The Polysaccharides of Chara *(a Fresh-water Alga). Part II.* The Isolation and Study of* Chara *Hemicellulose.*

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Chara hemicellulose (0·2%), on hydrolysis, gave D-glucose (85%), D-xylose (10·5%), L-arabinose (2·5%), and uronic acid (2%; probably D-glucuronic acid). Methylation of the hemicellulose or of its acetyl derivative and subsequent hydrolysis gave 2 : 3 : 4 : 6-tetra- (3·4%), 2 : 3 : 6-tri-, (90·5%), and 2 : 3-di-*O*-methyl-D-glucose (3·5%), and 2 : 3-di-*O*-methyl-D-xylose (2%).

Chara hemicellulose was extracted with dilute sodium hydroxide solution after primary extraction with hot water and dilute sodium carbonate (to remove carbohydrates other than hemicellulose). It was purified by several precipitations from alcohol, formation of an insoluble copper complex, and dialysis. The product was soluble in water, had a high positive optical rotation, and gave a purple colour with iodine changing to blue. It is hydrolysed by saliva. Hydrolysis by mineral acid and paper chromatography disclosed mainly glucose, with a little xylose and arabinose, identification being as glucosazone and di-*O*-benzylidene-D-xylose dimethyl acetal. The uronic acid (2%) was not galacturonic acid as it did not yield mucic acid. Further evidence was afforded by methylation of either the acetyl derivative or the hemicellulose with methyl sulphate and sodium hydroxide, followed by methyl iodide and silver oxide. The methylated material, after fractionation, had a high positive optical rotation. Examination of the fission products on the paper chromatogram showed mainly 2 : 3 : 6-tri- and small quantities of 2 : 3 : 4 : 6-tetra- and 2 : 4-di-*O*-methyl-D-glucose, and 2 : 3-di-*O*-methyl-D-xylose. The methylpentose arose from the contaminating xylan. Crystalline 2 : 3 : 6-tri-*O*-methyl-D-glucose and the crystalline aniline derivative of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose were isolated.

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* Part I, preceding paper.

EXPERIMENTAL

Chromatography, alkali extractions, and evaporations were done as in the preceding paper.

Preparation of Chara *Hemicellulose.*—*Chara* powder (2 kg.; cf. preceding paper) was extracted with 1·5% sodium carbonate solution (2 × 5 l.) at room temperature for 2 days, and with boiling water (20 × 20 l.) for 4 hr. until the extracts on evaporation and hydrolysis were non-reducing to Fehling's solution. The residue (850 g.) was dried and extracted at 100° with 4% sodium hydroxide solution (2 × 15 l.) for 3 hr. It was filtered through muslin, the filtrate centrifuged, the extract acidified with glacial acetic acid with ice-cooling and then centrifuged, and the clear centrifugate poured into 96% ethyl alcohol (5 parts) with stirring. The amorphous precipitate was separated in the centrifuge, then further purified by dissolution in ammonia and precipitation as the copper complex with 25% copper chloride solution. The copper complex was washed with water, then alcohol, followed by ethanolic hydrogen chloride (5%) until the washings were copper-free and finally with ethanol until chloride-free. The amorphous product was dried at 50° under reduced pressure (yield, 40 g., 0·2%); it had $[\alpha]_D^{28}$ +128·5° (*c*, 1 in N-NaOH), and contained sulphated ash 4%, N, 0·3%.

The finely powdered material (15 g.) was repeatedly dissolved in water and precipitated with acetone. The product was washed with alcohol and ether and dried to constant weight. Since analysis showed a high ash content (3·2%) and nitrogen content (0·3%), it was further purified by dialysis. The substance was dissolved in 0·01N-hydrochloric acid and dialysed for 48 hr. against water. After precipitation with acetone (5 parts), the hemicellulose was filtered off, redissolved, and reprecipitated with alcohol. The precipitate was washed with alcohol and ether and dried (yield, 8 g.).

This *Chara* hemicellulose was an amorphous material, dissolving slowly in hot water to form a viscous solution. It was insoluble in organic solvents, had $[\alpha]_D^{28}$ +130·5° (*c*, 0·6 in N-NaOH) and $\eta_{sp.}^{28}$ 0·047 (*c*, 0·2 in N-NaOH), and gave sulphated ash 0·01% and N 0·01%. It gave with iodine a purple colour, changing to blue. After the material had been treated with saliva, the iodine colour reaction was negative.

Hydrolysis of Chara *Hemicellulose.*—A portion (360 mg.) was heated with N-sulphuric acid (25 c.c.) at 100° for 12 hr. in a sealed tube. The acid, in which was suspended a small amount of flocculent material (9 mg.), was neutralised with barium carbonate and filtered, and the filtrate de-ionised with a mixture of Amberlite resin IR-120 and IR-400. It was then concentrated to a syrup (320 mg.). Examination of a portion on the paper chromatogram gave spots corresponding to glucose (R_G 0·09), arabinose (R_G 0·12), and xylose (R_G 0·15). A portion (102 mg.) of the hydrolysate was separated on a paper chromatogram; after being dried, the appropriate parts of the paper were extracted with hot methanol and the carbohydrates determined by Somogyi's copper reagent, giving glucose (85%), xylose (10·5%), and arabinose (2·5%). The amount (2%) of uronic acid was determined by Le Fèvre and Tollens's method (*Ber.*, 1907, **40**, 4513). The uronic acid was not galacturonic acid since with nitric acid it did not give mucic acid.

Another portion of the hydrolysate (150 mg.) was fractionated on the paper chromatogram and extracted with methanol. The first fraction had $[\alpha]_D^{25}$ +54·2° (equil.; *c*, 0·6 in H_2O). It gave an osazone, m. p. 205° undepressed on admixture with authentic glucosazone. The second fraction gave a dibenzylidene dimethyl acetal (Breddy-Jones, *J.*, 1945, 738), m. p. 211° undepressed on admixture with the D-xylose derivative.

Acetylation of Chara *Hemicellulose.*—A portion (4 g.) of the dry powdered polysaccharide was warmed with pyridine (100 c.c.) for 2 hr. at 70°. It was cooled and kept overnight at room temperature. Acetic anhydride (40 c.c.) was added dropwise during 0·5 hr. The mixture was incubated at 52° for 3 days. The clear solution was diluted with acetic acid (100 c.c.) and poured into ethyl alcohol (2 l.) with stirring. The white amorphous product was isolated by means of a centrifuge, washed with alcohol and ether, and dried (yield, 3·2 g.). Reacetylation was carried out as above, giving a product of $[\alpha]_D^{25}$ +128° (*c*, 0·8 in $CHCl_3$).

Methylation of Chara *Hemicellulose.*—(*a*) Hemicellulose (6 g.) was suspended in water (40 c.c.) and allowed to swell for 2 hr. The air was then displaced by nitrogen, a gentle stream of the gas being maintained throughout. The flask was cooled to 10° and 30% (w/w) sodium hydroxide (80 c.c.) was added with vigorous stirring. After 2 hr. methyl sulphate (40 c.c.) was added dropwise during 8 hr. Stirring was continued overnight, and the reaction was completed by heating at 50° for an hour. The mixture was cooled and treated with 30% sodium hydroxide solution (100 c.c.), followed by methyl sulphate (50 c.c.) as before. It was then neutralised with 0·5N-sulphuric acid and dialysed for 2 days. The methylation was repeated six times in presence of acetone (80 c.c.) at room temperature without cooling. The acidified mixture was extracted with chloroform, the solvent removed from the extract, and the product dissolved in 1 : 4 aqueous acetone. It was twice methylated with methyl sulphate and sodium hydroxide as above, giving a product of $[\alpha]_D^{25}$ +130° (*c*, 1 in $CHCl_3$) (Found : OMe, 38; sulphated ash, 0·2%). The methylated substance was treated with methyl iodide (80 c.c.) and silver oxide (40 g.) under reflux for 6 hr. and after the reaction was complete, had $[\alpha]_D^{25}$ +145° (*c*, 0·8 in $CHCl_3$) (Found : OMe, 41%).

(*b*) The acetylated hemicellulose was stirred with acetone (150 c.c.) and methylated with methyl sulphate (70 c.c.) and sodium hydroxide solution (140 c.c.) as above. After eight methylations the product was purified by precipitation from light petroleum (b. p. 40—60°), giving a white amorphous substance (3·6 g.). It was remethylated with methyl iodide and silver oxide, giving a product of $[\alpha]_D^{25}$ +150° (*c*, 1 in $CHCl_3$) (Found : OMe, 41·6%). The methylated product was fractionated by successive extractions under reflux with mixtures of light petroleum (b. p. 60—80°) and chloroform in the ratios 95 : 5, 90 : 10, and 85 : 15. The main product (80%) was found in the last solvent mixture; it had $[\alpha]_D^{25}$ +149° (*c*, 0·8 in $CHCl_3$) (Found : OMe, 41·5%).

Fission of Methylated Chara *Hemicellulose.*—(*a*) A portion (0·7 g.) was heated with methanolic hydrogen chloride (3%) in a sealed tube for 7 hr. at 100°. The product was neutralised with cold ethereal diazomethane, concentrated at normal temperature, and refluxed with 4% hydrochloric acid for 7 hr., then neutralised with silver carbonate, filtered, de-ionised with a mixture of Amberlite resins IR-120 and IR-400, and evaporated to a syrup (560 mg.). Paper chromatography indicated the presence of 2 : 3 : 4 : 6-tetra- (R_G 1·0), 2 : 3 : 6-tri- (R_G 0·83), and 2 : 3-di-*O*-methyl-D-glucose (R_G 0·57), and 2 : 3-di-*O*-methyl-D-xylose (R_G 0·74). The methyl sugars, determined by the alkaline hypoiodite micro-method of Hirst, Hough, and Jones (*loc. cit.*), were 2 : 3 : 4 : 6-tetra- (3·4%), 2 : 3 : 6-tri- (90·5%), and 2 : 3-di-*O*-methyl-D-glucose (3·5%), and 2 : 3-di-*O*-methyl-D-xylose (2%). Another portion of the hydrolysate was separated on the paper chromatogram, the methyl sugars located, and sections of the remainder of the chromatogram extracted with hot methanol, giving on evaporation 2 : 3 : 6-tri-*O*-methyl-D-glucose, m. p. and mixed m. p. 120° (from ether), $[\alpha]_D^{22}$ +66° (*c*, 1·8 in H_2O) (Found : OMe, 40·1. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

(*b*) The methylated polysaccharide (0·6 g.) was heated in methanolic 2% hydrochloric acid (50 c.c.) under reflux for 7 hr. The fully methylated sugar (27 mg.) was isolated from the mixture by Brown and Jones's quantitative procedure (*J.*, 1947, 1344). It was hydrolysed in N-sulphuric acid for 6 hr. to syrupy 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose (14 mg.), $[\alpha]_D^{20}$ +84° (*c*, 0·8 in H_2O). It gave an aniline derivative, m. p. and mixed m. p. 132° [from light petroleum (b. p. 60—80°)].

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