7.8 mg. of dibenz-9,10-anthraquinone,[6] the recovery was 68%.

Acetylation of Phenols.—The radioactive fraction containing hydrolyzed acids and phenols (Fig. 1) was acetylated as shown in Fig. 3.

Chromatographic Separation.—In model experiments, 20 μg. each of dibenzanthracene, dibenz-9,10-anthraquinone (II), dibenz-3,4-anthraquinone (III) and 4′,8′-diacetoxydibenz-9,10-anthraquinone (Va)[7] were separated chromatographically by the following procedure. The column, 3 × 180 mm., consisted of 0.7 g. of Florisil[31] (60/100 mesh) in a thistle tube. Gravity flow was used and the rate of elution was 0.25 ml. per minute. The dibenzanthracene was located by fluorescence and the three quinones were followed visually. The elution pattern is given in Table I.

In order to "clean up" the various radioactive neutral fractions, the solutions were concentrated and washed through the Florisil column with 25 ml. of ethyl acetate. This eluate was evaporated, Skelly B added and evaporated several times to remove traces of ethyl acetate. The residue was dissolved in 0.1 ml. of benzene diluted to 1 ml. with Skelly B and placed on the column. The elution was conducted as shown in Table I. Each chromatographic fraction was plated and counted. The results are given in Table II.

Purification of Carriers.—The appropriate quinone carrier (0.8–2 mg.) was added to the radioactive fractions obtained from chromatography. Each carrier was crystallized twice from butyl acetate and sublimed at 0.05 mm. The temperatures required were 180° for dibenz-9,10-anthraquinone, 250° for dibenz-3,4-anthraquinone and 280° for 4′,8′-dihydroxydibenz-9,10-anthraquinone. The carriers were then chromatographed on Florisil as shown in Table I and counted. One or more of the above operations was repeated until constant specific activity was achieved. The radioactivity of the carriers is reported as c.p.m. per total weight of carrier. The data are shown in Table III.

MADISON, WISCONSIN

(31) Obtained from the Floridin Co., Warren, Pa.

[CONTRIBUTION FROM THE LABORATORY OF CHEMICAL PHARMACOLOGY, NATIONAL CANCER INSTITUTE[1]]

Components of Podophyllin. XI. Isolation of Two New Compounds from *Podophyllum emodi* Wall.[2]

BY MORESHWAR V. NADKARNI,[3] JONATHAN L. HARTWELL, PRISCILLA B. MAURY AND J. LEITER

RECEIVED SEPTEMBER 13, 1952

Fractionation of the resin of *Podophyllum emodi* Wall., employing chromatographic adsorption on activated alumina yielded, beside podophyllotoxin, the new compounds 4′-demethylpodophyllotoxin and 1-O-(β-D-glucopyranosyl)-picropodophyllin, hereafter called demethylpodophyllotoxin and picropodophyllin glucoside. No α-peltatin or β-peltatin was found. Demethylpodophyllotoxin was active in causing damage to Sarcoma 37 in mice.

The isolation of α- and β-peltatin,[4] both active in producing damage to tumors in mice, from the resin of *Podophyllum peltatum* L. (American podophyllum), prompted an inquiry into the composition of the resin of *P. emodi* (Indian podophyllum, *Podophyllum indicum*).[5] Although the resins from both species of *Podophyllum* are official in the British Pharmacopoeia[6] and are used for the same purpose, and while earlier studies[7] had shown that only two components (podophyllotoxin and quercetin) were present in both species, the finding of α- and β-peltatin in the American resin[4a] and certain differences in the color reactions of the two resins[6,8] warranted the reinvestigation of the resin of *P. emodi* by the employment of chromatographic adsorption on alumina.

A modified alcohol–benzene process[4a] was used for the chromatographic fractionation. Unlike the American resin, when a solution of the Indian resin in alcohol was treated with 9 volumes of benzene, a flocculent suspension of tarry material was produced which could not be cleanly separated; the tar was always found to retain a substantial amount of tumor-damaging activity against Sarcoma 37 in mice. Consequently, this preliminary treatment was omitted and a solution of the resin in equal volumes of alcohol and benzene was used directly. It was also found that the cuts had to be taken at different points in the chromatogram from those in the *P. peltatum* process. The following substances were isolated, in the order of their appearance through the chromatogram: (1) podophyllotoxin, 45–48% yield, identified by m.p., m.p. of anhydrous form and acetyl derivative (m.p. and mixed m.p.)[9]; (2) a phenolic substance m.p. 250.0–251.6° cor., 1.7% yield, crystallizing in colorless, transparent prisms from ethanol, or as plates from 50% ethanol; and (3) a glycoside, m.p. 237.0–238.2° cor., 1.8% yield, crystallizing

(1) National Institutes of Health, Public Health Service, Federal Security Agency.

(2) (a) First reported as a Communication to the Editor by M. V. Nadkarni, P. B. Maury and J. L. Hartwell, THIS JOURNAL, **74**, 280 (1952); (b) for paper X in this series, see J. L. Hartwell, A. W. Schrecker and G. Y. Greenberg, THIS JOURNAL, **74**, 6285 (1952).

(3) Post-doctorate Research Fellow of the National Cancer Institute.

(4) (a) J. L. Hartwell and W. E. Detty, THIS JOURNAL, **72**, 246 (1950). For papers on the tumor-damaging action, *cf.* (b) J. Leiter, V. Downing, J. L. Hartwell and M. J. Shear, *J. Nat. Cancer Inst.*, **10**, 1273 (1950), and (c) E. M. Greenspan, J. Leiter and M. J. Shear, *ibid.*, **10**, 1295 (1950).

(5) We wish to thank Dr. W. G. Bywater of S. B. Penick and Co. for the procurement of the roots of this plant from India and the extraction of the resin therefrom, by the procedure given in "National Formulary," Vol. IX (1950).

(6) "British Pharmacopoeia," 1948.

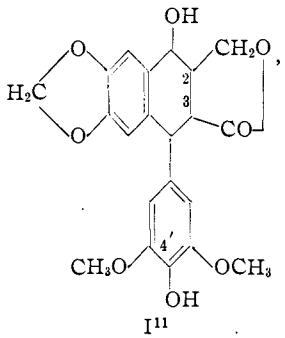
(7) (a) F. A. Thompson, *Am. J. Pharm.*, **62**, 245 (1890); (b) J. C. Umney, *Pharm. J.*, **23**, 207 (1892); (c) W. R. Dunstan and T. A. Henry, *J. Chem. Soc.*, **73**, 209 (1898); (d) W. Borsche and J. Niemann, *Ann.*, **494**, 126 (1932); (e) E. Späth, F. Wessely and L. Kornfeld, *Ber.*, **65**, 1536 (1932).

(8) "National Formulary," ninth ed., American Pharmaceutical Association, Washington, D. C., 1950.

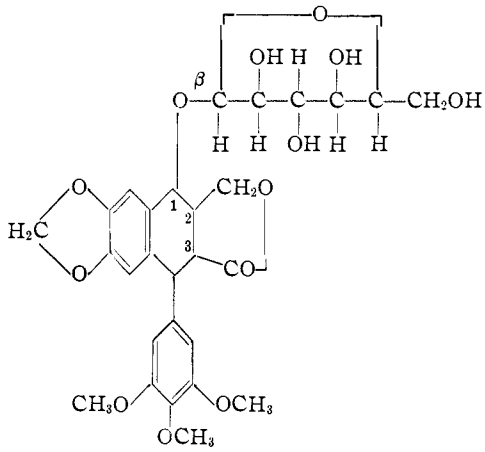
(9) From the mother liquor of the podophyllotoxin a small amount (0.4%) of picropodophyllin sometimes separated; it was identified by m.p. and acetyl derivative (m.p. and mixed m.p.). The small amount obtained (less than 1% of the podophyllotoxin) makes it uncertain whether this compound occurs as such in the resin or is an artifact produced during the chromatography, inasmuch as traces of weak alkali are known to epimerize podophyllotoxin to picropodophyllin. In an unpublished experiment, Mr. W. E. Detty was able to recover 0.49 g. from 0.50 g. (98%) of pure podophyllotoxin after passage through an alumina column using a mixture of 1:9 ethanol–benzene, indicating that less than 2%, if any, is converted into other products.

from 75% methanol or from water in long, thin, colorless needles, and non-reducing to Benedict solution.[10] No α- or β-peltatin could be isolated from any of the fractions.

The phenolic substance, m.p. 250.0–251.6° cor., was identified as 4′-demethylpodophyllotoxin. Methylation with diazomethane yielded podophyllotoxin; the position of the phenolic hydroxyl group was determined by permanganate oxidation of the ethyl ether (C_3-epimer or B-form) to syringic acid ethyl ether. The structure is thus given by I.



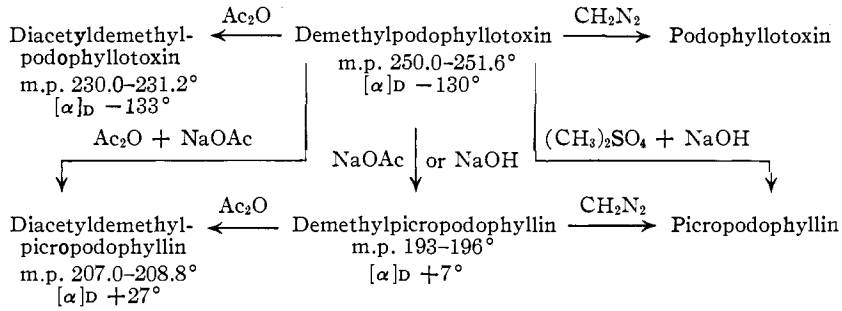
I[11]



II[11]

Its chemical behavior was found to be as expected, two series of isomeric derivatives being obtained depending upon the reaction conditions (*cf.* ref. 4a). These reactions are summarized in Chart I.[13]

CHART I



The substance, m.p. 237.0–238.2° cor., was identified as 1-O-(β-D-glucopyranosyl)-picropodophyllin (II) (or, less formally, picropodophyllin glucoside). Hydrolysis by acid or by emulsin yielded picropodophyllin[14] (m.p., optical rotation and acetyl derivative) and D-glucose (phenylosazone and benzimidazole derivative). Determination of reducing sugar produced during hydrolysis gave a value of 29% calculated as glucose (calcd. for 1 mole, 31%). Acetylation with acetic anhydride yielded a crystalline tetraacetate in good yield, m.p. 269–270.2°. Hydrolysis with emulsin and failure to hydrolyze with maltase showed the glucoside to have the β-configuration. This finding, together with the fact that the original glucoside was non-reducing to the usual copper reagents, indicates that the glucose is in the pyranose form.[15]

Compound I is active in producing hemorrhage and necrosis in Sarcoma 37 in mice, while II is inactive even in high doses.[16] Within the limits of the experimental technique, all the tumor-damaging activity of the resin was accounted for by the podophyllotoxin and demethylpodophyllotoxin obtained (see Table I).

TABLE I

PROPORTIONAL TUMOR-DAMAGING ACTIVITY OF THE RESIN OF *P. emodi* AND SOME OF ITS COMPONENTS

Substance	Yield, %	MED (approx.),[a] μg./g.	Proportion of total activity,[b] %
Original resin	100	6	100
Podophyllotoxin	47	3	94
Demethylpodophyllotoxin	1.7	8	1
Picropodophyllin	0.4	>500	0
Picropodophyllin glucoside	1.8	>1000	0

[a] Minimum effective dose for a single subcutaneous injection in mice bearing implants of Sarcoma 37; olive oil used as vehicle. [b] Yield X 6/MED.

It is of interest to note that the demethylpodophyllotoxin and podophyllotoxin found in *P. emodi* bear the same structural relationship to

(10) Although podophyllin has been reported (J. Guareschi, *Ber.*, **12**, 683 (1879); I. Guaresci, *Gazz. chim. ital.*, **10**, 16 (1880)) to yield glucose on treatment with emulsin or dilute sulfuric acid, this is the first report (*i.e.*, ref. 2a) of the isolation of a crystalline glycoside from the resin of either *P. peltatum* or *P. emodi*. Recently, R. Chatterjee and D. K. Datta, *Ind. J. Physiol. Allied Sci.*, **4**, 61 (1950), have reported the crystalline pigment 3-galactosidylquercetin from the resin of a related species, *P. sikkimensis*. A substance of high molecular weight believed to be a complex polysaccharide has been reported from podophyllin (source not given) by M. R. Loran and A. Towbin, *Federation Proc.*, **11**, No. 1, part 1, 370 (1952).

(11) No implication of steric configuration of the lactone ring is contained in these structures. Our conviction (*cf.* ref. 12) is that in I the configuration around C_2:C_3 is *trans*, while in II it is *cis*.

(12) J. L. Hartwell and A. W. Schrecker, THIS JOURNAL, **73**, 2909 (1951); paper XII, A. W. Schrecker and J. L. Hartwell, *ibid.*, in press.

(13) Rather than name the C_3-epimeric compounds as derivatives of demethylpodophyllotoxin-B, as was done analogously with the peltatins,[4b] they are named after demethylpicropodophyllin, since picropodophyllin is already established[12] as the C_3-epimer of podophyllotoxin.

(14) The possibility of the picropodophyllin glucoside being an artifact produced during the chromatography is believed to be remote. The formation of the 1.8% of glucoside obtained would require a large quantity of hypothetical podophyllotoxin glucoside as a precursor; none of the latter was isolated.

(15) *Cf.* E. Fischer, *Ber.*, **42**, 1980 (1914), and W. N. Haworth, *et al.*, *J. Chem. Soc.*, 2254 (1932).

(16) Details to be published elsewhere; the method of bioassay has been described in ref. 4b.

each other as the α- and β-peltatin found in *P. peltatum*.[17]

Experimental[18,19]

Chromatography.—The solution for chromatography was made by dissolving 60 g. of the resin of *P. emodi* in 150 cc. of absolute ethanol with warming, adding 150 cc. of benzene, and letting cool to room temperature. A glass chromatographic cylinder 7.5 cm. inside diameter and 33 cm. high was packed with alumina[20] to a depth of 20 cm. (about 835 g.). The column was wet with a 1:1 mixture of absolute ethanol and benzene. The solution of resin was added and the chromatogram developed with 1:1 ethanol–benzene. Fraction A was collected when the filtrate began to show a greenish tint. The fraction was cut when the eluate had become almost colorless and showed a minimum quantity of residual solids (700 cc.).[21] Fraction B was cut when the eluate had gone through a yellowish-green color and become almost colorless again (2.3 l.).

At this point the developing liquid was made more polar by the addition of 5% water (*i.e.*, 47.5:47.5:5 parts by volume). Fraction C was then cut (at 4 l.) when the filtrate showed a minimum quantity of residual solids. The developing liquid then was made more polar by increasing the water to 10% (*i.e.*, 45:45:10 parts by volume), and fraction D was taken at 8 l.

For the final developing liquid, a still more polar liquid, 50% ethanol, was used. Fraction E was cut at 4 l.

Isolation of Podophyllotoxin.—All fractions were evaporated to dryness on the steam-bath under a current of air. Fraction A gave an oil which solidified to a green cake (50% yield). Extraction with benzene at room temperature, by trituration in a mortar to a thin slurry, left a white solid, m.p. 180.8–181.8°, yield in several runs, 27.1–28.1 g. (45–47%). Concentration of the mother liquor yielded successive small crops of crystals, m.p. 110–116°, yield 0.24–0.67 g. (0.4–1.1%), total yield (unsolvated basis) 45–48%. Both products were identified as podophyllotoxin, the higher melting form unsolvated and the lower melting form solvated, by m.p.[12] and by acetylation with acetic anhydride to give acetylpodophyllotoxin m.p. 206–207°, no depression with authentic sample.[22,23]

Isolation of Demethylpodophyllotoxin.—After evaporation of fraction B to dryness (5.2%), the brownish solid residue was extracted with ethanol at room temperature. A nearly white product remained, m.p. 234–235° (shrinks 226°), yield 1.53 g. (2.5%). Repeated crystallization from ethanol gave colorless, transparent prisms (from 50% ethanol, plates), m.p. 250.0–251.6° (shr. 246°), yield 0.90 g. (1.5%); $[\alpha]^{20}D$ −130° (*c* 0.75, chloroform).

Anal. Calcd. for $C_{21}H_{20}O_8$: C, 63.0; H, 5.0; 2 OCH_3, 15.5. Found: C, 63.1; H, 5.1; OCH_3, 16.0.

Demethylpodophyllotoxin is fairly soluble in chloroform, acetone, hot ethanol and dilute caustic alkali; it is practically insoluble in water, hexane, cold methanol, cold ethanol, and sodium bicarbonate solution. It gives a deep green color with alcoholic ferric chloride, and an orange-red color with diazotized *p*-nitroaniline.

Isolation of Picropodophyllin Glucoside.—Fraction C was a transitional fraction, containing some demethylpodophyllotoxin carried over from fraction B and some glucoside most of which later appeared in fraction D. On evaporation to dryness, fraction C yielded a solid (5.0%) which was extracted with about 11 cc. of boiling absolute ethanol and filtered. The solution, on evaporating to small volume and standing in the refrigerator, slowly deposited colorless prisms of demethylpodophyllotoxin, m.p. 242.5–247° (shrinks 240°), yield 0.12 g. (0.2%); acetyl derivative, m.p. 230.0–231.2°, no depression with an authentic sample of diacetyldemethylpodophyllotoxin (see below). The insoluble portion was recrystallized from 75% methanol, yielding colorless needles of the glucoside, m.p. 237–238° (darkens), no depression with a sample of glucoside isolated from fraction D (see below); yield 0.30 g. (0.5%). Furthermore this product, on hydrolysis, gave 29% reducing sugar (calcd. as glucose) and picropodophyllin, identified by m.p. and mixed m.p. of its acetyl derivative.

Fraction D contained the bulk of the glucoside. It gave a dark brown residue on evaporation (10.5%). Extraction with cold methanol left a colorless solid which was crystallized from 75% methanol; yield 0.78 g. (1.3%). Recrystallization from 75% methanol or from water afforded long, thin, colorless needles of a pure product, m.p. 237.0–238.2° (darkens), $[\alpha]^{20}D$ −11.5° (*c* 0.5, pyridine).

Anal. Calcd. for $C_{28}H_{32}O_{13}\cdot 0.5H_2O$: C, 57.4; H, 5.7; 3 OCH_3, 15.9. Found: C, 57.4; H, 5.7; OCH_3, 15.2.

The glucoside is slightly soluble in boiling methanol, boiling water and pyridine; it is practically insoluble in cold methanol, water, acetone, chloroform, ethyl acetate and cyclohexane.

Fraction E left a dark brown tar (4.5%) from which no crystalline substance could be isolated and which was inactive against Sarcoma 37.

The results on the separation and isolation of the components are summarized in Table II.

TABLE II

COMPOSITION OF RESIN OF *P. emodi*

Fraction	Dry solids, %	Crystalline components	%
A	50	Podophyllotoxin	50
B	5.2	Demethylpodophyllotoxin	1.5
C	5.0	Demethylpodophyllotoxin	0.2
		Picropodophyllinglucoside	0.5
D	10.5	Picropodophyllinglucoside	1.3
E	4.5	.....	
Retained on tower	24.8 (by diff.)		
Total	100.0		53.5

Reactions and Structure of Demethylpodophyllotoxin (I). Methylation. (a) With Diazomethane.—To a solution of 1.0 g. of I in 25 cc. of methanol and 5 cc. of acetone was added a solution of freshly distilled diazomethane in ether[24]; an excess of diazomethane (yellow color) was maintained for 2.5 hours. Evaporation of the solvent to small volume gave a crop of colorless needles, m.p. 224–226°, no depression with an authentic sample of picropodophyllin; yield 0.095 g. (9%). On evaporation of the mother liquor to dryness and taking up the residue in hot benzene, white needles of podophyllotoxin were obtained, m.p. 115–116° (foaming), yield 0.95 g. (83%, on a dry basis). Drying in an Abderhalden pistol first at 78°, then at 110°, gave the unsolvated form, m.p. 180.1–181.5°, no depression on admixture with an authentic sample.[12] Acetylation with

(17) J. L. Hartwell, A. W. Schrecker and G. Y. Greenberg, THIS JOURNAL, **74**, 6285 (1952).

(18) M.ps. are corrected; determinations were carried out with the Hershberg apparatus.

(19) Analyses were performed by the Microanalytical Laboratory of the National Institutes of Health under the direction of Dr. W. C. Alford.

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

(21) These and subsequent color changes were best observed by frequently intercepting a few cc. of the eluate in a small separatory funnel with a filter paper as a background. Visual estimates of the solids content of the eluate were made regularly by evaporating a few drops on a glass slide.

(22) When hexane is used to extract the green cake, a similar yield of a light green product with inferior m.p. is obtained. The hexane mother liquors, after concentration, usually on standing, deposited needles of picropodophyllin in yields of 0.24–0.30 g. (0.4–0.5%). Recrystallization from ethanol gave needles, m.p. 226–227° (lit. 228°,[7e] 231.5–232.5°[12]); acetyl derivative, m.p. 215.0–215.2° (lit. 215–216°,[7b] 220–221°[12]), no depression on admixture with an authentic specimen.

(23) The higher yield of podophyllotoxin reported here over that of the previous communication (ref. 2a) is a result of improvement in the extraction of the green cake. A similarly high yield of podophyllotoxin from *P. emodi*, 56.6% of impure substance, has been reported in the literature.[7e] It may be noted that the yield reported by us is about five times that of podophyllotoxin in podophyllin derived from *P. peltatum*.[4a]

(24) An ethereal solution of diazomethane was prepared from nitrosomethylurea[25] and the solution distilled. It was found that if the undistilled solution was used, only picropodophyllin could be isolated in the reaction; apparently I is very sensitive to traces of base.

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

acetic anhydride yielded the acetate, m.p. 208.5–209.5°, no depression on admixture with an authentic sample.[12]

(b) **With Dimethyl Sulfate.**—A 1.0-g. portion was methylated in a similar fashion to α-peltatin[4a]; yield of crude 0.98 g. (95%). Recrystallization from ethanol gave needles of picropodophyllin, m.p. 224–225°. The acetyl derivative, prepared by means of acetic anhydride, had m.p. 214.5–215.2°, no depression with an authentic specimen.

Diacetyldemethylpodophyllotoxin.—Acetylation with acetic anhydride in the usual manner gave, after crystallization from ethanol, colorless needles, m.p. 230.0–231.2°, yield of pure product 71%, $[\alpha]^{20}D$ −133° (*c* 0.5, chloroform).

Anal. Calcd. for $C_{25}H_{24}O_{10}$: C, 62.0; H, 5.0; 2 OCH_3, 12.8; 2$COCH_3$, 17.8. Found: C, 62.2; H, 4.9; OCH_3, 13.2; $COCH_3$, 18.2.

Demethylpicropodophyllin. (a) **With Sodium Acetate.**—A solution of 0.5 g. of demethylpodophyllotoxin and 0.25 g. of anhydrous sodium acetate in 25 cc. of absolute ethanol was refluxed for 17 hours. After cooling, the aggregates of colorless needles were filtered off and dried; yield 0.45 g. (90%). Attempts to recrystallize the product from anhydrous solvents resulted in gelatinous precipitates. Recrystallization from 50% acetone or 50% ethanol gave fine colorless needles, m.p. 225–236° (softens 222°); dried in an Abderhalden pistol at 78°, m.p. 217–219°, $[\alpha]^{20}D$ +7.0° (*c* 0.75, acetone).

Anal. (dried at 78°). Calcd. for $C_{21}H_{20}O_8 \cdot 0.5H_2O$: C, 60.3; H, 5.3; 2 OCH_3, 14.8. Found: C, 60.5; H, 5.6; OCH_3, 15.3.

Dried at 140° in vacuum, the compound had m.p. 193–196°, $[\alpha]^{20}D$ +7.0° (*c* 0.75, acetone).

Anal. (dried at 140°). Calcd. for $C_{21}H_{20}O_8$: C, 63.0; H, 5.0; 2 OCH_3. 15.5. Found: C, 62.9; H, 5.4; OCH_3, 15.4.

The compound is soluble in acetone and in dilute caustic alkalies, difficultly soluble in chloroform and ethanol, and practically insoluble in water and sodium bicarbonate solution. It gives a green color with alcoholic ferric chloride, and a red color with diazotized *p*-nitroaniline.

(b) **With Sodium Hydroxide.**—A solution of 0.50 g. of demethylpodophyllotoxin in 5 cc. of 6% sodium hydroxide was warmed on the steam-bath for about 5 minutes. The clear yellow solution was cooled to room temperature, and acidified with 2 *N* hydrochloric acid. The gel, first formed, turned into a curdy precipitate after some time. Filtration, washing, and drying, afforded a product (98% yield) which, after recrystallization from 50% acetone, gave silky needles, m.p. 229–232° (shrinks 221°), yield 0.37 g. (74%).

Diacetyldemethylpicropodophyllin. (a) **Acetylation with Acetic Anhydride.**—Demethylpicropodophyllin was acetylated with acetic anhydride in the usual manner. Recrystallization from ethanol gave colorless needles, m.p. 206.8–208.0°[26]; yield of once-crystallized product 64%, $[\alpha]^{20}D$ +27.5° (*c* 0.84, chloroform).

Anal. Calcd. for $C_{25}H_{24}O_{10}$: C, 62.0; H, 5.0; 2 OCH_3, 12.8; 2 $COCH_3$, 17.8. Found: C, 61.8; H, 5.2; OCH_3, 13.0; $COCH_3$, 17.4.

(b) **Acetylation with Acetic Anhydride and Sodium Acetate.**—Demethylpodophyllotoxin (2.0 g.) was refluxed for 1 hour with acetic anhydride (40 cc.) and anhydrous sodium acetate (1.0 g.). Working up in the usual manner gave colorless needles, m.p. 207.0–208.8°, no depression with the product from (a) above; yield of once-crystallized material 1.65 g. (69%).

Methylation of Demethylpicropodophyllin. (a) **With Diazomethane.**—Methylation with diazomethane, similar to the procedure already described, gave colorless needles of picropodophyllin, m.p. 221–224.5°; yield, 72%. Acetylation of the product with acetic anhydride in the usual manner yielded the acetyl derivative, m.p. 214–215°, no depression with an authentic sample.

(b) **With Dimethyl Sulfate.**—Methylation with dimethyl sulfate in the usual manner gave crystals of picropodophyllin, m.p. 223.8–225.2°, yield 95%. The acetyl derivative had m.p. 214.5–215.2°; no depression with an authentic specimen.

(26) An earlier preparation had m.p. 193.0–194.5° and gave good analytical values: C, 62.2; H, 5.4; OCH_3, 13.5; and $COCH_3$, 19.0. The $[\alpha]^{20}D$ was +24.4° (*c* 0.45, chloroform). Possibly, this substance and the later one of m.p. 207–208° are polymorphs.

4′-Ethyldemethylpicropodophyllin.—Demethylpicropodophyllin (2.6 g.) was ethylated with diethyl sulfate in a manner similar to α-peltatin[4a]; crude yield 2.68 g. (96% yield). Recrystallization from ethanol gave 0.91 g. of colorless needles, m.p. 203.2–206.0°, $[\alpha]^{20}D$ −1.7° (*c* 0.84, chloroform).

Anal. Calcd. for $C_{23}H_{24}O_8$: C, 64.5; H, 5.6. Found: C, 64.2; H, 5.8.

The compound is soluble in chloroform and acetone, insoluble in water and cold dilute alkalies, and gives no color with alcoholic ferric chloride.

Oxidation of Demethylpicropodophyllin Ethyl Ether.—A permanganate oxidation was carried out essentially as with the peltatin ethers.[4a] One gram of ethyl ether consumed 172 cc. of 4% permanganate solution, and gave 120 mg. of crude, and 55 mg. of purified, crystalline syringic acid ethyl ether, m.p. 120.2–121.5°, mixed m.p. with an authentic specimen (m.p. 121.2–122.8°) gave no depression.

Reactions and Structure of Picropodophyllin Glucoside (II). Test for Reducing Power.—The usual test with Benedict solution was run. No trace of reducing action was observed.

Optimum Conditions of Acid Hydrolysis.—Samples of the glucoside were heated at 100° with 100 parts of dilute hydrochloric acid, varying the strength of the acid between 0.25 and 2 *N*, and varying the time between 0.5 and 4 hours. The resulting reducing sugar was determined by the method of Folin–Wu.[27] The maximum amount of reducing sugar obtained was 29% (calcd. as glucose), when hydrolysis was carried out with 0.5 *N* acid for 2 hours.

Action of Emulsin.—A suspension of 20 mg. of almond emulsin[28] ("Rohferment" of Helferich) and 100 mg. of glucoside in 10 cc. of water was incubated at a *p*H of 5.0 at 37° for 24 hours. The filtrate gave a positive test for reducing sugar, while control experiments with methyl α-D-glucoside[28] and with no glucoside added gave negative tests.

Action of Maltase.—A mixture of the glucoside (50 mg.), 2 cc. of water and 1 cc. of maltase preparation[29] was incubated at a *p*H of 7.0 at 34–37° for 2.5 hours. The test for reducing sugar was negative. The control experiment with methyl α-D-glucoside was positive and with no glucoside added was negative.

Identification of Aglycon. (a) **From Acid Hydrolysis.**—The glucoside (100 mg.) was hydrolyzed under the optimum conditions, the hydrolyzate cooled, the mineral acidity cut back with sodium acetate, and the precipitate collected; yield 60 mg. (85%). Crystallization from ethanol gave white needles of picropodophyllin, m.p. 224.5–226.5°; acetyl derivative m.p. 216°, no depression with admixture with an authentic sample.

(b) **From Emulsin.**—A mixture of 2.0 of glucoside and 1.0 g. of emulsin in 500 cc. of acetate buffer at a *p*H of 5.0 was stirred at 37° for 20 hours. The collected solids, dried at 60°, weighed 1.98 g. The mother liquor contained 241 mg. of reducing sugar[27] (corresponding to 39% hydrolysis). Extraction of the solid with chloroform, evaporation of chloroform solution to dryness, and crystallization of the white solid residue from absolute ethanol gave white silky needles of picropodophyllin; yield 167 mg. (12%), m.p. (after recrystallization from ethanol) 215–218°, $[\alpha]^{20}D$ +3.2° (*c* 0.77, $CHCl_3$).

Identification of Sugar. (a) **Phenylosazone.**—The filtrate, from the acid hydrolysis experiment above, was diluted with an equal volume of water, treated with a mixture of 0.75 g. each of phenylhydrazine hydrochloride and anhydrous sodium acetate, and the mixture heated on the steam-bath for 45 minutes. After 20 minutes, yellow crystals separated. These had the typical microscopic appearance of D-glucose phenylosazone.[30] Recrystallization from dilute ethanol gave a product, m.p. 208°, no depression with

(27) O. Folin and H. Wu, *J. Biol. Chem.*, **41**, 367 (1920).

(28) Obtained through the kindness of Dr. Nelson K. Richtmyer, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health.

(29) A maltase solution was prepared from fresh brewer's yeast by the method of Weidenhagen, *cf.* J. B. Sumner and K. Myrbäck, "The Enzymes," Vol. I, Part 1, Academic Press, Inc., New York, N. Y., 1950, p. 564.

(30) W. Z. Hassid and R. M. McCready, *Ind. Eng. Chem., Anal. Ed.*, **14**, 683 (1942).

an authentic sample of D-glucose phenylosazone (lit.[31] 204–205° uncor.).

(b) **Benzimidazole Derivative.**—An amount of hydrolyzate (filtered from the aglycon and neutralized with bicarbonate) estimated to contain 1.0 g. of hexose was evaporated to 50 cc. and oxidized with bromine, in portions[32]; one week was required for completing the reaction (negative test for reducing sugars, after removal of the bromine). The solution was concentrated to 5 cc. in vacuum and treated with 0.66 g. of *o*-phenylenediamine in acid medium.[33] After cuprammonium salt treatment and removal of the copper, a product was obtained which, after crystallization from water, gave off-white needles, m.p. 216–217.5° (lit.[33] 215° uncor.), yield 50 mg. A mixed m.p. with an authentic specimen of D-glucobenzimidazole prepared similarly from methyl α-D-glucoside showed no depression.

1-O-(β-D-Glucopyranosyl)-picropodophyllin Tetraacetate.—The glucoside (1.0 g.) was refluxed with 20 cc. of acetic anhydride and 0.5 g. of anhydrous sodium acetate for 2 hours. After cooling, and decomposing the excess acetic anhydride with water, an oil which slowly turned crystalline, was obtained; yield 1.16 g. (90%). Crystallization from 1:2 chloroform–methanol gave fine needles, m.p. 269–270.2°, yield 1.03 g. (79%), $[\alpha]^{20}D$ −5.2° (*c* 0.5, chloroform).

Anal. Calcd. for $C_{36}H_{40}O_{17}$: C, 58.1; H, 5.4; 3 OCH_3, 12.5; 4 $COCH_3$, 23.1. Found: C, 58.0; H, 5.4; OCH_3, 12.3; $COCH_3$, 23.2.

BETHESDA, MD.

(31) E. Fischer, *Ber.*, **17**, 579 (1884).

(32) C. S. Hudson and H. S. Isbell, *J. Research Nat. Bur. Standards*, **3**, 58 (1929).

(33) S. Moore and K. P. Link, *J. Biol. Chem.*, **133**, 302 (1940).

[CONTRIBUTION FROM THE CENTRAL RESEARCH DEPARTMENT, MONSANTO CHEMICAL COMPANY]

Bicyclic Dihydropyrans by the Diels–Alder Reaction

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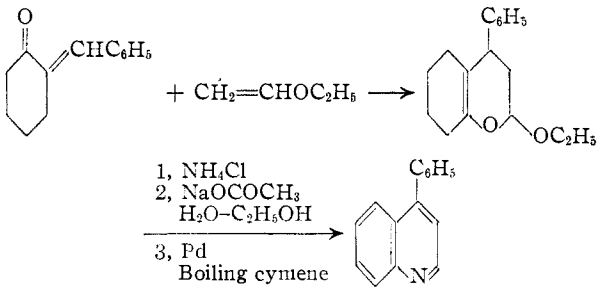
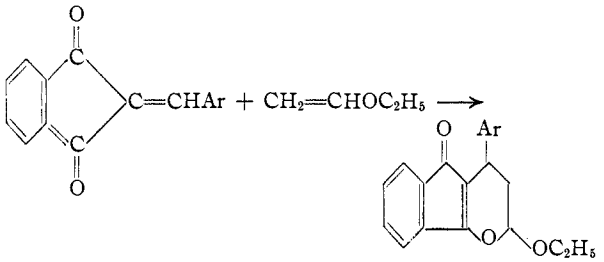
The synthesis of dihydropyrans by the Diels–Alder addition of vinyl ethers to α,β-unsaturated carbonyl compounds has been extended to the preparation of bi- and tricyclic dihydropyrans.

The successful preparation of dihydropyrans by using α,β-unsaturated carbonyl compounds as the diene component in the Diels–Alder reaction[1] has been extended to the synthesis of bicyclic dihydropyrans. When 2-benzylidenecyclopentanone, 2-benzylidenecyclohexanone, 2-piperonylidenecyclohexanone and 2-veratrylidenecyclohexanone were treated with ethyl vinyl ether, the corresponding 2-ethoxy-4-aryl-5,6-tri- or tetramethylene-3,4-dihydro-2H-pyrans were obtained in 22–52% yields.



These structures have been postulated by analogy with the similar monocyclic compounds and from the fact that treatment of 2-ethoxy-3,4,5,6,7,8-hexahydro-4-phenyl-2H-benzopyran with ammonium chloride and then with sodium acetate in aqueous ethanol followed by dehydrogenation of the product with palladium in boiling *p*-cymene yielded tar from which a picrate was isolated which melted at the same point as that recorded for 4-phenylquinoline.

With 2-piperonylidene-1,3-indandione and 2-veratrylidene-1,3-indandione, ethyl vinyl ether yielded compounds believed to be the corresponding 1-aryl-3-ethoxy-1,2,3,4-tetrahydro-4-oxa-9-fluorenones in 43–49% yields.



Under basic conditions the 1-(3,4-dimethoxyphenyl)-3-ethoxy-1,2,3,4-tetrahydro-4-oxa-9-fluorenone yielded a monoxime. Since these conditions would not favor the hydrolysis of the dihydropyran ring, the formation of a monoxime indicates the utilization of one carbonyl group in the Diels–Alder type reaction.

Experimental

Arylidene Ketones.—The method used was similar to that of Vorländer and Kunze.[2] The preparation of 2-benzylidenecyclohexanone is presented as an example of the procedure.

A mixture of 4.16 l. of water, 18.0 g. of sodium hydroxide, 88.0 g. (0.90 mole) of cyclohexanone and 32.0 g. (0.31 mole) of benzaldehyde was stirred at room temperature for 12 hours. Acetic acid (25 cc.) was added to neutralize the sodium hydroxide, and the mixture was extracted three times with benzene. The benzene solution was washed with water, filtered, and distilled to give 22.0 g. (39% yield) of 2-benzylidenecyclohexanone, b.p. 165–171° at 9 mm. Crystallization from aqueous ethanol gave a product melting at 51–53°.

In the preparation of 2-piperonylidene-1,3-indandione and 2-veratrylidene-1,3-indandione, neutralization of the reaction mixtures with acetic acid resulted in the separation of solid products. These products were removed by filtration, recrystallized from xylene and washed with hexane.

(1) (a) R. I. Longley, Jr., and W. S. Emerson, THIS JOURNAL, **72**, 3079 (1950); (b) W. E. Parham and H. E. Holmquist, *ibid.*, **73**, 913 (1951); (c) C. W. Smith, D. G. Norton and S. A. Ballard, *ibid.*, **73**, 5267 (1951).

(2) D. Vorländer and K. Kunze, *Ber.*, **59**, 2078 (1926).