[CONTRIBUTION FROM THE DIVISION OF CHEMISTRY, AGRICULTURAL EXPERIMENT STATION, STATE COLLEGE OF WASHINGTON]

# AN INVESTIGATION OF THE HYPOTHETICAL COMBINED PENTOSE AND THE SO-CALLED FREE PENTOSE WITH INFERENCES ON THE COMPOSITION OF PECTIN

BY RONALD B. MCKINNIS

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## Introduction

In connection with a study of the carbohydrate changes in apples, a question arose as to the pentose content. A study of the literature revealed the fact that very little had been done by other workers. The results obtained from this investigation point to some very significant and interesting facts concerning pectin and pentose.

## Occurrence

Pentoses are commonly assumed to be components of leaves and other portions of plants. Spoehr[1] states that the existence of free pentoses in leaves is entirely uncertain. Colin and Franquet[2] question the findings of Davis and Sawyer and state that pentoses, if present at all, are there in very slight quantity.

The pentoses are widely distributed in a hypothetical combined form as components of complex substances such as glucosides, nucleinic acids and most commonly as anhydride-like condensation products of unknown molecular weight and structure.

On preliminary inspection it seems probable that the main source of pentose in apples would be the pectins and hemicelluloses. The existence of free pentoses would, then, be dependent upon the hydrolysis of the pectins.

## Method of Determining Pentoses

Although many methods have been used for determining pentoses, none is entirely satisfactory. The method used in this work is similar to the Official Method,[3] with the exception that steam is slowly passed through the mixture to carry off the furfural, and at the same time maintain the volume of the mixture more constant. Distillation is stopped when the distillate fails to give a red color with freshly prepared aniline acetate test paper. Phloroglucinol is used to determine the furfural.

## Free Pentoses

It is evident that before estimating free pentoses there must first be made a separation from pectin, pectic acids and galacturonic acids, be-

[1] Spoehr, "Photosynthesis," A. C. S. Monograph, Chemical Catalog Co., New York, 1926, pp. 189–194.

[2] Colin and Franquet, *Bull. soc. chim. biol.*, 9, 114 (1927).

[3] "Official and Provisional Methods of Analysis," U. S. Dept. of Agr., Bur. of Chem., *Bull.* No. 107, 54–55 (1912).

cause these give a considerable yield of furfural. This separation can probably be best obtained by extraction with 95% alcohol, keeping the concentration of the alcohol on the apples at least 80%. The free pentoses will be found in the alcohol extract along with the hexoses. Such an extract, from Delicious apples, failed to give an estimable precipitate of furfuralphloroglucide. This is significant as it shows that free pentoses are absent, or are present in only small amounts.

### Combined Pentoses. Pentoses in Pectin

In estimating the total, or combined pentoses, an error will result due to the furfural from the pectin. Various workers, but especially Ehrlich[4] and Dore,[5] have shown that pectin and the galacturonic acids yield considerable quantities of furfural. All pectic substances contain galacturonic acid as an essential constituent.

Due to the fact that pectin yields furfural on distillation with hydrochloric acid, it has generally been assumed that pectin contains a pentose, probably arabinose. Nanji, Paton and Ling[6] include one arabinose molecule in their hexa-ring formula for the structure of pectin.

Ahmann and Hooker[7] state that the pentose of pectin probably does not exist as such, but is a decomposition product of galacturonic acid, which in turn is derived from pectin.

According to Ehrlich,[4] even so mild an hydrolysis as boiling with water splits off the araban portion of the pectin complex in a form soluble in 70% alcohol. Since all ordinary pectin preparations are produced by a treatment at least as drastic as boiling with water, it would appear that the arabinose-containing fraction of pectin could be leached out by digesting in 70% alcohol. The furfural yield of the residue might then be a measure of the galacturonic acid content and the furfural yield of the extract a measure of the pentose. Dore,[5] however, found that the furfural yield of the residue varied and that after two successive digestions the yield was much less and constant.

According to Nanji, Paton and Ling,[6] in their hexa-ring formula for pectin, arabinose is within the ring. For this reason it does not seem probable that arabinose within could be split off with 70% alcohol. According to Dore,[5] an extraction with 70% alcohol might extract arabinose extraneous to the ring. His results show that this is possible, as his furfural yields vary accordingly.

If the furfural error due to galacturonic acid could be found, an estimation of pentose would be possible. One molecule of galacturonic acid will

[4] Ehrlich, *Chem.-Ztg.*, **41**, 197 (1917).

[5] Dore, THIS JOURNAL, **48**, 232 (1926).

[6] Nanji, Paton and Ling, *J. Soc. Chem. Ind.*, **44**, 253T (1925).

[7] Ahmann and Hooker, *Univ. of Missouri Agr. Expt. Sta. Res. Bull.*, **1925**, p. 77.

theoretically yield either one molecule of mucic acid or one molecule of furfural.

$$COH(CHOH)_4COOH \xrightarrow{HNO_3} \underset{\text{mucic acid}}{COOH(CHOH)_4COOH}$$

$$COH(CHOH)_4COOH \xrightarrow{HCl} \underset{\text{furfural}}{C_4H_3O.CHO} + CO_2 + 3H_2O$$

The furfural yield of the galacturonic acid could be estimated from the yield of mucic acid if we knew what fraction of the theoretical yield would be obtained in practice and that no galactose would take part in the reaction. Interference is quite probable since galactose is included in the hexa-ring formula of the pectin molecule. The estimation of galacturonic acid by determining the mucic acid yield would, therefore, be unsatisfactory.

The amount of carbon dioxide evolved, however, is theoretical and provides an accurate estimation of the galacturonic acid. This method is specific for the glucuronic acids and it can be assumed that the other isomeric glucuronic acids are absent.

## Method of Determining Galacturonic Acid

For the determination of galacturonic acid, a modification of the Lefevre-Tollens method for glucuronic acid was used. Briefly stated, the method consists of decomposing the substance by heating with 12% hydrochloric acid and absorbing the evolved carbon dioxide in scrubbing towers containing a known volume of standard barium hydroxide solution. A current of carbon dioxide free air drawn by suction carried the carbon dioxide away from the surface of the liquid through a reflux condenser, which returned the condensable matter. For precaution, an absorption tower filled with granulated zinc was placed between the condenser and the carbon dioxide scrubbing towers. After the scrubbing towers came a guard tube of soda lime and an expansion chamber which was connected with the vacuum supply through a release valve which served to regulate the current.

## Correlation between Galacturonic Acid Content and Furfural Yield

The samples used in all determinations were from ripe Winesap apples, grated and thoroughly mixed to get comparable samples. These samples, of 25 g. each, were preserved in sufficient strong alcohol to keep the alcohol concentration above 75%. All determinations were made on the whole 25g. sample, the alcohol being slowly evaporated off just before use.

The following results were obtained with the furfural distillation method already mentioned, without alcohol extraction of the phloroglucide.

| Sample | 1 | 3 | 4 | 5 | Avg. |
|---|---|---|---|---|---|
| Furfural, g. | 0.1013 | 0.1163 | 0.1025 | 0.1132 | 0.1083 |

With alcohol extraction lower results were obtained.

| Sample | 6 | 7 | |
|---|---|---|---|
| Furfural, g. | 0.0950 | 0.0790 | Av. 0.0870 |

The galacturonic acid determinations gave the following results

| Sample | 9 | 10 | |
|---|---|---|---|
| Carbon dioxide, g. | 0.1125 | 0.1200 | Av. 0.1162 |

0.1162 g. of carbon dioxide would be derived from 0.4885 g. of digalacturonic acid.

To check the actual furfural yield, Ehrlich's digalacturonic acid was isolated as follows. A water extract of the apples was prepared by repeated digestion with hot water. The apple extract was saponified by the addition of sodium hydroxide and boiled with hydrochloric acid. This, according to Ehrlich,[4] gives digalacturonic acid. These processes were repeated and the precipitate was dried and weighed. The procedure was similar to that used by Rowell,[8] for the determination of pectin. To the 200 cc. of apple extract obtained from 25 g. of apples, was added 10 cc. of 10% sodium hydroxide and the mixture was allowed to stand for fifteen minutes. Twenty cc. of 10% hydrochloric acid was then added. The mixture was boiled for five minutes and filtered on a fluted filter paper; the precipitate, washed with a small amount of hot water, was returned to the beaker and made up to 100 cc. Treatment with base and acid, boiling and filtration was repeated. Two hundred cc. volume was used for the boiling with acid. The precipitate was washed with hot water until the filtrate showed only a slight acidity, transferred to a tared Gooch crucible, dried first at 55–60°, then overnight at 95° and weighed. The average yield from the 25g. samples of apples was 0.0955 g.

The acid has been shown by Ehrlich to be free from pentoses. It is prepared in the same way as the pectic acid of Wichmann and Chernoff, which Nelson[9] has shown to be identical with the digalacturonic acid of Ehrlich and Sommerfeld.[10]

Furfural determinations on the digalacturonic acid gave results as follows.

| Sample, g. | 0.0942 | 0.0968 | Av. 0.0955 |
|---|---|---|---|
| Furfural, g. | 0.0166 | 0.0164 | Av. 0.0165 |

0.0955 g. of digalacturonic acid would give a theoretical furfural yield of 0.0495 g., which makes the actual yield of 0.0165 g., 33.3% of the theoretical yield. From the 0.4885 g. of digalacturonic acid found by the carbon dioxide determination, a theoretical furfural yield of 0.2534 g. would be obtained. Actually the yield would be 33.3% of the theoretical or 0.0844 g. This checks well with the actual yield of 0.0870 g. found.

This relation of digalacturonic acid to pentose is more clearly shown by calculating the ratio of the furfural and carbon dioxide yields for the whole apple and the digalacturonic acid. The ratio, furfural/carbon dioxide

[8] Rowell, State College of Washington, Master's *Thesis*, **1926**.

[9] Nelson, THIS JOURNAL, **48**, 2412 (1926).

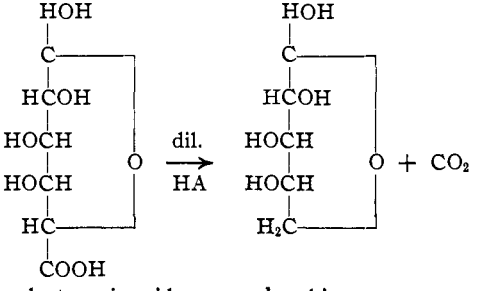
[10] Ehrlich and Sommerfeld, *Biochem. Z.*, **168**, 263 (1926).

for the apple sample was found to be 0.75, and for the digalacturonic acid, 0.74.

From this it seems evident that the apples contained no pentose whether free or combined and that the assumed pentose of pectin does not exist.

## The Significance of Galacturonic Acid

It has been shown that pectin does not contain a pentose sugar. Ehrlich, however, has isolated by boiling with dilute organic acids, *d*-rotatory *l*-arabinose. This statement is not contradictory to what has just been found. With dilute weak acid hydrolysis, *d*-galacturonic acid very probably gives *l*-arabinose, as follows.




*d*-galacturonic acid *l*-arabinose

If stronger acid is used, furfural is formed from the arabinose. This accounts for the pentose confusion as being due to the fact that galacturonic acid with dilute acids gives *l*-arabinose, while with more concentrated acid it gives furfural.

The digalacturonic acid of Ehrlich is probably a compound formed from *d*-galacturonic acid, as follows.

$$2\text{COH(CHOH)}_4\text{COOH} \longrightarrow [\text{COH(CHOH)}_4\text{COOH}]_2 - \text{H}_2\text{O}$$

*d*-Galacturonic acid Digalacturonic acid

Pectin is probably composed of a number of these groups, with part or all of the carboxyl groups occupied by methoxyl or other esterifying or salt-forming groups, though it is not certain. The work is being extended to other fruits and the isolation and properties of digalacturonic acid are being investigated.

## Summary

It has been shown that apples and apple pectin contain no pentose, either free or combined. The furfural comes from arabinose, which in turn is derived from the galacturonic acid. Arabinose is only an intermediate product in the formation of furfural from galacturonic acid. With weak acids some arabinose can be obtained before it is decomposed. The digalacturonic acid is probably the nucleus unit of the pectin molecule.

UNIVERSITY OF PITTSBURGH, PITTSBURGH, PENNSYLVANIA