TANNIC ACID, ETHYL GALLATE, AND THE SUPPOSED ESTER OF TANNIC ACID.

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The optical activity of tannic acid has led many investigators to seek an adequate explanation of this phenomenon. The original digallic acid formula of Schiff[1] contains no asymmetric carbon atom and consequently fails to explain the behavior of the substance towards polarized light. Schiff[2] suggested the existence of a ketone group in the digallic acid structure, while Walden[3] concluded that the optical activity is due to the presence of some other body or bodies associated with tannic acid and from which separation is very difficult. Rosenheim and Schidrowitz[4] found on the other hand that by certain methods of purification a product may be obtained from tannins of varying optical activity, which has a constant specific rotatory power of about +75.5°. They also noticed that the acetyl derivative as also the quinine salts obtained from tannic acid, showing a wide range of variation in optical activity, were almost identical in specific rotatory power, regardless of the source from which they were derived. Nierenstein[5] has devoted considerable study to this question with particular reference to certain derivatives of tannic acid and concludes from his investigations that tannic acid consists of equal parts of digallic acid and a substance called by him leucotannin which is supposed to contain an asymmetric carbon atom.

In the various investigations dealing with tannic acid from the middle of the last century down to the present time, it has been repeatedly noted that the acid contains glucose. Strecker,[6] for example, pointed out as early as 1852 that upon hydrolysis tannic acid yields gallic acid and glucose. Since that time similar observations have been made repeatedly. Of particular interest here from the synthetic standpoint is the recent preparation by Fischer[7] of penta-galloyl-glucose and similar substances.

In the preparation of so-called pure tannic acid the separation of impurities is extremely difficult from the nature of the substance. In fact it may well be doubted whether chemically pure tannic acid from natural sources has ever been obtained. The purification of colloids presents almost insurmountable difficulties. It seems, therefore, not at all impossible that the optical properties of tannic acid may after all be due to the presence of glucose whether occurring as an impurity or in some essential combination of the nature of a glucoside.

[1] Schiff, *Ber.*, **4**, 231, 967 (1871).

[2] Schiff, *Gazz. chim. ital.*, **25**, 437 (1895).

[3] Walden, *Ber.*, **30**, 3051 (1897).

[4] Rosenheim and Schidrowitz, *J. Chem. Soc.*, **73**, 878 (1898).

[5] Nierenstein, *Ber.*, **41**, 77, 3015 (1908); **43**, 628 (1910).

[6] Strecker, *Ann.*, **81**, 248 (1852).

[7] Fischer and Freudenberg, *Ber.*, **45**, 915 (1912).

As is well known the natural tannins are capable of fermentation.[1] One of the commercial methods for producing gallic acid, indeed, depends on this fact.[2] It, therefore, is of some interest to study the effect of fermentation under varying conditions on the optical properties of pure tannic acid. If the optical properties are due to the presence of glucose either as an impurity or as an essential part of the tannin, it is conceivable that fermentation of the glucose into carbon dioxide and alcohol might, under some conditions, lead to an extinction of the optical activity without completely hydrolyzing the tannic acid to gallic acid.

Action of Microörganisms.—The fact that various fungi will develop on solutions of tannic acid is a matter of common observation among pathologists. Pottevin and Fernback[3] claim that the fungus, *Aspergillus niger*, when grown in solutions containing tannic acid develops an enzyme, tannase, which can be extracted from it by means of alcohol and which has the property of splitting tannic acid into free gallic acid and glucose. Some preliminary study of the action of this fungus has been made. We have found that pure cultures of the fungus can be easily grown in sterile solutions of tannic acid, and that the solutions subsequent to the development of the mycelium still give the reactions of tannic acid, but do not contain large quantities of gallic acid.

A few preliminary experiments have been made regarding the action of yeast (*Saccharomyces cerevisiae*) upon tannic acid. Samples of the purest tannic acid obtainable, including a sample of Baker's highest purity and one from the German firm of Schering, were dissolved in water to a 4% solution, sterilized and then inoculated with pure cultures of yeast. After an incubation period of ten days, it was found that in every instance alcoholic fermentations had taken place. Portions of the solutions were distilled off and the distillate tested for alcohol by means of the iodoform reaction. The presence of alcohol was shown in every case. The specific rotatory powers of the several samples before fermentation varied from $+10°$ to $+55°$, but in every instance the action of the yeast cells totally destroyed the optical activity. Portions of the fermented solutions after being freed from aggregates of yeast cells were found to give the characteristic reactions of tannic acid, such, for example, as the precipitation of solutions of gelatin and ammoniacal solutions of copper sulfate. The content of gallic acid appeared to have increased to some extent as shown by the reaction with potassium cyanide, although the conversion to this acid was far from complete.[4]

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[1] Tieghem, *Z. Chem.*, 1868, 222.

[2] Allen's, "Com. Org. Analysis," 4th edition, Vol. 3, p. 526.

[3] Pottevin and Fernback, *Compt. rend.*, 131, 1214 (1900).

[4] The conversion of tannin into gallic acid by atmospheric fermentation, as it is practiced in the commercial production of gallic acid, probably results from the combined action of various microörganisms, rather than from that of a single species.

The preparation and isolation of a definit crystallin compound of tannin containing glucose as a necessary constituent of the molecule would bring strong evidence to support the glucoside character of natural tannin. In 1910 Manning[1] published an article in which he claims to have synthesized such a compound, ethyl tannate (m. p. 157°), and gives evidence showing apparently that glucose forms an integral part of the molecule. The importance of such an observation, if correct, in throwing light on the constitution of tannin itself is self evident. As the method of synthesizing the so-called ethyl tannate seemed a little extraordinary in view of the ready hydrolysis of tannin to gallic acid, we took occasion to repeat Manning's work. The results, however, were entirely different from those of Manning. The ester obtained was found to be invariably ethyl gallate, differing in no way from the ester synthesized from pure gallic acid. Further study of his work revealed the fact that his method for estimating glucose in the supposed ethyl tannate is unreliable. Moreover the peculiar properties of pure ethyl gallate when melting, as developed in our study, lead to the conclusion that Manning has simply mistaken this substance for ethyl tannate.

The Work of Manning.—In repeating Manning's synthesis the tannin used was that from Schering. This was purified and treated in exact accordance with the directions of Manning. The ester obtained from extraction with chloroform, we found as he states, forms pale yellow, spherical nodules, but an examination of the substance showed it to be identical, as we have indicated, with ethyl gallate prepared by similar methods from pure gallic acid. Thus, mixtures of the crystals of ethyl gallate and the supposed ethyl tannate obtained by crystallizing the two from ether-ligroin showed no variation in melting point or in any other property from ethyl gallate itself. An analysis of the supposed ethyl tannate recrystallized from ether-ligroin gave the following results:

0.2 gram substance gave 0.3990 gram CO_2 and 0.0946 gram H_2O.

Calculated for ethyl gallate: C, 54.54; H, 5.05.
Found: C, 54.4 ; H, 5.2.

In Manning's work[2] determination of glucose in tannin as well as in the supposed ethyl tannate was made by hydrolyzing these substances with hydrochloric acid and extracting with ether six times to remove gallic acid. The solution which was thus supposed to have been freed from all but traces of gallic acid was then used as a basis for the estimation of glucose, a certain correction being made for the glucose which had been dissolved in the ether during extraction.

In this method the mistake is made of assuming that six extractions with ether under the conditions given are sufficient to remove from the

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[1] Manning, THIS JOURNAL, 32, 1312 (1910).

[2] *Ibid.*, 32, 1313 (1910).

solution all but traces of gallic acid. If, for example, pure ethyl gallate is submitted to Manning's method of procedure, the solution remaining after extraction with ether gives vigorous reaction for gallic acid with potassium cyanide, providing excess of hydrochloric acid is *first* neutralized with sodium carbonate, and, furthermore, it contains ordinarily sufficient gallic acid to account for all the reducing action which Manning attributes to the presence of glucose. These results are not materially affected whether the ether used in extraction is the ordinary washed ether or that of the highest purity. Thus, an application of Manning's method to 0.2 gram of pure ethyl gallate synthesized from Kahlbaum's pure gallic acid led to a reduction of Fehling's solution which estimated as glucose amounted in one case to 16.4% and in another to 15.8%. (Manning[1] estimates glucose in his supposed ethyl tannate to be 15.38%)—and this in a substance absolutely devoid of glucose!

It follows from these considerations that the results of Manning's investigation must be regarded as unreliable and the supposed ethyl tannate becomes in all probability identical with ethyl gallate. The source of the confusion of ethyl gallate with a supposititious ethyl tannate lies in the failure of Manning to note the peculiar behavior of ethyl gallate on melting—a peculiarity apparently hitherto entirely unnoted by other investigators as well, in the case ofthis particular ester.

Ethyl Gallate.—In the literature three melting points are recorded for ethyl gallate. Grimaux[2] finds a melting point of 158°; Ernst and Zwenger[3] record a melting point of 150°; and Etti[4] states that the ester melts at 141°. The result of the last determination is assumed by Manning[5] to be correct and since he finds for his substance synthesized from tannic acid a melting point of 157° he reaches the conclusion that it is not ethyl gallate but ethyl tannate.

A study of ordinary ethyl gallate reveals peculiarities in its melting point which at first glance strongly suggests the possible phenomenon of liquid crystals, or the existence of two crystallin phases. As prepared from pure gallic acid and purified by crystallization from chloroform or by careful sublimation, the ester forms a mass of long, fine, white, silky needles. These when melted ordinarily begin to sinter down near 145° and melt to a turbid liquid at 149–150°, which only becomes clear at 157–158°. The supposed ethyl tannate of Manning behaves in precisely the same way. The possible contamination of ethyl gallate with an impurity led us to a comparison of our product, which had been synthesized from Kahlbaum's pure gallic acid, with products obtained

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[1] THIS JOURNAL, **32**, 1317 (1910).

[2] Grimaux, *Bl.*, **2**, 94 (1864).

[3] Ernst and Zwenger, *Ann.*, **149**, 29 (1871).

[4] Etti, *Ber.*, **11**, 1882 (1878).

[5] Manning, THIS JOURNAL, **32**, 1316 (1910).

from other sources. The same phenomena were observed in all specimens. We then submitted ethyl gallate to careful purification by recrystallizing it successively from chloroform, ether-ligroin and water. The hydrated ester was then freed from water of crystallization and carefully sublimed. A product was thus obtained which sinters down at about 148–149° and melts as before at 149–150° to a turbid liquid which clears at 157–158°. Through a range of fully 8° the liquid remains distinctly turbid, becoming somewhat less dense as it approaches 157–158°, but clearing fairly sharply at this temperature. On remelting the solidified ester, the same phenomena are repeated. If, however, the temperature is raised about 8° above 158° before the fused mass is cooled, there solidified ester now melts to a *clear* liquid at 149–150°. If, on the other hand, during the process of cooling from the higher temperature, the clear, fused mass is inoculated with a trace of the unfused substance near 150°, the turbid state is apparently slowly reinduced before solidification takes place.

The appearance of ethyl gallate between crossed nicols during the process of fusion strongly suggests a case of crystallin polymorphism not unlike that observed by Pope[1] in the case of chloral aud somewhat similar to that noted by Offret and Vittenet[2] in the case of di-*m*-nitro-*s*-diphenylcarbamide. A suitable hot stage for the study of the phenomena of fusion was found in a thinly platinized glass slide whose resistance to the passage of the electric current afforded ready production and control of the temperature desired.[3] In order to obtain the ethyl gallate in a form suitable for examination, a little is fused down on the microscope slide either with or without the addition of a cover glass, and the whole is allowed to cool. A casual inspection of the film under the polarizing microscope between crossed nicols shows ordinarily what appears to be a mixture of crystallin plates and hair like needles or sheaf like groups of needles. If the temperature is now gradually raised, very slight alterations are observed in the preliminary softening. These are followed by a melting process in which the plate like crystals fuse to a clear liquid, leaving the more or less intricate mass of hair like needles unchanged. As the temperature continues to rise these in turn disappear and the liquid becomes clear. On recrystallization during cooling, ordinarily both forms of crystals are reproduced, the needles and the plates. If, however, the fusion is carried to the point of removing from the slide every trace of needles, on cooling but one form of crystals, the plates,

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[1] Pope, *J. Chem. Soc.*, **75**, 455 (1899).

[2] Offret and Vittenet, *Bull. soc. fr. min.*, **22**, 69 (1899); *Z. Kryst.*, **34**, 627 (1901); *J. Chem. Soc.*, **76**, 886.

[3] We are indebted to Dr. F. G. Cottrell, formerly of this University, and now of the U. S. Bureau of Mines, for the apparatus here used, a description of which will appear soon.

are produced and these on fusion now melt to a *clear* liquid without showing any presence of needles. But if the crystallin plates are allowed to stand at the ordinary temperature for about twelve hours, they are largely converted into the hair like needles as may be shown by a repetition of the phenomena of fusion noted in the first instance.

It would seem, then, that ethyl gallate probably presents two crystallin phases, one the hair like needles, stable at ordinary temperatures, the other, the flat plates stable, if stable at all, at more elevated temperatures, and that further the turbid condition of the fused ester is apparenty due to the presence of masses of unfused needles.

These suggestions are advanced tentatively as the phenomena in question have as yet been only incompletely studied. The investigation, however, is being continued particularly to determin with certainty whether or not the case is actually one of crystallin dimorphism and, if so, to learn the habits of the two crystallin forms and the exact conditions under which one crystallin phase passes into the other.

The peculiar behavior of ethyl gallate on fusion, however interpreted,[1] serves not only to explain the divergence in melting points given for this substance in the literature, but also probably explains, in part at least, the mistake of Manning[2] who, assuming that ethyl gallate melted at 141°, concluded that the substance melting or changing at 157° must be another substance.

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A STUDY OF THE DERIVATIVES OF PHENYL AND ETHYL CYANOACETIC ETHER.

BY HARRY F. HADLEY.

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Phenyl- and ethylcyanoacetic acid contain a socalled asymmetric carbon atom. It was the purpose of these experiments to get a separation of each of these acids into the two enantiomorphic modifications.

Some of the salts of phenylcyanoacetic acid were prepared but they decomposed so easily that nothing could be done with them.

Two methods were used in attempting to separate monoethylcyanoacetic acid into its two modifications. First: Salts of this acid were crystallized

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[1] It is to be noted that the ethyl gallate prepared by Etti and melting at 141° was obtained from gallic acid derived from the socalled *kinvin*. A further study of gallic acid from this source to determin whether or not it is really idenical with ordinary gallic acid seems desirable.

[2] In a letter just received from Prof. W. Lash Miller (under whom Mr. Manning carried out his work) in response to a request for a specimen of the so-called ethyl tannate, Prof. Miller likewise states that he has repeated Manning's work and has been able to get only ethyl gallate, instead of ethyl tannate.