Carbohydrate Research, 125 (1984) 145–153
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

STRUCTURAL STUDIES ON THE POLYSACCHARIDE OF MAHUA *(Madhuca indica)* FLOWERS*

NUPUR SARKAR AND BISHNU P. CHATTERJEE
Department of Macromolecules, Indian Association for the Cultivation of Science, Jadavpur, Calcutta 700 032 (India)

(Received July 28th, 1983; accepted for publication, August 22nd, 1983)

ABSTRACT

Graded hydrolysis of purified mahua polysaccharide, PS-AI, afforded four neutral and three acidic oligosaccharides, together with monosaccharides. These oligosaccharides were characterized through hydrolysis, methylation, and reduction with lithium aluminum hydride. On methylation, Smith-degraded PS-AI gave 2,3,4,6-tetra-*O*-methyl-D-galactose (5.5 mol), 2,3,4-tri-*O*-methyl-D-galactose (1 mol), 2,4,6-tri-*O*-methyl-D-galactose (2.2 mol), and 2,3,4-tri-*O*-methyl-L-arabinose (0.9 mol). Based on these results, and those obtained from methylation, periodate oxidation, and chromium trioxide oxidation studies on the polysaccharide PS-AI, a tentative structure has been assigned to the average repeating-unit in the polysaccharide.

INTRODUCTION

In our previous communication[1], we reported the results of periodate oxidation, methylation, and chromium trioxide oxidation studies of one of the homogeneous fractions of mahua polysaccharide (PS-AI) and its carboxyl-reduced derivative. These studies reflect the structural pattern of the polysaccharide, which is highly branched. The results of a structural investigation on the PS-AI are now reported.

EXPERIMENTAL

General methods. — All evaporations were conducted under diminished pressure at 40° (bath temperature). Paper chromatography was performed with the following solvent systems (v/v): (*A*) 8:2:1 ethyl acetate–pyridine–water, (*B*) 9:2:2 ethyl acetate–acetic acid–water, and (*C*) 4:1:5 (upper layer) 1-butanol–acetic acid–water. Whatman No. 1 filter papers were used for qualitative, paper chromatography, and large amounts of sugar mixtures were separated on Whatman No. 3

*Part II. For Part I, see ref. 1.

0008-6215/84/$ 03.00

MM sheets. The spray reagents used were (*a*) alkaline silver nitrate and (*b*) aniline oxalate. Optical rotations were measured at 23 ±1° and 589.5 nm with a Perkin–Elmer model 241 MC spectropolarimeter. Colorimetric estimations of monosaccharides were made in a Hitachi model spectrophotometer, and i.r. spectra were recorded with a Beckman IR-20A instrument. Gas–liquid chromatography was performed with a Hewlett–Packard Model 5730 A gas chromatograph fitted with a flame-ionization detector and a glass column (1.8 m × 6 mm) packed with (*i*) 3% of ECNSS-M on Gas Chrom Q (100–200 mesh) and (*ii*) 3% of OV-225 on Gas-Chrom Q (100–200 mesh). All g.l.c. analyses were conducted (at 185° for unmethylated sugars, and at 165° for methylated sugars) by converting the sugars into their alditol acetates.

Isolation of oligosaccharides. — The mahua polysaccharide PS-AI (200 mg) was hydrolyzed with 0.05M sulfuric acid for 4 h at 100°, the optimum conditions for obtaining a good yield of oligosaccharides being determined by monitoring pilot experiments. The hydrolyzate was made neutral with barium carbonate, and the suspension was centrifuged. The precipitate was washed twice with distilled water, and the washings and supernatant liquor were combined and concentrated to ~2 mL. The neutral hydrolyzate was passed successively through columns of Dowex-50W X8 (H^+) and Dowex-1 X4 ($HCOO^-$) ion-exchange resins, in order to trap the acidic oligosaccharides. The column was washed thoroughly with water until free from neutral oligosaccharides (Molisch test). The washings containing neutral oligosaccharides were collected, and concentrated to a small volume. Finally, the column was eluted with 20% formic acid (100 mL). The formic acid was removed under diminished pressure. The mixtures containing the neutral and acidic oligosaccharides were resolved by preparative, paper chromatography on Whatman No. 3 MM papers, using solvent *C*, and the separated sugars were isolated by eluting the corresponding strips with water. The homogeneity of each oligosaccharide was checked by p.c.

Acid hydrolysis of the oligosaccharides. — The oligosaccharides (0.5 mg each) were hydrolyzed with 0.5M sulfuric acid for 16 h at 100°. The excess of acid was neutralized, and the sugars were converted into their alditol acetates, which were then analyzed by g.l.c. using column *i*.

Determination of reducing ends of oligosaccharides. — The oligosaccharides (1 mg each) were reduced with sodium borohydride (10–15 mg). The solutions were made neutral with acetic acid, and boric acid was removed as methyl borate. The reduced oligosaccharides were then hydrolyzed with 0.5M sulfuric acid for 16 h at 100°. The hydrolyzates were made neutral with barium carbonate, the suspension was filtered on a Celite bed, and the filtrate was evaporated under diminished pressure. The samples were then acetylated with acetic anhydride–pyridine, and the acetates analyzed by g.l.c. using column *i*.

Methylation of oligosaccharides. — The oligosaccharides were methylated by the Kuhn procedure[2]. To a solution of each of the oligosaccharides (1 mg) in *N,N*-dimethylformamide (1 mL) were added silver oxide (0.4 g) and Drierite (0.25 g).

The mixture was stirred for 30 min, and methyl iodide (0.3 mL) was added. Stirring was continued for 30 h in the dark, chloroform (10 mL) was added, and the mixture was vigorously stirred. The solids were filtered off through a bed of Celite, and the filtrate was washed with water (4 × 25 mL), dried (anhydrous sodium sulfate), and evaporated to dryness. The products were then hydrolyzed under the same conditions as before, and the alditol acetates were prepared. These were identified, and estimated, by g.l.c. in columns *i* and *ii*.

Reduction of methylated, acidic oligosaccharides. — Methylated, acidic oligosaccharides (1 mg each) were reduced with lithium aluminum hydride[3] in 1:4 diethyl ether–dichloromethane, and the reduced, methylated oligosaccharides were hydrolyzed with 0.5M sulfuric acid for 12 h at 100°, converted into alditol acetates, and analyzed by g.l.c. using columns *i* and *ii*.

Characterization of Smith-degraded product from PS-AI. — The polysaccharide PS-AI (50 mg) was subjected to Smith degradation[4] as described previously[1]. On paper-chromatographic examination in solvent *A*, the Smith-degraded material gave spots corresponding to galactose, arabinose, and three oligomers. The oligosaccharides were separated on Whatman No. 3 MM papers as usual, and their homogeneity was checked by p.c. The oligosaccharides were characterized through hydrolysis, and one of them was subjected to methylation by the Kuhn procedure. The methylated sugars were analyzed by g.l.c. as their alditol acetates.

In two separate experiments, the periodate oxidized-reduced PS-AI was methylated by the method of Hakomori, followed by the Kuhn procedure, and the mildly hydrolyzed product from the periodate oxidized-reduced PS-AI was methylated by the Kuhn method. In both cases, the methylated sugars were identified, and estimated, by g.l.c. as usual.

RESULTS AND DISCUSSION

In order to ascertain the sequence of the different sugar residues, the polysaccharide was subjected to graded hydrolysis, and, guided by the results of pilot experiments for maximal release of oligosaccharides, the polysaccharide was hydrolyzed with 0.05M sulfuric acid for 4 h at 100°. After neutralization ($BaCO_3$), the hydrolyzate was successively passed through columns of Dowex-50W X8 (H^+) and Dowex-1 X4 ($HCOO^-$) ion-exchange resin. The eluate and the washings, containing neutral oligosaccharides, were concentrated to a small volume. The column of anion-exchange resin was eluted with 20% formic acid (100 mL), and the eluate was evaporated to dryness, yielding the acidic oligosaccharides. The mixtures containing the neutral and acidic oligosaccharides were resolved on Whatman No. 3 MM papers, using solvent *C*; each fraction was found to be homogeneous in paper chromatography, and was characterized as follows.

Neutral oligosaccharides. — On hydrolysis, oligosaccharide **1** {4.8 mg; R_{Gal} 0.64 (solvent *C*); $[\alpha]_D^{23}$ +49° (*c* 0.4, water) (lit.[5] +52.7°)} gave galactose (by p.c. and g.l.c.). The material was fully methylated by the Kuhn method, and the prod-

uct was hydrolyzed. The methylated sugars were identified, and estimated, in the usual way by g.l.c. using columns *i* and *ii*; they were 2,3,4,6-tetra-*O*-methylgalactose (1.0 mol) and 2,3,6-tri-*O*-methylgalactose (0.95 mol). From the methylation results and the literature value of the specific rotation, the oligosaccharide was therefore assigned the following structure.

β-Gal-(1→4)-Gal
1

On hydrolysis, oligosaccharide **2** {6.9 mg; R_{Gal} 0.51 (solvent *C*); $[\alpha]_D^{23}$ +22.5° (*c* 0.5, water) (lit.[6] +25.1°)} gave galactose only. On methylation by the Kuhn method, hydrolysis, and g.l.c. analysis, the oligosaccharide yielded 2,3,4,6-tetra-*O*-methylgalactose (1.0 mol) and 2,3,4-tri-*O*-methylgalactose (0.83 mol). Based on these results, the structure assigned to this oligosaccharide was as follows.

β-Gal-(1→6)-Gal
2

On hydrolysis, oligosaccharide **3** {4.7 mg; R_{Gal} 0.26 (solvent *C*); $[\alpha]_D^{23}$ +29° (*c* 0.4, water)} gave galactose. The material was fully methylated, the product was hydrolyzed, and the alditol acetates of the resulting methylated sugars were identified by g.l.c. as being those from 2,3,4,6-tetra-*O*-methylgalactose (1.0 mol), 2,4,6-tri-*O*-methylgalactose (0.92 mol), and 2,3,4-tri-*O*-methylgalactose (0.90 mol). Hence, the oligosaccharide has either structure **3a** or **3b**.

Gal-(1→3)-Gal-(1→6)-Gal Gal-(1→6)-Gal-(1→3)-Gal
3a **3b**

On hydrolysis, oligosaccharide **4** {4.0 mg; R_{Gal} 0.16 (solvent *C*); $[\alpha]_D^{23}$ +47.5° (*c* 0.35, water)} gave a peak corresponding to galactose in g.l.c. On methylation and g.l.c. examination, this oligosaccharide yielded peaks corresponding to 2,3,4,6-tetra-*O*-methylgalactose (1.0 mol), 2,3,6-tri-*O*-methylgalactose (0.9 mol), and 2,3,4-tri-*O*-methylgalactose (0.92 mol). Borohydride reduction followed by methylation, hydrolysis, and g.l.c. analysis gave 2,3,4,6-tetra-*O*-methylgalactose (1.0 mol) and 2,3,6-tri-*O*-methylgalactose (0.98 mol). From these results, oligosaccharide **4** was identified as the following.

Gal-(1→4)-Gal-(1→6)-Gal
4

Acidic oligosaccharides. — On hydrolysis and p.c. examination, oligosaccharide **5** {5.6 mg; R_{GlcA} 0.78 (solvent *C*); $[\alpha]_D^{23}$ −6.6° (*c* 0.5, water) (lit.[7] − 2°)} was found to give galactose and glucuronic acid. On reduction with sodium boro-

hydride and hydrolysis, only glucuronic acid was detected. The galactose[8] and glucuronic acid[9] in it were estimated to be present in the mol. ratio of 1:0.9. On methylation, hydrolysis, and analysis by g.l.c., 2,3,4-tri-*O*-methyl galactose was identified. Hence aldobiouronic acid **5** is

β-GlcA-(1→6)-Gal

5

On hydrolysis, oligosaccharide **6** {5.3 mg; R_{GlcA} 0.54 (solvent *C*); $[\alpha]_D^{23}$ −14.2° (*c* 0.4, water) } yielded galactose and glucuronic acid, estimated to be in the mol. ratio of 2.0:0.95. On reduction ($NaBH_4$) and hydrolysis, it gave galactose and glucuronic acid. On hydrolysis, the fully methylated oligosaccharide yielded (g.l.c. examination) 2,3,4-tri-*O*-methylgalactose (1.0 mol) and 2,3,6-tri-*O*-methylgalactose (0.90 mol). The methylated oligosaccharide was reduced with $LiAlH_4$, and the product hydrolyzed. The hydrolyzate was found to contain 2,3,4-tri-*O*-methylglucose (0.9 mol), 2,3,4-tri-*O*-methylgalactose (1 mol), and 2,3,6-tri-*O*-methylgalactose (0.90 mol). All these results indicated that the structure of the aldotriouronic acid is either GlcA-(1→6)-Gal-(1→4)-Gal or GlcA-(1→4)-Gal-(1→6)-Gal. But the former structure is more appropriate than the latter as it contains the aldobiouronic acid 6-*O*-(β-D-glucopyranosyluronic)-D-galactose; this is glycosidically linked to O-4 of the galactosyl residue, as proved by the fact that, on mild hydrolysis with acid, the aldotriouronic acid yielded the aldobiouronic acid and galactose. Hence, the structure of aldotriouronic acid **6** is as follows.

GlcA-(1→6)-Gal-(1→4)-Gal

6

On hydrolysis, oligosaccharide **7** {4.0 mg; R_{GlcA} 0.34 (solvent *C*); $[\alpha]_D^{23}$ +4.2° (*c* 0.4, water) (lit.[10] +3.6°)} gave galactose, glucuronic acid (trace), and the aldobiouronic acid. The ratio of galactose to glucuronic acid in the acidic oligosaccharide **7** was estimated to be 2:1. On complete methylation, followed by reduction ($LiAlH_4$), and hydrolysis, it gave 2,3,4-tri-*O*-methylgalactose (1.95 mol) and 2,3,4-tri-*O*-methylglucose (1 mol). Based on these results, the structure of the aldotriouronic acid **7** was assigned as follows.

β-GlcA-(1→6)-β-Gal-(1→6)-Gal

7

Characterization of periodate-oxidized and Smith-degraded material from PS-AI. — The polysaccharide PS-AI was subjected to periodate oxidation followed by reduction with sodium borohydride[1]. On methylation analysis, this material gave peaks corresponding to 2,4-di-*O*-methylgalactose (7.7 mol) and 2,4-di-*O*-methylarabinose (1 mol) in g.l.c. The mildly hydrolyzed product of this material, on

methylation study, yielded 2,3,4,6-tetra-*O*-methylgalactose (5.5 mol), 2,3,4-tri-*O*-methylgalactose (1 mol), 2,4,6-tri-*O*-methylgalactose (2.2 mol), and 2,3,4-tri-*O*-methylarabinose (0.9 mol). On p.c. examination, the Smith-degraded material gave spots corresponding to galactose (a trace), arabinose, and three oligosaccharides[1].

The oligosaccharides were isolated by preparative paper-chromatography on 3 MM papers, using solvent *A*, and were designated OL-1, OL-2, and OL-3. The homogeneity of these oligosaccharides was checked by p.c. On hydrolysis and g.l.c. examination, OL-1 gave a peak corresponding to galactose only; this material could not be characterized further, due to lack of material. For oligosaccharide OL-2, $[\alpha]_D^{23}$ +20° (*c* 0.3, water), (lit.[6] +25.1°), $R_{I.ac}$ 1.2, p.c. and g.l.c. showed the presence of galactose. On methylation of OL-2 and hydrolysis, g.l.c. showed 2,3,4,6-tetra-*O*-methylgalactose (1 mol) and 2,3,4-tri-*O*-methylgalactose (0.98 mol). Hence, the structure of oligosaccharide OL-2 has been assigned as 6-*O*-β-D-galactopyranosyl-D-galactose. On hydrolysis, oligosaccharide OL-3, having $[\alpha]_D^{23}$ +52° (*c* 0.2, water) (lit.[11] +51°), yielded galactose as the only sugar. The material could not be studied further, due to the poor yield.

The structures of oligosaccharides **2** and **3** (**3a** or **3b**) indicate that the main chain of the polysaccharide is composed of (1→3)- and (1→6)-linked galactose residues to which are glycosidically linked galactose units at O-3, O-4, or O-6; no oligomer isolated had a galactose residue linked (1→3) from the main chain. The oligosaccharide **1**, which is glycosidically linked to O-4 or O-6 of galactose units in the backbone, is present as a side chain in the macromolecule. The latter sequence of galactose units holds for the structure of oligosaccharide **4**.

The structures of acidic oligosaccharides **6** and **7** show that the aldobiouronic acid **5** is glycosidically linked to O-4 of a galactose residue in the side chain and to O-6 of a galactose residue in the main chain. The structure of the former indicates that the aldotriouronic acid is glycosidically linked to O-4 of a galactose residue in the main chain, and that O-3 of the galactose residues in some of the aldobiouronic acid units is joined to an arabinofuranose residue.

The Smith-degradation product of PS-AI yielded, besides monosaccharides, three oligomers, one of which, OL-2, was fully characterized through hydrolysis, specific rotation value, and methylation analysis, and was found to be 6-*O*-β-D-galactopyranosyl-D-galactose. It is evident that this disaccharide is available from the main backbone of the macromolecule. The higher oligosaccharide OL-3 may have the structure 3-*O*-β-D-galactopyranosyl-3-*O*-β-D-galactopyranosyl-D-galactose, as determined by specific rotation and hydrolysis study. This material could not be further characterized due to the poor yield. The lower oligosaccharide OL-1 could not be studied in detail, except hydrolysis, due to paucity of the material. On methylation, periodate-oxidized and borohydride-reduced PS-AI gave 2,4-di-*O*-methylgalactose (7.7 mol) and 2,4-di-*O*-methylarabinose (1.0 mol). These results were substantiated by the methylation study of Smith-degraded PS-AI, which gave peaks corresponding to 2,3,4,6-tetra-*O*-methylgalactose (5.5 mol), 2,3,4-tri-*O*-

methylgalactose (1 mol), 2,4,6-tri-*O*-methylgalactose (2.2 mol), and 2,3,4-tri-*O*-methylarabinose (0.9 mol). These results give some insight into the structural details of the galactan framework of PS-AI.

Because the main chain of the polysaccharide molecule is (1→3)- and (1→6)-linked, it is obvious that the galactose-containing disaccharide **2** and trisaccharide **3** (**3a** or **3b**) originated from this part of the macromolecule. The generation of **2** is also possible from the side chain. The other galactose-containing disaccharide (**1**) and the trisaccharide **4** obviously originated from the side chain.

Isolation of an aldobiouronic acid, 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose and two aldotriouronic acids, 6-*O*-(β-D-glucopyranosyluronic acid)-4-*O*-β-D-galactopyranosyl-D-galactose and 6-*O*-(β-D-glucopyranosyluronic acid)-6-*O*-β-D-galactopyranosyl-D-galactose, from the acidic portion of the graded-hydrolysis product fully supported the presence of glucuronic acid at the nonreducing end, thus confirming the results of methylation studies[1] of carboxyl-reduced PS-AI.

However, it must be pointed out that the presence of an arabinose-containing disaccharide could not be determined from the graded-hydrolysis study. Such a grouping may be present in some side chains of polysaccharides from gums[12–14] and other plant sources[11]. Under the hydrolytic conditions used for graded hydrolysis of mahua polysaccharide PS-AI, arabinose-containing disaccharide could not be obtained. However, methylation studies[1] of carboxyl-reduced PS-AI gave 2,3,5-tri-*O*-methylarabinose (4.0 mol) and 2,4-di-*O*-methylgalactose (1.0 mol). From such a study it may be concluded that arabinose units present as the nonreducing end are in the furanose form, and that those present in the inner chain are in the pyranose form. The other three nonreducing arabinose units are glycosidically joined to O-3 of galactose residues in the aldobiouronic acid. Furthermore, this inner arabinose unit, in turn, is glycosidically joined to a rhamnopyranose unit, as evidenced by the presence of 3,4-di-*O*-methylrhamnose obtained by methylation analysis[1] of PS-AI. However, from such study it is not possible to know the exact sequence of arabinose and rhamnose units inside the side chain. An alternative sequence of these sugars cannot be precluded.

If only (1→6)-linked galactosyl units are present in the polysaccharide, a second periodate oxidation will completely cleave the molecule; if (1→3) linkages are present exclusively in the main chain, the nonreducing-end sugars will be attacked by the oxidant the second time, leaving a stable molecule containing D-galactosyl residues. If both types of linkage are present in the main chain, the reaction mixture so obtained will have galactose-containing oligosaccharides whose length depends on the frequency of the two types of such linkage in the macromolecule. Smith-degraded PS-AI gave rise to three galactose-containing oligosaccharides, of which OL-2 and OL-3 were generated from the main chain of the molecule. The generation of OL-1 could not be explained properly; it may arise from the side chain, in which is a periodate-resistant galactose unit linked glycosidically to O-4 of a lower polyalcohol, as revealed from its R_{Lac} value (1.73).

The galactose residues (1→6)-linked in the main chain, and (1→4)-linked in

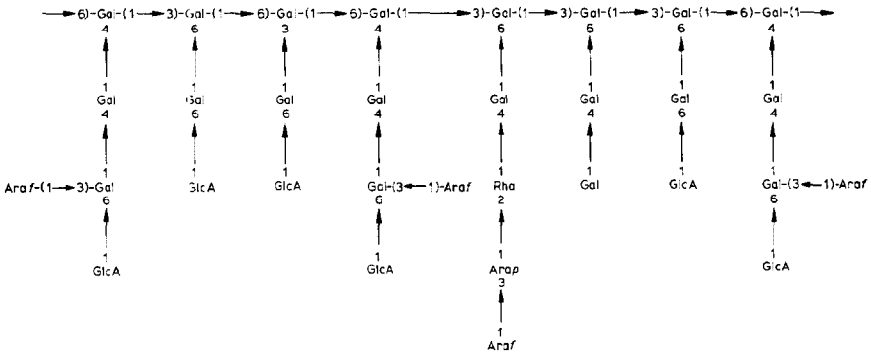

Fig. 1. Structure of the polysaccharide PS-AI of Mahua flowers.

the side chain, of PS-AI may be arranged alternatively, but, in that case, the main chain would be built with (1→3)-, (1→4)-, and (1→6)-linked galactose units, which is rather unlikely. In arranging the sequence of linkages of galactose units in the oligosaccharide **4**, alternative structures could be proposed in which a terminal, nonreducing galactosyl group is linked 1→6 to a galactose residue which, in turn, is linked to O-4 of a galactosyl unit in the main chain, and this is improbable, as otherwise, 2,6-di-*O*-methylgalactose would have been obtained in methylation studies[1].

From consideration of the results of analysis of the sugars of the polysaccharide PS-AI, and the carboxyl-reduced PS-AI, and their methylation analysis, Smith degradation, and graded hydrolysis, one of the plausible structures that can be assigned to the average repeating unit of mahua polysaccharide PS-AI is given in Fig. 1. From the results of chromium trioxide oxidation[1], it appears that galactose, glucose (formed from glucuronic acid), arabinose, and rhamnose were oxidized rapidly thus leading to the disappearance of all of the sugar residues having β-glycosidic linkages. However, from the rate at which arabinose residues were removed by graded hydrolysis of PS-AI, it may be concluded that it had the α configuration, and, as acetylated furanoses are nonspecifically oxidized by chromium trioxide[15], the anomeric configuration of the arabinose residues cannot be specified from that work. This structure merely represents the general nature of the linkages of the different monosaccharide units, but it well explains the different fragmentation products obtained, although it does not indicate the true sequence of branches present in the mahua polysaccharide PS-AI. One point that still remains unsolved is the occurrence of nonreducing xylose (giving 2,3,4-tri-*O*-methylxylose). This could not be explained from the structure of PS-AI. It may be assumed that this xylose unit is linked to some branch point in the polysaccharide molecule as a nonreducing end after a considerable length of repeating unit.

ACKNOWLEDGMENT

The authors express their grateful thanks to Prof. C. V. N. Rao, Head of the Department of Macromolecules, for helpful discussions during this work.

REFERENCES

1 N. SARKAR AND B. P. CHATTERJEE, *Carbohydr. Res.*, 112 (1983) 113–121.
2 H. G. WALKER, JR., M. GEE, AND R. M. MCREADY, *J. Org. Chem.*, 27 (1962) 2100–2102.
3 B. LINDBERG, J. LONNGREN, AND W. NIMMICH, *Carbohydr. Res.*, 23 (1972) 47–55.
4 M. ABDEL-AKHER, J. K. HAMILTON, R. MONTGOMERY, AND F. SMITH, *J. Am. Chem. Soc.*, 74 (1952) 4970–4971.
5 H. MASAMUNE AND S. KAMIYAMA, *Tohoku J. Exp. Med.*, 66 (1957) 43–49.
6 K. FREUDENBERG, A. WOLF, E. KNOPF, AND S. H. ZAHEER, *Ber.*, 61 (1929) 1743–1750.
7 G. O. ASPINALL, J. A. MOLLOY, AND C. C. WHITEHEAD, *Carbohydr. Res.*, 12 (1970) 143–146.
8 Z. DISCHE, L. B. SHETTLES, AND M. OSNOS, *Arch. Biochem.*, 22 (1949) 169–184.
9 Z. DISCHE, *J. Biol. Chem.*, 167 (1947) 189–198.
10 J. ROSÍK, V. ZITKO, Š. BAUER, AND J. KUBALA, *Collect. Czech. Chem. Commun.*, 31 (1966) 1072–1078.
11 S. HAQ AND G. A. ADAMS, *Can. J. Chem.*, 39 (1961) 1563–1573.
12 S. BASU AND C. V. N. RAO, *Indian. J. Chem.*, 21B (1982) 537–541.
13 D. M. W. ANDERSON AND A. HENDRIE, *Carbohydr. Res.*, 22 (1972) 265–279.
14 D. M. W. ANDERSON AND A. HENDRIE, *Carbohydr. Res.*, 26 (1973) 105–115.
15 S. J. ANGYAL AND K. JAMES, *Aust. J. Chem.*, 23 (1970) 1209–1221.