IV. THE DISTRIBUTION OF MALTASE IN PLANTS. II. THE PRESENCE OF MALTASE IN FOLIAGE LEAVES.

By ARTHUR JOHN DAISH.

Rothamsted Experimental Station.

(*Received December 17th, 1915.*)

In Brown and Morris' [1893] classical paper on the "Chemistry and Physiology of Foliage Leaves" the early history is traced of the question of the starch resolving enzymes present in plants. Brown and Morris proved in a conclusive manner that the starch in the leaf is dissolved by the action of a diastatic enzyme; they regarded the formation of this enzyme as a "starvation phenomenon." They showed that diastase was present in a large number of plants and concluded from a study of the sugars of the leaf, amongst which maltose was always found, that the degradation of starch is effected in the same way as by malt diastase, the end product being maltose.

As explained by Davis [1916, p. 31] the fact that maltose was never found among the sugars present in a large number of leaves examined at Rothamsted [Davis and Sawyer, 1916] led to a re-examination of the enzymes of the leaf. This has shown that in addition to the ordinary diastatic enzymes, which convert starch into dextrin and maltose, the enzyme *maltase* is also invariably present. The absence of maltose from plant leaves at all periods of the night and day is therefore easily explained. It is due to the fact that sufficient maltase is always present to convert the maltose, as fast as it is formed from starch, into the final product of hydrolysis, glucose.

It is interesting historically that Brasse [1884] in a paper cited by Brown and Morris [1893] showed that the diastatic action of leaves on starch paste at 63° gives rise to maltose[1] and dextrin just as does that of malt diastase.

[1] At 63°, the maltase was destroyed, leaving the ordinary diastatic enzymes: hence the production of maltose and dextrin [see Davis, 1916, p. 34]. At 34–42° the maltase remained and converted the maltose into glucose.

In a subsequent paper [Brasse, 1885] which Brown and Morris apparently overlooked, it is, however, shown that a "diastase" can be extracted from leaves and germinated barley *which at* 34° *to* 42° *converts starch into glucose.* As pointed out elsewhere [Davis, 1916, p. 35], Brasse realised very clearly the instability of the glucose-forming enzyme in presence of alcohol and at temperatures above 40°. He did not, however, identify it as a maltase (*glucase*), which enzyme was discovered by Cuisinier [1885] in the same year.

That Brown and Morris did not recognise the presence of maltase in leaves may be explained by the fact that, although they carried out their transformations at 30°, they used leaves which had previously been dried at 40–50° and chloroform as an antiseptic; the drying of the leaves under the conditions named would probably destroy much of the maltase whilst *ordinary* impure chloroform also renders it inactive.

The results obtained by Vines [1891] call for a word of comment. Vines showed that his leaf extracts acted on starch paste giving a cupric-reducing and fermentable sugar which he concluded was not maltose because it had no optical activity. The explanation is that Vines worked with a very large amount of leaf material so that the substance he finally obtained was a mixture of the leaf-sugars (largely consisting of invert sugar) with the products of starch transformation; by chance the positive and negative rotations of the sugars present balanced so as to give an apparently inactive product.

Robertson, Irvine and Dobson [1909] have recently stated that they found maltase as well as ordinary diastase in the leaf and root of the sugar beet. From the results given later it is clear that maltase is also present abundantly in the leaf of the closely allied mangold. This fact is very striking when taken in conjunction with the complete absence of starch from the mangold leaf during all its later stages of growth [Davis, Daish and Sawyer, 1916].

The experiments which are given below show that all the plants we have examined, which include the *Tropæolum majus* investigated by Brown and Morris, contain maltase in considerable quantities; this is true whether the leaves be plucked in the daytime or at night.

Experimental.

To avoid destroying the maltase the freshly picked leaf material was always used without drying; for the same reason toluene was used as antiseptic, not chloroform, and the starch conversions were carried out

at 38°, which is the optimum temperature for the maltase. The leaves were ground up in a mortar with a little water and the pulp was allowed to act on the starch paste or soluble starch during 24 hours or longer.

Action of leaf pulp on Soluble Starch at 38°.

Series I.

To 100 cc. of a soluble starch solution (= 2·2481 g. of anhydrous soluble starch dried *in vacuo* at 100° and having $[\alpha]_D^{20°} = 191·1°$) 3 g. of wet leaf previously pulped in water were added; the volume was made up to 200 cc. and after adding a little toluene was digested at 38° for 24 hours. The mixture was then boiled to destroy the enzymes, 10 cc. of alumina cream were added and the volume diluted to 500 cc. at 15°. A control experiment was made with 3 g. of leaf under the same conditions, so as to allow for the sugars in the leaf. The results are given in Table I.

TABLE I.

Leaves plucked at 11 *a.m., September* 10, 1913.

Expt.	Leaf	CuO from 50 cc. of the 500 cc. (corr.)	$\alpha_D^{20°}$ in 200 mm. tube (corr.)	Total rotation of solution, reducing sugars calculated as	
				Maltose	Glucose
1	*Tropæolum majus*	0·1264	1·459°	1·559°	1·474°
2	Potato	0·0389	1·599°	1·668°	1·643°
3	Dahlia	0·1450	1·424°	1·536°	1·450°

The following example shows the method of calculation; the methods of analysis are those adopted by Davis and Daish [1913], the reducing power being determined under Brown, Morris and Millar's conditions.

(*a*) *Reducing sugars assumed to be entirely maltose:*

Maltose in 50 cc. $= \frac{0·1264}{1·374} = 0·0920$ g.

Therefore total maltose in 500 cc. = 0·920 g.

Starch corresponding with this $= \frac{0·920}{1·055} = 0·8717$ g.

Soluble starch taken = 2·2481 g.

Therefore starch remaining = 2·2481 − 0·8717 = 1·3764 g.

Rotation in 200 mm. tube, $\alpha_D^{20°}$ due to maltose = 0·507°

Rotation in 200 mm. tube due to soluble starch ($[\alpha]_D^{20°} = 191·1°$) = 1·052°

Total rotation calculated = 1·559°

(*b*) *Reducing sugars assumed to be glucose:*

Glucose in 50 cc. $= \frac{0{\cdot}1264}{2{\cdot}578} = 0{\cdot}04903$.

Therefore total glucose in 500 cc. $= 0{\cdot}4903$ g. $\times\ 0{\cdot}9 = 0{\cdot}4413$ g. starch.

Therefore soluble starch remaining $= 2{\cdot}2481 - 0{\cdot}4413 = 1{\cdot}8068$ g.

Rotation due to glucose in 200 mm. tube ... $= 0{\cdot}093°$

Rotation due to soluble starch ... ... $= 1{\cdot}381°$

Total rotation $= 1{\cdot}474°$

In the case of *Tropæolum* and dahlia, which gave the highest proportion of reducing sugars and of which the diastatic activity was greatest, the rotation calculated on the assumption that the whole of the reducing sugar is glucose, is much closer to that actually observed than the value obtained assuming the sugar to be maltose. In these two cases the whole of the sugar appears to be glucose. In the case of the potato the diastatic action is far less and the calculation points to a mixture of maltose and glucose being present.

Analysis by means of Maltase-free yeasts.

Series II.

In order to make sure that glucose is formed from the soluble starch a series of experiments was carried out in which the product of transformation was analysed by means of the special yeasts (*S. marxianus* and *S. exiguus*) so as to obtain values showing the proportions of maltose and glucose actually formed [Davis and Daish, 1913].

50 cc. of a solution of soluble starch ($[\alpha]_D^{20°} = 192{\cdot}1°$), and corresponding with 1·0152 g. of soluble starch, were digested with 3 g. of wet leaf, after making the volume up to 200 cc., for 48 hours at 38° in the presence of toluene; at the end of this time 10 cc. of alumina cream were added and the mixture boiled and diluted to 500 cc. (solution *A*). For the fermentations, 150 cc. portions after adding 5 cc. of yeast-water were sterilised and inoculated with pure cultures of the yeasts. After 3 weeks, 5 cc. of alumina cream were added, the mixture was boiled and diluted to 200 cc. (solution *B*). 50 cc. portions were used for the cupric reductions (= 37·5 cc. of *A*) and the results obtained calculated back to 50 cc. of *A*.

In each experiment a duplicate control was made with 3 g. of the wet leaf so as to allow for the original sugars present in the leaf. The following table gives the corrected results.

Method of calculation: CuO from 50 cc. of *A* (corr. for leaf sugars) = 0·0888 g.
CuO due to maltose in 50 cc. of *A* = 0·0388 g.

CuO due to glucose in 50 cc. *A* = 0·0500 g., or $\frac{0{\cdot}0500}{2{\cdot}578} = 0{\cdot}0194$ g. glucose.

CuO due to maltose in 50 cc. *A* corresponds with $\frac{0{\cdot}0388}{1{\cdot}38} = 0{\cdot}0281$ g. maltose.

Total glucose in 500 cc. of *A* = 0·1940 g. = 0·1746 g. soluble starch
„ maltose „ = 0·2810 „ = 0·2664 „ „
Therefore total starch converted to sugars = 0·4410 g.
Total soluble starch taken = 1·0152 g.
Starch remaining in *A* = 1·0152 − 0·4410 = 0·5742 g.

Rotation of glucose in *A*, in 400 mm. tube ... ... = 0·082°
„ maltose „ „ ... ... = 0·310°
„ soluble starch in *A* in 400 mm. tube, taking $[\alpha]_D^{20^\circ} = 192{\cdot}1^\circ$ ... ... = 0·872°
Total = 1·264°
Actually observed in *A* = 1·222°

TABLE II.

Leaves picked at midnight, September 29, 1913.

Expt.	Leaf	CuO from 50 cc. of *A* (corr.)	CuO corr. with 50 cc. *A* after fermentation			$\alpha_D^{20^\circ}$ of *A* in 400 mm. tube	$\alpha_D^{20^\circ}$ calc. for glucose, maltose and unchanged starch
			S. exiguus	*S. marxianus*	Mean		
4	*Tropæolum*	0·0382	—	—	—	1·385°	—
5	Dahlia	0·0888	0·0364	0·0412	0·0388	1·222°	1·264°
6	Mangold	0·0873	0·0462	0·0412	0·0437	1·218°	1·280°

In the case of the dahlia and mangold, these results clearly show that more than half of the sugar produced from the starch is glucose. In the case of *Tropæolum* the reducing power was so small that fermentations were not carried out. The results given in Table III however show that with this leaf the greater proportion of the sugar formed is glucose.

It is interesting to note that the diastatic power of the *Tropæolum* and dahlia leaves picked at midnight appears to be smaller than in the case of the same leaves picked at 11 a.m. (Table I). Using the same weight of leaf in the digestion, less reducing sugars have been formed and a smaller proportion of glucose in 48 hours than in the earlier results in 24 hours. As, however, no special precautions to ensure neutrality of the soluble starch were taken and as it is well known, from the work of Ford and Guthrie and

of Sherman, that very small differences of reaction greatly influence the results obtained, no special significance can at present be attached to these data.

Action of leaf material on Gelatinised Starch.

Series III.

In this series a known weight of starch dried *in vacuo* at 130° was gelatinised by boiling for 15 minutes with 200 cc. of water; the paste was digested with 5 g. of the wet leaf during 32 hours at 38°. The solution was then boiled and made up to 500 cc. at 15° (solution *C*). In these experiments, although nearly all of the starch was liquefied, a small quantity was left undissolved in every case; on filtering the solution it was found on the filter-paper. On this account it was not possible to compare the rotation of the solution obtained with that calculated for the sugars present. Fermentations were carried out as in Series II and controls made with 5 g. of the leaf material.

TABLE III.

Action of leaf enzymes on Gelatinised Starch. Leaves picked 12 midnight, October 3, 1913.

Expt.	Leaf	Vacuum dried starch taken	CuO from 50 cc. of *C* (corr.)	CuO from 50 cc. of *C* after fermentation	
				S. exiguus	*S. marxianus*
7	*Tropæolum*	2·2340	0·0493	0·0061	0·0100
8	Dahlia	2·3250	0·2170	0·1595	0·1655
9	Turnip	2·1418	0·2110	0·1308	0·1376

In these experiments the *Tropæolum* leaf again shows a relatively low diastatic activity as compared with that of the dahlia and turnip; the greater part of the sugar, however, is seen to be glucose. The turnip leaf is particularly active, which is interesting in view of the high proportion of starch which is characteristic of this leaf [Davis and Sawyer, 1916]. In all cases the leaf enzymes have produced a mixture of maltose and glucose, the maltose generally predominating.

Action of leaves on Soluble Starch.

Series IV.

100 cc. of a soluble starch solution (= 1·6855 g. vacuum dried soluble starch, having $[\alpha]_D^{20°} = 193·7°$) were digested with 5 g. of wet leaf for 36 hours

at 38°. The solution was then boiled, 5 cc. of alumina cream were added and the volume made up to 500 cc. at 15° (solution *D*). Controls were made as usual. The calculations are similar to those of Series II.

TABLE IV.

Expt.	Leaf	CuO from 50 cc. solution *D*	CuO from 50 cc. *D* after fermentation		$\alpha_D^{20°}$ of solution *D*	
			S. exiguus	*S. marxianus*	Measured	Calc. for sugars and unchanged starch
10	Sunflower	0·0377	0·0123	—	0·612° (100 mm.)	0·612°
11	Dahlia	0·1449	0·0460	0·0532	1·065° (200 mm.)	1·060°

In both cases, especially in that of the dahlia which has the far higher diastatic activity, the larger part of the sugar consists of glucose. The agreement between the values observed and calculated for the mixture of sugars and unchanged soluble starch is very close.

SUMMARY.

1. It is shown that the crushed pulp of all the leaves examined (*Tropæolum*, potato, dahlia, turnip, sunflower and mangold) acts upon soluble starch or gelatinised starch, forming reducing sugars; of these the greater part consists of glucose, the rest being maltose. There is therefore no doubt as to the presence of maltase in these leaves, whether plucked at night or in the daytime.

2. When a relatively large excess of starch is used the conversion is generally incomplete. The action of the endo-cellular enzyme maltase being limited under these conditions of working, by its low solubility and low power of diffusion, maltose is nearly always found among the products.

REFERENCES.

Brasse (1884), *Compt. rend.* **99**, 878.
—— (1885), *Compt. rend.* **100**, 454.
Brown and Morris (1893), *J. Chem. Soc.* **63**, 604.
Cuisinier (1885), *French Patent*, No. 171958.
Davis (1916), *Biochem. J.* **10**, 31.
Davis and Daish (1913), *J. Agric. Sci.* **5**, 437.
Davis, Daish and Sawyer (1916), *J. Agric. Sci.* **7**, 255.
Davis and Sawyer (1916), *J. Agric. Sci.* **7**, 352.
Robertson, Irvine and Dobson (1909), *Biochem. J.* **4**, 258.
Vines (1891), *Brit. Assoc. Report*, 1891, 697.