Isolation and identification of the alkaloids of western Coptis

Thomas D. Rowe

The University of Montana

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ISOLATION AND IDENTIFICATION OF THE ALKALOIDS
OF WESTERN COPTIS

by

Thomas D. Rowe, Ph.C., B. S.

Presented in partial fulfillment of the
requirement for the degree of
Master of Science.

State University of Montana
1933

Approved:

[Signatures]

Chairman of Examining Committee

Chairman of Graduate Committee
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Introduction

There is considerable plant life in Western Montana and plants and trees of many various kinds are found thriving throughout this area. One of the plants which is found in great abundance, especially in the damp, mossy sections, is a small, dark green plant commonly known as "Geld Thread", and classified as *Coptis occidentalis*. This is a close relative to *Coptis trifolia* which is official in the National Formulary V. It is used in the treatment of canker sores in the mouth and as a stomach bitter. *Coptis trifolia* is known to contain two alkaloids, berberine and coptine, the former being a much-studied alkaloid, found in many plants; and the latter a rather unknown substance found, so far as is known, only in the various species of *Coptis*.

*Coptis occidentalis*, which is one of the many known species of *Coptis*, is also known to contain coptine and berberine but the quantity of coptine present is not definitely known. Qualitative tests for identifying coptine are few and rather indefinite. With this knowledge our problem of study of *Coptis occidentalis* was undertaken with three purposes:

First: To try to make quantitative analyses and qualitative tests on the contents of the roots and rhizomes of *Coptis occidentalis* so that coptine can be identified in the future.

Second: To determine if *Coptis occidentalis* is as valuable medicinally in regard to alkaloidal content as is *Coptis trifolia*.

Third: To determine if there are more than two alkaloids present in *Coptis occidentalis*. 
Historical

The genus *Coptis* is a member of the Ranunculaceae, commonly known as the Crow-foot or Buttercup family which contains a number of very important medicinal plants, among which are *Aconite, Hydrastis,* and *Cimifuga*.

The genus name "Coptis" is taken from the Greek word "Koptein" meaning "to cut" and referring to the cut appearance of the compound leaves. *C. trifolia* was first described in 1807 by Salisbury and in 1838 a new species, *C. occidentalis*, was described by Futtall. He further classified these plants by splitting the genus *Coptis* into *Coptis* and *Chrysocoptis*; the former for the one-flowered and ternately divided leaves, *C. trifolia*, and the latter for the 2-4-flowered and pinnately divided leaves, *C. occidentalis*. This last classification has not been accepted by Index Kewensis but "Chrysocoptis" is accepted as a synonym for *C. occidentalis*.

*Coptis occidentalis* is a small plant growing from 5 to 7 inches in height. The rhizomes are long and slender and when broken are quite yellow on the inside. They have an intensely bitter taste and a very slight odor. The rhizomes


Plate I

Coptis occidentalis

Photographed by Dean C. E. Mollett
of _C. occidentalis_ are considerably larger than those of _C. trifolium_ so in this study only the roots and rhizomes of the plant were used although in _Coptis trifolium_ the whole plant is official. _C. occidentalis_ corresponds to _C. Trilobata_ in general, but differs as follows: the leaves of _Coptis occidentalis_ are larger and more leathery; the margins are incisely toothed and more or less lobed. The flowers are smaller and generally two, sometimes three, on the scape. The flowers are light yellowish green in color while those of _Coptis trifolium_ are white.4

A further comparison may be tabulated as follows:

1. _C. trifolium_. Leaves obscurely 3-lobed, sepals oval or oblong-obtuse, petals enlarged at the summit.

2. _C. occidentalis_. Leaves ternate, all three leaflets long petioluled, leaf divisions obtuse, obtusely dentate, seed oblong.5

Lloyd's Bulletin states that the species found in the Rocky Mountains, _C. occidentalis_, is of little interest from

---


a medical standpoint. . . "as the common species, C. trifolium, is so very abundant that there will never be any demand nor occasion for collecting either of the Western plants". This seems to be a rather broad statement especially if the western Coptis is found to contain more active constituents than C. trifolium, and also if the western species is more readily utilized due to its larger rhizome. It will be attempted to show that C. occidentalis is of interest from a medical standpoint.

The plants used in this investigation were found growing in the damp mossy and boggy woods of Western Montana near Noxon. Their range extends from the Bitter Root Mountains where the plants are rather small, to the moist woods of the northwest ridges along the Continental Divide. The plants reach their largest size in the high moist ravines west of the Continental Divide. Although Coptis is a swamp plant it is not a mud plant, but generally selects dry knolls surrounded by wet soil.

The official plant is collected while in flower in May or June but this is nearly impossible in Montana as the plant flowers early, in April or May, when snow is still on the

ground. For this reason the plant is often called "Snow Flower". In this investigation the major portion of plant used was collected late in the fall, about the middle of October, and a small portion was used that was collected in June.

Very little work has been done on coptine although it was discovered by E. Z. Gross in an assay he made of *Coptis trifolia* as early as 1873. He assayed the plant as follows: First the plant was macerated in water for 24 hours producing a dark yellowish brown solution which when tested with litmus gave neither a blue nor red color change which indicated the absence of free acids and alkalies.

His next tests were for the presence of starch using potassium iodide and also iodine solutions both of which gave no color reactions which indicated the absence of starch. This was later found to be incorrect and will be mentioned subsequently.

He also tested for sugar using Trommer's test and obtained a red precipitate of copper oxide which indicated that sugar was present.

He obtained a resin which was insoluble in cold water but completely soluble in alcohol.

---

The next procedure was to examine the drug for alkaloids present and this was done by making an alcoholic tincture. Berberine was found to be present in this. He then separated the Berberine by acidulating the filtered alcoholic solution with dilute hydrochloric acid and then adding an excess of sodium carbonate until a precipitate was formed. This precipitate was washed with ammonia until nearly colorless, and then dissolved in water by the aid of hydrochloric acid. This solution was then evaporated to a syrupy consistency and allowed to crystallize. Colorless crystals were formed. These were treated with concentrated sulfuric acid and heated which caused the formation of a dark purple color. A solution of the alkaloid was tested with Mayer's reagent and a crystalline precipitate was formed.

From the above data Gross concluded that a new alkaloid was present and he named it "coptine".

J. J. Schultz was the next person to study Coptis. He assayed Coptis trifolia in 1884 and made more of a quantitative study than did Gross. He found first that it was unsatisfactory to separate berberine by precipitating it with hydrochloric acid so he used ammonium hydroxide (U. S. P.).

He added ammonium hydroxide to an alcoholic tincture which caused the formation of a dark brown precipitate. This was collected on a filter and the filtrate made acid with sulfuric acid, allowed to stand, and then made alkaline with ammonium hydroxide. A precipitate was again formed and was separated and the two precipitates mixed together, dried spontaneously, and washed with successive portions of chloroform. The chloroform was distilled and the residue exhausted with sulfuric acid. This was made alkaline and a precipitate was formed which was dried, weighed, and found to weigh 3.42 grains or .012 percent of the original sample of 4 pounds.

This precipitate was dissolved in water, acidulated with acetic acid, and gave precipitates with solutions of platinic chloride, ammonium molybdate, iodine, potassium iodide, and mercury potassium iodide.

A chloroformic solution of the remainder of the precipitate when evaporated on a slide gave microscopic crystals.

In conclusion he gave the following data:

1. 10 percent of C. trifolia is soluble in alcohol.
2. It contains an amount of berberine equivalent to 0.8 percent berberine sulfate.
3. 0.012 percent coptine is present

Since then very little work has been done on *Coptis trifolia* and none on *Coptis occidentalis*. It has been found that *Coptis* does contain starch and that it has from 4.28 to 5.07 percent ash.

A Japanese, Kitase, discovered a new alkaloid in *C. japonica* in 1926 and called it "coptisine". He gave it the formula $C_{19}H_{15}O_{5}N$. He did not state if this were the same as coptine or not, and inasmuch as the formula for coptine is not known this may be a new alkaloid and may be coptine.

In the research made by the writer some differences were found in his results and those obtained by Gross and by Schultz. These differences will be noted when they appear in this report.

**Experimental**

**I Quantitative**

Work on *Coptis occidentalis* was begun on a sample which had been gathered in June. The rhizomes and roots were separated from the rest of the plant and ground into a moderately fine powder. This had a greenish yellow color.

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The first work was to determine the amounts soluble in several of the various solvents. Samples of the plant were weighed out and extracted successively with several solvents and the amount of material was determined by direct weighing in each case after drying. The extractions were made with Soxhlet extractors and in all cases the solvents were allowed to percolate continually for eighteen hours. The specific solvents were used in the order given below, allowing the drug to dry after the removal of each solvent.

Sample #1 weighed 2.4245 gm.
Sample #2 weighed 2.2238 gm.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Sample #1</th>
<th>Sample #2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt. of Ext.</td>
<td>Percent</td>
</tr>
<tr>
<td>Petroleum Ether</td>
<td>0.0233 gm.</td>
<td>0.9610</td>
</tr>
<tr>
<td>Ether</td>
<td>0.0256 gm.</td>
<td>1.05</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0.5389 gm.</td>
<td>24.083</td>
</tr>
<tr>
<td>Water</td>
<td>0.2857 gm.</td>
<td>11.783</td>
</tr>
</tbody>
</table>

These results are somewhat different from those obtained by Schultz when he obtained 10 percent alcohol-soluble portion of *Coptis trifolia*. This probably does not indicate anything of a very important nature except to show that there is considerable difference between the plants *C. occidentalis* and *C. trifolia*.

The water-soluble portion was tested with litmus and found to turn red litmus blue, indicating the presence of free alkali. This result differed from that of Gross who obtained no free alkali.

All of the residues were redissolved into their original solvents, each one being soluble but rather slow. The resin obtained in the alcohol portion was slightly soluble in cold alcohol but when the alcohol was heated it became quite soluble. Both the water and alcohol solutions were colored. The alcohol gave a dark amber colored mixture and the water a dark, almost muddy, solution. Both the ether and petroleum ether gave colorless solutions.

Portions of each of the solutions were placed into acid solution by the usual process, except for alcohol and in this case the acid water was added to the alcohol and the mixture placed in a steam bath, and the alcohol driven off quite slowly with the result that the non-volatile substances remained in the acid water solution. Each of these solutions was tested with Mayer's, Wagner's, and Scheibler's reagents to determine the presence of alkaloidal material. The following results were obtained:

<table>
<thead>
<tr>
<th></th>
<th>Petroleum Ether</th>
<th>Ether</th>
<th>Alcohol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayer's</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Wagner's</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Scheibler's</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+ indicates precipitate formed, indicating presence of alkaloid.

- indicates no precipitate formed, indicating absence of alkaloid.

The above table shows that the coptine was all dissolved by petroleum ether and none was in the ether solution. The positive tests obtained on alcohol were for berberine and this was all removed by the alcohol with the result that no alkaloids were present which were soluble in water.

Having determined the soluble portions of the rhizomes in the various solvents and partially identifying portions of the extracted material to be alkaloidal in nature, the next step was to determine the percentage of ether-soluble alkaloid thought to be coptine.

First, four samples of the rhizomes from the June specimen were weighed out and assayed. Each of these samples was placed in a 250 cc. Erlenmyer flask and 100 cc. of ether, accurately measured, were added to each. The flasks were stoppered tightly and shaken vigorously for five minutes. Then 5 cc. of 10 percent ammonium hydroxide were added to each. Again the flasks were stoppered, shaken, and allowed to stand for twenty-four hours. The ether solution was amber in color. At the end of this time 50 cc. of ether, measured accurately, were decanted, run thru a filter of cotton, and placed in a separatory funnel. To the ether in the funnel was then added 20 cc. of 2 percent sulfuric acid.
solution. The mixture was shaken for several minutes and then the two liquids allowed to separate out. The acid was then drawn off into a second separatory funnel and a second portion of 15 cc. of acid added to the ethereal solution and shaken. Again the acid was drawn off and placed with the first acid washings. This procedure, addition of acid and drawing it off, was continued until five washings had been made, using 10 cc. portions of acid for the last three washings.

The acid solution in the separatory funnel was cooled by running water over the funnel and then the solution was made alkaline by the addition of sufficient 10 percent ammonium hydroxide so that red litmus turned blue. In most alkaloidal extractions in which this method is used a precipitate or cloudiness will form when the solution is made alkaline, but this did not occur in the extractions of Coptis, probably due to the small quantity of alkaloid that was present.

To the alkaline solution was then added 20 cc. of ether and the mixture shaken and allowed to stand. The ether was separated from the aqueous solution and a second portion of 15 cc. of ether was added to the aqueous solution. Again the two liquids were separated and the second ether portion mixed with the first. This was continued until five washings with ether had occurred, using 10 cc. of ether in the last three washings. A portion of the last ether solution
was tested for alkaloid by evaporating the ether nearly to
dryness, adding 2 percent sulfuric acid, and then adding a
drop of Mayer's reagent. No precipitate formed which in-
dicated that the alkaloid had been removed in the previous
washings.

The ethereal solution was then filtered thru a hard
filter paper into a tared glass evaporating dish and set
aside and allowed to evaporate spontaneously. The fil-
tering was done to separate any water present and also to
remove the scum which had formed due to partial emulsifi-
cation.

The ethereal solution in the tared dish was slightly
amber in color due to the color of the first two ether wash-
ings. Inasmuch as coptine is supposed to be a clear,
colorless alkaloid it was thought that the color was due to
the presence of a small quantity of berberine which may
have dissolved very slightly in the ether. In order to
remove this color and also any ammonium sulfate that may
have dissolved in the ether, the ether solutions were washed
three times with separate portions of distilled water.
The solution, when filtered, was clear and colorless.

A word of explanation regarding the procedure is prob-
ably needed at this time.

First. Ether is used as the solvent because berberine
forms soluble compounds with immiscible solvents such as
acetone, alcohol, and chloroform, leaving ether and petroleum
as the only practical solvents to be used and these were found to be practically the same in solvent ability on Coptis. Thus this procedure eliminates the presence of berberine in the calculations.

Second. It is necessary to make the first ether solution alkaline with ammonium hydroxide because alkaloids are known to be insoluble in immiscible liquids when in the form of salts. They are soluble in such liquids when in the uncombined form. Coptine is thought to be a typical alkaloid as it was necessary to apply the above and form free coptine by adding the alkali.

Third. Only 50 cc. of ether are removed as it is quite difficult to remove all of the solvent and by taking an aliquot part time can be saved and more accurate results obtained.

Fourth. The acid washing acts as a purification step removing the water-insoluble products, and obtaining an alkaloidal salt. It is necessary to make this solution alkaline for the reason given in the second statement above.

Fifth. The final ether solution contains only pure alkaloid and when evaporated the solid residue can be considered as the ether-soluble alkaloid of Coptis and is thought to be coptine.

The procedure already described was used throughout in the quantitative determinations of coptine and the results were obtained by the above method.
The amount of ether-soluble alkaloid or alkaloids in the roots and rhizomes was determined with the following results:

**#1. Weight of sample** 9.2100 gm.
- Weight of ether-soluble alkaloid .0044 gm.
- Percent of ether-soluble alkaloid .094

**#2. Weight of sample** 9.3246 gm.
- Weight of ether-soluble alkaloid .0047 gm.
- Percent of ether-soluble alkaloid .12

- Weight of ether-soluble alkaloid .0049 gm.
- Percent of ether-soluble alkaloid .104

**#4. Weight of sample** 8.6111 gm.
- Weight of ether-soluble alkaloid .0045 gm.
- Percent of ether-soluble alkaloid .1044

The weight of the ether-soluble portion is only one-half of the total amount present due to the aliquot part taken in each case. The ether-soluble alkaloid obtained in each case was colorless, but not crystalline although it did form in tree-like shapes.

At this time a fresh supply of *Coptis* was obtained which had been gathered in October of 1932. Part of the roots and rhizomes were separated, part of the leaves separated, and a part of the whole plant kept intact. These three portions were then ground separately and assays for the percentage of ether-soluble alkaloid run on each of the three.

First the roots and rhizomes were assayed, using four...
samples and obtaining the following results:

1. Weight of sample 5.0400 gm.
   Weight of ether-soluble alkaloid .0046 gm.
   Percent of ether-soluble alkaloid .1824

2. Weight of sample 5.0204 gm.
   Weight of ether-soluble alkaloid .0040 gm.
   Percent of ether-soluble alkaloid .1592

3. Weight of sample 7.6935 gm.
   Weight of ether-soluble alkaloid .0079 gm.
   Percent of ether-soluble alkaloid .2054

4. Weight of sample 7.9293 gm.
   Weight of ether-soluble alkaloid .0069 gm.
   Percent of ether-soluble alkaloid .1740

These results would tend to indicate that the plant has a higher ether-soluble alkaloid content in October than it has in June. A tabulation of the results shows this difference to be quite marked.

<table>
<thead>
<tr>
<th>June sample</th>
<th>October sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 0.094 percent</td>
<td>1. 0.1824 percent</td>
</tr>
<tr>
<td>2. 0.120 percent</td>
<td>2. 0.1592 percent</td>
</tr>
<tr>
<td>3. 0.104 percent</td>
<td>3. 0.2054 percent</td>
</tr>
<tr>
<td>4. 0.1044 percent</td>
<td>4. 0.1740 percent</td>
</tr>
<tr>
<td><strong>Average - 0.106 percent</strong></td>
<td><strong>Average - 0.1802 percent</strong></td>
</tr>
</tbody>
</table>

The above averages show that there is nearly twice as much ether-soluble alkaloid present in the rhizomes in October as in June. As yet there is no satisfactory explanation for this unless the plant builds up the alkaloid during its
earl y summer growth and stores it for later use.

Next the leaves were assayed using the same method as employed in determining the alkaloidal content of the rhizomes. Two samples were run and the following results were obtained:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight of sample</th>
<th>Weight of ether-soluble alkaloid</th>
<th>Percent of ether-soluble alkaloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>5.1540 gm.</td>
<td>0.0041 gm.</td>
<td>0.158</td>
</tr>
<tr>
<td>#2</td>
<td>5.1391 gm.</td>
<td>0.0023 gm.</td>
<td>0.108</td>
</tr>
</tbody>
</table>

These results show that the alkaloidal content of the leaves is approximately the same as the roots and rhizomes. Further samples would have been assayed but at this time no more leaves were available.

After assaying the leaves and roots the remaining portion left to be assayed was the whole plant. Four different samples were assayed, using the general procedure described before and the following results were obtained:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight of sample</th>
<th>Weight of ether-soluble alkaloid</th>
<th>Percent of ether-soluble alkaloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>9.0960 gm.</td>
<td>0.0049 gm.</td>
<td>0.1100</td>
</tr>
<tr>
<td>#2</td>
<td>8.5568 gm.</td>
<td>0.0065 gm.</td>
<td>0.1514</td>
</tr>
</tbody>
</table>
These results check well with each other but are somewhat lower than those obtained on the assays of the roots and rhizomes. The average of these four samples is .117 percent of ether-soluble material, in comparison with .180 percent which was found in the rhizomes. This shows that the rhizomes have the largest alkaloidal content and if this is proven to be coptine the author suggests that only the rhizomes should be official and not the whole plant.

Even though the whole plant of Coptis occidentalis contains less ether-soluble alkaloid than the rhizomes alone, the amount present is considerably more than the coptine content of Coptis trifolia which was found by Schults to be .012 percent. This indicates that C. occidentalis is higher in alkaloidal content of coptine than C. trifolia and would tend to prove that C. occidentalis is as useful medicinally, if not more so, than C. trifolia. This will be true especially if at sometime in the future it can be shown that coptine is one of the main active constituents of the various species of Coptis. As yet no definite use has been determined for coptine but perhaps the therapeutic values of Coptis are dependent on the coptine present. If
such is the case the author believes that *Coptis occidentalis* should replace *C. trifolia* in the National Formulary.

II Qualitative

It was deemed necessary at this time to prove conclusively that the ether extractive material discussed above was definitely alkaloidal in nature. Hence, dilute acid solutions of the substance in question were tested with Mayer's, Wagner's, Scheibler's, and Wormley's reagents and positive results were obtained in each case which showed the presence of alkaloidal material.

To further prove the presence of alkaloid the dried alkaloidal material, obtained from ether residue of quantitative determinations, was tested qualitatively for the presence of nitrogen. A small portion of the substance was fused with metallic sodium and the resultant cyanide thus formed plus an acid gave a blue precipitate. This positive test for nitrogen, coupled with the good results obtained with the alkaloidal precipitants gave proof of the presence of an ether-soluble alkaloid or alkaloids in *Coptis occidentalis*.

The outstanding difficulty in the study of the ether-soluble material known as "coptine" was the absence of qualitative tests which could be used in identifying the alkaloid. Therefore, it was decided to subject the substance in question to the common alkaloidal precipitants and reagents in order that coptine could be identified in the future.
The marc of all the *Coptis* rhizome samples on hand which had been kept stoppered continually was treated with several hundred cc. of ether, shaken, and allowed to stand for twenty-four hours. At the end of this time the ethereal solution had a marked fluorescence of a purplish tint. This fluorescence had not been noticed in any of the twenty-four hour samples run before and was thought to have developed on standing with ether over considerable period of time. The appearance of this fluorescence will be discussed more fully later on.

The ether solution was removed from the drug and this solution of about 70 cc. was split into two portions, one of which was to be used for the color tests to be made later, and the other sample was extracted with acid water in the usual manner. The free alkaloidal material was found to be dissolve with difficulty in the dilute sulfuric acid solution as shown by the fact that twelve acid washings were required before the acid solution failed to give a test with Mayer's reagent. These combined washings were extracted with ether which was observed to possess again the characteristic purplish fluorescence. There was a very slight yellow color in the first two washings but the fluorescence could be seen clearly.

As it was necessary that the alkaloidal substance be in an aqueous solution before the various alkaloidal reagents could react, a portion of the ether solution was washed with
2 percent sulfuric acid solution. Tests were made on this solution with the various alkaloidal reagents and the following results obtained:

1. Mayer's reagent. This gave a definite heavy white precipitate which formed immediately. This was allowed to stand for five days at the end of which time an increase in the precipitate was observed.

2. Wagner's reagent. This gave a slight precipitate which was yellowish in color. This was allowed to stand and at the end of five days there was considerable increase in the precipitate.

3. Picric acid. This gave a heavy white precipitate after about 30 seconds. There was no noticeable increase when allowed to stand for five days.

4. Marmee's reagent. This was rather indefinite although it did give a slight white precipitate. After standing five days this precipitate had increased but still was not nearly as copious as the previous three.

5. Scheibler's reagent. This gave a definite white precipitate when added. Upon standing for five days the whole solution became white and the precipitate very heavy. A check on this made by exposing Scheibler's solution to the air gave the same results showing that in this case it was the reagent that increased the pre-
cipitate, and not the alkaloid present.

6. Sonnenschein's reagent. A definite yellowish white precipitate was formed which increased a little on standing for five days.

7. Wormley's reagent. A very heavy brownish precipitate was formed immediately upon the addition of this solution. On standing this precipitate decreased a little and the color became lighter. This was probably due to the volatilization of the free bromine present.

8. Valsar's reagent. This gave a very definite light yellow precipitate, really more definite than any of the reagents so far. The precipitate was more flocculent than the others. Very little change occurred when allowed to stand.

9. Gold chloride. This gave a precipitate but not immediately. It was necessary to apply some heat and then only a slight precipitate was formed. There was no change in the precipitate after standing for five days.

10. Tannic acid solution. This gave no reaction whatsoever, even when heat was applied, and when the mixture was allowed to stand no precipitate was formed.

The above trials yielded better than average results with the reagents used. It must be remembered that the
quantity of alkaloidal material was rather small so that when it is said that the precipitate was definite or quite heavy this does not mean that a real large quantity was formed, but means that a precipitate was formed which was quite heavy considering the amount of alkaloidal material present. The results obtained can be used in identifying coptine.

The tests were run once more and confirmed. The remaining acid solution of the alkaloid was allowed to stand for several hours and the tests applied again. No positive results were obtained which indicated that the alkaloid may have been hydrolyzed. In order to determine this another acid solution of the alkaloid was made and tested. At 12 noon the tests tried with Mayer's, Wagner's, and Scheibler's reagents were positive. At 5 o'clock of the same day the tests were repeated and the results were negative. This may not prove definitely the occurrence of hydrolysis but this is the only logical explanation of the failure of the alkaloid to give tests. This reaction was noticed several times during the research and led the author to conclude that coptine is hydrolyzed slowly by dilute acid solutions.

The next step was to identify further the alkaloid. Portions of the previously mentioned fluorescent ether solution of the free alkaloid were evaporated to dryness on a white "spot" plate for the purpose of performing color tests. The common mineral acids, concentrated ammonium
hydroxide, and some of the more common reagents were used to this end. The following are the results obtained:

1. Concentrated sulfuric acid. This produced a blue color changing to light pink, then to a darker pink, and finally a very pale lavender.

2. Concentrated nitric acid. This gave a slight brown color which did not change.

3. Concentrated ammonium hydroxide. No reaction.

4. Hecke's reagent. This gave first a blue color which changed to slight pink and finally to lavendar.

5. Marquis' reagent. This gave a rather greenish blue color that did not change greatly.

6. Frohde's reagent. This gave first a green color which slowly changed to blue and finally to pink.

The final colors correspond a great deal to the color of the fluorescence and indicate that fluorescence is probably due to the alkaloid present.

These color reactions seem to set the alkaloid off from any other related ones, and to identify it as coptine. The alkaloid is not berberine as this gives entirely different reactions, producing an olive green color with sulfuric acid, a reddish brown with nitric acid, an olive green with Frohde's when heated, a greenish yellow turning to violet
and finally red with Mecke's reagent, and giving no reaction at all with Marquis' reagent.

Some writers claim that coptine is the same alkaloid as hydrastine but the color reactions of the two indicate that this is not the case. The color reactions of hydrastine which are given below can be seen to be greatly different from those of the ether-soluble alkaloid of Coptis, thus separating these two alkaloids from each other.

Hydrastine color reactions:
1. Concentrated sulfuric acid. Colorless, to red by heat.
2. Concentrated nitric acid. Orange color.
3. Frohde's reagent. Colorless, changing to green, to brown.

The qualitative data gathered so far certainly shows that a different alkaloid is present in Coptis and the author believes that it is coptine. In order to make further identity tests of this alkaloid it was necessary to determine if the fluorescence spoken of several times before was due to the presence of coptine or perhaps a third alkaloid. This fluorescence does not appear at first but

only after prolonged maceration. Because of this it was decided to see if the alkaloidal content increased with the appearance of the fluorescence and if so this would indicate further that the color was due to the alkaloid.

A sample weighing 11.5004 gm. was weighed out and 200 cc. of ether added and allowed to stand for twenty-four hours. At the end of this time the ether-soluble alkaloid was obtained from 50 cc. and weighed. No fluorescence was present in the ethereal solution. After four days the ether-soluble alkaloid was once more obtained and weighed. The ethereal solution had a purplish fluorescence but was not as definite as in some of the earlier samples. After six days the alkaloid was again obtained and weighed. At this time the ether solution had a definite pinkish blue fluorescence which was quite noticeable especially thru reflected light.

The results obtained were as follows:

#1. After one day

Weight of ether-soluble alkaloid .0039 gm.
Percent of ether-soluble alkaloid .118

#2. After four days

Weight of ether-soluble alkaloid .0046 gm.
Percent of ether-soluble alkaloid .160

#3. After six days

Weight of ether-soluble alkaloid .0067 gm.
Percent of ether-soluble alkaloid .223
These results show that the alkaloidal content increases on standing and that it also increases with the appearance of the fluorescence. Thus one of the characteristics of *Coptis occidentalis* can be said to be that the ether-soluble alkaloid material is slowly soluble in its solvent, but produces a purplish fluorescence when dissolved. This may or may not be due to coptine as a third alkaloid may be present which does not dissolve in ether until all of the coptine is removed. In order to determine this another experiment was run. In this the usual procedure was followed except that the drug was completely exhausted each time that the ether was withdrawn. Thus after macerating the sample for one day all of the ether was removed and the mare washed with portions of ether until no soluble alkaloid remained. Then a fresh sample of ether was placed on the drug and this macerated until a definite fluorescence was present. This was completely removed. Portions of both purified ether samples were then evaporated on a white "spot" plate and color tests made. The following results were obtained:

**One day sample (no fluorescence)**

1. Mecke's reagent

Bluish green color turning to blue, to pink, and finally to pale purple, mostly on edges.

**Fluorescent sample**

Bluish green turning to pink and finally obtaining purple background with slight green solution.
One day sample (no fluorescence)  Fluorescent sample

2. Sulfuric acid
   Green color becoming blue, then pink, and finally bluish pink
   Greenish blue, becoming blue, and finally bluish pink.

3. Frohde's reagent
   Slight green color changing to blue and finally pink
   Slight green color changing to blue and finally pink.

4. Marquis' reagent
   Definite greenish blue color that did not change greatly
   Definite greenish blue, no change.

These results are nearly identical and thus indicate that the alkaloid present the first day is the same as that which appears with the fluorescence, and that it is coptine. If there is a third alkaloid present in Coptis it is present in such small quantities that it fails to give any characteristic tests. Therefore, it would seem safe to conclude that there are only two alkaloids present in *Coptis occidentalis* in sufficient quantities to deal with, and that they are coptine and berberine.

The above color reactions differ slightly in the initial color from those given previously but this is probably due to the concentration of the alkaloid as the color changes in the previous tests were more rapid and also much more definite than these last ones. However, the final colors are prac-
tically identical and bluish pink color appearing when Mecke's solution is used is a very good test for identifying the presence of coptine.

Another sample was tested by letting it macerate for forty-eight hours and then adding acid to the ether removed from the marc. The ethereal solution had the characteristic fluorescence. The acid was not removed immediately as in the usual procedure but allowed to stay with the ether for twenty-four hours. At the end of this time the ether solution had lost its fluorescence and was quite clear and colorless. This indicated that the coptine was more soluble in the acid solution than in ether and in order to prove this "spot" plate tests were run on the ether portions. The reactions obtained were very poor and could not be classified. In none of them did the characteristic purple color appear.

A third sample was washed in the usual manner and the first two final ether washings which had fluorescence kept separate from the other three. Separate "spot" plate tests were made on each. The first two washings gave very good tests. An olive green color appeared at first but after standing for considerable time the characteristic purple color of coptine appeared. This was in the background and the liquid itself had a bluish green color. The last three washings, which were combined, did not respond very well although Frohde's and Mecke's reagents did give a slight purplish background on standing. This would indicate that
most of the alkaloid is removed by the first two washings so it is necessary to be sure to remove all of the berberine possible before making tests and not discard the first two washings. Nearly all the berberine can be removed by washing the final ether solution carefully with water and this has been done in the past so that no berberine was present.

One further test was made to try to identify further the cause of the fluorescence and this was as follows: a sample of Coptis trifolia and one of Berberis were placed in a flask and allowed to macerate with ether for seven days under the same conditions as the Coptis occidentalis had. Both of these had to be filtered at the end of this time as the original samples were fine powders. After filtering neither of these had a fluorescence at all. A fluorescence was not expected with Berberis but it was with Coptis trifolia. This would indicate that coptine does not cause the fluorescence, but if Schultz' work was correct and C. trifolia contained only .012 percent coptine in comparison with .14 percent in the Western species, the lack of fluorescence could be explained on the basis of there being too small an amount present in C. trifolia. Inasmuch as the color reactions show this purple color and that the fluorescence disappears when treated for a period of time with acid, and the resulting solution gives no characteristic color tests, it must be that the fluorescence is due to the presence of coptine in large enough quantities.
Color tests were made on the *Eastern Coptis* ether solution and the results were practically identical with those obtained before, the green or greenish blue color appearing first, and a final bluish pink background.

One more method was used to aid in the identification of coptine and this was the determination of its solubility in various solvents. Portions of the free alkaloid obtained in the quantitative tests were used and the results were as follows:

1. Very soluble in alcohol, dissolving readily and without the aid of mechanical means.
2. Quite soluble in chloroform.
3. Slowly soluble in petroleum ether. It was necessary to heat the solvent before solution was effected.
4. Slowly soluble in ether, heat being necessary to effect a solution.
5. Very slightly soluble in 2 percent sulfurous acid solution. Mechanical means were used and a solution obtained slowly.
6. Insoluble in both hot and cold water.

Having determined good qualitative means of identifying the alkaloid, the next procedure was to prepare the salts of coptine.

First, attempts were made to prepare the hydrochloride by passing a dry stream of hydrogen chloride gas for an hour into a petroleum ether solution of the alkaloid which had
been obtained by extracting the drug in a Soxhlet extractor with the above solvent and then applying the usual procedure, substituting petroleum ether for ether. During the period a turbidity was formed but not a definite precipitate. The petroleum ether was allowed to evaporate spontaneously with the result that a white solid substance gathered in the beaker. There was not a large amount of this but enough to be very easily seen. A portion was dissolved in water in which it was quite soluble. This latter solution was tested with Mayer’s. A precipitate formed which indicated that an alkaloid was present. This indicated that the hydrochloride of coptine could be formed and in order to confirm this a series of further tests were run.

First, a sample was weighed out and treated in the usual manner, being allowed to stand until a definite fluorescence appeared. This sample was washed and the first two portions of the final ether washings were kept separate and attempts made to form a hydrochloride. Hydrogen chloride gas was passed in as before. At first a very turbid solution was formed having a somewhat greenish color but on continual addition of the gas, this disappeared. The gas was run in for one hour and then the ether was allowed to evaporate. A white solid appeared when the solution was allowed to evaporate. A small quantity of ether was added and part of the solid dissolved. The ether was removed and water was added. This dissolved the remaining
solid quite readily, thus showing that this substance was partially soluble in ether and quite soluble in water. Again the acid solution was tested with Mayer's reagent and a precipitate formed, once more indicating that a hydrochloride had been formed. The ether-soluble portion did not have the fluorescence which had formerly been present, showing that only the alkaloid gave the color and not its hydrochloride.

One further attempt to make the hydrochloride was undertaken. The ether-soluble portions of two of the quantitative samples which had been run before were dissolved in alcohol, in which they were quite soluble. These two portions were kept separate and placed in evaporating dishes and to each were added 20 cc. of 2 percent sulfuric acid solution. These were placed in a steam bath and the alcohol evaporated slowly. When all of the alcohol was removed, a drop of each of the acid solutions was tested with Mayer's. In each case a very heavy white precipitate was formed. This was much heavier than any of the tests obtained before and suggested a method of obtaining even better qualitative results than had yet been obtained. This was done after finishing the attempt to make the coptine salts and will be taken up later.

The hot acid solution was allowed to cool and made alkaline. This alkaline solution was then washed with ether
with the result that the ether solution had the fluorescence which it had had before being allowed to evaporate to dryness. A portion of this ether was treated with hydrogen chloride gas as before and once again a solid was found when the ether had evaporated. This solid was tested in the usual manner and again positive results were obtained. A portion of the solid was kept undissolved and retained for the microscopic work that was to be done. These tests show definitely that a hydrochloride of coptine can be formed.

After formation of the hydrochloride attempts were made to form the hydroiodide of coptine. A portion of the ether solution obtained above was treated with an ether solution of hydroiodic acid. The acid solution was added directly to the ether and a precipitate was formed immediately. A slide was made of this precipitate and it was seen to be amorphous. It was allowed to stand in order to see if crystals would form, but no crystals appeared after 24 hours. The substance was slightly yellow in color. Microphotographs were taken of this and are shown in Plate III.

The hydrochloride portion saved from the preceding experiment was now examined under the microscope. This was shown to be crystalline in structure, the crystals forming together in mats. These crystals were monoclinic and showed pleochroism through 90°. They were colorless and quite small. Microphotographs were taken of these and
Plate III

Coptine Hydroiodide 16 diameters

Plate IV

Coptine Hydrochloride 16 diameters
are shown in Plate IV.

Attempts were made to form the bromide of coptine but these were not successful.

Microscopic Examination

Inasmuch as it was noted that the hot acid solution of the ether-soluble residue gave very good precipitation reactions, it was attempted to obtain these and study them so further identification could be made. A sample of acid solution was made as before and a drop of this was placed on each of four small watch glasses. A drop of Mayer's was added to one, Wagner's to another, Marmee's to a third, and Scheibler's to the fourth. Each one produced a heavy white precipitate indicating the presence of the alkaloid. At the same time, a drop of 2 percent acid water was placed on four different watch glasses and a drop of each of the four reagents added to one of the glasses. These eight watch glasses were then covered and allowed to stand for twenty-four hours. At the end of this time crystals were formed in each of the eight samples and they were then examined under the microscope in pairs, i.e., the solutions of Mayer's reagent - alkaloid - acid water was compared with the solution containing Mayer's reagent - acid water.

In this experiment it was the object to try to determine if crystals were formed by the alkaloid with the various reagents. There were many crystals in each pair, so
it was quite difficult to determine if the crystals were from the alkaloid or from the reagents combined with the acid water. Consequently a very detailed study was made of each slide and a written description made of each one. The following are the results obtained.

#1. Mayer's reagent + alkaloid.

In this slide square reddish-brown crystals were predominant. These showed little pleochroism and then only through 180°. These were undoubtedly inorganic crystals. Next in quantity were long monoclinic crystals that showed a strong pleochroism every 90°. These were colorless. A third group of crystals were present, quite similar to the ones just mentioned but not having as pronounced pleochroism. It may be that these were identical with the previous crystals, but they did not appear to be quite the same. Considerable amorphous material was present.

#1C. Mayer's Reagent + 2 percent acid solution.

This field was nearly identical with the previous one, having the same red square crystals showing slight pleochroism and long monoclinic crystals showing pleochroism every 90°. The third crystal was lacking in this one, but inasmuch as the two monoclinic crystals were thought to be alike it is doubtful if the third crystal was the alkaloid. No amorphous material was present as in #1.

Conclusion: Mayer's reagent forms a precipitate with
coptine which is amorphous at first and may become crystal-line but in all probability it does not. This will be proven by further work.

#2. Wagner's Reagent + alkaloid.

In this slide many monoclinic crystals were formed into long rope-like clusters. These were quite clear and showed pleochroism every 90°. There were also some small square crystals which were clear and gave the appearance of having a halo. These were monoclinic and showed pleochroism. There was considerable granular amorphous material present.

#2c. Wagner's Reagent - 2 percent acid solution.

This slide was nearly identical with the previous one except for the amorphous material being lacking in this one. The large monoclinic crystals were present and showed pleochroism every 90°. The same small square clear crystals were also present.

Conclusion. Coptine forms an amorphous precipitate with Wagner's reagent.

#3. Marmee's Reagent - alkaloid.

There were many monoclinic crystals in this slide which appeared very much like those in the slide with Mayer's reagent - alkaloid. These had no red color but were quite clear. Not a great deal of amorphous material was present.

#3c. Marmee's Reagent - 2 percent acid solution.

This slide was practically identical with #3, having
many long clear monoclinic crystals and some square crystals.

Conclusion: Coptine forms an amorphous precipitate with Marmee's reagent. Marmee's is not a very satisfactory reagent to use in testing for coptine. 

#4. Scheibler's Reagent - alkaloid.

There was a predominance of sheaf-like crystals which formed in clusters or bundles and had pleochroism every $90^\circ$. These were clear crystals.

#4C. Scheibler's Reagent - 2 percent acid solution.

There were no definite sheaf-like structures here but there were many clear tree-like structures radiating from a common center. These did not show pleochroism. Most of the material in this slide was floculent and some granular.

Conclusion: Scheibler's may form a crystalline precipitate with coptine, the crystals being formed in sheaf-like clusters. This is not definite and further studies will be made of this.

It was hoped to obtain more definite results from the above, but due to the fact that the alkaloidal reagents crystallize on standing, much interference was encountered. However, it was thought that microscope determination would be the most certain in determining the coptine so these methods were continued. Before proceeding, fresh solutions of each of the above four reagents were made up and also several others.
A fresh sample of drug was treated in the usual manner and the final purified ether sample was allowed to evaporate to dryness. This was dissolved in alcohol and treated with acid as before. A few drops of the acid solution were placed on each of nine slides and a few drops of the different reagents added to one of each alkaloidal sample. These were observed both macroscopically and microscopically with the following results:

#1. Mayer’s Reagent. This gave a milky looking solution and a precipitate which increased on standing. When viewed under the microscope the precipitate was not white but light brown in color and was in the form of fine granular material. Under cross nichols no color change was seen.

#2. Wagner’s Reagent. This gave immediate precipitation, which seemed to be white in the reddish brown solution. Under the microscope this precipitate looked nearly identical with that formed with Mayer’s reagent. The granules were quite small, but due to the color of the solution the color of them could not be determined. No color change was shown under cross nichols.

#3. Scheibler’s Reagent. A rather heavy white precipitate was formed here which increased when slight heat was added. This precipitate was light brown in color when viewed under the microscope and formed fine granules. No pleochroism was shown.
#4. Marmee's Reagent. This gave but a slight precipitate and when viewed under the microscope the results were too slight to be of any value.

#5. Gold Chloride. When first added, this gave no precipitate and after standing no precipitate was formed. On the addition of slight heat a precipitate was formed which was as the others - granular and showing no pleochroism.

#6. Picric Acid. This did not give a precipitate immediately, but on standing about 90 seconds a very heavy precipitate was formed. This appeared nearly colorless and looked crystalline to the eyes. Under the microscope beautiful monoclinic fishtail crystals were seen. These were colorless and showed marked color change under cross nichols. With polarized light these were very beautifully colored, being blue and green, especially with the gypsum plate present. These were the first definite crystals of coptine formed yet, and if not due to the reaction of the picric acid and sulphuric acid present, this will be one very definite method of identifying coptine. This will be determined.

#7. Sonnenschein's Reagent. This is slow to give a reaction but on the addition of heat a precipitate which seems to be slightly yellow is formed. This is granular and shows no pleochroism. Inorganic crystals were present in the field.

#8. Wormley's Reagent. Immediately upon adding this reagent a very heavy yellowish precipitate was formed. This became
white when allowed to stand and when viewed under the microscope a clear, granular substance showing no pleochroism was seen.

29. Valser's Reagent. A very definite yellow precipitate was formed, which, when viewed under the microscope, was amorphous.

In order to determine if picric acid formed a definite crystal with coptine a slide was prepared containing picric acid and 2 percent sulfuric acid solution which contained no alkaloid. This formed a crystalline precipitate but was not at all similar to the one of coptine picrate which had been obtained before. The latter showed much more pleochroism than the former and the colors under polarized light were more pronounced. Microphotographs were taken of both of these slides and are shown in Plates V and VI. The crystals of coptine picrate can be seen more in detail in Plate VII, which is an increased magnification of Plate VI.

Slides were also made of some of the other reagents and the alkaloid. These were allowed to stand for two hours and at the end of this time microphotographs were taken which may be seen in Plate VIII.
Plate V

Picric Acid and Sulfuric Acid 16 diameters

Plate VI

Coptine Picrate 16 diameters
Plate VII

Coptine Picrate 62 diameters
Plate VIII

Sonnenschein's Reagent and Coprine

Note clusters of amorphous coprine salt which is the typical form of all coprine amorphous salts. The large monoclinic crystals are those of Sodium Nitrate.
This concluded the work in assaying coptine both qualitatively and quantitatively. In the qualitative tests coptine and hydrastine were compared and found to be quite different in their reactions. It was now necessary to determine if the two alkaloids differed microscopically and if so this would definitely separate them. Literature referred to classed coptine and hydrastine together but the author doubted this. Consequently a slide was made of hydrastine in 2 percent sulfuric acid solution to which picric acid was added. A precipitate was formed and allowed to crystallize and then examined microscopically. The crystals formed were not at all like those of coptine picrate, and showed less pleochroism. The field in general was not the same, there being much more granular material in the hydrastine field. A check was run on this by repeating the experiment and the results were the same as before. Microphotographs were taken of the slide and the results shown in Plate XI. By comparing this with Plate VI it can easily be seen that coptine picrate is entirely different from hydrastine picrate. With these results it was definitely concluded that coptine was a very distinct alkaloid and unlike hydrastine in its reactions.

Plate XI

Hydrastine Picrate 16 diameters

Plate XII

Berberine Picrate 16 diameters
In order to make sure that the alkaloid was not berberine a slide was made of berberine and picric acid. This formed crystal-like plates and did not resemble the coptine picrate in any form. A microphotograph was taken of the berberine slide and this is seen in Plate XII. By comparing all three plates it can be seen that each of the alkaloids is quite different in crystalline form and they can therefore be identified from each other by forming the picrate of each.

This concluded the experimental work on the coptine of *G. occidentalis* and left the percentage of berberine to be determined. This was done, using the following procedure: a sample of the drug was weighed out and placed in a Soxhlet extractor and extracted for about six hours using alcohol as the solvent. The percolate was allowed to cool and diluted to 100 cc. with the same menstrum. 25 cc. of this were removed and placed in a suitable vessel to which were added 1.3 cc. hydrochloric acid (U. S. P.), 0.2 cc. sulfuric acid, and 12.5 cc. of ether. The mixture was allowed to stand for twenty-four hours in a cool place with occasional agitation. The crystals were collected on a tared Gooch crucible and washed with equal volumes of ether and alcohol until the filtrate gave no acid reaction with litmus paper. The crystals were then dried at 105°C and allowed to cool and were
then weighed. Bright orange crystals were obtained. The following results were obtained when the berberine was determined:

#1. Weight of Sample
Weight of berberine hydrochloride
Percent of berberine in C. occidentalis

#2. Weight of Sample
Weight of berberine hydrochloride
Percent of berberine in C. occidentalis

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Summary and Conclusions

1. The ether-soluble portion of *Coptis occidentalis* was obtained by a washing-out method and the quantity determined. This was found to be equivalent to 0.180 percent in an October sample and 0.106 percent in a June sample giving 0.14 percent as the average coptine content of the roots and rhizomes. This is considerably higher than the coptine content of *C. trifolia*.

2. The ether-soluble portion was tested for identity in the usual method followed for alkaloids. This portion was found to be typically alkaloidal, responding very well to alkaloidal reagents and color tests. By combining the qualitative tests the substance was identified as coptine. These tests can be used in future work in determining coptine.

3. Coptine produces a fluorescence when macerated in ether for sufficient time, but this fluorescence does not appear unless there is considerable coptine present. This color can be used in identifying coptine.

4. There is no third alkaloid present in *Coptis occidentalis*. This was determined by showing that the fluorescence was caused by coptine and that with the removal of coptine the remaining part gave no typical alkaloidal tests.

5. Microscopic crystals can be made of the salts of coptine. The hydrochloride is crystalline, and the picrate
is characteristic of coptine alone. The microscopic determinations can be used as methods of identifying coptine and to separate it from two closely related alkaloids, hydrastine and berberine.

6. *Coptis occidentalis* contains a higher percentage of berberine than *Coptis trifolia*, the latter containing only .8 percent of berberine as the sulfate while the former contains 1.79 percent as pure berberine.

7. The therapeutic value of *Coptis trifolia* is due mainly to the alkaloids, berberine and coptine, which are present. With the data gathered from this research it can easily be seen that *Coptis occidentalis* is a plant richer in alkaloidal content than *Coptis trifolia*. 
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