A comparison of the temperature coefficients for the formation of ethyl acetate over two aluminas gives further evidence that the aluminas differ in the space relationships of the molecules or "active points" of the catalyst.

MADISON, WISCONSIN

[CONTRIBUTION FROM THE INSTITUTE FOR ORGANIC CHEMISTRY OF CZECH UNIVERSITY]

PROTOPECTIN AND SOME OTHER CONSTITUENTS OF LEMON PEEL

BY RUDOLPH SUCHARIPA

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Various terms have been used in describing pectin, the jellying principle of fruits, and related substances. There has been much confusion in the literature because workers have not employed the same nomenclature. In this paper three classes of pectic substances will be distinguished, namely, protopectin, free pectin and pectic acid.

The pectic substance occurs in plants in two different forms, only one of which is soluble in water. The soluble or "free pectin" occurs in the fruit and in many other soft places of the plant, both in the juice and in the cell wall. It is located in the cell wall just on the periphery, where two cells meet. This "free pectin" has the highest jelly-making power of the entire pectic group. The water-insoluble class of pectic substances is called "protopectin," a name first proposed by Tschirsch.[1] It occurs only in the cell wall, lying closest to the cellulose layer, nearer the inner part of the wall, under the film of free pectin. It must be understood, however, that the free pectin layer merges into the protopectin layer without a sharp line of division, and in a similiar manner the protopectin merges into the pure cellulose wall inside the cell.

The third class of pectic substances is pectic acid, which is the ultimate product of the hydrolysis of any of the pectic substances. Free pectin is the fully methylated ester of this acid, which is practically insoluble in water.

It has been definitely established by Fellenberg[2] that protopectin is the methyl ester of pectic acid and not the calcium salt as a number of earlier investigators[3,4] had thought. This view has been confirmed by the work of D. Haynes[5] and Devaux.[6]

Julius Wiesner[7] found that protopectin contains a cellulose radical,

[1] Tschirsch, *Dissertation*, Berne, **1908.**

[2] Fellenberg, *Biochem. Z.*, **85**, 161 (1918).

[3] Payen, *Rec. sav. etrang.*, **9**, 148 (1846).

[4] Chodnew, *Ann.*, **51**, 355 (1844).

[5] Haynes, *Biochem. J.*, **8**, 553 (1914).

[6] Devaux, *Lineana*, Bordeaux, **1903.**

[7] Wiesner, *Jahrb. wiss. Bot.*, **1**, 61 (1861).

his views being derived from experiments on the different solubilities of various parts of the cell in Schweitzer's reagent and chromic acid and on certain staining reactions. Mangin[8] concluded also that protopectin is a cellulose compound; he showed that ammonium oxalate solution was an excellent solvent for removing pectin completely from the plant tissue so that the remaining cellulose would easily dissolve in Schweitzer's reagent.

It is the aim of the present paper to prove conclusively that protopectin is a pectin cellulose compound.

Experimental Part

Protopectin.—The material used in the preliminary work was a very fine powder of the white part of lemon peel. This material, the so-called "Albedo" of lemon peel, was first used by Harlay,[9] and has the advantage over any other pectous material, in that after extraction with alcohol and ether it contains practically no other substances except pectin, protopectin, hemicelluloses and cellulose. It contains no coloring matter and only traces of lignin.

The alcohol extract obtained in the preparation of the albedo contained ethereal oils, terpenes, resins, some tannic and bitter substances, and the glucoside hesperidin. It also contained methyl alcohol which, however, was not split off from pectin during the extraction, as an effort to obtain methyl alcohol by boiling pectin with ethyl alcohol failed to yield even traces of methyl alcohol.

The peel, after extraction with alcohol and ether, was dried and ground. By sifting the ground peel through silk screens of the finest obtainable mesh (such as are used by pharmacists), it was possible to prepare a powder that formed a suspension in liquids and under the microscope exhibited only broken cells and no whole parts of the parenchymous tissue of the lemon pericarp.

The powder was first extracted with cold, distilled water, until no trace of free pectin could be detected in the extracts. In order to be sure that no free pectin remained in the intercellular spaces, protected by two adjacent cell walls, the powder was ground in a mortar with a small quantity of water and left with more water for extraction. Pectin could not be detected by alcohol precipitation in the extract. The fine state of division of the material, which produced a uniform suspension in water, made the complete extraction very easy.

The powdered albedo was then gathered on a filter, washed several times with alcohol, allowed to dry, and ground in a mortar to prevent the formation of lumps. It is very important to use alcohol for washing and drying the material, as otherwise protopectin and hemicellulose will retain a little water which will cause the powder to stick together in a single hard mass. After the powder had been thoroughly dried at 100° in a vacuum, it was ground (in a porcelain mortar) with Schweitzer's reagent, and allowed to stand covered with the reagent for two days at several degrees below the freezing point. The Schweitzer's reagent was then filtered through a Büchner funnel, the powder ground again with freshly prepared reagent and again allowed to stand in an excess of reagent for two days under the same conditions. The whole operation was

[8] Mangin, *J. de Bot.*, **1893.**

[9] Harlay, *J. pharm. chim.*, **1912,** 344.

repeated four times, until the Schweitzer's reagent did not exhibit the least trace of cellulose upon addition of sulfuric acid. Further prolonged grinding with this reagent did not dissolve any more cellulose. Several microscopical examinations proved the absence of unbroken cells so that after the fifth grinding with Schweitzer's reagent it was assumed that no more free cellulose was contained in the treated tissue.

The material, now freed from free pectin and cellulose, was washed entirely free from copper by shaking it several times with a 2% solution of acetic acid, allowing it to stand for some time, and finally decanting the liquid. The product was used as pure protopectin.

Hydrolysis.—A preliminary experiment showed that the pure protopectin, containing neither free pectin nor free cellulose, could be decomposed into both of these constituents. When hydrolyzed by a hot 0.5% solution of ammonium oxalate the pectin split off completely and could be precipitated with alcohol from this solution. The remaining material, thoroughly washed with hot distilled water and with alcohol to dry it, dissolved almost completely in Schweitzer's reagent, leaving only traces of lignin and inorganic matter undissolved. Thus, there could be no mistake about its cellulose nature.

An attempt was next made to establish the nature of the linking of pectin and cellulose in protopectin. A preliminary experiment disclosed that not only did "free" pectin differ in methoxyl content from the pectin hydrolyzed out of protopectin but also that, if proper means were applied, fractions of varying methoxyl content could be obtained from protopectin; in these pectin fractions the percentage of methoxyl decreased in the order in which they were split off from the protopectin. Free pectin contains the highest percentage of methoxyl; the first pectin hydrolyzed was next richest, while the last fraction was the poorest. Another outstanding fact was that each time a pectin fraction was extracted from protopectin a certain amount of cellulose was set free and became soluble in Schweitzer's reagent. It seemed, therefore, that protopectin was not a homogeneous substance but contained a series of pectins in which the methoxyl groups were more or less completely replaced by the cellulose radical.

Comparison of Fresh and Dried Peel Pectins.—In order to judge of the importance of the changes in the pectic substances in the drying process, the same series of pectins was prepared from fresh and dried peel simultaneously. The amount of each hydrolyzed pectin was determined and the pectin analyzed. The estimation of cellulose, however, could naturally be carried out on the dried peel only, since it was impossible to dissolve all the free cellulose in Schweitzer's reagent unless the material was in the state of a fine dry powder.

The fresh peel (albedo), dried at 104–105°, lost 76.82% of its weight as the average of four determinations. The loss consisted of water, volatile esters and essential oils. On extracting thrice, on a water-bath under a reflux condenser, with about 1500 cc. of 96% alcohol per 100 g. of fresh peel, followed by extraction with 200 cc. of ether per 100 g. of peel, the dried peel lost 12.53% (2 determinations). It contained 3.32% of ash,

including calcium, ferrous iron, magnesium, potassium and phosphate, chloride and sulfate.

In the quantitative experiments, where simultaneous treatment of both dried and fresh peel was carried out, the weight of the latter was converted into "dry weight" on the basis of the loss of weight at 104–105°, and all the results were calculated on the basis of the dry weight before extraction with alcohol and ether.

Determination of Free Pectin.—The fresh white peel was prepared exactly like the albedo, but not dried.

It was ground in a meat-grinder and two samples of 40 g. each were soaked in 200 cc. of distilled water at laboratory temperature for 2 days. The liquid was then filtered through a paper filter and the remaining pulp soaked in 80 cc. of water each, left for a few hours, and filtered again. A third extraction with cold, distilled water failed to yield an appreciable amount of pectin. The pulp was allowed to drain and was never squeezed. this precaution being necessary to avoid the passage of cell particles into the filtrate from which it was nearly impossible to remove them.

The liquid was precipitated with more than twice its volume of 96.5% ethyl alcohol, containing about 0.1% hydrochloric acid, and allowed to stand for some time. The coagulum formed jelly-like lumps of elastic nature, not easily broken if handled with a glass rod. After a few hours it was poured on a special filtering device and allowed to drain; the filter was made of a dialysator glass over which a piece of smooth, closely-woven linen was stretched. After the coagulum had drained it could be washed into a beaker without loss. The precipitated pectin was transferred into absolute alcohol and left for several hours, then drained and transferred in the same way into ether. The latter hardened the coagulum considerably, allowing it to be easily freed from the liquid contained in its honeycomb structure by squeezing it with a porcelain spatula while it was still on the linen filter. The pectin dried quickly in air, when carefully spread out on the filter and, as it became brittle, was easily removed from the linen and gathered into a dish for final drying. The air-dried pectin was then ground to a uniform white powder. Air-drying was found to be preferable to oven-drying, the latter procedure causing horn-like hard lumps which were difficult to grind.

Determination of Hydrolyzed Pectins.—The second and all of the following pectins, being "combined," had to be hydrolyzed by various methods. The following methods were used in succession: (1) hydrolysis with water under pressure by Verdon's method;[10] (2) hydrolysis with 50% sucrose solution, according to Tschirch's method;[11] (3) hydrolysis with 0.5% ammonium oxalate solution according to the method of Mangin.[8]

Pressure Hydrolysis.—Before the second extraction was made, the pulp left from the cold water extraction was washed twice in alcohol, dried and weighed, in order to determine how much of other substances besides "free pectin" had been extracted in the first treatment. The albedo was then put into 150 cc. of water and held under a steam pressure of 0.5 atmosphere for 30 minutes. The solution was then filtered off, and the pectin precipitated, washed and dried as in the first case.

Hydrolysis by Sugar Solution.—The residue was washed again in alcohol, dried and weighed, in order to establish the loss in addition to "pressure pectin" as a result of the treatment under pressure. The weight of the residue was converted to the dry basis. The albedo was then heated with a 50% sucrose solution on a water-bath, the

[10] Verdon, *Chem. Zentr.*, **1912**, (II), 1726.

[11] Tschirch, *Ber. pharm. Ges.*, **1907**, 237.

water lost by evaporation being constantly replaced. About 25 g. of sugar solution was used per g. of dry albedo, and only after 8 hours of extraction was there an appreciable amount of alcohol precipitate in the extraction liquid. The amount of 75% alcohol necessary to keep in solution the whole amount of sugar contained in the extract was then calculated, and the extract was next precipitated with this amount of alcohol. The coagulum was freed from sugar by washing it several times with 96% alcohol. The wash water from the albedo was concentrated and combined with the sucrose extract. The pectin was dried in the usual way.

Hydrolysis with Ammonium Oxalates.—The residue from the treatment with sugar solution was washed in alcohol, dried, weighed and submitted to the final extraction, in which about 30 cc. of 0.5% ammonium oxalate solution was used for each gram of (dry) albedo. The extraction was made by heating the mixture on a water-bath for 12 hours, until the last trace of pectin was removed. The liquid was then filtered and precipitated with *hot* alcohol in order to prevent crystallization of ammonium oxalate, the precipitation vessel being kept on the water-bath for an hour before the pectin was filtered off. After the residue had been carefully washed with alcohol until no oxalate reaction was obtained in the washings, it was treated as usual.

A portion of the parenchymous tissue of the albedo in its original state (neither dried nor ground) was also subjected to this series of extractions, the effect of each succeeding

TABLE I

PECTINS OF FRESH PEEL

Weight of sample, 40 g.

Wt. free pectin G.	Wt. pressure pectin[a] G.	Free pectin %	Pressure pectin %
0.2890	0.8720	3.05 (dry)	9.21 (dry)
.2880	.8740		

[a] Since no difference was observed in quantity and quality of the pectins recovered in the succeeding processes with fresh and dried peel, the data are omitted; sucrose-pectin was 0.99%; $(COONH_4)_2$ pectin, 9.71%; total pectins, 22.96%.

TABLE II

PECTINS OF DRIED PEEL

	I	II
Original wt. of albedo, g	22.7340	16.1110
Residue fr. cold water extr., g	16.6700	11.8160
Free pectin, g	1.9505	1.3855
Free pectin, %		Av. 7.63 (dry basis)
Total loss to cold water, %		Av. 23.70 (dry basis)
Pulp fr. pres. extr., g	15.1525	10.2425
Pressure pectin, g	0.7130	0.4820
Pressure pectin, %		Av. 3.31 (dry basis)
Total loss on pres. extr., %		Av. 9.50 (dry basis)
Pulp for sugar soln. extr., g	12.2575	8.3200
Pectin, g	0.1995	0.1333
Pectin, %		Av. 0.99 (dry basis)
Total loss to sugar soln., %		Av. 7.72 (dry basis)
Pulp for $(COONH_4)_2$ extr., g	9.2005	6.1500
Pectin, g	1.5909	1.0960
Pectin, %		Av. 9.71 (dry basis)
Total loss to $(COONH_4)_2$ soln., %		Av. 10.15 (dry basis)
Total pectins, %		21.64

reagent being followed by microscopic examination. The intercellular spaces became larger with each successive extraction until finally the oxalate treatment completely disrupted the cells from one another.

Of the pulp remaining after each extraction a certain quantity was always reserved for analysis for cellulose.

The amounts of pectins obtained, and the losses which resulted in the cellular tissue as a result of each extraction, are shown in Tables I and II.

The pectins gave all the following characteristic reactions: the solutions gave precipitates with cupric and lead ions but no precipitate with silver nitrate, zinc sulfate, calcium chloride and mercurous chloride. When hydrolyzed with alkali, they formed pectic acid and calcium, barium, cupric and lead salts. The latter two salts dissolved in an excess of ammonium hydroxide and oxalic acid, respectively. When oxidized with nitric acid the pectins invariably gave mucic acid, under the same conditions as lactose.

Estimation of Methoxyl Content of Pectins.—The pectins were dissolved in water to form about a 0.5% solution, and an appropriate amount of the latter was then weighed (not pipetted) into a fractionating flask connected with a condenser. An equal volume of *N* potassium hydroxide solution was then gradually added from a funnel inserted into the stopper of the flask. The methyl alcohol formed was distilled into a graduated cylinder with a ground stopper and the distillate was then cohobated until the concentration of methyl alcohol was high enough for estimation. The methyl alcohol was determined by the colorimetric method of Denigès,[12] especially adapted by Fellenberg.[13] A Wolff colorimeter was used. In each pectin the content of ash was established before the determination of methoxyl.

The percentages of ash and methyl alcohol in the various pectins are given in Table III.

TABLE III

ASH AND METHYL ALCOHOL IN PECTINS

Fresh peel	Ash %	CH_3OH %	Dried peel	Ash %	CH_3OH %
a Free pectin.....	3.09	11.33	*a* Free pectin.....	4.60	11.00
b Pressure pectin....	3.71	10.20	*b* Pressure pectin....	5.05	10.26
			c Sucrose pectin.....	5.96	8.03
			d $(COONH_4)_2$ pectin.	6.75	2.05

Estimation of Cellulose in Peel and Pulps.—The cellulose soluble in Schweitzer's reagent was estimated in the original albedo and in each of the pulps remaining after the different extractions. The albedo was always dried thoroughly, finely powdered and then extracted several times with the reagent, first by grinding with a few cubic centimeters and then gradually increasing the amount of the reagent; the extraction was considered complete when the last two extractions showed no trace of cellulose.

The reagent was prepared as follows: a solution of pure cupric sulfate containing a small quantity of ammonium chloride was precipitated with sodium hydroxide solution, the precipitate washed several times by decantation, then filtered off and washed on the filter until no sulfate was detected in the washings; finally the precipitate was washed with alcohol, dried quickly, and dissolved in concd. ammonium hydroxide to form a

[12] Denigès, *Compt. rend.*, **150**, 832 (1910).

[13] Fellenberg, *Biochem. Z.*, **85**, 47 (1918).

saturated solution. The liquid had to be cooled to several degrees below zero during this process, in order to give an effective reagent. It was also found necessary to perform extractions at or below 0°, in order to obtain a considerable dissolving power for cellulose.

The determinations summarized in Table IV were carried out during the winter months when the temperature was sometimes as low as —25°, making it an ideal condition for this work. However, the long grinding at low temperature, the very slow filtration through the porcelain filter, etc., made this part of the work very tedious.

After the powdered albedo had been thoroughly ground with the reagent for 20 to 30 minutes, nearly all of the soluble cellulose was removed, the second extraction showing only traces. This ready solubility of the cellulose was due to the dryness and fine state of division of the peel.

The first two filtrates containing cellulose were united and dil. sulfuric acid was added until the mixture was slightly acid. The cellulose precipitate was gathered on filter paper, washed with 5% ammonium hydroxide, then with 1.5% sulfuric acid, and with water. The cellulose was finally transferred to a Gooch crucible, washed with alcohol and ether, and dried to constant weight at 105°. The ammonia dissolved some brown substances, probably hemicelluloses; the sulfuric acid was used for washing out the last traces of copper. It should be stated that this method of estimation does not give an accurate determination of the cellulose content; however, the analyses were carried out in order to obtain comparative figures on the various pulps and were not intended to give absolute values of the cellulose content. It was, therefore, very important to maintain the same conditions throughout, and this precaution was constantly kept in mind.

TABLE IV

SOLUBLE CELLULOSE IN PEEL AND PULPS

Sample	Wt. sample I G.	Wt. sample II G.	Wt. cellulose I G.	Wt. cellulose II G.	Cellulose I %	Cellulose II %	Av. %
Peel fr. alcohol and ether extr.	1.2112[a]	0.9046[b]	0.2059	0.1528	17.00	16.89	16.95
Pulp fr. "free pectin" detn.	1.5195	1.0947	.3105	.2250	20.44	20.50	20.47
Pulp fr. "pressure pectin" detn.	0.7945	0.5015	.2093	.1314	26.34	26.20	26.27
Pulp fr. "sucrose pectin" detn.	1.6205	1.1388	.4888	.3439	30.16	30.20	30.18
Pulp fr. $(COONH_4)_2$ extr.	1.1598	0.8780	.5862	.4502	51.31	51.29	51.30

[a] Extracted successively with 100, 80, 60 and 50 cc. of Schweitzer's reagent.

[b] Extracted successively with 80, 60, 50 and 40 cc. of Schweitzer's reagent.

TABLE V

SUMMARY

Pectin	Pectin %	CH_3OH %	Cellulose %	Increase in cellulose %
Free pectin	7.63	11.33	15.00	..
				1.30
Pressure pectin	3.31	10.20	16.30	
				0.44
Sucrose pectin	0.99	8.03	16.74	
				7.20
$(COONH_4)_2$ pectin	9.71	2.05	23.94	

In Table V are given the percentages of pectin, the corresponding methoxyl content and the corresponding soluble cellulose content, as obtained after each extraction, all calculated as percentage of original dry albedo.

Discussion

The results of the determinations of free and pressure pectin in fresh and dried peel (Tables I and II) indicate that drying increased the amount of free pectin considerably whereas the amount of pectin split off by pressure was decreased in nearly the same proportion. Drying evidently accomplished some of the work that otherwise would have been done by the pressure cooking. Heat is regarded as the responsible factor in this case for the hydrolysis of pectin from protopectin, the pectin thus hydrolyzed becoming soluble in cold water, so that this "free pectin" fraction was considerably higher in dried peel than in the fresh peel. The heat of the sun undoubtedly has a similar effect on the protopectin contained in unripe fruit, gradually producing free, water-soluble pectin and making cellular tissue softer as the fruit ripens.

The losses of other substances, besides pectin, amounted to 16.07% by cold-water extraction, 9.50% by steam-pressure extraction, 7.72% by 50% sucrose-solution extraction, and 0.44% by the 0.5% ammonium oxalate-solution extraction; a total of 33.73%. It is interesting to follow the gradual decrease from 16.07% to 0.44% of the substances which are loosened and dissolved as extraction progresses. It shows how important a part these saccharides, which as yet are little known, play in the cell wall in which they constitute more than one third of the dry weight. The small loss after the last pectin extraction, 0.44%, seems to indicate that after this extraction there would not be much left of these substances, which we may probably class among the hemicelluloses. This view is consistent with the results of chemical and microscopical examinations, according to which there remains, after ammonium oxalate treatment, only the true cellulose wall, in a nearly pure state.

The figures for the methoxyl content of the pectins a, b, c, of fresh and dried peel (Table III) are quite close to the results of Fellenberg,[2] who analyzed pectins from various sources, and called them "octamethoxypectin" if they contained over 11% of methoxyl radical, or "hepta-," "hexa-," "pentamethoxypectins" if their methoxyl content was lower. He did not obtain any pectin containing less than 7.41%. While it is not the aim of the present work to decide the chemical constitution of the various pectins, it seems, as a result of another experiment, that it may be possible to obtain a pectin containing more than 12% of methyl alcohol by careful preparation from white lemon peel, and the avoidance of any application of heat either during the water extraction or during the concen-

tration of the extract. Fellenberg assumes his "octamethoxypectin" to be the highest methoxylated pectin.

In the first part of this work it was found that in dried peel the quantity of free pectin increased while pressure pectin decreased, as compared with fresh peel, and that some pectin was probably decomposed by the heat. The figures on methoxyl content afford further evidence of the effect of drying, dried peel giving a "free pectin" poorer in methoxyl than the corresponding pectin from fresh peel. Two reasons are conceivable for this difference: the first may be decomposition by heat, by which some methoxyl groups may have been split off; the second, proved by the results of the quantitative determinations, is that some of the pectins are loosened from protopectin by the heat during drying, becoming soluble in cold water and then being extracted with the free pectin. The pectins hydrolyzed from protopectin have, however, a much lower methoxyl content than the original "free pectin." If the free pectin of highest methoxyl content is mixed with hydrolyzed pectin of lower methoxyl content, the product must contain less methoxyl than the *pure* free pectin, and this is distinctly shown in Table III.

The process by which heat converts an insoluble cell wall constituent into a soluble one, as proved above, is of great interest in the development of plant tissue. It may explain the vanishing of a parent cell wall when daughter cells are formed inside it. It may also explain how tubes are formed in plant tissue from a string of cells. The transverse cell walls in that line of cells become thinner and thinner because the cell sap dissolves the substances which gradually become soluble in the evolution of the cell-wall constituents. That it is not only pectins which undergo such a change is made evident by the results obtained in the course of the several extractions, where a considerable amount of other substances was removed from the cell wall during the extraction of pectin.

Considering the results of the determination of soluble cellulose (Table IV) it is seen that although the original dried peel gave 16.95%, after the removal of the first ("free") pectin it gave 20.47%. However, if we consider the loss sustained by the peel on extracting the free pectin, namely 26.67%, the first result should be increased by this amount, and then becomes 16.95 + 4.31 or 21.26%. In reality, then, the original peel contained 21.26% of free cellulose and the peel after the first extraction 20.47%; but the amounts should be the same in either case, since the removal of free pectin should produce no soluble cellulose. The difference of 0.79% in favor of the first peel is probably due to some hydrolysis of cellulose resulting from the extraction with water, washing, etc., causing the second peel to become slightly poorer in this substance. This corroborates the assumption that the first pectin extraction leaves the protopectin cellulose substance unhydrolyzed.

Similarly, the increase of 1.30% in soluble cellulose content after the steam-pressure extraction, and the increase of 0.44% after the sucrose solution extraction, indicate hydrolysis of protopectin. The yields of all the cellulose determinations should have been greater, as it has been shown in the first two determinations that the various extractions had a dissolving and hydrolyzing effect on the cellulose itself, probably changing it into some soluble polysaccharides and lowering the content of cellulose by each extraction. Nevertheless, even with this unfavorable circumstance the soluble cellulose is seen to increase with each pectin extraction, and is even roughly proportional to the amount of pectin. The increase of 7.20% after the last extraction is the largest hitherto obtained and is easily explained by the considerable amount of pectin hydrolyzed from protopectin by the last extraction (9.71%).

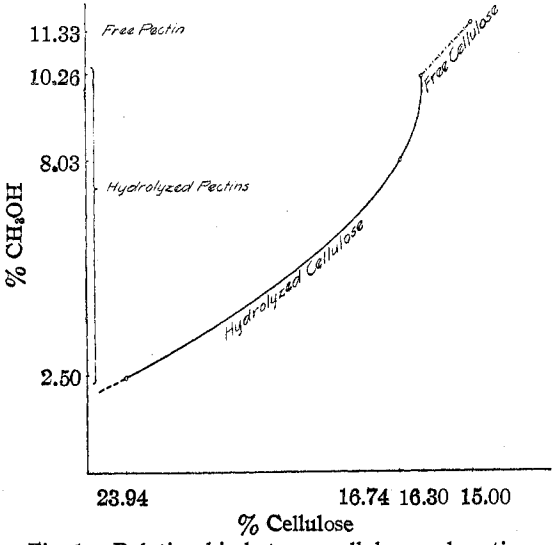

Fig. 1.—Relationship between cellulose and pectins

The relation of "pressure" pectin, "sucrose" pectin and ammonium oxalate pectin to the increase in "free" cellulose, as well as the relation of the latter to the methoxyl content, is shown in Table V, as well as in Figs. 1 and 2. With the decrease in methoxyl content the cellulose yield rises. The explanation for this fact is that the free pectin, being a fully esterified pectic acid with no free carboxyl groups and no acid function, is the highest in methoxyl, while the other pectins must represent only partially esterified pectic acids in which there are carboxyl groups not replaced by methoxyl. These carboxyls, however, certainly do not exist as free groups in the cell wall, the latter being absolutely neutral, with no trace of acidity. It seems very probable, therefore, that the carboxyls form the link between pectin and cellulose, which together form protopectin.

Thus, protopectin may be represented as a compound of pectin and cellulose in which the methoxyl groups are replaced in part by cellulose groups. From the experimental results it can be stated that there is quite a range of protopectins in which the content of cellulose decreases with the increase of pectin and its methoxyl groups. There is most probably a continuous transition between the pure cellulose cell wall and the pure pectin film, the first representing the innermost part of the wall and the second the outer layer. From the cellulose part there is a gradual transition into protopectin rich in cellulose, then the pectin content gradually increases as the outer layer of the cell-wall is approached, finally becoming pure or "free" pectin. Between the two extremes there must be quite a number of compounds, but no homogeneous mixture or system of layers results. Like the colors of the spectrum, there are no sharp boundaries. Similarly, the pectins prepared and discussed above are far from being chemical individuals, but are probably mixtures of differently methoxylated pectins, the determinations of methyl alcohol giving only the average content of several mixtures, for which no method of separation is known. The only pectin which may be claimed to be a chemical individual is "free" pectin.

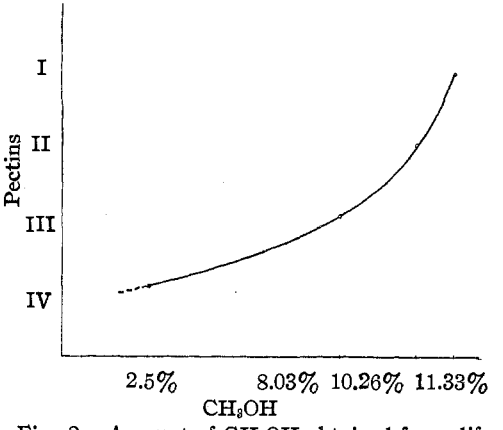

Fig. 2.—Amount of CH_3OH obtained from different pectins. I, Free pectin. II, Pressure pectin. III, Sucrose pectin. IV, Ammonium oxalate pectin

Table VI is a summary of the constituents of albedo.

The remainder of 9.13% is to be ascribed to hydrolysis causing losses of cellulose. The total of "hydrolyzed" cellulose is probably higher than

TABLE VI
CONSTITUENTS OF ALBEDO

	%
Ash	3.32
Soluble in ether and alcohol	12.54
Total pectin, incl. "free" and hydrolyzed	21.64
Total of substances extracted with pectin	29.43
Free cellulose	15.00
Total hydrolyzed cellulose	8.94
Total	90.87
Unaccounted for	9.13
	100.00

8.94%. There is also an insoluble residue consisting of lignocelluloses, the amount of which has not been estimated. As has been stated, the methods applied were not irreproachable, but were chosen for obtaining comparative results.

It is a current view that cellulose does not occur in plant tissue uncombined. Votocek,[14] the famous sugar investigator, found that celluloses separated from the sugar beet tissue contained galactose groups. Considering that the sugar beet tissue contains a very large amount of protopectin, it is very probable that the cellulose separated from the beet might carry some residual groups of protopectin, if it was not treated in such a way as to split off all the pectin, and since protopectin contains the nucleus of pectic acid with its galactan component and the araban and *d*-tetragalacturonic acid molecules,[15] the origin of the galactose groups in such a cellulose could easily be explained. On the other hand, this fact affords a confirmation of the views submitted above, since cellulose has been found combined with a "remainder" of pectin.

This work was carried out during the years 1921 and 1922 in the Institute for Organic Chemistry of the Czech University of Prague.

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Summary

1. Protopectin has been prepared from the white pericarp of *Citrus Limonum*. It gave pectin and cellulose on hydrolysis, besides a small amount of impurities, including lignocelluloses that could not be removed previously.

2. Pectins with different methoxyl content have been prepared from the same source.

3. It has been shown that the cell wall of the parenchymous tissue of *Citrus Limonum* contains "free" cellulose, which dissolves readily in Schweitzer's reagent, and also cellulose combined with pectin, which becomes soluble only after hydrolysis.

4. An hypothesis as to the linkage of the cellulose and pectin has been advanced, based on the replacement of the cellulose in protopectin by methoxyl groups.

5. A picture of the transition of protopectin to pectin in the cell wall has been presented, as well as a discussion of the part played by heat in this process.

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