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Structure of Polysaccharide from the Seeds of Kigelia Pinnata Part-I- Hydrolytic Studies

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Abstract

The ionic mobility of the polysaccharide occurring in the seeds of Kigelia Pinnata was determined by chromatographic studies. The partial specific volume of polysaccharide was also determined.

Keywords: Kigelia Pinnata, Hydrolytic Studies**.**

Introduction

Kigelia Pinnata commonly known as "Sausage Tree or Balam Kheera" belongs to the family Bignoniaceae and its bitter pulp of the fruit is used for the traditional medicines[1] preparation of active carbon, as dressing for ulcer, for syphilis and Rheumatism[2], dysentery and venereal diseases.[3-4]. The K. Pinnata plant has many medicinal properties due to the presence of numerous secondary metabolities[5] like alkaloids, glycosides and terpenoids. These compounds include irrioids, flavonoids and nahthaoquinones and volatile constituent, etc.[6-9].

Review of Literature

J.J. Baker and H.T.E. Hulton (1920) "The spray reagents used for detecting the sugars, p-anisidine phosphate to till date, Khursheed Siddiqui, Avijit Mazumder, Sept. 2015 it has many medicinal properties due to the presence of numerous secondary metabolites.

Aim of the Study

The main aim of this study was to a systematic chemical investigation of polysaccharide from the seeds of Kigelia Pinnata, which constitute the subject matter findings show promising results and can be used for further studies.

Methodology

Extraction of polysaccharide from the seed of K. Pinnata with water furnished crude polysaccharide which was subsequently purified by repeated fractionation with ethyl alcohol followed by deionization with ion-exchange resins. The polysaccharide isolated was neutral and free nitrogen, sulphur, halogen, methoxyl and uronic acid groups. Paper electrophoresis showed that the polysaccharide migrated as a single spot having ionic mobility $\mu = 0.24 \times 10^{-5}$ cm^2 $volt^{-1}$ $sec.^{-1}$. The sedimentation pattern of the compound as revealed from ultra centrifugal analysis showed a single symmetrical peak, thus establishing its homogeneity.

Complete acid hydrolysis of the polysaccharide gave a mixture of neutral sugars which was resolved into D–galactose and D–mannose by paper chromatography as well as on a cellulose column.

The individual sugars were further characterized on the basis of their m.ps. specific rotations and m.ps of their crystalline derivatives. Quantitative acid hydrolysis of the polysaccharide showed that galactose and mannose were present in a molar ratio 3:8:1.

Experimental and Discussion

Unless otherwise stated all evaporations were carried out at 40-50° under reduced pressure. Values of specific rotation at equilibrium and melting points are uncorrected Paper chromatographic analysis was carried out by using the following solvent system (v/v).

S_1-n-butanol-ethanol-water (4:1:5 upper layer)[10], S_2-n-butanol-acetic acid-water (4:1:5 upper layer)[11], S_3-ethyl-acetate-acetic acid-water-butanol (4:3:3:4)[12], S_4-2-butanol-water-azotrope[13], S_5-Benzone ethanol-water (167:47:15)[14], S_6-2-butanol-water ammonia (200:17:2)[15], S_7-ethylacetate-acetic acid-water (9:2:2)[16].

The spray reagents used for detecting the sugars were

R_1-acetonical silver nitrate alcoholic sodium hydroxide[17]; R_2-p-anisidine phosphate[18]; R_3-p-anisidine hydrochloride[19], R_4-diphenyl-p-anisidine[20-21]; R_5-sodium meta periodate benzidine[22].

Isolation and Purification

The seeds of K. Pinnata (260gm) were pulverised in a laboratory grinder and endosperm was separated form kernel and husk by winnowing. The endosperm (130gm) was finally powdered and extracted with water (2 litre) at 50-60° for 10 hrs. under constant stirring. The resulting highly viscous suspension was filtered through a muslin cloth and undissolved material again extracted with water as above. The combine filtrate was centrifuged (3000 r.p.m.) for 50 minutes and yellow supernatant solution decanted off. It was then acidified (pH-20.0–3.0) with glacial acetic acid and aq. acidic extract was poured slowly into four times its volume of rectified sprit with continuous stirring. When the polysaccharide was obtained as white flocculent precipitate.After the removal of aq. ethanol, the precipitate was found to be sticky in nature. At this stage, acetone (350ml) was added and solution kept overnight, subsequently, acetone was replaced by absolute alcohol (150ml.). This operation was repeated thrice when the precipitate become granular. Finally, the compound was purified by passing its aqueous solution successively through columns of freshly regenerated cation (Duolite-C-25) and anion (Duolite A-7) exchange resins. The compound effluent was treated with a large volume of ethanol to get the pure polysaccharide in the form of a white granular powder 30 grams $[\alpha]^{30}D$ + 26.20° (C.0.53 gm. in 1N NaOH). Its aqueous solution was almost neutral (pH-6.5). The polysaccharide was non-reducing and did not contain nitrogen, sulphur, halogens, methoxyl and anhydroinic acid. It formed an o-acetyl derivative $[\alpha]^{25}D$ + 38.62° (C.0.5 gm.) in acetone, found : acetyl, 43.93 calculated for acetylated polysaccharide : acetyl 44.30%). IR spectrum of polysaccharide showed a prominent band for –OH (3400 cm^{-1}) besides other bands at 2850, 2300, 880 and 800 cm^{-1}.

Polysaccharide did not move at all under various condition of paper chromatography. However when subjected to paper electrophoresis on strips (45.5 cms) of whatmann No.-1 filter paper in borate buffer (0.005M sod. tetraborate decahydrate pH–9.2), under a field strength of 700 volt for 7 hrs. the compound moved as a single spot. The presence of polysaccharide spot in the paper was detected by spray reagent R_5 and washing afterwards with acetone.

The ionic mobility of Polysaccharide was calculated from the equation :

$$\mu = \frac{dqak}{t\,I}$$

Where, d = distance moved = 15cm; qa = Cross section area of paper = 0.05cm^2 strip; K = conductivity of buffer = 1/18, 180; t = time in sec. = 420×60sec.; I = current in amp. = 0.020; μ = Ionic mobility = 0.24 ×10^{-5} cm^2 sec^{-1} $volt^{-1}$.

Ultracentrifugal analysis of the compound in 0.05m borate buffer at 5000 r.p.m. for appropriate time intervals showed only one peak confirming the homogeneity of the sample.

The partial specific volume of the compound was determined at 20° by the procedure described earlier at three difference concentration of the polysaccharide in buffer pH–8.4.

Table–1
Partial Specific Volume of Polysaccharide

Concentration Polysaccharide w/w	Weight of Pyknometer + Polysaccharide Solution (grams)	ρ_o 1/C	ρ W/C	Average Specific Vol.
2.46	83.5132	1.0018	1.0160	0.4789
1.80	83.3354	1.0018	1.0100	0.4880
1.07	83.1574	1.0018	1.0074	0.4758

Mean Value of the δ = 0.4809

Substituting value of specific volume in the following formula

M.W. = RTS/D (1– δ/d)

Where, M.W. = molecular weight of the polysaccharide; R = 8.317×10^7/ °K; D= Diffusion Coefficient = 8.04 × 10^{-7}; δ = Partial Specific Volume = 0.4809, d = density of water at 20°C = 0.9982

The molecular weight of a pure sample of Kigelia Pinnata Seed Polysaccharide was found to be in the order of 9978.

Complete acid hydrolysis of the Polysaccharide

Purified Polysaccharide (5.0gm) was subjected to hydrolysis with sulphuric acid (2N, 250ml) for 24 hrs. on a boiling water-bath. The course of hydrolysis was followed by Iodometric titration.[23]

After definite intervals of time aliquot (2ml) of the hydrolyte was withdrawn in a arlymeter flash and mixed with iodine solution (0.1N, 30 ml) and sodium hydroxide solution (0.1N 40 ml). The mixture was kept for 30 minutes in dark. The solution was acidified with sulphuric acid (15ml) and excess of iodine was titrated with standard sod. thiosulphate solution (0.05N). The time taken for complete hydrolysis alongwith the variation of iodine adsorption during the progress of the reaction is reordered in Table No. 2.

The hydrolyte was neutralized (barium carbonate) and evaporated to a thin syrup. Its paper chromatography in solvent S1 revealed the spots corresponding to galactose and mannose. Resolution of the sugars on a cellulose column using n-butanol half saturated with water as the eluant resulted isolation of two individual sugars. Sugars were identified as D-mannose from its MP 133°, optical rotation $(\alpha)^{30}D$ = + 18.2 and by preparing p-nitro-N-phenyl-mannosylamine derivative MP-212-225°). The slown moving sugar (MP-170°) was established as D-galactose from measurement of its optical rotation

$(\alpha)^{30}D$ = + 80.2 and also by preparing N = phenyl-galactosylamine derivative (MP-255°).

Conclusion

K. Pinnata is a multipurpose medicinal plant with many attributes. This work was promoted as a result of lack of recent.

Table-2
Process of Acid Hydrolysis of Polysaccharide

Time	Volume(ml) of the sulphate Solution (0.5) required for excess iodine	Volume (ml) of iodine solution consumed
0	14.6	0.00
4	14.0	1.00
8	13.7	1.60
12	13.1	2.10
16	12.8	2.20
20	12.2	2.50
24	12.0	2.9
28	11.8	3.0
32	11.7	3.1
36	11.6	3.2
40	11.6	3.2

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References

1. *Cordell, G.A., Biodiversity and drug discovery, a symbiotic relationship, phytochemistry, 2000, 56, 463-480.*
2. *Benthall, 347; Bhatia and Manohar Lal, J. Indian Chem. Soc., Industr. Edn.; **4**, 236 (1941).*
3. *Watt and Breyer Brandwijk; 171, Quisumbing; 877.*
4. *E. Anderson. Ind. Engi. Chem. **41**, 2887 (1949).*
5. *Khursheed Sidduqui, Avijit Mazumder, Gunosindhu Chakraborthy, International J. of Pharma Research & Review, September 2015; 4(9); 34-38.*
6. *Houghton P.J. The sausage tree (Kigelia Pinnata) ethnobotany and recent scientific work, South Aft. J. Bot. 2002, 68(1), 14-20.*
7. *Asckum OT, Oluscgun E. and Adebola O. The volatile constituents of the leaves and flowers of Kigelia africana Benth, Flav Frager J. 2006, 22(1), 21-23.*
8. *Gornann R. Schreiber L. and Kalod Ziej H., culticular wax profiles of leaves of some traditionally used African Bignoniaceae, Z Nature forsch, 2004, 59 (9-10), 631-635.*
9. *Sudhir Tripathi, Ph.D. Thesis, Kanpur University, Kanpur, India, 40 (1993).*
10. *E.L. Hirst and K.N. James, Dls. Faraday Soc. 268 (1949).*
11. *S.M. Patridge and R.C. Westwell, Biochem, J. **42**, 567 (1971).*
12. *S. Bose and P.L. Soni, J. Indian Chem. Soc., **46**, 567 (1971).*
13. *I.A. Boggs, L.S. Cuendit, I. Ehrenthal, R. Koch and F. Smith Nature, 166, 520 (1950).*
14. *P. Andrews, L. Hough and J.K.N. Jones. J. Chem., Soc. 1188 (1953).*
15. *D.M.W. Anderson, E. Hirst and J.F. Stodoort, J. Chem., Soc., 1476 (1969).*
16. *M. Biswas and S. Bose, India J. Bio. Chem. **7(1)**, 68-72 (1970).*
17. *W.E. Trevelyon, D.P. Procter. Harrison. J.S. Natwas 166, 444 (1950).*
18. *J.J. Baker and H.T.E. Hulton. Bio. Chem. J. **14**, 754 (1920).*
19. *L. Hough, J.K.N. Jones and W.H. Wadman, J. Chem. Soc., 1702 (1950).*
20. *R.W. Bailey and E.J. Bourne. J. Chromatory, 4206 (1960).*
21. *S. Schwinnier and A. Benell, Science, **123**, 543, (1956).*
22. *D.F. Smith and J. Robert, Methods in Carbohydr. Chemistry, Academic Press Inc. (London), Vol. 4, P. 41 (1961).*
23. *F. Ehrlich, Ber. **65**, 352 (1932).*