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SULPHATED POLYSACCHARIDES OF THE *Grateloupiaceae* FAMILY PART V[1]. A POLYSACCHARIDE FROM *Aeodes ulvoidea*

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ABSTRACT

Aeodes ulvoidea, a red seaweed of the *Grateloupiaceae*, yielded a highly sulphated polysaccharide which was shown to contain D-galactose, 4-*O*-methyl-L-galactose, and 2-*O*-methyl-D-galactose, together with chromatographic traces of 6-*O*-methylgalactose, xylose, and mannose. The sulphate was not labile to alkali, but it was largely removed with methanolic hydrogen chloride. Periodate oxidation of the polysaccharide and methylation of the desulphated polymer indicate (*a*) the presence of (1→3)- and (1→4)- glycosidic links in the macromolecule, (*b*) that the 2-*O*-methyl-D-galactose is either (1→4)- and/or (1→3)-linked, and (*c*) that the 4-*O*-methyl-L-galactose is probably present only as non-reducing end-group.

INTRODUCTION

In continuing our work on the water-soluble, sulphated polysaccharides of the *Grateloupiaceae*, we report an initial study of the polysaccharide from *Aeodes ulvoidea*.

RESULTS AND DISCUSSION

Hot-water extraction of fresh *Aeodes ulvoidea*, followed by precipitation of the mucilage into ethanol, yielded a sulphated polysaccharide which was purified in the usual way. Ultracentrifuge examination showed a large, sharp peak, indicating that it is probably an extended type of molecule; the presence of a very much smaller peak showed that the polymer was not completely pure. The infrared spectrum of the polysaccharide showed the characteristic sulphate ester absorption band at 1240 cm^{-1}, but only a broad shoulder in the 800–850 cm^{-1} region, giving no indication of the possible location of the sulphate ester groups[2].

Fractionation of a neutralised, acid hydrolysate by cellulose-column chromatography resulted in the isolation of crystalline D-galactose, 4-*O*-methyl-L-galactose, and 2-*O*-methyl-D-galactose. In addition, paper-chromatographic evidence for the presence of traces of 6-*O*-methylgalactose, xylose, and mannose was obtained. The

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molar proportion of D-galactose/4-*O*-methyl-L-galactose/2-*O*-methyl-D-galactose, as determined by g.l.c.[3] of their derived alditol acetates, was 10.5:1.1:1.0. This polysaccharide differs from those *Grateloupiaceae* polysaccharides already discussed in this series (*cf.* aeodan[4,5] and phyllymenan[1,9]), in that the 4-*O*-methyl-L-galactose is a major constituent.

Treatment of the polysaccharide with sodium hydroxide and sodium borohydride[6] resulted in the loss of only ~10% of the sulphate and the formation of an equivalently small amount of 3,6-anhydrogalactose. In this respect, the polysaccharide resembles aeodan[4] in having, possibly, a small proportion of (1→2)- or (1→4)-linked D-galactose 3(or 6)-sulphate.

Desulphation of the polysaccharide with methanolic hydrogen chloride[7] afforded a considerably degraded, desulphated polymer (SO_4^{2-}, 6.2%) in 65% yield. Paper chromatography of a neutralised, acid hydrolysate of the methanol-soluble material showed that desulphation had removed some galactose, 2-*O*-methylgalactose, and 4-*O*-methylgalactose residues. Paper chromatography of a neutralised, acid hydrolysate of the desulphated polymer showed that the 4-*O*-methylgalactose spot was less intense than the 2-*O*-methylgalactose spot, whereas, for the unmodified polysaccharide, the intensities of the spots were clearly reversed. This was confirmed by g.l.c. studies of the derived alditol acetates. Although exact measurements were not possible, the estimated molar ratios of galactose–4-*O*-methylgalactose–2-*O*-methylgalactose were 35:1:2 (*cf.* corresponding molar ratios of 10.5:1.1:1 in the unmodified polysaccharide).

Oxidations of polysaccharide and desulphated polysaccharide with sodium metaperiodate were followed spectrophotometrically[18] to completion (Table I). Although the desulphated polysaccharide consumed ~50% more periodate than the polysaccharide, this is readily explained by the extensive degradation which took place during desulphation of the polysaccharide. Paper chromatography of neutralised, acid hydrolysates of the oxopolysaccharides derived from both polysaccharide and desulphated polysaccharide revealed galactose and 2-*O*-methylgalactose, but no trace of 4-*O*-methylgalactose.

TABLE I

PERIODATE REDUCED (MMOLES) PER "ANHYDROHEXOSE" UNIT

Time (h)	*5*	*10*	*24*	*48*	*72*	*96*	*150*
Polysaccharide[a]	100	—	228	246	264	264	—
Polysaccharide[b]	194	229	263	280	288	300	300
Desulphated polysaccharide (SO_4^{2-}, 6.2%)[a]	295	—	361	395	430	430	—

[a]Spectrophotometric determination. [b]Titrimetric determination.

In order to obtain sufficient oxopolysaccharide for further examination, a second experiment was performed in which the oxidation with sodium metaperiodate

was followed titrimetrically[19] (Table I). G.l.c. studies of this oxopolysaccharide, using the same technique as mentioned earlier, showed no trace of 4-*O*-methyl-galactose, the molar ratio of D-galactose–2-*O*-methyl-D-galactose in the oxopolysaccharide being 6·1:1. If all the 4-*O*-methyl-L-galactose residues in the polysaccharide are oxidised by periodate, they must be either (*a*) (1→6)-linked or (*b*) linked only through position one, *i.e.*, the 4-*O*-methyl-L-galactose must be present as non-reducing end-group. The possibility of (1→6)-links is ruled out by the absence of 2,3,4-tri-*O*-methylgalactose in the hydrolysate of the methylated, desulphated polysaccharide (see later). The presence of 4-*O*-methyl-L-galactose as an end-group could account, in part, for its ready removal during desulphation of the polymer. The molar ratio of D-galactose to 2-*O*-methyl-D-galactose in the oxopolysaccharide is also significant. The reduction of 0.3 mole of periodate per "anhydrohexose" unit by all the 4-*O*-methyl-L-galactose and part of the D-galactose in the polysaccharide would leave the molar ratio of D-galactose–2-*O*-methyl-D-galactose at *ca.* 7–8:1, which is of the order of the ratio found. Hence, it appears that the 2-*O*-methyl-D-galactose is largely immune to periodate attack. The majority of the 2-*O*-methyl-D-galactose residues must therefore be either (1→3)- and/or (1→4)-linked. The possibility of (1→6)-linked 2-*O*-methyl-D-galactose, either without sulphate or sulphated in either position 3 or 4, is further ruled out by the absence of 2,3,4-tri-*O*-methylgalactose in the hydrolysate of the methylated, desulphated polysaccharide (see later). Finally, the low reduction of periodate (<0.3 mole of periodate) suggests that at least one half of the galactose residues are (1→3)-linked and/or a large proportion of the galactose residues contain other glycosidic linkages so as to render them immune to periodate. The low percentage of alkali-labile sulphate in the polysaccharide precludes the possibility of a large proportion of (1→4)-linked D-galactose residues protected from periodate attack by sulphate ester groups at positions 2 and/or 3.

Methylation of the desulphated polysaccharide (SO_4^{2-}, 6.2%) was effected by addition of solid sodium hydroxide and methyl sulphate to a solution of the polymer in methyl sulphoxide. Paper chromatography of a neutralised, acid hydrolysate showed mostly 2,3,6- and 2,4,6-tri-*O*-methylgalactoses, with smaller proportions of 2,3,4,6-tetra-*O*-methylgalactose, some di-*O*-methylgalactoses, and 2-*O*-methylgalactose. In view of the difficulty of methylating polysaccharides on a large scale with Purdie's reagents[8], only a small quantity of the methylated, desulphated polysaccharide (*A*) was exhaustively methylated in this way. The derived, solid foam (*B*) showed no hydroxyl peak in the infrared. Paper chromatography of a neutralised, acid hydrolysate of *B* showed all the saccharides present in *A*, including the mono- and di-*O*-methylgalactoses in much the same concentrations as for *A*. It was therefore decided not to treat the bulk of *A* with Purdie's reagents, since it appeared to be already fully methylated.

Hydrolysis of *A* and fractionation of the hydrolysate, by elution from a charcoal–Celite column with a linear gradient of 0–5% butanone in water, gave 2,3,6- and 2,4,6-tri-*O*-methyl-D-galactose as the major components, together with a smaller amount of a mixture of 2,3,4,6-tetra-*O*-methyl-D- and -L-galactose in the ratio of

~1:1 (inferred from an $[\alpha]_D$ value of +2°). In addition, a mixture of methylated saccharides, identified by paper chromatography as 2-*O*-methylgalactose and a number of di-*O*-methylgalactoses, all in trace quantities, was obtained. The presence of 2,4,6-tri-*O*-methyl-D-galactose as a major saccharide in the hydrolysate supports the evidence cited earlier for (1→3)-linked D-galactose residues, while the presence of 2,3,6-tri-*O*-methyl-D-galactose as the other major saccharide indicates a high proportion of (1→4)-links in the macromolecule. The 2,3,4,6-tetra-*O*-methyl-D-galactose is considered to have arisen mainly from D-galactose and possibly from some 2-*O*-methyl-D-galactose end-groups. The presence of 4-*O*-methyl-L-galactose as end group in the desulphated polymer accounts for at least some of the 2,3,4,6-tetra-*O*-methyl-L-galactose in the methylated, desulphated polymer.

EXPERIMENTAL

Evaporation of solutions was carried out below 50° at reduced pressure, using a rotary film evaporator. Specific rotations were measured in water. Paper chromatography was carried out on Whatman No. 1 paper, using the following solvent systems: (**1**) ethyl acetate–acetic acid–formic acid–water (18:3:1:4), (**2**) butyl alcohol–pyridine–water (9:2:2), (**3**) butyl alcohol–ethanol–water (40:11:9), (**4**) butanone saturated with water containing 1% of 0.88 ammonia, and (**5**) ethyl acetate–pyridine–water (8:2:1). R_{Gal} values refer to chromatographic mobilities relative to galactose. The spray reagents used were (*a*) *p*-anisidine hydrochloride[10] (*b*) aniline–diphenylamine–phosphoric acid[11], (*c*) 20% sulphuric acid in ethanol, and (*d*) triphenyltetrazolium chloride[15]. Gas–liquid chromatography (g.l.c.) was carried out on a Beckman GC-4 gas chromatograph, using dual flame-ionization detectors and nitrogen as carrier gas. For the quantitative determination of sugars as their alditol acetates, the column packing[3] used was 20% Apiezon M supported on Chromosorb W (80–100 mesh, acid-washed and chlorodimethylsilane treated), and the operating temperature was 175°. Infrared spectra were recorded on a Beckman IR-8 spectrophotometer, using KBr discs.

Aeodes ulvoidea is easily recognised by its broad, flat fronds which often have an endophyte (belonging to the green algae) growing on them, and, during the processing of the weed, great care was taken to remove all visible traces of this endophyte so as to ensure as homogeneous a product as possible.

Isolation and purification of the polysaccharide. — Wet *Aeodes ulvoidea* (2.5 kg) was half covered with water, and glacial acetic acid was added to pH 3–4. Steam was passed into the mixture, the acetic acid aiding in the complete disintegration of the weed. The pH rose to between 6 and 7 during the process. The extract was centrifuged hot, and the product was precipitated with ethanol (5 volumes). The crude polysaccharide was washed with ether and dried at 50° under reduced pressure (10% yield on a wet-weight basis). Purification of the polysaccharide was effected by dissolution in water, centrifugation, and precipitation with ethanol. After three such cycles, the polysaccharide was washed with ether and dried at 50° under reduced pressure (Found: $[\alpha]_D^{23}$ +34°; 3,6-anhydrogalactose[12], 1.4; N, 0.4; OMe, 2.07; SO_4^{2-} (ref. 13), 19.9%).

Both the sodium and the ammonium salts of the polysaccharide gave a peak in the infrared at 1240 cm^{-1}; only a broad shoulder was present in the 800–860 cm^{-1} region[2].

Separation and characterization of the components of the polysaccharide. — The polysaccharide (20 g) was hydrolysed with 0.5M sulphuric acid (100 ml) on a boiling water-bath overnight. The solution was neutralised ($BaCO_3$), centrifuged, and evaporated to a hygroscopic, solid foam (11.6 g). Paper chromatography (solvents **1**, **3**, and **4**) of the solid revealed the presence of three sugars with R_{Gal} 2.15, 1.75, and 1.00 (solvent **1**), corresponding to 2-*O*-methylgalactose, 4-*O*-methylgalactose, and galactose (major sugar), respectively. The solid was applied to a cellulose column (5.4 × 43 cm), and the monosaccharides were eluted with butyl alcohol–water (95:5). Fractions (50 ml) were analysed by paper chromatography and combined into six major fractions.

Fraction 1. The syrup (274 mg), after recrystallisation from ethyl acetate–methanol, yielded 2-*O*-methyl-D-galactose, m.p. and mixed m.p. 147–148°; $[\alpha]_D^{18}$ +52 (5 min) → +92° (*c* 0.58); lit.[4] m.p. 148–149°, $[\alpha]_D^{16}$ +84.9° (final) (*c* 0.53). The infrared spectrum of this sugar was identical with that of authentic 2-*O*-methyl-D-galactose.

Demethylation[10] of the sugar (5 mg) by heating on a boiling water-bath with 46% HBr (1 ml) gave (paper chromatography) galactose and a trace of 2-*O*-methylgalactose. Later investigation of the mother liquor from which the 2-*O*-methyl-D-galactose had crystallised showed the presence of a trace of 6-*O*-methylgalactose (paper chromatography, solvents **1** and **5**, and sprays *a* and *d*).

Fraction 2. A syrup (440 mg) which was shown by paper chromatography to be a mixture of 2-*O*-methylgalactose and 4-*O*-methylgalactose (solvents **1**, **2**, and **4**).

Fraction 3. The syrup (370 mg) was chromatographically identical with 4-*O*-methylgalactose (solvents **1**, **2**, and **4**). After recrystallisation from methanol, it yielded large, colourless crystals of m.p. 200–202°; $[\alpha]_D$ −65 (5 min) → −83° (*c* 0.42). The m.p. was depressed to 192° on admixture with 4-*O*-methyl-D-galactose. Araki *et al.*[14] reported m.p. 202–203°; $[\alpha]_D^{13}$ −74.8 (27 min) → −85.1° (24 h) (*c* 2.70), for authentic 4-*O*-methyl-L-galactose. Demethylation[10] gave (paper chromatography) galactose and a trace of starting material. The infrared spectrum of the sugar was identical with that of its enantiomorph. The derived 4-*O*-methyl-*N*-phenyl-L-galactosylamine had m.p. 167.5–168°; lit.[14] m.p. 167–168°.

Fraction 4. A syrup (92 mg), shown by paper chromatography (solvents **1**, **2**, and **4**) to contain traces of galactose, mannose (R_{Gal} 1.30), xylose (R_{Gal} 1.65), and 4-*O*-methylgalactose (R_{Gal} 1.67).

Fraction 5. An amorphous solid (4.7 g) which, after recrystallisation from ethanol, yielded D-galactose, m.p. and mixed m.p. 160–162°, $[\alpha]_D^{17}$ +116 (5 min) → +85° (*c* 0.52). The sugar was oxidized by heating for 1 h with nitric acid (1 ml of 1:1 nitric acid–water) at 100° to give mucic acid, m.p. and mixed m.p. 210–212°.

Fraction 6. This fraction, a hygroscopic solid (2.7 g), was eluted from the column with aqueous ethanol and ethanol, and not further investigated.

Quantitative determination of the hexose residues in the polysaccharide. — The

method used for the quantitative determination of the sugar residues in the polysaccharide, desulphated polysaccharide, and periodate-oxidized polysaccharide was similar to that of Bowker and Turvey[3].

Desulphation of the polysaccharide. — Polysaccharide (16 g) and 0.15M methanolic hydrogen chloride[7] (500 ml) were shaken for 48 h. Insoluble material was then filtered off, washed with a little dry methanol, and re-treated with fresh, 0.15M methanolic hydrogen chloride (500 ml). The insoluble material (11.0 g) was filtered off, washed with ethanol, and dried at 55°/0.1 mm (Found: N, <1; SO_4^{2-}, 6.2%). The combined, methanolic filtrates were neutralised (Ag_2CO_3), centrifuged, and evaporated to a syrup. Paper chromatography of a neutralised, acid hydrolysate showed the presence of galactose, 4-*O*-methylgalactose, and 2-*O*-methylgalactose. Paper chromatography of a neutralised, acid hydrolysate of the desulphated polymer revealed that the 4-*O*-methylgalactose spot (spray *a*) was less intense than the 2-*O*-methylgalactose spot. Although exact measurements were not possible, g.l.c. studies showed that the ratio of galactose–4-*O*-methylgalactose–2-*O*-methylgalactose was ~35:1:2.

Alkali-treatment of the polysaccharide. — To the polysaccharide (1.5 g) in water (200 ml) was added sodium borohydride[6] (0.5 g), and the mixture was stored for 48 h at room temperature. Aqueous sodium hydroxide (40%, 50 ml) and sodium borohydride (1.5 g) were then added, and the mixture was heated at 70–75° for 7 h, with additions of sodium borohydride (0.5 g) every hour. This process was repeated in a second experiment, but with the water bath maintained at 80 ±1°. The solutions were dialysed, the dialysates were centrifuged and concentrated, and the polysaccharides were isolated by freeze-drying (1.1 g in each case) [Found: 1st experiment; $[\alpha]_D^{26}$ +43° (*c* 0.7); 3,6-anhydrogalactose, 2.7; N, 0.0; SO_4^{2-}, 17.5%; 2nd experiment; $[\alpha]_D^{26}$ +53° (*c* 1.3); 3,6-anhydrogalactose, 3.5; SO_2^{4-}, 17.9%].

Periodate oxidation of polysaccharide and desulphated polysaccharide. — To the polysaccharide (19.2 mg) and desulphated polysaccharide (19.6 mg; SO_4^{2-}, 6.2%) in water (5 ml) were added equal volumes of 29.8mM sodium metaperiodate, and the oxidations were followed spectrophotometrically[18] (Table I). The solutions were then treated with excess of ethylene glycol, dialysed, and concentrated. Paper chromatography (solvents **1** and **2**) of a neutralised, acid hydrolysate showed only galactose and 2-*O*-methylgalactose in each case. In order to obtain sufficient oxopolysaccharide for further examination, the polysaccharide (387 mg) in water (50 ml) was treated with an equal volume of 59.4mM sodium metaperiodate, the reduction of periodate being followed titrimetrically[19] (Table I). After 150 h, the solution (now reduced to 35 ml; 65 ml having been used for the titrimetry) was treated with excess of ethylene glycol and dialysed. The oxopolysaccharide (40 mg) was isolated by concentration of the solution to a suitable volume and freeze-drying. Paper chromatography of a neutralised, acid hydrolysate of the oxopolysaccharide indicated galactose and 2-*O*-methylgalactose, but 4-*O*-methylgalactose and xylose were not detected. G.l.c. (conditions outlined earlier) showed that the molar ratio of D-galactose to 2-*O*-methyl-D-galactose in the oxopolysaccharide was 6.1:1, a peak corresponding to 4-*O*-methyl-L-galactose was not detected.

Methylation of desulphated polysaccharide. — To a solution of desulphated polysaccharide (9.0 g; SO_4^{2-}, 6.2%) in methyl sulphoxide (150 ml) was added solid sodium hydroxide (60 g) and methyl sulphate (30 ml) over a period of 6 h with vigorous stirring. The mixture was then stirred overnight. This treatment was repeated, after which the residual methyl sulphate was destroyed by heating on a boiling water-bath for 1.5 h. Water was added to dissolve the solids, and the alkaline solution was partially neutralised in the cold with 2.5M sulphuric acid, the neutralisation being completed with dilute acetic acid. This solution (1.5 l) was dialysed, concentrated to a suitable volume, and freeze-dried to a solid foam (12.6 g). After a second methylation, the methylated polymer was extracted into chloroform in the usual way, yielding a glassy foam (*A*) (1.1 g), which had no i.r. band for hydroxyl groups. Paper chromatography (solvents **1** and **5**, spray *a*) of a neutralised, acid hydrolysate showed 2,3,6- and 2,4,6-tri-*O*-methylgalactoses, with smaller amounts of 2,3,4,6-tetra-*O*-methylgalactose, some di-*O*-methylgalactoses, and 2-*O*-methylgalactose. In view of the difficulty of methylating on a large scale with Purdie's reagents[8], a small quantity of *A* (35 mg) was exhaustively methylated by stirring in methyl iodide (12 ml) with freshly prepared silver oxide (2 g added in portions over 4 days). The mixture was filtered and concentrated to a glass, and the above methylation procedure was repeated. Finally, the polymer was given two more treatments as above, the silver oxide (3 g each) being added while the solution was under reflux, to yield a glass (*B*, 24 mg). Paper chromatography (solvents **1** and **5**) of a neutralised, acid hydrolysate of *B* showed all the sugars present in the hydrolysate of *A*. The infrared spectrum of *B* showed no hydroxyl peak (Found: OMe, 37.4%).

Hydrolysis of the methylated, desulphated polysaccharide. — The methylated, desulphated polysaccharide (1 g; SO_4^{2-}, 6.2%) described above was hydrolysed by the formic acid–dilute sulphuric acid method[16], to yield a syrup which was applied to a charcoal–Celite (1:1) column (4.5 × 50 cm). The methylated sugars were eluted by applying a linear gradient of 0–5% butanone in water over a volume of 12 l. Fractions (30 ml) were collected, analysed by paper chromatography, and combined into the following main fractions.

Fraction 1. The syrup contained minute traces of galactose and was discarded.

Fraction 2. The syrup (130 mg) was further fractionated by paper chromatography (solvent **5**) into *2(a)* a syrup (60 mg) chromatographically (solvents **1** and **5**, sprays *a* and *d*) identical with 2-*O*-methylgalactose; and *2(b)* a syrup (60 mg) which was shown by paper chromatography (solvents **1** and **5**, sprays *a* and *d*) to be a mixture of di-*O*-methylgalactoses; this fraction was not further investigated.

Fraction 3. The syrup (20 mg) was shown by paper chromatography (solvents **1** and **5**) to be 2,4,6-tri-*O*-methylgalactose containing traces of di-*O*-methylgalactoses and 2-*O*-methylgalactose.

Fraction 4. The syrup (147 mg), which was chromatographically identical with 2,4,6-tri-*O*-methyl-D-galactose, had $[\alpha]_D^{19}$ +82° (*c* 0.98) and yielded an "anilide" which, after two recrystallisations from ethanol, had m.p. 171–172° alone and in admixture with 2,4,6-tri-*O*-methyl-*N*-phenyl-D-galactosylamine[4].

Fraction 5. The syrup (210 mg) was shown by paper chromatography (solvents **1** and **5**) to be a mixture of 2,3,6- and 2,4,6-tri-*O*-methylgalactose.

Fraction 6. The syrup (117 mg) was shown by paper chromatography (solvents **1** and **5**) to be 2,3,6-tri-*O*-methylgalactose containing traces of 2,4,6-tri-*O*-methylgalactose and 2,3,4,6-tetra-*O*-methylgalactose. The derived 2,3,6-tri-*O*-methyl-D-galactonolactone[17], after recrystallisation from dry ether, had m.p. and mixed m.p. 97–98°.

Fraction 7. The syrup (105 mg), $[\alpha]_D^{20}$ +74° (*c* 1.18), was shown by paper chromatography (solvents **1**, **4**, and **5**) to be pure 2,3,6-tri-*O*-methyl-D-galactose.

Fraction 8. The syrup (68 mg), $[\alpha]_D^{19}$ +2° (*c* 3.1), was shown by paper chromatography (solvents **1**, **4**, and **5**) to be 2,3,4,6-tetra-*O*-methylgalactose. The aniline derivative, after several recrystallisations from ethanol, had m.p. 179–180°, which could not be increased by further recrystallisations. The mixed m.p. (with authentic 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine of m.p. 189–190°) was 181°. This m.p. behaviour of the "anilide", together with the optical rotation of the syrup, suggests that this fraction is a mixture of approximately equal parts of tetra-*O*-methyl-D- and -L-galactoses.

Fraction 9. The syrup (53 mg) contained degradation products arising from the hydrolysis and was not further investigated.

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REFERENCES

1 Part IV: J. R. NUNN AND H. PAROLIS, *Carbohyd. Res*, 14 (1970) 145.
2 A. G. LLOYD AND K. S. DODGSON, *Biochim. Biophys. Acta*, 46 (1961) 116; A. G. LLOYD, K. S. DODGSON, R. G. PRICE, AND F. A. ROSE, *ibid*, 46 (1961) 108.
3 D. M. BOWKER AND J. R. TURVEY, *J. Chem. Soc. (C)*, (1968) 983.
4 J. R. NUNN AND H. PAROLIS, *Carbohyd. Res.*, 6 (1968) 1.
5 J. R. NUNN AND H. PAROLIS, *Carbohyd. Res.*, 8 (1968) 361.
6 D. A. REES, *J. Chem. Soc.*, (1961) 5168.
7 J. P. MCKINNELL AND E. PERCIVAL, *J. Chem. Soc.*, (1962) 3141.
8 T. PURDIE AND J. C. IRVINE, *J. Chem. Soc.*, 83 (1903) 1021.
9 J. R. NUNN AND H. PAROLIS, *Carbohyd. Res.*, 9 (1969) 265.
10 L. HOUGH, J. K. N. JONES, AND W. H. WADMAN, *J. Chem. Soc.*, (1950) 1702.
11 S. SCHWIMMER AND A. BEVENUE, *Science*, 123 (1956) 543.
12 W. YAPHE, *Anal. Chem.*, 32 (1960) 1327.
13 A. S. JONES AND D. S. LETHAM, *Analyst*, 81 (1956) 15.
14 C. ARAKI, K. ARAI, AND S. HIRASE, *Bull. Chem. Soc. Japan*, 40 (1967) 959.
15 K. WALLENFELS, *Naturwissenschaften*, 37 (1950) 491.
16 H. O. BOUVENG, H. KIESSLING, B. LINDBERG, AND J. E. MCKAY, *Acta Chem. Scand.*, 16 (1962) 615.
17 A. J. CHARLSON, J. R. NUNN, AND A. M. STEPHEN, *J. Chem. Soc.*, (1955) 1428.
18 G. O. ASPINALL AND R. J. FERRIER, *Chem. Ind.* (London), (1957) 1216.
19 P. FLEURY AND J. LANGE, *J. Pharm. Chim.*, 17 (1933) 107.