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Determination of polyalcohols by Smith degradation technique from *Withania somnifera* Dunal seeds oxo-polysaccharide

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ABSTRACT

Water soluble seeds polysaccharide were extracted from Withania somnifera Dunal as D-glucose and D-mannose in 1:3 molar ratio by GLC, column and paper chromatography. Periodate oxidized seeds polysaccharide was degraded with sodium borohydride followed by sulphuric acid with Smith degradation method. Hydrolysate yielded polyalcohols as glycerol and erythritol in 1:54:4.26 molar ratio with traces of thritol on paper chromatogram. Derivatives of polyalcohols were produced as glycerol-tri-O-p-nitrobenzoate and tetra-O-tosyl-erythritol. Absorbance of polyalcohols were recorded on 540 mμ in photoelectrocolorimeter. The earlier proposed seeds polysaccharide structure of Withania somnifera Dunal plant has been tentatively confirmed on the basis of above finding results.

Key words: Polyalcohols from *Withania somnifera* Dunal seeds oxo-polysaccharide by Smith degradation.

INTRODUCTION

Withania somnifera Dunal plant[1] belongs to the family – Solanaceae and commonly called as *Ashwagandha, Winter cherry or Indian ginseng.* It is an evergreen upto 30-170 cm in height. It occurs in sub Himalayan tract upto Punjab to Nepal, Rajasthan, Sind and other dried region of India, Eastern & Western ghats, Andaman & Nicobar Islands, Sri Lanka, Myanmar, Pakistan, Malaysia, Thailand, Java, Asian & African Tropics. Alkaloids extracted from leaves, roots, seeds and bark are medically important in therapeutic agent, disorder of nervous system, dysentery and also used in child birth in difficult cases. depression, astrigent, antioxidant, anticancer, etc. It is widely used in babies tonic and inhancing the reproductive function in man and women. It is also used for spermatorrhoea infertility in women. Seeds can be used as a substituted for rennet to curdle milk. Water soluble seeds contains a polysaccharode[2] as D-glucose and D-mannose in 1:3 molar ratio from the hydrolysed compound by paper chromatographic analysis. Nature of the constituent sugars were present in water soluble seeds extract have already been reported[2] and methylation study have also been carried out for tentative polysaccharide structure. Present investigation mainly deals with the identification of polyalcohols from the reduction of periodate oxidised *Withania somnifera* Dunal seeds polysaccharide by Smith degradation method[3] for the confirmation of earlier proposed polysaccharide structure.

MATERIALS AND METHODS

Polyalcohols obtained from *Withania somnifera* Dunal seeds polysaccharide were separated on Whatman No. 3 MM filter paper sheet by descending paper chromatographic technique[4]. The following upper phase solvent mixture

(v/v) were used for the identification of polyalcohols as (A) *n*-butanol-ethanol-water (4:1:5)[5] and (B) ethyl acetate-pyridine-water (2:1:2)[6]. Spray reagent (R) acetonical silver nitrate-alcoholic sodium hydroxide[7] was used for the detection of polyalcohols on paper chromatogram. Hydrolysed syrupy liquid yielded glycerol, erythritol and traces of thritol were separated by paper chromatographic analysis.

Polysaccharide (1.5 gm) was oxidised[8] by Smith degradation methods with sodium metaperiodate (0.125 M, 10 ml) for 36 hrs in refrigerator at 4-8^{0}C. The remaining periodate oxidised compound was treated with ethylene glycol (5 ml) to decomposed the excess of periodate then it was dialysed against running water upto 48 hrs and concentrated to 50 ml. The resulting solution was reduced[9] with sodium borohydride (1gm) with mechanical stirring for 24 hrs. Excess sodium borohydride was acidified with glacial acetic acid (5 ml) and resulting solution was dialysed against running water then evaporated to dryness. Residue was distilled with methyl alcohol to remove the borate ions as methyl borate. Borate free reduction product was dialysed against running water for 48 hrs to remove inorganic ions. It was concentrated to a thin syrup which was hydrolysed with sulphuric acid (0.5 N, 10 ml) for 12 hrs. Hydrolysed product was neutralized with barium carbonate slurry with the help of mechanical stirrer then content left for 24 hrs. It was then filtered off and obtained filtrate was deionised by Amberlite ion exchange resins[10] IR-120 (H^+) and IR-45 (OH^-) then concentrated to a syrup.

Hydrolysed product was resolved into its components by paper chromatographic analysis on Whatman No 3 MM filter paper sheet using solvent mixture (A) and used (R) as spray reagent for the detection of polyalcohols. Sugar strips were cut out with the help of guide spots parallel to the authentic sample of polyalcohols. It was eluted with water according to the Dent's method[11] and polyalcohols were identified and characterised as glycerol, erythritol and traces of thritol.

Glycerol: Sugar syrup (320 mg) was dissolved in 5 ml ethanol, filtered and filtrate concentrated to a syrup. It moved a single spot parallel to the authentic sample of glycerol on paper chromatogram, Derivative was prepared with sugar residue (250 mg) in pyridine (5 ml) and *p*-nitrobenzoyl chloride (3 gm). Content was heated over water-bath for 1 hr at 85^{0}C, cooled and added saturated solution of sodium bicarbonate then finally filtered. After cooling the filtrate yielded the crystals of glycerol-tri-O-*p*-nitrobenzoate after recrystallisation with acetone having m.p. & mixed m.p. 184-186^{0}C, Lit. m.p. 186-1880C[12].

Erythritol: Sugar syrup (580 mg) was treated with 20 ml water then it purified by animal charcoal for 24 hrs, filtered and filtrate concentrated to a syrup. It dissolved in 5 ml ethanol, upon cooling the solution of erythritol was crystallized out from the solution. Crystals of erythritol were filtered and on recrystallisation with ethanol had m.p. & mixed m.p. 120-121^{0}C, Lit m.p. 120-1210C[13].

Derivative of erythritol was prepared by dissolving the syrup (280 mg) in 5 ml of anhydrous pyridine solution and 1.5 gm of *p*-toluene sulphonyl chloride for 24 hrs. Content was poured into ice cold water, derivative were crystallized out as crystals, filtered and washed with water followed by ethanol. On recrystallisation with acetone and ethanol gave crystals of tetra-O-tosyl-erythritol having m.p. & mixed m.p. 167-169^{0}C, Lit. m.p. 166-1680C[13].
Thritol: It was obtained in traces (20 mg) on paper chromatogram having *Rf* values more than D-glucose and D-mannose. It was identified as thritol and spot was visible only in ultraviolet light in UV-spectrum[14].

Polyalcohols were quantitatively estimated by chromotropic acid method[15] and respective polyalcohols were separated by paper chromatographic analysis on Whatman No. 3 MM filter paper sheet in solvent mixture (B) and used (R) as spray reagent. Polyalcohols were characterized as glycerol and erythritol in 1.54:4.26 molar ratio with traces of thritol.

Seeds polysaccharide (780 mg) was dissolved in 50 ml water and aliquot were put into six test tubes and each tubes adjusted with water (2 ml). Phenol solution (5%, 1 ml) was added to each solution followed by sulphuric acid (0.5 N, 5 ml) then tubes were allowed to stand for 20 min and cooled it in running water. A blank reading for each sugar was also prepared in the same way. The colour intensity and absorbance of polyalcohols were recorded on 540 mμ colour filter in a Klett Summerson photoelectrocolorimeter[16] for each sugars. The analytical data of polyalcohols from *Withania somnifera* Dunal seeds polysaccharide are given in table 1.

Table-1 : Absorbance of polyalcohols from *Withania somnifera* Dunal seeds polysaccharide.

S.No.	Wt. in microgram		Phenol (ml)	H_2SO_4 (ml)	Klett reading (Absorbance)	
	Glycerol	Erythritol			Glycerol	Erythritol
1.	2	2	1	5	24	20
2.	4	4	1	5	48	38
3.	6	6	1	5	73	60
4.	8	8	1	5	96	75
5.	10	10	1	5	118	98
6.	12	12	1	5	110	119

RESULTS AND DISCUSSION

Periodate oxidized seeds polysaccharide from *Withania somnifera* Dunal plants was reduced with sodium borohydride and sulphuric acid, yielded glycerol, erythritol in 1.54:4.26 molar ratio with traces of thritol on Whatman No 3MM filter paper sheet by paper chromatography. A large proportion of erythritol released by acid hydrolysis (H_2SO_4) was obtained by the reduction of borohydride. The evidence of linkages[17] in polysaccharide structure showed that the main polymer linkages are of (1→4)-β-type and branching point with (1→6)-α-type. Ratio of erythritol to the amount of glycerol indicates that the branching point after every 8 hexoses repeating units of one reputting unit was obtained due to the presence of D-glucose at non-reducing end in the main polymer chain. It indicated a branching points on the average of 7 hexoses units are in the backbone and 1 hexose unit in the non-reducing end for the support of the earlier proposed seeds polysaccharide structure of *Withania somnifera* Dunal plant as shown in Figure-1. The molar ratio of D-glucose and D-mannose was found to be 1:3 moles by GLC, column and paper chromatography of the hydrolysed products.

REFERENCES

[1]. Chadha Y.R. (**1976**). *Wealth of India Raw Materials.* Publication & Information Directorate, CSIR, New Delhi (India), *X (SP-W)*: 581-585.
[2]. Singh R.B. (**2011**), *National Seminar on Conservation, Cultivation and Utilization of medicinal plants*, R B S College, Agra on 26-27th February.
[3]. Smith, F. (**1940**). *J. Chem. Soc.* 1035.
[4]. Partridge, S. M. (**1946**), *Nature (London)*, *158*:270.
[5]. Partridge, S. M. and Westall, R.G. (**1948**), *Biochem. J., 42*: 238.
[6]. Jermyn, M. A. and Isherwood, F.A. (**1949**), *Biochem. J., 44*: 402.
[7]. Trevelyan, W. E., Procter, D.P. and Harrison, J.S. (**1950**), *Nature (London)*, *166*:444.
[8]. Abdel, A. M., Hamilton, J.K., Montogomery, R. and Smith, F. (**1952**), *J. Amer. Chem. Soc., 74:* 4970.
[9]. Morimoto, I. J., Unrau, C.J. and Unrau, A.M. (**1960**), *J. Agri.Food.Chem.,10:* 134.
[10]. Adams, B. A. and Halmes, E. L. (**1956**), *J. Soc. Chem. Ind., 54*: 17.
[11]. Dent, C. E. (**1947**), *Biochem. J., 41*: 240.
[12]. Unrau, A. M. (**1961**), *Journal Org. Chem. 26 :* 3097.
[13]. Hamilton, J. K. and Smith, F. (**1956**), *J. Amer. Chem. Soc., 75:* 5907.
[14]. Jaffe, H. H, and Orchin, M. (**1962**), *Theory and practical application of ultraviolet spectroscopy*. John Wiley & Sons, New York.
[15]. Lanbert, M., Neish, A. (**1950**), *Can. J. Res., 28-B:* 83.
[16]. Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A. and Smith, F. (**1956**). *Anal. Chem., 28*: 350.
[17]. Baker, S. A., Bourne S. J. and Whiffen, O.H. (**1956**). *Methods Biochem. Anal.* 3: 313.