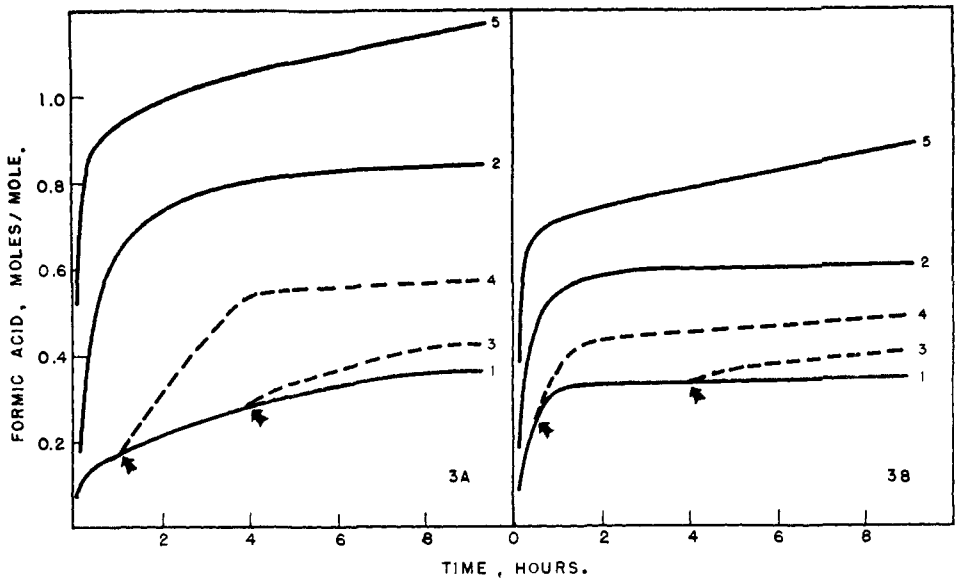
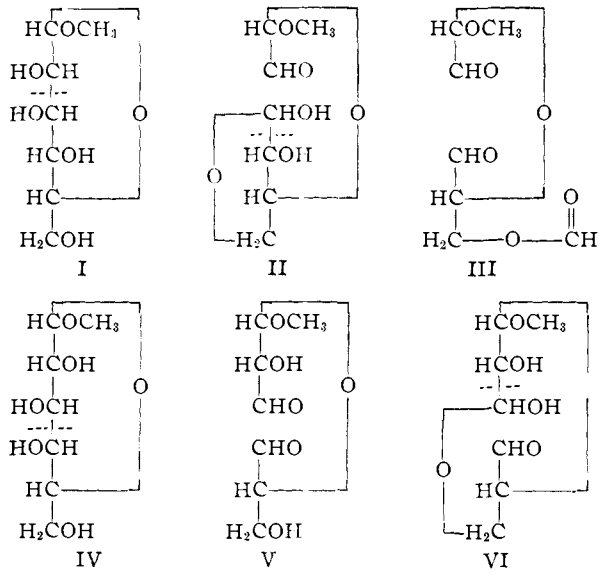


Fig. 3.—Effect of water on the rate of formic acid production (CO_2 evolution) during oxidation of methyl α-D-galactopyranoside (3A) and methyl α-D-mannopyranoside (3B) with lead tetraacetate. Acid production (1) in glacial acetic acid, (2) in 98%, acetic acid, (3) 2% water added to glacial acetic acid after 2 moles of oxidant consumed (↑), (4) 2% water added to glacial acetic acid after 1.5 moles of oxidant consumed (↑), (5) in 90% acetic acid.

(IV), Hockett and McClenahan[11] found that one mole of oxidant was consumed rapidly, and the second mole more slowly. As above, the *cis*-diol was expected to account for the first mole, yielding the dialdehyde V. However, it was considered unlikely that carbon 3 (from which the formic acid is derived) would become involved in ring-formation as in II, and therefore would be oxidized at the relatively slow rate characteristic of hydroxyaldehydes. From these assumptions of Hockett and McClenahan, it was now expected that the yield of free formic acid would far exceed the quantity esterified. In fact, the converse was found (Fig. 2, curve 2).



Although the theoretical 2 moles of lead tetraacetate were consumed by the glycoside (Table I) only 0.3–0.4 mole of free acid could be accounted for. One possible explanation of this apparent anomaly was indicated by examination of a molecular model of IV. On cleavage of the 3,4-diol, the dialdehyde V was favorably oriented for ring-formation through carbon 3 and the primary hydroxyl group of carbon 6, with a resulting seven-membered cyclic hemiacetal VI. The relatively slow rate of oxidation of V would favor cyclization, and oxidation of VI would yield the formyl ester III with a consequent small yield of free acid.

The effect of water on the oxidations provided additional evidence of formyl ester formation. For example, the yield of free formic acid from methyl galactoside was markedly increased from 0.35 mole in glacial acetic acid (Fig. 3A, curve 1) to about 0.85 mole in 98% acetic acid (curve 2). However, when the water was added after the theoretical two moles of oxidant had been consumed by the glycoside, *i.e.*, after ester formation presumably was completed, only a small increase in acid production was observed (curve 3). On the other hand, when the water was added after about 1.5 moles of oxidant had been consumed by the glycoside, *i.e.*, when ester formation presumably was half completed, a very rapid increase in the rate of acid production was found (curve 4). The water therefore chiefly appeared to reduce the extent of ester formation but it may also have promoted some hydrolysis of the esters (curve 3). Results with methyl mannoside were similar (Fig. 3B), but the increases in acid yield were usually smaller by about 20%. The greater sensitivity of the galactoside to these changes in the solvent was in agreement with the above suggestion that ester formation must have required a favorably-oriented intermediate. In 90% acetic acid, the yield of formic acid more nearly approached the theoretical one mole but over-oxidation also was evident (Fig. 3A, curve 5). The evolution of carbon dioxide from both the galactoside and mannoside continued at an almost linear

rate for at least 45 hours when the consumption of lead tetraacetate by each had reached approximately 4.5 moles per mole.

The assumption that formyl ester formation involved the primary hydroxyl group of carbon atom 6 was supported by results from the oxidation of the methyl pyranosides of arabinose, lyxose and xylose. Since these compounds differ from the corresponding hexosides only in that carbon atom 6 is absent, it was expected that the oxidations would not involve intermediate ester formation and that the production of formic acid would proceed smoothly to completion. In agreement with these expectations, the rate of formic acid production closely followed the rate of lead tetraacetate consumption for each compound (*e.g.*, Fig. 4), and the theoretical one mole of acid was produced. The production of formic acid was more rapid in 90% acetic acid, which resulted from a faster rate of oxidation of the glycosides themselves and, as with the hexosides, over-oxidation was eventually evident.

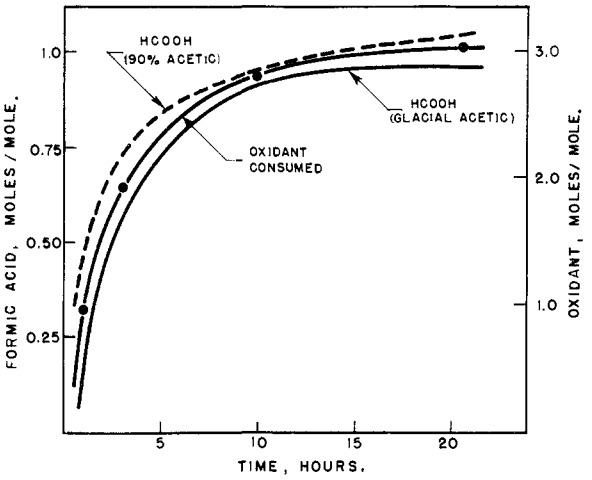


Fig. 4.—Formic acid production (CO_2 evolution) and consumption of oxidant during oxidation of methyl β-D-xylopyranoside with lead tetraacetate in glacial acetic acid. Broken-line plot represents formic acid production (CO_2 evolution) in 90% acetic acid.

A generally acceptable mechanism for lead tetraacetate oxidations has not as yet been advanced (see ref. 13). The current view[13,14] appears to favor an ionic rather than a radical[15] mechanism, although Barron, *et al.*,[16] indicated recently that both types of reaction may occur in glacial acetic acid. The apparent catalysis described above strongly suggests an ionic mechanism in the present oxidations.

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(14) M. S. Kharasch, H. N. Friedlander and W. H. Urry, *J. Org. Chem.*, **14**, 91 (1949).

(15) W. A. Waters, *Trans. Faraday Soc*, **42**, 184 (1946).

(16) H. G. Barron, G. W. K. Cavill, E. R. Cole, P. T. Gilham and D. H. Soloman, *Chem. and Ind.*, No. 3, 76 (1954).

Experimental

Materials.—The glycosides were prepared according to the method of Fischer,[17] and were crystalline compounds having melting points and specific rotations in agreement with those reported in the literature. Solution in glacial acetic acid was facilitated by first grinding samples to a fine powder.[3]

Lead tetraacetate was prepared by the procedure recommended by Vogel.[18] All other compounds were of reagent grade. The glacial acetic acid was used without further drying.

Apparatus.—A conventional constant volume type of Warburg respirometer was used. The apparatus and method for its use are described in detail by Umbreit, *et al.*[19] The respirometer was calibrated by lead tetraacetate oxidation of a standard solution of formic acid under a given set of conditions (see below) and subsequent oxidation of the materials described was carried out in the same manner. The bath temperature was 27 ± 0.05°.

Oxidation with Lead Tetraacetate in the Warburg Respirometer.—A typical oxidation was carried out as follows: 1.0 ml. of glacial acetic acid containing 20 mg. (0.045 mmole) of lead tetraacetate and 20 mg. (0.2 mmole) of potassium acetate was pipetted into the vessel chamber. To the side arm was added 0.2 ml. of glacial acetic acid containing 1.28 mg. (0.0078 mmole) of methyl α-D-arabopyranoside. A second vessel, which served as the reagent blank, contained glacial acetic acid (0.2 ml.) in place of the glycoside solution. The apparatus was equilibrated for at least 10 minutes,[20] the contents of the chamber and side-arm were mixed, and changes in pressure were noted at intervals as desired. At 20 hours, the difference in pressure between the sample and blank manometers virtually was constant at 82 mm. which, according to the calibration, corresponded to 0.0075 mmole of formic acid or 0.97 mole per mole of glycoside.

The contents of the vessel were transferred to a 125-ml. flask with 6 ml. of "stopping" solution[13] (10 g. of potassium iodide and 50 g. of sodium acetate in 100 ml. of water), and the iodine released was titrated with 0.005 *N* thiosulfate to the starch end-point. The difference in titer between the blank and the sample was 9.70 ml. of thiosulfate, which corresponded to a lead tetraacetate consumption of 3.03 moles per mole.

Acknowledgment.—The technical assistance of Mr. J. Giroux is gratefully acknowledged. The author also expresses his thanks to Dr. Morris Kates for many very helpful discussions, to Dr. R. Hochster and Dr. R. Baxter for the loan of Warburg equipment and for their kind interest in this work, and to Dr. E. O. Hughes for reviewing the manuscript.

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(19) W. W. Umbreit, R. H. Burris and J. F. Stauffer, "Manometric Techniques and Tissue Metabolism," Burgess Publishing Co., Minneapolis, Minn., 1949.

(20) In the original calibration of the respirometer with formic acid the equilibration was carried out for only 10 minutes to minimize diffusion of formic acid vapor into the chamber, but in other runs longer equilibration periods also were used.