512. *Constitution of Tamarind-seed Polysaccharides, and the Structure of the Xylan.*

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The xylan from decorticated tamarind seeds is devoid of arabinose residues. Chromatography of the methylated derivatives on paper and on cellulose columns reveals that the polysaccharide consists of about 80 ± 5 β-D-xylopyranose units, linked through 1 : 4-positions and disposed in a singly branched structure with bifurcation involving the 3-hydroxyl group of one of the xylopyranose residues. The molecule has one reducing and two non-reducing terminal groups. These observations are supported by oxidations with periodate, the reducing power of the xylan, and its molecular weight.

SAVUR and SREENIVASAN [1] showed that the gel-forming constituent obtained from aqueous extracts of decorticated tamarind-kernel powder by precipitation with alcohol differs fundamentally from pectins, although it forms excellent jellies with sugar both in acidic and neutral media. The powder contains three types of material,[2] *viz.*: P_1 (2—4%), soluble in water within 2—3 minutes at 5°; P_2 (20—22%), soluble at room temperature on vigorous stirring with water for 35 minutes; and P_3 (30—35%), insoluble in cold but soluble in boiling water. Fraction P_1 has no jellying properties, while fractions P_2 and P_3 have excellent jellying and sizing properties.

The xylan [3] from fraction P_3 was extracted with dilute sodium hydroxide solution at room temperature, isolated by acidification with acetic acid and precipitation with acetone, and purified by repeated precipitation as the copper complex. On hydrolysis it gave D-xylose, the yield being determined as 97·6% by determination of furfuraldehyde phloroglucide and 97·4% by filter paper chromatography.[4]

[1] Savur and Sreenivasan, *Current Sci.*, 1945, **14**, 129; 1946, **15**, 43, 134, 168; *J. Biol. Chem.*, 1948, **172**, 501; *J. Soc. Chem. Ind.*, 1948, **67**, 190.
[2] Savur, *Current Sci.*, 1955, **24**, 235.
[3] Savur, Indian P. 53,429/1954.
[4] Flood, Hirst, and Jones, *J.*, 1948, 1679.

Paper chromatography of the hydrolysate of the methylated xylan indicated the presence of 2 : 3 : 4-tri-*O*-methylxylose (2·5 mol.%), 2 : 3-di-*O*-methylxylose, mono-*O*-methylxylose (5·2%), and xylose (a trace). The sugars were identified by the R_G values, the proportions being determined by hypoiodite oxidation [5] in sodium hydroxide–phosphate buffer at pH 11·4. These experiments indicate one non-reducing end group per 40 residues.

The amount of tri-*O*-methylxylose obtained on cellulose columns after quantitative methylation of the xylan hydrolysate corresponds to 35 ± 5 residues for each non-reducing end group. The molecular weight of the xylan, determined by measurements of viscosity in *m*-cresol, was 11,500. The tri- and mono-*O*-methyl sugars were identified as 2 : 3 : 4-tri-*O*-methyl- and 2-*O*-methyl-D-xylopyranose respectively, the results being confirmed by formation of the anilides. The 2 : 3-di-*O*-methyl-D-xylose was identified as its anilide, lactone, and amide.

Since more than one molar proportion of 2-*O*-methylxylopyranose was separated and identified in the hydrolysate, branching occurred through the position $C_{(3)}$ of the xylose residue in the 1 : 4-linked chain. Determination of formic acid liberated from the end groups by periodate oxidation and determination of the reducing groups [6] agree with the observations that the xylan molecule comprises of about 80 ± 5 β-D-xylopyranose units linked through 1 : 4-linkages and terminated by one reducing and two non-reducing groups.

Experimental

Preparation of the Xylan.—The polysaccharide P_3 (500 g.) was extracted with 4% sodium hydroxide solution (1 l.) at room temperature; the solution was acidified and precipitation effected with acetone, the operation being repeated on the residual solid. The crude xylan was collected on a centrifuge, washed with 1 : 1 acetone–water, then with alcohol and with ether, and dried at 55° and then over P_2O_5 (yield, 125 g.). Chromatography of the hydrolysate [4] disclosed xylose 86%, glucose 8·5%, and galactose 2·4%.

Crude xylan (30 g.) was dissolved in 2% sodium hydroxide solution (2 l.) and treated with freshly prepared Fehling's solution (2 l.) and a little acetone. The precipitated copper complex was collected on muslin, suspended in water (1 l.), and treated with 2N-hydrochloric acid, the acidity of solution being kept below N. The xylan was precipitated with acetone as a white flocculent material which was collected on a centrifuge and washed with acidified acetone–water until free from copper, then with neutral acetone–water to remove the acid, and finally with alcohol and ether. It was suspended in water (1 l.) and shaken for 12 hr. The insoluble product (yield 25 g.) contained no glucose or galactose and gave a 95% yield of xylose on hydrolysis, estimated as di-*O*-benzylidenexylose dimethyl acetal.[7]

Filter-paper chromatography of the hydrolysate showed the presence of only xylose. The product on hydrolysis with 0·5N-sulphuric acid and estimation with Somogyi's copper reagent accounted for 97—98% of the xylan.

Methylation.—Purified xylan (25 g.) was suspended in water (200 c.c.) for about 12 hr. under a stream of nitrogen. 40% Sodium hydroxide solution (300 c.c.) was added and the whole stirred for 4 hr. Methyl sulphate (200 c.c.) was added dropwise during 8 hr. with ice-cooling. Next morning the mixture was heated for 1 hr. on a water-bath. The mixture was cooled and treated with 40% sodium hydroxide solution (300 c.c.), followed by methyl sulphate (250 c.c.), this treatment being repeated 4 times at room temperature in nitrogen; 200 c.c. of acetone were added before the last methylation and distilled off on completion of the methylation. The mixture was cooled and its pH adjusted to 8 by 0·5N-sulphuric acid. The precipitate was boiled with water, dissolved in 4 : 1 aqueous acetone, and treated with 40% sodium hydroxide solution (400 c.c.) for 2 hr. with stirring in nitrogen, and then with methyl sulphate, this methylation being repeated four times. The final product (28·5 g.) had $[\alpha]_D^{20}$ −82·5° (*c* 0·8 in $CHCl_3$) (Found : OMe, 36·2%).

Fractionation of the Methylated Xylan.—The preceding product was fractionated according to the method of Chanda *et al.*[8] The seventh fraction (eluted with 35 : 65 v/v chloroform–light petroleum) amounted to 36·5% and had $[\alpha]_D^{20}$ −84° (Found : C, 52·0; H, 7·7; OMe, 36·8%. Calc. for $C_7H_{12}O_4$: C, 52·5; H, 7·5; OMe, 37·2%).

[5] Hirst, Hough, and Jones, *J.*, 1949, 928.
[6] Ingles and Israel, *J.*, 1948, 810.
[7] Breddy and Jones, *J.*, 1945, 738.
[8] Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289.

Molecular Weight.—The molecular weight of the xylan was determined in 0·1N-sodium hydroxide, and those of its derivatives in *m*-cresol by measuring the viscosity in an Ostwald viscometer, the relation used being $\eta_{sp.} = K_m mc$. Values of 14,835 for the methylated xylan and 17,660 for the acetylated xylan [9] were obtained.

Hydrolysis of the Methylated Xylan.—(a) *By paper chromatography.* Hydrolysis was as described by Chanda *et al.*[8] Paper chromatography of the hydrolysis products with butanol–ethanol–water and development with aniline oxalate gave spots corresponding to tri-*O*- (R_G 0·94—0·96), 2 : 3-di-*O*- (R_G 0·75—0·76), and mono-*O*-methylxylose (R_G 0·38—0·40), and traces of xylose. The methylated sugars were estimated by alkaline hypoiodite after separation on the paper chromatogram. The following results were obtained: 2 : 3 : 4-tri-*O*-methylxylose, 1·00 mg. gave 0·98 mg.; 2 : 3-di-*O*-methylxylose, 0·95 mg. gave 0·94 mg.; 2-*O*-methylxylose, 1·2 mg. gave 1·16 mg. 2 : 4-Di-*O*-methylxylose was not found.[10]

50 mg. of the methylated xylan, hydrolysed as before, gave on a paper chromatogram: 2 : 3 : 4-tri-, 0·25 mg.; 2 : 3-di-, 8·64 mg.; and 2-*O*-mono-methylxylose, 0·5 mg.; and xylose, 0·03 mg.

(b) *On a cellulose column.* When the methylated xylan (6 g.) was heated on a water-bath with 1% methanolic hydrogen chloride (600 c.c.) and then neutralised, the following rotations were observed: $[\alpha]_D^{18}$ 4° (1 hr.); 13·5° (2 hr.); 20° (3 hr.); 36° (4 hr.); 50·5° (5 hr.); 60° (6 hr.); 68° (7 hr.); 78·5° (8 hr., constant). Subsequent procedure was as described by Chanda *et al.*,[8] but a column of powdered cellulose [11] (60 × 4 cm.) was used with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3) saturated with water containing 1% of ammonia as solvent. The eluates were concentrated, treated with methanol, and distilled, yielding: (1) tri-*O*-methylpentose (268 mg.); (2) di-*O*-methylpentose (620 mg.); (3) monomethyl pentose (260 mg.); and (4) xylose (5 mg.).

Fraction 1 was purified by crystallisation {yield 100 mg.; $[\alpha]_D^{20}$ 22·5 (*c* 1·5 in H_2O), m. p. 90°, not depressed on admixture with tri-*O*-methyl-D-xylopyranose (Found: C, 50·3; H, 8·2; OMe, 48·8. Calc. for $C_8H_{16}O_5$: C, 50·0; H, 8·3; OMe, 48·4%)}. The total quantity of tri-*O*-methyl-D-xylopyranose was 212 ± 10 mg., corresponding to 35 ± 3 residues.

Fraction 2 was a syrup, $[\alpha]_D^{20}$ 24° (*c* 2·4 in H_2O) (Found: OMe, 35·4. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). The presence of 2 : 3-di-*O*-methylxylose was confirmed by preparation of the lactone. This with methanolic ammonia yielded 2 : 3-di-*O*-methyl-D-xylonamide, m. p. and mixed m. p. 134·7°, $[\alpha]_D^{20}$ +48·6° (*c* 0·8 in H_2O) (Found: C, 43·3; H, 7·5; N, 7·2; OMe, 32·2. Calc. for $C_7H_{15}O_5N$: C, 43·5; H, 7·7; N, 7·25; OMe, 32·1%). The anilide had m. p. and mixed m. p. 145·2°, $[\alpha]$ +191° (*c* 1·2 in ethyl acetate; const. after 24 hr.) (Found: C, 61·8; H, 7·7; N, 5·8; OMe, 24·1. Calc. for $C_{13}H_{19}O_4N$: C, 61·6; H, 7·6; N, 5·5; OMe, 24·5%).

Fraction 3 was a syrup; it crystallised on inoculation with 2-*O*-methyl-D-xylose. Paper chromatography showed the presence of 2-*O*-methylxylose, m. p. and mixed m. p. 133·8°, $[\alpha]_D^{20}$ +6° (after 15 min.), 25° (1 hr.), 34·5° (3 hr., const.) (*c* 1·0 in H_2O) (Found: C, 43·7; H, 7·5; OMe, 17·8. Calc. for $C_6H_{12}O_5$: C, 43·9; H, 7·3; OMe, 18·9%).

Fraction 4, also a syrup, had $[\alpha]_D^{20}$ +13·0° (*c* 3·0 in H_2O) and contained xylose (paper chromatography).

Periodate Oxidation.—The method of Chanda *et al.*[8] gave the following amounts (10^{-2} mole) of formic acid per $C_5H_{10}O_5$ unit: 1 hr., 0·17; 2 hr., 0·38; 72 hr., 4·0; 96 hr., 4·5; 150 hr., 4·7; 200 hr., 4·9; 300 hr., 5·0. The results indicate that the amount of formic acid liberated was practically constant after one week, corresponding to one mole per 20 xylose residues.

The amount of periodate consumed per $C_5H_8O_4$ unit, determined by the method of Fleury and Lang,[12] was 0·90 (24 hr.), 1·2 (48 hr.), and 1·3 (74 hr.).

Reducing Power.—The polysaccharide (0·1 g.) was treated with N-sodium hydroxide (20 c.c.), followed by 0·1N-iodine (10 c.c.). The solution was kept in the dark for 16 hr., acidified with 2N-sulphuric acid (25 c.c.), and titrated with sodium thiosulphate, blanks being run concurrently. The results correspond to one reducing group per 48 xylose residues.

Discussion

It is evident that the xylan is a separate entity which exists along with hexosans in close physical union. The starting material used by earlier workers was prepared by

[9] Rao and Beri, *Proc. Indian Acad. Sci.*, 1956, **42**, 199.
[10] Rao and White, *J. Amer. Chem. Soc.*, 1953, **75**, 2617.
[11] Hough, Jones, and Wadman, *J.*, 1949, 2511.
[12] Fleury and Lange, *J. Pharm. Chim.*, 1933, **17**, 107.

alcohol precipitation and contained at least 17% of proteins, in distinction from the present work where the pure polysaccharide constituent was isolated. It is thus not surprising that earlier workers obtained contradictory results.[13] For example, Sarkar and Muzumdar [14] stated that tamarind-kernel powder contains no sugars other than glucose and xylose, which is contradictory to the experience of Savur and Sreenivasan [1] or Rao and Krishna.[15] The statement by Das *et al.*[16] that " tamarind seed polysaccharide is not only composed of glucose, galactose, and xylose but also arabinose and uronic acid, which have escaped the detection of by previous workers " is incorrect as Savur and Sreenivasan [1] had already stated that tamarind-kernel preparation contains 3·44% of uronic acid. Damadaran and Rangachari [17] detected arabinose among the hydrolytic products of the polyose, but this was not confirmed by other investigators.

Rao and Dickey's observation [18,19] that " in some cases arabinose was also detected when the acid hydrolysate of the whole tamarind-kernel powder (not the polysaccharide) was analysed chromatographically on filter paper " could also not be confirmed. The present investigation reveals that the uronic acid is not an integral part of the gel-forming substance and that neither the alcohol-insoluble fraction nor the non-polysaccharide fraction yields arabinose on hydrolysis, in disagreement with Das *et al.*[16] and Rao and Dickey.[18]

The only sugars identified in acidic or enzymic hydrolysates of the gel-forming constituent were glucose, galactose, and xylose. In view of the observations by Rao and Dickey,[18,19] the non-polysaccharide fraction from tamarind-kernel powder was examined chromatographically; uronic acid but no arabinose or other sugar was detected. Rao and White's work [10] is based on the products obtained from a mixture of polysaccharides which they considered as a single entity. Contrary to their findings 3 : 4-di-*O*-methylxylose was not detected.

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[13] Savur, *Sci. and Cult.*, 1956, **21**, 464.
[14] Sarkar and Muzumdar, *J. Textile Inst.*, 1952, **43**, T453.
[15] Rao and Krishna, *Current Sci.*, 1946, **15**, 133.
[16] Das, Roy, and Wareham, *J. Textile Inst.*, 1953, **44**, T402.
[17] Damadaran and Rangachari, *Current Sci.*, 1945, **14**, 203; 1946, **15**, 20.
[18] Rao and Dickey, *J. Textile Inst.*, 1953, **44**, T401.
[19] *Idem, Sci. and Cult.*, 1955, **21**, 102.