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OXIDATION OF GALACTURONIC ACID AND OF 5-KETO- GLUCONIC ACID IN ALKALINE SOLUTION

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ABSTRACT

In the present investigation determinations were made of the quantities of oxalate, tartrate, and trihydroxyglutarate formed by the oxidation of galacturonic acid and of 5-keto-gluconic acid in the presence of various bases and various concentrations of the base. Although preliminary measurements indicate that the proportions of the products can be altered by use of catalysts, this report is restricted to the results obtained without the addition of catalytic substances. Oxidation of sodium galacturonate with oxygen in 1-*N* sodium hydroxide solution gave oxalate, *D*-tartrate (levo), and *D-arabo*-trihydroxyglutarate in yields of 0.10, 0.10, and 0.45 mole, respectively, per mole of galacturonate. Oxidation of sodium 5-keto-gluconate under like conditions gave oxalate, *L*-tartrate (dextro), and *xylo*-trihydroxyglutarate in yields of 0.29, 0.10, and 0.45 mole, respectively, per mole of sodium 5-keto-gluconate. Directions are included for the preparation of potassium *D-arabo*-trihydroxyglutarate from galacturonic acid.

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I. INTRODUCTION

The development of relatively simple methods for the preparation of salts of galacturonic acid [1]¹ and of 5-keto-gluconic acid [2] makes these substances readily available for the investigation of possible uses. The preparation of *L*-tartaric acid (dextrorotatory)² from 5-keto-gluconic acid and the preparation of *D-arabo*-trihydroxyglutaric acid from galacturonic acid are reported in the patent literature [3, 4]. The processes for the preparation of the two products are

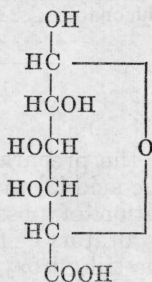
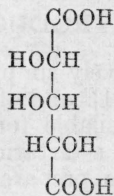
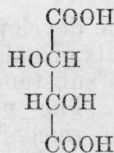
¹ Figures in brackets indicate the literature references at the end of this paper.

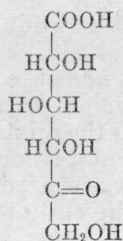
² Dextrorotatory tartaric acid is commonly called *d*-tartaric acid. The "*d*" refers to the direction of optical rotation, and should not be confused with the "*D*" used to indicate configuration in the carbohydrate field. Dextrorotatory tartaric acid is configurationally related to *L*-glyceraldehyde, the substance used for the *D* and *L* classification of the carbohydrates. In this paper it is designated *L*-tartaric acid.

similar, but it would appear from the patent specifications that 5-keto-gluconic acid on oxidative cleavage yields largely a 4-carbon dibasic acid (tartaric), whereas galacturonic acid yields largely a 5-carbon dibasic acid (trihydroxyglutaric). Since both materials might be expected to yield both 4- and 5-carbon dibasic acids, further investigation of the oxidative cleavage of the two substances seemed desirable.

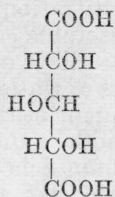
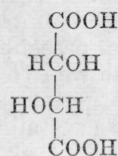
5-Keto-gluconic acid and galacturonic acid are sugars having a terminal carboxyl group in place of the terminal CH_2OH group characteristic of the simple sugars. Thus galacturonic acid is a substituted *D*-galactose and 5-keto-gluconic acid is a substituted *L*-sorbose. The investigations of Nef, Spoehr, Anderson, Evans, Glattfeld, and others [5] have shown that the oxidation of reducing sugars in alkaline solution gives rise to numerous products. In the galactose series, it has been shown that oxidative cleavage of the bond between carbons 1 and 2 yields *D*-lyxonic and formic acids; cleavage of the 2,-3-bond yields *D*-threonic and glycolic acids; and cleavage of the 3,-4-bond yields two molecules of glyceric acid. In addition to these acids, small quantities of carbon dioxide and oxalic acid are formed.

With galacturonic acid, I, oxidative cleavage of the 1,2-bond would be expected to yield formic acid and *D-arabo*-trihydroxyglutaric acid, II, and cleavage of the 2,3-bond, glycolic acid, and *D*-tartaric acid, III. With 5-keto-gluconic acid, IV, oxidative cleavage of the bond between carbons 5 and 6 or between carbons 4 and 5, would be expected to yield products quite similar to those formed from galacturonic acid. The 5-carbon dibasic acid in this case would be *xylo*-trihydroxyglutaric acid, V, and the 4-carbon dibasic acid, *L*-tartaric acid, VI. Oxidative cleavage of the 3,4-bond of either galacturonic acid or 5-keto-gluconic acid would be expected to yield *D,L*-glyceric acid and tartronic acid. The separation and quantitative determination of all the products formed by the oxidation of the two materials would constitute a long investigation, which does not seem warranted at this time. The present study has been restricted to the determination of the amounts of oxalic, tartaric, and trihydroxyglutaric acid formed in the reaction.

I. *D*-Galacturonic acid.II. *D-arabo*-Trihydroxyglutaric acid.III. *D*-Tartaric acid.



IV. 5-Keto-D-gluconic acid.

V. *xyl*-Trihydroxy-glutaric acid.

VI. L-Tartaric acid.

II. EXPERIMENTAL METHODS

1. OXIDATION METHOD

The oxidation reactions were conducted in a Parr apparatus of the type used for low-pressure hydrogenation. The aqueous alkali required for each experiment was placed in the glass reaction flask and saturated with oxygen at room temperature. The material to be oxidized, usually 0.05 equivalent, was added quickly, connections were made with the oxygen supply, and the mixture was shaken vigorously with oxygen at a pressure of 20 pounds. Most of the oxidation occurred in the course of several hours, but in order to maintain uniform conditions and be certain that the reaction was complete, shaking was continued for a period of 48 hours. The reaction mixture was then analyzed or used for the preparation of the desired compounds. At the end of the reaction period a test for copper-reducing substances by means of Benedict's reagent was negative.

2. DETERMINATION OF OXALATE, TARTRATE, AND TRIHYDROXYGLUTARATE

In the experiments in which calcium hydroxide was used as a base a precipitate was formed which consisted largely of calcium oxalate and basic calcium salts of tartaric acid and trihydroxyglutaric acid. When a soluble hydroxide was used, an equivalent amount of calcium chloride was added to the solution containing the oxidation products to precipitate the insoluble calcium salts. The precipitate was collected on a filter, washed with lime water, dried, and weighed. A calcium determination was made on the dried material to facilitate subsequent work.

Oxalate was determined on a sample of the dried calcium salts by dissolving them in acid and precipitating calcium oxalate by the addition of ammonium hydroxide to neutrality. The simultaneous precipitation of calcium tartrate was prevented by the addition of 5 g of ammonium acetate per 100 ml of solution.³ The calcium oxalate was precipitated and titrated with potassium permanganate in the usual manner.

³ Boric acid may be used also to avoid interference of tartaric acid in the determination of oxalate. See "Analysis of Foods" by A. L. Winton and K. B. Winton, page 216 (John Wiley & Sons, Inc., New York N. Y., 1945).

The determination of tartrate was based upon the separation of potassium acid tartrate and its titration with standard alkali. Two grams of the dried mixture of insoluble calcium salts was added to an amount of finely powdered crystalline oxalic acid equivalent to the calcium present. Four milliliters of water was added, and the mixture was allowed to stand with frequent stirring. After about 24 hours, 1.8 g of potassium acetate was added, and the material was stirred to induce crystallization of potassium acid tartrate. At the end of an hour 18 ml of glacial acetic acid was added, and the mixture was allowed to stand for 24 hours. Fifteen milliliters of 95-percent alcohol was then added, and after an additional 24 hours the mixture was filtered on a Gooch crucible, washed with 5 ml of 95-percent aqueous acetic acid, 5 ml of glacial acetic acid, and finally with 95-percent alcohol. The filtrate and washings were set aside for the determination of trihydroxyglutaric acid. The crucible and contents were dried at 105° C in a vacuum oven until free from volatile acids. The dry material, largely calcium oxalate and potassium acid tartrate, was suspended in water, and the potassium acid tartrate was determined by titration with potassium hydroxide.

The determination of trihydroxyglutarate was based upon precipitation as the insoluble lead trihydroxyglutarate. The filtrate from the tartrate determination was evaporated to dryness at room temperature in a stream of air. The residue was dissolved in a few milliliters of water, and an aqueous solution of lead acetate was added in amount sufficient to convert the potassium trihydroxyglutarate to the insoluble lead salt. A large excess of lead acetate was avoided. After 18 hours, the lead salt was filtered on a weighed Gooch crucible, washed with water, dried at 60° C in a vacuum oven, and weighed.

In order to establish the identity of the lead salt derived from galacturonic acid, it was converted to the characteristic crystalline potassium salt. The optical rotation and analysis showed that the product was potassium *D-arabo*-trihydroxyglutarate.

To identify the lead salt from 5-keto-gluconic acid, it was decomposed with hydrogen sulfide, and the acid was converted to the bisphenylhydrazide. The melting point and analysis agreed with those of the previously known bisphenylhydrazide of *xylo*-trihydroxyglutaric acid.

3. PREPARATION OF POTASSIUM ACID D-TARTRATE (LEVO) AND POTASSIUM D-ARABO-TRIHYDROXYGLUTARATE FROM SODIUM GALACTURONATE

For the separation of potassium trihydroxyglutarate a method was employed which is somewhat similar to that used for the analysis of the oxidation mixture. The sodium galacturonate (0.15 mole) was dissolved in 600 ml of normal sodium hydroxide and oxidized in the manner described on page 435. When oxidation was complete, the reaction product was treated with 45 g of calcium chloride. After several hours, the resulting mixture of difficultly soluble calcium salts was separated by filtration, washed with lime water, and finally suspended in water. Sufficient oxalic acid was added to convert the salts to calcium oxalate and the free acids. It was necessary to digest the mixture on the steam bath for several hours to effect complete conversion of the salts. The calcium oxalate was separated by filtra-

tion and discarded. The filtrate was divided into two equal parts, one of which was neutralized with potassium hydroxide. The two portions were then combined, and the solution was concentrated to a volume of 100 ml. Methyl alcohol was added to the point of turbidity, and the solution was allowed to stand until crystallization of potassium acid tartrate was complete (2 days). The crystals were collected on a filter, washed with 50-percent aqueous alcohol, and dried. The crude potassium acid tartrate weighed 4.22 g. After one recrystallization from water, 2.8 g of pure potassium acid *D*-tartrate was obtained. This corresponds to 0.10 mole of tartrate per mole of sodium galacturonate.

The filtrate and mother liquor remaining after separation of the potassium acid tartrate were combined, and the resulting solution was neutralized with potassium hydroxide and evaporated nearly to dryness. The crystals that formed were separated by filtration, washed with 50-percent aqueous alcohol, and dried. The material thus obtained weighed 17.3 g, corresponding to a yield of 0.45 mole of potassium trihydroxyglutarate per mole of sodium galacturonate. The crude material had a specific rotation of -8.4° . After recrystallization of the compound from water, $[\alpha]_D^{20} = -8.6^\circ$ (water, $c=4$). The specific rotation of potassium *D*-*arabo*-trihydroxyglutarate reported in the literature is -8.7° [6]. *Analysis*: Calculated for $K_2C_5H_6O_7$: C, 23.43; H, 2.36; K, 30.51. Found: C, 23.4; H, 2.7; K, 30.5.

4. PREPARATION OF POTASSIUM ACID L-TARTRATE (DEXTRO) AND POTASSIUM XyLO-TRIHYDROXYGLUTARATE FROM CALCIUM 5-KETO-GLUCONATE

Sodium carbonate (5.3 g) was added to 24 g (0.1 equivalent) of calcium 5-keto-gluconate suspended in 200 ml of water. After removal of the resulting calcium carbonate by filtration, 200 ml of 2-*N* sodium hydroxide was added and the sodium 5-keto-gluconate was oxidized with oxygen. Potassium acid *L*-tartrate and potassium *xylo*-trihydroxyglutarate were prepared from the reaction mixture by the procedure described in the previous section for the preparation of potassium acid *D*-tartrate and potassium *D*-*arabo*-trihydroxyglutarate. The potassium acid *L*-tartrate weighed 0.95 g and was substantially pure. The potassium *xylo*-trihydroxyglutarate did not crystallize and was obtained only as an amorphous solid. By treatment with lead acetate it was converted to lead *xylo*-trihydroxyglutarate. The lead salt (17 g) was decomposed with hydrogen sulfide, and the resulting acid was converted to the characteristic bisphenylhydrazide. The latter substance melted at 210° C in accordance with the value reported by Fischer [7].

III. DISCUSSION OF RESULTS

The results obtained by application of the foregoing methods to a number of salts of galacturonic acid and of 5-keto-gluconic acid are given in table 1. It will be recalled that oxidation of 5-keto-gluconic acid is reported to yield dextrorotatory tartaric acid [3], whereas galacturonic acid is reported to yield *D*-*arabo*-trihydroxyglutarate [4]. In the experiments given here, trihydroxyglutaric acid was obtained from both materials in approximately 45-percent yield, and tartaric

acid in approximately 10-percent yield. Thus, on oxidation with oxygen in alkaline solution, in the absence of a catalyst, both 5-keto-gluconic acid and galacturonic acid yield the corresponding 5-carbon dibasic acids in substantial quantities. Four-carbon dibasic acids are formed from the same substances in smaller yields.

TABLE 1.—*Oxidation of galacturonic acid and 5-keto-gluconic acid in alkaline solution*

Substance oxidized (0.05 equivalent)	Alkaline medium	Products (moles per equivalent of substance oxidized)		
		Oxalate	Tartrate	Trihydroxyglutarate
Potassium galacturonate.....	200 ml of 1-N KOH.....	0.08	0.13	0.47
Sodium galacturonate.....	200 ml of 1-N NaOH.....	.10	.10	.45
Do.....	200 ml of 2-N NaOH.....	.10	.09	.48
Sodium calcium galacturonate.....	200 ml of 1-N NaOH.....	.17	.09	.41
Calcium galacturonate.....	200 ml of water + 15 g of CaO.....	.41	.11	.34
Do.....	200 ml of water + 15 g of CaO + 10 g of calcium acetate.....	.44	.10	.20
Sodium 5-keto-gluconate.....	200 ml of 1-N NaOH.....	.29	.10	.45
Calcium 5-keto-gluconate.....	200 ml of water + 15 g of CaO.....	.31	.11	.20

In all the experiments oxalic acid was formed in addition to tartaric acid and trihydroxyglutaric acids. Power and Upson [8] have found that air oxidation of both glucose and fructose in the presence of calcium hydroxide yields calcium oxalate in relatively large quantities, whereas air oxidation in the presence of sodium hydroxide produces little or none. Our results with galacturonic acid and 5-keto-gluconic acid show that these substances also give higher yields of oxalate with calcium hydroxide than with either sodium or potassium hydroxide. The yields of tartaric acid and trihydroxyglutaric acid obtained with calcium hydroxide are less than those obtained with alkali metal hydroxides. The addition of calcium acetate to the calcium hydroxide mixture caused only a small change in the amounts of oxalate and tartrate, but there was a substantial decrease in the amount of trihydroxyglutarate formed.

IV. REFERENCES

- [1] H. L. Frush and H. S. Isbell, *J. Research NBS* **33**, 401 (1944) RP1617.
H. S. Isbell and H. L. Frush, *J. Research NBS* **33**, 389 (1944) RP1616; **32**, 77 (1944) RP1576.
- [2] J. J. Stubbs, L. B. Lockwood, E. T. Roe, and G. E. Ward, U. S. Patent No. 2,318,641, May 11 (1944).
A. J. Kluyver and A. I. J. Boezaardt, *Rec. trav. chim.* **57**, 609 (1938).
- [3] R. Pasternack, U. S. Patent No. 2,197,021, April 16, 1940.
- [4] H. S. Isbell, U. S. Patent No. 2,338,114, January 4, 1944.
- [5] J. U. Nef, *Liebigs Ann. Chem.* **403**, 204 (1914).
H. A. Spoehr, *Am. Chem. J.* **43**, 238 (1910).
E. Anderson, *Am. Chem. J.* **42**, 423 (1909).
W. L. Evans, *Chem. Revs.* **6**, 281 (1929).
J. W. E. Glattfeld, *Am. Chem. J.* **50**, 135 (1913).
J. U. Nef, O. F. Hedenburg, and J. W. E. Glattfeld, *J. Am. Chem. Soc.* **39**, 1638 (1917).
- [6] B. Tollens and F. Rorive, *Z. Ver. deut. Zucker-Ind.* **59**, 579 (1909).
- [7] E. Fischer, *Ber. deut. chem. Ges.* **24**, 1836 (1891).
- [8] M. H. Power and F. W. Upson, *J. Am. Chem. Soc.* **43**, 195 (1926)

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