BIBLIOGRAPHY

(1) Allen-Doisy, *J. Am. Med. Assoc.*, 81 (1923), 1808.

(2) Butenandt, *Z. physiol. Chem.*, 218 (1933), 104.

(3) Dorn, Faure, etc., *Med. Klin.*, 22 (1886), 1626.

(4) Frank, "The Female Sex Hormone" (1929).

(5) Harrow-Sherwin, "The Chemistry of Hormones" (1934).

(6) Loewe-Lange, *Biochem. Z.*, 180 (1927), 1.

(7) Lunge-Keane, "Technical Methods of Analysis" (1924).

(8) Walker-Janney, *Endocrinology*, 14 (1930), 389.

Pectin Studies, V. Organic Base Derivatives of Pectinic and Pectic Acids

By Reinhold F. Stuewer* and Aksel G. Olsen*

The present conception of pectin as a chain molecule made up largely, if not entirely, of galacturonic acid anhydride units, part of which are methylated, greatly simplifies an interpretation of pectin properties (2), (6), (8), (9). Acid properties are conferred by the free carboxyl groups resulting from partial demethoxylation. The reactions of pure pectin correctly called pectinic acid are, therefore, essentially those of a typical organic acid of high molecular weight (1). The colloidal properties presumably are indicative of the number of units in the chain, and the only difference between pectic acid and pectinic acid is that the former is more completely demethoxylated than the latter. Both pectins and pectic acid of greatly varying molecular size, as indicated by specific viscosity and gel properties, do exist; but usually pectic acid will be found to be of considerably lower molecular weight as indicated by specific viscosity, than is normal for pectin. This is a normal result of conditions which cause demethoxylation. However, by slow, strong acid, low temperature demethoxylation one can avoid the splitting of the long chains (7). In this manner pectic acid of high molecular weight can be prepared,

* General Foods Corporation, Hoboken, New Jersey.

while by the usual alkaline method of demethoxylation low molecular weight pectic acid usually is obtained.

The reaction of pectin and pectic acid with certain metal ions to form insoluble compounds has long been known. Such insoluble precipitates have been described by a number of writers from Braconnot to von Fellenberg. From a study of several pectins differing in methoxyl content, von Fellenberg correctly concluded that "the more methoxyl groups split off the more acid groups present in the pectin molecule and the easier does coagulation with metal salts occur" (5).

Nevertheless, even more recently, some have failed to recognize the essentially stoichiometric relationships which control reactions that occur between pectin and either inorganic or organic bases. Some commercial pectin preparations may be but slightly acid in reaction due to a neutralization of the free acid groups. However, the combined cations are readily removed by washing with acidified alcohol and the excess acid can be removed by washing with dilute alcohol. Such pectin in 1% solution will have a p_H well below 3.0, and should preferably be referred to as pectinic acid. It is questionable whether a "neutral" pectin, that is a completely methylated pectin, exists in the free state. Most pectin samples observed by the authors have equivalent weights of not over 1200, and some commercial preparations run as low as 400 equivalent weight while pectic acid has an equivalent weight of about 205. It is not logical to assume a difference in type of reactions entered into by pectic acid and such pectinic acids. The difference is rather one of degree due to the difference in equivalent weight. Naturally one may expect pectic acid to form insoluble precipitates with many salts which do not precipitate pectins of high equivalent weight because the latter do not combine with enough of the precipitating ion to become insoluble. For example, pectin of high combining weight reacts with calcium to form calcium pectinate although it does not form a precipitate with calcium, whereas insoluble calcium pectate provides the well-known method for determining pectic acid.

Nevertheless, Chodnew (4) many years ago prepared a pectinic acid, which he assumed to be pure pectin, and which was precipitable with calcium. In corroboration of this, the present authors have observed that pectins of about 475 equivalent weight or less are readily precipitable by calcium. Other metallic ions may form precipitates with pectins of higher equivalent weight, for example aluminum, copper, iron, nickel and others. This, of course, is what might be inferred from von Fellenberg's general statement quoted above. The maximum equivalent weight permitting precipitation differs to some extent, and inasmuch as such precipitates tend to be soluble in acids, the
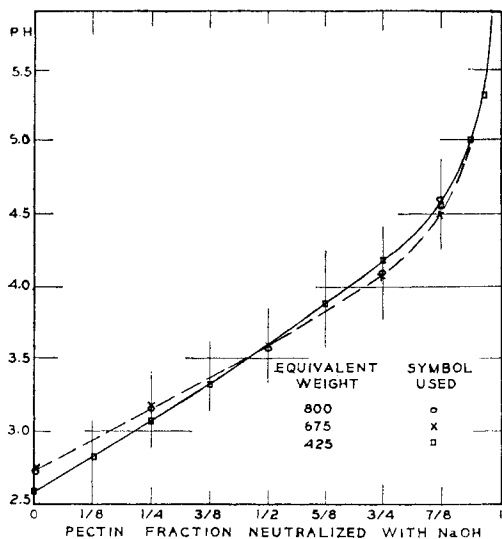

Fig. 1.—Electrometric Titration Curves for 1% Pectin Solution.

maximum equivalent weight at which precipitation occurs will vary with the p_H of the solution, the higher the p_H the easier is the precipitation.

None of these reactions of pectin need to be explained in any but common stoichiometric terms. Under some conditions adsorption may occur in addition to chemical combination (11); but adsorption is not needed to explain and by itself certainly does not explain the reactions of pectin with metallic ions. Pectin is essentially a typical organic acid of high molecular weight. Typical titration curves for one per cent water solutions of pure pectins are shown in Fig. 1.

Some heavy metal ions, as, for example, lead, react so readily with pectin that they can be quantitatively removed from solutions in the pectin precipitate. In fact lead will replace other ions such as calcium so that finely divided calcium pectate preparations if used as a filter bed, or if dispersed in a solution and again filtered off will effectively remove lead ions from the solution. This is readily applicable to other heavy metal ions and it reminds one of Braconnot's early suggestion that pectic acid be taken as an antidote for heavy metal poisoning (3). It also suggests how the ever present pectin in fruits may greatly minimize the danger of lead poisoning from spray residue (10).

The alkali metals and ammonia also react with pectin in stoichiometric proportions but form only soluble salts. These are, therefore, most conveniently obtained by adding calculated amounts of the ions in the form of salts of weak acids to a suspension of the pure pectinic acid, or pectic acid as the case may be, in dilute alcohol. For this purpose the carbonates are most desirable. In 50% alcohol the pectin particles swell sufficiently so that a rather rapid reaction will occur. After the reaction is complete the pectin can be recovered by filtering, then washed with additional alcohol and dried. This method of reacting on powdered pectin while suspended in a solvent in which the pectin particles swell but are not soluble but in which the other reagent is soluble is a very convenient procedure, in particular in the preparation of several organic derivatives of pectin and pectic acid.

The solubilities in water of metal base pectates and pectinates, as already indicated, differ greatly one from another, all the way from completely insoluble to completely soluble. It was, therefore, considered of some interest to observe the differences introduced by forming various organic base derivatives.

EXPERIMENTAL

Although there is nothing particularly novel about neutralizing an organic acid by an organic base we have failed to find any published record of preparations of organic base pectinates and pectates of the general form $R_x(COOCH_3)_n(COOB)_{x-n}$ in which R is the galacturonic acid anhydride unit and B the

organic base. With x the number of galacturonic acid anhydride units in the molecule and n the number of methoxyl groups there would be $x - n$ carboxyl groups free to react with B. In partially neutralized products hydrogen replaces some of the B groups.

We have prepared a number of such compounds and many of the organic base derivatives have been found to have interesting properties. For example, the dispersibility of different pectates in dilute alcohol is greatly modified by the base. The maximum per cent of alcohol in which the common sodium salt of pectic acid can be dispersed to form a clear solution is 6–8%; for the potassium salt, it is 10–12%; for triethanolamine pectate, it is about 60% and for propyl amine pectate, it is about 75%. It is readily apparent that this permits the use of pectic substances under conditions not otherwise possible, as for example in cosmetic, or pharmaceutical preparations containing alcohol in appreciable concentration. Propyl amine pectate also has interesting emulsifying and foam producing properties, and even partial neutralization of pectinic or pectic acids by different organic bases may confer attributes quite different from those of the original material. The now well established value of pectin in the treatment of infected wounds, burns, diarrhea, etc., suggests its utility also in combination with therapeutically important organic bases.

Among the compounds we have prepared are the pectic salts of the following:

Simple aliphatic amines

1. *N*-Propylamine
2. *n*-Butylamine
3. Di-*n*-butylamine
4. Tri-*n*-butylamine
5. Tri-iso-amylamine

Aromatic amines

1. Aniline
2. *o*-Anisidine
3. *p*-Toluidine

Guanidines

Diphenyl guanidine

Substituted aliphatic amines

1. Diethanolamine
2. Triethanolamine
3. Ephedrine
4. Benzylamine
5. Methyl glucamine

Heterocyclic bases

1. Pyridine
2. Piperidine
3. Quinine

Substituted hydrazines

Phenyl hydrazine

In general the dissociation constants of all organic bases suitable for reaction with pectic or pectinic acids fall between the limits of $K = 1 \times 10^{-3}$ and $K = 1 \times 10^{-10}$. Some substituted ammonium hydroxides are even stronger than $K = 1 \times 10^{-3}$ and of course will react with either pectic or pectinic acid.

The reactivity of most of the compounds listed above is readily demonstrated by their ability to disperse pectic acid. That is, while pectic acid is quite insoluble in water nearly all the organic derivatives are very soluble, and the solubilizing of the pectic acid is therefore a definite indication that the reaction has taken place. With some compounds such as quinine and cinchonine, the derivatives although

formed are not soluble. The limitations of reactivity may be summarized as follows:

Aliphatic Amines.—The common aliphatic amines are all fairly strong bases. Their dissociation constants fall roughly between 1×10^{-3} and 1×10^{-6}. They react readily with either pectic or pectinic acids to give practically neutral salts.

Some of the higher aliphatic amines, e. g., tributylamine are practically insoluble in water, yet will react with pectic acid to form soluble pectates. Amines of this type which are completely insoluble in water may react readily by use of the dilute alcohol suspension method, since in general they are soluble in alcohol solutions.

Solubility in alcohol or acetone solutions of rather high concentration appears to be a characteristic of all pectic salts of aliphatic amines.

Other Bases.—The strictly aromatic amines, e. g., aniline and toluidine are considerably weaker bases than are those of the simple aliphatic series. Aniline has a dissociation constant of 4.6×10^{-10} but regardless of its low basicity, it reacts to a sufficiently great extent to give a water soluble product with pectic acid, as is readily shown by addition of aniline to a water suspension of pectic acid. Pyridine, which shows all the characteristics of aromatic compounds, behaves in much the same way as aniline, while piperidine, its reduction product, is typically aliphatic. *p*-Phenylene diamine, while of about the basic strength of aniline does not cause appreciable dispersion of pectic acid. Di- and tri-arylamines, e. g., di-phenylamine, are almost without basic properties and do not react. As with other properties benzyl amine behaves as an aliphatic amine. Ephedrine also shows the reactivity one would expect of an aliphatic amine.

Quinine which has a K value of 2.2×10^{-7} does not cause dispersion of pectic acid. That reaction takes place to some extent may, however, be shown by use of pectinic acid of low equivalent weight. When a dispersion of pectinic acid in 60% alcohol was allowed to absorb quinine and was then washed free of excess reagent with alcohol solution, the p_H of a 1% solution of the product was about 3.2 indicating $^5/_{16}$ neutralization. A similar experiment with pyridine, $K = 2.3 \times 10^{-9}$, gave a product with pectic acid which was not completely dispersible in the cold. The p_H was about 3.0. Excess pyridine causes complete dispersion of pectic acid. The obvious conclusion is that a difference in solubilities rather than in reactivities of the bases is responsible for the dispersion of pectic acid by pyridine but not by quinine.

With methyl glucamine, complete dispersion takes place. Solubility in alcohol is not so great as in the case of simple aliphatic amines.

The above compounds are prepared by common chemical procedures whereby basic and acid reacting materials are caused to combine. The exact procedure may vary with the different compounds because of their having greatly varying solubilities; for instance, such substances as are water soluble

may be added directly to a water suspension of finely divided pectic acid or to a water solution of the pectinic acid. In other cases where the organic reagent is not water soluble, it may be used in the form of a solution in a suitable organic solvent. Care must be taken not to have present any great excess of the base. Preferably, the total amount of base added should be slightly less than equivalent to the pectic or pectinic acid. A few specific examples of organic derivatives prepared are given below:

Triethanolamine Salt of Pectinic Acid.—To 45 Gm. of pectinic acid (about 450 eq. wt.) suspended in 80% alcohol are added 14.9 Gm. of triethanolamine as a 20% solution in alcohol. The triethanolamine solution is added slowly with constant stirring, and stirring is continued for 15 to 30 minutes after the addition has been completed. The liquid is filtered off, the product washed with alcohol, and dried.

n-Propylamine Salt of Pectic Acid.—To 44 Gm. of pectic acid (about 220 eq. wt.) suspended in 85% acetone are added 11.8 Gm. of n-propylamine as a 10% solution in acetone. The n-propylamine solution is added slowly with constant stirring, and stirring is continued for 15 to 30 minutes after the addition has been completed. The liquid is filtered off, the product washed with alcohol and dried.

Methylglucamine Salt of Pectic Acid.—To 22 Gm. of pectic acid suspended in water are added 19.4 Gm. of methylglucamine as a water solution with constant stirring. The resultant solution of methylglucamine pectate is poured slowly into two volumes of 95% alcohol. The precipitate produced is filtered off, washed with alcohol, pressed and dried.

SUMMARY

Purified pectins (free pectinic acids) and pectic acid are pictured as typical organic acids of high molecular weight. Differences in behavior result from the differences in equivalent weight which regulate the amounts of basic reagents combining with a pectin or with pectic acid. Typical titration curves for pectins are given. Reactions with either organic or inorganic bases are described.

Triethanolamine pectate is soluble in as high as 60% alcohol, and the propyl amine in 75% alcohol.

Other interesting properties of the organic base derivatives are suggested.

REFERENCES

(1) Bonner, J., *Proc. Acad. Sci., Amsterdam,* 38 (1935), 346–359.

(2) Bonner, J., *Botan. Rev.,* 2 (1936), 475–497.

(3) Braconnot, H., *Ann. chim. phys.,* 30 (1825), 96–102.

(4) Chodnew, A., *Ann.,* 51 (1844), 355–395.

(5) Fellenberg, T. von., *Biochem. Z.,* 85 (1918), 118–161.

(6) Hirst, E. L., and Jones, J. K. N., *J. Chem. Soc.* (March 1939), pages 454–460.

(7) Olsen, Aksel G., and Stuewer, R., U. S. Patent 2,132,577 (Oct. 11, 1938).

(8) Olsen, Aksel G., *et al., Ind. Eng. Chem.,* 31 (1939), 1015–1020.

(9) Schneider, G. G., and Boch, H., *Angew. Chem.,* 51 (1938), 94–97.

(10) Shields, J. B., Mitchell, H. H., and Ruth, W. A., *J. Nutrition,* 18 (1939) 87–97.

(11) Stuewer, R. F., *J. Phys. Chem.,* 42 (1938) 305–315.

Elkonite, A Colloidal Clay*

Properties, Actions and Possible Medicinal Uses of

By *M. L. Tainter, M.D., G. Kulchar, M.D., and A. B. Stockton, M.D.*

Elkonite is a naturally occurring clay, laid down in terrestrial beds near Elko, Nevada. When placed in water, it swells to many times its original volume, forming a firm jelly-like mass. Accordingly, it is a hydrophilic colloid with certain characteristics that set it apart from other similar materials commonly available in medicine. This paper presents the results of a study of the scope of possible clinical applicability and usefulness of Elkonite.

EXPERIMENTAL

Physical and Chemical Properties.—In the native state, Elkonite has the appearance of a light olive-green or greyish piece of soapstone. It is rather friable, so that pieces can be broken in the hand. Dispersed through it are small particles of sand or quartz comprising something less than 5 per cent of the total mass. When a piece of this clay is put in water, the surface softens and swells, taking on a gummy gelatinous consistency. If water is added in the proportion of 6 cc. for each Gm. of Elkonite, the swelling continues for several days until finally a homogeneous colloidal gel is secured, of the general consistency of library paste. This paste quivers when the container is struck a sharp blow, thus indicating an elasticity of an organized colloidal gel. Its physical state is not affected by normal temperature changes, the paste being stable under ordinary conditions as long as the evaporation of water is prevented.