# *The Polysaccharides of* Chara *(a Fresh-water Alga). Part I. The Isolation and Study of* Chara *Cellulose.*

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*Chara* cellulose gives, on hydrolysis, only D-glucose. The preparation of cellobiose octa-acetate indicates a β-1 : 4-linkage, confirmed by the optical activity of the methylated polysaccharide which on hydrolysis gave 2 : 3 : 6-tri- and 2 : 3-di-*O*-methyl-D-glucose. The results indicate that *Chara* cellulose is similar to cotton cellulose.

Few of the carbohydrates occurring in fresh-water algae have been investigated, in contrast to those of marine algae (Whistler and Smart, "Polysaccharide Chemistry," Academic Press, New York, 1953; Hough, Jones, and Wadman, *J.*, 1952, 3393).

*Chara* (class Chlorophyceae; order Charales; family Characeae), freshly harvested in December, 1952, from Lake Idku, Egypt, was extracted successively with alcohol (to remove fats and chlorophyll), water, methanol, cold dilute sodium carbonate, and hot sodium hydroxide. The remaining insoluble product was neutral, and soluble in Schweizer's reagent, and gave a blue colour with iodine after treatment with zinc chloride. Hydrolysis gave 85% of D-glucose, the identity of which was confirmed by paper chromatography and osazone formation. Cellobiose octa-acetate was prepared, proving the β-1 : 4-linkage. Oxidation with periodate indicated a chain length of 180 glucose units. *Chara* cellulose was then exhaustively methylated with methyl sulphate and sodium hydroxide in an atmosphere of nitrogen. The specific rotation of the methylated product was similar to that of methylated cotton cellulose, thus supporting the β-1 : 4-linkage, and its hydrolysis products were mainly 2 : 3 : 6-tri-*O*-methyl-D-glucose (90%) with 4% of 2 : 3-di-*O*-methyl-D-glucose. The absence of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose suggests a chain length of not less than 100 glucose units. The presence of the small amount of di-*O*-methyl-D-glucose may be due to demethylation during the hydrolysis (cf. Hirst, Hough, and Jones, *J.*, 1949, 928; Barker, Bourne, and Wilkinson, *J.*, 1950, 3027) or to incomplete methylation. The above results support those of Hough, Jones, and Wadman (*loc. cit.*) and of Percival and Ross (*J.*, 1949, 3041) concerning the relation of algal cellulose to cotton cellulose.

## Experimental

Evaporations were done at 50° under reduced pressure unless otherwise stated. Chromatography was carried out on Whatman No. 1 filter paper by the descending method (Partridge, *Biochem. J.*, 1948, **42**, 238) with *n*-butanol–ethanol–water (5 : 1 : 4 v/v). Sugars were detected on the paper chromatogram with *p*-anisidine hydrochloride. All treatments with alkali were done in a nitrogen atmosphere.

*Preparation of* Chara *Cellulose.*—The alga (10 kg.) was a tangled mass of long green filaments (2 mm. in diameter). It was cleaned and washed in water. After being drained from excess of moisture the filaments were steeped in ethyl alcohol (6 + 6 + 7 l.) for 3 days. The insoluble product was dried in a vacuum at room temperature, ground to a fine pale green powder (yield, 1 kg.), and extracted with hot water (4 l.), methyl alcohol (4 l.; 6 hr.), 1·5% sodium carbonate solution (4 l.; 3 days; removed a trace of polysaccharide), 4% sodium hydroxide solution at 100° (4 l.; 3 hr.; removed 0·2% of polysaccharide) and 25% sodium hydroxide at 100° (4 l.; 3 hr.; removed 0·04% of polysaccharide). The extracts were clarified on the centrifuge (3500 r.p.m.) and then poured into ethyl alcohol (5 parts) with vigorous stirring. An appreciable precipitate appeared from the 4% sodium hydroxide extract and was proved to contain carbohydrate. The residual insoluble fluffy material was washed with water and 2N-acetic acid and extracted with ethyl alcohol and then acetone (Soxhlet) for 12 hr. The insoluble material was dried to constant wt. (yield, 400 g., 4% of the fresh alga) and gave sulphated ash 2·5%, N 0·1%.

*Hydrolysis of* Chara *Cellulose.*—A portion (0·2 g.) was hydrolysed with 72% sulphuric acid according to Monier-Williams's directions (*J.*, 1921, **119**, 803), giving 85% of D-glucose (cf. 86%

for cotton cellulose), determined by Somogyi's method (*J. Biol. Chem.*, 1933, **100**, 695) and by polarimetry. Paper chromatography showed only glucose ($R_G$ 0·09). An osazone was prepared in pale yellow needles of the same shape as glucosazone and having m. p. and mixed m. p. 205°.

*Chain Length of* Chara *Cellulose*.—A portion (80 mg.) in each of five bottles was oxidised with potassium periodate (Brown *et al.*, *Nature*, 1945, **156**, 785). The amount of formic acid corresponds to a chain length of 180 glucose units (cf. 200 units for cotton cellulose).

*Cellobiose Octa-acetate*.—This was prepared by Hibbert and Barsha's method (*Canad. J. Res.*, 1934, **10**, 178). Recrystallised from ethyl alcohol, it had m. p. and mixed m. p. 221°, $[\alpha]_D^{25}$ +39° (*c*, 0·5 in $CHCl_3$).

*Methylated* Chara *Cellulose*.—The cellulose (12 g.) was methylated eight times with methyl sulphate and 30% sodium hydroxide solution by the method of Haworth, Montonna, and Peat (*J.*, 1939, 1899) (yield, 9·2 g.) (Found : OMe, 42·5; sulphated ash, 0·4%); $[\alpha]_D^{25}$ −15·2° (*c*, 1·8 in $CHCl_3$).

A portion (560 mg.) was hydrolysed by the method of Hough, Jones, and Wadman (*loc. cit.*) (yield, 520 mg.). Paper chromatography gave spots corresponding to 2 : 3 : 6-tri- ($R_G$ 0·83) and 2 : 3-di-*O*-methyl-D-glucose ($R_G$ 0·57). The two methyl sugars were estimated quantitatively by alkaline hypoiodite (Hirst, Hough, and Jones, *loc. cit.*), a phosphate buffer being used according to Chanda *et al.* (*J.*, 1950, 1289), giving 90% of tri- and 4% of di-*O*-methyl-D-glucose.

A portion (150 mg.) of the hydrolysate was separated on the paper chromatogram and the methyl sugars were extracted (Hough, Jones, and Wadman, *loc. cit.*). The first fraction gave 2 : 3 : 6-tri-*O*-methyl-D-glucose, m. p. and mixed m. p. 120°, $[\alpha]_D^{22}$ +69° (*c*, 1·5 in $H_2O$) (Found : OMe, 39·5. Calc. for $C_9H_{18}O_6$ : 41·9%).

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