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# Studies on the Products Obtained from the Hydrolysis of Methylated Oxidized Cellulose Acetate[1a,b]

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In studies on the decarboxylation of oxidized cellulose acetate[3] it was found that although the decarboxylation of oxidized cellulose acetate led to a concurrent extensive degradation of the polymer (as shown by viscosity measurements), the decarboxylation of oxidized cellulose acetate that had first been reduced with sodium borohydride did not lead to any extensive degradation. In continuation of these studies, oxidized cellulose acetate was deacetylated and methylated with sodium hydroxide and dimethyl sulfate. The methylated product was found to possess properties analogous to that of the oxidized cellulose acetate, in that it was easily degraded during decarboxylation or by treatment with dilute acid. When the methylated product was first reduced with sodium borohydride, however, it was found to be comparatively stable. Hydrolysis of the methylated oxidized cellulose in the presence of 2,4-dinitrophenylhydrazine and chromatography of the 2,4-dinitrophenylhydrazones led to the isolation of the hydrazone of 2-methoxymalonaldehyde. The methylated product was reduced and then hydrolyzed with formic acid, and the acidic constituents were separated as barium salts. The free acids were obtained by treating the barium salts with an ion exchange resin. The methyl esters of the methyl glycosides were formed and reduced with lithium aluminum hydride. 2-*O*-Methyl-D-glucose was isolated. We suggest that oxidation of cellulose leads to the formation of a 3-*keto*-uronic acid which is an unstable unit in the cellulose chain.

In a previous communication[3] it was reported that although the decarboxylation of oxidized cellulose acetate by the reaction of the acid chloride with silver oxide and bromine led to extensive depolymerization of the oxycellulose chain, decarboxylation was achieved without any marked depolymerization, if the oxidized cellulose acetate was first reduced with sodium borohydride.

It is known that even mild oxidation of cellulose results in a change in its physical and chemical properties. Oxycellulose or oxidized cellulose formation is always accompanied by a fall in the tensile strength of the fiber,[4] there is an enhanced absorption of basic dyes, probably due to the formation of carboxylic acid groups and the oxidized cellulose becomes more sensitive to the action of acidic or alkaline conditions than the unoxidized cellulose. That the degradation does not take place to any great extent, as a direct consequence of the oxidation reaction itself, is strongly suggested by the fact[5] that when cellulose is oxidized particularly at a neutral *p*H and the viscosity of the nitrate esters of the oxidized and unoxidized celluloses are compared (prepared by the action of an anhydrous nitric-phosphoric acid mixture), there is apparently little if any degradation found as the result of oxidation and nitration. On the other hand, if the oxycellulose is treated with dilute alkali at elevated temperatures before it is nitrated, extensive degradation takes place as is shown by a decrease in the viscosity of the cellulose nitrate. A similar decrease in viscosity is observed when the oxidized cellulose is directly dissolved in cupraammonia. It would seem that although oxidation does not necessarily cause a scission of the polysaccharide chain it does lead to the formation of chemical groups that are particularly sensitive to conditions of *p*H.

The instability to alkali that is conferred on the cellulose molecule by the action of oxidizing agents is apparently related to the formation of reducing groups. If oxycellulose is boiled with 1% sodium hydroxide,[6] there is a loss in weight and this loss is accompanied by a reduction in the copper number, that is, in the number of reducing groups. Indeed, the number of reducing groups can be virtually reduced to zero on prolonged boiling with dilute alkali. An oxidized cellulose with few reducing groups is relatively stable to alkali. It is interesting, in agreement with the concept that the formation of reducing groups is responsible for the formation of chemically labile groups, that the loss in weight that oxidized cellulose undergoes after boiling with 1% sodium hydroxide is considerably decreased when the oxidized cellulose is first treated with a reducing agent such as sodium borohydride.[7]

Although it might be expected that oxidation, particularly low degrees of oxidation, would cause the random formation of carboxylic acid, aldehyde and ketone groups and therefore the presence of ketone groups could be used to explain the sensitivity of oxidized cellulose acetate to decarboxylation, it was difficult to see how the reduction of a ketone group in a glucose unit, even one adjacent to the uronic acid, could make it possible to decarboxylate the uronic acid while the presence of the ketone group made it impossible to do so without extensive depolymerization taking place at the same time. One possibility was that at least a fraction of the glucose units that had undergone oxidation had been oxidized at two carbon atoms; carbon 6 to yield a carboxylic acid and carbon 2 or 3 to yield a ketone. Decarboxylation would substitute a strongly negative halide for a carboxylic acid group and although the mechanism was not clear, this in conjunction with the ketone already present could form a chemically labile group which would further break down and result in a split in the cellulose chain. At the same time fragments derived from the splitting of the glucose unit might be expected as integral parts of the split cellulose chains. The isolation and identification of such fragments should substantiate our hypothesis.

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(3) F. A. H. Rice and A. R. Johnson, THIS JOURNAL, **79**, 5049 (1957).

(4) G. F. Davidson, *Soc. Dyers and Colourists J.*, **56**, 58 (1940).

(5) G. F. Davidson, *J. Textile Institute*, **29**, T195 (1938).

(6) G. F. Davidson, *J. Textile Institute*, **27**, P162 (1936).

(7) A. Meller, *Chemistry & Industry*, 1204 (1953).

It was considered that if methylated oxidized cellulose behaved similarly to oxidized cellulose acetate it would be of interest to study "fragments" that might arise on hydrolysis of the methylated product since, although the non-sugar components that could be expected from the action of acid on the hexoses were known[8] and hence no importance would be attached to these compounds, it seemed that the presence of methoxy groups in the "fragments" might serve to stabilize them and also the position of a methoxy group in a "fragment" should help to establish its original position in the cellulose chain.

It was also considered that an examination of the acidic fraction that could be obtained by the hydrolysis of methylated oxidized cellulose after the ketone groups had been reduced would be of interest.

Accordingly 30-g. lots of oxidized cellulose acetate were methylated[9] in an atmosphere of nitrogen with dimethyl sulfate and sodium hydroxide at slightly above room temperature. The methylated product still retained, although in somewhat decreased amounts, carboxylic acid and ketonic groups. The number of carboxylic acid groups, as determined from the halogen content of samples of the methylated products that had been treated with thionyl chloride to form the acid chloride, varied from 0.37 to 1.0%. The carboxylic acid content was determined in one sample by directly titrating a chloroform solution of the methylated polysaccharide with sodium methoxide to the end-point of thymol blue[10] and was found to be in agreement with the figure obtained from the halogen determination. The methylated product before and after reduction showed changes in intrinsic viscosity completely analogous to those found with oxidized cellulose acetate.[8]

The methylated oxidized cellulose was hydrolyzed with 2 *N* HCl in the presence of 2,4-dinitrophenylhydrazine and the insoluble hydrazones separated by filtration. A considerable portion of polymeric material (as indicated by the formation of a quite viscous solution in chloroform) was also found in the insoluble portion; however, this material was separable from the true hydrazones by extracting the mixture with benzene in which the polymeric material was insoluble. Initially the polymeric material was thought to consist solely of incompletely hydrolyzed polysaccharide. Further hydrolysis, however, with hydrochloric acid led to the separation of small amounts of colored material while at the same time the rest of the polysaccharide dissolved. The aqueous solution yielded 2,3,6-tri-*O*-methyl-α-D-glucose on concentration and extraction with ether. The insoluble material was not identified. The results could be accounted for by considering that the insoluble polymeric material consisted of a chain of methylated glucose units at the end of which chain a "fragment" that had not been removed by hydrolysis had reacted to form a hydrazone.

The hydrazones which were soluble in benzene were chromatographed and yielded a compound that on the basis of analysis and comparison of its infrared spectrum with an authentic sample proved to be the di-2,4-dinitrophenylhydrazone of 2-methoxymalonaldehyde. If a ketone group were on carbon 3 of a glucose molecule (hence carbon 2 would be methylated), the compound could be expected to result from a split in the carbon chain between carbons 3 and 4.

When the methylated oxidized cellulose was reduced with sodium borohydride, ketonic groups were no longer found.[10] On the other hand there was little if any change in the number of carboxylic acid groups (one sample showed 0.37 and 0.31%, respectively[10]). The reduced methylated oxidized cellulose was hydrolyzed with formic acid in the manner used for the hydrolysis of alginic acid[11] and the acidic components of the hydrolysate isolated as the barium salts.

The free acids were obtained from the barium salts by the use of ion exchange resins and the methyl glycosides of the methyl esters formed and then reduced with lithium aluminum hydride. Lithium aluminum hydride is known to reduce the methyl esters; on the other hand it will not reduce a methyl glycoside[12] and hence the reduction could be expected to yield the methyl methylated glycoside corresponding to the methylated uronic acid. Chromatography led to the isolation and identification of the β-methyl glycoside of 2-*O*-methyl-D-glucose, which would indicate the presence of a 2-*O*-methyl-D-glucuronic acid in the reduced methylated oxidized cellulose. This compound could arise from the reduction of the corresponding 3-*keto* compound since the 3-*keto*uronic acid would only be methylated in the 2-position and would on reduction with sodium borohydride yield a mixture of 2-*O*-methyl-D-glucuronic and 2-*O*-methyl-D-alluronic acids. Since the methyl glycoside methyl esters were formed and then reduced with lithium aluminum hydride, it might be expected that both the α- and β-forms of 2-*O*-methyl-D-allose and 2-*O*-methyl-D-glucose would be found on chromatography. Interestingly enough four zones were found on chromatography of the acetylated mixture and one of these zones, although it contained a negligible amount of material, fell in a position corresponding to methyl-2-*O*-methyl-α-D-glucose triacetate. It might be anticipated that two of the zones consist of the corresponding α- and β-forms of allose.

It is of more than considerable interest that Lindberg and Theander[13] have been able to isolate and identify D-allose in the hydrolysate obtained from cellulose which had been first oxidized with chromate and subsequently reduced with borohydride. No D-allose was found in unoxidized

(8) F. A. H. Rice and L. Fishbein, THIS JOURNAL, **78**, 3731 (1956).

(9) W. N. Haworth, E. L. Hirst, L. N. Owen, S. Peat and F. J. Averill, *J. Chem. Soc.*, 1885 (1939); W. N. Haworth, E. L. Hirst and H. A. Thomas, *ibid.*, 821 (1931); W. N. Haworth and H. Machemer, *ibid.*, 2270 (1932); W. N. Haworth, R. E. Montonna and S. Peat, *ibid.*, 1899 (1939); G. G. Johnston, THIS JOURNAL, **63**, 1043 (1941).

(10) We are pleased to acknowledge our indebtedness to Dr. A. Patchornik of the National Institutes of Health for the determination.

(11) S. K. Chanda, E. L. Hirst, E. G. V. Percival and A. G. Ross, *J. Chem. Soc.*, 1833 (1952).

(12) M. Abdel-Akher and F. Smith, *Nature*, **166**, 1037 (1950); B. Lythgoe and S. Trippett, *J. Chem. Soc.*, 1983 (1950).

(13) B. Lindberg and O. Theander, *Acta Chem. Scand.*, **11**, 1355 (1957).

cellulose. It would seem that there is little doubt that for some reason, oxidation of cellulose leads to a preferential oxidation at carbon 3 as compared to carbon 2 in the glucose monomer of cellulose. There is also evidence[13] that carbon 6 is readily oxidized. Although oxidation at carbon 3 to form a 3-*keto*-D-glucose in the cellulose chain might explain the general sensitivity of oxidized cellulose to agents such as dilute sodium hydroxide, we consider that the formation of a 3-*keto*-D-glucose alone will not explain our results on the decarboxylation of oxidized cellulose. The results reported here indicate that to some extent in any case, chromate oxidation of cellulose leads to the oxidation of carbons 3 and 6 on the same glucose monomer.

A determination of the carboxylic acid and ketone groups in celluloses that had been oxidized under various conditions[14] have shown that in many cases, particularly when the cellulose is oxidized under mild conditions, there was a one-to-one ratio between carboxylic acid and ketone groups (*e.g.*, purified cotton; 0.04% carboxylic acid and 0.04% ketone; cotton oxidized by hypochlorite at *p*H 6.8; 0.21% carboxylic acid and 0.22% ketone). It is tempting therefore to consider that mild oxidation of cellulose leads to the formation of ketone and carboxylic groups on the same glucose monomer and that this particular form of oxidation is responsible for the chemical lability of oxidized cellulose. It is known that under certain conditions an aldonic lactone is reduced to the corresponding alcohol[15] and it is therefore possible that treatment of oxidized cellulose with potassium borohydride in an aqueous medium not only leads to the reduction of the 3-*keto* groups but also the carboxylic acid, perhaps *via* the lactone. This would make it possible to isolate D-allose from the hydrolytic products of reduced oxidized cellulose as reported by Lindberg and Theander. When the oxidized cellulose, as the methylated derivative, was reduced in dioxane solution certainly very few, if any, carboxylic acid groups were lost and hence in our case it was possible to isolate, after reduction, the 2-*O*-methyluronic acid. It is also possible that both 3-*keto*-D-glucoses and 3-*keto*-D-glucuronic acids are formed as separate entities in the cellulose chain as a consequence of oxidation and that the uronic acid was either removed as a barium salt when the acid hydrolysate was neutralized with barium carbonate, or destroyed by the enzyme, and hence not noted in the chromatograms of Lindberg and Theander.

## Experimental

Viscosities were measured with a modified Ubbelohde type viscometer[16] with a flow time of 118.6 sec. for dioxane. Solutions of approximately 1% (g./100 ml.) concentration were made by dissolving an accurately weighed sample in an accurately weighed quantity (approximately 25 ml.) of solvent. Dilutions were made directly in the viscometer by adding weighed quantities of solvent. The temperature was maintained at 25 ± 0.1°.

**Methylation of Oxidized Cellulose Acetate.**—Oxidized cellulose acetate[17] (30 g.) was dissolved in 500 ml. of acetone and methylated[9] in an atmosphere of nitrogen, with dimethyl sulfate and sodium hydroxide. Ten portions of dimethyl sulfate (15 ml.) and 30% sodium hydroxide (30 ml.) were added concurrently at approximately equal intervals over a period of eight hours and the mixture stirred with an efficient mechanical stirrer. No attempt was made to control the temperature by external cooling, but the rate at which the reagents were added was such that the temperature was maintained between 40 and 45°. After the addition of the reagents, stirring was continued for a period of 24 hours. At the end of this time the acetone was removed under reduced pressure at room temperature, the mixture acidified to *p*H 3 (alkacid test paper[18]) with aqueous sulfuric acid (1:1 by vol.) and the insoluble material which consisted essentially of methylated oxidized cellulose and sodium sulfate immediately separated by filtration on a fritted glass Büchner funnel. The insoluble material was washed with approximately 500 ml. of petroleum ether (b.p. 30–60°) and then dried under reduced pressure (mechanical pump). The methylated oxidized cellulose was separated from the inorganic salts by extracting the dried insoluble material with chloroform (400 ml. × 5) and then blowing off the chloroform by a stream of air to yield a thick film. The film was redissolved in 500 ml. of chloroform and remethylated with sodium hydroxide and dimethyl sulfate as above without, however, the addition of acetone.

Final purification of the methylated oxidized cellulose was achieved by dissolving the methylated product in 500 ml. of dioxane, filtering the solution through a bed of Celite[19] and precipitating the methylated product by pouring the dioxane solution into 1 to 2 liters of petroleum ether (b.p. 30–60°). The final product was dried in a high vacuum; yield 9–10 g. (50–60% of theoretical). A small amount of additional material could be obtained by extracting the aqueous filtrate from the first methylation with chloroform, $[\alpha]^{22}D$ −14° (dioxane, *c* 1).

*Anal.* C, 50.25; H, 7.75; ash, 1.1; OMe,[20] 43; intrinsic viscosity, 0.75.

**Reduction of Methylated Oxidized Cellulose with Sodium Borohydride.**—Ten grams of methylated oxidized cellulose was dissolved in 100 ml. of dioxane (freshly distilled from sodium metal), 10 ml. of water was added together with a large excess of sodium borohydride (1 g.) and the mixture stirred at room temperature for a period of approximately 48 hours. The mixture was then acidified in order to destroy the excess sodium borohydride, by adding 10 ml. of glacial acetic acid drop by drop and 200 ml. of water. The reduced methylated oxidized cellulose was separated by extracting the aqueous solution with chloroform (25 ml. × 5). The chloroform solution was washed with a small amount of water (10 ml. × 2) and evaporated to dryness in a current of air. The film that formed was redissolved in 10 ml. of chloroform, filtered through a fritted glass Büchner funnel and poured into 1 liter of petroleum ether (b.p. 30–60°). The precipitate of reduced methylated oxidized cellulose was collected by filtration; $[\alpha]^{22}D$ −8.6° (dioxane, *c* 1), intrinsic viscosity 0.76.

**Acetylation of Methylated Oxidized Cellulose and Reduced Methylated Oxidized Cellulose.**—One gram of methylated oxidized cellulose was suspended in a mixture of 20 ml. of pyridine and 30 ml. of acetic anhydride and stirred at room temperature for 24 hours. The mixture was then poured into ice and water (500 ml.). After several hours the product was extracted into chloroform and the chloroform removed in a current of air at room temperature. The product, a film, was redissolved in chloroform (10 ml.) filtered and precipitated as a fibrous mass by pouring it into 10 volumes of petroleum ether (b.p. 30–60°). The methylated oxidized cellulose had $[\alpha]^{22}D$ −15.7° (dioxane, *c* 1) after acetylation. The reduced methylated oxidized

(14) E. D. Stalkcheeva-Kaverzneva, *Doklady Akad. Nauk SSSR.*, **68**, 865 (1949); *C. A.*, **44**, 1257 (1950).

(15) M. L. Wolfrom and H. B. Wood, THIS JOURNAL, **73**, 2933 (1951).

(16) W. E. Davis and J. H. Elliott, *J. Colloid Sci.*, **4**, 313 (1949); S. Rothman, R. Simha and S. G. Weissberg, *J. Polymer Sci.*, **5**, 141 (1950); S. G. Weissberg, R. Simha and S. Rothman, *J. Research Natl. Bur. Stand.*, **47**, 298 (1951).

(17) Obtained through the courtesy of Mr. C. H. Penning of Eastman Products, Inc., Kingsport, Tenn.

(18) Obtained from the Fisher Scientific Co., New York, N. Y.

(19) No. 535 obtained from the Johns-Manville Co., New York, N. Y.

(20) D. O. Hoffman and M. L. Wolfrom, *Anal. Chem.*, **19**, 225 (1947).

cellulose after acetylation had $[\alpha]^{22}D$ −10.5° (dioxane, *c* 1), intrinsic viscosity, 0.70.

*Anal.* Product from methylated oxidized cellulose: OMe, 43; OAc,[21] 0.19; Product from reduced methylated oxidized cellulose: OMe, 43; OAc, 0.76.

**Preparation of Acid Chlorides.**—The acid chlorides were prepared by treating a solution of the polysaccharide in dioxane with thionyl chloride[3] and precipitating the resulting product by pouring the solution into petroleum ether (b.p. 30–60°). The acid chlorides obtained from one sample had the following properties:

| | $[\eta_{sp}/c]$ | $[\alpha]^{22}D$ | Cl, % |
|---|---|---|---|
| Methylated | 0.76 | +10.5 | 1.05 |
| Methylated and acetylated | .74 | +10.6 | 1.06 |
| Methylated and reduced | | +10.5 | 0.98 |
| Methylated, reduced and acetylated | .76 | + 4.1 | 1.04 |

The chloride content of the methylated oxidized cellulose acetate and the derivatives that were obtained from it varied with each lot of oxidized cellulose that was methylated (0.3 to 1%). Apparently some degradation occurred during methylation that was not possible to control.

**Hydrolysis of Methylated Oxidized Cellulose in the Presence of 2,4-Dinitrophenylhydrazine.**—Methylated oxidized cellulose (5. g.) in the form of a fine powder was suspended in 500 ml. of 2 *N* HCl containing 1 g. of 2,4-dinitrophenylhydrazine that had been repeatedly washed with ether and then recrystallized several times from methanol. The mixture was stirred at room temperature for 72 hours and then heated to reflux temperature for 1 hour. The mixture was then filtered on a fritted glass Büchner funnel and the insoluble material washed with distilled water until the filtrate reacted neutral to alkacid test paper[18] (approximately 1 liter). The filtrate was extracted 5 to 6 times in a separatory funnel with 200-ml. portions of benzene and the benzene solution evaporated under reduced pressure at room temperature to dryness to give fraction A, weight approximately 300 mg. The insoluble material that had been removed by filtration was extracted ten times with 25-ml. portions of benzene and the extracts combined and evaporated to dryness under reduced pressure at room temperature to give fraction B, weight 300 mg. The material insoluble in benzene (fraction C) was apparently polymeric in nature, since it formed a highly viscous solution. It weighed approximately 1 g.

**Chromatography of Fraction A.**—Three-hundred milligrams of fraction A dissolved in 20 ml. of benzene was chromatographed on a mixture of silicic acid[22] and Celite[19] (3:1 by weight) contained in a 3.3 by 45 cm. column. The column was developed with 600 ml. of a mixture of benzene and *n*-heptane (1:1 by volume). Seven zones were discernible at the following distances (in centimeters) from the top of the column. 1, 0–1; 2, 1.0–3.5; 3, 3.5–5.5; 4, 7.0–9.0; 5, 10–13; 6, 17–21; 7, 25–28. The contents of each zone was eluted with ethanol and the ethanol extract concentrated to dryness at room temperature under reduced pressure. Only zones 2, 3 and 6 could be obtained crystalline. Zone 2 melted at 197–200° and analyzed for 2,4-dinitrophenylhydrazine. Comparison of the infrared spectrum with that of authentic 2,4-dinitrophenylhydrazine completed the identification of the compound. Zone 3 melted at 152° and analyzed for $C_9H_{10}O_4N_4$. By comparing its infrared spectrum with a known compound it was identified as the 2,4-dinitrophenylhydrazone of acetaldehyde. Zone 6 melted at 164° and analyzed for $C_7H_6O_4N_4$. A comparison of infrared spectra completed its identification as the 2,4-dinitrophenylhydrazone of formaldehyde.

**Chromatography of Fraction B.**—An amount of 300 mg. of fraction B in benzene was chromatographed on a 3.3 cm. × 45 cm. column. The column was developed with 1500 ml. of a mixture of *n*-heptane and benzene (3:7 by vol.). Four zones were observed at the following distances (cm.) from the top of the column; 1, 0–3; 2, 5–8; 3, 11–20; 4, 24–40. The contents of each zone was eluted with ethanol and the ethanol solution concentrated to dryness at room temperature under reduced pressure. The contents of zones 1, 2 and 3 gave a blue color when a drop of 10% aqueous KOH was added to a dilute ethanolic solution of the zone indicating the presence of a di-2,4-dinitrophenylhydrazone. Zones 1 and 2 contained insufficient material (a sirup) to attempt any identification. Zone 3, however, although it could not be crystallized, was obtained as an amorphous powder (m.p. 90° dec.) after it had been precipitated several times from chloroform by the addition of petroleum ether (b.p. 30–60°). Analyses suggested that it was the hydrazone of 2-methoxymalonaldehyde. Comparison with an authentic sample (see below) of the di-2,4-dinitrophenylhydrazone of 2-methoxymalonaldehyde completed its identification.

**Preparation of an Authentic Sample of the 2,4-Dinitrophenylhydrazone of 2-Methoxymalonaldehyde.**—3-*O*-Methyl-α-D-glucose[23] (m.p. 157°) was prepared after the manner of Irvine and co-workers and was reduced in aqueous solution with excess sodium borohydride to yield 3-*O*-methyl-D-glucitol. The aqueous solution was acidified with glacial acetic acid, passed through ion exchange resins[24,24a] and concentrated to dryness. The 3-*O*-methyl-D-glucitol was obtained as a sirup, $[\alpha]^{20}D$ +3° ($H_2O$, *c* 2).

*Anal.* Calcd. for $C_7H_{16}O_6$: C, 42.85; H, 8.22; OMe, 15.82. Found: C, 42.85; H, 7.76; OMe, 15.42.

Neither acetylation nor benzoylation yielded crystalline derivatives; treatment with benzaldehyde did, however, yield a crystalline dibenzylidene derivative.

**Preparation of Dibenzylidene 3-*O*-Methyl-D-glucitol.**—Five hundred milligrams of 3-*O*-methyl-D-glucitol was dissolved in 2 ml. of freshly distilled benzaldehyde to which had been added 1 drop (from a 1-ml. capacity pipet) of *concd.* sulfuric acid. The mixture was allowed to stand overnight at room temperature and then diluted with 100 ml. of anhydrous diethyl ether. The acid was removed by shaking the ether solution first with saturated aqueous sodium bicarbonate (5 × 50 ml.) and then with water (5 × 50 ml.). The ether phase was concentrated to a thick sirup at room temperature under a high vacuum and the sirup extracted several times with petroleum ether (b.p. 30–60°). The insoluble residue was crystallized from a mixture of ether and *n*-heptane and recrystallized from ethanol, m.p. 130–131°, $[\alpha]^{20}D$ +24° (EtOH, *c* 3).

*Anal.* Calcd. for $C_{21}H_{24}O_6$: C, 67.8; H, 6.5. Found: C, 67.1; H, 6.6.

The 3-*O*-methyl-D-glucitol was treated with lead tetraacetate in glacial acetic acid. After 10–15 minutes the solution was treated with hydrogen sulfide (gas) and filtered. The filtrate was concentrated to dryness at room temperature under a high vacuum and yielded an oil which when treated with a solution of 2,4-dinitrophenylhydrazine in 2 *N* HCl gave a product which could not be obtained crystalline but after being several times precipitated from a chloroform solution by the addition of petroleum ether (b.p. 30–60°) had m.p. 90° dec.

*Anal.* Calcd. for $C_{16}H_{14}O_9N_8$: C, 41.6; H, 3.05; N, 24.2. Found: C, 41.0; H, 2.95; N, 23.7.

The product had the same chromatographic properties as the product obtained from the hydrolysis of the methylated oxidized cellulose and also showed the same infrared absorption spectrum (Fig. 1).

**Hydrolysis of Methylated Oxidized Cellulose after it had been Reduced with Sodium Borohydride.**—An amount of 100 g. of methylated oxidized cellulose, which had first been reduced with sodium borohydride (see above) was dissolved in 1500 ml. of 98% formic acid and heated under reflux for 36 hours.[11] The solution was then concentrated to dryness at room temperature under reduced pressure and the thick sirup that resulted further dried overnight in a high vacuum over solid sodium hydroxide. The sirup was then dissolved in 100 ml. of saturated aqueous barium hydroxide. Additional saturated barium hydroxide was added from time to time over a period of 1 to 2 hours, until the *p*H remained constant at *p*H 8 (test paper). The solution, now approximately 600–700 ml., was allowed to stand at room temperature for 5 to 6 hours, and was then extracted in a separatory funnel with 1500 ml. (5 × 300 ml.) of chloroform (fraction I). The aqueous phase was filtered, treated with carbon

(21) A. Chaney and M. L. Wolfrom, *Anal. Chem.*, **28**, 1614 (1956).

(22) Merck reagent grade.

(23) J. C. Irvine and T. P. Hogg, *J. Chem. Soc.*, **105**, 1386 (1914); J. C. Irvine and J. Patterson, *ibid.*, **103**, 564 (1913).

(24) IR-120, a product of the Rohm and Haas Co., Resinous Products Division, Philadelphia, Penna.

(24a) Duolite A-4, a product of The Chemical Process Co., Redwood City, Calif.

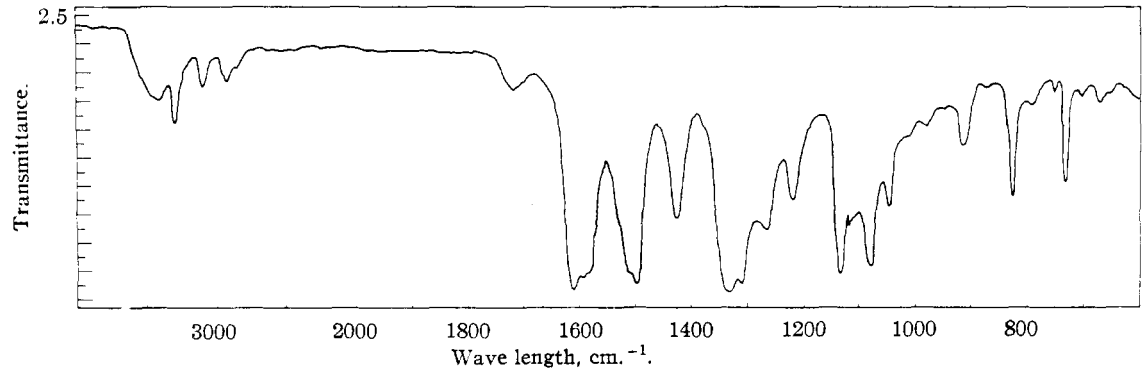


Fig.1.—Infrared absorption spectrum of the di-2,4-dinitrophenylhydrazone of 2-methoxymalonaldehyde in KBr pressed disk.

dioxide (gas), refiltered and then concentrated to dryness under reduced pressure at room temperature. The resulting material was extracted with chloroform (fraction II) and then with ethanol (fraction III). The material that was insoluble in ethanol and should consist of barium salts was dissolved in 100 ml. of water and passed through an ion exchange resin[24] to remove the barium and thus liberate the free acids. The ion exchange resin was washed with an additional 1200–1300 ml. of water which was added to the solution that had first passed through the exchange resin and the solution was concentrated to dryness at room temperature under reduced pressure. The resulting viscous sirup was soluble in ethanol or methanol. Essentially all the material could be precipitated from 5 ml. of ethanol on the addition of a 10% ethanolic NaOH solution and consisted therefore in all probability solely of a mixture of uronic acids.

**Preparation of the Methyl Glycoside Methyl Esters of the Uronic Acids.**—The sirupy (between 75–100 mg.) mixture of uronic acids was then dissolved in 250 ml. of anhydrous methanol and 1 ml. of concd. sulfuric acid added. After standing for 24 hours at room temperature, the sulfuric acid was neutralized with barium carbonate and the solution filtered and concentrated to dryness at room temperature under reduced pressure.

**Reduction of the Methyl Ester Groups of the Methyl Glycoside Methyl Esters with Lithium Aluminum Hydride.**—The mixture of the methyl esters (approximately 500 mg.) was dissolved in 15 ml. of anhydrous tetrahydrofuran and excess lithium aluminum hydride (125 mg.) added.[12] The solution was allowed to stand in an atmosphere of nitrogen for two hours at room temperature and then the excess lithium aluminum hydride was decomposed by the addition of glacial acetic acid (10 ml.) followed by the addition of water (10 ml.). The mixture (without separating the insoluble precipitate) was concentrated to dryness at room temperature under reduced pressure and dried overnight under a high vacuum over phosphorus pentoxide.

**Acetylation of the Mixture of Reduced Uronic Acids.**—Four milliliters of acetic anhydride together with 5 ml. of pyridine were added to the dried powder obtained from the reduction with lithium aluminum hydride described above and the mixture allowed to stand for 24 hours at room temperature. The solution was then poured into 250 ml. of ice-water and after standing for 4 to 6 hours extracted in a separatory funnel with chloroform (100 ml. × 5). The chloroform extract was dried over *anhydrous* sodium sulfate and then concentrated to dryness under reduced pressure at room temperature. The sirup that resulted was redissolved in 25 ml. of chloroform and treated with 5 ml. of a 50% aqueous solution of cadmium chloride to remove traces of pyridine, the chloroform layer was separated, dried over *anhydrous* sodium sulfate and concentrated to dryness; weight 300–600 mg.

**Chromatography of the Material Obtained by the Reduction and Acetylation of the Methyl Glycoside Methyl Esters of the Uronic Acid Fraction.**—Approximately 500 mg. of the reduced and acetylated material was dissolved in 50 ml. of benzene and chromatographed on a mixture of Magnesol[25] and Celite[19] (5:1 by weight, 7 × 32 cm. column) using 4 liters of benzene containing 0.5% (by vol.) of *t*-butyl alcohol, to develop the column. The column was extruded and streaked with alkaline permanganate.[26] Two zones were readily apparent; zone 1, in the upper half of the column, and zone II, in the lower half of the column. Two other zones, one between zone I and II and the other between zone II and the bottom of the column, could also be seen. The zones were eluted with ethanol and the ethanol extract concentrated to dryness under reduced pressure at room temperature. Some inorganic material derived from the column was removed by dissolving the ethanol eluate of the zone in benzene or ether, filtering and again concentrating to dryness.

**Identification of the Contents of Zone I as Methyl 2-*O*-Methyl-β-D-glucose Triacetate.**—The contents of zone I (a sirup, weight approximately 100 mg.) had an optical rotation of $[\alpha]^{22}D$ +6° (chloroform, *c* 1) and analyzed for the triacetate of a monomethyl, methyl glycoside.

*Anal.* Calcd. for $C_{14}H_{22}O_9$: C, 50.34; H, 6.63; OMe, 18.5; OAc, 9 ml. of 0.1 *N* NaOH per 100 mg. Found: C, 50.93; H, 7.02; OMe, 18.2; OAc, 8.5 ml. of 0.1 *N* NaOH per 100 mg.

The optical rotation is that given for methyl 2-*O*-methyl-tri-*O*-acetyl-β-D-glucopyranoside.[27] The substance was deacetylated by dissolving 50 mg. in 10 ml. of acetone, adding 25 ml. of 0.1 *N* NaOH and allowing the solution to stand overnight at 0°. The acetone was removed at room temperature under reduced pressure and the aqueous solution passed over an ion exchange resin[24] to yield approximately 20 mg. of a sirup that crystallized from ethyl acetate; m.p. 92–94°, $[\alpha]^{22}D$ −22° (EtOH, *c* 1) in agreement with the reported values for methyl-2-*O*-methyl-β-D-glucose[27] of m.p. 95–97°, $[\alpha]^{17}D$ −23.9°.

*Anal.* Calcd. for $C_8H_{16}O_6$: C, 46.47; H, 7.7. Found: C, 47.2; H, 7.5.

The methyl-2-*O*-methyl-β-D-glucose (15 mg.) was heated under reflux with 5 ml. of 0.2 *N* aqueous sulfuric acid[27,28] for 12 hours and then the acid removed by adding excess solid barium carbonate and filtering. The filtrate upon concentration under reduced pressure at room temperature yielded a sirup (2-O-Me-glucose) which upon treatment with phenylhydrazine and acetic acid at room temperature after the manner of Haworth, Hirst and Teece[28] gave a phenylhydrazone, m.p. 174°, indistinguishable from the phenylhydrazone prepared from 2-O-methyl-β-D-glucose.

**Chromatography of the Synthetic Methyl 2-O-Methyl-α- and β-D-glucopyranoside Triacetates.**—The methyl 2-*O*-methyl-α-D-glucopyranoside triacetate was prepared after the manner of Haworth, Hirst and Teece[28] and the methyl 2-*O*-methyl-β-D-glucopyranoside triacetate was prepared after the manner of Brigl and Schinle[27] and chromatographed on a mixture of Magnesol[25]–Celite[19] under the same conditions as used for the material originating from the uronic fraction of methylated oxidized cellulose. The α- and β-forms of the methyl 2-*O*-methyl-D-glucopyranoside triacetates were separable and the β-form corresponded in position

(25) A product of the Westvaco Chlor-Alkali Division of Food Machinery and Chemical Corp., South Charleston, W. Va. The regular grade was used.

(26) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, THIS JOURNAL, **67**, 527 (1945).

(27) P. Brigl and R. Schinle, *Ber.*, **62**, 1716 (1929).

(28) W. N. Haworth, E. L. Hirst and E. G. Teece, *J. Chem. Soc.*, 2858 (1931); W. J. Hickinbottom, *ibid.*, 3140 (1928); P. Brigl and R. Schinle, *Ber.*, **63**, 2884 (1930).

to that of the material originating from the methylated oxidized cellulose. Specifically, the β-form was found 4–6.2 cm. from the top of the column and the α-form 9.5 to 13 cm. from the top of the column.

**Examination of Fraction A.**—Fraction A, the chloroform extract of the aqueous solution of the hydrolyzed methylated oxidized cellulose was concentrated to dryness under reduced pressure at room temperature and then extracted with petroleum ether (b.p. 30–60°). The petroleum ether extract on being concentrated to dryness and treated with aniline in ethanol yielded the anilide of tetramethyl-D-glucose, m.p. 97°, in agreement with reported constants[29] (yield 10 mg.). The compound showed no depression in melting point on admixture with an authentic sample. The material that was insoluble in petroleum ether was dissolved in ether, filtered and allowed to stand several weeks at 0°. The solution deposited crystals (1 g.) which were identified as 2,3,6-tri-*O*-methyl-α-D glucose, m.p. 120–123°, $[\alpha]^{20}D$ +70° ($H_2O$, *c* 1),[30] after recrystallization from ether.

(29) J. C. Irvine and Agnes M. Moodie, *J. Chem. Soc.*, **93**, 95 (1908); M. L. Wolfrom and W. L. Lewis, THIS JOURNAL, **50**, 837 (1928).

(30) H. C. Carrington, W. N. Haworth and E. L. Hirst, *ibid.*, **55**, 1084 (1933).

*Anal.* Calcd. for $C_9H_{18}O_6$: C, 48.6; H, 8.12. Found: C, 48.7; H, 8.03.

**Examination of Fraction B.**—Fraction B was dissolved in ether, filtered and allowed to stand several weeks at 0°. The solution deposited crystals (16 g.) which were identified as 2,3,6-tri-*O*-methyl-α-D-glucose, m.p. 119–121°, $[\alpha]^{20}D$ +70° ($H_2O$, *c* 1). The filtrate from the crystals on being allowed to stand at 0° continued to deposit additional quantities of 2,3,6-tri-*O*-methyl-α-D-glucose over a period of weeks.

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[CONTRIBUTION FROM THE BOTANICAL INSTITUTE, FACULTY OF SCIENCE, UNIVERSITY OF TOKYO]

# A New Phenolic Glycoside in *Viburnum furcatum* Blume[1]

BY SHIZUO HATTORI AND HIDEMASA IMASEKI

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A phenolic glycoside was isolated from the leaves of *Viburnum furcatum* Blume. The chemical structure of the glycoside has been shown to be *p*-vinylphenol D-apio-(D or L)-furanosyl-1,6-β-D-glucopyranoside, and the name "furcatin" is proposed for it.

In the course of experiments designed to isolate chlorogenic acid isomers as the agents responsible for the color change to brown of the leaves of the genus *Viburnum* (Caprifoliaceae), a phenolic glycoside has been isolated from the leaves of *Viburnum furcatum* Blume. This glycoside is new and the authors wish to propose for it the name "furcatin."

Furcatin crystallizes as colorless needles of bitter taste and is very easily soluble in ethanol, methanol and water and insoluble in ether, benzene and petroleum ether. It is also soluble in ethyl acetate saturated with water, but is slightly soluble in water-free ethyl acetate. Concentrated sulfuric acid dissolved furcatin and gave a deep red solution which did not fluoresce under ultraviolet light. Furcatin decolorized dilute potassium permanganate and bromine solutions, thus showing the presence of an aliphatic unsaturated bond in the molecule. On the other hand, negative reactions with ferric chloride or diazotized *p*-sulfanilic acid, 2,4-dinitrophenylhydrazine or acid fuchsin decolorized with sodium sulfite, hydroxylamine–ferric salt and hydroiodic acid by Zeisel's method indicated that furcatin did not possess free phenolic, carbonyl, carboxyl and methoxyl groups. Lead acetate did not give an insoluble lead salt with furcatin.

Furcatin was easily hydrolyzed by dilute mineral acids into reducing sugars and an oily aglycone, but was not hydrolyzable by apricot emulsin. The aglycone purified by steam distillation was a colorless oil of a peculiar aromatic odor and turned brown in the air in a few days. It has free phenolic hydroxyl group, since it reacted with diazotized sulfanilic acid and ferric chloride yielding orange and faint blue-violet colors, respectively. The aglycone also gave a deep red coloration with concd. sulfuric acid and a cornflower blue coloration with concd. hydrochloric acid. By catalytic hydrogenation, one mole of furcatin absorbed one mole of hydrogen. This also showed that furcatin has a double bond.

Methylation of the aglycone with dimethyl sulfate gave an oily methyl ether of an anise-like odor, which yielded *p*-methoxybenzoic acid and an alkaline-soluble, acid-insoluble gas by oxidation with dilute potassium permanganate. This indicates that the aglycone is a simple phenol which has an unsaturated side chain at the *p*-position. Although the gas evolved was not examined qualitatively, it must be carbon dioxide. Oxidation of the methyl ether with Beckmann mixture and ozone gave anisaldehyde and formaldehyde. Therefore it can be deduced that the carbon dioxide or formaldehyde formed by oxidation of the methyl ether arose from a terminal carbon atom of the two-carbon side chain. On the other hand, the carboxyl carbon atom of anisic acid is derived from the second carbon atom of the side chain. These results mean that the aglycone has a vinyl group as a side chain at the *p*-position. Therefore, it should be considered that the aglycone was *p*-vinylphenol.

H. Schmidt and P. Karrer[2] are the first to isolate from plant material (*Papaver somniferum* L.)

(1) Part of a thesis by H. Imaseki in partial fulfillment of the requirement for a D.Sc. degree, December, 1958.

(2) H. Schmidt and P. Karrer, *Helv. Chim. Acta*, **28**, 722 (1945).