THE AMYLOLYTIC ACTION OF MAPLE AND BIRCH SAPS

BY

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INTRODUCTION

Canadian workers (1, 2, 3) have claimed the presence in maple sap of "sucrogène-amylase" and "cellobiogène-amylase" which transform starch into sucrose and cellobiose, respectively, and in birch sap, of a similar "cellobiogène-amylase" as well as a glucose-forming enzyme. In previous communications (5, 6), it was pointed out that these results could not be confirmed in Holland. In saps from 4 species of Dutch maple, only "ordinary" amylase, which converted starch to maltose and dextrin, was detected. Similarly, 5 species of birch possessed "ordinary" amylase and α-glucosidase (maltase) activity, but "cellobiogène" and "sucrogène-amylases" were absent. This and other evidence was apparently sufficient to render the Canadians' point of view untenable; the possibility remained, however, that minute quantities of cellobiose, more or less masked by the much larger amounts of sugar present in fresh saps, might have been overlooked. Attempts to eliminate the latter by dialysis through collodion membranes were not very successful.

The present report concerns further attempts to elucidate the phenomenon with the aid of filter paper chromatography and dialysis through cellulose membranes.

MATERIAL AND METHODS

A. Saps

The following species and hybrids were investigated: *Acer campestre* L., *A. cappadocicum* Gled., *A. dasycarpum* Ehrh., *A. Negundo* L. and *A. platanoides* L.; *Betula lenta* L., *B. papyrifera* Marsh and various *Betula* hybrids. In 1951 alone, 10 individual birch-trees were investigated, one of which was clearly a specimen of *Betula lenta*, while two of the others were identified as *Betula papyrifera*. The remaining seven were hybrids that were very difficult to identify.

The saps were collected as previously described (5, 6), care being taken to avoid contamination by bacteria, yeasts or saliva. Active enzyme preparations were prepared from the saps by precipitation with alcohol in the cold followed by rapid drying.

B. Dialysis

Fresh birch saps, as well as some concentrated by freezing, were dialyzed through cellulose membranes against (1) tap water, (2) neutral phosphate buffer and (3) a fluid obtained from concentrated saps by fermentation procedures (the latter, because co-enzymes might be functional in α-amylase action). There was little difference in activity among the various preparations, however, and the tedious fermentation method was soon discarded; dialysis against cold phosphate buffers was subsequently adapted as a routine method.

C. Filter paper chromatography

A mixture of *n*-butanol, acetic acid and water (160 : 40 : 200) was allowed to ascend during 20—24 hrs. in 20 by 40 cm pieces of Whatman No. 1 filter paper dotted with the unknown sugars; the sugar-spots were developed with the aid of *m*-phenylenediamine at a temperature of 110° C. This is essentially PARTRIDGE's procedure (7). It was found that distinction between maltose and cellobiose is very difficult by this method, the R_F-values being quite close together. For that reason, fermentation-methods were combined with filter paper chromatography, as will become apparent further on.

D. Quantitative sugar-determinations

The micro-fermentation method described by VAN LUTSENBURG MAAS and VAN ITERSON (4) was used throughout. One of the yeast-species applied fermented only hexoses, a second one hexoses plus sucrose, and a third one hexoses, sucrose and "maltose". It is probable that fermentable trisaccharides are included in the "maltose"-fraction.

E. Differentiation between α- and β-amylase

WIJSMAN's plate test (8, 9) was used but the results were difficult to interpret. Because of the relative heat-stability of the α-amylase expected to be present, concentrated saps were also kept at elevated temperatures (e.g. 65° C.) for extended periods; by means of this method definite conclusions as to the nature of the amylase could be arrived at.

EXPERIMENTAL RESULTS

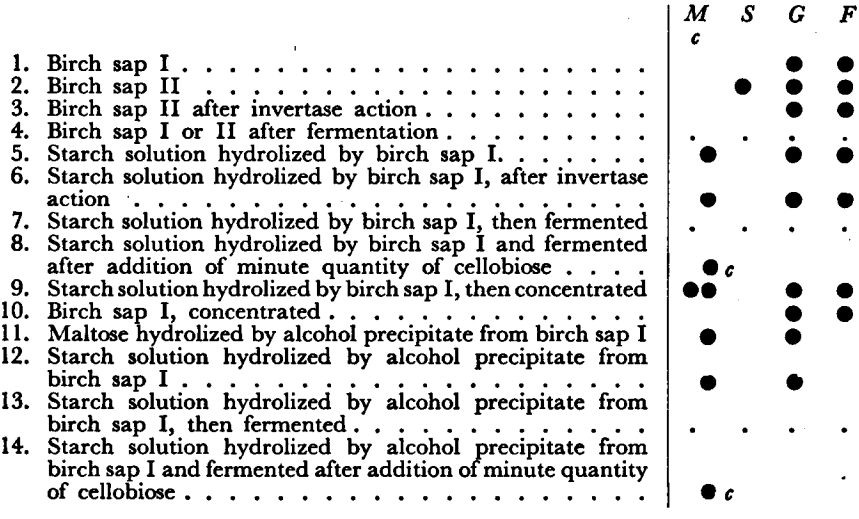
A. Chromatography

The results are illustrated in figures I and II, idealized representations of the lower portions of developed filter-paper strips (actually, glucose and fructose spots partly overlap in the form of a figure-8). Only essential details are included; results from some control experiments with boiled enzyme, for example, have been omitted.

Birch saps. 6 of the 10 saps collected in 1951 contained only fructose and glucose (diagram I, No. 1), while 4 showed the presence of an additional sugar which, according to its position on the chromatograms, its vulnerability to invertase (diagram I, No. 3) and

its fermentability (diagram I, No. 4) must be sucrose (diagram I, No. 2). It is worthy of note that 2 of the trees with sucrose in their saps were *papyriferas*. Cellobiose, which is nonfermentable, never appeared in the strips. Small quantities, however, when added to saps and sap-starch digests, survived the baker's yeast treatment and

Diagram I. For explanation, see text

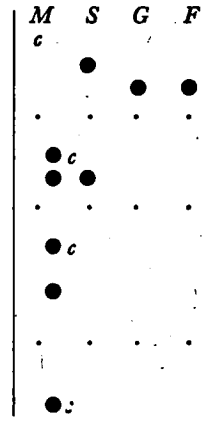


were recovered as expected (diagram I, No. 8). Starch solutions, hydrolyzed by birch sap, always gave rise to a sugar which corresponded in position to maltose or cellobiose (diagram I, No. 5). Since it was inert to invertase action (diagram I, No. 6) and disappeared following the yeast treatment (diagram I, No. 7), thus excluding sucrose and cellobiose, respectively, it is most probably identifiable with maltose. In concentrated digests, yet another sugar was found, vulnerable to yeast and possessing a very low R_F value (diagram I, No. 9). The assumption that this sugar is a trisaccharide is very well compatible with the conclusion (arrived at in our earlier papers) that an α-amylase is active in the birch saps.

By the use of alcohol-precipitates from birch saps, it could be shown that both glucose and maltose are produced from starch (diagram I, No. 12). Whether the presence of these two sugars is due to the action of one single enzyme, an α-glucosidase with a great specificity-range, remains to be decided. The fact that the glucose-spots in chromatograms of starch/enzyme digests appeared stronger than those obtained from experiments with enzyme plus maltose (diagram I, No. 11) seems to favor the one enzyme concept.

Maple saps. These saps contained sucrose and amylase, but were devoid of hexose sugars and enzymes producing glucose from maltose or starch. For particulars, see diagram II.

Diagram II. For explanation, see text



B. Quantitative sugar-determinations

Some of the results have been summarized in table III:

TABLE III

Enzyme	Substrate, initial concentration in digest	Maltose (incl. fermentable trisaccharide) produced after 4 days at 35° C. and pH 6.0	Fermentable free hexose, produced after 4 days at 35° C. and pH 6.0
1. Birch sap, concentrated by freezing, then dialyzed against phosphate buffer pH 6.2 in the cold	Starch 2.1 %	0.24 %	0.70 %
2. Birch sap, concentrated by freezing, heated at 65° C. for 20 min., and dialyzed against phosphate buffer pH 6.2 in the cold	Starch 2.7 %	0.16 %	0.66 %
3. Same as 2, but unheated	Starch 2.7 %	0.32 %	0.55 %
4. Same as 3	Maltose hydrate 2.2 %		0.68 %
5. Alcohol precipitate from birch sap	Starch 2.7 %	0.35 %	0.70 %
6. Same as 5	Maltose hydrate 2.2 %		0.51 %

Since the iodine reaction in the starch digests had in all cases disappeared at the time of examination, while the amounts of sugars formed at that time were fairly small, the presence of an α-amylase relatively free from β-amylase must be assumed here. In concentrated birch saps it seems to possess a more marked insensitivity to heat than in fresh saps (table III, items 2 and 3; compare ref. 6).

To investigate the possibility that cellobiose might be present in

the large fraction of the starch-enzyme digests not accounted for in the fermentation, one of the experiments carried out in 1947 with the aid of fresh maple sap was now repeated with dialyzed birch sap.

The sap was allowed to act on a starch solution till the iodine reaction had disappeared; then a little saliva was added and allowed to work. Under these conditions, 0.18 % of fermentable hexose and 2.25 % of "maltose" (incl. some trisaccharides) were produced from 2.5 % starch. Even though we have to make certain allowances for the relative inaccuracy of the micro-fermentation method, it is quite clear that, at the achroic point, only noncoloring dextrins can have been present besides the free sugars produced from the starch by the birch-enzymes: the unfermentable sugar, cellobiose, is not transformed into maltose by saliva. In other words: appreciable quantities of cellobiose simply cannot be present among the products of starch degradation.

Discussion

In accordance with previous findings (5, 6), "ordinary" amylase activity yielding maltose from starch was demonstrated in both maple and birch sap. At the achroic point, the quantity of maltose is low, a fact which can be interpreted as evidence for an α-amylase. This would be in agreement with the relative thermostability noticed in birch amylase when present in concentrated saps.

When salivary amylase is allowed to act on a starch/tree sap digest after disappearance of the iodine reaction, the starch is converted almost quantitatively to fermentable sugars. From this it follows that there cannot be an appreciable amount of cellobiose among the initial products of starch degradation. The presence of cellobiose in tree saps and sap/starch digests, claimed by the Canadian workers Bois et al. (1, 2, 3) could not be confirmed for Dutch trees. However, the possibility must be left open that birch trees yielding cellobiose in "commercial" quantities (1) are among the natural assets of Canada and not of Holland.

In starch/birch sap digests glucose was formed in addition to maltose. The question whether these two sugars are formed by different enzymes or by a single one, will be the subject of a separate paper.

SUMMARY

By means of filter paper chromatography and dialysis experiments combined with fermentation procedures, it was shown that maple sap contains an enzyme producing maltose but not cellobiose from starch. Birch sap contains an enzyme system converting starch into a mixture of maltose, glucose and dextrins, again without production of cellobiose. The amylases of maple and birch sap probably belong to the α-group.

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REFERENCES

1. Bois, E. & W. O. Chubb, (1942). The sap of the birch tree, *Betula papyrifera* Marsh. I. The amylase system. *Canad. J. Res.* B, *20*, 114.
2. Bois, E. & A. Nadeau, (1938*a*). Contributions à l'étude d'*Acer saccharum*. II. La présence d'amylases dans la sève d'érable et les produits d'hydrolyse. *Canad. J. Res.* B, *16*, 114.
3. Bois, E. & A. Nadeau, (1938*b*). III. Action des amylases de la sève d'érable. *Canad. J. Res.* B, *16*, 121.
4. Lutsenburg Maas, H. J. van & G. van Iterson Jr., (1915). A micro-saccharimeter. *Proc. Kon. Akad. Wet. Amsterdam*, *18*, 258.
5. Meeuse, B. J. D. (1943). Oriënterende onderzoekingen over de vorming van rietsuiker uit zetmeel in planten bij lage temperatuur. Diss. Delft.
6. Meeuse, B. J. D. (1949). Observations on the enzymatic action of maple and birch saps. *New Phytologist*, *48*, 125.
7. Partridge, S. M. & R. G. Westall (1948). Filter-paper partition chromatography of sugars. I. General description and application to the qualitative analysis of sugars in apple juice, egg white, and fetal blood of sheep. *Biochem. J.* *42*, 238.
8. Wijsman, H. P. (1889*a*). De diastase, beschouwd als mengsel van maltase en dextrinase. Diss. Amsterdam.
9. Wijsman, H. P. (1889*b*). La diastase considérée comme un mélange de maltase et dextrinase. *Rec. Trav. Chim. Pays-Bas*, *9*, 1.