SCIENTIFIC SECTION

BIOCHEMISTRY OF MAY APPLE ROOT (PODOPHYLLUM PELTATUM) I.*

BY ARNO VIEHOEVER AND HARRY MACK.**

INTRODUCTION.

The medical purgative properties of the creeping root of the May Apple, so prominent a plant in the spring, were known to the Indians before the settling of this country by the white race. The use of this plant has been recorded in literature well over one hundred years ago. While the edibility of the fruit has been generally accepted by the layman, and the leaves have been reported to contain various amounts of resin, the part of the plant used is restricted to the rhizome. The so-called "Mandrake pills" contain considerable quantities of the resin of podophyllum. The drug has been recommended as a drastic purgative, as well as in diseases of the liver.

On account of the drastic character of podophyllum and its resin, the drug has lost favor with some of the medical profession. No standard exists for its evaluation other than a minimum limit for the amount of alcohol-chloroform soluble material extracted in presence of acidulated water. This extract is a crude mixture.

This study, a continuation of previous work (1, 2, 3), aims at the isolation of every ingredient and the determination of the physical, chemical and physiological properties of each one.

The results should clarify our understanding of the composition of the drug, the mechanism of action of its constituents, and thus assist in improving their usefulness as medicinal agents.

I. THE RESIN "PODOPHYLLIN."

Occurrence.—In 1832, Hodgson (4) obtained a mixture of resinous substances by precipitating a concentrated alcoholic extract of the rhizome with water. Professor John King, in 1844, first described the material now known as the resin and affixed the term "resin of podophyllum" to the mixture of substances. In 1846, Merrell presented the drug before the Eclectic medical profession and gave it the name of podophyllin which it still bears to-day. There seems to be some evidence that the name was acceptable to the authors of the United States Dispensary as early as 1833, but only upon the basis that the resin "should be found to be the purgative principle of the plant."

The amount of resin, as obtained by the U. S. P. method, may vary in amount (3–5%) depending upon the age of the plant, locality, time of year collected and storage. Russell (5) reports that the greatest percentage of resin was found in the early spring collected drug, which amount conformed with the pharmacopœial requirements. He states, "the spring collected drug gathered just as the plants begin to show above the ground, and the late fall collected drug, are both worthy of consideration." He notes further, that "to secure the maximum amount of resin, the rhizome and the roots of the plant should be collected in the fall of the year after the aerial

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part of the plant has died down. If the spring collection is attempted it should be done before the plant begins to send out aerial shoots."

Preparation (U. S. P. Method).—Exhaust with alcohol, by slow percolation, 1000 Gm. of finely powdered podophyllum; evaporate the percolate to thin syrup; pour syrup into 1000 cc. of H_2O containing 10 cc. of HCl, cooled below 10° C.; decant clear liquid from settled precipitate; wash preparation twice with two portions (each 1000 cc.) of cold distilled H_2O; dry resin and powder.

PROPERTIES.

Physical.—Podophyllin, an amorphous powder, is colored light brown to greenish yellow, turning dark upon exposure to light or when heated above 25° C. Its slight peculiar taste is faintly bitter. It forms a slightly opalescent, and faintly acid (to litmus) solution with alcohol, is only partially soluble in chloroform and ether, and practically insoluble in cold water. It is soluble in normal solutions (T.S.) of potassium or sodium hydroxide, producing a deep yellow liquid which darkens on standing. Acids precipitate the resin again.

Chemical.—The complex composition of the resin makes it impossible to formulate its precise properties or to work out an adequate satisfactory method of chemical evaluation. Dohme and Kelly (6) state that the resinous products obtained by three different methods, upon analysis showed the following characteristics:

TABLE I.—COMPOSITION OF PODOPHYLLIN.

Method.	Alc. Insol.	Ash.	Chloroform Sol.	Ether Sol.	P. Toxin.	Resin. Loh.	Resin. D. & K.
I	0.45%	0.18%	66%	68.5%	30.2%	4%	5.0%
II	Com. sol.	None	59%	64.2%	30.0%	9%	5.5%
III	0.475%	0.3%	68%	74.5%	28.2%	15%	4.9%

PHYSIOLOGICAL.

External.—Podophyllin greatly irritates eyes and mucous membranes in general. Chiani (7) indicates that the symptoms of podophyllin poisoning are severe pains in the eyes, edema of the lids, chemosis, slight superficial capacity of the cornea myosis, hyperemia of the iris, decreased visual power and paracentral scotoma. The Encyclopedia Britannica notes that the resin does not affect the unbroken skin but may be absorbed from a raw surface and will then cause purging. The skin of the face of one of the students, working with extracts of podophyllum, became severely inflamed.

Internal.—According to the U. S. P. it is an active purgative, is used as the basis of several proprietary "liver pills," and is administered in average doses of 0.01 Gm. "It is both a secretory and an excretory cholagogue," states the Encyclopedia Britannica, "and is used as an auxiliary to aloes." In toxic doses it produces intense entiritis which may end in death.

Lohmann (8) administered a nearly white resinous substance obtained by precipitation of the alcohol extract of podophyllum with water. Violent pains in the intestines and convulsions were produced in a sixteen-year-old boy with $^1/_{150}$ Gm. Much larger doses ($^1/_4$–$^1/_2$ grain) could be given when the precipitation was effected with water acidulated with hydrochloric acid, or still more (1–2-grain doses) when the resin was precipitated by acidulated water containing 5% alum.

Podwyssotski (9) recorded the following pharmacological data upon administering commercial podophyllin to cats.

To a white cat (weight not given) was administered 0.06 Gm. orally or in water; after $5^1/_2$ hours this dose was followed alternately by vomiting of mucus and later by an evacuation of the intestines. Recovery resulted only after forty-eight hours.

Another animal was given a 0.02-Gm. dose, which was followed six and one-half hours later by vomiting. No further symptoms were exhibited forty-eight hours after administration.

Ludwig Disque (10) submits the following data. When 1% alcoholic solutions were injected subcutaneously into cats, or given in pill form by mouth, amounts equivalent to 0.01 Gm. produced enteritis and gastritis, increased the secretion of mucus, vomiting and paralysis, followed by death in one or two days. With dogs 0.01 Gm. of pure podophyllin by mouth was uniformly cathartic; with frogs, however, 0.01 Gm. by mouth and subcutaneously produced no effect.

To offer a means for standardization of the gross constituents Munch (11) states, as a result of his experiments, that in general the human cathartic dose of podophyllin is considered to be 160 times the dose which is effective upon mice. The action of podophyllin was obtained after twelve to twenty-four hours and the cathartic dose for mice was about $^1/_{100}$ of the human dose. In our own experiments, giving a male dog, weighing 4660 Gm., podophyllin 0.05 Gm., administered with 200 Gm. food, caused vomiting after $4^1/_4$ hours, and purgative action after 4 hours and 41 minutes and 5 hours. Given a food containing 0.2 Gm. in 200 Gm. food one dog, tasting the resin, refused to eat the food.

Two facts are obvious: *First*, that the drug is essentially unfit for human consumption until it can be freed from its highly toxic, irritating principles and subsequent harmful reactions; *second*, that should the drug prove satisfactory, after refinement, for administration, an infallible method for regulating its medicinal use should be established.

II. The Toxic "Podophyllotoxin."

Historical.—Podwyssotski (9) carried out the first important chemical research upon the constituents of the rhizome in 1880. While he was unable to verify the presence, previously reported, of the alkaloid berberine, he obtained three substances. To the first, which he named podophyllotoxin, though not obtained in crystalline condition and melting at 93–95° C., he ascribed the laxative action.

Kuersten (12) who conducted, for his time, a very thorough research, succeeded in crystallizing podophyllotoxin and gave it the empirical formula of $C_{28}H_{24}O_9\ 2H_2O$.

Preparation.—After drying the pulverized rhizome in a drying oven at a temperature not exceeding 70° C. approximately 5 Kg. were extracted in a large copper extractor for one hour with 3 liters of petroleum ether. The liquor containing waxes, fatty acids, pigments and sterol-like constituents was set aside for further study.

Four successive chloroform extractions were then made for one-hour periods using three liters of liquid with each successive operation. (Upon testing the residual drug with more chloroform, a pale yellow coloration resulted and the liquor, upon evaporation, left a negligible quantity of residue.)

1. Amorphous Podophyllotoxin.

The combined chloroform liquors were distilled to 1500 cc. and added slowly, with continuous agitation to five times their volume of anhydrous, alcohol-free diethyl ether. After separating the precipitate (Podwyssotski; amorphous podophyllic acid) the combined chloroform, ether liquors were distilled to a volume of 500 cc. and added to twenty times their volume of petroleum ether. A yellow, amorphous, flocculent precipitate separated out which was partially purified by repeatedly dissolving in chloroform and precipitating with petroleum ether. (By the evaporation of the petroleum-ether chloroform liquors a fatty matter was obtained.)

The substance, obtained after collection and drying of the precipitate, was not crystalline, nor obviously pure enough to determine other physical characteristics, as optical rotation, or to carry out combustion analyses. Further purification by the method advocated by previous workers, seemed unpromising.

2. Crystalline Podophyllotoxin.

A sufficient quantity of the chloroform extract was evaporated to dryness. Approximately 80.0 Gm. of the pulverized residue were extracted in three successive operations with 25 cc. of anhydrous boiling benzene under a reflux condenser.

The combined extracts were permitted to cool and the supernatant brown liquor was decanted into a crystallizing dish from the yellow-brown residue of insolubles. This operation had to be repeated several times throughout the day. After standing ten days almost colorless crystals were deposited from this crude benzene extract representing impure podophyllotoxin.

Physical.—While these crystals showed in definite crystalline outline they were laminated, more or less ovate and showed striking-polarizing properties (see Fig. 1). On further purification they showed marked polymorphism; namely, single prismatic crystals (see Fig. 2) growing greatly

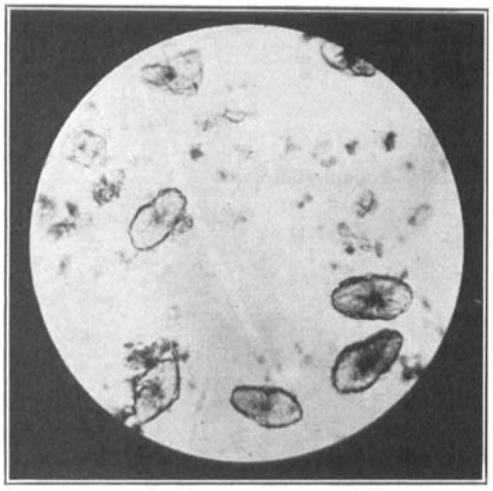


Fig. 1.—Podophyllotoxin (× 35) (from impure benzene extract).

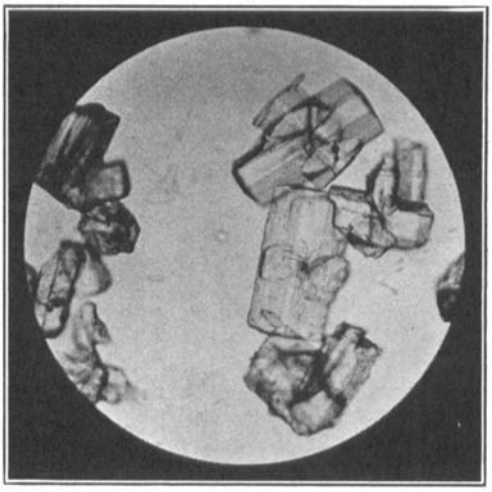


Fig. 2.—Podophyllotoxin (× 35) (from hot, dilute solution in benzene).

on standing, when recrystallized from dilute hot benzene solutions (using animal charcoal for decolorization and dense clusters or short needles (see Fig. 3), when recrystallized) from a hot concentrated solution in benzene.

The amorphous substance, obtained in the first method of preparation, softened at 112° C., melted partially at 118° C. and completely at 130° C.

The pure crystals of podophyllotoxin showed a corrected melting point of 118° C. The specific rotation from alcohol was:

$$\frac{-4.2°SS \times 0.3466 \times 100}{1 \times 1.504} =$$

$$-96.84° \text{ A. R. at } 19° \text{ C.}$$

The podophyllotoxin was very soluble in absolute alcohol, chloroform, acetone and hot benzene; slightly soluble in cold benzene and very slightly soluble in water. A red-brown to orange coloration was imparted to the sulfuric-phosphoric acid test solution (1 + 1).

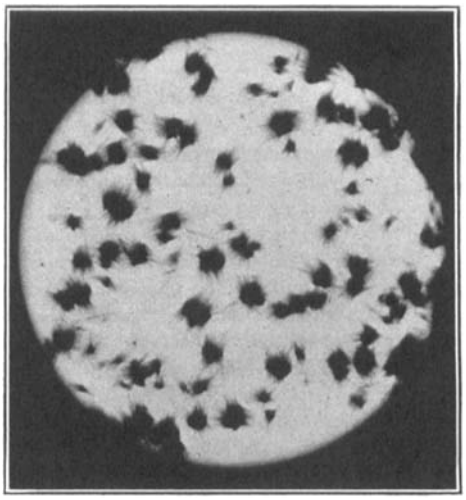
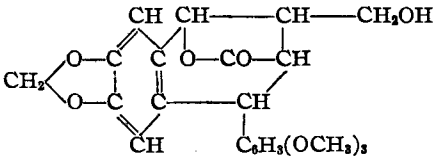


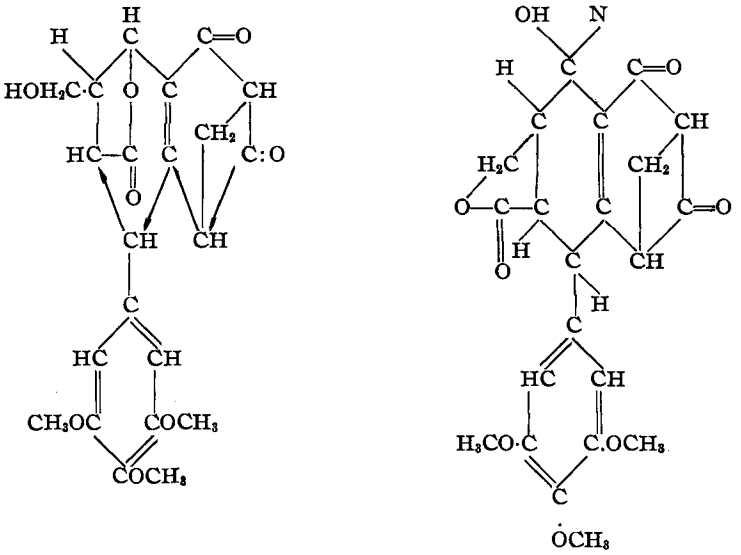
Fig. 3.—Podophyllotoxin (× 40) (from hot, concentrated solution in benzene and crystallization upon cooling).

These findings agree well with those of Dunstan and Henry (13) who reported a melting point of 117° C. and a specific rotation of −94°48′ for the hydrated podophyllotoxin, and a melting point of 157° and a specific rotation of −78°4′ for anhydrous podophyllotoxin.

Chemical.—While Dunstan and Henry (13) assigned the formula $C_{15}H_{14}O_6 2H_2O$ to podophyllotoxin, Borsche and Nieman (14) and Spathe, Wessely and Kornfeld (15) established the empirical formula $C_{22}H_{22}O_8$. As decomposition products they obtained benzene beta carboxylic, hydrastic, pyromellitic, trimethylgallic and podophyllomeronic acids as well as podophyllomerol. They suggested the following formulations:



Borsche and Nieman Formulation for Podophyllotoxin.



Spathe, Wessely and Kornfeld Formulation for Podophyllotoxin.

When podophyllotoxin was oxidized with nitric acid, oxalic acid was the principal product; when fused with fixed alkalies, orcinol and acetic acid were formed. It contains two methoxyl groups, but no hydoxyl group.

A red-brown to orange coloration was produced by pure crystalline podophyllotoxin, and a yellow color by impure amorphous podophyllotoxin, when treated with a mixture of equal parts of concentrated sulfuric and phosphoric acids.

Upon alkaline hydrolysis two crystalline products, picropodophyllin and podophyllic acid, are formed, which will be discussed below.

Physiological.—Podwyssotski (9) attributed the laxative action of the drug solely to podophyllotoxin. He admits that it acts strongest, and is intensively toxic in the crystalline modification to dogs and cats—0.005 Gm. subcutaneously injected, killed a cat without fail. Upon subcutaneous injection the following symptoms were observed by him in dogs:

1. Effects on the nervous system become obvious very soon ($1^1/_2$–2 hours) after injection with disturbances of coördination in posterior extremities. Animals, in walking, throw hind legs awkwardly and in confusion together, falling down easily.
2. Rapidly increasing weakness becomes noticeable, not always standing in direct relation to the violence of the gastro-intestinal symptoms.
3. Respiration becomes greatly increased.
4. The temperature is greatly lowered.
5. Before termination of life: several times violent colonic cramps have been observed.
6. Death occurs in comatous state.

Dissection immediately after death shows:

(*a*) The heart still beats.

(*b*) The mucous membrane of the stomach is almost completely reddened in obvious red spots, very moist and moderately swollen.

(*c*) The intestines are mostly strongly contracted.

(*d*) The mucous membrane of the intestine is, as a rule, less hyperemic than that of the stomach, but very succulent in its entire length covered with mucus and thrown-off epithelium.

(*e*) In dogs occurred several times small losses of substance in the ilium.

(*f*) The liver is markedly small and squashed, very dark and full of blood.

(*g*) The gall-bladder is frequently overfilled.

TABLE II.—EFFECT OF SUBCUTANEOUS ADMINISTRATION OF AMORPHOUS PODOPHYLLOTOXIN (.02 GM.) ON DACHSHUND (PODWYSSOTSKI (9)).

Time.	Symptoms.
After 2 hrs.	Vomiting of slime
After $2^1/_3$ hrs. until 7 hrs.	Repeated vomiting of slime. Slime colorless, water-clear
After 7 hrs.	Intestinal evacuation
During night	Continued vomiting, animal becomes very weak
After 24 hrs.	Animal takes no food
After $28^1/_2$ hrs.	Vomiting of much yellow slime
After 30 hrs.	Second intestinal evacuation
During night	Abundant yellow liquid evacuations
After 48 hrs.	Animal takes no food, but drinks water
During day	Abundant greenish, slimy evacuations
After 60 hrs.	Animal takes little food
After 72 hrs.	Animal takes little food, becomes more active
After 96 hrs.	Animal takes little food, evacuations with blood continue
In approx. 8 days	Animal recovers

DAPHNIA EXPERIMENTS.

Amorphous podophyllotoxin exerts in daphnia a slight laxative action in the time necessary to produce some solution. All test animals were obviously incapacitated within fifteen minutes after the application of the substance. They showed twitching of the swimming legs, slowed heart action and arrested breathing. The animals were dead in twenty-five minutes.

Crystalline podophyllotoxin, in a saturated aqueous solution, caused collapse of the test animals in eleven minutes. Partial evacuation was noted before general paralysis set in. When the liquid used in the last experiment was introduced to fresh animals, complete debility occurred within forty-five seconds. The heartbeats were shallow and irregular. No evacuations were noted. The animals were dead after two hours.

III. HYDROLYSIS PRODUCTS.

1. PICROPODOPHYLLIN.

Historical.—Upon alkaline hydrolysis of the podophyllotoxin, Podwyssotski (9) obtained two substances, one crystalline, which he called picropodophyllin and another gelatinous, which he named picropodophyllic acid. Podwyssotski even reported evidence of the presence of picropodophyllin in the drug, as he found it in the chloroform-extract residue, after treatment with cold absolute alcohol. Kuersten (12) corroborated Podwyssotski's findings of the formation of picropodophyllin. Dunstan and Henry (13) obtained it upon boiling with ethyl alcohol of the isolated gelatinous "podophyllic acid" (formed upon acidification with dilute acetic acid) in the alkaline hydrolysis mixture of podophyllotoxin.

Preparation.—To pure crystalline podophyllotoxin, dissolved in 25 cc. of absolute alcohol, 5 cc. of strong (28%) ammonium hydroxide were added. The mixture was heated on the water-bath until all the ammonia fumes were driven off, diluted with absolute alcohol and set aside to cool. Crystals of picropodophyllin were deposited which were filtered by suction and washed with cold alcohol.

PROPERTIES.

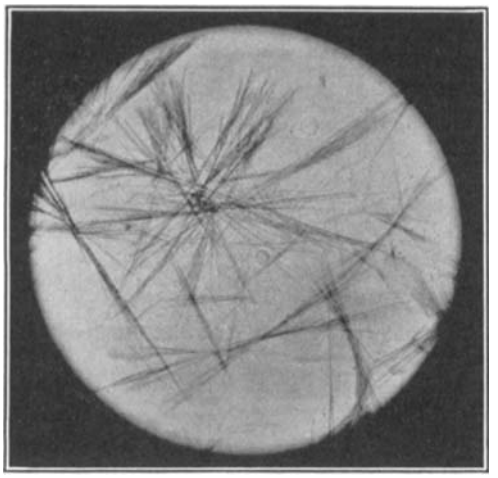
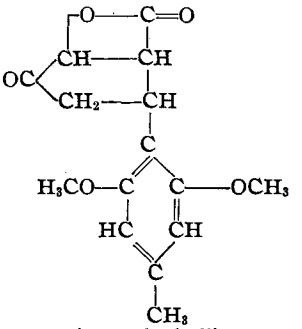


Fig. 4.—Picropodophyllin (× 40) (from treatment of podophyllotoxin in alcohol (hot) with ammonium hydroxide, and crystallization upon cooling).

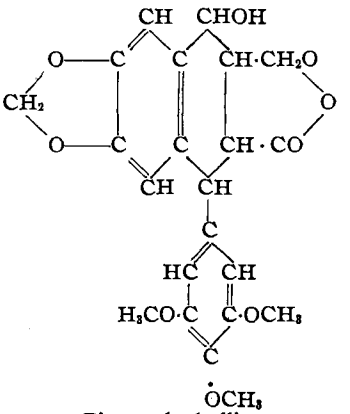
Physical.—The crystals consist of long, fine, colorless, silky needles (see Fig. 4). They melted at 228° C., uncorrected, with slight decomposition. The specific rotation in pyridine was found to be zero. Dunstan and Henry (13) had reported a melting point of 227° C. and optical inactivity.

Picropodophyllin crystals were found soluble in chloroform, acetone, hot ethyl alcohol, hot benzene and pyridine, slightly soluble in ethyl ether and insoluble in cold alcohol and water.

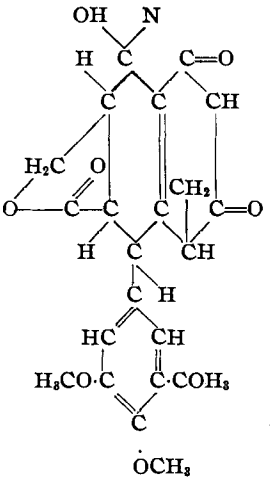
Chemical.—Dunstan and Henry (13) assigned to it the empirical formula of $C_{15}H_{14}O_9$. Picropodophyllin was found to furnish the identical decomposition products as podophyllotoxin, yielding oxalic acid, upon oxidation with nitric acid, and orcinol and acetic acid upon fusion with fixed alkalis. It also contains two methoxyl groups and no hydroxyl. Picropodophyllin finally was considered as the lactone of podophyllic acid with the following formulations suggested by the various workers.



Picropodophyllin.
Borsche and Nieman Formulations.



Picropodophyllin.
Spathe, Wessely and Kornfeld Formulations.



Picropodophyllin.

Enough discrepancy exists between the three proposals, the nature of their formulas and isomerism, to warrant that the problem be given further attention.

Physiological.—Picropodophyllin, practically insoluble in body fluids, is obviously highly irritating on account of the extremely pointed, needle-like crystals, resembling fine glass.

TABLE III.—EFFECT OF ORAL ADMINISTRATION OF PICROPODOPHYLLIN UPON LARGE CAT (AMT. 0.05 GM. CRYSTALS DISSOLVED IN OIL) (9).

Time.	Symptoms.
3 hrs.	Evacuation of old hard masses
3 1/2 hrs.	Vomiting of food remains and white slime, marked weakness
4 hrs.	Vomiting
5 1/6 hrs.	Vomiting with yellow-colored slimy liquid
5 1/3 hrs.	Strong evacuation
5 1/2 hrs.	Strong evaucation
5 3/4 hrs.	Strong evacuation, greenish
6 hrs.	Slimy vomiting with great effort, no bile
6 1/4 hrs.	Vomiting of very small amount with white slimy liquid
7 hrs.	Strong evacuation
8 1/4 hrs.	Vomiting, as before
12–20 hrs.	Vomiting, diarrhea
24 hrs.	Death (during night)

Post Mortem.

Liver: Very dark red, gall-bladder much filled with bile.
Stomach: Large curvature.
Duodenum: Middle portion shows red spots.
Intestine: Much contracted, filled with slime.
Mucous membranes: Stomach and intestine very red and swollen.

EFFECT ON DAPHNIA.

The crystals, practically insoluble in the culture water, and physiologically apparently inert, cause severe irritation and destruction through the sharply pointed ends of the mucous lining of the digestive canal. This irritation caused cramps and slimy evacuations in the daphnia, without being toxic. Thus, some daphnia survived for 24 hours and longer in the presence of picropodophyllin in their intestine.

2. PODOPHYLLIC ACID.

Historical.—Upon alkaline hydrolysis of podophyllotoxin Podwyssotski (9), as indicated before, obtained a gelatinous substance, which he could not isolate in a crystalline condition and which he called picropodophyllic acid. Kuersten (12) concluded that a crystalline substance, obtained by the alkaline oxidation of podophyllotoxin with permanganate, was identical with this picropodophyllic acid. He ascertained its general composition and called it podophyllic acid.

Dunstan and Henry (13) obtained the gelatinous substance after acidification of the alkaline hydrolysis product of podophyllotoxin with dilute acetic acid. All attempts to isolate this gelatinous substance failed and the name podophyllic acid was retained. Its acid reaction and salt-forming properties were established.

Preparation of the Amorphous Podophyllic Acid.—The light brown substance which is precipitated in great flocculent curds was described in connection with the preparation of amorphous podophyllotoxin (in the chloroform extract of the drug upon addition of alcohol free diethyl ether). After resolution in chloroform and reprecipitation with diethyl ether the substance was still obviously impure. Only by dissolving in hot isoamyl alcohol, decolorization by animal charcoal and reprecipitation by petroleum ether was an amorphous compound free from pigmentation obtained.

Preparation of the Crystalline Podophyllic Acid.—After many disappointing results the following new method was devised: 10 Gm. of Podwyssotski's amorphous podophyllotoxin were dissolved in 100 cc. in $N/2$ NaOH and heated at the boiling point for one-half hour. The solution undergoes a transition of color from yellow to a deep red brown. After cooling, it is acidified with $N/2$ aqueous HCl until faintly acid to litmus, then five cc. of more acid are added. The solution changes to a deep yellow to orange color. This transition in the coloration of the hydrolyzed solution is largely due to the presence of quercetin. Precipitation by 20% acetic acid was found inadequate and incomplete.

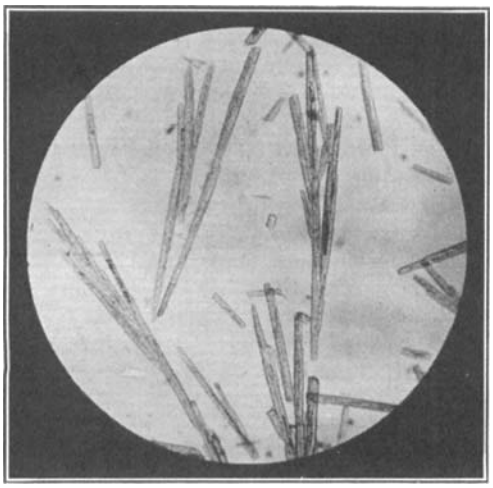


Fig. 5.—Podophyllic acid (× 35).

As the result of acidification a yellow, flocculent, gelatinous precipitate is produced which was taken up with isoamyl alcohol in a large separatory funnel. The mixture was placed aside for a short time to permit separation and the lower, clear aqueous layer drawn off and discarded. The alcoholic solution acquired a deep yellow color which was discharged by heating and filtration with good grade of animal charcoal. Upon cooling, the solution deposited no crystalline precipitate.

Enough anhydrous petroleum ether was now added to produce a faint turbidity and the mixture set aside over night, at room temperature, to permit crystallization.

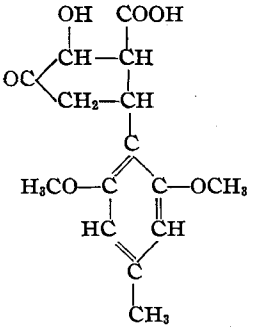
TABLE IV.—PHYSICAL PROPERTIES OF PODOPHYLLIC ACID.

	Amorphous.	Crystalline.	Anhydride.
Melting point	150° with slight decomposition	147° corrected with decomposition	208° with slight decomposition
Specific rotation	−80.36 at 19° C. in alcohol	−50.67 A. R. at 20° C. in absol. ethyl alcohol	....

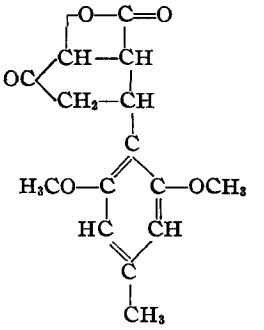
Both the amorphous and crystalline podophyllic acid preparations are soluble in cold, absolute and 95% alcohol, acetone, slightly soluble in ethyl ether and practically insoluble in petroleum ether and water. The amorphous substance was more soluble in cold and hot chloroform.

The crystalline substance consists of colorless prismatic needles (see Fig. 5) much larger in diameter than the thin-pointed needles of picropodophyllin.

Chemical.—Kuersten (12), by combustion analysis, ascertained the formula $C_{20}H_{24}O_2$. Dunstan and Henry (13) suggested the following structural formulation.



Podophyllic Acid.



Picropodophyllin.

They prepared the sodium, silver and copper salts. The rotation of the sodium salt was found to be −83°85.

When a crystal of the material is placed upon a piece of blue litmus paper and a drop of distilled water applied, a circular red area is formed about the crystal which is indicative of an acid reaction. When treated with the acid sulfuric-phosphoric mixture (1 + 1), a transition of color from yellow through green and finally blue occurs, which is later followed by a limpid liquid with a white turbidity.

The solid resulting on cooling of the molten crystals of podophyllic acid, represents small scalpel-shaped crystals melting, upon reheating at 208° C., with slight decomposition. From this melting-point observation it can be inferred that by heating the substance above its fusing point it is dehydrated and converted to the anhydride, picropodophyllin. Borsche and Nieman (14) intimate such an occurrence after obtaining the acid by another method. The oxidation method of Kuersten (12) for the preparation of podophyllic acid was attempted without success. Upon evaporation of the ethereal solution, a foul, evil-smelling, brownish red, resinous mass remained.

Physiological.—1. Amorphous Podophyllic Acid.

Podwyssotski considered the amorphous podophyllic acid prepared by him physiologically inert. When applied, in solution, to daphnia it exhibited no laxative action. It exerted a slight depressive effect on the breathing organs and the heart. The animals survived.

2. Crystalline Podophyllic Acid.

When several daphnia were placed into a saturated aqueous solution of the acid, evacuation was noted within 10 minutes. The abdominal organs moved freely. The breathing was somewhat hampered, locomotion was somewhat depressed, the animals showed slight toxic symptoms. However, two hours and ten minutes after administration the animals were still alive and moving freely.

IV. The Pigment Quercetin.

Historical.—Podwyssotski (9) obtained among the substances isolated from the rhizomes of podophyllum a crystalline pigment, which he named podophylloquercetin, pointing out its difference from quercetin. Kuersten (12) also studied its composition, and established its formula as $C_{23}H_{16}O_{10}$. He prepared hexacetyl and hexabenzoyl derivatives and concluded that podophylloquercetin was not identical to quercetin from quercitron bark. Dunstan and Henry (13), however, proved its identity with quercetin, much distributed in plants and plant products.

Properties.—The physical and chemical properties as well as the physiological inertness testify to the identity of podophylloquercetin. Whether quercetin occurs in partial combination with other constituents of the drug, in the form of a glucoside or otherwise, has not been finally decided. Thus far the results speak against the presence of a quercetin glucoside in podophyllum root.

CONCLUSIONS.

The extract of podophyllum, official as the resin podophyllin is a mixture of physiologically active and inactive substances. There is further evidence that this mixture contains a predominatingly toxic, and thus undesirable substance, and also a markedly laxative substance. Thus the pharmacopœial method of extraction as well as the official standard are in need of revision.

Experiments, undertaken with the aim of solving the problems which confront us even to-day, although podophyllum is a long known, important domestic drug, have thus far yielded the following results:

1. Only one active crystallizable substance, occurring as such, has thus far been isolated from either podophyllum or podophyllin, namely, podophyllotoxin.

2. Podophyllotoxin is characterized physically by its melting point of 118° C. and marked polymorphism: (*a*) Laminated, more or less ovate, crystals, showing striking polarizing properties, crystallizing from crude benzene extract; (*b*) dense clusters of short needles, crystallizing from hot concentrated benzene solution; (*c*) single prismatic crystals, growing greatly in size on standing, crystallizing from dilute benzene solutions; characterized chemically by 2 crystalline products of alkaline hydrolysis: (*a*) Picropodophyllin from alcoholic, alkaline solution; (*b*) podophyllic acid, from aqueous, alkaline solution; physiologically by its marked toxic and limited laxative properties when tested upon daphnia. The peculiar physiological effects of podophyllotoxin make it obviously unsuitable as a basis for a standard.

3. Commercial podophyllotoxin is an impure, only partially crystalline substance which exhibits both toxic and laxative properties. The pure crystalline podophyllotoxin exhibits predominantly toxic properties.

4. Picropodophyllin is characterized physically by its colorless needles, melting at 228° C., its insolubility in cold alcohol and water, and physiologically by the swelling, irritant and destructive action of the sharply pointed needles upon the mucous membrane of the digestive canal, causing cramps and slimy evacuation in daphnia.

5. Podophyllic acid is characterized physically by its colorless needles, melting at 147° C., its solubility in isoamyl alcohol, physiologically by its mild toxic effect in saturated aqueous solution and by its limited and uncertain laxative properties; when tested on daphnia.

6. Tannin has not been found by us. Quercetin, widely distributed in the plant, is obviously responsible for the reaction with iron chloride (resembling tannin-iron precipitation), observed by previous workers.

7. Chloroform completely frees the original drug of its toxic podophyllotoxin; this extraction leaves a laxative, less toxic drug residue.

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A PHARMACOGNOSTIC STUDY OF *CHRYSANTHEMUM BALSAMITA* L., VAR. *TANACETOIDES* BOISS., TOGETHER WITH A STUDY OF ITS VOLATILE OIL.*[1]

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The overground parts of *Chrysanthemum Balsamita* L., var. *tanacetoides* Boiss. (Family, *Compositæ*), contain a volatile oil, the odor of which is almost indistinguishable from that of oil of Spearmint. The striking similarity of the two oils, together with the fact that the plants yielding them come from different families, induced us to seek information in the literature concerning the composition of the oil of *Chrysanthemum Balsamita* L., var. *tanacetoides* Boiss. as compared to that of Spearmint. Very few references were found reporting on the chemistry of the oil, the ones that are available giving information only on physical constants. A number of references of a botanical nature are recorded and a few of historical interest.

The purpose of this study was to investigate in part the chemistry of the volatile oil, to determine the yields at various seasons of the year, and to study the morphology and histology of the plant. Material employed was obtained from plants collected in Michigan, Wisconsin and Minnesota and cultivated in the Medicinal Plant Garden of the College of Pharmacy, University of Minnesota.

Chrysanthemum Balsamita L., var *tanacetoides* Boiss. is an herbaceous, woody perennial native to western Asia. It was introduced into Europe and from there found its way to the United States. It is commonly known as Costmary, Old Maid, Sweet Susan, Sweet Mary and Mint Geranium. History reveals that the plant has been used in the treatment of many common ailments. In the British Pharmacopœia of 1788 it was recognized and used as an aperient. Some of its other uses have been as a diuretic, astringent, antiseptic, stomachic and anthelmintic.

PHARMACOGNOSTIC STUDY—EXTERNAL MORPHOLOGY.

Flower.—The florets are assembled into small closed heads, which form a corymbose inflorescence. The individual heads vary up to 8 mm. in diameter. They appear cup-shaped

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