colored liquids gave water-white liquids. The 3-ethyl compound (XXIV) had $n^{24}D$ 1.5072, with the nmr showing τ^{neat} = 9.32 (t, 3, CH_3), 7.2–9.0 (cm, 6), 6.25 (q, 1), 4.38, 3.95, 3.64 ppm (m, 1, pyrrole H). *Anal.* Calcd for $C_9H_{13}N$: C, 79.95; H, 9.69; N, 10.36. Found: C, 80.11; H, 9.79; N, 10.29.

The 4-ethyl compound (XXV) had $n^{24}D$ 1.5205, with the nmr showing τ^{neat} = 8.89 (t, 3, CH_3), 7.66 (m, 4), 6.56 (t, 2), 4.45 and 4.25 ppm (m, 1, pyrrole H).

Anal. Calcd for $C_9H_{13}N$: C, 79.95; H, 9.69; N, 10.36. Found: C, 80.02; H, 9.64; N, 10.16.

Preparation of Cinnamyltriphenylphosphonium Bromide (XXVI).—In a three-neck, 1-l. flask fitted with a stirrer and a reflux condenser was placed 500 ml of anhydrous benzene. To this was added 50 g of cinnamyl bromide (0.25 mole). To the stirred mixture was added 66 g (0.25 mole) of triphenylphosphine. The mixture was stirred at room temperature and a slight heating accompanied by slow precipitation of the salt was observed. After stirring overnight, the mixture was refluxed for 2 hr, followed by filtration, washing with benzene, and refluxing with an additional 500 ml of benzene for 1 hr. The salt was filtered, washed with benzene, and air dried overnight, yielding 90 g (77.5%), mp 256–258° (lit.[13] mp 240°).

(13) K. Friedrich and H. Mirback, *Chem. Ber.*, **92**, 2756 (1959).

Reaction of Pyrrole-2-carboxaldehyde with Cinnamyltriphenylphosphonium Bromide (XXVI).—Into a 500-ml flask fitted with a magnetic stirrer, a condenser, and a drying tube was placed 2.29 g of a 52.6% dispersion of sodium hydride in mineral oil (1.2 g of NaH, 0.05 mole) and 200 ml of anhydrous ether. To the stirred suspension was added 4.75 g of pyrrole-2-carboxaldehyde (0.05 mole). When evolution of hydrogen ceased, 23 g of the cinnamyl salt (XXVI) (0.05 mole) was added. Reaction occurred immediately, and it was necessary to temper with a cool water bath. The mixture was allowed to stir overnight. The salts were removed by filtration, and the ether was allowed to evaporate, leaving a dark oil which was chromatographed on alumina with hexane–benzene (1:5), resulting in an oily solid. This was chromatographed again using a 2:1 mixture of benzene–hexane, resulting in a light yellow solid which when sublimed at 100° at 0.3 mm gave 3.36 g of 1-(2-pyrryl)-4-phenylbutadiene (XXVII), mp 192–195°. The nmr spectrum showed τ(pyridine-d_5) = 3.8–2.5 (cm, 13), −1.86 ppm (s, 1, NH).

Anal. Calcd for $C_{14}H_{13}N$: C, 86.12; H, 6.71; N, 7.18. Found: C, 86.12; H, 6.78; N, 7.00.

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Studies on the Anomalous Linkages in Glycogen and Amylopectin[1]

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The problem of anomalous linkages in glycogen and amylopectin has been investigated by studying the periodate oxidation products of these polysaccharides. Glycogen and amylopectin polyaldehydes having degrees of oxidation 96.8 and 98.5%, respectively, have been reduced with sodium borohydride to the corresponding polyalcohols. After a complete methylation of the polyalcohols, the resulting methylated derivatives have been hydrolyzed. The major components, namely, methoxyacetaldehyde, 1,3-di-*O*-methylglyceritol, 1,4-di-*O*-methylerythritol, and 1-*O*-methyl-D-erythritol, and the minor components 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, 2,6-di-*O*-methyl-D-glucose, 2,3-di-*O*-methyl-D-glucose, and mono-*O*-methyl-D-glucose have been separated, identified, and quantitatively determined. The proportion of 2,6-di-*O*-methyl-D-glucose from both glycogen and amylopectin polyaldehydes amounting to only 0.02% is very small and does not favor the presence of the so-called anomalous linkages of (1→3) type.

Glycogen and amylopectin have long been recognized as high molecular weight polymers having branched-chain structures composed of D-glucopyranose residues. These residues are joined by (1→4)-α-D-glucosidic linkages and the branch points are located at position 6 of one out of about 12 residues in the case of glycogen and one of about 20 glucose residues in the case of amylopectin. The number of glucose units in the inner and outer branches amounts to 4 and 8 for glycogen and 12 and 8 for amylopectin, respectively. These structural concepts have emerged from the application of various techniques such as methylation,[3] periodate oxidation,[4] enzymic degradation,[5] and partial acid hydrolysis.[6] Although the general structures of glycogen and amylopectin stated above have been accepted by most investigators, there remains a difference of opinion as to the presence of linkages other than those of (1→4) and (1→6) types. The evidence that has so far accumulated from various studies discussed below indicates the possible existence of (1→3)-α-D linkages in both glycogen and amylopectin.

The original suggestion as to the presence of (1→3) linkages came from the methylation studies. Bell[7] obtained, from the degradation products of methylated glycogen, 2,3,4,6-tetra-*O*-methyl-D-glucose (I), 2,3,6-tri-*O*-methyl-D-glucose (II), and di-*O*-methyl-D-glucose in a molar ratio of 1:9:2. Subsequently, this di-*O*-methyl-D-glucose fraction was found to contain an appreciable amount of 2,6-di-*O*-methyl-D-glucose[8] (III) in addition to 2,3-di-*O*-methyl isomer (IV), a finding which prompted the suggestion that (1→3) linkages were present in glycogen. It was, however, found later[9] that most of the component III resulted from incomplete methylation and some by demethylation of the higher methyl ethers of glucose during hydrolysis.[10] Similar studies in our laboratory have also confirmed that the hydroxyl groups at positions C-3 are more difficult to methylate than those at C-2 and C-6. Evi-

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dently, because of these limitations inherent in the methylation technique, such a procedure would not be suitable for ascertaining minor linkages present in polysaccharides.

A second line of evidence has come from the periodate oxidation studies. Periodic acid is a specific reagent for α,β-glycol grouping. All of the units in a polymer containing 1,4-di- and 1,4,6-trisubstituted D-glucopyranose residues would have free hydroxyl groups at C-2 and C-3 and, thus, would be susceptible to attack by periodic acid or its salts. The resulting polyaldehyde would not be expected to yield any glucose on hydrolysis. On the other hand if branching occurs through C-2 or C-3, these glucose residues would not contain adjacent hydroxyl groups and consequently would not be attacked by periodate. Hamilton and Smith[11] subjected waxy corn starch and its β-amylase limit dextrin to prolonged oxidation under drastic conditions of relatively high temperature and high concentration of periodate. The resulting polyaldehyde was reduced with sodium borohydride to the corresponding polyalcohol which on hydrolysis afforded 0.2–0.5% glucose. It was pointed out that this glucose could arise because of incomplete oxidation or because of fixed *trans* hydroxyl groups.[12] However, since the glucose survived even when the polyalcohol was treated with periodate, it was concluded that waxy corn starch probably contained linkages other than those of the (1→4) and (1→6) types. Similar studies on glycogen[13] also indicated the presence of periodate-stable glucose units to the extent of 1%. On the other hand, Bell and Manners[9] oxidized four samples of glycogen and by subsequent hydrolysis of the polyaldehydes found glucose only in one case. These authors also oxidized dextrins obtained by the action of α-amylase on glycogen and found no free glucose in the hydrolysate. It appears, however, that the conditions selected for oxidation of the reducing oligosaccharides present in α-amylolysis mixture were too drastic inasmuch as there was evidence of over-consumption of periodate as revealed by the excessive liberation of iodine. More recently Manners and Mercer[14] subjected potato amylopectin to rather drastic conditions of oxidation and the polyaldehyde thus formed was reduced with sodium borohydride. The resulting polyalcohol on acid hydrolysis followed by an examination of the products by paper chromatography failed to reveal any glucose. Again, the conditions selected for oxidation were such that the extent of reduction of the priodate and the production of formic acid was greatly in excess of that expected from a Malapradian periodate oxidation. Hence, any conclusions with regard to the anomalous linkages drawn from such studies would be open to question.

Partial acid hydrolysis has been used effectively in the determination of glycosidic linkages in polysaccharides. Wolfrom and his associates reported that the partial acid hydrolysis of waxy starch[15] and beef liver glycogen[16] yielded a small portion of *O*-α-D-glucopyranosyl-(1→3)-β-D-glucose (nigerose). They isolated 350 and 2 mg of nigerose octaacetate from 130.0 g of amylopectin and 92 g of beef liver glycogen, respectively. These authors also carried out a control experiment by heating glucose with dilute acid under the same conditions as used for the polysaccharides and concluded that nigerose was not formed by "acid reversion." On the basis of these results, they concluded that a small number of (1→3)-α-D-glucosidic linkages were present in glycogen and amylopectin. Although it was shown that nigerose did not arise from free glucose by acid reversion, it is, however, possible that acid-catalyzed transglucosylation might have been responsible for the formation of nigerose. In support of this may be cited an experiment of Pazur and Budovich[17] who prepared nigerose to the extent of 1% by the action of 0.1 *N* hydrochloric acid at 100° on a mixture of glucose and maltose. Manners and Mercer[18a,b] have more recently shown, by employing the same conditions for hydrolysis as used by Wolfrom and his associates, that maltose alone can also form nigerose. The latter authors, however, on the basis of their recent work,[18c] maintain that the nigerose isolated by the fragmentative hydrolysis of glycogen and amylopectin is not an artifact.

It follows from the above discussion that techniques so far used to investigate the possible presence of linkages other than those of the (1→4) and (1→6) types suffer from one or the other disadvantage so that no clear-cut decision can be made concerning the presence or absence of the so-called anomalous linkages in glycogen and amylopectin. For example, the difficulty with the periodate oxidation is that it may not go to completion under mild conditions and may result in overoxidation under severe conditions. In the case of the methylation procedure, the degree of reliance which one can place on the results, would entirely depend both on the extent of methylation and the absence of demethylation of the higher methyl ethers of glucose during hydrolysis. Similarly, the danger in partial hydrolysis procedure is the possibility of the products formed by acid-catalyzed transglucosylation. The procedure adopted in this work tends to eliminate some of these difficulties.

Briefly, the technique consists in oxidizing glycogen or amylopectin with periodate in the cold at 4°. Under such mild conditions the oxidation does not go to completion. Consequently, the danger of overoxidation is minimized. The polyaldehyde is reduced with sodium borohydride to the polyalcohol in which the proportion of (1→3) linked glucose residues is considerably increased because almost all of the (1→4)- and (1→6)-linked residues are destroyed by periodate. The polyalcohol is then subjected to methylation which proceeds more readily than with the parent polysaccharide because of the acyclic nature of the residues. The methylated polyalcohol is methanolyzed and the resulting degradation products are fractionated and identified. Any glucose residue linked through C-1, C-3, and C-4 should yield 2,6-di-*O*-methyl-D-glucose, whereas glucose residues linked through C-1 and C-3 should furnish 2,4,6-tri-*O*-methyl-D-glucose. Nearly all of

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(12) M. Abdel-Akher and F. Smith, *ibid.*, **81**, 1818 (1959).
(13) M. Abdel-Akher and F. Smith, *Arch. Biochem. Biophys.*, **78**, 451 (1958).
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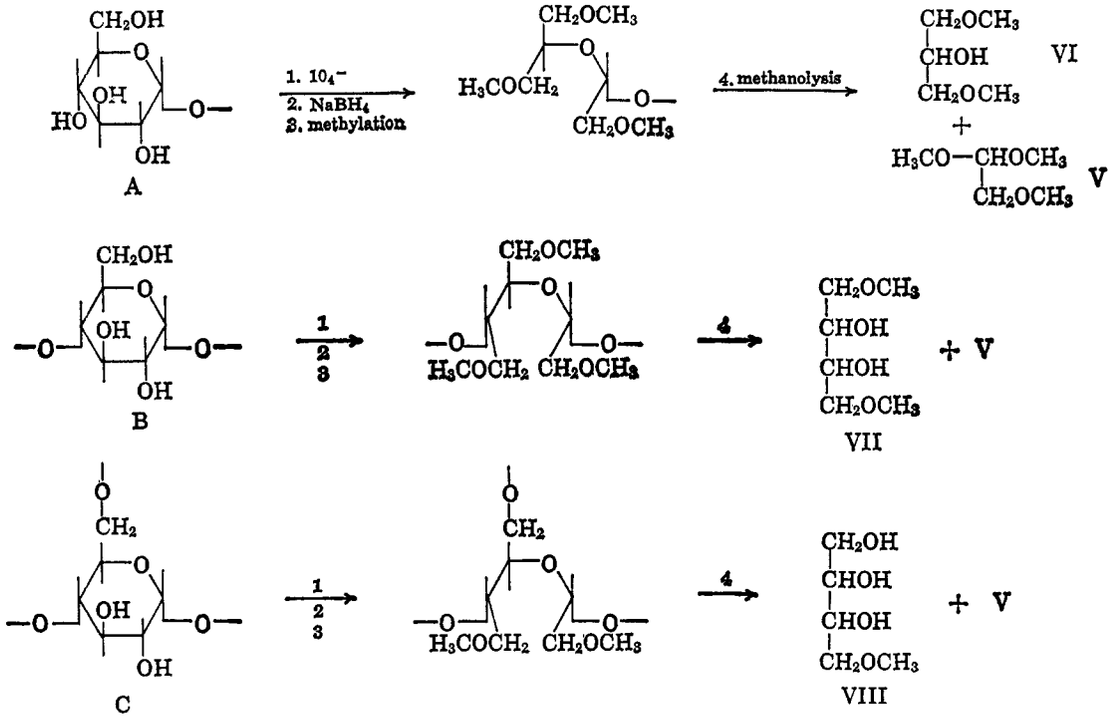
(15) M. L. Wolfrom and A. Thompson, *J. Am. Chem. Soc.*, **77**, 6403 (1955); **78**, 4116 (1956).
(16) M. L. Wolfrom and A. Thompson, *ibid.*, **79**, 4212 (1957).
(17) J. H. Pazur and T. Budovich, *ibid.*, **78**, 1885 (1956).
(18) (a) D. J. Manners and G. A. Mercer, *Biochem. J.*, **89**, 34P, (1963); (b) D. J. Manners, G. A. Mercer, and J. J. M. Rowe, *J. Chem. Soc.*, 2150 (1965); (c) M. L. Wolfrom, A. Thompson, and R. H. Moore, *Cereal Chem.* **40**, 182 (1963).

CHART I

STUDIES ON THE METHYLATED GLYCOGEN AND AMYLOPECTIN POLYALCOHOLS



(A represents glucose residues linked at C-1, B at C-1, and C-4, and C at C-1, C-4, and C-6 positions)

the residues linked through C-1, or C-1 and C-4, or C-1, C-4, and C-6 would be oxidized and would give rise to methoxyacetaldehyde dimethylacetal (V), 1,3-di-*O*-methylglyceritol (VI), 1,4-di-*O*-methylerythritol (VII), and 1-*O*-methyl-D-erythritol (VIII) (Chart I). In the event of incomplete oxidation, other components such as 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, and 2,3-di-*O*-methyl-D-glucose would also be expected to appear in the hydrolysate. Separation of the cleavage products is facilitated by the volatility of the major components (V–VIII) which can be removed by distillation under diminished pressure. Glycogen polyaldehyde, containing 3.2% glucose (about 97% degree of oxidation) was reduced with sodium borohydride to the polyalcohol which was fully methylated by the application of Haworth,[3] Purdie,[19] and Kuhn methods.[20] The methylated polyalcohol was methanolyzed and the resulting mixture containing V, VI, VII, and VIII as well as methylated glucosides was subjected to distillation under reduced pressure to remove components V–VIII. The residues from the distillation were subjected to acid hydrolysis; the products so formed were separated by a combination of paper and column chromatography. The recovery of these methylated glucose derivatives was about 90% of the expected amount based on 3.2% of glucose in the polyaldehyde. Now if (1→3) linkages were present to the extent of 1% of the total linkages, as reported earlier,[13] then out of 3% glucose present in the parent polyaldehyde, one-third would give rise to 2,6-di-*O*-methyl-D-glucose and the remaining two-thirds would yield the other methylated glucose derivatives; thus the ratio of 2,6-di-*O*-D-glucose to the other methylated glucose derivatives should be approximately 1:2. But the ratio between 2,6-di-*O*-methyl-D-glucose and all other methyl glucose derivatives actually obtained was 1:128.

Amylopectin polyaldehyde containing 1.5% glucose was treated according to the method described above. The recovery of the methylated glucose derivatives was about 91% of the expected amount. The molar ratio of 2,6-di-*O*-methyl-D-glucose to all other methylated glucose derivatives was found to be 1:70. If the polysaccharide had contained 0.5% (1→3) linkages as has been suggested,[11] the ratio of 2,6-di-*O*-methyl-D-glucose to the other methyl glucose derivatives to be expected would be 1:2. All the methylated glucose derivatives obtained from the methylated glycogen and amylopectin polyalcohols are recorded in Table I.

TABLE I

METHYLATED GLUCOSE DERIVATIVES IN THE HYDROLYSATES OF METHYLATED POLYALCOHOLS

Component	Glycogen (6.2 g), mg	Amylopectin (10.5 g), mg
2,3,4,6-Tetra-*O*-methyl-D-glucose	1.9	3.3
2,3,6-Tri-*O*-methyl-D-glucose	164.6	135.4
2,3-Di-*O*-methyl-D-glucose	8.9	1.4
2,6-Di-*O*-methyl-D-glucose	1.3	1.8
Mono-*O*-methyl-D-glucose (as glucose)	0.3	1.2

The amount of 2,6-di-*O*-methyl-D-glucose isolated from the methylated glycogen and amylopectin polyalcohol is only 0.02% and is believed to have arisen owing to incomplete methylation or demethylation of the higher methyl ethers during hydrolysis.[10] It is clearly much less than that required if all the periodate-stable glucose residues were due to the presence of (1→3) linkages as proposed earlier. It is concluded from

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(20) R. Kuhn, H. Trischmann, and I. Low, *Angew. Chem.*, **67**, 32 (1955).

these experiments that the glucose residues in glycogen and amylopectin which resist periodate oxidation do not arise from glucose units linked through C-3. A further investigation as to the causes of resistance to periodate of certain glucose residues is in progress.

It is quite surprising to note as indicated by the presence of a small quantity of tetra-*O*-methyl-D-glucose in the hydrolytic mixture that some nonreducing terminal glucose units would survive periodate attack even after the oxidation had gone as far as 97–99%. It appears that a small number of terminal residues are inaccessible to periodate.

Experimental Section

Solutions were concentrated under reduced pressure below 40° in a rotary evaporator. Paper chromatography was done by the descending technique at room temperature on Whatman No. 1 and 3MM papers in the following solvent systems (v/v): (A) 1-butanol–ethanol–water, 4:1:5 upper layer;[21] (B) pyridine–ethyl acetate–water, 1:2.5:3.5 upper layer;[22] (C) butanone–water azeotrope;[23] (D) benzene–ethanol–water–ammonium hydroxide,[24] 200:47:14:1 upper layer. The sugars were detected on the paper chromatograms by the following spray reagents: (E) ammoniacal silver nitrate[25] and concentrated ammonium hydroxide; (F) *p*-anisidine trichloroacetate,[26] prepared by dissolving *p*-anisidine (0.4 g) and trichloroacetic acid (2.0 g) in water (100 ml).

All the hydrolyses of methylated glucosides were performed by refluxing with 1 *N* hydrochloric or sulfuric acids for 5–6 hr followed by neutralization of the acid with silver or barium carbonate.

Periodic Acid Oxidation of Calf Liver Glycogen.—A solution of calf liver glycogen (71.56 g), $[\alpha]^{23}D$ +194.4° (*c* 0.8), in water (2.8 l.) was treated with 0.65 *M* periodic acid (900 ml) and the volume was adjusted to 4 l. The solution was kept in the cold at 4–5°. A blank was carried out concurrently under the same conditions. At suitable intervals the consumption of periodate[27] was determined. After 5 weeks, when the periodate uptake became constant at 1.07 moles per mole of glucose unit, the calf liver glycogen polyaldehyde (70 g) was recovered from the oxidation mixture by freezing and thawing.[12] The polyaldehyde was found to contain 3.2% glucose.[28]

Methylation Studies on Glycogen Polyalcohol.—The glycogen polyaldehyde (18 g) as obtained above was reduced with a solution (400 ml) of sodium borohydride (9 g) at room temperature. The resulting polyalcohol was methylated as reported earlier.[29] The methylated polyalcohol was a clear transparent syrup which was optically inactive in chloroform (*c* 0.5). *Anal.* Calcd: OCH_3, 44.7. Found: OCH_3, 44.4.

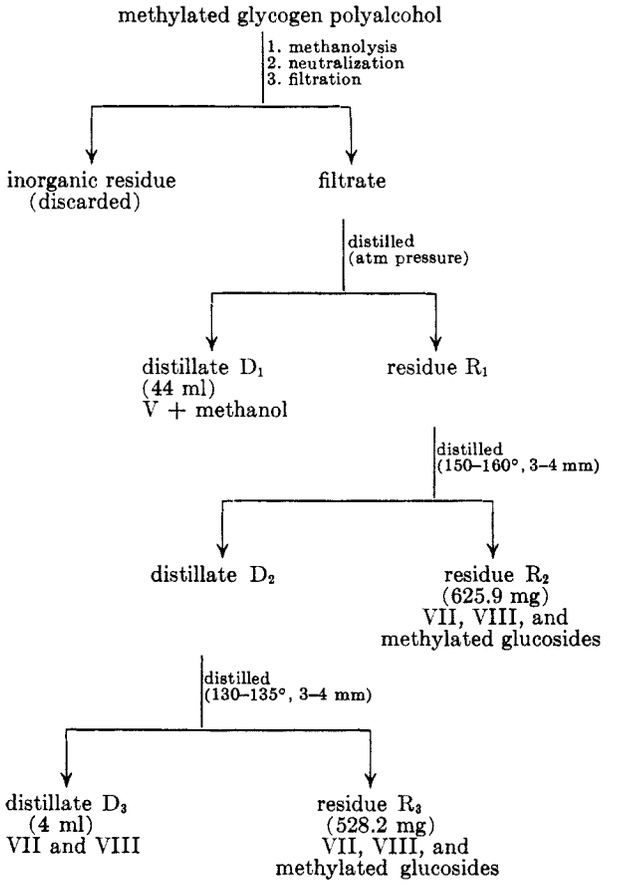
Fractionation and Estimation of the Methanolysis Products of Methylated Glycogen Polyalcohol.—A solution of methylated glycogen polyalcohol (6.206 g) in 2.5% methanolic hydrogen chloride was refluxed for 4 hr. The reaction mixture was neutralized with silver carbonate, filtered and the filtrate distilled at atmospheric pressure giving a distillate, D_1 (45 ml), and a residue R_1 (6 ml).

The distillate D_1 possessed an aldehyde-like smell and a portion of it when treated with *p*-nitrophenylhydrazine hydrochloride[30] gave a yellow, crystalline *p*-nitrophenylhydrazone of methoxyacetaldehyde. After recrystallization from aqueous ethanol, the methoxyacetaldehyde-*p*-nitrophenylhydrazone had mp and mmp 115–116°.

Fractional Distillation of the Methanolysate.—The residue R_1 consisting of a mixture of 1,4-di-*O*-methylerythritol (VII), 1-*O*-methyl-D-erythritol (VIII) and methylated glucosides was subjected to distillation under reduced pressure (3–4 mm) at 150–160° (bath temperature) giving a residue, R_2 (625.9 mg), and a distillate, D_2 (4.5 ml). To ensure complete recovery of the relatively volatile glucosides such as methyl tri- and methyl tetra-*O*-methyl-D-glucopyranosides, the distillate D_2 was again distilled at a lower temperature (130–135°) under reduced pressure (3–4 mm) yielding distillate D_3 (4 ml) and residue R_3 (528.2 mg). Chart II outlines the fractionation procedure employed.

CHART II

FRACTIONAL DISTILLATION OF THE METHANOLYSIS PRODUCTS OF METHYLATED GLYCOGEN POLYALCOHOL (6.206 g)



Preliminary examination of the distillate D_3 by paper chromatography using solvent C and spray reagent E showed the presence of 1,4-di-*O*-methylerythritol (VII) and 1-*O*-methyl-D-erythritol (VIII). A portion of D_3 (0.5 ml) was fractionated by preparative paper chromatography in solvent C. Component VII was identified as its di-*O*-(*p*-tolylsulfonyl)[30] and VIII as its tris(*p*-nitrobenzoyl)[31] derivative.

Residue R_2 (625.9 mg) was hydrolyzed be refluxing it in a solution of 1 *N* sulfuric acid (20 ml) for 6 hr. The hydrolysate, by paper chromatography in solvent C and using spray F, was shown to contain 2,3,4,6-tetra-*O*-methyl-D-glucose (R_f 0.8), 2,3,6-tri-*O*-methyl-D-glucose (R_f 0.58), 2,3-di-*O*-methyl-D-glucose (R_f 0.28), and 2,6-di-*O*-methyl-D-glucose (R_f 0.20). When a second paper chromatogram prepared in the above manner was sprayed with reagent E the two components, 1,4-di-*O*-methylerythritol (R_f 0.58) and 1-*O*-methyl-D-erythritol (R_f 0.28) were also detected. The spray E alone failed to distinguish between 2,3-di-*O*-methyl-D-glucose and 1-*O*-methyl-D-erythritol since both have the same mobility in solvent C.

Fractionation of the Acid Hydrolysate of Residue R_2.—The syrupy mixture (528.3 mg) obtained by the acid hydrolysis of residue R_2 was separated by preparative paper chromatography using 3 MM paper and solvent D for 4–5 hr, the components being located by spray reagent E. The paper was eluted with water

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(31) I. J. Glodstein, J. K. Hamilton, and F. Smith, *ibid.*, **81**, 6252 (1959).

TABLE II

PAPER CHROMATOGRAPHIC FRACTION OF THE ACID HYDROLYSATE OF RESIDUE R_2

Fraction	Component	R_f	Wt, mg
A	Methyl-tri-*O*-methyl-D-glucopyranoside	>0.6	
	Methyl-tetra-*O*-methyl-D-glucopyranoside		19.8
B	2,3,6-Tri-*O*-methyl-D-glucose	0.6	
	1,4-Di-*O*-methyleryth-ritol		170.0
C	2,3-Di-*O*-methyl-D-glucose		
	2,6-Di-*O*-methyl-D-glucose	0.2	238.9
D	1-*O*-Methyl-D-erythritol		
	Unhydrolyzed material		
	Mono-*O*-methyl-D-glucose	0.2	5.2

and the resulting eluates were evaporated to dryness. In this manner four fractions listed in Table II were obtained.

Analyses of Fractions A, B, C, and D.—Fraction A (19.8 mg) was hydrolyzed by refluxing with 1 *N* sulfuric acid (5 ml) for 6 hr. The hydrolysate was found to be a mixture of 2,3,6-tri-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose by paper chromatography in solvents C and D and using spray reagent F. The mixture was separated on paper and the amounts of 2,3,6-tri-*O*-methyl-D-glucose (11.7 mg) and 2,3,4,6-tetra-*O*-methyl-D-glucose (1.9 mg) were determined by the phenol–sulfuric acid method.[32]

Fraction B (170.1 mg) consisting of 2,3,6-tri-*O*-methyl-D-glucose and 1,4-di-*O*-methylerythritol, as indicated by paper chromatography using solvent C and spray reagent E and F. The amount of 2,3,6-tri-*O*-methyl-D-glucose as determined by phenol–sulfuric acid method[32] was 118.7 mg and hence by difference (170.1 − 118.7) the amount of 1,4-di-*O*-methylerythritol would be 51.4 mg.

Fraction C (238.9 mg) was dissolved in a small quantity of methanol (0.5 ml) and was chromatographed on two sheets of paper using solvent C. One of the chromatograms was sprayed with reagent E and the other with reagent F. By the combined use of these two spray reagents, fraction C was found to be mixture of 2,3-di-*O*-methyl-D-glucose (R_f 0.28), 2,6-di-*O*-methyl-D-glucose (R_f 0.20), and 1-*O*-methyl-D-erythritol (R_f 0.28).

Separation of 2,3,-Di-*O*-methyl-D-glucose, 2,6-Di-*O*-methyl-D-glucose, and 1-*O*-Methyl-D-erythritol.—Fraction C (238.9 mg), containing the above components, was refluxed with 2% methanolic hydrogen chloride (15 ml) to convert the methylated sugars into the corresponding glucosides. The reaction mixture was neutralized with silver carbonate and filtered and the filtrate was evaporated to dryness to give a light yellow, syrupy residue (216.5 mg) consisting of methylated glucosides and 1-*O*-methyl-D-erythritol. This mixture was separated on Whatman 3 MM paper using solvent C and spray reagent E. The strip of paper in front of 1-*O*-methyl-D-erythritol was eluted with water and the aqueous solution was concentrated to dryness to give a yellow, syrupy residue (13.5 mg) consisting of a mixture of methyl 2,3-di-*O*-methyl-D-glucopyranoside and methyl 2,6-di-*O*-methyl-D-glucopyranoside. The paper strip containing 1-*O*-methyl-D-erythritol on elution with water and evaporation of the solvent gave 184.2 mg of this compound.

The mixture of the above glucosides (13.5 mg) was hydrolyzed by refluxing with 1 *N* sulfuric acid for 4–5 hr. The hydrolysate containing the corresponding methylated glucose derivatives was separated by paper chromatography and quantitatively determined by the phenol–sulfuric method.[32] The amount of 2,3-di-*O*-methyl-D-glucose (IV) and 2,6-di-*O*-methyl-D-glucose (III) were found to be 8.9 and 1.3 mg, respectively.

Paper chromatographic examination of fraction D (5.2 mg) using solvent C and spray reagent E showed the presence of mono-*O*-methyl-D-glucose (R_f 0.05). The amount of mono-*O*-methyl-D-glucose in the fraction determined as glucose by the phenol–sulfuric acid method was 0.3 mg. This fraction was not investigated any further.

Fractionation of Residue R_3.—Residue R_3 (528.3 mg) was dissolved in butanone–water azeotrope (about 1 ml) and transferred to a hydrocellulose–cellulose column.[33] The following three fractions were detected: fraction A_1, 40.8 mg; fraction B_1, 228.5 mg; and fraction C_1, 25.7 mg. Fraction B_1 and C_1 were found, by paper chromatography using solvents C and D and spray reagent E, to be 1,4-di-*O*-methylerythritol and 1-*O*-methyl-D-erythritiol, respectively.

Fraction A_1 was hydrolyzed by heating in a boiling-water bath with 1 *N* sulfuric acid for 5–6 hr. Examination of the hydrolysate by paper chromatography in solvent C and spray reagent F indicated the presence of 2,3,6-tri-*O*-methyl-D-glucose. The residue was extracted with methanol and the solvent removed *in vacuo* giving a syrup (34.2 mg) which crystallized on keeping.

Studies on Methylated Amylopectin Polyalcohol.—Amylopectin polyaldehyde[34] (37 g) containing 1.5% glucose[29] was reduced with sodium borohydride (20 g) to the polyalcohol which was subsequently methylated as described above. The methylated polyalcohol (21.9 g) was optically inactive in chloroform (*c* 0.5). *Anal.* Calcd: OCH_3, 45.1. Found: OCH_3, 44.0.

Separation and Estimation of Methanolysis Products of Methylated Amylopectin Polyalcohol.—A solution of the methylated amylopectin polyalcohol (10.5 g) in methanol (50 ml) was refluxed for 3 hr with 8% methanolic hydrogen chloride (25 ml). After neutralization of the reaction mixture with silver carbonate and filtration, the filtrate was distilled at atmospheric pressure giving a distillate, D_4 (960 ml), and a residue R_4, (10 ml). The distillate was found to contain methoxyacetaldehyde which was characterized by preparing its *p*-nitrophenylhydrazone.[30]

The residue R_4 was subjected to distillation under reduced pressure (1–1.5 mm) at 110–115° (bath temperature). From the distillate (8 ml) 1-*O*-methyl-D-erythritol and 1,4-di-*O*-methylerythritol were isolated by paper chromatography, the former being identified as the tris(*p*-nitrobenzoate)[31] and the latter as its di-*O*-(*p*-tolylsulfonyl) derivative.[30] The residue (1.14 g) obtained from the distillation of the residue R_4 under diminished pressure contained 1-*O*-methyl-D-erythritol, 1,4-di-*O*-methylerythritol, and methylated glucosides. This mixture was fractionated into five fractions as recorded in Table III on a hydrocellulose–cellulose column[33] using butanone–water azeotrope as an eluent.

TABLE III

FRACTIONS FROM THE METHANOLYSIS PRODUCTS OF METHYLATED AMYLOPECTIN POLYALCOHOL

Fraction	Component	Wt, mg
F 1	Methyl 2,3,6-tri-*O*-methyl-D-glucopyranoside	
	Methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside	526.8
	1-*O*-Methyl-D-erythritol	
	1,4-Di-*O*-methylerythritol	
	Noncarbohydrate oily material	
F 2	Methyl 2,3,6-tri-*O*-methyl-D-glucopyranoside	254.2
	1-*O*-Methyl-D-erythritol	
F 3	Methyl 2,3-6-tri-*O*-methyl-D-glucopyranoside	
	Methyl 2,3-di-*O*-methyl-D-glucopyranoside	77.5
	Methyl 2,6-di-*O*-methyl-D-glucopyranoside	
	1-*O*-Methyl-D-erythritol	
F 4	1-*O*-Methyl-D-erythritol	142.2
F 5	Methyl mono-*O*-methyl-D-glucopyranoside and high molecular weight organic material	11.1

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(33) J. D. Geerdes, B. A. Lewis, R. Montgomery, and F. Smith, *ibid.*, **26**, 264 (1964).

(34) Amylopectin polyaldehyde was kindly provided by the American Maize Products Co.

Although the hydrocellulose–cellulose column had previously been used successfully in our laboratory in the separation of a similar mixture, the results shown in Table III indicate that in this case it failed to resolve the various components. Probably, it was due to the deterioration of the column.

Analyses of Fraction F_1–F_5.—Fraction F_1 was hydrolyzed by refluxing with 1 *N* sulfuric acid (25 ml) for 5 hr. A major portion of the fraction (about 320 mg) which did not go in solution during hydrolysis was removed, methanolyzed with methanolic hydrogen chloride, and finally was subjected to acid hydrolysis; despite this treatment, the yellow oily product remained insoluble. Examination of this insoluble oily product by paper chromatography in solvent C showed that it moved with the solvent front as a yellow spot and did not give a color with either spray reagent E or F. It was also found to be optically inactive and did not reduce Fehling's solution. These facts point to the noncarbohydrate nature of the material and so it was discarded.

The hydrolysate of the soluble portion (190.1 mg), by paper chromatography using solvent C and spray reagent E and F, showed the presence of 1-*O*-methyl-D-erythritol and traces of 1,4-di-*O*-methylerythritol, 2,3,6-tri-*O*-methyl-D-glucose, and 2,3,4,6-tetra-*O*-methyl-D-glucose. The mixture was separated on paper into three fractions and the amount of each fraction was determined either by the phenol–sulfuric acid method or gravimetrically. The results are recorded in Table IV.

TABLE IV

COMPONENTS IN FRACTION F_1

Component	Wt,[a] mg
Crystalline 2,3,4,6-tetra-*O*-methyl-D-glucose	3.3 (p)
Crystalline 2,3,6-tri-*O*-methyl-D-glucose	94.7 (g)
1-*O*-methyl-D-erythritol	76.8 (g)

[a] g, gravimetrically; p, phenol–sulfuric acid method.

Fraction F_2 (254.2 mg) was hydrolyzed for 5–6 hr with 1 *N* sulfuric acid (10 ml). The hydrolysate afforded a syrupy residue (231 mg) which consisted of 2,3,6-tri-*O*-methyl-D-glucose and 1-*O*-methyl-D-erythritol as shown by paper chromatography using solvent C and spray reagents E and F. The amounts of 2,3,6 tri-*O*-methyl-D-glucose and 1-*O*-methyl-D-erythritol obtained by preparative paper chromatography were 26 mg and 195 mg, respectively.

Fraction F_3 (77.5 mg) was hydrolyzed by refluxing with 1 *N* hydrochloric acid (5 ml) for 5–6 hr. The syrupy product (68 mg) was shown by paper chromatography in solvent C using spray reagents E and F to contain 2,3,6-tri-*O*-methyl-D-glucose, 2,3-di-*O*-methyl-D-glucose, 2,6-di-*O*-methyl-D-glucose, and 1-*O*-methyl-D-erythritol. The mixture was separated on paper into three fractions: (a) 2,3,6-tri-*O*-methyl-D-glucose (14.7 mg), (b) a mixture of 2,3-di-*O*-methyl-D-glucose and 1-*O*-methyl-D-erythritol (44 mg), and (c) 2,6-di-*O*-methyl-D-glucose (1.8 mg). The amount of 2,3-di-*O*-methyl-D-glucose in b, determined by the phenol–sulfuric acid method, was 1.4 mg.

After hydrolysis, fraction F_4 (142.2 mg) was identified as 1-*O*-methyl-D-erythritol by paper chromatography using solvent C and spray reagents E and F.

Fraction F_5 (11.1 mg) was hydrolyzed with 1 *N* sulfuric acid (5 ml) by heating in a boiling-water bath for 5–6 hr. Examination of the resulting syrupy residue by paper chromatography in solvent C using spray reagents E and F revealed the presence of mono-*O*-methyl-D-glucose (R_f 0.05). The residue was dissolved in water (50 ml) and the amount of mono-*O*-methyl-D-glucose determined as glucose by the phenol–sulfuric acid method was 1.2 mg.

Identification of Methylated Glucose Derivatives.—The various methylated glucose derivatives including 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, and 2,3-di-*O*-methyl-D-glucose were characterized as reported earlier.[29] Component 2,6-di-*O*-methyl-D-glucose was found to be chromatographically identical, in two solvent systems C and D, with an authentic specimen. It gave 2,6-di-*O*-methyl-D-glucose 1,3,4-tris(*p*-azobenzoate) with *p*-phenylazobenzoyl chloride in pyridine, mp 203–205°, lit.[35] mp 205–207°.

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The Structural Characterization of Tetrangomycin and Tetrangulol

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Two new benz[*a*]anthraquinone derivatives have been isolated from fermentations of *Streptomyces rimosus*. Experiments are reported which lead to the assignment of structure **1** to tetrangomycin and **3** to tetrangulol.

Despite the intensive examinations of microbial metabolites carried out in recent years and the remarkably diverse structural types already uncovered, new types are coming to light with increasing frequency.[1] We would like to describe two metabolites belonging to a new structural type which were discovered in the course of our antibiotic screening program. These compounds, tetrangomycin and tetrangulol, represent the first recorded isolation of benz[*a*]-anthraquinone derivatives from a living system. The finding is particularly intriguing in light of the well-known carcinogenic properties of hydrocarbons of this ring type.[2]

Tetrangomycin (**1**) was isolated from cultures of a variant strain of *Streptomyces rimosus* as an optically active, yellow quinone melting at 182–184° and having the molecular formula $C_{19}H_{14}O_5$ (M = 322 ± 0).[3,4] The ultraviolet and visible absorption spectra were similar to those reported for 1-hydroxyanthraquinone.[5] The infrared spectrum is consistent with an aromatic quinone in which one of the quinone carbonyls is chelated (1642 cm^{-1}) and the other is not (1678 cm^{-1}).[6] A third carbonyl band is present whose frequency (1705 cm^{-1}) is intermediate between that of an aromatic-conjugated ketone (1690 cm^{-1}) and a noncon-

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(3) The molecular weight was determined mass spectrometrically, using an Atlas CH4 instrument with a direct inlet system.

(4) A preliminary report emphasizing the biological properties of these metabolites was presented before the International Congress of Chemotherapy at Washington, D. C., Oct 21, 1965, and will appear in *Antimicrobial Agents Chemotherapy*–1965.

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