

- (1) Menthol or borneol are the only alcohols present; that
- (2) Menthyl or bornyl acetates are the only esters present, and
- (3) That menthol or borneol are the only constituents reacting with acetic anhydride.

Inasmuch as none of these assumptions is strictly correct,¹ all of the calculations incident to correction of assay data could readily be obviated, by employing the simpler expedient as proposed by (E. J.) Parry.² Proceed in the following manner with an oil containing both esters and alcohols:

(I) Estimate the amount of combined alcohols by a preliminary saponification of the esters.

(II) Saponify 20.0 Gm. of the sample with alcoholic KOH solution, add H₂O and wash the separated oil now free from esters, dry over KHSO₄ and

(III) Estimate the total alcohols (now all free) in 2.0 to 5.0 Gm. of (II).

(IV) The amount of alcohols as found in (III) refers to the *saponified* sample; to calculate the total alcohols in the original sample necessitates a correction by allowing for the reduction in weight by saponification of the original oil. This reduction can be ascertained by calculation from the amount of alkali used for saponification in (I).

(V) Deduct the combined alcohols found in I from the total alcohols determined in (IV); the difference represents the amount of free alcohols.

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FURTHER STUDY OF THE TANNIN OF HEUCHERA AMERICANA, LINNE.*

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This paper is intended to review, and will be found to revise in some particulars, the results of an investigation of the root of *Heuchera Americana*, published by one of us (J. C. P.) in *American Journal of Pharmacy*, April 1891.

Working then with the methods in general use for isolating and purifying tannins, as outlined in that paper, there was obtained a dark brown mass, which was but slowly and only partly soluble in cold water; and while readily soluble in hot water a large proportion of the dissolved matter separated when the solution was allowed to cool.

¹ This is readily apparent on consideration of the complex character of American oil of peppermint in particular, which according to "Schimmel & Co.'s Report" (April 1897) consists essentially of the following:

Acetaldehyde,	Limonene,
Iso-valeric aldehyde,	Menthone (C ₁₀ H ₁₈ O),
Dimethyl sulphide,	Menthol,
Amyl alcohol,	Menthyl acetate,
Iso-valeric acid,	Menthyl iso-valerate,
Pinene,	a-lactone (C ₁₀ H ₁₆ O ₂),
Phellandrene,	Cadinene.
Cineol,	

² "Allen's Commercial Org. Analysis," 5th edition, Vol. IV, p. 596.

* Read before Pennsylvania Pharmaceutical Association, 1927.

The color of this educt and its solubility behaviors toward water taken into consideration with its reactions to gelatin and ferric salts marked it plainly as a phlobaphene. A specimen of a similar substance from the present work is here shown¹ for the opportunity to contrast the result of 1927 with that of 1891.

Within two years after the publication of these results, Henry Trimble and Josiah C. Peacock adapted certain suggestions, which had been made by others in the literature of the tannins, into a decidedly improved and simplified method of isolating and purifying certain of these principles.

The most noteworthy of these utilizations was that of acetic ether (ethyl acetate) for the removal of tannin from water solution into which it is repeatedly brought as a means to gradual separation from the less soluble plant constituents, especially from resins and cold water insoluble phlobaphenes.

Although such use of acetic ether had been proposed by Löwe in 1872, through lack of application, its several very practical advantages over the troublesome and often disappointing fractional precipitation with lead salts (which procedure was so thoroughly ingrained in plant chemistry methods) had not been recognized. The prime utility and function of the acetic ether is simply that of an immiscible liquid with selective solvent action, while its other particular advantage is its easy manageability in recovery of solvent to obtain the extracted matter.

Reports on the satisfactory use of this simplified method were made in the following joint contributions by Trimble and Peacock: "Canaigre Tannin," *A. J. P.* (April 1893); and "The Preparation of the Oak Tannins, etc.," *PROCEEDINGS A. PH. A.*, p. 110 (1893), and *A. J. P.* (Sept. 1893); while other references to its successful use and practical value were made by Trimble in the second volume of his monograph "The Tannins" published in 1894. Also during the next few years, it was used by him and by his students on a considerable variety and number of tannin-bearing materials as attested by the pages of the *American Journal of Pharmacy* over that period.

To return now to the stated object of this paper, as the proximate source of phlobaphene is expected to be a cold water or plant-sap soluble tannin, ever since the aforementioned adoption of acetic ether, it has been thought desirable to try the process on a commercial sample of this drug in order to ascertain the presence of a tannin of that nature; especially since, at that time, observation of the freshly collected root while drying noted the likelihood of much of the phlobaphene of the air-dried drug being an alteration product which is formed as the moisture is gradually dissipated.

For the purpose of this work, a quantity of the identified drug was reduced to dust-like powder. In milling, there was a loss of approximately 8 per cent of unground drug, which was discarded.

The powdered drug showed 10.55 per cent of moisture and 11.11 per cent of ash.

To examine the drug for cold water soluble tannin, one kilo of the dust-like powder was macerated at room temperature with five times its weight of water, with frequent stirring during twenty-four hours. The mixture was then strained and the resulting fluid filtered to obtain a brilliantly clear cold water infusion.

This infusion was shaken with several successive portions of acetic ether, which

¹ Shown at the meeting.

removed tannin and some other substance that at this stage prevented the tannin, in recovery of solvent, *in vacuo*, from drying out as a porous or puffed-up residue; instead, a varnish-like formation was obtained.

By treating this residue with cold water, filtering with the aid of paper pulp to clear, shaking out with acetic ether and removal of this solvent by distillation under reduced pressure, the quality of the tannin through frequent repetition of this process was gradually improved until the degree of refinement exemplified by the sample submitted herewith was attained.

Next, this purified tannin was dissolved in cold water and the resulting solution shaken in succession with petroleum ether, chloroform, benzol and ether, in attempt to further purify the tannin, especially through removal of crystalline acids which are frequently associated with the tannins. But only a minute amount of such substance was removed by any or by all of these solvents. From aqueous solutions this tannin is not removed in any considerable quantity by ether. After these treatments the tannin was again taken from the water solution by means of acetic ether. The sample here shown was obtained by evaporating the acetic ether from such solution, after exhaustion of air from the flask, and thereafter dissolving the then similar residue in a small amount of acetone and repeating the distillation, *in vacuo*, as a means of expelling an acetic odor which clings to tannin that is dried out of acetic ether.

The outcome of this experiment clearly shows that the root of *Heuchera Americana* yields to cold water a tannin that is distinctly different in appearance and solubility from the phlobaphene material which was reported in 1891, and which was the best that the then used methods would afford.

The specimen from the present work conforms to the description given in sensible properties by the U. S. P. X, for gallotannic acid, from which tannin, however, it differs in certain physical and chemical effects as hereinafter mentioned. If this specimen of it is contaminated at all with crystalline or other substance, the amount of such is extremely minute.

Like the official gallotannic acid this tannin from *Heuchera Americana* is readily soluble in cold water, hot water, glycerin, acetic acid, alcohol, acetone and acetic ether. It is insoluble in chloroform, benzol or petroleum ether. It is dissolved by both ammonium and potassium hydroxides.

Its aqueous solutions are acid to litmus. They are promptly reddened and made turbid by addition of hydrochloric acid through production of phlobaphene; but are not so affected by oxalic, tartaric or gallic acids, nor by acetic acid in which, even in weak dilutions, all phlobaphene formations encountered in this work were soluble, the resulting solutions withstanding addition of water without precipitation.

Its aqueous solution gives flesh-colored precipitates with gelatin, dark-blue colors and precipitates with ferric chloride solution, and whitish flocculent precipitate with lead acetate. An excess of the ferric reagent, or a solution of it that contains uncombined hydrochloric acid produces greenish colors and precipitates.

This tannin decomposes barium carbonate with production of a soluble salt of that base.

Such are some of its properties in common with gallotannic acid, but this tannin from *Heuchera Americana* gives a precipitate with bromine water; it also gives

a whitish precipitate with calcium hydroxide which upon addition of more of the reagent becomes bluish or slate-colored and finally assumes a reddish-brown or rusty color. Again, unlike gallotannic acid, it dissolves readily and freely in isopropyl alcohol.

The ease and degree of its solubility in ether appear to depend upon its state of hydration, or it may be that the occurrence of solution depends largely upon the fact that the cold induced through the evaporation of the ether applied to it brings about the condensation of sufficient moisture to cause the tannin to dissolve. An exact explanation is difficult to reach, since this tannin upon application of temperature above 100° C. tends readily toward phlobaphene formation. On account of that, it is not feasible to dehydrate the tannin without experiencing some change to phlobaphene; but, when thus dried, the tannin was much less disposed to dissolve in ether.

While this tannin, in common with gallotannic acid, gives blue colors and precipitates with ferric salts, it differs under the effect of mineral acids in yielding a red insoluble phlobaphene as shown by the following experiment: Five Gm. of the tannin were dissolved in water, this solution shaken with ether to remove any contained crystalline principles (such as the acid derivatives of phenols which are frequently found associated with the tannins); the liquid was warmed to dissipate the last portions of ether, and then mixed with enough hydrochloric acid to represent 10 per cent of HCl gas in the mixture; after which the liquid was heated on a boiling water-bath until the brick-red phlobaphene that was gradually formed seemed to have reached a fixed amount. The filtered liquid even upon cooling had a wine-red color; upon shaking it with petroleum ether, chloroform and ether in succession, only a trifling amount of crystalline matter was extracted, which result would seem to indicate the likelihood that this substance (which was revealed at several junctures in the work, but never in more than minutest trace) is not an hydrolysis product of the tannin but evidently a principle that exists in the dry drug. From any of its solvents, it appeared almost invariably as needles radially arranged or interlaced. Similar crystals will be mentioned with other phases of this work. The brick-red phlobaphene was soluble in acetic ether, acetone and alcohol.

When an aqueous solution of this tannin is saturated with sodium chloride, the tannin is thrown out as a flesh-colored precipitate, which remains undissolved in contact with acetic ether, but the jelly-like formation that obtains upon addition of the acetic ether, when drained to remove any superfluity of the last, is found to be almost entirely soluble in acetone, as elsewhere mentioned for other phlobaphene formations from this drug.

In 1891, it was customary to group with gallotannic acid those tannins which give blue colors and precipitates with ferric salts. And, on that basis, at that time, colorless crystalline extractive which was removed by ether, sp. gr. 0.725, and which in water solution gave a blue color with ferric salts was generally interpreted as gallic acid. As the result of following these impressions, it was stated in the paper then published that such ether extracted traces of gallic acid from the freshly gathered roots and a greater proportion from the dried root.

But, upon finding that the cold water soluble tannin of this drug is under certain circumstances soluble in such ether, it at once became desirable to review the

formerly used method and the statement as regards the finding of gallic acid.

Later experience has taught that, in the presence of a tannin which gives a blue color with ferric salts, it is extremely difficult, if indeed possible, by use of such ether alone, to determine with certainty the presence or absence of minute amounts of gallic acid, since agitation of aqueous solutions containing only tannin with immiscible solvents disposed to remove gallic acid may remove enough tannin through inclusion of some of the aqueous layer by the other solvent to give a blue color, and thereby mislead. And, now with the knowledge before us that the cold water soluble tannin of *Heuchera Americana* is capable of solution in ether, sp. gr. 0.725, it is clear why evidence of gallic acid was then found. But since other evidence hereinafter adduced shows far more plainly that gallic acid is not present in the drug, it is fully realized that its presence as stated 1891, was a wrongly drawn inference and one that we would now correct.

Moreover, the occurrence or production of phlobaphene with accompaniment of gallic acid has grown to be more and more improbable because correspondingly inconsistent with observed fact, as acquaintance with a greater variety of tannins has been acquired.

Again, in 1891, precipitation of tannin with gelatin and weighing of precipitate was the usual procedure for determining the amount of tannin. But soon thereafter the hide-powder method of assay was adopted as preferable for comparing tanning values.

As the hide-powder method, which frequently sheds light on the constituents of tannin-bearing materials, had not been applied in the previous work, it was turned to as another approach to the question of the presence of gallic acid, and toward the settling of this matter it proved to be particularly helpful.

For this purpose, infusions of ten per cent of the dust-like powder was prepared with both cold and hot water. The cold infusion was made by repeated stirring during six hours. The hot extraction was had by boiling the powder with water for one hour. Both liquids were filtered. The hot one was allowed to cool; whereupon it deposited considerable sediment and was therefore clarified with the aid of paper pulp.

Both solutions were brilliant, wine-red (that prepared with hot water had the more color), had odor of the drug, were strongly astringent and showed a strongly acid reaction to litmus. Both possessed a pronounced disposition to froth when shaken.

Both solutions gave abundant flesh-colored precipitates with gelatin solution; blue and blue-black colors and precipitates with ferric chloride; and heavy pale-buff precipitates with lead acetate. Hydrochloric acid slowly caused precipitation in both liquids. Acetic acid did not precipitate either of the solutions.

The cold water infusion showed 19 per cent of dissolved solids; while the clarified hot water decoction was found to contain 20 per cent. From the cold water preparation, hide-powder removed solids which indicated 10.85 per cent of tannin or tanning value; from the hot water solution 12.82 per cent. All four of these figures refer to the air-dried powder.

The detannating effect of the hide-powder was rapidly realized and was very striking. Upon contact with the hide, the wine-red liquids almost instantly lost their colors; after maceration for an hour, the fluids were practically water-white,

were found to have given up their astringency and almost all of their taste, as well as approximately five-sixths of their original titratable acidity. Both the original and the hide-treated liquids rapidly decolorized potassium permanganate solution, the former to several times the extent to which the latter did this.

When the fluids from this hide treatment were filtered entirely clear, they showed no precipitate with gelatin, but a slight precipitate with lead acetate solution, and only a very slight and extremely fleeting color reaction with ferric chloride, as follows: First, a mere flash of purple or violet, changing instantly to blue and passing with almost equal quickness into green or greenish yellow. The blue color produced by ferric chloride and gallic acid is distinctly different from this behavior through being appreciably permanent.

The liquids thus detannated were again treated with hide-powder to ascertain whether all reaction to ferric chloride would be removed by further effect of this agent. Such second treatment made no difference in the behaviors toward gelatin, lead acetate or ferric chloride, thus necessitating the attribution of these behaviors to causes other than tannin, unless they are due to a trace of tannin that is held in solution by other constituents of the drug.

However, this result is especially interesting as evidence of the absence of gallic acid. In addition there will be other evidence which shows that gallic acid is not present in this drug.

Attempts to observe by dialysis the behavior of infusions of the root and of solutions of the purified cold water soluble tannin were vitiated through prompt formations of phlobaphene, presumably from effect of some disturber in the parchment that was used as septum; but, in every instance, whether with infusion or solution of the tannin, the outer liquid promptly showed, with ferric chloride, the same or a similar reaction as that described under the experiment of detannating: namely, a fleeting tinge of blue (here usually without precedence by purple or violet) that quickly passed into a greenish or into a yellowish tint, when the latter, being little more than what the color of the reagent itself imparted.

To study further the nature and amount of the substance to which this ferric chloride reaction is due, and to learn more regarding the behavior of the tannin constituents toward solvents, as also to note the amounts of extractive removed, a kilo of the dust-like powder was exhausted with the following solvents: chloroform, ether, acetic ether and acetone. These were used in the order given; the drug being freed of the adhering solvent before the next was applied.

Chloroform Extract.—The chloroformic percolate was of a pinkish color, and upon evaporation left a blood-red residue amounting to 0.75 per cent of the drug. When this extract was treated with petroleum ether of low boiling point one-half of its amount dissolved, and upon evaporation of this solvent, was found to consist largely of fatty semi-solid of dark amber color. This mass was entirely soluble in ether, and water removed from it a minute amount of colorless needle-shaped crystals, an aqueous solution of which did not precipitate gelatin but gave with ferric chloride the transient color reaction which has been described in mention of similar crystals obtained by other means.

The frothing of the fatty portion when shaken with water strongly suggested the presence of saponin.

The other half of the chloroformic extract was a dark reddish brown residue

of mixed substances, which were entirely soluble in benzol and alcohol, but insoluble in water; behaviors which indicate resinous matter.

Ether Extract.—Applied after chloroform, ether U. S. P. removed but a trifle of extract, approximately 0.07 per cent, or one-tenth of that removed by chloroform. The percolate exhibited but little color until concentrated by distillation for recovery of solvent, when it showed a golden-yellow, varnish-like residue. Petroleum ether dissolved about one-fifth of this residue; after which benzol removed but a trifle; and water applied next took into solution the minute amount of tannin which had been extracted.

It is interesting to note that while U. S. P. ether extracts but a minute amount of tannin from the air-dried drug, the purified cold water soluble tannin will dissolve in that ether, as already mentioned.

Acetic Ether Extract.—Following chloroform and ether, acetic ether of 99 per cent strength was applied. The percolate was of light amber tint and yielded but 0.26 per cent of a flesh-colored powdery, scaly or granular residue upon recovery of solvent. Toward water this substance behaved like phlobaphene, being but slightly soluble and imparting but a tinge of color to the liquid but which gave reactions for tannin with ferric chloride and gelatin. This extractive was not soluble in benzol, but dissolved readily in acetone or alcohol.

Now, strangely enough, the purified cold water soluble tannin is instantly and freely soluble in acetic ether, as though again, as in the case of ether, pointing to hydration as a likely essential to solubility.

Acetone Extract.—After extracting the drug with the three mentioned solvents, acetone U. S. P. was applied. From the start of percolation, the solvent had a sherry-wine color, but this gradually decreased in intensity until the percolate was very pale amber. By this time but little solids were being removed, as learned by recovering of solvent from the successive lots of percolate. Up to this stage, acetone had extracted 7.17 per cent of solids, which later proved to be almost entirely cold water soluble tannin.

But at this juncture, with everything pointing to completion of practical exhaustion, the percolate suddenly regained its original color and in greater depth, and by continuing the process until exhaustion was again apparently accomplished, fully three times as much solids were removed. As extraction progressed, the solids so removed showed increasing tendency toward phlobaphene nature.

From the extractive which followed this change in the action of acetone, petroleum ether removed a minute amount of substance that formed colorless needles, which were soluble in water and gave the transient bluish and greenish colors to which reference has been made.

The extractive obtained by means of acetone before this unusual occurrence was of a slightly reddish color, porous and had the appearance of a mass of yellowish white tannin with some admixture of reddish phlobaphene; and this it proved to be upon treatment with water which dissolved it with the exception of 0.40 per cent of phlobaphene and resin.

After this strange occurrence, the continuance of percolation to exhaustion was a protracted process. It was carried on over several weeks during the greater part of which period there was removed an apparently fixed quantity of dissolved solids per volume of solvent, which, though trifling in each portion of percolate,

totalled a considerable amount, and seemed to imply some peculiar change in the drug with production of extractive that was but sparingly soluble in acetone, each lot of which was accordingly saturated—the most plausible explanation for the unending supply of what upon recovery of solvent appeared to be a mixture of porous phlobaphene and a varnish of other substance.

Percolation was eventually discontinued for the reason that only a minute amount of tannin which would dissolve in water was being extracted. But it should be noted that acetone was still removing matter which, treated with cold water and this solution filtered, continued to precipitate gelatin and react characteristically also with ferric chloride for tannin.

In order to confirm this peculiar behavior with acetone, another portion of the original drug powder was percolated with another lot of chemically pure acetone; the result was identical. As this behavior was suspected as due to a gradual accumulation of water from this solvent with an ultimate disruption of the cells of the drug, or some similarly occurring other change that favored extraction, another portion of the original drug powder was moistened with just sufficient water to make it damp to the touch, and then percolated with acetone. With this preliminary treatment made, acetone forthwith exhausted the drug to the limit of its solvent effect.

In the purification of the tannin extracted by means of acetone, a phase was reached wherein acetic ether perceptibly lessened or lost its ability to remove all of the tannin from water solution; the situation seems to indicate that there was a portion of the tannin (obviously most closely approximating the cold water soluble tannin) which was readily removable while another portion (of more distinctly phlobaphene nature) was not readily given up to acetic ether by the water solution.

When such juncture was reached, the addition of sodium chloride to saturation instantly brought about phlobaphene formation and precipitated the phlobaphene, which remained undissolved in contact with acetic ether, but which dissolved almost completely in acetone, and, upon recovery of same, showed as a porous residue with red-brown color that tended to deepen with each repetition of the process.

After prolonged extraction with acetone, the marc was freed of this solvent and then treated first with cold water and thereafter with boiling water. Both treatments removed tannin-reacting matter as shown by gelatin and ferric chloride. There was an abundance of starch in the hot water extract.

It is remarkable that while acetic ether readily dissolved every form of water soluble tannin so far obtained from *Heuchera* and most of the phlobaphene formations, still when it is applied directly to the drug it removes but little of either, as stated. And, again, while ether dissolves the purified cold water soluble tannin, it does not dissolve the phlobaphene forms which result therefrom, nor, as pointed out, does it extract any considerable amount of either form of tannin from the drug. But, as proved by actual trial with both acetic ether and ether, if the powdered drug is first moistened with water, as in a similar experiment mentioned for acetone, tannin is removed in far greater proportion—further confirmation of hydration as requisite to these solubilities.

As compared with these behaviors acetone eventually extracts and, subsequent to any purification or phlobaphene formation, dissolves all forms of tannin from *Heuchera*, which (as the specimens show) vary in color and physical form assumed

from pale-buff or pinkish flesh-colored powder through porous masses (that possess the appearance of incinerated ferric oxide) to a condition of transparent gum-like formation which suggests kino or red (eucalyptus) gum.

The commercial ether, sp. gr. 0.750, used in 1891 to extract the drug for tannin was favored by the presence of the ten per cent of alcohol which it contained, as all of the tannin and phlobaphene of *Heuchera* are soluble in alcohol.

As said in the beginning of this paper it was for the purpose of reviewing, in the light of improved methods, the inferences drawn in 1891, that this work was undertaken. From the evidence set forth herein, it is plain that the reaction on which the presence of gallic acid was then predicated was due to a trace of tannin or to an associated crystalline substance that is present in minute amount and which gives a fleeting bluish color reaction with ferric chloride instead of the more permanent color caused by gallic acid. Gallic acid is not present.

Phlobaphene production shows that the tannin of *Heuchera Americana* is not gallotannic acid.

The isolation from *Heuchera* of a cold water soluble tannin of its type, in the highly refined state which the accompanying specimen reflects, affords much encouragement for the separation and study of this class of plant principles, as a practical way through which to comprehend some of the changes which occur in the curing of astringent drugs, and as the means to a better understanding of the preparations which are made from them.

CZECHOSLOVAK PHARMACOPŒIA.

The chemical sub-committee of the Czechoslovak Pharmacopœia commission has reached agreement on the following points: (1) Solubilities are to be expressed in terms of weight, and not of volume. (2) As the standard temperature for specific gravities 20° C. has been adopted, and for water 4° C.; measuring vessels are to be graduated at 20° C. (3) Volumetric solution of potassium hydroxide is to be used in acidimetry; as indicators, phenolphthalein, methyl orange and methyl red. (4) Structural formulas of organic compounds are to be included. The pharmacological and biological sub-committee proposed the inclusion in Czechoslovakia's first national Pharmacopœia of the following serums: Diphtheria antitoxin, tetanus antitoxin, anthrax, meningococcus, scarlet fever and fowl cholera serum. Two of the members of this sub-committee were entrusted with the task of drawing up a report in which the serums to be stocked in all pharmacies are specified.—*Chem. & Drug.*, June 11, 1927.

DRUGGISTS' RESEARCH BUREAU.

It is said that approximately 20,000 people in the drug industry have already enrolled as members of the Druggists' Research Bureau. Over one-half of these members are retail drug-store proprietors, about one-quarter are clerks and the remaining number represent wholesalers, manufacturers, their salesmen, pharmaceutical journalists, college men, advertising agencies and other interests, according to the August 2nd bulletin issued by the National Wholesale Druggists' Association. The membership campaign is progressing rapidly and the directing committee of the Bureau is confident that the total membership will reach their fondest hopes.

Druggists' Research Bureau booklets, application cards and other information may be obtained by addressing the Bureau at 51 Maiden Lane, this city.