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The Estimation of Pectin  
and  
A Study of the Constitution of Pectin

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# The Estimation of Pectin and A Study of the Constitution of Pectin

C. F. AHMANN AND H. D. HOOKER

**Abstract.**—The method depends upon the production of acidity during the treatment of pectin with NaOH. The excess of NaOH after saponification of pectin is titrated with HCl. The difference between the original amount of NaOH used and the excess represents the amount which has combined with the pectin to form sodium pectate. The temperature at which the saponification is carried on affects the degree of saponification. At 23°C. one gram of NaOH combines with 6.4 grams of pectin while at 55°C. one gram of NaOH combines with 5.2 grams of pectin. The length of time of saponification also influences the reaction. Pectic acid precipitated after saponification at 23°C. has a neutral equivalent of 251.0 while pectic acid precipitated with HCl after saponification at 55°C. has a neutral equivalent of 194.9. Electro-metric titrations of pectic acid indicate an acid of eleven carboxyl groups. The composition of pectic acid obtained by standard combustion methods indicates that it may be an acid with twelve carboxyl groups. On the basis of eleven free carboxyl groups and one unsaponified group the molecular weight of pectic acid is 2,138. Pectin dissolves in concentrated HCl and undergoes decomposition and hydrolysis. The effect of temperature and concentration upon the products formed is shown. A method of separating the decomposition products is described.

## INTRODUCTION

Pectin, the jelling principle of fruits was found in 1831 by Braconot.<sup>1</sup> Pectic substances represent a class of naturally occurring plant compounds that are found as widely distributed as cellulose. Like cellulose they are essential constituents of the cell walls of all higher plants being the main constituent of the middle lamella. They have recently been found also in the cell walls of algae<sup>2</sup>. They are present in the bark, leaves, stalks, roots and tubers of all plants and more abundantly in fruits such as apples, pears, gooseberries, currants, the peel of citrus fruits and in fleshy roots of vegetables such as sweet potatoes, parsnips, sugar beets and carrots.

Pectin is commercially important for its jelling power. Pectic substances are likewise physiologically important. The middle lamella has been shown by Mangin<sup>3</sup> to be a salt of pectic acid, and Sampson<sup>4</sup> has shown that pectic bodies are important in the formation of abscission layers. Rosa<sup>5</sup> has shown the probable relation of so-called hot water soluble pentosans, which undoubtedly include pectic substances, to

<sup>1</sup>Annales de Chem. et de Phys. V. 47, p 226, 1831.

<sup>2</sup>Wurdack, Ohio Jour. Sci. V. 23, No. 4, p 181, 1923.

<sup>3</sup>Comp. Rend. V. 110, p 295.

<sup>4</sup>Bot. Gaz. V. 66, p 32, 1918.

<sup>5</sup>Res. Bul. No. 48, U. of Mo. 1921.

water-retaining capacity and to winter hardiness. According to Howe, the root hairs of plants have a layer of pectic material on the outside. The work of True and Eckerson<sup>7</sup> indicates that pectic compounds are essential for the absorption of mineral salts from the soil. Moreover, a detailed study of pectin is chemically important, as it would probably throw considerable light on the composition and structure of related gums, mucilages, galactoarabans and the so-called hemi-celluloses.

## LITERATURE

**Classification of Pectic Substances.**—Various terms have been used to designate pectin and its related compounds. There has been much confusion in the literature due to the fact that the various investigators have not employed the same nomenclature.

A critical study of the properties of the pectic substances indicates that there are three types of compounds: pectic acid, its salts, and its esters. Pectic acid is very slightly soluble in water. The calcium and magnesium salts are insoluble in water. The esters of pectic acid are of two types: soluble esters, pectin; and insoluble esters, protopectin. Table 1 shows the variety of names given to these compounds.

The earlier workers have also ascribed various formulae to pectic acid and have recognized various kinds of pectic acid. Fremy<sup>8</sup> considers pectic acid an isomer of pectin. According to other investigators acidic pectic compounds, such as pectosic acid, pectic acid, metapeptic acid, parapectic acid, perpectic acid and cytopectic acid were obtained. Moreover, the formulae given by the various investigators for pectic acid differ. Table 2 shows the formulae ascribed to so-called pectic acid by various investigators.

These investigators have assigned to pectic acid their respective formulae on the basis of the analyses of the lead, silver, barium, or calcium salts.

Fellenberg<sup>9</sup> points out that such variations might be expected since in many cases the ash of the original pectic acid was high. Mulder<sup>9a</sup> shows that drying the material for combustion at different temperatures changes the composition of pectic acid. However, the empirical formula of pectic acid has very little significance without some knowledge of the constitution of pectin and of pectic acid.

<sup>6</sup>Science, V. 55, p 1-7, 1922.

<sup>7</sup>Bot. Gaz. V. 72, p 313, 1921.

<sup>8</sup>Annal de Chemie et Phy. V. 24, p 5.

<sup>9</sup>Biochem. Zeits. V. 85, p 118.

<sup>9a</sup>J. prak. Chem. V. 16, p 246.

TABLE 1.—NAMES GIVEN BY VARIOUS AUTHORS TO PECTIC SUBSTANCES

Investigator	Pectic acid slightly sol. in H <sub>2</sub> O	Ca, Mg salts Insol. in H <sub>2</sub> O	Pectin H <sub>2</sub> O sol. esters	Protopectin esters insol. in H <sub>2</sub> O
Braconnot <sup>10</sup>	Gallertsaure	Pectic acid salts	Pectin	Pectin
Fremy <sup>11</sup>	Pectic acid Pectin Metapectin Parapectin	Pectose Protopectin, Ca-pectinate	Pectin an isomer of pectic acid, Pectosic acid, Parapectin	Pectose
Chodnew <sup>12</sup>	Pectic acid Perpectic acid		Pectinic acid Pectosic acid	
Payen <sup>13</sup> Mangin <sup>14</sup> Wiesner <sup>15</sup> Tschirch <sup>16</sup> Ehrlich <sup>17</sup>		Ca pectate Pectose  Protopectin		Protopectin Protopectin Pectose
	Tetra-galacturonic acid		Pectic acid	
Tromp de Haas, and Tollens <sup>18</sup>	Pectin	Pectin	Pectin	Pectin
Onslow <sup>19</sup> Fellenberg <sup>20</sup>	Pectin Pectic acid		Pectinogen Pectin	Pectocellulose Protopectin Pectose
Carre and Haynes <sup>21</sup> Clayson, Norris and Schryver <sup>22</sup> Sucharipa <sup>23</sup>	Pectic acid Cyto-pectic acid Pectic acid	Ca pectate Pectinogen	Pectin  Protopectin, Free pectin	 Pectinogen Protopectin
Caldwell <sup>24</sup> Wurdack <sup>25</sup> Mulder <sup>26</sup>	Pectic acid	Ca pectate Ca pectate Ca pectate Pectin		Pectose Pectose
Farnell <sup>27</sup>			Pectinogen	

<sup>10</sup>loc. cit.<sup>11</sup>loc. cit.<sup>12</sup>Berzelius Jahresbr. V. 25, p 566, 1846.<sup>13</sup>Comp. Rend. V. 43, p 769, 1856.<sup>14</sup>loc. cit.<sup>15</sup>J. Am. Chem. Soc. V. 46, p. 145, 1924.<sup>16</sup>Biochem. Zeits. V. 85, p 59, 1918.<sup>17</sup>Chem. Zeitung, V. 41, p. 197, 1917.<sup>18</sup>Liebig's Annal. V. 286, p. 282, 1895.<sup>19</sup>Practical Plant Biochem. Cambridge, Eng. 1920.<sup>20</sup>Biochem. Zeits V. 85, p 118, 1918.<sup>21</sup>Biochem. J. V. 16 p 60.<sup>22</sup>Biochem. J. V. 15, p. 643, 1921.<sup>23</sup>J. Am. Chem. Soc. V 46, p 145.<sup>24</sup>Bul. 147, State Col. Wash. p. 5, 1917.<sup>25</sup>loc. cit.<sup>26</sup>J. fur Pract. Chem. V. 14, p 277, 1838.<sup>27</sup>Intern, Sugar J., V. 25, p. 248, 1923.

TABLE 2.—FORMULAE ASCRIBED TO SO-CALLED PECTIC ACID BY VARIOUS INVESTIGATORS

Investigator	Compound	Formula	Composition		
			C	H	O
Fremy <sup>28</sup>	Pectosic acid	C <sub>32</sub> H <sub>46</sub> O <sub>31</sub>	41.46	4.96	53.58
Fremy	Parapectic acid	C <sub>24</sub> H <sub>34</sub> O <sub>23</sub>	41.73	4.92	53.35
Fremy	Metapectic acid	C <sub>8</sub> H <sub>14</sub> O <sub>9</sub>	37.79	5.51	56.70
Fremy	Pectic acid	C <sub>32</sub> H <sub>44</sub> O <sub>30</sub>	42.29	4.84	52.87
Mulder <sup>29</sup>	Pectic acid	C <sub>6</sub> H <sub>8</sub> O <sub>5</sub>	45.31	5.21	49.42
Regnauld <sup>30</sup>	Pectic acid	C <sub>11</sub> H <sub>14</sub> O <sub>10</sub>	43.13	4.57	52.30
Fromberg <sup>31</sup>	Pectic acid	C <sub>22</sub> H <sub>28</sub> O <sub>20</sub>	43.13	4.57	52.30
Berzelius <sup>32</sup>	Pectic acid	C <sub>12</sub> H <sub>16</sub> O <sub>10</sub>	45.00	5.00	50.00*
Berzelius	Metapectic acid	C <sub>6</sub> H <sub>8</sub> O <sub>5</sub>	45.00	5.00	50.00*
Chodnew <sup>33</sup>	Pectic acid	C <sub>28</sub> H <sub>40</sub> O <sub>24</sub>	42.42	5.05	52.53
Chodnew	Pectosic acid	C <sub>28</sub> H <sub>40</sub> O <sub>25</sub>	43.29	5.15	51.56
Chodnew	Perpectic acid	C <sub>28</sub> H <sub>38</sub> O <sub>27</sub>	41.68	4.71	53.61
Ehrlich <sup>34</sup>	Tetra-galacturonic acid	C <sub>24</sub> H <sub>34</sub> O <sub>25</sub>	39.94	4.98	55.08
Fellenberg <sup>35</sup>	Pectic acid	C <sub>90</sub> H <sub>104</sub> O <sub>86</sub>	41.33	5.11	53.56
Clayson, Norris and Schryver <sup>36</sup>	Cytopectic acid	C <sub>7</sub> H <sub>11</sub> O <sub>6</sub> *	42.33	5.54	52.13
Schryver and Haynes <sup>37</sup>	Pectic acid	C <sub>17</sub> H <sub>24</sub> O <sub>16</sub>	42.14	4.95	52.90
Gaertner <sup>38</sup>	Pectic acid	C <sub>12</sub> H <sub>20</sub> O <sub>10</sub>	41.35*	6.10*	52.49

\*Calculated from data given in literature.

Scheibler<sup>39</sup> made a study of the metapectic acid from beet pulp, as reported by Fremy<sup>40</sup>, and found that the products of hydrolysis were a sugar and an acid. The sugar he identified as arabinose. He considered that metapectic acid was not a pectic body, but arabin or arabic acid.

Herzfeld<sup>41</sup> found that during the hydrolysis of pectic substances arabinose was formed. Oxidation led to large yields of mucic acid, from which he concluded galactose was present.

According to Tollens<sup>42</sup> the naturally occurring pectin is neutral and the acid groups are present as lactones or esterified carboxyl groups. On hydrolysis hexoses, pentoses and acids were formed.

A more complete study of pectin and its decomposition products was made by Ehrlich.<sup>43</sup> Working with beet pulp he obtained a raw pectin which consisted of two substances, an araban and a calcium-magnesium salt of pectic acid. A solution of this calcium-magnesium salt was pre-

<sup>28</sup>loc. cit.

<sup>29</sup>J. fur pract. Chem. V. 14, p 277, 1838.

<sup>30</sup>J. fur pract. Chem. V. 14, p 270, 1838.

<sup>31</sup>Berzelius Jahresbr. V. 24, p 371, 1845.

<sup>32</sup>loc. cit. (31).

<sup>33</sup>loc. cit. (32).

<sup>34</sup>loc. cit. (17).

<sup>35</sup>loc. cit.

<sup>36</sup>loc. cit.

<sup>37</sup>Biochem. J. V., 10, p 539, 1916.

<sup>38</sup>Chem. Ab. V., 14, p 1455, 1920.

<sup>39</sup>Ber. d. deutsch. Chem. Ges. V. 1, p 59.

<sup>40</sup>loc. cit. (9).

<sup>41</sup>Liebig and Kopp, Jahresbr. fur Fortsch. Chem. p 2189, 1890.

<sup>42</sup>Liebig's Annal. V. 286, p 292, 1895.

<sup>43</sup>loc. cit.

precipitated by alcohol and hydrochloric acid. It was a weak acid, one gram requiring fifteen cc. 0.1 *N.* sodium hydroxide for neutralization. He found also that this substance was free from pentoses even though it yielded much furfural on distillation with hydrochloric acid. On further treatment of the weak acid with alkali, he obtained an acid considerably stronger than the acid first produced. This acid he concluded was the result of splitting off methyl and galactose groups. He called this acid tetragalacturonic acid and assigned it the formula  $C_{24}H_{34}O_{25}$ . This substance on hydrolysis with oxalic acid yielded galacturonic acid. Other products of oxalic acid hydrolysis which he isolated were galactose and galacto-galacturonic acid. Heretofore, galactose has been assumed to be present since on oxidation pectic compounds yielded mucic acid but Ehrlich isolated it as a product of oxalic acid hydrolysis.

It has been established by Fellenberg<sup>44</sup> that pectin is the methyl ester of pectic acid. According to Ehrlich pectin treated with alkali yields methyl alcohol, galactose and pectic acid. In order to confirm the work of Ehrlich, Fellenberg treated pectin with various concentrations of alkali and found only methyl alcohol. The methyl alcohol obtained by hydrolysis ranged from 7 per cent to 11.7 per cent. The pectin yielding 11.7 per cent, the highest found, he called octamethylpectic acid. From the proportion of methyl alcohol produced and from various salts formed he concluded that pectic acid had eight reactive carboxyl groups. The different pectic acids in Table 2 would represent, according to Fellenberg, various degrees of demethylation since they were prepared by different methods. He showed that under varying conditions there is a possibility of having from one to eight methyl groups attached to pectic acid. According to Fellenberg the pectin molecule is a combination of the following: two molecules of arabinose, one molecule of methyl pentose, one molecule of galactose and eight molecules of methylated galacturonic acid with the elimination of ten molecules of water. Table 3 gives his analyses of pectin from various sources.

TABLE 3.—FELLENBERG'S ANALYSES OF PECTIN FROM VARIOUS SOURCES

Calculated as octamethylpectic acid	Sources of Pectin			
	Currants	Apples	Orange a	Orange b
Ash .....	2.9	.25	-----	1.4
C .....	43.65	43.58	-----	-----
Furfural formed cal. as arabinose .14.0	46.7	45.7	39.4	40.2
Methylpentose .....	8.4	-----	9.2	10.2
Mucic acid cal. as galactose .....	75.6	-----	-----	54.8
Methyl alcohol .....	11.94	9.3	10.57	11.33
			11.33	10.3

<sup>44</sup>loc. cit.

Fellenberg did not consider that these results represented the true composition of the pectin molecule. It has been shown by Ehrlich<sup>45</sup> that galacturonic acid yielded considerable quantities of furfural and consequently the entire yield of furfural could not be due to arabinose.

According to Spoehr<sup>46</sup> pentoses are not the only substances that yield furfural on treatment with mineral acids. The hexoses, cane sugar, starch, cellulose as well as glucuronic acid form furfural under these conditions. In view of these facts the estimation of pentoses is reliable only when these sugars are separated from other substances.

Gaertner<sup>47</sup> working with sugar-beet marc found an araban pectin complex. The araban appeared to be only loosely combined with pectin since it was detached on boiling with water alone. He obtained from 100 grams of pectin by prolonged boiling with oxalic acid and neutralization with lime about 20 grams of crystallized arabinose, some noncrystallizable syrup, methyl alcohol and about 26 grams of a calcium salt of pectic acid to which he ascribed the formula  $C_{12}H_{20}O_{10}$ . On heating this pectic acid with dilute sulphuric acid under pressure it yielded a small quantity of galactose and galacturonic acid. This acid is syrupy, reduces Fehling's solution in the cold and yields a crystallizable salt with cinchonine.

Tutin<sup>48</sup> studying the effect of alkalis and pectase on pectin found methyl alcohol, acetone and a salt of pectic acid as the decomposition products. He concludes that pectin is probably the dimethylisopropenyl ester of pectic acid.

A recent article by Sucharipa<sup>49</sup> shows a possibility of a higher percentage of methyl alcohol in pectin than was found by Fellenberg. The highest degree of methylation found by Fellenberg was 11.7 per cent corresponding to the esterification of eight carboxyl groups. Sucharipa finds more than 12 per cent of methyl alcohol which indicates the presence of more carboxyl groups than were found by Fellenberg.

**Methods of Determining Pectin.**—In studying the pectin content of plant material many difficulties have been encountered in complete extraction and separation of the various pectin constituents. It has been shown by Bigelow, Gore and Howard<sup>50</sup> that six hours boiling under a reflux with a change of water every hour does not completely remove all pectin from apple pulp.

Goldthwaite<sup>51</sup> has also shown that when apples were crushed, covered with water, brought to a boil, drained through cheesecloth, returned to a fresh quantity of water and the extraction repeated, five successive

<sup>45</sup>loc. cit.

<sup>46</sup>Carnegie Inst. of Wash. Pub. 287, p 37, 1918.

<sup>47</sup>loc. cit.

<sup>48</sup>Biochem. J. V. 15, pp 494—497, 1921.

<sup>49</sup>loc. cit.

<sup>50</sup>U. S. Dept. Agr. Bur. Chem. Bul. 94, p 100, 1905.

<sup>51</sup>J. Ind. and Eng. Chem. V. 2, p 457, 1910.

extractions were necessary in order to remove all the pectin. These results were confirmed by Caldwell<sup>52</sup>. Carre and Haynes<sup>53</sup> found that warm water could not be used for extraction since it increased the amount of pectin obtained, presumably by promoting hydrolysis. Their method of extraction is as follows: the finely minced material was frozen to kill the pulp completely, allowed to come to room temperature and pressed. The residue was then ground with purified sand and extracted with cold water. Sixty to eighty extractions were usually necessary.

It has been the general practice to estimate the pectin content by precipitation with alcohol. This method is lacking in accuracy since at low concentrations no precipitate is formed even after long standing with a large excess of alcohol. In the method of Carre and Haynes<sup>54</sup>, the difficulty of complete precipitation was avoided since calcium pectate is an insoluble compound. This method consists of the hydrolysis of pectin, forming pectic acid, acidification with acetic acid, and the precipitation of the calcium pectate by the addition of calcium chloride. Filtration of the calcium pectate is rapid and subsequent washing is easy. The precipitate is then transferred to a Gooch crucible and dried to constant weight.

The method of determining pectin according to Fellenberg<sup>55</sup> is as follows: a pectin solution is treated with a small excess of alkali, after a few minutes all the methyl alcohol being split off. The quantity of methyl alcohol is then determined. According to Fellenberg the methyl alcohol content of pectin from spices is approximately one per cent. By multiplying the percentage of alcohol by ten he obtained the percentage of pectin present.

According to the method of Wichmann and Chernoff<sup>56</sup> pectin is saponified with alkali. The pectic acid is precipitated with hydrochloric acid and boiled with hydrochloric acid. It is filtered, transferred to a crucible, dried and weighed. The dried pectic acid is ashed. The difference before and after ashing represents the amount of pectic acid present.

<sup>52</sup>loc. cit. (24).

<sup>53</sup>loc. cit. (21).

<sup>54</sup>Loc. cit.

<sup>55</sup>Biochem. Zts. V. 85, pp. 61-74, 1918.

<sup>56</sup>J. Assoc. Official Agr. Chemists, V. 6, p 34, 1922.

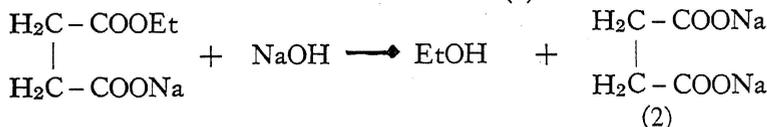
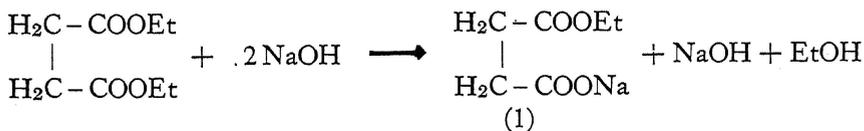
## EXPERIMENTAL

**Method of Determining Pectin.**—As pointed out by Carre and Haynes<sup>57</sup> the method of estimating pectin by precipitation with alcohol is lacking in accuracy since the pectin is of variable composition existing in forms intermediate between pectin and pectic acid. Moreover in dilute solution no precipitate is formed after long standing with a large excess of alcohol. In consequence of these difficulties they have developed a method of estimating pectin as the calcium salt of pectic acid. In this method they point out the possibility of error due to the fact that pectin readily absorbs other substances which once occluded are removed with great difficulty. This is especially true in the precipitation of calcium pectate from water extracts of plant material which also contains inorganic and organic salts. The process of washing the precipitate free from occluded material is of necessity laborious.

However, there is another factor which might make this method unreliable. It is assumed that the calcium pectate is of uniform composition. There is a possibility of the salt being a sodium-calcium salt of pectic acid. Unless the controlling factors namely time, temperature and concentrations, were uniform it would be expected that a product of variable composition would be formed. On the basis of pectic acid being an octocarboxylic acid, as Fellenberg suggested, it is impossible to say that in each case all the carboxyl groups are covered or what number is covered. For this reason more calcium may be combined with a given quantity of pectic acid in some cases than under other conditions making the estimation of pectin unreliable.

There is still another factor in this determination which may introduce an error. It is apparently assumed that the hydrolysis of pectin takes place with equal ease at varying temperatures.

If the hydrolysis of ethyl succinate be considered, a number of equilibria are found depending on the temperature:



The ratio of the products of hydrolysis will be different as the temperature varies. At one given temperature the ratio of products (1) and (2) may be one to three, at a different temperature one to one and

<sup>57</sup>loc. cit.

so on, the equilibrium in each case depending on the hydrolysis constant of the different reaction products.

It is evident from this that a substance with eight or more esterified carboxyl groups will have a number of products in equilibrium at any given temperature. The composition of the product formed is not known since the equilibrium constants for pectin have not been determined. Unless pure sodium pectate has been obtained and not methyl-sodium pectate as a result of hydrolysis, the calcium pectate weighed would be a compound of variable composition of methyl-sodium-calcium pectate. Other factors which might influence this hydrolysis are time and the concentration of alkali and of pectin.

The method developed by Fellenberg<sup>58</sup> for estimating pectin from the amount of methyl alcohol obtained is questionable since this assumes pectin from different sources to be equally methylated. However, pectin from various sources is methylated to different degrees, as pointed out by Fellenberg<sup>59</sup> which would lead to unreliable results.

The method of Wichmann and Chernoff<sup>60</sup> may also lead to unreliable results. The authors point out that by boiling pectic acid with hydrochloric acid a furfural-forming body is split off. In the light of the results of the present study of pectin this treatment of pectic acid with hydrochloric acid would yield a series of decomposition products of pectic acid depending on the length of time the material is boiled and the strength of the acid. The production of a furfural-forming body indicates pectic acid decomposition. From this it appears that the authors are determining the amount of one of the decomposition products of pectin. Moreover they state that their pectic acid is a simpler compound than pectin. This also indicates that the substance they call pectic acid is one of the decomposition products of pectin and not pectic acid.

In order to avoid the error due to the occlusion of foreign material in the precipitated calcium pectate and the laborious task of washing the precipitate free from occluded material as is necessary in the method of Carre and Haynes; also in order to avoid the possible error in the method of Fellenberg due to pectin being methylated to different degrees and further to avoid the possibility of the formation in different amounts of the decomposition product of pectic acid as produced in the method of Wichmann and Chernoff a method of estimating the pectin in the plant material by titrating the acidity produced on saponification of pectin was developed.

**Materials Used.**—The pectin used for this investigation was pectin obtained from apples and supplied by the Leo Greenwald Vinegar Company of Topeka, Kansas. The commercial product was dissolved in

<sup>58</sup>Biochem. Zts. V. 85, pp 61-74, 1918.

<sup>60</sup>J. Assoc. Official Agr. Chemists, V. 6, p 38, 1922.

<sup>59</sup>Biochem. Zts. V. 85, p 118, 1918.

water, precipitated by alcohol, redissolved in dilute hydrochloric acid, reprecipitated, the process being repeated until a fairly white material was obtained. This material yielded 3.75 per cent ash. By standard combustion methods this material showed the following composition: C. 42.73 per cent, H. 5.57 per cent, O. 51.70 per cent on an ash free basis. A water solution containing 0.2 gram in 100 cc. of water with a pH value of 6.2 to 6.4 has a pH value of 5.3, showing that the naturally occurring pectin is an incompletely esterified pectic acid. Precautions were taken to remove all traces of hydrochloric acid as shown by an alcohol extract which gave no test for hydrochloric acid with silver nitrate. In the earlier part of this investigation triply distilled water with a pH range of 6 to 6.4 was used. Later boiled distilled water was used for comparison. A carbon dioxide free sodium hydroxide solution was used. This solution was prepared according to the method of Carnog,<sup>61</sup> from sodium and carbon dioxide free water. In the earlier part of the work the titrations were made electrometrically with a Bovie Hydrogen Ion Potentiometer. Titrations were made also using phenolphthalein as an indicator. During saponification all samples were protected from carbon dioxide by paraffined stoppers or by soda lime tubes.

**Methods of the Investigations.**—*Effect of temperature.*—In order to study the effect of temperature on the saponification of pectin the following investigation was made. Aqueous solutions containing 1.00, 0.75, 0.50, and 0.25 gram respectively in 200 cc. were treated with 50 cc. of 0.424 *N.* sodium hydroxide for twelve hours at 23°C., 50°C., 55°C. and 65°C. At the end of twelve hours they were cooled to 23°C. and made up to 250 cc. Aliquots of 25 cc. were taken and titrated with 0.08 *N.* hydrochloric acid. The titrations were made electrometrically. The end point used was the pH of sodium pectate. The sodium pectate was prepared by saponification of pectin with sodium hydroxide at 55°C. for twelve hours. The sodium salt of pectic acid was precipitated with alcohol and washed with alcohol until no test for alkalinity was obtained. This material was then dissolved in water and the pH taken.

These titrations were repeated using phenolphthalein as an indicator. These check the results obtained by the electrometric titration since the pH of the sodium pectate used was 8.6. The difference between the amount of alkali added and the excess is represented by the difference in the amount of hydrochloric acid needed if there were no pectin present and the amount actually used. This difference in cubic centimeters calculated to cubic centimeters of normal sodium hydroxide represents the amount of alkali combined with the pectin to form sodium pectate. The effect of temperature would be represented by different amounts of

<sup>61</sup>J. Am. Chem. Soc. V. 43, p 2575, 1921.

alkali used for a given weight at varying temperatures. These results are shown in Table 4, and in Figs. 2 and 3.

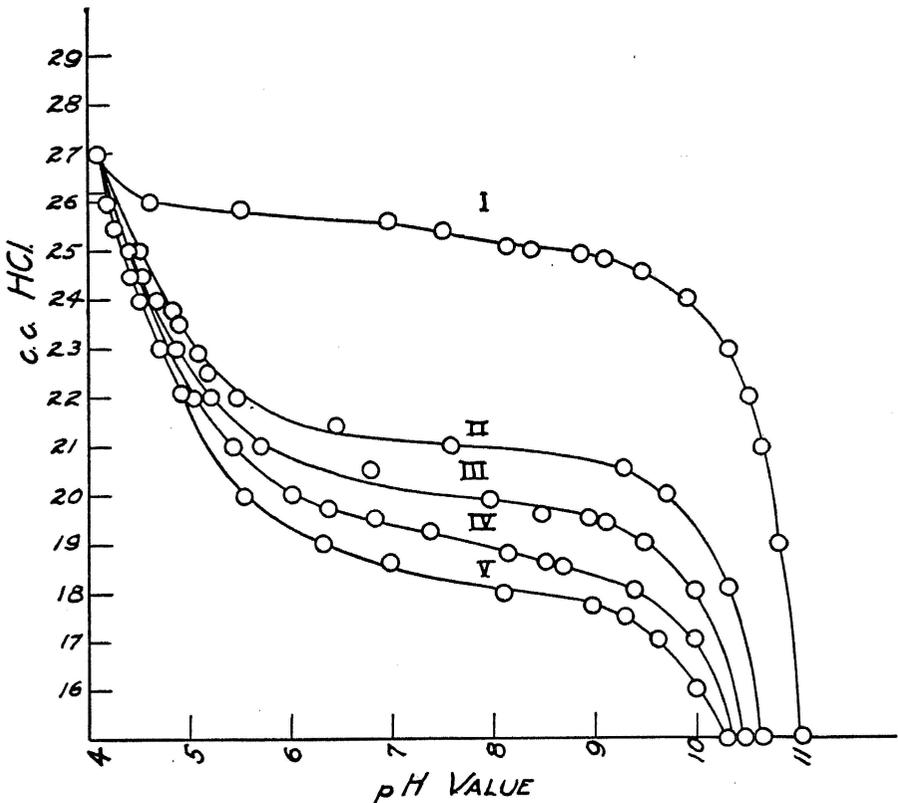
TABLE 4.—EFFECT OF TEMPERATURE ON SAPONIFICATION OF PECTIN

Wt. of Pectin	Temp. degrees C.	.424N NaOH cc.	.08N HCl cc.	Difference	.1N NaOH Eq. cc.
0.0	50	5.0	26.6	0.0	0.0
0.10	23	5.0	22.1	4.5	3.6
0.10	50	5.0	21.0	5.6	4.5
0.10	55	5.0	20.6	6.0	4.8
0.10	65	5.0	18.9	7.7	6.2
0.075	23	5.0	23.2	3.4	2.7
0.075	55	5.0	22.2	4.4	3.5
0.050	23	5.0	24.3	2.3	1.8
0.050	55	5.0	23.5	3.1	2.5
0.025	23	5.0	25.6	1.0	0.8
0.025	55	5.0	25.1	1.5	1.2

TABLE 5.—EFFECT OF TIME ON SAPONIFICATION OF PECTIN

Wt. of Pectin	Time hrs.	Temp. Deg. C.	.424N NaOH cc.	.08N HCl cc.	Difference	.1N NaOH Eq. cc.
0.0	24	50	5.0	26.6	0.0	0.0
0.10	3	23	5.0	22.3	4.3	3.4
0.10	12	23	5.0	22.2	4.4	3.5
0.10	24	23	5.0	22.1	4.5	3.6
0.075	3	23	5.0	23.2	3.4	2.7
0.075	12	23	5.0	23.2	3.4	2.7
0.075	24	23	5.0	23.1	3.5	2.8
0.050	3	23	5.0	24.3	2.3	1.8
0.050	12	23	5.0	24.3	2.3	1.8
0.050	36	23	5.0	24.1	2.5	2.0
0.075	3	55	5.0	22.7	3.9	3.1
0.075	12	55	5.0	22.2	4.4	3.5
0.075	24	55	5.0	21.0	6.6	5.2
0.050	3	55	5.0	23.9	2.7	2.1
0.050	12	55	5.0	23.5	3.1	2.5
0.050	24	55	5.0	23.0	3.6	2.8

Fig. 1.—Titration curves showing the development of acidity during saponification as the time varies.

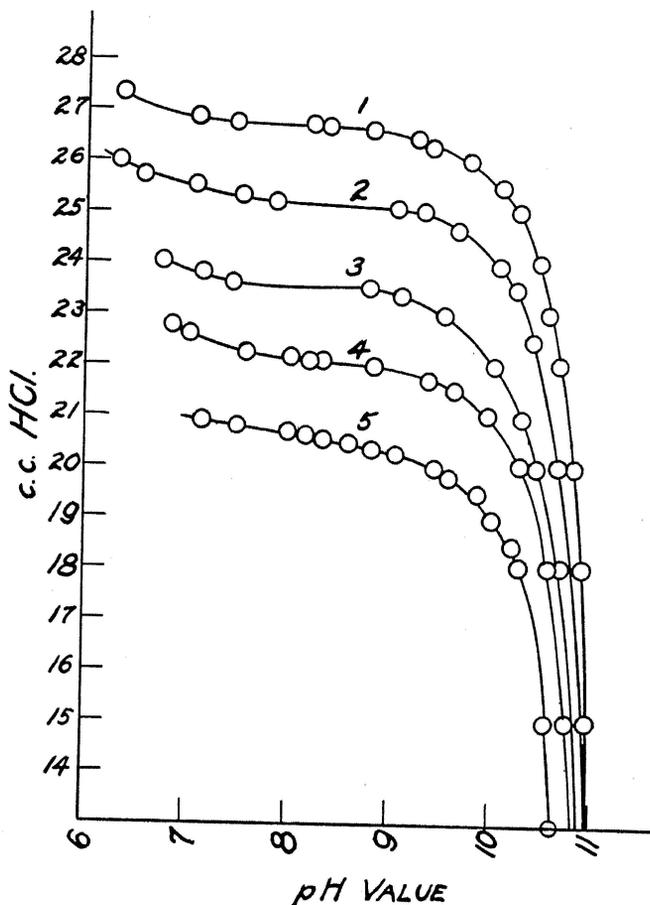


Weight of pectin 0.075 gr. Temperature 55°C.  
 Curve I.—Blank 10 cc. NaOH 25.10 cc. HCl.  
 Curve II.—4 hours 10 cc. NaOH 20.80 cc. HCl.  
 Curve III.—8 hours 10 cc. NaOH 19.80 cc. HCl.  
 Curve IV.—12 hours 10 cc. NaOH 18.75 cc. HCl.  
 Curve V.—24 hours 10 cc. NaOH 18.00 cc. HCl.

*Effect of time.*—Pectin solutions prepared similarly to those described in the preceding paragraph were allowed to saponify for different lengths of time. After the saponification had proceeded as before for three hours the solutions were made up to 250 cc. Aliquots of 25 cc. were taken and titrated. At intervals of 12, 24 and 36 hours the titrations were repeated. The amount of alkali combined with the pectin as before is represented by the difference in amount of hydrochloric acid needed when no pectin was present and the amount used in the respective samples. These results are shown in Table 5, and in Fig. 1.

*Pectic acid produced under different conditions.*—Pectic acid was produced by the saponification of pectin at 23°C. with sodium hydroxide for twelve hours forming sodium pectate. The solution of sodium

Fig. 2.—Titration curves of acidity developed during saponification of pectin show that as the amount of pectin in solution varies, the acidity developed changes proportionally under constant conditions.



Time 12 hours. Temperature 55°C.

Curve 1.—0.000 gr. Blank 8.0 cc. NaOH 26.60 cc. HCl.

Curve 2.—0.025 gr. pectin 8.0 cc. NaOH 25.10 cc. HCl.

Curve 3.—0.050 gr. pectin 8.0 cc. NaOH 23.50 cc. HCl.

Curve 4.—0.075 gr. pectin 8.0 cc. NaOH 22.10 cc. HCl.

Curve 5.—0.100 gr. pectin 8.0 cc. NaOH 20.55 cc. HCl.

pectate was then acidified with hydrochloric acid, precipitating the pectic acid. The pectic acid thus formed was dissolved and reprecipitated, the process being repeated until the ash content was less than 0.1 per cent, and an alcoholic extract gave no test for chlorides with silver nitrate.

Another sample of pectic acid was prepared by a more drastic saponification, the temperature being kept at 55°C. for twelve hours. The pectic acid obtained by acidifying this solution was purified as described above.

These samples of pectic acid were then titrated with sodium hydroxide. From the values obtained by titration the neutral equivalents based on a monocarboxylic acid were calculated. The effect of varying the conditions during saponification would be shown by the difference in the neutral equivalents of these pectic acids.

**Results of the Investigation.**—*Effect of temperature.*—Solutions containing 1.00, 0.75, 0.50 and 0.25 gram of pectin in 200 cc. were treated with 50 cc. of 0.424 *N.* sodium hydroxide for twelve hours at 23°C., 50°C., 55°C. and 65°C. From the results in Table 4 it is quite evident that temperature influences the reaction. There is a large variation in the apparent amount of pectin present when determined at 23°C. or 55°C. The pectin in the various solutions was determined by saponification for three hours at 23°C. Compared with these results are the values which would result on the basis of hydrolysis at 55°C. From the results of hydrolysis at 55°C., 1.00 gram of sodium hydroxide combines with 5.2 grams of pectin. Using this to estimate pectin at 23°C. the effect of temperature on the degree of saponification is shown and the variation in results is evident if temperature is not controlled.

However, comparable results are obtained for a given series if the temperature is held constant as shown by the titration curves Figs. 2 and 3. At 23°C. as the amount of pectin is increased the amount of alkali used increases proportionally but gives an incorrect value for the amount

TABLE 6A.—COMPARISON OF FACTORS FOR VARYING AMOUNTS OF PECTIN AT 23°C. AND 55°C.

Temp. degrees C.	Wt. of pectin	0.1 N. NaOH cc.	Factor
23	0.100	3.6	6.93
23	0.075	2.7	6.97
23	0.050	1.8	6.94
23	0.025	0.8	7.81
55	0.100	4.8	5.20
55	0.075	3.5	5.35
55	0.050	2.5	5.00
55	0.025	1.2	5.20

TABLE 6B.—COMPARISON OF SAPONIFICATION OF PECTIN AT 23°C. AND 55°C. CALCULATED ON THE BASIS OF SAPONIFICATION AT 55°C.

Temp. degrees C.	Wt. Cal. Sapon. 55°C.	Wt. found Sapon. 23°C.	Difference 23°C.-55°C.	Per cent Error
23	0.096	0.072	0.024	25.00
23	0.070	0.059	0.011	15.70
23	0.050	0.036	0.014	28.00
23	0.024	0.017	0.007	29.18

of pectin present if the factor for any other temperature is used as shown by Table 6A. If, however, the factor at any given temperature is determined and a saponification is carried on at that temperature comparable results are obtained as shown by Table 6B.

The effect of temperature is also shown by the change in the apparent molecular weight of pectin as the temperature varies. These calculations are based on pectic acid as a monocarboxylic acid, as shown in Table 7.

TABLE 7.—APPARENT MOLECULAR WEIGHT WITH CHANGE IN TEMPERATURE

Temperature degrees C.	Saponification equivalent of pectin	Neutral equivalent of pectic acid.*
23	285.6	271.6
35	250.2	236.2
55	208.9	194.9
65	175.0	161.0

\*Obtained by subtracting 14, result of demethylation.

*Effect of time.*—Solutions similar to those used for the study of temperature on the degree of saponification were used in studying the effect of time. Table 5, shows there is a little variation in the saponification at 23°C. with changes in time. At 55°C. however, there is a marked change with time. These results are also shown by titration curves in Fig. 3. Saponification at 65°C. or even higher temperatures for twelve hours or more would insure complete saponification, but carbohydrates at temperatures of 60°C. or 70°C. on prolonged standing give rise to acids of small molecular weight. The method proposed for the determination of pectin depends on the increased acidity due to the formation of pectic acid from pectin, hence the results will be unreliable if other acids are formed in addition to pectic acids. Those solutions kept at 60°C. or 70°C. for twelve hours or more develop an odor of caramel which indicates a decomposition of the pectin. It was therefore necessary to determine the length of time required for saponification to produce a pectic acid having a neutral equivalent like that obtained from the purified pectic acid produced by hydrolysis at 55°C. From Table 7, pectin solutions kept at 55°C. for twelve hours approach most closely the neutral equivalent of the purified pectic acid. At the end of twelve hours saponification at 55°C. there was no odor of caramel indicating that the decomposition of pectin for this length of time is negligible. Table 8, shows the equivalent weight of pectin saponified at 55°C. for different lengths of time.

Fig. 3.—Titration curves of acidity developed during saponification of pectin show that as the amount of pectin in solution varies, the acidity developed changes proportionally under constant conditions.

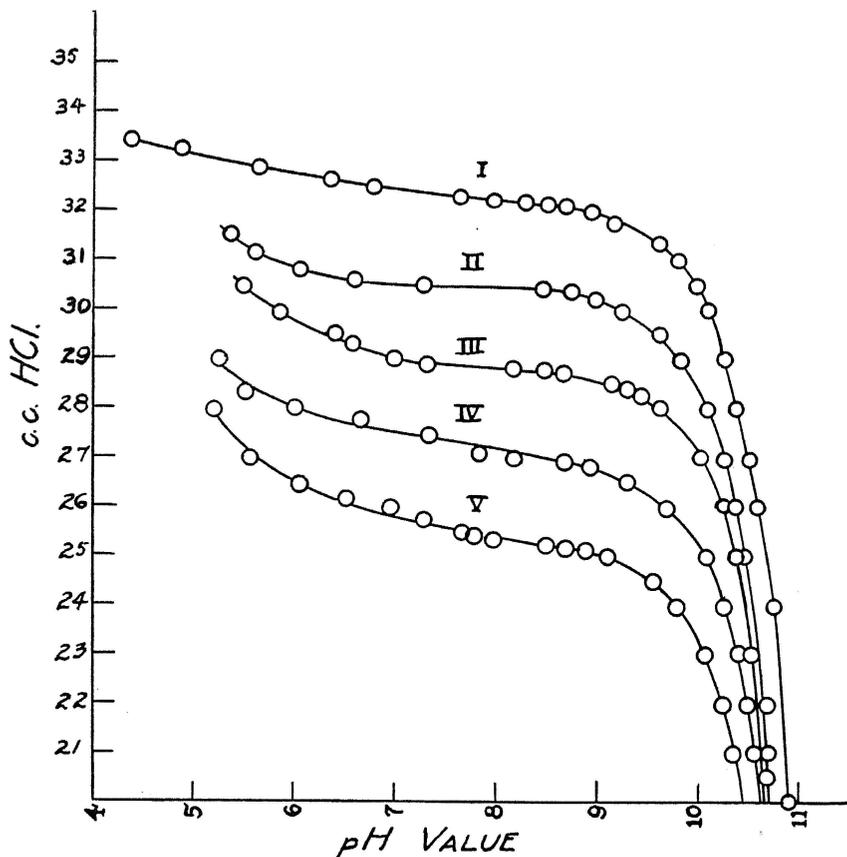


TABLE 8.—EFFECT OF TIME ON THE SAPONIFICATION OF PECTIN AT 55°C.

Temperature degrees C.	Saponification equivalent of pectin*	Neutral equivalent of pectic acid	Time
55	234.0	220.0	3 hrs.
55	208.9	194.9	12 hrs.
55	170.0	156.0	24 hrs.

\*Obtained by adding 14, result of methylation of COOH group.

*Pectic acid produced at 23°C. and 55°C.*—In order to determine the conditions which give results that represent most accurately the quantity of pectin present, pectic acid was prepared by treating pectin at 23°C. for twelve hours and also at 55°C. for twelve hours with sodium hydroxide according to the method previously described. If there is a difference in degree of saponification there will be a difference in the amount of alkali necessary to neutralize a given weight of acid. As pointed out before there may be an increased acidity produced with time due to further breaking down of pectin to acids of low molecular weight but these would be removed in the process of purifying the pectic acid. The acid prepared in this way represents pectic acid produced by saponification at 55°C. Two tenths of a gram of pectic acid prepared by saponification at 23°C. required 7.9 cc. of 0.1*N.* NaOH. Two tenths of a gram of pectic acid prepared at 55°C. required 10.3 cc. 0.1*N.* NaOH. The neutral equivalent of pectic acid at 23°C. is 251.0; at 55°C is 194.9. The conditions during saponification which produce a pectic acid of a neutral equivalent nearest 194.9 would be best suited. As seen in Table 8, saponification at 55°C. for twelve hours yields a pectic acid with a neutral equivalent 194.9. Continued saponification at 55°C, will produce inaccurate results due to the action of alkali on carbohydrates for long periods of time by producing acids of low molecular weight.

Titration curves of pectin solutions saponified at 23°C. and 55°C., Fig. 2 and Fig. 3, also show that when conditions are held constant comparable results are obtained. The effect of varying the time of saponification is shown in Fig. 1. However, reliable results cannot be obtained unless the neutral equivalent has been determined for the conditions under which saponifications are carried out.

**Method of Determining Pectin.**—From the results of the preceding investigations the following method of estimating pectin was adopted. To pectin solutions containing from 0.25 gram to 1.0 gram in 200 cc. of solution a known amount of alkali (50 cc.) was added from a pipette so that the concentration of the alkali would be about 0.1 normal. The solutions were then made up to volume (250 cc.) and allowed to stand at 55°C. for twelve hours. The flasks were sealed during hydrolysis in order to prevent the entrance of carbon dioxide. Aliquots were then pipetted off and titrated with hydrochloric acid. The sodium hydroxide should be about four times as strong as the hydrochloric acid used for the back titration in order that the difference will be large and so reduce the percentage error. From the number of cubic centimeters of alkali combined with the pectin the amount of pectin is calculated. Taking the neutral equivalent of pectic acid at 55°C. as 194.9 which is equiva-

lent to 208.9 grams of pectin, the following method can be used to calculate the amount of pectin from the amount of alkali used:

$$\begin{array}{rclcl} \text{NaOH} & : & \text{Pectin} & : : & \text{Wt. of alkali combined} & : & X. \\ 40 & & 208.9 & & & & \end{array}$$

The following precautions must be taken to insure comparable results. In case very small quantities of pectin are found (0.010 grams in 50 cc. of solution) about a 200 cc. sample should be taken. As the alkali is four times as strong as the acid, care must be taken in pipetting the alkali for saponification since a drop more in one than in another makes an error of about 0.2 of a cubic centimeter.

#### Comparison of the Precipitation Method with the Titration Method.

—The method of determining pectin as described in the preceding paragraph was checked against the method developed by Carre and Haynes.

The samples for comparison were taken from a stock solution of pectin containing 2.00 grams per liter. The pectin was estimated in 25 cc. aliquots. According to the method of Carre and Haynes the pectin was saponified at room temperature, however the precaution was taken to keep the temperature at 29°C. during the saponification. The pectin in the samples to be determined by the titration method was saponified at 55°C. Table 9 shows the results obtained by the two methods

TABLE 9.—COMPARISON OF THE PRECIPITATION METHOD WITH THE TITRATION METHOD.

Wt. of Pectin by precipitation method	Wt. of Pectin by titration method	Wt. of Pectin.
0.0450	0.0520	0.050
0.0484	0.0525	0.050
0.0486	0.0515	0.050
0.0473	0.0512	0.050

As shown by this table each method gives reliable estimates of pectin when working with pure pectin. However, when working with plant material which contains salts which are occluded by the calcium pectate gel, as pointed out by Carre and Haynes, the precipitation method becomes less reliable while by the titration method the difficulty of occluded material interfering is avoided. The titration method as developed in this study is a more rapid method than the precipitation method.

### A STUDY OF PECTIC ACID

In connection with the development of the method for the quantitative determination of pectin a series of investigations were made to determine the number of carboxyl groups in pectic acid. Fellenberg<sup>62</sup> considered pectic acid to be an octocarboxylic acid. Work of Sucharipa<sup>63</sup> indicated that there are more than eight carboxyl groups in pectic acid. Carre and Haynes<sup>64</sup> suggest that pectic acid be considered a dicarboxylic acid until its structure is accurately determined.

**Method of Attack.**—Since the various salts of a polycarboxylic acid yields aqueous solutions of different hydrogen ion concentrations, the number of carboxyl groups might be detected from the hydrogen ion values found by titrating pectic acid with sodium hydroxide electrometrically, in case the dissociation constants of successive hydrogen ions are sufficiently far apart.

Purified pectic acid was dissolved and titrated electrometrically, using a Bovie Hydrogen Ion Potentiometer. By adding the alkali, 0.5 cc. at a time, as rapidly as a constant reading could be established no abrupt changes in the pH values of the solution could be detected. A smooth curve shown in Fig. 5 was obtained. However, it was observed that when longer time intervals were allowed between additions of the alkali, there were some points on the curve which did not remain constant, but after standing became constant at some point different from that shown by the original reading. This change in hydrogen ion concentration with time suggested that for any given salt of pectic acid a number of hours are required for an equilibrium to be established. As it would be impracticable to continue a titration over a period of time long enough for each point to come to equilibrium, the following method of titration was used.

The number of cubic centimeters of alkali needed to neutralize a given weight of pectic acid was determined by titration, using an indicator to determine the end point. A series of samples containing this weight of pectic acid was then made up and placed in weighing bottles. To the first a fraction of a cubic centimeter of sodium hydroxide was added, to the second sample twice this amount, to the third three times this amount, and so on until the total amount of alkali as found by the preliminary titration was added. These bottles were then stoppered and sealed to prevent absorption of carbon dioxide and allowed to remain for twelve hours for the solutions to come to equilibrium. At the end of this time the pH value of each solution was determined and all these values were plotted together. The different dissociation constants of the salts of pectic acid formed breaks in the curve. These curves are shown in

<sup>62</sup>Loc. cit. (Ref. 20).

<sup>64</sup>Loc. cit.

<sup>63</sup>Loc. cit.

Figs. 4 and 5. Each of these breaks in the curve represents a salt of a definite pH value which in turn represents a carboxyl group. It will be observed that there are no breaks in the curve on the acid side of the pH 5.3. In the pH range 5.3 to 8.3 there are distinct breaks. From the neu-

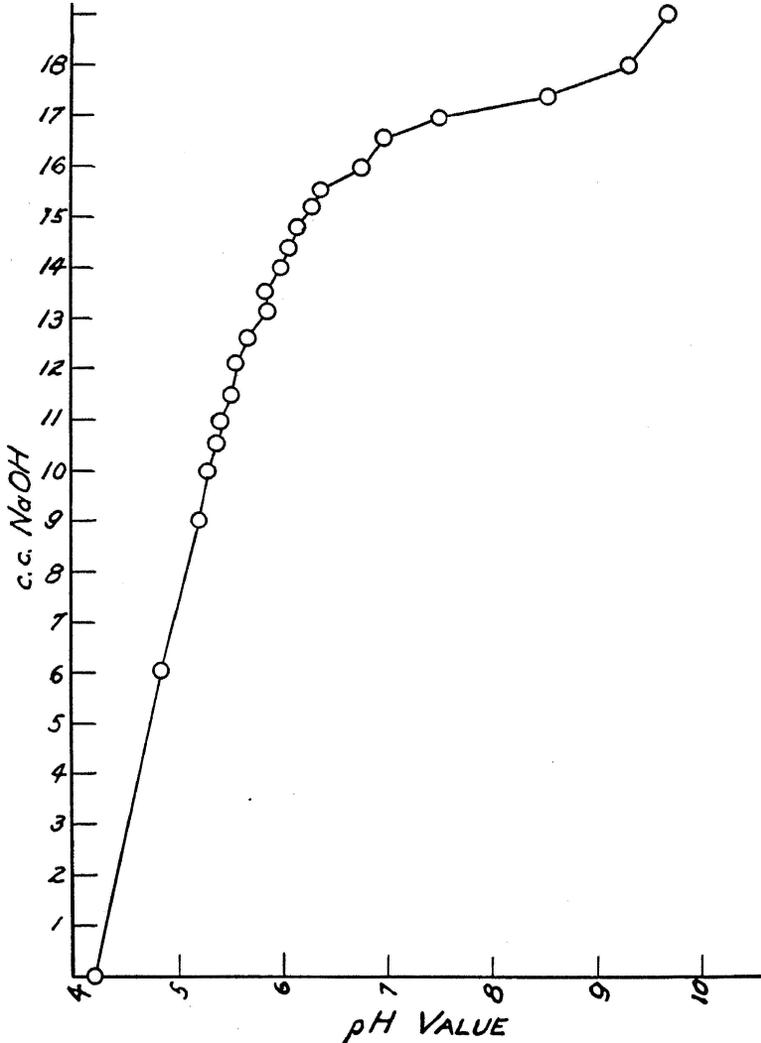


Fig. 4.—Titration curve of pectic acid

Weight of pectic acid per sample, 0.049 gram 16.8 cc. NaOH needed—0.0149 Normal.

Curve 1. Titration curve of pectic acid obtained by allowing sealed aliquots containing 0.3 cc., 0.5 cc. etc. up to 20 cc. of alkali to remain for 12 hours before taking the pH. Each point represents an average of three samples of like aliquots of three series. The maximum variation for the experimental points on the curve is 0.05 of a pH from the average.

tral point of each of these three breaks the number of cubic centimeters of alkali needed for the neutralization of the equivalent of one carboxyl group was calculated. The number of carboxyl groups in pectic acid can

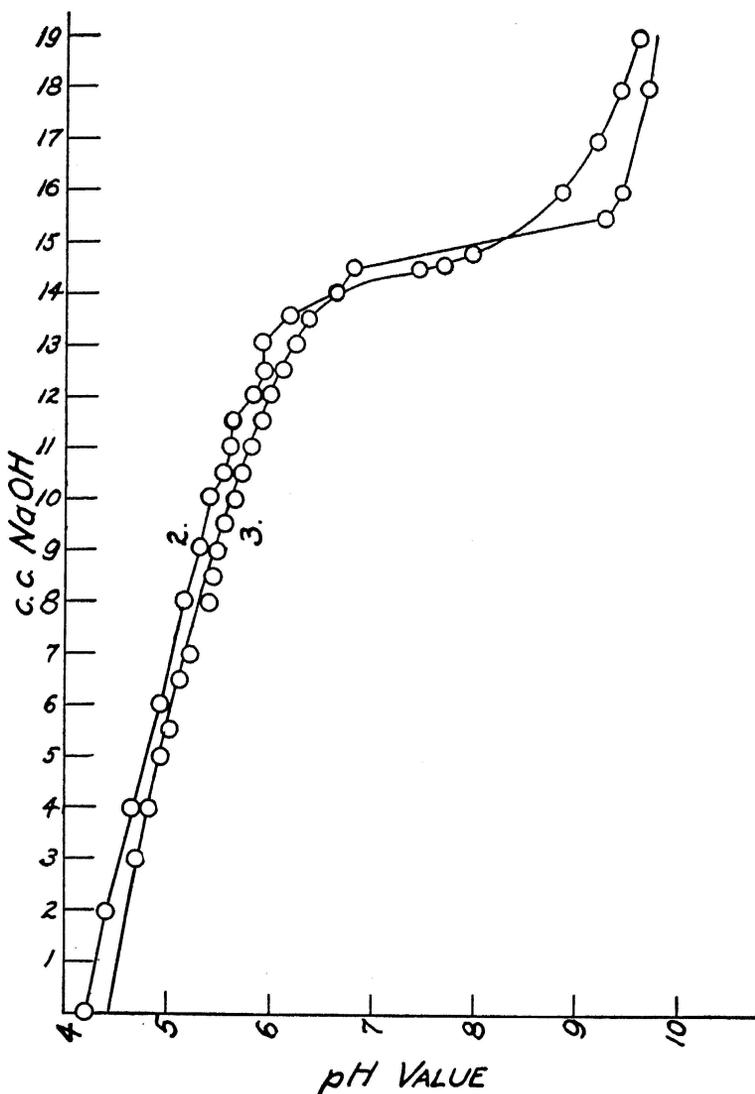


Fig. 5.—Titration curves of pectic acid

Weight of pectic acid per sample, 0.043 gram.

Curve 2. Titration curve of pectic acid obtained by allowing sealed aliquots containing 0.5, 1.0 cc. etc. up to 20 cc. of alkali to remain 12 hours before taking the pH. Each point represents an average of three samples of like aliquots of three series. The maximum variation for the experimental points on the curve is 0.05 of a pH from the average.

Curve 3. Titration curve of pectic acid obtained by adding the alkali 0.5 cc. at a time rapidly.

be determined by dividing the total number of cubic centimeters used for the neutralization of a given weight of pectic acid by the number required for one carboxyl group. Thus from the curve, Fig. 4, it is seen that salts of pectic acid are formed at pH 5.5 and at pH 5.70 respectively. Also there is the possibility of another break occurring at a pH of about 6.2. The difference in cubic centimeters of alkali between the pH at 6.2 and 8.3 is the same as the amount between 5.5 and 5.7. The difference between pH 5.7 and 6.2 would be the same as between pH 6.2 and 8.3. The difference between each of these points is 1.5 cubic centimeters of alkali. Since 16.8 cc. of alkali were needed to neutralize the weight of pectic acid used,  $\frac{16.8}{1.5}$  or 11.2 represents the number of carboxyl groups in this pectic acid.

A close study of these curves might suggest that it would be more legitimate to use the average of the points determined and draw a smooth curve for the titration with no breaks since the changes in pH are quite small. However, this curve was determined in triplicate, each point representing three samples with the breaks occurring at the same pH value. If these breaks were due to experimental error they would not occur at the same pH values and at regular intervals. Moreover, when solutions of varying concentrations were titrated the breaks in the curve occurred at the same pH value, as shown in curve 2, Fig. 5.

The neutral equivalent, on the basis of a monocarboxylic acid, of this substance is 194.9. By standard methods of combustion, on the ash-free basis, pectic acid is shown to have the following percentage composition:

C 40.90      H 5.28      O 53.82.

These values are expressed by the empirical formula  $C_6H_9O_6$ , with a molecular weight of 177.0. Compared to this value the neutral equivalent obtained by titration was 194.9. This difference may be accounted for by assuming completely demethylated pectic acid to have twelve carboxyl groups. On this basis the molecular weight of pectic acid is represented by twelve times the empirical formula  $C_6H_9O_6$  or 2124. The pectic acid used in this investigation has eleven free carboxyl groups as shown by the curve in Fig. 4. Assuming pectic acid to have twelve carboxyl groups there was one unsaponified methyl group remaining in the pectic acid studied. This incompletely saponified pectic acid would have a molecular weight as given for pectic acid above plus one methyl group or 2138 with a neutral equivalent  $\frac{2138}{11}$  or 194.4, the calculated value as compared to 194.9, the value found.

The presence of an unsaponified methyl group does not materially alter the combustion as shown by the following calculation. On the basis of the empirical formula adopted a completely methylated pectic acid has the following composition:  $C_{84}H_{132}O_{72}$ , pectic acid  $C_{72}H_{108}O_{72}$  and pectic acid with one unsaponified group  $C_{73}H_{110}O_{72}$ , with the calculated percentage composition as shown in Table 10. The tetragalacturonic acid reported by Ehrlich which Fellenberg<sup>65</sup> considers pectic acid also corresponds closely to the empirical formula  $C_6H_9O_6$ .

TABLE 10.—COMPARISON OF VARIOUS DEGREES OF METHYLATION OF PECTIC ACID

Material	Per cent C.	Per cent H.	Per cent O.
Completely methylated pectin.....	43.97	5.75	50.28
Completely saponified pectin.....	40.67	5.08	54.26
Partially saponified pectin (one group unsaponified)	41.00	5.18	53.18
Tetragalacturonic acid.....	39.94	4.98	55.08
Pectic Acid, (page 26).....	40.90	5.28	53.82

From Table 10 it will be seen that the presence of an unsaponified ester group cannot be established by combustion. On the basis of the combustion and the values obtained by titration, as shown before, the nucleus of pectic acid is represented by some multiple of  $C_6H_9O_6$ .

**Development of Acidity During Titration of Pectic Acid.**—As the electrometric titration of pectic acid proceeded to a pH value of 7.5, acidity developed changing the pH value to 7.0 and on longer standing to 6.0. Addition of more sodium hydroxide brought the pH value to 8.0 but this changed again quickly to about 7.0. This process repeated itself on further addition of sodium hydroxide. What caused the development of this acidity is yet undetermined. This phenomenon was not observed during titration with an indicator, but occurred only in the presence of the platinum black of the hydrogen electrode.

Solutions of pectic acid were neutralized with sodium hydroxide, a few drops were added in excess and allowed to stand for twelve hours. At the end of this time these solutions were distinctly alkaline to phenolphthalein. However by determining electrometrically the pH value of the solutions, which were distinctly colored, the pink color soon faded out as acidity developed, showing that this acidity developed only when the solutions came in contact with the hydrogen electrode. Similar solutions held their color for several days but when placed in contact with the hydrogen electrode the color was soon discharged. That this is not a reduction due to the hydrogen of the hydrogen electrode is shown by the following experiment. To six pectic acid solutions of the

<sup>65</sup>Loc. cit. (Ref. 20).

same concentration enough sodium hydroxide was added to give a deep coloration with phenolphthalein. Three of these solutions were stoppered and shaken. The color did not disappear. Into the remaining three solutions platinum black electrodes were introduced and stoppered. After only a few minutes the pink color of the phenolphthalein was discharged, the solutions were again made alkaline and in a short time the color again disappeared. This development of acidity was also observed when platinized tips of platinum wire were introduced into alkaline pectin solutions. A similar study was made of the effect of platinum black on alkaline solutions of galactose. To a series of alkaline solutions of galactose phenolphthalein was added. Some of these solutions were allowed to stand in contact with platinum black and others without the platinum black. Only those in contact with the platinum black developed enough acidity to discharge the color of the phenolphthalein. No explanation of this phenomenon can be offered at the present time.

### CONCENTRATED HYDROCHLORIC ACID HYDROLYSIS OF PECTIN

In connection with this investigation an attempt was made to obtain by hydrolysis of pectin with concentrated hydrochloric acid a series of decomposition products ranging from the more complex to the simple nucleus. From the products of hydrolysis information concerning the structure of pectic acid could be obtained.

*Effect of concentrated HCl on pectin.*—Pectin was found to dissolve with decomposition in concentrated hydrochloric acid at room temperature. With varying amounts of pectin in a given amount of hydrochloric acid different products were obtained. It was also found that by varying the duration of hydrolysis different series of decomposition products could be obtained.

**Method of Hydrolysis.**—To a concentrated solution of hydrochloric acid pectin was added, with stirring. This formed a gelatinous mass which on continued stirring dissolved after a few hours giving a very dark brown solution that afterwards turned black. The solutions were then allowed to stand for different lengths of time in closed flasks. At the end of the time allotted for the hydrolysis a current of air was drawn through the liquid to remove part of the hydrochloric acid and the solutions were then diluted. On dilution a precipitate formed. The material was then filtered and the different products of hydrolysis were separated from the residue and the filtrate.

**Method of Separation of the Products of Hydrolysis of Pectin Contained in the Residue.**—On addition of water to the hydrolytic mixture a black or brown precipitate formed depending on the length of time of the hydrolysis. This solution was allowed to stand several

hours to permit the precipitate to settle. The solution was then filtered, the filtrate having a reddish-brown color. The residue was washed with water until free from acid. The washings were combined with the filtrate.

The residue obtained in the first case studied was a black pasty substance. It was found that on treatment with hot alcohol a brown substance could be extracted. After complete extraction with alcohol a black powder remained which was insoluble in cold water. This black powder was then treated with hot water and filtered. Filtration was possible only when the solution was kept hot, indicating that in the

TABLE 11.—SEPARATION OF THE HYDROLYTIC PRODUCTS OF PECTIN CONTAINED IN THE RESIDUE

400 cc. Conc. HCl, 100 grams of Pectin, Time 24 hours.  
Add 200 cc. of water. Filter.

Black residue, wash with H <sub>2</sub> O. Extract with hot C <sub>2</sub> H <sub>5</sub> OH.		Reddish brown filtrate. (Method of separation, page 38, Table 12)
Dark brown extract. Evaporate to dryness. Black powder.	Black residue. Extract with hot H <sub>2</sub> O.	
	Brown powder. Dissolve in KOH Precipitate with acid.	Filtrate colorless. Cool, white precipitate.

TABLE 12.—SEPARATION OF THE HYDROLYTIC PRODUCTS OF PECTIN CONTAINED IN THE FILTRATE.

400 cc. Conc. HCl, 100 grams of Pectin, Time 24 hours.  
Add 200 cc. of water. Filter.

Black residue (Method of separation, page 38, Table 11)	Reddish brown filtrate, add five times its volume of C <sub>2</sub> H <sub>5</sub> OH. Filter.	
	White precipitate.	Reddish brown filtrate. Evaporate to dryness. Black residue. Ext. with alcohol-ether.
	Brown extract. Evap. to dryness.	Black residue, dissolve in KOH. Acidify, black precipitate forms.

black powder there was present a hot-water-soluble substance. The filtrate was colorless and the residue, insoluble in hot water, was a very dark brown powder. On cooling the filtrate, a white precipitate formed. The residue was soluble in sodium hydroxide. The separation of the hydrolytic products is shown in Table 11.

**Method of Separation of the Products of Hydrolysis of Pectin Contained in the Filtrate.**—To the original filtrate alcohol was added giving a precipitate that dissolved on stirring. On the addition of larger

quantities of alcohol a permanent white precipitate was obtained. The material was filtered giving a brown filtrate and leaving a residue which became white on washing with alcohol. The filtrate was then evaporated to a small volume under diminished pressure and finally on a water bath to dryness. The residue was a black substance. Extraction with alcohol and ether removed a substance giving a brown color to the alcohol extract and leaving a black residue. The residue was soluble in potassium hydroxide. A precipitate formed on acidifying the alkaline solution. Table 12 shows the method used in separating the products in the filtrate.

After developing a method of separating the different products of hydrolysis, the effect of time on hydrolysis was studied. The method of separation had to be altered, however, since by changing the duration of hydrolysis different products formed.

**Effect of Length of Time of Hydrolysis.**—In the study of the effect of time on hydrolysis the work was carried on at room temperature. A solution of pectin in concentrated hydrochloric acid was made by grinding pectin in hydrochloric acid. In this way 200 grams of pectin were dissolved in 800 cc. of concentrated hydrochloric acid. A series was then made to study the effect of time. The solution was divided in four aliquots of 200 cc. each, the first allowed to stand 8 hours, the second 16 hours, the third 24 hours and the fourth 48 hours.

At the end of 8 hours the material formed a gelatinous mass, completely soluble in cold water. On addition of alcohol a solid gelatinous precipitate formed similar to the precipitated pectin. No attempt was made to separate this material.

At the end of 16 hours another sample of the series was studied. The pectin in this case was dissolved giving a viscous solution. On addition of water to this material a light brown precipitate formed. After allowing this to settle it was filtered. Only the residue in this case was studied. The residue was washed with water to free it from acid. An alcohol extract was then made which removed a dark brown substance. The residue, after extraction with alcohol was a cocoa-brown powder fairly soluble in cold water and very soluble in hot water. A very small amount of black residue remained after the hot water extraction. The extract was a light brown solution. On cooling a gray precipitate formed leaving a viscous brown solution. This was decanted since it was too viscous to filter. The gray residue was washed in a centrifuge with cold water to remove all acid. The washings gave a brown solution leaving a small amount of white material insoluble in cold water. There was, however, only a very small amount of this material formed. Alcohol was then added to the light brown filtrate. A brown precipitate formed. This precipitate, a single substance probably of high molecular weight as indicated by its colloidal nature, was then treated with concentrated

TABLE 13.—SEPARATION OF THE HYDROLYTIC PRODUCTS OF PECTIN TREATED WITH CONC. HCl ACID FOR 16 HOURS.

400 cc. Conc. HCl, 100 grams of Pectin.  
Time 16 hours, Temp. 25°C.

Add 200 cc. H <sub>2</sub> O. Filter.	
Light brown ppt. Wash with H <sub>2</sub> O. Extract with C <sub>2</sub> H <sub>5</sub> OH.	Light brown filtrate.
Cocoa-brown residue. Extract with hot H <sub>2</sub> O.	Dark brown solution. Evap. to dryness. Light-brown residue.
Black residue. Sol. in KOH	Light-brown extract. Cool. Gray ppt. forms. Separate and wash by centrifuging.
White ppt. Sol. in hot H <sub>2</sub> O, sparingly sol. in cold H <sub>2</sub> O.	Light-brown solution. Add C <sub>2</sub> H <sub>5</sub> OH, brown ppt. forms. Sol. in H <sub>2</sub> O. An acid. Treat 25 gr. with 100 cc. of conc. HCl for 12 hours.
Add H <sub>2</sub> O. Filter.	
Black residue, wash with H <sub>2</sub> O. Extract with hot C <sub>2</sub> H <sub>5</sub> OH.	Reddish-brown filtrate.
Black residue, extract with hot H <sub>2</sub> O.	
Black residue. Soluble in KOH. An acid.	Colorless filtrate. Cool, white ppt. forms. An acid.

hydrochloric acid for twelve hours. A black solution was obtained from which was precipitated a black material on the addition of water. The solution was filtered, and the residue washed with water and hot alcohol. The alcohol extract was colored brown. A black residue remained which was washed with hot water. On cooling a white precipitate formed in the filtrate. The white amorphous substance has the same properties and titration value as the white material separated above. The residue was soluble only in alkali. The method of separation of the products of this treatment is shown in Table 13.

At the end of 24 hours another sample of the series was diluted with water. The results of this study have already been given in Tables 11 and 12. The white material here obtained was insoluble in cold water but soluble in hot water and is the same as the one isolated in the preceding treatment.

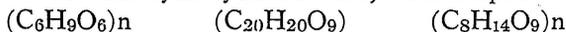
At the end of 48 hours another sample of the series was diluted. A fine black precipitate formed. The solution was filtered, washed free from acid and extracted with alcohol. A very dark brown extract was obtained and on evaporation to dryness left a black solid. No attempt has been made to separate it. The residue from the alcohol extract was washed with hot water. A black residue remained. On cooling the filtrate there was no precipitate as was found in the other treatments and on evaporation of the filtrate there was no residue. The white substance insoluble in cold water but soluble in hot water seemingly had been decomposed. Nothing was done with the original filtrate in this series. The results obtained after 48 hours treatment are shown in Table 14.

TABLE 14.—PECTIN TREATED WITH CONC. HCl. TIME 48 HOURS.  
Add water. Filter.

Black residue, wash with H <sub>2</sub> O Extract with hot C <sub>2</sub> H <sub>5</sub> OH		Reddish brown filtrate.
Dark brown extract. Evap. to dryness. Black powder.	Black residue Extract with hot H <sub>2</sub> O	
	Black residue	Colorless extract, cool. No precipitate.

**A Study of the Hydrolytic Products of Pectin.**—In this investigation three of the hydrolytic products shown in Tables 11 and 12 were studied. These tables are condensed in Table 15 to show the method of separating these products.

As shown in Table 15 products have been isolated from the concentrated hydrochloric acid hydrolysis mixture, with empirical formulae:



**A Study of the Compound (C<sub>6</sub>H<sub>9</sub>O<sub>6</sub>)<sub>n</sub>**—This substance was isolated from a mixture of concentrated hydrochloric acid hydrolytic products. Pectin treated with concentrated hydrochloric acid for 16 to 24 hours at room temperature forms a black viscous solution. On addition of water to this solution a black precipitate was obtained. After filtering and washing the precipitate free from acid it was extracted with hot alcohol. The residue was then transferred to water and boiled. The hot solution was filtered and the colorless filtrate was cooled whereupon a white

precipitate formed. The solution unless kept at the boiling point is syrupy and will not filter. As the precipitate formed it separated as a white pasty mass. The filtrate and precipitate were separated by centrifuging. On washing the precipitate with alcohol and ether it dried to a white amorphous powder. This material does not reduce Fehling's solution. It is an acid very soluble in hot water, sparingly soluble in cold water. Its calcium and barium salts are insoluble, forming gelatinous precipitates. From the colloidal nature of the solution and the gelatinous appearance of the calcium and barium salts the substance is a compound of high molecular weight. On titration with sodium hydroxide this compound has a neutral equivalent of 210. The combustion gives the following values:

C 40.77,            H 5.28,            O 53.95.

This is expressed by the empirical formula  $C_6H_9O_6$ . With a neutral equivalent of 210 the formula for this substance must be represented by a multiple of  $C_6H_9O_6$ .

TABLE 15.—SUMMARY OF TABLES 11 AND 12, SHOWING METHODS OF SEPARATION OF THE THREE HYDROLYTIC PRODUCTS WHICH WERE STUDIED.

Pectin (50 gr.) dissolved in 200 cc. Conc. HCl  
Time 24 hours. Temp. 25°C.

Add 200 cc. H <sub>2</sub> O. Filter.			
Black residue, wash with H <sub>2</sub> O. Extract with hot C <sub>2</sub> H <sub>5</sub> OH		Reddish-brown filtrate, add C <sub>2</sub> H <sub>5</sub> OH. Ppt. forms. Filter.	
Black residue, extract with hot H <sub>2</sub> O.		White residue. Wash with C <sub>2</sub> H <sub>5</sub> OH. An acid. (C <sub>8</sub> H <sub>14</sub> O <sub>9</sub> ) <sub>n</sub>	
Black residue. Soluble in in KOH. An acid. C <sub>20</sub> H <sub>20</sub> O <sub>9</sub> .	Colorless. filtrate. Cool, white ppt. forms. An acid. (C <sub>6</sub> H <sub>9</sub> O <sub>6</sub> ) <sub>n</sub>	Reddish-brown filtrate. Evap. to dryness. Extract with ether.	
		Brown extract.	Black residue. Sol. in KOH. ppt. by acid. An acid. (C <sub>20</sub> H <sub>20</sub> O <sub>9</sub> ) <sub>n</sub>

**A Study of the Compound (C<sub>8</sub>H<sub>14</sub>O<sub>9</sub>)<sub>n</sub>.**—As shown in Table 15, this compound was separated from a mixture of compounds in the filtrate obtained from the concentrated hydrochloric acid hydrolysis. This material is very soluble in water, requiring five volumes of alcohol to one of aqueous solution for complete precipitation. The precipitate is white and pasty in alcohol. When dried with ether it formed a white

amorphous powder. When prepared by evaporation of a water solution of this material a brown glassy mass formed. It reduces Fehling's solution. It is an acid, its calcium and barium salts being insoluble in water. The neutral equivalent obtained by titration with sodium hydroxide is 308. The combustion gives the following results:

C 37.80            H 5.5            O 56.65

which is expressed by the following empirical formula  $C_8H_{14}O_9$ . A substance of this empirical formula was also isolated by Fremy<sup>66</sup> and termed by him metapectic acid. This formula however does not agree with the neutral equivalent, hence must be expressed as a multiple  $(C_8H_{14}O_9)_n$ .

**A Study of the Compound  $C_{20}H_{20}O_9$ .**—On continued hydrolysis of pectin with concentrated hydrochloric acid a black compound is formed. This substance is slightly soluble in hot alcohol. The alcoholic solution on evaporation forms an oily liquid having an odor characteristic of furfural. This material is insoluble in water or ether but soluble in sodium hydroxide. On addition of acid to the alkaline solution the black material is reprecipitated. It reduces Fehling's solution. The combustion of this substance is as follows:

C 59.30            H 4.95            O 35.65

The empirical formula corresponding to this combustion is  $C_{20}H_{20}O_9$ .

**Summary of the Compounds Obtained from Pectin.**—The following compounds have been derived from pectin as shown by the review of literature.

(1) Methyl alcohol and pectic acid were obtained by alkaline hydrolysis.

(2) Metapectic acid and tetragalacturonic acid were obtained by acid hydrolysis.

(3) Acetone was obtained by the action of enzymes.

(4) Arabinose and galactose have been found as products of hydrolysis.

(5) Strong reducing substances result during hydrolysis.

(6) Mucic acid has been obtained as the result of oxidation.

(7) Galacturonic acid and galacto-galacturonic acid have also been obtained as hydrolytic products.

In addition to these compounds the following were obtained from pectin during this study.

(8) The compound  $C_8H_{14}O_9$  designated by Fremy as metapectic acid was isolated from a mixture of hydrolytic products obtained by concentrated hydrochloric acid.

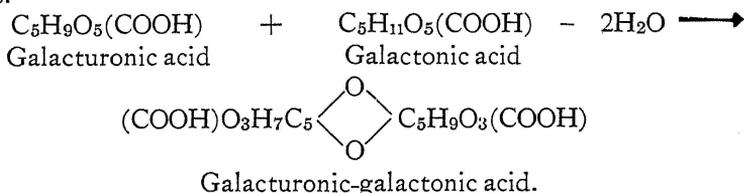
(9) Compounds with the empirical formula  $C_6H_9O_6$  and  $C_{20}H_{20}O_9$  were isolated as hydrolytic products.

<sup>66</sup>Loc. cit.

(10) The combustion of pectic acid shows it to have the empirical formula  $C_6H_9O_6$ .

Titration values of pectic acid incompletely methylated give a neutral equivalent of 194.9. That of the completely demethylated pectin would be 177.0 on the basis of twelve carboxyl groups. This neutral equivalent corresponds to the formula  $C_6H_9O_6$ .

Any proposed formula for pectic acid must be capable of accounting for the formation of the compounds just listed. The following formula is proposed for the unit nucleus of pectic acid as best explaining the known facts.



Pectic acid would be represented by a number of these units linked together to form a ring structure. Pectin would be represented by covering the carboxyl group with methyl groups.

During acid hydrolysis of pectic acid arabinose has been isolated. This might arise from two different sources. According to Ehrlich<sup>67</sup> arabinose is not a part of the pectic acid molecule but is found only in a mixture of araban and pectin. However, it might arise from galacturonic acid by decarboxylation. Some investigators have concluded that pentoses were present since furfural was obtained. However, both Spoehr and Ehrlich pointed out that hexoses also yield furfural. Hence the formation of furfural is not conclusive evidence that pentoses are present. The proposed formula would account for the formation of pentoses during acid hydrolysis by decarboxylation of galacturonic acid. Ehrlich first isolated galactose from pectin by hydrolysis with oxalic acid. The proposed formula would account for the formation of galactose by the hydrolysis with oxalic acid, a strong reducing agent.

The presence of strong reducing substances in the hydrolytic products of pectin can be explained as due to the splitting of the galacturonic-galactonic acid to galacturonic acid and galactonic acid, the former being an acid that reduces Fehling's solution in the cold. Furfural-like compounds which are strongly reducing substances, are also formed.

On oxidation galacturonic and galactonic acids give mucic acid.

On hydrolysis galacturonic-galactonic acid yields galacturonic acid. Galacto-galacturonic acid would be formed by reduction during hydrolysis of pectin with oxalic acid.

<sup>67</sup>Loc. cit.

The compound  $C_8H_{14}O_9$  designated by Fremy<sup>68</sup> as metapectic acid was isolated in this work. This substance has a neutral equivalent of 308. Hence the simple formula  $C_8H_{14}O_9$  does not represent the compound.

This substance might be derived from galacturonic-galactonic acid by hydration.



Assuming that three of the carboxyl groups recombined during hydration, leaving five free acid radicals, this compound would have a neutral equivalent of  $\frac{1524}{5} = 305.0$  as compared to 308, the value found.

During this investigation a compound with the following composition was isolated:

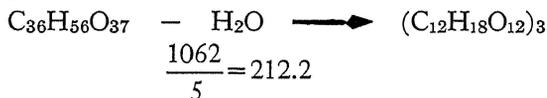
C 40.77            H 5.28            O 53.95

This substance is an acid having a neutral equivalent of 209. This acid is very soluble in hot water, sparingly soluble in cold water and hot alcohol. Its calcium and barium salts form gelatinous precipitates indicating that it is a compound of high molecular weight. It does not reduce Fehling's solution. The composition of this substance is almost the same as that of pectic acid. However, its appearance, a pure white amorphous substance, its solubility in hot alcohol and the solubility of its sodium salt, distinguish it from pectic acid.

Moreover, this substance cannot be expressed by the simple formula  $C_6H_9O_6$  as shown by its neutral equivalent. Assuming that pectic acid is split in two by hydrolysis and that the hydrolytic products regain a cyclic structure by esterification of one of the carboxyl groups, a compound would result having the empirical formula  $C_6H_9O_6$  and a neutral equivalent of 212 as only five of the six carboxyl groups are free. This is shown by the following equation:



By esterification:



By treatment of pectin with concentrated hydrochloric acid for 24 hours or longer a large yield of a black substance sparingly soluble in hot alcohol and soluble in alkali was formed. The combustion of this material gives the following values:

C 59.38            H 4.95            O 35.67

The empirical formula corresponding to this combustion is  $C_{20}H_{20}O_9$ . This substance has physical properties similar to furfural compounds and

<sup>68</sup>Loc. cit.

is probably derived from the galacturonic-galactonic acid by dehydration and decarboxylation, in the same way that furfural derivatives are generally produced from carbohydrates by the action of strong hydrochloric acid.

### SUMMARY

A method for estimating pectin by titration of the acidity developed on saponification of pectin and the effect of time and temperature on the process has been described. Under constant conditions the amount of pectin found is proportional to the acidity developed.

Titration curves of pectic acid show eleven carboxyl groups, but evidence is presented to show that saponification was not complete, which would indicate that there are more than eleven carboxyl groups in pectic acid. On the basis of twelve carboxyl groups the smallest molecular weight of pectic acid would be 354 multiplied by six, or 2,124. The nucleus proposed for pectic acid is a dibasic acid galacturonic-galactonic acid. At least six of these nuclei are linked together to form pectic acid.

Pectin dissolves in concentrated hydrochloric acid and undergoes hydrolysis and decomposition. A method of separating the hydrolytic products produced by the cold concentrated hydrochloric acid has been described and a preliminary study of some has been made.

### ACKNOWLEDGMENTS

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