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# ACTION OF MANGANESE DIOXIDE ON CARBOHYDRATES—I

## SOME DISACCHARIDES

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**Abstract**—Manganese dioxide degrades 1 → 3, 1 → 4 and 1 → 6 linked hexose disaccharides to, *inter alia*, 1 → 2, 1 → 3 and 1 → 5 linked hexosyl-pentoses, respectively. The yields (18–31 per cent) compare favourably with those recorded for other degradations. The methods which have been worked out for the isolation of these products are experimentally straightforward, and should be generally applicable to mixtures obtained by oxidation of 3-, 4- and 6-substituted hexoses with manganese dioxide.

PREVIOUS studies[1] have shown that the principal pathway of oxidation for aldohexoses with manganese dioxide involves elimination of Cl to give the corresponding aldopentoses; some acidic products are also formed. Tetroses are probably produced from the pentoses, but they do not accumulate, being rapidly oxidized further to yield acid products. Similarly, hexosyl-hexoses give hexosyl-pentoses, with no accumulation of hexosyl-tetroses in those cases (1 → 4 and 1 → 6 linked disaccharides) where further oxidation is possible.

The direct conversion of hexose to pentose is not a common oxidation pathway of sugars, although it can be achieved using glycol cleavage reagents,[2,3] especially lead tetra-acetate. This oxidant can be used with unsubstituted hexoses,[4] when accompanying formation of tetrose necessitates chromatographic isolation of the pentose; yields vary widely with different hexoses, but are generally in the range 15–35 per cent. With a suitably substituted hexose, the yield of pentose is quantitative.[3] Hexosyl-pentoses have not been prepared by this method since, except for 1 → 3 linked disaccharides, hexosyl-tetroses are also produced.[5] Standard methods for shortening the carbon-chain of a sugar, such as the Ruff[6] or Wohl–Zemplén[7] degradations, and glycal oxidation[8] involve two or more stages, with isolation of intermediates and a consequent lowering of overall yields. Recently,[9] D-glucose has been degraded to D-arabinose in a two-stage, but single batch oxidation with hypochlorite. Because of experimental simplicity, oxidation with manganese dioxide appeared to be an attractive method for obtaining hexosyl-, O-methyl-, and deoxy-pentoses from the appropriate hexose

[1] J. L. Bose, A. B. Foster, M. Stacey and J. M. Webber, *Nature, Lond.* **184,** 1301 (1959).
[2] A. S. Perlin, *J. Amer. Chem. Soc.* **76,** 2595 (1954).
[3] J. Fried and D. E. Walz, *J. Amer. Chem. Soc.* **74,** 5468 (1952).
[4] A. S. Perlin and C. Brice, *Canad. J. Chem.* **34,** 541 (1956); A. S. Perlin, *Adv. Carbohyd. Chem.* **14,** 9 (1959).
[5] A. J. Charlson, P. A. J. Gorin and A. S. Perlin, *Canad. J. Chem.* **34,** 1811 (1956).
[6] H. G. Fletcher, Jr., H. W. Diehl and C. S. Hudson, *J. Amer. Chem. Soc.* **72,** 4546 (1950).
[7] G. Zemplén, *Ber. Dtsch. Chem. Ges.* **60,** 1555 (1927).
[8] G. E. Felton and W. Freudenberg, *J. Amer. Chem. Soc.* **57,** 1637 (1935); A. M. Gakhokidze, *J. Gen. Chem. U.S.S.R.*) **18,** 60 (1948); *Chem. Abstr.* **42,** 4948 (1948).
[9] R. L. Whistler and R. Schweiger, *J. Amer. Chem. Soc.* **81,** 5190 (1959).

derivatives. The method should be especially applicable to hexoses having a substituent or deoxy group at C3, since further oxidation of pentose products would be minimized by the absence of a C2 hydroxyl group. The oxidation of a series of model compounds was therefore examined.

The manganese dioxide used throughout was prepared from manganese sulphate and potassium permanganate under alkaline conditions.[10] Although this oxide repeatedly gave an alkaline (pH ca. 10) extract with water, it caused oxidation patterns qualitatively similar to those of the product obtained by decomposition of manganese carbonate at 250° (neutral aqueous extract), and moreover, was a better oxidant under standard conditions. Oxidations were usually performed with equal weights of disaccharide and oxidant, in water for 1 hr at 100°. In oxidations carried out at 50°, acid formation was significantly reduced. After removal of cations from an oxidation mixture, the acidic products were removed using a strongly basic anion exchanger.[11]

It soon became clear that even a large excess of manganese dioxide did not completely oxidise an aldohexose, so that an efficient fractionation procedure was required if the method was to be of preparative value. With 1 → 3 and 1 → 4 linked hexosyl-glucoses, the principal oxidation products were hexosyl-arabinoses, linked 1 → 2, and 1 → 3, respectively. In both cases, the hexosyl-glucose and hexosyl-arabinose had markedly different paper electrophoretic mobilities ($M_G$ values) in borate buffer (pH 10)[12], and were therefore favourable mixtures for the application of borate-charcoal chromatography, a method which makes use of the reduced affinity of sugar-borate complexes for charcoal. The method was developed[13] with mixtures of melibiose and maltose, and isomaltose and maltose, but its application to mixtures containing alkali-sensitive,[14] 1 → 3 linked disaccharides, such as laminaribiose or nigerose, has not been reported. These disaccharides are degraded in solution at pH 10, but are stable while adsorbed on paper during electrophoresis in alkaline buffers. It has now been found that they are similarly stabilized whilst adsorbed on charcoal in the presence of borate at pH 10. Oxidation of maltose with manganese dioxide at 100° gave neutral products (69 per cent) comprizing arabinose, glucose, maltose ($M_G$ 0·34) and 3-O-α-D-glucopyranosyl-D-arabinose (Compound A, $M_G$ 0·56); this mixture was fractionated by charcoal-borate chromatography. Immediate neutralization of the column eluate prevented decomposition of Compound A, which had been eluted from the column immediately following the monosaccharides, with some overlapping of components (Fig. 1); elution of maltose did not commence until removal of Compound A was complete. Compound A was easily freed from inorganic material and monosaccharides by charcoal column chromatography,[15] and hence was recovered completely (18 per cent overall yield) from the reaction mixture. The disaccharide, which was very hydroscopic and failed to yield a crystalline heptaacetate, gave glucose and arabinose (chromatographic detection) on acidic hydrolysis. The reduced disaccharide, which was also very hygroscopic, gave glucose and arabitol on acidic hydrolysis, and a periodate oxidation pattern which agreed with that for a 3-O-D-glucopyranosyl-D-arabitol (I). The initial, very rapid uptake of periodate by a disaccharide alcohol

[10] J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jansen and T. Walker, *J. Chem. Soc.* 1094 (1952).
[11] G. Machell, *J. Chem. Soc.* 3389 (1957).
[12] A. B. Foster, *J. Chem. Soc.* 982 (1953).
[13] S. A. Barker, E. J. Bourne and O. Theander, *J. Chem. Soc.* 4276 (1955).
[14] W. M. Corbett and J. Kenner, *J. Chem. Soc.* 3274 (1954).
[15] R. L. Whistler and D. F. Durso, *J. Amer. Chem. Soc.* **72**, 677 (1950).

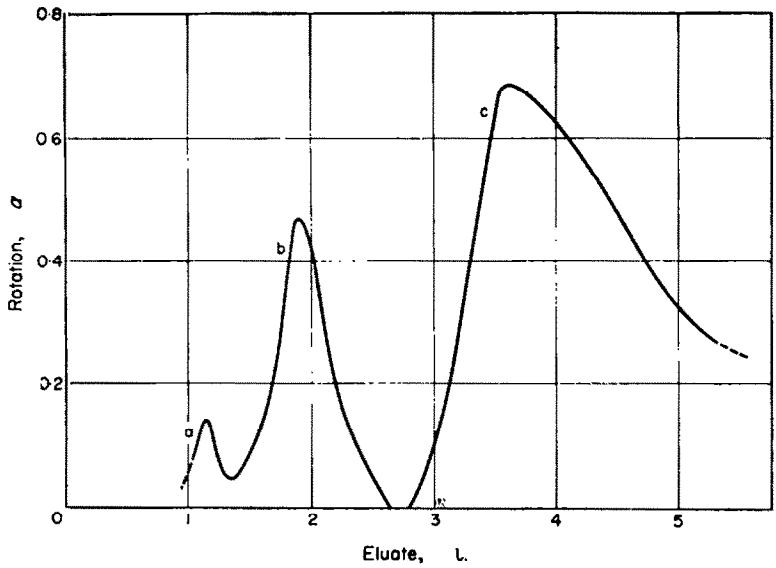
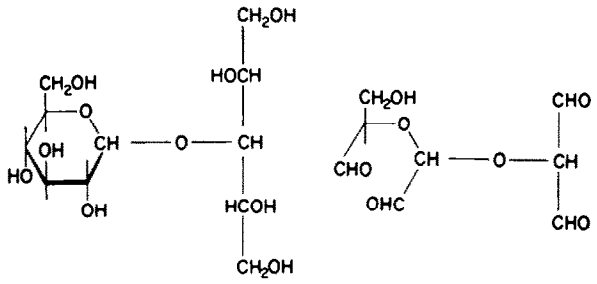



FIG. 1. Borate-charcoal fractionation of neutral products from oxidation of maltose with manganese dioxide.
(a) monosaccharides, (b) 3-O-α-D-glucopyranosyl-D-arabinose, (c) maltose.

results from attack of the acyclic moiety. With the glucosyl-arabitol, the accompanying release of 2 mole of formaldehyde (with an uptake of less than 3 mole of periodate) reveals a 1 → 3 glycosidic linkage; other glucosyl-arabitols would give only 1 mole of formaldehyde at this stage of oxidation. The slower oxidation of the cyclic moiety




would give II which would be subject to overoxidation through its malondialdehyde group.[16]

The borate-charcoal method of fractionation is applicable to oxidation mixtures obtained from 1 → 3 linked hexosyl-glucoses, and it is the method of choice if recovery of unoxidized starting-material is desirable. However, the alkali-sensitivity of 1 → 3 linked disaccharides[14] permits a simpler approach in which the unoxidized substrate is selectively degraded. Thus, when the neutral products from a laminaribiose oxidation were treated with saturated lime-water, the residual laminaribiose was completely destroyed giving monosaccharide and acidic components, while the principal oxidation product, 2-O-β-D-glucopyranosyl-D-arabinose, was essentially

[16] J. L. Bose, A. B. Foster and R. W. Stephens, *J. Chem. Soc.* 3314 (1959) and references cited therein.

unaffected. Fractionation of the mixture on a charcoal column then furnished the glucosyl-arabinose in an overall yield of 31 per cent. The glucosyl-arabinose was identified by conversion to known 1,3,5-tri-O-acetyl-2-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-D-arabinose.[17] Confirmatory evidence for the 1 → 2 linkage was provided by the periodate oxidation pattern of the reduced disaccharide which consumed 3·8 mole of the oxidant with release of 1·8 mole of formic acid and 0·9 mole of formaldehyde. The theoretical values for 2-O-β-D-glucopyranosyl-D-arabitol are 4, 2, and 1, respectively.

The $M_G$ values of 1 → 6 linked hexosyl-glucoses and their 1 → 5 linked hexosyl-arabinose oxidation products are not appreciably different, so that borate-charcoal chromatography is not feasible. Moreover, a 1 → 6 linked disaccharide cannot be selectively destroyed with alkali; alternative methods of fractionation were therefore

TABLE 1. ACIDIC PRODUCTS FROM OXIDATION OF MELIBIOSE

| Acid | Reference acid | $M_{GA}$ | Galactosyl-aldonic acid ($M_{GA}$) |
|---|---|---|---|
| 2C | glycollic | 1·77 | |
| 3C | glyceric | 1·54 | 0·88 |
| 4C | erythronic | 1·27 | 0·67 |
| 5C | arabonic | 1·09 | 0·47 |
| 6C | gluconic | 1·00 | 0·42 |

sought. The neutral products from a melibiose oxidation were fractionated on a charcoal column (gradient elution) to give melibiose (22 per cent overall recovery), and a mixture (33 per cent) containing melibiose and a galactosyl-arabinose; all fractions containing the galactosyl-arabinose also contained melibiose. Homogeneous galactosyl-arabinose (21 per cent overall yield) was obtained from this mixture in a small-scale (50 mg) fractionation using thick-paper chromatography. Although this disaccharide was not rigorously identified, it gave galactose and arabinose on acidic hydrolysis and, by analogy with the preceding oxidations, must be 5-O-α-D-galacto-pyranosyl-D-arabinose.

The procedures evolved for fractionation of mixtures obtained by oxidation of laminaribiose and maltose are straightforward, and should be applicable to a range of 3- and 4-substituted hexoses. Complete fractionation of the oxidation mixture from the 1 → 6 linked disaccharide was less easily achieved. Since the possible variations in oxidation conditions were not exhaustively examined, the recorded yields (18–31 per cent) of oxidation products cannot be considered as maximal; even so, they compare favourably with those obtained by other degradation methods.

The formation of acids during oxidation of disaccharides with manganese dioxide was greater than had previously[1] been supposed, for example, at 100°, the weight of acidic products was 18 per cent of that of the maltose substrate. Oxidation of melibiose gave the series of aldonic and galactosyl-aldonic acids listed in Table 1. The latter acids were identified by hydrolysis, after removal of aldonic acids by charcoal column chromatography. With maltose and melibiose, the principal acidic products were the corresponding glycosyl-tetronic and glycosyl-pentonic acids.

[17] B. Weissmann and K. Meyer, *J. Amer. Chem. Soc.* **76**, 1753 (1954).

## EXPERIMENTAL

Wherever possible, optical rotations were measured in 2 dm tubes. Paper chromatography was performed on Whatman No. 1 paper by downward irrigation with the organic phase of butanol–ethanol–water (4:1:5). Paper electrophoresis was carried out on Whatman No. 3 paper using the enclosed strip technique,[18] with borate (pH 10),[19] or acetate (pH 5) buffers. Detection was effected with aniline hydrogen phthalate[20] or silver nitrate.[21] The mobilities of acid products in acetate buffer are expressed as $M_{GA}$ values (GA = gluconic acid). Charcoal/Celite columns were prepared from an acid-treated[22] mixture containing equal parts of B.D.H. activated charcoal and Celite No. 545.

Pyrex glass vessels and stirring rods were used in all oxidations in order to avoid the alkali-induced side reactions which result from the use of soda-glass. Manganese dioxide was prepared from manganese sulphate and potassium permanganate under alkaline conditions.[10]

*Oxidation of maltose*

Manganese dioxide (12·3 g) was added to a solution of maltose (12·3 g) in water (370 ml) at 95–100° and the mixture was stirred vigorously during 1 hr at the same temp. The cooled and filtered solution was passed through a column of Amberlite IR 120 resin ($H^+$ form; 100 ml) and dried in the frozen state. A solution of the residue (10·7 g) in water (300 ml) was stirred overnight with Deacidite FF resin ($CO_3^{2-}$ form, 100 ml), and the mixture was then transferred to a column containing the same resin (50 ml). The combined resins were washed with water (3 l.), and the total eluate was concentrated to give a residue (8·5 g) which was free from acid products (electrophoresis in acetate buffer).

After being washed with more water (4 l.), the resin column was eluted with 4% aqueous ammonium carbonate (300 ml). The eluate was concentrated at 40° (bath)/12–15 mm to ca. 20 ml and stirred with Amberlite IR 120 ($H^+$ form; 20 ml). When effervescence had ceased, the mixture was transferred to a column containing an equal volume of the same resin, the combined resins were washed with water (100 ml), and the total eluate was dried in the frozen state. Paper electrophoresis showed the residue (2·2 g) to contain hexonic ($M_{GA}$ 1·0), pentonic (1·18), and tetronic (1·48) acids, as well as glucosyl-pentonic (0·62) and glucosyl-tetronic (0·43) acids; the last of these acids was the principal component.

Paper chromatographic and electrophoretic analyses of the neutral residue revealed arabinose, glucose, maltose ($R_G$ 0·37, $M_G$ 0·34) and Compound A ($R_G$ 0·53, $M_G$ 0·56). A solution of this mixture (8·5 g) in water (85 ml) was introduced on to a charcoal/Celite column (42 × 5·5 cm) which had been washed with borate buffer (pH 10, ca. 3·6 l.),[19] until the pH values of the solutions entering and leaving the column were the same, and then with water (100 ml). After the material had been washed on to the column with water (100 ml), elution was commenced using ethanolic borate buffer and the gradient technique;[22] the ethanol concentration was increased at a rate of 1·25% per litre. The eluate was collected in 50 ml fractions which were siphoned into tubes containing sufficient acetic acid (0·35 ml, 90%) to lower the pH of the solution to ca. 6. The fractions were analysed polarimetrically (Fig. 1), and by paper electrophoresis (borate buffer), which revealed that peaks a, b and c could be attributed to monosaccharides (mainly glucose), Compound A, and maltose, respectively. The first few fractions containing Compound A were contaminated with traces of glucose; there was no overlapping of disaccharides.

The fractions (24–51) containing Compound A were concentrated at 40° (bath)/12–15 mm to 600 ml (pH ca. 6) and introduced on to a charcoal/Celite column (16 × 3·5 cm) which was then washed with water (500 ml) to remove inorganic material and glucose. Elution with 5% aqueous ethanol gave chromatographically homogeneous Compound A (2·02 g; 18%), $[\alpha]_D$ + 47·1° (*c* 4·04 in $H_2O$). The product could be crystallized from aqueous ethanol, but was so hygroscopic that the m.p. could not be determined. Acetylation gave a syrup which could not be crystallized.

*Characterization of compound* A.

(a) *Acidic hydrolysis.* A solution of Compound A (10 mg) in N HCl (2 ml) was heated at 95–100° for 2 hr, then cooled, diluted with water (3 ml), neutralized with methyl di-n-octylamine,[23] and

[18] A. B. Foster, *Chem. & Ind.* 1050 (1952).
[19] A. B. Foster, P. A. Newton-Hearn and M. Stacey, *J. Chem. Soc.* 30 (1956).
[20] S. M. Partridge, *Nature, Lond.* **164**, 443 (1949).
[21] W. E. Trevelyan, D. P. Proctor and J. S. Harrison, *Nature, Lond.* **166**, 444 (1950).
[22] B. Lindberg and B. Wickberg, *Acta Chem. Scand.* **8**, 569 (1954).
[23] E. Lester Smith and J. E. Page, *J. Soc. Chem. Ind.* **67**, 48 (1948).

concentrated. Chromatographic examination of the residue revealed a mixture of glucose and arabinose.

(b) *Reduction with sodium borohydride.* A solution of sodium borohydride (0·6 g) in water (20 ml) was added to a solution of Compound A (1·0 g) in water (20 ml). After 3 hr, when $\alpha_D$ was constant, the solution was acidified with acetic acid, neutralized (pH 8) with sodium hydroxide, and introduced on to a charcoal/Celite column (8 × 1·8 cm) which was then washed with water (100 ml). Elution with 15% aqueous ethanol gave a chromatographically homogeneous ($R_G$ 0·61, $M_G$ 0·54), non-reducing residue (0·94 g) which was crystallized from aqueous ethanol. The colourless crystals which had $[\alpha]_D$ + 113° (*c* 1·48 in $H_2O$) were very hygroscopic and the m.p. could not be determined. Hydrolysis of the material as described in (a) gave a mixture of glucose and arabitol.

(c) *Periodate oxidation of reduced compound* A. A solution of the reduced product (100 mg) in water (10 ml) was treated with 2·8% sodium metaperiodate solution (50 ml), and the volume was rapidly adjusted to 100 ml. The consumption of oxidant was followed by the standard method;[24] formic acid liberation was determined, after destruction of excess periodate with ethylene glycol, by direct titration with 0·01N NaOH, and formaldehyde by the chromotropic acid method.[25] The following results were obtained:

| Time (hr) | 0·07 | 0·16 | 0·25 | 0·33 | 1·0 | 1·5 | 2·5 | 4·0 | 5·5 | 20 | 44 | 92 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Oxidant consumption (moles) | 2·82 | 3·48 | — | 4·66 | 5·82 | 6·09 | 6·26 | 6·66 | 7·02 | 7·14 | 7·52 | 7·85 |
| Acidity, as formic acid (moles) | — | — | 0·79 | — | 1·75 | — | — | — | 3·47 | 4·05 | 4·44* | — |
| Formaldehyde liberation (moles) | 2·0 | — | 2·1 | — | 2·1 | — | — | 2·1 | — | — | — | — |

* 4·94 moles by back titration

*Oxidation of laminaribiose*

A solution of laminaribiose[26] (1 g) in water (30 ml) was stirred with manganese dioxide (1 g) for 1 hr at 50°, and then processed as in the maltose oxidation. Chromatographic examination of the neutral residue (0·98 g) revealed arabinose, glucose, laminaribiose ($R_G$ 0·48, $M_G$ 0·66) and Compound B ($R_G$ 0·51, $M_G$ 0·33). The solution obtained by dropwise addition of water (ca. 5 ml) to a mixture of the neutral products (0·895 g) and saturated lime-water (160 ml) was freed from oxygen by passage of nitrogen gas for 15 min. After storage at 25° for 30 hr, the solution was passed through Amberlite IR-120 ($H^+$ form, ca. 70 ml) and concentrated to yield a solid residue (0·89 g). Chromatographic and electrophoretic analyses showed that Compound B was largely unaffected, and that complete destruction of laminaribiose had occurred with formation of glucose, fructose, mannose and acidic components.

A solution of this product in water (10 ml) was introduced on to a charcoal/Celite column (13 × 3·5 cm) which was then washed with water (1550 ml) to remove acidic and monosaccharide components; elution of Compound B, commenced by the water, was continued using an aqueous-ethanol gradient (1·6% per litre). Concentration of the aqueous-ethanol eluate (1750 ml) gave chromatographically-homogeneous Compound B (0·28 g; 31%) which had $[\alpha]_D$ −46·4° (*c* 5·6 in $H_2O$, 1 dm tube).

*Characterization of compound* B

(a) Chromatographic analysis of a neutralized (methyl di-n-octylamine) hydrolysate (N HCl, 95–100°, 2 hr) revealed glucose and arabinose. A similar hydrolysis of the reduced (sodium borohydride) Compound B gave a mixture of glucose and arabitol. Oxidation of reduced Compound B with an excess (12·5 moles) of 0·43% sodium metaperiodate gave the following results:

| Time (hr) | 0·33 | 0·66 | 1·5 | 3·0 | 5·0 | 20 |
|---|---|---|---|---|---|---|
| Oxidant consumption (moles) | 2·2 | 2·5 | 2·8 | 3·2 | 3·9 | 3·8 |
| Formaldehyde liberation (moles) | — | 0·9 | — | — | — | 0·9 |

After 20 hr, acidity corresponding to 1·8 moles of formic acid had developed.

(b) Compound B (72 mg), fused sodium acetate (40 mg), and acetic anhydride (0·5 ml) were heated at 95–100° for 2·5 hr, with occasional shaking. After being cooled, the mixture was poured into

[24] E. L. Jackson, *Org. Reactions* **2**, 341 (1944).
[25] J. F. O'Dea and R. A. Gibbons, *Biochem. J.* **55**, 580 (1953).
[26] Prepared by acidic hydrolysis of insoluble laminarin, following S. Peat, W. J. Whelan and H. G. Lawley, *J. Chem. Soc.* 724 (1958).

ice-water (8 ml) and stored at 0° overnight. Decantation left a syrup which was washed with water and dried *in vacuo* ($P_2O_5$) to give a solid product (55 mg). Chloroform extraction of the decanted solution furnished additional syrupy material (57 mg). The crude products were separately crystallized from ethanol–light petroleum (b.p. 60–80°) to give 1,3,5-tri-O-acetyl-2-O-[2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl]-D-arabinose (14 mg, and 4 mg, respectively). In both cases, the product was obtained as colourless needles, m.p. 196·5–198°, alone, and 196–199°, in admixture with an authentic sample of the compound (m.p. 200·5–201°).[17] The infra-red spectrum of the product and that of a sample of the authentic compound were indistinguishable.

*Oxidation of melibiose*

A solution of melibiose (9·3 g) in water (280 ml) was stirred with manganese dioxide (9·3 g) for 1 hr at 95–100°, and then processed, as in the previous oxidations, to separate the neutral and acidic products.

(a) *Examination of acidic products.* Paper electrophoresis of this material (1·35 g) showed the series of galactosyl-aldonic acids and aldonic acids listed in Table 1.

Aqueous elution of the sodium salts of the acidic products (1 g) from a charcoal/Celite column (15 × 3·5 cm) furnished the following fractions (25 ml): IV—3C and 4C acids; V—3C to 6C acids; VI, VII—4C to 6C acids and galactosyl-aldonic acids; VIII to XI—galactosyl-aldonic acids. Hydrolysis (N HCl, 1 hr) of the material recovered from fractions VIII to XI yielded galactose and a mixture of 3C, 4C, and 5C aldonic acids.

A solution of the acidic products (0·35 g) in water (50 ml) was passed through a column (14 × 2·5 cm) of IR-4B resin (HO′ form) which, after being washed with water (200 ml), was subjected to gradient (7·4% per litre) elution with aqueous formic acid. The following fractions (25 ml) were obtained: 36 to 38—galactosyl-aldonic acids; 39 to 60—galactosyl-aldonic and aldonic acids.

(b) *Fractionation of neutral products.* Paper chromatography and paper electrophoresis showed the presence of lyxose, galactose, melibiose ($R_F$ 0·043, $M_G$ 0·74) and Compound C ($R_F$ 0·090, $M_G$ 0·87).

A solution of the neutral products (5·5 g) in water was introduced on to a charcoal/Celite column (34 × 6 cm). Elution was commenced with water (2250 ml), continued with an aqueous ethanol gradient (5 l.; 0–5%), and completed with 6% aqueous ethanol (2·5 l.). Paper chromatography showed the following fractionation pattern.

| Fraction (25 ml) | Components | Yield (g) |
|---|---|---|
| 29–34 | lyxose, galactose | — |
| 35–50 | galactose | 0·09 |
| 59–108 | melibiose | 2·03 |
| 109–195 | melibiose, compound C | 3·01 |

A portion (50 mg) of the combined fractions 109–195 was further fractionated on a sheet of Whatman No. 17 paper (57 × 46 cm) by overnight irrigation with the butanol–ethanol–water (4:1:5) solvent. Chromatographically homogeneous Compound C (30 mg) was extracted from the appropriate strip with hot water.

*Characterization of compound* C

(a) *Acidic hydrolysis.* Chromatographic examination of a neutralized (methyl di-n-octylamine) hydrolysate (N HCl, 95–100°, 2 hr) showed the presence of galactose and arabinose.

(b) *Reduction with sodium borohydride.* A solution of the melibiose-Compound C mixture was treated with sodium borohydride and processed as described for Compound A. Paper chromatography showed melibiitol ($R_G$ 0·36) and a second, non-reducing component ($R_G$ 0·50). Paper electrophoresis in molybdate buffer (pH 5)[27] also revealed two components, having $M_S$ 0·75 (melibiitol), and 0·50, respectively ($M_S$ = mobility with respect to that of sorbitol). Reduced Compound C was thereby identified as a 5-O- or 4-O-substituted arabitol.

[27] E. J. Bourne, D. H. Hutson and H. Weigel, *J. Chem. Soc.* 35 (1961).