International Journal of Chemical Science
Online ISSN: 2523-2843, Print ISSN: 2523-6075; Impact Factor: RJIF 5.22
Received: 05-12-2020; Accepted: 22-12-2020; Published: 11-01-2021
www.chemicaljournals.com
Volume 5; Issue 1; 2021; Page No. 01-05

Structural elucidation of oligosaccharides by partial acid hydrolysis studies from seeds polysaccharide of *Cassia glauca* Lam. plant

RB Singh
Scientist 'C' UGC, Department of Zoology, School of Life Sciences, Dr. Bhimrao Ambedkar University, Khandari campus, Agra, Uttar Pradesh, India

Abstract
Partial acid hydrolysis of water soluble *Cassia glauca* Lam. seeds polysaccharide afforded four oligosaccharides as: [I] 4-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose, [II] 6-O-α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranose, [III] 6-O-α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose and [IV] 6-O-α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose. Oligosaccharides were characterized and identified by optical rotation, degree of polymerization, derivatives (disaccharides only), reduction with sodium borohydride, acid hydrolysis and periodate oxidation studies. These oligosaccharides results were corroborated the earlier proposed seeds polysaccharides structure of *Cassia glauca* Lam. plant.

Keywords: oligosaccharides, partial acid hydrolysis, *cassia glauca* seeds polysaccharide

Introduction

Cassia glauca Lam. Plant [1] belongs to the family-Caesalpiniaceae is a large shrub upto 10m in height. It is an evergreen shrub and occurs in Northern India, Malaysia, Peninsula, Australia, Pakistan, Sri Lanka, China, South America and Tropical Asia. Bark and leaves are medically used [2] for the treatment in diabetes, skin infection, asthma and other human diseases. Seed oils are used in indigenous system of medicine for skin and leucoderma diseases. Seeds contain a water soluble polysaccharide as D-galactose and D-mannose in the molar ratio of 1:4 confirmed by GLC, TLC, Column and Paper chromatographic analysis [3]. Present manuscript mainly deals with the isolation, characterization, identification and structural elucidation of oligosaccharide obtained after partial acid hydrolysis studies of seeds polysaccharide for the confirmation of earlier proposed seeds polysaccharide structure of *Cassia glauca* Lam. plant. In our earlier communications, the nature of the constituent sugars [4], methylation studies [5], periodate oxidation studies [6]. Smith degradation studies of periodate oxidised seeds polysaccharide for the confirmation of seeds polysaccharide structure have already been studied. It showed that the D-galactopyranose units mostly occupy the terminal position in the main chain which consist predominantly of D-mannopyranose units.

Partial acid hydrolysis of seeds polysaccharide followed by column chromatography over charcoal-celite column [7] and paper chromatography [3] of hydrolysate afforded four oligosaccharides. Each oligosaccharide was purified separately and characterized by optical rotation, formation of crystalline derivatives (disaccharides only), degree of polymerization (D.P.) by Timell's method [8], reduction with sodium borohydride, complete acid hydrolysis and periodate oxidation studies[9]. Oligosaccharides were characterized and identified as: [I] 4-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose, [II] 6-O-α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranose, [III] 6-O-α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose and [IV] 6-O-α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose. Recently the oligosacchraides were isolated from seeds polysaccharide of *Wrightia tinctoria* R.Br. (Roxb.) [10], *Withania somnifera* Dunal [11], *Cassia alata* Linn. [12] etc.

Materials and methods

Oligosaccharides were separated from the hydrolysed compound of *Cassia glauca* Lam. water soluble seeds polysaccharides was carried out by descending techniques on Whatman No. 3MM filter paper sheet by paper chromatography [3]. Upper phase of the following solvent mixture (v/v) were used for the detection of monosaccharides and oligosaccharides as: (A) *n*-butanol, acetic acid, water (4:1:5) [13], (B) ethyl acetate, acetic acid, water (9:2:2) [14] and (C) ethyl acetate, pyridine, water (10:4:3) [15]. The spray reagent (R) *p*-anisidine phosphate [16] was used for the detection of oligosaccharides and monosaccharides from the hydrolysed compound of polysaccharide. Unless otherwise stated that all evaporations were carried out under reduced pressure at 45-50^{0}C. Optical rotation values were recorded after equilibrium and melting points are uncorrected and *R*gal and *R*glu refer to the rate of movement of sugars relative to D-galactose and D-glucose. Oligosaccharides mixture were separated on charcoal-celite column chromatography (1:1, w/w) using 2.5,5.0,7.5 and 10.0% (v/v) aqueous ethanol as eluants which were further separated by paper chromatography on Whatman No. 3MM filter paper. Degree of Polymerization (DP) was determined by Timell's method [8] and Deionization was done with Amberlite ion-exchange resin [17] at IR-45 (OH^-) and IR-120 (H^+) ions.

Partial acid hydrolysis of oligosaccharides

After a long series of trial experiments the following method

was carried out for partial acid hydrolysis [18] to obtain maximum yield of oligosaccharides from seeds polysaccharide (20gm) was partially hydrolysed with sulphuric acid (1.5N, 900ml) then the reaction mixture was left for 24hrs at room temperature and content was heated over boiling water bath for 45min. The hydrolysate was cooled, filtered and neutralized with barium carbonate slurry. It was again filtered and filtrate concentrated to a small volume (40ml). Concentrated syrup was added to ethanol (350ml) with the help of mechanical stirrer when the degraded polysaccharide was precipitated out as white course powder after filtration and washing with ethanol and dried. Paper chromatographic analysis [3] of the hydrolysate in solvent mixture (A) after concentration of ethanolic extract showed the presence of D-galactose and D-mannose together with traces of lower oligosaccharides.

Degraded polysaccharides was again hydrolysed by keeping it in sulphuric acid (1.5N, 600ml) at R.T. for 72hrs. Mixture was heated on boiling water bath for 40min. followed by cooling in the same bath for 30 min. then concentrated to a thin syrup. The syrup was deionized with Amberlite ion-exchange resin IR-45 (OH^-) and IR-120 (H^+) ions then the effluent was concentrated to a thin syrup.

Separation of oligosaccharides

Oligosaccharides sugar mixture were separated by chromatographic adsorption method in charcoal-celite (1:1, w/w) glass column (60×2.5cm) employing the graded elution method [19]. Glass column was eluted with water (2ltr) under 6lbs/sq. inch pressure to remove the monosaccharides then subsequently with 2ltr each of 2.5, 5.0, 7.5 and 10.0% aqueous ethanol (v/v) as eluant. Each oligosaccharide fraction (100ml) was concentrated and examined by paper chromatographic analysis on Whatman No. 3MM filter paper sheet using solvent mixture (A) and used (R) as spray reagent. The corresponding oligosaccharide [20] sugar strips were cut out with the help of guide spots and eluated with water according the Dent's method [21], then filtered and filtrate concentrated to a syrup. It observed that each fraction was a single component but a mixture of four oligosaccharides. This led to the isolation of oligosaccharides [20] were identified as two disaccharides, one trisaccharide and one tetrasaccharide in authentic form which were characterized and identified as follows

Fraction-I:4-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose

Oligosaccharide syrup (290mg) was dissolved in water-ethanol mixture (1:1, v/v) and filtered then *n*-butanol (4ml) was added to the cold filtrate. The resulting solution was evaporated on a boiling water-bath to a slight turbidity. Upon cooling the oligosaccharide was crystallized out in the form of cubes, yield (160mg). It had m.p. 204-205^{0}C, Lit. m.p. 203-206^{0}C [22] and optical rotation $[\alpha]_D^{20}$ -7.4-2.4^{0}C (H_2O), Lit. $[\alpha]_D$ -7.7-2.5^{0}C [23]. It had *R*gal 0.59 in solvent (B) and *R*glu 0.52 in solvent (C). It moved one sugar spots by paper chromatography showed D-mannose only on Whatman No. 3MM filter paper sheet. The Degree of Polymerization (DP) was found to be 1.98 by Timell's method [8]. Upon acid hydrolysis of disaccharide with sulphuric acid (1N) afforded D-mannose only by phenol sulphuric acid method [24]. Disaccharide (20mg) was reduced with sodium borohydride solution (0.2N, 3ml) followed by sulphuric acid (1N, 3ml) showed the presence of D-mannose was indicating the reducing end of the disaccharide is to be D-mannose. Its phenyl hydrazine derivative [25] was prepared by usual manner, had m.p. 174-175^{0}C, Lit. m.p. 175-176^{0}C [26].

The periodate oxidation[9] of disaccharide (50mg) was dissolved in water (80ml) and oxidised with sodium metaperiodate solution (0.25M, 15ml) in refrigerator at 4-5^{0}C. It showed the consumption of 5.75 moles of periodate with simultaneous liberation of 3.05 moles of formic acid per mole of disaccharide after 45hrs and results are shown in Table-1.

Table 1: Periodate oxidation of oligosaccharide-1

S. No.	Sugar Component	Time (hrs)						
		10	20	25	30	35	40	45
1.	Periodate consumption of disaccharide (moles/mole)	4.10	4.45	4.90	5.50	5.75	5.75	5.75
2.	Formic acid liberation of disaccharide (moles/mole)	1.90	2.20	2.55	2.85	3.05	3.05	3.05

Fraction-II: 6-O-α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranose

Oligosaccharide syrup (200mg) was dissolved in aqueous methanol (50ml), filtered and filtrate concentrated to a syrup. It was decolourised by charcoal to obtain glassy solid, had *R*gal 0.53 in solvent (B) and *R*glu 0.44 in solvent (C), optical rotation $[\alpha]_D^{25}$ +120.7^{0}C (H_2O), Lit. $[\alpha]_D$ +120^{0}C [9, 23], having m.p. 156-158^{0}C. It moved as a single spot on paper chromatogram in solvent mixture (A). The D.P. was found to be 1.75, indicating that the oligosaccharide was a disaccharide. Complete acid hydrolysis of syrup with sulphuric acid (1N), filtered and filtrate was neutralized with barium carbonate then concentrated to a syrup was subjected to paper chromatographically in solvent (A) showed the presence of D-galactose and D-mannose in equilibrium value as determined by phenol sulphuric acid method [24]. Its phenyl hydrazone derivative of disaccharide (50mg) was prepared by usual manner as: 6-O-α-D-galactopyranosyl-D-mannopyranose octa-acetate, having m.p. 172-174^{0}C, Lit. m.p. 175-176^{0}C [26, 27].

Periodate oxidation studies [28] of disaccharide (60mg) was carried out with sodium metaperiodate (0.25M, 15ml) in dark at 4-8^{0}Cin refrigerator for 40hrs. It consumed 5.54 moles of periodate and liberated 3.83 moles of formic acid per mole of disaccharide after 40 hrs and results are given in Table-2.

Table-2: Periodate oxidation of oligosaccharide-II

S. No.	Sugar Component	Time (hrs)							
		05	10	15	20	25	30	35	40
1.	Periodate consumption of disaccharide (moles/mole)	3.96	4.22	4.52	4.86	5.18	5.54	5.54	5.54
2.	Formic acid liberation of disaccharide (moles/mole)	2.16	2.34	2.56	2.94	3.48	3.83	3.83	3.83

Fraction-III: 6-O-α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose

Oligosaccharide fraction (160mg), had *R*gal 0.36 in solvent (B) and *R*glu 0.16 in solvent (C), optical rotation $[\alpha]_D^{25}$ +31.5^{0}C (H_2O), Lit. $[\alpha]_D$ +31^{0}C (H_2O) [29]. DP was found to be 2.94 indicating that this oligosaccharide was a trisaccharide. Acid hydrolysis of trisaccharide with sulphuric acid (1N) and obtained hydrolysate by paper chromatography on Whatman No. 3MM filter paper sheet

showed the presence of D-galactose and D-mannose in 1:2 molar ratio as determined by phenol sulphuric acid method[24]. It showed the presence of D-galactose on paper chromatogram indicating that the D-mannose sugar units are at the reducing end in the main polymer chain. Phenylhydrazone derivative of trisaccharide (50mg) was prepared by usual manner having m.p. 172-174^{0}C, Lit. m.p. 175-176^{0}C [26, 30]. Periodate oxidation of trisaccharide (80mg) was oxidised with water (50ml) and sodium metaperiodate (0.25M, 15ml) in dark at 4-8^{0}C in refrigerator. It consumed 7.40 moles of periodate with simultaneous liberation of 4.50 moles of formic acid per mole of trisaccharide after 50hrs. It was found to liberate 1.95 moles of formaldehyde and its dimedone derivative of formaldehyde had m.p. 186-188^{O}C, Lit. m.p. 189-190^{O}C [31] and results are shown in Table-3

Table 3: Periodate oxidation of oligosaccharide-III.

S. No.	Sugar Component	Time (hrs)						
		10	20	30	35	40	45	50
1.	Periodate consumption of trisaccharide (moles/mole)	5.40	5.90	6.40	6.95	7.40	7.40	7.40
2.	Formic acid liberation of trisaccharide (moles/mole)	2.84	3.26	3.84	4.12	4.50	4.50	4.50

Fraction-IV: 6-O-α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose

Oligosaccharide syrup (220mg) was dissolved in aqueous methanol (60ml), filtered and filtrate concentrated to a syrup. It was decolourized by charcoal to obtain a glassy solid and found chromatographically pure. It had optical rotation $[a]_D^{30}$ + 25^{O}C (H_2O), Lit. $[a]_D$ +26^{O}C [29]. The degree of polymerization (D.P.) was found to be 3.80 indicating that the oligosaccharide was a tetrasaccharide. Acid hydrolysis of tetrasaccharide with sulphuric acid (1N) and obtained hydrolysate on Whatman No. 3MM filter paper by paper chromatography showed the presence of D-galactose and D-mannose in 1:3 molar ratio as determined by phenol sulphuric acid method [24]. Periodate oxidation of tetrasaccharide (60mg) was oxidized with water (80ml) and sodium metaperiodate (0.25M, 20ml) in dark at 4.5^{O}C in refrigerator. It consumed 7.58 moles of periodate with simultaneous liberation of 3.54 moles of formic acid per mole of tetrasaccharide after 50 hrs. It liberated 0.78 moles of formaldehyde and possess α- and β- linkages on the basis of fragmentation analysis. The α-linkages linked through D-galactopyranosyl-D-mannopyranose while β-type linkages are linked through D-mannopyranosyl-D- mannopyranose units and results are shown in Table-4.

Table 4: Periodate oxidation of oligosaccharide-IV.

S. No.	Sugar Component	Time (hrs)						
		10	20	30	35	40	45	50
1.	Periodate consumption of tetrasaccharide (moles/mole)	5.10	5.86	6.20	6.90	7.58	7.58	7.58
2.	Formic acid liberation of tetrasaccharide (moles/mole)	1.96	2.24	2.82	3.20	3.54	3.54	3.54

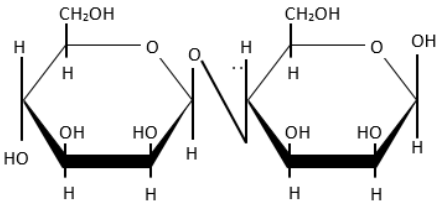
Results and discussion

Water soluble seeds polysaccharide was extracted from *Cassia glauca* Lam. plant to obtained D-galactose and D-mannose in 1:4 molar ratio by TLC, Column and Paper chromatographic analysis of hydrolysed compound by usual manner. Present manuscript mainly deals with the partial acid hydrolysis of purified seeds polysaccharide was carried out with sulphuric acid (1N) followed by charcoal-celite column chromatography and paper chromatography of hydrolysate on Whatman No. 3MM filter paper sheet which afforded two disaccharides, one trisaccharide and one tetrasaccharide. Each oligosaccharide was purified separately and characterized by their optical rotation, melting points, formation of crystalline derivatives (disaccharide only), Degree of Polymerization (D.P.), reduction with sodium borohydride, complete acid hydrolysis and periodate oxidation studies. Oligosaccharides (Figure-1) were identified as: (I) 4-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose, (II) 6-O-α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranose, (III) 6-O-α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose and (IV) 6-O-α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose.

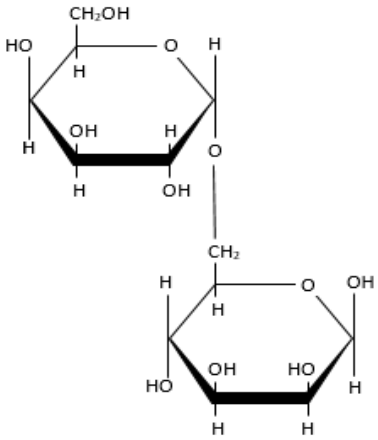
Oligosaccharide (I) clearly indicates that the main polymer chain of the polysaccharide is made up of D-mannopyranose units only which are linked through (1→4)-β-type linkages. Formation of oligosaccharide (II), (III) and (IV) supports the fact that the branches of main chain consists of single non-reducing D-galactopyranose units which are glycosidically attached through (1→6)-α-type linkages with D-galactopyranose and D-mannopyranose residues at the non-reducing terminal position of the backbone of the seeds polysaccharide structure. Characterization of trisaccharide (III) also confirms that the D-mannopyranose units at which the branches strarts is linked to other D-mannopyranose units of the main chain by (1→4)-β-type linkages and D-galactopyranose unit by (1→6)-α-type linkages. The oligosaccharide (IV) indicates that the D-galactopyranose units is glycosidically attached through (1→6)-α-type linkage with D-mannopyranose unit at a branch of a single repeating unit after several repeating unit of non-reducing residue of D-galactopyranose.

The D-mannopyranose units are attached through (1→4)-β-type linkages of the main polymer chain with other D-mannopyranose units.

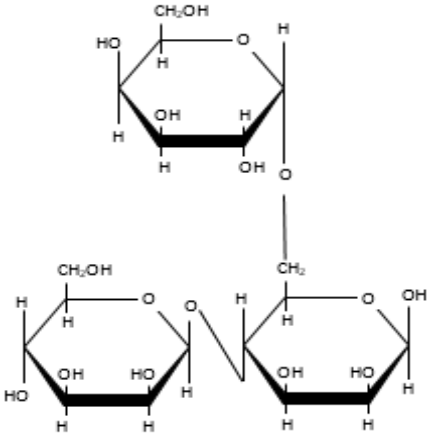
Its significance can only be adjusted in the earlier proposed seeds polysaccharide structure of *Cassia glauca* Lam. plant (Figure-2) is fully supported by the above oligosaccharides results.



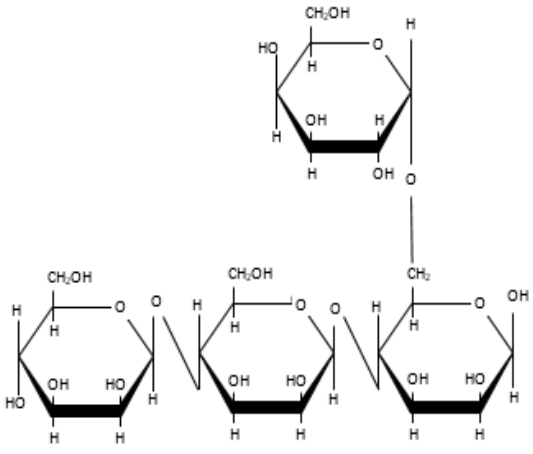
4-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose



6-O-α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranose



6-O-α-D-galactopyranosyl-(1→6)-O-α-D mannopyranosyl-(1→4)-O-β-D-mannopyranose



6-O-α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose

Fig 1: Oligosaccharide structure from *Cassia alata* Linn. Seeds polysaccharide

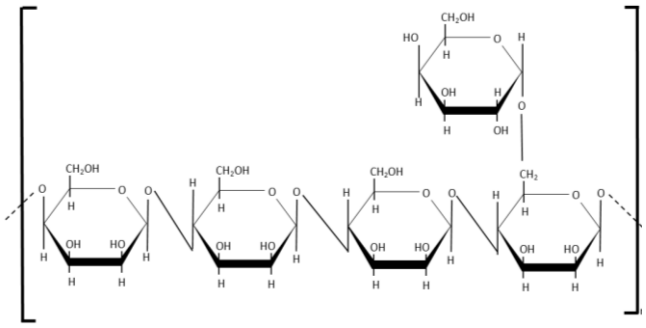


Fig 2: Seeds polysaccharide structure from *Cassia alata* Linn. Plant.

References

1. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants, CSIR Pub., New Delhi (India). 1956; 1:54-56.
2. Kirtikar KR, Basu BD. Indian Medicinal Plants, Periodical Export, New Delhi (India). 1990; 2:23-33.
3. Partridge SM, Nature (London), 1946; 158:270-272.
4. Singh RB. International Journal of Multidisciplinary Sciences. 2018; 1(1):01-03.
5. Singh RB, Jindal VK. Acta Ciencia Indica (Chemistry). 1989; 15(1):37-48.
6. Singh RB. International Journal of Academic Research and Development. 2019; 4(5):80-81.
7. Kowkobany GN. Advances in Carbohydrate Chemistry. 1950; 9:304-306.
8. Timell TE, Svensk V. Papp. Stid. 1960; 63:688-690.
9. Fluery P, Lange JJ. Pharma. Chem. 1933; 17:107-110.
10. Singh RB, Meenakshi. Eduved, International Journal of Interdisciplinary Research. 2016; 3(4):37-42.
11. Singh RB. Advances in Applied Science Research. Pelagia Research Library. 2014; 5(6):04-07.
12. Singh RB. American International Journal of Research in Formal, Applied and Natural Sciences. 2018 24(1):38-41.
13. Partridge SM, Westall RG, Biochem. J. 1948; 42:238-240.
14. Jermyn MA, Isherwood FA. Biochem. J., 1949; 44:402-404.
15. Meir H. Acta Chem. Scand. 1960; 14:749-752.
16. Mukherjee S, Srivastava HC. Nature (London). 1952; 169:330-333.
17. Adams BA, Halmes EL. J. Soc. Chem. Ind. 1935; 54:17-20.
18. Parikh VM, Jones JKN. Can. J. Chem. 1966; 44:1531-1534.
19. Whistler RL. Durso DF. J. Amer. Chem. Soc. 1951; 73:4189-4191.
20. Andrews P, Hough L, Jones JKN. J. Amer. Chem. Soc. 1952; 74:4029-4031.
21. Dent CE. Biochem. J. 1947; 41:240-242.
22. Aspinall GO, Rashbrook RB, Kessler G. J. Chem. Soc, 1958, 215-218.
23. Whistler RL, Durso DF. J. Amer. Chem. Soc. 1952; 74:3795-3798.
24. Dubois M, Gilles KA, Hamilton JK, Roberts PA, Smith F. Analytical Chem. 1956; 28:350-356.
25. Rafique CM, Smith F. J. Amer. Chem. Soc., 1950; 72:4034-4036.
26. Sommie R. Acta Chem. Scand. 1967; 21:685-687.
27. Wolform ML, Word HB. J. Amer. Chem. Soc. 1957

73:2933-2937.
28. Halsall TG, Hirst EL, Jones JKN. J. Chem. Soc. 1947; 1427-1430.
29. Aspinall GO, Whyte JNC. J. Chem. Soc. 1964; 972:5058-5061.
30. Lambert M, Neish A. Can. J. Res. 1950; 28-B:83-86.
31. Reeves RE. J. Amer. Chem. Soc.1941; 63:1476-1479.