CIV. A WATER-SOLUBLE POLYSACCHARIDE FROM BARLEY LEAVES

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By cold water extraction of dried barley leaves, Archbold & Barter [1935] isolated a non-reducing polysaccharide which was described as a fructose anhydride. A small amount of this polysaccharide has been placed at our disposal by Prof. V. H. Blackman and we now record the result of an investigation into its chemical constitution.

We confirm the properties ascribed to the polysaccharide by Archbold & Barter and, in particular, the ease of its hydrolysis by means of dilute acid, *N*/50 aqueous HCl being sufficient to effect complete hydrolysis at 50°. We were further able to confirm the view that fructose is possibly not the only sugar liberated on hydrolysis. A total sugar estimation on the acid hydrolysate gave a value of 99 %, whereas an estimation of ketose by Nyn's method [1924] showed only 93 % of the total sugar to be present as fructose. The amount of material available precluded a complete investigation into the nature of the non-ketose sugar, although it was possible to establish the absence of a pentose.

It appeared, from its properties, that the polysaccharide was closely related to, if not identical with, laevan, and a study of the acetyl and methyl derivatives of the barley polysaccharide confirmed this view. The acetylation with pyridine and acetic anhydride was carried out in the presence of water [Challinor *et al.* 1934, 2] and it was found that acetates of widely different rotations could be produced by varying the proportion of water in the acetylation mixture. It appears probable that this is a property peculiar to laevan (unpublished work) whether it be prepared by bacterial action or obtained from plant sources.

The polysaccharide was methylated directly in aqueous acetone solution and yielded, after three methylations, a granular powder containing 43.0 % methoxyl. The specific rotation of the methylated derivative (−50° in chloroform) is only slightly less than that of the laevan from meadow grass [Challinor *et al.* 1934, 2] or from the synthetic action of *B. mesentericus* [Challinor *et al.* 1934, 1].

The hydrolysis of the methylated laevan was effected by the use of aqueous-methyl alcoholic oxalic acid and from the syrupy product crystals separated, which were crystallographically identical with those of an authentic specimen of 1:3:4-trimethyl fructofuranose. It is clear, therefore, that the polysaccharide under discussion is constituted on a plan similar to that of laevan, namely, that it consists mainly of a chain of fructofuranose residues united by the reducing group of one unit engaging with position 6 of the contiguous unit, thus

The total amount of methylated polysaccharide used was 0.5 g.; it is therefore impossible to make any statement, from experimental evidence, on the nature of the end groups or on the number of fructofuranose units in the chain. Nevertheless, the close similarity in physical properties between the polysaccharide under discussion and the laevan synthesized by bacteria favours the assumption that the number of fructofuranose units constituting the chain molecule of the barley polysaccharide is not very different from that of laevan (10 units).

EXPERIMENTAL

The polysaccharide was a white powder, soluble in water, non-reducing and having $[\alpha]_D^{20} - 35^\circ$ in water. It contained 6.0 % moisture and 0.3–0.4 % ash.

Hydrolysis of the polysaccharide. In *N*/50 aqueous HCl at 50° complete hydrolysis was achieved, the specific rotation increasing during the hydrolysis from $[\alpha]_D^{20} - 40^\circ$ to $[\alpha]_D^{20} - 82^\circ$ (constant value). Hydrolysis also occurred when the substance was heated with phenylhydrazine and acetic acid, glucosazone being recognized as one of the products.

The total sugar liberated by acid hydrolysis was estimated by titration against Fehling's solution (methylene blue indicator) and fructose according to Nyn [1924]. If allowance is made for the ash and moisture contents of the original polysaccharide, the total sugar in the acid hydrolysate was estimated as 99 %, the fructose as 93 % (calculated in terms of an anhydrohexose), indicating the presence of a reducing substance (equivalent to 6 % of an aldose) other than fructose. That this substance was not a pentose was established by distillation of the polysaccharide with 12 % aqueous HCl and treatment of the distillate with barbituric acid. No precipitate was formed, indicating the absence of furfuraldehyde and therefore of pentose residues in the polysaccharide. Control experiments on mixtures of fructose and arabinose showed that 0.02 g. of pentose could be detected by this method.

Acetylation of the polysaccharide. (a) A solution of the polysaccharide (0.25 g.) in water (0.5 ml.) was treated with pyridine (5 ml.) and acetic anhydride (5 ml.) at 0° . After standing at room temperature for 8 hr. the solution was poured into a large volume of cold water, whereby the polysaccharide acetate was precipitated as a fine white powder. The precipitate, washed free from acid and dried, was purified by precipitation from its solution in chloroform by the addition of light petroleum. The pure, dry acetate (yield, 0.25 g.) showed $[\alpha]_D^{20} + 11^\circ$ in chloroform.

(b) The polysaccharide (0.25 g.) was dissolved in 1 ml. of water and treated as under (a) with pyridine (5 ml.) and acetic anhydride (5 ml.). The acetate so obtained (yield, 0.28 g.) had $[\alpha]_D^{20} - 27^\circ$ in chloroform.

Methylation of the polysaccharide. A solution of the polysaccharide (1 g.) in water (5 ml.) and acetone was treated at 55° with methyl sulphate (5 ml.) and 30 % aqueous KOH (15 ml.) over the course of 2.5 hr. Thereafter, the solution was boiled, neutralized by the addition of dilute H_2SO_4 and concentrated to small volume. This concentrated solution was submitted to further methylation in the same manner and the methylated product (now only partially soluble in hot water) extracted with chloroform. After three such methylations, the product was completely insoluble in hot water but easily soluble in chloroform. From the chloroform solution it was precipitated by the addition of light petroleum as a powder which had $[\alpha]_D^{20} - 50^\circ$ in chloroform. (Found: OMe, 43.0 %; calc. for $C_6H_{16}O_5$, 45.6 %.)

Hydrolysis of the methylated polysaccharide. The methylated derivative (0.5 g.) was hydrolysed by treatment with a solution of oxalic acid (0.5 g.) in water

(10 ml.) and methyl alcohol (25 ml.) at 80° for 8 hr. The solution was neutralized (CaCO_3), filtered and evaporated to dryness in a vacuum. The solid residue was extracted with ether and the ether extract evaporated. The residual syrup was strongly reducing and crystallized slowly. Crystallographic measurement of this product established its identity with 1:3:4-trimethyl fructofuranose.

SUMMARY

A fructose anhydride extracted by cold water from barley leaves has been shown to be a laevan, that is, a polysaccharide constituted of fructofuranose units linked together by bonds each of which engages the reducing group of one fructofuranose unit (C_2) and the C_6 position of the contiguous fructofuranose unit.

REFERENCES

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