SUGAR OXIDATION

Saccharic and Oxalic Acids by the Nitric Acid Oxidation of Dextrose

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As part of a program for the extended utilization of products from corn, a systematic study was made of the preparation of saccharic acid by the nitric acid oxidation of corn sugar. Saccharic acid can be prepared in a yield of 41% by oxidizing 1 mole of dextrose with 4 moles of concentrated nitric acid at 55° to 75° C. for 1 hour. After the removal of saccharic acid as its insoluble potassium acid salt, the by-product sugar acids formed in the oxidation may be converted to oxalic acid in a yield of 44% of theory. For this purpose nitric acid and vanadium catalyst are employed. Saccharic acid, if available at sufficiently low cost, would be an important addition to the list of organic acids now being used in industrial processes. Preliminary work has shown saccharic acid to be particularly effective as a sequestrant for metal ions in alkaline solutions. As saccharic acid is nontoxic, it also has potential value for application in the food industry.

SACCHARIC ACID, if available at sufficiently low cost, would be an important addition to the expanding list of organic acids now being used as raw materials in manufacturing processes. In sirup form or as the crystalline monolactone it is a potential substitute for citric, tartaric, lactic, and gluconic acids in the food acid field. Several of its salts have found use in therapeutics (*15*, *18*), but the industrial possibilities of others have not yet been investigated. Potassium acid saccharate, which is stable to moisture, might well supplement potassium bitartrate as an acidifier in baking powder. It also has potential value as a retarding agent for metallic mordants in the dyeing of textiles and as a sequestrant in cleaning compositions.
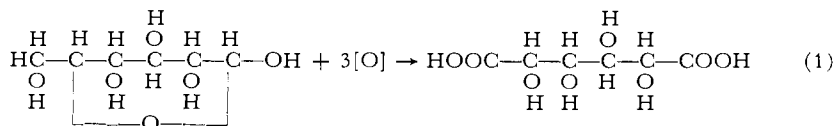
Although saccharic acid has been prepared from many carbohydrate compounds by oxidation (*2*), only starch and dextrose have the combination of favorable chemical structure, availability, and low cost to make them practical raw materials for such a conversion. Previous methods for preparing saccharic acid from these substances by oxidation with nitric acid have involved long periods of time for completion of the reaction and the use of relatively weak nitric acid (*5-7*, *9*, *10*, *13*). The yields reported have varied from several per cent to a maximum of 45%, obtained by Haworth and Jones (*9*) in 1944. Apparently, however, no systematic study was ever reported with the view of dis-

closing optimum conditions for its production.

The present investigation was undertaken to determine the effect of time, temperature, and concentration of dextrose and nitric acid on the yield of saccharic acid. Conditions were also found for the efficient oxidation of the by-product sugar acids to oxalic acid. The course of the oxidations was also studied.

Oxidation

In the conversion of dextrose to saccharic acid it is necessary to oxidize the terminal groups of the former as illustrated in Equation 1.

$$\begin{array}{c}\text{H}\\ \text{H H H O H H}\\ \text{HC—C—C—C—C—C—OH} + 3[\text{O}] \rightarrow \text{HOOC—C—C—C—C—COOH} \qquad (1)\\ \text{O} \quad | \quad \text{O H O}\\ \text{H} \quad | \quad \text{H} \quad \text{H}\\ \text{L——O——J}\end{array}$$

Reaction of the secondary alcohol groups also occurs to a great extent (*1*, *11*), however, with a corresponding lowering of the yield of the desired product.

A standard procedure was developed in which dextrose was added gradually to nitric acid in a three-necked flask fitted with a liquid-sealed stirrer, thermometer, and reflux condenser. Simultaneous agitation and external cooling allowed more rapid addition of the dextrose and ready control of the reaction. In each experiment a small amount of sodium nitrite first reacted with the nitric acid to

produce brown oxides of nitrogen which initiate the oxidation. All of the dextrose was then introduced over a period of 15 to 20 minutes.

Systematic experiments were conducted using from 2 to 8 moles of nitric acid per mole of dextrose. The acid concentration was varied from 20 to 90%, the reaction time from 15 minutes to 10 hours, and the temperature from 45° to 95° C. Saccharic acid was conveniently separated from the oxidation mixture as its crystalline potassium acid salt, which was obtained in an exceptionally pure condition. Maximum recovery of this compound was achieved when the oxidation liquor was first neu-

tralized with an excess of potassium hydroxide solution to convert the saccharolactone–saccharic acid mixture to potassium saccharate. Potassium acid saccharate was then precipitated by adjusting the slightly alkaline solution to pH 3.4 with nitric acid. Oxalic acid, a by-product of the reaction, was isolated quantitatively as its calcium salt by the addition of calcium chloride to the final mother liquor. The calcium oxalate was precipitated at pH 2.5 to prevent contamination with calcium salts of other by-product acids which separate

from solutions of higher pH. The yield of oxalic acid dihydrate was calculated from the weight of calcium oxalate obtained. The experimental data are presented in Table I.

Optimum Conditions

The optimum temperature for the reaction, with 70% nitric acid, was 55° to 75° C. as illustrated in Figure 1. The optimum concentration of nitric acid was 60 to 70% when a 4 to 1 mole ratio of acid to dextrose was employed (Figure 2). The use of acid concentrations below 50% at temperatures of oxidation of 55° to 95° for 1 to 10 hours produced inferior yields of potassium acid saccharate. Figure 3 shows the effect of the mole ratios of the reactants at 55° to 60° C.; the yield of potassium acid saccharate reached 41% when the mole ratio of 70% nitric acid to dextrose was 4 to 1. Increasing the ratio did not affect the yield of product appreciably. Periods of oxidation longer than 1 hour were not markedly effective (Figure 4). Anhydrous dextrose and dextrose hydrate gave approximately the same results under analogous conditions of oxidation.

Several experiments were conducted in which the temperature of the reaction was maintained at 55° to 65° C. for 20 minutes and then allowed to rise spontaneously to 90° C. for another 30 minutes (Table I). In general, this procedure did not improve the yield of the desired product.

On the basis of these results the following procedure for the preparation of saccharic acid was adopted. This method has repeatedly given yields of 40 to 43% of the theoretical (14).
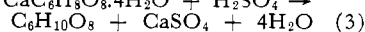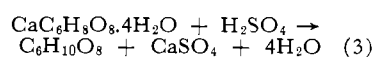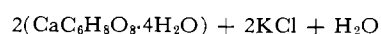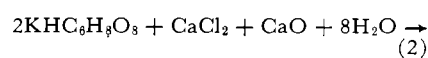
Preparation of Potassium Acid Saccharate In a 500-ml. three-necked flask (ground-glass joints) fitted with a mechanical liquid-sealed stirrer, thermometer, and reflux condenser is placed 128 ml. (2 moles) of 70% nitric acid. The acid is heated to 60° C. with constant stirring, 0.1 gram of sodium nitrite is added, 99 grams (0.5 mole) of dextrose hydrate is then introduced over a 20-minute period, and the temperature is maintained at 55° to 60° C. by means of external cooling with cold water. The reaction is allowed to continue at this temperature for 1 hour. The oxidation mixture is then cooled to room temperature and 45% potassium hydroxide solution is gradually added with stirring to obtain a pH of at least 9 and a temperature of 60° to 80° C. After cooling to 20° C. the solution is adjusted to pH 3.4 with 70% nitric acid. Crystallization of potassium acid saccharate begins immediately. The mixture is allowed to stand overnight at room temperature and the precipitate is collected by filtration. After being washed free of mother liquor with a minimum of cold water, the product is dried at 50° C. The potas-

sium acid saccharate weighs 51 grams and is 99.2% pure. Yield is 41% of theory.

Analysis Calculated for $C_6H_9O_8K$. Neutralization equivalent, 248; K, 15.7. Found. Neutralization equivalent, 250; K, 15.6.

Saccharic Acid and Saccharolactone

In the commercial production of tartaric acid from potassium bitartrate the first step is the conversion of the latter compound to insoluble calcium tartrate by the addition of calcium chloride and lime (8). This simple and inexpensive procedure was applied directly to potassium acid saccharate for the preparation of calcium saccharate from which saccharic acid and saccharolactone were subsequently made. The following equations illustrate these reactions:

$$2KHC_6H_8O_8 + CaCl_2 + CaO + 8H_2O \rightarrow \quad (2)$$
$$2(CaC_6H_8O_8 \cdot 4H_2O) + 2KCl + H_2O$$

$$CaC_6H_8O_8 \cdot 4H_2O + H_2SO_4 \rightarrow$$
$$C_6H_{10}O_8 + CaSO_4 + 4H_2O \quad (3)$$

Two hundred and forty-eight grams (1 mole) of potassium acid saccharate was suspended in 500 ml. of water at 35° C. with continuous stirring and 56

grams (0.5 mole) of calcium chloride in solution in 100 ml. of water was added. A slurry of 39 grams (0.5 mole) of USP calcium hydroxide in 20 ml. of water was next introduced and the temperature of the reaction was maintained at about 40° C. for 1 hour. The calcium saccharate precipitate was removed by filtration, washed with water, and air-dried overnight. A yield of 312 grams (97% of theory) was obtained. Analysis showed the presence of 12.6% calcium. The calculated amount of calcium in calcium saccharate tetrahydrate is 12.5%.

A slurry of 640.5 grams (2 moles) of calcium saccharate tetrahydrate in 1200 ml. of water was gradually added to a stirred solution of 204.6 grams (2 moles) of concentrated sulfuric acid in 1250 ml. of water. After 1 hour the pH of the mixture was 1.4. The precipitated calcium sulfate was removed by filtration and washed with hot water. The combined filtrate and washings were concentrated in vacuo to about 1500 ml. and deposited calcium sulfate was filtered off. The filtrate was analyzed for calcium and sulfate ions. Calculated amounts of oxalic acid and barium hydroxide were then added to remove the soluble calcium sulfate as insoluble calcium oxalate and barium sulfate. The filtrate from

Table I. Effect of Reaction Variables on Yield of Potassium Acid Saccharate and Oxalic Acid

Nitric Acid/Dextrose Mole Ratio	Nitric Acid Concn., Weight %	Oxidation Time, hours	Oxidation Temp., °C.	Weight % (Based on Dextrose Used) Reducing power[a]	Weight % (Based on Dextrose Used) Yield, oxalic acid dihydrate	Potassium Acid Saccharate Yield, % of Theory
4	50	1.0	60–65	. . .	2.8	35
8[b]	50	0.7	70–75	27.2	. . .	44
4[b]	60	'0.3]	55–65	. . .	. . .	. . .
		[0.5]	65–90	25.4	3.9	43
4	62 (tech.)	1.0	60–65	24.5	3.0	43
3	70	1.0	55–60	. . .	1.8	28
3.5[b]	70	1.0	55–60	. . .	2.1	32
4	70	1.0	85–90	31.9	. . .	20
4[b]	70	1.0	75–80	25.7	4.9	38
4[b]	70	1.0	70–75	26.6	4.1	40
4[b]	70	5.0	70–75	15.3	10.1	41
4[b]	70	1.0	65–70	28.2	4.4	44
4[b]	70	1.0	60–65	25.7	3.3	43
4[c]	70	1.0	60–65	25.9	2.9	43
4[d]	70	1.0	60–65	26.5	3.3	44
4	70	0.3	55–60	. . .	1.6	25
4	70	1.0	55–60	26.7	1.9	41
4	70	2.5	55–60	. . .	. . .	43
4	70	5.0	55–60	24.0	6.6	44
5	70	1.0	55–60	27.2	3.3	43
6	70	1.0	55–60	29.0	3.5	46
7	70	1.0	55–60	29.0	4.6	43
8	70	1.0	55–60	28.1	5.1	44
4[e]	70	1.0	55–60	1.3	. . .	91
4	70	1.0	45–50	27.4	3.2	37
4	70	5.0	45–50	24.7	6.5	44
4	80	1.0	55–65	34.0	3.5	39
4	90	1.0	55–65	31.7	3.3	35

The experiments were run with 0.25 to 0.75 mole of dextrose. Commercial dextrose hydrate was used unless otherwise noted. Its purity was determined by optical rotation and was at least 99.5%.
[a] Reducing substances in oxidation mixture calculated as dextrose.
[b] Commercial anhydrous dextrose (99.9% pure).
[c] c.p. anhydrous dextrose.
[d] Glucono-Δ-lactone.
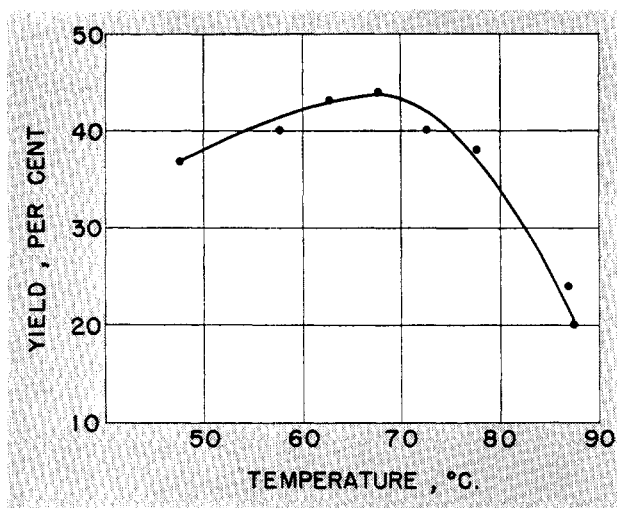[e] 3,6-Saccharolactone.

Figure 1. Effect of temperature on yield of potassium acid saccharate

Reactants. 4 moles of 70% nitric acid per mole of dextrose
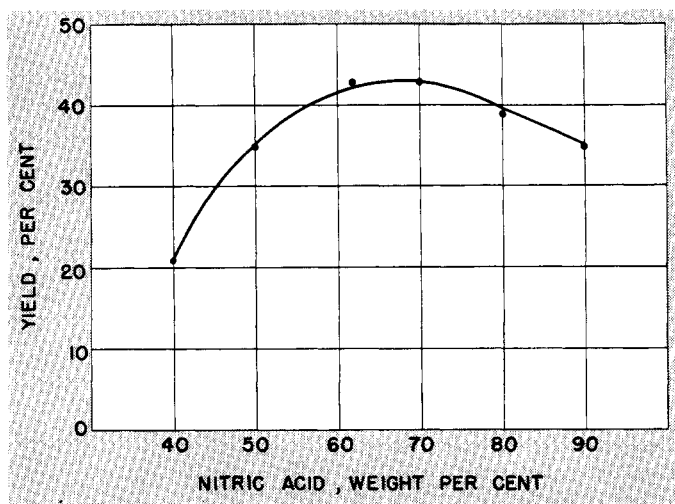Reaction time. 1 hour

Figure 2. Effect of concentration of nitric acid on yield of potassium acid saccharate

Reactants. 4 moles of nitric acid per mole of dextrose
Reaction temperature. 55°–60°
Time. 1 hour

these preciptates was a solution of practically pure saccharic acid of about 20% concentration.

This solution was further vacuum evaporated to a thick light amber colored sirup which contained about 85% saccharic acid. Seeding the sirup caused a thick mass of saccharolactone to crystallize. After several hours the lactone was triturated with ethyl alcohol and isolated by filtration. It was washed with absolute ethyl alcohol and air-dried when it weighed 310 grams (81% of theory) and melted at 130°–132°. Analysis by titration with standard alkali showed 94% of the product to be saccharolactone.

No doubt it is a mixture of 3,6-saccharolactone (melting point 149°) and 1,4-saccharolactone (melting point 98°) (17) in which the former substance predominates.

By-Products of the Oxidation

Oxalic Acid The potassium acid saccharate mother liquor was adjusted to pH 2.5 with hydrochloric acid and treated with 5 grams of calcium chloride at 60° to 70° C. After digesting for several hours, the calcium oxalate was filtered off, washed with water, and dried in vacuo at 140° C. A yield of 2.6 grams was obtained, which calculates as 2.9% of oxalic acid dihydrate when based on the weight of dextrose used. Analysis of the product showed the presence of 30.0% calcium. Theory for calcium oxalate is 31.3%.

L-Tartaric Acid Three hundred and sixty grams (2 moles) of anhydrous dextrose was oxidized with 511 ml. (8 moles) of 70% nitric acid under the conditions previously described. The potassium acid saccharate was precipitated and removed by filtration. The filtrate was treated with 40 grams of anhydrous calcium chloride and then neutralized to pH 9 with calcium hydroxide. The brown calcium salts that precipitated were filtered off, washed with water, and dried at 60° C. The

mixture weighed 125 grams. The solution of organic acids formed from the calcium salts by acidification with the equivalent amount of sulfuric acid was filtered from calcium sulfate and concentrated in vacuo to a thick sirup. This was leached four times with 175-ml. portions of hot anhydrous ethyl acetate and the amorphous residue was further extracted in a Soxhlet apparatus for 5 hours. The combined ethyl acetate solutions were concentrated in vacuo to a thick sirup which was extracted with boiling ether. When the ether solution was evaporated, a residue of 15 grams of dark sirup was obtained.

To 2.0 grams of the dark sirup dissolved in 60 ml. of water was added 12.5 grams of brucine. The mixture was heated on the steam bath for 4 hours when solution occurred. The solution was concentrated to dryness and the residue dissolved in 100 ml. of hot 95% ethyl alcohol. After cooling for several minutes, the supernatant liquor was decanted from deposited oil. The clear liquor was treated with activated carbon and concentrated in vacuo to a yellow glassy solid. Repeated crystallization of this material from ethyl alcohol gave 0.1 gram of a product which melted at 245°–246°. It was identified as brucine-L-bitartrate by its melting point (12), a mixed melting point with an authentic sample and elementary analysis.

5-Ketogluconic Acid Ninety grams (0.5 mole) of anhydrous dextrose was oxidized with 128 ml. (2 moles) of 70% nitric acid for 1 hour at 60° C. (1). The solution was cooled and after the addition of 150 grams of sodium acetate trihydrate and 30 grams of anhydrous calcium chloride dissolved in a minimum of water, the reaction mixture stood at room temperature for 40 hours. The crude calcium-5-ketogluconate that deposited was collected and treated with hydrochloric acid, and the resulting solution was decolorized with carbon. By adjusting

the solution to pH 1.5 with ammonium hydroxide a small amount of calcium oxalate precipitated, which was removed by filtration. The colorless filtrate was neutralized to pH 4.7 with ammonium hydroxide and calcium-5-ketogluconate crystallized rapidly. After several hours the product was removed by filtration and recrystallized as before. Five grams of white product was obtained. (Yield was 5% of theory.) An analysis showed the presence of 8.5% calcium. The theoretical amount of calcium in calcium-5-ketogluconate trihydrate is 8.3%.

A solution formed by treating 1.0357 grams of the product with 1 ml. of 6 N hydrochloric acid and diluting to 25 ml. with water had a specific rotation of −14.1° when calculated as the free acid. The value reported by Barch (1) is −14.43°.

Oxalic Acid from Potassium Acid Saccharate Mother Liquor

The potassium acid saccharate mother liquor from the nitric acid oxidation of dextrose contains by-products which include 5-ketogluconic, tartaric, and oxalic acids, approximately equivalent to 59% of the dextrose used. It is impractical to separate these acids individually. Most of them, however, can be oxidized with nitric acid to produce commercially valuable oxalic acid in relatively high yield. Conditions favorable to such a conversion comprise the use of vanadium catalyst and careful temperature control (3, 4, 16, 19).

The oxidation of the potassium acid saccharate mother liquor was most effectively carried out using a mole ratio of 60% nitric acid to by-product acids (calculated as dextrose) of 5 to 1, 0.005% vanadium pentoxide, and a temperature of 40° C. for 30 hours.

In a typical run, the mother liquor of the potassium acid saccharate precipitate from the oxidation of 2 moles of dextrose with 8 moles of 60% nitric acid was treated with concentrated nitric acid to

obtain a pH of 1. The by-product organic acids were thus liberated from their potassium salts with the formation of potassium nitrate. The solution was concentrated in vacuo to approximately one half its volume to crystallization of potassium nitrate. After cooling, the latter was separated by filtration, washed with a minimum of cold water, and dried at 50° C. when it weighed 203 grams (60% recovery). The combined filtrate and washings were then concentrated in vacuo to a brown sirup which was calculated to contain by-product sugar acids equivalent to 1.24 moles of dextrose. To this sirup was added 478 ml. of 60% nitric acid (6.20 moles) to obtain a total volume of oxidation mixture of 690 ml. Vanadium pentoxide amounting to 0.035 gram was then introduced to the oxidation mixture and the whole was stirred and kept at 40° C. for 30 hours. During the oxidation oxalic acid dihydrate crystallized from solution. After cooling to 10° C. for 2 hours and to −10° C. for 1 hour the oxalic acid was isolated by filtration and washed with cold water. The white product was air-dried overnight and weighed 209.5 grams. It had a purity of 91% as oxalic acid dihydrate. The filtrate contained 14.8 grams of oxalic acid dihydrate which could be returned to the process. The total amount of oxalic acid produced was thus 44% of theory based on 1.24 moles of dextrose or 25% based on original dextrose.

Course of the Oxidation

The nature of the principal consecutive reactions that occurred in the initial oxidation was established by comparing the yields of potassium acid saccharate and by-product oxalic acid and the reducing values of the mixtures obtained by the oxidation of dextrose and glucono-Δ-lactone (Table I). This information was supplemented by the isolation of the by-products L-tartaric and 5-ketogluconic
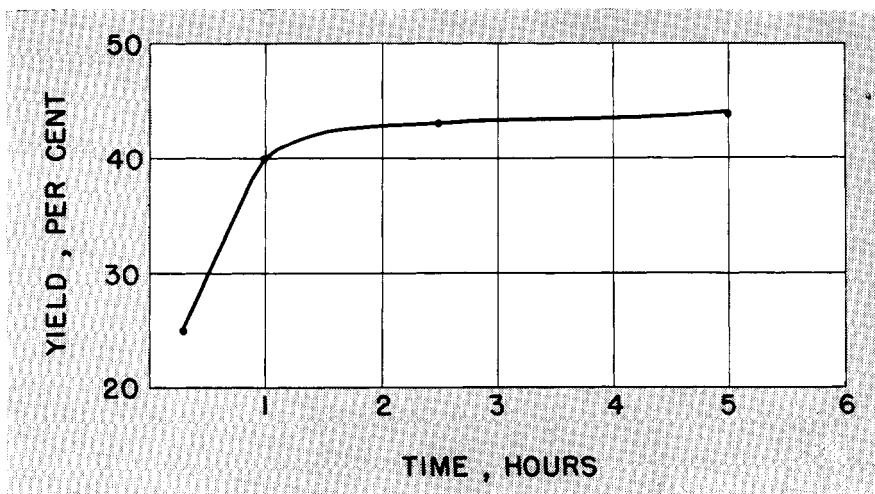

Figure 4. Effect of reaction time on yield of potassium acid saccharate

Reactants. 4 moles of 70% nitric acid per mole of dextrose
Reaction temperature. 55°–60°

acids as their brucine and calcium salts, respectively.

The probable extent of saccharic acid recovery is indicated by the precipitation of 88% of potassium acid saccharate from a synthetic mixture containing known amounts of potassium nitrate, and 5-ketogluconic, L-tartaric, oxalic, and saccharic acids. Potassium acid saccharate was also isolated in 91% yield from an unsuccessful attempt to oxidize saccharolactone under the conditions found to be optimum for conversion of dextrose to saccharic acid (Table I, [e]). While yields of 43% of potassium acid saccharate were actually isolated, this probably represents but 90% of what was present in the oxidation mixture. Oxalic acid was precipitated quantitatively as its calcium salt from the above synthetic mixture.

Total reducing power was estimated on a suitable aliquot of the oxidation mixture by the Munson-Walker method, the cuprous oxide formed being determined by permanganate titration (20). Since total reducing power would include both unreacted dextrose and its oxidation products, an independent analysis for true dextrose was conducted. The cal-

cium salts of the organic acids were first precipitated with 80% ethyl alcohol and the dextrose was determined in the filtrate, after removal of ethyl alcohol by vacuum evaporation. No dextrose was found in the reaction mixtures produced under the best conditions for saccharic acid production. Thus, the reducing power observed is a measure only of the keturonic acids and other reducing substances formed. Reducing power was conveniently calculated as dextrose. For comparative purposes, reducing power calculated as dextrose and the by-product oxalic acid were reported in Table I as weight per cent of the dextrose used.

Theoretically, 3 moles of nitric acid are required for the oxidation of dextrose to saccharic acid. However, under the optimum conditions for producing saccharic acid, a portion of the nitric acid also oxidized secondary alcohol groups to keto forms. Oxidative fission and nitration reactions (7) accounted for more of the oxidant, so that at least 4 moles of nitric acid were required to obtain maximum yields of saccharic acid.

The experimental data indicate that the oxidation followed the course

Dextrose → glucono-Δ-lactone

saccharic acid   keturonic acids   tartaric a
                                     oxalic ac
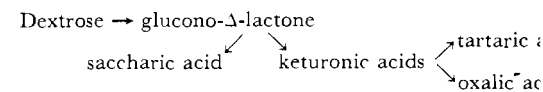
Evidently the first reaction was the rapid conversion of dextrose to glucono-Δ-lactone, as the latter was oxidized to saccharic acid (Table I, [d]) in a yield almost identical to that obtained with dextrose under similar conditions (Table I, [c]). Likewise, a comparison of the reducing values of their reaction mixtures shows nearly analogous rates of oxidation of the secondary alcohol groups. Oxalic acid formation in both cases was approximately the same. The second reaction consisted of the simultaneous oxidation of glucono-Δ-lactone to saccharic and keturonic acids. Practically all of the saccharic acid was formed from dextrose in 1 hour with the production of only a slight amount of

Figure 3. Effect of mole ratio of 70% nitric acid to dextrose on yield of potassium acid saccharate

Reaction temperature. 55°–60°
Time. 1 hour
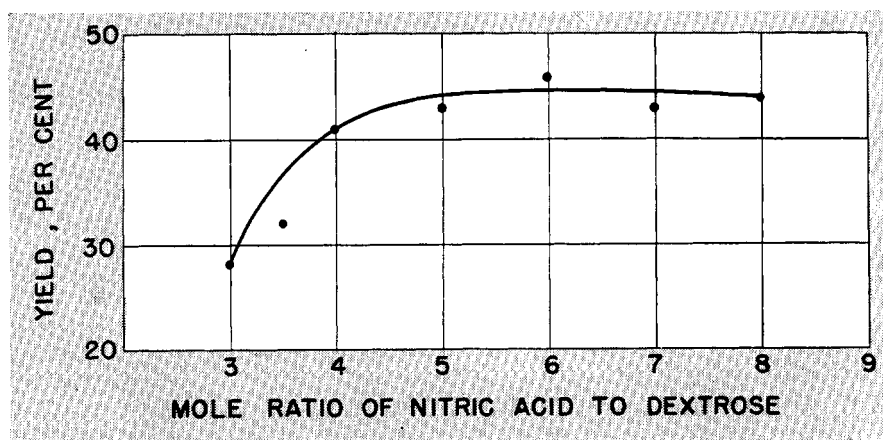
carbon dioxide. The fact that very little carbon dioxide was evolved indicated that essentially all of the dextrose that had not been oxidized to saccharic acid had been converted to keturonic acids. Oxidation with 70% nitric acid at 55° to 60° C. for 5 hours did not affect the yield of potassium acid saccharate appreciably. The stability of saccharic acid toward further oxidation is shown in Table I, [e]. Of the keturonic acids only 5-ketogluconic acid could be isolated and identified. The presence of oxalic and L-tartaric acids, however, shows that early oxidative degradation of the keturonic acids had occurred. Longer periods of reaction at higher temperatures (Table I) increased this degradation, as is evidenced by the increase in oxalic acid formation and the decrease in reducing value of the oxidation mixture. A considerable quantity of carbon dioxide also was produced during the latter oxidations.

The course of the nitric acid oxidation of the by-product sugar acids in the potassium acid saccharate mother liquor was changed by the use of vanadium catalyst. Apparently the oxidation was directed toward the keto groups in the sugar acids (7) with cleavage at such points to produce acids of lower molecular weight together with carbon dioxide and eventually oxalic acid. Oxidative degradation of oxalic acid also occurred to some extent.

Acknowledgment

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Literature Cited

(1) Barch, W. E., J. Am. Chem. Soc., 55, 3653 (1953).
(2) "Beilstein's Handbuch der Organischen Chemie," 4th ed., Vol. 3, p. 577, Berlin, Julius Springer, 1921.
(3) Brooks, M. J. (to General Chemical Co.), U. S. Patent 2,322,915 (June 29, 1934).
(4) Dominik, W., and Janczak, St., Roczniki Chem., 14, 141 (1934).
(5) Fischer, E., "Anleitung zur Darstellung Organischer Präparate," p. 80, 1905.
(6) Gans, R., and Tollens, B., Ann., 249, 215 (1888).
(7) Hachihama, Y., and Fujita, H., J. Soc. Chem. Ind., Japan, 38, Suppl. Binding 744 (1935).
(8) Halperin, Z., Chem. and Met. Eng., 52, 116 (September 1945).
(9) Haworth, W. N., and Jones, W. G. M., J. Chem. Soc., 1944, 65.
(10) Kiliani, H., Ann., 205, 145 (1880); Ber., 56, 2016 (1923); 58, 2344 (1925).
(11) Ibid., 55, 2817 (1922).
(12) Ladenburg, K., Ann., 364, 227 (1908).
(13) Liebig, J., Ibid., 113, 1 (1860).
(14) Mehltretter, C. L. (to U. S. of America represented by Secretary of Agriculture), U. S. Patent 2,436,659 (Feb. 24, 1948).
(15) Pasternack, R., and Giles, W. R. (to Chas. Pfizer & Co.), Ibid., 1,965,535 (July 3, 1934).
(16) Simpson, G. S. (to General Chemical Co.), Ibid., 2,057,119 (Oct. 13, 1936).
(17) Smith, F., J. Chem. Soc., 1944, 633.
(18) Sondern, C. W., and Doak, G. O. (to Geo. A. Breon and Co.), U. S. Patent 2,232,411 (Feb. 18, 1941).
(19) Tang, T. H., and Kuo, F. C., J. Chem. Eng., China, 6, 32 (1939).
(20) U. S. Dept. Commerce, Natl. Bur. Standards, Circ. C-440, 170, 182 (1942).

FOOD ANALYSIS

Detection of Ethylvanillin in Vanilla Extract

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A SIMPLE METHOD of detecting the addition of ethylvanillin to pure or imitation vanilla extract has long been desired. Several more or less adequate methods have been devised, but each has its drawbacks.

The most widely used of these procedures is probably the Chenoweth peroxide test (2). Utilizing readily available reagents, this color test takes about 45 minutes [after the usual extraction procedure of the Association of Official Agricultural Chemists (7)]. The time and temperatures used are critical, and even slight deviations from the procedure may inhibit proper color development. The need for a more reliable method has been felt by analysts. The Gailey chromatographic method (3) is excellent, but requires 7 hours or more to perform. Another test frequently referred to is the Nechamkin copper–iron thiocyanate test (4), which employs three special reagents to obtain a color reaction.

The present test requires only one reagent, alkaline 1% p-aminophenol solution.

Procedure

The unknown is extracted with ether according to the usual AOAC method (7). The vanillin-ethylvanillin fraction is dried, preferably over sulfuric acid, and a 1% alcoholic solution is made. To 2 ml. of this unknown, 0.5 ml. of 1N sodium hydroxide and 0.25 ml. of fresh colorless 1% p-aminophenol alcoholic solution are added and the solution is shaken.

If the unknown is ethylvanillin, a dark purplish color will develop. Vanillin gives only a pale yellow or olive tint. A 1 plus 1 mixture of vanillin and ethylvanillin will give an intermediate shade. Mixtures containing considerably less than 50% ethylvanillin do not give enough distinction to be of much practical value.

It is important that the p-aminophenol reagent be freshly prepared, as it is rapidly oxidized to dark-colored products, especially in alkaline media. The reagent should be prepared in a full container, and air excluded as much as possible.

Interfering substances are removed by the usual AOAC extraction (7). Coumarin and heliotropin are among the substances normally present in imitation vanilla, which give this color reaction with p-aminophenol. Both these compounds are eliminated during the ether extraction and hence give no difficulty.

A known control should be run for color comparison purposes, especially where a mixture of vanillin and ethylvanillin is suspected.

Literature Cited

(1) Assoc. Offic. Agr. Chemists, "Methods of Analysis," 7th ed., p. 305, 1950.
(2) Chenoweth, H. W., Ind. Eng. Chem., Anal. Ed., 12, 98 (1940).
(3) Gailey, W. R., Chemist-Analyst, 39, 59 (1950).
(4) Nechamkin, H., Ind. Eng. Chem., Anal. Ed., 15, 268 (1943).