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## THE ESTIMATION OF CARBOHYDRATES. IV.

### THE PRESENCE OF FREE PENTOSE IN PLANT EXTRACTS AND THE INFLUENCE OF OTHER SUGARS ON THEIR ESTIMATION.

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PREVIOUS papers of this series have dealt with the estimation of the hexoses, disaccharides and starch present in plant material. In the present paper we bring forward evidence to show that free pentoses are also usually present in the alcoholic extracts, and have to be taken into account in the scheme of analysis<sup>1</sup>. The existence of free pentoses in plants has not hitherto been recognised; the evidence that they are actually present may briefly be summarised as follows.

Substances undoubtedly exist in these extracts which are soluble in 80 % alcohol, are not precipitated by basic lead acetate, are unfermentable by ordinary yeasts and exercise a certain reducing power after all other sugars have been fermented away. This reducing power (calculated as a mixture of arabinose and xylose) corresponds with a proportion of pentose very nearly identical with that calculated from the weight of phloroglucide obtained on subjecting the purified aqueous solution used in the analysis to the ordinary Kröber-Tollens distillation process. These facts taken together can only be explained by assuming that *free* pentoses are actually present and not by any assumption that the furfural obtained on distilling with acid originates from gums or pentosans or from the other sugars present.

It is true that, when cane sugar or certain hexoses are distilled with hydrochloric acid, small quantities of furfural-like compounds are formed which yield an insoluble phloroglucide and that, in consequence, the estimation of *small* quantities of pentose in presence of large

<sup>1</sup> *J. Agric. Science*, 1913, v. 465.

quantities of these sugars is not strictly accurate. But we show below that the proportion of other sugars present in such extracts as we have dealt with can give rise only to a relatively small proportion of the phloroglucide actually found and does not materially interfere with the pentose figure obtained by the ordinary process.

The occurrence of pentoses in plants would explain the accumulation of free pentoses in the "vinasses" remaining after distilling off the alcohol from the fermented liquors of distilleries which make use of the molasses of the beet-root sugar industry<sup>1</sup>. Other sugars are completely fermented away by the ordinary distillery yeasts but, as we have found, the pentoses remain completely unfermented, and therefore form a very large proportion of the total carbohydrates existing in these liquors after the fermentation is complete.

*Presence of Pentoses in Alcoholic Extracts of Foliage Leaves.*

In a large number of estimations made with the leaves of different plants (mangolds, turnips, *Tropaeolum majus*, *Helianthus*, carrot, potato, etc.) the percentage of pentose obtained by the distillation method, when applied to the alcoholic extract treated according to our scheme of analysis, was found to range from 0.3 to 1 %, calculated on the total vacuum-dried matter. The fresh leaf material (about 1000 grms.) used in our analyses was dropped into boiling alcohol (2 litres) containing a little ammonia to stop enzyme action, and was then extracted for 18 hours in a large specially constructed metal form of Soxhlet extractor. After the operations outlined in our scheme<sup>2</sup> 50 c.c. of the 2 litres of solution A were distilled with acid according to the Kröber-Tollens method. The phloroglucide frequently weighed from 0.015 to 0.026 gm.; thus, for instance, in one case (*Mangold Leaf*, 1 a.m. Oct. 12, 1912) when the total pentose was 0.78 %, the phloroglucide weighed 0.0192 gm. Now the total sugars in this case did not exceed 20 grms. in the 2 litres of solution, so that the amount of sugars other than pentoses in the 50 c.c. used for the analysis did not exceed 0.5 gm. We have found (see p. 411) that 0.5 gm. of carefully purified cane sugar, dextrose or maltose, when subjected to Kröber's distillation process gives only 0.0036 to 0.0047 gm. phloroglucide, so that although it is perfectly true, as contended by Kluyster<sup>3</sup>, that the

<sup>1</sup> Matignon, *Bull. Soc. d'Encouragement*, 1914, cxxi. 445.

<sup>2</sup> *loc. cit.* p. 466.

<sup>3</sup> *Biochemische Suikerbepalingen*, Leiden, 1914, pp. 181 and 190.

hexoses etc. are a source of error when estimating small quantities of pentose, their presence does not account for the greater part of the phloroglucide actually obtained. We show below (p. 411) that when 0.01 gram of pure arabinose is mixed with 25 times its weight of cane sugar (0.25 gm., a proportion of total sugar to pentose far greater than usually occurs in these extracts) the weight of phloroglucide obtained being 0.0094 gm., the result obtained was about 20 % higher than in the absence of cane sugar (0.0162 gm. arabinose, as compared with 0.0128 gm.). When 0.02 gm. arabinose is distilled with 0.25 gm. cane sugar (a proportion corresponding with that usually found in our extracts) so that 0.018 gm. phloroglucide is weighed, the error in the pentose estimation is smaller, the result being about 15 % high (0.0256 gm. found instead of 0.0222).

That *free* pentoses actually exist in these alcoholic extracts is also borne out by the following facts. When portions of the aqueous solution A of our scheme are fermented as completely as possible, and the reducing power of the fermented solutions estimated after purification with alumina cream, the residual reducing power agrees fairly closely with that calculated for the proportion of pentoses present, as determined by the ordinary distillation method. This residual reducing power can indeed be used as a means of estimating the pentoses present, as the following example shows :

*Turnip Leaves, July 9, 1913.*

The pentose found by distilling 50 c.c. of solution A = 0.60 % on the total dry matter.

One litre of solution A was then evaporated *in vacuo* to about 175 c.c. and made up to 250 c.c.

Five portions of 50 c.c. (each representing 200 c.c. of the original solution A) were then sterilised, and fermented as completely as possible for three to five weeks with a pure culture of baker's yeast<sup>1</sup>. 5 c.c. of alumina cream was then added to each portion and the solution filtered and washed to 100 c.c. with boiling water, the volume being finally made up to 100 c.c. at 15°. 50 c.c. of the filtrate of each (=100 c.c. of the original solution A) was then used to ascertain the reducing power.

<sup>1</sup> A large number of experiments, details of which will be published later, have shown that baker's yeast does not ferment or assimilate the pentoses (xylose and arabinose) ; on the other hand the maltase-free yeasts (*S. Marxianus*, *S. exiguus* and *S. anomalus*) gradually but slowly destroy these sugars.

The average value for the CuO found = 0.0512 gm. Now if the whole of the reducing power is due to pentoses, assuming the mean value found for arabinose and xylose under these conditions<sup>1</sup> we have:

Percentage of pentose calculated on the total dry matter

$$= \frac{0.0512}{2.53} \times \frac{2000}{100} \times \frac{500}{440} \times \frac{100}{89.4} = 0.51 \%$$

There is thus a fairly close agreement between the result obtained for pentose by direct distillation and that obtained on the assumption that the slight residual reducing power which remains after fermentation is due solely to pentoses. It is true that the result obtained by the direct distillation by the Kröber-Tollens method (0.60 %) is slightly higher than the other value (0.51 %) but the difference is exactly of the order (18 % higher) that would be expected owing to the production of phloroglucide from the sugars other than pentoses present in solution A. The value 0.51 % probably more correctly gives the true proportion of pentoses present, but for practical purposes the difference between 0.5 and 0.6 % is of little importance, as the error introduced in correcting the calculation for the reducing sugars present by such a difference does not exceed 1 milligram of CuO. For most purposes therefore little error is introduced by estimating the pentoses by the distillation process, but when it is desired to estimate the pentoses present with the highest possible degree of accuracy the process suggested by Kluyver may be employed: the other sugars (dextrose, laevulose, cane sugar, etc.) should be completely fermented away with *S. cerevisiae* and the pentose determination carried out on the fermented material, after adding alumina cream and diluting to a known volume.

From the above example with turnip leaves it appears that in the case of alcoholic leaf extracts prepared by our method the slight residual reducing power which always remains after complete fermentation of the solution with yeast is due to the pentoses present. In nearly 400 fermentation experiments made with extracts of leaves of many different kinds, the amount of this residual reducing power has been found to be proportional to the pentoses present as estimated by the Kröber method, so that it is highly probable that it is to be attributed solely to free pentoses.

<sup>1</sup> Daish, *J. Agric. Sci.* 1914, vi. 255.

*Effect of other Sugars on the Kröber-Tollens Method of Estimating Pentoses.*

It has been generally recognised since the early days of this method that the ordinary carbohydrates such as cane sugar, galactose and dextrose give small quantities of furfural-like substances<sup>1</sup> when subjected to distillation with hydrochloric acid<sup>2</sup>, but the amounts are, for practical purposes, so small that they can generally be disregarded<sup>3</sup>. Stoklasa<sup>4</sup> states that 100 grms. of cane sugar gives about 0.22 gm. of furfural, and Kröber and Rimbach<sup>5</sup> give this figure as about 0.5 gm. In the ordinary estimation of pentoses and pentosans these amounts can generally be disregarded, but when very small amounts of pentose have to be estimated accurately, Kluyver's experiments and our own show that a considerable error may be introduced owing to the presence of excess of hexose sugars; in such cases, the pentose should be estimated after completely fermenting away the other sugars by ordinary yeast. As a rule, however, in the scheme of analysis we have adopted, such a refinement is unnecessary as such differences, as, for example, between 0.5 and 0.6 % of pentose, do not materially interfere in calculating the results obtained for the other sugars.

*Experiments with pure sugars.*

The sugar was dissolved in 50 c.c. of water and subjected to Kröber's distillation process in Jena glass flasks fitted with rubber stoppers<sup>6</sup>.

<sup>1</sup> It is probable that these sugars give, not furfural, but hydroxymethylfurfural, which also forms a sparingly soluble phloroglucide. When these sugars are distilled with acid, there is a marked difference between the colour changes shown on adding the phloroglucide from those given with pure arabinose, or pure furfural. In the latter cases, the solution is first yellow and then turns green. With the hexoses, the solution changes from yellow to a *claret red* and then finally to green. With mixtures of pure arabinose and hexoses the claret colour is always observed. [*Note added Oct. 16, 1914.* Since this paper was written, Cunningham and Dorée in a communication to the *Biochemical Journal*, 1914, VIII. 433, dated July 13, have dealt with several of the points raised above somewhat more fully than we have done; they give qualitative evidence that hydroxymethylfurfural is actually formed when hexoses are distilled with hydrochloric acid under the Kröber-Tollens conditions.]

<sup>2</sup> Compare Gunther, *Gött. Dissert.* 1891, p. 19.

<sup>3</sup> Cp. Kruger, *Rostock Dissert.* 1895, p. 29.

<sup>4</sup> *Zeit. Zuckerind. Böhmen*, XXIII. 291.

<sup>5</sup> *Zeit. angew. Chem.* 1902, 508.

<sup>6</sup> A necessary precaution in the estimation of pentoses and pentosans which is not emphasized in the standard text-books is that the distillation flasks should be fitted with rubber stoppers and *not* with ordinary corks. The latter are invariably attacked by the

*Pure hexoses* (several times recrystallised).

0.5 gm. maltose (free from dextrin, etc.), weight of phloroglucide  
= 0.0036 gm.

0.5 gm. dextrose, weight of phloroglucide = 0.0040 gm.

0.5 gm. cane sugar, weight of phloroglucide = 0.0047 gm.

0.01 gm. *arabinose* (specially prepared from gum arabic and carefully purified by several crystallisations).

1. Gave 0.0068 gm. phloroglucide.

2. Gave 0.0060 gm. phloroglucide.

Average  $\frac{0.0064}{2}$  gm. phloroglucide = 0.0129 gm. pure arabinose.

0.01 gm. *arabinose* + 0.25 gm. *cane sugar*.

1. Gave 0.0097 phloroglucide.

2. Gave 0.0090 phloroglucide.

Average  $\frac{0.0094}{2}$  gm. phloroglucide = 0.0162 gm. arabinose.

Here the addition of 0.25 gm. cane sugar has increased the yield of phloroglucide by 0.0030 gm. and the apparent pentose by almost 25 %.

0.02 gm. *arabinose alone*.

1. Gave 0.0150 gm. phloroglucide.

2. Gave 0.0146 gm. phloroglucide.

Average  $\frac{0.0148}{2}$  gm. phloroglucide = 0.0222 gm. arabinose.

0.02 gm. *arabinose* + 0.25 gm. *cane sugar*.

1. Gave 0.0175 gm. phloroglucide.

2. Gave 0.0185 gm. phloroglucide.

3. Gave 0.0178 gm. phloroglucide.

Average  $\frac{0.0179}{3}$  gm. phloroglucide = 0.0256 gm. arabinose.

Here the cane sugar (0.25 gm.) has caused an increase of 0.0031 gm. on the phloroglucide and an increase of 16 % on the pentose.

With larger quantities (0.2 gm.) of arabinose, cane sugar or dextrose still give rise to an increase in the phloroglucide of about 2 milligrams, but the error in the pentose estimation is diminished proportionately to the larger quantity of arabinose and becomes approximately 1 % on the pentose present.

hydrochloric acid and give appreciable quantities of furfural. The following example shows this. A series of "blanks" was carried out with hydrochloric acid in a flask fitted with ordinary corks. The results were as follows: 1st distillation gave 0.0022 phloroglucide; 2nd distillation gave 0.0042; 3rd distillation 0.0045; 4th 0.0035; 5th distillation gave 0.0013 gm. phloroglucide. When similar distillations were made in a flask fitted with rubber stoppers there was no change of colour after adding the phloroglucinol and the weight of phloroglucide obtained was *nil*.

*Arabinose alone.*

Taken 20 c.c. of an approximately 1 % solution of arabinose.

1. Gave 0.1716 phloroglucide.
2. Gave 0.1726 phloroglucide.
3. Gave 0.1705 phloroglucide.

Average  $\frac{0.1716 + 0.1726 + 0.1705}{3}$  phloroglucide = 0.1964 grm. arabinose.

20 c.c. of the same solution + 0.2 grm. cane sugar.

Phloroglucide weighed 0.1735 grm. = 0.1985 grm. arabinose.

20 c.c. of the same solution + 0.2 grm. dextrose.

Phloroglucide weighed 0.1735 grm. = 0.1985 grm. arabinose.

*Discussion.*

The results given above can be explained only by the assumption that free pentoses are present in the leaf-extracts we have examined. Kluver<sup>1</sup> in discussing our first paper<sup>2</sup> suggested that pentoses are absent in such cases and that their estimation by the Kröber-Tollens method may give rise to considerable error owing to the production of furfural-like compounds from the other sugars present. We have shown above that, although some error does arise from this cause, it is not significant in the majority of cases. Kluver emphasized the fact that we had merely stated in our previous paper that pentoses are present without giving any experimental evidence. This we have now done.

## SUMMARY.

Evidence is brought forward to show that free pentoses exist in the alcoholic extracts of foliage leaves of different plants. Their amount can be estimated with a fair degree of accuracy by the ordinary distillation process or by the reducing power of the purified liquor after other sugars have been fermented away.

When, however, small amounts of pentose have to be estimated accurately in presence of large quantities of other sugars, it is advisable, as suggested by Kluver, to ferment away these sugars before applying Kröber's process.

<sup>1</sup> *loc. cit.*

<sup>2</sup> Davis and Daish, *J. Agric. Sci.* 1913, v. 465.

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