THE PHLOBAPHENES.*

BY MORITZ DITTMAR.

In the year 1844 two German chemists, Stähelin and Hofstetter, made the observation that when plant extracts containing tannins were heated or treated with acids, brown or red brown substances separated out to which they gave the name phlobaphenes. The word phlobaphene is derived from two Greek words meaning bark and color. Stähelin and Hofstetter however further noted that the phlobaphenes were not only to be found in the bark but also in fruits, seeds and other parts of the plant.

The naming of a group of substances is a comparatively easy matter. Their exact chemical identification and study is generally more difficult. The phlobaphenes have proved no exception to this proposition.

During the years following the first observations of the latter substances, chemists have not concerned themselves to any great extent with them. The reasons for this are fairly evident. The phlobaphenes are derived from the tannins through certain changes which the latter undergo. These changes will be discussed later on.

It would be natural to expect that chemists would rather busy themselves with the more simply constructed tannins than the complicated end-products, the phlobaphenes. But the practical reason why the attention of chemists has been concentrated upon the tannins is their great economic importance which the phlobaphenes do not possess.

With their study of the tannins scientific investigators have had their hands amply full. The problem of trying to answer the question. "What is a tannin, chemically and physiologically considered?" has been no easy one. Up to the present day no absolutely satisfactory answer has been found. Scientists of equal eminence and standing differ widely in their views. It required the full genius, of that master chemist Emil Fischer to synthesize penta-methyldigalloyl-glucose, now almost universally accepted as a tannin corresponding to the natural tannin of nutgall. Even here a few dissenters will be found.

The phlobaphenes are so intimately related to the tannins that a brief consideration of the latter in the light of our present knowledge is necessary before arriving at an understanding of the phlobaphenes.

There have been many classifications of the tannins made. Most of these have been unwieldy and complicated.

Prof. Henry Trimble of the Philadelphia College of Pharmacy made an interesting classification of the tannins based on the results of their elementary analyses. Thus he found that the tannins fell into two groups. In the first group he placed those tannins which upon combustion yielded 52 per cent. C. This group, called the nutgall tannin group contains the tannins from nutgalls, chestnut leaves, and pomegranate. The members of the second group called the oak-tannin group, includes tannins from oakbark, mangrove, canaigre, rhatany and kino.

To my mind the best classification of the natural tannins is that of Karl Freudenberg. He divides, like Trimble, the tannins into two groups.

In the first group he places those tannins in which a number of benzol nuclei are united over oxygen to form a complex compound. In this group would fall the so-called depsides of Emil Fischer which are formed by the inter-reaction of two or more phenol or oxyaromatic acids. The simplest depside is the digallic acid which for a long time was believed to correspond to the natural tannin of nutgall. This view predominated until the synthesis of penta-methyldigalloyl-glucose by Emil Fischer. The dominant phenolic component in this group is gallic acid.

However caffeic acid, which is a dioxycinnamic acid, is present to a certain extent in members of this group. Chlorogenic acid which is the mother-substance for the coffee tannins represents a compound containing a caffeic acid and quinic acid nucleus. A group characteristic is the ease with which ferments such as emulsin and tannase split these tannins into their component parts.

The second group of tannins is designated by Freudenberg as the group of condensed tannins. In this group the benzol nuclei are united by carbon atoms. These tannins are not broken up into simpler components by the action of ferments. They are nearly always precipitated by bromine. Through the agency of heat, oxygen, or acids, the tannins of this class go into the form of amorphous, so-called tannin reds or phlobaphenes. Phlobaphenes are then products of the tannins of this group.

This second group is further subdivided into two groups. The tannins of one sub-group contain a phloroglucin nucleus, those of the second sub-group do not. Both sub-groups are characterized by the common tendency to precipitate with bromine and to go over into the phlobaphene stage. This group of tannins is economically the most important.

Gambir tannin is a tannin of great economic importance. It is derived from a crystalline, colorless mother substance, gambir catechin. If gambir catechin is heated it is transformed into colored amorphous gambir tannin. If the gambir tannin is further heated a gambir red or phlobaphene results. Thus in the case of gambir we have a progression from a crystallizable colorless substance, a proto-tannid, to the amorphous deeply red or red-brown colored phlobaphene. The formula of gambir catechin as given by Kostanecki is the following:
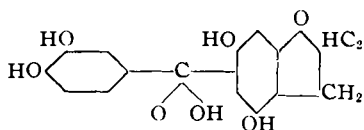
After this brief consideration of the tannins we may proceed to a more detailed discussion of the phlobaphenes themselves. Chemical literature dealing with the tannins is rich and plentiful, but as little attention has been paid to the phlobaphenes, the phlobaphene literature is accordingly scarce and unsatisfactory.

Freudenberg's definition of the phlobaphenes as being the water-insoluble deeply colored products of the oxidation or condensation of tannins, which result through the agency of dry heat, hot water, diluted acids or the oxygen of the air is the most satisfactory one to be found.

Kunz-Krause's definition of the phlobaphenes as being the red or red-brown amorphous substances found in the outer cellular layers of the bark is an older and less satisfactory one.

A point of confusion when reading literature dealing with the phlobaphenes is the uncertain usage of the expressions phlobaphene and tannin red. Some authors will use both interchangeably. Thus Etti in his work with oak tannin, differentiates between an oak-tannin red and an oak phlobaphene. Böttinger on the other hand who studied the same substances regarded both as identical.

Dekker made the suggestion of calling all the phlobaphenes Reds. In the German literature the word "Rot" is very often used to designate the red-colored oxidation or condensation products of the tannins. But if this suggestion was carried out, we would have to call a lot of substances "Reds" which were really of brown color. The simplest thing to do would be to retain the name phlobaphene which, after Freudenberg, includes the condensation or oxidation products of the tannins, and regard the so-called reds as red-colored phlobaphenes.

Another question of dispute among various investigators is the one having to do with the manner of formation of the phlobaphenes. All are agreed that the phlobaphenes are products of the tannins. But the manner in which the tannins are converted into phlobaphenes has led to different views. There are two main theories concerning the formation of the phlobaphenes—the oxidation theory and the dehydration theory.

According to the oxidation theory the phlobaphenes are simply the result of an oxidation of the tannins. This oxidation can take place directly through the agency of the oxygen of the air, or with the aid of oxidizing ferments found in the plants themselves.

According to the dehydration theory water is split off by condensing tannins, anhydrides of the tannins resulting, which constitute the phlobaphenes. This anhydridization of the tannins can be progressive as Etti showed, by treating oak-tannin with sulphuric acid under varying conditions. He obtained amorphous red-colored phlobaphenes of the composition $C_{20}H_{36}O_{16}$, $C_{20}H_{34}O_{15}$ and $C_{20}H_{32}O_{14}$. The three differ progressively by one molecule of water.

Kunz-Krause does not believe that the phlobaphenes of the bark result from an oxidation process. He argues that in the cork tissue which is richest in phlobaphenes, the cells are the dryest and most airtight. He remarks that the localization of such a sensitive alkaloid as Curarin in the cork alongside of phlobaphene masses, is an argument against the theory that phlobaphenes result from an oxidation process. If oxygen had access to the cork cells, such an unstable alkaloid as curarin could no longer be detected.

Tschirch (1891) advanced the proposition that the red-brown coloring of the cell walls of the barks of many drug plants as cinchona and cinnamon, resulted from an absorption of the prototannids from the cell contents. These were then oxidized while in the membrane, to the red-colored phlobaphenes. Tschirch made the observation that when fresh cinchona bark or cola nuts were subjected to a steam sterilization the reddening of the cell walls did not occur. Ordinarily, without steam sterilization a freshly removed cinchona bark will redden in 15 to 20 seconds. This observation led Tschirch to believe that enzymes (oxidases) played a rôle here.

Lindet (1898) supported Tschirch's observations. He observed that a steam sterilized apple would not turn brown when cut. The preparation of green tea by heating supports this theory also.

Tschirch divides the phlobaphenes into two groups. The phlobaphenes of the first group are formed during the life of the plant. These are to be found as part of the cellular contents. They build red-brown or brown masses and are particularly localized in physiologically dead tissue.
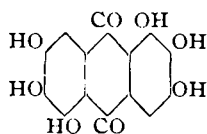
The phlobaphenes of the second group are formed postmortally and are especially to be found in the cell membranes. Tschirch calls these the "membrane phlobaphenes." Since the membranes which contain the postmortally formed phlobaphenes were originally colorless, we can account for the coloration as follows:

The soluble tannin penetrates from the cell into the cell membrane and is there oxidized to phlobaphene. It is possible that the tannin penetrates the membrane in the form of a glucoside. However it is not necessary to presuppose the existence of a glucoside.

There are a great many drugs whose red or red-brown color is due to postmortally formed phlobaphenes. Some of these are, cinchona, cinnamon, sassafras, oak and quebracho barks, rhatany, rhizome of male fern and tormentilla, cola nuts, and cloves.
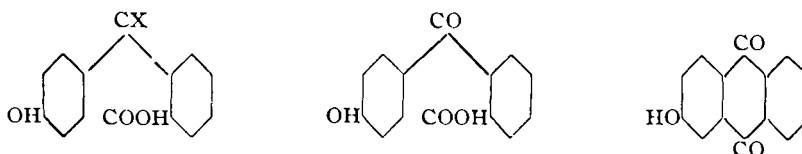
Next to nothing is known of the exact chemical structure of the phlobaphenes. Through the application of strong reagents products are obtained which give us only a comparatively poor idea of their real constitution. Thus by fusion with potassium hydroxide products such as phloroglucin, protocatechuic acid, acetic, and butyric acids are obtained. But by such destructive methods, not very much of the inner structure of the phlobaphenes can be learned.

Nierenstein was of the opinion that the phlobaphenes might possibly be constructed similar to ruffigallic acid.

$$\text{HO} \quad \overset{\text{CO OH}}{\diagup\!\!\diagdown} \quad \text{OH} \\ \text{HO} \quad \diagdown\!\!\diagup \quad \text{OH} \\ \overset{}{\text{HO CO}}$$

In this case the phlobaphenes would be anthracene derivatives. Nierenstein subjected the phlobaphene from Quebracho-tannin to a distillation with powdered zinc. He actually obtained anthracene.

Nierenstein and Webster formulated the formation of phlobaphene from mangrove tannin as follows:

$$\overset{\text{CX}}{\text{OH}\diagdown\text{COOH}\diagup} \qquad \overset{\text{CO}}{\text{OH}\diagdown\text{COOH}\diagup} \qquad \overset{\text{CO}}{\text{HO}\diagup\diagdown\underset{\text{CO}}{}}$$

The tannin is first oxidized to the alpha-phlobaphene 2, which latter goes over into beta-phlobaphene upon further oxidation. By distilling the phlobaphenes 2 and 3 with zinc dust, diphenyl and anthracene were obtained respectively.

Dekker subjected a phlobaphene from *Eucalyptus occidentalis* to a similar reducing distillation and obtained thereby diphenyl.

At the Pharmaceutical Institute at Berne under the guidance of Professor Tschirch, I made a comparative study of a number of phlobaphenes obtained from drugs. I will briefly describe some of the work which we did.

The following phlobaphene-containing drugs were examined: Krameria, Tormentilla, Bistorta, Cinnamon Bark, Cinchona, Cloves, Kola Nuts and Horse-chestnut Shells.

The method of obtaining the phlobaphenes is the following one. The coarsely ground drug was percolated with distilled water after the method of interrupted percolation. The percolation with water was continued until the percolate ceased to precipitate with 1% hydrochloric acid. This precipitation took place either in the cold or was first induced by warming on a water-bath.

In the aqueous percolate the phlobaphenes are held in colloidal solution by the peptizing action of water soluble tannins. By the addition of a mineral acid this peptizing influence is destroyed and the phlobaphenes precipitate out. The latter may be then collected on a suction filter, washed free of mineral acid, and dried at 70° C. in a drying oven.

After filtering off the first phlobaphene precipitate an interesting observation is made in regard to the filtrates. In a short time the clear filtrate becomes cloudy. A second precipitate of phlobaphene occurs. If this is filtered off and the clear filtrate allowed to stand the same phenomenon is repeated. This reprecipitation is especially abundant in aqueous percolates obtained from the root of Krameria and the rhizome of Tormentilla.

This reprecipitation can be accounted for in the following manner. After the initial phlobaphene precipitate, the tannins which are in solution are acted upon by the mineral acid present and so condense to phlobaphenes. The latter being insoluble naturally precipitate out.

The initial precipitate is always a great deal less than the sum of the subsequent precipitates. Thus the phlobaphene precipitates of the first five aqueous percolates of 500 grams of Krameria weighed 9.189 grams. The weight of the phlobaphenes subsequently precipitating out during the course of one year equaled 31,386 grams. In the case of Tormentilla the subsequent precipitation was nearly five times as much as the initial precipitate.

In connection with the reprecipitation it was noticed that the color of each succeeding precipitate was darker than its predecessor.

The aqueous percolate of the rhizome of Bistorta represents a dark red colloidal solution. Upon the addition of dilute hydrochloric acid in the cold no precipitation occurs. Despite the presence of hydrochloric acid molds thrive readily in such a solution. This is readily accounted for by the fact that the rhizome of Bistorta contains an enormous amount of sugar which is naturally found in the aqueous percolate.

If such an aqueous percolate be permitted to stand several weeks with the molds, and then after filtering off the molds, allowed further to stand, it will be found that within a week a red amorphous precipitate of phlobaphene will precipitate out. If this be filtered off a reprecipitation in the filtrate will occur. This reprecipitation takes place several times.

It is possible in this case that the large amount of sugar present in the aqueous extract prevents the immediate precipitation of phlobaphene by the hydrochloric

acid. Only then when the sugar has to a certain extent been devoured by the molds can the precipitation proceed in a normal manner.

This explanation however sounds unsatisfactory when one considers that in fresh aqueous extract of Tormentilla a great deal of sugar is also found. Here however one obtains an immediate precipitation upon the addition of hydrochloric acid. This behavior may be due to a peculiarity in the structure of the Bistorta phlobaphene concerning which next to nothing is now known. Bjalobrsheski was able to obtain ellagic acid from Bistorta tannin by treating the latter with dilute hydrochloric acid. Now ellagic acid represents two benzol nuclei which are united not only by oxygen but also directly by carbon. This would place the Bistorta tannin, if we assume that ellagic acid is its nucleus, in a special class lying between the two groups of Freudenberg's classification.

When the fresh aqueous percolate of Bistorta is warmed on a water-bath with dilute hydrochloric acid a brownish precipitate of phlobaphene is immediately obtained.

After exhausting the various drugs with water, percolation with a dilute sodium hydroxide solution (1–1000) is carried out. The percolates are dark red in color and readily give a voluminous precipitate with hydrochloric acid. The phenomenon of reprecipitation does not take place with these alkaline percolates. This is easily accounted for. In the alkaline percolate the phlobaphenes are no longer held in solution by the peptizing action of soluble tannins. They are doubtlessly present as soluble sodium salts. Upon the addition of a mineral acid all the phlobaphenes would precipitate out at once and reprecipitation would not occur.

The phlobaphenes can also be salted out. By removing the electrolyte the phlobaphene goes into solution again. However if the electrolyte hydrochloric acid is used in precipitating the phlobaphenes, the latter are then practically insoluble in water.

If the Krameria is first subjected to a steam sterilization before percolating, some differences occur which are worthy of mention. In an experiment with equal lots of Krameria the following differences were noted.

In the percolate of the sterilized drug the peculiarly aromatic odor present in the percolate of the unsterilized drug is missing.

The percolate of the sterilized drug is clear. That of the unsterilized drug is somewhat cloudy.

If the percolate of the unsterilized drug is exposed to the air molds develop on its surface in a short time. The percolate of the sterilized drug does not develop mold colonies when exposed to the air. Even when infected with molds the percolate from a sterilized drug does not permit their growth. This would indicate that the drug contains some volatile odorous principle which perhaps supplies nourishment for molds. This volatile substance is removed by a steam-sterilization of the drug.

That the tannin of Krameria has antiseptic properties and justifies the usage to which the natives of Peru have put it for many centuries, namely as a preservative of the teeth and as a mouth wash and gargle, is indicated by the fact that molds cannot grow in its aqueous solution.

The yield of phlobaphene from the aqueous percolates of the sterilized drug

is less than that from the unsterilized drug. The yield from the combined aqueous percolates of 500 grams of sterilized Krameria equalled about 76 per cent. of the yield obtained from the same quantity of unsterilized drug. The yields from the first thirty alkaline percolates of each were quite similar being for the unsterilized drug 13.87 per cent. for the sterilized drug 13.56 per cent. of the total weight of the respective drugs.

Doubtlessly, in the steam sterilization of the drug, the heat of sterilization affects part of the water-soluble tannin converting it into an insoluble condensation product of phlobaphene nature.

The following table shows the yield of phlobaphene from several phlobaphene containing drugs.

	Weight of drug.	Phlobaphene ppt. aqueous extract.	Per cent.	Phlobaphene ppt. alkaline extract.	Per cent.	Total Per cent.
Krameria	500 Gm.	41.8 Gm.	8.36	90.1 Gm.	18.02	26.48
Tormentilla	1000 Gm.	95.7 Gm.	9.57	92.2 Gm.	9.22	18.79
Bistort	1000 Gm.	41.1 Gm.	4.16	103.74 Gm.	10.37	14.53
Cinchona (red)	300 Gm.	0.73 Gm.	0.26	34.4 Gm.	11.46	11.72
Cinnamon	300 Gm.	3.12 Gm.	1.04	11.65 Gm.	3.88	4.92

The phlobaphenes may be reduced. Experiments in regard to the reduction of the phlobaphenes were carried out with powdered zinc in neutral, alkaline and acid alcoholic solution.

When a dark red, alcoholic solution of phlobaphene (the phlobaphenes from the eight drugs under examination acted similarly) is boiled with zinc dust, a nearly perfect decolorization takes place. This is however not due to a reduction of phlobaphene. The phlobaphene in alcoholic solution is adsorbed by, or enters into combination with the zinc. If the zinc residue is treated with dilute sodium hydroxide solution the latter leaches out the phlobaphene from the zinc and is colored red. Water or alcohol will no do this.

By boiling an alkaline alcoholic solution of the phlobaphene with zinc dust only a partial decolorization takes place. In this case again a combination with the zinc seems to have taken place and not a true reduction.

By boiling an acid alcoholic solution of the phlobaphene with zinc dust a real reduction is obtained. A dilute sulphuric acid is used. The boiling is carried out in a hydrogen atmosphere. The dark red solutions of the eight phlobaphenes permitted reduction to solutions of a faintly yellow color. If the still warm practically colorless solution of reduced phlobaphene is exposed to the air, after filtering off the zinc an interesting phenomenon is observed. In a very few minutes the solution becomes yellowish red in color. This color changes to a dark red and finally a dark brown-red.

On the other hand if the reduced phlobaphene solution is kept under a hydrogen atmosphere, no color change occurs. Solutions of the various reduced phlobaphenes were kept in this manner for over 24 hours.

This reduction experiment would indicate that the oxygen of the air is at least partly concerned with the formation of the phlobaphenes.

By simply adding water to an alcoholic solution of reduced phlobaphene, a greyish white precipitate is obtained which rapidly turns black-brown when ex-

posed to the air. I tried to obtain an ashless precipitate but succeeded in one case only, that of a reduced phlobaphene from Krameria. This precipitate was dried in a CO_2 atmosphere and combusted to determine the C, O, and H content.

In boiling the phlobaphenes with dilute sulphuric acid no splitting off of glucosidal sugar can be detected. Nor could any splitting off of a part of the phlobaphene structure be noted. But that the phlobaphenes do undergo changes when boiled with dilute sulphuric acid is evidenced by the color changes which the lighter colored phlobaphenes undergo.

For instance the phlobaphene from an aqueous percolate of Krameria is colored light yellow-brown. By boiling a half hour with dilute sulphuric acid (2%) the color is changed to a bright red. The dilute acid has also taken on a deep red color showing that part of the phlobaphene has gone into solution. Both the red colored phlobaphene and the red solution reduce Fehling's solution. Sugar is not present as indicated by a negative osazone reaction. The reduction of the Fehling's solution is due to the phlobaphene itself. If the latter is filtered off, the filtrate will in a few days grow cloudy and soon a deposit of red phlobaphene occurs. If the filtrate from this is allowed to stand, further deposition of red phlobaphene occurs. This deposition takes place several times.

This phenomenon can be accounted for as follows. By boiling the amorphous phlobaphene with dilute sulphuric acid the former, despite the fact that it is normally insoluble in an acid solution, enters into colloidal solution in a state of fine dispersion. But this highly disperse phase of the phlobaphene is unstable. The colloidal particles enter into large aggregates as shown by the initial clouding and final precipitation.

The effect of nitric acid upon the phlobaphenes was studied. Nitric acid of various concentrations and at differing temperatures was used. All the phlobaphenes from the eight drugs examined yield oxalic acid. In the case of a phlobaphene from Tormentilla, styphnic acid (tri-nitro resorcin) was obtained. From Krameria, in addition to the oxalic and styphnic acids, picric acid was found.

As was mentioned before, products such as phloroglucin, protocatechuic acid and lower fatty acids are obtained by fusion with potassium hydroxide. Going to the other extreme and boiling the phlobaphenes with dilute sodium hydroxide (2% and 5%) gives negative results. The structure of the phlobaphenes is resistent to such attacks.

A number of elementary analyses of the phlobaphenes were made. The phlobaphenes are not chemical individuals. Since we assume that the phlobaphenes are a result of a progressive oxidation or condensation of the tannins they cannot themselves be pure chemical substances. We must regard them as mixtures of substances of closely allied structure.

To set up a formula for such a substance is foolish. An elementary analysis has therefore only a relative value. It is, however, interesting to know in just what proportions the carbon, hydrogen and oxygen are present.

In preparing the phlobaphenes, for combustion, great difficulty is experienced in removing small amounts of inorganic matter which cling tenaciously to them. Salting out the phlobaphenes was tried but without success. As the phlobaphenes are but sparingly soluble in water, removal of impurities by simply washing with distilled water was successful in several cases.

Another method used was to dissolve the phlobaphene in alcohol. From its alcoholic solution the phlobaphene could be precipitated by the addition of slightly acidulated water.

The phlobaphenes so obtained were first dried in an oven at 70° C. and then kept in desiccators until their weight remained constant. The following figures representing the results of some of the combustions carried out show how widely the various phlobaphenes differ.

	%	%	%
Krameria	C 62	H 5	O 33
Tormentilla	C 59	H 5	O 36
Bistorta	C 57	H 7.8	O 38.2
Kola Nuts	C 56.7	H 5	O 38.3

The phlobaphenes are the end products of a large number of natural tannins. They are the last step in a long chain of transformations, starting with simple crystallizable substances and ending with an amorphous complicated condensation product.

In concluding it might be interesting to mention an economic value, if only an indirect one of certain phlobaphenes. The beautifully attractive brown color of chocolate is in great measure due to a phlobaphene derived from a tannin originally present in the fresh cacao seed. We know that in the fresh cacao seed an alkaloid-tannoglucoside is present which during the process of fermentation to which the seeds are subjected is broken up into its component parts. The tannin is oxidized by oxidases present to cacao-red. The oxidation is progressive, the end product being the brown colored phlobaphene.

ON 3-AMINO-4:4'-DIHYDROXYARSENOBENZENE AND ITS N-METHYLENESULPHINATE AND N-METHYLENESULPHONATE DERIVATIVES.[*][†]

BY MERRILL C. HART AND WILBUR B. PAYNE.

For the production of unsymmetrical arseno compounds three general methods are given in the literature. According to Bertheim[1] the reduction of equimolecular mixtures of two different arsinic acids or oxides, or a mixture of an acid with an oxide leads only to the production of the unsymmetrical derivative and not to a mixture of the three possible products. Limiting the reduction agent used to sodium hydrosulphite we find Fargher[2] describing the unsymmetrical compound 3'-amino-4'-hydroxy-1:3-diazole-5:1'-arsenobenzene by reduction of the acids. Christiansen[3] also produces by this method 3-amino-4:4'-dihydroxyarsenobenzene and 3-5-3'-triamino-4:4'-dihydroxyarsenobenzene. To test the purity of these compounds Christiansen relied on the solubility tests and the percentage of arsenic.

[1] Chem. Zeit., 38, 756, 1914.

[2] J. Chem. Soc., 117, 866, 1920.

[3] J. Am. Chem. Soc., 43, 2202, 1921.