CCXLVIII. THE PREPARATION OF METHYL ESTERS OF PECTIC ACID.

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It has been generally accepted that the naturally occurring water-soluble pectins are methyl esters of pectic acid, this latter substance, which is insoluble in water, being regarded as containing 4 galacturonic anhydride units, whose carboxyl groups are intact and are involved in the formation of the methyl esters. This conception has been based on the fact that the soluble pectins, on treatment with very dilute alkali in the cold, are rapidly converted into free pectic acid, with liberation of methyl alcohol. Further, the methoxyl-content of samples of soluble pectin prepared from fruits, *etc.* frequently corresponds closely with that required for a tri- or tetra-methyl ester of pectic acid [*cf.* Norris, 1926; Norman, 1928]. So far as can be ascertained, however, no attempt has been made to confirm this view by converting pectic acid into its methyl esters. The accomplishment of this reaction is necessary before the relation between pectin and pectic acid can be regarded as firmly established, for observations have been reported from time to time which suggest that the relationship may not be so simple. For instance, Tutin [1921; 1923] reported the liberation of acetone during treatment of pectin by weak alkali, while Ehrlich and Schubert [1926] consider that acetyl groups are also present.

Experiments have accordingly been made in an attempt to prepare the methyl esters of pectic acid; soluble methyl esters have been obtained and have been found to resemble the natural soluble pectins in all respects. There seems therefore no reason to suggest that any reaction other than hydrolysis of the methyl ester is involved in the formation of pectic acid from pectin.

Similar attempts to prepare other (*e.g.* ethyl) esters of pectic acid were, however, entirely unsuccessful. It appears, in fact, that the methyl derivatives are possibly the only stable esters of pectic acid, and that, if other esters are formed, they undergo ready decomposition, not only with loss of the ester group, but actually with scission of the basal pectic molecule.

EXPERIMENTAL.

Preparation of the methyl esters.

Since the object of the experiments was to methylate only the free carboxyl groups of the pectic acid, and not the hydroxyl groups present in the uronic anhydride residues, it was obviously impossible to adopt any of the methods used in the exhaustive methylation of sugars. The following methods suggested themselves:

- (a) treatment of calcium pectate with methyl oxalate;
- (b) treatment of silver pectate with methyl iodide;
- (c) treatment of sodium pectate with methyl sulphate.

For these experiments, calcium pectate was prepared by precipitating a solution of pectin, after treatment for 4 hours with dilute sodium hydroxide, with calcium chloride solution in the presence of acetic acid. Sodium pectate was prepared by the method of Candlin and Schryver [1928] from the same sample of commercial pectin used by these workers. Silver pectate was prepared by addition of aqueous silver nitrate to a 1 % solution of sodium pectate; the silver compound was precipitated as a clear gel, which was thoroughly washed with cold water, and then with graded strengths of alcohol, and finally dried *in vacuo*. The washing and drying were carried out in weak artificial light, since the compound was found to darken rapidly in daylight. The dry silver pectate was obtained as a cream-coloured powder; the Ag content of different samples varied from 30.3 to 32.1 %, the highest figure being obtained in the case of a sample in the preparation of which a large excess of silver nitrate had been used. Calculated on the basis of the Nanji, Paton and Ling [1925] formula for pectic acid, the Ag content of silver pectate should be 30.3 %. The purity of the samples of calcium and sodium pectate was also checked by ash determinations.

Method (a). 1 g. of powdered calcium pectate, suspended in 25 cc. dry methyl alcohol, was heated with 5 g. methyl oxalate for 3 hours in a pressure bottle immersed in boiling water. At the end of this time, the mixture was added to 200 cc. hot water, filtered and the filtrate poured into 500 cc. alcohol. A small gelatinous precipitate was obtained, which, after washing with dilute alcohol and drying, weighed 0.09 g. Its methoxyl content, determined by micro-Zeisel, was 3.4 %. It seemed therefore that some esterification had taken place, but the poor yield and low methoxyl content of the product led to the abandonment of the method.

Method (b). A number of attempts were made to prepare the methyl esters by the action of methyl iodide on silver pectate under different conditions; the results are summarised in Table I. Usually, 1–2 g. of dry silver pectate were heated with 10–15 g. methyl iodide in 25 cc. methyl alcohol; at the end of the period of heating, the mixture was added to about 300 cc. water, boiled for a short time, the residue of silver iodide and unchanged silver pectate filtered or centrifuged off and the filtrate treated with excess of alcohol to

precipitate the soluble pectins formed. Considerable difficulty was experienced in removing colloidal silver iodide, the presence of which rendered filtration extremely slow and resulted in contamination of the alcohol precipitate with as much as 25 % of inorganic matter. The most satisfactory method of removing this colloidal material was by adding a few cc. of 5 % potassium bromide and allowing the liquid to stand on the steam-bath for an hour. The silver iodide was thus coagulated; from the clear filtrate, the pectic substance was precipitated as a transparent gel which, when dry, contained only about 3 % ash in most cases.

Table I.

Exp. No.	Treatment of silver pectate	Yield of sol. pectin (ash-free) per g. silver pectate g.	Ash in soluble pectin %	Methoxyl content of sol. pectin (corr. ash) %
1	Boiled with CH_3I under reflux, 8 hours	0.1	—	<3
2	Heated with CH_3I at 96° in pressure bottle, 1 hour	0.35	13*	4.2
3	Heated as above, $4\frac{1}{2}$ hours	0.30	3.5	7.28
4a	Heated as above, 12 hours	—	2.3	10.50
4b	„	0.42	6.0	8.60
5	Heated in sealed tube at 100° , 12 hours	—	2.3	5.95
6	Heated as above, 48 hours	—	—	6.0

* Silver iodide incompletely coagulated; boiled with $N/75$ HCl in place of potassium bromide.

From the results recorded above, it appeared that the greatest degree of esterification was produced by heating silver pectate with methyl iodide and methyl alcohol under pressure at 96° for several hours; heating at slightly higher temperatures, or for prolonged periods, resulted in much charring. The products obtained resembled in appearance and solubility the natural soluble pectins; further tests of their identity are described below.

Since a rather wide variation in the methoxyl content of the various products was met with, it was of interest to ascertain whether this was due to the fact that only partial esterification of the carboxyl groups had taken place, or whether substances other than pectins had been formed and carried down in the alcohol precipitate along with the pectins. Accordingly the calcium pectate values for certain of the products were determined; from these a calculation was made of the true methoxyl content of the actual pectic substance present [cf. Norman, 1928]. The results (Table II) suggested that even when conditions were apparently most satisfactory there was some production of non-pectin degradation products, possibly hemicellulose-like bodies. As will be seen later, these may in some cases comprise the major part of the products. It may be pointed out that in the case of sample 2, the apparent 25 % of non-pectic material includes the 13 % ash present in the sample; hence the actual amount of organic contaminant is only about 10 %.

Table II.

Sample No.	Methoxyl-content %	Ca pectate value %	MeO, corr. by Ca pectate %
2	3.65	75	4.85
4a	10.26	91	11.51
4b	8.09	81	9.98
5	5.82	68	8.55

In column 2, the methoxyl content of the sample, uncorrected for ash, is recorded, since the correction applied on the basis of the calcium pectate value will cover this.

Since this sample was prepared by heating only for 1 hour, the low methoxyl value (4.85 %) indicated incomplete esterification, and not extensive decomposition. In the case of sample 5, the prolonged heating of the reaction mixture led to the formation of large amounts of degradation products, reflected in the low calcium pectate value.

With regard to the yield of soluble pectin, in Exp. 4b the amount of true pectic substance obtained from 1 g. silver pectate was 0.35 g.; since the theoretical yield of tetramethyl ester per g. of silver pectate is 0.74 g., this represented a yield of approximately 47 %. The solid residue in the pressure bottle was found to contain 0.18 g. unchanged silver pectate.

Method (c). A number of attempts to convert sodium pectate into soluble methyl esters by the action of dry methyl sulphate proved entirely unsuccessful. When the substances were heated together at 60–70°, with precautions to exclude moisture, slight darkening of the sodium pectate took place, but on examining the residue (after washing away the methyl sulphate with alcohol), this was found to consist of unchanged sodium pectate. In the presence of moisture, pectic acid was formed from the sodium salt, and this was further attacked by the methyl sulphate (or by sulphuric acid produced by partial hydrolysis thereof) with liberation of carbon dioxide and complete breakdown of the pectin molecule. In no case could any substance resembling pectin be isolated from the mixtures.

Properties of the methyl esters obtained.

As stated above, the appearance and solubilities of the products obtained by the silver pectate-methyl iodide reaction resembled those of the natural soluble pectins. Treatment with lime water produced an immediate precipitate of a typical calcium pectate gel. In the presence of *N*/10 sodium hydroxide, methyl alcohol was rapidly liberated, de-esterification being complete within 15 minutes—a rate comparable with that observed in the case of natural soluble pectin. The soluble pectins were strongly dextrorotatory.

A marked characteristic of the natural soluble pectins of high methoxyl content is their ability to form jellies with sucrose in the presence of traces of acid; among the pectic substances, soluble pectin alone possesses this property [Branfoot, 1929, p. 128]. On this account, sucrose-acid jellies were made from the synthetic methyl esters, and their strengths compared with those

of similar jellies prepared from natural pectin. Jelly strengths were estimated in an apparatus similar to the "pectinometer" of Luers and Lochmüller [1927], but adapted to deal with quantities of 10 cc. of jelly, since the amounts of soluble pectin available would not permit of the preparation of larger quantities. The results are shown in Table III (methoxyl contents shown are corrected on the basis of the calcium pectate value of the sample).

Table III.

Sample	Methoxyl- content %	Jelly strength	
		1.5 % pectin g.	0.75 % pectin g.
Grape-fruit pectin	11.60	320	—
Commercial citrus pectin*	9.1	270	175
Methyl ester No. 4a	11.51	310	180
Methyl ester No. 4b	9.98	285	—
Methyl ester No. 5	8.55	180	105

* Pectin from which sodium pectate and silver pectate used in these experiments was prepared.

The results indicated quite clearly that the esters now prepared were comparable in jelling properties with the original natural soluble pectins.

Examination of the figures for methoxyl content of the various products suggested that in each case mixtures of the esters were formed, since none of the figures approximated to the methoxyl-content of a definite di-, tri- or tetra-methyl ester of pectic acid.

Attempted preparation of ethyl esters.

So far as is known, only the methyl esters of pectic acid occur naturally [von Fellenberg, 1918]. In order to see whether other esters resembled the methyl derivatives in their properties, an attempt was made to prepare the ethyl esters by heating silver pectate and ethyl iodide together under the same conditions as were found successful in producing methylation.

During the reaction, the mixture was observed to become very dark in colour. The aqueous extract after the reaction gave no precipitate with alcohol, but on evaporation left a brownish gum, which gave a strong reduction with Fehling's solution. Since an examination of the solid residue in the pressure bottle showed this to consist almost entirely of silver iodide, with a little unchanged silver pectate, it seemed likely that the reaction between the silver pectate and ethyl iodide had actually taken place with formation of ethyl pectates, and that it was the esters which underwent subsequent decomposition, with formation of the gummy reducing substances. This view was further supported by the observation that pectic acid itself was not appreciably destroyed by heating for some hours at 96° with ethyl iodide, alone or in the presence of absolute alcohol. It may be suggested, tentatively, that the introduction of a heavier group into the pectic molecule (even the substitution of C_2H_5 — for CH_3 —) results in an instability of the pectic ring, and its consequent breakdown to simpler substances. In this connection one may recall

the comparative ease with which pectins have been found to break down into hemicellulose-like bodies, sugars, *etc.* [*cf.* Candlin and Schryver, 1928; Linggood, 1930; Norman and Norris, 1930].

SUMMARY.

1. Pectic acid is readily converted into its methyl esters by heating silver pectate with methyl iodide under slight pressure. The products resemble the natural soluble pectins in all respects.

2. There is no reason to suppose that the soluble pectins are other than simple methyl esters of pectic acid.

3. The ethyl esters of pectic acid are apparently unstable, undergoing extensive decomposition under the experimental conditions used in their preparation.

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APPENDIX.

Small-scale "pectinometer."

The small-scale "pectinometer" used in testing the strengths of the sugar-pectin jellies in the above work was devised and made by our laboratory assistant, Mr G. Howkins. It was designed to deal with one-tenth the amount of jelly used in the ordinary pectinometer, *i.e.* 0.15 g. pectin in 10 g. jelly.

The vessel in which the jelly is allowed to set consists of a piece of thin copper tubing, 6.5 cm. long and 2.4 cm. internal diameter closed at the bottom. Into the sides of this are drilled and soldered 8 pieces of brass wire (s.w.g. 16), projecting 5 mm. into the tube; these are arranged in two circles, the lower being 1 cm. and the upper 1.5 cm. from the bottom of the tube, and serve to hold the jelly firmly in the tube. Along the axis of the tube is fixed a piece of similar wire,

Fig. 1. Small-scale "pectinometer"; arrangement of vessel in which jelly is "set," and device for "tearing" jelly.

8 cm. long, to serve as a guide for the jelly-tearing device. This latter consists of a thin copper tube, 10 cm. long and as narrow as possible, which just slides easily over the guide-wire; to its lower end is fixed a circle of brass, 12 mm. diameter, perforated with 7 holes at its periphery. The upper end of the inner tube carries a hook, to which is attached a fine chain, passing over two pulley-wheels to a counterpoised balance-pan, as in the ordinary pectinometer.

In making the test, sufficient of the pectin sample to yield 0.15 g. calcium pectate is dissolved in 10 cc. of 0.2 % tartaric acid solution, together with 6 g. sucrose. The mixture is boiled in a 30 cc. beaker until the weight of the mixture is reduced to 10 g. (By means of a preliminary test, a mark is made on the beaker to indicate this point.) The boiling is completed within 15 minutes. The hot mixture is transferred immediately to the setting-tube of the pectinometer, in which it forms a layer about 2 cm. deep. The subsequent procedure in estimating the strength of the jelly is exactly the same as in the case of the ordinary pectinometer. The apparatus has proved sufficiently satisfactory, giving comparable results within about 5 %.