2. The carbonate formed is determined, in the case of compounds not containing halogen, by titration with standard hydrochloric acid using phenolphthalein and methyl orange as indicators.
3. The amount of sulfur in a sulfur compound may be determined gravimetrically on the titrated solution used in the carbon analysis.
4. In the case of halogen compounds, any oxyhalogen compounds formed are destroyed by some reducing agent such as hydrazine hydrate, the solution is then acidified and boiled, and the carbon dioxide thus liberated is reabsorbed by barium hydroxide and titrated in the usual manner.
5. Nitrogen from nitrogen compounds does not interfere with the carbon determination and seems to escape almost entirely as free nitrogen.

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF ILLINOIS]

THE OCCURRENCE OF FREE PENTOSES IN PLANTS. THE EFFECT OF EXTRACTION OF THE SUGARS WITH AMMONIACAL ALCOHOL

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Introduction

In 1914 Davis and Sawyer[1] offered the first evidence for the occurrence of pentoses in the free state. Further evidence by Spoehr[2] confirmed such occurrence. Still, Davis and Sawyer based their conclusions on the fact that a non-fermentable reducing sugar residue was obtained from a plant extract after fermentation with yeast and also on the fact that by running the Krober-Tollens determination of pentoses by distillation with hydrochloric acid, a greater precipitate was obtained with phloroglucinol than could be accounted for as due to hexoses. They also showed that the furfural-like compounds produced from sugars other than pentoses are of very little significance in the majority of cases.

However, for the purpose of neutralizing any organic acids that were present, these investigators added to the alcoholic extract of the sugars ammonium hydroxide (d., 0.88) in a proportion of 1% by volume. This treatment would tend to throw some doubt as to the conclusiveness of their evidence. Perhaps enough isomerization of fructose to glutose or some other sugar, non-fermentable by yeast, could have taken place to give a residue after fermentation which would reduce Fehling's solution. Furthermore, it is possible that some intramolecular change or some degradation of the hexose sugars could have given rise to a product which

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[1] Davis and Sawyer, *J. Agr. Sci.*, **6**, 406 (1914).

[2] Spoehr, *Carnegie Inst. Pub.*, **1919**, 287.

would lead to the formation of more furfural or furfural-like compounds than the original hexose.

The use of some weak alkali to neutralize organic acids is a detail of common practise in the extraction of sugars from plants. Hence with more comprehensive studies of carbohydrate metabolism in which pentoses are to be determined, some definite information as to the effect upon pure sugars of such a treatment as was employed by Davis, Daish and Sawyer is necessary.

Experimental Part

Preparation of the Solution.—Twenty g. of pure sucrose was dissolved in 220 cc. of distilled water. A 100cc. portion of this solution was inverted with invertase and made up to 220 cc. (Solution A). The invertase was prepared according to Hudson[3] and was dialyzed to remove any sugars. Another 100cc. portion of the original solution was diluted to 220 cc. (Solution B). Our blank solution was prepared by diluting 100 cc. each of Solutions A and B to two liters with water and therefore contained sucrose and invert sugar in about equal proportions and a total sugar content slightly less than 10 g.

Extraction Procedure. Expt. I.—Into 1500 cc. of boiling alcohol 100 cc. each of Solutions A and B were measured, followed by 5 cc.[4] of ammonium hydroxide (d. 0.90). This solution was refluxed in a Pyrex flask on the water-bath for 18 hours, concentrated under diminished pressure to about 150 cc., transferred to a smaller flask and diluted with water to 500 cc. During the refluxing the sugar solution became quite yellowish and after the process of concentration a very small quantity of resin-like material remained insoluble in water. The final solution was cleared with basic lead acetate, filtered and the residue washed until the volume of filtrate was about 1900 cc. The excess of lead was removed with sodium carbonate and the precipitate filtered off and washed until the volume of the filtrate was 2 liters.

Expt. II.—Expt. I was repeated without introduction of any air, inasmuch as it seemed possible that the introduction of air to lessen the bumping during concentration might have caused an increased oxidation of the sugars and increased formation of pentose.

Expt. III.—This experiment was identical with Expt. I except that the refluxing for 18 hours was omitted.

Determination of Pentoses by Distillation.—Fifty-cc. portions of the final filtrate, after removal of the lead, were analyzed for pentoses according to the usual Krober-Tollens[5] method. The rate of distillation was maintained as high as possible (30 cc. every 10 minutes) since, according

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[3] Hudson, This Journal, **36**, 1566 (1914).

[4] Assuming the use of an initial concentration of 1% by volume of the ammonia in an actual plant extraction. This seemed a reasonable excess to expect.

[5] "Methods of Analysis," Assoc. Offic. Agr. Chem., **1919**, p. 96.

to Van Haarst and Olivier,[6] continued boiling with hydrochloric acid tends to decompose any furfural.

Reduction of Hexoses and Determination of Pentoses by the Reduction Method.—For this determination a 1000cc. portion of the 2-liter filtrate was concentrated under diminished pressure to 250 cc. Of this 50cc. portions were taken, sterilized and fermented with brewers' yeast for several weeks. They were then cleared with alumina cream and washed with boiling water until the total volume was 100 cc. The reduction was carried out according to the method of Brown, Morris and Millar[7] on 50cc. portions of the final solutions. The amount of reduced copper was estimated following the method of Peters,[8] by titrating the excess of copper in the filtrate and subtracting from a blank. The apparent pentose was calculated from a table by Daish.[9]

The results of the experiments are recorded in Table I.

TABLE I

APPARENT PENTOSES PER 50 CC. OF ORIGINAL SOLUTION

	{By distillation method}		{By reduction after fermentation}	
	Phloroglucide G.	Pentose G.	CuO G.	Arabinose[a] G.
Blank a	0.0020	0.0073	0.000	0.0000
b	.0024	.0077	.0000	.0000
Expt. I a	.0191	.0247	.0255	.0046
b	.0188	.0244	.0324	.0060
Expt. II a	.0163	.0221	.0117	.020[b]
b	.0162	.0218		
Expt. III a	.0097	.0151	.114	.0021[b]
b	.0091	.0145		

[a] Calculated to original solution.

[b] Average of five determinations.

Discussion

From Table I it will be noted that the long refluxing with ammonium hydroxide gives a very considerable increase in apparent pentose by the distillation method.

By the reduction method only a fraction of the quantity of the apparent pentoses indicated by the Krober-Tollens method is found. A part of the difference may be explained as due to the high results from the latter method because of the prolonged distillation. It has also been assumed that the brewers' yeast (*Saccharomyces cerevesiae*) is without effect on pentoses even under prolonged action and in the absence of other food.

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[6] Van Haarst and Olivier, *Chem. Weekblad*, **11,** 918 (1914).

[7] Brown, Morris and Millar, *J. Chem. Soc.*, 71, 72 (1897).

[8] Peters, THIS JOURNAL, **34,** 928 (1912).

[9] Daish, *J. Agr. Sci.*, **6,** 255 (1914).

In Expts. II and III the solutions were allowed to ferment for eight weeks but it is not probable that any pentose was assimilated. A slightly higher value in favor of the distillation method is of course to be expected owing to the production of phloroglucide from other substances than pentoses.

It is significant that the blank gave no apparent pentose by the reduction method. The quantities found in the other cases were small and insufficient to account for the total free pentose found by Davis and co-workers. Still, they are of sufficient amount to introduce a very appreciable error in the determination of free pentose if its real amount is only slightly greater. Consequently, any extraction procedure involving the use of ammonia and perhaps other weak alkalies should be avoided, or used with caution.

Summary

It has been shown that refluxing with ammoniacal alcohol and extensive concentration of an alcoholic solution of pure sucrose and invert sugar give appreciable amounts of apparent pentoses by both the Krober-Tollens and the fermentation methods. These are not in sufficient quantity to account for the total quantities reported in plant extracts. In the absence of ammonia some apparent pentose is indicated by the Krober-Tollens method but none by the fermentation method.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF MISSOURI]

ACTION OF PHENYLMAGNESIUM BROMIDE ON ORGANIC ACIDS

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The investigation of the action of the Grignard reagent on free organic acids was undertaken as a result of an unsuccessful attempt to synthesize benzopinacol from oxalic acid and phenylmagnesium bromide. A review of the literature showed that little work had been done on the free acids, and such results as were published seemed to indicate that the reaction would be a general one. As a rule, the reactions discussed in the literature yielded tertiary alcohols as the chief products, although the conditions of the experiments often governed the products of the reactions. Thus it was found that in the action of carbon dioxide on arylmagnesium halides, low temperatures favored the formation of acids while higher temperatures tended to bring about the formation of ketones and tertiary alcohols.[1]

[1] Grignard, *Compt. rend.*, **138**, 152 (1904); *Bull. soc. chim.*, [3] **31**, 751 (1904). Shroeter, *Ber.*, **40**, 1585 (1907). Bodroux, *Compt. rend.*, **137**, 710 (1903). Houben, *Chem.-Ztg.*, **29**, 667 (1905). Zelinsky, *ibid.*, **28**, 303 (1904). Simmonis and Arand, *Ber.*, **42**, 3721 (1909). Ger. pat. 166,898.