## 1041. *The Hemicelluloses of Roselle Fibre* (Hibiscus sabdariffa).

By P. C. Das Gupta.

Roselle fibre holocellulose was extracted with alkaline solutions of successively increasing concentration and finally with alkaline borate solution. Hemicellulose fractions (1—4) were isolated from the solutions. Fractions 2 and 3 have molecular weights of 23,000 ± 500 and 33,600 ± 500, respectively. Analytical data are recorded for each fraction.

Partial acid hydrolysis of roselle hemicellulose yields (2-D-xylose 4-*O*-methyl-α-D-glucopyranosid)uronic acid. The main hemicellulose fraction (2) was methylated and then hydrolysed, affording 2,3,4-tri-, 2,3-di-, and 3-mono-*O*-methyl-D-xylose, and (3-*O*-methyl-2-D-xylose 2,3,4-tri-*O*-methyl-α-D-glucopyranosid)uronic acid in the approximate molar proportions 1 : 34·5 : 2·1 : 6·9. The methylated polysaccharide has a degree of polymerisation 124 ± 3. It is concluded that the polysaccharide is composed of chains of *ca.* 107 1,4-linked β-D-xylopyranose residues, approximately every sixth residue carrying a terminal 4-*O*-methyl-α-D-glucopyranosiduronic acid residue linked through position 2. A small degree of branching in the backbone of D-xylose residue is indicated.

The structural studies of hemicelluloses of land plants [1] from different sources have revealed their general characteristics. The hemicelluloses of bast fibres so far studied were

[1] Aspinall, *Adv. Carbohydrate Chem.*, 1959, **14**, 429.

those from New Zealand flax,[2] from jute,[3] and from common flax.[4] Roselle fibre, a lignified tissue, is used as a substitute for jute. An attempt was made to fractionate the total carbohydrate constituent of roselle fibre, and this paper describes the structure of the main polysaccharide component of the hemicellulose fraction.

The extractive-free fibre was delignified by the chlorite method.[5] Stepwise extraction [6] (see Experimental section) of the resulting holocellulose led to four hemicellulose fractions (1—4) and a cellulose-rich residue (see Table). Fraction 2A was obtained by treatment of fraction 2 with Fehling's solution.

Physical constants of hemicellulose fractions.

| Fraction | Yield * (%) | Xylan † (%) | Uronic anhydride (%) | MeO (%) | Equiv. | D.P. | $[\alpha]_D$ (in N-NaOH) |
|---|---|---|---|---|---|---|---|
| 1 | 0·95 | 47·9 | 23·5 | 2·8 | 705 | — | −4·9° |
| 2 | 9·65 | 70·6 | 17·1 | 3·4 | 1071 | — | −46·9 |
| 2A | — | 76·6 | 17·5 | 3·6 | 1040 | 163 ± 3 | −46·6 |
| 3 | 6·74 | 65·2 | 16·6 | 3·2 | 1170 | 241 ± 3 | −37·7 |
| 4 | 1·39 | 50·4 | 13·4 | 2·8 | 1374 | — | −39·6 |

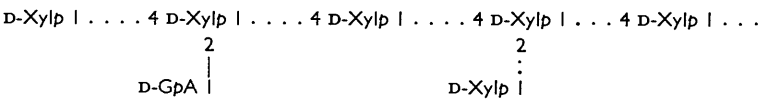
* On the weight of dry fibre. † Corrected for uronic anhydride [uronic anhydride gives 16·2% of its weight of furfuraldehyde (Sen and Das Gupta, unpublished work)].

Partial acid hydrolysis of roselle hemicellulose yields xylose and a main acidic component, characterised as (2-D-xylose 4-*O*-methyl-α-D-glucopyranosid)uronic acid.

Fraction 2 was converted into its fully methylated derivative which on hydrolysis gave 2,3,4-tri-, 2,3-di-, and 3-mono-*O*-methyl-D-xylose, and (3-*O*-methyl-2-D-xylose 2,3,4-tri-*O*-methyl-α-D-glucopyranosid)uronic acid in the approximate molar proportions 1 : 34·5 : 2·1 : 6·9. These results and the negative rotation of the original hemicellulose and its methylated derivative indicate that the polysaccharide contains a chain of 1,4-linked β-D-xylopyranose residues, approximately every sixth xylose residue carrying a terminal 4-*O*-methyl-D-glucuronic acid residue attached as a side chain to position 2. The methoxyl content, uronic anhydride content, and equivalent weight of roselle hemicellulose fraction (2A) also indicate the presence of approximately six xylose residues for each 4-*O*-methylglucuronic acid residue.

The number-average molecular weight of the methylated polysaccharide, as determined by osmometry, was found to be 20,800 ± 500 (degree of polymerisation, 124 ± 3). Since the methylation analysis indicated the presence of 2·5 non-reducing xylose end groups for a molecule of this size, there will be on the average 1·5 branches in the main chain of xylose residues per molecule. The corresponding figure for the original polysaccharide will be 2. The amount of formic acid liberated by periodate oxidation of fraction (2A) also suggests a number of branch points in the polysaccharide molecule. The presence of mainly 3-*O*-methylxylose as the monomethyl ether of xylose in the hydrolysis product of methylated hemicellulose indicates that the branch points must involve 1,2-linkages. The proportion of 3-*O*-methylxylose is slightly higher than could be accounted for the branch points and may arise from partial hydrolysis of the partially methylated aldobiouronic acid or incomplete methylation or demethylation during hydrolysis of the methylated polysaccharide. The accompanying partial structure for the xylan indicates the main features of the molecule. The consumption of 0·82 mol. of periodate per sugar residue also supports this structure.

[2] McIlroy, Holmes, and Mauger, *J.*, 1945, 796; McIlroy, *J.*, 1949, 121.
[3] (*a*) Sarkar, Mazumdar, and Pal, *Textile Res. J.*, 1952, **22**, 529; (*b*) Aspinall and Das Gupta, *J.*, 1958, 3627.
[4] Geerdes and Smith, *J. Amer. Chem. Soc.*, 1955, **77**, 3569, 3572.
[5] Chattopadhyaya and Sarkar, *Proc. Nat. Inst. Sci., India*, 1946, **12**, 23.
[6] Jones, Wise, and Jappe, *TAPPI*, 1956, **39**, 139.




(D-Xylp = D-Xylopyranose; D-GpA = 4-*O*-methyl-D-glucuronic acid)

Fraction 3 appears to have a structure similar to that of fraction 2 but a higher molecular weight. The major part of roselle hemicellulose thus consists of 4-*O*-methylglucuronoxylan whose structure is similar to that from jute [3] but differs from that of flax-straw hemicellulose [4] in having a higher proportion of uronic acid and in the absence of L-rhamnopyranose as the integral part of its molecule.

Roselle α-cellulose and the cellulose-residue both contain, besides glucose, a certain proportion of other sugars. It is not possible to ascertain with certainty whether they are associated with, or an integral part of, the cellulose molecule.

## Experimental

Paper partition chromatography was carried out on Whatman No. 1 and No. 3MM filter papers with the following solvent systems (v/v): (A) butan-1-ol–ethanol–water (40 : 11 : 19); (B) butan-1-ol–pyridine–water (10 : 3 : 3); (C) ethyl acetate–acetic acid–water (9 : 2 : 2); (D) butan-2-one, two-thirds saturated with water, containing 1% of ammonia; (E) benzene–ethanol–water (169 : 47 : 15, upper layer). Saturated aqueous solution of aniline oxalate was used as the spray reagent. Paper ionophoresis was in borate buffer at pH 10. Evaporations were carried out *in vacuo* at 40—45°.

*Composition of Roselle Fibre.*—Analysis by standard methods [7] indicated the following composition: α-cellulose 59·7, lignin 9·87, pentosan 15·0, Ac 4·76, ash 0·47, uronic anhydride 4·2, and fat and wax 0·58; residues of galactose 3·35, glucose 64·8, mannose 1·36, arabinose 0·42, rhamnose 0·77, and xylose 11·63%.

*Isolation and Purification of the Hemicellulose Fractions.*—Roselle fibre, in small pieces, was extracted with 1 : 2 v/v alcohol–benzene. The extractive-free fibre was delignified with sodium chlorite to give holocellulose [90·0%; lignin (Schwalbe's), 3·0; Ac, 4·7%). The holocellulose (450 g.) was treated successively with 10 parts of 2% sodium carbonate solution, 4% and 10% sodium hydroxide solution, and 16% sodium hydroxide solution containing 4% of boric acid for 48, 2, 2, and 72 hr., respectively. Hemicellulose fractions (1—4) were isolated from the solutions as described previously.[3a] Hemicellulose fraction 1 was brown owing to the presence of lignin. It was purified to a white powder by treatment with sodium chlorite in hot methanol.[8] Fraction 2 (10 g.) was treated thrice with Fehling's solution according to the method of Chanda *et al.*,[9] giving fraction 2A (8·6 g.).

Samples of the hemicellulose fractions, cellulose residue, and α-cellulose were hydrolysed as described by Saeman *et al.*,[10] and the neutral sugars in the hydrolysate were estimated by quantitative paper chromatography [11] with solvents B and C. The results are summarised in the Table.

| Fraction | A | Gal | Glu | Xyl | Man | Rham |
|---|---|---|---|---|---|---|
| 1 | 2·5 | 7·7 | 0·55 | 82·5 | — | 6·7 |
| 2 | 0·6 | 1·2 | 0·6 | 96·4 | — | 1·3 |
| 2A | — | — | 0·15 | 99·1 | — | 0·7 |
| 3 | — | 0·9 | 6·3 | 89·0 | 2·6 | 1·1ᴅ |
| 4 | — | 1·1 | 14·3 | 71·0 | 12·75 | 0·9 |
| Cellulose residue | — | — | 98·45 | 1·1 | 0·3 | 0·2 |
| α-Cellulose | — | — | 95·7 | 3·7 | 0·4 | 0·1 |

*Isolation of the Aldobiouronic Acid.*—The hemicellulose (mixture of fractions 2 and 3; 60 g.) was heated with N-sulphuric acid (1800 ml.) at 100° for 7 hr. ($[\alpha]_D$ +39·6°, const.). The cooled solution was neutralised with barium hydroxide and barium carbonate, and the filtrate was

[7] Das Gupta, *J. Sci. Ind. Res., India*, in the press.
[8] Rogers, Mitchell, and Ritter, *Analyt. Chem.*, 1947, **19**, 1029.
[9] Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289.
[10] Saeman, Moore, Mitchell, and Millett, *TAPPI*, 1954, **37**, 336.
[11] Flood, Hirst, and Jones, *J.*, 1948, 1679.

concentrated to a small volume and then poured into ethanol. The precipitated barium salts were extracted with boiling ethanol until the extract was more or less free from xylose. The barium salts were then dissolved in water, barium ions were removed with Amberlite resin I.R.-120($H^+$), and the solution was concentrated to a syrup. Chromatography in solvent C showed a main component with $R_{xyl}$ 0·83. The acidic syrup (5·4 g.) was dissolved in a little water, absorbed on cellulose powder, and dried in a vacuum-desiccator, and the mixture was placed on a cellulose column (70 × 4·5 cm.).[12] Elution of the column with solvent C yielded a chromatographically pure aldobiouronic acid (1·4 g.), $[\alpha]_D^{25}$ +105° (*c* 1·0 in $H_2O$) (Found: OMe, 9·3%; equiv., 352. Calc. for $C_{12}H_{20}O_{11}$: OMe, 9·1%; equiv., 340).

*Characterisation of Aldobiouronic Acid as* (2-D-*Xylose* 4-O-*Methyl*-α-D-*glucopyranosid*)*uronic Acid.*—The aldobiouronic acid (0·4 g.) was refluxed with methanolic 2% hydrogen chloride, and the resulting methyl ester methyl glycoside was reduced with lithium aluminium hydride in tetrahydrofuran.[13] The product was hydrolysed with N-sulphuric acid at 100° for 7 hr. and, after neutralisation with barium carbonate, the sugars were separated on filter sheets with solvent A, giving fractions *a* and *b*. Chromatography of the sugar and the product of periodate oxidation [14] indicated that fraction *a* (190 mg.), $[\alpha]_D^{27}$ +61° (*c* 1·1 in $H_2O$) (Found: OMe, 15·5. Calc. for $C_7H_{14}O_6$: OMe, 16·0%), was 4-*O*-methyl-D-glucose. The derived glucosazone had m. p. and mixed m. p. 159—160°, $[\alpha]_D^{27}$ −17·4° (*c* 0·64 in EtOH) (Found: OMe, 8·6; N, 15·3. Calc. for $C_{19}H_{24}N_4O_4$: OMe, 8·3; N, 15·0%). Fraction *b* (142 mg.), m. p. and mixed m. p. (with D-xylose) 144°, $[\alpha]_D^{25}$ +18° (*c* 1·0 in $H_2O$), was characterised by conversion into the di-*O*-benzylidene dimethyl acetal, m. p. 210°, $[\alpha]_D^{30}$ −8·2° (*c* 0·5 in $CHCl_3$).

Treatment of the methyl ester methyl glycoside (760 mg.) of the aldobiouronic acid first with methyl sulphate and sodium hydroxide and then with methyl iodide and silver oxide gave the methylated derivative (700 mg.). This with lithium aluminium hydride in ether [13] and then methyl iodide and silver oxide furnished the fully methylated neutral disaccharide (475 mg.), $[\alpha]_D^{25}$ +109° (*c* 1·0 in $CHCl_3$) (Found: OMe, 52·0. Calc. for $C_{18}H_{34}O_{10}$: OMe, 52·9%). Hydrolysis of this (400 mg.) with N-sulphuric acid at 100° for 6 hr. yielded a syrup which was separated by paper chromatography with solvent A into two fractions. Fraction (i) (112 mg.) was 2,3,4,6-tetra-*O*-methyl-D-glucose, m. p. and mixed m. p. 88—90°, $[\alpha]_D^{30}$ +84° (equil.) (*c* 1·0 in $H_2O$) (Found: OMe, 51·9. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%). The 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-glucopyranosylamine, after recrystallisation from light petroleum, had m. p. 135—136°, $[\alpha]_D^{30}$ +208° (*c* 0·5 in $COMe_2$). Chromatography with solvent E and ionophoresis of fraction (ii) (80 mg.) showed only 3,4-di-*O*-methyl-D-xylose, $[\alpha]_D^{30}$ +21·8° (*c* 1·1 in $H_2O$), characterised by conversion into 3,4-di-*O*-methyl-D-xylonolactone, m. p. 66—67°, $[\alpha]_D^{30}$ −22° (equil.) (*c* 1·0 in $H_2O$) (Found: OMe, 34·75. Calc. for $C_7H_{12}O_5$: OMe, 35·2%).

*Periodate Oxidation.*—Hemicellulose fractions 2A and 3 (900 mg.) were shaken with water (61 ml.) to form colloidal solutions, and 0·31M-sodium metaperiodate (29·5 ml.) was added. Oxidation was allowed to take place at 10° in the dark. The uptake of periodate, determined by the arsenite method, was constant after 268 hr. at values of 0·82 and 0·80 mol. of periodate consumed per sugar residue for fractions 2A and 3 respectively.

Oxidation of sodium salts of hemicellulose fractions 2A and 3 with potassium metaperiodate rapidly liberated one mole of formic acid per 4 kg. of hemicellulose in both cases in 150 hr.

*Methylation of Roselle Hemicellulose.*—The hemicellulose (fraction 2) (19 g.) was methylated by methyl sulphate and sodium hydroxide and then with methyl iodide and silver oxide, to give a methylated hemicellulose (20 g.), $[\alpha]_D^{25}$ −39° (*c* 1·0 in $CHCl_3$) (Found: OMe, 38·3%), whose infrared spectrum indicated the absence of hydroxyl groups. This (18 g.) was fractionated by dissolution in boiling chloroform–light petroleum (b. p. 60—65°). The main fraction (16·27 g.) was soluble in this mixture (3 : 7) and had $[\alpha]_D^{30}$ −43·8° (*c* 1·5 in $CHCl_3$) (Found: OMe, 39·0%). Hydrolysis of this fraction and quantitative chromatography [9] of the resulting neutral methylated sugars showed the tri-, di-, and mono-*O*-methylxylose in the proportion of 1 : 32 : 2.

*Hydrolysis of Methylated Hemicellulose and Separation of the Methylated Sugars.*—The methylated hemicellulose (8 g.) was hydrolysed successively with boiling methanolic 1·26% hydrogen chloride (700 ml.) for 10 hr. ($[\alpha]_D$ +86°, const.) and 0·5N-hydrochloric acid (700 ml.)

[12] Hough, Jones, and Wadman, *J.*, 1949, 2511; Whistler, Conrad, and Hough, *J. Amer. Chem. Soc.*, 1954, **76**, 1663.
[13] Abdel-Akher and Smith, *Nature*, 1950, **166**, 1037; Lythgoe and Trippett, *J.*, 1950, 1983.
[14] Lemieux and Bauer, *Canad. J. Chem.*, 1953, **31**, 814.

at 100° for 8 hr. ($[\alpha]_D$ +52°, const.). The solution was neutralised with silver carbonate, silver ion removed with hydrogen sulphide, and the solution concentrated to a syrup (8·5 g.). The methylated sugars (6 g.) were fractionated on a cellulose column (75 × 4·5 cm.) by solvent D into three fractions (1—3); eluting the column with water gave a fourth fraction (containing barium salts), which was treated with Amberlite resin I.R.-120($H^+$) and concentrated to a syrup.

*Fraction* 1. Chromatography of the syrup (120·5 mg.) showed only 2,3,4-tri-*O*-methyl-D-xylose, m. p. and mixed m. p. 84—85°, $[\alpha]_D^{25}$ +19·5° (equil.) (*c* 0·5 in $H_2O$) (Found: OMe, 48·0. Calc. for $C_8H_{16}O_5$: OMe, 48·4%).

*Fraction* 2. The syrup (3·85 g.) crystallised when seeded with 2,3-di-*O*-methyl-β-D-xylose and, recrystallised from ether, had m. p. 91—92°, $[\alpha]_D^{21}$ −34° (3 min.) ⟶ +23·5° (60 min., const.) (*c* 1·5 in $H_2O$) (Found: OMe, 35·0. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). The derived 2,3-di-*O*-methyl-*N*-phenyl-D-xylopyranosylamine, recrystallised from ethyl acetate, had m. p. 121°, $[\alpha]_D^{25}$ +181° (*c* 0·8 in EtOAc) (Found: OMe, 24·6. Calc. for $C_{13}H_{19}NO_4$: OMe, 24·5%), and 2,3-di-*O*-methyl-D-xylonamide had m. p. 131—132°, $[\alpha]_D^{29}$ +46·3° (*c* 1·1 in $H_2O$) (Found: OMe, 31·8. Calc. for $C_7H_{15}NO_5$: OMe, 32·1%).

*Fraction* 3. Chromatography and ionophoresis of the syrup (214·5 mg.), $[\alpha]_D^{30}$ +15·9° (*c* 0·67 in $H_2O$), showed 3-*O*-methyl-D-xylose and a trace of the 2-*O*-methyl ether (Found: OMe, 18·7. Calc. for $C_6H_{12}O_5$: OMe, 18·9%).

*Fraction* 4. Chromatography of the syrup (1·66 g.) in solvent C showed a main component and a trace of 2,3,4-tri-*O*-methylglucuronic acid. The syrup (550 mg.) was converted into its methyl ester methyl glycoside and then reduced in ether solution with lithium aluminium hydride in ether. The product (470 mg.) was hydrolysed with N-sulphuric acid at 100° for 7 hr. The hydrolysate on chromatography and ionophoresis showed 2,3,4-tri-*O*-methyl-D-glucose, 3-*O*-methyl-D-xylose, and traces of 2,3-di-*O*-methyl-D-xylose. The sugars were separated on filter sheets with solvent A into two main fractions *a* and *b*. Fraction *a* (236·7 mg.) was 2,3,4-tri-*O*-methyl-D-glucose, $[\alpha]_D^{30}$ +70·1° (*c* 1·2 in $H_2O$) (Found: OMe, 41·6. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). The derived 2,3,4-tri-*O*-methyl-*N*-phenyl-D-glucosylamine had m. p. 143—144° (Found: OMe, 31·6. Calc. for $C_{15}H_{23}NO_5$: OMe, 31·3%). Fraction *b* (185·5 mg.) was shown by ionophoresis to be 3-*O*-methyl-D-xylose, $[\alpha]_D^{30}$ +14° (*c* 1·85 in $H_2O$) [derived 3-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. 135° (from ethyl acetate), $[\alpha]_D^{25}$ +81·3° (*c* 0·5 in EtOAc) (Found: OMe, 12·75; N, 5·6. Calc. for $C_{12}H_{17}NO_4$: OMe, 13·0; N, 5·9%).

*Molecular-weight Determinations.*—The number-average molecular weights of fractions 2A and 3 were determined by measuring the osmotic pressure of solutions of their sodium salts in 0·4M-aqueous sodium chloride.[15] The measurements were made by the static method at at 35° ± 0·02° with the Hellfritz osmometer [16] and a Gel Cellophane membrane. The molecular weight of the methylated hemicellulose was determined in butyl acetate with a membrane " Ultrazellafilter allerfeinst " supplied by Membrane Filter Gesellschaft, Göttingen.

The molecular weight of α-cellulose and cellulose residue were determined from the viscosity of their solutions in cuprammonium hydroxide by using the equation,[17] $[\eta] = 5 \times 10^{-4}M$. The measurements were made at 20° ± 0·05° in the British Cotton Industries Research Association standard capillary viscometer. The values obtained were 155,000 ± 1000 and 148,000 ± 1000 respectively.

The author thanks Dr. P. B. Sarkar for his interest and Dr. N. C. Ghosh, University College of Science and Technology, Calcutta, for doing the ionophoresis work.

TECHNOLOGICAL RESEARCH LABORATORIES,
INDIAN CENTRAL JUTE COMMITTEE, CALCUTTA, INDIA.
[Present address: DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY,
LAFAYETTE, INDIANA, U.S.A. [*Received, June 26th,* 1961.]

[15] Das, Das Gupta, and Basu, *J. Sci. Ind. Res., India,* 1953, **12**, *B*, 146.
[16] Hellfritz, *Makromol. Chem.*, 1951, **7**, 184.
[17] Staudinger and Daumiller, *Annalen*, 1937, **529**, 219.