Gilvarg[15] and Sowden, *et al.*,[16] isolated mannose from polysaccharides of yeast grown on glucose-1-C^{14}. The former investigator working with *S. cerevisiae* found essentially the same activity in the mannose as in the original glucose, and the activity distribution indicated a direct conversion with little disruption of the carbon chain. In the same experiment, dilution of added acetate by acetate from glucose carbon was such as to indicate that essentially all of the acetate was derived via the E.M. process. In contrast, the latter investigators found no radioactivity in the mannose from *T. utilis*. This indicated that there was no direct conversion of glucose to mannose and that in this process carbon 1 of glucose was lost. The authors concluded from these results that the shunt was greatly preponderant in their organism.

In the present method of estimation the assumption is made that the E.M. process is the only one which yields labeled C2 units from glucose-1-C^{14}. Another possibility remains to be considered. Racker, de la Haba and Leder[17] recently reported that transketolase catalyzed the transfer of a "diose" from carbons one and two of fructose-6-phosphate to glyceraldehyde-3-phosphate or to ribose-5-phosphate, with the formation of the corresponding pentulose and heptulose phosphates. This would result in labeling of these sugars in the 1-position from glucose-1-C^{14}. Since the pentose thus labeled may add another "diose" to form a 1,3-labeled heptulose, and since these variously labeled heptuloses may transfer a triose moiety to another molecule of triose phosphate to form fructose-6-phosphate[18] a mechanism is apparent whereby labeling originally present in carbon 1 of glucose would appear in carbons 1 and 3 of fructose-6-phosphate. If these processes are rapid in comparison with the utilization of fructose-6-phosphate *via* the E.M. process, and if phosphohexose isomerase activity is sufficiently rapid, it is conceivable that glucose-6-phosphate would become labeled in carbon 3 and the further metabolism of this compound *via* the shunt would result in the formation of pentose labeled in carbon 2.

These reactions thus provide a means whereby labeled glucose carbon 1 can appear in pentose carbons 1 and 2. It is as yet unknown whether, in yeast, carbons 1 and 2 of pentose can yield acetate, as occurs in other organisms[12,13,19] or whether acetate may be formed from the "diose" split directly from fructose-6-phosphate. The possibility must be kept under consideration, therefore, that part of the C2 units calculated to have arisen *via* the E.M. pathway, in reality arose from "diose" units. This seems unlikely in the present experiments, however. If the "diose" pathway represented an important source of acetyl groups, we should expect a falsely high value for the E.M. pathway calculated from data obtained with glucose-1-C^{14}. However, somewhat lower values were obtained with glucose-1-C^{14} than were obtained with glucose-6-C^{14}, a result inconsistent with the formation of acetyl groups from glucose carbon 1 by a pathway other than that of Embden and Meyerhof.

(15) C. Gilvarg, *J. Biol. Chem.*, **199**, 57 (1952).

(16) J. C. Sowden, S. Frankel, B. H. Moore and J. E. McClary, *ibid.*, **206**, 547 (1954).

(17) E. Racker, G. de la Haba and I. G. Leder, *Arch. Biochem. and Biophys.*, **48**, 238 (1954).

(18) B. L. Horecker and P. Z. Smyrniotis, THIS JOURNAL, **75**, 2021 (1953).

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[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

The Constitution of Iles Mannan[1]

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Iles Mannan, the polysaccharide extracted from tubers of *Amorphophallus* plants is shown herein to be composed of two polysaccharides, one being a polyglucosan closely resembling amylose and the other a glucomannan. The latter is a linear polymer containing two parts of D-mannose and one part of D-glucose, the sugar residues being of the pyranose form and joined by 1,4-β-glycosidic bonds. The glucomannan exhibits retrogradation when aqueous solutions of it are heated. Precipitated in this manner, the glucomannan became insoluble in water and in alkali. It could be dissolved, however, in strong aqueous solutions of sodium xylenesulfonate. Prolonged drying of the glucomannan also rendered it insoluble in water and alkali.

Iles mannan meal is prepared from the tubers of the *Amorphophallus oncophyllus* and *Amorphophallus variabilis* plants which are native to and cultivated in Indonesia. It is a complex mixture containing a glucomannan and a smaller amount of a polyglucosan as its major carbohydrate constituents. Some cellulose, protein, lignin and minerals are also present in the meal. The mannose content is reported to vary from 43 to 75%, depending upon the species and growing conditions.[2,3] Although an aqueous solution of the mixture can be prepared, the glucomannan can be precipitated in a water-insoluble form by heating of its solution to 100°. Partly as a result of this peculiar solubility behavior it finds use in paper manufacture, as an adhesive and as a flocculating agent in rubber manufacture.[2]

This paper is concerned with the constitution of the glucomannan and the polyglucosan

(1) Extracted from a thesis submitted by P. A. Rebers to the University of Minnesota in partial fulfillment for the degree of Ph.D. (1953). Paper No. 3203, Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul.

(2) C. J. Van Hulssen and D. R. Koolhaas, *De Ingenieur in Nederlandsch-Indie*, **7**, 30 (1940).

(3) L. E. Wise, *Arch. Biochem.*, **23**, 127 (1949).

of Iles mannan as revealed by methylation studies.

Inasmuch as the Iles mannan meal was only sparingly soluble in hot or cold water or sodium hydroxide solutions, it was necessary at the outset to devise a method of dissolving the polysaccharide material more nearly completely. This was achieved by the action of a 50% solution of sodium xylenesulfonate which effected gelation, so that the resulting gel became soluble in 30% sodium hydroxide. This solution was stable to dilution with water and to neutralization by acetic acid. Treatment of a dilute alkaline solution of Iles mannan with Fehling solution effected a partial separation,[4] the glucomannan being precipitated while the polyglucosan remained in solution. Aqueous solutions of the glucomannan component purified in this way are stable but the polysaccharide is precipitated when the solutions are boiled.

Separation of the Iles mannan polysaccharides was obtained by methylation followed by fractional precipitation of the mixture of methylated polysaccharides. This precipitation was carried out[5] by dissolving the methylated polysaccharide in ethanol or acetone and cooling the solution to 0°. After standing for several hours a partial precipitation occurred, the precipitate being richer in the glucomannan than the supernatant. Repetition of this "crystallization" process with different solvents finally furnished two fractions which resisted further purification. The first, a methylated polyglucosan, showed $[\alpha]_D$ +163° in ethanol and the second, a methylated glucomannan, showed $[\alpha]_D$ −41° in ethanol.

Since the methylated polysaccharide before fractional precipitation showed $[\alpha]_D$ −12° in ethanol the ratio of the glucomannan to the polyglucosan in the original methyl Iles mannan was approximately 6:1.

The polyglucosan and its methyl derivative gave a blue color with iodine similar to that given by amylose and methanolysis of the methyl polyglucosan followed by hydrolysis yielded only 2,3,6-tri-*O*-methyl-D-glucose. No tetramethyl- or dimethylglucose was detected. The 2,3,6-tri-*O*-methyl-D-glucose was isolated in the crystalline state and further identified by its transformation into a crystalline di-*O*-*p*-nitrobenzoate, a derivative which is recommended for the identification of this trimethyl sugar.

Since the methylated polyglucosan had a high positive rotation which decreased during hydrolysis it seemed reasonable to deduce that the glycosidic linkages of the polymer were of the α variety and since the methanolysis step required about six hours, the trimethyl sugar residue was most probably of the pyranose type.

These data strongly suggest that the polyglucosan is composed of linear chains of α-glucopyranose units joined by 1,4-glycosidic bonds thus: [-4-α-Gp 1-]$_x$. Since no dimethylglucose was formed by cleavage of the methyl polysaccharide the latter cannot be of the branched chain type. Furthermore, the failure to detect any tetramethylglucose shows that the molecule is either cyclic in form or of sufficient length that the amount of terminal tetramethylglucose is so small that it cannot be detected by chromatographic methods. This is the formula presently accepted for amylose, the linear component of starch and although the methylated polyglucosan has a specific rotation (+180°) in chloroform which differs somewhat from that (+214°) shown by methylated amylose,[5] the characteristic blue color it gives with iodine and the nature of its cleavage product together with its specific rotation (+163°) in ethanol, which is very nearly the same as that (+167°) shown by methylated amylose, strongly suggest that if it is not identical with the amylose of starch it is similar to it. This finding is rather surprising since heretofore amylose has been found only in association with amylopectin.

Investigations into the second polysaccharide of Iles mannan which afforded a methyl derivative with a specific rotation of $[\alpha]_D$ −41° ($CHCl_3$) have shown that it is a linear polymer composed of pyranose units of D-glucose and D-mannose joined principally by 1,4-glycosidic bonds.

The experimental evidence for this conclusion is as follows. Hydrolysis of the polysaccharide itself gave rise to D-glucose and D-mannose. The stability of the polysaccharide indicated that the sugar units had a pyranose and not a furanose structure.

Cleavage of the methyl derivative of the polysaccharide proceeded at a slow rate characteristic of methyl polysaccharides built of pyranose sugar residues. The reducing sirupy product so formed appeared to be a trimethylhexose. Using a variety of irrigating solvents partition chromatographic analysis revealed only one component. By itself this evidence indicated that the product was either a trimethylhexose or a mixture of trimethylhexoses not separable by chromatographic techniques. No tetramethyl or dimethyl sugars were detected and hence it was deduced that the polysaccharide had essentially a linear structure.

Application of selective methyl furanoside formation coupled with partition chromatography on a hydrocellulose–cellulose column[6] afforded two fractions. The faster moving one was non-reducing and showed $[\alpha]_D$ +47° (water) while the slower moving component was reducing to Fehling solution and showed $[\alpha]_D$ −12° (water).

The reducing component proved to be almost pure 2,3,6-tri-*O*-methyl-D-mannose (the other three possible trimethylmannose and the four possible trimethyl-D-glucoses all show a positive rotation (see Table I)) and this was confirmed by its conversion into the characteristic crystalline di-*O*-*p*-nitrobenzoate.

When the faster moving furanoside component was hydrolyzed and the resulting trimethyl sugar treated with *p*-nitrobenzoyl chloride, there was formed the crystalline di-*O*-*p*-nitrobenzoate of 2,3,6-tri-*O*-methyl-D-glucose.

From the specific rotations of 2,3,6-tri-*O*-methyl-D-mannose and of 2,3,6-tri-*O*-methyl-D-glucose, the ratio of these two trimethyl sugars in the mixture derived from the methylated glucomannan was found to be 2:1, respectively.

(4) *Cf.* E. Salkowski, *Ber.*, **27**, 497 (1894).

(5) *Cf.* J. E. Hodge, S. A. Karjala and G. E. Hilbert, THIS JOURNAL, **73**, 3312 (1951).

(6) J. D. Geerdes, Bertha Lewis, R. Montgomery and F. Smith, *Anal. Chem.*, **26**, 264 (1954).

TABLE I

Sugar	Tri-*O*-methyl derivative	$[\alpha]_D$ (water)	Reference
D-Mannose	2,3,4-	+7°	7
D-Mannose	2,4,6-	+19°	8
D-Mannose	3,4,6-	+8°	9
D-Mannose	2,3,6-	−7°	10
D-Glucose	2,3,4-	+50°	11
D-Glucose	2,3,6-	+70.5°	12
D-Glucose	2,4,6-	+72°	13
D-Glucose	3,4,6-	+71°	14

Inasmuch as the building units in this negatively rotating polysaccharide are of the pyranose type, the polysaccharide is assigned the linear structure I.

—[4 β-D-Man*p* 1—4 β-D-Man*p* 1—4 β-D-G*p* 1]$_n$—

I

Clearly this is not the only formula to be deduced from the experimental data for other linear structures such as, for example, those having repeating units shown in II and III would also explain the results equally as well.

—[4 β-D-G*p* 1]$_2$—[4 β-D-Man*p* 1]$_4$—

II

—[4 β-D-G*p* 1—4 β-D-Man*p* 1]$_2$—[4 β-D-Man*p* 1]$_2$—

III

Since this hetero-polysaccharide composed of D-glucose and D-mannose has a negative rotation changing upon hydrolysis to a positive value, it is believed that a large proportion of its glycosidic bonds are of the β-type.

Further clarification of the constitution of the glucomannan of Iles mannan will be forthcoming from the preparation and characterization of oligosaccharides. Experiments with this end in view are now in progress.

The separation of the 2,3,6-tri-*O*-methyl derivatives of D-mannose and D-glucose by means of preferential furanoside formation was based on preliminary studies carried out with authentic specimens of these two trimethyl sugars. The glucose derivative was found to undergo furanoside formation at a much greater rate than the mannose derivative. At first sight this is a strange phenomenon since both trimethyl sugars carry methyl substituents at position 2, 3 and 6 and have a free –OH group at C_4 and might be expected to give rise to furanosides with equal facility. The marked difference in behavior of the mannose derivative appears to be due to steric inhibition. It is also apparent that, in the mannose series, the failure to form a methyl furanoside when treated with methanolic hydrogen chloride, a diagnostic structural test not infrequently used, does not justify the conclusion that a hydroxyl group is lacking at C_4.

(7) W. N. Haworth, E. L. Hirst and F. A. Isherwood, *J. Chem. Soc.*, 784 (1937).

(8) W. N. Haworth, R. L. Heath and S. Peat, *ibid.*, 833 (1941).

(9) H. G. Bott, W. N. Haworth and E. L. Hirst, *ibid.*, 1395, 2653 (1930).

(10) F. Smith, THIS JOURNAL, **70**, 3249 (1948).

(11) W. Charlton, W. N. Haworth and R. W. Herbert, *J. Chem. Soc.*, 2855 (1931).

(12) J. C. Irvine and J. K. Rutherford, THIS JOURNAL, **54**, 1491 (1932).

(13) W. N. Haworth and W. G. Sedgwick, *J. Chem. Soc.*, 2573 (1926).

(14) W. N. Haworth, E. L. Hirst and L. Panizzon, *ibid.*, 154 (1934).

Furanoside formation requires that carbon atoms 1, 2, 3 and 4 lie in one plane. Now it has been established that even the H atoms of one CH_3 group in ethane prefer to assume a position in which they are at the greatest possible distance from the H atoms of the other CH_3 group, so that the molecular constellation has minimum energy.[15] Similarly in *n*-butane the molecule prefers a constellation in which the Me groups are *trans* to each other.[15] It may be deduced, therefore, by analogy, that in tri-*O*-methyl derivatives of D-mannose and D-glucose the methoxyl groups at positions C_2 and C_3 may well play a major role as far as furanoside ring formation is concerned. Mutual repulsion will tend to make them assume a *trans* position with respect to each other. If this is the controlling factor, it may be seen from molecular models that the planar furanoside ring formation in the case of 2,3,6-tri-*O*-methyl-D-glucose will be more readily achieved for the –OMe groups will be in the relatively favorable *trans* position to each other after the ring is formed whereas, in the case of the mannose derivative, the –OMe groups would be in the unfavorable *cis* position to each other. In the pyranose non-planar ring systems the mutual effect of the –OMe groups at C_2 and C_3 is probably small.

The importance of ring conformation and the disposition of large groups is further illustrated by reference to the di-*O*-*p*-nitrobenzoates of the 2,3,6-tri-*O*-methyl-derivatives of D-glucose and D-mannose (see Table II).

TABLE II

Di-*O*-*p*-nitrobenzoate of	M.p., °C.	$[\alpha]_D$ ($CHCl_3$)	Anomeric form
2,3,6-Tri-*O*-methyl-D-glucose	189–190	−33°	β
2,3,6-Tri-*O*-methyl-D-mannose	187–188	+33°	α

Comparison of these derivatives with other derivatives of D-glucose and D-mannose shows that the di-*o*-*p*-nitrobenzoate of the tri-*O*-methyl-D-mannose has the α-configuration while the D-glucose derivative has the β-configuration. The particular anomeric form of the mannose and of the glucose derivative appears to be formed to the almost complete exclusion of the other. This seems to indicate that the *O*-*p*-nitrobenzoyl group at C_1 is constrained to take up the more favorable *trans* position in relation to the methoxyl group at C_2.

Some examples of this phenomenon of molecular constellation have already been revealed by the pioneering experiments of Hassel[16] and later by those of Reeves[17,18] but apart from the work of the Hudson school[19] its extensive application especially in synthetic work still awaits exploitation.

Experimental

Preparation of Solutions of Iles Mannan.—The Iles mannan[20] was a light tan free-flowing powder which was insoluble

(15) J. C. McCoubrey and A. R. Ubbelohde, *Quart. Rev.*, **4**, 364 (1951).

(16) O. Hassel and B. Ottar, *Acta Chem. Scand.*, **1**, 929 (1947).

(17) R. E. Reeves, THIS JOURNAL, **72**, 1499 (1950).

(18) *Cf.* S. A. Barker and E. J. Bourne, *J. Chem. Soc.*, 3865 (1952).

(19) R. K. Ness, H. G. Fletcher, Jr., and C. S. Hudson, THIS JOURNAL, **72**, 2200 (1952); R. K. Ness and H. G. Fletcher, Jr., *ibid.*, **76**, 1665 (1954).

(20) The authors thank General Mills, Inc., Minneapolis, for the sample of Iles mannan.

in water and aqueous alkali. It was dissolved as follows. To air-dried Iles mannan (10 g.) was added dropwise with stirring, 50% (w./w.) sodium xylenesulfonate (20 ml.) (prepared by dissolving 50 g. of the salt[21] in water (50 ml.), adding charcoal and filtering). The temperature rose from 24 to 30° during the first stages and remained at 30° until the addition was complete. To the gel formed in this way a solution of sodium hydroxide (30 g.) in water (100 ml.) was added. The mixture was heated at 60° for a few minutes to complete solution of the gel and diluted with water to 1 liter. The solution was filtered to remove an insoluble residue (0.52 g.), acidified with acetic acid (no precipitate was formed) and then poured with stirring into ethanol (2 liters). The polysaccharide obtained as a white stringy precipitate was washed with ethanol (twice), petroleum ether (once) and dried *in vacuo* over phosphorus pentoxide. The material (7.93 g.) showed $[\alpha]^{24}D$ −14° approx. (*c* 0.1) in water containing 5% sodium hydroxide and 1% sodium xylenesulfonate.

Fractionation of Iles Mannan.—Iles mannan meal (2.27 g.) was dissolved as described above, the final volume being 200 ml. To the clear solution were added Fehling solution B (40 ml.) and Fehling solution A (30 ml.). The dark blue precipitate of the copper hydroxide–glucomannan complex was centrifuged and washed with water (200 ml.) containing Fehling solution A (10 ml.) and Fehling solution B (10 ml.).

The copper–polysaccharide complex was dissolved in concentrated (28%) ammonium hydroxide and the solution dialyzed until free from color. The solution was acidified with acetic acid and the dialysis continued until the solution was neutral. When this solution, which showed $[\alpha]^{24}D$ −21.3°, was heated, the glucomannan began to separate and the rotation became less and less negative until after 80 minutes it was zero; at this stage the clear liquid gave a negative Molisch test. The polysaccharide precipitate (0.616 g.) did not give a blue color with iodine and it was insoluble in water and in 0.5 *N* potassium hydroxide.

Hydrolysis of the Glucomannan.—The polysaccharide (0.396 g.) was treated with 70% sulfuric acid (2 ml.) and allowed to stand overnight. The solution was diluted with water (13 parts) and heated on the boiling water-bath until the rotation became constant (28 hours). The solution was neutralized ($BaCO_3$), filtered, passed through a cation-exchange column (Amberlite IR 120) and evaporated *in vacuo* to a sirup which showed $[\alpha]^{24}D$ +28° in water (*c* 2.7). Partition chromatographic analysis of this sirup using 1-butanol–ethanol–water (4:1:5) and phenol saturated with water as irrigating solvents and ammoniacal silver nitrate as the spray reagent indicated the presence of mannose, glucose and a slow moving minor component. Isolation of this component by sheet paper chromatography followed by rehydrolysis showed that it was an oligosaccharide, probably a disaccharide, composed of mannose and glucose; evidently it arose as a result of incomplete hydrolysis.

Separation of the sirup (271 mg.) into its components was effected by partition chromatography on Whatman No. 3 paper for 3 days using butanol–ethanol–water (4:1:5). After locating the sugars by cutting out thin (1/4") vertical strips of paper and spraying with Tollens reagent they were eluted from the appropriate unsprayed zones of the paper with water and freed from solvent by evaporation *in vacuo*. The two sirupy products corresponding to glucose and mannose were each treated with *p*-nitroaniline (1.1 moles) and 0.2 ml. of methanol containing a trace of hydrochloric acid (0.15 ml. of concd. hydrochloric acid added to 200 ml. of methanol) according to the procedure of Weygand, *et al.*[22] The faster moving component gave D-glucose-*p*-nitroanilide m.p. and mixed m.p. 180–181°, after recrystallization from methanol, while the slower moving component yielded D-mannose-*p*-nitroanilide m.p. and mixed m.p. 213° after recrystallization from methanol.

Isolation of the Polyglucosan.—After removing the glucomannan by precipitation with Fehling solution as described above the mother liquors were dialyzed until colorless to remove the excess of the copper, acidified with acetic acid and redialyzed to remove acid. The polysaccharide in solution was precipitated with ethanol and washed first with acidified ethanol (1 part *N* HCl and 4 parts ethanol) to remove a blue tinge from the precipitate, and then with ethanol and finally dried *in vacuo*. The polysaccharide thus obtained (54 mg.) showed $[\alpha]^{25}D$ +84° in 0.5 *N* KOH (*c* 0.1); it gave a blue color with iodine and readily dissolved in water or alkali.

Hydrolysis of the Polyglucosan.—Hydrolysis of the polysaccharide with *N* sulfuric acid for 36 hours at 95° gave a reducing sirup which showed $[\alpha]^{24}D$ +28° in water (*c* 0.9). Paper partition chromatographic analysis using 1-butanol–ethanol–water (4:1:5) indicated a strong glucose spot, traces of mannose and traces of what appeared to be glucuronic acid as shown by a slow moving spot due to glucuronic acid and a faster one due to D-glucuronolactone; to confirm this the component giving the faster moving spot was eluted and rechromatographed when there were produced a slow moving and a fast moving spot; this is characteristic of glucuronic acid. Isolation of the component whose R_F value corresponded to glucose followed by treatment with *p*-nitroaniline, afforded D-glucose-*p*-nitroanilide m.p. and mixed m.p. 180°.

Methylation of Iles Mannan.—The polysaccharide mixture (7 g.) purified as described above was dissolved in 50% sodium xylenesulfonate (20 ml.) and 30% sodium hydroxide (160 ml.). The solution was treated dropwise with methyl sulfate (100 ml.) and 30% sodium hydroxide (150 ml.) at 55° with vigorous stirring during 3 hours. The reaction was completed by heating the mixture for 1 hour on the boiling water-bath. The excess of the sodium hydroxide was partially neutralized with 10% sulfuric acid (50 ml.) after which glacial acetic acid (30 ml.) was added to bring the *p*H to 7.5. Alcohol (1.2 vol.) was added and the precipitated sodium sulfate removed (centrifuge). The aqueous alcohol containing the partially methylated polysaccharides was evaporated to dryness *in vacuo*.

The residue was treated with acetic anhydride (200 ml.) in 20-ml. portions, cooling being applied when necessary to keep the temperature at or below 60°. The mixture was shaken overnight and the homogeneous solution poured with stirring into water (600 ml.). No precipitate formed but upon saturation of the solution with ammonium sulfate the acetate of the mixture of partially methylated polysaccharides separated as a sticky solid. The latter was removed, dissolved in acetone (100 ml.) and methylated with methyl sulfate (120 ml.) and 30% sodium hydroxide (360 ml.), the reagents being added dropwise during 1.5 hours at 50°. After removing the acetone by heating the reaction mixture on the boiling water-bath the methylated compound separated on the surface of the solution as a pasty solid. The material was removed and subjected to three more methylation treatments under the same conditions. The mixture of methylated polysaccharides thus obtained (3.1 g.) showed $[\alpha]^{27}D$ −12° in ethanol (*c* 0.7) (found: OMe, 38.0). The low yield is almost certainly due to the solubility of the compound in the cold water used to wash out the salts after methylation.

Separation of the Methylated Polysaccharides. (a) Isolation of the Methylated Glucomannan.—The methylated material consisting of a mixture of the methylated glucomannan and the methylated glucosan (3.0 g.) was subjected to the following fractionation procedure shown.

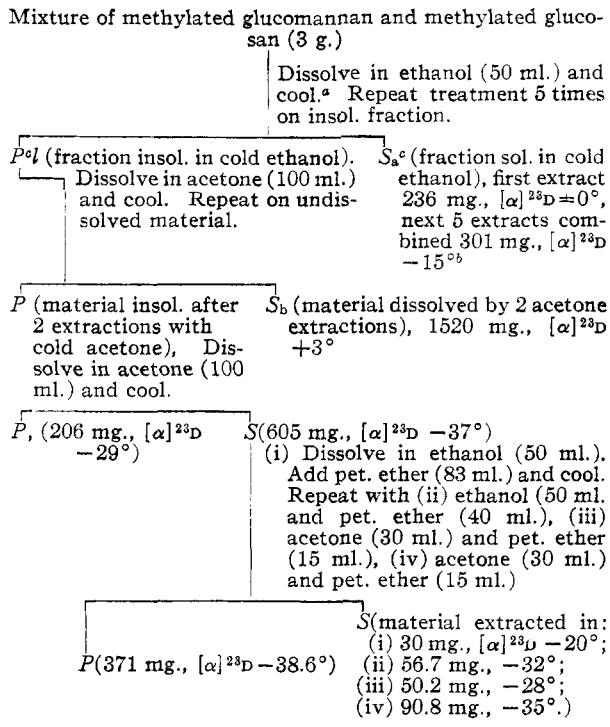
The two fractions, 90.8 mg. ($[\alpha]^{23}D$ −35°) and 371 mg. ($[\alpha]^{23}D$ −38.6°), were combined, freed from solvent and subjected to two treatments with silver oxide (5 g.) and methyl iodide (15 ml.). Fractional precipitation of 305 mg. of the methylated material from benzene (10 ml.) with petroleum ether (15 ml.) at room temperature gave a precipitate which showed $[\alpha]^{22}D$ −41° in ethanol (*c* 0.6) (found: OMe, 44.2) while the material in the supernatant liquid had $[\alpha]^{22}D$ −40° in ethanol (*c* 0.5) (found: OMe, 44.5). It was assumed at this stage that the negatively rotating methyl polysaccharide was essentially homogeneous.

Isolation of the Methyl Polyglucosan.—The two fractions, S_a and S_b) (see the above fractional precipitation scheme) having the lowest negative specific rotations, were combined and the mixture (1.65 g.) showing $[\alpha]^{23}D$ −2.6° (ethanol), was fractionated according to the following scheme.

All fractionations were carried out at 0° for the times shown and rotations were determined in ethanol unless stated otherwise. *P* denotes, as in the previous fractionation, the fraction precipitated and *S* the fraction remaining in solution; after cooling the separation was effected by centrifuging. It was noted during the fractional precipitation that the positively rotating methylated polyglucosan became less

(21) The authors thank the Wyandotte Chemical Co., for the sodium xylenesulfonate ("Naxonate G").

(22) F. Weygand, W. Perkow and P. Kuhner, *Ber.*, **84**, 594 (1951).



[a] The solution was kept for about 12 hr. at 0° and then centrifuged. [b] The rotations were determined in ethanol at 23°. [c] *P* denotes fraction precipitated and *S* the fraction dissolved.

soluble in ethanol as its purity increased and it tended to form cloudy solutions in ethanol.

The two final fractions *S* (45 mg.) and *P* (95 mg.) were combined and subjected to two methylations with silver oxide (5 g.) and methyl iodide (10 ml.) using benzene (10 ml.) as an additional solvent. The methylated polyglucosan, isolated by means of acetone and dried *in vacuo* showed $[\alpha]^{23}D$ +180° in chloroform (*c* 0.5).

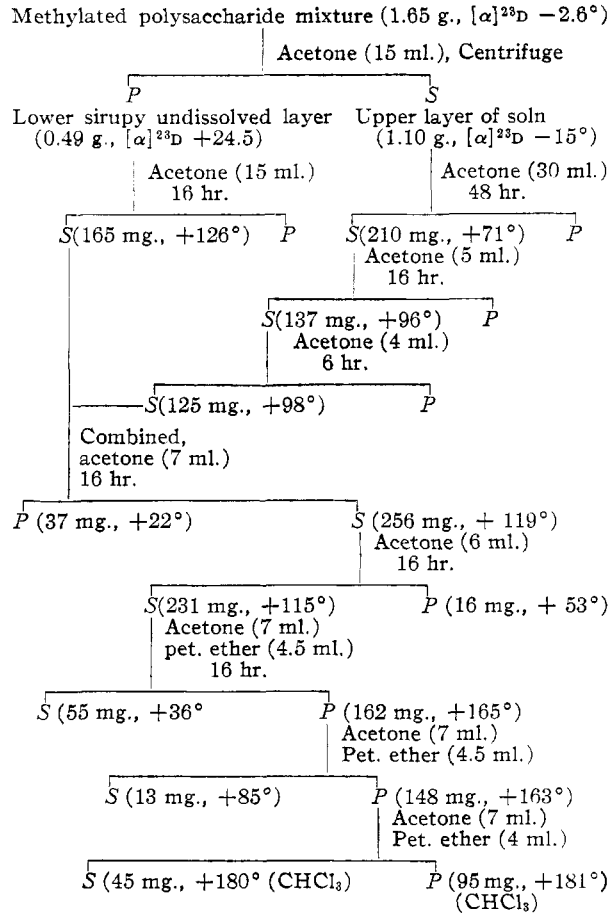
Anal. Calcd. for $C_9H_{16}O_5$: OCH_3, 45.6. Found: OCH_3, 45.7.

Hydrolysis of the Methylated Glucomannan and Identification of 2,3,6-Tri-*O*-methyl-D-glucose and 2,3,6-Tri-*O*-methyl-D-mannose.—When the methylated glucomannan ($[\alpha]^{23}D$ −40° (ethanol) (227 mg.) was boiled with 3% methyl alcoholic hydrogen chloride (20 ml.), the rotation changed in 5 hours from $[\alpha]^{23}D$ −38 to +66° (constant value). Neutralization of the acid (Ag_2CO_3), filtration and evaporation *in vacuo* yielded a sirupy mixture of glycosides (238 mg.) which showed $[\alpha]^{21}D$ +48.5° in water (*c* 0.5).

Treatment of this mixture of glycosides with *N* sulfuric acid (50 ml.) on the boiling water-bath until the rotation became constant, 72 hours, removal of the acid ($BaCO_3$) and the solvent yielded a mixture of trimethyl sugars (228 mg.) showing $[\alpha]^{24}D$ +13.4° in water (*c* 2.2).

Paper chromatographic analysis, using three different solvent developers (methyl ethyl ketone–water azeotrope, butanol–ethanol–water (4:1:5), or benzene–ethanol–water (167:47:15)) showed only one spot when sprayed with *p*-anisidine.[23]

Separation of the 2,3,6-Tri-*O*-methyl Derivatives of D-Mannose and D-Glucose by Selective Furanoside Formation.—When the mixture of trimethyl sugars (199 mg.) was treated at room temperature with 1% methanolic hydrogen chloride (45 mg.), the change in specific rotation was as follows: +24° (initial value); +17° (after 3 hr.); +11.3° (6 hr.); +2.5° (10 hr.); −4.5° (22 hr.). The solution was neutralized with silver carbonate, filtered and freed from solvent and the mixture of 2,3,6-tri-*O*-methyl-D-mannose and methyl 2,3,6-tri-*O*-methyl-D-glucofuranoside resolved on the hydrocellulose-cellulose column[6] using methyl ethyl ketone–water azeotrope as the solvent. The solvent front



was marked by a dye (Sudan IV) which was put on the column with the mixture of methyl sugars and fractions were collected by an automatic device at 10-minute intervals. The reducing component, tri-*O*-methyl-D-mannose was located in the usual way.[24] The results of the separation are given in Table III.

TABLE III

Fraction tube no.	Component	Weight, mg.	$[\alpha]^{22}D$ H_2O
1–2	Dye marking front	..	.....
3–33	Methyl 2,3,6-tri-*O*-methyl-D-glucofuranoside (non-reducing)	97	−19.5°
34–66	2,3,6-Tri-*O*-methyl-D-mannose (reducing)	103	−11.6°

Identification of 2,3,6-Tri-*O*-methyl-D-mannose.—The reducing component (103 mg.) showing $[\alpha]^{22}D$ −11.6° in water (*c* 1.0), suggesting 2,3,6-trimethyl-D-mannose[10] was dissolved in dry pyridine (6 ml.) and treated with *p*-nitrobenzoyl chloride (426 mg.) for 30 minutes at 65–75° and left overnight at room temperature. A saturated solution of sodium bicarbonate was added dropwise to the reaction mixture until no further effervescence occurred. Water (10 ml.) was added and the product was extracted with chloroform (three 25-ml. portions). The extract was dried (Na_2SO_4) and evaporated *in vacuo* to 2–3 ml. Addition of petroleum ether induced crystallization of the di-*O*-*p*-nitrobenzoate of 2,3,6-tri-*o*-methyl-D-mannose (86 mg.), m.p. 187–188°, $[\alpha]^{23}D$ +33° in chloroform (*c* 0.6) (after recrystallization from methanol). A specimen of this hitherto unknown compound prepared from the 2,3,6-tri-*O*-methyl-D-mannose of methylated guar galactomannan[10] had m.p. 187°, $[\alpha]^{23}D$ +32° in chloroform (*c* 0.6). A mixture of the di-*O*-*p*-nitrobenzoates showed no depression of the m.p.

(23) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 1702 (1950).

(24) L. A. Boggs, L. S. Cleudet, M. Dubois and F. Smith, *Anal. Chem.*, **24**, 1148 (1952).

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]

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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.

(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).

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