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

DECARBOXYLATION STUDIES ON PECTINS AND CALCIUM PECTATES[1]

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The acid hydrolysis of pectin and its various derivatives is accompanied by a certain amount of decarboxylation. With 12% hydrochloric acid the decarboxylation is complete and quantitative in five hours or less. However, the extent of decarboxylation in concentrations of mineral acids ordinarily used in hydrolysis of complex carbohydrates has not been reported except in a few isolated cases. Thus, Link and Niemann,[2] using lemon pectin, noted that 33.85% of the galacturonic acid present was destroyed in fifteen hours by boiling 2% sulfuric acid and Link and Dickson,[3] with the same material and conditions except using 2.5% sulfuric acid, noted the same loss; again, Nelson and Cretcher,[4] noted a loss of carbon dioxide during the hydrolysis of algin.

In connection with certain studies it was desirable to know the extent of decarboxylation of pectins when boiled with different concentrations of hydrochloric and sulfuric acids. The data obtained are believed to be of sufficient interest to warrant publication.

Experimental Part

Materials.—Two samples of pectin were used. A purified apple pectin was kindly furnished by the Pectin Sales Company, since incorporated as General Food Sales Company of Fairport, N. Y. A rather pure lemon pectin was kindly supplied by Messrs. C. P. Wilson and H. W. Hall of the California Fruit Growers Exchange. These pectins gave the following analyses.

	Apple pectin	Lemon pectin
Moisture	11.55	10.50
Ash	7.80	7.85
Galacturonic acid (dry, ash-free)	74.30	85.70

Besides the pectins, several samples of calcium pectate were used. These were obtained in the course of the quantitative determination of pectin in fruit or vegetable tissues.

Apparatus and Method of Procedure

Figure 1 shows the apparatus employed to follow the decarboxylation. It was modeled somewhat after that described by Dickson, Otterson and Link,[5] differing es-

[1] Published with the permission of the Director of the Maryland Agricultural Experiment Station.

[2] Link and Niemann, THIS JOURNAL, **52**, 2474 (1930).

[3] Link and Dickson, *J. Biol. Chem.*, **86**, 491 (1930).

[4] Nelson and Cretcher, THIS JOURNAL, **51**, 1914 (1929).

[5] Dickson, Otterson and Link, *ibid.*, **52**, 775 (1930).

sentially in the following features. Absorption of carbon dioxide occurs in an inclined 11-mm. glass tube without glass beads, platinum gauze, or other inclusions. Since the inclination of the tube from horizontal is only slight the bubbles travel up very slowly and absorption of carbon dioxide is complete. The reaction flask, usually a 300-cc. Erlenmeyer, rests on a 3-mm. sand-bath in a 20-cm. sand dish and is heated by a small carefully adjusted gas flame. A rubber stopper is used in the reaction flask. No appreciable error was found to result. It is renewed at intervals as it begins to show wear. All the bottles containing solutions used in the determination, together with burets, are permanently attached to the apparatus so that exposure to the air is avoided.

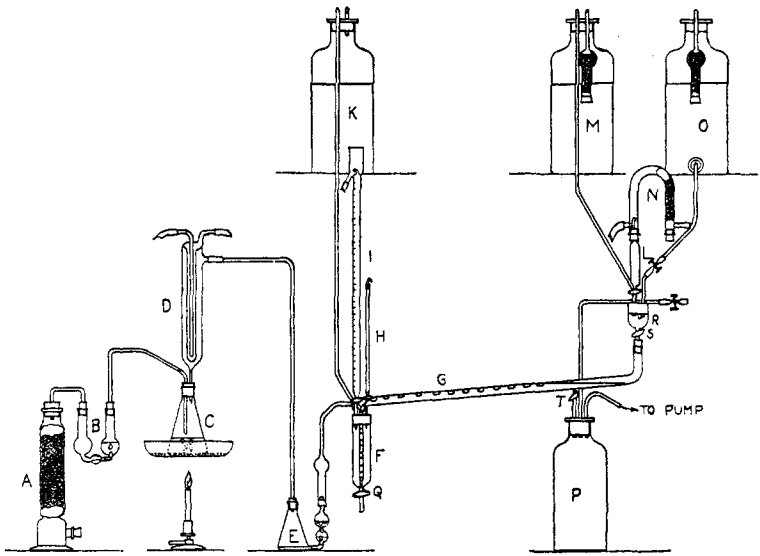

Fig. 1.—Apparatus used for decarboxylation studies: A, soda lime tower; B, trap with barium hydroxide solution; C, reaction flask; D, condenser; E, trap with 10% silver nitrate solution; F, titration chamber; G, absorption tube; H, buret for indicator; I, buret for standard hydrochloric acid; K, reservoir for standard hycrochloric acid; L, automatic pipet; M, reservoir for standard barium hydroxide; N, soda lime guard tube; O, reservoir for CO_2-free water; P, air chamber.

In determining the extent of decarboxylation the sample is placed in the reaction flask C and covered with 100 cc. of the acid solution desired. The flask is tightly fitted to its stopper and the sand-bath raised until the sand makes good contact with the bottom of the flask. With stopcock Q closed and S open, 15 to 20 cc. of carbon dioxide-free water from reservoir O is allowed to flow through absorption tube G into the titration chamber F. Stopcock S is closed and the water pump turned on. T is almost closed, causing a partial vacuum in the trap P. S is now cautiously opened so that the distilled water is drawn from chamber F slowly up into tube G. As the last of the water passes into the tube, bubbles follow and the number and size of these are regulated by adjusting S. Carbon dioxide-free air is thus drawn through the system for fifteen minutes to sweep out all carbon dioxide. Stopcocks T and Q are then opened in the order named, allowing the distilled water to flow out. Stopcock Q is closed and a measured quantity of approximately 0.2 *N* barium hydroxide is allowed to flow from

pipet L through chamber R into the absorption tube G. The stopcock of L is closed and the barium hydroxide is rinsed out of R with 4–5 cc. of water from O. Stopcock S is then closed and T again adjusted until only a small amount of air passes into P. Stopcock S is again cautiously opened and the barium hydroxide solution is drawn up into G, followed by a slow stream of bubbles. Cold water is passed through condenser D and the burner is lighted under the reaction flask. In actual runs boiling began in about twelve minutes. The flame was not changed after once having been adjusted.

When the reaction is finished, the flame is turned out and the stream of air drawn through the apparatus for about fifteen minutes longer. Stopcock T is then opened, followed by S, allowing the barium hydroxide carrying any carbonate to flow into the titration chamber. Buret I is filled with standard 0.2 *N* hydrochloric acid from the reservoir K. A drop or two of phenolphthalein solution is introduced from H and the residual alkali in F is titrated. Frequently during the titration stopcock T is momentarily closed, thus drawing part of the titration mixture up into G. As T is opened, the mixture flows back into the chamber and mixing is readily accomplished. By proper manipulation of stopcock T in conjunction with stopcock S it is easy to control the movement of liquid in G. Especially near the end-point the liquid should be drawn entirely into G several times so that neutralization shall be complete. The end-point having been reached, the buret reading is taken and the titrated mixture discharged. The apparatus is then ready for another determination. The presence of a small amount of barium carbonate in G is not detrimental. However, this may be removed by means of water and acid from the reservoirs.

Repeated blank determinations with 100 cc. of 12% hydrochloric acid over the period used showed that barium hydroxide was neutralized equivalent to 0.0013 g. of carbon dioxide. All determinations were corrected for this amount.

Results

Decarboxylation of Pectins with Different Concentrations of Hydrochloric and Sulfuric Acids.—Samples of both apple and lemon pectins weighing from 0.3–1.0 g. were gently boiled with different concentrations of hydrochloric and sulfuric acids for sixteen hours in the apparatus previously described. The amounts of carbon dioxide produced, corrected for blank and calculated to one gram of dry ash-free pectin are shown in Tables I-III.

TABLE I

DECARBOXYLATION OF APPLE PECTIN BOILED WITH DIFFERENT CONCENTRATIONS OF HYDROCHLORIC ACID FOR SIXTEEN HOURS

Concn. of acid, %	CO_2 evolved, g.	Galacturonic acid decomposed	
		Actual, g.	Part of total, %
0.12	0.0149	0.0656	8.8
2.0	.0719	.3163	42.6
5.0	.1619	.7123	95.9
8.0	.1651	.7264	97.8
12.0	.1707	.7511	101.1
18.0	.1725	.7590	102.1

According to Ehrlich[6] and others pectins contain a complex consisting of four molecules of galacturonic acid joined in a ring. As the carboxyl groups are not concerned in the linkage, it is conceivable that decarboxyl-

[6] Ehrlich and Schubert, *Ber.*, **62,** 1974 (1929); Ehrlich and Kosmahly, *Biochem. Z.*, **212,** 162 (1929).

TABLE II

DECARBOXYLATION OF APPLE PECTIN BOILED WITH DIFFERENT CONCENTRATIONS OF SULFURIC ACID FOR SIXTEEN HOURS

Concn. of acid, %	CO_2 evolved, g.	Galacturonic acid decomposed Actual, g.	Part of total, %
0.1	0.0070	0.0308	4.1
2.0	.0494	.2174	29.3
5.0	.0982	.4321	58.2
8.0	.1214	.5341	71.9
12.0	.1478	.6503	87.5
18.0	.1615	.7106	95.6

TABLE III

DECARBOXYLATION OF LEMON PECTIN BOILED WITH DIFFERENT CONCENTRATIONS OF SULFURIC ACID FOR SIXTEEN HOURS

Concn. of acid, %	CO_2 evolved, g.	Galacturonic acid decomposed Actual, g.	Part of total, %
0.1	0.0102	0.0449	5.2
2.0	.0612	.2693	31.4
5.0	.1147	.5047	58.9
8.0	.1340	.5896	68.8
12.0	.1769	.7784	90.8

ation of pectin might take place entirely independent of the hydrolysis of pectin into galacturonic acid. However, it was observed that practically no carbon dioxide was formed until after some minutes of heating. Then if the acid was sufficiently strong it began to be evolved more rapidly, as was shown by the separation of barium carbonate in the absorption tube. This would suggest that hydrolysis precedes decarboxylation; however, it is not impossible that the hydrolytic products act catalytically.

It is seen from the tables that decarboxylation occurs already in very weak concentrations of hydrochloric and sulfuric acids and increases rapidly with increasing concentration of acid. Hydrochloric acid of a given percentage concentration is much more effective than sulfuric acid of the same percentage value but this is due principally to the greater hydrogen-ion concentration of the hydrochloric acid. Hydrochloric acid of 12% or more probably yields a little carbon dioxide from other sources than the carboxyl groups when the pectin is heated for sixteen hours. This is indicated by the high calculated values for galacturonic acid. In the case of sulfuric acid even as high as 18% fails to give the theoretical yields of carbon dioxide. It is therefore not appropriate for use in place of hydrochloric acid for determining uronic acids.

Decarboxylation of Calcium Pectate from Several Sources.—Pectins plant gums and similar complex polysaccharides are very generally hydrolyzed with 2% sulfuric acid.[7] Periods of heating vary from a few

[7] Ehrlich and Sommerfeld, *Biochem. Z*, **168**, 263 (1926); Butler and Cretcher, THIS JOURNAL, **51**, 1519 (1929); Sands and Klass, *ibid.*, **51**, 3441 (1929); Bowman and McKinnis, *ibid.*, **52**, 1209 (1930); Link and Niemann, *ibid.*, **52**, 2474 (1930).

hours to as many as thirty, with a majority approximating sixteen hours. Inasmuch as the foregoing results showed so large a decarboxylation of apple and lemon pectin with this concentration of acid it was interesting to inquire whether this was a general property of the pectic substances from various sources. Therefore samples of calcium pectate which had been obtained in a rather pure condition in the course of other investigations were boiled with 2% sulfuric acid under the conditions previously described for pectins. The results are brought together in Table IV.

TABLE IV

DECARBOXYLATION OF CALCIUM PECTATE FROM VARIOUS SOURCES BY BOILING WITH 2% SULFURIC ACID FOR SIXTEEN HOURS

Calcium pectate from	Sample, g.	CO_2 obtained, g.	Galacturonic acid decomposed Weight, g.	Part of sample, %
Peach fruit	0.2665	0.0101	0.0444	16.7
Apple fruit	.3892	.0130	.0572	14.7
Tomato fruit	.1528	.0066	.0290	19.0
Strawberry fruit	.1999	.0075	.0330	16.5
Carrot root	.1674	.0053	.0233	13.9
Beet root	.1653	.0068	.0299	18.1

The results indicate that within the experimental error about the same percentage of galacturonic acid is decomposed in each case.

Summary and Conclusions

1. Decarboxylation of apple and lemon pectins with mineral acids occurs at low concentration of acid and is very appreciable with as weak as 0.1% sulfuric acid. The extent of decarboxylation of apple pectin in various concentrations of sulfuric and hydrochloric acids and of lemon pectin in various concentrations of sulfuric acid has been determined for a sixteen hour period of boiling.

2. A comparative study of the calcium pectate from several sources shows that the rate of decarboxylation with 2% sulfuric acid is approximately the same in each.

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