side (4 g.) was dissolved in 100 cc. of methyl alcohol and 100 cc. of 2 N potassium hydroxide in methyl alcohol solution was added. About 10 cc. of water was added and the solution was heated on the water-bath for forty hours. The sirupy residue was extracted several times with chloroform. The combined chloroform extracts were washed with water until free from alkali and then dried over anhydrous sodium sulfate. The chloroform was removed by vaporization under reduced pressure. A yellow sirup (2.0 g.) remained, which distilled at 80° under 3 mm. pressure. It absorbed bromine readily, n^{25}_{D} 1.4485, $[\alpha]^{25}_{\mathrm{D}}$ +14° (in methyl alcohol, about 1% solution).

Anal. Calcd. for $C_{10}H_{16}O_4$: C, 59.97; H, 8.0; OCH_3, 15.49. Found: C, 59.56; H, 8.2; OCH_3, 15.98.

The author wishes to express his appreciation to Drs. P. A. Levene and R. S. Tipson for their many helpful suggestions during the progress of this work.

Summary

A theory is presented to explain Walden inversions in sugars. The theory has been tested by a study of the hydrolysis of the *p*-toluenesulfonyl esters of rhamnose. These esters yielded inverted products on alkaline hydrolysis. It should be possible, in this manner, to cause the inversion of any hydroxyl group the *p*-toluenesulfonyl ester of which can be prepared, and thus lead to a simple method for the preparation of the rarer sugars and their derivatives, from the more commonly occurring sugars.

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF IOWA STATE COLLEGE]

Alkaline Oxidation of Lignin

BY A. W. WALDE AND R. M. HIXON

The use of ammonia as a pulping agent for the grasses and the recovery of lignin from the black liquor has been described previously.[1] Where the preparation of lignin is the main objective, it has been found more convenient to remove the hemicelluloses from the tissue by dilute acid hydrolysis before extraction of the lignin with ammonia. This modification avoids the filtration difficulties associated with the colloidal properties of the hemicelluloses.

Freshly prepared ammonia lignin, like other alkali lignins, softens and coalesces when heated in water to about 60°. On cooling, it solidifies to a brittle, friable mass. This characteristic property is gradually lost within four to six weeks after preparation. In an effort to correlate this physical change with chemical properties, a study has been made of the alkaline oxidation reactions of lignin.

While the ease of oxidation[2] of lignin has been recognized for many years, it was surprising to find that the reaction with alkaline iodine solutions could be made quantitative. Iodoform was identified as one of the oxidation products as previously reported by Harris, Sherrard and Mitchell.[3] The oxidized lignin residue contains iodine and carboxy groups. This oxidized lignin derivative is tentatively designated as "iodo-carboxy-lignin," the term being used with the indefinite connotations still necessary with the term lignin.

Experimental

Preparation of Ammonia Lignin from Oat Hulls.—Three kilograms of oat hulls were hydrolyzed for four hours at 90° with 0.6 N hydrochloric acid, rendering approximately 50% of the tissue soluble. These conditions of time, temperature and acid concentration give maximum yields of reducing sugars with minimum decomposition.[4] The washed, hydrolyzed hulls were transferred to a steel digester, covered with aqueous ammonia (one volume of sp. gr. 0.90 with an equal volume of water) and digested for eight hours under 100 pounds pressure. After cooking, most of the ammonia was recovered by blowing the hot ammonia vapors into cold water. The ammoniacal liquor was drained through cloth directly into a stone filter; the oat hull residues were washed twice with water and the wash water combined with the filtrate. A second filtration of the liquor removed any suspended cellulosic material. The lignin in the ammoniacal liquor was recovered in one of two ways: by evaporation and aeration to reduce the basicity of the solution, or by precipitation with dilute hydrochloric acid at about 50°. After allowing time to settle and decanting the supernatant liquor, the lignin was filtered on a large Büchner funnel and washed free from chlorides. It was dried at 40° until it lost its plastic character, and then at 105° for two hours; yield, 135–150 g.

Anal. (Calcd. to ash-free basis): OCH_3, 13.1; C, 63.78; H, 6.02; N, 3.04; ash, 3.7.

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[1] Peterson and Hixon, *Iowa State Coll. J. Sci.*, **7**, 25 (1932).

[2] A discussion of the studies on oxidation reactions of lignin will be found in the literature summary by Phillips, *Chem. Rev.*, **14**, 103–170 (1934).

[3] Harris, Sherrard and Mitchell, THIS JOURNAL, **56**, 889 (1934).

[4] Bryner, Thesis, Iowa State Coll., June, 1934.

The 3% of nitrogen may be associated as an impurity, since caustic alkali lignin from the same plant source contained but 1.9% N. Lignin prepared from cornstalks contains 14.8% OCH_3 and only 0.1% ash. The quality of the lignin depends, to a large extent, upon the conditions of precipitation and the technique of filtration; modification of the procedure by change in the concentration of ammonia, the concentration of acid in the preliminary hydrolysis, and the time of heating does not change the properties of the product appreciably.

Ammonia lignin is a light brown, amorphous powder, insoluble in most organic solvents, water, and 5% sodium acetate. It is very slightly soluble in sodium carbonate solution, the solubility decreasing with increase in time of drying of the lignin at 105°. It is soluble in cold 1.0 *N* sodium hydroxide. While dry lignin appears to be insoluble in water, 15% is lost during recovery from alkaline solution due to its colloidal properties.

Quantitative Alkaline Iodine Oxidation of Lignin.—Samples of lignin weighing 0.1500 g. were moistened with 10 cc. of water to facilitate solution, then dissolved by adding 25 cc. of 1.0 *N* sodium hydroxide. They were oxidized with a 0.2 *N* iodine solution and back titrated with sodium thiosulfate according to the standard acetone determination of Goodwin.[5]

With ammonia lignin, the oxidation was complete in thirty-five to forty minutes, and the titration value underwent no further change in ninety hours. Oxidation at 75° gave inconsistent results. Acid solutions of iodine, in contrast with those of chlorine and bromine, do not oxidize lignin at room temperature.

The titration value for several different preparations of ammonia lignin varied from 150 to 190 cc. of 0.1 *N* iodine per gram sample. The titration value decreased from 188 cc. to 154 cc. when one ammonia lignin preparation was treated with Fehling's solution. The values for two different preparations of caustic alkali lignin from oat hulls[6] were 110 and 123. Ammonia lignin from cornstalks gave oxidation values of about 150. Sulfuric acid lignins[7] from oat hulls, spruce and aspen gave oxidation values of 159.6, 68.4 and 112.0 cc. of 0.1 *N* iodine per gram, respectively.

Effect of Acid Hydrolysis on the Alkaline Oxidation Equivalent of Lignin in Various Tissues.—Iodoform could be detected, by its characteristic odor, as a product of oxidation of the original tissue (not acid hydrolyzed) of rye straw, artichoke stalks and cornstalks. A study was made of the change in the alkaline iodine equivalent for five different tissues when they were subjected to acid hydrolysis. The oxidation values of the original tissue may be as high as, or lower than, the value for hydrolyzed tissue. Acid hydrolysis apparently removes a certain amount of lignin from the tissue. The decrease in methoxy content of oat hulls upon acid hydrolysis is the same as the decrease in lignin content, indicating that no pectic substances were present. The decrease in methoxy content of the other tissues was considerably greater than the decrease in lignin content.[8]

Isolation of Iodoform as an Oxidation Product of Lignin.—To establish the formation of iodoform from the alkaline iodine oxidation of lignin, five grams of ammonia lignin was treated with a concentrated solution (containing 25 g.) of sodium hydroxide, the solution cooled to room temperature, and slightly more than the theoretical amount of iodine (8.5 g.) added with constant shaking. The solution stood for one hour with occasional shaking, and was then extracted twice with alcohol-free ether. After evaporation of the ether, the residue was dissolved in alcohol or a mixture of alcohol and petroleum ether and a solid fractionally crystallized. The substance so obtained was identified and characterized as iodoform. In the case of alkali-soluble lignins, the iodoform may be recovered by filtering the reaction mixture through an alundum crucible; this method does not permit quantitative recovery of iodoform, but it does prove that the iodoform has not been formed from organic solvents.

Preparation of Iodo-carboxy-lignin.—Ten grams of ammonia lignin (13.1% OCH_3) was dissolved in about 400 cc. of 1.0 *N* sodium hydroxide. The theoretical amount of 0.2 *N* iodine solution was added and the mixture allowed to stand for two hours, after which it was acidified with 1.0 *N* sulfuric acid. The precipitated oxidized lignin was filtered by suction and washed free from sulfates, the latter process requiring about two days. The product was dried as described in the preparation of ammonia lignin; yield, 8.5 g. Chlorine water liberated no iodine from the oxidized lignin.

Anal. Found: ash, 0.1; OCH_3, 7.40; N, 3.0; C, 54.48; H, 5.01; I, 13.3.

Iodo-carboxy-lignin can also be prepared without first isolating the lignin. Approximately 450 g. of ground, acid hydrolyzed oat hulls containing 27.46% lignin was treated with one liter of 25% sodium hydroxide. An iodine solution containing 192 g. of iodine, 315 g. of potassium iodide and made up to 2.5 liters was added slowly over an hour's time to the alkaline oat hull mixture. As soon as the oxidation was complete, the solution was filtered and washed twice with water. The combined filtrates were acidified with hydrochloric acid, and the precipitated iodo-carboxy-lignin was filtered and dried as above.

Anal. Found: ash, 0.54; OCH_3, 7.24.

In the preparation of iodo-carboxy-lignin from acid hydrolyzed tissues, several precautions have been found to be necessary. A preliminary quantitative determination of the amounts of reagents used should be made so that an excess of oxidizing agent can be avoided. Excess iodine is difficult to remove, sodium thiosulfate cannot be used for this purpose since free sulfur and sulfur dioxide are liberated before the lignin can be separated. About a day's time is required to wash iodides from the lignin.

Iodo-carboxy-lignin is amorphous and resembles the original ammonia lignin in color. It is soluble in sodium carbonate solution and can be salted out of its alkaline solution.

Methylation of Ammonia Lignin and Iodo-carboxy-lignin.—Methylation of ammonia lignin with dimethyl sulfate decreased the alkaline iodine oxidation values from 188 cc. to 7.0 cc. of 0.1 *N* iodine per gram. The methoxy content of the methylated ammonia lignin was 26.0%.

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[5] Goodwin, THIS JOURNAL, **42**, 39 (1920).

[6] Phillips, *ibid.*, **49**, 2037 (1927).

[7] Peterson, Walde and Hixon, *Ind. Eng. Chem., Anal. Ed.*, **4**, 216 (1932).

[8] Walde, Thesis, Iowa State Coll., Dec., 1933.

Methylation of iodo-carboxy-lignin yielded a product having the *analysis* OCH_3, 22.2; C, 56.03; H, 5.53; I, 10.2. This product was insoluble in cold 10% sodium hydroxide, but when warmed to about 80° it began to go into solution. At 90° it dissolved completely in the alkali in less than one minute. The soluble product was reprecipitated with dilute sulfuric acid and washed free from sulfates.

Anal. Found: OCH_3, 17.4; C, 56.71; H, 5.31; I, 10.48.

The latter product will liberate carbon dioxide from a carbonate solution and therefore contains carboxyl groups. On the assumption of one atom of iodine in the completely methylated and in the saponified iodo-carboxy-lignin, the molecular weight for the two products can be calculated as 1240 and 1210, respectively. A similar calculation based on methoxy content assuming 9 methoxy groups in the former and 7 in the latter gives 1260 and 1250. The saponification reaction could be represented by the equation

$$RI(OCH_3)_7(COOCH_3)_2 \xrightarrow{\text{Saponification}} RI(OCH_3)_7(COOH)_2$$

where the radical R represents a portion of the original lignin complex.

Alkaline Bromine Oxidation of Lignin.—The alkaline bromine oxidation of lignin proceeds farther than that of iodine, as shown by the fact that iodo-carboxy-lignin oxidizes in alkaline bromine solution. Several hours are required for complete oxidation. Ammonia lignin oxidized for thirteen hours required 730 cc. of 0.1 *N* bromine per gram. The product was isolated in a manner similar to that for iodo-carboxy-lignin. It decomposes below 100° and contains bromine, no nitrogen, about 38% C and 3.7% H. A more complete analysis of this substance and its derivatives will be published later. Carbon tetrabromide was isolated and characterized as previously reported by Konschegg for a similar oxidation of humic acid.[9]

Alkaline Chlorine Oxidation of Lignin.—The procedure was similar to the alkaline iodine oxidation. Special precautions were taken to prevent the solutions from becoming acidic since chlorine substitution takes place in acid media. As the oxidation proceeds, the oxidized lignin goes into solution and remains in solution after acidification. There is considerable evolution of carbon dioxide when the reaction mixture is acidified. The reaction appears to have no definite end-point.

Discussion

There can be no question as to the formation of iodoform from the lignin complex. The absence of sugars or acid hydrolyzable carbohydrates in the lignin samples used was checked by several independent reactions. It is of interest to note that iodoform could also be isolated from the alkaline iodine oxidation of nitrolignin, showing that the grouping from which it arises had not been destroyed during nitration. The reaction in this case could not be followed quantitatively due to conflicting color reactions.

The data clearly show that the oxidation values for lignin preparations vary considerably according to the method of preparation. The formation of iodoform from the alkaline iodine treatment of lignin already oxidized by Fehling's solution indicates the presence of an aldehyde group, or some other easily oxidized group, in addition to the group producing iodoform. The amount of oxidation due to Fehling's solution corresponds to one aldehyde group to every 3300 units of molecular weight; the amount of copper reduced does not parallel the loss in iodine oxidation value.

The oxidation of lignin with alkaline solutions is peculiar in that the methoxyl content decreases from 12.5 to approximately 7.4%, while the iodine entering the molecule accounts for but 1.1% of the decrease. This fact supports the contention that the methoxyl groups are not all similarly linked in the lignin molecule.

Although methylated lignin cannot be oxidized by alkaline iodine, the oxidized lignin can be methylated. Unlike the methylated lignin before oxidation, the methylated oxidized lignin is soluble in hot alkali with loss of methoxy groups; it is therefore known to contain an ester linkage. This total solubility in alkali and the presence of the carboxyl group indicates, in agreement with Harris, Sherrard and Mitchell,[3] that a secondary alcohol group, which was a constituent part of the lignin molecule, has been the seat of at least a portion of the oxidation. Assuming that there is one carboxyl group to the molecule of oxidized lignin, the average minimum molecular weight can be calculated as approximately 600, while with the assumption of one atom of iodine per molecule, the minimum molecular weight can be calculated as about 1200. There are, therefore, two carboxyl groups present to every one of iodine. The relative ease with which a strong green fluorescence is produced, when the oxidized lignin is heated with resorcinol and concentrated sulfuric acid, indicates that the two carboxyl groups are near enough together to form an anhydride.

Summary

1. The alkaline halogen oxidation of ammonia lignin has been studied. Alkaline iodine oxidation proceeds quantitatively and forms a product which contains carboxyl groups and iodine. What appears to be the same compound can be made directly from the acid-hydrolyzed

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(9) Konschegg, *Z. physiol. Chem.*, **69**, 390 (1910).

tissue by cold alkaline iodine oxidation without first isolating the lignin.

2. Iodoform has been isolated as one of the products resulting from alkaline iodine oxidation of lignins prepared from different plant materials by several different methods.

3. Carbon tetrabromide resulted from the alkaline bromine oxidation of lignin.

4. A secondary group of the type $CH_3CH(OH)$— is probably present in the lignin molecule.

AMES, IOWA RECEIVED JULY 20, 1934

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF NEBRASKA]

A Study of the Types of Lactones Formed from the Monobasic Sugar Acids under Varying Conditions

BY JOHN M. BRACKENBURY AND FRED W. UPSON

The salt of a monobasic sugar acid can in some cases be treated so as to yield either the free acid, the normal lactone (butylene oxide ring), or the abnormal lactone (amylene oxide ring).[1] This paper presents data to show that the rate, extent and type of lactone formation is dependent on the solvent, the temperature, and the concentration, as well as the particular nature or configuration of the acid under consideration. The data were obtained by means of the polariscope from solutions of the free sugar acids in water, dilute mineral acid and concentrated acetic acid. The concentration and temperature have been varied in some cases.

Hudson[2] has shown that the sign of rotation of a lactone is governed by the position of the ring and this in turn is dependent upon the position of the hydroxyl group involved in lactone formation. Therefore, if a free acid in solution undergoes lactone formation involving first a hydroxyl group to the right of the carbon chain and then lactone formation involving a hydroxyl group to the left of the carbon chain, the rotation of the solution should become first more positive and then more negative than the free acid. Since *d*-mannonic and *d*-gluconic acids were known to form two isomeric lactones, Levene and Simms[3] undertook a quantitative study of the rate and type of lactone formation of these and a number of other sugar acids. By applying Hudson's rule to the data obtained on observing the change of rotation in solution of the free acids prepared from their salts, they concluded that the δ-lactone is formed first with a much greater velocity but in smaller amount than the γ-lactone. Isbell and Frush[4] have recently published the results of an extensive study of several sugar acids and their lactones. Their paper deals primarily with methods of preparation of these compounds, using dioxane as a new solvent in this type of work. Their study includes polarimetric readings in water solution only.

The following table, graphs and discussion deal with the types and extent of lactone formation from the free sugar acids in water, in the presence of aqueous acids, and in acetic acid solutions. In some cases the influence of temperature and concentration has also been considered.

Experimental

Acetic acid solutions of *d*-mannonic, *d*-gluconic and α-*d*-glucoheptonic acids were prepared in the following manner. One gram of the anhydrous crystalline sodium salt of the sugar acid was weighed into a 25-cc. volumetric flask. After solution of the salt in 4 cc. of distilled water, sufficient glacial acetic acid was added to make a total volume of 25 cc. Polarimetric readings on the solutions were made in a two-decimeter tube.

The acetic acid solution of *d*-galactonic acid was made from sodium galactonate having two molecules of water of crystallization. The solution was made in the same manner as described above with the exception that 6 cc. of water was added instead of 4 cc. This was found necessary to prevent the galactonic acid from immediately crystallizing out of solution. Similarly, 10 cc. of water was used in making the acetic acid solution of α-*l*-rhamnohexonic acid from the crystalline sodium rhamnohexonate.

The concentrated solution (14.60%) of glucoheptonic acid was made by dissolving 4.000 g. of sodium glucoheptonate in 5 cc. of water and then adding sufficient glacial acetic acid to make a total volume of 25 cc.

The other acetic acid solutions were made from the calculated weight of the crystalline lactone or free acid dissolved in sufficient 1 *N* sodium hydroxide to effect complete neutralization. To this solution was added sufficient glacial acetic acid to make a total volume of 25 cc.

The solution in excess of mineral acid was made by dissolving the sodium salt in 0.5 *N* sulfuric acid in the cases

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[1] Brackenbury and Upson, THIS JOURNAL, **55**, 2512 (1933).

[2] Hudson, *ibid.*, **32**, 338 (1910).

[3] Levene and Simms, *J. Biol. Chem.*, **65**, 31 (1925).

[4] Isbell and Frush, *Bur. Standards J. of Res.*, **11**, 649 (1933).