made. The conditions and results were similar to those for 2-ketogluconic acid. The presence of 2,5-diketogluconic acid was again indicated by the presence of a yellow spot on chromatograms developed in developer C. The spot had an $R_g$ value of 0.6 and gave a yellow color with *p*-anisidine hydrochloride. The acid was very unstable and neither the free acid nor the calcium salt could be isolated. Therefore positive identification was impossible. The quantity of this acid in the oxidation solutions was probably very small.

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# Assignment of Structure to Cellulose 3,6-Dinitrate[1]

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A cellulose dinitrate, degree of substitution (D.S.) 1.72 (N, 10%), was prepared by the action of pyridine–hydroxylamine on the trinitrate (D.S., 2.88; N, 13.8%), and was methylated to a mono-*O*-methylcellulose dinitrate. Almost complete denitration then yielded an *O*-methylcellulose, D.S., 1.12, which on hydrolysis gave 2-*O*-methyl-D-glucose (84%) and 2,3-di-*O*-methyl-D-glucose (11%). Hence the nitrate groups in the original dinitrate were exclusively or almost exclusively in the third and sixth positions of the cellulose.

Although cellulose trinitrate was rapidly degraded to an amorphous powder by solution in dry pyridine at room temperature, the presence of an excess of free hydroxylamine base markedly altered the course of the reaction[2]; almost exactly one molar equivalent of nitrogen gas of 99% purity was evolved, and the near-white, fibrous product had the composition of a dinitrate (D.S. 1.7). Since the product had a degree of polymerization of about 120, not more than 1.7% of the original glucosidic bonds had been cleaved, and the reaction was therefore a denitration rather than a degradation. This dinitrate was remarkable not only in being soluble in an unusually wide range of organic liquids, but also in its relatively great stability toward alkali. It could be methylated with dimethyl sulfate and 30% sodium hydroxide to soluble, brittle white fibers with unchanged nitrate substitution and only a moderate decrease in intrinsic viscosity (Table I). The same stability, however, defeated early attempts to determine the structure of the mono-*O*-methylcellulose dinitrate by denitration to the corresponding methylcellulose. The nitrate groups could not be removed by hydrogenation over a palladium–calcium carbonate catalyst,[2,3] while reduction with iron and acetic acid,[4] or reductive acetylation with zinc and acetic anhydride,[5] yielded highly degraded gums. Very little denitration was achieved by employing a low concentration (3%) of ammonium hydrosulfide in aqueous acetone at less than 20°, as recommended by Bock,[6] Riechel[7,8] and their respective coworkers. An increase in the concentration of the hydrosulfide led to increased decomposition, and to the formation in large amounts of mercaptans and other sulfur compounds (perhaps condensation products of the acetone and ammonium hydrosulfide) which could not be effectively separated from the product. These experiments have been omitted from the present article.

TABLE I

YIELDS, VISCOSITIES AND DEGREES OF SUBSTITUTION IN THE CELLULOSE TRINITRATE, *O*-METHYLGLUCOSE SEQUENCE

| Cellulose | Yield,[a] % | $NO_3$ D.S. | $OCH_3$ D.S. | $[\eta]$,%[b] |
|---|---|---|---|---|
| Trinitrate | .. | 2.88 | 0 | 18.7 |
| 3,6-Dinitrate | 99 | 1.72 | 0 | 1.22[c] |
| Methyl dinitrate | 95 | 1.70 | 1.22 | 0.24 |
| Monomethyl | 91 | 0.14 | 1.12 | 0.21[d] |
| Methylglucoses | 89 | .. | 1.16 | .. |
| 2-*O*-Methyl[e] | 84 | .. | 1.0 | .. |
| 2,3-Di-*O*-methyl[e] | 11.3 | .. | 2.0 | .. |
| 2,6-Di-*O*-methyl[e] (?) | 1.7 | .. | 1.97 | .. |

[a] From preceding substance, corrected for change in D.S. [b] Intrinsic viscosities in ethyl acetate uncorrected for variability in nitrate substitution; observed relative viscosities corrected for kinetic energy effect. [c] After renitration, presumably without degradation, to D.S. 2.75. [d] In cuprietylenediamine, according to K. Wilson, *Svensk Papperstidn.*, **55**, 125 (1952). [e] As percentage of methylglucose mixture.

Satisfactory denitrations eventually were achieved in aqueous dioxane by using a large excess of ammonium hydrosulfide in high concentration, and by adding water to the system to keep the product in solution for the most part as denitration proceeded. The reaction was slow and required more than 24 hours, perhaps because it was not catalyzed sufficiently by polysulfide ions.[9] The methylcellulose was isolated as a light yellow, degraded powder which was partially soluble in water and completely so in 4% sodium hydroxide. The intrinsic viscosity (Table I), however, suggested that the additional degradation incurred during the denitration was not severe. No way was found to eliminate a residual nitrogen content of about 1% from the product, and this negative result was in accord with those of other denitra-

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tions.[10,11] Although the methylcellulose, D.S. 1.12, appeared to dissolve in a 4:3 mixture of carbon disulfide and acetone, the system formed proved to be only a very highly swollen gel. A significant variation in the ratio of carbon disulfide to acetone decreased the swelling, and little or none occurred with either liquid when tested separately. It was interesting to note that carbon disulfide and acetone absorbed the maximum amount of heat when mixed in equal amount,[12] or in about the proportion necessary to cause maximum swelling in the above case. A 4:3 mixture of carbon disulfide and ethanol also produced great swelling, although ethanol itself had little effect. The probable relationship between the swelling and solubility of polymers and the "internal pressure" or "cohesive energy density" of solvent mixtures was adequately discussed elsewhere.[13]

The hydrolysis of the methycellulose was with hydrochloric or sulfuric acid, and the best yield of partly methylated glucoses was 89% of theory. Partition chromatography of the sugars was carried out on a column of powdered cellulose with a mixture of methyl ethyl ketone–ethanol–water as the eluting solvent. The efficiency of this system compared favorably with that of the better-known mixture based on butanol. The hydrolyzate proved to contain only one mono-*O*-methylglucose, the 2-isomer, which was isolated in a crystalline condition and which was thoroughly identified; 2,3-di-*O*-methyl-D-glucose was identified as the crystalline anilide, and a more mobile isomer was present in small amount. Since the hydrolyzate of the methylcellulose contained 84% of 2-*O*-methylglucose and 11.3% of 2,3-di-*O*-methylglucose (Table I), no less than 95% of the methyl groups occupied the second positions in the parent methylcellulose. These were the positions from which pyridine–hydroxylamine had removed nitrate groups from the cellulose trinitrate; this reaction therefore had a high degree of selectivity and yielded what was substantially 3,6-cellulose dinitrate. The di-*O*-methylglucoses probably originated from free hydroxyl groups in the cellulose "trinitrate" whose substitution was only 2.88 instead of 3.00. If it were assumed that all the nitrate groups in the second positions were eliminated, then the 1.7% of the unidentified di-*O*-methylglucose could only be the 2,6-isomer. Although a faint spot that might have corresponded to 2,3,6-tri-*O*-methylglucose was detected on one paper chromatogram, the failure of attempts to confirm this observation made it probable that all of the anhydroglucose units in the original cellulose suffered at least some nitration.

The 2-nitrate group in many glucose derivatives has been cleaved preferentially by various reagents. Thus the one in methyl-3,4,6-tri-*O*-acetyl-β-D-glucoside-2-nitrate was removed more easily by alkaline hydrolysis than was that in the isomeric 6-nitrate[14]; strong alkali, or sodium iodide in a ketonic solvent, or in methanol, acetic anhydride or pyridine, afforded 3-nitrate derivatives from the 2,3-dinitrates of various methyl glucosides[15–19]; sodium nitrite in aqueous ethanol was used with similar results.[17] Although the partial denitration of methyl β-glucopyranoside tetranitrate by pyridine–hydroxylamine was to some extent of a random nature, the nitrate group in the fourth, and not the second, position, was most affected.[20] No explanation is offered to account for the failure of methyl glucoside tetranitrate to serve as a model of cellulose trinitrate in this reaction, or for the much more specific behavior of the latter compound.

The above conversion of cellulose to pure, crystalline 2-*O*-methyl-β-D-glucose in 63% over-all yield can be recommended as a laboratory synthesis if adequate precautions are observed in the handling of the dangerously explosive, bone-dry cellulose trinitrate. Weygand and Trauth[21] obtained 2-*O*-methyl-β-D-glucose in 41% yield from 1,2-*O*-isopropylidene-D-glucofuranose, and earlier methods of preparation were reviewed.[22] The most recent involved a separation from a mixture of *O*-methylglucoses on the borate form of a strongly basic ion-exchange resin.[23]

## Experimental

**Materials.**—Published methods[2] were followed in preparing cellulose trinitrate (D.S. 2.88) from 25 g. of dry, de-waxed cotton linters and in causing 50 g. of the dry product to react with pyridine–hydroxylamine. Great care was taken in handling the dry trinitrate in order to reduce the risk of deflagration or detonation. A solution of 36 g. of the resulting cellulose 3,6-dinitrate in 2100 ml. of peroxide-free dioxane was then methylated in a nitrogen atmosphere by the slow, simultaneous addition of 173 ml. of 40% aqueous sodium hydroxide and of 230 ml. of dimethyl sulfate. The mono-*O*-methylcellulose 3,6-dinitrate was isolated[2] as short, yellow-brown fibers. The analytical methods were those previously used, and the previous analyses and intrinsic viscosities in ethyl acetate at 25° were confirmed for the above materials. No account was taken of the slight oxime substitution in calculating the nitrate D.S. (Table I).

Hydrogen sulfide gas, after being washed with water, was bubbled into 400 g. (450 ml.) of concentrated ammonium hydroxide (28% $NH_3$) at 5–10° until the weight had increased by 142 g. This solution had a concentration of ammonium hydrosulfide ($NH_4HS$) of approximately 39%, and the molar ratio of ammonia to hydrogen sulfide was about 1.6:1. Storage was at 5° in a bottle previously flushed with hydrogen sulfide gas.

**Denitration to a Methylcellulose.**—Dry dioxane, 190 ml. and free of peroxides, and 15 g. of the above mono-*O*-methyl cellulose 3,6-dinitrate were stirred together in a 1-l. three-necked flask until solution was almost complete after several hours. The flask was equipped with a mechanical stirrer and mercury seal. A highly swollen gel resulted when 375 ml. of the above solution of ammonium hydrosulfide was slowly added at room temperature, but almost all of this gel redissolved after stirring for 24 hr. The slightly turbid, orange solution was then diluted with 190 ml. of water and was stirred for a further 29 hr. before being centrifuged.

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A small, highly swollen deposit was separated, was washed with water, alcohol, acetone, carbon disulfide–acetone (4 vol.:3 vol.) and ligroin. Drying was *in vacuo* at room temperature over phosphorus pentoxide; yield of light yellow-green powder, 0.51 g.

*Anal.* Calcd. for cellulose substituted with 0.44 nitrate and 1.07 methoxyl groups: N, 3.13; $OCH_3$, 16.8. Found: N, 3.11, 3.16; $OCH_3$, 16.7, 17.0.

The clear mother liquor was concentrated at 45° under reduced pressure to about 100 ml. to eliminate most of the ammonium hydrosulfide, small amounts of butanol being added to prevent foaming. The addition of 250 ml. of ethanol precipitated much of the product, which was recovered on the centrifuge and was washed with two 100-ml. volumes of ethanol. Evaporation of the combined liquors *in vacuo* to 50 ml., and dilution with acetone, yielded a second fraction. After combining the two fractions, they were thoroughly washed with acetone and were exhaustively extracted with the carbon disulfide–acetone mixture. The transparent, orange gel was washed three times with ligroin before being dried; yield of light yellow powder, 9.4 g., or 90.8%.

*Anal.* Calcd. for cellulose substituted with 0.14 nitrate and 1.12 methoxyl groups: N, 1.07; $OCH_3$, 18.9. Found: N, 1.08, 1.06; $OCH_3$, 19.0, 18.8.

**Hydrolysis of the Methylcellulose.**—A 5-g. sample was stirred with 50 ml. of 72% sulfuric acid at 5° for 16 hr., after which time the dark sirup was diluted with 1150 ml. of cold distilled water (to 5% acid) and boiled under reflux for 10 hr. Neutralization of the cold liquor was effected partly with solid barium hydroxide and finally with barium carbonate. The filtrate and washings were clarified with adsorbent carbon, were concentrated, de-ionized by passage through a bed of Amberlite IR-4B and IR-120 exchange resins, and evaporated to a light yellow sirup which was thoroughly dried *in vacuo* over phosphorus pentoxide; yield, 4.75 g., or 89% on the basis of the methoxyl content.

*Anal.* Calcd. for a methylglucose of D.S. 1.16; $OCH_3$, 18.3. Found: $OCH_3$, 18.3, 18.4.

Five grams of the *O*-methylcellulose was also dissolved in 20 ml. of cold, 20% hydrochloric acid and after dilution with 90 ml. of water the solution was boiled under reflux for 7 hr. Boiling for a further 3 hr. produced no change in the optical rotation of the light brown liquor. The brown sirup isolated from this hydrolysis partly crystallized when diluted with a little ethanol, but excessive manipulation reduced the yield to 84% of the theoretical.

*Anal.* Found: $OCH_3$, 17.8, 18.0.

**Chromatography of the Hydrolyzate.**—A small amount of the sirup from the hydrolysis with sulfuric acid was deposited from methanol solution, together with adjacent reference spots of 3-*O*-methyl-D-glucose and glucose, on the starting line of a paper chromatogram. The chromatogram was developed with the upper layer of a mixture of butanol, ethanol, water and ammonia in the ratio 40:10:49:1[24] and was sprayed with aniline acid phthalate solution.[25] When $R_g$ 0.27 was assumed for the brown spot of 3-*O*-methyl-D-glucose, a brick-red spot corresponding to another mono-*O*-methylglucose occurred at $R_g$ 0.28, with other very faint spots at $R_g$ values of 0.16 (glucose) and of 0.40 and 0.45. No spot for 3-*O*-methyl-D-glucose could be detected.

Because development with the above solvent mixture required as long as 24 hr., and the butanol component might prove to be a persistent contaminant of fractions separated in column chromatography, a search was made for a better developing solvent. Finally, a mixture of ethanol (1 vol.) and methyl ethyl ketone saturated with water (4 vol.) was found to be about five times as fast as the previous mixture but was extremely sensitive to change in temperature. The presence of 0.1% of concentrated ammonium hydroxide produced more compact spots, but the solvent then discolored in a few days and was less stable than the butanol mixture. The methyl ethyl ketone system, however, produced a better separation of the *O*-methylglucoses, as evidenced by $R_f$ values of 0.11, 0.26, 0.46 and 0.60 for glucose, the mono-*O*-methyl-D-glucose and for two di-*O*-methyl-D-glucoses, respectively. Authentic 2,3-di-*O*-methyl-D-glucose showed a chromatographic behavior identical with that of the substance with $R_f$ 0.46.

A tightly packed 70 × 4 cm. cellulose column was washed with the ethanol–water-saturated methyl ethyl ketone (1:4 vol.) mixture, and 3.00 g. of the sirup from the above hydrolysis, diluted with a little methanol, was added. The progress of the elution with the methyl ethyl ketone mixture was followed in the eluates at 30- or 60-min. intervals by paper chromatography. Di-*O*-methyl-D-glucose was detected in the eluate after 8 hr., and after 19 hr. traces of mono-*O*-methylglucose appeared. After no more of this component was discharged, the fractions containing the individual sugars were concentrated to sirups, which were redissolved in small volumes of water. These solutions were clarified with adsorbent carbon, and on evaporation yielded colorless sirups. Drying was by adding and evaporating absolute ethanol, and finally *in vacuo* at 60°. Each sirup was shown by paper chromatography to give a single spot. The most mobile fraction, $R_f$ 0.60, 0.05 g. (1.7%), had a methoxyl content (29.4%) near the value for a di-*O*-methylglucose.

**2-*O*-Methyl-β-D-glucose.**—The sirup of $R_f$ 0.26, 2.37 g. (84%), crystallized solidly, and recrystallization from absolute ethanol yielded 2.17 g. with the methoxyl content (16.0%) of a mono-*O*-methylglucose. The m.p., 158–159°, and the specific mutarotation in water, $[\alpha]^{25}D$ +38° (5 min.) → 65° (final) (*c* 3.1%), agreed with the values reported for 2-*O*-methyl-β-D-glucose.[21,22] Condensation with phenylhydrazine first yielded the colorless, flat needles of the phenylhydrazone, with the correct methoxyl content (10.9%) and melting point of 176–77°[22]; then methoxyl-free D-glucosazone decomp. 204–205°; the corresponding phenyl glucosatriazole had m.p. 195–196°.[26]

**2,3-Di-*O*-Methyl-D-glucose.**—The sirup of $R_f$ 0.46, 0.34 g. (11.3%), had the methoxyl content ($OCH_3$, 29.8%) of a dimethylglucose, and the equilibrium specific rotation in water $[\alpha]^{20}D$ 63.3° (*c* 1.1%) agreed with that of the 2,3-isomer.[27] The anilide melted at 133–134°, the value reported[28] for the anilide of 2,3-di-*O*-methyl-D-glucose.

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