

[CONTRIBUTION FROM THE LABORATORY OF PLANT PHYSIOLOGY, UNIVERSITY OF MARYLAND, AND THE AGRICULTURAL EXPERIMENT STATION]

## THE DECARBOXYLATION OF *d*-GALACTURONIC ACID WITH SPECIAL REFERENCE TO THE HYPOTHETICAL FORMATION OF *l*-ARABINOSE<sup>1</sup>

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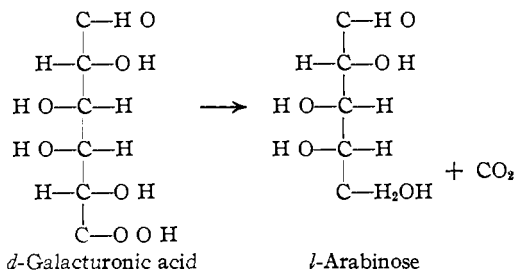
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### Introduction

*l*-Arabinose has long been considered to be a component in the molecular structure of pectins.<sup>2</sup> Recently, several investigators<sup>3</sup> have cast doubt on this belief. They think that *l*-arabinose may arise during dilute acid hydrolysis of pectin by decarboxylation of *d*-galacturonic acid.

When *d*-galacturonic acid is heated with 12% hydrochloric acid it decomposes into carbon dioxide and furfural.<sup>4</sup> Theoretically, *l*-arabinose should be formed momentarily, thus



McKinnis<sup>5</sup> predicted that in dilute mineral acids decarboxylation would occur and that the arabinose produced would not be decomposed. Link and Niemann<sup>6</sup> have shown that *d*-galacturonic acid (also *d*-glucuronic acid) actually is decarboxylated with dilute mineral acids. However, it has not been determined whether arabinose occurs among the reaction products.

Proof that arabinose or other pentoses are produced from uronic acids during the hydrolysis of plant materials would be of considerable importance with respect to carbohydrate chemistry. First, it would discredit the occurrence of arabinose, as such, in pectins. Second, it would render more

<sup>1</sup> Published with the permission of the Director of the Maryland Agricultural Experiment Station.

<sup>2</sup> Scheibler, *Ber.*, 1, 108 (1868); von Fellenberg, *Biochem. Z.*, 85, 118 (1918); Nanji, Paton and Ling, *J. Soc. Chem. Ind.*, 44, 253T (1925); Ehrlich and Sommerfeld, *Biochem. Z.*, 168, 263 (1926).

<sup>3</sup> Ahmann and Hooker, University of Missouri Agr. Expt. Sta., *Research Bull.*, 77 (1925); McKinnis, *THIS JOURNAL*, 50, 1911 (1928); Willaman, in Gortner's "Outlines of Biochemistry," Chap. XXVII, p. 585.

<sup>4</sup> Ehrlich and Schubert, *Ber.*, 62, 1974 (1929).

<sup>5</sup> McKinnis, *THIS JOURNAL*, 50, 1911 (1928).

<sup>6</sup> Link and Niemann, *ibid.*, 52, 2474 (1930).

or less uncertain the occurrence of the pentoses reported in many gums, mucilages and hemicelluloses. The pentoses assumed to have been hydrolytic products could have been decarboxylation products of a complex uronide. Therefore, it is important to determine, if possible, whether arabinose is formed as a decarboxylation product of galacturonic acid in dilute mineral acids.

### Experimental Part

In order to test the production of arabinose by decarboxylation it is necessary to work with galacturonic acid or with its arabinose-free derivatives. For this purpose barium galacturonate, and Ehrlich's<sup>4</sup> tetra acid "a" were chosen.

**Preparation of Materials.**—Barium galacturonate was prepared from lemon pectin,<sup>7</sup> according to the procedure of Link and Dickson.<sup>8</sup> On a dry weight basis the product yielded 16.50% carbon dioxide when boiled with 12% hydrochloric acid and contained 25.15% of barium. The theoretical values are 16.81 and 26.3%, respectively.

Tetra acid "a" was prepared from lemon pectin according to Ehrlich's<sup>4</sup> directions. One part of pectin is heated at 80° with 10 parts of 5% hydrochloric acid for six hours. The grayish white precipitate which forms is washed exhaustively, first with 1% hydrochloric acid, then with water, 95% alcohol, and finally ether. The material is sucked as dry as possible on the Büchner funnel and spread out to dry in the air. The material thus prepared contained 13.61% moisture, 0.61% ash and yielded 24.55% carbon dioxide on the dry ash-free basis. Ehrlich found for the tetra acid "a" of sugar beets, 0.19% ash and an average of 24.81% of carbon dioxide.

**Apparatus.**—The apparatus used to measure the extent of decarboxylation has been described previously by Conrad.<sup>9</sup>

**Selection of 4% Sulfuric Acid for Decarboxylation.**—Sulfuric acid has been used more than any other mineral acid for hydrolysis and isolation of sugars. This is because of the ease with which it can be removed from the hydrolytic products. In the case of gums, mucilages and hemicelluloses a 4% concentration is more often employed.<sup>10</sup> This concentration does not appear to be very injurious to the liberated arabinose if the heating is not longer than eight to ten hours.<sup>11</sup> By preliminary experiments it was found that within this time sufficient decarboxylation could be obtained to detect arabinose if it is produced.

**Method for the Determination of Arabinose.**—Since the recovery of arabinose in crystalline condition is usually attended by much loss, it was thought preferable to obtain it as the diphenylhydrazone compound according to the method of Neuberg and Wohlgemuth.<sup>12</sup> Instead of using the free base, as these investigators did, the hydrochloride salt was used. This was purified by once recrystallizing the commercial prepa-

<sup>7</sup> The lemon pectin was kindly furnished by Messrs. C. P. Wilson and H. W. Hall of the California Fruit Growers Exchange.

<sup>8</sup> Link and Dickson, *J. Biol. Chem.*, **86**, 491 (1930).

<sup>9</sup> Conrad, *THIS JOURNAL*, **53**, 1999–2003 (1931).

<sup>10</sup> Abderhalden, "Handbuch der Biochemischen Arbeitsmethoden," Urban and Schwarzenberg, Berlin, 1910, Vol. II, pp. 59–64.

<sup>11</sup> Anderson and Sands, *Ind. Eng. Chem.*, **17**, 1257 (1925); Brown, "Handbook of Sugar Analysis," John Wiley and Sons, New York, 1912, p. 548; Muther and Tollens, *Ber.*, **37**, 298 (1904); Mauenbrecher and Tollens, *ibid.*, **39**, 3576 (1906).

<sup>12</sup> Neuberg and Wohlgemuth, *Z. physiol. Chem.*, **35**, 31 (1902).

ration from 95% alcohol containing a little hydrochloric acid. It was used in 50% alcoholic solution, either with 1.5 parts of sodium acetate, or after addition of almost the calculated quantity of *N* sodium hydroxide.

In a few cases  $\alpha$ -benzylphenylhydrazine hydrochloride was employed in parallel tests with the diphenylhydrazine hydrochloride. It was always used with 1.5 parts of sodium acetate in 75% alcohol.

## Results

**Failure to Obtain Arabinose from Galacturonic Acid.**—When barium galacturonate is treated with 4% sulfuric acid the barium is at once precipitated as the sulfate, and the concentration of sulfuric acid is slightly reduced. The liberated galacturonic acid, on heating, yields carbon dioxide and other decomposition products. Among these, arabinose was sought in a number of experiments of which the following is a typical example.

Five grams of barium galacturonate in an Erlenmeyer flask was covered with 100 cc. of 4% sulfuric acid and gently boiled for five hours on the sand-bath. At the end of this period the evolved carbon dioxide was found to be 0.1633 g., or theoretically equivalent to 0.5565 g. of arabinose. The solution was removed from the apparatus, cooled, and treated with a very slight excess of barium hydroxide solution. The excess of alkali was immediately neutralized with a stream of carbon dioxide, and the mixture heated on the steam-bath for about an hour to enlarge the barium sulfate particles. After standing overnight the mixture was warmed with animal charcoal and filtered through a thin layer of infusorial earth. The clear filtrate was evaporated to dryness at 25-mm. pressure in a water-bath heated to 55°. The residue was dissolved in 10 cc. of water and the residual barium galacturonate precipitated with 190 cc. of 95% alcohol. The salt was flocculated by heating somewhat and separated by means of a hardened paper on a suction filter. The filtrate was clarified once again with animal charcoal and divided into two equal parts. These were evaporated to dryness, separately. To one was added 15 cc. of 50% alcoholic solution containing 0.259 g. of diphenylhydrazine hydrochloride and 0.4 g. of sodium acetate. The mixture was heated under the reflux condenser for thirty minutes and set aside. To the other portion was added 2 cc. of 75% alcoholic solution of 0.277 g. of benzylphenylhydrazine hydrochloride and 0.415 g. of sodium acetate. It was heated under the reflux condenser for twenty minutes. In both cases a dark resinous substance separated immediately on cooling. After standing for seven days no hydrazone crystals could be seen in either test. The gums dissolved completely in cold 50% aqueous pyridine and could therefore contain no hydrazone.

In a control experiment 0.4 g. of arabinose was dissolved in a mixture of 5 g. of barium galacturonate in 100 cc. of 4% sulfuric acid. The mixture was not heated, the object being only to determine how much of the arabinose would be lost due to adsorption, transfers and the like. Although the solution was not colored, animal charcoal and infusorial earth were used exactly as in the previous experiment. The final residue was not divided. It was treated with 36 cc. of an alcoholic solution containing 0.62 g. of diphenylhydrazine hydrochloride and 0.93 g. of sodium acetate. The mixture was refluxed as before and there was obtained 0.5256 g. of beautiful *l*-arabinose diphenylhydrazone, equivalent to 0.249 g. of arabinose. This is 62.3% of the amount originally added.

In a second control experiment it was sought to determine whether certain decomposition products of galacturonic acid might prevent the separation of the hydrazones. The 5 g. of barium galacturonate was boiled for five hours on the sand-bath. A

quantity of arabinose, 0.556 g., equivalent to that calculated to have been produced in the principal experiment was divided into two equal portions. One portion was added at the beginning of the heating period, the other when it was half through. There was collected 0.1805 g. of carbon dioxide theoretically equivalent to 0.615 g. of additional arabinose. On removing the sulfuric acid, clarifying, concentrating, removing the remaining barium galacturonate and dividing the final alcoholic solution into two equal parts, there was finally obtained from one portion 0.3401 g. of *l*-arabinose diphenylhydrazone. Allowing for the division of alcoholic solution this accounts for 58% of the added *l*-arabinose. After purifying once from 95% alcohol and twice from 50% aqueous pyridine the hydrazone melted at 202.5° (uncorr.). From the other portion there were obtained crystals which after twice recrystallizing from 75% alcohol melted at 173–174° (uncorr.). Van der Haar<sup>13</sup> gives corrected values of 204 and 174°, respectively, for the diphenylhydrazone and  $\alpha$ -benzylphenylhydrazone of *l*-arabinose. It is therefore evident that the decomposition products of galacturonic acid do not prevent the separation of these hydrazones.

**Failure to Obtain Arabinose from Ehrlich's Tetra Acid "A."**—According to Ehrlich and Schubert<sup>4</sup> tetra acid "a" is a polymer containing four units of galacturonic acid. It contains no pentose units. On heating with acids it is hydrolyzed into galacturonic acid which in turn undergoes decarboxylation.

In repeated trials arabinose was sought in the decarboxylation products from tetra acid "a." The following is a typical experiment. A 5-g. sample of tetra acid "a" in 100 cc. of 4% sulfuric acid was boiled gently for nine hours. There was collected 0.1309 g. of carbon dioxide equivalent to 0.446 g. of arabinose. The sulfuric acid was precipitated with barium hydroxide and the mixture heated for an hour on the steam-bath. It was filtered through paper pulp and the filtrate clarified with bone charcoal. The almost colorless filtrate was evaporated at 25–35-mm. pressure to about 10 cc. Barium galacturonate was precipitated with five volumes of 95% alcohol, and removed by filtration. The clear filtrate was evaporated to 2–3 cc. Some material which had separated was again removed by adding 95% alcohol and filtering. The clear filtrate was evaporated completely to dryness. The residue was extracted three times with 10, 10 and 6 cc. of hot 95% alcohol. The combined extracts were filtered and evaporated to about 2 cc. In testing for arabinose no diphenylhydrazone could be obtained, although a dark resinous substance separated.

In a control experiment 5 g. of tetra acid "a" was heated with 100 cc. of 4% sulfuric acid in a boiling water-bath (instead of the sand-bath) for fifteen and one-half hours. Only 0.0620 g. of carbon dioxide equivalent to 0.2114 g. of arabinose was obtained and no diphenylhydrazone could be recovered. Again, 15 g. of tetra acid "a" was heated with 500 cc. of 1% sulfuric acid in the autoclave at 25-lb. pressure for one hour. In this case it was impossible to measure the amount of carbon dioxide formed. After removal of the sulfuric acid and concentration as before no *l*-arabinose diphenylhydrazone could be obtained. On the other hand, the light colored sirupy residue which remained reacted with the diphenylhydrazine to give a dark colored resinous mass.

**Identification of Arabinose in the Hydrolytic Products of Apple and Lemon Pectins.**—McKinnis,<sup>5</sup> in a study of apple pulp, concluded that apple pectin contains no arabinose. In view of the results obtained above with galacturonic acid and tetra acid "a" it was of interest to subject

<sup>13</sup> Van der Haar, "Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide und Aldehydsäuren," Gebrüder Borntraeger, Berlin, 1920.

pectins to the same conditions of experimentation and determine whether arabinose is to be found among the products.

The apple pectin<sup>14</sup> used was prepared from "Certo" by alcoholic precipitation and thorough washing with additional alcohol. It contained 74.3% of galacturonic acid on the dry, ash-free basis. The lemon pectin was the same as that used for the preparation of barium galacturonate and tetra acid "a."

Ten grams of pure apple pectin was mixed with 200 cc. of 4% sulfuric acid in a 500-cc. Florence flask and heated on the steam-bath for a short time until dissolved. The flask was removed to the sand-bath and the solution boiled very gently for five hours. During this period 0.0688 g. of carbon dioxide theoretically equivalent to 0.2350 g. of arabinose was produced. After cooling somewhat the acids were neutralized with barium hydroxide solution. The mixture was digested on the steam-bath for a time and set aside overnight. Next morning the supernatant liquid was poured off and the residue filtered on a hardened paper in a Büchner funnel. The decanted liquid and filtrate were combined, stirred with animal charcoal, and filtered by means of suction through a thin layer of infusorial earth on a hardened paper. The clear, practically colorless filtrate was evaporated at 20-mm. pressure in a water-bath at 50° to about 200 cc. Some white substance, probably mineral, was filtered off after first adding some animal charcoal. The filtrate was now evaporated to dryness. The residue was dissolved in 10 cc. of water and barium galacturonate precipitated by addition of 190 cc. of 95% alcohol. The precipitate was flocculated by gentle heating and filtered off on a hardened paper. The clear filtrate was again evaporated to a stiff sirup under reduced pressure. The sirup was dissolved in 33 cc. of water and treated with a solution of 1.55 g. of diphenylhydrazine hydrochloride and 6.50 cc. of *N* sodium hydroxide in 50 cc. of 95% alcohol. The mixture was heated under a reflux condenser for thirty minutes and set aside. After two days there had separated *l*-arabinose diphenylhydrazone which, when dried and weighed, amounted to 1.151 g. This is equivalent to 0.5465 g. of arabinose or over twice the amount that could be calculated from the carbon dioxide obtained. After twice recrystallizing from 50% aqueous pyridine, the hydrazone melted, on rapid heating, at 204.2° (corr.). Van der Haar<sup>15</sup> gives 204° as the melting point.

Using practically the same procedure there was obtained from lemon pectin 0.0668 g. of carbon dioxide equivalent to 0.2280 g. of arabinose. However, only 0.1892 g. of arabinose in the form of the diphenylhydrazone was isolated. In this case all the arabinose found could have been produced by decarboxylation. Nevertheless, from results obtained above, its generation through decarboxylation is unlikely. The hydrazone melted at 204° (corr.).

### Discussion of Results

The results of the foregoing experiments render it very doubtful that *l*-arabinose arises as an intermediate product in the decarboxylation of galacturonic acid by mineral acids. Instead there are formed one or more sirupy substances which readily react with diphenylhydrazine and  $\alpha$ -benzylphenylhydrazine to give dark resinous products.

There is another argument which militates against the theory that

<sup>14</sup> This was kindly furnished by the Pectin Sales Co., since incorporated as the General Food Sales Co., of Fairport, N. Y.

<sup>15</sup> Van der Haar, Ref. 13, p. 178.

arabinose arises from galacturonic acid. As is well known, arabinose, when distilled with 12% hydrochloric acid, gives 70–80% of the theoretical quantity of furfural. Galacturonic acid, distilled under these conditions, loses practically the theoretical quantity of carbon dioxide.<sup>4,16</sup> If, in this process, arabinose is first formed and gives rise to furfural, we should expect to recover 70–80% of the theoretical amount of furfural. However, Ehrlich and Schubert<sup>4</sup> obtained only 41.1–42.6%<sup>17</sup> under these conditions and McKinnis,<sup>5</sup> working with digalacturonic acid, obtained only 33.3% of the theoretical yield of furfural. These results, therefore, suggest that some substance other than arabinose is formed at the intermediate stage and that this substance does not decompose into furfural as completely as arabinose does.

If *d*-galacturonic acid on decarboxylation with mineral acids does not give arabinose, we may well ask what is the intermediate substance. This must remain for further investigation to disclose. In the meantime we must conclude that the recovery of arabinose from the hydrolytic products of plant materials is indicative of the occurrence of arabinose as such and not of galacturonic acid.

### Summary and Conclusions

1. A test was made of the theory that *l*-arabinose arises in acid decomposition of *d*-galacturonic acid as an intermediate product in the formation of furfural. Galacturonic acid in the form of its barium salt was decarboxylated with 4% sulfuric acid but no arabinose could be recovered.

2. Ehrlich's tetra acid "a," a polymer of *d*-galacturonic acid, was also studied but in no case could arabinose be recovered from the decarboxylation products.

3. Carefully conducted controls showed that added arabinose could always be recovered.

4. *l*-Arabinose was found in the hydrolytic products of both apple and lemon pectins. In the case of apple pectin over twice the amount of arabinose that could have been due to decarboxylation was found.

5. The arabinose recovered from the hydrolytic products of plant substances comes from arabinose units in the substances and not from decarboxylation of galacturonic acid.

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<sup>16</sup> Dickson, Otterson and Link, THIS JOURNAL, 52, 775 (1930).

<sup>17</sup> Calculated by the writer from Ehrlich and Schubert's data.