

STRUCTURAL INVESTIGATION OF OXALATE-SOLUBLE RAPESEED (*Brassica campestris*) POLYSACCHARIDES PART III. AN ARABINAN*

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ABSTRACT

An arabinan isolated from rapeseed was shown by sedimentation studies to be essentially homogeneous, and methylation analysis revealed a highly branched structure. Hydrolysis of the methylated polysaccharide yielded 2,3,5-tri-*O*-methyl-L-arabinose (11 mol.), 2,3-di-*O*-methyl-L-arabinose (7 mol.), 3-*O*-methyl-L-arabinose (trace), 2-*O*-methyl-L-arabinose (7 mol.), and L-arabinose (2 mol.). Periodate-oxidation data substantiate the methylation results. The general, structural features of the arabinan are discussed.

INTRODUCTION

In previous reports^{1,2}, the isolation and characterization of an amyloid and an acidic arabinogalactan from rapeseed were described. Further work on the oxalate-soluble fraction of the rapeseed cotyledon meal has resulted in the isolation of material which has been fractionated into an acidic polysaccharide complex and an arabinan; and we now report on the main structural features of the latter polysaccharide.

RESULTS AND DISCUSSION

The residue from the hot-water extraction² of rapeseed was extracted with 2% EDTA, yielding material which, after purification, gave only traces of sugars on hydrolysis. Subsequently, the EDTA-insoluble residue was repeatedly treated with 0.5% ammonium oxalate until free of oxalate-soluble material. A portion of the total oxalate-soluble material (14.2%) was deproteinized³, yielding a polysaccharide fraction which was largely free from proteins and, on acid hydrolysis, gave galactose, glucose, arabinose, xylose, galacturonic acid, small amounts of fucose and rhamnose, and traces of a mono-*O*-methylhexose.

Fractionation of the polysaccharide material on DEAE-cellulose (carbonate form)⁴ yielded a neutral fraction (5.9%, eluted with water), and acidic fractions

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comprising 59.3, 5.1, and 5.6% of the deproteinized polysaccharide-fraction. Acid hydrolysis of the water-eluted fraction gave mainly glucose, galactose, and xylose, together with traces of arabinose and possibly fucose; uronic acid was not detected. This fraction therefore appeared to be similar to the amyloid fraction characterized earlier. The three acidic fractions were essentially similar on the basis of acid hydrolysis, which gave galactose, arabinose xylose, and galacturonic acid, together with traces of rhamnose, fucose, and a mono-*O*-methylhexose.

Further fractionation of the major, acidic fraction on DEAE-cellulose (phosphate form)⁵ yielded a water-eluted fraction (13.6%) and a series of phosphate-eluted fractions (3.3, 62.3, 3.2, and 1.9%). The minor fractions eluted with phosphate were essentially identical to the major fraction but contained fractionally higher amounts of glucose and lower amounts of rhamnose. The water-eluted fraction, on acid hydrolysis, gave mainly arabinose with small proportions of galactose, glucose, and xylose.

The crude arabinan thus obtained was partially purified by fractionation on DEAE-cellulose (borate form)⁶. Elution with water yielded a minor fraction (0.5%), which on hydrolysis gave mainly galactose together with a small proportion of arabinose, and a major fraction (70%), which on hydrolysis gave a large proportion of arabinose together with traces of galactose, glucose, and xylose. Paper electrophoresis revealed traces of galacturonic acid (tentative identification) in the former, and traces of an unidentified acid in the latter. On hydrolysis, the borate-eluted fractions (11.2 and 2.1%) gave mainly glucose, arabinose, and xylose, together with slightly lesser amounts of galactose.

A final purification of the arabinan was effected on Sephadex⁷ G-75, giving two partially separated peaks from which three fractions (20.7, 15.4, and 54.7%) were collected. The first fraction, on acid hydrolysis, gave arabinose with appreciable proportions of galactose, glucose, and xylose. The last two fractions were essentially identical and, on hydrolysis, gave mainly arabinose with traces of galactose.

A carefully dried sample of rapeseed arabinan had $[\alpha]_D^{23} - 181^\circ$. Sedimentation analysis^{2,3} showed a major, symmetrical peak with a small shoulder amounting to 3–4% of the total. The formation of traces of galactose on hydrolysis suggested that the minor peak might have originated from a galactan or an arabinogalactan contaminant. The results of complete and partial hydrolysis with acid suggested the presence of an arabinan in which the arabinose residues were almost exclusively present in the furanoid form. After mild hydrolysis with acid, crystalline L-arabinose was recovered in ~75% yield.

The polysaccharide was methylated, and then methanolysed and hydrolysed. The identities and the percentages of the products were determined by g.l.c.⁸, and the quantitative results for a number of arabinans are compared in Table I.

The identities of the above sugars were established by g.l.c.–m.s.^{8,10} by comparison with appropriately prepared standards. The identification of 2-*O*- and 3-*O*-methylarabinoses, for which standards were not available, was confirmed by the presence of primary fragments having *m/e* 117 and 216 in the mass spectrum of the

TABLE I

PERCENTAGE COMPOSITION (BY G.L.C.) OF METHYLATED ARABINITOL ACETATES FROM METHYLATED ARABINANS

Acetate derivative	Soybean arabinan ^a	Lemon-peel arabinan ^a	Mustard-seed arabinan ^a	Rapeseed arabinan
2,3,5-Tri- <i>O</i> -methylarabinitol	39.2	30.0	39.6	34.0
2,3-Di- <i>O</i> -methylarabinitol	30.0	38.4	25.4	25.7
2- <i>O</i> -Methylarabinitol	14.2	15.0	28.6	31.5
3- <i>O</i> -Methylarabinitol	6.0	4.6	tr.	tr.
Arabinitol	10.5	12.0	6.3	8.7

ANALYTICAL DATA FOR THE RAPESEED ARABINAN AFTER CONVERSION INTO THE NEAREST WHOLE NUMBERS

Component	Molar ratio of alditol acetates by g.l.c.	Molar ratio by weight of sugars	Mode of linkage
2,3,5-Tri- <i>O</i> -methyl-L-arabinose	11	11	L-Araf-(1→
2,3-Di- <i>O</i> -methyl-L-arabinose	7	7	→5)-L-Araf-(1→
2- <i>O</i> -Methyl-L-arabinose	7	7	→3,5)-L-Araf-(1→
3- <i>O</i> -Methyl-L-arabinose	trace	trace	→2,5)-L-Araf-(1→
L-Arabinose	2	2	→2,3,5)-L-Araf-(1→

arabinitol acetate derived from the former compound, and m/e 189 in that of the latter. All of the above sugars, with the exception of 3-*O*-methylarabinose, were either obtained crystalline or characterised as crystalline derivatives.

Methylation analysis showed that the molecule has a highly branched structure with an average repeating-unit containing 27 sugar residues, including 11 terminal, non-reducing L-arabinose residues. There were also 9 L-arabinose residues involved in branching, including 7 through positions 3 and 5, and 2 through positions 2, 3, and 5. The non-terminal residues consist of (1→5)-linked L-arabinose residues. The highly negative specific rotations of the unmethylated ($[\alpha]_D -181^\circ$) and methylated ($[\alpha]_D -165.7^\circ$) polysaccharide suggest that the majority, if not all, of the sugar residues are those of the α -L type.

The repeating unit proposed above requires a periodate consumption of 0.66 mol. per "anhydro sugar" residue. The experimentally determined figure (0.69), although in excellent agreement with that expected, is based on an end point which was unstable. The figure refers to an end point which persisted for ~60 seconds. Smith degradation of the oxopolysaccharide yielded, *inter alia*, mainly arabinose and glycerol.

The differences between the various arabinans (Table I) are, on the whole, minor. The configuration of the linkages is the same in each polysaccharide, but there

are some minor, quantitative differences in the proportion of linkages. The detection of traces only of 3-*O*-methylarabinitol, however, may provide a distinction between the mustard/rape family arabinan and those from other sources.

Arabinans of plant or seed origin, which have been studied in some detail, tend to fall into two classes. The first class includes those that are associated with pectins and supposedly are released by alkaline degradation during the isolation and fractionation procedures¹²⁻¹⁴. The second group consists of what are believed to be natural homoglycans. The arabinans from mustard seed¹⁵⁻¹⁷, soybean⁹, and rapeseed belong to the latter type.

The role of arabinans and other cell-wall polysaccharides in the control of plant growth has been a subject of much speculation in recent years. The cell-wall changes associated with growth appear to be guided by a process of secondary interactions or entanglement^{17,18}. It is suggested that the cell wall is held together by these non-covalent interactions between the various macromolecules. Contrary to this view, and based on a study of similar cell-wall polymeric species from suspension-cultured sycamore cells¹⁹⁻²¹, it has been claimed that, with the exception of cellulose, the macromolecules of the cell are not free entities but a matrix of covalently cross-linked polymers, where even the links between cellulosic and non-cellulosic materials or polymers possess the strength of a covalent bond.

EXPERIMENTAL

The general experimental methods have been reported previously².

Extraction with EDTA. — The water-insoluble residue² (5194 g) was extracted with 2% EDTA (110 l), yielding an insoluble residue (4300 g, 82.7%; N, 8.18%; ash, 2.6%) and a soluble extract which was adjusted to pH 4.5 with 50% acetic acid. The precipitated material (183.4 g, 3.5%; N, 6.8%; ash, 10.06%), recovered by centrifugation, gave only traces of sugars after hydrolysis (M sulphuric acid, 100°, 3 h). The supernatant solution was mixed with ethanol (2 vol.), yielding a material (55.2 g, 10.7%) which also gave only traces of sugars following deproteinization and hydrolysis.

Extraction with ammonium oxalate. — The EDTA-insoluble residue (4300 g) was extracted 6 times with 0.5% ammonium oxalate (225 l) at $90 \pm 5^\circ$ for 5 h. Acidification of the first extract to pH 4.5 with 50% aqueous acetic acid gave a precipitate (16.8 g) which gave negligible amounts of sugars on hydrolysis. This step was therefore omitted in further extractions. The clarified extracts were mixed with ethanol (2 vol.) to yield the ammonium oxalate-soluble solids. The precipitates were recovered and dried by solvent exchange with ethanol and ether. The yields and the nitrogen and ash contents of the ammonium oxalate-soluble solids are shown in Table II. On hydrolysis, all the precipitates gave similar mixtures of sugars, namely, galacturonic acid, galactose, glucose, arabinose, xylose, fucose, rhamnose, and traces of an unidentified mono-*O*-methylhexose.

Removal of protein. — A portion (58.9 g) of the combined, oxalate-soluble

TABLE II

AMMONIUM OXALATE EXTRACTS FROM RAPESEED COTYLEDON MEAL

<i>Precipitate</i>	<i>Weight (g)</i>	<i>Nitrogen (%)</i>	<i>Ash (%)</i>
1	288.7	6.53	4.42
2	142.5	6.59	2.53
3	126.3	5.17	1.05
4	24.5	7.33	1.07
5	19.8	5.51	2.42
6	10.0	6.36	1.83
Final residuc	1414.0	9.49	1.33

solids was deproteinized³ with 90% aqueous phenol, yielding a protein fraction (22.4 g; N, 7.92%; ash, 4.65%; moisture, 8.7%) and a polysaccharide fraction (20.2 g; N, 1.42%; ash, 4.24%; moisture, 9.73%).

Fractionation of polysaccharide. — A solution of the polysaccharide (12 g) in water (600 ml) was mixed with Rexyn-101(H⁺) resin. The filtrate was mixed with ethanol (4 vol.) and centrifuged, the precipitate was dissolved in water, and the solution was concentrated to remove traces of ethanol. The solution (250 ml) was added to a column (4 × 15 in.) of DEAE-cellulose (carbonate form)⁴. Elution with water (5.5 l) removed the neutral fraction *N* (0.71 g, 5.9%; N, 2.5%; ash, 0.98%; moisture, 9.4%), and elution with the first volume of 0.5M ammonium carbonate removed the bulk of the major, acidic fraction *A*₁ (7.12 g, 59.3%; N, 0.26%; ash, 1.52%; moisture, 9.18%). The material slowly removed by a further batch of 0.5M ammonium carbonate (4.5 l) was recovered to yield fraction *A*₂ (0.61 g, 5.08%; N, 1.13%; ash, 1.84%; moisture, 9.93%). A final washing with 0.5M sodium hydroxide (2 l) yielded fraction *A*₃ (0.67 g, 5.6%; N, 2.44%; ash, 12.8%; moisture, 9.4%). Paper chromatography of the hydrolysates prepared from fractions *N*, *A*₁, *A*₂, and *A*₃ showed glucose, galactose, and xylose, with traces of arabinose and fucose in *N*, and galacturonic acid, galactose, arabinose, xylose, small proportions of rhamnose and fucose, and traces of an unidentified mono-*O*-methylhexose in *A*₁–*A*₃.

Fractionation of acidic polysaccharide (Fraction A₁). — Fraction *A*₁ (6 g) was fractionated on a column (1.8 × 26 in.) of DEAE-cellulose (phosphate form)⁵. Elution with water (1.5 l) removed a neutral fraction (0.82 g, 13.6%; N, 0.57%; ash, 0.58%; moisture, 3.24%), and gradient elution with phosphate buffer (pH 5.5) 0–0.25M (2 l), 0.25–0.5M (3 l), and 0.5–1.0M (3 l) yielded 3 fractions, which were concentrated, dialysed for 48 h against running tap-water and for 4 h against two changes of distilled water, and freeze-dried to yield the respective products (0.19 g, 3.3%; N, 1.33%; ash, 5.39%; moisture, 9.03%), (3.7 g, 62.3%; N, 0.45%; ash, 5.76%; moisture, 7.95%), and (0.19 g, 3.2%; N, 0.91%; ash, 5.0%; moisture, 6.65%).

Acid hydrolysis of the water-eluted (arabinan) fraction gave largely arabinose, with minor amounts of galactose, glucose, and xylose, and possibly traces of uronic

acid. The hydrolysates of each fraction eluted with phosphate yielded the same sugars, namely, galacturonic acid, galactose, arabinose, and xylose, with trace amounts of glucose, rhamnose, fucose, and an unidentified mono-*O*-methylhexose.

Fractionation of the arabinan. — The crude arabinan (0.89 g) was fractionated on a column (1.2 × 18 in.) of DEAE-cellulose (borate form)⁶. Elution with water (600 ml) followed by a continuous gradient of 0–0.25M (1400 ml) and 0.25–0.5M sodium metaborate (1200 ml), with collection of effluent in 10-ml fractions, yielded two water-eluted fractions (W_1 and W_2) and a borate-eluted fraction. Fractions W_1 and W_2 were dialyzed against running tap-water for 20 h and then for 4 h against two changes of distilled water, and freeze-dried to yield materials W_1 (4.1 mg, 0.47%) and W_2 (0.62 g, 70%). The borate fraction was deionized with Rexyn-101(H⁺) resin, filtered, dialyzed for 65 h against running tap-water and for 4 h against two changes of distilled water, and freeze-dried (yield, 119 mg, 13.3%).

Paper chromatography of the hydrolysates of W_1 and W_2 showed mainly galactose with minor amounts of arabinose in the former, and major amounts of arabinose with minor amounts of galactose, glucose, and xylose in the latter. Paper electrophoresis²² (acetate buffer, pH 5) revealed faint traces of galacturonic acid in the former hydrolysate, and faint traces of a uronic acid moving faster than either galacturonic acid or glucuronic acid in the latter.

Fraction W_2 (0.43 g) was fractionated on a column (1 × 25 in.) of Sephadex G-75. Elution with water (5-ml fractions) at 5 ml/h yielded fractions 1 (91 mg, 20.7%), 2 (67 mg, 15.4%), and 3 (237 mg, 54.7%).

Acid hydrolysis of fraction 1 gave arabinose, galactose, glucose, and xylose. Hydrolysis of fractions 2 and 3 gave arabinose with traces of galactose.

Analysis of rape-seed arabinan. — The arabinan was free from nitrogen and ash, and had $[\alpha]_D^{23} -181^\circ$ (*c* 1.12, water). Sedimentation analysis²³, using a synthetic boundary-cell and a 1.2% solution in 0.1M sodium chloride at 29,500 rev./min, showed a major, symmetrical peak with a tiny shoulder. The respective areas, estimated by triangulation, showed that the shoulder amounted to *ca.* 3–4% of the total area under the peaks. The arabinan (2.0 mg) was hydrolysed with 0.5M sulphuric acid (0.5 ml) for 5 h at 100°. The hydrolysate (1 ml) was treated with sodium borohydride (5 mg), and the resulting mixture of alditols, recovered in the usual way, was acetylated and examined by g.l.c. on a Pye 104 Gas Chromatograph, using dual columns (5 ft) of 4% OV-225 on Gas Chrom Q (80–100 mesh) with temperature programming at 5°/min from 180–250°, and a nitrogen flow-rate of 60 ml/min. The results showed that the recovery of galactose was 5% of the total polysaccharide. Partial hydrolysis of the arabinan samples with 5 and 10mM sulphuric acid, followed by reduction, acetylation, and examination of the resulting alditol acetates, showed that the percentage recoveries of arabinose from the polysaccharide were 6 and 72%, respectively, with the corresponding recoveries of galactose being 0.8 and 1.2%.

Acid hydrolysis of the arabinan. — The arabinan (50 mg) was hydrolysed with 10mM sulphuric acid (3 ml) at 100° for 6 h. Neutralization of the hydrolysate (BaCO₃), filtration, and evaporation yielded a syrup which crystallized from 95% aqueous

ethanol, giving L-arabinose (43 mg, ~75%), m.p. and mixture m.p. 152–154°, $[\alpha]_D^{25} + 134.3$ (4 min) $\rightarrow +104.6^\circ$ (equil.) (*c* 1.23, water).

Methylation analyses. — A solution of the arabinan (140 mg) in water (10 ml) was cooled and treated dropwise, in an atmosphere of nitrogen, with 40% aqueous sodium hydroxide (15 ml) and methyl sulphate (5 ml), so that the pH of the mixture remained nearly neutral for the first few hours. The addition lasted several hours, after which time the solution was stirred for 24 h. After 9 such additions during 9 days, the reaction mixture was neutralized with 3M sulphuric acid, under cooling and in an atmosphere of nitrogen. The solution (300 ml) was extracted with chloroform (4 \times 150 ml), and the extract was dried (Na₂SO₄), filtered, and concentrated. The syrupy residue (180 mg) was dissolved in methyl iodide (20 ml), silver oxide (2 g) was added, and the mixture was stirred and boiled under reflux for 24 h. After 4 such methylations, the product showed a very weak i.r. absorption for hydroxyl, and this was not diminished by four further methylations by Purdie's method. The final product (170 mg) had $[\alpha]_D^{24} - 165.7^\circ$ (*c* 1.1, chloroform).

A solution of the methylated arabinan (170 mg) in 3% methanolic hydrogen chloride (25 ml) was boiled under reflux for 20 h. After neutralization (silver carbonate), filtration, and evaporation, the syrupy product was hydrolysed with 0.5M sulphuric acid (5 ml) for 32 h. The hydrolysate was neutralized with barium carbonate, filtered, and concentrated to a syrup (166 mg).

A portion (10 mg) of the methylated sugars was reduced with sodium borohydride (20 mg) in water (1 ml) for 24 h. The excess of borohydride was destroyed with glacial acetic acid, and the solution, after removal of sodium ions with Rexyn-101(H⁺) resin, was freed from borate by repeat evaporations with methanol. The resulting mixture of methylated arabinitols was acetylated with acetic anhydride (0.3 ml) and pyridine (0.7 ml) for 1 h at 100° and overnight at room temperature. Water was added to the reaction mixture which, after removal of solvent, was taken up in chloroform for analysis by g.l.c. G.l.c. was carried out on a Pye 104 Gas Chromatograph, using dual columns (5 ft \times 0.25 in.) of 4% OV-225 on Gas Chrom Q (80–100 mesh) with temperature programming from 180–250° at 5°/min, and a nitrogen flow-rate of 45 ml/min. The following results were obtained (retention times are with respect to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol):

Compound	Retention time (min) of acetate	Molar ratio
2,3,5-Me ₃ -Arabinitol	0.96	11
2,3-Me ₂ -Arabinitol	1.84	7
2-Me-Arabinitol	2.43	7
Arabinitol	2.87	2

G.l.c.-m.s. of the methylated arabinitol acetates^{8,10}. — The identities of the methylated arabinitol acetates were confirmed by g.l.c.-m.s. by comparison with authentic

standards. G.l.c.-m.s. was effected with temperature programming from 180–240° at 6°/min, using a column (5 ft) of OV-17 on Gas Chrom Q (80–100 mesh) coupled to a Dupont 21-490 mass spectrometer. The mass spectra were recorded at an inlet temperature of 180°, an ionizing potential of 70 eV, and an ion-source temperature of 200°. Although no standard was available for 2-*O*-methylarabinitol, the presence of primary fragment ions m/e 117 and 261 confirmed the identity of this component.

Further characterization of the methyl sugars. — The remaining mixture of methylated sugars was fractionated on sheets of Whatman No. 1 paper with 1-butanol-ethanol-water (40:11:19) to give fractions 1, 49.6 mg; 2, 32.8 mg; 3, 35.1 mg; 4, traces; and 5, 11.2 mg.

Fraction 1. Fraction 1 was paper chromatographically and electrophoretically identical with 2,3,5-tri-*O*-methylarabinose. A solution of fraction 1 (49 mg) in water (2 ml) was oxidised with an excess of bromine in the presence of barium carbonate (50 mg) for 72 h. Bromine was removed by aeration, and the acidified solution was extracted with chloroform for 48 h. The chloroform extract was dried and concentrated, and the residual syrup was distilled [90–95°(bath)/0.02 mmHg] to yield a product which was kept in methanol saturated with ammonia. After 48 h in the cold, removal of the methanol in a desiccator produced crystals. Recrystallization from cold ethanol gave 2,3,5-tri-*O*-methyl-L-arabinonamide, m.p. and mixture m.p. 139–140°, $[\alpha]_D^{27} +14.8^\circ$ (c 0.4, water); lit.²⁴ m.p. 138°, $[\alpha]_D +16^\circ$.

Fraction 2. Fraction 2 was paper chromatographically and electrophoretically identical with 2,3-di-*O*-methylarabinose. It was treated with a boiling solution of aniline (16 mg) in ethanol (1 ml) for 5 h. Removal of the ethanol and crystallization from acetone gave 2,3-di-*O*-methyl-*N*-phenyl-L-arabinosylamine, m.p. and mixture m.p. 135–138°, $[\alpha]_D^{22} +160.1$ (10 min) $\rightarrow +8.9^\circ$ (29 h, equil.; c 0.67, methanol); lit.²⁵ m.p. 138°.

Fraction 3. On paper chromatography in 1-butanol-ethanol-water (40:11:19), this fraction showed a single component (R_G 0.57 and M_G 0.35). A solution of fraction 3 (35 mg) in water (1 ml) was oxidized with bromine, as for fraction 1, to give a product which was extracted with acetone-chloroform (1:1). The soluble portion was purified by t.l.c., using the same irrigant, to give a syrup which was kept in methanol saturated with ammonia. Crystallization of the product from cold ethanol gave 2-*O*-methyl-L-arabinonamide, m.p. 129–130°; lit.²⁵ m.p. 130°.

Fraction 4. Fraction 4 showed two components on paper chromatography and electrophoresis. The faster-moving component (p.c.) was identical with 2-*O*-methylarabinose (M_G 0.35), and the slower-moving component had M_G 0.79. G.l.c. of the acetylated alditols derived from fraction 4 was performed as already described, but on columns of OV-225 on Chromosorb W-HP (80–100 mesh). Two peaks were revealed, one identical with 2-*O*-methyl arabinitol tetra-acetate, and the other having T 1.04 relative to 2-*O*-methyl arabinitol tetra-acetate. G.l.c.-m.s.^{8,10}, as already described, showed a single, unsymmetrical peak. Mass spectra recorded on the leading and trailing slopes of the peak showed primary fragments of high intensities at m/e 117 and 261 in the former slope, and m/e 189 in the latter.

Fraction 5. This fraction was paper chromatographically and electrophoretically identical with arabinose, and crystallized from 95% aqueous ethanol to give L-arabinose, m.p. 149–151°, undepressed on admixture with an authentic specimen.

Periodate oxidation. — Rapeseed arabinan (18.3 mg) was oxidized with 20mm sodium metaperiodate (50 ml) at room temperature in the dark. Aliquots (5 ml) were removed after 40 h, and the consumption of periodate and production of formic acid were determined^{27,28}. The results (based on 132 g of polysaccharide) were: periodate reduced, 0.69 mole; formic acid produced, 0.05 mole.

The remaining solution was treated with an excess of barium carbonate, filtered, concentrated to 1 ml, and reduced with sodium borohydride (25 mg). After 18 h at room temperature, excess of borohydride was destroyed with acetic acid, the solution was deionized with Rexyn-101(H⁺) resin, and borate ions were removed by repeated distillation of methanol from the residue. After hydrolysis of the residue with 0.5M sulphuric acid for 3 h at 100°, examination of the neutralized (BaCO₃) hydrolysate by paper chromatography showed mainly arabinose and glycerol, with traces of what appeared to be threitol and glyceraldehyde.

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