Carbohydrate Research, 39 (1975) 115–123

WATER- AND ALKALI-SOLUBLE GLUCANS FROM OAK LICHEN*

MIRJANA HRANISAVLJEVIĆ-JAKOVLJEVIĆ, JELENA MILJKOVIĆ-STOJANOVIĆ, RADMILA DIMITRIJEVIĆ, AND VUKIC MIĆOVIĆ

Department of Chemistry, Faculty of Science, University of Belgrade, and Institute of Chemistry, Technology and Metallurgy, Belgrade (Yugoslavia)

(Received April 12th, 1974; accepted for publication, June 24th, 1974)

ABSTRACT

A water-soluble glucan, $[\alpha]_D^{20}$ +217° (water), and an alkali-soluble glucan, $[\alpha]_D^{20}$ +152° (sodium hydroxide), have been isolated from the oak lichen *Evernia prunastri* (L.) Ach. On the basis of methylation analysis, periodate oxidation, and partial acid hydrolysis, the water-soluble polysaccharide has been shown to be a neutral, slightly branched glucan with a main chain composed of (1→3)- and (1→4)-linked glucopyranose residues in the ratio ~1:1. Branching occurs most probably at position 2 of (1→4)-linked glucopyranose residues. On the basis of optical rotation and i.r. spectral data, and enzymic hydrolysis, the α-D configuration has been assigned to the glycosidic linkages. Likewise, the alkali-soluble polysaccharide was shown to be a neutral, branched glucan with a main chain composed of (1→3)- and (1→4)-linked α-D-glucopyranose residues in the ratio 6:1. Each of the (1→4)-linked units was a branch point involving position 6. The presence of some β-D linkages is not excluded since hydrolysis with β-D-glucosidase occurred to a small extent.

INTRODUCTION

The most thoroughly investigated glucans from the oak lichens are those from *Cetraria islandica*[1]. The major structural feature is a chain of (1→3)- and (1→4)-linked D-glucopyranose residues mostly of the β-type, although isolichenan[1] is an α-D-glucan. The oak lichen *Evernia prunastri* (L.) Ach. has been little investigated. Except for studies on evernan (the hot water-soluble glucan)[2] and the alkali-soluble galactomannan[3], the study of any other polysaccharide has not been reported. We now report on a water-soluble glucan and an alkali-soluble glucan.

RESULTS AND DISCUSSION

The finely powdered, oak lichen *E. prunastri* was treated with ether and methanol, prior to extraction with hot water. Hot water-soluble glucan (evernan) and

*Structural Studies of Polysaccharides from the Oak Lichen *Evernia prunastri* (L.) Ach.: Part II.

heteroglycans were removed by freezing and thawing followed by precipitation with barium hydroxide[4] and Fehling's solution[5]. Soluble copper complexes were fractionally precipitated with ethanol. After several fractionations, the glucan (3.2% on the basis of dry lichen) had $[\alpha]_D^{20}$ +217° (water), and on acidic hydrolysis gave glucose as the sole product characterised as *N*-*p*-nitrophenyl-D-glucopyranosylamine[6]. The molecular weight of the glucan obtained by viscometry[7] and hypoiodite oxidation[8] is ~8,000. The high, positive optical rotation indicated that the glycosidic linkages were mainly α-D and this was confirmed by the i.r. absorption at 845 cm^{-1}, which is characteristic[9] of α-D linkages, and hydrolysis by α-amylase (apparent conversion into glucose 45% after 24 h).

Hydrolysis of the fully methylated glucan, $[\alpha]_D^{20}$ +155° (chloroform), gave four components which were separated by column and paper chromatography and identified (physical constants, crystalline derivatives) as 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, 2,4,6-tri-*O*-methyl-D-glucose, and 3,6-di-*O*-methyl-D-glucose in the molar proportions 1.8:22:20:1.

On the basis of methylation analysis, it is concluded that the polysaccharide has a linear chain of (1→3)- and (1→4)-linked α-D-glucopyranose residues with branching occurring (one per 42 units) most probably at position 2 of (1→4)-linked residues. The approximate ratios of the structural units are as follows:

$$[\text{Glc}p\text{-}(1\rightarrow]_2 \quad [\rightarrow 4)\text{-Glc}p\text{-}(1\rightarrow]_{22} \quad [\rightarrow 3)\text{-Glc}p\text{-}(1\rightarrow]_{20} \quad [\rightarrow 4)\text{-Glc}p\text{-}(1\rightarrow]_1$$
$$\begin{array}{c} | \\ \widehat{2} \\ \uparrow \end{array}$$

The reduction of 0.53 mol. of periodate by the glucan with the production of 0.07 mol. of formic acid per anhydrohexose unit accorded with the structural units shown above. Hydrolysis of the reduced oxopolysaccharide[10] afforded glucose, erythritol, and glycerol in agreement with the results of periodate oxidation and methylation analysis.

The mixture of mono- and oligo-saccharides obtained by partial acid hydrolysis of the glucan was resolved by preparative paper chromatography. The main fractions obtained were maltose and nigerose (identified as the octa-acetates[11,12]), thereby confirming the presence of α-(1→3)- and α-(1→4)-linkages in the glucan. The trisaccharide fraction contained only one component which was identified as *O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→3)-D-glucose on the basis of partial acid hydrolysis and methylation data.

A second glucan having $[\alpha]_D^{20}$ +152° (M sodium hydroxide) was extracted with 5% sodium hydroxide from the oak lichen previously freed from fatty materials, lichen acids, and water-soluble carbohydrates. Contaminating polysaccharides were removed as insoluble barium and copper complexes[4,5]. Further purification was effected by fractional precipitation with ethanol. The fraction precipitated in 40% ethanol (yield 3.5–4%) had, after several reprecipitations, a constant optical rotation, $[\alpha]_D^{20}$ +152° (M sodium hydroxide), and on acid hydrolysis gave only glucose which

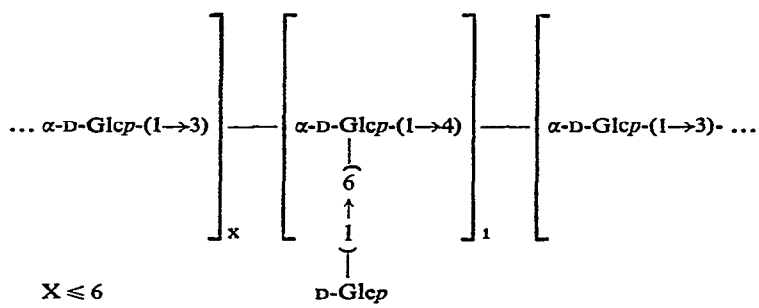
was characterised as *N-p*-nitrophenyl-D-glucopyranosylamine[6]. The specific rotation of the glucan changed on acid hydrolysis from +152 ±1° to +63 ±2°, indicating the presence of α-D linkages. Incubation of the polysaccharide with α-amylase in phosphate buffer resulted in 50% conversion of the polysaccharide into glucose after 24 h. During the same period, only 10% of the glucan was hydrolysed by β-D-glucosidase. The presence of an i.r. band at 870 cm^{-1} indicated[9] the presence of α-D linkages in the glucan, but the bands characteristic for β-D linkages were not observed. The molecular weight as estimated by viscometry was ~12,000, and hypoiodite oxidation indicated the number-average degree of polymerization to be 64.

The methylated polysaccharide, $[\alpha]_D^{20}$ +119° (chloroform), on acid hydrolysis afforded 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,4,6-tri-*O*-methyl-D-glucose, and 2,3-di-*O*-methyl-D-glucose in the molar ratios 2:6:1. The methylated derivatives were separated by cellulose column chromatography[13] and by preparative layer chromatography, and identified (physical constants, crystalline derivatives).

The alkali-soluble glucan reduced 0.36 mol. of periodate and liberated 0.17 mol. of formic acid per anhydrohexose unit, which is in reasonable agreement with methylation data. Smith degradation[10] of the polysaccharide gave glucose, erythritol, and glycerol in agreement with the foregoing data.

Partial acid hydrolysis of the glucan gave four products which were isolated by chromatography[14] on charcoal–Celite and by preparative paper chromatography as appropriate. The disaccharides maltose and nigerose were identified as the octa-acetates[12,13]. One trisaccharide was not characterised because of the small amount available, but the second had $[\alpha]_D^{20}$ +170° (water) and was identified as *O*-α-D-glucopyranosyl-(1→3)-*O*-α-D-glucopyranosyl-(1→4)-D-glucose[15].

On the basis of the foregoing data, the most probable structure for the alkali-soluble glucan is as follows:



Thus, the glucans from the oak lichen *Evernia prunastri* (L.) Ach. are slightly branched and have a backbone chain made up of (1→3)- and (1→4)-linked units. The glucans isolated from the related oak lichen *Cetraria islandica*[1] have a similar backbone. Besides differences in branching between these related types of polysaccharides, there are differences in the proportions of α and β linkages. Glucans from *Evernia prunastri* have mainly or exclusively α-D linkages whereas, for those from *Cetraria islandica*, with the exception of isolichenan, β-D linkages preponderate.

EXPERIMENTAL

Paper chromatography was performed by the descending method on Whatman No. 1 paper, using *A* ethyl acetate–pyridine–water (2.5:1:2.5), *B* 1-butanol–pyridine–water (3:1:1.5), *C* 1-butanol–ethanol–water (3:1:1), *D* 1-butanol–ethanol–water (4:1:5), and *E* butanone saturated with water. For preparative purposes, Whatman 3MM paper was used. Thin-layer chromatograms were run on silica gel H (Merck), using *F* 1-butanol–acetic acid–water–ether (9:6:1:3), *G* benzene–acetone (1:1), *H* 1-butanol–acetic acid–ethyl acetate–water (9:6:3:1), and *I* ethyl acetate–pyridine–water (10:9:3). Sugars were detected on paper with silver nitrate–sodium hydroxide[16], aniline hydrogen phthalate[17], *p*-anisidine hydrochloride[18], or triphenyltetrazolium chloride[19]. Visualisation of sugars after t.l.c. was achieved with aniline hydrogen phthalate, *p*-anisidine hydrochloride, or 50% sulphuric acid. Melting points are uncorrected. Optical rotations were recorded with a Perkin–Elmer 141 polarimeter and i.r. spectra with a Perkin–Elmer Model 421 spectrophotometer.

Isolation and fractionation of the water-soluble polysaccharides. — Finely powdered oak lichen (500 g) was extracted with ether and methanol (Soxhlet) to remove fatty materials and lichen acids. The dried residue was exhaustively extracted (anthrone test) with distilled water at 50°. The combined extracts were concentrated to 10 l, saturated, aqueous barium hydroxide was added until precipitation was complete, and the precipitate was removed by centrifugation. The remaining solution was acidified with acetic acid (to pH 5), dialyzed against running tap-water and distilled water, and concentrated to 5 l (at 35° *in vacuo*). The concentrate was then poured into ethanol (2.5 l) with vigorous stirring, and the precipitated polysaccharide was collected by centrifugation, washed with ethanol, and dried *in vacuo* (yield, 3.2% on the basis of dry lichen).

To an aqueous solution of the crude polysaccharide preparation (8 g), Fehling's solution was added dropwise with continuous stirring. The precipitated complex was removed by centrifugation and shown by paper chromatography (solvent *A*) of an acid hydrolysate to be composed of glucose, mannose, and galactose. The water-soluble copper complexes were then fractionated by the dropwise addition of ethanol. The fraction which precipitated in 20% ethanol was treated in the usual way with 5% methanolic hydrogen chloride to decompose the complex, and the polysaccharide was dried by solvent exchange. Purification of the polysaccharide was effected by repetition of the procedure six times. The resulting polysaccharide (3.5 g) was a creamy powder (Found: ash, 0.31%; N, 0%) having $[\alpha]_D^{20}$ +217° (*c* 0.5, water), which reduced Fehling's solution. On acid hydrolysis, glucose was the only product (paper chromatography, solvent *A*), identified as *N*-*p*-nitrophenyl-D-glucopyranosylamine[6], m.p. 182–183°.

The number-average degree of polymerization of the glucan obtained by hypoiodite oxidation was found to be 44 ± 1, and the molecular weight as estimated by viscometry was ~8,000.

Methylation analysis of the water-soluble glucan. — The glucan (3 g) was

methylated twice by the Haworth procedure[20] and six times with the Purdie reagent[21] to give fully methylated product (no i.r. absorption for hydroxyl), $[\alpha]_D^{20}$ +155° (*c* 0.7, chloroform).

A solution of the methylated glucan (0.6 g) in ice-cold 72% sulphuric acid (10 ml) was stored at room temperature for 4 h, and then diluted (to 70 ml) and heated for 4 h on a boiling-water bath. The hydrolysate was neutralised with barium carbonate, filtered, and concentrated to dryness at 35° *in vacuo*. The residue was successively extracted with methanol and chloroform, the combined extracts were concentrated, and the residue was examined by paper chromatography (solvents *D* and *E*) and t.l.c. (solvents *E* and *G*). Four components were revealed which were isolated by preparative paper chromatography (solvent *E*).

Fraction 1 was a chromatographically homogeneous syrup (11.2 mg, 3.6%) with a mobility corresponding to that of 2,3,4,6-tetra-*O*-methyl-D-glucose and which crystallized on storage. After recrystallization from hexane, it had m.p. and mixture m.p. 92–93°, $[\alpha]_D^{20}$ +82° (*c* 0.35, water)[22].

Fraction 2 (0.1404 g, 50%) contained mainly a product with R_G 0.83 and R_F 0.56 (solvents *D* and *E*, respectively), and which was indistinguishable from 2,3,6-tri-*O*-methyl-D-glucose. The contaminant appeared (R_G and R_F) to be 2,4,6-tri-*O*-methyl-D-glucose. When recrystallized from ether, the product had m.p. 122°, $[\alpha]_D^{20}$ +70° (*c* 0.92, water).

Fraction 3 (0.1354 g, 45%) had the same R_G and R_F values (0.76 and 0.48 in solvents *D* and *E*, respectively) as authentic 2,4,6-tri-*O*-methyl-D-glucose. The compound, when crystallized from ether, had m.p. 120–122°, $[\alpha]_D^{20}$ +70° (*c* 0.38, water), and the anilide had m.p. 160–161°, $[\alpha]_D^{20}$ −111° (*c* 0.22, methanol), in good agreement with literature data[24,25].

Fraction 4 (8.3 mg, 2.6%) had the same mobility in paper chromatography (solvents *D* and *E*) and t.l.c. (solvent *E*) as reported for 3,6-di-*O*-methyl-D-glucose. A positive test with the triphenyltetrazolium reagent indicated HO-2 to be unsubstituted. The compound had $[\alpha]_D^{20}$ +101°→+60° (*c* 0.14, water), in accordance with the reported data[26]. The small amount of material precluded derivatisation.

Periodate oxidation. — The glucan (0.113 g) was dissolved in water (50 ml), and 0.1M sodium periodate (50 ml) was added. Oxidation was allowed to proceed at 20° in the dark. At intervals, the periodate uptake was determined by the arsenite procedure, and formic acid production by iodometric titration[27]. The results are recorded in Table I.

After oxidation was complete, barium acetate was added and the insoluble salts were removed. The solution was deionized with Amberlite IR-45(HO^-) and IR-120(H^+) resins, concentrated to 5 ml, and treated with sodium borohydride (100 mg). An additional amount (50 mg) of reductant was added after 12 h. After a further 6 h, the excess of borohydride was destroyed with Amberlite IR-120(H^+) resin, the solution was concentrated, and borate was removed by distillation of methanol from the residue. The polyalcohol was hydrolysed with M sulphuric acid (10 h, 100°) and,

TABLE I

PERIODATE OXIDATION OF THE WATER-SOLUBLE GLUCAN

Time (h)	20	44	68	92	120
Periodate[a]	0.28	0.36	0.41	0.43	0.44
Formic acid[a]	0.02	0.04	0.06	0.08	0.08

[a]In mole per mole of "anhydrohexose".

after neutralization with barium carbonate, the hydrolysate was concentrated and the residue was examined by paper chromatography (solvent *C*) and t.l.c. (solvent *H*). By comparison with authentic samples, the main components appeared to be glucose (R_F 0.16 and 0.43), erythritol (R_F 0.31 and 0.45), and glycerol (R_F 0.39 and 0.43).

Partial acid hydrolysis. — The polysaccharide (1 g) was treated with 0.165M sulphuric acid (50 ml) for 2 h on a boiling-water bath. The clear solution was neutralised with barium carbonate (to pH 6), deionized with Amberlite IR-120(H^+) resin, and concentrated. The residue (0.5 g/10 ml) was fractionated on a column (15 × 3.5 cm) of charcoal–Celite by gradual elution with 1, 5, 10, 15, and 20% ethanol[14]. 1% Ethanol eluted glucose (0.163 g) as proved by paper chromatography and t.l.c. The 5% alcoholic eluate was judged by t.l.c. (solvent *H*) and paper chromatography (solvent *C*) to contain two components (R_F 0.32 and 0.20, R_G 0.65 and 0.38). These components were separated by preparative paper chromatography and, after acetylation, were characterized as maltose octa-acetate, m.p. 160°, $[\alpha]_D^{20}$ +63° (*c* 0.26, chloroform), and nigerose octa-acetate, m.p. 151°, $[\alpha]_D^{20}$ +84° (*c* 0.11, chloroform), which is in accordance with literature data[11,12].

The component eluted with 10% ethanol had $[\alpha]_D^{20}$ +100° ±1° (*c* 0.06, water), was chromatographically homogeneous (R_F 0.1 and R_G 0.57 in solvents *C* and *I*, respectively), and was shown by hypoiodite oxidation to be a trisaccharide. Graded, acid hydrolysis gave glucose and maltose as the main components. Methylation analysis gave 2,3,6- and 2,4,6-tri-*O*-methyl-D-glucoses in addition to 2,3,4,6-tetra-*O*-methyl-D-glucose. The most probable structure of the trisaccharide was therefore *O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→3)-D-glucose.

Isolation and fractionation of the alkali-soluble polysaccharides. — After hot water-extraction of the lichen, the residual material was extracted with 4% sodium hydroxide under nitrogen as described previously[3]. The crude polysaccharide preparation (10 g) was then treated with barium hydroxide to remove galactomannan. The solution was acidified, dialysed against running tap-water (3 days) and distilled water (1 day), and poured into the same volume of ethanol. The precipitated polysaccharide was collected by centrifugation and dried by solvent exchange. Further purification was effected by the formation of a copper complex with Fehling's solution. The solution was centrifuged to remove a small quantity of insoluble copper complex. Subsequent, gradual addition of ethanol resulted in precipitation of the main quantity of soluble copper complex at 20% ethanol. After destruction of the complex (5% methanolic hydrogen chloride), glucose was found (paper chromatography, solvents *A*

and *B*) as the main component in the acid hydrolysate. Glucose was characterized as *N*-*p*-nitrophenyl-D-glucopyranosylamine[6], m.p. 183°.

The pure glucan, $[\alpha]_D^{20}$ +152° (*c* 1.2, 0.1M NaOH), obtained after several repeated fractionations with Fehling's solution, reduced Fehling's solution. On acid hydrolysis, the optical rotation changed from +152° ±1° to +63° ±1°, indicating α-D-glycosidic linkages. Incubation of the polysaccharide with α-amylase in phosphate buffer (pH 4.95) at 37° showed that, after 5 h, 20% of polysaccharide was hydrolyzed. β-D-Glucosidase caused only 10% conversion into glucose during the same incubation period. The i.r. spectrum contained a band at 870 cm^{-1} indicative[9] of α-D linkages. The molecular weight determined by viscometry was 12,400, but a smaller value (d.p. ~58) was obtained by hypoiodite oxidation.

Methylation analysis. — Methylation was performed by the Hakomori procedure[28], and hydrolysis was performed as described for the water-soluble glucan. Separation of *O*-methylglucoses was effected[13] on a column of cellulose powder (Machery and Nagel), using solvent *E*. The purity of fractions was monitored by t.l.c. (solvents *E* and *G*) and by paper chromatography (solvents *D* and *E*). In a separate experiment, a smaller quantity was resolved by preparative-layer chromatography.

A hydrolysate of fully methylated glucan (220 mg), $[\alpha]_D^{20}$ +119° (*c* 0.87, chloroform), was applied as a 10% solution in chloroform to a column (3.5 × 50 cm) of cellulose.

Fraction 1 (4.7 mg, 21.4%) had R_F 0.78 (solvent *E*), $[\alpha]_D^{20}$ +85° (*c* 1, water)[22], and was identical with 2,3,4,6-tetra-*O*-methyl-D-glucose isolated from the water-soluble glucan. The derived 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-glucosylamine[29] had m.p. 136–138°.

Fraction 2 (145 mg, 65.9%) was chromatographically homogeneous (R_G 0.76 and R_F 0.48 in solvents *D* and *E*, respectively) and had m.p. 120°, $[\alpha]_D^{20}$ +110° → +70° (*c* 1.5, methanol), corresponding to 2,4,6-tri-*O*-methyl-D-glucose[24].

Fraction 3 (24 mg, 11%) was identified as a di-*O*-methylglucose on the basis of chromatographic mobilities (R_F 0.28, solvent *E*, R_G 0.57, solvent *D*). A negative reaction with the triphenyltetrazolium reagent proved HO-2 to be substituted. It had m.p. 84°, $[\alpha]_D^{20}$ +51° (*c* 0.15, acetone), in fairly good agreement with the data for 2,3-di-*O*-methyl-D-glucopyranose[30]. The derived methyl glycoside had $[\alpha]_D^{20}$ +138 ±1° (*c* 0.08, water), a somewhat smaller value than reported and which could indicate traces of impurities.

Periodate oxidation. — The glucan (531.8 mg) was oxidized with 0.1M sodium periodate as described above. The data are given in Table II.

When extrapolated to zero time, the data indicated a net value of 0.65 mol. of periodate reduced and 0.17 mol. of formic acid released per anhydrohexose unit.

Acid hydrolysis of the reduced oxopolysaccharide gave three components (paper chromatography, R_F 0.16, 0.31, and 0.39, solvent *C*; t.l.c., R_F 0.43, 0.50, and 0.58, solvent *F*), which were identified as glucose, erythritol, and glycerol by comparison with authentic compounds.

Partial acid hydrolysis. — Hydrolysis was performed as described above.

TABLE II

PERIODATE OXIDATION OF ALKALI-SOLUBLE GLUCAN

Time (h)	24	44	64	90	120
Periodate[a]	0.60	0.64	0.65	0.65	0.67
Formic acid[a]	0.14	0.16	0.17	0.17	0.17

[a]In mole per mole of "anhydrohexose".

Fractionation of the hydrolysate (0.5 g) was effected on a column (3.5 × 45 cm) of charcoal–Celite, using an aqueous ethanol gradient[14].

Fraction 1 (257 mg), eluted with 1% alcohol, contained only glucose[6].

Fraction 2 (351 mg), eluted with 5 and 10% ethanol, contained two components. Preparative paper chromatography (solvent *D*) gave *Fraction 2a* (107 mg), R_F 0.32 (solvent *D*), which, on acetylation, gave maltose octa-acetate[11], m.p. 160°, $[\alpha]_D^{20}$ +64° (*c* 1, chloroform), and *Fraction 2b* (12 mg) which, on acetylation, gave nigerose octa-acetate[12], m.p. 150°, $[\alpha]_D^{20}$ +83° ±1° (*c* 0.42, chloroform).

Fraction 3 (12 mg), eluted with 20% ethanol, was chromatographically homogeneous and had $[\alpha]_D$ +170° ±1° (*c* 0.5, water). On the basis of hypoiodite oxidation, controlled acid hydrolysis, and methylation analysis, the oligosaccharide was identified as *O*-α-D-glucopyranosyl-(1→3)-*O*-α-D-glucopyranosyl-(1→4)-D-glucose[15].

ACKNOWLEDGMENT

The financial support of the Serbian Research Fund and Serbian Academy of Sciences and Arts is thankfully acknowledged.

REFERENCES

1 S. PEAT, W. J. WHELAN, AND J. G. ROBERTS, *J. Chem. Soc.*, (1957) 3916, and references cited therein; S. PEAT, W. J. WHELAN, J. R. TURVEY, AND K. MORGAN, *J. Chem. Soc.*, (1961) 623; W. L. CUNNINGHAM AND D. J. MANNERS, *Biochem. J.*, 90 (1964) 696; A. S. PERLIN AND S. SUZUKI, *Can. J. Chem.*, 40 (1962) 50.
2 F. STÜDE, *Ann.*, 131 (1864) 241; K. MÜLLER, *Z. Physiol. Chem.*, 45 (1905) 265; A. ULLANDER AND B. TOLLENS, *Ber.*, 39 (1906) 401; B. DRAKE, *Biochem. Z.*, 313 (1943) 388; V. STEFANOVIĆ, Ph.D Thesis, Faculty of Sciences, University of Belgrade, Beograd, 1960.
3 V. M. MIĆOVIĆ, M. HRANISAVLJEVIĆ-JAKOVLJEVIĆ, AND J. MILJKOVIĆ-STOJANOVIĆ, *Carbohyd. Res.*, 10 (1969) 525.
4 H. MEIER, *Acta Chem. Scand.*, 12 (1958) 144.
5 E. SALKOWSKI, *Ber.*, 27 (1894) 497.
6 E. WEYGAND, W. PERKOW, AND P. KUHNER, *Chem. Ber.*, 84 (1951) 594.
7 H. STAUDINGER AND V. SCHWEITZER, *Ber.*, 63 (1930) 2317.
8 R. WILLSTÄTTER AND G. SCHUDEL, *Ber.*, 51 (1918) 780.
9 S. A. BARKER, E. J. BOURNE, R. STEPHENS, AND D. H. WHIFFEN, *J. Chem. Soc.*, (1954) 3468.
10 M. ABDEL-AKHER, J. K. HAMILTON, R. MONTGOMERY, AND F. SMITH, *J. Amer. Chem. Soc.*, 74 (1952) 4970.
11 M. L. WOLFROM AND A. THOMPSON, *Methods Carbohyd. Chem.*, 1 (1962) 334.
12 S. HAQ AND W. J. WHELAN, *J. Chem. Soc.*, (1958) 1342.
13 L. HOUGH, J. K. N. JONES, AND W. H. WADMAN, *J. Chem. Soc.*, (1949) 2511.

14 R. L. WHISTLER AND D. F. DURSO, *J. Amer. Chem. Soc.*, 72 (1950) 677.
15 S. A. BARKER, E. J. BOURNE, D. M. O'MANT, AND M. STACEY, *J. Chem. Soc.*, (1957) 2448.
16 W. E. TREVELYAN, D. P. PROCTER, AND J. G. HARRISON, *Nature (London)*, 166 (1950) 444.
17 S. M. PARTRIDGE, *Nature (London)*, 164 (1949) 443.
18 L. HOUGH, J. K. N. JONES, AND W. H. WADMAN, *J. Chem. Soc.*, (1950) 1702.
19 K. WALLENFELS, *Naturwissenschaften*, 37 (1950) 491.
20 W. N. HAWORTH, *J. Chem. Soc.*, 107 (1915) 8.
21 T. PURDIE AND J. C. IRVINE, *J. Chem. Soc.*, 83 (1903) 1021.
22 J. C. IRVINE AND J. W. H. OLDHAM, *J. Chem. Soc.*, 119 (1921) 1744.
23 J. C. IRVINE AND E. L. HIRST, *J. Chem. Soc.*, 121 (1922) 1213.
24 W. N. HAWORTH AND W. G. SEDGWICK, *J. Chem. Soc.*, (1926) 2573.
25 H. GRANICHSTÄDTEN AND E. G. V. PERCIVAL, *J. Chem. Soc.*, (1943) 54.
26 D. J. BELL, *J. Chem. Soc.*, (1935) 175.
27 P. FLEURY AND J. LANGE, *J. Pharm.*, 17 (1933) 107.
28 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205.
29 J. C. IRVINE AND A. M. MOODIE, *J. Chem. Soc.*, 93 (1908) 95.
30 J. C. IRVINE AND J. P. SCOTT, *J. Chem. Soc.*, 103 (1913) 575.