colchicine was boiled with 15 cc. of water, leaving a residue (A) which partly crystallized on cooling. From the filtrate was obtained by evaporation and trituration of the residue with ethyl acetate–ether some 125° hexahydro compound; the ethyl acetate–ether washings on standing slowly deposited crystals of the desmethoxy compound. The residue A was dried and separated into 125° hexahydro and 171° hexahydro by chromatography on alumina, using benzene and chloroform as eluents.


## Summary

Hydrogenation of purified colchicine in methanol with Adams catalyst gives two stereoisomeric hexahydrocolchicines, which have been characterized. A desmethoxyhexahydrocolchicine is also formed in small amount.


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[CONTRIBUTION FROM THE CHEMOTHERAPY SECTION, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

# Components of Podophyllin. III. Isolation of α- and β-Peltatin. Structure Studies[1]

BY JONATHAN L. HARTWELL* AND WENDELL E. DETTY[2]

The report[3] that local application of podophyllin causes complete regression of *condylomata acuminata* (venereal warts), along with the observation[4] that this drug produces mitotic abnormalities in cells of rabbit and human skin similar to those produced by colchicine, prompted a test of this drug with the finding[5] that it exerts a strong destructive action on sarcoma 37 in mice. Belkin,[6] also prompted by these reports,[3,4] obtained reduction in size of sarcoma 180 and of a transplated mouse mammary carcinoma following subcutaneous injection of podophyllin, demonstrating for the first time that this crude drug can affect the growth rate of malignant tumors. Reich, *et al.*,[7] described the regression of soft papillomas of the female urethra by local applications of podophyllin.

Podophyllin N. F. (or resina podophylli, U. S. P. XI), a drug previously sued as a purgative, is a brown powder with a peculiar odor, a sharp taste, and an irritating effect on mucous membranes. It is derived, in the United States, from the alcohol-soluble portion of the dried roots and rhizomes of *Podophyllum peltatum* L. (Fam. *Berberidaceae*), the mandrake or May apple. A search of the literature revealed that podophyllin is a complex mixture of oil, pigments, and noncrystallizable resins from which two definite compounds have been isolated, one colorless and one colored, namely, podophyllotoxin and quercetin.[8] Podophyllotoxin has been the subject of several investigations and the structure, I, has been proposed for it independently by Borsche[9] and by Späth.[10] Bioassays with sarcoma 37 showed[5] that podophyllotoxin (prepared as described below) had several times the tumor-necrotizing action of podophyllin while quercetin[5,11] (prepared from rutin[12]) was inactive except in very much higher doses.

In a search for other components it seemed reasonable to try chromatography both because podophyllin is a highly-colored complex mixture and because in all previous chemical studies of the drug this technique had never been employed. Application of this technique has resulted so far in the isolation of podophyllotoxin and two other colorless, crystalline compounds[1a,1b] of roughly equal biological activity[5,11] against tumors, which have been named α-peltatin and β-peltatin. The isolation and progress in the structure determination of the last two substances comprise the subject of this communication.

## Structural Studies

Consideration of the elementary analysis and molecular weight values for the peltatins and their derivatives indicates an empirical formula of $C_{22}H_{22}O_8$ as the most likely for both peltatins. The latter would thus be isomeric with podophyllotoxin. It may be mentioned, however, the the analyses of α-peltatin and several of its derivatives are equally consistent with the formula $C_{21}H_{20}O_8$. Until more evidence is obtained on this point, the two peltatins will be considered to be isomers. If the alternate formula for α-peltatin should be correct, the following discussion will not be invalidated.

The functional groups containing all the oxygen are accounted for as follows. Both peltatins

* Harvard University Ph.D. 1935.

(1) Presented, in part, at the Detroit meeting of the American Association for Cancer Research Inc., April 16, 1949. (a) For paper I in this series, see Hartwell, THIS JOURNAL, **69**, 2918 (1947); (b) for paper II, see Hartwell and Detty, *ibid.*, **70**, 2833 (1948).

(2) The isolation studies constituted a thesis presented by Wendell E. Detty to the Department of Chemistry of the Graduate Division of Georgetown University, in partial fulfillment of the requirements for the degree of Master of Science, 1949. Present address: University of Oklahoma, Norman, Oklahoma.

(3) Kaplan, *New Orleans Med. Surg. J.*, **94**, 388 (1942).

(4) King and Sullivan, *Science*, **104**, 244 (1946).

(5) Hartwell and Shear, *Cancer Research*, **7**, 716 (1947). In this paper α-peltatin is referred to as NCI-1074.

(6) Belkin, *Fed. Proc.*, **6**, 308 (1947); *J. Pharm. Exp. Therap.*, **93**, 18 (1948).

(7) Reich, Nechtow and Rubenstein, *Am. J. Obstet. Gyn.*, **53**, 658 (1947).

(8) Podwyssotzki, *Arch. exp. path.*, **13**, 29 (1881).

(9) Borsche and Niemann, *Ber.*, **65**, 1633 (1932).

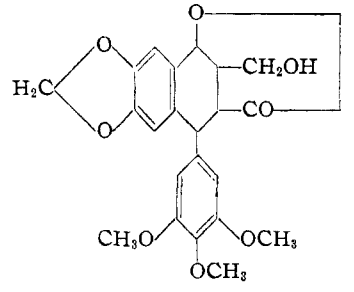
(10) Späth, Wessely and Nadler, *ibid.*, **66**, 125 (1933).

(11) Leiter, Downing, Hartwell and Shear, *Cancer Research*, **9**, 597 (1949).

(12) Wunderlich, *Arch. pharm.*, **246**, 224, 244 (1908).

gave the Gaebel test[13] for the methylenedioxy group; methoxyl determination (Zeisel) indicated two such groups for α-peltatin and three for β-peltatin; acetylation and alkylation indicated two hydroxyl groups for α-peltatin and one for β-peltatin; finally, the presence of a lactone group in each peltatin was strongly suggested by the behavior toward alkalies. Both peltatins gave indications of a masked acidic group; the compounds were insoluble in cold but soluble in hot sodium carbonate solution; the methyl ethers were soluble in hot sodium hydroxide solution. These indications of an ester grouping pointed more specifically to a lactone group when it was determined that the products of the action of hot alkalies had the same number of carbon atoms as the original starting materials. Acidification of the alkali-treated peltatins or their methyl ethers frequently yielded gelatinous products, which may well have been free acids, but low-temperature drying and recrystallization yielded the starting materials or their isomers. Although attempts to prepare derivatives establishing the masked carboxyl group have so far not met with success, a hydrazine derivative has been obtained from β-peltatin. This evidence for ketonic function seems to be less in favor of a ketone than of a lactone group, since no evidence of keto–enol tautomerism was obtained in the bromine test for either the peltatins or their methyl ethers. The hydroxyl groups were all shown to be phenolic by solubility in cold dilute caustic alkalies, methylation with diazomethane, color (blue) with alcoholic ferric chloride, and ready coupling with diazonium salts. For comparison, podophyllotoxin (I) has[9,10] a methylenedioxy group, three methoxyl groups, one alcoholic hydroxyl group and one lactone group. The peltatins can now be written: for α-, $C_{18}H_{12}(O_2CH_2)(OCH_3)_2(OH)_2(COO)$ and for β- (and podophyllotoxin), $C_{17}H_{10}(O_2CH_2)(OCH_3)_3(OH)(COO)$.

Some deductions may be made as to the nature of the nuclei. Replacing the substituents by hydrogens, the parent hydrocarbons become, respectively, $C_{18}H_{20}$ and $C_{17}H_{18}$, both $C_nH_{2n-16}$. $C_{17}H_{18}$ may similarly be derived from podophyllotoxin. A methylenedioxy group implies a phenyl group and evidence from oxidation given below shows that there is present another phenyl group bearing only hydroxyl or methoxyl groups. Inability of the peltatins to form double compounds with 1,3,5-trinitrobenzene makes the presence of a naphthalene residue improbable. That the two phenyl groups are not united in a diphenyl residue is also indicated by the oxidation studies. If the hydrocarbon formulas given above are diminished by two phenyl groups and if two hydrogens are added for replacement, there remain $C_6H_{12}$ and $C_5H_{10}$, respectively, to be accounted for. These moieties, $C_nH_{2n}$, must represent either an olefinic residue or a single saturated ring. The presence of a double bond is made improbable by the failure of the peltatins or their methyl ethers to add bromine in the usual test[14]; the peltatins (and podophyllotoxin) are unreactive toward bromine, and their methyl ethers substitute bromine with the evolution of hydrogen bromide. No chemical evidence is at present available for distinguishing between the many possibilities involving a single saturated ring. But from the similarity in the course of the permanganate oxidation reaction of the peltatins (described below) with that of podophyllotoxin, the presence of the peltatins and podophyllotoxin together in the plant, the isomerism of all three substances along with the similarity in substituents, and the similarity of the ultraviolet absorption spectra of all three, it seems reasonable tentatively to consider the peltatins

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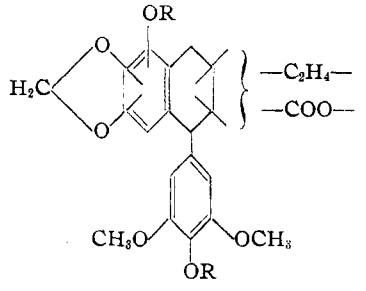
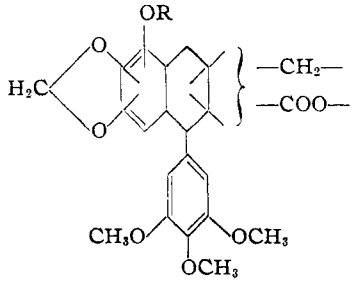
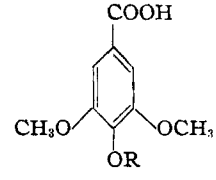



IIa and IIb, R = H
IIc and IId, R = $COCH_3$
IIe and IIf, R = $CH_3$
IIg, R = $C_2H_5$




IIIa and IIIb, R = H
IIIc and IIId, R = $COCH_3$
IIIe and IIIf, R = $CH_3$
IIIg, R = $C_2H_5$




IVa, R = $CH_3$
IVb, R = $C_2H_5$

(13) Gaebel, *Arch. pharm.*, **250**, 617 (1916).

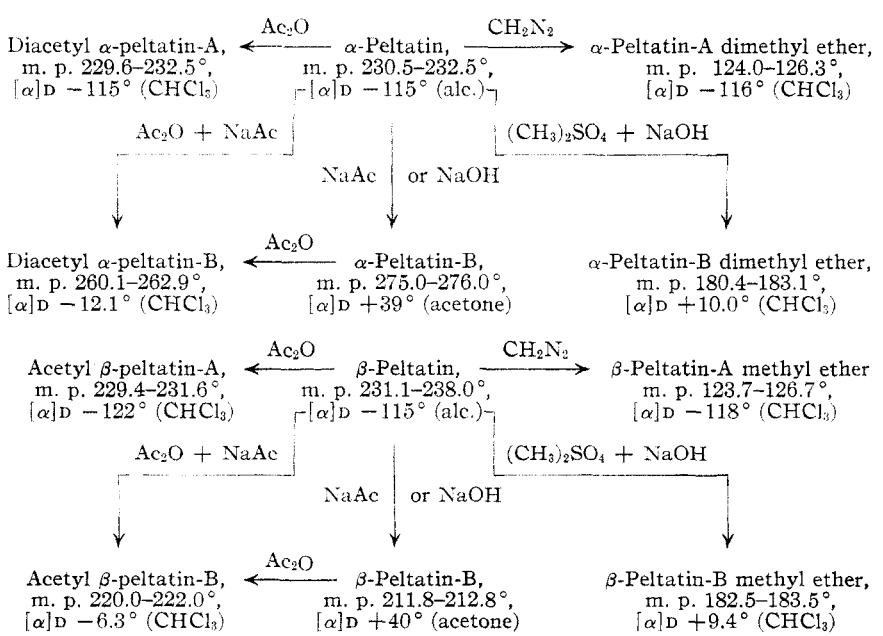
(14) Shriner and Fuson, "Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 3rd ed., 1948, p. 93.

as derivatives of 1-phenyl-1,2,3,4-tetrahydronaphthalene. Consequently, as best representing the facts so far known, the formulas shown are provisionally written for α-peltatin (IIa) and β-peltatin (IIIa), with podophyllotoxin (I) given for comparison.

The positions of the substituents in the isolated benzene ring have been located. When the hydroxyl groups in the peltatins were protected and tagged by ethylation, and the ethyl ethers were oxidized with hot permanganate in a manner which has given 3,4,5-trimethoxybenzoic acid (IVa) with picropodophyllin[15] (an isomerization product of podophyllotoxin, involving only the substituents on the saturated ring), α-peltatin diethyl ether (IIg) yielded syringic acid ethyl ether (IVb) and β-peltatin ethyl ether (IIIg) gave the same product (IVa) as picropodophyllin. These facts show that in α-peltatin one hydroxyl group must be in the ring bearing the two methoxyl groups as indicated (IIa) while the other hydroxyl group, which is also phenolic, must be in the ring bearing the methylenedioxy group; and in β-peltatin (IIIa) the three methoxyl groups are arranged as in podophyllotoxin while the one hydroxyl group must be in the ring bearing the methylenedioxy group. With regard to the position of the hydroxyl group in the ring bearing the methylenedioxy group, it can be said that it must have a free *o*- or *p*-position since both peltatins couple readily with diazonium salts and the other hydroxyl group in α-peltatin has no open *o*- or *p*-position for coupling.

No implication is meant, in formulas II and III, that the reduced ring of the naphthalene nucleus must have three substituents (including the phenyl group). The evidence favors three substituents for both compounds, however. The absence in both peltatins of the C-methyl group makes four substituents in III a virtual impossibility and in II an improbability. But, until the presence of the reduced naphthalene ring is proved, there is the possibility that the reduced ring may be five-membered, with the resulting possibility of more than three substituents in the reduced ring.

**Optical Isomerism.**—Both peltatins form two sets of derivatives, depending on the condition of formation. Thus, α-peltatin forms two diacetates (IIc and IId), one with acetic anhydride and the other with acetic anhydride in the presence of sodium acetate, and two dimethyl ethers (IIe and IIf), one with diazomethane and the other with dimethyl sulfate and alkali. Similarly, β-peltatin forms two monoacetates (IIIc and IIId) and two monomethyl ethers (IIIe and IIIf). Both acetates prepared with acetic anhydride alone and both methyl ethers made with diazomethane were highly levorotatory like the parent peltatins, while the other acetates and methyl ethers were either dextrorotatory or had only a small levorotation. Furthermore, dextrorotatory isomers of the peltatins themselves could be obtained by refluxing alcoholic solutions with sodium acetate or by short heating with dilute caustic alkalies. That the dextrorotatory peltatins are the parent substances of the dextrorotatory (or less levorotatory) acetates and (probably) methyl ethers was indicated by the identity of the acetates prepared by acetylating the dextrorotatory peltatins with acetic anhydride alone with the dextrorotatory acetates prepared from the levorotatory peltatins by means of acetic anhydride and sodium acetate. These two series of diastereoisomeric peltatins and derivatives are named A for the levo series and B for the dextro. A probable explanation of these facts is that under the influence of the sodium acetate or the sodium hydroxide one of the several asymmetric carbon atoms, probably one alpha to the carbonyl group of the lactone ring, undergoes inversion. Inasmuch as yields are sometimes nearly quantitative, complete inversion rather than racemization is postulated. It may be recorded here that the A derivatives are active against tumors in mice, while the B compounds are




(15) Borsche and Niemann, *Ann.*, **499**, 59 (1932).

inactive[16,17]; these are probably the first reported instances of such differences in the action of optical isomers on tumors. The chemical relationships in the two series are shown in the schemes given.

**Acknowledgments.**—The authors wish to express their appreciation to Gertrude Yarchin Greenberg and to Wendell Lucas for technical assistance. The aid of Margaret M. Ledyard in carrying out the methoxyl determinations is gratefully acknowledged.

## Experimental[18-21]

### Isolation

Separation by chromatography proceeded better if much of the biologically inert material was first removed. This could be done in two ways, by chloroform extraction or by benzene precipitation of an alcohol solution. The latter method gave a better yield of products, was easier to carry out on a large scale and involved less solvent manipulation; it will be described here in detail. The chloroform method resulted in the spontaneous crystallization of α-peltatin, without the intervention of chromatography; it will be described only as far as the crystallization.

**Chloroform Extraction.**—When podophyllin is shaken with chloroform at room temperature, most of the biological activity goes into the soluble portion[22] leaving, in one large run, a dark insoluble residue amounting to 37%. A hot extraction was not feasible as the residue became gummy and difficult to extract further. In practice, 1 kg. of podophyllin was shaken a few minutes with 5 l. of chloroform, the mixture filtered by suction, the residue washed with chloroform on the funnel and air-dried. The ground residue was extracted with 2 l. of fresh chloroform. A third extraction was found to remove a negligible amount of soluble matter. The combined extracts were evaporated to dryness on the steam-bath, vacuum being applied at the end. While the dark gummy residue was still hot, it was made up to a total volume of 2.6 l. with a hot 1:1 mixture of absolute alcohol and benzene. The solution was now ready for chromatographing.

In one run, when the chloroform-soluble residue was being washed with about 400 cc. of alcohol into another flask, some difficultly-soluble white solid was obtained in the first flask; and when the alcohol solution had stood for several days, another crop of white solid was obtained. This solid proved to be crude α-peltatin and amounted to 2.2% of the original podophyllin; its separation here proves its presence, as such, in podophyllin, and indicates that it is not formed as an artifact as a result of chemical action during the adsorption.

**Alcohol-Benzene Extraction.**—Podophyllin is completely soluble in alcohol. When more than one volume of benzene is added to the alcoholic solution a dark tar separates which contains only a minor part of the biological activity of the drug.[22]

One kg. of podophyllin was dissolved in 1.2 l. of absolute alcohol by warming. To the warm solution was added 10.8 l. of benzene. After standing long enough for the mixture to cool to room temperature, the clear brown supernatant solution was decanted from the heavier tar (which was rejected) and evaporated to dryness. This tar, insoluble in alcohol-benzene, after removing all volatile solvents under vacuum at 100°, amounted to 16 to 35% for four different samples of the original podophyllin. While still hot, the viscous residue from the evaporation was taken up in 2.5 l. of absolute alcohol, and 2.5 l. of benzene added. After cooling to room temperature, the solution was ready for chromatographing.

**Chromatography.**—The best combination of adsorbent and solvent was found to be alumina (Alcoa activated alumina, grade F-20,[23] used without treatment) with 1:1 absolute alcohol-benzene. A gravity-feed, flowing or liquid chromatogram was used. In the procedure about to be described, podophyllotoxin is least strongly adsorbed, followed by β- and α-peltatin. None of these compounds is fluorescent in ultraviolet light[24] or was found to have a characteristic color reaction that could be used to follow the separation. The method of separation necessarily adopted was the empirical one of taking cuts at regular intervals, evaporating off most of the solvent, and identifying the crystals which separated. Podophyllotoxin was identified by its melting-point (114–116°). α-Peltatin and β-peltatin have similar m. p.'s (about 225°), solubilities and optical rotation, and the mixed m. p. is not definitive. They could be readily distinguished only by methoxyl determination. The two peltatins crystallize together but the percentage composition of mixtures could be determined by analysis for methoxyl. By standardizing the conditions and taking cuts at empirically determined points, a reproducible procedure was evolved which would produce podophyllotoxin in a state of purity and the two peltatins containing small percentages of one another. It should be emphasized that if this procedure is repeated in another laboratory with different materials, especially with different specimens of alumina, the composition of the peltatin mixtures should be checked by methoxyl determinations. The procedure follows.

A glass chromatographic cylinder 7.5 cm. in inside diameter and 33 cm. high was packed with alumina to a depth of 20 cm. (about 835 g.). The column was wet with a 1:1 mixture of absolute alcohol and benzene. The solution to be chromatographed (an amount corresponding to 105 g. of podophyllin for the chloroform process and 60 g. for the alcohol-benzene process) was added to the column and the chromatogram developed with 1:1 alcohol-benzene. Hereafter, only data from the alcohol-benzene process will be given. The first fraction was collected when the filtrate began to show a greenish tint. This and subsequent color changes were best seen if the filtrate was allowed partly to fill a small separatory funnel before collection. When the green color definitely began to turn yellowish or brownish, usually after about 315–560 cc., the cut for the first (podophyllotoxin) fraction was taken and collection of the second (β-peltatin) fraction was begun. The first fraction was evaporated to a thick green oil and taken up in about 75 cc. of hot benzene. On cooling, podophyllotoxin separated as fine white needles, m. p. 114–116° (foaming) after drying at not over 50°. The yield was 9.1–10.2% of the original podophyllin for four different batches of podophyllin; the yields of the whole solvent-free fraction before separation of the podophyllotoxin, and of the other crude fractions, are given in Table II.

The second fraction was cut when the color had deepened to brownish-yellow then lightened until the solution became almost colorless (1.2 to 1.6 l.). The solution was evaporated to about 50 cc. and allowed to crystallize at room temperature. β-Peltatin separated as colorless, thick prisms, m. p. 225–228° (shr. 220°) usually contain-

(16) Leiter and Hartwell, *Cancer Research*, **9**, 625 (1949). These studies also showed that the oxidation products IVa and IVb as well as syringic acid were inactive against tumors in mice.

(17) The effect of these compounds on the activity of cytochrome oxidase and other enzymes in mouse tumors and organs *in vivo* and *in vitro* is being studied in this laboratory; Waravdekar and Leiter, *ibid.*, **9**, 625 (1949).

(18) All m. p.'s are corrected. Determinations were made with the Hershberg apparatus[19] containing Aroclor 1248.[20]

(19) Hershberg, *Ind. Eng. Chem., Anal. Ed.*, **8**, 312 (1936).

(20) Hartwell, *Anal. Chem.*, **20**, 374 (1948).

(21) Analyses were carried out by the Microanalytical Laboratory of the National Institutes of Health (Mr. C. A. Kinser, Mr. W. C. Alford, Margaret M. Ledyard, and Mrs. E. Peake) and by Oakwold Laboratories, Alexandria, Va.

(22) Unpublished results of Hartwell and Leiter.

(23) Stated by the manufacturer, Aluminum Co. of America, to be between 80 and 200 mesh.

(24) The statement in ref. 1a that solutions of podophyllotoxin and α-peltatin are fluorescent should be amended in the light of further observations.

ing less than 3% α-peltatin, but often containing no α-peltatin at all. The yield was 4.2–5.5% for four different batches of podophyllin.

To bring down α-peltatin, a solvent mixture consisting of 1:1 alcohol–benzene containing 5% water (*i. e.*, 47.5:47.5:5 parts by volume) was used for development of the chromatogram. The filtrate progressed in color through a maximum brownish-yellow to a very pale yellow. Most of the α-peltatin was brought down in the first 2 l. of filtrate; continued development appeared to bring down resin which interfered with crystallization. The third fraction was evaporated on the steam-bath to about 60 cc. and allowed to crystallize. α-Peltatin separated as light buff aggregates, not obviously crystalline, m. p. 223–226° (red). A second crop could be obtained by concentrating the mother liquor. Methoxyl determination showed the presence of up to 20% of β-peltatin. The yield from four different batches of podophyllin was 4.0–7.8%. Recrystallization from alcohol (charcoal) gave colorless prisms.

**Caution.**—Podophyllin, its crude fractions and its three pure colorless components are irritating to the skin and mucous membranes. Unnecessary inhalation or contact with the skin should be avoided. Material on the skin should be wiped off with swabs wet with alcohol.

TABLE I

CORRECTED YIELDS IN % OF CRYSTALLINE COMPONENTS OF PODOPHYLLIN

| Lot | Podophyllotoxin | β-Peltatin | α-Peltatin | Total |
|---|---|---|---|---|
| 1[a] | 9.8 | 6.1 | 6.2 | 22.1 |
| 2[b] | 10.2 | 5.4 | 4.1 | 19.7 |
| 3[b] | 9.1 | 5.5 | 5.6 | 20.2 |
| 4[b] | 9.3 | 7.1 | 5.0 | 21.4 |
| Av. | 9.6 | 6.0 | 5.2 | 20.8 |

[a] Source J. T. Baker Chemical Co. [b] Source S. B. Penick and Co.

**Discussion.**—Inasmuch as the peltatins, as isolated, contain varying amounts of one another, it was of interest to correct the yield values for the presence of each component and to calculate the absolute yields of each peltatin. Table I gives the corrected yield values for four different lots of podophyllin.

Table II gives a summary of the percentage of total solids in the different fractions obtained from each of the same four lots of podophyllin.

TABLE II

YIELDS IN % OF CRUDE FRACTIONS OF PODOPHYLLIN OBTAINED IN THE ALCOHOL–BENZENE PROCESS

| Lot | Podophyllotoxin fraction | β-Peltatin fraction | α-Peltatin fraction | Total | Tar insoluble in alcohol-benzene | Total |
|---|---|---|---|---|---|---|
| 1 | 21 | 7 | 14 | 42 | 26 | 68 |
| 2 | 19 | 10 | 11 | 40 | 16 | 56 |
| 3 | 16 | 9 | 13 | 38 | 35 | 73 |
| 4 | 21 | 9 | 11 | 41 | 24 | 65 |
| Av. | 19 | 9 | 12 | 40 | 25 | 65 |

The general picture of the fractionation of podophyllin that arises from this process is as follows: an average of 25% is precipitated in the preliminary alcohol–benzene treatment and rejected; 40%, of which 21% is crystalline, passes through the adsorption column; 35% (by difference) is retained on the column. Since the greater part of the biological activity of the drug can be accounted for in the very active crystalline fractions, the remainder would appear to contain either minor amounts of very active components, or larger amounts of less active components.

It is worth noting that the methods for the estimation of podophyllotoxin in podophyllin,[25] depending on conversion to the sparingly soluble picropodophyllin and weighing the latter, may be considerably in error due to the unsuspected presence of the peltatins in the precipitate. Thus Dunstan and Henry[25a] reported about 20% podophyllotoxin in American podophyllin; this figure corresponds closely with the total of the three components obtained in the present work (Table I). Podophyllotoxin is so weakly adsorbed on alumina that it is improbable that the amount isolated here differs far from the actual amount present.

**Simplified Chromatographic Method for Isolation of Podophyllotoxin.**—In the course of this work a short method for the isolation of podophyllotoxin alone was evolved. Although the method is less suitable for the separation of the peltatins than the one already given, it is included here because it represents the simplest method known to the writers for the production of pure podophyllotoxin.

Fifty-five grams of podophyllin was dissolved in 59 cc. of absolute alcohol with warming and 59 cc. of benzene added. The solution was chromatographed on a column of alumina prepared as described above, using the same wetting and development solvent. The podophyllotoxin fraction was taken when the green color gave way to brown, usually after 330–350 cc. The fraction was worked up as above. This brown material, or other resinous matter accompanying the brown material, interfered with crystallization of the β-peltatin and was the chief reason for adopting the procedure involving preliminary precipitation of part of the podophyllin.

## Characterization

**Melting Point.**—The melting points of the two peltatins are not sharp. The values recorded before, 230.5–232.5° (shrinks at 222.5° and darkens at m. p.) for α-peltatin,[1a] 231.1–238.0° (shrinks at 225.5°) for β-peltatin,[1b] are the highest obtained. Usually a value of around 225° is obtained, and there is evidence that the melting points of samples with a high original value drop to around 225° after a few weeks. The mixed melting point of the peltatins shows no depression but the shrinking temperature is depressed to about 200°. It is evident that identity and purity must be determined by other means. This is best done by methoxyl determination, as discussed in the next paragraph.

**Analysis.**—Since the theoretical methoxyl values for α- and β-peltatin are 14.98 and 22.47%, respectively, the composition of a mixture of the two is readily calculated. By this criterion, β-peltatin has been obtained 100% pure,

(25) (a) Dunstan and Henry, *J. Chem. Soc.*, **73**, 209 (1898); (b) Tanzen, *Arch. pharm.*, **254**, 44 (1916); (c) U. S. Pharmacopoeia, Vol. XI, 1936.

but α-peltatin has never been obtained with less than 6% β-peltatin as impurity. β-Peltatin frequently separates out of the second fraction of the chromatogram in a form yielding the theoretical methoxyl figure for pure β-. The best samples of α-peltatin, containing 6% β-peltatin, were obtained by crystallization directly from the third fraction of the chromatogram, followed by recrystallization from alcohol. Attempts are being made by different means to obtain 100% pure α-peltatin from the 94% product. The derivatives of α-peltatin reported in this paper were prepared with material containing up to 12% β-peltatin.

The elementary analyses of the peltatins are repeated[1] here. *Anal.* Calcd. for $C_{22}H_{22}O_8$: C, 63.75; H, 5.35; 2-$OCH_3$, 15.0; 3-$OCH_3$, 22.5. Found: α-peltatin, C, 63.4; H, 5.3; $OCH_3$, 15.7; for β-peltatin, C, 64.0, H, 5.6; $OCH_3$, 22.2.

**Color Test.**—The color test with concentrated sulfuric acid reported before,[1b] whereby the acid turns reddish-brown with α-peltatin and green with β-peltatin, is not suitable for the identification of β-peltatin containing more than the small amounts of α-peltatin. The green color is given only by relatively large amounts of β-peltatin and this color is obscured if a small percentage of α-peltatin is present. Use could not be made of this test in isolation procedure.

However, if a chloroform solution containing a mixture of podophyllotoxin and the two peltatins was shaken with 2% sodium hydroxide, the peltatins were quantitatively removed into the aqueous layer, leaving the podophyllotoxin completely in the chloroform. If the aqueous layer were acidified, the peltatins could be transferred to a fresh chloroform solution. When treated with concentrated sulfuric acid, the chloroform solution of the podophyllotoxin and of the peltatins both gave red colors. These facts were made use of in the isolation procedure to determine when to cut the podophyllotoxin fraction and start collecting for β-peltatin.

**Solubility.**—Both peltatins are fairly soluble in chloroform, hot ethyl alcohol, acetic acid, acetone, and dilute caustic alkalies; less soluble in benzene, ether, carbon tetrachloride, propylene glycol and cold alcohol; and practically insoluble in petroleum ether and water. In general, the peltatins are less soluble in organic solvents and water than podophyllotoxin, and β-peltatin is somewhat less soluble than α-peltatin.

A determination of the solubility of podophyllotoxin and β-peltatin in distilled water at 23° gave 120 mg./l. and 13 mg./l., respectively. The values for the solubilities calculated from the amounts undissolved and from the amounts dissolved agreed closely, and the figures given are the averages of the two sets of values.

**Optical Rotation.**—The best value for $[\alpha]^{20}D$ of both peltatins, in absolute alcohol, is −115°.[1] Table III gives the values of $[\alpha]D$ for different solvents; the α-peltatin used had a slightly lower value than the best obtained.

TABLE III

OPTICAL ROTATION OF α- AND β-PELTATIN IN DIFFERENT SOLVENTS

| | α-Peltatin | | | β-Peltatin | | |
|---|---|---|---|---|---|---|
| Solvent | $[\alpha]D$ | $t$, °C. | $c$, g./100 cc. | $[\alpha]D$ | $t$, °C. | $c$, g./100 cc. |
| Ethanol | −111 | 21.8 | 1.008 | −115 | 20.0 | 1.009 |
| Chloroform | −120 | 20.2 | 1.014 | −119 | 20.2 | 0.998 |
| Acetone | − 96 | 19.4 | 1.005 | − 95 | 19.7 | 1.025 |
| 0.100 *N* sodium hydroxide | −160 | 20.6 | 1.012 | −141 | 20.6 | 1.008 |

**Ultraviolet Absorption.**—The ultraviolet absorption spectra of the two peltatins are very similar and have a general resemblance to the spectrum of podophyllotoxin. The absorption spectra of the three compounds are shown in Fig. 1. α-Peltatin had a minimum at 263 mμ (*E*, 2000), and maxima at 213 mμ (*E*, 59200) and 274 mμ (*E*, 2700): β-peltatin had a minimum at 263 mμ (*E*, 1500), and maxima at 213 mμ (*E*, 58900) and 273 mμ (*E*, 1880); and podophyllotoxin showed a minimum at 260 mμ (*E*, 1150) and maximum at 292 mμ (*E*, 4430), with a shoulder or possible maximum at 208 mμ (*E* 55500).

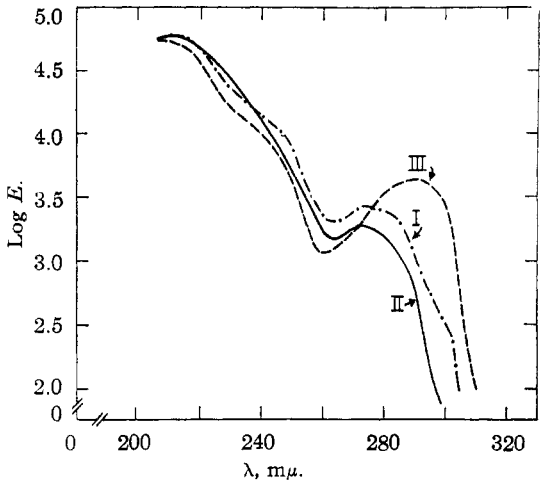



Fig. 1.—Ultraviolet absorption curves in 95% ethyl alcohol; I, α-peltatin; II, β-peltatin; III, podophyllotoxin.

Absorption spectra were taken in 95% ethyl alcohol using the Cary recording spectrophotometer.[26] Molecular extinction coefficients were calculated for molecular weight of 414.4 for the peltatins ($C_{22}H_{22}O_8$) and 455.5 for podophyllotoxin ($C_{22}H_{22}O_8 \cdot H_2O \cdot {}^1/_2C_2H_5OH$). Dr. Friedel writes: "In general, these spectra would be expected of highly substituted alkyl phenols or their methyl ethers. Intensities of the bands in the 270–290 range corroborate this and at the same time preclude the presence of the more intensely absorbing phenylnaphthalene nucleus."

## Derivatives

**α-Peltatin (IIa).**—Analyses for acetyl and for C-methyl were negative. Determinations of mol. wt. by the Rast method and by a vapor pressure method were erratic; Rast mol. wt. determinations on the methyl ether and acetate, however, extablished the formula as given.

The Gaebel test[13] for methylenedioxy group was positive; picropodophyllin was used as a control and also gave a positive test. The Labat test[27] for the methylenedioxy group was negative with both α-peltatin and the control.

Solutions of α-peltatin in dilute sodium hydroxide solution gave an immediate deep red color with diazotized *p*-nitroaniline, *o*-anisidine, or sulfanilic acid.

α-Peltatin was insoluble in cold but soluble in hot dilute sodium carbonate solution.

**α-Peltatin-B (IIb).**—α-Peltatin (2.0 g.) was refluxed overnight with 40 cc. of absolute alcohol and 0.4 g. of anhydrous sodium acetate. Sometimes needles separated and sometimes a solid, usually gelatinous, separated only on cooling. While the mixture was still hot, 200 cc. of hot water was added, and the whole boiled for a few seconds. On cooling, the needles were collected, washed with water, and dried; yield 1.63 g. (82%), m. p. 271–275°. Recrystallization from alcohol gave white needles, 0.90 g., m. p. 275.0–276.5° (dark).

The same compound was obtained by warming a solution of 1.0 g. of α-peltatin with 5 cc. of 15% sodium hydroxide for a few minutes, acidifying with mineral acid, cooling, collecting, washing, and recrystallizing from alcohol; yield, 0.55 g. (55%).

The product was nearly insoluble in chloroform, less than 1% soluble in alcohol, at least 1% soluble in acetone;

(26) We wish to acknowledge the kindness of Drs. Milton Orchin and R. A. Friedel of the Bureau of Mines, Pittsburgh, Pa., in providing these spectra.

(27) Labat, *Bull. soc. chim.*, **5**, 745 (1909).

it was insoluble in hot water, hot sodium bicarbonate solution, but soluble in hot sodium carbonate solution. It gave a green color with alcoholic ferric chloride.

*Anal.* Calcd. for $C_{22}H_{22}O_8$: C, 63.75; H, 5.35; 2-$OCH_3$, 15.0. Found: C, 63.3, 63.1; H, 4.9, 5.1; $OCH_3$, 15.8; $[\alpha]^{20}D$ + 39° (*c*, 1.000, acetone).

**α-Peltatin-A Dimethyl Ether (IIe).**—To a solution of 1.0 g. of α-peltatin in 53 cc. of absolute alcohol was added an ethereal solution of diazomethane until, after standing a few minutes, the color of diazomethane persisted and a sample failed to give a color with diazotized *p*-nitroaniline in the presence of alkali. The diazomethane from 1.9 g. of wet nitrosomethylurea[28] was required. On evaporation to a few cc. and after standing several weeks, large, colorless, transparent prisms separated. These were collected and washed with cold alcohol; yield 0.30 g. (28%), m. p. 121.0–123.0°. Recrystallization from alcohol presented no difficulty and gave 0.24 g. of long, colorless, transparent prisms, m. p. 124.0–126.3°. The compound was soluble in alcohol, chloroform and acetone.

*Anal.* Calcd. for $C_{24}H_{26}O_8$: C, 65.1; H, 5.9; 4-$OCH_3$, 28.05; mol. wt., 442.5. Found: C, 64.6; H, 5.6; $OCH_3$, 28.9; mol. wt. (Rast, camphor), 438; $[\alpha]^{20}D$ −116° (*c*, 1.006, chloroform).

**α-Peltatin-B Dimethyl Ether (IIf).**—A solution of 5.0 g. of α-peltatin in 145 cc. of 2% sodium hydroxide was warmed to 60–65° and treated with dimethyl sulfate in 3-cc. portions, swirling continuously and adding more alkali when necessary, until a spot test on filter paper gave a negligible color with diazotized *p*-nitroaniline. About 12 cc. of dimethyl sulfate was required. A white solid separated. On boiling for ten minutes, adding 16 cc. of 5% sodium hydroxide and boiling ten minutes longer, nearly all went into solution. The solid was filtered off, washed, dried, and identified as crude dimethyl ether; yield 0.57 g. The filtrate was acidified with mineral acid, boiled a few minutes to make the gel granular, cooled, and the product collected, washed and dried; yield 4.69 g.; total yield, 5.26 g. (99%). Crystallization from alcohol gave white needles, 4.23 g., m. p. 179.0–180.5° (shr. 176°). A recrystallization from alcohol afforded an analytically pure product, m. p. 180.4–183.1°.

The compound was slightly soluble in alcohol and carbon tetrachloride, and more than 2% soluble in chloroform. The compound in carbon tetrachloride suspension rapidly absorbed about 3 moles of bromine with evolution of hydrogen bromide. *Anal.* Calcd. for $C_{24}H_{26}O_8$: C, 65.1; H, 5.9; 4-$OCH_3$, 28.05. Found: C, 65.0; H, 5.8; $OCH_3$, 28.7; $[\alpha]^{20}D$ + 10.0° (*c*, 1.997, chloroform).

**α-Peltatin-B Diethyl Ether (IIg).**—This preparation was carried out in a manner similar to that of the B dimethyl ether (preceding) except that diethyl sulfate (15 cc.) was employed, and a temperature of 70° was found to be preferable. The insoluble part consisted of 0.94 g. of crude product and 4.69 g. was obtained from the filtrate; total yield, 5.63 g. (99%). Crystallization from alcohol gave 4.06 g. in two crops, m. p. 138.1–139.5 (shr. 137°). Recrystallization from alcohol gave pure white needles, m. p. 138.8–141.1°. The product was soluble in alcohol.

The determination of optical rotation was not carried out on this compound. Its assignment to the B series is made by analogy with the dimethyl ether and the known effect of alkalies to invert the peltatins. *Anal.* Calcd. for $C_{26}H_{30}O_8$: C, 66.4; H, 6.4; 4 alkoxyls calcd. as $OCH_3$, 26.4. *Found:* C, 65.4, 65.3; H, 6.4, 6.5; alkoxyl calcd. as $OCH_3$, 26.5.

**Diacetyl α-Peltatin-A (IIc).**—α-Peltatin (0.5 g.) was refluxed one-half hour with 5 cc. of acetic anhydride. After decomposing the excess acetic anhydride with water, the product, which first separated as an oil, crystallized. After collecting and washing with water, the wet cake was crystallized from alcohol. The product separated in white needles, m. p. 229.1–231.4°; yield, 0.60 g. (100%). *Anal.* Calcd. for $C_{26}H_{26}O_{10}$: C, 62.6; H, 5.3; 2-$OCH_3$, 12.5; 2-$COCH_3$, 17.3; mol. wt., 498.5. Found: C, 62.4; H, 5.45; $OCH_3$, 13.2; $COCH_3$, 16.4; mol. wt. (Rast, camphor), 540; $[\alpha]^{22}D$ −115° (*c*, 1.007, chloroform). The compound was very soluble in chloroform.

**Diacetyl α-Peltatin-B (IId).**—When the foregoing procedure was repeated with the addition of 0.1 g. of anhydrous sodium acetate, and crystallizing the wet cake from alcohol, 0.33 g. (54%) of large flat prisms was obtained, m. p. 253.8–262.1° (turns sl. yellow). Recrystallization from alcohol gave colorless, transparent, flat needles, m. p. 260.1–262.9° (sl. yellow); yield 0.29 g. (48%). *Anal.* Calcd. for $C_{26}H_{26}O_{10}$: C, 62.6; H, 5.3; 2-$OCH_3$, 12.5; 2-$COCH_3$, 17.3. Found: C, 62.5; H, 5.2; $OCH_3$, 12.9; $COCH_3$, 17.1, 17.7; $[\alpha]^{22}D$ −12.1° (*c*, 3.05, chloroform).

The compound was more than 3% soluble in cold chloroform and was less soluble in alcohol than was the isomeric A-diacetate.

The same compound (by mixed m. p.) was formed when α-peltatin-B was acetylated with acetic anhydride alone as under the procedure for diacetyl-α-peltatin-A (yield, 82% of recrystallized product).

**β-Peltatin (IIIa).**—Analyses for acetyl and for C-methyl were negative. Mol. wt. determination calcd., 414.5; found (Rast, camphor), 385, 367. Better correspondence with theory was found in the derivatives.

Like α-peltatin and picropodophyllin, the Gaebel test for the methylenedioxy group was positive while the Labat test was negative.

Solutions of β-peltatin in dilute sodium hydroxide gave an immediate red color, like α-peltatin, with diazonium salts.

β-Peltatin was insoluble in cold but soluble in hot dilute sodium carbonate solution.

**β-Peltatin-A Hydrazide.**—β-Peltatin (0.50 g.) was dissolved in 15 cc. of absolute alcohol. After cooling to room temperature, 0.5 cc. of hydrazine hydrate was added and the mixture let stand. After a few days, small globular aggregates began to form. After a few more days the product was collected, washed with alcohol, and dried; yield 0.07 g. (14%), m. p. 211.0–212.0° (↑). The compound was sparingly soluble in alcohol, acetone and chloroform; more than 1% soluble in pyridine. *Anal.* Calcd. for $C_{22}H_{24}O_7N_2$: N, 6.5. Found: N, 6.6; $[\alpha]^{21}D$ −141° (*c*, 1.031, pyridine).

**β-Peltatin-B (IIIb).**—β-Peltatin (0.5 g.) was refluxed overnight with 10 cc. of absolute alcohol containing 0.1 g. of anhydrous sodium acetate. When 50 cc. of cold water was added, a gelatinous product separated. Boiling for a few seconds converted it to a crystalline form; yield 0.50 g. (100%), m. p. 207.9–210.2° Crystallization from alcohol gave long white needles, m. p. 212.3–213.3°. *Anal.* Calcd. for $C_{22}H_{22}O_8$: C, 63.75; H, 5.35; 3-$OCH_3$, 22.5. Found: C, 63.4; H, 5.6; $OCH_3$, 22.3; $[\alpha]^{21}D$ + 40° (*c*, 1.002, acetone).

The product was more soluble in alcohol than was α-peltatin-B, less than 1% soluble in chloroform, more than 1% soluble in acetone, and insoluble in cold sodium carbonate solution.

The compound could also be prepared (identical by mixed m. p.), in a less easily purifiable form, by short heating of β-peltatin in 2% sodium hydroxide solution, and acidification with mineral acids; yield of crude, 100%.

**β-Peltatin-A Methyl Ether (IIIe).**—β-Peltatin (1.5 g.) was dissolved in 33 cc. of absolute alcohol, cooled to room temperature and ethereal diazomethane added until, after about two hours of standing, there persisted an excess of diazomethane as judged by color and odor, and a drop failed to give a color with diazotized *p*-nitroaniline in the presence of alkali. The diazomethane from 2.0 g. of wet nitrosomethylurea was required. On evaporating most of the solvent and setting in the ice-box, large prisms were obtained after several weeks, m. p. 122.0–124.5°; yield, 1.1 g. (72%). Crystallization from alcohol gave a pure product, m. p. 123.7–126.7°; mixed m. p. with α-peltatin-A dimethyl ether showed a small depression. *Anal.* Calcd. for $C_{23}H_{24}O_8$: C, 64.5; H, 5.65; 4-$OCH_3$, 29.0. Found: C, 64.7; H, 5.8; $OCH_3$, 29.0; $[\alpha]^{20}D$ −118° (*c*, 1.005, chloroform).

(28) "Organic Reactions," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1942, p. 50.

The compound was soluble in carbon tetrachloride. A solution in this solvent slowly absorbed about two moles of bromine with the evolution of hydrogen bromide.

**β-Peltatin-B Methyl Ether (IIIf).**—β-Peltatin (2.0 g.) was methylated with dimethyl sulfate in a manner similar to that for the preparation of α-peltatin-B dimethyl ether. A total of 3 cc. of dimethyl sulfate was required. After acidification and boiling, the gelatinous precipitate became partly granular; yield, 1.96 g. (95%); m. p. 178.8–180.8°. Crystallization from alcohol gave long fine needles, m. p. 183.0–184.3°; yield, 1.67 g. (81%). Mixed m. p. with α-peltatin-B dimethyl ether showed a small depression. *Anal.* Calcd. for $C_{23}H_{24}O_8$: C, 64.5; H, 5.65; 4-$OCH_3$, 29.0. Found: C, 64.7; H, 5.8; $OCH_3$, 28.6; $[\alpha]^{20}D$ +9.4° (*c*, 1.011, chloroform).

The compound was insoluble in cold, but soluble in hot sodium hydroxide solution; sparingly soluble in carbon tetrachloride but more than 1% soluble in chloroform. Acidification of the alkaline solution precipitated a gel.

A suspension in carbon tetrachloride rapidly absorbed nearly three mols of bromine with the evolution of hydrogen bromide.

Acetylation with acetic anhydride containing sodium acetate resulted in a quantitative yield of starting material.

An attempt was made to isolate the free acid derived from the lactone by acidifying the cold solution in sodium hydroxide. The gelatinous precipitate was washed, and dried at room temperature. Crystallization from alcohol yielded needles of the original lactone.

**β-Peltatin-B Ethyl Ether (IIIg).**—This preparation was carried out in a manner similar to that of α-peltatin-B diethyl ether. The insoluble solid amounted to 0.80 g. and the filtrate yielded 4.53 g. of the product; total yield 5.33 g. (100%). Crystallization from alcohol gave white needles, m. p. 197.9–199.3°, in a yield of 3.70 g. (69%). A second crop was obtained from the mother liquor. *Anal.* Calcd. for $C_{24}H_{26}O_8$: C, 65.1; H, 5.9; 4 alkoxyls calcd. as $OCH_3$, 28.05. Found: C, 65.3; H, 6.1; alkoxyl calcd. as $OCH_3$, 28.2.

This compound is assigned to the B series by analogy with the dimethyl sulfate product.

The product was soluble in hot alcoholic sodium hydroxide solution; acidification gave a gel which soon spontaneously crystallized to needles of the starting material, m. p. 197–198°.

**Acetyl β-Peltatin-A (IIIc).**—This compound was prepared in a manner similar to diacetyl α-peltatin-A. Crystallization of the wet cake from alcohol gave colorless, transparent prisms, m. p. 227.3–229.2°; yield, 0.55 g. (100%). Two recrystallizations from alcohol gave the pure compound, m. p. 229.4–231.6°. Mixed m. p. with diacetyl α-peltatin-A showed a large depression. *Anal.* Calcd. for $C_{24}H_{24}O_9$: C, 63.15; H, 5.3; 3-$OCH_3$, 20.4; 1-$COCH_3$, 9.43; mol. wt., 456.4. Found: C, 62.95, 63.1; H, 5.4, 5.7; $OCH_3$, 20.1; $COCH_3$, 8.62; mol. wt. (Rast, camphor), 429, 436; $[\alpha]^{21}D$ −122° (*c*, 0.999, chloroform).

**Acetyl β-Peltatin-B (IIId).**—When β-peltatin was acetylated by the method described for diacetyl α-peltatin-B, a mixture of two forms of crystals, was obtained which could be separated by hand—colorless, flat prisms and white needles. Sometimes, advantage could be taken of the fact that the prisms separated first in a nearly pure state. The latter were identified (mixed m. p.) as the A-diacetate. The needles, after purification by recrystallization from alcohol, had m. p. 220.0–222.0°. *Anal.* Calcd. for $C_{24}H_{24}O_9$: C, 63.15; H, 5.3; 3-$OCH_3$, 20.4; 1-$COCH_3$, 9.43; mol. wt., 456.4. Found: C, 63.3; H, 5.55; $OCH_3$, 20.5; $COCH_3$, 9.49; mol. wt. (Rast, camphor), 431; $[\alpha]^{21}D$ −6.3° (*c*, 3.01, chloroform).

This compound was prepared without the necessity for separating isomers by acetylating β-peltatin-B according to the procedure for acetyl β-peltatin-A. β-Peltatin-B (0.20 g.) gave 0.22 g. (100% yield) of acetate m. p. 217.0–220.0°, which, after crystallization, afforded 0.20 g. (91% yield) of pure product identical with the above by mixed m. p.

**Oxidation of Ethers.**—α-Peltatin-B dimethyl ether (IIf) (1.0 g.) was suspended in 10 cc. of *N* sodium hydroxide and kept at nearly boiling while 4% potassium permanganate solution was slowly added with stirring to a 10-minute end-point. About 140 cc. was required. The manganese dioxide was filtered off and a slight pink color in the filtrate reduced with bisulfite. The filtrate was acidified with hydrochloric acid and extracted thoroughly with ether; the combined ether layers were extracted with sodium carbonate solution, the aqueous solution acidified and the precipitated oil or solid extracted with ether. The ether solution after washing, drying and evaporating to dryness, yielded crystals, which, after crystallization (charcoal) from water, were obtained as colorless needles, m. p. 166.0–170.0°; yield, 56 mg. Mixed m. p. with an authentic specimen of 3,4,5-trimethoxybenzoic acid (m. p. 168.5–170.7°) showed no depression.

α-Peltatin-B ethyl ether (IIg) (1.0 g.), by a similar procedure, consumed 110 cc. of permanganate and yielded 20 mg. of nearly white needles, m. p. 119–121°. A mixed m. p. with a sample of syringic acid ethyl ether prepared from syringic acid and diethyl sulfate,[29] melting at 123–124°, showed no depression.

β-Peltatin-B methyl ether (IIIf) (1.0 g.) required 158 cc. of permanganate and gave 117 mg. of crude and 18 mg. of pure needles identical (mixed m. p.) with 3,4,5-trimethoxybenzoic acid.

β-Peltatin-B ethyl ether (IIIg) (1.0 g.) required 166 cc. of permanganate solution and gave 220 mg. of crude and 42 mg. of needles identical (mixed m. p.) with 3,4,5-trimethoxybenzoic acid.

## Summary

1. A procedure is given for the isolation, by chromatography on alumina, of podophyllotoxin, α-peltatin, and β-peltatin from the drug podophyllin N. F.

2. Some chemical and physical properties of the peltatins, and the preparation of several derivatives, are described.

3. Tentative structural formulas for the peltatins are proposed and the evidence for their assignment as derivatives of 1-phenyl-1,2,3,4-tetrahydronaphthalene is presented and discussed.

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(29) Bogert and Ehrlich, THIS JOURNAL, **41**, 801 (1919).