TABLE I

Compounds	Solvent for recrystn.	Cryst. form	Yield, %	M. p., °C.	Formula	% Nitrogen Calcd.	% Nitrogen Found	Toxicity: *Lactobacillus arabinosus 17-5*	Toxicity: *Streptococcus lactis R*	Toxicity: *Staphylococcus aureus*	Toxicity: *Escherichia coli*
N^4-(*p*-Nitrobenzoyl)-sulfanilamide	Acet. acid.	Fine need.	89[a], 75[b]	263 dec.[c]	$C_{13}H_{11}N_3O_5S$	...	...	†NR[g]	...	...	...
albucid	Pyridine	Prisms	78[d], 100[e]	279–280 dec.	$C_{15}H_{15}N_3O_5S$	11.57	11.53	†NR	...	...	..
sulfapyridine	Pyridine	Prisms	82	272 dec.	$C_{18}H_{14}N_4O_4S$	...	...	††R	O	†NR	†NR
sulfathiazole	Pyridine	Prisms	90	281–282 dec.	$C_{16}H_{12}N_4O_4S_2$	13.86	13.74	††R	††NR	††NR	††NR
sulfadiazine	Pyrid. + alc.	Needles	74	282 dec.	$C_{17}H_{14}N_5O_4S$	17.54	17.51	††R	††NR	††NR	††NR
sulfaguanidine	Pyridine	Prisms	78	266–267	$C_{14}H_{13}N_5O_4S$	19.28	19.14	O	...	...	...
acetylsulfaguanidine	Acetone	Plates	82	238–239 dec.	$C_{16}H_{15}N_5O_5S$	17.28	17.15	...	...	...	...
N^4-(*p*-Aminobenzoyl)-sulfanilamide	Acetone	Prisms	50	276, 313 dec.	$C_{13}H_{13}N_3O_3S$	14.43	14.21	†NR	...	...	...
albucid	Pyridine	Prisms	67	230	$C_{15}H_{17}N_3O_3S$	12.61	12.80	O	...	...	...
sulfapyridine	Acetone	Prisms	78	255–256	$C_{18}H_{16}N_4O_2S$	15.21	15.10	††R	††NR	††NR	††NR
sulfathiazole	.....	....	90	265 dec.	$C_{16}H_{14}N_4O_2S_2$						
sulfadiazine	Acet. + pyrid.	Cubes	49	233 dec.	$C_{17}H_{16}N_5O_2S$	18.97	18.71	††R	††NR	††NR	††NR
sulfaguanidine	Pyridine	Prisms	67	253–254	$C_{14}H_{15}N_5O_2S$	21.01	20.98	O	...	...	...
N^4-(*p*-Acetylaminobenzoyl)-sulfathiazole	Acet. acid	Prisms	..	314 dec.	$C_{18}H_{18}N_4O_4S_2$	13.46	13.30	...	...	...	..

[a] From *p*-nitrobenzoyl chloride and sulfanilamide. [b] From *p*-nitrobenzanilide. [c] Siebenmann and Schnitzer[4] reported a m. p. 260°. [d] From *p*-nitrobenzoyl chloride and albucid. [e] Acetylation of N^4-(*p*-nitrobenzoyl)-sulfanilamide. [f] Mistry and Guha[5] reported a m. p. 293°. [g] † = slightly toxic; †† = toxic; R = reversed and NR = not reversed by *p*-aminobenzoic acid.

addition of 1 γ (gamma) of *p*-aminobenzoic acid per 10 ml. For *Streptococcus lactis R*, *Staphylococcus aureus* and *Escherichia coli* the same medium was modified by addition of 1 γ of *p*-aminobenzoic acid and 1 γ of folic acid concentrate per 10 ml. The medium containing the testing substance (200 γ per 10 ml.) was inoculated with the respective organism, incubated at 30° for twenty-four hours and the turbidity was read as usual.

The properties, analyses and toxicity action of these compounds are collected in the following table.

Summary

N^4-(*p*-Aminobenzoyl)-sulfanilamide and analogs derived from albucid, sulfapyridine, sulfadiazine, sulfathizole and sulfaguanidine have been synthesized by reduction of corresponding nitro compounds. Among reducing agents tried, Raney nickel in alcohol or pyridine is the most satisfactory.

N^4-(*p*-Nitrobenzoyl) derivatives of sulfapyridine, sulfadiazine, sulfathiazole and N^4-(*p*-aminobenzoyl) derivatives of sulfapyridine and sulfadiazine inhibit growth of *Lactobacillus arabinosus 17-5* and the inhibition action is reversed by *p*-aminobenzoic acid. The action of N^4-(*p*-nitrobenzoyl) derivatives of sulfanilamide and of albucid and N^4-(*p*-aminobenzoyl)-sulfanilamide are not reversed by *p*-aminobenzoic acid, while N^4-(*p*-nitrobenzoyl)-sulfaguanidine, N^4-(*p*-am-inobenzoyl)-albucid and N^4-(*p*-aminobenzoyl-sulfaguanidine are indifferent.

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[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY,[1] ALBANY, CALIFORNIA]

The Thermal Degradation of Pectin

BY REYNOLD C. MERRILL[2] AND MARY WEEKS

The most obvious change that occurs when a solution of pectin is heated is the rapid irreversible decrease in viscosity[3] which is denoted in this paper by the term degradation. Kertesz[4] has shown that most of this change in viscosity occurs before appreciable changes in the methoxyl content and reducing power of the pectin solutions are detected. He postulated a structure for pectin in solution described by the formula $[(G)_m]_n$. $(G)_m$ represents a polymer of *m* galacturonic acid units which forms aggregates containing *n* of these units held together by secondary valence forces. These "secondary aggregates," he believes, are mostly responsible for the high viscosity of pectin solutions, and the rapid initial decrease in viscosity on heating is due to the destruction of these aggregates, held together by secondary forces.

One method of testing this theory and of establishing the type of bond broken during the rapid initial decrease in viscosity on heating is to measure the activation energy of the process. The secondary forces holding such an aggregate to-

(1) Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

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(3) See *e. g.*, P. B. Myers and G. L. Baker, *Agr. Expt. Sta. (Del.) Bull.*, **149**, 26 (1927).

(4) Z. I. Kertesz, THIS JOURNAL, **61**, 2544 (1939).

gether would almost certainly be hydrogen bonds. The activation energy for the breaking of a hydrogen bond is not over 9,000 cal./mole and that for the destruction of aggregates held together by van der Waals or other secondary forces is considerably less. On the other hand, activation energies for the breaking of primary chemical valence bonds are from 11,000 to 35,000 cal./mole.

Another method is to measure the intrinsic viscosity as a function of time. Intrinsic viscosity is related to molecular weight and, since it is obtained by extrapolation to infinite dilution, it is probably not influenced by aggregation. Decreases in intrinsic viscosity are assumed to represent decreases in molecular weight, which can occur only by the breaking of primary chemical valence bonds.

This paper presents a study of the viscosity measured at 25.00° of 1.00% solutions of purified apple and citrus pectins and of a commercial citrus pectin, as a function of heating time at 70, 80, 90 and 100°. The changes in intrinsic viscosity of a purified citrus pectin when heated in 1.00% solution at 90° are also given. From these data the activation energy for the process has been calculated.

Experimental

Materials.—The characteristics of the pectin used are summarized in Table I. The ash contents of commercial apple[5] and two lots of commercial citrus pectin[6] were reduced by means of ion exchange resins.[7,8] The deashed citrus pectin A and the apple pectin were identical with those used in recent viscosity studies.[9] Both contained about 10% of material unaccounted for as ester groups and anhydrogalacturonic acid residues. Comparison of the viscosity data[7] with the results of Saverborn[10] suggests molecular weights of the order of 70,000 to 80,000 for the deashed apple pectin and 50,000 to 60,000 for the citrus pectins. Since pectins readily sorb water, all samples were dried at 70° in a vacuum oven overnight and kept in a desiccator. All other chemicals used were C. P. grade.

TABLE I

CHARACTERISTICS OF THE PECTINS

Pectin	Ash, %	Equivalent weight, pectin	Equiv. wt., pectic acid[a]	Methoxyl, %	Natural pH of 1.00% pectin soln. at 25°[b]	η_r of 1.00% solns. at 25.00°
Deashed citrus A	0.2	720	198	10.7	2.69	19.5
Deashed citrus B	0.17	686	206	10.5	2.71	18.1
Deashed apple	0.2	830	203	10.9	2.77	64.3
Commercial citrus[c]	0.77	1280	240	10.7	3.65	19.1

[a] That is, of completely saponified pectin. [b] By "natural pH" is meant the unadjusted pH of the solution. [c] The commercial citrus pectin contained 1.38% nitrogen, probably as ammonium ion, since it was completely removed by passage through an ion exchange column. Correction for the alkalinity of the ash (less than 2%) and for the partial neutralization of the carboxyl groups gave an equivalent weight of 560.

Technique.—Dry pectin was sprinkled on water, which was thoroughly stirred by mechanical means. The solutions were then heated to desired temperatures and immersed in an oil-bath; for the 100° runs solutions were boiled gently under a reflux condenser. Bath temperatures, read with a thermometer calibrated by the National Bureau of Standards, were accurate to ±0.1° and constant to ±0.03°. One per cent. pectin solutions boiled at 100.0 ± 0.4°.

The solutions were heated so rapidly that reaction during heating could have been neglected; however, zero time was arbitrarily selected at mid-point in the time necessary to heat the solution. For short reaction periods, it was necessary, in order to arrive at the desired temperature rapidly, to dilute concentrated pectin solutions with boiling water and then pass the solution through a steam-jacketed heating coil into the flask in the thermostat. At the end of the reaction time, each solution was rapidly cooled in an ice-bath in order to reduce the extent of reaction during cooling.

Viscosities were measured on 1% solutions at 25.00° with Ostwald-Cannon-Fenske viscosimeters. No difficulty was experienced in obtaining reproducible results after slight degradation. No incipient gelation or precipitation was observed except for a slight precipitate of pectic acid after two to eight hours of boiling at pH 1.30. Longer boiling degrades the molecule sufficiently to make it soluble at room temperature. Relative viscosity, η_r, is expressed as the ratio of the viscosity of the pectin solution to that of the solvent (salt or buffer solution or water). The weight intrinsic viscosity values, $[\eta]$, were obtained by extrapolation of the linear (below 0.1% pectin) η_{sp}/C–*vs.*-concentration curves in 0.9% sodium chloride to minimize electroviscous effects.[7] Solid salt was added after the heating period.

Beckman glass electrode apparatus was used to obtain pH values.

Results

The changes in relative viscosity measured at 25.00° of a 1.00% solution of commercial citrus pectin as a function of the time of heating at 70, 80, 90 and 100° are shown in Fig. 1. Similar data were also obtained for the other three pectin samples of Table I and for commercial citrus pectin with hydrochloric acid or sodium hydroxide added to give an initial pH of 2.02 and 4.11, respectively. In each of these cases the relative viscosity changed with time of heating at a given temperature in a manner very similar to that shown in Fig. 1.

The pH and relative viscosity at 25.00° of 1.00% solutions, and the weight intrinsic viscosity and titratable acidity of deashed citrus B as a function of time at 90° are given in Table II. Only small changes in the optical rotation or reducing power of the pectin solution were detected in the time shown.

The rapid decrease in viscosity during heating at the natural pH was also accompanied by a slow decrease in the pH of the pectin solutions and a parallel increase in the titratable acidity (Table II). Methoxyl determinations by both saponification and Zeisel methods showed that these changes were due to concomitant demethylation. However, for deashed pectin A at 100°, demethyl-

(5) Certo No. 1, General Foods, Inc., Hoboken, N. J.

(6) Pectinum NF VII, California Fruit Growers Exchange, Ontario, Calif.

(7) H. S. Owens, H. Lotzkar, R. C. Merrill and M. Peterson, THIS JOURNAL, 66, 1178 (1944).

(8) Amberlite IR-4 and IR-100, Resinous Products and Chemicals Co., Inc., Philadelphia, Pa.

(9) We express our thanks to H. Lotzkar of this Laboratory for samples of these two pectins.

(10) S. Saverborn, *Kolloid.-Z.*, 90, 41 (1940).

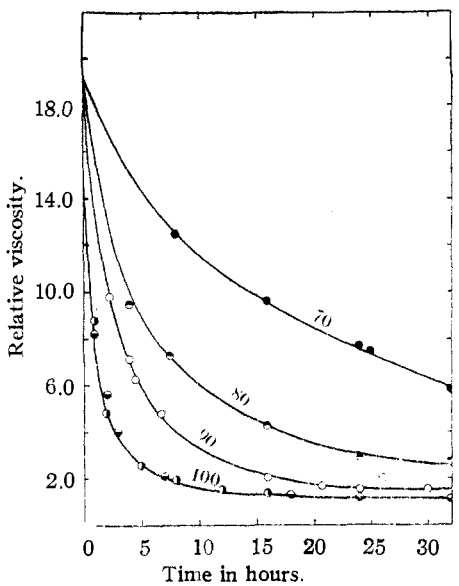

Fig. 1.—Relative viscosity at 25° of 1% commercial citrus pectin as a function of heating time at different temperatures: initial *p*H 3.65; ◒, 100°; ○, 90°; ◓, 80°; ●, 70°; ◑, 100°, heated 2% solution, then diluted to 1%.

ation was found to be only 7% complete when the change in viscosity was 96% complete. The

TABLE II

CHANGES IN DEASHED CITRUS PECTIN B AS A FUNCTION OF TIME AT 90°

Time, hr.	*p*H[a]	Titratable acidity[b]	η_r[a]	[η]
0	2.71	1.47	18.1	3.43
2	..	..	7.49	2.10
4	..	..	4.50	1.49
8	2.67	1.67	3.19	0.73
16	2.62	1.92	1.92	0.38
24	2.57	2.11	1.48	0.15

[a] Measurements made on 1.00% solutions at 25.00°. [b] Milliequivalents of sodium hydroxide per gram of pectin to bring the 1.00% solution to *p*H 7.5.

corresponding figures for 90 and 80° are 4 and 2% demethylation for 84 and 61% change in viscosity.[11] Likewise only a small increase in iodine number occurred during the time required for a large change in viscosity[12] in agreement with the prior results of Kertesz.[4] It is therefore evident that large changes in viscosity occur before much change in methyl ester content or reducing power.[4,13,14]

(11) R. C. Merrill and Mary Weeks, unpublished data.

(12) Most of the iodine number determinations were made by S. W. Waisbrot, formerly of this Laboratory.

(13) C. L. Hinton, "Fruit Pectins. Their Chemical Behavior and Jellying Properties," Special Report No. 48 of the Department of Scientific and Industrial Research, His Majesty's Stationery Office, London, 1939, p. 47.

(14) E. F. Jansen and L. R. MacDonnell, *Arch. Biochem.*, **8**, 97 (1945); H. S. Owens, H. Lotzkar, S. W. Waisbrot and W. D. Maclay, unpublished work.

Discussion

Accurate values for the order and velocity constants of the degradation reaction cannot be calculated from our data, since the exact relationship to molecular weight of neither the relative viscosity of 1.00% pectin solutions nor the intrinsic viscosity at different degrees of degradation is known. However, activation energies can be calculated from our viscosity data, on the assumption that degradation is a first-order process. One of the tests for a first-order process is that the fraction of the process completed at a given time is independent of the concentration. If the degradation of pectin is a first-order process, the viscosity of a given concentration of pectin after different periods of heating will be independent of the concentration during the time of heating. Figure 1 shows that the viscosity of a 1.00% pectin solution after different times of heating was the same, whether it was heated as a 1.00 or a 2.00% solution. These results indicate that the degradation of pectin can be regarded as a first-order process, especially since viscosity in this range is very sensitive to extent of completion of the reaction. This interpretation is in agreement with the statement of Schulz[15] that one can generally assume that the oxidative or hydrolytic degradation of macromolecules follows the equation for a first-order process.

Activation energies for the degradation of pectin were calculated from the following equation, valid for a first-order process

$$E = \frac{RT_2T_1}{T_2 - T_1} \ln \frac{t_1}{t_2}$$

where E is the activation energy, R the gas constant, t_1 the time necessary for the viscosity of the solution measured at 25° to decrease to a specified viscosity at temperature T_1, and t_2 the time necessary for the viscosity to decrease to this same value at the higher temperature T_2.

Calculation of activation energies by this method also involves the reasonable assumption that solutions of the same pectin degraded at two different temperatures to the same viscosity have about the same molecular weight distributions. This is equivalent to the assumption that the activation energies of the bonds split during degradation are closely similar. Evidence that this assumption is reasonable may be obtained from researches on cellulose, which show that the viscosity method gives results for the activation energy which agree with those obtained by the hypoiodite titration and polarimetric methods.[16,17]

Activation energies for the degradation of pectin calculated from the above equation at relative viscosities of 6.0 and 3.0 are listed in Table III.

(15) G. V. Schulz, *Z. physik. Chem.*, **51B**, 127 (1942).

(16) (a) G. V. Schulz and H. J. Lohmann, *J. prakt. Chem.*, **157**, 238 (1941); (b) A. af Ekenstam, *Ber.*, **69B**, 553 (1936); (c) K. Freudenberg and G. Blomquist, *ibid.*, **68B**, 2070 (1935).

(17) I. Sakurada and S. Okamura, *Z. physik. Chem.*, **187A**, 289 (1940).

TABLE III

ACTIVATION ENERGIES FOR THE DEGRADATION OF PECTIN

		Time to reach specified viscosity hrs.			Apparent activation energy (cal./mole)[a]	
	η_r	80°	90°	100°	80–90°	90–100°
Deashed citrus A	6.0	8.0	3.0	1.1	24,300	27,000
Deashed citrus A	3.0	24.0	8.0	2.5	28,000	31,300
Deashed citrus B	6.0	..	2.9	1.1	....	26,100
Deashed citrus B	3.0	..	8.4	2.6	....	31,400
Deashed apple	6.0	13.9	4.7	1.3	27,600	34,600
Deashed apple	3.0	27.1	9.7	3.1	26,100	30,700
Commercial citrus	6.0	10.1	4.9	1.4	18,700	33,700
Commercial citrus	3.0	26.6	11.0	4.1	22,400	26,600
Commercial citrus	6.0	5.5	2.2	0.8	23,300	27,200
pectin + HCl, *p*H 2.02	3.0	13.5	5.2	1.6	24,300	31,700
Commercial citrus pectin + NaOH, *p*H 4.11	6.0	..	5.9	2.0	....	29,100

[a] Data for the deashed pectins at 70–80° indicate an apparent activation energy of approximately the same order of magnitude. Viscosities were not measured over sufficiently long periods of time at 70° to permit more accurate calculations.

It is seen that similar values were obtained at both these viscosities for all the various pectin samples, although the values calculated for the 90 to 100° temperature interval are systematically somewhat higher than those for the 80 to 90° interval. The data of Sauer and Sanzenbacher[18] on the decrease in viscosity of pectin solutions at 90 and 98° also give an activation energy of approximately 30,000 cal./mole when calculated by the above equation. We conclude that an activation energy for the degradation of pectin of 28,000 cal./mole is probably correct as to order of magnitude, although this figure may be in error by as much as 6,000 cal./mole.

The magnitude of the activation energy for the degradation of pectin (28,000 cal./mole) indicates that the degradation process involves the breaking of primary chemical valence bonds. This conclusion is also supported by the fact that the large increase in relative viscosity is accompanied by a marked lowering of the molecular weight as indicated by the large decrease in intrinsic viscosity (Table II). The observations[7] that the relative viscosities of dilute pectin solutions are not appreciably altered either by the presence of urea (a hydrogen bond-splitting agent) or by an increase in temperature to 50° also indicate that thermal degradation of pectin solutions is not primarily a destruction of aggregates held together by secondary forces, such as hydrogen bonds or van der Waals forces. Furthermore, the rapid, initial decrease in viscosity of pectin solutions on heating is irreversible, whereas it should be reversible if the only process which occurs is the dissociation of secondary aggregates.

The activation energies in Table III are comparable to the values of 30,500 and 29,500 cal./mole obtained by iodometric titration for the hydrolysis in *N* hydrochloric acid at 85, 95 and 100° of a comparatively low-molecular-weight polygalacturonide[19] and for α-methyl-*d*-galacturonide.[20] The values of 27,300, 29,800, 29,000 and 30,970 for the acid-catalyzed hydrolysis of the glycosidic linkages in cellobiose,[16d] cellulose,[16d] amylose[21] and maltose[22] are also comparable. Comparison of these values with those listed in Table III suggests that the main change involved in degradation is probably the splitting of the glycosidic linkages between the galacturonic acid units. Failure to detect an appreciable change in the reducing power may be due to the insensitiveness of these methods for measuring number average molecular weights in the high polymer range. Sakurada and Okamura[17] have calculated that the entirely random cleavage of about 2.8 glycosidic bonds in a dissolved linear macromolecule will reduce the average chain length and hence the Staudinger viscosity of the solution by one-half. For a polymer of 280 monomeric units, a reasonable value for pectins,[23] this corresponds to only about 1% of the total possible change in reducing power.

Summary

The large initial decrease in viscosity of pectin solutions on heating is due mainly to the breaking of primary chemical valence bonds rather than to the destruction of a secondary aggregate. This is shown by the irreversibility of degradation, the absence of a pronounced effect of urea and temperature (up to 50%) on the viscosity of dilute pectin solutions, the fact that the loss in relative viscosity is closely associated with a decrease in the intrinsic viscosity of the pectin, and the magnitude of the value for the activation energy of the loss in viscosity with heating time (28,000 ± 6,000 cal./mole).

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(18) E. Sauer and K. Sanzenbacher, *Kolloid-Z.*, **79**, 55 (1937).

(19) S. Morell, L. Baur and K. P. Link, *J. Biol. Chem.*, **105**, 1 (1934).

(20) S. Morell and K. P. Link, *ibid.*, **104**, 183 (1934).

(21) K. H. Meyer, H. Hopff and H. Mark, *Ber.*, **62B**, 1103 (1929).

(22) E. A. Moelwynn-Hughes, *Trans. Faraday Soc.*, **25**, 81 (1929).

(23) H. S. Owens and E. F. Jansen; private communications, based on unpublished work at this Laboratory.

(24) Original manuscript received February 3, 1945.