The Polysaccharides of Colocasia antiquorum (*Taro or Colocass*).

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The starch of *Colocasia* tubers contains 28% of amylose. Periodate oxidation, and methylation and hydrolysis, indicate a chain length of 490 glucose units for amylose and 22 units for amylopectin. These results are similar to those for potato starch.

Colocasia mucilage yields D-galactose (88%), L-arabinose (8%), and uronic acid (2%). Hydrolysis of the main methylated fraction gave 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose (10%), 2 : 3 : 6-tri-*O*-methyl-D-galactose (87%), and 2 : 4-di-*O*-methyl-D-galactose (2·5%).

Colocasia (family Araceae) is widely cultivated and used as common food in Egypt. Its polysaccharides can be roughly distinguished as a water-soluble mucilage and insoluble starch granules.

The starch was isolated from the tubers after complete removal of the mucilage; its blue value corresponded to an average amylose content of 28·0%. It was then separated into amylose (30%) and amylopectin. Oxidation with periodate suggested a chain length of about 490 glucose units for amylose and 22 units per end-group for amylopectin. The latter value was found to be in agreement with the result obtained by hydrolysis of methylated amylopectin. Examination of the fission products of the methylated amylose and amylopectin on a paper chromatogram showed mainly 2 : 3 : 6-tri-*O*-methylglucose and a little 2 : 3-di-*O*-methylglucose. 2 : 3 : 4 : 6-Tetra-*O*-methylglucose was found only in the hydrolysate of methylated amylopectin, suggesting a chain length of not less than 100 units for amylose and 22 units for amylopectin (Hough, Jones, and Wadman, *J.*, 1952, 3393). The dimethylglucose may have arisen from incomplete methylation or from demethylation during hydrolysis (Hirst, Hough, and Jones, *J.*, 1949, 928; Barker, Bourne, and Wilkinson, *J.*, 1950, 3027). Confirmation of the above results was obtained by the isolation of crystalline glucosazone, 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-glucosylamine, 2 : 3 : 6-tri-*O*-methyl-D-glucose, and syrupy 2 : 3-di-*O*-methyl-D-glucose.

Colocasia mucilage was extracted from the tubers with water at room temperature and purified by several reprecipitations from alcohol and formation of a water-insoluble copper complex. It had a low positive optical rotation and exhibited a slight reducing action on

Fehling's solution. Attempts to separate the pentose and uronic acid by fractional precipitation were unsuccessful. Hydrolysis of the mucilage by mineral acid and paper chromatography gave galactose (88%) and arabinose (8%), identification being as D-galactose methylphenylhydrazone, mucic acid, and L-arabinose α-benzoylphenylhydrazone. The uronic acid content was 2%. The occurrence of D-galactose and L-arabinose is common in a wide variety of plant materials. Further information concerning the mode of linkage of the various components in *Colocasia* mucilage was obtained by methylation of the acetyl derivative with methyl sulphate and sodium hydroxide, followed by methyl iodide and silver oxide and fractionation. The low negative specific rotation of the main fraction indicates that the sugar units are joined by β-linkages. Hydrolysis and examination of the fission products on the paper chromatogram showed 2 : 3 : 4 : 6-tetra-*O*-methylgalactose (10%), 2 : 3 : 6-tri-*O*-methylgalactose (87%), and 2 : 4-di-*O*-methylgalactose (2·5%). This suggests that the main linkage in the polysaccharide is through carbon atoms 1 and 4. Hydrolysis of the second fraction gave 2 : 3 : 5-tri-*O*-methylarabinose (19%), besides the above-mentioned methyl-sugars. In confirmation, crystalline 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine, 2 : 3 : 6-tri-*O*-methyl-D-galactonolactone, 2 : 3 : 5-tri-*O*-methyl-L-arabonolactone, and 2 : 3 : 5-tri-*O*-methyl-L-arabonamide were prepared, as well as syrupy 2 : 4-di-*O*-methyl-D-galactose.

Experimental

Chromatography was carried out on Whatman No. 1 filter paper sheets by the descending method (Partridge, *Biochem. J.*, 1948, **42**, 238), *n*-butanol–ethanol–water (5 : 1 : 4 v/v) being used. Sugars and methyl-sugars were detected with *p*-anisidine hydrochloride as spray reagent. Specific rotations and viscosity determinations were carried out at 28°. Evaporations were done under reduced pressure at 50° unless otherwise stated. The results were calculated on an ash-free basis and on an actual starch content. Treatments with alkali were done in nitrogen atmosphere.

Preparation of Colocasia *Starch.*—*Colocasia* tubers (10 kg.) were freshly harvested in February, 1953 (Alexandria, Egypt). The average weight of each tuber was ½ kg. The tubers were washed thoroughly, the outer fibrous material was removed, and the flesh cut into thin slices and soaked in water (20 l.) containing sulphur dioxide for a week. The starch was isolated according to directions of Hirst, Jones, and Roudier (*J.*, 1948, 1779). The fatty acids were removed by Schoch's method (*J. Amer. Chem. Soc.*, 1942, **64**, 2954), and the product was dried at room temperature and pressure (yield 200 g., 2%). The yield was not quantitative as much of the original starch remained enclosed in the unruptured cells.

Properties of Colocasia *Starch.*—Microscopic examination revealed traces of fibrous material admixed with the granules (size 3—10 μ). The blue value was 0·47 (procedure of Wilson, Schoch, and Hudson, *ibid.*, 1943, **65**, 1380). Based on a blue value of 1·64 for amylose and 0·01 for amylopectin, this suggests an amylose content of 28% (Found : H_2O, 14%; sulphated ash, 0·3%; N, 0·05%). $[\alpha]_D$ was +160° (*c*, 0·5 in 4% NaOH). The presence of anthocyanins was indicated according to the procedure of Campbell, Frahn, Hirst, Packman, and Percival (*J.*, 1951, 3489) : the pink colour changed to green on addition of a slight excess of sodium hydroxide solution. The gelation point was 70—75°, above which the starch formed a pale buff paste. The granules dispersed with difficulty in N-sodium hydroxide to give a pale brown solution.

According to a report by Prof. Dr. M. S. Ridi, Kasr El Aini, Faculty of Medicine, Cairo, the composition of *Colocasia* and potato tubers was as tabulated for duplicate analyses.

	Colocasia	Potato		*Colocasia*	Potato
Water (%)	70·2, 74·0	76·2, 69·6	Fibre (%)	0·69, —	— 1·4
Protein (%)	1·9, 2·0	1·9, 0·35	Ash (%)	1·6, 0·8	1·1, 0·53
Fat (%)	0·15, 0·04	0·2, 0·2	Caloric value	89·9, 98·0	90·0, 113·6
Carbohydrate (%) ...	21·5, 21·8	20·1, 27·6			

If the percentage (86·0%) of edible matter is considered, the amount of carbohydrate and the calorific value of *Colocasia* exceed that of potato.

Hydrolysis of Colocasia *Starch.*—A portion (60 mg.) was hydrolysed according to the procedure of Hough, Jones, and Wadman (*loc. cit.*), giving a syrup (52 mg.). Examination on the paper chromatogram showed only glucose (R_G 0·09), $[\alpha]_D$ +54° (equil.; *c*, 0·5 in H_2O). It gave glucosazone, m. p. and mixed m. p. 205°. Quantitative determination (Flood, Hirst, and Jones, *J.*, 1948, 1679) accounted for 85% of starch.

Fractionation of Colocasia *Starch*.—This was done according to the procedure of Bourne, Donnison, Haworth, and Peat (*J.*, 1948, 1687), with 60 g. of defatted material. The amylose was then further purified by four reprecipitations with butanol (yield 18 g.).

Amylose. This was a pale brown amorphous substance. It dissolved with difficulty in sodium hydroxide solution, giving a pale brown opaque solution. It had $[\alpha]_D$ +150° (*c*, 0·4 in 4% NaOH) (Found : H_2O, 14%; sulphated ash, 0·4%; N, 0·06%).

The amylopectin was isolated from the mother-liquor after precipitation of amylose, by addition of ethanol (5 parts) (yield 38 g.). It was a white amorphous substance, dissolved in sodium hydroxide solution to a clear solution, and had $[\alpha]_D$ +152° (*c*, 0·9 in 4% NaOH), and H_2O 15%.

The viscosity was measured according to the procedure of Potter, Hassid, and Joslyn (*J. Amer. Chem. Soc.*, 1949, **71**, 4075), being 0·95 for amylose and 0·92 for amylopectin.

Chain Length of Colocasia *Amylose and Amylopectin*.—A series of five portions each of amylose and amylopectin (200 mg. each) were treated with sodium periodate (Potter and Hassid, *ibid.*, 1948, **70**, 3488). The amount of 0·01N-barium hydroxide (0·75 and 5·57 ml.) after 25 hr. was taken as the end-point : it gave 22 glucose units as an average number per end-group for amylopectin and 490 units for amylose.

Acetylation of Colocasia *Amylose and Amylopectin*.—Each polysaccharide (4 g.) was twice acetylated by Hirst, Jones, and Roudier's method (*loc. cit.*), giving white amorphous substances (yield, 80% and 82% respectively), $[\alpha]_D$ (amylose) +172° (*c*, 1 in $CHCl_3$), (amylopectin) +168° (*c*, 1 in $CHCl_3$).

Methylation of Colocasia *Amylose*.—A portion of acetylated derivative (2 g.) was stirred with acetone (50 c.c.) and methylated eight times with methyl sulphate and sodium hydroxide (Clinton, Ballou, and Percival, *J.*, 1952, 1054). The final product was fractionated by successive extractions with light petroleum (b. p. 60—80°)–chloroform in the proportions, 90 : 10, 85 : 15, and 80 : 20. The main product (1·3 g.), obtained from the middle solvent mixture, had $[\alpha]_D$ +210° (*c*, 0·5 in $CHCl_3$) (Found : OMe, 41. Calc. for $C_9H_{16}O_5$: OMe, 45·6%), $\eta_{sp.}$ 0·4 (*c*, 0·5 in *m*-cresol). The other two fractions had the same methoxyl content and viscosity as above.

Hydrolysis of Methylated Colocasia *Amylose*.—A portion (400 mg.) was hydrolysed according to the procedure of Hough, Jones, and Wadman (*loc. cit.*) to a syrup (340 mg.) which on the paper chromatogram showed spots corresponding to 2 : 3 : 6-tri- (R_G 0·83) and 2 : 3-di-*O*-methylglucose (R_G 0·57). Quantitative estimation of the methyl sugars (Hirst, Hough, and Jones, *loc. cit.*), a phosphate buffer being used (Chanda *et al.*, *J.*, 1950, 1289), gave 2 : 3 : 6-tri- (94%) and 2 : 3-di-*O*-methylglucose (3%), the first of which was isolated crystalline from the paper chromatogram {yield 70 mg.; m. p. and mixed m. p. 120—121°, $[\alpha]_D$ +69° (equil.; *c*, 2·0 in H_2O)}.

Methylation of Colocasia *Amylopectin*.—The acetylated substance (2 g.) was methylated eight times as above. Attempts to fractionate the product with light petroleum and chloroform, as above, gave a main product (1·6 g.) from the 85 : 15 mixture, having $[\alpha]_D$ +212° (*c*, 0·5 in $CHCl_3$), $\eta_{sp.}$ 0·3 (*c*, 0·5 in *m*-cresol) (Found : OMe 42·5%). The two other fractions had the same methoxyl content and viscosity.

Hydrolysis of Methylated Colocasia *Amylopectin*.—(*a*) A portion (350 mg.) was hydrolysed as above, giving a syrup (320 mg.) the components of which were separated on the paper chromatogram, giving spots of 2 : 3 : 4 : 6-tetra- (R_G 1·00), 2 : 3 : 6-tri- (R_G 0·83), and 2 : 3-di-*O*-methylglucose (R_G 0·57). The methyl-sugars were determined as above : 2 : 3 : 4 : 6-tetra- (4·5%), 2 : 3 : 6-tri- (89%), and 2 : 3-di-*O*-methylglucose (5%). Another portion of the hydrolysate (170 mg.) was fractionated on the paper chromatogram, and crystalline 2 : 3 : 6-tri-*O*-methyl-D-glucose (85 mg.) isolated, with m. p. and mixed m. p. 121°, $[\alpha]_D$ +70° (equil.; *c*, 2·0 in H_2O) (Found : OMe, 41·0. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

(*b*) Methylated amylopectin (0·7 g.) was heated in methanolic 2% hydrochloric acid (50 c.c.) under reflux for 7 hr. The fully methylated sugar (42 mg.) was isolated from the mixture by Brown and Jones's method (*J.*, 1947, 1344). It was hydrolysed in N-sulphuric acid for 6 hr. to syrupy 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose (29 mg.), $[\alpha]_D$ +82° (*c*, 1 in H_2O) (Found : OMe, 52·0. Calc. : OMe, 52·5%). It gave an aniline derivative (8 mg.), m. p. and mixed m. p. 132° by the method of Hough, Jones, and Wadman (*loc. cit.*).

Colocasia *Mucilage*.—Tubers (12 kg.) were cleaned thoroughly, the fibrous outer skin was removed, and the flesh cut into thin slices. After 24 hours' soaking in N-hydrochloric acid (18 l.) the mucilage was dispersed in the aqueous solution. It was filtered through a sieve (50-cm. diameter, mesh 2 mm.). Extraction was repeated and the slices were then discarded. The whole extract was shaken with charcoal for 4 hr. at room temperature, then twice centrifuged

(3500, then 24,000 r.p.m.). The clear centrifugate was poured into 96% ethyl alcohol (5 parts) with stirring, giving a snow-white fibrous material. To reduce the high ash content (4·6%) the amorphous product was redispersed in water, 5% hydrochloric acid was added to a final normality of N, and the mucilage dialysed for 24 hr. (yield 360 g., 3%). The substance was further purified by dissolution in N-sodium hydroxide, acidification with acetic acid, centrifugation, and precipitation with alcohol. A portion (50 g.) was suspended in water (2·5 l.) and ammonia (*ca.* 2N) was added, with shaking, until the solution was neutral. A little undissolved material was removed on the centrifuge. A 10% solution of copper chloride (1 l.) was added dropwise and the resultant insoluble complex was removed on the centrifuge, and washed with water and alcohol, followed by ethanolic hydrogen chloride (5%) until the washings were copper-free and finally with ethanol until chloride-free. The product was dried to constant wt. in a vacuum at 50° (yield 42 g.). The mother-liquor after precipitation of the copper complex was concentrated to 100 c.c. and treated with alcohol (5 parts). The precipitate was treated as above, giving amorphous white material (1·5 g.), proved to be a polysaccharide.

Properties of Colocasia *Mucilage*.—It was a white amorphous substance insoluble in organic solvents, dissolved with difficulty in water but easily in alkali, had little reducing action on Fehling's reagent, was unaffected by saliva during 10 min. (Found: sulphated ash, 0·2; N, 0·05%), had $[\alpha]_D$ +13·4° (*c*, 0·6 in N-NaOH), $\eta_{sp.}$ 0·2 (c, 0·1 in N-NaOH), and gave 2% of uronic acid according to Lefèvre and Tollens's method (*Ber.*, 1907, **40**, 4513).

Acid Hydrolysis of Colocasia *Mucilage*.—A portion (650 mg.) was heated with N-sulphuric acid (5 c.c.) at 100° in a sealed tube for 12 hr. Flocculent material (15 mg.) was filtered off, and the filtrate neutralised with barium carbonate, de-ionised with a mixture of Amberlite resins IR-120 and IR-400 and concentrated to a syrup (580 mg.). Examination of the hydrolysate on the paper chromatogram showed spots of galactose (R_G 0·07) and arabinose (R_G 0·12). Quantitative determination of these sugars (Flood, Hirst, and Jones, *loc. cit.*) indicated galactose 88% and arabinose 8% (calc. as $C_6H_{10}O_5$ and $C_5H_8O_4$ respectively). Another portion of the hydrolysate (140 mg.) was fractionated on the paper chromatogram and the fractions were extracted with methyl alcohol. The first fraction had $[\alpha]_D$ +82° (equil.; *c*, 0·5 in H_2O), and gave D-galactose methylphenylhydrazone, m. p. and mixed m. p. 185° (decomp.) (Hirst, Jones, and Woods, *J.*, 1947, 1048), and mucic acid, m. p. and mixed m. p. 215° (Heyne and Whistler, *J. Amer. Chem. Soc.*, 1948, **70**, 2249). The second fraction had $[\alpha]_D$ +105° (equil.; *c*, 0·5 in H_2O), and gave L-arabinose α-benzoylphenylhydrazone (8 mg.), m. p. and mixed m. p. 170—171° (Hirst, Jones, and Woods, *loc. cit.*).

Acetylation of Colocasia *Mucilage*.—A portion (4·3 g.) of the dry powdered polysaccharide was warmed with pyridine (100 c.c.) for 2 hr. at 70°, then cooled and kept overnight at room temperature. Acetic anhydride (40 c.c.) was added dropwise during 1 hr. The mixture was incubated at 52° for 3 days. The solution was diluted with glacial acetic acid (100 c.c.) and poured into ethyl alcohol (2 l.; 96%) with stirring. The white amorphous product was isolated at the centrifuge, washed with alcohol and ether, and dried (yield 3·5 g.). Acetylation was repeated as above, giving a product (2·9 g.) of $[\alpha]_D$ +35° (*c*, 1 in $CHCl_3$).

Methylation of Colocasia *Mucilage*.—The acetylated material (2·8 g.) was stirred with acetone (150 c.c.) and methylated with methyl sulphate (70 c.c.) and 30% sodium hydroxide solution (140 c.c.) at 10° during 8 hr. Stirring was continued overnight at room temperature, and the reaction was completed by heating at 50° for ½ hr. After eight methylations, the product was purified by precipitation from light petroleum (b. p. 40—60°), giving a white amorphous substance (1·9 g.). It was remethylated twice with methyl iodide and silver oxide, giving a product (1·8 g.) of $[\alpha]_D$ −15° (*c*, 1 in acetone) (Found: OMe, 42%), $\eta_{sp.}$ 0·3 (*c*, 1 in $CHCl_3$). An attempt to fractionate the methyl derivative with light petroleum (b. p. 40—60°)–chloroform in the ratios 95 : 5, 90 : 10, and 85 : 15 gave mainly two fractions. The fraction (1·2 g.) from the last solvent mixture (85 : 15) had $[\alpha]_D$ −13° and gave furfuraldehyde 1·2%. The middle fraction (0·6 g.) had $[\alpha]_D$ −15° and gave furfuraldehyde 14·5%. The first fraction (600 mg.) 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 room 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 (450 mg.). Examination on the paper chromatogram showed spots of 2 : 3 : 4 : 6 tetra- (R_G 0·88), 2 : 3 : 6 tri- (R_G 0·71), and 2 : 4-di-*O*-methylgalactose (R_G 0·41). In a quantitative experiment the proportion of the methyl sugars was determined by the alkaline hypoiodite procedure of Hirst, Hough, and Jones (*loc. cit.*), giving 2 : 3 : 4 : 6-tetra- 10%, 2 : 3 : 6-tri- 87%, and di-*O*-methylgalactose 2·5%.

Another portion of the hydrolysate (160 mg.) was separated on the paper chromatogram, the methyl sugars were located, and sections of the different fractions extracted with hot methanol. The first fraction (Found : OMe, 50·5. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%) gave 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine (12 mg.), m. p. and mixed m. p. 194° (Found : OMe, 38·9. Calc. for $C_{16}H_{25}O_5N$: OMe, 39·9%) (Hough and Jones, *J.*, 1950, 1199). The second fraction had $[\alpha]_D$ +88° (*c*, 1 in H_2O) (Found : OMe, 39·0. Calc. for $C_9H_{18}O_6$: OMe, 41·8%) and gave crystalline 2 : 3 : 6-tri-*O*-methyl-γ-D-galactonolactone (50 mg.), m. p. and mixed m. p. 98—99° (Hough and Jones, *loc. cit.*).

A portion (500 mg.) of the second methylated polysaccharide fraction was hydrolysed and the hydrolysate examined on the paper chromatogram as above. Spots of the above mentioned sugars were observed besides a spot of 2 : 3 : 5-tri-*O*-methylarabinose (R_G 0·64). That fraction was isolated from the paper chromatogram (95 mg.). It gave 2 : 3 : 5-tri-*O*-methyl-L-arabonolactone (30 mg.), m. p. and mixed m. p. 28°, and 2 : 3 : 5-tri-*O*-methyl-L-arabonamide (22 mg.), m. p. and mixed m. p. 134°, according to the procedure of Hirst, Jones, and Walder (*J.*, 1947, 1225).

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