LXII. ON THE HYDROLYSIS OF THE ENDOSPERM OF *PHYTELEPHAS MACROCARPA* BY ITS OWN ENZYMES.

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(Received March 4th, 1924.)

INTRODUCTORY.

OUR knowledge of the enzymes which bring about the hydrolysis of the mannans is far less extensive than of those by which other polysaccharides are hydrolysed. There is no doubt, however, that they play an important role in the metabolism of certain plants, more especially during the germination process. Moreover since mannans are readily digested in the animal organism, it is more than likely that such digestion is the result of enzyme action. It would seem therefore that enzymes capable of hydrolysing mannans are widely disseminated in nature.

The first recorded observation of the hydrolysis of a mannan through the agency of an enzyme is that of Bourquelot and Herissey [1899] who showed that during the germination of the carob bean an enzyme is produced which converts the polysaccharides of the bean into mannose and galactose. In later communications these observers showed [1900] that enzymes (which they refer to as seminases), capable of hydrolysing mannogalactans and galactans into their derivative monosaccharides, occur in the fruits of fenugreek, lucerne, *Trifolium repens* and other plants. Subsequently they were found in orchid tubers [1901]. In some cases the enzyme action is comparatively slow, but it appears invariably to be accelerated by the presence of sodium fluoride. Grüss [1902] has observed the presence of similar enzymes in germinating date seeds.

Bierry and Giaja [1912] show that enzymes capable of hydrolysing mannans, galactans and celluloses are present in the alimentary canal of certain invertebrates, this being the first recorded instance of the presence of these enzymes in the animal kingdom.

Vegetable ivory (*Phytelephas macrocarpa*) is the usual source of mannose which is obtained by hydrolysing it with sulphuric acid. However, it will

be shown that the ivory nuts themselves contain an enzyme capable of bringing about the hydrolysis of the mannan, with the production of considerable quantities of mannose.

Pringsheim [1912] showed that if an infusion of certain bacteria be added to a suspension of ivory nut shavings the mannan is hydrolysed, giving mannose and a trisaccharide, mannotriose. This is presumably composed of three molecules of mannose and gives a characteristic osazone, forming stellate aggregates of needles, which are soluble in water and decompose at 196°.

EXPERIMENTAL.

Whilst investigating the action of malt diastase on a suspension of ivory nut shavings it was observed that whereas diastase had no action if the suspension had been boiled, yet when added to the unboiled suspension, a considerable amount of reducing sugar was formed. This led to the discovery that the shavings contained an enzyme capable of hydrolysing the insoluble mannans and a series of experiments were carried out in order to determine the best conditions for this enzyme action.

Ten grams of the shavings were suspended in 200 cc. of water containing toluene, and the conversion was followed by withdrawing at definite intervals some of the solution, making up to a suitable volume and titrating with Fehling's solution. It was found that the maximum quantity of reducing sugar was formed when the temperature of incubation was 45°, whilst at a temperature of 60° the action appeared to be almost completely annulled. Further experiments indicated that the quantity of reducing sugars produced was approximately proportional to the concentration of the suspension, at least during the first 24 hours.

In order to determine the influence of hydrogen ion concentration on this enzyme action, a series of conversions were carried out in aqueous media at different hydrogen ion concentrations, the latter being varied by the addition of dilute sodium hydroxide or acetic acid to the initial liquid. The course of hydrolysis at 45° was followed, all other conditions being kept constant. It was found, however, that during hydrolysis there was an increase in acidity and that the suspensions attained a constant p_H value of 4.2 after two hours. This at once suggested the possibility that the shavings contained an appreciable amount of fatty matter, which was simultaneously hydrolysed by a lipoclastic enzyme also present. In one experiment an acidity corresponding to as much as 15 cc. *N*/10 NaOH was developed from 20 g. of the shavings. In order therefore to determine the concentration of hydrogen ions corresponding with the optimum activity of the enzyme, it was obviously necessary to extract the shavings with ether.

The fat-free shavings thus obtained were then used in a similar series of conversions at definite initial p_H values which in this case remained constant during the conversion. Four grams of the shavings were suspended in 100 cc.

of water and the conversions were carried out in exactly the same manner for two hours as in the previous series. The reducing powers of the different solutions after filtering were determined by titration with Fehling's solution and are shown in the diagram calculated as Cu_2O .

From the above diagram it will be seen that the p_{H} of optimum activity is about that produced by the formation of fatty acid during the conversion, viz. 4.2.

200 g. of shavings were allowed to hydrolyse in presence of toluene for six days in 1250 cc. of water at 45° when the solution was found to contain about 45 g. of reducing sugar, calculated as mannose. The liquid was pressed out, filtered, treated with lead acetate and the excess of lead removed by hydrogen sulphide.

After decolorising with norit it was concentrated *in vacuo* to a syrup. Its specific rotatory power was found to be $[\alpha]_D +13.3^\circ$ which is just less than that of mannose $[\alpha]_D +14.2$.

When treated with phenylhydrazine in the cold a bulky precipitate of mannose hydrazone was formed, and after filtration and heating with phenylhydrazine acetate, two osazones were observed. One of these, which was soluble in water, gave the characteristics of Pringsheim's mannotriosazone but decomposed at about 170° . The other, which was insoluble in water, was shown to be glucosazone.

A portion of the sugar solution was used for the estimation of the tri-saccharide present. It was found that after heating with dilute hydrochloric

acid for some time on a water-bath there was an increase in reducing power which corresponded to 7.5 % of mannotriose, assuming this to give three molecules of mannose on hydrolysis. Attempts were then made to isolate this polysaccharide but were not successful, the amount of osazone obtained being always very small. It was thought possible to effect a partial separation of the mannotriose by its sparing solubility in 92 % alcohol. Accordingly fractions were isolated, cryoscopic determinations of which gave values which although higher than the molecular weight of mannose were only indicative of a partial separation.

In these conversions it is highly probable that the trisaccharide is produced as an intermediate product which is finally hydrolysed to mannose. It was found that by allowing the enzyme action to continue until no further hydrolysis was observed (about 10 days) only mannose was produced. Attempts were then made to isolate mannotriose by stopping the reaction at an earlier stage. Accordingly a large scale conversion was carried out, the reaction being stopped by boiling after two days. The reducing power towards Fehling's solution before and after boiling with 1 % hydrogen chloride solution, appeared to indicate the presence of 10 % of trisaccharide. It was still however not possible to isolate this body.

It has not been found possible to obtain crystalline mannose direct from the solution after allowing the conversion to proceed under optimum conditions until the maximum reducing power was observed. Although syrups were obtained agreeing very closely with the optical constants of mannose they were not of sufficient degree of purity to crystallise from glacial acetic acid, possibly owing to the presence of galactose and laevulose produced by the hydrolysis of laevulo-mannan and galacto-mannan which have been shown to be present in the ivory nuts by Baker and Pope [1900].

BIBLIOGRAPHY.

- Baker and Pope (1900). *J. Chem. Soc.* **77**, 696.
Bierry and Giaja (1912). *Biochem. Z.* **40**, 370.
Bourquelot and Herissey (1899). *Compt. Rend. Acad. Sci.* **129**, 614.
—— (1900). *Compt. Rend. Acad. Sci.* **130**, 42, 1719.
—— (1901). *Compt. Rend. Acad. Sci.* **133**, 49, 1902; **134**, 721
Grüss (1902). *Ber. deutsch. bot. Ges.* **20**, 36.
Pringsheim (1912). *Z. physiol. Chem.* **80**, 376.