The Oxidation of 5-Keto-gluconic Acid with Nitric Acid in the Presence of Vanadium

BY W. E. BARCH

5-Keto-gluconic acid has been obtained by Boutroux and others[1] from glucose by means of certain oxidizing organisms (bacterium xylinum, bacterium gluconium, etc.). Kiliani[2] obtained it by chemical means from glucose, by mild oxidation with nitric acid. His method of preparation has been the source of the material used in the following.

Boutroux[2b] oxidized the acid with nitric acid and identified among the products of oxidation racemic acid, trihydroxyxyloglutaric acid, glyoxylic acid, and others.

It has been shown by Odell[3] that *d*-tartaric acid may be obtained by nitric acid oxidation, in the presence of vanadium, of common starch.

There will be an attempt to show, in future publications, that this means of oxidation offers certain advantages over that using nitric acid alone, or other oxidizing agents, in the study of the oxidative degradation of the monosaccharides, their acids, and other derivatives. A major portion of the original molecule may be recovered in the form of four carbon acids, (*d*-tartaric, *l*-tartaric, racemic or mesotartaric acids, dependent on the configuration of the starting material). These acids are readily followed analytically in the presence of each other and other products of oxidation which may be present.

5-Keto-gluconic acid was chosen as the subject of this preliminary paper because of the relative simplicity of the oxidation, and because of its application as a partial picture of the more complex oxidation of glucose itself.

It was the original intention to conduct the experiments with the free acid. It was found that the sirup obtained from the calcium salt of the acid by decomposition with oxalic acid was too unstable in the high concentration necessary for the oxidation. It rapidly turns black on standing a short time in the desiccator over sulfuric acid, swelling and evolving gas.

It was found, however, that the calcium salt itself was suitable for the purpose. It is quite easily obtained in the pure condition, and dissolves readily in the nitric acid used in the experiments. The calcium of the salt appears early in the oxidation as calcium oxalate and may be removed by simple filtration.

Discussion

The results of twelve oxidations are tabulated according to the analytical methods described in the experimental part.

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(1) Boutroux, (a) *Compt. rend.*, **102**, 924 (1880); (b) **127**, 1224 (1898); (c) Bertrand, *Ann. chim.* [8] **3**, 281 (1904); (d) Bernhauer and Schon, *Z. physiol. Chem.*, **180**, 232–240 (1929); (e) Herman, *Biochem. Z.*, **214**, 357–367 (1929); (f) Takahashi and Asai, *J. Agr. Chem. Soc. Japan*, **6**, 223–241 (1930).

(2) Kiliani, *Ber.*, **55**, 2817 (1922).

(3) A. F. Odell, U. S. Patent 1,425,605.

The experiments are calculated on the basis of percentage of total carbon recovered from the original starting material as 100%.

It will be noted that as much as two-thirds of the original acid may be recoverable in readily determined non-gaseous products.

The main products are tartaric, racemic, trihydroxyxyloglutaric, and oxalic acids as shown. The column labeled carbon dioxide may be considered a rough estimation only, as it includes only that gas formed under the reflux and does not include that formed on evaporating off the nitric acid *in vacuo*. It is of interest chiefly in Part 1 in which the nitric acid was entirely consumed during the refluxing. One carbon of the six original carbons in the ketogluconic acid being 16.67%, it will be noted that the percentage of the gas with different molecular proportions of nitric acid closely approximates molecular proportions.

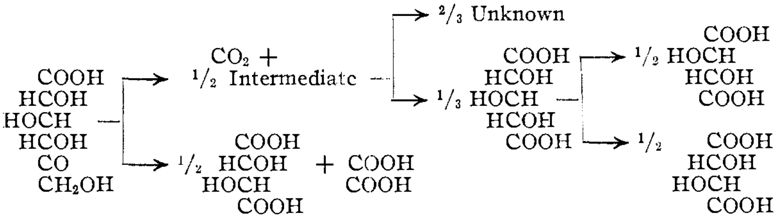
Referring first to Part 1, here the amount of available oxygen has been varied by varying the amount of nitric acid in each experiment. It has been a matter of observation, in this work, that at the boiling temperature nitric acid is deprived of oxygen by a simple reduction to nitrous acid, and therefore furnishes one molecular proportion of oxygen to one of nitric acid. This is true only at the higher temperatures, longer contact with the acid producing further reduction. In order to break the carbon chain at the keto group to tartaric and oxalic acids, or to trihydroxyxyloglutaric acid and carbon dioxide, three molecular proportions of oxygen are required. In this first series of four experiments, the following significant observations may be made. The greater bulk of the tartaric acid appears before any racemic or trihydroxyxyloglutaric acid can be detected. However, carbon dioxide, one-twelfth of the molecule (necessary for the postulation of one-half the original molecule breaking between carbon five and six) is formed at once. The amount shows no increase on increasing the molecular proportions of nitric acid from 2 to 2.5, indicating it as an early phenomenon. As tartaric acid continues to increase, the tryhydroxyxyloglutaric acid appears, oxidizing rapidly further to racemic acid. In the fourth experiment, with the splitting of the carbon chain presumably complete, the trihydroxyxyloglutaric acid has almost disappeared, the earlier formed tartaric acid has suffered by further oxidation, and the racemic acid is at its maximum point.

In Part 2, the available oxygen has been varied by time of contact with the nitric acid, using the same amount of nitric acid in each experiment. Here the peak for trihydroxyxyloglutaric acid is clearly shown, with its subsequent rapid disappearance.

All the products of the oxidation are themselves subject to further oxidation, making it probable that none of the observed results represent maxima. This is shown, to some extent, in Part 4, where the conditions of the oxidation are more mild than the foregoing. The amount of tartaric acid obtained here is near the maximum (33.3%) required by theory, and the trihydroxyxyloglutaric acid is also increased.

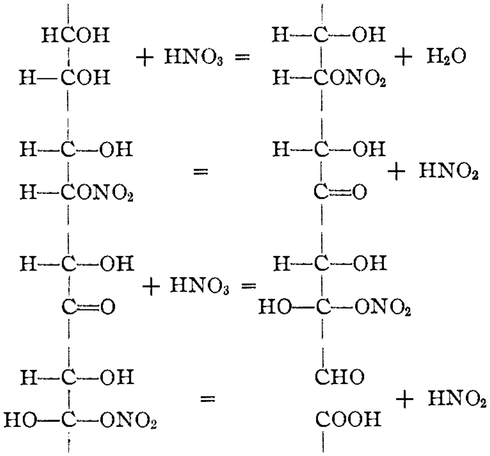
Summarizing, briefly, the following conclusions may be drawn on the breakdown of the carbon chain in 5-keto-gluconic acid on oxidation with nitric acid in the presence of vanadium.

The chain breaks on either side of keto group, equally between the break between carbon four and five and the break between carbon five and six; 50% of the molecule appears as tartaric and oxalic acids. Only one-third of the five carbon fragment appears as trihydroxyxyloglutaric acid, which is subsequently further oxidized to racemic acid. In this way, two-thirds of the original keto-gluconic molecule has been accounted for, the fate of the remaining one-third being unknown.



It is probable that vanadium is catalytically manifested only by the early break of the carbon chain between carbon four and five. Although four-carbon acids have been reported as products of oxidation of hexose derivatives, without the use of this catalyst, it is probable that they are the result of a secondary oxidation of the first formed five-carbon acid, as illustrated by racemic acid in these experiments.

There is indirect evidence for the following hypothesis as to the reaction mechanism.



$$\begin{array}{c}|\\ \text{HCOH}\\ |\\ \text{H—COH}\\ |\end{array} + HNO_3 = \begin{array}{c}|\\ \text{H—C—OH}\\ |\\ \text{H—CONO}_2\\ |\end{array} + H_2O$$

$$\begin{array}{c}|\\ \text{H—C—OH}\\ |\\ \text{H—CONO}_2\\ |\end{array} = \begin{array}{c}|\\ \text{H—C—OH}\\ |\\ \text{C=O}\\ |\end{array} + HNO_2$$

$$\begin{array}{c}|\\ \text{H—C—OH}\\ |\\ \text{C=O}\\ |\end{array} + HNO_3 = \begin{array}{c}|\\ \text{H—C—OH}\\ |\\ \text{HO—C—ONO}_2\\ |\end{array}$$

$$\begin{array}{c}|\\ \text{H—C—OH}\\ |\\ \text{HO—C—ONO}_2\\ |\end{array} = \begin{array}{c}|\\ \text{CHO}\\ \\ \text{COOH}\\ |\end{array} + HNO_2$$

When glucose is oxidized under the conditions described in this paper, and the reaction slowed, by cooling, after the first violent phase, the unreacted nitric acid may be removed by repeated extraction with ether. The resulting liquor, free of nitric acid, when allowed to stand a short time in sunlight gradually warms until there is again a violent reaction with vigorous evolution of nitrogen oxides.

The hypothesis of successive formation of unstable nitrates and their decomposition by loss of nitrous acid is also supported by the predominance of nitrogen peroxide over nitrogen monoxide in the early stages of the reaction and the evolution of nitrogen monoxide only, in the latter stages when the free nitric acid has been consumed.

(Early stage) $HNO_2 + HNO_3 = H_2O + 2NO_2$
(Latter stage) $3HNO_2 = HNO_3 + H_2O + 2NO$

CARBON DISTRIBUTION %

Part 1. Experiments refluxed to completion

Moles HNO_3	Acid Tartaric	Acid Racemic	Acid trihydroxy-xyloglutaric	Acid Oxalic	Solids Detd.	Reaction CO_2
2	17.3	0.0	0.0	11.4	28.7	8.3
2.5	20.4	0.2	4.1	14.2	38.9	8.1
3	27.8	4.0	5.9	17.3	55.0	16.7
4	14.2	8.5	1.1	16.8	40.6	33.1

Part 2. Experiments with 4 moles of nitric acid refluxed for different periods with the excess nitric acid quickly evaporated off *in vacuo*

Time, minutes						
30	24.3	0.0	0.0	17.2	41.5	1.7
60	22.2	1.8	11.7	16.3	52.0	8.0
120	26.7	2.1	8.7	19.0	56.5	13.2
960	14.2	8.5	1.1	16.8	40.6	33.1

Part 3. Experiments refluxed for thirty minutes with nitric acid and evaporated *in vacuo*

Moles HNO_3						
4	24.3	0	0	17.2	41.5	1.7
5	21.1	4.0	11.7	16.3	53.1	8.4

Part 4. Experiment with 6 moles nitric acid refluxed for ten minutes and the excess nitric acid evaporated at 45° *in vacuo*

	29.1	2.7	13.9	20.5	66.2	....

Part 5. Experiment allowed to stand at 45° to completion

4	19.4	7.6	0.6	37.4	65.0	....

Part 6. Experiment with 6 moles of nitric acid refluxed for ten minutes, then the excess nitric acid removed with formic acid

	20.9	0.5	0	16.0	37.4	9.2

Experimental

Preparation of Calcium 5-Keto-gluconate.—The method of Kiliani is repeated here, with some variation.

One hundred grams of glucose is dissolved in 28 cc. of water in a 300-cc. Erlenmeyer flask. To this is added 52 cc. of concentrated nitric acid (this is approximately

1.5 mole). Any number of these preparations as described may be placed in a slowly circulating shallow bath at 20 to 25°. Working with larger quantities in a single lot is not advisable because of difficulties in cooling.

Fumes of nitrogen peroxide appear slowly after twenty-four hours and continue to evolve for several days. In a week's time the reaction has practically ceased, and the flasks may be removed.

One hundred grams of solid sodium acetate is added and stirred into the acid solution, until it has dissolved more or less completely, and then 25 g. of calcium chloride dihydrate, dissolved in a little water. The solution becomes rapidly darker in color and may show a haze of calcium oxalate. Crystallization begins after four or five hours and continues almost indefinitely. The product obtained after long standing, however, contains large quantities of calcium saccharate. The subsequent purification is easier, if the precipitate is filtered off after forty-eight hours.

The brown amorphous precipitate is redissolved in sufficient hydrochloric acid to be equivalent to the calcium present in the precipitate. The calcium oxalate will remain undissolved in the cold, and is filtered off, after adding norite. The filtered and decolorized solution is then neutralized to 85% of its total acidity with aqueous ammonia, care being taken to avoid local alkalinity which will again color the solution. The calcium keto-gluconate appears rapidly as a coarse crystalline meal and may be filtered in a few hours.

After a second recrystallization in this manner, a product was obtained, pure white in color, containing 11.66% CaO, calcd. for $Ca(C_6H_9O_7)_2$—$3H_2O$, 11.67%. One gram dissolved in the theoretical amount of hydrochloric acid plus 5%, and made up to 25 cc., shows α^{20}_{D} of $-11.69°$, which calculated to the free acid is $-14.43°$. It shows no loss in weight on drying in the oven at 100°; yield, 10–12%.

Oxidation.—All experiments were conducted in the same manner, using 10 g. of the above prepared salt in a 250-cc. round-bottomed flask with a reflux condenser ground to fit the flask. The salt was covered with sufficient water to be equivalent, by volume, to 25% of the nitric acid used in the experiment. 0.001 g. of sodium orthovanadate was present in each experiment. The top of the reflux condenser was connected to two wash bottles containing concentrated sulfuric acid to absorb the bulk of the nitrogen oxides and then to a bottle containing 40% potassium hydroxide, protected from the atmosphere with a long tube coarsely packed with soda lime. This last was for a rough estimation of the carbon dioxide formed, which was determined on an aliquot of the potassium hydroxide solution by the usual volumetric method.

The flask containing the nitric acid, salt, water and sodium orthovanadate was immersed completely in a water-bath and heated to 100° (except where otherwise mentioned).

The reaction proceeds in three stages. The colorless solution very slowly turns green, a few desultory bubbles appear, increasing rapidly in number until a violent reaction ensues, with large quantities of red nitrogen peroxide evolving from the surface of the solution. The violent reaction is very brief and is succeeded by a slower reaction which continues for several hours. In this phase the nitrogen peroxide ceases to be evolved and is replaced by a slower evolution of nitrogen monoxide. The reaction is complete after all evolution of gas has ceased. Early in the reaction, soon after the violent phase, calcium oxalate begins to appear, in unusually coarse, granular crystals. To ensure the complete removal of the nitric acid, each experiment was evaporated to dryness in the vacuum without removing from the reaction flask.

Analysis.—The dry material is dissolved in a small quantity of hot water and the insoluble calcium oxalate filtered off. The filtrate is collected in a 10-cc. volumetric flask; 1 cc. is taken for titration with $N/5$ alkali. The same titration sample is then used for an oxalic acid determination. The remaining portion of the filtrate is then neutralized with the calculated quantity of a strong solution of potassium hydroxide (cooling) and rendered acid again with concentrated hydrochloric acid equivalent to exactly one-half the amount of the potassium hydroxide. The potassium acid salts of tartaric and racemic acids precipitate immediately and are filtered off after standing for forty-eight hours. The precipitate is washed with 60% alcohol.

The resulting precipitate is dried in the oven at 100° for twelve hours. A 1-g. sample is titrated with $N/5$ potassium hydroxide, then made up to 25 cc. and polarized. From the titration of pure potassium acid tartrate (26.58 cc.), and the specific rotation of potassium tartrate (α_D^{20} + 27.58°), the relative amounts of potassium acid tartrate and racemate may be calculated, together with inert material (potassium chloride). The weight of the equivalent potassium used in neutralizing is, of course, added to the original 1 g., in calculating the specific rotation of the unknown salt.

The filtrate from the potassium acid salts is evaporated to remove the alcohol, and sufficient calcium chloride added to be roughly equivalent to the oxalic acid as determined previously. This solution is immediately filtered hot and the filtrate neutralized. To this filtrate is added a solution equivalent to 5 g. of calcium chloride and allowed to crystallize for two days. The calcium trihydroxyxyloglutarate crystallizes rather slowly, and should only be filtered after no more crystals appear on the sides of the beaker after scraping down. The salt contains two molecules of water. It is not quite pure, but for purposes of analysis was considered as such because of the rather large loss on recrystallization: CaO determined, 21.87%; calcd. 22.06%. The free acid obtained by breaking up the salt with oxalic acid has no polarization and readily crystallizes from water; it melts at 142–144° and titrates 55.1 cc. of $^1/_5N$ KOH, calcd. 55.5.

Summary

1. The probable mechanism of the breakdown of 5-keto-gluconic acid on oxidation with nitric acid in the presence of vanadium is shown.

2. The application of the method as a means for the study of the oxidation of other carbohydrate derivatives is suggested.

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Solubility of Inulin

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Such expressions as "insoluble" and "slightly soluble" applied to inulin are very common in the literature, but actual measurements recorded are very few. Some of the old fragmentary results on solubility of inulin in water and alcoholic solutions are recorded by Dragendorff,[1] von Lippmann,[2] and Prantl.[3] Wolff and Geslin[4] investigated the comparative solubilities of inulin from chicory and dahlia with the results in Table I.

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(1) Dragendorff, "Materialien zu einer Monographie des Inulins," St. Petersburg, 1870, pp. 54 and 66.

(2) Von Lippmann, "Chemie der Zuckerarten," 1904, p. 797.

(3) Prantl, "Das Inulin," München, 1870, p. 21.

(4) Wolff and Geslin, *Bull. soc. chim. biol.*, **2**, 19 (1920).