Anal. Calcd. for $C_{23}H_{24}O_{12}N_2$: C, 53.1; H, 4.65; N, 5.4. Found: C, 52.6; H, 4.5; N, 5.2.

Identification of 2,3,6-Tri-*O*-methyl-D-glucose.—The non-reducing component (77 mg.) from the cellulose–hydrocellulose column was hydrolyzed with 0.1 *N* sulfuric acid. The solution (final rotation $[\alpha]^{23}D$ +47°) was neutralized ($BaCO_3$), freed from solvent and treated with *p*-nitrobenzoyl chloride as described above. The 1,4-di-*O*-*p*-nitrobenzoate of 2,3,6-tri-*O*-methyl-D-glucose thus produced had m.p. 189–190°, $[\alpha]^{23}D$ −33° in chloroform (*c* 0.7) (after recrystallization from methanol). A specimen prepared from an authentic sample of 2,3,6-tri-*O*-methyl-D-glucose had m.p. 189–190°, $[\alpha]^{23}D$ −33° in chloroform (*c* 0.8).

Anal. Calcd. for $C_{23}H_{24}O_{12}N_2$: C, 53.1; H, 4.65; N, 5.4. Found: C, 53.2; H, 5.2; N, 5.5.

Determination of the Composition of the Methylated Glucomannan. Reaction of 2,3,6-Tri-*O*-methyl-D-glucose with Methanolic Hydrogen Chloride.—A solution of crystalline 2,3,6-tri-*O*-methyl-D-glucose in 1% methanolic hydrogen chloride (75 ml.), at room temperature (25°), showed $[\alpha]^{23}D$ +61° (initial value) changing in 22 hr. to −33.7°. Neutralization of the reaction mixture with silver carbonate, removal of solvent, paper partition chromatographic analysis using benzene–ethanol–water as the irrigating solvent and the phenol–sulfuric acid method for the analysis, showed that 90% of the 2,3,6-tri-*O*-methyl-D-glucose was converted into a glycoside of which 90% was furanoside, hydrolyzable with 0.1 *N* sulfuric acid.

Reaction of 2,3,6-Tri-*O*-methyl-D-mannose with Methanolic Hydrogen Chloride.—A solution of tri-*O*-methyl-D-mannose (64.4 mg.) in 1% methanolic hydrogen chloride (25 ml.), when kept at room temperature, showed $[\alpha]^{23}D$ +1° (initial value) and no change in rotation was noted after 22 hours. Analysis of the reaction product by chromatographic analysis on paper as described above showed that 46% of the tri-*O*-methyl-D-mannose had been converted into glycosides.

Ratio of 2,3,6-Tri-*O*-methyl-D-glucose to 2,3,6-Tri-*O*-methyl-D-mannose. (a) From the Rotation of the Methylated Reducing Sugars.—Since the 2,3,6-tri-*O*-methyl derivatives of D-glucose and D-mannose have specific rotations of +70.5 and −11.6°, respectively, in water and the hydrolysate of the methylated glucomannan showed $[\alpha]^{23}D$ +13.4° in water it may be deduced that the ratio of the glucose to the mannose derivative is 30.5:69.5.

(b) From the Change in Rotation upon Reaction with Methanolic Hydrogen Chloride for 22 hours.—The 2,3,6-trimethyl-D-glucose changes from $[\alpha]^{23}D$ +61° to −33.7°, 2,3,6-trimethyl-D-mannose shows no change from $[\alpha]^{23}D$ +1° while the mixture of trimethyl sugars from the glucomannan changed from $[\alpha]^{23}D$ +23 to −4.5°. From the final rotations it may be calculated that the ratio of the glucose to the mannose derivative is 29:71, respectively, while from the initial specific rotations the ratio is 38.3:61.7.

The average of the three values showed that the hydrolysate contains about two parts of 2,3,6-tri-*O*-methyl-D-mannose to one part of 2,3,6-tri-*O*-methyl-D-glucose. This corresponds to the ratio of D-mannose to D-glucose in the original glucomannan component of Iles mannan (hydrolysate showed $[\alpha]^{23}D$ +28°, H_2O).

Hydrolysis of the Methylated Glucosan and Identification of 2,3,6-Tri-*O*-methyl-D-glucose.—The methylated polysaccharide (131 mg., $[\alpha]^{23}D$ +180° ($CHCl_3$)), isolated by fractionation of the mixture of methylated polysaccharides as described above, was treated for 8 hours under reflux with 1% methanolic hydrogen chloride (40 ml.) until the rotation became constant, $[\alpha]^{23}D$ +42.6°. Removal of the acid with silver carbonate and the solvent by evaporation *in vacuo* gave sirupy methyl 2,3,6-tri-*O*-methyl-D-glucoside (138 mg.). This sirup was treated for 12 hours with *N* sulfuric acid (30 ml.) on the boiling water-bath when the rotation became constant ($[\alpha]^{23}D$ +54.5°). After neutralization ($BaCO_3$) and removal of solvent *in vacuo* the 2,3,6-tri-*O*-methyl-D-glucose (105 mg.) crystallized spontaneously. Before recrystallization it had $[\alpha]^{23}D$ +61° in water (*c* 0.5) and after recrystallization from ether it showed $[\alpha]^{23}D$ +66° and had m.p. 106–107°.

When treated with *p*-nitrobenzoyl chloride in pyridine as described above it readily afforded the di-*O*-1,4-*p*-nitrobenzoate of 2,3,6-tri-*O*-methyl-D-glucose, m.p. and mixed m.p. 190°, $[\alpha]^{23}D$ −31° in chloroform (*c* 0.3) (after recrystallization from methanol).

ST. PAUL, MINNESOTA

[CONTRIBUTION FROM THE INSTITUTE OF PAPER CHEMISTRY]

Study of Alcoholysis and Hydrolysis of Cellulose Triacetate in Chloroform[1]

BY KYLE WARD, JR., CHEN-CHUAN TU AND MAIJA LAKSTIGALA

RECEIVED JUNE 3, 1954

Ethanolysis and methanolysis of cellulose triacetate in chloroform in the presence of benzenesulfonic acid yielded a series of methyl and ethyl glycosides, respectively. The water-soluble glycosides were highly dextrorotatory. Hydrolysis of the methyl glycosides indicated that the glucosidic linkage of the glycoside was not uniform. Hydrolysis of cellulose triacetate in chloroform produced a water-soluble and highly dextrorotatory polysaccharide. Partial hydrolysis of the polysaccharide, chromatography of the hydrolyzate of the polysaccharide, and the isolation of gentiobiose indicated that the polysaccharide contained several types of glucosidic linkages.

A re-examination of the reaction of cellulose triacetate with benzenesulfonic acid in chloroform solution makes it necessary to modify previous hypotheses about the reactions involved.[2] The main reactions concerned are deacetylation, degradation such as alcoholysis (ethanol is commonly used as a stabilizer in chloroform), or hydrolysis and condensation of some of the sugar fragments produced in the breakdown. These reactions have been studied in the presence of ethanol, of methanol, and of small amounts of water.

The course of the reaction using ethanol is as follows. When a solution of cellulose triacetate in chloroform containing ethanol is refluxed with benzenesulfonic acid, a precipitate is formed which still contains 10 to 20% acetyl. If this product is further deacetylated with ammonia or with sodium methylate, the product is a white water-soluble powder which can be purified by precipitation of the aqueous solution with alcohol. Adsorbed alcohol is hard to remove and products for analysis were reprecipitated with acetone. Evaporation to dryness from aqueous solution also removes most of the adsorbed alcohol. If all reagents are anhydrous, the materials are non-reducing to Fehling solution, but in the presence of even a trace of water, there is a slight reducing power to

(1) This article is based upon papers presented at the 124th and 125th Meetings of the American Chemical Society, September, 1953, and March, 1954.

(2) H. Pringsheim, E. Kasten and E. Schapiro, *Ber.*, **61**, 2019 (1928); H. Pringsheim, G. Otto and J. R. Katz, *Cellulosechemie*, **11**, 137 (1930); H. Pringsheim and K. Ward, Jr., *ibid.*, **13**, 65 (1932).

Benedict solution. Depending on the details of the treatment, the end-product contains from 0.4 to 3% ethoxyl.

It is, of course, possible that the alkoxyl is present as an ether, but the method of preparation makes this unlikely. Moreover, no ethers are indicated in chromatography of the hydrolyzed material. The method of preparation seems rather to indicate the formation of ethyl glycosides of cellulose breakdown products.

The reaction was carried out with various concentrations of methanol and ethanol. The use of water instead of an alcohol gives what appears to be a polysaccharide, produced similarly to the glycosides with alcohol.

The apparent mechanism of the process seems to be as follows. At first there are two simultaneous homogeneous reactions, deacetylation and chain-splitting (alcoholysis or hydrolysis). The deacetylation eventually results in chloroform insolubility and the material precipitates out. This simple picture is, however, complicated by the condensation reaction to be discussed later. This condensation will hinder the use of the reaction in structural studies, but it can be prevented or kept very low; however, the method may be useful for it will presumably apply to any polysaccharide ester if carried out in an inert medium which will dissolve the ester, but not the partially deacetylated product. The yields are high (60 to 90% of theory based on the original ester) and the product is free of mono- and disaccharides after purification, even if any had been formed.

The products are highly dextrorotatory. This might indicate a preponderance of α-glycoside over β-glycoside, but this explanation will not suffice, although it may be a factor. The amount of alkoxyl group in the product will vary with the concentration of alcohol in the chloroform. In both the methanolyzed and ethanolyzed material, rotation increases as the alkoxyl content decreases. This is the reverse of what might be expected if rotation depended solely on the terminal alkoxyl group, for optical rotation is known to decrease from cellobiose to celloheptaose.[3]

Another factor which may contribute to high dextrorotation was discovered in a study of the hydrolysis products of the methyl glycosides. If the methyl glycoside mixture is hydrolyzed with dilute acid and the hydrolyzate chromatographed, unusual results are obtained. There is one monosaccharide spot corresponding to glucose. There are, however, two strong spots in the disaccharide region, one corresponding to cellobiose and the other moving at the same rate as gentiobiose. This indicates that the alcoholysis product is not uniformly 1,4-linked.

No similar spot was found in the hydrolysis of the original cellulose triacetate nor from its acetolysis products. Neither was one found in the hydrolysis of methyl glycosides produced by heterogeneous methanolysis of cellulose with methanol and hydrogen chloride.[4] This indicates that the new linkages are unique to the reaction under study.

(3) M. L. Wolfrom and J. C. Dacons, THIS JOURNAL, **74**, 5331 (1952).

(4) R. E. Reeves, L. W. Mazzeuo, Jr., and C. L. Hoffpauir, *ibid.*, **72**, 4773 (1950).

Results with paper partition chromatography are indicative, but more positive identification of gentiobiose was desirable. For this purpose the polysaccharide produced by using water instead of alcohol proved suitable. Paper-partition chromatography of this material gave results similar to the methanolyzed material. No monosaccharides are found except glucose, but there were several spots between glucose and cellotriose.

One such spot traveling slightly slower than cellobiose appears relatively resistant to hydrolysis. A large amount of the original oligosaccharide mixture was therefore subjected to prolonged hydrolysis and separated on a charcoal column. Several hundred milligrams of a disaccharide was isolated, crystallized, and identified as gentiobiose by melting point, rotation, hydrolysis to glucose, and mixed melting point of the octaacetate with an authentic sample.

These new glucosidic linkages may have a marked effect on optical rotation. Gentiobiose has about the same rotation as cellobiose, but other glucose linkages have probably been formed and some of these, especially the α-forms, are higher in rotation than cellobiose. One would expect condensation to occur randomly and this is indicated by other less sharp unidentified spots in the chromatograms. The identification of gentiobiose is made possible by its relative resistance to hydrolysis and by the probability of its formation in relatively greater amounts since the 6-hydroxyl is made available to a greater extent than any of the others during deacetylation.[5] The optical rotation would be affected also by branching, which probably occurs although we have no direct evidence to substantiate it.

There still remains the question of the nature of condensation in the alcoholyses, where, presumably, no free 1-hydroxyls are formed. We have no direct evidence on this point but several possibilities exist.

One is that traces of water may be found in the reaction mixture (as by the formation of esters of benzenesulfonic acid or by the dehydration of the carbohydrate material). Another is that it may not be necessary to have a free 1-hydroxyl to produce condensation, but that the alkyl glycosides may condense with free hydroxyls in other positions or even, by splitting out alkyl acetate, with the acetylated hydroxyl.

A similar position actually exists in the case of the alcoholyses. The alkoxyl may not necessarily be introduced by direct alcoholysis, but by alkylation of a hydrolysis product.

There seems to be little doubt that condensation of sugar units has occurred, but this may have been either simple reversion of glucose formed from the cellulose triacetate or condensation of glucose or its higher homologs and derivatives with long-chain material. Ordinary reversion of glucose to polymeric materials as, for example, described by Pacsu and Mora[6] is not ruled out, but we favor the other mechanism for the following reasons.

The reaction product from water and cellulose triacetate is water soluble like the products from

(5) T. S. Gardner and C. B. Purves, *ibid.*, **64**, 1539 (1942).

(6) E. Pacsu and P. T. Mora, *ibid.*, **72**, 1045 (1950).

alcoholysis, but is strongly reducing. Glucose pentaacetate in chloroform does not form this type of reversion product under the same conditions of treatment. If the amount of water is increased several fold, a reversion product is indeed obtained from glucose pentaacetate, but it has a lower reducing value and can be fractionated on a charcoal column to give an appreciable non-reducing fraction. The corresponding product from cellulose triacetate is less mobile on the column and all fractions are reducing. The product from glucose pentaacetate contains no 1–4 linkages; at least, neither cellobiose nor maltose appears on chromatography of the hydrolyzate.

As for the reversion of glucose itself in the presence of acids, this process can be carried out so as to give a mixture in which disaccharides predominate or one in which larger polymers predominate. The former has recently been reviewed by Thompson[7] and co-workers, who have shown the presence in the mixture of a great many disaccharides as well as dehydration products of glucose. The latter has been discussed by Pacsu and Mora[6]. Their methylation studies gave no evidence of a gentiobiose linkage, but did not entirely rule out the possibility.

So far, only the water-soluble glycoside or polysaccharide has been mentioned. With increasing amounts of water or alcohol, the solubility of the deacetylated product in water decreases. In the case of the ethanol reaction, complete water solubility results from 1.5 to 2.5% ethanol in chloroform and complete water insolubility above 5% ethanol. The water-insoluble fractions have not been studied to any great extent. It is interesting to note, however, that in the intermediate range some of the partially deacetylated primary products are water soluble, but become partially insoluble on further deacetylation. This solubility of the substance with 14 to 20% acetyl is perhaps a similar phenomenon to the solubility of partially methylated cellulose and is also reminiscent of the results of Fordyce.[8] No detailed study of the water-insoluble products has been made. Such a study and an investigation of the relative rates of the various competing reactions may result in more information on the nature of this reaction and the resultant product.

Experimental

Starting Materials.—The starting materials were commercial cellulose triacetate (Eastman Kodak), benzenesulfonic acid monohydrate (Eastman Kodak), ethanol, methanol and chloroform (U.S.P.) containing 0.5% ethanol by weight. The cellulose triacetate was dried in a vacuum oven for 24 hours at 105°. The dehydration of benzenesulfonic acid monohydrate was carried out by mixing 8 g. of the acid with 750 g. of chloroform in a 1000-ml. round-bottomed flask equipped with a water trap for continuous removal of water. The mixture was refluxed for 6 hours and the separated water was removed from the apparatus. The chloroform was distilled off until 100 ml. was left in the flask. Chloroform, ethanol and methanol were purified by the usual methods.[9]

(7) A. Thompson, K. Anno, M. L. Wolfrom and M. Inatome, THIS JOURNAL, **76**, 1309 (1954).

(8) C. R. Fordyce and Eastman Kodak Co., U. S. Patent 2,129,052 (1938).

(9) A. Weissberger, "Organic Solvents," The Clarendon Press, Oxford, 1935.

I. Alcoholysis. Preparation of Alkyl Glycosides.—For a typical run, 30 g. of the oven-dried cellulose triacetate was dissolved by shaking with 600 g. of chloroform in a 1000-ml. two-necked round-bottomed flask. The flask was equipped with a water trap and the solution was refluxed on a steam-bath for 6 hours. Usually a few drops of water were removed in this way. To this chloroform solution was added a solution of 100 ml. of chloroform, 15 g. of anhydrous ethanol and the dehydrated benzenesulfonic acid obtained from 8.0 g. of the monohydrate. The flask was equipped with a motor-driven mercury-sealed stirrer and a reflux condenser, fitted with a calcium chloride tube to eliminate atmospheric moisture. The solution was heated slowly to boiling in an oil-bath (68°) and refluxed with constant stirring for 48 hours. Precipitation began in five hours and was completed after about 8 hours. The originally gelatinous precipitate, quite hard by this time, was then broken into small pieces with a spatula and refluxing continued. Toward the end of the reaction the chloroform solution became brownish-green. After 48 hours, the reaction mixture was cooled to room temperature, and the separated precipitate ground with alcohol, filtered, washed with 300 ml. of absolute ethanol and finally dried *in vacuo*. The yield of dried product was about 16 g.

For deacetylation, the dried product was dissolved in five times its weight of water. To the cold solution was added 80 ml. of 5% aqueous ammonia for every 10 g. of sample. The solution was allowed to stand for 24 hours and concentrated *in vacuo* to a sirup. Precipitation and flocculation occurred when the sirup was stirred into 500 ml. of absolute ethanol. The precipitate was separated and dried.

Other 30-g. batches of cellulose triacetate were ethanolyzed, varying the ethanol content of the chloroform. Runs were made with 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5% ethanol by weight of the chloroform. In another series, the methanolysis of cellulose triacetate was carried out using 2.0, 3.0, 4.0 and 5.0% of methanol by weight of the chloroform. Each run was conducted in the same manner as described above. In order to remove the adsorbed alcohol the products were dissolved in water and reprecipitated with acetone.[10] In one case, this was checked by simply evaporating the aqueous solution. The original material precipitated with alcohol had 3.47% methoxyl. Evaporation of the water solution reduced this to 1.70%, and precipitation with acetone to 1.50%.

Most of the partially deacetylated materials were completely water soluble. However, after deacetylation with ammonia, samples prepared with 3% or more alcohol were only partly soluble. The ammonia solutions either gelled or precipitated. The solubility decreases with increasing amounts of alcohol. In these less soluble products, the insoluble fractions were removed by filtration of the aqueous solutions and the soluble portions were worked up as described above.

The yields and the properties of these products are shown in Tables I and II.

TABLE I

YIELDS AND PROPERTIES OF ETHYL GLYCOSIDES FROM CELLULOSE TRIACETATE

Ethanol content of chloroform, %	Yield, as % of original cellulose triacetate: Before deacetyln.	After deacetyln. with ammonia: Water sol.	After deacetyln. with ammonia: Water insol.	Props. of water-soluble fraction: Acetyl content,[a] %	Ethoxyl content,[b] %	Reducing power,[c] mg. reducing sugar	$[\alpha]^{25}_{D}$
1.5	46.7	40.0	..	0.58	0.40	8.3	+86.7°
2.0	53.3	50.7	..	.85	0.80	7.5	76.0
2.5	53.3	36.8	..	.26	1.00	2.2	69.0
3.0	51.7	26.8	12.4	.74	1.45	1.9	45.2
3.5	51.0	10.7	30.0	.23	1.90	..	31.2
4.0	56.0	12.3	30.3	.56	2.0	1.1	27.5
4.5	56.7	4.0	39.8	.21	2.28	..	38.4

[a] R. L. Whistler and A. Jeanes, *Anal. Chem.*, **15**, 317 (1943). [b] TAPPI Standard T 209 m-45. [c] G. Luff and N. Schoorl, described on p. 195, Bates, *et al.*, "Polarimetry. Saccharimetry and the Sugars." Natl. Bur. Standards Circular C440 (1942), 1.25 g. table used.

(10) L. Steffens and D. P. Heath, *Ind. Eng. Chem., Anal. Ed.*, **16**, 525 (1944).

TABLE II

YIELDS AND PROPERTIES OF METHYL GLYCOSIDES FROM CELLULOSE TRIACETATE

Methanol content of chloroform, %	Yields, as % of original cellulose triacetate: Before complete deacetyln.	After deacetyln. with ammonia: Water sol.	After deacetyln. with ammonia: Water insol.	Properties of the water-soluble glycoside: Acetyl content, %	Methoxyl content, %	Reducing power, mg. reducing sugar	$[\alpha]^{25}D$
2	55.0	45.0	..	0.67	1.50	6.5	76.3
3	50.0	35.0	..	.67	2.00	8.3	69.3
4	48.3	11.0	23.3	.26	2.56	2.6	45.1
5	55.0	6.7	39.0	.23	2.99	2.9	40.6

Hydrolysis of Methyl Glycosides.—The sample used for hydrolysis was prepared as described, using 3.3% methanol. This sample was a little lower in methoxyl content (1.76%) than those described in Table II. The reason for this is not clear: the purification differed slightly, however, in that this sample was worked up after deacetylation by a single precipitation with acetone. One and one-half grams was dissolved in 50 ml. of 0.1 *N* hydrochloric acid and the solution refluxed in a two-necked round-bottomed flask equipped with a stirrer and reflux condenser. The flask was immersed in an oil-bath at 100°. Samples were withdrawn at intervals and reducing power[11] and optical rotation were determined. All samples were spot-checked by paper chromatography as follows.

Each time a sample was withdrawn from the reaction flask five drops were placed on a strip of Whatman No. 1 filter paper. The strips were developed downward for 24 hours with a mixture of pyridine:butanol-1:water (4:6:3), then air-dried and sprayed with hydrogen aniline phthalate reagent. The most noteworthy spot on the chromatograms was a strong spot between cellobiose and cellotriose. For comparative purposes a methyl glycoside,[12] produced by heterogeneous methanolysis of cellulose with methanol and hydrogen chloride, was hydrolyzed as follows. Two grams was dissolved in 72% sulfuric acid in about one-half hour. The solution was diluted with one liter of water and boiled under reflux for 6 hours. A 25-ml. sample was then withdrawn and neutralized with barium carbonate. The solution was filtered, concentrated to about 5 ml. and spot-checked by paper chromatography as described above. Three distinct spots were noticed, corresponding to glucose, cellobiose and probably cellotriose. No spots were observed between the latter and cellobiose.

II. Hydrolysis

Preparation of Water-soluble Polysaccharide from Cellulose Triacetate.—One hundred and twenty grams of cellulose triacetate was dissolved in 2000 ml. of chloroform in a 3000-ml. two-necked round-bottomed flask. To the solution were added 30 g. of benzenesulfonic acid, 16 ml. of water and 100 ml. of benzene. The flask, fitted with a condenser and a mercury-sealed motor-driven stirrer, was heated in an oil-bath and the solution was refluxed. Degradation and deacetylation occurred, and the partially deacetylated material precipitated. After 20 hours, the reaction mixture was cooled to room temperature and the precipitate was filtered, washed with 1000 ml. of acetone and dried in a vacuum desiccator; yield 70 g. The whole amount of the dried precipitate was suspended in 1600 ml. of 0.2 *M* sodium methoxide in a 2000-ml. small-mouthed reagent bottle. The bottle was rotated mechanically overnight at room temperature. The suspension was made slightly acidic with acetic acid, filtered through a sintered glass funnel, and the precipitate washed on the funnel with five 100-ml. portions of absolute ethanol. The precipitate was dissolved in water and the residue was separated by centrifuging. The solution was deionized,[13] concentrated and evaporated under vacuum, adding absolute ethanol, and repeating the evaporation to dryness; yield 30 g. The product thus prepared gave $[\alpha]^{24}D$ +80°.

The paper chromatogram of the water-soluble polysaccharide showed that there were no trisaccharides or lower sugars present.

Partial Hydrolysis of the Water-soluble Polysaccharide and Isolation of Gentiobiose from the Hydrolyzate.—Preliminary hydrolyses of the water-soluble polysaccharide gave indications of the presence of two disaccharides other than cellobiose, one of which was fairly resistant to further hydrolysis and could, therefore, be isolated. The other was present in a smaller amount and disappeared on further hydrolysis, as did the cellobiose present. The resistant sugar appeared to be gentiobiose. This conclusion was verified as follows: Seventy grams of the water-soluble polysaccharide was dissolved in 2000 ml. of 0.1 *N* hydrochloric acid and the hydrolysis conducted as described previously. After heating for 100 hours, the solution was cooled, neutralized, deionized and concentrated to 700 ml. The concentrated solution was transferred to the top of a charcoal column, 60 by 630 mm.[14] The column was washed with 2000 ml. of water to remove glucose, and successively washed with two 4000-ml. portions of 5% ethanol. The first and second 4-l. portions were separately concentrated, evaporated to dryness and spot-checked; yield 0.3 g. each. It was found that the first portion consisted only of the resistant disaccharide, later identified as gentiobiose, in addition to some insoluble impurities obtained probably from the charcoal column. The second portion was found to be a mixture of cellobiose and gentiobiose with some impurities.

Characterization of Gentiobiose. a. Crystallization.—Five-hundredths gram of the crude gentiobiose was dissolved in a few drops of water. To the solution was added 10 ml. of absolute methanol. The solution was warmed and filtered. The filtrate was allowed to stand for two days at 5°; crystallization occurred. The yield was 0.020 g. of crystals which softened at 83° and melted at 88°, $[\alpha]^{25}D$ +12.0° (*c* 0.5 water). Gentiobiose is reported to melt from 85 to 86° with $[\alpha]^{20}D$ +21.4 → +8.7.[15]

b. Hydrolysis of Gentiobiose.—Forty-four thousandths gram of the crude gentiobiose was dissolved in 1.5 ml. of 0.2 *N* hydrochloric acid. The hydrolysis was conducted as previously described. One-tenth milliliter portions of the solution were withdrawn periodically and spot-checked. The chromatograms showed the presence of gentiobiose and glucose. As the hydrolysis proceeded to completion, gentiobiose gradually disappeared, leaving glucose on the chromatogram and a very faint spot moving much faster than a hexose. Direct evidence of the nature of this spot is lacking, but it may be a dehydration product of glucose as these are known to be formed easily.[7,16]

c. Preparation of Gentiobiose Octaacetate.—Two-tenths gram of the crude gentiobiose was mixed with 0.4 g. of fused sodium acetate and 5 ml. of acetic anhydride in a two-necked round-bottomed flask, fitted with a condenser and a mercury-sealed motor-driven stirrer. The flask was heated in a boiling water-bath for three hours. Then the warm solution was poured with vigorous stirring into 100 ml. of ice-water. The white powder which formed was separated, washed, dried and purified by crystallization from ethanol; yield 0.080 g. The acetate was purified by recrystallization from absolute ethanol; yield 0.050 g., m.p. 191 to 192° alone or when mixed with an authentic specimen of gentiobiose octaacetate, $[\alpha]^{23}D$ −4.8 (*c* 1.5, chloroform). (Previous workers have found that the β-octaacetate of gentiobiose melts at 193° and has $[\alpha]^{20}D$ −5.3°.)[17]

Reversion Product from Glucose Pentaacetate.—Five grams of α-glucose pentaacetate was dissolved in 100 ml. of alcohol-free chloroform. To the solution were added 1.5 ml. of water and 1 g. of *p*-toluenesulfonic acid monohydrate. The mixture was refluxed for 46 hours. The resulting precipitate was separated and completely deacetylated in 100 ml. of 0.2 *M* sodium methoxide in methanol. To the neutralized methanol solution was added 100 ml. of methanol. The mixture was filtered. The methanol-insoluble portion, 1.2 g., was dissolved in 10 ml. of water and transferred to the top of a charcoal column, 17 by 70 mm. After washing with 1000 ml. of water and 1000 ml. of 5% ethanol, the

(11) R. L. Whistler and C. C. Tu, THIS JOURNAL, **74**, 3609 (1952).

(12) This sample was kindly furnished by Dr. R. E. Reeves of the Southern Regional Research Laboratory.

(13) Amberlite IR-120 and IR-4B produced by The Rohm and Haas Co., Resinous Products Div., Philadelphia, Penna.

(14) R. L. Whistler and D. F. Durso, THIS JOURNAL, **72**, 677 (1950); R. L. Whistler and C. C. Tu, *ibid.*, **74**, 3609 (1952).

(15) F. J. Bates, *et al.*, "Polarimetry, Saccharimetry and the Sugars," N. B. S. Circular C440, 1942, Government Printing Office, Washington.

(16) M. L. Wolfrom, R. D. Schuetz and L. F. Cavalieri, THIS JOURNAL, **70**, 514 (1948).

(17) G. Zemplēn, *Z. physiol. Chem.*, **85**, 402 (1913).

column was eluted with 1500 ml. of 30% ethanol. The effluent was evaporated to dryness, repeating after addition of absolute ethanol; yield 0.42 g., $[\alpha]^{25}D$ +88°, non-reducing. Three-hundredths gram of the obtained material was hydrolyzed in 1 ml. of 0.05 N hydrochloric acid and spot-checked. As the hydrolysis proceeded, a gentiobiose spot appeared on the paper chromatogram but no cellobiose spot was found.

Acetolysis of Cellulose Triacetate.—Ten grams of the cellulose triacetate used for the production of the water-soluble polysaccharide was dissolved in a mixture of 34 ml. of glacial acetic acid, 10 ml. of acetic anhydride and 6 ml. of concentrated sulfuric acid. The solution was allowed to stand at room temperature for 140 hours, and filtered through a sintered glass funnel. The filtrate was poured with vigorous stirring into 500 ml. of ice-water. The precipitate was filtered, washed, and dissolved in 400 ml. of chloroform. The solution was washed thrice with 500 ml. of water, 400 ml. of 1% sodium bicarbonate solution and again with 500 ml. of water. The chloroform was removed by distillation under vacuum. The acetolyzate was separated and dried as usual; yield 7 g.

One gram of the acetolyzate was deacetylated in 14 ml. of 0.2 M sodium methoxide. After complete deacetylation the mixture was neutralized with dilute acetic acid, diluted with water, de-ionized and concentrated under vacuum to a thin sirup. To the sirup was added 70 ml. of absolute ethanol. The precipitate was separated and the filtrate evaporated to dryness, repeatedly adding absolute ethanol. Both the precipitate and the residue were spot-checked. On the chromatogram the precipitate from ethanol was found to be a mixture of cellobiose, cellotriose and their higher homologs, and the residue, a mixture of glucose, cellobiose and cellotriose. No gentiobiose was indicated on the chromatogram.

Hydrolysis of Cellulose Regenerated from Cellulose Triacetate.—Two grams of cellulose triacetate was deacetylated completely in 30 ml. of 0.2 M sodium methoxide in methanol. The regenerated cellulose was dissolved in 72% sulfuric acid at room temperature. The solution was diluted with water to 1 l., and refluxed for 4 hours. Twenty-five milliliters of the solution was withdrawn and neutralized with barium carbonate. The solution was filtered and the filtrate concentrated to 5 ml. The solution was spot-checked. On the chromatogram the solution was found to be a mixture of glucose, cellobiose and cellotriose.

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[CONTRIBUTION FROM THE INSTITUTE OF PAPER CHEMISTRY]

Studies on Lignin and Related Products. X.[1] Further Studies on the Isolation of Compounds from Lignin Oxidation Mixtures by Chromatographic Techniques[2,3]

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RECEIVED JUNE 17, 1954

Further studies have been made of chromatographic fractionation of lignin oxidation mixtures, with separation of a variety of products.

In an attempt to obtain better preliminary separation of the acid fraction alkaline cupric oxide oxidations of lignosulfonate materials a large scale experiment was performed on the use of an anion-exchange resin in ethanolic solution as described earlier.[4] An ethanol solution comprising the combined total ether extracts from the many individual oxidations for fermented spent sulfite liquor reported earlier,[5] was filtered to remove dehydrodivanillin and then passed through a column of Duolite A-2.[4] The column was washed with ethanol and then with water, collecting each washing separately. Finally the column was washed with 4% sodium hydroxide solution followed by water. A flow sheet giving the preliminary separations employed is pictured in Fig. 1.

Fractions IV, V and VII were fractionated by means of techniques reported earlier,[6,7] but it soon became obvious that such techniques employing only adsorption chromatography on Magnesol were not sufficient for the complete fractionation of the phenolic and acidic mixtures. The original mixture contained a great many phenolic and carboxylic compounds in small concentration in the presence of an overwhelming amount of vanillic acid. Under these conditions the vanillic acid was dispersed in all of the above fractions and was obtained along with vanillin, 5-formylvanillin, 5-carboxyvanillin, vanillil, a compound melting at 110–120° which yielded only vanillic acid upon further chromatography, and several unidentified crystalline compounds. It was at this point that recourse was made to cellulose column and paper chromatographic methods.

Fractions I, II and III were separated by chromatography on Magnesol into fractions which were analyzed by means of cellulose powder and paper chromatography employing butanol saturated with 2% aqueous ammonia. Vanillin and acetovanillone were found to be the chief components of these fractions along with an unidentified compound having an R_f value of 0.87.

It soon became apparent from these paper chromatographic studies and from analogous studies on the oxidation of lignin model substances[1] that, for a particular developer, R_f values of a pure compound and the compound in admixture are usually different. This difference might be of considerable magnitude, especially with compounds of similar R_f values. The adulterants appear to act as components of the developing system until they are completely removed as independent spots by development. When the R_f values are very close, the adulterant may merely push the desired compound to a higher R_f without any separation whatsoever. Thus, simultaneous paper chromatography of a lignosulfonate oxidation fraction and known pure compounds will yield spots for identical

(1) For paper IX of this series, see THIS JOURNAL, **76**, 2224 (1954).

(2) Presented before the Division of Cellulose Chemistry at the 126th Meeting of the American Chemical Society, New York, N. Y., September 12–17, 1954.

(3) The results reported here are from a research program at this Institute sponsored by the Sulphite Pulp Manufacturers' Research League. Acknowledgment is made for their permission to publish these results.

(4) I. A. Pearl and D. L. Beyer, THIS JOURNAL, **75**, 2630 (1953).

(5) I. A. Pearl and D. L. Beyer, *Tappi*, **33**, 544 (1950).

(6) I. A. Pearl, THIS JOURNAL, **71**, 2196 (1949).

(7) I. A. Pearl and E. E. Dickey, *ibid.*, **74**, 614 (1952).