sulfite waste liquor, other calcium lignosulfonates, and isolated lignins including studies of reactant ratios, temperatures and pressures of reaction, type of silver oxide, etc., will be the subjects of future papers.

Summary

Basic calcium lignosulfonate has been treated with mercuric oxide in the presence of excess alkali at atmospheric pressure to give increased yields of ether-soluble materials, which have been separated to bisulfite-, bicarbonate- and alkali-soluble and neutral fractions. Vanillic acid has been obtained in high yield. Other compounds isolated were vanillin, guaiacol, acetovanillone and 5-hydroxymercurivanillin. A new procedure for the isolation of pure vanillic acid from bicarbonate-soluble fractions obtained upon oxidation of lignin is described.

Oxidation of BCLS with silver oxide and excess alkali gives high yields of ether-soluble materials. The chief product is vanillic acid. Other products isolated and identified were vanillin, acetovanillone, guaiacol, eicosanol and 4-nitroguaiacol.

APPLETON, WISCONSIN RECEIVED OCTOBER 16, 1948

[CONTRIBUTION FROM THE INSTITUTE OF PAPER CHEMISTRY, APPLETON, WISCONSIN]

Oxidation of D-Galactose and Cellulose with Nitric Acid, Nitrous Acid and Nitrogen Oxides

BY W. W. PIGMAN, B. L. BROWNING, W. H. MCPHERSON, C. R. CALKINS AND R. L. LEAF, JR.[1]

The marked specific oxidation by nitrogen dioxide (NO_2) of primary alcoholic groups as compared with secondary alcoholic groups in carbohydrates has stimulated considerable interest in this type of reaction.[2] Certain experimental work in this laboratory has indicated that the course of the oxidation by nitrogen dioxide is markedly influenced by the amount of water present in the system. A consideration of the method through which water might be effective led to the view that nitric acid with added nitrites or nitric oxide would be an effective oxidant. The present paper presents the results obtained in the oxidation of D-galactose. In addition, the probable course of the oxidation of galactose to mucic acid is described. The oxidation methods used were also applied to cellulose.

Results and Discussion

Oxidations with Nitrogen Dioxide.—When air-dry cotton linters were oxidized by gaseous nitrogen dioxide in an apparatus similar to that of Yackel and Kenyon,[2] it was found that the rate of oxidation was retarded by the presence of a desiccant (phosphorus pentoxide) in the system. The degree of oxidation was judged by application of the carbon dioxide-evolution method to the oxidized product. When no desiccant was present the carbon dioxide yield from the product obtained by ninety-six hours of oxidation was 25.5%, as compared with a theoretical value of 25.0%. When the reaction system was subjected to the action of the desiccant, the oxidized product yielded only 13% of carbon dioxide after ninety-six hours of oxidation and very slightly more after 120 hours of oxidation.

When solid D-galactose was subjected to oxidation by gaseous nitrogen dioxide, a similar inhibiting effect caused by desiccation was found. In this case, the extent of oxidation was judged by the amount of insoluble mucic acid formed. For periods of oxidation from twelve to ninety-six hours, the yields were from 60 to 80% of theoretical in the absence of added water, and from 80 to 86% when small amounts of water were added. The results were erratic because of the very heterogeneous nature of the reaction.

It is difficult to control the amount of water present during reactions of the kind described, because water is one of the products of the reaction and because the oxidation systems are heterogeneous. However, the results obtained indicate that oxidations by nitrogen dioxide are markedly influenced by the presence of moisture, which may be present in the original materials or formed as a product of the oxidation. Riebsomer[3] has indicated the importance of the maintenance of anhydrous conditions if nitrogen dioxide is to be considered the actual reactant.

Although the equilibria in the system nitrogen oxides–water undoubtedly are complex,[4] a possible explanation of the results obtained with nitrogen dioxide is that a mixture of nitrous and nitric acids are formed according to the known reaction

$$2NO_2 + H_2O \rightleftarrows HNO_3 + HNO_2$$

Since such a mixture would be provided by the addition of nitrites to concentrated nitric acid,

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[1] Presented before the Division of Sugar Chemistry and Technology, American Chemical Society, New York, 1947. Taken in part from theses presented by W. H. McPherson and C. R. Calkins in partial fulfillment of the requirements of The Institute of Paper Chemistry for the degree of Master of Science from Lawrence College, Appleton, Wisconsin.

[2] E. C. Yackel and W. O. Kenyon, THIS JOURNAL, **64**, 121 (1942); C. C. Unruh and W. O. Kenyon, *ibid.*, **64**, 127 (1942); K. Maurer and G. Drefahl, *Ber.*, **75**, 1489 (1942).

[3] J. L. Riebsomer, *Chem. Rev.*, **36**, 157 (1945).

[4] See D. M. Yost and H. Russel, Jr., "Systematic Inorganic Chemistry of Fifth- and Sixth-group Non-metallic Elements," Prentice-Hall, New York, N. Y., 1944. The kinetics and equilibria involved have been studied particularly by E. Abel and associates, *Z. physik. Chem.*, **A132**, 55 (1928); **A148**, 337 (1930).

a study of this oxidant system[5] was also made on galactose and cellulose with the results described in the following sections.

Yield of Mucic Acid from Galactose after Oxidation by Nitric Acid and Nitrites.—According to the Tollens–Kent[6] procedure for the determination of D-galactose by oxidation to mucic acid, the process is carried out by partial evaporation at 100° of a nitric acid solution, diluted to about 25% nitric acid (sp. gr. 1.15), containing the galactose. The yield under this condition is far from quantitative and usually falls in the range of 70 to 80%.[6,7]

Since somewhat higher yields were obtained by nitrogen dioxide oxidations at room temperatures, oxidations with nitric acid in the presence of added sodium nitrite were carried out. For these experiments, galactose and sodium nitrite were mixed and treated for twenty-four hours at 0–3° with concentrated nitric acid (C. P., 70%). It was found that, under suitable conditions of time and temperature, reproducible yields of 88 to 90% could be obtained.

The effect of several other substances in place of sodium nitrite was tested under the conditions indicated above. The yields of mucic acid shown were obtained when the nitric acid solution was: (1) saturated initially at 0° with nitric oxide (86.5–86.6%); (2) treated for thirty minutes at 0–3° with gaseous nitrogen dioxide (87.2–88.0%); (3) treated with 1 g. of copper turnings (87%); (4) treated with 1 g. of silver or barium nitrite (88 and 89%). When nitric acid was used alone, the yields were extremely variable (in the range 75 to 85%).

This type of procedure possibly may provide the basis for an improved method for the determination of galactose and galacturonic acid. The possibility of the application as an analytical procedure has not yet been further explored.

About 10% of the galactose is not recovered as mucic acid. Only a small part of this loss results from the slight solubility of mucic acid in the oxidation solution. Tests of the residual liquid showed the absence of oxalic acid, the most likely product of chain cleavage. The solution had a reducing power corresponding to 5 to 6% galactose. This reducing material might be unchanged galactose but, in view of the results obtained from the analysis of partially oxidized solutions (see later), it is more likely to be L-galacturonic acid. Undoubtedly, some of the residual material is composed of lactones of mucic acid. Mucic acid is not attacked by the oxidation mixture and could be recovered in yields of 97 to 99% after application of the oxidation procedure described above.

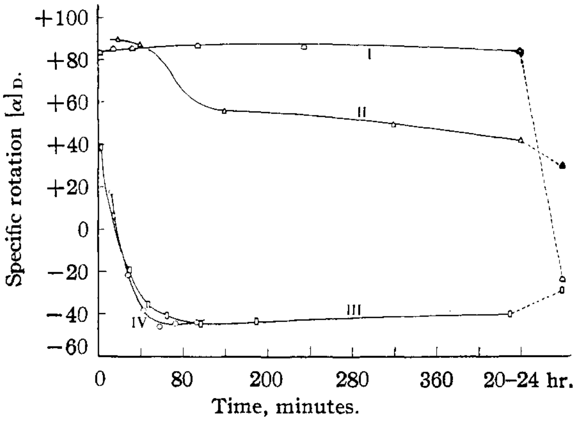

Fig. 1.—Effect of nitrites and nitrogen oxides on the rate of oxidation of D-galactose: I, HNO_3 alone (dilution for rotations, 1:3); II, HNO_2 ($NaNO_2$ + 50% H_2SO_4) (dilution for rotations, 1:3); III, HNO_3 + $NaNO_2$ (dilution for rotations, 1:3); IV, HNO_3 + NO (dilution for rotations, 1:2.5).

Qualitative Studies of the Rate of Oxidation of Galactose.—For these studies, D-galactose was oxidized by concentrated nitric acid, nitric plus nitrous acids, and nitrous acid solutions at 0 to 3°, and the rate of oxidation was followed by polarimetric measurements. In Fig. 1, the changes in specific rotation (based on the galactose originally added) during the oxidation reaction are illustrated. It will be seen that in fresh C. P. concentrated nitric acid, no appreciable change in optical rotation occurred in 200 minutes. Subsequently, oxidation did occur, probably because the reaction products of an initial very slow reaction resulted in an oxidation mixture different from pure nitric acid. When sodium nitrite was added to the concentrated nitric acid, a rapid change from a positive to a negative rotation occurred. When the nitric acid was saturated with nitric oxide prior to the addition of galactose, the oxidation reaction commenced immediately, and the rate was similar to that for the experiment in which sodium nitrite was added. In the previous section, the effect of nitrites and nitric oxide on the yield of mucic acid formed from galactose was shown to follow a similar pattern.[8] Urea, which is known to remove

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(5) A plausible explanation of the results obtained in the present work is that the active oxidant is nitrous acid, but insufficient work has been done to establish this mechanism. Nevertheless, all the solutions in which active oxidation occurred must have contained appreciable amounts of nitrous acid from the nitrites added or formed from nitric acid by reaction of nitric oxide (a by-product of the reaction) according to the established equilibrium:

$$HNO_3 + 2NO + H_2O \rightleftarrows 3HNO_2$$

The mechanism of the oxidation of cellulose by nitrogen dioxide has been previously considered by P. A. McGee, *et al.*, THIS JOURNAL, **69**, 355 (1947). The results of these workers are not necessarily in conflict with the above suggestion of the role of nitrous acid since their analytical methods would not distinguish between nitrites and nitrate esters or even possible isonitroso compounds.

(6) W. H. Kent and B. Tollens, *Ann.*, **227**, 221 (1885).

(7) A. W. van der Haar, "Anleitung zum Nachweis der Monosaccharide und Aldehydsäuren," Gebrüder Borntraeger, Berlin, 1920, p. 123.

(8) The catalytic effect of sodium nitrite on the reaction was first reported by B. L. Browning and W. H. McPherson in a master's thesis presented by the latter in 1944. Subsequently, the effect was reported in the oxidation of δ-hydroxyvaleraldehyde by J. G. M. Bremner, *et al.*, U. S. Patent 2,389,950 (1945).

nitrous acid, was found to be an effective inhibitor of the reaction.

Intermediates in the Oxidation of Galactose to Mucic Acid.—The early work by Kiliani[9] established the presence of D-galactonic acid or lactones in the products obtained by the treatment of D-galactose with nitric acid. Militzer and Angier[10] isolated salts of L-galacturonic acid. D-Galactonic acid in the form of its hydrazide was also isolated in the present work, in which the oxidant was nitric acid saturated with nitric oxide.

The formation of these products makes it probable that the oxidation proceeds generally through the series

$$\text{D-galactose} \longrightarrow \text{D-galactonic acid} \longrightarrow \text{L-galacturonic acid} \longrightarrow \text{mucic acid}$$

The course of the oxidation of D-galactose by nitric acid plus nitric oxide is shown in Fig. 2, which illustrates the changes in rotation of the solution during the period of oxidation. Initially, there is a rapid change in the specific rotation from a strongly positive value to a negative value of −40 to −50° (based on the original galactose present). The rotation remains fairly constant and then slowly approaches zero. A final rotation of zero would be expected if mucic acid is the final product, since the compound has meso symmetry.

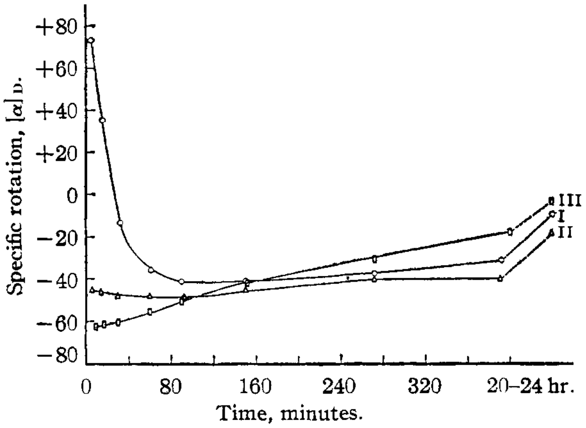
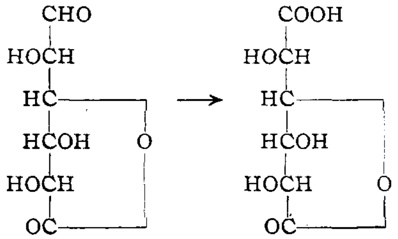


Fig. 2.—Polarimetric observations of nitric acid oxidation mixtures at 0–3°: I, D-galactose; II, cadmium D-galactonate; III, sodium calcium L-galacturonate.

As is shown also in Fig. 2, the oxidation curve for D-galactonic acid (added as cadmium galactonate in amounts molecularly equivalent to the D-galactose) follows closely the final stage of the curve for the oxidation of galactose. This result, as well as the known negative rotations of D-galactonic acid and the γ-lactone, indicates strongly that the rapid initial stage of oxidation of D-galactose is a conversion of the aldehyde or hemiacetal group to a carboxyl or lactone group, respectively; and galactonic acid or a lactone is the first product. The presence of much δ-lactone seems precluded at this point because of its probable high dextrorotation.

The oxidation curve for L-galacturonic acid is also shown in Fig. 2. (Actually, the sodium calcium salt of D-galacturonic acid was studied, and the actual signs of the rotations were reversed in the preparation of the curve.) Two interpretations of this curve are possible. It may represent the continuous oxidation of the aldehyde group with the formation of optically inactive mucic acid, or there may be a rapid initial oxidation of the γ-lactone of L-galacturonic acid (I) to a γ-monolactone of mucic acid (II), which hydrolyzes slowly to mucic acid. (According to the lactone rule of Hudson, the corresponding δ-lactone of mucic acid would be dextrorotatory, whereas the γ-lactone would be levorotatory.) In view of the apparent rapid oxidation of the aldehyde group in galactose, the latter interpretation seems the more probable.



(I) (II)

TABLE I

COMPOSITION OF PRODUCTS OBTAINED BY THE OXIDATION OF GALACTOSE WITH NITRIC ACID SATURATED WITH NITRIC OXIDE

Component	Amount present (%) I[a]	 II[b]
Free carboxylic acids[c]	27	35
Monobasic lactones[d]	64	16
Reducing materials[e]	10	14
Mucic acid lactone[f]	7	35
Total	108	100
Uronic acid[g]	9	12
Mucic acid[h]	..	14[j]
Total recovered solids[i]	60[j]	72[j]

[a] Nitric acid removed by passage through an anion-exchange column. [b] Nitric acid removed by precipitation of product with dry ether. [c] By potentiometric titration to *p*H 6.5. A correction for the presence of nitric acid was made on the assumption that the acidity determined by titration to *p*H 2 was due only to nitric acid. [d] Difference after neutralization with excess alkali and correction for mucic acid lactones and carboxylic acids. [e] Munson-Walker method, using galactose factor. [f] From the amount of mucic acid formed after hydrolysis with hydrochloric acid at 100°. [g] By carbon dioxide evolution with 12% hydrochloric acid. [h] Mucic acid which separated from the solution and was removed by filtration before removal of the nitric acid. [i] By evaporation of aliquots in vacuum desiccator over phosphorus pentoxide including any mucic acid which separated previously. [j] Calculated on the basis of galactose originally used. All other percentages are on the basis of solids recovered from the solution after removal of the nitric acid.

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(9) H. Kiliani, *Ber.*, **54**, 456 (1921); **55**, 75 (1922); **56**, 2016 (1923); **58**, 2344 (1925).

(10) M. Militzer and R. Angier, *Archives Biochem.*, **10**, 291 (1946).

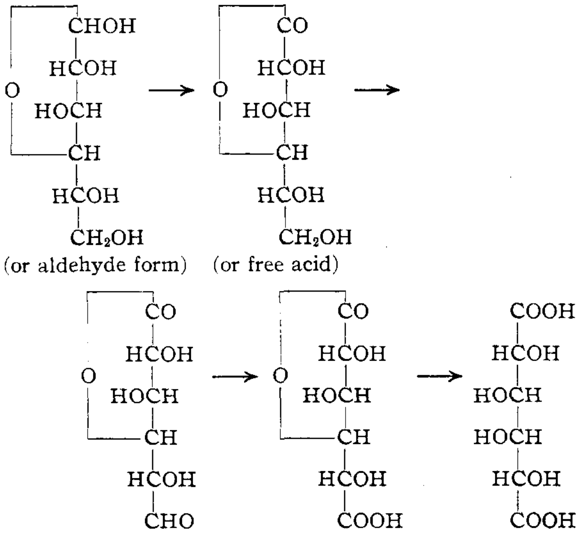
Further information was obtained by analysis of the solution at stages of the oxidation corresponding to the maximum negative rotations. For this purpose, the solutions were freed of nitric acid by precipitation of the solids with large volumes of absolute ether (II) or by dilution and passage through an anion-exchange column (I). The apparent composition of two such products is indicated in Table I.

There is a considerable loss of material during the oxidation or in the course of removal of the nitric acid; this loss amounted to 40 and 28%, respectively, for the two methods of purification employed. Satisfactory analytical methods for accurate characterization of the recovered products are not available. Although the over-all summations appear to be satisfactory, no more than qualitative significance should be attached to the individual analyses at this time.

The values for uronic acid, in particular, may be too high, because it has been shown[11] that D-gluconic acid and its γ-lactone yield considerable quantities of carbon dioxide under the conditions of the uronic acid determinations. Work in this laboratory has shown that the same is true of mucic acid.

The above data show that considerable quantities of lactones of mucic acid are present. The amount of monobasic acid was calculated on the assumption that the mucic acid lactone exists as the monolactone. This seems probable for, if the mucic acid occurs as the dilactone, the amount of carboxylic acids will be doubled and the total amount of material would be much greater than that actually found to be present.

In view of the above evidence, it seems probable that the oxidation of galactose to mucic acid proceeds principally through the stages



Catalyzed Oxidation of Cellulose by Nitric Acid.—Experiments were carried out to determine whether nitric acid with added sodium nitrite is applicable to the oxidation of cellulose to celluronic acids (polyglucuronic acids). Products of this type have been made by oxidation with nitrogen dioxide.[2,12]

For this purpose, cotton linters were suspended in nitric acid containing sodium nitrite and, after one hour at 0°, the suspension was rotated slowly at room temperature for one, two or three days. The fibrous product obtained had a uronic anhydride content of 26.4, 27.2 and 31.3% after one, two and three days of oxidation, respectively.

A cellulose activated by a swelling treatment with 85% phosphoric acid was treated similarly. The properties are shown in Table II.

TABLE II

ACTIVATED CELLULOSE OXIDIZED WITH NITRIC ACID FOR SEVENTY-TWO HOURS

	In presence of $NaNO_2$	In absence of $NaNO_2$
Recovery, % unit	50	45
Combined nitrogen (as N_2), %	0.15	1.5
Uronic anhydride, %	60.2	26.0

As shown in Table II, the oxidation proceeded to 60% with activated cellulose in the presence of sodium nitrite. The effect of the nitrite is particularly noticeable in the experiments summarized in this table. Kenyon and associates[2] reported some instances in which the oxidation approached 100%, but most of their products were oxidized to the extent observed in the present work.

The use of nitric acid would be advantageous in reducing the hazard involved in working with the extremely toxic nitrogen dioxide, but it gives lower yields under the conditions used. As in the case of the nitrogen dioxide oxidation, the products obtained by the use of nitric acid also contain small amounts of combined nitrogen.

Experimental

Oxidation with Gaseous Nitrogen Dioxide.—For these studies, the apparatus was very similar to that used by Yackel and Kenyon[2] in their cyclic method. However, the oxidation chamber was a 12 × 9-inch desiccator with a special ground glass joint in the lid which carried a connection to the boiling flask with a tube extending to the desiccator plate and another connecting with the condenser, the condensed nitrogen dioxide being returned to the boiling flask. This apparatus made it fairly easy to remove samples and to have a desiccant present. For the experiments in which desiccants were used, phosphorus pentoxide was placed in a layer at the bottom of the desiccator and was also kept in the reservoir of liquid nitrogen dioxide.

The cotton linters for this work were provided by the Hercules Powder Co., had a degree of polymerization of 1400 (viscometric results on the nitrate), and a moisture content of 5.63%.

The Yield of Mucic Acid from Galactose after Oxidation by Nitric Acid and Nitrites.—The following typical procedure represents the optimal conditions for mucic acid production. Ten grams (±0.01 g.) of galactose and 1 g. of sodium nitrite were mixed dry in a 250-ml. beaker. The beaker was placed in a water-bath maintained at 0

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[11] E. W. Taylor, W. F. Fowler, P. A. McGee and W. O. Kenyon, THIS JOURNAL, **69**, 342 (1947).

[12] K. Mauer and G. Reiff, *J. Macromol. Chem.*, **1**, 27 (1943).

to 3°, and 30 ml. of ice-cold nitric acid (C. P., 70%) was added with stirring, which was continued until all solid material had dissolved. The reaction mixture was kept at 0–3° for four hours; it was then removed from the bath and allowed to warm spontaneously to room temperature and kept at this temperature for about twenty hours, during which time crystalline mucic acid appeared. The solution was diluted with 20 ml. of a saturated solution of mucic acid and decanted through a weighed fritted-glass crucible. The beaker and mucic acid crystals were washed with portions, totaling about 130 ml., of a saturated solution of mucic acid. The product was dried to constant weight *in vacuo* at 50°. The yields of mucic acid obtained varied from 88 to 90% of the theoretical value.

Under the above conditions, a variation in the amount of nitric acid from 30 to 50 ml. and of the amount of sodium nitrite from 1 to 10 g. had no appreciable effect on the yield of mucic acid. When a smaller amount of sodium nitrite or none at all was used, the yields were erratic and fell in the range of 75 to 85%. Dilution of the oxidation mixture with water also decreased the yields markedly. When the reaction was carried out entirely at room temperature (20–25°), the yield dropped markedly to as low as 70%.

Use of Optical Rotations in Following the Course of the Oxidations.—For these experiments, a 38 × 200-mm. test-tube was mounted in an ice-bath, and a glass stirrer and a thermometer were inserted. A solution was prepared by adding about 25 ml. of cold concentrated nitric acid to a 50-ml. volumetric flask containing 5 g. of D-galactose and 2 g. of sodium nitrite; when active evolution of gases stopped, the solution was made up to 50 ml. with the concentrated acid (C. P., 70%) and poured into the test-tube. The solution was stirred and kept at 0°.[13] At intervals, 5-ml. portions of the solution were removed by a pipet, and transferred to a 25-ml. graduate and immediately diluted to 15 ml. with distilled water,[14] and the diluted solution filtered through a fritted glass crucible. The optical rotation of the filtrate was determined in a 2-dm. tube using a saccharimeter. The specific rotations given in this paper are calculated from the data on the basis of the galactose originally added.

Analysis of Oxidation Products.—One-hundred grams of D-galactose was dissolved in 200 ml. of cold concentrated nitric acid; the solution was saturated with nitric oxide and kept at 0° for six hours. Half of the solution was poured slowly into 700 ml. of dry ether, and the resulting precipitated sirup was washed three times with 200 ml. portions of dry ether. The sirup was taken up in water, filtered to remove mucic acid, and made up to a volume of 500 ml. Aliquots of this solution were used for analysis.

The remaining half of the solution was diluted to a volume of 2.5 l. with distilled water and passed through an anion-exchange column (Amberlite I-R-4) until the *p*H of the solution was about 3.0. The solution then was evaporated to a volume of 500 ml., and aliquots were used for analysis.

The methods of analysis are outlined in the footnotes to Table I.

Catalyzed Oxidation of Cellulose with Nitric Acid.—Hercules Powder Co. cotton linters (10 g.) were placed in a 2-liter bottle with 10 g. of sodium nitrite. The bottle was placed in an ice-bath and 250 ml. of cold concentrated nitric acid (C. P. 70%) added. After one hour, the bottle was removed from the ice-bath, placed in a rotator and rotated slowly at room temperature. After one, two, or three days, the suspension was diluted with one liter of water, and filtered. The insoluble oxycellulose was suspended in 2 l. of distilled water, stirred occasionally, and kept at least twelve hours; the solution was then removed by decantation, and the washing repeated in the same manner six times. The uronic anhydride content of the products was 26.4, 27.2 and 31.3% for the samples oxidized one, two and three days, respectively. By use of the nitrometer, the nitrogen content of the three-day sample was determined as 0.62%.

A reactive cellulose was prepared from cotton linters by the following procedure. Purified cotton linters (30 g.) were placed in 500 ml. of 85% phosphoric acid (at 0 to 5°), and the suspension was kept cold and stirred for fifteen minutes. The phosphoric acid suspension was then poured slowly into 2 l. of water stirred as vigorously as possible. The suspension was filtered. The cellulose was washed several times with distilled water, several times with acetone, and finally with dry ether. The product was dried over sodium hydroxide in a vacuum desiccator. Products prepared in this way in this laboratory have been shown to have a degree of polymerization of 1000 to 1200.

The activated cellulose was treated with nitric acid for three days in the same manner as for the cotton linters. Two experiments were carried out, in one of which the sodium nitrite was omitted. The properties of the products are shown in Table II. The products dissolved almost completely in 1% ammonium hydroxide and were precipitated upon acidification of the alkaline solution.

Summary

1. By adding nitrites, nitric oxide, or nitrogen dioxide to concentrated nitric acid, the oxidation of galactose proceeds readily at 0–3°, and yields of mucic acid in the range 88–90% are obtained.

2. Evidence is given that the oxidation of galactose to mucic acid by nitric–nitrous acid proceeds through the following stages: D-galactose → D-galactonic acid (or γ-lactone) → L-galacturonic acid (or γ-lactone) → γ-lactone of mucic acid → mucic acid.

3. By the oxidation of cellulose with concentrated nitric acid containing nitrites, fibrous polyuronides are obtained.

APPLETON, WISCONSIN

RECEIVED MAY 29, 1948

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[13] In most cases, an initial rise of temperature of several degrees occurred, but after a few minutes, the temperature fell to 0°. When nitric oxide was used as a catalyst, the galactose was dissolved in concentrated nitric acid and, after transfer to the test-tube, the solution was saturated with nitric oxide, which was passed through the solution during the entire reaction.

[14] At this dilution, the reaction is very slow. Apparently, the concentration of acid is sufficiently great so that the rotations are equilibrium values. No mutarotation was observed.