490. *The Constitution of a Glucosan from the Fungus* Polyporus betulinus.

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The flesh of the bracket fungus *Polyporus betulinus* gave glucose (*ca.* 60%) and a residue (*ca.* 30%) on hydrolysis. Only traces of sugars other than glucose were present.

Examination of the products of hydrolysis of the methylated fungus flesh indicated a ratio of approximately 1 mole of tetra- to 12—14 moles of tri-, 4 moles of di- and 1 mole of mono-methyl glucose. 2 : 3 : 4 : 6-Tetramethyl, 2 : 4 : 6-trimethyl, and 4 : 6-dimethyl glucose were obtained crystalline.

The flesh of the fungus *Polyporus betulinus* (class Basidiomycetes, order Hymenomycetes) is white and soft, becoming cork-like in the mature fungus. The present preliminary work suggests that this flesh consists, in part at least, of a glucosan with 1 : 3-linkages, of which a proportion may be of the α-type.

In the laboratory (unpublished work by T. M. Forrester, Microbiological Section), and probably under natural conditions, the fungus can obtain energy for synthetical activities by breakdown of cellulose, a β-1 : 4-linked glucose polymer. The fungus is said to cause a "brown rot" of timber since cellulose is decomposed and a brown lignin-like material remains.

Previous work on the "cellulose" obtained from this fungus includes that of Winterstein (*Ber.*, 1895, **28**, 774) and Proskuriakow (*Biochem. Z.*, 1926, **68**, 167); it seems possible that a pure glucosan was not prepared by these workers since a yield of only 78% of glucose was obtained on hydrolysis, and in the present preliminary work no attempt was made to isolate a similar glucosan. The fungus flesh was purified by removal of water-soluble materials, and paper chromatography of the hydrolysate showed that sugars other than glucose were present in negligible amount only. A yield of 60% of glucose was obtained from this crude polysaccharide. The methylation procedure adopted was expected to remove impurities, and the nitrogen content (0·5%) of the material was reduced (to 0·14%) by this procedure, showing that a considerable fractionation had taken place. Furthermore, the methoxyl content (42%) of the methylated material was reasonably close to that required for a fully methylated hexosan, and only tetramethyl glucose and partially methylated glucoses could be distinguished in the hydrolysate by paper chromatography and by partition chromatography on a cellulose column (Hough, Jones, and Wadman, *J.*, 1949, 2511). In the latter case, 2 : 3 : 4 : 6-tetramethyl, 2 : 4 : 6-trimethyl, and 4 : 6-dimethyl glucose were obtained crystalline in amounts corresponding approximately to the ratios obtained by estimation of the above sugars on the paper chromatogram with hypoiodite solution (Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289). A certain amount of decomposition was apparent when the methylated material was hydrolysed, and only 75% recovery was obtained. 85% of the material added to the cellulose column was recovered in the eluate. The isolation of 2 : 4 : 6-trimethyl glucose indicates the presence of 1 : 3-linkages in the polysaccharide but other modes of linkage cannot at present be excluded. The proportion of tetramethyl glucose "end group" indicates a repeating unit of about 19 glucose residues, but this value can only be regarded as provisional.

The methylated fungus, $[\alpha]_D^{18}$ +68° (*c*, 1·98 in chloroform), may contain a proportion of α-linkages, as methylated yeast glucan with $[\alpha]_D$ +3·3° (*c*, 1·3 in chloroform) (Bell and Northcote, *J.*, 1950, 1944) and methylated laminarin with $[\alpha]_D^{15}$ −7·5° (*c*, 1·0 in chloroform) (Connell, Hirst, and Percival, *J.*, 1950, 3494) have β-linkages. The rotation of Winterstein's "fungus cellulose" from *Polyporus betulinus* {$[\alpha]_D$ +240° (*c*, 4·0 in 5% sodium hydroxide)} and the rotation (+210°) of that part of the fungus flesh dissolved initially by sodium hydroxide solution support this suggestion. The preparation of a pure glucosan from the larger supply of fungus flesh now available is being attempted.

Experimental

Preparation of a Crude Polysaccharide from Polyporus betulinus *Flesh.*—The fruiting bodies of the fungus (4—8″ diam.) were cut into large pieces and the stem, the spore-bearing tissue, and the brown outer cuticle removed. The white cork-like flesh was cut into smaller strips, dried in air at room temperature, and milled to pass a 2-mm. mesh sieve. After removal of of material soluble in hot water, the flesh was dried *in vacuo*.

Chemical Properties of the Flesh.—Analyses were carried out on several samples collected at different times [Found : H_2O, 8—13; N, 0·46—0·52; ash, 1·2—1·5; alcohol–benzene extract, 16·4—18·4; uronic anhydride, negligible; chitin, 2·9—3·4% (Black and Schwartz, *Analyst*, 1950, **75**, 185)], $[\alpha]_D^{17}$ +210° [*c*, 2·52, in 5% sodium hydroxide solution; in 10 minutes only 60% of the material dissolved in the alkali (determined by separating the residue on the centrifuge, washing it, and drying it to constant weight at 100°) and the rotation is calculated on the basis of the material dissolved]. The material gave no blue colour with zinc chloride and iodine (as distinct from cellulose). The flesh was practically insoluble in water and in dilute acids. Schweizer's reagent dissolved 18% of the material.

Hydrolysis of the Fungus Flesh.—The flesh was hydrolysed by Monier-Williams's method, and a yield of 60% of the theoretical amount of glucose was obtained. An insoluble residue (30% of the starting material) was left after the acid hydrolysis. Glucose was determined gravimetrically, by polarimetric measurement, and by copper reduction. Paper chromatography of the hydrolysate showed that glucose was the only sugar present in quantity although traces of fructose, galactose, and xylose were observed. Paper chromatograms were run in a thermostatically controlled cabinet at 32° (Hough, Jones, and Wadman, *loc. cit.*), Whatman No. 1 paper and butanol saturated with water being used.

Methylation.—The flesh (15·0 g.; 8·5% H_2O) was methylated with 30% sodium hydroxide (1000 ml.), methyl sulphate (150 ml.), and dioxan (100 ml.) at room temperature and in an atmosphere of nitrogen. After destruction of methyl sulphate, the neutral solution was evaporated, dialysed against tap water until free from sulphates, and evaporated to a small bulk. After nine such methylations, the aqueous solution was extracted with chloroform, the extract evaporated and dried (Na_2SO_4), and light petroleum (b. p. 60—80°) added until no further precipitate was obtained. The methylated polysaccharide was dried *in vacuo* (overall yield, 35%) and then had $[\alpha]_D^{18}$ +68° (*c*, 1·98 in chloroform) [Found : N, 0·14 (microKjeldahl); OMe, 39·7; ash, 5·2%]. Three methylations with Purdie's reagents raised the methoxyl content to 42%.

Hydrolysis of the Methylated Polysaccharide.—The methylated glucosan (2·570 g.) was left overnight at room temperature in contact with glacial acetic acid (35 ml.). Hydrochloric acid (8%; 35 ml.) was added to the jelly, and the mixture was boiled for 7 hours. After neutralisation of the mixture with barium carbonate, barium salts and insoluble decomposition products were removed by filtration, the solution de-ionised by passage through large columns of Amberlite resins, and the effluent evaporated to dryness, ethanol and benzene being used to remove the last traces of water. The residue was exhaustively extracted with chloroform, and the extract evaporated to a syrup which was dried (P_4O_{10}) to constant weight (1·712 g., 74% yield; allowing for moisture and ash in the starting material) at room temperature/0·2 mm.

The hydrolysate was examined by paper chromatography, and the identity of the partially methylated sugars was established by comparison with authentic specimens. R_G values are not given, since experience with the apparatus used for paper chromatography at 32° has shown that reproducible values are difficult to obtain, although separations are usually better than those achieved at room temperature. The presence of tetramethyl glucose, 2 : 4 : 6-trimethyl glucose (easily distinguished from the 2 : 3 : 6-isomer), dimethyl glucose, and smaller amounts of monomethyl glucose and glucose was shown. Estimation by Chanda, Hirst, Jones, and Percival's method (*loc. cit.*) gave the molar ratio of the first three of the above sugars as 1 : 12 : 4. As mentioned by these authors, heavy spotting was necessary to obtain sufficient " end group " for estimation with the above reagents. The operation was facilitated by supporting the paper about 1 cm. above an electric heating pad on strips of asbestos. The pad was constructed with a resistance mat (25 × 16 cm.; 200 watts) wrapped in three thicknesses of asbestos paper and mounted on a suitable heat-resisting base. The heating effect was controlled so that solvent evaporated rapidly but the residue on the paper was not subjected to undue heat.

Separation and Identification of Methylated Glucoses from the Hydrolysate.—The syrupy hydrolysate (1·689 g.) was transferred with *n*-butanol to a column (115 × 2·5 cm.) packed with

Whatman's ashless cellulose powder and enclosed in a water jacket, the contents of which were circulated and kept at 32° by an electrical device. The column was washed with a mixture of *n*-butanol (2 vol.) and light petroleum (b. p. 100—120°; 3 vol.), and fractions (4—5 ml.) collected at 30-minute intervals with the aid of an automatic fraction cutter, similar to that described by Hough, Jones, and Wadman (*loc. cit.*). The distribution of the sugars was followed by paper chromatography.

The eluates were grouped appropriately, and the solvent removed at 35°/20 mm. The residues crystallised and, after being dissolved in water and filtered to remove impurities, were dried to constant weight at room temperature/0·2 mm. over phosphoric oxide.

Fraction A (0·1425 g.). Paper chromatography showed that only one sugar was present since the material travelled as a single compact spot with an R_G value identical with that of an authentic specimen of 2 : 3 : 4 : 6-tetramethyl glucose. The fraction was contaminated with dark impurities due probably to the somewhat drastic hydrolytic conditions employed. The amount of tetramethyl glucose present was calculated from the weight and methoxyl content of the crude material (Found : OMe, 29·0. Calc. for $C_{10}H_{20}O_6$: 52·5%) and amounted to 0·080 g.

After suitable purification and one recrystallisation from light petroleum (b. p. 60—80°), material was obtained with $[\alpha]_D^{12}$ +82° (*c*, 0·39 in water) and m. p. 89°, and did not depress the melting point of an authentic specimen.

Fraction B (0·997 g.). The material travelled on the paper chromatogram as a single compact spot with an R_G value identical with that of 2 : 4 : 6-trimethyl glucose and easily distinguishable from the spot from 2 : 3 : 6-trimethyl glucose (Found : OMe, 40·2. Calc. for $C_9H_{18}O_6$: 41·8%) (weight 0·959 g., corrected in accordance with methoxyl content). After three recrystallisations from ether, the material had m. p. 121—123°, unchanged when mixed with authentic 2 : 4 : 6-trimethyl glucose and depressed to 93° when mixed with 2 : 3 : 6-trimethyl glucose. The purified material had $[\alpha]_D^{10}$ + 72° at equilibrium (*c*, 3·0 in water; apart from initial mutarotation, no change in rotation occurred in 1% methanolic hydrochloric acid (*c*, 2·6) during 4 days, showing that a furanoside derivative was not formed (Haworth and Sedgwick, *J.*, 1926, 2573); $[\alpha]_D^{14}$ +75° (2 hours); +70° (1—4 days). The identification of the fraction as 2 : 4 : 6-trimethyl glucose was confirmed by its conversion into the β-methylglycoside, m. p. 67—69°, by Oldham's method (*J. Amer. Chem. Soc.*, 1934, **56**, 1360).

Fraction C (0·370 g.). This material appeared to be a mixture of 4 : 6- and 2 : 6-dimethyl glucose (cf. Connell, Hirst, and Percival, *loc. cit.*) (Found : OMe, 24·9. Calc. for $C_8H_{16}O_6$: 29·8%) (weight 0·308 g., corrected in accordance with methoxyl content). After two recrystallisations from ethyl acetate–light petroleum (b. p. 40—60°) crystals were obtained with $[\alpha]_D^{15}$ +60·5° at equilibrium (*c*, 6·0 in water), m. p. 157—159° alone or mixed with an authentic specimen of 4 : 6-dimethyl glucose.

Fraction D (0·090 g.). Paper chromatography showed the presence of monomethyl glucose and glucose. No further examination was made.

Summary of corrected yields. 2 : 3 : 4 : 6-Tetramethyl glucose (0·080 g., 4·6%); 2 : 4 : 6-trimethyl glucose (0·959 g., 57%); dimethyl glucose (0·308 g., 18%); monomethyl glucose and glucose (0·090 g., 5·3%). Total recovery 85%. The molar ratio of the above sugars is approximately 1 : 12 : 4 : 1.

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