I. OCCURRENCE OF RAFFINOSE IN THE SEED OF THE JUTE PLANT (*CORCHORUS CAPSULARIS*).

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Raffinose has been shown to occur in beet molasses, cotton seed, barley grains, wheat sprouts and eucalyptus manna. It is now shown to be present in jute seeds.

Preparation. In the course of an examination of jute seed for a glucoside Mr R. S. Finlow, Fibre Expert to the Government of Bengal, obtained a substance, which had a sweet taste, and handed the material to me for further investigation. The jute seed had been finely ground and then exhausted with ether and with petrol. The residue was next exhausted with alcohol. From this alcoholic extract the substance under investigation was obtained as a copious white precipitate on the addition of ether.

The impure substance was dissolved in hot 80 % alcohol. On filtering and cooling, the solution deposited after several days rosettes of white needles. The crystals were collected on a filter and recrystallised from 80 % alcohol. About 3 g. of the pure crystals were finally obtained.

General Physical and Chemical Properties.

The substance had an extremely sweet taste, was very soluble in water, and crystallised from alcohol in characteristic rosettes of white needles. It gave no osazone and did not reduce Fehling's solution. 10 cc. of a 1 % solution gave only 2.5 milligrams of CuO under Brown and Morris's conditions. On hydrolysis with acid or treatment with emulsin or invertase, reducing sugars were formed and then an osazone was readily obtained. It gave Selivanoff's reaction and on boiling with concentrated hydrochloric acid gave a red colour indicating the presence of fructose.

Water of crystallisation. On heating a small portion of the crystals in the steam oven they melted indicating the presence of much water of crystallisation. Some freshly prepared crystals were well washed with absolute alcohol on a small Hirsch funnel under suction and then dried thoroughly between filter paper. About 0.5 g. was then dried *in vacuo* in a toluene bath in presence of phosphorus pentoxide. For the first few hours the temperature was kept below 60° C. but it was finally increased to 105° C. and maintained at that temperature until a constant weight was obtained. This took about 24 hours.

0.5420 g. substance gave water of crystallisation = 15.09 %. For pure raffinose the theory is 15.15 %. A sample of recrystallised raffinose gave 15.54 %.

Specific rotatory power. This had to be determined in a Schmidt-Haensch saccharimeter using ordinary white light. The substance showed no mutarotation.

0.4602 g. of anhydrous sugar was dissolved in water and made up to 50 cc. This gave a reading on the sugar scale of + 6.64. Raffinose should have given + 6.51. The agreement is within the error due to the unfavourable conditions under which the rotation had to be determined.

Action of enzymes. These tests were carried out in conjunction with parallel tests on pure raffinose.

(a) *Emulsin.* When emulsin acts on raffinose, cane sugar and galactose are produced according to the equations:

Whence 100 parts of raffinose give 30.3 parts of galactose and 57.57 parts of saccharose.

Since $[\alpha]_D^{20}$ for raffinose = + 104°, for galactose = + 81°, and for sucrose = + 66.5°.

30.3 % galactose will give a rotation of $0.303 \times 81 = + 24.54^\circ$,

57.57 % sucrose will give a rotation of $0.5757 \times 66.5 = + 38.28^\circ$,

$\therefore [\alpha]_D^{20}$ of the mixture will give a rotation of = + 62.82°,

the $[\alpha]_D^{20}$ falling from + 104° to + 62.82°.

Exp. 1. 1.0103 g. of pure raffinose were dissolved in water and the volume made up to 100 cc. This gave a reading of + 5.94.

To 50 cc. of this solution 0.1 g. emulsin + 0.5 cc. toluene were added. The reading became + 3.50 (corrected for emulsin).

Exp. 2. 1.2280 g. of the jute sugar in the anhydrous condition were dissolved in water and the volume made up to 100 cc. This gave a reading

in the saccharimeter of $+8.51$. To 50 cc. 0.2 g. emulsin and 0.5 cc. toluene were added. The reading became $+5.32$ (corrected for emulsin).

The reading of the pure raffinose solution after emulsin action should have been $62.8/104 \times 5.94 = +3.59$, and that of the jute sugar $62.8/104 \times 8.51 = +5.14$.

It must be noted here that with the emulsin used some 10 days were required before a constant saccharimeter reading was obtained.

(b) *Invertase + Melibiase.* When invertase acts on raffinose, d-fructose and melibiose are produced.

It can readily be shown that the $[\alpha]_D^{20}$ of raffinose $+104^\circ$ falls to $+54.4^\circ$ as a result of the action of invertase. The action of the melibiase in addition to the invertase, produces from one molecule of raffinose, one molecule each of d-glucose, d-galactose and d-fructose. Hence it follows that the $[\alpha]_D^{20}$ of raffinose should fall after the action of invertase + melibiase from $+104^\circ$ to $+12.5^\circ$.

The invertase used in these tests was prepared according to Hudson's method [1914] which gives a highly pure and active product which even in this climate has retained its activity for more than a year. It was made from bottom yeast and contained melibiase.

Exp. 3. To 30 cc. of the pure raffinose solution which had been treated with emulsin (*Exp. 1*), 0.25 cc. of invertase + melibiase solution was added together with a few drops of toluene. A further fall in rotation was observed and no further fall took place after 24 hours. The original reading of the raffinose solution was $+5.94$, the action of emulsin reduced the reading to $+3.50$, and the action of invertase further reduced the reading to $+0.87$ (corrected for invertase control).

Theoretically the final reading should have been $12.5/104 \times 5.94 = +0.71$.

Since the readings were made at about 30° , it is to be expected that the actual reading would be too high owing to the large increase in $[\alpha]_D^{20}$ of fructose caused by rise of temperature.

Exp. 4. 0.9320 g. of roughly anhydrous jute sugar was dissolved and made up to 100 cc. The solution gave a reading in the saccharimeter (200 mm. tube) of $+6.80$. To 60 cc. of this solution, 1 cc. of invertase + melibiase solution and 0.5 cc. of toluene were added and the mixture incubated at 37° . The rotation became constant at $+0.94$ (corrected for invertase) after 48 hours.

Assuming the sugar to be raffinose this final reading should have been $12.5/104 \times 6.80 = +0.85$.

Osazone formation. *Exp. 5.* 20 cc. of a 1 % solution of the jute sugar were mixed with 0.4 g. of phenylhydrazine hydrochloride and 0.6 g. of sodium acetate and heated in the water bath. No osazone was obtained after 1 hour even on cooling. 0.411 g. of partly dehydrated jute sugar was dissolved in about 4 cc. of water, 0.5 cc. invertase and a few drops of toluene were added, and the mixture incubated at 30° for two days. 5 cc. of a solution containing 1 g. phenylhydrazine hydrochloride and 1.5 g. sodium acetate were then added.

The mixture was heated in a water bath. Under these conditions fructose should form an osazone in 3 minutes, glucose in 4—5 minutes and galactose in 15—19 minutes [1912].

An osazone formed in 3 minutes. After 15 minutes the liquid was filtered and the osazone recrystallised twice from alcohol (Crop 1).

The filtrate was again heated in the water bath for 1 hour when a second crop of crystals was filtered off. These were recrystallised twice from alcohol to form Crop 2. The filtrate was again heated for $\frac{3}{4}$ hour and a third crop of crystals was obtained and recrystallised twice from alcohol (Crop 3).

As a control 0.15 g. each of galactose, glucose and fructose were dissolved in about 4—5 cc. of water and 5 cc. of the phenylhydrazine hydrochloride and sodium acetate mixture added. Three crops of crystals were obtained exactly as with the jute sugar.

Crop 3 in each case was very small in amount.

The melting points of these crops of crystals were taken. Duplicate determinations being done in each case.

They were found to be:

Crop 1. M.P. (a) 203, (b) 202°.

Crop 2. M.P. (a) 202.5, (b) 203°·5.

Crop 3. M.P. (a) 196.5, (b) 195°·5.

The melting points of the crystals from the mixture of the sugars were (corrected):

Crop 1. M.P. (a) 204, (b) 203°.

Crop 2. M.P. (a) 201.5, (b) 203°·5.

Crop 3. M.P. (a) 193.5, (b) 194°·5.

Pure glucosazone under the same conditions had a M.P. (corrected) of 205 to 206°. The M.P. of galactose d-phenylosazone is given as 194—196° [1912].

The crystals were also examined microscopically, after mounting in xylene balsam.

The crystals of Crop 1 from the jute sugar were all of one kind collected

into the characteristic bundles of needles generally seen with phenylglucosazone.

Those of Crop 2 resembled Crop 1, but showed a few square ended yellow leaflets.

Those of Crop 3 consisted mostly of square ended bright yellow leaflets.

The microscopic appearance of the crops of crystals from the artificial mixture of sugars agreed in appearance with that of Crops 1, 2, 3 obtained from jute sugar.

The above experiments prove that the sugar obtained from jute seed is raffinose. By way of confirmation a 1 % solution of the sugar was treated with emulsin in presence of toluene. Emulsin hydrolyses raffinose to galactose and cane sugar. After 10 days the liquid was heated with phenylhydrazine hydrochloride and sodium acetate. An osazone was produced which contained many yellow square ended platelets characteristic of galactose phenylosazone.

The amount of raffinose in jute seed. 200 g. of finely ground seed were well shaken with 500 cc. distilled water, containing 10 cc. toluene, for one hour and filtered.

To 50 cc. of the filtrate 1 cc. of basic lead acetate was added and the liquid filtered. A bright solution was obtained from which lead was removed by means of solid sodium phosphate. This gave a reading in the 100 mm. tube of the saccharimeter of + 8.1.

25 cc. of this solution were slightly acidified by the addition of four drops of acetic acid and then 0.5 cc. invertase + melibiase and 0.5 cc. toluene were added and the mixture incubated at 37° for two days. Four drops concentrated NH_4OH were then added and the liquid filtered. A reading of + 5.2 was obtained in the 100 mm. tube of the saccharimeter.

To 25 cc. of the original water extract (which was faintly acid) 0.5 cc. invertase + melibiase and 0.5 cc. toluene were added and it was then incubated as above. After two days 0.5 cc. of basic lead acetate was added, the liquid being filtered after shaking. Lead was separated from the filtrate with sodium phosphate and the filtrate gave a reading in the 100 mm. tube of + 5.2.

The invertase had thus caused a fall in rotation, measured in the 100 mm. tube of + 8.1 to 5.2 or 2.9 degrees on the sugar scale. For the 200 mm. tube this equals 5.8.

The invertase and melibiase solution will cause a fall in rotation for raffinose of 91.5° from + 104° to + 12.5°.

Assuming that raffinose is the only sugar present in the jute seed capable

of being acted on by invertase, the original rotation due to raffinose for the 200 mm. tube would have been $5.8 \times 104/91.5 = 6.6$; this corresponds to 2.29 angular degrees and gives a content of about 1.1 g. raffinose per 100 cc. of the original water extract from 200 g. jute seed. Since there were 500 cc. of water added; the jute seed contains about 2.25 % raffinose.

CONCLUSIONS.

Raffinose has been shown to occur in the seed of the jute plant *Corchorus capsularis*. In the particular seed sample examined, the sugar occurs to the extent of about 2.25 %.

REFERENCES.

- Browne, C. A. (1912), *A Handbook of Sugar Analysis*, London, 1912.
Hudson, C. S. (1914), *J. Amer. Chem. Soc.* **36**, 1566.