Anal. Calcd. for $C_{23}H_{36}O_5$: C, 70.38; H, 9.25; CH_3O, 15.82. Found: C, 70.09; H, 8.95; CH_3O, 15.84.

(b) From Δ^4-Pregnene-11β-ol-3,20-dione-21-al Dimethylacetal (IX).—The ol-dione acetal X (100 mg.) was reduced with lithium aluminum hydride and treated with manganese dioxide as above. Two recrystallizations of the crude product (70 mg., m.p. 150–153°) from acetone–petroleum ether gave needles, m.p. 157.5–158.5°, undepressed on admixture with IV prepared from the trione acetal II. The respective infrared spectra were likewise identical.

Anal. Calcd. for $C_{23}H_{36}O_5$: C, 70.38; H, 9.25. Found: C, 70.70; H, 9.00.

Δ^4-Pregnene-11β,20β-diol-3-one-21-al (V).—The diol-one acetal IV (1.00 g.) in 50% aqueous acetic acid (20 ml.) was kept at 90–95° for three hours. The mixture was cooled to 20°, water was added and the mixture extracted with chloroform. The chloroform extract was washed with aqueous sodium bicarbonate, water and dried over sodium sulfate. The amorphous residue (990 mg.), after removal of the solvent, gave a negative tetranitromethane test for unsaturation. The BT assay[20] run *vs.* corticosterone (VII) showed the slow generation of a reducing system—28% after one hour; 43% after two hours. Normally (corticosterone) the full dye color was produced within 20 to 40 minutes, $\lambda\lambda_{max}^{nujol}$ 2.9 μ (O–H); 5.8 μ (very weak) (C=O); 6.00, 6.15 μ (conj. C=O).

Anal. Found: CH_3O, 0.1.

The product partially crystallized from ethyl acetate to give micro-crystalline material m.p. 165–175° with infrared spectrum similar to that of the total material.

Δ^4-Pregnene-11β,20β-diol-3-one-21-al Sodium Bisulfite Addition Product (VI).—To the crude hydrolysis product V (600 mg.) in methanol (40 ml.) was added sodium bisulfite (250 mg.) in water (15 ml.). The slightly turbid mixture was kept at 25° one hour and concentrated under vacuum at 20–30° until a granular precipitate appeared. Water (50 ml.) was added, and the concentration continued until the methanol had been removed completely. The precipitate was filtered, washed with water and dried (170 mg., m.p. 165–170°, of recovered polymeric forms of V). The colorless filtrate and washings were concentrated under vacuum to dryness. The water-soluble amorphous precipitate was triturated with absolute ethanol and unreacted sodium bisulfite (80 mg.) was removed by filtration. Concentration of the filtrate gave the sodium bisulfite addition product VI in three crops of hygroscopic micro-crystalline material (350 mg., 63% corrected for recovered reusable V), m.p. 165° dec. Recrystallization from absolute ethanol did not change the decomposition point. On addition of dilute hydrochloric acid or dilute sodium carbonate solution to a water solution of the derivative VI, the water-insoluble aldehyde V precipitated.

Anal. Calcd. for $C_{21}H_{31}O_7NaS$: C, 55.98; H, 6.94; S, 7.05. Found: C, 55.44; H, 7.16; S, 7.50.

Corticosterone (VII). (a) From the Hydrolysis Product (V).—The crude hydrolysis product V (160 mg.) was dissolved in dry pyridine (5.00 ml.) and the solution refluxed gently (115°) under nitrogen 5.5 hours.[13] The solvent was removed under vacuum, and the residue crystallized from ethyl acetate–ether. Corticosterone (VII) (35 mg., 20%), micro m.p. 165–175°, was obtained. Recrystallization from ethyl acetate–petroleum ether gave material with micro m.p. 176–180° (capillary m.p. 180–183°). Authentic corticosterone (VII) had micro m.p. 176–181° and the mixture, 176–180°. The respective infrared spectra were identical.

Treatment of the hydrolysis product V (70 mg.) with sodium methoxide (30 mg.) in methanol (7 ml.) under nitrogen at 25° for 30 minutes gave a residue (67 mg.) the infrared spectrum of which was very similar to that of the starting material except for a slight intensification of the weak saturated carbonyl band, indicating that no significant change had occurred.

(b) From the Sodium Bisulfite Addition Product (VI).—To the sodium bisulfite addition product VI (80 mg.) in methanol (5.00 ml.) was added freshly prepared methanolic sodium methoxide (10 ml., 0.5 *N*) under nitrogen. The pale yellow solution was stirred at 22° for one-half hour. Addition of acetic acid (0.5 ml.) neutralized the alkali and discharged the color. Most of the solvent was removed under vacuum, water was added, and the mixture was extracted with chloroform. The chloroform extract was washed with dilute aqueous sodium bicarbonate and water and dried over sodium sulfate. The residue (61 mg.) was crystallized from acetone–ether and gave the characteristic stout prisms[11] of corticosterone; 37 mg. (60%) in several crops, m.p. 174–178° and higher, raised to 179–182° in one recrystallization. The mixed melting point with an authentic sample (m.p. 180–183°) was 179–182° and the respective infrared spectra in chloroform were identical.

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.71; H, 8.95.

RAHWAY, NEW JERSEY

[CONTRIBUTION FROM THE DEPARTMENT OF PLANT BIOCHEMISTRY AND THE FOREST PRODUCTS LABORATORY, UNIVERSITY OF CALIFORNIA]

The Structure of an Arabogalactan from Jeffrey Pine (*Pinus Jeffreyi*)

BY W. H. WADMAN, ARTHUR B. ANDERSON AND W. Z. HASSID

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A water-soluble polysaccharide consisting of L-arabinose and D-galactose has been isolated from Jeffrey pine heartwood. Methylation and subsequent hydrolysis of this arabogalactan yielded 2,3,5-tri-*O*-methyl-L-arabinose, a di-*O*-methyl-L-arabinose (undetermined), 2,3,4-tri-*O*-methyl-D-galactose, and 2,4-di-*O*-methyl-D-galactose. A relatively small amount of 2,3,4,6-tetra-*O*-methyl-D-galactose, and a trace of partially methylated uronic acid also were present. On the basis of these results, the polysaccharide appears to be a highly branched molecule in which only L-arabinose residues occupy terminal positions. Ultracentrifuge measurements indicate that the polysaccharide is polydisperse, having an average molecular weight of approximately 100,000.

Arabogalactans have been found in a number of coniferous woods, particularly in larch woods. These polysaccharides are of interest in connection with wood formation and have been studied by a number of investigators.[1–5] Hydrolysis of specimens of arabogalactan from different species of larch wood yields L-arabinose and D-galactose in an approximate ratio of 1:6 moles.[2,6,7]

In studying the fractions obtained by fractional precipitation of solutions of arabogalactan and of derivatives from *Larix occidentalis*, Peterson, *et al.*,[6] demonstrated that the fractions exhibited appreciable difference in their properties, indicating that the polysaccharide is non-homogeneous. Simi-

(1) A. W. Schorger and D. F. Smith, *Ind. Eng. Chem.*, **8**, 494 (1916).

(2) L. E. Wise and F. C. Peterson, *ibid.*, **22**, 362 (1930).

(3) F. C. Peterson, M. Maughan and L. E. Wise, *Cellulosechemie*, **15**, 109 (1934).

(4) N. I. Nikitin and I. A. Soloviev, *J. Applied Chem. (U.S.S.R.)*, **8**, 1016 (1935).

(5) For review see: L. E. Wise and E. C. Jahn, "Wood Chemistry," Vol. I, Reinhold Publ. Corp., New York, N. Y., 2nd Ed., 1952, pp. 644–646.

(6) F. C. Peterson, A. J. Barry, H. Unkauf and L. E. Wise, THIS JOURNAL, **62**, 2361 (1940).

(7) E. V. White, *ibid.*, **64**, 2838 (1942).

larly, the results of Campbell, *et al.*,[8] show that the arabogalactan from European larch wood, *Larix decidua*, is heterogeneous, consisting of a mixture of galactan and galactoaraban. On the other hand, White,[7,9] who made an extensive study of the arabogalactan from *Larix occidentalis*, interpreted his results as indicating that the polysaccharide is an arabogalactan and not a mixture. He proposed a tentative structure for its repeating unit containing six D-galactose and one L-arabinose residue.

Similar polysaccharide fractions, which on hydrolysis yield uronic acid in addition to D-galactose and L-arabinose, have been reported to be present in southern yellow pine (*Pinus palustris*)[10] in black spruce (*Picea mariana*),[11] and in jack pine (*Pinus banksiana*).[4]

In the present investigation the structure of an arabogalactan isolated from Jeffrey pine (*Pinus jeffreyi*) has been studied. Paper chromatographic analysis showed that it consists entirely of D-galactose and L-arabinose. Its specific rotation in water is $[\alpha]^{23}D$ +17°. The final specific rotation of the polysaccharide after hydrolysis with 0.1 *N* sulfuric acid was +88°, which is consistent with that of a mixture of 5 moles of D-galactose and 4 moles of L-arabinose.

The position of linkage between the individual monosaccharide residues in the polysaccharide was determined from the identification of the methylated D-galactose and L-arabinose derivatives of the degraded methylated polysaccharide. The arabogalactan was methylated with dimethyl sulfate and sodium hydroxide and, after methanolysis and subsequent hydrolysis, the product was separated by the aid of a powdered cellulose column into eight fractions (A through H).

Fraction A consisted of a relatively small amount of methyl 2,3,4,6-tetra-*O*-methyl-D-galactose, which apparently escaped hydrolysis. When subjected to another hydrolysis with 1 *N* sulfuric acid, tetra-*O*-methyl-D-galactose was obtained which was identified by its 2,3,4,6-tetra-*O*-methyl-D-galactose anilide derivative.

Fraction B contained tri-*O*-methyl-L-arabinose and tetra-*O*-methyl-D-galactose. Since these two compounds possess the same R_f value, they could not be separated by column or paper chromatography. Advantage was therefore taken of the fact that methyl furanosides are formed more readily than pyranosides and also that they are hydrolyzed more readily than pyranosides.[12] From the rate of the disappearance of the reducing power of the sirup (B fraction) containing the mixture of tri-*O*-methyl-L-arabofuranose and tetra-*O*-methyl-D-galactopyranose when subjected to glycoside formation, the methylated arabinose component to that of the methylated galactose component was found to be 9 to 1.

The products of fraction B were identified by subjecting a portion of this material to partial glycoside formation under mild conditions (heating with 0.5% methanolic hydrogen chloride for 15 minutes at 100°). After separation of the glycosidic fraction from the non-glycosidic fraction by means of paper chromatography, and subsequent hydrolysis of the former, the 2,3,5-tri-*O*-methyl-L-arabinose was identified as 2,3,5-tri-*O*-methyl-L-arabonamide. The non-glycosidic fraction was identified as 2,3,4,6-tetra-*O*-methyl-D-galactose by forming its 2,3,4,6-tetra-*O*-methyl-D-galactose anilide derivative.

The relative proportions of the methylated derivatives of D-galactose and L-arabinose in fraction C were similarly determined by following the course of hydrolysis of their glycosides. Their rate of hydrolysis indicated that this fraction consisted of approximately 30% labile methylated glycoside of arabinose and 70% of the more stable methylated glycoside of galactose. The latter component was identified as 2,3,4-tri-*O*-methyl-D-galactose from its anilide derivative. The methylated arabinose derivative was identified as di-*O*-methylarabinose only by the methoxyl content of its glycoside derivative, and by the fact that demethylation with hydrobromic acid[13] produced free arabinose. The position of its methyl groups was not determined.

Fractions D, E and F consisted of di-*O*-methyl-D-galactose, which was obtained as crystalline material, and identified as 2,4-di-*O*-methyl-D-galactose by its anilide derivative.

Fractions G and H contained chiefly mono-*O*-methyl-D-galactose and a small amount of unidentified sugar acids.

The proportion of the various methylated derivatives of D-galactose and L-arabinose obtained from the hydrolysis of the methylated arabogalactan are given in Table I.

TABLE I

SUMMARY OF MOLECULAR CONSTITUENTS IN THE ARABOGALACTAN

Fraction	Wt. of fraction	Methylated constituent[a]	Wt. of constituent	Mol. wt. of constituent	Molecular parts		Ratio of molecular constituents
A	41	Me^4 G	41	250	0.16	0.51	0.33
B	925	Me^4 G	83	236	0.35		
		Me^3 A	842	192	4.40	4.40	3
C	860	Me^2 A	258	178	1.44	1.44	1
		Me^3 G	602	222	2.71	2.71	2
D + E + F	749	Me^2 G	749	208	3.60	3.60	3
G	62	Me^1 G	62	194	0.32	1.17	1
H	166	Me^1 G	166	194	0.85		
	40	acids	40	..			

[a] Me^4 G = tetra-*O*-methyl-D-galactopyranose; Me^3 A = tri-*O*-methyl-L-arabinofuranose, etc.

Making a slight adjustment so as to compensate for systematic errors, such as incomplete methylation and demethylation[14] known to occur during methanolysis, it was calculated that the methylated arabogalactan is composed of the following parts:

(8) W. G. Campbell, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 774 (1948).

(9) R. V. White, THIS JOURNAL, (a) **63**, 2871 (1941); (b) **64**, 302, 1507 (1942).

(10) E. L. Foreman and D. T. Englis, *Ind. Eng. Chem.*, **23**, 415 (1931).

(11) F. E. Brauns, *Science*, **102**, 155 (1945).

(12) W. N. Haworth, *Ber.*, **65A**, 50 (1932).

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

(14) R. A. Laidlaw and E. G. V. Percival, *ibid.*, 1600 (1949).

2,3,5-tri-*O*-methyl-L-arabinose (3 parts)
2,3,4,6-tetra-*O*-methyl-D-galactose (0.3 part)
di-*O*-methyl-L-arabinose (1 part)
2,3,4-tri-*O*-methyl-D-galactose (2 parts)
2,4-di-*O*-methyl-D-galactose (3 parts)
mono-*O*-methyl-D-galactose (1 part)
A partially methylated uronic acid (trace)

There is no information available at present to show how these residues are related in the polysaccharide molecule. No definite structural formula can, therefore, be assigned to the arabogalactan. However, identification of the methylated derivatives of D-galactose and L-arabinose in the hydrolysis products of the methylated polysaccharide discloses the various positions through which the monosaccharides are linked in the polysaccharide molecule. On the basis of the above proportions of the methylated monosaccharides, one of the many possible variations in which the monosaccharide residues might be combined in the polysaccharide can be visualized as follows: D-galactose residues are mutually attached through 1,3-glycosidic linkages. These residues are combined by their primary alcohol groups with position 1 of other D-galactose residues which are in turn substituted at the 6-position by terminal L-arabinose residues. The presence of di-*O*-methyl-L-arabinose indicates that some of the L-arabinose residues also exist in the polysaccharide in non-terminal positions.

The relatively large proportions of tri-*O*-methyl-L-arabinose and di-*O*-methyl-D-galactose present in the methylated polysaccharide indicate that the polysaccharide is highly branched. The small amount of D-galactose end-group (0.33 part tetra-*O*-methyl-D-galactose) relative to the other methylated derivatives indicates that one terminal D-galactose residue is probably combined with a number of other D-galactose and L-arabinose residues to form a unit of about 30 monosaccharide residues. This combination may constitute the repeating unit in the large polysaccharide molecule of approximately 100,000 molecular weight found from ultracentrifuge determinations.

It is to be observed that the hydrolysis products of the methylated polysaccharide contain only a small proportion of tetra-*O*-methyl-D-galactose; also no mono-*O*-methyl-L-arabinose could be detected, but the proportion of tri-*O*-methyl-L-arabinose is high. These data can best be interpreted to mean that the arabogalactan is probably a single compound. However, the possibility that the polysaccharide is a mixture of a galactan and an arabogalactan is not excluded.

Experimental

Isolation and Purification of Polysaccharide.—Five hundred grams of benzene-extracted Jeffrey pine butt heartwood sawdust was placed in a 4-liter glass percolator and 3 liters of water was added. The polysaccharide was extracted by circulating the water through the sawdust overnight and then decanting the solution. The aqueous extraction was repeated a total of three times and the residue washed with 3 liters of water after the final extraction. The solutions were combined and concentrated to about 400 ml. in a flask evaporator under reduced pressure. The concentrated solution was cooled, centrifuged to remove a small amount of water-insoluble material, and poured with stirring into 900 ml. of 95% ethanol. The resulting flocculent precipitate was removed by centrifugation. A small amount of ethanol was added to the precipitate, the mixture stirred and again centrifuged. The residue was then dispersed in ethanol, filtered, washed with absolute alcohol, ether and then dried in a vacuum oven at 60°; yield 6.8 g., or 1.5% based on the benzene extracted heartwood (dry basis). Similar extractions of various lots of Jeffrey pine butt heartwood gave comparable yields of the crude polysaccharide.

The crude product (6.0 g.) was dissolved in 150 ml. of water by warming slightly on a water-bath, the solution cooled to room temperature and centrifuged for about half an hour at 20,000 r.p.m. The small quantity of brown precipitate was discarded and the polysaccharide precipitated by the addition of an equal volume of 95% ethanol. The moist precipitate was dissolved in water and the procedure of purification by centrifuging the solution was repeated two more times. Finally, the dissolved material was dialyzed against distilled water for 24 hours and precipitated by the addition of an equal volume of 95% ethanol. The polysaccharide was thoroughly washed with acetone and dried in a vacuum oven at 50°, yield 4.9 g.

The white powdered polysaccharide was readily soluble in cold water, yielding a solution that showed a slight opalescence. It did not reduce Fehling solution, its ash content was 0.51% and nitrogen 0.14%. Its specific rotation (*c* 1) in water was $[\alpha]^{23}D$ +17°.

Identification of Hydrolysis Products.—When the polysaccharide was oxidized with nitric acid, crystals were obtained that were identified as mucic acid, showing that the compound contained galactose. After hydrolysis of the polysaccharide and subsequent neutralization, it gave the orcinol–hydrochloric acid reaction (Bial), characteristic for pentose sugars. Paper chromatographic analysis of this solution showed two spots on the filter paper, one corresponding to galactose and the other to arabinose.

A 1-g. sample of the polysaccharide was heated under reflux with 30 ml. of 1 *N* sulfuric acid at 100° for 13 hours. The solution was neutralized with barium carbonate and after filtration concentrated to a sirup under reduced pressure. The sirup was dissolved in 8 ml. of water, the solution equally divided and applied on 7 chromatogram filter papers, placing 32 0.01-ml. spots plus the necessary marker spots on each paper. The papers were chromatographed with a mixture of 52.5% *n*-butanol, 32.0% ethanol and 15.5% water. After development of the test strip, the two paper strips containing the carbohydrate material were located and excised, and the carbohydrates extracted with 95% methanol in Soxhlets for 8 hours. On evaporation of the methanol, the sirup obtained from the arabinose strip produced 82 mg. of L-arabinose with a specific rotation $[\alpha]^{23}D$ +101° (in water, *c* 3). The sirup obtained from the galactose strip yielded 210 mg. of D-galactose, specific rotation, $[\alpha]^{23}D$ +78° (in water, *c* 2).

Complete hydrolysis of the polysaccharide with 0.1 *N* sulfuric acid for 30 hours at 100° gave a specific rotation $[\alpha]^{23}D$ +88° (*c* 1.58). This specific rotation is in fair agreement with that of a mixture of 5 moles D-galacotose and 4 moles of L-arabinose, assuming $[\alpha]D$ of the former sugar to be +80.2° and $[\alpha]D$ of the latter +104.5°.

Methylation of Polysaccharide.—The crude material (12 g.) was dissolved in 12 ml. of water by prolonged stirring, the solution transferred to a 1500-ml. flask equipped with a mechanical stirrer and an inlet funnel, and the flask with the contents immersed in a water-bath at 30°. Twelve ml. of concentrated sodium hydroxide solution (made by dissolving 50 g. of the alkali in 100 ml. of water) was added, and at approximately half hour intervals 2-ml. aliquots of both dimethyl sulfate and the sodium hydroxide solution were added. Excess heating was prevented by maintaining a small volume of ether in the flask. Addition of the reagents was continued until 75 ml. of each of the reagents had been added, by which time the mixture was so thick that efficient stirring was impossible. The mixture was neutralized by the addition of sulfuric acid and water was added until solution occurred, and the density of the solution was less than that of chloroform. The solution was then extracted 6 times with 100-ml. portions of chloroform; the combined extracts were evaporated to a sirup and the product was methylated, as above, twice more.

The final chloroform extract was dried over anhydrous sodium sulfate, shaken with 5 g. of a dry mixture of the ion-exchange resins, Amberlites, IR-4B and IR-120. The solution was then filtered through a thin bed of charcoal,

concentrated *in vacuo* to 100 ml. and poured slowly with stirring into approximately 900 ml. of petroleum ether. The supernatant liquid was decanted and discarded, and the precipitate was dried *in vacuo* at 50°. A yield of 9.7 g. of methylated product was obtained. Its methoxyl content, OCH_3, was 40.1%; N, 1.2%; and ash, 1.7%. The OCH_3 content, calculated on the ash and protein free basis, was 43.1%.

Methanolysis and Subsequent Hydrolysis of Methylated Polysaccharide.—The methylated material (7.2 g.) was heated in 9 sealed tubes for 6 hours at 100° with 4% methanolic hydrogen chloride (150 ml.). The tubes were cooled to 0°, opened, and the contents filtered through a little charcoal. The filtrate was evaporated to a clear sirup *in vacuo* in a desiccator over solid sodium hydroxide, and the resultant sirup heated with 150 ml. of 1 *N* hydrochloric acid at 100° under reflux. The solution was cooled, neutralized with an excess of silver oxide, filtered, and the filtrate concentrated *in vacuo* to a sirup, weighing 7.05 g.

Separation of Hydrolysis Products on a Powdered Cellulose Column.—A portion (2.97 g.) of the hydrolysis products was separated on a column of powdered cellulose (3 cm. by 40 cm.) using benzene–ethanol–water (169–47–15; top layer) as eluent.[15] Elution was continued until 345 tubes, each containing approximately 6 ml., had been collected. The residue on the column was then removed by washing with 250 ml. of methanol.

The contents of the tubes were examined by paper chromatography and grouped into the following fractions: A, B, C, D, E, F, G and H. After concentration of the solutions, the total weight of sugars removed from column was 3.044 g. The recovery of the sugars was 101%.

Constitution of the Fractions. Fraction A (41 mg.).—Paper chromatographic examination of this fraction, using *p*-anisidine hydrochloride as a spray, did not produce any color reaction, indicating that the compound was non-reducing, and therefore probably a glycoside. The sirup was hydrolyzed with 1 *N* sulfuric acid for 6 hours, neutralized with barium carbonate, filtered, and concentrated to a sirup *in vacuo*. This material on chromatographic examination corresponded to tetra-*O*-methylgalactose, and yielded crystalline 2,3,4,6-tetra-*O*-methyl-D-galactose anilide on warming with an equal weight of aniline in ethanolic solution. The anilide had a melting point of 193°. Its methoxyl content, OCH_3, was 39.50%, and N content, 4.50%. Calculated theoretical value for $C_{16}H_{25}O_5N$: OCH_3, 39.86; N, 4.50.

Fraction B (925 mg.).—The relative proportions of the methyl derivatives of D-galactose and L-arabinose in fraction B were determined as follows.

A portion of this fraction (66.1 mg.) was dissolved in 25 ml. of methanol. To 20 ml. of the solution was added 2 ml. of a 4% solution of hydrogen chloride in methanol. Two aliquots (2 ml. each) of this mixture were immediately placed into two tubes each containing 1 ml. of sodium carbonate–bicarbonate buffer, *p*H 10.6. Further eight aliquots (2 ml. each) were placed in sealed tubes, and all the tubes were simultaneously placed in a water-bath at 100°. At noted intervals of time tubes were removed, cooled, and the contents quantitatively washed into the buffer solution, and reducing power estimated by the alkaline iodine procedure of Hirst, *et al.*[16] From a graph of the reducing values plotted aginst time, the approximate molecular ratio of tetra-*O*-methyl-D-galactopyranose to the relatively unstable tri-*O*-methyl-L-arabofuranose, was found to be as 1:9.

Identification of Components of Fraction B.—A small portion (60 mg.) of fraction B was subjected to partial glycoside formation by heating with 0.5% methanolic hydrogen chloride for 15 minutes at 100°. The solution was neutralized and reduced under pressure to a sirup which was separated on a paper chromatogram using the benzene solvent. The glycosidic fraction was excised, eluted and hydrolyzed with hydrochloric acid. The product was then oxidized, lactonized and treated with ammonia[9a] to yield the crystalline 2,3,5-tri-*O*-methyl-L-arabonamide, having a methoxyl content, OCH_3 of 44.3% (calculated OCH_3 for $C_6H_{17}O_5N$, 44.9%).

A further portion (270 mg.) was similarly subjected to partial glycosidation and the unreacted material (47 mg.) isolated on the paper chromatogram. This material was refluxed with an equal quantity of aniline in ethanolic solution, which after four hours was concentrated under reduced pressure to yield 2,3,4,6-tetra-*O*-methyl-D-galactose anilide (27 mg.). On recrystallizing from *n*-propyl alcohol it had a melting point of 193°. Its melting point was not depressed on admixture with an authentic specimen of this anilide.

Fraction C (860 mg.).—The relative proportion of the methylated derivatives of D-galactose and L-arabinose was determined by following the course of hydrolysis of the glycosides on a portion of fraction C. A portion of this fraction was heated with 2% methanolic hydrogen chloride for 6 hours at 100°. After neutralization with silver oxide, the solution was concentrated to a sirup (14.7 mg.), treated with 25 ml. of 0.1 *N* sulfuric acid, and the solution heated at 100°. Aliquots were withdrawn at intervals and their reducing value estimated by means of alkaline iodine titration.[16]

A graph constructed for the rate of hydrolysis indicated that fraction C consisted of two components: a labile material representing approximately 30% of the ultimate reducing power, and a more stable material representing some 70% of the reducing power.

Identification of Components of Fraction C.—A further portion of fraction C (56 mg.) was heated with 0.5% methanolic hydrogen chloride for 15 minutes, neutralized and separated on a paper chromatogram. Excision of the tri-*O*-methyl-D-galactose strip, elution and formation of the anilide yielded crystalline 2,3,4-tri-*O*-methyl-D-galactose anilide. After two recrystallizations from ether the anilide melted at 166°. No depression of melting point was observed when mixed with an authentic specimen of melting point 167°. Analysis of the anilide for its methoxyl content, OCH_3, gave a value of 32.0% and for N, 5.2%. Calculated for $C_{15}H_{24}O_5N$: OCH_3, 31.4%; N, 4.8%.

The second component, presumably di-*O*-methyl-L-arabinose, was not positively identified but elution of the glycoside area of the above chromatogram followed by demethylation with hydrobromic acid[13] gave largely arabinose with a trace of galactose.

Fractions D, E and F (749 mg.).—These fractions had the same R_f values and therefore were indistinguishable on a paper chromatogram. Fraction E crystallized spontaneously; fractions D and F crystallized partially and slowly.

Fraction E was recrystallized from ether and a portion of this material (99 mg.) was heated on a water-bath with aniline (0.08 ml.) in ethanol (10 ml.) for 4 hours. After remaining for 12 hours at room temperature, the 2,4-di-*O*-methyl-D-galactose anilide crystals were removed and recrystallized from ethanol. The crystals melted at 218–228°. The methoxyl content of the anilide, OCH_3, was 21.7%, and N content 4.8% (calculated for $C_{14}H_{21}O_5N$: OCH_3, 21.9%; N, 5.2%).

Fraction G (62 mg.).—The fraction was chromatographically homogeneous and had an R_f value corresponding to a monomethyl galactose. This fraction was not further investigated.

Fraction H (206 mg.).—The material obtained by concentration of the eluate contained a number of impurities and was separated into an acidic and a neutral fraction by means of ion-exchange resins.

The sirup was taken up in water and the solution was passed down a column (10 ml. volume) of Amberlite IR-4B. After thoroughly washing the column, the eluate was stirred for one hour with Amberlite IR-100, decanted and reduced to a sirup (120 mg.). On chromatographic examination, the sugar was found to be largely a monomethyl galactose with a trace of free galactose. The Amberlite IR-4B column was then eluted with 4% hydrochloric acid (100 ml.) and the eluate concentrated to a sirup *in vacuo* at room temperature over sodium hydroxide pellets. The sirup was taken up in water, filtered through charcoal, and once more reduced to a sirup (81 mg.). Chromatographic examination of this product indicated it to be a mixture of a monomethyl-galactose and an unidentified sugar acid.

Molecular Weight.—The sedimentation constant, determined by H. K. Schachman, Department of Biochemistry, measured in a Spinco ultracentrifuge using a 1% solution of arabogalactan in water was found to be about 4.6 *S*. From the measured viscosity $\eta_{sp}/C = 0.52$ deceliter/g., and an assumed partial specific volume $\bar{V} = 0.63$, a molecular weight of approximately 100,000 was calculated. The ultracentrifuge patterns showed that the polysaccharide was markedly polydisperse.

(15) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 2511 (1949).

(16) E. L. Hirst, L. Hough and J. K. N. Jones, *ibid.*, 928 (1949).

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BERKELEY 4, CALIFORNIA

[CONTRIBUTION FROM DIVISION OF APPLIED BIOLOGY, NATIONAL RESEARCH LABORATORIES]

Oxidation of Carbohydrates with Periodate in the Warburg Respirometer[1,2]

BY A. S. PERLIN

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A modified periodate oxidation of carbohydrates is described, the reaction being carried out in dilute bicarbonate solution and an atmosphere of carbon dioxide in the Warburg respirometer. Formic acid produced in the oxidations is thereby conveniently estimated on the micro scale as an increase in pressure due to the carbon dioxide displaced from solution. The results obtained with a wide variety of simple carbohydrates and polysaccharides are in good agreement with theory or with the results obtained by established methods. The modified reaction may be especially useful in conjunction with new isolation and fractionation techniques of carbohydrate chemistry when often only small quantities of material are available.

Carbohydrates which contain at least three adjacent hydroxyl groups or an aldehyde adjacent to a hydroxyl group yield formic acid when oxidized with periodate.[3-5] A knowledge of the quantity of acid produced, therefore, is often of assistance in elucidating structure and affords an index of the number of end groups present in many polysaccharides, including starch and glycogen.[6] Several methods, recently reviewed by Morrison, *et al.*,[7] have been devised for direct titration of formic acid, but they generally require macro to semi-micro quantities of the carbohydrate.

The manometric technique used for measuring acid production in biochemical studies[8] suggested that the formic acid released during periodate oxidation of carbohydrates might also be estimated in the Warburg respirometer.[8] Thus, if the oxidation were performed in bicarbonate buffer the quantity of acid produced should be indicated by the observed increase in pressure due to carbon dioxide displaced from solution. Because minute amounts of gas exchange are measurable with the respirometer, the reaction would permit oxidations on the micro scale and would provide a continuous record of acid production.

Periodate oxidations are usually carried out at a hydrogen-ion concentration below *p*H 5, because in alkaline media over-oxidation and other side-effects have been observed.[9-12] Since no appreciable concentration of bicarbonate exists below *p*H 5[13] more alkaline conditions were necessary in our experiments. However, it was found possible to maintain the *p*H slightly above 5 by use of dilute bicarbonate (<0.02 *M*) and an atmosphere of pure carbon dioxide. This small departure from the more usual hydrogen-ion concentration had no apparent effect on the oxidation of a wide range of carbohydrates. Figure 1, illustrating rates of formic acid production (carbon dioxide evolved) at *p*H 5.7 and 16.7° in the Warburg respirometer, shows that all of the oxidations proceeded smoothly to completion with evolution of close to the theoretical quantities of carbon dioxide. The results were typical of all aldoses, hexitols, uronic acids, glycosides and non-reducing oligosaccharides examined. The scale of the oxidations is indicated by the fact that samples of from 0.2 mg. (glucose) to 2 mg. (raffinose) were sufficient. The oxidation of glucose, representative of the aldoses, incidentally constitutes a novel manometric estimation of these reducing sugars. In agreement with results obtained by the titration method,[14-17] a reducing disaccharide having a 1:6 linkage (*e.g.*, melibiose) yielded the theoretical quantity of acid but those

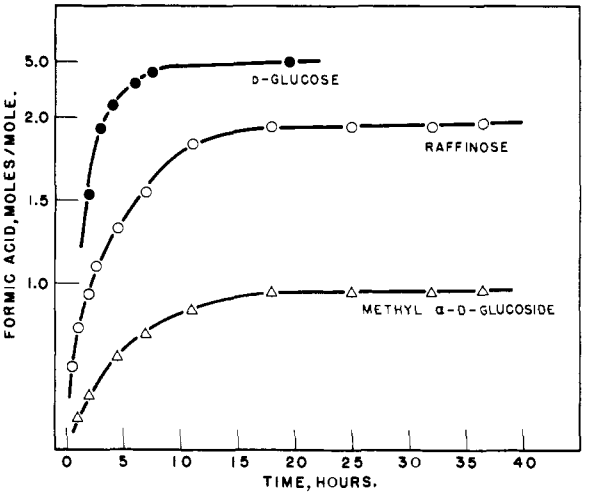

Fig. 1.—Rates of formic acid production in the oxidation of some simple carbohydrates at *p*H 5.7.

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