the theoretical amount of the potassium salt of the required phenol, the mixture evaporated to dryness and then heated on an oil-bath for three hours at 150–170°. Unchanged nitrobenzene derivative was then removed by distillation with steam, and the non-volatile residue (ether) purified in a suitable manner. When two or more nitro radicals were present in the starting material, the "wet method" described by Raiford and Colbert[13] gave best results. With the exceptions noted above, other derivatives were prepared by standard methods that will not be described here. The yields, general properties and analytical data for these substances are given in the tables.

Summary

1. When 4-nitrodiphenyl ether is brominated as here described, the halogen atoms enter positions 2′ and 4′ only, although excess of bromine may be present. Position 2′ is probably attacked first.

2. Nitration of 4-nitro-2′,4′-dibromodiphenyl ether gave two products: the 2,4,2′,4′-tetranitro compound, and a trinitro derivative in which all bromine is retained.

3. Increase in the number of substituents seems to interfere with diazotization of an aminophenyl ether.

4. Several new derivatives of diphenyl ether have been synthesized and their structures established.

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A STUDY OF THE PENTOSE AND URONIC ACID CONTENT OF ORANGE ALBEDO, AND AN ARABINO-GALACTURONIC ACID DERIVED FROM ORANGE PECTIN

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I. Introduction

This work was undertaken to determine the relative amounts of combined pentose and uronic acid in orange pectin, as it occurs in the albedo of commercial ripe oranges, and subsequently to see if the relation of these substances is similar to that in apple pectin.

Apple tissue has been found previously to contain no free pentose and the pectin to contain no combined pentose.[1] The furfural obtained on distillation with hydrochloric acid was quantitatively attributed to the galacturonic acid part of the pectin, arabinose being an intermediate stage in the decomposition.

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[13] Ref. 1, p. 2659.

[1] Ronald B. McKinnis, THIS JOURNAL, **50**, 1911 (1928).

The present analytical procedure has a decided advantage in that it does not involve the use of empirical constants. However, the results justify the conclusions arrived at by the use of the former method.[1] Dore[2] has used a similar method, but did not apply it in the same manner.

II. The Simultaneous Determination of Pentose and Uronic Acid

A. Theoretical.—As is well known, pentoses split off water on boiling with dilute acids, giving furfural. This distils and can be precipitated almost quantitatively with phloroglucinol. The maximum yield of furfural is not obtained, but the results are practically constant and therefore comparable. Uronic acids first split off carbon dioxide to form a pentose; then the pentose splits off water to form furfural. A 100% yield of carbon dioxide and about 34% of the possible theoretical yield of furfural are obtained from combined galacturonic acid.

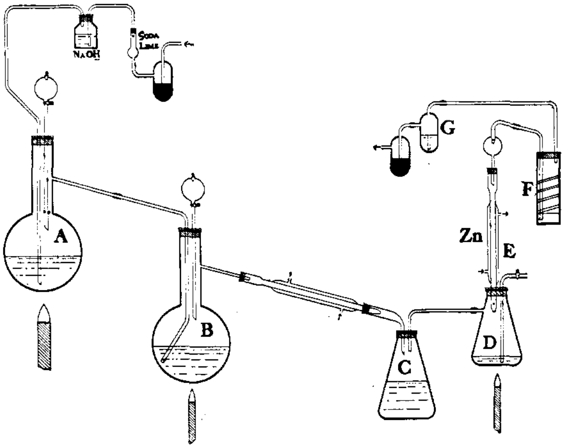

Fig. 1.

Certain α-hydroxy acids under the action of mineral acids break down into aldehydes with the elimination of formic acid, which would decompose forming carbon monoxide, but in this case no carbon monoxide could be detected in a typical experiment. This result, together with the good yields of carbon dioxide obtained from digalacturonic acid and barium galacturonate, indicates that the formic acid type of decomposition does not take place to any appreciable extent under the conditions of the experiment.

B. Apparatus and Procedure.—Most of the details of the distillation train are evident from Fig. 1. The steam generator, A, contains a trace of sodium hydroxide, and the steam inlet tube to the main reaction flask, B, is bent so as to give the contents a swirling motion. The furfural distillate is collected in C at 0°. The condenser, E, is filled with fine mossy zinc kept moist by refluxing from D to prevent any hydrochloric

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[2] W. H. Dore, *ibid.*, **48**, 232 (1926).

acid from distilling into the barium hydroxide in F and G. G remained clear throughout the work. A slow current of air previously freed of carbon dioxide is drawn from A to G. At each end of the apparatus is a small trap filled with mercury.

The sample and 200 cc. of exactly 12% hydrochloric acid are placed in Flask B. The carbon dioxide is swept out by applying suction to D, and the water in D is brought to a boil. The suction is now started at G, the reaction flask heated, steam blown through and the flame adjusted so that the mixture remains at constant volume. The receiver, C, is fitted with a device (not shown in the figure) which allows sampling of the distillate without an influx of carbon dioxide. A glass tube inserted obliquely in the stopper and closed with rubber tubing and a clamp allows sampling the distillate without an influx of carbon dioxide. The distillation is stopped half an hour after a practically colorless test is obtained with aniline acetate paper. This usually requires three and one-half to four hours, but a faint pink color persists even after six hours.

Carbon dioxide is determined by titration of the barium hydroxide with 0.1 *N* hydrochloric acid, both before and after the distillation. Furfural is determined as the phloroglucide, without washing with alcohol, the difference being insignificant.

C. Results Obtained on Digalacturonic Acid.—The digalacturonic acid was prepared from apple pectin by the procedure already reported.[1] The results, Table I, show that the actual yields of carbon dioxide and furfural are 100 and 34%, respectively, of the theoretical.

TABLE I

FURFURAL AND CARBON DIOXIDE YIELDS FROM DIGALACTURONIC ACID

Sample, g.	CO_2, g.	Yield, %	Furfural, g.	Yield, %	$\frac{\text{Moles furfural}}{\text{Moles CO}_2}$
0.3012	0.0719	100	0.0541	34.6	0.34
.3025	.0716	99.5	.0526	33.5	.33

D. Results Obtained on Orange Albedo.—Ripe commercial oranges were used throughout the work. The pulp and flavedo were removed and the albedo rinsed in cold water, then squeezed out and stored in absolute alcohol. It is interesting that the alcohol extracted no pentoses. Just before use it was washed in fresh alcohol, dried in a current of air at room temperature and crushed to a powder. Table II shows the results with this material.

TABLE II

FURFURAL AND CARBON DIOXIDE YIELDS FROM ORANGE ALBEDO

Sample, g.	CO_2, g.	Furfural, g.	$\frac{\text{Moles furfural}}{\text{Moles CO}_2}$
7.02	0.0292	0.0438	0.68
8.21	.0315	.0433	.64
8.45	.0390	.0563	.66

Comparison of this ratio with that of Table I shows that there was twice as much furfural as carbon dioxide in the orange albedo. Since uronic acids give one mole each of carbon dioxide and furfural, and pentoses give one of furfural only, this result clearly indicates an equal proportion of combined pentose and uronic acid in the orange albedo.

III. Arabino-galacturonic Acid from Orange Albedo

A. Preparation.—Five hundred grams of preparedo range albedo was covered with 1% sodium hydroxide solution and heated for an hour with frequent stirring. The extract was made slightly acid with hydrochloric acid, raised to the boiling point and the crude arabino-galacturonic acid filtered off. The precipitate was then redissolved in a liter of 1% sodium hydroxide solution, allowed to stand for a few moments, then neutralized, warmed and filtered off again. The process was repeated through at least seven reprecipitations, decolorizing carbon being added to the alkaline solution the last few times and filtered off before neutralization. After the last precipitation, the precipitate was washed with hot water until no longer acid, then dried in a vacuum at 35 to 40° and ground. The yield of 1 g. was less than 10%, calculated on the basis of carbon dioxide determinations on the original albedo.

B. General Properties.—The compound is light tan to buff in color, practically odorless and tasteless, and has no melting point, decomposition beginning at rather low temperatures. It is insoluble in water and all the common organic solvents, but dissolves easily in dilute aqueous alkalies. The alkali is almost completely removed by dialysis, giving a sol which is immediately coagulated by small quantities of inorganic acids and salts. The action on ammoniacal silver and Fehling's solution is very slow, indicating that the aldehyde groups are not active. Definite salts of barium, potassium or sodium could not be made.

IV. Evidence for the Constitution of Arabino-galacturonic Acid

A. Combined Pentose and Uronic Acid Determination.—When the acid was first prepared, it was expected to be digalacturonic acid, but Table III shows that such was not the case. The compound gave the same ratio as did the albedo, showing that it also contains equal amounts of combined pentose and uronic acid.

TABLE III

FURFURAL AND CARBON DIOXIDE YIELDS FROM ARABINO-GALACTURONIC ACID

Sample, g.	CO_2, g.	Yield, %	Furfural, g.	Yield, %	$\frac{\text{Moles furfural}}{\text{Moles } CO_2}$
0.2983	0.0402	99.7	0.0533	30.3	0.61
.3113	.0419	99.6	.0625	34.1	.68
.0155	.0021	100.9	.0031	34.0	.68

B. Hydrolysis of Arabino-galacturonic Acid.—Hydrolysis was accomplished by a method similar to that used by Ehrlich and Sommerfeld[3] for the preparation of galacturonic acid from digalacturonic acid.

Three or four grams of arabino-galacturonic acid was refluxed for seventeen and one-half hours with 2% sulfuric acid and decolorized with charcoal.

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[3] F. Ehrlich and R. V. Sommerfeld, *Biochem. Z.*, **168**, 263 (1926).

A simultaneous determination of the furfural and carbon dioxide evolved during the hydrolysis revealed the fact that furfural was evolved but only a trace of carbon dioxide. This showed that the pentose was partially broken down, but that the uronic acid was almost untouched by the treatment.

C. Separation and Identification of Galacturonic Acid.—The hydrolyzed solution was exactly neutralized with barium hydroxide, filtered, concentrated to 100 cc. and poured into three times its volume of alcohol. The precipitate was filtered off, washed with alcohol, then redissolved in water and reprecipitated with alcohol three times. The product, dried in a vacuum and ground, was a straw-colored powder. The yield of about 0.2 g. gave 27.5% barium, compared with 26.3% calculated for $Ba(C_6H_9O_7)_2$.

The carbon dioxide and furfural determinations given in Table IV show that the compound was barium galacturonate.

TABLE IV

FURFURAL AND CARBON DIOXIDE YIELDS FROM BARIUM GALACTURONATE

Sample, g.	CO_2, g.	Yield, %	Furfural, g.	Yield, %
0.1325	0.0214	96	0.0171	35
.1941	.0310	95	.0244	34

Neutralization of the barium salt with sulfuric acid gave a solution of galacturonic acid. Oxidation with bromine at room temperature for four days gave 60.5% of the theoretical yield of mucic acid, and oxidation for nine days at room temperature and then for seven hours at 35–40° gave a 69.1% yield. Ehrlich and Sommerfeld[8] report 67.9% when at room temperature for seven days. The optical rotation of the galacturonic acid solution, $[\alpha]_D^{22}$ +53.6, compares favorably with Ehrlich's value, $[\alpha]_D^{20}$ +53.4. These results indicate that the uronic acid left after the combined pentose had been split off with the 2% sulfuric acid is galacturonic acid.

D. Separation and Identification of Arabinose.—A slight excess of barium hydroxide was added to another portion of the hydrolyzed material with sufficient alcohol to precipitate the organic acids. Barium was removed with sulfuric acid. Crystallization was impossible, but a hydrazone was prepared which melted at 204°. The reported value for *l*-arabinose diphenylhydrazone is 203–205°. The hydrazone from Eastman's *l*-arabinose melted at 204°. Only a small amount was available for a carbon dioxide and furfural determination, but a 27% yield of furfural was obtained and no carbon dioxide.

These results show that the pentose is *l*-arabinose and that the pentose-uronic acid is arabino-galacturonic acid or a polymer of it.

V. Discussion of Results

Ratios of moles of carbon dioxide and furfural obtained indicate the existence of two acid compounds in pectin, one giving half as much carbon dioxide as the other. The yield of carbon dioxide is 100% and of furfural 34% for galacturonic acid as determined on barium galacturonate. These figures when applied to the two acids indicate that one may be digalacturonic acid and the other arabino-galacturonic acid. Further proof of the composition of arabino-galacturonic acid is given by the production of furfural without carbon dioxide by means of weak acids. Galacturonic acid remains, and was identified by its barium salt, optical rotation and yields of furfural, carbon dioxide and mucic acid. Arabinose was isolated from the same solution and identified as *l*-arabinose diphenylhydrazone.

It was previously found that apple tissue gives the same ratio as does digalacturonic acid isolated from it, but this work shows that orange albedo gives the same ratio as arabino-galacturonic acid. Since no free arabinose is present, it can be concluded that the pectin in orange albedo contains groups giving furfural and carbon dioxide in the same ratio as in arabino-galacturonic acid, which probably is the real nucleus to which the methyl groups, etc., are attached. A polymer or association compound of either of the acids is possible, since our work does not indicate the molecular weight.

The absence of pentoses in the alcoholic washings of the albedo, and the ratio of the moles of furfural to carbon dioxide in the albedo and in the arabino-galacturonic acid show that free pentoses are not present in orange albedo. There is little doubt that the oxidation of galactose to galacturonic acid and subsequent decarboxylation forms arabinose in nature, but it is still doubtful whether free arabinose ever occurs naturally.

The arabino-galacturonic acid could readily be formed from the digalacturonic acid by mere splitting off of one mole of carbon dioxide. Further changes need not necessarily be like the hydrolysis with 2% sulfuric acid.

Smolenski and Wlostowska[4] have studied the hydrolysis products of beet pectin. They found araban, galactan and a digalacturonate as the main products, but no proof was given that the araban or galactan or both were not formed from the galacturonate during hydrolysis.

Since one acid may be derived from the other, it is possible that both occur in some pectins. Nanji, Paton and Ling[5] obtained 20% of furfural and 17.64% of carbon dioxide from pectic acid. A mixture of 40% of digalacturonic acid and 60% of arabino-galacturonic acid would give 19.2% of furfural and 17.56% carbon dioxide. The present results do not confirm the hexa ring formula of Nanji, Paton and Ling.[5] No reliable data for galactose in pectin is available, so this point is still in doubt.

Ehrlich and Schubert[6] obtained 0.0958 g. of furfural from 0.4998 g.

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[4] K. Smolenski and W. Wlostowska, *Roczniki Chem.*, **7**, 591 (1927), *C. A.*, **22**, 4109 (1928).

[5] D. R. Nanji, F. J. Paton and A. R. Ling, *J. Soc. Chem. Ind.*, **44**, 253T (1925).

[6] F. Ehrlich and F. Schubert, *Biochem. Z.*, **169**, 13 (1926).

of pectic acid and 0.1605 g. of carbon dioxide from 1.2364 g. of the same material. From these figures the ratio is found to be 0.676, closely agreeing with the ratio found for arabino-galacturonic acid.

Norris and Schryver[7] obtained 20.32% of furfural from pectinogen extracted by ammonium oxalate, and found 50.9% of galactose as determined by mucic acid. Mucic acid, however, is also derived from galacturonic acid; arabino-galacturonic acid would give a theoretical value of 52.3% for such a determination, and their figures agree with this value within the limits of experimental error.

The present results and the results of others indicate that pectin can vary in composition, and that its hydrolysis products may be the cause of the confusion in regard to its composition.

The work seems promising and is being continued with the hope that order may be brought out of the present confusion.

The authors wish to express their appreciation to Dr. C. G. King for his suggestions and interest in the work.

VI. Summary

1. A method has been found for simultaneously determining the relative number of furfural yielding and uronic acid components, and has been used to study the composition of orange albedo pectin and its nucleus.

2. An arabino-galacturonic acid has been isolated from orange albedo and shown to yield two moles of furfural to one of carbon dioxide, as did the original orange albedo.

3. Orange albedo has been shown to contain no free pentose.

4. Hydrolysis of arabino-galacturonic acid with a dilute acid splits off only the arabinose, which is further hydrolyzed to form furfural. With stronger acids the carboxyl of the galacturonic acid is also split off to form more arabinose, which also is hydrolyzed to furfural. Arabinose and galacturonic acid were isolated from the products of dilute acid hydrolysis. These facts and the furfural and carbon dioxide yields establish the composition of the acid nucleus of orange albedo pectin.

5. Digalacturonic acid and arabino-galacturonic acid were probably successive steps in the production of arabinose.

6. Arabino-galacturonic acid or a polymer is probably the nuclear unit of orange albedo pectin.

7. Since digalacturonic acid has been found in apple pectin, it is probable that some pectins contain both digalacturonic acid and arabino-galacturonic acid in varying proportions. The present results and the results of others indicate that pectin varies in composition and that its hydrolysis products cause the confusion in regard to its composition.

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[7] F. W. Norris and S. B. Schryver, *Biochem. J.*, 19, 676 (1925).