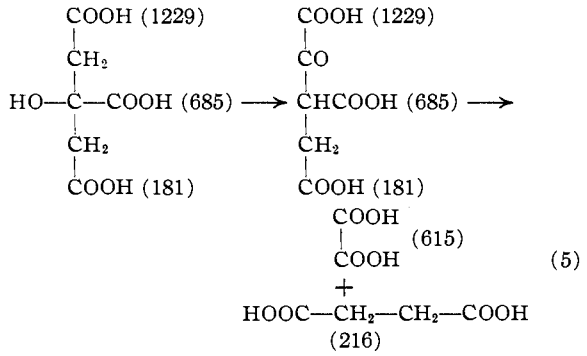
in eliminating the endogenous metabolism of the molds. Despite reasonable efforts to wash out endogenous nutrients the average specific activity of the metabolic CO_2 was 114 c./m. which is only $^1/_3$ of that of the citrate carbons. Apparently endogenous substrates supplied the major quantity of carbon for complete combustion to CO_2. As shown in equation 5, if breakdown of citrate proceeded by way of oxalosuccinate to oxalate and



(5)

succinate the former would have an over-all specific activity of 615 as compared with an over-all activity of 216 for the succinate. Although interpretation is rendered somewhat ambiguous by the high endogenous metabolism, the fact that the oxalate activity of 90 c./m. is even less than that of the average of the metabolic CO_2 (114 c./m.) is taken to indicate that a direct conversion of oxalosuccinic to oxalic acid is not a major pathway of oxalate biosynthesis.[15]

Direct Oxidation of Acetate.—Although the relatively low activity in acetate isolated from these experiments as compared with that of oxalate apparently excludes acetate as the sole or even major precursor of oxalate, the possibility remains that a minor source of oxalate is a direct oxidation of acetate *via* glycolic and glyoxylic acids, as suggested by Challenger, *et al.*,[2] and more recently by Nord and Vitucci.[3] This process is now under investigation by us. The very low relative activity of formic acid isolated from these experiments quite definitely eliminates formate as a direct precursor of oxalate.[5,6]

(15) In a recent study of α-ketoglutarate metabolism in the wood-destroying molds *Trametes cinnabarina* and *Lentinus lepideus*, De Baun, Kudzin and Schubert, *Arch. Biochem.* **26**, 375 (1950), conclude that the conversion of this keto acid to oxalate occurs *via* succinate, fumarate and malate, in substantial agreement with the data of this paper.

Experimental

Enzymatic Degradation of Citric to α-Ketoglutaric Acid.—The procedure used for citrate breakdown was essentially that of Potter and Heidelberger.[12] A washed homogenate of rat liver was prepared by the method of Lehninger and Kennedy[16] and 2.0 ml. suspended in 3 ml. total volume of a *p*H 7.4 phosphate-buffered 0.1 *M* KCl solution containing Mg^{++} ions, 0.005 *M*, adenosine triphosphate, 0.001 *M*, arsenite, 0.001 *M* and 20 μM. of radioactive citrate. After incubation of this mixture at 37° in air for one hour, approximately 7 μM. of α-ketoglutarate is formed; the arsenite prevents further breakdown of the α-ketoglutarate, so that virtually all of the citrate which disappears under these conditions can be accounted for as the keto acid. In the degradation of labeled citrate the contents of 4 such flasks were combined, and after centrifugation, the supernatant solution and washings were treated with an excess of 2,4-dinitrophenylhydrazine. There was then added 100 mg. of non-isotopic α-ketoglutarate 2,4-dinitrophenylhydrazone as carrier and the mixture exhaustively extracted with ethyl acetate. The hydrazone was separated from the excess hydrazine by extraction with 10% sodium carbonate and was recovered by reëxtraction in ethyl acetate and evaporation to dryness. The residue was then crystallized 5 times to constant specific activity. Approximately 100 mg. of the hydrazone was oxidized to CO_2 and succinic acid by the procedure of Krebs.[17] The CO_2 was recovered in essentially quantitative yield by absorption in a bead tower with CO_2-free sodium hydroxide and was isolated by precipitation as barium carbonate. The residual solution was filtered from manganese dioxide, 60 mg. of non-isotopic succinic acid was added as carrier, and the solution extracted with ether. The succinate was recovered in pure form by successive precipitations as the silver salt and barium salt. Its activity was established by oxidation with persulfate and counting as barium carbonate. All activities given in Table II are corrected for added carrier.

(16) A. L. Lehninger and E. P. Kennedy, *J. Biol. Chem.*, **173**, 753 (1948).

(17) H. A. Krebs, *Biochem. J.*, **32**, 108 (1938).

PHILADELPHIA 11, PENNA. RECEIVED DECEMBER 21, 1950

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

Components of Podophyllin. V. The Constitution of Podophyllotoxin[1]

BY JONATHAN L. HARTWELL AND ANTHONY W. SCHRECKER[2]

New evidence is given for the location of the free hydroxyl group in picropodophyllin at C_1, as in formula II. Podophyllotoxin is formulated as a diastereoisomer, not a structural isomer, of picropodophyllin, differing only in the configuration around C_3. Podophyllotoxin chloride and bromide are described and their hydrolysis and ethanolysis studied. Two new diastereoisomers of podophyllotoxin are reported.

The finding that podophyllin N.F. exerts a strong destructive action against sarcoma 37 in mice[3] has led to the isolation of three active components, α-peltatin, β-peltatin and podophyllotoxin. The first two of these are new,[4] but podophyllotoxin has been known for seventy years[5] and has been the subject of many investigations. Certain aspects of the chemistry of the peltatins[4] have suggested to us that revision of the generally accepted structural formula for podophyllotoxin might be indicated. New experimental data have been obtained which are difficult to explain by the older structure. The evidence for a new formula forms the subject of this communication.

(1) This paper was presented in part before the Medicinal Chemistry Division of the American Chemical Society, in Philadelphia, April 10, 1950, and summarized in a Communication to the Editor (Paper IV, J. L. Hartwell and A. W. Schrecker, THIS JOURNAL, **72**, 3320 (1950)).

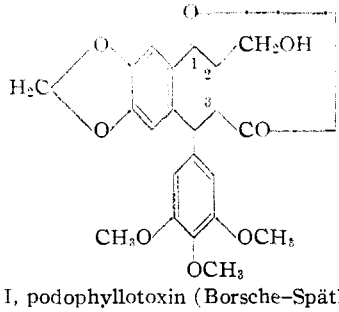
(2) Postdoctoral research fellow, National Cancer Institute.

(3) J. L. Hartwell and M. J. Shear, *Cancer Research*, **7**, 716 (1947).

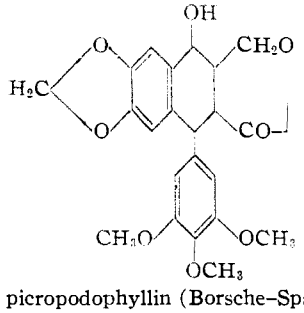
(4) J. L. Hartwell and W. E. Detty, THIS JOURNAL, **72**, 246 (1950).

(5) V. Podwyssotzki, *Arch. exp. Path.*, **13**, 29 (1881).

In 1932, Borsche and Niemann[6] and Späth, Wessely and Nadler[7] independently proposed formulas for the isomers podophyllotoxin (I) and picropodophyllin (II) which have become generally accepted as correct.

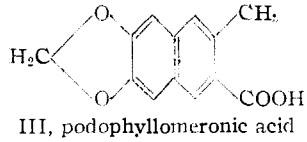


I, podophyllotoxin (Borsche–Späth)

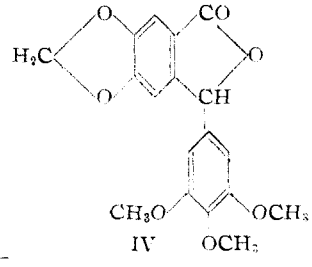


II, picropodophyllin (Borsche–Späth)

An account of their evidence for the location of the substituents on the hydroaromatic ring is necessary at this point. The fact that podophyllotoxin was isomerized to picropodophyllin by means of basic reagents (ammonia,[5] sodium acetate or carbonate,[8] or piperidine[9]) in alcoholic or aqueous alcoholic solution, combined with the fact that the same dihydroxy acid (podophyllic acid) was obtained[8,10] from either compound by opening the lactone ring, found an explanation in the assumption that the two lactones were structural isomers formed by ring closure between the carboxyl and either of the two alcoholic hydroxyl groups. Proof of the location of the carbon-containing substituents (carboxyl and hydroxymethyl) was found in the degradation of podophyllotoxin and picropodophyllin to podophyllomeronic acid (III).[8,10,6b]



III, podophyllomeronic acid



IV

(6) (a) W. Borsche and J. Niemann, *Ann.*, **499**, 59 (1932); (b) *Ber.*, **65**, 1633 (1932); (c) *ibid.*, **65**, 1790 (1932).

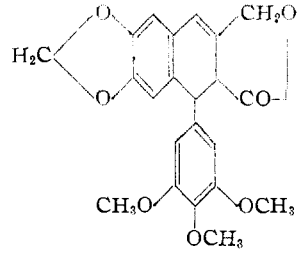
(7) E. Späth, F. Wessely and E. Nadler, *ibid.*, **65**, 1773 (1932).

(8) W. Borsche and J. Niemann, *Ann.*, **494**, 126 (1932).

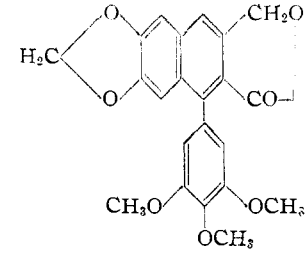
(9) A. Robertson and R. B. Waters, *J. Chem. Soc.*, 83 (1933).

(10) E. Späth, F. Wessely and L. Kornfeld, *Ber.*, **65**, 1536 (1932).

No direct chemical evidence has been put forth for locating the other hydroxyl group at C_1 as shown in I and II. However, Borsche and Niemann[6a] obtained, in the alkaline permanganate oxidation of podophyllic acid, a lactone, IV, whose structure was proved by synthesis.[11] It was recognized that formation of this compound would be difficult to explain if the hydroxyl group were placed along with the trimethoxyphenyl group at C_4. Placing the hydroxyl group at C_1 not only permitted the formulation of two γ-lactones (I and II), but afforded a ready explanation of the dehydration reaction[8,9,10] which picropodophyllin but not podophyllotoxin underwent. Since dehydrating agents removed the elements of water from picropodophyllin to yield an unsaturated compound (apopicropodophyllin (V)), but had no such action on



V, apopicropodophyllin (Borsche–Späth)



VI, dehydroanhydropicropodophyllin

podophyllotoxin, the formula with the free secondary alcohol group (II) was assigned to the former and the one with the primary alcohol group (I) to the latter. These formulas received strong support from the work of Haworth and Richardson[12] who synthesized a compound of the structure VI and found it to be identical with dehydroanhydropicropodophyllin, prepared[10] by dehydrogenation of V.

The Lactone Ring.—Our recent work[4] has shown that α- and β-peltatin are optically active lactones, which are probably closely related to the compounds mentioned. Both of these compounds can be converted in good yield by basic reagents to diastereoisomers with opposite sign of rotation. Acetylation with acetic anhydride alone yields acetyl derivatives which are diastereoisomeric with other acetyl derivatives prepared in the presence of sodium acetate. This stereochemical behavior is strikingly similar to the published behavior of podophyllotoxin, as Table I shows. It is

TABLE I

OPTICAL ROTATIONS

	$[\alpha]_D$[a]	Prod. with NaOH or NaOAc $[\alpha]_D$[d]	Acetate with Ac_2O $[\alpha]_D$[e]	Acetate with Ac_2O + NaOAc $[\alpha]_D$[e]
α-Peltatin	$-115°$[b,c]	$+39°$[c]	$-115°$[c]	$-12.1°$[c]
β-Peltatin	$-115°$[c,f]	$+40°$[c]	$-122°$[c]	$-6.3°$[c]
Podophyllotoxin	$-111.4°$[g]	$+9.6°$[h]	$-134.9°$[i,j]	$+18.9°$[k,j]

[a] In ethanol. [b] J. L. Hartwell, THIS JOURNAL, **69**, 2918 (1947). [c] Ref. 4. [d] In acetone. [e] In chloroform. [f] J. L. Hartwell and W. E. Detty, THIS JOURNAL, **70**, 2833 (1948). [g] H. Thoms and E. Pupko, *Arbeit. Pharmazeut. Inst. Univ. Berlin*, **13**, 110 (1927). [h] Picropodophyllin, E. Späth, F. Wessely and L. Kornfeld, ref. 10. [i] Acetylpodophyllotoxin. [j] Ref. 8. [k] Acetylpicropodophyllin.

(11) E. Späth, F. Wessely and E. Nadler, *ibid.*, **66**, 125 (1933).

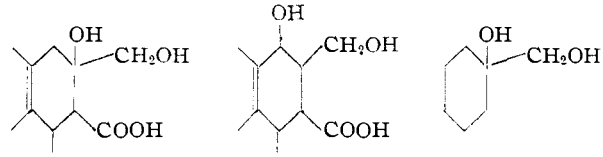
(12) R. D. Haworth and T. Richardson, *J. Chem. Soc.*, 348 (1936).

clear from the formulas[4] that, in the case of the peltatins, there is no free alcoholic hydroxyl group with which to construct an alternate lactone ring, and the isomers obtained by the action of basic reagents must therefore be stereoisomers. It was assumed, therefore, that inversion took place by enolization on the asymmetric carbon atom α- to the lactone carbonyl group.[4,13]

Similarly, podophyllotoxin and picropodophyllin may be diastereoisomers and not structural isomers. Strong evidence for this explanation has been obtained by demonstrating isomerization with derivatives in which the free hydroxyl group was blocked. It was found, in fact, that when benzoylpodophyllotoxin was refluxed in ethyl alcoholic solution containing sodium acetate,[14] benzoylpicropodophyllin separated in a 90% yield, and when acetic anhydride was used as the solvent instead of ethanol, a 75% yield was obtained. Also, acetylpodophyllotoxin gave acetylpicropodophyllin when heated with an isoamyl alcohol or acetic anhydride solution of sodium acetate. It is difficult to conceive of these isomerizations on the basis of known reactions or of known rearrangements involving structures represented by formulas I and II. A more reasonable explanation, and one for which a precise analogy exists (*cf.* footnote 14), is that both compounds possess the same lactone ring and that inversion or epimerization takes place at C_3, the asymmetric carbon atom α- to the lactone carbonyl group.[15] That the primary alcohol group and not the secondary is involved in the formation of the lactone ring is proved by the identification of VI already mentioned, and by the properties of the halides obtained from podophyllotoxin, which are discussed below.

A study of Fisher–Hirschfelder–Taylor atomic models and of the older spring-peg type models, alone, does not permit of a final decision as to the *cis* or *trans* configuration of the lactone ring at C_2:C_3 in podophyllotoxin and picropodophyllin. According to these models, one of the two possible *trans* lactones possesses as little strain as the two possible *cis* lactones, due to the puckering of the hydroaromatic ring. Since, however, one of the two compounds must be *cis* and the other *trans*, and since the lactone ring in podophyllotoxin represents the condition of lesser stability, we can tentatively assign a *trans* configuration (X) (according to models, the only one having considerable strain) to podophyllotoxin and a *cis* configuration (XI) to picropodophyllin.[16]

The Ring Hydroxyl Group.—The assignment of the same points of attachment of the lactone ring in both podophyllotoxin and picropodophyllin makes necessary a new inquiry into the position of the free hydroxyl group. Present evidence favors C_1 as in II. The production of the lactone, IV, as mentioned above, excludes C_4. The epimerization discussed in this paper requires an enolizable hydrogen atom on C_3 so that this position is eliminated. There remain positions C_1 and C_2. Our evidence against C_2 is the failure of podophyllic acid or its sodium salt to yield any trace of formaldehyde even on prolonged periodate oxidation. The two structures under consideration for podophyllic acid, partial formulas VII and VIII, represent a 1,2-glycol and a 1,3-glycol, respectively.



VII VIII IX

That the failure of podophyllic acid to yield formaldehyde with periodate was not due to non-reactivity of this type of 1,2-glycol[18] with periodate was shown by the ready formation of formaldehyde from the model compound 1-hydroxymethylcyclohexanol (IX).

Only position C_1 remains under consideration. Although serious attempts to obtain positive evidence for a secondary alcohol group by oxidation to a ketone were not made by us,[19] several reactions were successful which might be expected from either tertiary or from benzyl-type secondary alcohols. Since the tertiary alcohol formulation (hydroxyl at C_2) is ruled out on the basis of the non-reactivity of podophyllic acid with periodate, these reactions may be considered evidence for a secondary alcohol group (C_1). Thus the action of phosphorus trichloride, thionyl chloride or acetyl chloride on podophyllotoxin yielded a chloride, m.p. 190–191° (effervescence), by replacement of the hydroxyl group, while phosphorus trichloride or thionyl chloride gave the dehydration product α-apopicropodophyllin (V)[20] with picropodophyllin. Similarly, phosphorus tribromide or thionyl bromide yielded a bromide, m.p. 157.5–159° (effervescence), with podophyllotoxin. These halides gave an immediate precipitate of silver halide with alcoholic silver nitrate in the cold[21] and were rapidly hy-

(13) A similar example of epimerization in the analogous conidendrin series has been described by B. Holmberg and M. Sjöberg, *Ber.*, **54**, 2406 (1921), and by W. M. Hearon, H. B. Lackey and W. W. Moyer *Abstracts Papers, Am. Chem. Soc.*, **116**, 2R (1949).

(14) Conditions under which diacetyl α-peltatin-A and acetyl β-peltatin-A are converted to the disastereoisomeric B-compounds (ref. 4).

(15) Independent and different evidence that the configuration around C_3 is opposite in podophyllotoxin from what it is in picropodophyllin has been obtained by E. H. Price and presented in his thesis, University of Maryland, 1949. Price, however, interpreted his findings in terms of the Borsche–Späth formula and offered no evidence that configurational difference around C_3 was the *sole* difference between podophyllotoxin and picropodophyllin; *cf.* N. L. Drake and E. H. Price, THIS JOURNAL, **73**, 201 (1951).

(16) This is consistent with the ease of lactonization of podophyllic acid to picropodophyllin[8] (exclusively) which, according to the Alder-Stein rule,[17] indicates a *cis* relationship between the OH and the COOH group taking part in the lactone formation.

(17) K. Alder and G. Stein, *Ann.*, **504**, 229 (1933).

(18) The only literature references to the periodate oxidation of tertiary-primary 1,2-glycols that were found were N. G. Brink, *et al.*, THIS JOURNAL, **70**, 2085 (1948) (dihydrodesoxystreptose), and R. Adams and T. R. Govindachari, *ibid.*, **72**, 158 (1950) (three derivatives of monocrotalic acid). The structures assigned to all four glycols are supported by strong experimental evidence. The glycol of Brink, *et al.*, is heterocyclic, while those of Adams and Govindachari are aliphatic.

(19) However, many such attempts were carried out without success by Drake and Price, ref. 15.

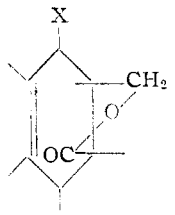
(20) The isomers of apopicropodophyllin will be discussed in a later paper.

(21) R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," 3rd ed., John Wiley and Sons, Inc., New York, N. Y., 1948, p. 121.

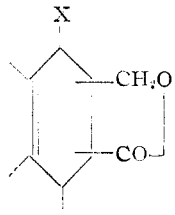
drolyzed by water, as indicated by the fact that their acetone solutions gave the congo red test immediately upon the addition of water. The action of acetyl chloride in forming a chloride instead of an acetate[22] is one more piece of evidence against the primary alcohol formula, I, and the ready hydrolysis of the halides with water would be most unexpected in a primary halide, but consistent with the behavior of a secondary benzyl halide.

At this point attention should be called to the conversion of podophyllotoxin into picropodophyllin by heating with palladium black in methanol[8] or with phenol.[23] This reaction has never been explained on the basis of the Borsche–Späth formulas, since rearrangement of I to II by such reagents is quite improbable. The reaction becomes understandable in the light of the present work, since examples of stereochemical inversion by these reagents are well-known. Racemization is greatly accelerated by solvents of high dielectric constant, especially phenols,[24] while palladium and platinum black have brought about many steric inversions.[25]

Hydrolysis and Ethanolysis of the Halides.— The ready hydrolysis of the podophyllotoxin halides provides evidence in favor of formula II and is incompatible with formula I for podophyllotoxin. When podophyllotoxin chloride or bromide (partial formula Xa) was refluxed with aqueous acetone in the presence or absence of calcium carbonate, there was obtained, in excellent yield, a new stereoisomer of podophyllotoxin (Xb), m.p. 159–161°, $[\alpha]D$ − 75°. That podophyllotoxin bromide is in the "toxin" series[26] was proved by its reaction with silver acetate to give acetylpodophyllotoxin (Xc). That the product, Xb, is also



Xa, X = Cl, Br
Xb, X = OH
Xc,e, X = $OCOCH_3$
Xd, X = OC_2H_5



XIa, X = OH
XIb, X = OC_2H_5
XIc, X = $OCOCH_3$

in the "toxin" series was proved by its conversion with piperidine in alcohol[27] into another new stereoisomer, XIa, m.p. 158–159°, $[\alpha]D$ +84°. Since XIa is thus evidently in the "picro" series, yet different from picropodophyllin, the difference must lie in the configuration of the hydroxyl group at C_1; XIa is, therefore, named epipicropodophyllin. It was also found that treatment with mineral acid converts epipicropodophyllin to picropodophyllin, a reaction of importance in the consideration of possible mechanisms. Since the only difference between XIa and Xb is the configuration around C_3, Xb must be in the "toxin" series; and since Xb is different from podophyllotoxin, the difference must also be in the configuration around C_1. Therefore, Xb is named epipodophyllotoxin. The conversion of podophyllotoxin into epipodophyllotoxin through the chloride or bromide represents a Walden inversion at C_1; such an inversion is an impossibility in a substance of structure I.

The halides, Xa, could be converted into an ethyl ether, Xd, m.p. 195°, $[\alpha]D$ − 88°, by refluxing with ethanol. That this ether is in the "toxin" series was proved by its conversion, by means of piperidine, into a stereoisomeric ether, XIb, m.p. 151–153°, $[\alpha]D$ + 45°, of the "picro" series.[28] Since ethanol reacts with the halides, Xa, probably by the same mechanism as does water, the ethyl ethers should have the same configuration around C_1 as epipodophyllotoxin and epipicropodophyllin and may, therefore, be called epipodophyllotoxin ethyl ether and epipicropodophyllin ethyl ether, respectively. These reactions are summarized in Chart I (all optical rotations in chloroform and melting points corrected). The values for optical rotation are consistent. In proceeding from the "toxin" to the "picro" series, there is a change of +141° between podophyllotoxin and picropodophyllin, +159° from epipodophyllotoxin (Xb) to epipicropodophyllin (XIa), and +133° for the corresponding ethyl ethers (Xd to XIb). In going from the normal to the "epi" series, there is a change of +57° from podophyllotoxin to epipodophyllotoxin (Xb), and +75° from picropodophyllin to epipicropodophyllin (XIa). The existence of the four possible diastereoisomers differing only in configuration around the carbon atom bearing the hydroxyl group and the carbon atom α- to the lactone carbonyl group is incompatible with formula I but consistent with formula II.

An unexpected reaction was encountered in an attempt to prepare the unknown C_1-epimeric bromide by the action of phosphorus tribromide on Xb; the bromide Xa was obtained. In other words, the same bromide was obtained by the action of phosphorus tribromide on either podophyllotoxin or epipodophyllotoxin. Therefore, one of the reactions must have proceeded with, and the other without, Walden inversion.

(22) A. McKenzie and G. W. Clough, *J. Chem. Soc.*, **103**, 687 (1913), found that phenyl methyl carbinol formed mainly the corresponding chloride when treated with acetyl chloride; *cf.* B. Radziszewski, *Ber.*, **7**, 140 (1874).

(23) H. Thoms and E. Pupko, see ref. *g*, Table I.

(24) J. Houben, "Die Methoden der organischen Chemie," 3rd ed., Vol. II, Georg Thieme, Leipzig, 1925, p. 591.

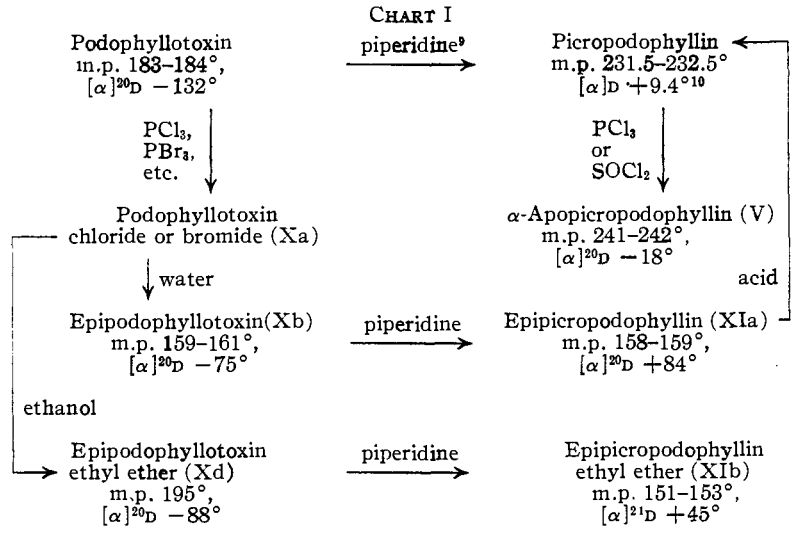
(25) Palladium and platinum black have induced *cis–trans* interconversions at temperatures under 100°; *cf.* O. Loew and K. Aso, *Bull. Coll. Agric., Tokyo Imp. Univ.*, **7**, 1 (1906); B. Tamamushi and H. Akiyama, *Bull. Chem. Soc. Japan*, **12**, 382 (1937); R. C. Fuson, *et al.*, THIS JOURNAL, **62**, 2091 (1940). Palladium black has brought about epimerizations in several steroids at temperatures of 250–260°, but in these steroids the hydrogen atom involved was not activated; *cf.* A. Butenandt, A. Wolff and P. Karlson, *Ber.*, **74**, 1308 (1941), and W. E. Bachmann and A. S. Dreiding, THIS JOURNAL, **72**, 1323 (1950).

(26) For convenience, compounds that have the same configuration around C_2 and C_3 (provisionally assigned *trans*) as podophyllotoxin are said to be in the "toxin" series. Similarly, compounds that have the same configuration around C_2 and C_3 (provisionally assigned *cis*) as picropodophyllin are said to be in the "picro" series.

(27) A reagent characterized by its ready ability to effect epimerization at C_3, *i.e.*, to convert podophyllotoxin into picropodophyllin.[9]

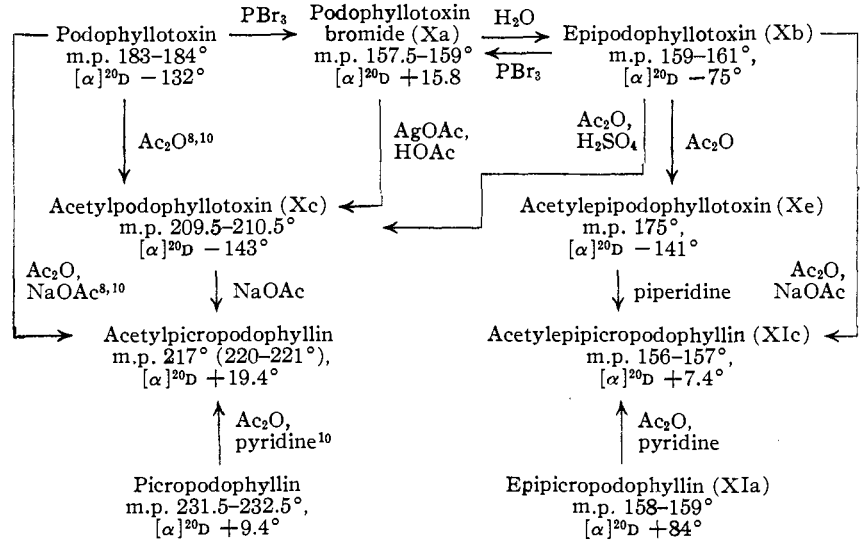
(28) The conversion of Xd into XIb proceeds much less readily by means of sodium acetate. This seems to be due to the ethoxyl group, not to the difference in configuration at C_1, since epipodophyllotoxin can be readily converted into epipicropodophyllin with sodium acetate, although in a somewhat lower yield than with piperidine.

CHART I



Both of the new diastereoisomers, Xb and XIa, formed the corresponding acetates (Xe, m.p. 175°, $[\alpha]_D$ − 141°, and XIc, m.p. 156–157°, $[\alpha]_D$ + 7.4°, respectively) when acetylated with acetic anhydride. It is interesting to note that, unlike the alcohols, the acetates of the "epi" series have optical rotations that are very similar to those of the normal series. In the presence of traces of sulfuric acid, however, Xb gave podophyllotoxin acetate; this different reaction path may indicate a different mechanism of acetate formation. The formation of the four diastereoisomeric acetates is summarized in Chart II.

CHART II



Studies on the action of dehydrating agents on podophyllotoxin and its isomers, and on the dehydrohalogenation of the podophyllotoxin halides, have been carried out and will be reported at a later date.

Lactone Ring Opening and Relactonization.—Because of the possibility (inferred from a consideration of models) that certain of the four diastereoisomers represented by II might relactonize in a different manner if the lactone ring were once opened to the hydroxy acid (a structure represented by I might even be formed), this possibility was tested. Podophyllotoxin and epipodophyllotoxin were unsuitable for the test since they epimerize with alkalies to the corresponding members of the "picro" series. Picropodophyllin is already well-known[11] to hydrolyze with sodium hydroxide to podophyllic acid, which, in turn, is lactonized exclusively to the original picropodophyllin. When epipicropodophyllin was saponified, a new acid, epipodophyllic acid, m.p. 174° (effervescence), $[\alpha]_D$ − 44°, was obtained.[29] On heating the acid above its m.p., epipicropodophyllin was formed. These results tentatively confirm a *cis* configuration about $C_2:C_3$ for epipicropodophyllin[30] and show that the two lactones of the "picro" series represent conditions of least strain in the cyclization of the corresponding hydroxy acids.

Acknowledgments.—The authors wish to thank Gertrude Y. Greenberg and Priscilla B. Maury for technical assistance, and to express their appreciation for the unfailing cooperation of the Microanalytical Laboratory of the National Institutes of Health (Mr. W. C. Alford, Mrs. Margaret M. Ledyard and Mrs. Evelyn Peake) in carrying out the analyses. They also wish to express their indebtedness to Professor Paul D. Bartlett of Harvard University for reading the manuscript and offering valuable suggestions.

Experimental[31]

Podophyllotoxin.—Anhydrous podophyllotoxin was prepared from the solvated product[4] (m.p. 114–116°, foaming), by heating at 100° (1–2 mm.) for 24 hours. The colorless powder melted at 183–184° (lit. 179°,[23] 157–158°[10])[32]; $[\alpha]^{20}_D$ −132° (*c*, 1.0, chloroform), −108° (*c*, 1.0, ethanol) [lit. −109° (*c*, 1.2, ethanol)[10]].

Anal. Calcd. for $C_{22}H_{22}O_8$: C, 63.75; H, 5.35; OCH_3, 22.47. Found: C, 63.70; H, 5.46; OCH_3, 22.44.

(29) This was obtained only if acetic was used to acidify the solution of the sodium salt. If a mineral acid was used, inversion at C_1 occurred and picropodophyllin was formed.

(30) *Cf.* discussion, above, regarding podophyllotoxin and picropodophyllin.

(31) All our melting points are corrected.

(32) The product m.p. 157–158° has never been obtained in this Laboratory. When our compound, m.p. 183–184°, is recrystallized from a mixture of ethanol, benzene and water, colorless needles are obtained which, after air-drying at room temperature, melt at 116–118° (foaming). Podophyllotoxin, m.p. 183–184° and 114–116° (foaming) yield identical acetates. The products melting at 183–184° and 157–158° are probably polymorphic forms of the anhydrous substance.

Acetylpodophyllotoxin (Xc).—This compound was prepared according to Borsche and Niemann[8] by treatment of podophyllotoxin with acetic anhydride: colorless needles from absolute ethanol, m.p. 209.5–210.5° (lit. 210°,[23] 204°[10]); $[\alpha]^{20}D$ −143° (*c*, 1.0, chloroform) [lit. −134.9° (*c*, 1.2, chloroform)[8]].

Anal. Calcd. for $C_{24}H_{24}O_9$: C, 63.15; H, 5.30. Found: C, 62.87; H, 5.38.

Acetylpicropodophyllin.—A mixture of 0.80 g. of acetylpodophyllotoxin, 0.8 g. of anhydrous sodium acetate and 16 cc. of acetic anhydride was refluxed for 2 hours. Cooling, filtering and washing with ethanol and water gave 0.48 g. of material, m.p. 211–214°. The mother liquor yielded a second crop of 0.18 g., m.p. 209°; total yield 0.66 g. (83%). Recrystallization from ethyl acetate gave colorless needles, m.p. 216.5–217°, no depression with authentic acetylpicropodophyllin[8] (m.p. 219.7–221.0°, lit. 215–216°[8]), large depression with acetylpodophyllotoxin or with picropodophyllin (m.p. 226–230°); $[\alpha]^{20}D$ +19.4° (*c*, 1.0, chloroform) [lit. +18.9° (*c*, 1.06, chloroform)[8]].

When isoamyl alcohol was substituted for acetic anhydride, a 47% yield of material melting at 208–211° was obtained; m.p. 213–215° after crystallization from ethyl acetate.

Benzoylpodophyllotoxin.—This compound was prepared according to Drake and Price[15] by direct benzoylation of podophyllotoxin. A 74% yield of crude product was obtained; m.p. 92–111° (effervescence at 110°). Recrystallization from absolute ethanol gave colorless crystals, m.p. 111–115° (sintering at 108.5°); $[\alpha]^{20}D$ −116° (*c*, 1, chloroform). Drake and Price[15] list m.p. 112.6–116.6°; $[\alpha]^{25}D$ −118° (*c*, 0.49, chloroform).

Benzoylpicropodophyllin. (a) By Direct Benzoylation of Picropodophyllin. (Drake and Price[15].)—An 86% yield of crude product was obtained, m.p. 188–195°. One crystallization from ethyl acetate followed by two from chloroform–ethanol gave colorless needles, m.p. 209.5–211°; $[\alpha]^{20}D$ +16.3° (*c*, 1.0, chloroform). Drake and Price[15] list m.p. 200.1–201.6°; $[\alpha]^{25}D$ +13° (*c*, 0.57, chloroform).

(b) By **Epimerization of Benzoylpodophyllotoxin.**—A mixture of 150 mg. of benzoylpodophyllotoxin, 75 mg. of crystallized sodium acetate and 3 cc. of absolute ethanol was refluxed for 2 hr. Crystallization began after 10 min. and was completed by diluting the reaction mixture with water; yield 135 mg. (90%), m.p. 211–212°. Recrystallization from chloroform–ethanol gave colorless needles, m.p. 213.2–214.4°, no depression with the product prepared from picropodophyllin, large depression with acetylpicropodophyllin; $[\alpha]^{20}D$ +16.0° (*c*, 1, chloroform).

Anal. Calcd. for $C_{22}H_{26}O_9$: C, 67.17; H, 5.06. Found: C, 66.98; H, 5.13.

By refluxing a mixture of 0.55 g. of benzoylpodophyllotoxin, 0.5 g. of sodium acetate and 10 cc. of acetic anhydride for 2 hr., followed by diluting with water, there was obtained 0.41 g. (75%) of solid, m.p. 180–195°. Recrystallization from chloroform–ethanol gave needles melting at 202–206°, no depression with material obtained from picropodophyllin, large depression with acetylpicropodophyllin.

Podophyllotoxin Chloride (Xa, X = Cl)[33] **(a) With Phosphorus Trichloride.**—This method consistently gave the purest material and was, therefore, used for preparative purposes. A suspension of 10.33 g. of anhydrous podophyllotoxin in 60 cc. of benzene containing 1.20 g. of phosphorus trichloride was refluxed for 1 hr. The clear yellowish solution was decanted from a dark-colored tar, which was washed with 10 cc. of hot benzene. The combined solutions were diluted with 40 cc. of anhydrous ether, then, while boiling, with 120 cc. of *n*-hexane. Crystallization was started by scratching the walls of the vessel and completed by cooling very slowly and finally keeping at room temperature for three days. The nearly colorless needles were washed with anhydrous ether; yield 4.99 g. (46%), m.p. 179–180.5° (effervescence). In a large number of similar preparations, the yields varied between 38 and 48%. One recrystallization from benzene–ether–hexane, and two from acetone–hexane gave long, colorless, hexagonal prisms which melted at 190–191° (effervescence), when immersed at 180°; the decomposition point varied with the temperature of immersion and the rate of heating. The compound was soluble in benzene and acetone, difficultly soluble in ether, insoluble in hexane. It was decomposed by ethanol. When water was added to its solution in acetone, free hydrochloric acid was formed immediately, as shown by the congo red paper test and the reaction with silver nitrate; $[\alpha]^{20}D$ −27.1° (*c*, 1, ethanol-free chloroform).

Anal. Calcd. for $C_{22}H_{21}O_7Cl$: C, 61.04; H, 4.89; Cl, 8.19. Found: C, 61.11; H, 4.86; Cl, 8.23.

(b) **With Thionyl Chloride.**—Ten grams of anhydrous podophyllotoxin was refluxed with 25 cc. of benzene and 8 cc. of thionyl chloride for 15 minutes. The solution was evaporated under reduced pressure and the residue dissolved in 25 cc. of hot benzene. Addition of 25 cc. of *n*-hexane to the hot solution gave, upon seeding and slow cooling, a partly gummy material. Recrystallization from benzene–hexane yielded 3.19 g. (31%) of needles, m.p. 181.5–182° (effervescence).

(c) **With Acetyl Chloride.**—A mixture of 8 g. of anhydrous podophyllotoxin and 8 cc. of acetyl chloride was refluxed for 30 minutes, then 80 cc. of *n*-hexane was added. The heavy oil, on trituration with more hexane, solidified. Recrystallization from acetone–hexane gave 4.5 g. (54%) of impure chloride, m.p. 177–179° (effervescence). Repeated recrystallization produced needles, m.p. 182–183° (effervescence). Mixed m.p. with samples obtained by the other methods showed no depression. Ethanolysis (see below) yielded the same ethyl ether (Xd) that was obtained from the chloride prepared with phosphorus trichloride.

Podophyllotoxin Bromide (Xa, X = Br) from Podophyllotoxin. (a) **With Phosphorus Tribromide.**—A suspension of 10.1 g. of anhydrous podophyllotoxin in 60 cc. of benzene and 2.32 g. of phosphorus tribromide was refluxed for 1 hr. The supernatant yellow solution was decanted from tarry material, which was washed with 20 cc. of hot benzene. To the hot solution was added 50 cc. of anhydrous ether and 120 cc. of hexane. Crystallization proceeded very slowly. The tan crystalline solid was filtered after 4 days at room temperature and washed with anhydrous ether; weight 8.05 g. (69%), m.p. 154.5° (effervescence). Similar preparations gave yields ranging from 60 to 76%. Two recrystallizations from benzene–ether gave long, colorless, rectangular prisms which melted between 157.5 and 159° (effervescence and darkening), depending on the temperature of immersion and rate of heating. The bromide was somewhat less soluble than the corresponding chloride and reacted with ethanol or with aqueous acetone in the same manner as the latter; $[\alpha]^{21}D$ +15.8° (*c*, 1, ethanol-free chloroform).

Anal. Calcd. for $C_{22}H_{21}O_7Br$: C, 55.36; H, 4.44; Br, 16.74. Found: C, 55.39; H, 4.47; Br, 16.53.

(b) **With Thionyl Bromide.**[34]—A mixture of 4.85 g. of anhydrous podophyllotoxin, 2.70 g. of thionyl bromide and 30 cc. of benzene was refluxed for 1 hr., then diluted with 25 cc. of anhydrous ether and 60 cc. of hexane. The turbid solution was clarified with another 10 cc. of benzene. Crystallization was induced by scratching. The tan-colored crystalline material was filtered after a week at room temperature and washed with anhydrous ether; yield 2.70 g. (48%), m.p. 154° (effervescence). Recrystallization from benzene–ether gave colorless prisms, m.p. 156° to 159° (effervescence), depending on the temperature of immersion.

Reaction of Picropodophyllin with Inorganic Acid Chlorides. (a) **With Phosphorus Trichloride.**—A suspension of 2.0 g. of picropodophyllin in 12 cc. of benzene was refluxed for 1 hr. with 0.24 g. of phosphorus trichloride. After cooling, the colorless solid was filtered, washed with ethanol, water and ethanol, then dried; yield 1.28 g. (67%); m.p. 239–243° (sinters 235°) (lit. 244–245°[9]); $[\alpha]^{20}D$ −18.0° (*c*, 0.50, chloroform) [lit. $[\alpha]^{21}_{5461}$ −17.5° (*c*, 0.49, chloroform)[9]]. There was no m.p. depression with α-apopicropodophyllin prepared[9] from picropodophyllin with acetic anhydride and sulfuric acid, $[\alpha]^{20}D$ −19.3° (*c*, 0.5, chloroform).

(b) **With Thionyl Chloride.**—A mixture of 4.0 g. of picropodophyllin, 100 cc. of chloroform and 4 cc. of thionyl chloride was refluxed for one-half hour. The solution was

(33) Thoms and Pupko, Table I, ref. *g*, apparently obtained this compound in a grossly impure state by treating an alcoholic solution of podophyllotoxin with dry hydrogen chloride gas. Their crystalline product, which they named "podophyllotoxin hydrochloride," melted at 179° and contained only 3.55% Cl.

(34) Obtained from Delta Chemical Works, 23 West 60th St., New York 23, N. Y.

evaporated under reduced pressure and the residue crystallized from 50 cc. of hot benzene. The colorless crystalline product weighed 1.66 g. (43%), m.p. 225–230°. Recrystallization from methyl ethyl ketone yielded colorless felt-like needles of α-apopicropodophyllin, m.p. 241–242°.

Anal. Calcd. for $C_{22}H_{20}O_7$: C, 66.66; H, 5.09. Found: C, 66.61; H, 5.22.

Podophyllic Acid (VIII).—This compound was prepared by the method of Borsche and Niemann,[8] except that the alkaline solution of podophyllotoxin was acidified with acetic acid, instead of hydrochloric acid; small colorless needles, m.p. 162.5–163° (effervescence) [lit. 163–165° (foaming)[8]].

Attempted Periodate Oxidation of Podophyllic Acid.[35]—To a solution of 0.864 g. of podophyllic acid in 8 cc. of 1 *N* sodium bicarbonate were added 6 cc. of 0.4 *M* sodium periodate solution, then, after 2 hours, 8.1 cc. of 1.2 *M* sodium arsenite and 18 cc. of 2 *N* HCl. The solid which precipitated was removed by filtration. To 20 cc. of the filtrate were added 10 cc. of 1 *M* sodium acetate and 8.6 cc. of an 8% solution of methone (dimedon) in ethanol. No condensation product with formaldehyde was obtained upon heating for 15 minutes, followed by standing at room temperature.

In a similar experiment, the reaction mixture was allowed to stand for 20 hours. No formaldehyde could be detected with methone. The same was true when podophyllic acid was dissolved in ethanol and treated with periodate in the absence of bicarbonate for 2 hours and for 45 hours.

Periodate Oxidation of 1-Hydroxymethylcyclohexanol (IX).—1-Hydroxymethylcyclohexanol, m.p. 77.6–79.7° (lit. 76.5°[36]), was prepared by a series of reactions published by Favorsky and Borgmann[36] and by Wallach,[37] with the last step as indicated by Favorsky.

A solution of 0.130 g. of this compound in 2 cc. of water was treated with 3 cc. of bicarbonate and 3 cc. of periodate, then, after 75 minutes, with 4 cc. of arsenite, 5.3 cc. of hydrochloric acid, 6 cc. of sodium acetate and finally with 10 cc. of methone reagent. The solution was then heated on the steam-bath for 10 minutes, and kept at room temperature for 1 hour. The formaldehyde–methone condensation product weighed 0.178 g. (61%); m.p. and mixed m.p. with an authentic sample, 190–191°.

Epipodophyllotoxin (Xb).—Podophyllotoxin chloride (4.15 g.) was refluxed with 17 cc. of acetone and 17 cc. of water in the presence of 1.7 g. of powdered calcium carbonate for 45 minutes. Calcium carbonate was removed by filtering the hot mixture (Celite) and washed with boiling acetone. The combined filtrate and washings were concentrated until the solution became milky, and then diluted with water. The oil solidified partly when kept in the ice-box for a short time and crystallized completely upon boiling. The mixture was then kept in the ice-box overnight and the colorless solid filtered and washed with water. After drying, it weighed 3.86 g. (97%) and melted at 158.4–161.2°. Purification by dissolving in 15 cc. of hot ethanol, adding 30 cc. of hot water and cooling very slowly gave colorless needles, m.p. 159.4–161.2°; $[\alpha]^{20}D$ −75.0° (*c*, 1, chloroform).

Anal. Calcd. for $C_{22}H_{22}O_8$: C, 63.75; H, 5.35. Found: C, 63.69; H, 5.63.

A mixture of this compound with anhydrous podophyllotoxin melted gradually between 158 and 182°. That pure epipodophyllotoxin and not a mixture was obtained in this reaction was, however, demonstrated by the constancy of the melting point, of the optical rotation, and of the activity against sarcoma 37 in mice[38] with successive recrystallizations of the product.

The same material was obtained in 95% yield when 2.5 g. of podophyllotoxin bromide was refluxed with 2.5 g. of calcium carbonate in 20 cc. of acetone and 20 cc. of water for one hour.

The reaction proceeded as readily in the absence of calcium carbonate, but the product obtained was somewhat less pure.

Epipicropodophyllin (XIa).—A solution of 3.16 g. of epipodophyllotoxin in 9.5 cc. of ethanol was refluxed with 3.8 cc. of water and 0.06 cc. of piperidine for one hour. An oil separated when 32 cc. of water was added, and the mixture solidified to a gel upon standing in the ice-box. The gel consisted of a suspension of tiny solid globules. The solid was filtered, dissolved in chloroform and the solution evaporated on the steam-bath. The oil was taken up in hot benzene. On scratching, glistening small rhombic or hexagonal plates separated which appear to contain two moles of benzene of crystallization; yield 3.08 g. (72%), m.p. 95–102° (sintering at 93°, effervescence at 102°).

Anal. Calcd. for $C_{22}H_{22}O_8 \cdot 2C_6H_6$: C, 71.56; H, 6.01; OCH_3, 16.35; loss on drying, 27.4. Found: C, 71.73; H, 6.14; OCH_3, 16.49; loss on drying (110°, vacuum), 26.3.

The solvent-free substance was prepared by drying the solvated product in a vacuum at 115°, dissolving the colorless glass in hot chloroform and adding hexane to incipient cloudiness. Tiny needles separated on scratching; m.p. 158.3–158.6° (no change on further recrystallization); $[\alpha]^{20}D$ + 84.0° (*c*, 1, chloroform).

Anal. Calcd. for $C_{22}H_{22}O_8$: C, 63.75; H, 5.35; OCH_3, 22.47. Found: C, 63.35; H, 5.51; OCH_3, 22.23.

The same compound was prepared by refluxing 2.5 g. of epipodophyllotoxin with 10 cc. of absolute ethanol and 0.9 g. of crystallized sodium acetate for 2.5 hours. Dilution with 38 cc. of water, isolation by chloroform extraction and crystallization from benzene gave 1.97 g. (58%) of colorless plates, m.p. 97–102° (sintering at 95°, effervescence at 102°). The solvent-free material, prepared as above, melted at 158.0–159.0°.

Conversion of Epipicropodophyllin to Picropodophyllin.—A solution of 0.30 g. of epipicropodophyllin in 3 cc. of acetone and 6 cc. of 1 *N* hydrochloric acid was refluxed for 30 minutes. A felt of colorless needles began to separate after a short while. Dilution with 6 cc. of water, and cooling, gave 0.24 g. (80%) of material melting at 227–230° [lit.[10] 228°]; $[\alpha]^{20}D$ +4.8° (*c*, 1, chloroform) [lit.[10] +9.4°]. It gave picropodophyllin acetate by treatment with acetic anhydride and pyridine on the steam-bath for 2 hours. After dilution with water there was obtained a 76% yield of colorless solid, m.p. 200–206°; colorless needles from ethanol, m.p. 214.2–215.6°, no depression with an authentic sample of acetylpicropodophyllin.

Picropodophyllin was also obtained when a solution of 0.40 g. of epipicropodophyllin in 25 cc. of hot 1.25 *N* aqueous sodium hydroxide was acidified with 20 cc. of 2 *N* hydrochloric acid and the mixture boiled for several minutes. After cooling, there was obtained 0.39 g. of material, m.p. 212–216°, which was identified as the acetate in the manner described above.

Reaction of Podophyllotoxin Bromide with Silver Acetate.—A mixture of 1.00 g. of podophyllotoxin bromide and 0.37 g. of silver acetate in 15 cc. of glacial acetic acid was refluxed with exclusion of moisture for 75 minutes. The light yellow solution was filtered from silver bromide, which was washed with warm chloroform. The combined filtrate and washings were added slowly to 200 cc. of boiling water. The oil thus obtained solidified when standing in the ice-box. The solid was dissolved in hot ethanol, water added to incipient cloudiness and the mixture allowed to cool slowly. There was obtained 0.32 g. (33%) of colorless needles, m.p. 202–204°. Recrystallization from absolute ethanol gave material melting at 207–209°; no depression with an authentic sample of acetylpodophyllotoxin.

The mother liquor gave on dilution with water some low-melting material, very soluble in ethanol, which could not be further purified.

Epipodophyllotoxin Ethyl Ether (Xd).—To a suspension of 2.5 g. of powdered calcium carbonate in 42 cc. of absolute ethanol was added 2.5 g. of podophyllotoxin chloride and the mixture refluxed with exclusion of moisture for one hour. It was then filtered hot (Celite) and the residue washed with boiling ethanol. The combined filtrate and washings were concentrated to a small volume and hot water added to incipient cloudiness. When the solution was boiled for a short time, colorless needles began to separate. The yield of material obtained after cooling was 2.22 g. (87%), m.p. 187–191° (softening at 184°). Three recrystallizations from 50% ethanol gave material, m.p. 194.8–195.0°.

(35) We are indebted to Dr. Ernest L. Jackson of the Experimental Biology and Medicine Institute, National Institutes of Health, for his helpful suggestions in regard to this experiment; *cf.* R. E. Reeves, THIS JOURNAL, **63**, 1476 (1941).

(36) A. Favorsky and I. Borgmann, *Ber.*, **40**, 4863 (1907).

(37) O. Wallach, *Ann.*, **347**, 329 (1906); **365**, 255 (1909).

(38) J. Leiter, J. L. Hartwell and A. W. Schrecker, unpublished results.

The compound crystallized either as needles or leaflets and was moderately soluble in cold ethanol; $[\alpha]^{20}D$ −88° (*c*, 1, chloroform).

Anal. Calcd. for $C_{24}H_{26}O_8$: C, 65.15; H, 5.92. Found: C, 65.10; H, 5.84.

The same compound was obtained in 87% yield by substituting podophyllotoxin bromide for the chloride; m.p. 185–191° (softening at 177°); after two recrystallizations from 50% ethanol, m.p. 193.5–194.2°; $[\alpha]^{20}D$ −86° (*c*, 0.5, chloroform).

Epipicropodophyllin Ethyl Ether (XIb).—A solution of 1.90 g. of epipodophyllotoxin ethyl ether in 9.5 cc. of ethanol, 3.8 cc. of water and 0.2 cc. of piperidine was refluxed for one hour. When it was diluted very gradually with 30 cc. of hot water, a felt of colorless hairlike needles separated. The solid was filtered, after standing in the ice-box, and washed with water; m.p. 151–153.5°. The yield was 1.27 g. (67%); in similar preparations the yields varied between 57 and 75%. Recrystallization from 50% ethanol gave material melting at 151.2–152.7°; $[\alpha]^{21}D$ +45.2° (*c*, 1, chloroform).

Anal. Calcd. for $C_{24}H_{26}O_8$: C, 65.15; H, 5.92. Found: C, 65.37; H, 6.18.

When 1.0 g. of epipodophyllotoxin ethyl ether was refluxed with 0.5 g. of crystallized sodium acetate in 10 cc. of ethanol for 2.5 hours, and the solution diluted with water, 0.75 g. (75%) of starting material, m.p. 191–194°, was recovered; $[\alpha]^{20}D$ −88° (*c*, 1, chloroform). If the time of refluxing was increased to 16 hours, 0.9 g. of a material was obtained which melted between 80 and 130° and apparently consisted of a mixture of the isomeric ethyl ethers.

Refluxing 0.50 g. of epipodophyllotoxin ethyl ether with 0.5 cc. of concd. aqueous ammonia in 2.5 cc. of ethanol for one hour yielded 0.45 g. (90%) of starting material, m.p. 191–194°, while refluxing for 16 hours yielded 0.44 g. of hairlike needles, m.p. 132–144°, which on recrystallization gave material melting between 139 and 147°, evidently impure epipicropodophyllin ethyl ether.

Reaction of Epipodophyllotoxin with Phosphorus Tribromide.—When epipodophyllotoxin (1.39 g.) was treated with phosphorus tribromide under the conditions described for the preparation of podophyllotoxin bromide from podophyllotoxin, there was obtained 0.72 g. (45%) of colorless crystals, m.p. 150° (effervescence), which after two recrystallizations from benzene–ether gave colorless prisms, m.p. 156.5–157° (effervescence), no depression with podophyllotoxin bromide from podophyllotoxin; $[\alpha]^{20}D$ +11° (*c*, 1, ethanol-free chloroform). The mother liquor gave, after dilution with ether and hexane and further standing, another 0.21 g. of less pure material, m.p. 149° (effervescence), bringing the total yield to 0.93 g. (58%).

Acetylepipodophyllotoxin (Xe).—Epipodophyllotoxin (3.0 g.) was refluxed with 30 cc. of acetic anhydride for 30 minutes. The solution was diluted with 15 cc. of acetic acid, then with 300 cc. of water and allowed to stand at room temperature for an hour. Boiling for a few minutes caused the oil to solidify. The crude material (2.58 g.) melted between 135 and 169°. Crystallization from absolute ethanol gave 2.05 g. (62%) of colorless needles, m.p. 172.6–173.2° (sinters 171.8°). Three further recrystallizations gave material melting at 174.4–175.4°; $[\alpha]^{20}D$ −141° (*c*, 1, chloroform).

Anal. Calcd. for $C_{24}H_{24}O_9$: C, 63.15; H, 5.30. Found: C, 63.24; H, 5.36.

Acetylepipicropodophyllin (XIc). (a) **From Epipicropodophyllin.**—A solution of 0.50 g. of epipicropodophyllin in 7.5 cc. of acetic anhydride and 5 cc. of dry pyridine was allowed to stand at room temperature for 18 hours, then heated on the steam-bath for 1 hour. Addition of 200 cc. of water gave a flocculent solid, which was crystallized from dilute ethanol to give 0.42 g. (76%) of colorless small hexagonal pyramids, m.p. 157.0–157.4° (shrinking at 151°). Recrystallization from 50% ethanol gave material, m.p. 156.0–156.8°, $[\alpha]^{20}D$ +7.4° (*c*, 1, chloroform).

Anal. Calcd. for $C_{24}H_{24}O_9$: C, 63.15; H, 5.30; sapn. equiv., 228. Found: C, 63.46; H, 5.68; sapn. equiv., 230.

(b) **From Acetylepipodophyllotoxin.**—A solution of 1.67 g. of acetylepipodophyllotoxin in 12 cc. of ethanol was refluxed with 0.06 cc. of piperidine and 4 cc. of water for one hour, then diluted with 16 cc. of hot water. The solution was seeded with material obtained from epipicropodophyllin and cooled very slowly. There was obtained 0.95 g. (57%) of crystals, m.p. 154.0–155.8° (sintering at 152.4°), which on recrystallization from 50% ethanol gave hexagonal pyramids, m.p. 156.0–156.8°.

The mother liquor from the first crop of crystals gave, when heated, diluted with water to incipient cloudiness and kept at 60° for 3 days, 0.48 g. of a solid melting below 150°. This, when recrystallized from 50% ethanol, then from absolute ethanol, gave small colorless needles, which were appreciably hygroscopic and melted at 198 to 208° (after drying at 110° *in vacuo*); $[\alpha]^{20}D$ + 10.6° (*c*, 1, chloroform). Repeated recrystallization did not narrow the melting range. The material might possibly be impure picropodophyllin.

Anal. Calcd. for $C_{22}H_{22}O_8$: C, 63.75; H, 5.35; sapn. equiv., 414. Found: C, 63.13; H, 5.60; sapn. equiv., 414.

Both acetylepipicropodophyllin, m.p. 156.0–156.8°, and the needles, m.p. 198–208°, were also obtained when epipodophyllotoxin was refluxed with sodium acetate in acetic anhydride for 1 to 2 hours. However, isolation of either was difficult and the yields were low.

When 0.20 g. of acetylepipicropodophyllin, m.p. 156.0–156.8°, was saponified by refluxing with 7.7 g. of 10% aqueous potassium hydroxide for 1 hour, the solution cooled in ice and acidified with acetic acid, a solid was obtained (evidently epipodophyllic acid, see below). This was lactonized by melting, and the residue crystallized from benzene (seeding) to give the characteristic crystals of epipicropodophyllin, m.p. 96–101° (sintering at 93°, effervescence at 101°). The solvent-free material (tiny needles from chloroform–hexane) melted at 158.0–158.2°.

Reaction of Epipodophyllotoxin with Acetic Anhydride and Sulfuric Acid.—A solution of 1.0 g. of epipodophyllotoxin in 6 cc. of acetic anhydride containing 0.007 cc. of concd. sulfuric acid was heated on the steam-bath for 40 minutes. The red-brown solution was heated with 10 cc. of water, then diluted with a large amount of water. The oil solidified in the ice-box. The orange-colored solid was washed with water and crystallized from 10 cc. of ethanol to give 0.46 g. (42%) of a yellow crystalline mass, m.p. 171–185°. Several recrystallizations from absolute ethanol (Norit) gave nearly colorless needles, m.p. 208.4–209.5°, no depression with an authentic sample of acetylpodophyllotoxin.

Epipodophyllic Acid.—A solution of 0.50 g. of epipicropodophyllin in 25 cc. of boiling 1 *N* aqueous sodium hydroxide was cooled in ice and acidified with 10 cc. of 3 *N* acetic acid. The yield of small colorless needles, which were isolated after keeping in ice and washing with ice-cold water, was 0.43 g. (82%). The material melted with effervescence between 166 and 171°, depending on the rate of heating. Recrystallization from dilute ethanol gave feathery fine needles, m.p. 174° (effervescence, immersion at 170°, rate of heating 3° per minute), m.p. 167° (effervescence, immersion at room temperature); $[\alpha]^{20}D$ −43.9° (*c*, 1, abs. ethanol).

Anal. Calcd. for $C_{22}H_{24}O_9$: C, 61.11; H, 5.59; neut. equiv., 432. Found: C, 60.43; H, 5.72; neut. equiv., 438.

Lactonization of Epipodophyllic Acid.—When 0.28 g. of this acid was heated at 175°, and the resulting oil dissolved in benzene, 0.20 g. (75%) of epipicropodophyllin separated on seeding as colorless plates, m.p. 96–101° (effervescence at 101°). Melting at 110° *in vacuo* gave tiny needles, m.p. 157.0–157.4° (from chloroform–hexane).

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