

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Pectic (Poly-D-galacturonic) Hydrazide¹

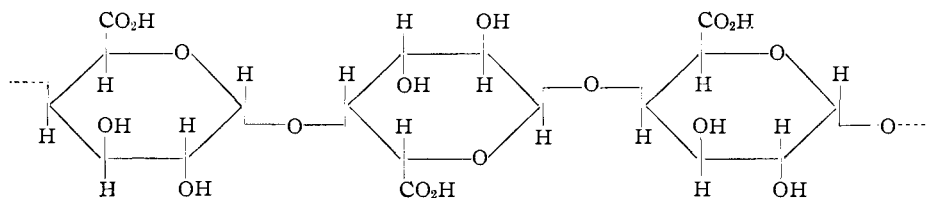
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The hydrazides of pectic (poly-D-galacturonic) and of methyl α -D-galactopyranosiduronic acids and their perchlorate salts are described.

Functional group transformation constitutes an interesting facet of carbohydrate chemistry. Of especial interest are those polymers which contain available carboxyl groups. For certain investigative purposes, the hydrazide perchlorate of a polysaccharide was desired. The polysaccharide selected was citrus pectic acid which is considered to be a polymer of D-galactopyranuronic acid units linked 1 \rightarrow 4 (I). This was converted to its degraded methyl ester according to the procedure of Link and co-workers.² On reaction with an excess of hydrazine, this polyester yielded pectic hydrazide. This polymeric hydrazide was obtained in several fractions of like analysis and closely agreeing optical rotation but with widely differing solubilities. It is probable that these represented some crude fractionation into groups of differing degrees of polymerization. The products formed water-soluble perchlorate salts of good stability. As a model compound, the crystalline hydrazide of methyl α -D-galactopyranosiduronic acid was synthesized in like manner and its perchlorate salt was prepared from it. The neutral point for this salt was found to be at pH 1.95 and that for the polymeric hydrazide salt at pH 1.84 (Figs. 1-2). The potentiometric titer curve for the polymer (Fig. 2) exhibited an inflection point appearing prior to the main plateau. This inflection point was probably due to salt formation with the small amount of free carboxyl present.

After the completion of this work, it was noted that Micheel and Millé³ had prepared the hydrazide of alginic acid, a β -D-(1 \rightarrow 4)-linked mannopyranuronic acid polymer, and Solms and Deuel⁴ had reported the conversion of pectic acid to its polymeric amide.



I, Pectic acid (probable structure); see also G. H. Joseph, A. H. Kieser and E. F. Bryant, *Food Technol.*, **3**, 85 (1949).

Experimental

Pectic Acid.—A commercial preparation of citrus pectic acid⁵ was purified by the procedure of Morell and Link.⁶

(1) This work was carried out under contract (DA-33-019-ord-727; supervising agency, Ballistic Research Laboratories, Aberdeen Proving Ground, Maryland) between the Ordnance Corps and The Ohio State University Research Foundation (Project 496).

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

(3) F. Micheel and E. Millé, *J. physiol. Chem.*, **293**, 121 (1953).

(4) J. Solms and H. Deuel, *J. Polymer Sci.*, **12**, 559 (1954);

(5) A product of the California Fruit Growers Exchange, Ontario, Calif.

(6) S. Morell and K. P. Link, *J. Biol. Chem.*, **100**, 388 (1933).

The product contained 0.80 $-\text{CO}_2\text{H}$ group per anhydro-D-galacturonic acid unit (by acidimetry).

Methyl Pectate.—An amount of 50.0 g. of the above purified pectic acid was esterified with methanolic hydrochloric acid by the method of Morell, Bauer and Link.² The yield of ester was 21.5 g., $[\alpha]^{25}_D +216^\circ$ (c 1.0, water). Morell, Baur and Link² report $+198^\circ$ for methyl pectate.

Anal. Found: C, 44.06; H, 5.36; $-\text{CO}_2\text{H}$, 2.60 ml. of 0.01 N NaOH per 100 mg.; $-\text{CO}_2\text{CH}_3$, 46.40 ml. This corresponds to 0.95 $-\text{CO}_2\text{CH}_3$ and 0.05 $-\text{CO}_2\text{H}$ groups per anhydro-D-galacturonic acid unit.

Pectic Hydrazide.—An amount of 7.0 g. of the above methyl pectate dissolved in 42 ml. of water was allowed to react with 7 ml. of 64% hydrazine for 112 hr. at 30° . The reactants were stirred mechanically; intense turbidity developed early in the reaction period. The reaction mixture was centrifuged and the precipitate (fraction I) was washed with two 3.5-ml. portions of water. The combined supernatants were cooled to $0-2^\circ$ and a second precipitate (fraction II) was obtained. The supernatant (44 ml.) was cooled to $0-2^\circ$ and diluted with 22 ml. of cold 95% ethanol. A third precipitate (fraction III) formed; the addition of 44 ml. of cold 95% ethanol to the cooled supernatant produced the final precipitate (fraction IV). These precipitates were washed with absolute ethanol until the filtrates were neutral to litmus, followed by a final wash with 15 ml. of ether; they were dried under reduced pressure over phosphoric anhydride at 30° ; yields 4.2 g., 0.8 g., 1.0 g., 0.2 g., respectively, for fractions I to IV; $[\alpha]^{25}_D +208^\circ$, $+189^\circ$, $+166^\circ$ (c 0.4, water) for fractions II, III and IV, respectively. Fraction I was water-insoluble but was soluble in dilute acid; $[\alpha]^{25}_D +206^\circ$ (c 1.0, 0.5 N HCl). Infrared absorption spectra⁷ of pectic hydrazide in Nujol mull showed bands at 6.5 (N-H), 3.1 (N-H and O-H) and 6.1 (C=O) microns.

Anal. Calcd. for 0.89 $-\text{CO}-\text{NH}-\text{NH}_2 \cdot \text{H}_2\text{O}$, 0.05 $[-\text{CO}_2]^-$ $[\text{NH}_2-\text{NH}_3]^+ \cdot \text{H}_2\text{O}$ and 0.06 $-\text{CO}_2\text{CH}_3$ group per polymerizing unit: C, 35.03; H, 5.77; N, 12.57. Found for fractions I and III, respectively: C, 34.70, 35.10; H, 5.99, 6.36; N, 12.38, 12.60.

The titration of fraction I with perchloric acid is shown in Figs. 1-2.

Pectic Hydrazide Perchlorate.—An amount of 75 mg. of each of the above pectic hydrazide fractions was allowed

to react with 1 ml. of 60% perchloric acid for 16 hr. at 12° . The reaction mixtures were each diluted with 10 ml. of glacial acetic acid, stirred thoroughly and centrifuged. The supernatant was removed and the decantate was washed twice with acetic acid, 25 times with ether and dried at 25° under reduced pressure and over phosphoric anhydride; yield 97-109 mg.; $[\alpha]^{25}_D +143^\circ$, $+139^\circ$, $+141^\circ$, $+129^\circ$ (c 1.0, water) for fractions I to IV, respectively. The pectic hydrazide perchlorates displayed infrared absorption bands⁷ at 6.5 (weak, N-H), 3.1 (N-H and O-H), 5.8 (C=O, shifted from 6.1) and 8.5-10.5 (ClO_4^-)⁸ microns. The

(7) Measured on a Baird Model B recording spectrophotometer by Messrs. Robert Leiberman and Albert Antoine of this Laboratory.

(8) F. A. Miller and C. H. Wilkins, *Anal. Chem.*, **24**, 1253 (1952).

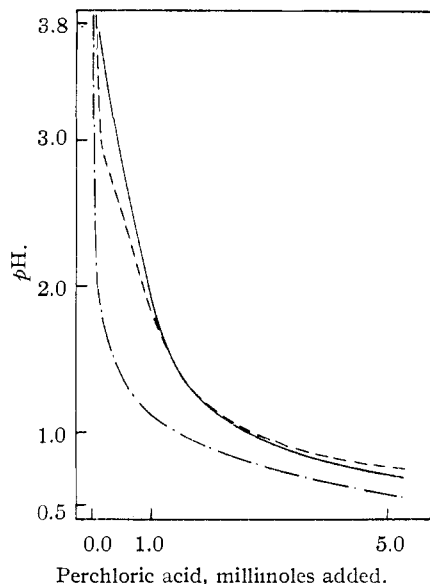


Fig. 1.—Electrometric titration at 23–25° with 0.5 *N* perchloric acid of (methyl α -D-galactopyranosid)-uronic hydrazide (—) and pectic hydrazide (— — —) in 0.1 *M* aqueous solution 0.2 *M* in sodium chloride, and of the solution medium alone (— · —); Beckman pH meter (model G).

products were cream colored solids; their aqueous solutions were acidic. On sudden heating, the solid produced a shooting flame, a whistling sound, and a small carbonaceous residue.

Anal. Calcd. for 0.89 $-\text{CONHNH}_2 + \text{ClO}_4^-$, 0.05 $-\text{CO}_2\text{H}$ and 0.06 $-\text{CO}_2\text{CH}_3$ group per polymerizing unit: Cl, 11.32; $-\text{CO}_2\text{H}$, 3.38 ml. of 0.01 *N* NaOH per 100 mg. Found: Cl (fraction I), 11.09; $-\text{CO}_2\text{H}$, 3.34, 3.28, 3.38, 3.44 ml., for fractions I to IV, respectively.

(Methyl α -D-Galactopyranosid)-uronic Hydrazide.—The methyl (methyl α -D-galactopyranosid)-uronate used in the preparation of this hydrazide was obtained by the procedure outlined by Jones and Stacey.⁹ An amount of 4.45 g. (0.02 mole) of this ester in 10 ml. of methanol was added slowly under stirring to a solution of 3.2 g. (0.10 mole) of hydrazine¹⁰ in 10 ml. of methanol. Within an hour the reaction mixture had become a mass of crystals. It was maintained at 28° for 20 hr. and was cooled then to 4°. The crystals were collected on a filter and were washed with a cold methanol-

(9) J. K. N. Jones and M. Stacey, *J. Chem. Soc.*, 1340 (1947).

(10) Minimum assay, 95% hydrazine; a product of the Mathieson Chemical Corp., Baltimore 3, Md.

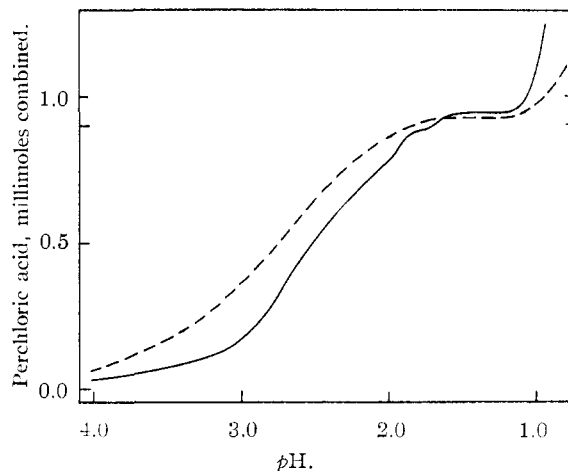


Fig. 2.—Perchloric acid combined at 23–25° with (methyl α -D-galactopyranosid)-uronic hydrazide (— — —) and pectic hydrazide (—) as calculated from the data in Fig. 1 according to E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, p. 455.

ethyl ether solvent mixture; yield 4.08 g., m.p. 184–186°. These crystals were recrystallized twice from methanol, m.p. 185.5–186°, $[\alpha]^{23}_D +104^\circ$ (*c* 1.3, H₂O). X-Ray powder diffraction data: 2.07¹¹ d,¹² 3.05 d, 3.59 mb, 3.82 d, 4.29 mb, 4.46 mb, 4.75 vb, 5.55 mb, 6.22 mb. The hydrazide had a sweet taste followed by a bitter one.

Anal. Calcd. for C₇H₁₄O₆N₂: C, 37.84; H, 6.35; N, 12.61. Found: C, 37.93; H, 6.44; N, 12.57.

The titration of this substance with perchloric acid is shown in Figs. 1–2. The perchlorate salt from an exactly neutralized aqueous solution was isolated as a white amorphous solid on lyophilization. On sudden heating, the salt puffed mildly and left a carbonaceous residue.

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(11) Interplanar spacing, Å.; CuK α radiation.

(12) Relative intensity, estimated visually; b = bright, d = dim, m = moderately, v = very.