

A METHOD FOR THE ESTIMATION OF ALOIN IN ALOES.

BY C. H. BRIGGS.

In the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION, Volume 12, No. 8, page 695, Engelhardt and Crosbie call attention to the fact that there is no reliable method available for the estimation of aloin in aloes. Some twenty years ago the writer had occasion to assay aloes and devised a process which has been in use in this laboratory ever since. While this method is not ideal in that it is somewhat long and tedious, it serves as a very good indication of the amount of aloin which can be obtained from aloes in the commercial manufacture of aloin. It is with the hope that this method may be of service to others that it is offered for publication.

The method is as follows:

Dissolve 50 grams of powdered aloes in 300 cc of boiling water; to the solution add 4 cc of 25% acetic acid; mix well and allow to cool. Decant the clear solution into a 500-cc graduate and wash the resin with water by decantation or on a filter, according to its nature. To the filtrate add a solution of 10 grams of lead acetate (if the aloes is very black use 15 grams) in water and dilute to 500 cc. Shake well and filter with suction. Remove the lead from the filtrate by hydrogen sulphide and filter again with suction. Measure off exactly 400 cc of the clear filtrate representing 40 grams of aloes, concentrate *in vacuo* to about 30 cc; transfer to a 50-cc graduate washing out the flask with small portions of water until the solution equals 40 to 43 cc. Allow the aloin to crystallize for one day at ordinary temperature and then place in a refrigerator for another 24 hours. Filter with suction and wash crystals with a saturated aqueous solution of aloin until the mother liquor has been all washed out. Transfer crystals to a tared watch glass and dry. After weighing, the crystals should be exposed to the air for several hours in order that they may absorb hygroscopic moisture and then be re-weighed and thus furnish a representative assay of the aloin as ordinarily manufactured.

As this assay is dependent upon a process of crystallization, three duplicate assays should be made of each sample and the highest results reported.

ANALYTICAL DEPARTMENT,
PARKE, DAVIS & COMPANY,
DETROIT, MICHIGAN.
August 22, 1923.

THE TANNIN OF WILD CHERRY BARK.*

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In 1834, Stephen Proctor published in *The American Journal of Pharmacy* the results of an investigation which he had made of the constituents of wild cherry bark. In this report, besides the presence of certain other substances, he inferred that this drug contains tannin and gallic acid.

His analysis appears to have been made the basis of nearly every later reference by the textbooks to the constituents of this bark. But the presence of gallic acid has not been mentioned by all, nor would it likely be credited without question by any who consult the original article, for the inference was drawn contrary to the evidence afforded by an appropriate test—the iron salt he used gave a green color, not the blue one which gallic acid produces.

The astringency of wild cherry bark seems to have been attributed to "tannin," with little, if any, regard to possible presence of gallic acid.

* Read before Pennsylvania Pharmaceutical Association, 1923.

The list of constituents given for the bark almost invariably includes "a bitter principle."

While agreed on the source of the astringency, there has been a division of opinion regarding the substance which gives the bitterness. This property has been ascribed, in part at least, to amygdalin; or to a similar substance, but amorphous; or to an entirely different material. By some, the bitter principle has been considered to be the fluorescent principle.

Until the suggestion now made by the writers, no one has viewed the tannin as contributing bitterness as well as astringency; and only through isolation of the tannin could such conclusion well be reached.

Although Stephen Proctor pointed out that the tannin of wild cherry bark gave a green color and precipitate with an iron salt, the books have used the word "tannin" without reference to this fact, nor have they by other qualification distinguished this tannin from that so well known to pharmacists; while those references which have given gallic acid as a constituent have offered opportunity, if not indeed inclined, to the drawing of the incorrect inference that the tannin of wild cherry bark is tannin from which gallic acid is derivable.

It would be better if such uses of the word were more restricted or defined, especially since the United States Pharmacopœia gives the term "tannin" as synonym of Acidum Tannicum or Gallotannic Acid, and qualifies this use by stating that tannic acid is "a tannin usually obtained from nutgalls." Employment of the words "a tannin" is suggested, when mentioning the presence in drugs of substances of this class, particularly when the composition or characteristics are not sufficiently known to enable a statement which will differentiate from gallotannic acid.

The tannin of wild cherry bark has been blamed for most, if not all, of the troublesomeness of the preparations of this drug. It has been customary to say that certain menstrua extract more or less of the tannin; the opinion in general being that the less tannin removed the better the taste and keeping qualities of the preparation. The color of the cold-water infusion has been thought to be darker or lighter as the amount of the tannin was more or less. The addition of glycerin to the water used for percolating the drug in the preparation of the syrup has been opposed by some on the ground that this addition extracts more of the tannin, while others have insisted that only by this use of glycerin can a satisfactory product be had.

The tannin has been estimated by several investigators, who have employed different methods. Its amount is given as varying from 2.5 to 4.5 per cent. By some it is stated to be most abundant in autumn, while others find that "in the spring when the bark is least valuable it is richest in the tannin." The U. S. P. IX defines wild cherry bark as "the stem-bark of *Prunus serotina* Ehrhart (*Prunus virginiana* Miller) (Fam. Rosaceae), collected in autumn and carefully dried."

An estimation of the tannin in the ground bark used in this work was made by the hide-powder method. This determination, which is regarded as only approximate, showed 3.33 per cent. for the air-dry drug. Loss upon drying to constant weight amounted to 2.14 per cent.; which, taken for the purpose as moisture, would indicate 3.4 per cent. of tannin in an absolutely dry sample.

A striking feature which was recognized in the application of this method of estimation was the fact that one-third of the total hot-water soluble matter taken from the bark was absorbed by hide. It was found impracticable to make a complete extraction of the cold-water-soluble solids before evidence of change became noticeable in the liquid. Such relatively large representation of the soluble solids and the readily alterable nature of the tannin make very evident the potentialities of this constituent for trouble in attempts to make and preserve uniform preparations of this long-used drug.

Our intention in undertaking this work was to extract and purify the tannin in order to learn something of it as an isolated substance. To obtain the tannin, a quantity of ground wild cherry bark was moistened with cold (but not distilled) water, macerated for twenty-four hours, and percolated with same solvent until the liquid coming through was pale yellow. The percolate was concentrated by evaporation until a dark wine-red liquid resulted. This was allowed to cool, and was then freed by filtration from the insoluble matter which separated during concentration; this was composed very largely of phlobaphene, but there was present also a decided showing of microscopic octahedra of calcium oxalate (a substance long known as present in the bark). Aside from determining the phlobaphene nature of the bulk of the insoluble matter, no further attention was paid here to these substances.

The clear filtrate was shaken with acetic ether to remove the tannin; the solvent layer was collected and distilled under reduced pressure in order to get the tannin as a solid.

THE PRESENCE OF BENZOIC ACID.

In the last-named effort, it was found that the presence of some accompanying substance was preventing the tannin from "puffing" into the porous or spongy condition usually assumed at the stage of the distillation when all but a little of the acetic ether has been driven off. Upon continued application of the water-bath heat to thoroughly dry the residue which had been obtained, white flakes of feathery crystals were observed to be accumulating on the cork and in the delivery tube. Their behavior and form was so immediately suggestive of benzoic acid that for the moment our attention was diverted to a review of the circumstances which would permit benzoic acid to be present. Until this interference, we were unacquainted with the occurrence of benzoic acid in wild cherry bark. But, in 1909, Power and Moore reported finding benzoic acid and a number of other substances not previously known to exist in this drug. (See *Jour. Chem. Society (English)* (95), 1909, page 243; or abstract in *PROC. AMER. PHARM. ASSOC.*, 1910, page 208.)

Our reflection ran as follows: why might not benzoic acid be present here, since it results from oxidation of benzaldehyde, and this tendency of benzaldehyde is a constant proclivity? Benzaldehyde was present in the original percolate, but not, if judged by odor, in the concentrated liquid. Perhaps it has been oxidized to benzoic acid, but here is evidence of a much larger amount of the acid than that source and change could supply even if all of it were oxidized, which is unlikely, because a portion would have been volatilized unchanged from the liquid as this became warm. From where, then, does this amount of it come? The simultaneous production of benzaldehyde and hydrocyanic acid when the bark is moistened with water is well proved. There has been widely quoted the statement that the dry

bark of commerce does not contain either hydrocyanic acid or benzaldehyde, and an examination of the dry bark, without moistening, strengthens this impression. However, Stevens while collecting the bark found it to have an odor of bitter almond. In this instance, the odor would scarcely be attributed entirely to hydrocyanic acid, but rather to both it and benzaldehyde as the products of the well-known reaction which takes place when the dry bark is moistened; for is not the bark on the tree functioning under the influence of its life moisture, its sap? The removal of the bark interrupts the plant process by discontinuing the moist condition essential to it. The loss of the odor of bitter almond when the fresh bark is dried must be due to volatilization of the hydrocyanic acid and benzaldehyde, or to alteration into something which differs in odor. The extremely volatile hydrocyanic acid may well be regarded as dissipated with little or no change, but the less volatile benzaldehyde would be inclined to undergo more or less oxidation to benzoic acid, besides in part passing off unchanged. And, is it not likely that this change is in constant operation in the living bark, with production and accumulation of benzoic acid therein? As regards the detached bark, the contained benzaldehyde perhaps dries off in part, but because its comparatively minute quantity is through relatively wide distribution so effectively given surface exposure, it is quite as likely oxidized *in situ* to benzoic acid. This conception appears as highly probable, indeed. Again, when the dried bark is chewed, or otherwise moistened, with cold water, does not the bark merely resume (to the extent possible with the now limited supply of the essential materials) the production of the substances which gave the living bark its odor of bitter almond? Therefore, would not the bark when collected probably contain benzoic acid, and would not the amount of this be increased by permitting the interrupted process of its production to resume by restoring the moisture necessary to this function? These crystals might well indeed be benzoic acid.

And subsequent examination showed them to be benzoic acid.

The foregoing thought led also to recognition of the probability that benzoic acid may well be expected to be found in every plant substance, wherein benzaldehyde has existed—the scope of which suggestion points to many possibilities of its occurrence.

In further examination of the thought that the greater part of the benzoic acid must exist prior to concentration of the percolate, it was recalled that considerable needles of benzoic acid had been observed to separate when some of the final portions of the water percolate, which had come through with but little color, had been boiled down. Their appearance was made long after the odor of the percolating liquid had ceased to suggest the presence of benzaldehyde. But, to learn of the possible influence of the heat used for evaporation of the percolate as a factor in the production of benzoic acid, another lot of the ground bark was macerated and percolated with cold water. The percolate was not concentrated, but at once shaken with acetic ether. The amount of benzoic acid which was encountered in this process was apparently as great as that in the case of the concentrated percolate. It thus became more evident that the bulk of the benzoic acid exists in the bark before the moistening process.

Finally, the existence of free benzoic acid in the bark was proved by direct extraction with ether and with chloroform; removal of solvent; treatment of ex-

tracted matter with water; shaking of aqueous solutions with ether and with chloroform, and separation and evaporation of these solvents.

Returning to the process of extraction of the tannin from the concentrated percolate, the residue left upon evaporation of the acetic ether was, at first, red, resin-like in appearance, nearly transparent, but, when it had become cold, was found to be largely composed of needle-shaped crystals. Each successive shaking of the concentrated percolate with acetic ether gave a similar mass; and these residues gradually showed an increasing proportion of the crystals. These residues were stirred with cold ether for the purpose of removing from the tannin such substances as would be dissolved. The greater part of the benzoic acid entered solution. Along with it, the ether dissolved an appreciable amount of a plastic, red material. When warm, this substance seemed to mix with the benzoic acid in such way as to obscure its crystals; but when cold, the needles became plainly visible throughout the plastic, resin-like material. Boiled with water, this mass gave up benzoic acid, which readily crystallized from solution upon cooling; but not without the red portion entering solution to a considerable extent, for concentration of the reddish liquid so obtained resulted in the settling out of a dark red substance, which, when cold, varied in consistency from fluid to semi-solid.

Both the hot- and cold-water solutions of the ether-dissolved substances displayed decided fluorescence. No further work was done on the last-named materials.

Under treatment with cold ether, the residue containing the tannin took at first the form of a granular, red, porous powder; but with accumulation of moisture, through constant condensation brought about by the cold resulting from evaporation of the ether, the undissolved matter became plastic. To relieve this situation, the residue was redissolved in acetic ether, and reobtained by distilling off the solvent *in vacuo*. This material was then stirred with cold chloroform; to appearance, it removed a trifle of yellow color. Upon recovery, the chloroform left an oily looking liquid. When this liquid was boiled with water, it separated into much benzoic acid and a small amount of oily drops. The solutions so obtained showed much fluorescence. Beyond identifying the benzoic acid, nothing was undertaken with these materials.

When the now partly purified tannin had dried free from chloroform, it was found to be a finely divided, reddish yellow powder. This powder was treated with water which left a considerable undissolved portion. The aqueous solution was clarified by means of paper pulp and filtered. The clear reddish amber solution was shaken with acetic ether, its layer separated and the solvent removed by distillation *in vacuo*. The residues thus had were subjected to separate successive treatments with ether and chloroform as outlined; after which, the tannin was again dissolved in water, and the whole process of purification repeated over and over, until the ether and chloroform failed to remove appreciable matter.

Judging by the bulk of benzoic acid which separated as this work was carried on, it is felt that there may be quite as much of free benzoic acid in wild cherry bark as there is of the tannin. Though now but speculation, the possible presence of benzoates also is a matter for investigation.

The many repetitions of the foregoing process of purification finally gave the tannin in straw-yellow or faintly pinkish porous masses, with satiny luster or

highly iridescent effect; these spongy masses were readily reducible to powder. The variations in color depended upon the extent of alteration which had occurred when the acetic ether solution was evaporated *in vacuo* to obtain the tannin in solid form. Snow-white porous masses which at first formed in the distilling flask, when the solution in acetic ether had reached a degree of concentration where solid showed above and about the boiling liquid, are believed to indicate the true color of this tannin; but, while the vaporization of the last portion of the solvent was being effected, the color of these masses changed to straw-yellow or faintly pinkish, it is thought, because of slight decomposition of the solvent with production of acetic acid, which is now known to exert an altering effect on the tannin. And it does not seem improbable that traces of benzoic acid, still adhering, caused or increased this behavior of the acetic ether, or this effect upon the tannin. It was not found to be practicable to disconnect the flask and remove the white masses, for immediate condensation of the acetic ether vapor caused their instant disappearance through solution. Nor did there seem to be any other way of overcoming this objectionable action which invariably rendered part of the tannin insoluble in cold water.

The tannin of wild cherry bark extracted and purified by the given method is a bitter as well as astringent substance. It was readily and completely soluble in hot water; when treated with cold water, a quantity entered solution, at once, imparting an amber color, while a greater amount remained undissolved as a peculiar, plastic phlobaphene. The one per cent. solution made with hot water had an amber color. Solutions of the tannin in hot water, when allowed to cool, acquired yellowish turbidities. Both hot- and cold-water solutions gave white precipitates with gelatin. When cold, the solutions of the tannin gave dark green colors and precipitates with ferric chloride and ferric acetate, the green shades becoming blackish on standing, or upon use of excess of reagents. Lead acetate caused a canary-yellow precipitate in the solutions. The solution of the tannin, like the substance itself, had both bitter and astringent tastes, which were appreciable in this order of effect, the bitterness was experienced first; it persisted, almost to the exclusion of the astringency, which was felt only after the bitterness had passed; recurrences of the taste seemed to be entirely those of bitterness. Blue litmus paper was intensely reddened by the solutions. The amber colors of the solutions were destroyed by addition of either diluted sulphuric, oxalic or benzoic acid; no fluorescence was manifested thereupon. When portions of solution, acidified with these acids, were heated in a boiling water-bath, reddish colors developed, and later red substances were deposited. As control, an unacidified portion of the tannin solution showed only the slightest reddening. This or a similar change was found to take place with benzoic acid even in cold dilute solutions, after a few days' standing. This effect of benzoic acid on the tannin is noteworthy by those concerned with the preparations of wild cherry bark.

Six per cent. acetic acid, at first, behaved toward the tannin like cold water, but soon dissolved the substance entirely. Furthermore, the insoluble substance produced by contact with water was found to be at once dissolved by six per cent. acetic acid. When solutions made with acetic acid were heated on a water-bath, no precipitation took place, but their amber colors were reddened. Both weak and strong acetic acids dissolved the red substances referred to above as produced by the action

of diluted sulphuric, oxalic or benzoic acid. The tannin was at once freely soluble in 36% acetic acid with formation of amber-colored solution; dilution of this fluid, or of any other containing acetic acid, with water did not cause precipitation. The tannin was readily soluble in alcohol, weak or strong; insoluble in ether free from alcohol, otherwise soluble; insoluble in chloroform or benzol. The tannin was freely soluble in glycerin, as were also both water-produced and acid-produced insoluble red phlobaphenes; these glycerin solutions remained brilliant upon dilution with water.

When the tannin had been found to be bitter as well as astringent, attention turned to the question whether this constituent contributes all or but part of the bitterness of the drug. In an effort to get information on this matter, infusions of the bark (some representing as much as 30 per cent. of the drug) were treated with well-washed hide-powder. The following facts were noted. Before maceration with hide, the freshly prepared infusions (light wine-red or reddish amber in color, when viewed in half-liter flasks) were immediately and decidedly acid to litmus paper; pronouncedly bitter and astringent as already described. After maceration with two successive treatments of hide-powder, these liquids, except for the distinct blue fluorescence, were almost entirely free from color; the odor was changed, but not removed; acidity to litmus paper was shown only upon standing; neither bitterness nor astringency could be detected; ferric chloride gave but the slightest greenish tinge that could have been discerned; there was no precipitate with either gelatin or lead acetate solution.

These treatments with hide-powder were made on infusions prepared with both hot and cold water, but the hot liquids were allowed to cool before being brought into contact with the hide. The results were uniform when the infusions were used without delay; but not so when old infusions were treated. A single treatment with hide-powder did not appear to effect complete removal of the tannin within a period not likely to admit of the variance just referred to as attendant upon delay. The several steps in the treatments with hide-powder were controlled by using an equal amount of this material with water alone. All detannated liquids were filtered before results were noted.

Some of the filtered, clear, fluorescent liquid from the hide treatment was shaken with chloroform; the layer of the latter separated, and the solvent evaporated. A small amount of white crystalline residue was obtained. This was but sparingly soluble in water; the solution displayed blue fluorescence. Upon the addition of ammonium hydrate, to alkalinity, a very high degree of fluorescence resulted. Appearances, however, pointed to the probability that the greater part of the crystalline residue was benzoic acid. The natural fluorescence in the infusions of the bark, or other solutions which exhibit it, should not be confused with like phenomena artificially brought about with outside agents, such as alkalies.

Because of the presence of so much calcium oxalate in the concentrated percolate, an effort was made to learn if free oxalic acid was present in the bark. To avoid any opportunity for it to combine with calcium or other bases, distilled water was used to percolate the bark. After concentration, the liquid was detannated by maceration with well-washed hide-powder, filtered, then made alkaline with ammonium hydrate, again filtered, then acidified with hydrochloric acid, again

made alkaline with ammonium hydrate, acidified now with acetic acid, and tested with calcium acetate solution. There was no precipitation by the calcium compound at this point to indicate the presence of oxalates; but that the conditions were favorable to their detection was proved by a control test, which, besides other matters, took into consideration the presence of free benzoic acid in the solution. The result of the experiment would seem to indicate that uncombined oxalic acid is not present in the bark.

GALLIC ACID NOT PRESENT.

As ferric chloride caused no blue color in the infusion after the latter had been detannated, the absence of gallic acid is regarded as now proved, especially since the addition of a minute amount of this substance, by giving the characteristic blue color, showed that conditions were favorable to its detection. Gallic acid should, therefore, be omitted from the list of constituents of wild cherry bark.

That the fluorescence of the infusion was not removed by hide-powder while the astringency and bitterness were completely absorbed are facts clearly established by that treatment; and, since fluorescence remained in the fluid from which all bitterness and astringency had been removed, it seems proper to regard the sources of these properties as different. Again, since the purified tannin was bitter as well as astringent, and no bitterness or astringency remained in the fluid from which all tannin had been absorbed (as proved by means of ferric chloride, gelatin and lead acetate), the conclusion that the tannin furnishes all of the bitterness and astringency seems justified. And, if, in the light of the determined characters of the tannin of wild cherry bark, we review the descriptions of efforts which have been made to isolate "a bitter principle," and compare the comments on the observed nature of the substance sought, there seems to be every reason to believe that, because of the inherence of bitterness in the tannin, it has all of the qualifications needed to elude isolation by the methods which have been employed to find another substance representative of this property of the drug.

The tannin of wild cherry bark having been found to be bitter, there arose the question: Is it the bitter tannin found in red and pale rose petals—the drugs coming from the same natural order of plants? The answer is in the negative, for the tannin of wild cherry bark does not yield the peculiar burr-like aggregates characteristic of the rose tannin when its solution is boiled with acids.

SUMMARY.

The results of this work may be summarized as follows:

Gallic acid is not present in wild cherry bark.

Cold water extracts only part of the tannin, for there is also a considerable proportion of cold-water-insoluble phlobaphene.

The tannin of this bark, as pointed out by Stephen Proctor, is an "iron-greening" tannin.

This tannin yields a red phlobaphene by action of acids, including that of benzoic acid.

There is a considerable proportion of free benzoic acid in this bark, the occurrence of which was first reported by Power and Moore.

The occurrence of benzaldehyde in living plant material may well be taken as indication of possible presence of benzoic acid in any subsequently dried form of the part.

The tannin is the bitter principle as well as the astringent constituent.

The bitter principle, which is the tannin, is not the fluorescent principle.

It permits of better understanding of the troublesomeness of the preparations of wild cherry bark to know that the free benzoic acid contained in the drug decomposes the cold-water-soluble tannin with production of cloudiness or sediment; and, because of this action, the blame, which in the past has been placed entirely upon the tannin, may now be equally accorded to the benzoic acid.

The addition of glycerin to the water used for extraction of the bark in the preparation of the syrup will now be fully appreciated as warranted by the association in the bark of the tannin and benzoic acid, as well as by the teaching of experience with its use.

Also, due allowance must be made for variation in the products of those vegetable drugs which bring into the course of preparation the resumption of an interrupted plant process. For this reason, variation in the preparations of wild cherry bark must be expected, even though glycerin or acetic acid (both solvents for the decomposition products of the tannin by the benzoic acid) or both, be employed in the menstruum.

A STUDY OF PACIFIC COAST PEPPERMINT.

BY C. W. JOHNSON AND ROBIN WILKES.

During the last two years a study of Pacific Coast peppermint has been made at the University of Washington. This study was made to show the advantages that Washington and Oregon have over eastern states in the production of peppermint oil. The age of the industry in the northwest is very young, but old enough, especially in Oregon, to show us that it is a very profitable and promising industry in these parts of the United States.

The peppermint industry appears to have been introduced into the United States by the early settlers of New England. The plants were first grown in Wayne County, New York. Then gradually the industry extended west to northern Ohio and from there into Indiana and Michigan. At the present day there are about 25,000 acres under mint cultivation in the United States, nearly all of which are in the states of Michigan, Indiana, New York and Oregon. This country furnishes about 250,000 pounds of peppermint oil yearly and, at the present time, we also import 88 per cent. of Japan's crop.

Peppermint was first introduced into Oregon about 1908 by Mr. O. H. Todd of Eugene and it has been produced in the state for the last fifteen years. Mr. Todd imported the roots for the first planting from England. Oregon is especially adapted to peppermint cultivation because it possesses the valuable and rich beaver-dam land of the Willamette Valley. This swamp-like land is rich in humus and retentive of moisture but fairly open in texture and when well drained, either naturally or artificially, is most suitable for peppermint cultivation. The district of Lake Labish contains several hundred acres of this type of land. The average