methylenediamine in 6 ml. of anhydrous ether was added to 7.4 g. (0.078 mole) of trifluoroacetonitrile. The excess nitrile was allowed to reflux for 45 min. then removed by distillation. Dry hexane (40 ml.) was added and the mixture filtered to give 4.4 g. (88%) of product. This solid was dissolved in 50 ml. of anhydrous ether followed by the addition of 70 ml. of hexane. After 34 hr., a white solid had formed on the bottom of the flask. It melted at 90–91.5°.

Anal. Calcd. for $C_{11}H_{18}N_4F_6$: C, 41.24; H, 5.66; N, 17.49; F, 35.59. Found: C, 41.21; H, 5.56; N, 17.65; F, 35.42.

The Hydrochloride Salt.—Dry hydrogen chloride was bubbled into a solution of 1.0 g. (0.0031 mole) of the free base in 5 ml. of anhydrous ethanol and 20 ml. of anhydrous ether. Then, 50 ml. of anhydrous ether was added to bring about the separation of a colorless oil. After 20 hr., as much as possible of the solvent was decanted. The remainder was removed under vacuum leaving a white solid. It appeared to be extremely hygroscopic and slowly evolved a gas when heated. The analytical sample was dried at 100° *in vacuo* over phosphorus pentoxide.

Anal. Calcd. for $C_{11}H_{20}N_4Cl_2F_6$: Cl, 18.03. Found: Cl, 18.31.

N,N-Dimethylethylenediamine. (a) **N-(β-Dimethylaminoethyl)trifluoroacetamidine** [$CF_3C(=NH)NH(CH_2)_2N(CH_3)_2$].—In a flask equipped with a Dry Ice–acetone reflux condenser and a magnetic stirrer was placed 3.1 g. (0.035 mole) of N,N-dimethylethylenediamine in 10 ml. of anhydrous ether. Trifluoroacetonitrile (5.1 g.; 0.054 mole) was distilled into the system. The nitrile was refluxed for 90 min., then removed. The solid product was recrystallized from chloroform and then sublimed to give 5.1 g. (78%) of a white substance melting at 108–109° (with sublimation). The solid was quite unstable, decomposing into a brown tar.

Anal. Calcd. for $C_6H_{12}N_3F_3$: C, 39.34; H, 6.60; N, 22.94; F, 31.11. Found: C, 36.60; H, 6.68; N, 22.63; F, 29.65.

The **hydrochloride salt** was prepared as follows: A solution containing 285 mg. (0.00156 mole) of the amidine dissolved in 50 ml. of anhydrous ether was added to a solution of anhydrous ether saturated with dry hydrogen chloride. Immediately a white precipitate formed. The mixture was stirred for 20 min. before the solvent was decanted. The product was dried under vacuum. The salt decomposed at approximately 205° (with evolution of a gas). It was hygroscopic.

Anal. Calcd. for $C_6H_{14}N_3Cl_2F_3$: C, 28.14; H, 5.10; Cl, 27.69; F, 22.26. Found: C, 25.38; H, 6.40; Cl, 27.5; F, 18.83.

N,N-Di-*n*-butylethylenediamine. (a) **N-(β-di-*n*-butylaminoethyl)trifluoroacetamidine** [$CF_3C(=NH)NH(CH_2)_2N(C_4H_9)_2$].—Trifluoroacetonitrile (10.5 g.; 0.11 mole) was distilled into a flask (equipped with a Dry Ice–acetone reflux condenser and a magnetic stirrer) containing 8.6 g. (0.05 mole) of N,N-di-*n*-butylethylenediamine dissolved in 10 ml. of anhydrous ether. The excess nitrile was allowed to reflux for 1 hr. and then removed. The solution, light yellow in color, was kept at about 4° for 2 hr. whereupon light yellow crystals formed. The filtrate was evaporated to yield more of the product. The solid was recrystallized from hexane, the analytical sample from a hexane–ether mixture (5:1). The over-all yield was 10.9 g. (81%) of material melting at 50–51°.

Anal. Calcd. for $C_{12}H_{24}N_3F_3$: C, 53.90; H, 9.05; N, 15.73; F, 21.32. Found: C, 53.65; H, 8.86; N, 15.53; F, 21.06.

After a few months, the solid had decomposed into a light yellow liquid.

The **hydrochloride salt** was prepared as above. It melted at 165.5–167°.

Anal. Calcd. for $C_{12}H_{26}N_3Cl_2F_3$: Cl, 20.81. Found: 20.84.

Reduction of the Products of Periodate Oxidation of Carbohydrates. XII. Methylation Studies on Cellulose Polyalcohol[1]

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Cellulose has been oxidized with sodium periodate and the resulting polyaldehyde reduced with sodium borohydride to cellulose polyalcohol. Methylation of this polyalcohol has yielded the corresponding trimethyl ether which upon hydrolysis gave 1,4-di-*O*-methylerythritol and methoxyacetaldehyde. A small proportion of racemic 1-*O*-methylerythritol was also produced, probably as a result of demethylation and its formation is not believed to be of any constitutional significance.

In previous communications[2,3] it was shown that the alcohols derived from the periodate oxidation products (the "dialdehydes") of methyl glycosides by reduction, undergo smooth methylation to give good yields of methylated derivatives whose structures were readily determined by hydrolysis and identification of the products so formed. Thus D′-methoxy-D-hydroxymethyl diethylene glycol, obtained[4] from methyl α-D-glucopyranoside by periodate oxidation followed by reduction, afforded the corresponding tri-*O*-methyl ether which upon hydrolysis furnished 1,3-di-*O*-methylglycerol and methoxyacetaldehyde. As a result of these studies it was pointed out[2,3] that the same general technique should be applicable to the polyalcohols derived from polysaccharides by periodate oxidation followed by reduction and that the procedure might prove useful for investigating the structure of polysaccharides. This technique, already applied to amylopectin polyalcohol,[5] has now been extended to cellulose.

Cellulose (I), in the form of defatted native

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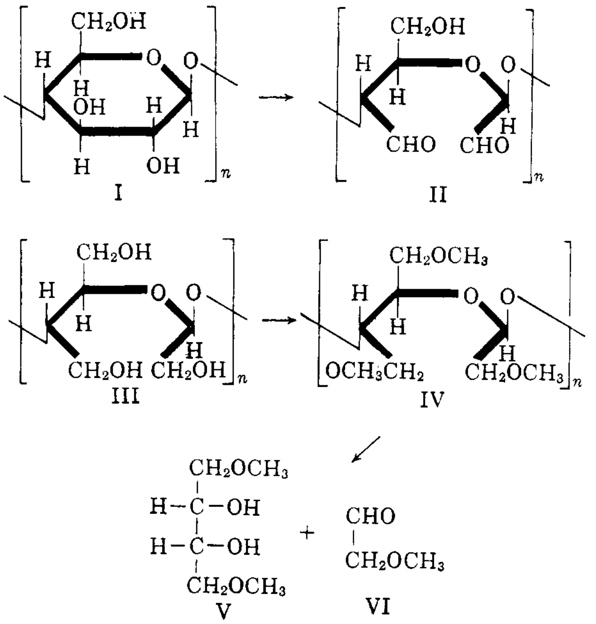
[1] Paper No. 4865 Scientific Journal Series, Minnesota Agricultural Experiment Station. This work was sponsored by the Office of Ordnance Research, U.S. Army.

[2] J. K. Hamilton, G. W. Huffman, and F. Smith, *J. Am. Chem. Soc.*, **81**, 2173 (1959).

[3] J. K. Hamilton, G. W. Huffman, and F. Smith, *ibid.*, **81**, 2176 (1959).

[4] F. Smith and J. W. Van Cleve, *ibid.*, **77**, 3091 (1955).

[5] I. J. Goldstein, J. K. Hamilton, and F. Smith, *ibid.*, **81**, 6252 (1959).



cotton, was oxidized with 0.2 *N* sodium periodate as formerly described.[6] The cellulose polyaldehyde (II) which remained undissolved was readily isolated and converted into the corresponding polyalcohol[5,7] (III) by reduction with sodium borohydride.[8] The cellulose polyalcohol thus obtained was a water-soluble, white amorphous powder which could be precipitated from aqueous solution with ethanol. Cellulose polyalcohol has been shown to yield upon hydrolysis erythritol, glycolic aldehyde, and a small proportion (0.1 to 0.2%) of D-glucose.[6]

Treatment of III first with methyl sulfate and sodium hydroxide solution and then with methyl iodide and silver oxide in the presence of *N*,*N*′-dimethylformamide,[9] yielded the corresponding trimethyl ether (IV) (OMe 45.15%).

Methanolysis of the tri-*O*-methylcellulose polyalcohol gave methoxyacetaldehyde dimethylacetal, 1,4-di-*O*-methylerythritol and a small proportion of racemic 1-*O*-methylerythritol. The methoxyacetaldehyde, which distilled upon concentrating the methanolyzate of the methylated cellulose polyalcohol, and which clearly arises from C-1 and C-2 of the glucose residues, was readily transformed into the crystalline *p*-nitrophenylhydrazone by treatment with an acidified solution of *p*-nitrophenylhydrazine.[10]

The 1,4-di-*O*-methylerythritol, which arises from the four-carbon fragment of the D-glucopyranose units was recognized as its crystalline 2,3-di-*O*-tosyl derivative.[3] The racemic 1-*O*-methylerythritol was separated from the 1,4-di-*O*-methylerythritol by cellulose column chromatography and identified as its crystalline tri-*p*-nitrobenzoate. It is believed that this racemic 1-*O*-methylerythritol which arises by demethylation of 1,4-di-*O*-methylerythritol[5] or as a result of incomplete methylation of the cellulose polyalcohol is of no constitutional significance. These findings are in accord with the view that cellulose is a linear polymer composed of D-glucopyranose units joined by 1 → 4 β-glycosidic bonds.

In a previous communication in this series[6] it was shown that after exhaustive oxidation of cellulose by periodate the resulting product still contained a small proportion (0.1 to 0.2%) of intact glucose residues. This observation prompted the suggestion that perhaps one out of about 500 of the nonterminal D-glucopyranose residues there was either a branch point or a 1 → 3 bond. In this study, using a relatively small amount of methylated cellulose polyalcohol, no evidence was obtained to indicate that the hydrolyzate of the methylated cellulose polyalcohol contained any methylated glucose derivatives. A larger amount of methylated cellulose polyalcohol is now being investigated.

Experimental

Unless stated otherwise, all evaporations were conducted *in vacuo* at 35–40°. Solvents used in chromatography were: *A*, 1-butanol–ethanol–water (4:1:5) and *B*, butanone–water azeotrope.

A. **Periodate Oxidation of Cellulose.**—Cellulose (10.0 g.) in the form of native cotton which had been defatted with acetone was oxidized with 0.2 *N* sodium periodate (1 l.) in the dark at room temperature (20°). At the end of 8 days, titration[11] revealed the cellulose had consumed 1.01 moles of periodate per glucose residue.

During the oxidation the cellulose, which had filled the reaction flask, underwent contraction to a relatively small mass. After standing for an additional 5 days the cellulose polyaldehyde (9.9 g.) was isolated by decantation of liquid and all traces of periodate removed by repeated washing with water.

B. **Reduction of Cellulose Polyaldehyde with Sodium Borohydride.**—To a suspension of the polyaldehyde (5.0 g.) in cold (5°) water (300 ml.), a cold (5°) aqueous solution (200 ml.) of sodium borohydride (4.0 g.) was rapidly added with stirring. A vigorous reaction ensued with much foaming. The exothermic reaction was moderated by cooling in an ice bath. After about 20 min. the cellulose derivative had dispersed giving an opalescent solution. After 5 hr. an additional portion of sodium borohydride (1.0 g.) was added and the solution was allowed to stand overnight. The reaction mixture was neutralized with acetic acid (tested with indicator paper), and the cellulose polyalcohol precipitated with ethanol as a white gummy mass. After decantation, the cellulose polyalcohol was triturated with 95% ethanol to remove sodium acetate.

The polyalcohol was dissolved in water (200 ml.) with gentle heating. The solution was cooled, acetic acid was added until slightly acid, and the polyalcohol precipitated by the addition of an excess of ethanol. After a third pre-

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(6) I. J. Goldstein, J. K. Hamilton, R. Montgomery, and F. Smith, *J. Am. Chem. Soc.*, **79**, 6469 (1957).

(7) M. Abdel-Akher, J. K. Hamilton, R. Montgomery, and F. Smith, *ibid.*, **74**, 4970 (1952).

(8) M. Abdel-Akher, J. K. Hamilton, and F. Smith, *ibid.*, **73**, 4691 (1951).

(9) R. Kuhn, H. Trischman, and I. Löw, *Angew. Chem.*, **67**, 32 (1955).

(10) N. L. Drake, H. M. Duwall, T. L. Jacobs, H. T. Thompson, and H. M. Sonnichsen, *J. Am. Chem. Soc.*, **60**, 73 (1938).

(11) P. Fleury and J. Lange, *J. pharm. chim.*, (8) **17**, 107 (1933).

cipitation from water in the same way, the cellulose polyalcohol, obtained as a white powder, was washed successively with ethanol, ether, and petroleum ether and finally dried *in vacuo*. The cellulose polyalcohol (4.08 g.) showed $[\alpha]^{25}\text{D}$ ±0 in water (*c*, 5).

Anal. Calcd. for $(C_6H_{12}O_5)n$: C, 42.9; H, 7.32. Found: C, 42.67; H, 7.29.

C. Methylation of the Cellulose Polyalcohol with Methyl Sulfate and Sodium Hydroxide.—Cellulose polyalcohol (3.25 g.) dissolved in water (50 ml.) was methylated with 30% sodium hydroxide (300 ml.) and methyl sulfate (100 ml.) in the usual manner. The reagents were added in ten equal portions over a period of 2.5 hr. while stirring the mixture vigorously at 55°. Acetone was added periodically to maintain a homogeneous reaction mixture. After destroying methyl sulfate by heating for 1 hr. on a boiling water bath the reaction mixture was stirred with chloroform, (300 ml.), filtered and the residue of salts was washed with chloroform. The chloroform layer was separated and the aqueous phase extracted three times with chloroform (200 ml.). The combined chloroform extracts were evaporated and the resulting sirupy residue containing the cellulose polyalcohol methyl ether was extracted with acetone. The acetone extract was filtered, concentrated (vol. 25 ml.), and remethylated as before using 30% sodium hydroxide (300 ml.) and methyl sulfate (100 ml.). Isolation of the methylated cellulose polyalcohol as before gave a clear, orange, viscous sirup (yield 2.75 g.).

The sirupy product was subjected to a third and fourth methylation using the method of Kuhn, *et al.*[9] To a solution of the methylated cellulose polyalcohol in *N*,*N*-dimethylformamide (50 ml.) was added methyl iodide (15 ml.) and silver oxide (10 g.) and the resulting mixture shaken for 24 hr. The silver salts were centrifuged and washed with *N*,*N'*-dimethylformamide, methyl iodide (25 ml.) and silver oxide (10 g.) were added to the solution, and the reaction was shaken for an additional 24 hr. The salts were then centrifuged and chloroform (100 ml.) was added. The copious yellow precipitate so formed was filtered and the chloroform solution was washed three times with 2% aqueous potassium cyanide solution, twice with water, and dried (Na_2SO_4). Evaporation of the chloroform solution gave a sirup which was remethylated in the same way. Final removal of the *N*,*N'*-dimethylformamide which necessitated heating at 90° *in vacuo*, gave a sirup which was dissolved in ether, filtered, and concentrated *in vacuo* to constant weight at 75° (bath temp.). The stiff orange sirup (2.6 g.), which became granular upon standing, had $[\alpha]^{23}\text{D}$ ±0° in chloroform (*c*, 4).

Anal. Calcd. for $C_9H_{18}O_5$: OCH_3 45.1. Found: (by the method of Gran[12]) OCH_3, 45.1, 45.2.

D. Methanolysis of the Methylated Cellulose Polyalcohol.—A solution of the methylated cellulose polyalcohol (0.39 g.) in methanol (10 ml.) containing hydrogen chloride (0.2 g.) was boiled under reflux for 3 hr., the solution was neutralized (Ag_2CO_3), filtered, and concentrated at 35–40° to give a residue *A* and a distillate *B*.

E. Identification of Methoxyacetaldehyde.—Treatment of the distillate *B* from the previous experiment with a solution of *p*-nitrophenylhydrazine in hydrochloric acid gave a yellow precipitate within a few seconds. The crystals were filtered within 5 min. to prevent the formation of the bis-*p*-nitrophenylhydrazone of glyoxal. Recrystallization of the precipitate from ethanol–water (2:3) gave the methoxyacetaldehyde *p*-nitrophenylhydrazone as yellow needles m.p. and mixed m.p. 115–116°, lit.,[10] 115–115.5°.

In another experiment, a portion of the methylated cellulose polyalcohol (0.30 g.) which had been treated with 2% methanolic hydrogen chloride and neutralized, was concentrated to about 3 ml. and *N* sulfuric acid (10 ml.) was added. The solution was boiled and the first 5–6 ml. of distillate containing the methoxyacetaldehyde–water azeotrope was collected. Treatment of the distillate with *p*-nitrophenylhydrazine gave the characteristic yellow crystals of methoxyacetaldehyde *p*-nitrophenylhydrazone, m.p. and mixed m.p. 114–115.5°.

F. Separation and Identification of 1-*O*-Methyl and 1,4-Di-*O*-methylerythritol.—The residue *A* was extracted with acetone and concentrated to give a clear orange sirup (0.238 g.). Paper chromatographic analysis using solvent *B* and Tollens spray reagent revealed the presence of two components, one corresponding to 1,4-di-*O*-methylerythritol (R_f 0.56) and the other (traces only) to 1-*o*-methylerythritol (R_f 0.25). In solvent *A*, the R_f values were 0.64 and 0.44, respectively.

The sirupy residue *A* containing the mixture of 1,4-di- and 1-*O*-methylerythritol was separated by column chromatography on cellulose–hydrocellulose[13] using solvent *B* and an automatic fraction collector which was adjusted to collect 18-ml. fractions every 30 min. Examination of the contents of the tubes by spotting on filter paper and spraying with Tollens reagent showed the presence of 1,4-di-*O*-methylerythritol in tubes 19–29 and 1-*O*-methylerythritol in tubes 45–70. The contents of tubes 19–29 were combined and concentrated to give chromatographically pure 1,4-di-*O*-methylerythritol (0.153 g.). The low yield is due to its volatility.[5] To a solution of 1,4-di-*O*-methylerythritol (0.04 g.) in dry pyridine (3 ml.) was added *p*-toluenesulfonyl chloride (0.15 g.) and the mixture heated for 30 min. at 90°. The cooled reaction mixture was poured into ice water containing 5% sodium bicarbonate. The white precipitate thus formed was filtered, washed, and dried. Recrystallization from ethanol gave 1,4-di-*O*-methyl-2,3-di-*O*-tosylerythritol, m.p. and mixed m.p. 139–140°, lit.,[3] m.p. 140°.

The 1-*O*-methylerythritol (0.005 g.) obtained upon concentrating the contents of tubes 45–70 showed $[\alpha]^{24}\text{D}$ ±0° in ethanol (*c*, 1.7). Treatment with *p*-nitrobenzoyl chloride in pyridine in the usual manner gave racemic 1-*O*-methyl-2,3,4-tri-*O*-*p*-nitrobenzoylerythritol as white crystals, m.p. 169–169.5° after recrystallization from acetone–ethanol. The tri-*p*-nitrobenzoate was optically inactive (tested in $CHCl_3$ *c*, 2).

Anal. Calcd. for $C_{26}H_{21}O_{13}N_3$: C, 53.52; H, 3.63; N, 7.20. Found: C, 53.92; H, 3.75; N, 6.8.

G. Attempts to Isolate a Methyl Derivative of D-Glucose.—In another experiment the fractions of 1,4-di-*O*-methylerythritol (0.76 g.) and 1-*O*-methylerythritol (0.012 g.) which had been isolated by column chromatography as above were separately subjected to treatment with *N* sulfuric acid in a sealed tube for 12 hr. at 100°. Neutralization $(BaCO_3)$, filtration, and concentration gave sirups which were subjected to paper chromatographic analysis using solvent *B* and *p*-anisidine trichloroacetate as the spray reagent. No spots indicating the presence of methylated derivatives of glucose were detected.

In another experiment, the total methanolyzate (0.30 g.) from methylated cellulose polyalcohol was subjected to hydrolysis with *N* sulfuric acid on a boiling water bath. After neutralization $(BaCO_3)$, filtration, and concentration the sirup was examined by paper chromatography on solvent *B*. Spraying with *p*-anisidine trichloroacetic acid and heating as usual revealed no methylated sugars.

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[12] G. Gran, *Svensk Papperstidn.*, **56**, 179 (1953).

[13] J. D. Geerdes, B. A. Lewis, R. Montgomery, and F. Smith *Anal. Chem.*, **26**, 264 (1954).