

activity of the digitalis. If you are going to put out an infusion of digitalis, uniform in therapeutically active constituents each time you fill a prescription, you must have the drug in such a form that you can withdraw uniform samples.

In the new Pharmacopœia instead of specifying that one shall use so much of the bruised digitalis, it is directed that one shall use so much of the No. 60 powder. One may withdraw typical samples, each of which will represent practically an identical amount of the therapeutic qualities in the drug.

The old method also directs to strain the infusion. If one uses a No. 60 powder, the strainer must be very fine. The best procedure is to weigh out the digitalis in powdered form; introduce hot water and allow it to stand, and then filter it. We do not know whether the change from the whole drug to the powdered drug is going to make the problem of the pharmacist in supplying the official infusion any more difficult than to use the whole drug. Certainly every pharmacist can quickly tell the difference between a nice whole digitalis leaf and one which is of inferior quality.

In the future you are going to have the powder to work with. What will you do about determining the quality of the powder? We want to issue a word of warning about powdered digitalis which is brown or gray. If prepared from select green leaf, it should be of a nice, rich, green color. If it is, you may rest assured a preparation made from it, providing it has been standardized, will give an infusion which will give the result the doctor has a right to expect.

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THE TANNIN OF RHUS GLABRA.*

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The title "Rhus Glabra," previously used by the United States Pharmacopœia to designate the fruit of that plant, is now employed for the same purpose in the National Formulary by which authority the drug is recognized.

That *Rhus glabra* contains an astringent principle—a tannin—has long been known, and on the recognition of this quality it has found use.

This drug has been the subject of several investigations, references to which were given by Henry Kraemer in *American Journal of Pharmacy* for September 1913, in an article explaining that in commerce the fruit of *Rhus glabra* was being replaced by the fruit of *Rhus typhina*, and, in which paper, besides summarizing the reports of those who had contributed to an understanding of the constituents, he gave means for distinguishing these fruits by examining their hairs under the microscope.

As mentioned by Kraemer, Watson in 1853 (*Amer. Jour. Phar.*, p. 193) published the results of his examination of the fruit of *Rhus glabra*, substantiating earlier statements by Cozzens and Rogers, the first of whom was concerned chiefly with establishing the presence of gallic and malic acids, while the latter pertained more to determining the form in which the malic acid occurs.

It is mainly on the statements of Watson that the references to the constituents of *Rhus glabra* found in the books which American pharmacists study and consult are based.

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The experiments of Stenhouse, to which Kraemer referred, were not made on *Rhus glabra* nor on any other of the species of *Rhus* dealt with by Kraemer, but on what is known in the leather industry as "sumac" of commerce, which is the ground leaves and twigs of *Rhus coriaria*, obtained usually from Southern Europe.

While from that date the belief that the tannin of this drug is gallotannic acid has not only held but has been strengthened, as for instance by the work of Trimble in 1892 and 1894 (reported by J. C. P. in *Amer. Jour. Phar.*, 1900, p. 334), still until now the tannin of the fruit of this particular species has not to our knowledge been isolated and purified as a means of studying it apart from its association with other constituents of the drug always present in the decoctions and only by use of which *Rhus glabra* has heretofore been observed.

This tannin has engaged our attention for some years past; there has long existed the desirability of knowing more definitely its nature and thereby supplementing the results of Trimble's work which showed by ultimate analysis and otherwise that the tannin in the several plant parts of the related species investigated by him is gallotannic acid. Again, in the exigencies of some recent work on the iron-greening factor of digitalis, it occurred that *Rhus glabra* might afford opportunity for studying the isolation and purification of a tannin associated with crystalline acid substance; and, since the presence of some crystallizable acids or of caffeine has been found by us to militate against the securing of tannins in porous or "puffed" condition, there was the thought that possibly experience might be gained with *Rhus glabra* which would be helpful in further consideration of the digitalis problem reported to this Association a year ago.

To find a solvent, or means, that will select either the tannin or the crystallizable substance and thereby enable separation is, in every such instance, obviously necessary as the only hope of effecting the purification of the tannin.

In this report, we confine our consideration almost entirely to the tannin, giving outlines of the methods used to isolate and purify it; but, because of the intimate interest which directs attention to gallic acid, the matter of its presence in sumach berries has been given thought, for some of the reference books used by American pharmacists do not mention gallic acid as present.

The drug used for this work was taken from a market lot. After maceration of a portion in glycerin for twenty-four hours, it was compared microscopically with Kraemer's drawings of the hairs of the fruits of the species *glabra*, *typhina* and *glabra borealis* and found to be both genuine and unmixed.

The sensible properties of a decoction of sumach berries and its behavior toward iron test solutions, while well known, are mentioned in order to contrast certain effects which were noted in this work.

A rich red, freshly prepared, ten per cent decoction gave with ferric chloride test solution a strong bluish black color and precipitate, that upon addition of an excess of the reagent became greenish black. With gelatin solution, the decoction produced an abundant pinkish or deep flesh-colored precipitate; and with lead acetate a drab precipitate.

In contrast with these effects, when another portion of the same decoction was mixed with enough well-washed rasped raw-hide to give a jelly-like consistency, and this mixture was allowed to stand for two hours, the expressed liquid, after filtration, was found to be colorless, free from astringent taste, to give but a slight,

if any, color with ferric chloride or ferric acetate solution beyond that of the reagent itself, and no precipitate with gelatin solution.

When this experiment was repeated, keeping the decoction in contact with the hide for but one hour, the result was the same. It was then found that, as soon as the color had been absorbed by the hide, the behavior of the filtered liquid toward ferric compounds and the other reagents was as stated for the two-hour contact.

Because of the absence of a reaction by the hide-treated decoction with ferric chloride and ferric acetate, there is reason to believe that gallic acid is not present in more than a minute amount, if at all. Furthermore, realizing that such small amounts might have been absorbed, the experiments were repeated using decoctions to which trifling quantities of gallic acid had been added. In every such instance, the filtered liquid from the hide treatment gave the characteristic blue color for gallic acid with both ferric chloride and ferric acetate, and, by comparison with a water solution of the same strength of gallic acid, no diminution of intensity of color reaction was manifested to indicate that any gallic acid originally present in the decoction had been removed by the hide.

When some of this same lot of decoction was allowed to stand for several days, by which time it usually became moldy, and was then treated with hide in the same way, the filtered liquid immediately showed the characteristic blue color with ferric chloride and ferric acetate indicating gallic acid and suggesting its presence as an alteration product of the tannin. (When the description of Watson's isolation and recognition of gallic acid in *Rhus glabra* is read with definite knowledge of the tannin and acid constituents of *Rhus glabra*, it is plainly evident that the latter furnished the crystalline form and the former the color reaction with iron salts; from which two observations he inferred the presence of gallic acid in accordance with earlier writers on this drug.)

But repetitions of this experiment with different lots of decoction showed no constancy in either the production or the amount of gallic acid.

Therefore, contrary to some authorities, it would seem that gallic acid is not a constant, if indeed ever a considerable, constituent of sumach berries, although it may at times be found in decoctions which have stood for several days.

In all of the filtered liquids from the hide treatments, there was acidity markedly demonstrable with litmus paper, and these liquids still gave precipitates with lead acetate, although now white and of much less amount.

All but three per cent of the titratable acidity of the decoction was retained by the hide.

The absorption of malic acid by hide was mentioned by Trimble, where cited and in same for 1903, p. 343 (reported for him by William E. Ridenour).

Turning now to the isolation and purification of the tannin, trials were made on small lots of sumach berries using for each an equal portion of the drug. Decoction with water and percolations with ether and acetic ether were the three variations of these efforts. These three solvents removed substances other than tannin; all took out the tannin about equally well, but none gave more than a small yield.

When ether or acetic ether was used on the drug, fat was extracted, but it was readily got rid of by evaporating these solvents and treating the residue with water, pulping with paper and filtering. Also, small quantities of matter suggesting resin

were thus removed, with production of brilliant solutions which could be diluted with water without producing turbidity.

Solutions of gallotannic acid, if not too dilute, form with ether a milky turbidity that soon becomes droplets which coalesce and settle as a subnatant layer. This formation approximates a saturated solution of the tannin in water-saturated ether. This behavior, which has not been observed with any other tannin and is therefore regarded as peculiarly characteristic of gallotannic acid, was soon noted as an attribute of the tannin of *Rhus glabra*. As long ago as 1837, advantage was taken of this behavior by Pelouzé as a step in the preparation of commercial gallotannic acid from galls. We made use of it in this work, but with little if any benefit through it serving to separate other constituents; a supernatant layer of ether, or ether quickly applied to the "puffed" tannin in process of purification, seemed as hereinafter mentioned in detail, much more effective in that capacity as evidenced by the relative amounts of non-tannin which were removed. And following such method of purification, shaking out from a water solution with acetic ether was done to obtain the tannin in "puffed" or porous form.

When ether is applied to porous *Rhus glabra* tannin, even when quickly done, the tannin at first acts as though it would not be soluble, but soon the rapidly evaporating ether has brought about condensation of sufficient moisture from the atmosphere to change this aspect, and solution becomes unmistakably evident, with eventual fulfillment of this tendency; in actuality this behavior is nothing more or less than the properties recognized and utilized by Pelouzé, merely somewhat differently induced.

Neither chloroform nor benzol applied to either the porous crude tannin or its solution in water were helpful as purifying agents.

Extraction of the fruit with hot water, concentration of the resulting extract and shaking out of this liquid with acetic ether as the primary process for securing the crude tannin was decided upon as the method to be applied to a two kilo. lot of the drug. And thus obtained from the concentrated decoction, the crude tannin was purified by the processes already outlined and as follows: repeated solution of the substance in water, dilution to a degree that permitted the aqueous liquid to be shaken with ether without formation of a water-ether-tannin layer, and subsequent removal of the tannin from the water solution by agitation with acetic ether. These several treatments were repeated until all appreciable non-tannin had been removed and the color, texture and solubility in water of the tannin seemed constant.

With the non-tannin, some tannin always went, so that the purification was a wasteful method, although in other respects quite satisfactory. Nowhere in these processes was evidence of gallic acid revealed.

The specimen of tannin from *Rhus glabra* that is shown to this meeting represents as high a degree of purity as was attained. Except for a tendency toward tan color, the properties of this tannin are those recited by the United States Pharmacopœia as requirements for the official tannin or gallotannic acid; for otherwise it complies, in form, odor, taste, solubilities, acidity and miscibility of aqueous solution, behavior toward ferric chloride, gelatin, albumen, starch and alkaloids, limit of moisture, and freedom from non-volatile matter, to the text of the Pharmacopœia; which may therefore be taken as description of the tannin of *Rhus glabra*.

This tint of tan is not native to the gallotannic acid of commerce which comes from material devoid of red coloring, but which frequently bears a tinge of the green incident to its source.

But whether this tan color is or is not due to the red color of the drug is a question that has not been settled. Bearing upon the matter, it is noteworthy that this color tends to remain in the mother-liquor as the tannin is reclaimed from solvents through evaporation of these by distillation, even under reduced pressure; also, that during these processes, up to the point of moderate concentration there appears on the flask at the edge of the boiling liquid a porous, almost snow-white, residue as the first portion of the tannin to separate from the lessening bulk. But, without interruption, the evaporation of the solvent must be carried to completion *in vacuo* in order to get a porous or even a dry product; since, otherwise, upon admission of air to the flask, the vapor of the solvent condenses to liquid and immediately dissolves the white mass of tannin. There appears to be no simple way to get a dry and permanent tannin except to sacrifice this whiteness, which, for its duration at least, indicates a tannin of commensurate purity; for with the volatilization of the last of the solvent, the red color, which until then has kept to the mother-liquor, instantly spreads throughout the entire contents of the vacuum flask. Inferentially, there is at least that much reason to regard the tannin of *Rhus glabra* as white, which is typical of a state of purity to which gallotannic acid has been brought by others and ourselves.

That this tendency to redden is not due to formation of a phlobaphene was several times demonstrated by use of mineral and vegetable acids on appreciable amounts of the tannin. It is our impression that this tinge of red will be traced to the same coloring in the drug.

Under the effect of diluted sulphuric acid, as in the customary process of preparing gallic acid from gallotannic acid, the tannin of *Rhus glabra* yielded a white, crystalline, acid substance in interlaced needles that responded to tests characteristic of gallic acid. (A small sample of the gallic acid thus made is exhibited.)

In view of the properties exhibited by this tannin it must be regarded as gallotannic acid, which Trimble showed to be the tannin in the several plant parts of the closely related species of *Rhus* that he investigated.

That conclusion having been reached, and freshly prepared decoctions having failed to show gallic acid, it was decided to subject some of the decoction to dialysis, primarily to learn whether the crystalline, vegetable acid substances present in the drug might have an exosmotic effect on the tannin, and, secondarily, as a further test for any gallic acid possibly existent in the drug.

Accordingly, a portion of ten per cent decoction was placed on a septum, and after twelve hours the outside liquid was tested with ferric chloride and ferric acetate, neither of which showed more than a faint green color in the liquid; but these tests upon addition of a drop of a weak solution of gallic acid gave at once the characteristic blue color. The liquid on the diaphragm had retained its strong reaction to these iron compounds. The outer liquid was already acid to litmus, showing that the crystalloid acids of the drug were diffusing through the membrane, and the successive lots of this liquid exhibited acidity up to the last portion, which was free from acid reaction to litmus. This examination with ferric salts and litmus

was made every twelve hours; and at the end of eighty-four hours, the characteristic blue color reaction of gallic acid was, for the first, observable, also, the liquid was without clouding effect on gelatin solution. The fluid on the membrane still possessed semblances of the decoction which it represented, including an ability to precipitate gelatin solution.

The next thirty-six hours revealed an increase in this evidence of gallic acid formation from the tannin, and then a decrease, until a test made at the end of five days and twelve hours proved that no more gallic acid was passing. At this juncture, examination of the fluid on the septum found it to be without iron-bluing effect, although at once coagulated by either iron chloride or iron acetate solution.

This residuum was without the acidity to litmus and the astringent taste which marked the decoction; and it had lost practically all of the original red color.

Repetitions of this experiment gave the same final result, but varied greatly in the length of time required for both the initiation and the progress to completion of the change to gallic acid.

These experiments proved to be unusually interesting, as they quite conclusively explain the occurrence of any gallic acid found in decoctions and other aqueous preparations of this drug. These experiments also show that gallotannic acid is not conducted through the septum by the crystalline acid substances associated with it in *Rhus glabra*; in these instances, it changed to gallic acid, which passed through as crystalloids normally do.

It is therefore plain to be seen that gallic acid may sometimes be present in *Rhus glabra*, since it is possible of derivation from the tannin through changes likely to occur in the drying or curing of this drug; but, if present as such result, it will probably be in very minute amount. And for these reasons, statements of its presence should be qualified accordingly.

These dialytic experiments were made also on weak solutions of purified *Rhus glabra* tannin, and of a purified sample of commercial gallotannic acid, but without crystalline acids. The results were concordant in so far as the tannin from either source was concerned, both paralleling the behaviors noted for the decoction, with variations in time required for the change to gallic acid and its diffusion.

Finally, though this investigation of the tannin *Rhus glabra* has shed but little light on the digitalis problem to which reference has been made, the isolation and purification of the tannin have added something of certainty to our understanding of this constituent, and of its deportment in aqueous solution, by definitely confirming what until now was but the assumption that this tannin is gallotannic acid. And, to that extent, when such astringent is needed and circumstances permit, this knowledge justifies the present-day inclination to use official tannin in definite amount for dependable activity, instead of decoctions of drugs of the uncertain strength that attaches to sumach berries. If wanted, malic, citric or tartaric acids may be added to such tannin solution to give it the acidulous palatability that is so commonly stated as an advantage to be had from *Rhus glabra*; and a pleasing color may be imparted with suitable tinctorial substance. With such dispensing ability available to the physician, there seems to be neither need nor good reason for employing *Rhus glabra*.
