[CONTRIBUTION FROM THE LABORATORY OF CHEMICAL PHARMACOLOGY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE, U. S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE]

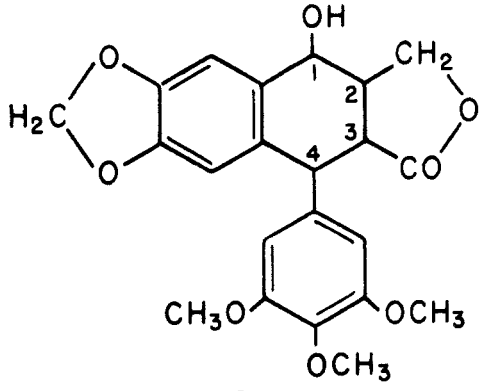
Components of Podophyllin. XII.[1] The Configuration of Podophyllotoxin

BY ANTHONY W. SCHRECKER AND JONATHAN L. HARTWELL

RECEIVED MAY 18, 1953

The configuration of podophyllotoxin (PT) (I) has been established as *trans*-(1:2)-*trans*-(2:3)-*cis*-(3:4) as in Ib, as follows: Esters of PT and picropodophyllin (PP) (II) are pyrolyzed more readily than those of their respective C_1-epimers (III, IV), indicating a *cis* relation of OH at C_1 and H at C_2. Hydrogenation of β- (VI) and of γ-apopicropodophyllin (VII), assumed to proceed by *cis–syn*-addition, yields a stereoisomer XI different from that (VIII) formed by hydrogenolysis of PT chloride and the ones (IX, X) obtained in hydrogenation of α-apopicropodophyllin (V), proving the relative configurations at C_2, C_3 and C_4.

In a preceding paper of this series[2] it was shown that podophyllotoxin (PT) and picropodophyllin (PP) are stereoisomers of structure Ia, differing only in the configuration at C_3. Two additional stereoisomers, named epipodophyllotoxin (EPT) and epipicropodophyllin (EPP) were obtained *via* the halides of PT. The experimental evidence was best explained by postulating that conversion from the "toxin" (PT, EPT) to the "picro" (PP, EPP) series involved inversion at C_3, while the change from the "normal" (PT, PP) to the "epi" (EPT, EPP) series resulted from inversion at C_1. As regards the activity of the four isomers against Sarcoma 37 in mice, considerable differences were found[3]; the change from PT to EPT was accompanied by a marked decrease, while both PP and EPP had negligible potency. To obtain further correlations between stereochemical features and pharmacologic action, an investigation of the relative configuration of the various substituents was undertaken. The results are reported in this communication.



Ia

In 1942, Haworth[4] proposed a *trans*-(1:2)-*trans*-(2:3)-*trans*-(3:4) relationship (formula Ia)[5] for PT. His assignment of a *cis* arrangement of the 1:3-positions[5] was, however, predicated upon the abandoned bridged lactonic structure,[2] while the *trans* relation for the substituents at C_3 and C_4 was deduced from the relative stability of certain model compounds[6] and from the assumption that naturally occurring lignans possess the most stable configuration. Price[7] suggested, on the contrary, that the carboxyl and trimethoxyphenyl groups were *cis*, on the basis that repulsion of these substituents would favor the epimerization at C_3, which is responsible for the conversion of PT to PP. Drake and Price[8] postulated a *trans*-(1:2) relation in PP from the results of the pyrolysis of its benzoate. The assumptions of Price[7] and of Drake and Price[8] have now been confirmed on the basis of more complete experimental data which show definitely that the configuration of PT is *trans*-(1:2)-*trans*-(2:3)-*cis*-(3:4) (partial formula Ib). Some of these data, now to be discussed, are summarized in Chart I.

Configuration at 1:2

Ester Pyrolysis.—Drake and Price's assignment[8] of the relative configuration at C_1 and C_2 was based on the assumption that the easy decomposition of PP benzoate (II, R = C_6H_5CO) proved *cis* elimination of substituents attached to those carbon atoms. It has been shown[9] that pyrolysis of xanthates, halides and carboxylic esters proceeds with *preferential cis* elimination; however, only comparison of a pair of appropriate epimers warrants a definite assignment of configuration. Moreover, analogous eliminations may involve substituents attached to non-neighboring carbon atoms. Decomposition of xanthates may lead to the formation of cyclopropane rings in bicyclic terpenes if the xanthate radical and the β-hydrogen are simultaneously *endo* and *cis*.[10,11] Since not α- (V), but β-apopicropodophyllin (VI) was isolated in the pyrolysis of PP benzoate,[8,12] there was no certainty that 1,2-elimination had occurred. Further investigation has now shown, however, that α-apoPP is indeed the primary decomposition product of PP benzoate. In addition, comparison of the decomposition temperatures and rates of pairs of esters differing only

(1) Paper XI: M. V. Nadkarni, J. L. Hartwell, P. B. Maury and J. Leiter, THIS JOURNAL, **75**, 1308 (1953).

(2) J. L. Hartwell and A. W. Schrecker, *ibid.*, **73**, 2909 (1951).

(3) J. Leiter, *et al.*, unpublished results.

(4) R. D. Haworth, *J. Chem. Soc.*, 448 (1942).

(5) Our numbering. It should be noted that Haworth used a different numbering system.

(6) R. D. Haworth and F. H. Slinger, *J. Chem. Soc.*, 1321 (1940).

(7) E. H. Price, Ph.D. thesis, University of Maryland, 1949.

(8) N. L. Drake and E. H. Price, THIS JOURNAL, **73**, 201 (1951).

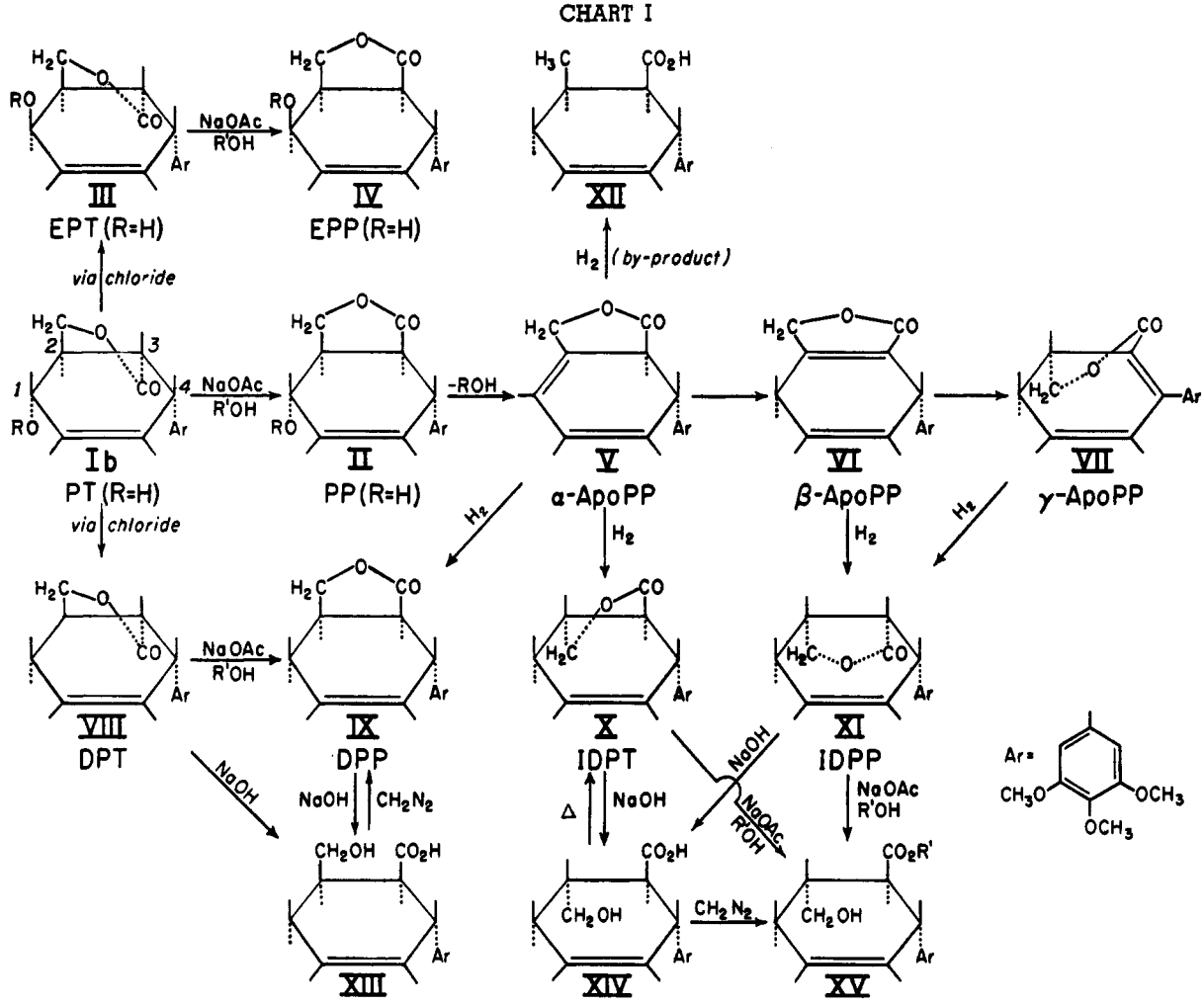
(9) (a) D. H. R. Barton, *J. Chem. Soc.*, 2174 (1949); (b) E. R. Alexander and A. Mudrak, THIS JOURNAL, **72**, 1810, 3194 (1950); **73**, 59 (1951).

(10) W. Hückel, W. Tappe and G. Legutke, *Ann.*, **543**, 191 (1940).

(11) Tricyclene, cyclofenchene and apocyclene (1,3-elimination), in addition to bornylene, fenchene and apobornylene (1,2-elimination), were isolated from the pyrolysis of bornyl, fenchyl and camphenilyl xanthates, respectively. See L. Tschugaeff and W. Budrick, *ibid.*, **388**, 280 (1912); G. Komppa and R. H. Roschier, *ibid.*, **429**, 175 (1922); *cf.* W. Qvist, *ibid.*, **417**, 278 (1918); S. Nametkin, *J. prakt. Chem.*, [2] **106**, 25 (1923).

(12) A. W. Schrecker and J. L. Hartwell, THIS JOURNAL, **74**, 5676 (1952).

CHART I



in the configuration at C_1 has yielded a definite assignment of the relative configuration at this carbon atom.

Since α-apoPP was converted to the β-isomer by heating at 240–260°,[12] it appeared not unlikely that this shift of the double bond could account for the isolation of the latter compound upon pyrolysis of PP benzoate. In order to minimize the effect of traces of alkali, possibly present on a glass surface, PP benzoate was pyrolyzed in a quartz vessel,[13] and the time of exposure to high temperature was reduced. While heating at 215–220° during ten minutes produced essentially pure β-apoPP in 76 to 81% yield,[14] a mixture of V and VI, consisting largely of the α-isomer, was isolated under the same conditions after five minutes. In control experiments, equimolecular mixtures of α-apoPP and benzoic acid gave essentially pure β-apoPP when heated in quartz at 220° for ten minutes. These results are evidence for a 1,2-elimination mechanism.

Comparing the pyrolysis rates of the benzoates of PP and of EPP[15] has substantiated the correctness of the configurational assignment of Drake and Price.[8] The rates of decomposition were measured by titrating the benzoic acid formed both on pyrolyzing samples in quartz vessels and on heating in inert solvents. The results thus obtained are shown in Table I.

TABLE I

THERMAL DECOMPOSITION OF PICROPODOPHYLLIN AND EPIPICROPODOPHYLLIN BENZOATES

Benzoate of	Solvent	Temp., °C.	Time, min.	Benzoic acid formed, %
Picropodophyllin (II)	Decalin	190–193	5	12
	Tetralin	205–207	20	50
	None	215–220	6	85[a]
Epipicropodophyllin (IV)	Decalin	190–193	5	1.7
	Tetralin	205–207	20	3.5
	None	215–220	6	30[a]

[a] Some benzoic acid may have been lost by sublimation.

On the basis of preferential *cis* elimination, the benzoxyl group at C_1 must be *cis* relative to the hydrogen at C_2 in the more readily decomposed PP benzoate (II, R = C_6H_5CO) and *trans* in EPP benzoate (IV, R = C_6H_5CO). This conclusion has been substantiated and extended to the "toxin" series by a comparison of the decomposition temperatures of the acetates of PT, EPT, PP and EPP,

(13) This was suggested by Dr. D. H. R. Barton.

(14) N. L. Drake and E. H. Price, ref. 8, isolated β-apoPP in 95% yield by heating at 240° for 20 minutes.

(15) EPP benzoate (IV, R = C_6H_5CO), which has now been synthesized, was decomposed to β-apoPP (74% yield) and benzoic acid when heated at 245° for 20 minutes. In view of the greater stability of this benzoate toward pyrolysis, no attempt was made to obtain α-apoPP by employing less drastic conditions.

measured in quartz capillaries.[16] These data are summarized in Table II.

TABLE II

TEMPERATURES OF VISIBLE DECOMPOSITION OF ACETATES

Acetate of	Decomposition temperature, °C. Slow heating[a]	Rapid heating[b]
Picropodophyllin (II)	218–219	220–223
Epipicropodophyllin (IV)	231–235	245–248
Podophyllotoxin (Ib)	240–245	245–250
Epipodophyllotoxin (III)	255–258	269–273

[a] 1°/min., immersed at 215°. [b] 3–5°/min., immersed at 215°.

Thus the substituents eliminated are *cis* in the esters of both PP (II) and PT (Ib), and the non-hydrogen substituents at C_1 and C_2 are therefore *trans*, while the precisely opposite relationship holds true for EPP (IV) and EPT (III). This evidence also disproves the ideas of Chatterjee and Chakravarti[17] concerning the configurations of PT and its isomers.

Ionic Elimination.—Since ionic elimination reactions proceed preferentially when the substituents removed are *trans*,[18] as contrasted with pyrolytic eliminations, it should be expected that EPT (III, R = H) would undergo dehydration more readily than PT (Ib, R = H) and EPP (IV, R = H) more easily than PP (II, R = H). When PP and EPP were treated with phosphorus oxychloride in pyridine,[13,18b] β-apoPP (VI)[19] was isolated in 3 and 8% yields, respectively, after 24 hours at room temperature.[21] This is consistent with *trans* elimination of hydroxyl and hydrogen in EPP. In other experiments,[22] no essential difference in the rates of dehydration of PP and EPP was observed when these compounds were treated with mixtures of acetic and hydrochloric acids. A common carbonium ion mechanism[22] may be postulated, with the loss of a proton as the rate-determining step. PT and EPT do not undergo ionic elimination at all; features other than the relative configurations at C_1 and C_2 are certainly responsible for this fact.[22]

Configuration at 2:3 and 3:4

Hydrogenation of the Apopicropodophyllins.—By hydrogenation of α-apoPP (V)[12] with platinum black in acetic acid, Borsche and Niemann[23] obtained a compound melting at 169–170°, which they named "desoxypicropodophyllin." Drake and Price[8] prepared a different desoxypicropodophyllin, m.p. 200–201°, $[\alpha]_D$ −114° (chloroform), by hydrogenating β-apoPP (VI) with Raney nickel in ethanol. The available evidence indicated that both compounds were stereoisomers of the structure obtained formally by replacement of the hydroxyl group in Ia with hydrogen. Hydrogenolysis of podophyllotoxin chloride[2] yielded another stereoisomer, desoxypodophyllotoxin (DPT) (VIII), m.p. 168–169°, $[\alpha]_D$ −115° (chloroform.)[24] This compound, which by its method of formation must have the same configuration at C_2, C_3 and C_4 as PT (Ib) itself,[25] was converted by base-catalysts to a substance, m.p. 171–172°, $[\alpha]_D$ +32° (chloroform). Since this conversion parallels that of PT (Ib) to PP (II) (epimerization at C_3), the new substance must have the same configuration at C_2, C_3 and C_4 as PP, and is therefore properly named desoxypicropodophyllin[24] (DPP) (IX). When Borsche and Niemann's hydrogenation of α-apoPP[23] was repeated, their "desoxypicropodophyllin" was found to be identical[26] with the DPP formed in the epimerization of DPT. Careful fractionation of the product obtained by hydrogenating α-apoPP with platinum black (prepared by the method of Willstätter[27] or by *in situ* reduction of platinum oxide) in acetic acid afforded, however, in addition to a 75 to 85% yield of DPP (IX), the methylcarboxylic acid (XII) formed in *ca.* 7% yield by hydrogenolysis of V, and furthermore a small amount (about 3%) of still another stereoisomer of DPT and DPP, m.p. 251–252°, $[\alpha]_D$ +82° (chloroform), named isodesoxypodophyllotoxin (IDPT) (X).[28] Using palladium-on-charcoal in acetic acid, the crude lactone fraction (92% yield) was found to be composed of 65% of DPP and 35% of IDPT.[29] Dehydration of PP (II) to α-apoPP (V) and the subsequent hydrogenation of V involve only C_1 and C_2. Therefore, the two possible hydrogenation products (IX and X) must have the same configurations at C_3 and C_4 as V and II. Thus one of those two products was expected to be identical with the DPP obtained from DPT.

Four diastereoisomeric desoxylactones (and their optical antipodes) are theoretically capable of existence. Comparison of the infrared absorption spectrum (Fig. 1) and of the optical rotations in chloroform and pyridine of Drake and Price's "desoxypicropodophyllin" with those of DPT, DPP and IDPT proves that all four compounds are different diastereoisomers. It is proposed that the compound obtained in the hydrogenation of β-apoPP[8] be renamed "isodesoxypicropodophyllin" (IDPP) (XI) to distinguish it from DPP.[28]

The relative configurations at C_2, C_3 and C_4 were assigned on the basis of preferential *cis* and

(16) Essentially similar results were obtained in Pyrex capillaries.

(17) R. Chatterjee and S. C. Chakravarti, *Science and Culture*, **17**, 136 (1951); **18**, 197 (1952). Their views are, furthermore, hardly consistent with generally accepted reaction mechanisms. Contrary to their statement, it was shown in our previous paper (ref. 2) that EPP was converted to PP by mineral acids and not by base-catalysts, since a solution of EPP prepared by boiling with aqueous alkali yielded epipodophyllic acid when acidified in the cold with acetic acid, and gave PP only when acidified with hydrochloric acid and then heated.

(18) (a) D. H. R. Barton, *Experientia*, **6**, 316 (1950); (b) D. H. R. Barton and W. J. Rosenfelder, *J. Chem. Soc.*, 1048 (1951); (c) C. C. Price and J. V. Karabinos, THIS JOURNAL, **62**, 1159 (1940).

(19) Since V is converted to VI in pyridine solution,[20] it is not surprising that the β-isomer was obtained.

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

(21) Most of the starting material was converted in both instances to a phosphate ester, which was not isolated but hydrolyzed to PP by heating with mineral acid, a treatment which is known to epimerize EPP to PP.[2]

(22) To be reported at a later date.

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

(24) J. L. Hartwell, A. W. Schrecker and J. M. Johnson, THIS JOURNAL, **75**, 2138 (1953).

(25) *Cf.* footnote 35.

(26) Identity proven by mixed melting point and infrared absorption spectra.

(27) R. Willstätter and E. Waldschmidt-Leitz, *Ber.*, **54**, 113 (1921).

(28) With regard to the nomenclature of the isodesoxylactones, it was decided to adopt the term "toxin" for the *trans*- and "picro" for the *cis*-lactones. These assignments are discussed below.

(29) Substitution of ethyl acetate for acetic acid produced a decrease in the proportion of IDPT without changing the results essentially.

syn addition[30] in catalytic hydrogenation. In the formation of IDPP, hydrogen should add to β-apoPP (VI) at C_2 and C_3 opposite the trimethoxyphenyl group at C_4. IDPP (XI) should, therefore, be *cis*-(2:3)-*cis*-(3:4). Since DPP and IDPT have identical configurations at C_3 and C_4 and are both different from IDPP, they must be *trans*-(3:4). DPT (and PT) can, therefore, be only *trans*-(2:3)-*cis*-(3:4). Since DPP, by its formation from DPT, must then be *cis*-(2:3)-*trans*-(3:4), IDPT is, finally, *trans*-(2:3)-*trans*-(3:4).

This reasoning is open to the criticism that *cis* and *syn* addition in hydrogenation is the rule only when platinum catalysts in acidic medium are employed.[30] IDPP was obtained with Raney nickel in a neutral medium,[8] and although the *cis–syn*-addition rule still holds in the majority of cases under these conditions, exceptions are known. Such exceptions can be accounted for by catalytic conversion of primarily formed enolizable compounds into thermodynamically stable ones.[31] While enolization (at C_3) is possible in the desoxylactones, it was found that base-catalysts, which should effect exactly analogous conversions, epimerize neither DPT, DPP nor IDPT to IDPP, which therefore should be the primary hydrogenation product. The *cis*-(2:3)-*cis*-(3:4) arrangement of IDPP is substantiated by the isolation of this compound as a by-product, together with the failure to isolate any of its stereoisomers, in the hydrogenation of β-apoPP (VI) with palladium-on-charcoal in warm acetic acid, to be discussed below in greater detail. It is rendered even more certain by the results of the hydrogenation of γ-apoPP[12] (VII). With this compound, *cis–syn*-addition should also yield the desoxylactone XI. Even if *syn* addition is not postulated, *cis* addition of hydrogen to both VI and VII could yield one and the same compound only if this compound has the all-*cis* configuration XI proposed for IDPP. Indeed, IDPP,[32] but none of its diastereoisomers, could be isolated from the hydrogenation of γ-apoPP with Raney nickel in ethanol or with platinum oxide and palladium-on-charcoal in acetic acid at 75°. Since all four desoxylactones (VIII, IX, X, XI) were found to be stable to similar treatment with palladium catalyst in acetic acid, it is quite certain that neither DPT, DPP nor IDPT were the precursors of the IDPP formed under such conditions in the hydrogenation of β- and γ-apoPP.[35]

(30) R. P. Linstead, *et al.*, THIS JOURNAL, **64**, 1985–2026 (1942); *J. Chem. Soc.*, 1423–1432 (1950).

(31) A. Farkas and L. Farkas, *Trans. Faraday Soc.*, **33**, 837 (1937).

(32) Since synthetic γ-apoPP[12,33,34] was employed, racemic IDPP was obtained. This substance, m.p. 203–204°, gave a mixed melting point depression with optically active IDPP, m.p. 202–203°. However, both IDPP prepared from VI and the racemic compound had completely identical infrared spectra.

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

(34) A. W. Schrecker and J. L. Hartwell, THIS JOURNAL, **74**, 5672 (1952).

(35) For the same reason, DPP and IDPT are certainly primary hydrogenation products of α-apoPP. In the preparation of DPT from PT chloride,[24] a non-polar solvent was employed, rendering inversion at C_3 very unlikely. Moreover, the epimerization of DPT to DPP parallels that of PT to PP, which makes it certain that the hydrogenolysis leading to DPT itself had not been accompanied by such epimerization. The failure of these compounds to undergo inversion at C_3 in the presence of platinum or palladium catalysts may be contrasted with Borsche and Niemann's observation[28] that PT could be converted to PP with colloidal palladium in methanol. It is conceivable that traces of alkali might have been present in their catalyst.

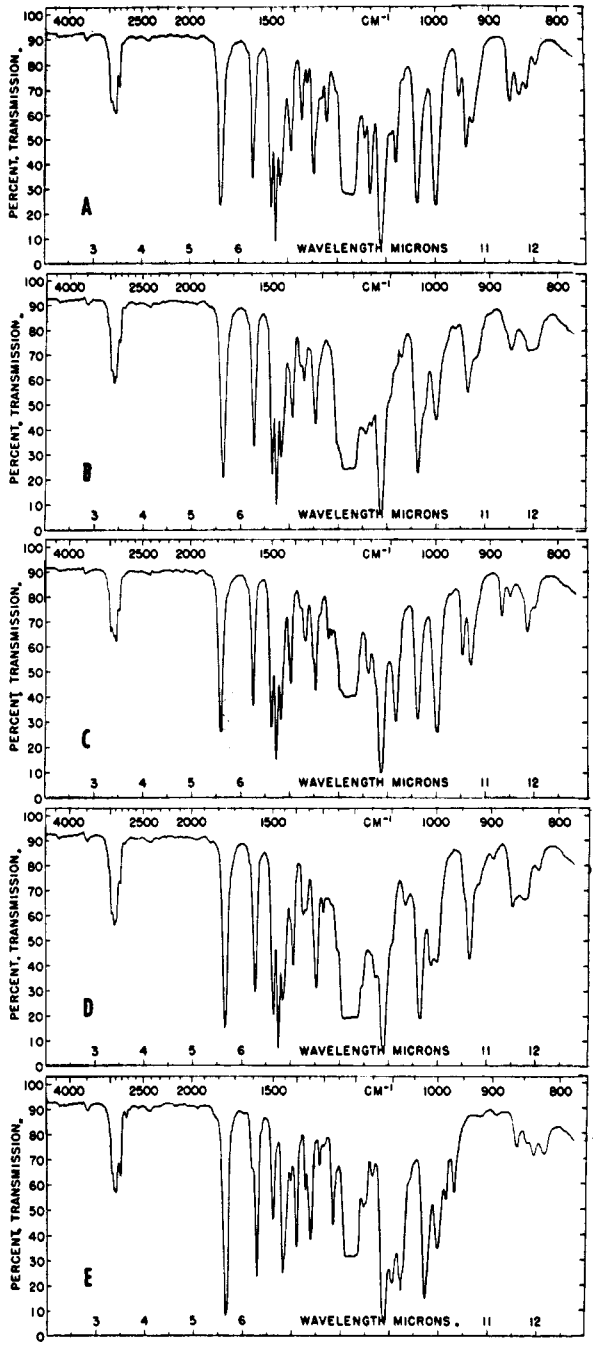


Fig. 1.—Infrared absorption spectra in chloroform of: A, desoxypodophyllotoxin (DPT, VIII); B, desoxypicropodophyllin (DPP, IX); C, isodesoxypodophyllotoxin (IDPT, X); D, isodesoxypicropodophyllin (IDPP, XI); E, 6,7-methylenedioxy-1-(3,4,5-trimethoxyphenyl)-3-hydroxymethyl-5,6,7,8-tetrahydro-2-naphthoic acid lactone (XVII).

Attempts to effect hydrogenation of the double bond in β-apoPP with platinum or palladium catalysts in acidic medium were unsuccessful in most instances. Price[7] already showed that no hydrogen uptake could be obtained with platinum oxide in ethanol. Similar failures were encountered in this Laboratory when using platinum oxide

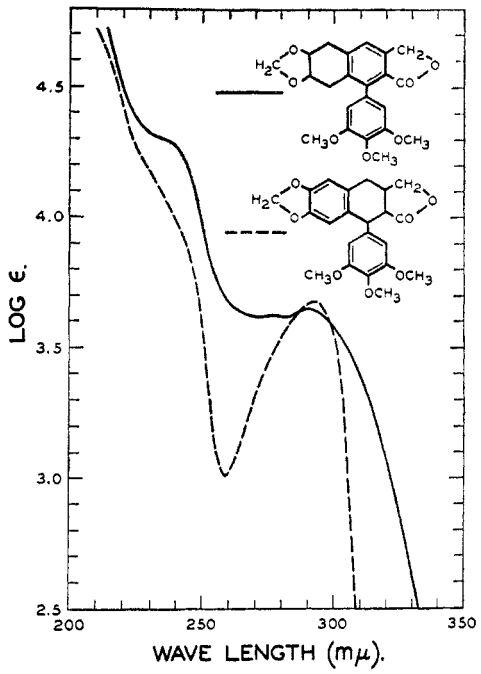
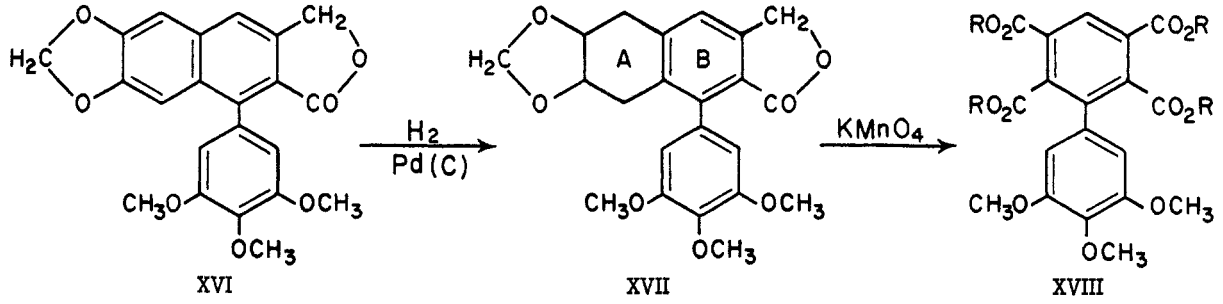


Fig. 2.—Ultraviolet absorption spectra in 95% ethanol of: ——, 6,7-methylenedioxy-1-(3,4,5-trimethoxyphenyl)-3-hydroxymethyl-5,6,7,8-tetrahydro-2-naphthoic acid lactone (XVII); – – – –, desoxypodophyllotoxin (DPT, VIII).

apoPP by aromatization of the B- and hydrogenation of the A-ring. It appears likely that aromatization preceded hydrogenation. On the one hand, DPT, DPP, IDPT and IDPP were recovered unchanged from attempted hydrogenations with palladium-on-charcoal in acetic acid at 75°, and on the other the compound XVII, m.p. 177.5–178.5°, was obtained in 80% yield from dehydroanhydropicropodophyllin (XVI)[12,33,36] under the same conditions.

Reactions of the Desoxylactones.—It has been mentioned that DPT (VIII) is isomerized to DPP (IX) by base-catalysts, such as sodium acetate in methanol or ethanol,[24] a reaction that parallels the conversion of PT (Ib) to PP (II).[2] Similarly, PT and DPT are saponified to podophyllic acid[2,23] and desoxypodophyllic acid[24] (XIII), respectively. Relactonization yields PP and DPP, indicating that the acids are in the *cis*-(2:3) series. With the iso compounds, these reactions took quite a different course. Saponification of both IDPT (X) and IDPP (XI) produced the same isodesoxypodophyllic acid (XIV),[37] which was lactonized by heating to IDPT and has therefore the *trans*-(2:3) configuration.[38] While desoxypodophyllic acid (XIII) was lactonized to DPP by treatment with diazomethane,[40] isodesoxypodophyllic acid (XIV) yielded methyl isodesoxypodophyllate (XV, R′ = CH_3). The same[26] methyl ester was obtained by refluxing



XVI XVII XVIII

in acetic acid at room temperature (in the presence or absence of perchloric acid) or at 80°, and also with palladium-on-barium sulfate in acetic acid at 80°. With palladium-on-charcoal in acetic acid at 75°, hydrogen uptake was quite rapid. In addition to IDPP (XI) (1.7% yield), a compound, $C_{22}H_{22}O_7$, m.p. 177.5–178.5°, was isolated in 73% yield. The same substance and racemic[32] IDPP were obtained in the hydrogenation of γ-apoPP in acetic acid in yields of 30 and 19%, respectively. The compound, m.p. 177.5–178.5°, by its empirical formula, is an isomer of DPT, DPP, IDPT and IDPP, but it is not a stereoisomer. Its infrared (Fig. 1) and ultraviolet (Fig. 2) absorption spectra were essentially different from those of the other desoxylactones. It was identified as 6,7-methylenedioxy-1-(3,4,5-trimethoxyphenyl)-3-hydroxymethyl-5,6,7,8-tetrahydro-2-naphthoic acid lactone (XVII) by its oxidation with permanganate to 3′,4′,5′-trimethoxybiphenyl-2,3,5,6-tetracarboxylic acid (XVIII, R = H), which was characterized as the tetramethyl ester. Since hydroaromatic nuclei are degraded in preference to aromatic nuclei in permanganate oxidations, there can be no doubt that the new lactone was obtained from β- and γ-

either IDPT (X) or IDPP (XI) with sodium acetate in methanol. Sodium acetate in ethanol gave the analogous ethyl ester (XV, R′ = C_2H_5). On the other hand, IDPT and IDPP were recovered unchanged when refluxed with sodium acetate in acetic anhydride, a reaction in which the acetate of PT is converted to that of PP.[2] These findings greatly strengthen our conclusions about the relative configurations at C_2, C_3 and C_4 for the various lactones.[41] The strain inherent in the *trans*-lactone

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

(37) Samples of XIV obtained from X and XI had optical rotations of the same sign and magnitude. This proves that the configuration of C_4 remained unchanged, as expected, in the series of reactions leading from α-apoPP (V) through β-apoPP (VI) to IDPP (XI).

(38) This might be considered as an exception to the Alder–Stein rule,[39] which was previously cited[2] in support of the *cis*-(2:3) configuration of PP. However, the *cis*-hydroxy acid XIII is lactonized at a lower temperature (about 172°)[24] than the *trans*-hydroxy acid XIV (213°), in accordance with that rule. When epimerization at C_3 takes place, it occurs certainly only during saponification of the lactones and not during relactonization of the hydroxy acids by heating.

(39) K. Alder and G. Stein, *Ann.*, **504**, 216 (1933).

(40) Similar lactonizations under the influence of diazomethane have been reported previously; *cf.* ref. 39.

(41) The all-*trans* configuration of IDPT (X) is also consistent with the higher melting point as compared with those of its diastereoisomers. *Cf.* A. Skita, *Ann.*, **431**, 1 (1923).

rings can be relieved either by ring opening or by epimerization to a less strained *cis*-lactone. In the case of DPT (VIII) (or PT, Ib), such epimerization takes place readily because it is also promoted by repulsion of the bulky substituents at C_3 and C_4.[7] There is no tendency toward ring opening. On the other hand, the tendency toward a conceivable isomerization of IDPT to IDPP is opposed by the same repulsive force of the substituents at C_3 and C_4. The strain in the *trans*-lactone ring of IDPT and the repulsion of the substituents in IDPP can, however, be relieved by ring opening with formation of the stable all-*trans* isodesoxypodophyllic esters.

Infrared Absorption Spectra.—The assignments of respective *cis*- and *trans*-lactone configurations are also corroborated by comparison of the carbonyl frequencies in the infrared, which are listed in Table III. The frequencies observed in the "picro" series are consistently lower than those of the corresponding "toxin" compounds. This is in agreement with the postulated decrease of strain which accompanies the change of the γ-lactone ring from the *trans* to the *cis* configuration. The configuration of the methylcarboxylic acid (XII) isolated as a by-product in the hydrogenation of α-apoPP was assumed to be *cis*-(2:3)-*trans*-(3:4) when the general shape of its infrared curve (Fig. 3) and the position of its carbonyl peak (1707 cm.$^{-1}$, Nujol) were compared with those of desoxypodophyllic[24] (XIII) (1705 cm.$^{-1}$) and isodesoxypodophyllic acid (XIV) (1730 cm.$^{-1}$).

TABLE III

C=O STRETCHING FREQUENCIES OF VARIOUS LACTONES

Compound	Position of max., cm.$^{-1}$ ($CHCl_3$)
Podophyllotoxin (PT) (Ib, R = H)	1785
Picropodophyllin (PP) (II, R = H)	1775
Epipodophyllotoxin (EPT) (III, R = H)	1780
Epipicropodophyllin (EPP) (IV, R = H)	1768
EPT ethyl ether[2] (III, R = C_2H_5)	1780
EPP ethyl ether[2] (IV, R = C_2H_5)	1770
Desoxypodophyllotoxin (DPT) (VIII)	1780
Desoxypicropodophyllin (DPP) (IX)	1765
Isodesoxypodophyllotoxin (IDPT) (X)	1780
Isodesoxypicropodophyllin (IDPP) (XI)	1765

Experimental[42,43]

Purification of Esters for Pyrolysis Experiments.—Special care was exercised to avoid the possible presence of traces of base-catalysts in the final products. The acetates and benzoates of PP (II) and EPP (IV) were obtained by acylation in pyridine[2,8,36] at room temperature and purified by the procedure outlined for the previously unknown EPP benzoate (except that sodium carbonate was not used in the case of the acetates and that the more insoluble esters of PP were crystallized from chloroform–ethanol). The acetates of PT (Ib) and EPT (III) were prepared by refluxing with acetic anhydride.[2,23,36] All these compounds were subjected to two final recrystallizations in quartz vessels.

Epipicropodophyllin Benzoate (IV, R = C_6H_5CO).—A solution of 4.0 g. of solvent-free EPP[2] (IV, R = H) in 40 ml. of anhydrous pyridine was treated with 3 ml. of benzoyl chloride, then kept at room temperature for 21 hours and diluted with aqueous sodium carbonate. The yellow oil which separated solidified in the refrigerator.[44] It was washed with water, dissolved in chloroform, and the solution washed with dilute sodium carbonate, 2 *N* hydrochloric acid, then twice with distilled water and dried over sodium sulfate. Removal of solvent and crystallization from dilute ethanol gave 4.40 g. (88%) of colorless needles, m.p. 191–193°. Further recrystallization in quartz vessels from dilute ethanol, then from 95% ethanol provided material, m.p. 193–194°, $[\alpha]^{21}D$ +94° (*c* 1.01, chloroform).

Anal. Calcd. for $C_{29}H_{26}O_9$: C, 67.17; H, 5.05. Found: C, 67.22; H, 5.30.

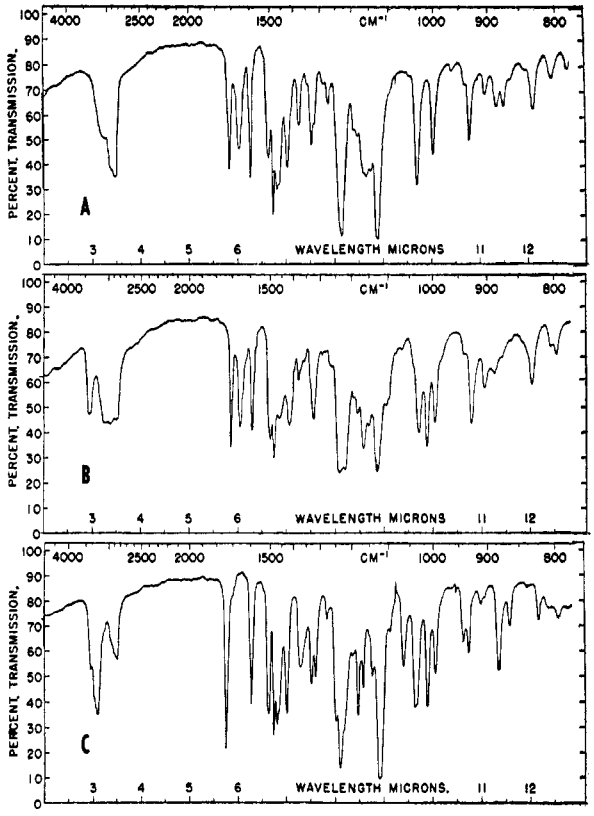


Fig. 3.—Infrared absorption spectra (Nujol mulls) of: A, 6,7-methylenedioxy-1-(3,4,5-trimethoxyphenyl)-3-methyl-1,2,3,4-tetrahydro-2-naphthoic acid (XII); B, desoxypodophyllic acid (XIII); C, isodesoxypodophyllic acid (XIV).

Pyrolysis of Picropodophyllin Benzoate.—Samples (300–500 mg.) of PP benzoate (II, R = C_6H_5CO) were placed in 25-ml. quartz erlenmeyer flasks and immersed in a metal-bath heated at 215–220°. The solid melted within approximately two minutes. The melt was boiled for a short time with 95% ethanol (distilled from tartaric acid), and the crystalline material collected after cooling. Thus heating under nitrogen at 220° for ten minutes produced a 76 to 81% yield of β-apoPP (VI), m.p. 215–218°, which gave an orange color with concd. sulfuric acid[20] and did not decolorize bromine in chloroform solution.[12] Heating under nitrogen at 215–220° for five minutes yielded 59% of material, m.p. 210–223°, which gave a purple color with sulfuric acid[20] and decolorized bromine rapidly,[12] indicating the presence of α-apoPP (V). Quantitative analysis of the product by measurement of the ultraviolet absorption at several wave lengths[45,46] showed that it contained 65% of α-apoPP.

(42) Melting points are corrected and were determined in Pyrex capillaries of 1.5 to 2 mm. o.d. with the Hershberg apparatus; *cf.* E. B. Hershberg, *Ind. Eng. Chem., Anal. Ed.*, **8**, 312 (1936).

(43) Infrared spectra were determined with a Perkin–Elmer model 21 spectrometer, using 0.2-mm. sodium chloride cells for chloroform solutions. Ultraviolet absorptions were measured with a Beckman model DU spectrophotometer in 1-cm. stoppered quartz cells.

(44) In a preliminary experiment, the crude material was recrystallized from dilute ethanol to yield 98% of product, m.p. 186–190°.

(45) R. A. Friedel and M. Orchin, "Ultraviolet Spectra of Aromatic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1951, p. 29.

(46) Absorptions were measured with solutions prepared by dissolving a sample in chloroform and diluting with 95% ethanol distilled from tartaric acid. Values for α-apoPP, β-apoPP and dehydroan-

Heating in air at 220° for five minutes gave 45% of a product, m.p. 228–231°, which contained 87% of α-apoPP and also 2% of dehydroanhydroPP (XVI).

When an intimate equimolecular mixture of α-apoPP and benzoic acid was heated similarly in a quartz vessel under nitrogen at 220° for ten minutes and the melt treated as above, β-apoPP, m.p. 215–219°, $[\alpha]^{21}D$ +98° (*c* 0.50, chloroform)[12] was isolated in 95% yield. No α-apoPP could be detected, even when the melt was crystallized from acetic acid containing a trace of hydrochloric acid.[12]

Pyrolysis of Epipicropodophyllin Benzoate.—Following the procedure of Drake and Price[8] for the decomposition of PP benzoate, 0.88 g. of EPP benzoate was heated under nitrogen at 245° for 20 minutes, and the sublimate identified as benzoic acid by its m.p. (122–123°) and mixed m.p. The yellow melt was dissolved in benzene and chromatographed on alumina.[47] Eluting with chloroform and concentrating with addition of ethanol yielded 0.50 g. (74%) of β-apoPP (VI), m.p. 217–219°. Recrystallization from chloroform–ethanol produced material which had m.p. and mixed m.p. 219–220°, $[\alpha]^{21}D$ +102° (*c* 0.53, chloroform).

Measurement of the Rates of Decomposition of the Benzoates of Picropodophyllin and Epipicropodophyllin. (a) In a Solvent.—The sample (0.12 to 0.15 g.) was refluxed in a 50-ml. round-bottom flask with 2 ml. of decalin (b.p. 190–193°) or tetralin (b.p. 205–207°) as indicated in Table I. A clear solution formed at the boiling point, and an oil or solid separated again on cooling. The suspension was then diluted with 25 ml. of ethanol, boiled for a few moments, cooled and titrated with 0.1 *N* sodium hydroxide against phenolphthalein. A blank in which PP benzoate was treated identically, but with omission of the refluxing period, was subtracted; its value was very small.

(b) **Without Solvent.**—Samples (0.4 g.) of the two compounds were placed in 100-ml. quartz erlenmeyer flasks, which were covered and immersed simultaneously for six minutes in a metal-bath heated at 215–220°. After cooling, the sublimed benzoic acid was rinsed down with 20 ml. of ethanol, the mixture boiled for a short time, cooled and titrated as above.

Measurement of the Visible Decomposition Temperatures of the Acetates.—The standard melting point technique[42] was employed, except for the use of quartz capillaries.[16] The samples were immersed at a bath temperature of 215°, which then was raised gradually. The first values of each range shown in Table II correspond to the temperatures at which bubbles started to appear, and the second to the points at which the column of liquid broke and was pushed up by the gas pressure. All figures are averages of several runs. Acetic acid was detected by placing a moistened strip of indicator paper over the mouth of the capillary and observing the color change. This test was slightly positive at about 3–5° below the temperatures listed, and strongly positive at those temperatures.

Reaction of Picropodophyllin (II, R = H) **and of Epipicropodophyllin** (IV, R = H) **with Phosphorus Oxychloride.**—To an ice-cold solution of 1.0 g. of PP (or EPP) in 10 ml. of anhydrous pyridine was added dropwise with swirling 1.0 ml. of phosphorus oxychloride. The reaction mixture, which after 24 hours at room temperature had become amber in the case of PP and brown in that of EPP, was decomposed with ice and water, then extracted repeatedly with chloroform. The extracts were washed with dilute hydrochloric acid, then with water, dried over magnesium sulfate and evaporated to dryness. The residue was boiled for a few moments with 3 ml. of ethanol, the supension cooled in the refrigerator and the felt-like needles collected and washed with a little chilled ethanol. PP yielded 30 mg. (3.1%) of material melting at 215–217.5°, and EPP 81 mg. (8.5%) of product melting at 213–217°. Recrystallization provided 23 mg., m.p. 219–220° (from PP), and 69 mg., m.p. 217–219° (from EPP), both samples identical with β-apoPP (VI) by mixed melting point determination.

The aqueous solutions which had been extracted with chloroform were cooled in ice and acidified strongly with hydrochloric acid. They remained clear after standing in the refrigerator for 24 hours. Heating on the steam-bath for one hour caused separation of solid material, m.p. 200–202°. The yield was 543 mg. from PP and 505 mg. from EPP. The product was identified in both instances as PP by conversion to PP acetate (II, R = CH_3CO), m.p. and mixed m.p. 213–216°.

Hydrogenation of α-Apopicropodophyllin. (a) With Palladium-on-charcoal.—A suspension of 2.0 g. of α-apoPP (V) and 0.4 g. of 10% palladium-on-charcoal[48] in 240 ml. of glacial acetic acid[29] was hydrogenated at room temperature and slightly above atmospheric pressure for 25 minutes, at which time the starting material had dissolved and absorption ceased. The catalyst was removed with suction (Celite), washed with hot acetic acid, and the colorless filtrate evaporated to complete dryness *in vacuo*. The solid residue was dissolved in hot ethyl acetate, chromatographed on alumina[49] and eluted with ethyl acetate (*lactone fraction*), then with chloroform–ethanol (9:1) (*acid fraction*). The lactone fraction, m.p. 176–215°, weighed 1.85 g. (92%) and had $[\alpha]^{20}D$ +50° (*c* 0.52, chloroform), indicating that it contained about 64% of DPP and 36% of IDPT. Fractional crystallization from ethyl acetate provided the less soluble **isodesoxypodophyllotoxin (IDPT)** (X) as tiny colorless needles in felt-like aggregates, m.p. 251–252°, $[\alpha]^{21}D$ +82° (*c* 0.52, chloroform), $[\alpha]^{20}D$ −3° (*c* 0.99, pyridine).

Anal. Calcd. for $C_{22}H_{22}O_7$: C, 66.32; H, 5.57. Found: C, 66.17; H, 5.74.

The mother liquors from IDPT yielded, by recrystallization from ethanol, **desoxypicropodophyllin (DPP)** (IX) as strongly electrified small colorless needles, m.p. 168–170°, $[\alpha]^{19}D$ +39° (*c* 0.53, chloroform), $[\alpha]^{19}D$ +42° (*c* 0.48, pyridine), identical[26] with DPP obtained by epimerization of DPT (VIII).[24,50] The actual amounts of DPP and IDPT isolated were in the ratio of 0.66 to 0.34.

The acid fraction provided, by recrystallization from dilute ethanol, 49 mg. (2.4%) of colorless needles, m.p. 230–235°. In a similar experiment, the acid fraction was purified by dissolving in aqueous sodium carbonate, filtering from a slight impurity, acidifying and recrystallizing twice from 50% ethanol to yield material, m.p. 238.5–239.5°, identical[26] with the methylcarboxylic acid XII, which was more readily obtained by using platinum black in the hydrogenation.

(b) **With Platinum Black.**—Hydrogenation of 2.5 g. of α-apoPP with 125 mg. of platinum oxide[48,51] in 300 ml. of acetic acid[29] for one hour, followed by chromatography as above, yielded a lactone fraction (86%) and an acid fraction (11%). The lactone fraction consisted mainly of DPP; less than 3% of IDPT (X) could be isolated by repeated crystallizations from ethyl acetate. The **desoxypicropodophyllin (DPP)** (IX),[26] after purification from ethanol, had m.p. 169.5–171°, $[\alpha]^{20}D$ +37° (*c* 1.0, chloroform), $[\alpha]^{20}D$ +41° (*c* 0.63, pyridine).[50]

Anal. Calcd. for $C_{22}H_{22}O_7$: C, 66.32; H, 5.57. Found: C, 65.98; H, 5.61.

Crystallization of the acid fraction from 50% ethanol afforded 0.19 g. (7.5%) of **6,7-methylenedioxy-1-(3,4,5-trimethoxyphenyl)-3-methyl-1,2,3,4-tetrahydro-2-naphthoic acid (XII)** as colorless needles, m.p. 236–237.5°, $[\alpha]^{20}D$ −144° (*c* 0.55, pyridine).

Anal. Calcd. for $C_{22}H_{24}O_7$: C, 65.99; H, 6.04; OCH_3, 23.25; neut. equiv., 400.4. Found: C, 65.83; H, 6.01; OCH_3, 22.97; neut. equiv., 403.

Hydrogenation of β-Apopicropodophyllin. (a) With Raney Nickel.—Following the procedure of Drake and Price,[8] a 79% yield of **isodesoxypicropodophyllin (IDPP)** (XI), m.p. 202–202.5° (lit.[8] 200.0–201.4°), was obtained after 18 hours at 60° and 1300 p.s.i. Further purification by chromatography on alumina,[49] elution with benzene and recrystallization from chloroform–ethanol provided hair-like colorless needles, m.p. 202.3–203.2°, $[\alpha]^{20}D$ −114° (*c* 0.51, chloroform) (lit.[8] −114°), $[\alpha]^{20}D$ −64° (*c* 0.45, pyridine).

(b) **With Palladium-on-charcoal.**—A solution of 1.50 g. of β-apoPP (VI) in 50 ml. of glacial acetic acid was hydrogenated with 1.25 g. of 10% palladium-on-charcoal at 75° and atmospheric pressure for 80 minutes, at which time up-

hydroPP were those previously reported.[12,34] PP benzoate had λ_{max}^{EtOH} 284.5 mμ (log ε 3.70).

(47) Alcoa activated alumina, grade F-20.

(48) Obtained from Baker & Co., Inc., Newark, N. J.

(49) The commercial product[47] was washed with dilute hydrochloric acid, then with water and dried at 200° overnight.

(50) The DPP from DPT[24] had m.p. 170.7–172.0°, $[\alpha]^{21}D$ +32° (*c* 0.50, chloroform), $[\alpha]^{21}D$ +43° (*c* 0.52, pyridine).

(51) Hydrogenation with 1.25 g. of Willstätter's platinum black[23,27] for two hours gave analogous results.

take had become very slow. The mixture was worked up as in the case of α-apoPP. No pure compound could be obtained from the "acid fraction." The lactone fraction was crystallized from 30 ml. of ethanol to yield 1.10 g. (73%) of **6,7-methylenedioxy-1-(3,4,5-trimethoxyphenyl)-3-hydroxymethyl-5,6,7,8-tetrahydro-2-naphthoic acid lactone (XVII)** as colorless prisms or needles, m.p. 177–178°. In some cases, the needles melted on rapid heating at 150° (foaming), resolidified and remelted at 177–178°, while the prisms did not melt below 177°. The needles seemed to represent a polymorphic modification with a tendency to retain solvent of crystallization; their infrared spectrum in solution could not be distinguished from that of the prisms. Further purification by melting the material in quartz under nitrogen, chromatography in chloroform on alumina and recrystallization from ethanol provided only prisms, m.p. 177.5–178.5°, $[\alpha]^{20}D$ 0° (*c* 1.0, chloroform).

Anal. Calcd. for $C_{22}H_{22}O_7$: C, 66.32; H, 5.57; OCH_3, 23.37. Found: C, 66.57, 66.46; H, 5.65, 5.84; OCH_3, 23.55.

The mother liquor from XVII was evaporated and crystallized from 30% ethanol to yield 26 mg. (1.7%) of **IDPP (XI)** as long colorless needles, m.p. 198–202°. Recrystallization from 30% ethanol gave material melting at 202.2–203.1°, identical[26] with the product obtained with Raney nickel.

(c) With Other Catalysts.—When β-apoPP in acetic acid was stirred under hydrogen in the presence of platinum oxide at room temperature, hydrogen uptake ceased with reduction of the catalyst. After 70 hours, only starting material could be isolated from the mixture. Similar failures were encountered when hydrogenation was attempted at 80°, at room temperature in the presence of perchloric acid for 11 hours, and with palladium-on-barium sulfate at 80°.

Hydrogenation of γ-Apopicropodophyllin.—A suspension of 134 mg. of synthetic γ-apoPP[12,33,34] (VII) and of 67 mg. of platinum oxide in 17 ml. of acetic acid was hydrogenated at room temperature and atmospheric pressure for two hours, at which time hydrogen uptake had almost ceased at one-half the theoretical level. Therefore 130 mg. of 10% palladium-on-charcoal was added and hydrogenation completed during one hour at 75°. The reaction mixture, worked up as in the case of α-apoPP, yielded an oily "acid fraction" (30 mg.), which could not be crystallized, and a colorless solid lactone fraction (93 mg.). The latter was recrystallized from 30% ethanol to give 26 mg. (19%) of **racemic isodesoxypicropodophyllin (IDPP) (XI)** as small colorless needles, m.p. 203.0–203.8°. Another recrystallization from absolute ethanol provided material melting at 203.3–204.3°. The substance gave a large mixed melting point depression with levorotatory IDPP, m.p. 202.3–203.2°, but both had completely identical infrared absorption spectra in chloroform.

Anal. Calcd. for $C_{22}H_{22}O_7$: C, 66.32; H, 5.57. Found: C, 66.51; H, 5.69.

When the mother liquor from the racemic IDPP was diluted with water and kept at room temperature for several days, 40 mg. (30%) of crystalline material, m.p. 169–172°, separated gradually. Several recrystallizations from 30% ethanol provided needles or prisms, m.p. 172–175°, which had the same infrared spectrum as the compound, m.p. 177.5–178.5°, obtained from β-apoPP and were therefore impure 5,6,7,8-tetrahydrolactone XVII.

Racemic IDPP, m.p. 196–200° (198.5–202° after recrystallization), was also obtained in 37% yield when γ-apoPP was hydrogenated with Raney nickel in ethanol at 70° and 1800 p.s.i. for 22 hours. No other compounds could be isolated from the reaction mixture.

Hydrogenation of Dehydroanhydropicropodophyllin.—DehydroanhydroPP (XVI) (308 mg.) was hydrogenated with 400 mg. of 10% palladium-on-charcoal in 16 ml. of acetic acid at 75° and atmospheric pressure for one hour, then worked up as usual. Crystallization of the lactone fraction from 5 ml. of ethanol gave 218 mg. of colorless needles, m.p. 176.5–177.5°. Another 31 mg., m.p. 174.5–176°, was isolated from the mother liquor, raising the yield to 80%. Recrystallization from ethanol provided needles, which melted at 150–153° (effervescence), resolidified and remelted at 176.7–178.1°. After drying at 137° *in vacuo* the material had m.p. 176.7–178.1 and was found to be identical[26] with the 5,6,7,8-tetrahydrolactone XVII obtained from β-apoPP.

Treatment of the Desoxylactones with Hydrogen and Palladium.—When VIII, IX, X or XI was stirred under hydrogen with palladium-on-charcoal in acetic acid at 75° for 1.5 hours and the mixtures worked up as usual, starting materials[26] were recovered in the following yields: DPT (m.p. 167–169°), 72%; DPP (m.p. 168–170°), 86%; IDPT (m.p. 249–250.5°), 72%; IDPP (m.p. 202–203°), 78%. No other compounds could be isolated from the lactone fractions, while the "acid fractions" were not worked up.

Tetramethyl 3′,4′,5′-Trimethoxybiphenyl-2,3,5,6-tetracarboxylate (XVIII, R = CH_3).—A solution of 786 mg. of the 5,6,7,8-tetrahydrolactone XVII, m.p. 177.5–178.5°, was prepared by saponification with 0.18 g. of sodium hydroxide in 10 ml. of 50% ethanol. After removal of ethanol by boiling, 58 ml. of 4% aqueous potassium permanganate was added with stirring at 100° in *ca.* 5-ml. portions, the last of which was not decolorized after 30 minutes. The clear yellow solution obtained by treatment with sulfur dioxide was extracted continuously with ether for 16 hours. The yellow ether extract was concentrated to about 20 ml., treated with 1 ml. of methanol and, at 5-hour intervals, with two 50-ml. portions of ethereal diazomethane solution (each distilled from 4.8 g. of nitrosomethylurea). The mixture was kept at room temperature for another 16 hours, filtered from polymeric gelatinous material and evaporated to dryness. Chromatography on alumina[47] using ethyl acetate, followed by crystallization from methanol yielded 168 mg. (18%) of yellowish prismatic needles, m.p. 155–165°. Further recrystallization from methanol, benzene–hexane and again methanol provided pale yellow needle-shaped prisms, m.p. 168–172.5°.

Anal. Calcd. for $C_{23}H_{24}O_{11}$: C, 57.98; H, 5.08; 7 OCH_3, 45.60. Found: C, 57.75; H, 5.00; OCH_3, 45.86.

Isodesoxypodophyllic Acid (XIV).—The solution obtained by boiling 350 mg. of IDPT (X) with 310 mg. of sodium hydroxide in 6 ml. of 50% ethanol for five minutes was diluted with 15 ml. of water, cooled in ice and, after addition of 15 ml. of chloroform, acidified with swirling by adding 4 ml. of 2 *N* hydrochloric acid. The solid (285 mg., 78%), which was collected after standing in the refrigerator for one hour and washed with chilled water and chloroform, melted with foaming at 202–205° (immersion at 200°), resolidified and remelted at 248–251°. Recrystallization from 35% ethanol gave colorless electrified prismatic needles, which melted at 251–252° when immersed at room temperature (no depression with IDPT), but melted with foaming at 213° when immersed at 200° and remelted at 250–252°. The acid had $[\alpha]^{20}D$ −110° (*c* 0.53, pyridine).

Anal. Calcd. for $C_{22}H_{24}O_8$: C, 63.45; H, 5.81. Found: C, 63.51; H, 5.81.

When 1.0 g. of IDPP (XI) was treated analogously, the crude acid (0.71 g., 68%) melted over a wide range. It was crystallized from ethanol, then from benzene–methanol to yield 0.22 g. (21%) of material which melted with foaming when immersed at 205° and remelted after resolidification at 244–250°. Another recrystallization from 35% ethanol gave colorless prismatic needles, m.p. 216° (foaming, immersion at 200°), remelting at 251–252°, $[\alpha]^{21}D$ −104° (*c* 0.50 pyridine). The resolidified material did not depress the m.p. of IDPT, and the infrared spectra of the acids prepared from IDPT and IDPP were identical. No pure compound could be isolated from the mother liquors.

Methyl Isodesoxypodophyllate (XV, R′ = CH_3).—A suspension of 155 mg. of isodesoxypodophyllic acid (XIV) in 8 ml. of acetone and 0.4 ml. of methanol, to which 3 ml. of ethereal diazomethane solution (distilled from 0.27 g. of nitrosomethylurea) had been added, was kept at room temperature for two days, then evaporated to dryness. Crystallization from dilute methanol yielded 153 mg. (96%) of colorless needles, m.p. 194–199°. Recrystallization from chloroform–hexane, then from methanol provided thin needles, m.p. 200.7–201.6°, $[\alpha]^{20}D$ −23° (*c* 0.62, chloroform).

Anal. Calcd. for $C_{23}H_{26}O_8$: C, 64.17; H, 6.09; 4 OCH_3, 28.84. Found: C, 64.09; H, 6.20; OCH_3, 28.89.

The same compound[26] was obtained when 160 mg. of IDPT (X) or 230 mg. of IDPP (XI) was refluxed with 700 mg. of anhydrous sodium acetate in 14 ml. of absolute methanol for 48 hours. The solutions were concentrated, diluted with hot water, and the colorless needles collected after cooling. The yields from IDPT and IDPP were 85

and 89% and the melting points 191–194° and 188–191°, respectively. Recrystallization as above gave in both instances thin needles, m.p. 200.4–201.0°.

Ethyl Isodesoxypodophyllate (XV, R′ = C_2H_5).—When IDPT (X) or IDPP (XI) was treated as in the preparation of the methyl ester, substituting ethanol for methanol, the crude products were amorphous solids. The yields were 78% and 88%, and the melting points 128–134° and 128–135°, respectively. Purification proved somewhat difficult because the compound tended to separate as a gel in the presence of impurities. It was dissolved in chloroform, adsorbed in alumina,[47] unchanged starting material eluted with chloroform, and the ester then eluted with chloroform–ethanol (9:1). Crystallization from 50% ethanol gave tiny colorless needles, m.p. 140–148°, and recrystallization from benzene–hexane (2:1) material melting at 148.8–149.6°, $[\alpha]^{21}D$ −22° (*c* 1.30, chloroform). The infrared spectra of the crude products were identical with that of the pure compound, except for the presence of a weak lactone band.

Anal. Calcd. for $C_{24}H_{28}O_8$: C, 64.85; H, 6.35; 4 alkoxyl calcd. as OCH_3, 27.93. Found: C, 64.72; H, 6.42; OCH_3, 28.10.

Attempted Methylation of Desoxypodophyllic Acid.—When 400 mg. of desoxypodophyllic acid[24] (XIII) was treated with diazomethane as in the preparation of methyl isodesoxypodophyllate, 360 mg. (94%) of crystalline material, m.p. 164–171°, was isolated. Recrystallization from chloroform–hexane, then from methanol gave DPP (IX)[26] as strongly electrified thin needles, m.p. 171–172°. In another experiment, 450 mg. of XIII in 10 ml. of methanol was treated at 0° with 40 ml. of ethereal diazomethane (distilled from 2 g. of nitrosomethylurea) and kept in ice with occasional shaking for two hours. The long colorless needles of DPP[26] (330 mg.) were collected and washed with ether; m.p. 172–173°. Another 53 mg. of DPP, m.p. 171–172°, was obtained from the mother liquor, bringing the yield to 89%.

Attempted Epimerization of the Isodesoxylactones.—When 150 mg. of IDPT (X) or IDPP (XI) was refluxed with 150 mg. of anhydrous sodium acetate in 3 ml. of acetic anhydride for one hour, and the solutions gradually diluted with water, starting materials[26] (m.p. 250–251 and 199–202°) were recovered in 94% and 97% yields, respectively.

Acknowledgments.—The authors are greatly indebted to Dr. D. H. R. Barton for reviewing part of the manuscript and for offering valuable suggestions. They want to thank Mrs. Priscilla B. Maury for determining some of the optical rotations, Mrs. Iris J. Siewers and Miss Alice M. Bernardi of the National Heart Institute for the infrared spectra, and Dr. W. C. Alford and coworkers for the microanalyses. They also wish to acknowledge stimulating discussions with several staff members of the National Institutes of Health.

BETHESDA 14, MARYLAND

[CONTRIBUTION FROM THE LABORATORY OF CHEMICAL PHARMACOLOGY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Components of Podophyllin. XIII.[1] The Structure of the Peltatins[2]

BY ANTHONY W. SCHRECKER AND JONATHAN L. HARTWELL

RECEIVED JUNE 29, 1953

The location of the substituents in ring A and the point of attachment of ring C of α- and β-peltatin (I) have been established by permanganate oxidation of their methyl ether (I, R = R′ = CH_3) to the keto acid II and to cotarnic acid (VI). Further degradation of II yielded myristicinic acid (IV). The positions at which the lactone ring is attached are postulated by analogy with desoxypodophyllotoxin.

In preceding publications of this series[3,4] the general features of the carbon skeleton of α- and β-peltatin (I) have been established. The positions at which the substituents in rings A and B are attached were, however, yet unknown. The present paper provides evidence required for the complete structure determination of the peltatins. While this evidence does not include proof for the positions of attachment of the lactone ring, they are made quite probable by analogy with podophyllotoxin and some of its derivatives.

Permanganate oxidation at 100° of α-peltatin-B dimethyl ether (β-peltatin-B methyl ether[4]) (I, R = R′ = CH_3) and also of β-peltatin-B ethyl ether (I, R = C_2H_5, R′ = CH_3) had previously provided 3,4,5-trimethoxybenzoic acid (V), while α-peltatin-B diethyl ether (I, R = R′ = C_2H_5) yielded similarly syringic acid ethyl ether.[3] This established the presence of a 4-hydroxy-3,5-dimethoxyphenyl and of a 3,4,5-trimethoxyphenyl residue in α- and β-peltatin, respectively. Using milder conditions in the permanganate oxidation of I (R = R′ = CH_3), two additional degradation products have now been isolated. One of them was a substituted benzoylbenzoic acid, $C_{19}H_{18}O_9$, m.p. 183.5–185°. Decarboxylation of this acid by boiling with copper powder in quinoline[5] gave the corresponding benzophenone, $C_{18}H_{18}O_7$, m.p. 164–165°, which was subjected to cleavage with potassium *t*-butoxide.[6] From the resulting mixture of acids, myristicinic acid (IV)[7] was isolated, in addition to 3,4,5-trimethoxybenzoic acid (V), thus establishing structure III for the benzophenone. In view of the previous isolation of V from I (R = R′ = CH_3),[3] there remain two possible keto acids which could lead to III by decarboxylation. However, the other new degradation product obtained was cotarnic acid (VI), which was characterized as the anhydride and N-methylimide, both identical[7] with authentic samples.[8,9] This demonstrates that the

(1) Paper XII, A. W. Schrecker and J. L. Hartwell, THIS JOURNAL, **75**, 5916 (1953).

(2) Presented in part before the Medicinal Chemistry Division of the American Chemical Society at Chicago, Ill., Sept. 7, 1953; *cf.* Abstracts of Papers, Am. Chem. Soc., **124**, 8N (1953).

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

(4) J. L. Hartwell, A. W. Schrecker and G. Y. Greenberg, *ibid.*, **74**, 6285 (1952).

(5) E. Späth, F. Wessely and E. Nadler, *Ber.*, **66**, 125 (1933).

(6) G. A. Swan, *J. Chem. Soc.*, 1408 (1948).

(7) Identity proven by mixed melting point determination and comparison of infrared spectra.

(8) (a) W. Roser, *Ann.*, **249**, 156 (1888); **254**, 334 (1889); (b) M. Freund and G. Wulff, *Ber.*, **35**, 1737 (1902); (c) E. Späth, L. Schmid and H. Sternberg, *ibid.*, **67**, 2095 (1934).

(9) Direct oxidation of cotarnine[8b] proved unsatisfactory. Oxidation of cotarnolactone[8a] yielded cotarnic acid, which was converted to the anhydride by vacuum sublimation[8c] and to the methylimide by heating with methylamine, as described in the Experimental section