THE ISOLATION AND IDENTIFICATION OF AN ETHER-SOLUBLE ALKALOID IN *COPTIS OCCIDENTALIS*.*,[1]

BY T. D. ROWE.[2]

One of the plants found in abundance in the damp mossy sections of Western Montana is *Coptis occidentalis, Ranunculaceæ*. This is a dark green plant averaging about 20 cm. in height, and was first described by Nuttall in 1838 (1). Although small, it is nearly twice as large as its close relative, *Coptis trifolia*, which was official in N. F. V.

In 1873, Gross assayed *C. trifolia* and concluded that a heretofore undiscovered alkaloid was present. This he named Coptine (2). In 1884, Schulze made a quantitative study of *C. trifolia*, and found it to contain 0.012% coptine (3). Since then very little work has been done on either species.

Neither plant seems to have great medicinal value, although in some sections of the west considerable quantities of the dried whole plant of *C. trifolia* are used by the laity in the treatment of various gastrointestinal disorders.

The purposes of this study were: (*a*) to determine the ether-soluble alkaloidal content of *C. occidentalis*; (*b*) to identify this alkaloid by means of qualitative tests. The pharmacology of coptine is yet to be accurately determined.

EXPERIMENTAL.

The major portion of *C. occidentalis* used in this study was collected in October; a small quantity was collected in June. Both portions were air-dried and ground to moderately fine powders.

Extraction with Selective Solvents.—Samples of the whole plant collected in October, weighing about five Gm. each, were extracted by successive extraction in Soxhlet extractors by continuous percolation for 18 hours. The drug was dried to constant weight after the removal of each solvent. The solvents were used in the order listed below; and the following was the average per cent of extractive material obtained:

Solvent.	Per Cent of Extractive.
1. Petroleum Ether	1.0065
2. Ether	1.075
3. Alcohol	23.166
4. Water	11.9215

QUANTITATIVE DETERMINATIONS.

These were made first on four samples each of the rhizomes of the October and June portions. The samples weighed about ten Gm. each. The U. S. P. maceration and aliquot part procedure for proximate assay was followed, using ether and two per cent sulfuric acid as the solvents. The results gave an average of 0.1055% ether-soluble material in the June samples, and 0.1802% in the October portions. Thus the fall samples were shown to contain over 70% more ether-soluble material than the drug collected in the early summer. This was over fourteen times the quantity of alkaloidal material found by Schultz in the whole plant of *C. trifolia*.

Five-gram samples of the leaves were assayed, and yielded 0.133% ether-soluble material. Ten-gram samples of the whole plant were found to contain 0.117%.

Because of the higher content of ether-soluble material found in the rhizomes of the October portion, this was used in most of the experimental work.

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QUALITATIVE TESTS.

Qualitative tests showed the material obtained in the quantitative work to be alkaloidal. The tests consisted of adding several alkaloidal precipitants to acid solution samples of the ether-soluble material. Mayer's, Wagner's, Scheibler's and Wormley's reagents were added to separate portions, and a precipitate occurred in each case. Portions of the dried residue obtained in the quantitative determinations were tested for nitrogen by fusing the residue with metallic sodium and testing for the formation of a cyanide. The test was positive on each of several portions. Because of the good results obtained with the precipitants, and with positive nitrogen tests, it was concluded that the ether-soluble material in *C. occidentalis* was alkaloidal.

The residues of the rhizome samples used in the previous work were combined and exhausted with ether. Only one-half of the ether originally used to extract the drug had been removed for the quantitative work, so the remaining ether contained alkaloidal material from the drug. Flasks containing the residues had been well stoppered since the quantitative tests were made, and the drug had been covered with ether. When removed from the drug, the ethereal solutions had a marked purple fluorescence.

The solutions from the residues were combined and divided into two parts, A and B. Solution A was extracted by shaking out in the usual manner, adding portions of two per cent sulfuric acid and then ether, and lastly two per cent sulfuric acid. Solution B was kept unchanged.

Tests were then run on a portion of the acid-water solution obtained from Solution A, with the following results:

1. Mayer's reagent—Definite white precipitate which formed immediately.
2. Wagner's reagent—Slight precipitate, yellowish in color.
3. Picric Acid Solution—Definite white precipitate after 30 seconds.
4. Marmee's Reagent—Indefinite. Slight white precipitate.
5. Scheibler's reagent—Definite white precipitate.
6. Sonnenschein's reagent—Definite yellowish white precipitate.
7. Wormley's reagent—Definite brownish precipitate which formed immediately.
8. Valsar's reagent—Definite white precipitate.
9. Gold Chloride Solution—Precipitate only when heat was applied.
10. Tannic Acid Solution—No reaction, even when heat was applied.

There was a very slight increase in the quantity of each precipitate after standing for five days.

The remaining acid-water solution was allowed to stand for several hours, and the tests applied again. No positive results were obtained. This indicated slow hydrolysis of the alkaloid and for confirmation a fresh acid solution was prepared and tested with the reagents listed above. At twelve noon the results were positive. At five o'clock of the same day the tests were repeated and negative results were obtained. This reaction was noticed several times during the investigation, and indicates that this alkaloid is hydrolyzed slowly by dilute sulfuric acid.

Portions of Solution B were evaporated to dryness on a white "spot" plate and color tests performed. The results were as follows:

1. Concentrated H_2SO_4—Blue color, changing to light pink to darker pink and finally to a very pale lavender.
2. Concentrated HNO_3—Light brown color which did not change.
3. Concentrated NH_4OH—No reaction.
4. Mecke's reagent—Blue color, changing to pink and finally to lavender.
5. Marquis' reagent—Greenish blue color which did not change.
6. Fröhde's reagent—Green color, slowly turning to blue and finally to pink.

The final colors in most of these resembled the color of the fluorescence previously mentioned, and indicate that the fluorescence may be due to the alkaloid present. Confirming results were obtained by repeating the above tests several times.

These "spot plate" tests were run on samples of ether-soluble alkaloid extracted from *Coptis trifolia* and the same results obtained. This indicated that the ether-soluble alkaloids in *C. trifolia* and *C. occidentalis* were the same, *i. e.*, coptine.

These color reactions appear to be characteristic of coptine. Tests tried on berberine gave entirely different reactions, and those listed in Merck's Index for hydrastine separate the latter from the alkaloid studied.

Fluorescent solutions which had been obtained by extracting the drug with ether were tested by allowing acid water to remain in contact with the ether solution for twenty-four hours. The fluorescence disappeared, and alkaloidal tests run on both portions, (1) ether, and (2) two per cent acid solution, gave negative results. This definitely showed that the fluorescence was caused by the alkaloidal material, and that the alkaloid was hydrolyzed by dilute sulfuric acid.

Tests for solubility of the free alkaloid gave the following results:

1. Alcohol—very soluble.
2. Chloroform—soluble.
3. Petroleum ether—slowly soluble; slight heat necessary.
4. Ether—slowly soluble.
5. Two per cent H_2SO_4—very slightly soluble.
6. Water—insoluble; both hot and cold.

FORMATION OF SALTS.

Hydrogen chloride was passed into an ether solution of the alkaloid for an hour. On evaporation of the ether, a white solid appeared. Ether was added and a very small quantity of

Fig. 1.—Coptine + 2% sulfuric acid + 1% picric acid. 16×.

the solid dissolved. Upon addition of water to the remaining part, the material dissolved rapidly. This solution was tested with Mayer's reagent and a precipitate formed immediately. These procedures were repeated as above, and also with slight modifications. Each time a water-soluble salt was obtained which gave characteristic alkaloidal reactions. No fluorescence was noted when the salt was dissolved in water, but when the solution was made alkaline and the alkaloid dissolved in ether, the fluorescence appeared.

Microscopic examination of the hydrochloride showed it to be crystalline in structure. The formation was in mats, composed of small, colorless crystals, monoclinic in structure.

The hydroiodide was formed by adding an ether solution of hydriodic acid to the ether solution of the alkaloid. A precipitate formed immediately, and when tested in the same manner as was the hydrochloride, corresponding results were obtained, indicating that a hydroiodide of coptine was formed. This salt was amorphous and slightly yellow in color. Attempts to form the hydrobromide were unsuccessful.

MICROSCOPIC EXAMINATION OF THE SALTS.

The free alkaloid in ether solution was dissolved in two per cent sulfuric acid, and a drop of this solution placed on each of several slides. A drop of Mayer's reagent was added to one slide, Wagner's to another, Marmee's to a third and Scheibler's to a fourth. A heavy precipi-

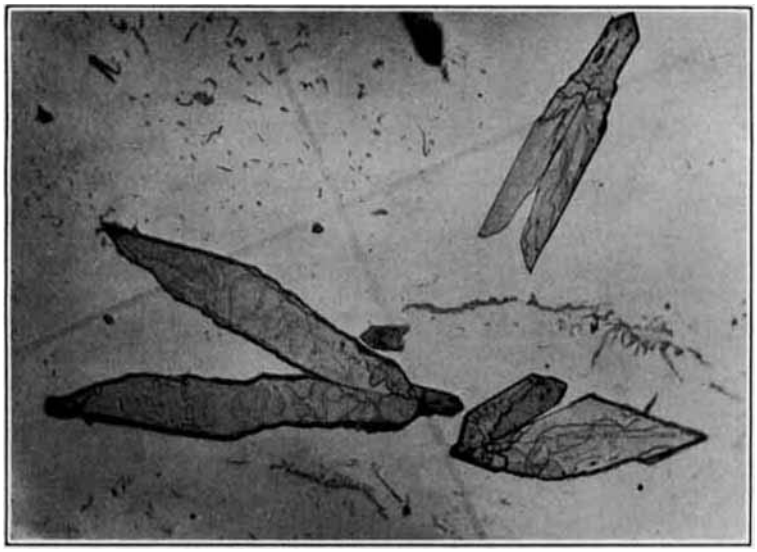

Fig. 2.—Figure 1 enlarged to 62 diameters.

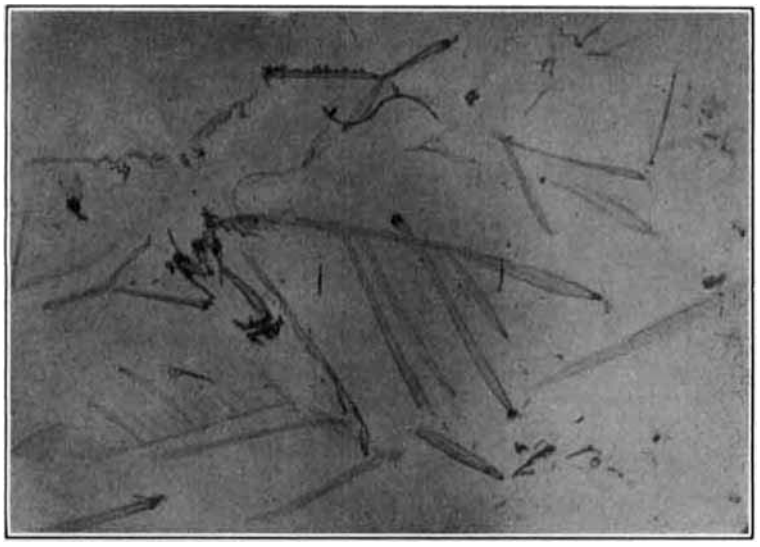

Fig. 3.—Control: 1% picric acid + 2% sulfuric acid. 16×.

tate appeared on each of the slides. To each of four more slides were added the above-named reagents, and a drop of acid solution which contained no alkaloid. The eight slides were covered and allowed to stand for twenty-four hours. Crystals formed on each of the slides. They were then examined in pairs, *i. e.*, Mayer's reagent and alkaloidal salt in acid water was compared with Mayer's reagent and acid water. A study was made of each slide, and the following conclusions drawn: With coptine and 2 per cent sulfuric acid (1) Mayer's, Marmee's and Wagner's reagents

produce an amorphous precipitate; (2) Scheibler's reagent forms clear sheaf crystals. These results were obtained uniformly in a series of comparisons.

Further tests were made using a freshly prepared sample of the alkaloid material, and additional reagents. Of ten reagents used, one per cent picric acid solution gave by far the most

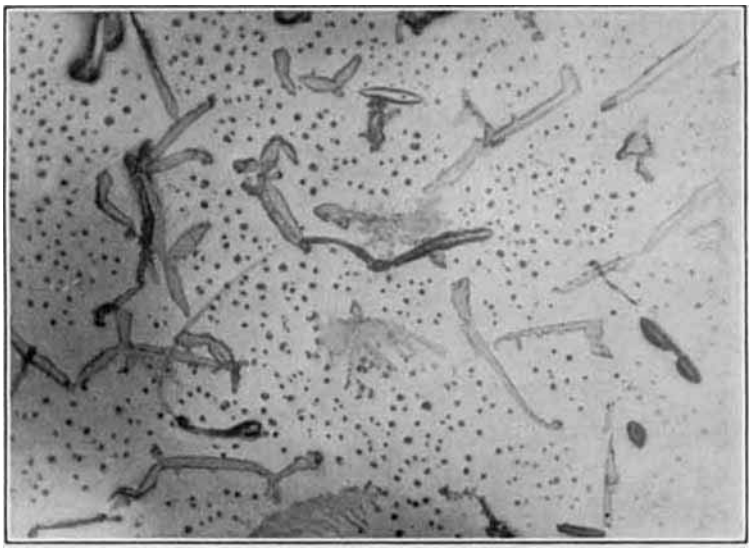

Fig. 4.—Hydrastine + 2% sulfuric acid + 1% picric acid. 16×.

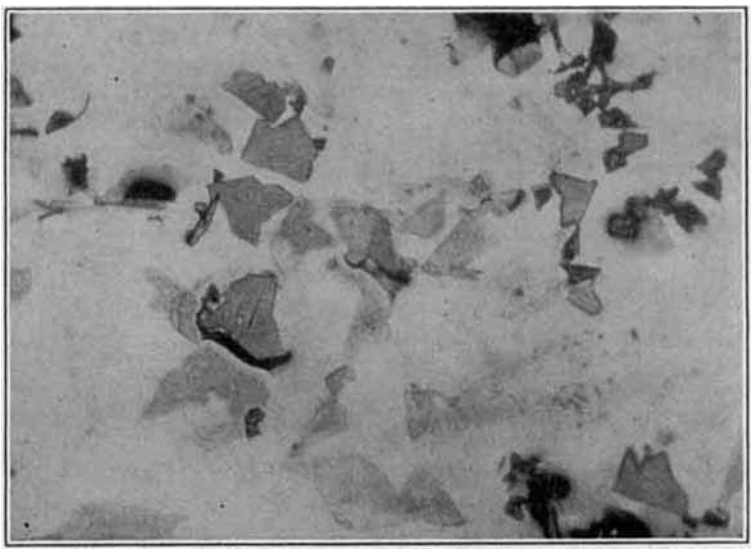

Fig. 5.—Berberine + 2% sulfuric acid + 1% picric acid. 16×.

conclusive results. With the alkaloid material dissolved in H_2SO_4, the picric acid produced definite monoclinic fishtail crystals. They were colorless under ordinary light, but with polarized light they gave striking blue and green colors. The controls of one per cent picric acid solutions with two per cent sulfuric acid contained crystals, but not like the ones obtained with coptine and two per cent sulfuric acid. No fishtails were seen, and the coloring was not as marked under

polarized light. The results can best be compared by a study of the photomicrographs shown in Figs. 1, 2 and 3.

Slides were made of hydrastine in two per cent sulfuric acid, and picric acid solution. The crystals formed were entirely unlike those of coptine and picric acid solution. Photomicrographs of these, and of the crystals obtained with berberine in two per cent sulfuric acid and picric acid, show the marked differences in the three, as seen in Figs. 4 and 5. These tests conclusively set coptine apart from berberine and hydrastine.

SUMMARY AND CONCLUSIONS.

1. *Coptis occidentalis* contains coptine, an ether-soluble alkaloid which is the same as the one found in *Coptis trifolia*. This alkaloid produces a marked purple fluorescence when dissolved in ether solutions.

2. Coptine is hydrolyzed slowly by dilute solutions of sulfuric acid to a form which gives no characteristic alkaloidal tests.

3. Microscopic examinations are practical to identify coptine and to distinguish it from two related alkaloids, hydrastine and berberine; the crystals formed with picric acid solution and coptine in sulfuric acid being particularly efficient for this identification.

REFERENCES.

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BIOASSAY OF LAXATIVES ON MONKEYS (RHESUS) AND ON LOWER MAMMALIANS (DYEMEAL METHODS).*

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The potency of many of the laxative preparations in use cannot be measured by chemical methods. Biological procedures must be employed, but the need for dependable bioassay methods for laxatives is unsatisfied.

In 1925, Loewe and Faure (1) devised a "dyemeal" procedure for measuring the passage of the intestinal contents, and applied this procedure to the assay of laxatives. Since that report, these studies have been continued with the objects of improving the bioassay methods implicated in those observations and of finding the most suitable test-animal for laxatives as well as the most appropriate test-function.

In the search for test-animals, our attention was originally directed to the smaller laboratory animals, particularly the albino mouse, which is most appropriate for dyemeal methods. As these studies went on, it became apparent that for many purposes the monkey was superior to all other test-animals. From the numerous manifestations of laxative action, increase in rate of intestinal progression was finally found most appropriate for the dyemeal assays in the mouse, and change in stool consistency for the assay in the monkey. Therefore, the present report on useful routine methods omits all our attempts with other devices of testing the functioning of the intestine, and is restricted to the applications of the dyemeal

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