CCCXIX. A FRUCTOSE ANHYDRIDE FROM THE LEAVES OF THE BARLEY PLANT.

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(Received October 11th, 1935.)

IN determining the sugar content of a cold water extract of dried barley leaves, it was found that the estimate of total sugar was considerably higher if hydrolysis was effected by heating with $N/5$ sulphuric acid in a boiling water-bath for 10 min., than if treatment with 0.1% invertase was employed. The sample of invertase was obtained from the British Drug Houses and under the conditions employed inversion of sucrose was complete. In one experiment the value for total sugar obtained after acid hydrolysis was 2.72% of the fresh weight of the leaves, whilst after enzymic hydrolysis it was only 1.33%. Separate estimates of fructose and glucose showed that the excess of reducing sugar resulting from the acid treatment was almost entirely fructose. These observations led to an attempt to isolate a fructose anhydride from the leaves.

It has long been known that fructose anhydrides occur in cereals [Müntz, 1878] and in bulbs [Wallach, 1886]. More recently their presence in the Gramineae has been reported by de Cugnac [1931], and by Kizel and Kretovitsch [1934], and their constitution has been discussed by Challinor *et al.* [1934] and by Schlubach and Koenig [1934]. The presence of these compounds in the barley grain was recorded by Tanret [1891], and in the stems and leaf sheaths of the barley plant by Colin [1925] and Belval [1924; 1933]. As far as can be ascertained their presence in the leaf lamina has not hitherto been demonstrated, in fact both Colin [1925] and de Cugnac [1931] state that these compounds do not invade the leaf parenchyma, although they have been found in the leaves of the iris [Augem, 1928], and of the yucca [Schlubach and Florsheim, 1931].¹

The barley leaves from which a fructose anhydride has now been isolated were detached from plants which had received a full manurial treatment. The seeds were sown on 3. iv. 33, and the collection of leaves was made 10–11 weeks later, when the ears were emerging from their sheaths. Subsequent work has shown that it is unlikely that fructose anhydrides accumulate in the leaves before this stage of the plant's development.

Preparation of material for water extraction. Green leaves (7 kg.) were detached from the plants at the stage described above and plunged into boiling 95% alcohol in such quantity that the final alcohol concentration was 75–80%. After extracting under a reflux condenser for 5–6 hours, the alcoholic extract was poured off and the residual leaf material dried at 80–90°. It was then ground to a powder and again extracted with 95% alcohol for 4 hours and dried at 95°. The product, a straw-coloured powder, was now free from sugars, waxes and green pigments and constituted 60% of the total dry weight of the leaves.

¹ Since this work was completed a paper has appeared by Yemm [1935] in which the presence of a fructose anhydride in some barley leaves is inferred from the results of sugar determinations.

The bulk of the fructose anhydride remains undissolved by this treatment, but a small amount was later recovered from the alcoholic extract.

Preparation and precipitation of the water extract. 100 g. lots of the dried powder were shaken in 3 litres of cold water for 10 hours, and the extract was then filtered off at the pump. An amount equivalent to 12 % of the total dry weight of the leaves was extracted by this treatment. The aqueous extract was evaporated *in vacuo*, below 40°, to an eighth of the original volume and an equal volume of 95 % alcohol then added in order to remove the bulk of the inorganic material. The precipitate so obtained had an ash content of 60–70 %; the remaining 30–40 % of organic material was not further examined.

The filtrate from this precipitate was evaporated to a twelfth of its volume and then poured slowly and with stirring into a large excess of 95 % alcohol, the final concentration of alcohol being about 80 %; the crude fructose anhydride was precipitated as a gum by this treatment. After removal of the supernatant liquid the gum was taken up in a little water and the solution poured cautiously into a large excess of absolute alcohol. A creamy white precipitate separated, which was washed with absolute alcohol by decantation and then dried *in vacuo* over sulphuric acid for several days and finally at 100° for 1 hour. The yield of crude material was 6.7 % of the total dry weight, or half the total amount of material extracted by water. Determination of the reducing power of the original aqueous extract, after hydrolysis with *N/5* sulphuric acid, showed a total sugar content equivalent to 8.4 % of the dry weight. Therefore, allowing for the ash content of the precipitate (16 %), about 66 % of the fructose anhydride in the water extract was precipitated by 80 % alcohol. The yield of crude product can be somewhat improved by further recovery as barium salt from the combined alcoholic filtrates.

Treatment of the crude fructose anhydride. In the first instance purification of the product was attempted by repeated reprecipitation from alcohol and by dialysis. It was found, however, that fractional precipitation with baryta was a much more satisfactory procedure. Consequently 93 g. of the crude product were dissolved in 50 % alcohol and saturated baryta was added in amounts of about 70 ml. at a time. After each addition alcohol was added to maintain the concentration at 50 %. The first five fractions were contaminated with a substance giving a bright yellow barium salt and varied in colour from yellow to cream. These were set aside. After the addition of 370 ml. of baryta the next addition gave a white precipitate. Three white fractions were obtained using 250 ml. baryta for each fraction.

After removal of barium in the usual way the aqueous solutions of these fractions (nos. 6, 7 and 8) were evaporated to small bulk and the purified product was precipitated by pouring the solution into absolute alcohol. The precipitates were quite white, and after drying a pure white powder was obtained in each case.

Hydrolysis of fractions 6, 7 and 8. Hydrolysis was effected by heating in a boiling water-bath with *N/5* sulphuric acid, and the total reducing power and fructose content of the hydrolysate were determined by a modification of the Harding and Downs copper reduction micro-method, and by the Kolthoff method as described by Van der Plank [1935].

The reducing power of the hydrolysate reached a maximum after 5 min. heating and remained constant for half an hour, after which it began to fall owing to destruction of fructose. A standard time of 15 min. was used in testing the various samples. The results are shown in Table I, together with the values obtained from a sample purified by dialysis and alcohol precipitation, followed by separation as the barium salt.

Table I. *Ash content, specific rotation, and sugar content after hydrolysis of samples of fructose anhydride.*

Method of preparation	Yield (g.) (from 93 g. see p. 2690)	Ash %	Specific rotation	% sugar after hydrolysis. Calculated as fructose	Fructose as % total reducing power after hydrolysis
Alcohol* precipitation and dialysis	—	3-5.5	$[\alpha]_D^{18} - 25^\circ$ to -29°	88-95	90
Alcohol precipitation, dialysis and baryta fractionation	—	0.16	$[\alpha]_D^{15} - 35^\circ$	105.5	94
Baryta fractionation:					
Fraction 6	11.9	3.1	—	98	—
Fraction 7	10.4	4.1	—	98	95
Fraction 8	3.8	1.9	—	100	89

* This sample gave on combustion C 42.7; H 6.0%. $C_{12}H_{22}O_{11}$ requires C 42.1; H 6.4%. $(C_6H_{10}O_5)_n$ requires C 44.4; H 6.2%.

Reprecipitation of fractions 6 and 7. Fractions 6 and 7 were again precipitated by three separate additions of baryta. The first two fractions (20 ml. baryta) were of a faint cream colour and rather small in amount. The fructose anhydride was regenerated in the usual way from the third fractions, which were pure white, and from the second fraction of no. 6, the other fractions being discarded. The ash content was considerably reduced by this procedure and a higher value obtained for the total sugar content after hydrolysis (Table II).

Table II.

Fraction	Yield (g.)	Ash (%)	% sugar in the hydrolysed pro- duct. Calculated as fructose	Fructose as % of total reducing power after hydrolysis
6 ₂	1.4	4.0	102	95
6 ₃	3.6	0.15	103	95
7 ₃	2.6	0.40	104	92
A	3.8 (from 5.8 g.)	None	105	95
B	1.3 (from 5.8 g.)	None	104	94

Since there appeared to be no material difference between the two pure white samples 6₃ and 7₃ they were combined and refractionated using two 10 ml. lots of baryta. The two samples, A and B, obtained by this final fractionation gave on hydrolysis the results shown in Table II. All the remaining ash had been removed, but the percentage of sugar obtained on hydrolysis was not sensibly altered.

The final product was a pure white amorphous powder, very soluble in cold water, insoluble in absolute alcohol, non-reducing towards copper and precipitated slowly by basic lead acetate. The lead precipitate was redissolved by a slight excess of the reagent.

The maximum yield of reducing sugar on hydrolysis was 105 % of the weight of fructose anhydride taken. This would correspond to complete hydrolysis of a disaccharide or to 94 % hydrolysis of the molecule $(C_6H_{10}O_5)_n$. In no case was the fructose found equivalent to more than 95 % of the total reducing power after hydrolysis. There appears therefore to be a small amount of glucose (or other reducing substance) produced on hydrolysis in addition to the fructose. There may also be a little non-reducing material but this remains uncertain until the size of the molecule is known.

The specific rotation of samples of fructose anhydride. Although the samples A and B discussed above showed no difference in chemical properties they did not show the same specific rotation. The values obtained were $[\alpha]_D^{15}$ A -37.8° , and B -28.1° . The value for sample A agrees fairly well with that for the sample prepared by alcohol precipitation, dialysis and baryta treatment (-35° ; see Table I), and also with the value for fraction 6₂ which was -37° . A low value, -26° , similar to that found for B, was obtained from a sample prepared from the filtrates remaining after the precipitation of the crude product with 80 % alcohol.

The action of invertase on samples of fructose anhydride. All the samples were slowly attacked by invertase, but the rates of hydrolysis of the different preparations varied considerably. Thus after an incubation period of 4 hours at 38° , with an enzyme concentration of 0.02 %, the degree of hydrolysis of samples A and B was 11.7 and 17.2 % respectively. Under the same conditions only 4 % of fraction 6₂ was hydrolysed, whilst values of the order of 20 % were found for fractions isolated from the alcoholic extract of the green leaves and from the filtrates after precipitation of the aqueous extract by 80 % alcohol (see p. 2690). All these samples gave the same results on acid hydrolysis as those already quoted for samples A and B.

The results of the measurement of specific rotation and of susceptibility to invertase action suggest that a mixture of two or more fructose anhydrides was present in these barley leaves; further, the more soluble fractions have a lower negative rotation and are more readily attacked by invertase than the less soluble.

A sample of the fructose anhydride is now in the hands of Prof. Haworth, who is studying the constitution of these compounds obtained from different sources. Further investigation is therefore suspended until these results are available.

SUMMARY.

A fructose anhydride has been isolated from a water extract of leaves obtained from barley plants at the time of emergence of the ear.

The product, a pure white amorphous powder, readily soluble in cold water, was obtained by precipitation of the aqueous solution with alcohol, followed by fractional precipitations with baryta until there was no change in the amount of sugar produced on hydrolysis of the regenerated product.

Hydrolysis of the final product yielded a solution containing reducing sugar equivalent to 105 % of the weight of anhydride taken, of which 94 % was fructose.

Samples were obtained which differed both in specific rotation and in susceptibility to invertase action, although otherwise exhibiting the same properties as those quoted above. The more soluble fractions had a specific rotation of about $[\alpha]_D^{15} -27^\circ$ and were more readily attacked by invertase than the less soluble, which had a specific rotation of about -37° .

It is concluded that a mixture of two or more anhydrides was present in the leaves.

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