Although the analytical evidence indicated that $C_{36}H_{30}O_2$ was a diketone, no derivatives of the carbonyl group were obtained. Furthermore, the reduction product did not give reactions of the hydroxyl group although four atoms of hydrogen were apparently added to the diketone.

Summary

1. The polyine, tetraphenyl-di-(3-methyl-3-ethylpentinyl-1)-ethane has been prepared. It is oxidized by air, cleaved by 40% sodium amalgam, and rearranges spontaneously to give a more stable isomeric hydrocarbon, $C_{42}H_{46}$.

2. Some reactions of the rearranged hydrocarbon $C_{42}H_{46}$ have been studied in the hope of establishing its structure. Cleavage by sodium amalgam has furnished evidence that at least half of the molecule retains the original carbon skeleton.

3. The question of final structure of the isomeric hydrocarbon $C_{42}H_{46}$ and the exact nature of the rearrangement by which it is formed must be left open until further evidence can be obtained.

URBANA, ILLINOIS

RECEIVED NOVEMBER 21, 1932
PUBLISHED APRIL 6, 1933

[CONTRIBUTION FROM THE BIOCHEMICAL LABORATORIES, STATE UNIVERSITY OF IOWA]

The Oxidation of Citric Acid

BY ADRIAN C. KUYPER

Except for the questionable procedure of Nordbo and Schersten[1] the only method for the quantitative determination of minute amounts of citric acid is that of Thunberg[2] which is based on the speed of decolorization of methylene blue in the presence of citric acid and the specific citric acid dehydrogenase of cucumber seed. With certain modifications,[3] this method, although time-consuming, has proved satisfactory but, as with other biological methods, trustworthy results are obtainable only at the cost of continuous checking of the uniformity of reagents and procedure. For the study of the metabolism of citric acid a precise microchemical method is greatly needed and the oxidation of citric acid by permanganate, with manometric measurement of the carbon dioxide evolved, offered some promise.

By analogy with the permanganate oxidation of lactic acid by Avery and Hastings,[4] in which 1 mole of carbon dioxide is obtained from each mole of lactic acid, the production of 3 moles of carbon dioxide per mole of citric acid should be expected, especially since the commonly used quanti-

---

(1) Nordbo and Schersten, *Skand. Arch. Physiol.*, **63**, 124–132 (1931).

(2) Thunberg, *Biochem. Z.*, **206**, 109 (1929).

(3) Kuyper and Mattill, *Proc. Soc. Expt. Biol. Med.*, **28**, 863 (1931).

(4) Avery and Hastings, *J. Biol. Chem.*, **94**, 273 (1931).

tative methods for citric acid are based on the formation of acetone[5] or pentabromoacetone[6] by permanganate oxidation. It is specified in these methods that the solution must be kept boiling[5] or that the temperature may not rise above 60°[6] but even with this clue as to the possible influence of temperature it was surprising to find (Table I) that the oxidation of citric acid by 0.1 *N* and 0.2 *N* permanganate at room temperature in the Van Slyke constant volume apparatus gave not 3 moles but 4.64 moles of carbon dioxide per mole of citric acid, and with *N* permanganate 4.94 moles of carbon dioxide. As shown by Avery and Hastings with lactic acid, the smaller the concentration of citric acid, the more complete is the oxidation, until as many as five of the carbons appear as carbon dioxide. With very small amounts of citric acid the experimental error is, of course, magnified.

TABLE I

PERMANGANATE OXIDATION OF LACTIC AND CITRIC ACIDS IN VAN SLYKE MANOMETRIC APPARATUS

Acid used	Mml. per liter	Temp., °C.	$KMnO_4$, normal	Carbon dioxide, mml.	$\frac{\text{Mml. } CO_2}{\text{mml. acid}}$ ($C \rightarrow CO_2$)
Lactic	0.652	25.3	0.1	0.643	0.985
Lactic	.590	23.2	.2	.592	1.004
Citric	.0944	25.0	.1	.438	4.64
Citric	.180	27.0	.2	.836	4.64
Citric	.0918	23.0	1.0	.454	4.94
Citric	.510	26.3	0.1	2.270	4.45
Citric	.255	26.4	.1	1.171	4.59
Citric	.1275	27.0	.1	0.609	4.78
Citric	.0637	27.0	.1	.303	4.75
Citric	.0318	27.3	.1	.154	4.85
Citric	.0159	27.2	.1	.0655	4.12
Citric	.00795	27.2	.1	.0406	5.10

This paradox, more complete oxidation at room temperatures than at higher temperature, was demonstrated in another way by titration of citric acid with permanganate in sulfuric acid at various temperatures within the range of 20–100°. The data in Table II show that between 60 and 70° the nature of the oxidation reaction changes. Below this point 1 mml. of citric acid consumed 3 mml. of permanganate oxygen, with a small temperature gradient. Above that point the permanganate oxygen consumption per mml. of citric acid declined, until at boiling temperature the amount of permanganate used per mml. of citric acid was about half that used at 57–63°. Also acetone appeared above that point (qualitative colorimetric estimation), but none below it. The less vigorous oxidation in acetic acid solution (Table II) revealed the same behavior; at boiling temperature some citrate was still oxidized beyond the acetone

---

(5) Kogan, *Z. anal. Chem.*, **80**, 112 (1930).

(6) Hartmann and Hillig, *J. Assn. Off. Agric. Chemists*, **10**, 264 (1927).

TABLE II

PERMANGANATE OXIDATION OF CITRIC ACID AT DIFFERENT TEMPERATURES

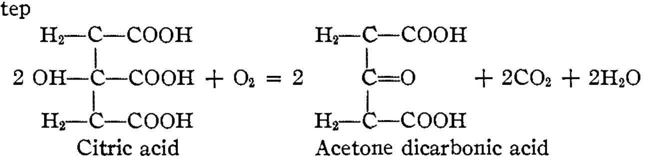
Acid oxidized	Mml. per liter	Acid	Temp., °C.	$KMnO_4$, cc.	Oxygen, mml.	$\frac{\text{Mml. oxygen}}{\text{Mml. acid}}$	Acetone
Citric	2.285	H_2SO_4	15–20	61.50	6.96	3.05	0
Citric	2.285	H_2SO_4	27–30	63.00	7.13	3.12	0
Citric	2.285	H_2SO_4	37–40	63.25	7.18	3.14	0
Citric	2.285	H_2SO_4	57–63	64.20	7.28	3.19	0
Citric	2.285	H_2SO_4	77–83	51.00	5.78	2.53	2+
Citric	2.285	H_2SO_4	87–93	46.80	5.33	2.33	5+
Citric	2.285	H_2SO_4	93–103	32.60	3.70	1.62	5+
Citric	0.815	Acetic	60–65	7.10	0.814	1.00	
Citric	1.63	Acetic	Boiling	8.10	.930	0.571	
Acetone dicarbonic	0.699	H_2SO_4	26	15.70	1.795	2.57	0
Acetone dicarbonic	.699	H_2SO_4	65	14.30	1.632	2.34	Trace
Acetone dicarbonic	.699	H_2SO_4	Boiling	0.00	0.000	0.00	5+

stage (0.571 instead of the theoretical 0.500) which explains why Kogan's method recovers only 89% of the theoretical amount of acetone.

An insight into the mechanism of the two different reactions above and below 60° was provided by the behavior of an intermediary product,[7] acetone dicarbonic acid (Table II), which paralleled that of citric acid, except that at boiling temperature acetone dicarbonic acid consumed no permanganate oxygen and decomposed spontaneously into carbon dioxide and acetone.

The following equations have been formulated to explain the divergent course of citric acid oxidation as influenced by temperature.

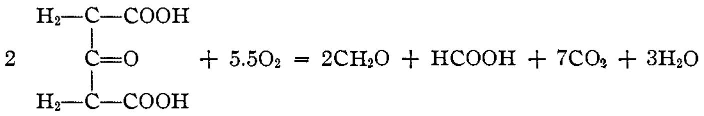
First Step



$$2\ \begin{array}{l} H_2\text{—}C\text{—}COOH \\ \quad\ | \\ OH\text{—}C\text{—}COOH \\ \quad\ | \\ H_2\text{—}C\text{—}COOH \end{array} + O_2 = 2\ \begin{array}{l} H_2\text{—}C\text{—}COOH \\ \quad\ | \\ \quad\ C{=}O \\ \quad\ | \\ H_2\text{—}C\text{—}COOH \end{array} + 2CO_2 + 2H_2O$$

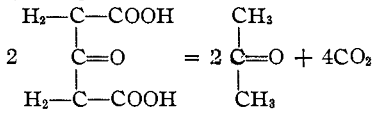
Second Step

At low temperature



$$2\ \begin{array}{l} H_2\text{—}C\text{—}COOH \\ \quad\ | \\ \quad\ C{=}O \\ \quad\ | \\ H_2\text{—}C\text{—}COOH \end{array} + 5.5O_2 = 2CH_2O + HCOOH + 7CO_2 + 3H_2O$$

At high temperature



$$2\ \begin{array}{l} H_2\text{—}C\text{—}COOH \\ \quad\ | \\ \quad\ C{=}O \\ \quad\ | \\ H_2\text{—}C\text{—}COOH \end{array} = 2\ \begin{array}{l} CH_3 \\ | \\ C{=}O \\ | \\ CH_3 \end{array} + 4CO_2$$

The first stage in the oxidation at any temperature is the formation of acetone dicarbonic acid. Additional evidence for this statement is that

---

(7) Wöhlk, *Z. anal. Chem.*, **41**, 77 (1902).

this acid and citric acid are the only substances of this general structure which quantitatively yield a pentabromoacetone derivative when oxidized in the presence of potassium bromide.[8] The nature of the second step depends on the relationship between the temperature and the speed of oxidation. At high temperature acetone dicarbonic acid is decomposed to acetone but if the oxidizing potential is high some of it may be oxidized. The oxidative reaction is accelerated by the use of sulfuric instead of acetic acid, or by increasing the strength of the permanganate solution. Indeed, an almost purely oxidative reaction can be secured even at high temperatures by very rapid addition of permanganate.

The reaction at room temperatures is equally variable and because of its immediate relation to the original purpose of the work it was studied in greater detail. Apparently the only products are formaldehyde, formic acid and carbon dioxide in variable amounts. Citric acid was titrated with acid permanganate in a closed flask, such that the carbon dioxide could be aerated from the solution and weighed. The formic acid remaining in the reaction mixture was determined by the weight of mercurous chloride produced by the reducing action on mercuric chloride.[9] No means was found for making simultaneous determinations of formaldehyde and carbon dioxide, and since separate determinations in different runs have no value because of the variability of the reaction, the amount of formaldehyde formed was calculated as representing the carbon which entered the reaction less that found as formic acid and carbon dioxide at its completion, a procedure which was later justified. From the observed and calculated data the oxygen entering the reaction in citrate and permanganate was compared with that found in the oxidation products. Table III presents the results of a series of titrations at room temperature in which the rate of addition of permanganate was gradually reduced. With rapid addition of permanganate the amount of oxygen found in the reaction products was equal (within 0.5%) to that present in the original citrate plus that derived from the permanganate used. As the speed of permanganate addition was reduced (Experiments 2 to 8) the reaction products contained increasingly more oxygen (finally 28% more) than is known to have entered the reaction as citrate and permanganate. The formation of less highly oxidized products is improbable; neither acetone nor other partial oxidation products could be detected and any calculation on the basis of their formation still fails to account for the additional oxygen present. When nitrogen was used for aeration (Experiments 9, 10), whether permanganate was added fast or slowly, no excess oxygen was recovered; on the other hand, when the solution was aerated very slowly with oxygen (Experiment 11), the products contained almost 36% excess

---

(8) Amberg and McClure, *Am. J. Physiol.*, **44**, 453 (1917).

(9) Autenrieth and Warren, "Detection of Poisons," P. Blakiston's Son and Co., Philadelphia, 1928, 6th ed., p. 98.

TABLE III

OXYGEN BALANCE IN CITRIC ACID OXIDATION AT ROOM TEMPERATURE

	Oxygen entering the reaction			Oxygen in the end products of the reaction						Remarks	
Expt.	Citrate[a] mml.	$KMnO_4$, mml.	Total, mml.	Formic acid, mml.	Carbon dioxide, mml.	Formaldehyde[b] mml.	Water[c] mml.	Total	Oxygen "recovery," %	Aerated with	$KMnO_4$ added
1	3.500	3.626	7.127	0.978	4.674	0.173	1.337	7.163	100.49	Air	Fast
2	3.500	3.545	7.044	1.016	4.706	.138	1.332	7.215	102.44	Air	
3	3.500	3.336	6.835	0.607	4.788	.302	1.394	7.094	103.78	Air	
4	3.500	3.440	6.942	.721	4.822	.229	1.411	7.182	103.47	Air	
5	3.500	3.334	6.833	.627	4.939	.216	1.470	7.252	106.12	Air	to
6	3.500	3.108	6.608	.589	4.985	.213	1.492	7.280	110.15	Air	
7	3.500	2.405	5.905	.265	4.808	.462	1.404	6.940	117.55	Air	
8	3.500	2.467	5.967	.266	5.512	.110	1.757	7.645	128.13	Air	Slowly
9	3.500	3.628	7.129	.730	4.653	.308	1.326	7.019	98.46	Nitrogen	Slowly
10	3.500	3.775	7.276	1.116	4.565	.160	1.282	7.124	97.93	Nitrogen	Fast
11	3.500	2.170	5.670	0.206	5.590	.102	1.795	7.694	135.70	Oxygen	Slowly

[a] From 0.9 to 1.7 mml. of citrate was used in these experiments. The results were all recalculated to 1 mml. of citric acid which contains 3.5 mml. of oxygen.

[b] The oxygen present in formaldehyde was calculated as equal to one-half the difference between the carbon entering the reaction and that recovered as formic acid and carbon dioxide, all expressed as millimoles.

[c] This value was obtained by considering the oxidation of citric acid as occurring in three separate reactions involving the formation of the determined amounts of formic acid, carbon dioxide and formaldehyde, respectively. The water taking part in each reaction was then calculated, the values were summated, and the oxygen equivalents obtained.

oxygen. Molecular oxygen therefore enters the reaction and in part supplants permanganate. An almost 100% recovery in the reactions aerated with nitrogen confirms the original assumption that permanganate oxidation of citric acid at room temperature produces only carbon dioxide, formaldehyde, formic acid and water. The small amount of oxygen unaccounted for in the nitrogen aerations may have been lost in volatilized formic acid.

The distribution of oxygen in the various end-products requires comment. The total amount of formaldehyde and formic acid produced per mole of citrate oxidized decreases with increasing participation of molecular oxygen (from considerably above 1 mole to less than half a mole); the amounts of carbon dioxide and water produced increase with increasing participation of molecular oxygen. Furthermore, when molecular oxygen is most instrumental in oxidation (Experiment 11), it enters the reaction in the ratio of two moles for each mole of citrate oxidized. This amount is just sufficient to oxidize one mole of acetone dicarbonic acid to three moles of carbon dioxide, one of water and two of formaldehyde. Three of its carbon atoms are already oxidized to or beyond the formaldehyde stage; the remaining two are probably oxidized by the same path. Acetone dicarbonic acid, perhaps in an active state, is therefore the most likely intermediate substance to react with molecular oxygen; permanganate is thereby released for the more complete oxidation of formaldehyde and formic acid to carbon dioxide.

These observations explain some of the difficulties encountered in the quantitative estimation of citric acid by permanganate oxidation. Duplicate results are obtainable only under exactly similar conditions. In Kogan's method slight changes in temperature, acidity and rate of addition of permanganate influence the amount of acetone produced. In the oxidation to pentabromoacetone an empirical conversion factor of 1.05 indicates that some citrate is oxidized beyond this stage. Oxidation by ceric sulfate[10] apparently also requires careful control of acidity, volumes and concentrations of the reacting substances, and according to its sponsors "the conversion factor indicates very extensive oxidation of acetone." By means of the conversion factors given in Table I citric acid in pure solutions can also be determined satisfactorily by the Van Slyke constant volume apparatus, provided standardized conditions are maintained.

The association of molecular oxygen with permanganate in the *in vitro* oxidation of citric acid at temperatures below 60° is interesting in the light of the rapid oxidation of this substance in the animal body. Its failure to produce acetone at body temperature *in vitro* agrees with the observation[11] that it is an antiketogenic substance.

The author wishes to thank Professor H. A. Mattill for his interest and assistance.

Summary

The acid permanganate oxidation of citric acid is more complete at room temperatures than at high temperatures. Acetone dicarbonic acid is the first intermediary product. At temperatures below 60°, depending on the concentration of permanganate and the rate of its addition, this intermediary product is oxidized to formaldehyde, formic acid and carbon dioxide; molecular oxygen takes part in this oxidation and no acetone is formed. The higher the temperature above 60° the more acetone dicarbonic acid is decomposed into acetone before it can be oxidized.

By means of proper conversion factors small amounts of citric acid in pure solution may be determined by oxidation with permanganate in the Van Slyke constant volume apparatus.

IOWA CITY, IOWA

RECEIVED NOVEMBER 21, 1932
PUBLISHED APRIL 6, 1933

---

[10] Willard and Young, THIS JOURNAL, **52**, 132 (1930).

[11] Greenwald, *J. Biol. Chem.*, **18**, 115 (1914).