Synthesis and Configurational Significance of 3-(O-Methyl)neoepipicropodophyllin[1,2]

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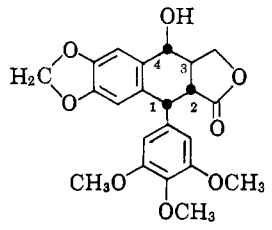
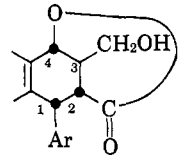
The methyl ether of neoepipicropodophyllin has been prepared in two ways. Dimethyl sulfate with alkali methylates picropodophyllic acid as well as epipicropodophyllic acid selectively on the primary alcohol group. The resulting methylated compounds lactonize on warming to give the same lactone, 3-(O-methyl)neoepipicropodophyllin. Proof that the ring closures involve the 4-position was obtained by hydrogenating the lactone to 3-(O-methyl)-4-desoxypicropodophyllic acid, with the hydroxyl group missing but with methoxyl retained. Mild saponification of the lactone furnishes 3-(O-methyl)epipicropodophyllic acid. Since the lactone can exist only in a rigid well-defined form, the configuration of the derived 3-(O-methyl)epipicropodophyllic acid is unambiguously fixed. This information allows the configuration of hydroxyl in podophyllotoxin and picropodophyllin to be determined unequivocally. The new results support the original assignment.

An early proposal[3] for the structure of podophyllotoxin (I) had the lactone ring 2,4 as in II instead of 2,3 as in I. This proposal was incorrect,[4a] and compound II, which may be called neopodophyllotoxin, has in fact never been obtained.[4b] The present paper describes



podophyllotoxin
I



neopodophyllotoxin
II

the methyl ether VII of a stereoisomer of neopodophyllotoxin and shows how this compound provides evidence confirming the original configurational assignment[5,6] for the secondary hydroxyl group of podophyllotoxin (I).

Picropodophyllin (IV), which is epimeric with podophyllotoxin (III = I) at the 2-position, saponifies readily to picropodophyllic acid (V).[7] The saponification does not affect the stereochemistry at any of the four centers of asymmetry, since picropodophyllic acid (V) can be shown to be stable to alkali and is known to relactonize readily to picropodophyllin (IV). No sign of lactonization involving the secondary instead of the primary hydroxyl group of acid V (*i.e.*, 2,4-lactonization) has been observed. To realize this mode of lactonization, we tried to block reaction at the preferred primary hydroxyl group by selective methylation. Treatment of picropodophyllic acid (V) with dimethyl sulfate and alkali gave product VI, which contained one hydroxyl group and one new methoxyl group and which lost water on heating to give lactone VII.

Proof that methylation had occurred as expected on the primary and not on the secondary hydroxyl group was obtained by hydrogenolysis of the monomethyl compound VI. Evidence for lactonization to VII during the course of the hydrogenolysis was obtained, but the relative importance of hydrogenolysis *via* VI or *via* VII was not determined. Under the conditions used, the only bond susceptible to reductive cleavage was the benzylic carbon-to-oxygen bond at the 4-position. The hydrogenolysis product VIII, which proved to be the same as 3-(O-methyl)desoxypicropodophyllic acid (VIII)[8] prepared from desoxypodophyllotoxin, retained the new methoxyl group. Accordingly, acid VI, with hydroxyl and not methoxyl at the 4-position, is 3-(O-methyl)picropodophyllic acid, and the derived lactone VII must be a 2,4-lactone.

Mild alkaline hydrolysis of lactone VII did not regenerate acid VI, but instead gave acid XII isomeric with VI. The new acid XII, on warming, was smoothly recyclized to lactone VII; in fact, ring closure occurred more readily with XII than with VI. No configurational changes could have occurred in the hydrolysis of lactone VII to acid XII because, first, no change can occur before ring opening, since there is no reason to expect action with alkali at the 1- or 3-positions and no possibility of change in the bridged cyclic system at the 2- and 4-positions (*cf.* XIII). Note, in this connection, that by Bredt's rule enolization at the 2-position of the lactone is precluded. Second, experiment shows that after ring opening product XII is not changed by alkali. Third, the possibility that alkyl-to-oxygen instead of the more familiar carbonyl-to-oxygen bond cleavage occurs in the lactone hydrolysis[9] was considered but dismissed. In a methanol-rich solvent, alkyl-to-oxygen cleavage, whether by monomolecular ionization or by bimolecular displacement, could be expected to produce appreciable amounts of open product carrying methoxy at the 4-position. The fact that no such product could be detected rules out the alkyl-to-oxygen bond breaking. Accordingly, the stereochemistry of lactone VII and acid XII must be the same. Both are epipicropodophyllin derivatives, in which the orienta-

(1) This is paper XV in the series entitled "Compounds Related to Podophyllotoxin." The preceding paper is by W. J. Gensler and F. Johnson, *J. Am. Chem. Soc.*, **85**, 3670 (1963).

(2) The work was supported by a grant from National Cancer Institute, U. S. Public Health Service (CA-02891).

(3) W. Borsche and J. Niemann, *Ann.*, **499**, 59 (1932); *Ber.*, **65**, 1633 (1932); E. F. Späth, F. Wessely, and E. Nadler, *ibid.*, **65**, 1773 (1932).

(4) (a) J. L. Hartwell and A. W. Schrecker, *J. Am. Chem. Soc.*, **73**, 2909 (1951). (b) This is no longer true; *cf.* M. Kuhn and A. von Wartburg, *Experientia*, **19**, 391 (1963). The neo designation used by Kuhn and von Wartburg for the 2,4-lactones has been adopted here.

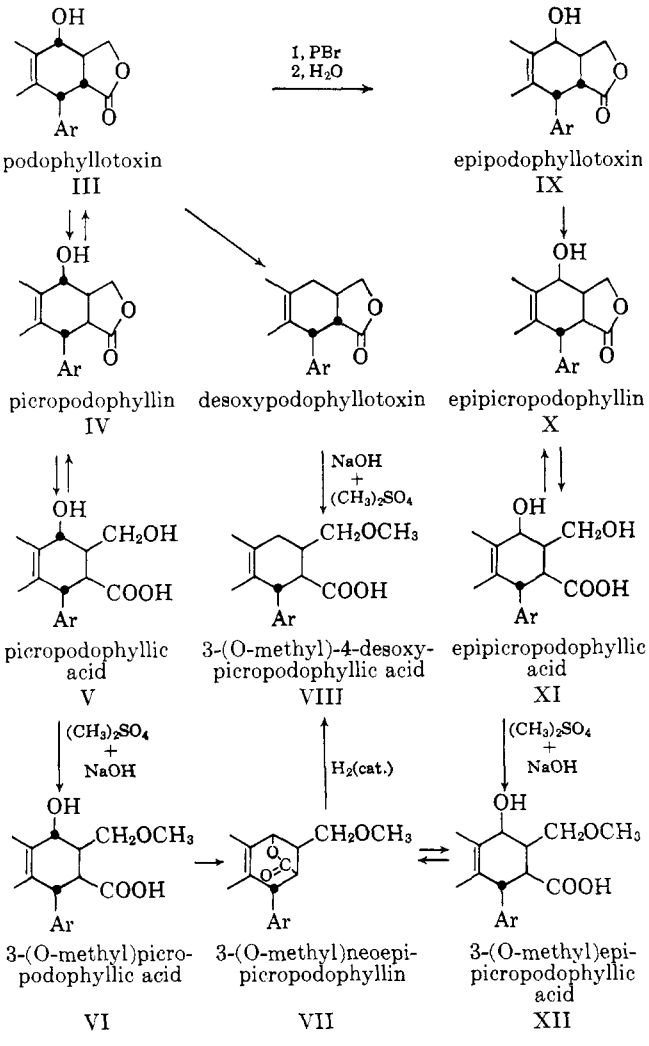
(5) N. L. Drake and E. H. Price, *J. Am. Chem. Soc.*, **73**, 201 (1951).

(6) A. W. Schrecker and J. L. Hartwell, *ibid.*, **75**, 5916 (1953).

(7) A comprehensive review of the chemistry of these compounds has been presented by J. L. Hartwell and A. W. Schrecker, *Progr. Chem. Org. Nat. Prod.*, **15**, 83 (1958).

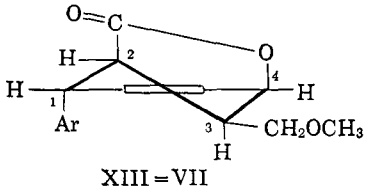
(8) A. W. Schrecker and M. M. Trail [*J. Org. Chem.*, **23**, 767 (1958)] reported m.p. 180–184° (foaming) for 3-(O-methyl)desoxypicropodophyllic acid (VIII), but this value must be low. L. Marion [*Can. J. Research*, **20B**, 157 (1942)] described the same compound with m.p. 194–195°; K. Noguchi and M. Kawanami, [*J. Pharm. Soc. Japan*, **60**, 629 (1940); *Chem. Abstr.*, **47**, 6386 (1953)] found 205°.

(9) E. S. Gould, "Mechanism and Structure in Organic Chemistry," Henry Holt and Co., New York, N. Y., 1959, p. 342; A. G. Davies, *Quart. Rev.* (London), **9**, 203 (1955); *cf.* J. Kenyon and P. R. Sharan, *J. Chem. Soc.*, 4084 (1963).



tion at the 4-position is opposite that in the picro series. Clearly, inversion occurs at the 4-position when 3-(O-methyl)picropodophyllic acid (VI) lactonizes to VII. Support for these assignments was obtained when epipicropodophyllic acid (XI),[4a] derived from podophyllotoxin (III) by way of epipodophyllotoxin (IX) and epipicropodophyllin (X), gave the same monomethylated acid XII on treatment with dimethyl sulfate and alkali.

The relative orientation of the groups at the 1-, 2-, and 3-positions of picropodophyllin (IV) and epipicropodophyllin (X) is well established as *trans*-1,2-*cis*-2,3[6] and, consequently, neolactone VII is constrained to the rigid ring system as in XIII. Here the groups at positions 2 and 4 must both be axial, or in other words, the arrangement must be *cis*-2,4. Since the epi acid XII is the one that is configurationally related to the neolactone, this acid XII also has *cis*-2,4 groups. The stereo chemistry is conveniently traced back by means of the 1 and 4 groups, which are seen to be *trans*-1,4 in 3-(O-methyl)epipicropodophyllic acid (XII), epipicropodophyllic acid (XI), and epipodophyllotoxin (IX), but *cis*-1,4 in podophyllotoxin (III). Corresponding results are obtained through the picro compounds. Since 3-(O-methyl)picropodophyllic acid (VI) lactonizes to VII with inversion at the 4-position, derivative VI must have *trans*-2,4 and, therefore, the *cis*-1,4 arrangement. The *cis*-1,4 relation may now be traced back through picropodophyllic acid (V), picropodophyllin (IV), and finally podophyllotoxin (III). The original stereochemical assignment for the 4-hydroxyl group, based on comparisons of the ease of elimination of 4-acyloxy derivatives,[5,6] is thus substantiated.[10]



Experimental

General.—Melting points were determined in capillary tubes and were not corrected. Infrared absorption curves were taken with Perkin-Elmer spectrophotometers; ultraviolet absorption curves were obtained with a Bausch and Lomb instrument. Elementary analysis were furnished by Scandinavian Microanalytical Laboratory, Herlev, Denmark.

Thin layer chromatograms were run on plates coated with 0.3-mm. layers of silica gel containing 5% calcium sulfate (Camag, Merck). The two solvent mixtures used in this work are: system A, methylene chloride–acetone, 4:1; and system B, methylene chloride–ethyl acetate–acetic acid, 5:2:1. Spots were developed by spraying the plate with a 1:1 mixture of ethanol–concentrated sulfuric acid and warming the treated plate for about 10 min. at 120°. The observed R_f values were found to vary somewhat from plate to plate and with the age of the solvent mixtures, but the separation of spots was always reproducible and reliable.

3-(O-Methyl)picropodophyllic Acid (VI) from Podophyllotoxin (III).—Heating a mixture of podophyllotoxin (2.5 g., 6.0 m-moles), potassium hydroxide (180 g. of 85% material, 2.7 moles), and water (500 ml.) on the steam bath for 25 min. gave a clear solution. An atmosphere of nitrogen was maintained over this solution throughout the reaction. To the stirred mixture at 0° freshly distilled dimethyl sulfate (115 ml., 1.20 moles) was added by drops over a period of 4 hr. After standing at room temperature for a day, the mixture was brought to 0°, and more dimethyl sulfate (80 ml., 0.84 mole) was added over 1 hr. The reaction mixture was then allowed to stand for 2 days. In some of the reactions, the total reaction period was 2 days.

Trace amounts of alkali-insoluble material were removed by extraction with two 100-ml. portions of chloroform. The extracted aqueous solution, cooled to 0°, was acidified with ice-cold 4 *N* hydrochloric acid to pH 3. The chloroform extract (four 100-ml. portions) of the acidified mixture was washed with water, dried with sodium sulfate, and distilled under reduced pressure to remove solvent.

Two crops were taken from the residual material (2.5 g.), the first (0.6 g.) from chloroform and the second (0.3 g.) from methylene chloride–cyclohexane. The combined solids were crystallized from aqueous acetone, two crops again being taken (0.6 g.). Finally, pure 3-(O-methyl)picropodophyllic acid (VI), m.p. 159–160° (foaming), was obtained (0.52 g., 19%) by crystallization from ethyl acetate–hexane.

The same compound VI, m.p. 159.5–160.5° and m.m.p. 159–160°, was obtained in about the same yield by methylating picropodophyllin (IV) essentially according to the above procedure.

Anal. Calcd. for $C_{23}H_{26}O_9$: C, 61.89; H, 5.87; 4(CH_3O), 27.80. Found: C, 61.73; H, 5.85; CH_3O, 27.38.

A 2×10^{-4} *M* solution of 3-(O-methyl)picropodophyllic acid (VI) in 95% alcohol showed an absorption maximum at 292–293 mμ (log ϵ 3.65). The compound as a mull with mineral oil showed infrared absorption peaks at 3250, 1710, and 1585 cm.$^{-1}$; and as a solution in chloroform, at 3440 (m), 1740 (m), 1705 (s), and 1588 (s) cm.$^{-1}$. The optical rotation was determined as $[\alpha]^{25}D$ −91° (*c* 4.0, acetone) or −95° (*c* 0.62, absolute alcohol). Thin layer chromatography with system B gave a single spot with R_f 0.34.

(10) Recent n.m.r. work has provided further confirmation for the *cis*-1,4 orientation in podophyllotoxin (III) and the *trans*-1,4 orientation in epipodophyllotoxin (IX) [E. Schreier, *Helv. Chim. Acta*, **46**, 75 (1963)].

When the material remaining in the mother liquors from the original two crops was examined by thin layer chromatography, three spots appeared corresponding to picropodophyllic acid (V), picropodophyllin (IV), and 3-(O-methyl)picropodophyllic acid (VI). The infrared absorption spectrum determined with the material in chloroform showed peaks at 3500 (hydroxyl), 1775 (lactone carbonyl), 1750, and 1710 cm.$^{-1}$ (carboxylic acid). Approximately half of the mixture consisted of picropodophyllin (IV).

Recovery of Picropodophyllic Acid (V) after Its Exposure to Concentrated Alkali.—A mixture of picropodophyllin (0.50 g., 1.2 mmoles) and 100 ml. of 7.1 *N* aqueous potassium hydroxide solution was heated on the steam bath for 20 min. under nitrogen. The homogeneous solution, still under nitrogen, was set aside at room temperature for 2 days.

Acidification at −5° with ice-cold 4 *N* hydrochloric acid brought the solution to pH 3. After addition of a few milliliters of chloroform to the cold solution, it was stirred vigorously for 10 min. The white crystalline precipitate was collected, washed quickly on the funnel with small portions of cold water to remove hydrochloric acid, and dried in a vacuum desiccator over calcium sulfate (Drierite) for 2 days. This material (471 mg.) showed m.p. 151.5–152° (foaming) by itself or admixed with authentic picropodophyllic acid (V). Drying the product over phosphorus pentoxide for 30 hr. at 100° and a pressure of 0.005 mm. gave 460 mg. (88%) of solvent-free picropodophyllic acid (V), m.p. 210–213°, on increasing the temperature *ca.* 2°/min. from 140°. Further drying for 48 hr. at 100° resulted in no loss of weight. The solid, as a mineral oil mull, showed the expected infrared absorption peaks at 3450 (hydroxyl) and 1704 cm.$^{-1}$ (carboxylic acid). A weak absorption at 1763 cm.$^{-1}$ (lactone) suggested the presence of a trace of picropodophyllin (IV). In agreement, while the product before drying at 100° gave only one spot on thin layer chromatography with system B, the final material showed the same spot plus a second minor spot corresponding to picropodophyllin.

Methoxy Lactone VII from 3-(O-Methyl)picropodophyllic Acid (VI).—A 165-mg. sample of 3-(O-methyl)picropodophyllic acid was sublimed under 0.01-mm. pressure in a test tube (27-mm. diameter). A cold finger cooled with acetone–Dry Ice served as condenser. The bath temperature was raised from 130 to 185° over a period of 0.5 hr. and was kept at this temperature for 45 min. The solid sublimate (113 mg.) became oily on standing overnight in a desiccator. This material plus the product (49 mg.) from an 83-mg. sublimation was combined, and 150 mg. of the sublimate in 10 ml. of benzene was added to a chromatography tube containing 15 g. of acid-washed alumina (Merck). Three 50-ml. portions of benzene were passed through the column followed by two 50-ml. portions of benzene–chloroform (9:1). The solvent-free fractions were examined with the help of thin layer chromatography (system B). The materials in the last three fractions (102 mg.) were combined and were crystalized twice from benzene–hexane to give 77 mg. (35% yield from acid VI) of methoxy lactone VII. The melting point, determined after heating the product at 60° (0.005 mm.) over phosphorus pentoxide for 2 hr., depended on the procedure. A capillary sample immersed at 68° and heated at 1°/min. softened at 80° and melted at 90–100°. Under the same conditions but in an evacuated sealed capillary, the product softened at 86° and liquified at 93–99°. Further exposure of this lactone product VII to temperatures around 65° (0.005 mm.) for 38 hr. brought the melting point in a sealed evacuated capillary to 93–96° with very little preliminary softening.

Anal. Calcd. for $C_{23}H_{24}O_8$: C, 64.48; H, 5.65; $4CH_3O$, 28.97. Found: C, 64.26; H, 5.46; CH_3O, 28.47.

Note that a carefully purified sample of podophyllotoxone showed about a 0.5% discrepancy in the methoxyl analysis.

Anal. Calcd. for $C_{22}H_{20}O_8$: C, 64.07; H, 4.89; $3CH_3O$, 22.58. Found: C, 64.23; H, 4.98; CH_3O, 22.07.

Methoxy lactone VII as a mull with mineral oil showed an absorption peak at 1767 cm.$^{-1}$, corresponding to lactone, but none in the hydroxyl region. The lactone in chloroform solution showed this peak at 1765 cm.$^{-1}$. The optical rotation in chloroform was $[\alpha]_D$ +50° (*c* 0.22) and +81° (*c* 0.12, absolute alcohol). Thin layer chromatography (system B) gave only a single spot with R_f 0.63; 3-(O-methyl)picropodophyllic acid under same conditions showed R_f 0.32 and could be detected at levels corresponding to 1% of the lactone. The ultraviolet absorption curve for a 2×10^{-4} *M* absolute ethanol solution of lactone VII gave a maximum at 293 mμ (log ε 3.58).

3-(O-Methyl)epipicropodophyllic Acid (XII) by Saponification of Methoxy Lactone VII.—A sample of 3-(O-methyl)picropodophyllic acid (VI) was sublimed as before at 180–185° (0.005 mm.) to give lactone VII in 78% yield. The sublimate (lactone carbonyl peak at 1767 cm.$^{-1}$) was hydrolyzed with hot 6% methanolic potassium hydroxide. The hydrolysis product (51%) gave a single spot on a thin layer chromatogram with the proper R_f value for 3-(O-methyl)epipicropodophyllic acid (XII). The melting point was 179.5–181° (softening at 178°) either with or without admixture of 3-(O-methyl)epipicropodophyllic acid (XII) from epipicropodophyllin (X). This acid in chloroform showed absorption peaks at 3480 (m), 1737 (s), 1704 (s), and 1590 (s) cm.$^{-1}$.

No sign of any product other than 3-(O-methyl)epipicropodophyllic acid (XII) was obtained when sodium methoxide in methanol containing trace amounts of water was used for the hydrolysis. Thus, a solution of 4 mg. of lactone VII in 3 ml. of absolute methanol containing 80 mg. of sodium was boiled for 2 hr. under nitrogen. Aliquots removed at various times were examined by thin layer chromatography (solvent system B). At no time was more than two spots—corresponding to lactone VII (R_f 0.83) and acid XII (R_f 0.60)—observed. The identifications were made by comparing the movement of authentic material spotted on the same plate. The intensity of the lactone spot rapidly decreased until, at the end of 0.5 hr., it became and remained very faint. The spot corresponding to acid XII was dark minutes after the start of the reflux period. After the 2-hr. period, several drops of water were added, and the mixture was allowed to stand overnight at room temperature. Aliquots of the solution as it was at the end of the 2-hr. period and after treatment with water were examined with the use of solvent system A. Both aliquots showed only two spots corresponding to the lactone (R_f 0.87, very faint) and the acid (R_f 0.02, dark).

3-(O-Methyl)epipicropodophyllic Acid (XII) from Epipicropodophyllin (X).—Epipicopodophyllin (X) was hydrolyzed with aqueous alkali and then was methylated with dimethyl sulfate in a manner completely analogous to that described above for the conversion of podophyllotoxin (III) or picropodophyllin (IV) to 3-(O-methyl)picropodophyllic acid (VI). The chloroform extract of the acidified aqueous solution (pH 3) contained 1.78 g. of crude product when 2.07 g. (5.0 mmoles) of epipicropodophyllin (X) was taken as starting material. The crude solvent-free product consisted of a gum mixed with crystals. Addition of benzene dissolved the gum and allowed the crystals to be collected and rinsed with benzene.

The crystals weighed 90 mg. and showed m.p. 175–179°. The infrared absorption curve of this material dissolved in chloroform was identical with that of the 3-(O-methyl)epipicropodophyllic acid (XII) obtained by hydrolysis of lactone VII. Thin layer chromatography (system B) produced a dark spot with R_f 0.43, the same as that obtained from material derived from the lactone; a very faint spot remaining at the origin was probably epipicropodophyllic acid (XI). On the same plate, 3-(O-methyl)picropodophyllic acid (VI) showed R_f 0.38, 3-(O-methyl)epipicropodophyllic acid (XII) showed R_f 0.48, and a mixture produced two spots with R_f 0.41 and 0.50.

Crystallization from ethyl acetate–hexane afforded 68 mg. of 3-(O-methyl)epipicropodophyllic acid (XII), m.p. 179–180° and $[\alpha]_D$ −41 ± 3° (*c* 0.07, chloroform) and −45° (*c* 0.17, absolute alcohol). A mixture of methylated acids VI (m.p. 159.7–160.5° with shrinking at 158°) and XII melted at 157.5–160.5° with shrinking at 155°; the capillary sample here was placed in the bath at 145° and the temperature was raised 2°/min.

Anal. Calcd. for $C_{23}H_{26}O_9$: C, 61.89; H, 5.87; $4CH_3O$, 27.80. Found: C, 61.67; H, 6.06; CH_3O, 27.51.

The infrared absorption curves of acids VI and XII in chloroform solutions showed considerable differences especially in the 1500–800-cm.$^{-1}$ region. The ultraviolet absorption spectrum of 3-(O-methyl)epipicropodophyllic acid (XII) in 2×10^{-4} *M* alcohol solution showed λ_{max} 292 mμ (log ε 3.64).

When the benzene solution of the gummy product was checked by thin layer chromatography (system B), the major spot (R_f 0.72) fell close to the spot for lactone VII (R_f 0.71). The infrared absorption spectrum of the solute in chloroform solution was very similar to that of the lactone. The benzene solution was passed through a 60-g. column of Merck acid-washed alumina, and the following eluent fractions were collected: 700 ml. of benzene, 200 ml. of 9:1 benzene–chloroform, 200 ml. of 4:1 benzene–chloroform, two 100-ml. portions of chloroform,

and one 100-ml. portion of 9:1 chloroform–ethanol. The last two portions contained 1.37 g. of lactone VII giving a single spot on thin layer chromatography (R_f 0.68). A portion of this material (0.52 g.) was exposed for 1.5 hr. to a boiling solution of potassium hydroxide (4.25 g.) in 85% aqueous methanol (100 ml.). Two crystallizations of the resulting organic acids from ethyl acetate–hexane gave 0.34 g. (44%) of 3-(O-methyl)-epipicropodophyllic acid (XII), m.p. 178–180°. The melting point did not change when this material was mixed with the crystals described above.

Stability of Methylated Acids VI and XII to Alkali.—A solution of 0.7 mg. of 3-(O-methyl)picropodophyllic acid (VI) in 0.16 ml. of 35% aqueous potassium hydroxide was heated on the steam bath under nitrogen for 20 min. and then allowed to stand under nitrogen for 50 hr. A solution of 0.6 mg. of 3-(O-methyl)epipicropodophyllic acid (XII) in 0.14 ml. of alkali was treated the same way. For thin layer chromatography, a 0.01-ml. aliquot was mixed with 0.1 ml. of acetic acid and 0.1 ml. of acetone, and half of this solution was spotted on a plate and run with system B. Each solution gave only one spot whose R_f value corresponded to the respective starting material. A mixture of the two solutions showed the expected two spots. Tripling the amount of methoxy acid spotted brought out some very faint spots but none that corresponded to interconversion of the two materials.

Lactone VII from 3-(O-Methyl)epipicropodophyllic Acid (XII).—A sample (46 mg.) of 3-(O-methyl)epipicropodophyllic acid (XII) was sublimed at 0.005 mm. in the apparatus used for the lactonization of 3-(O-methyl)picropodophyllic acid (VI). The temperature was held at 155° for 20 min. (no sublimate) and then at 183–185° for 50 min. The white sublimate (40 mg.) gave an infrared absorption spectrum identical with that obtained with the lactone VII from 3-(O-methyl)picropodophyllic acid (VI), and a thin layer chromatogram (system A) showing spots with R_f 0.71 (intense; lactone VII has R_f 0.73), 0.41 (very weak; unidentified), and 0.03 (very weak; 3-(O-methyl)epipicropodophyllic acid has R_f 0.02).

The product in 5 ml. of benzene was placed on a column of Merck acid-washed alumina (5 g.) and was eluted with 300 ml. of benzene, 200 ml. of benzene–chloroform (9:1), and 100 ml. of benzene–chloroform (4:1). Since all fractions showed only a single spot at the lactone position on thin layer chromatograms, they were combined. The eluted material (34 mg.) after two crystallizations from benzene–hexane gave a product (24 mg., 55%) that melted at 93–95.5° either with or without admixture of lactone VII from 3-(O-methyl)picropodophyllic acid (VI).

Ease of Lactonization of 3-(O-Methyl)picropodophyllic Acid (VI) and 3-(O-Methyl)epipicropodophyllic Acid (XII).—Separate solutions of the two acids VI and XII were boiled under a reflux condenser. From time to time aliquots were removed and examined by thin layer chromatography (system B). The pure compounds involved here showed the following R_f values: 3-(O-methyl)picropodophyllic acid (VI), 0.26; 3-(O-methyl)-epipicropodophyllic acid (XII), 0.33; and lactone VII, 0.62. No other spots were noted on any of the plates.

Boiling acid VI or acid XII in benzene for 5 hr. resulted in no lactonization whatever.

Better results were obtained with boiling solutions of the acids (1.5 mg.) in toluene (2 ml.). Thin layer chromatographic examination of the toluene reaction mixtures showed a more rapid lactonization of acid XII than of acid VI. Thus, after 3.75 hr., the lactone spot from VI was just discernable, while the lactone spot from acid XII was intense; and after 9.25 hr., the spot corresponding to unchanged XII was barely detectable, while the spot for unchanged VI remained darker than the lactone spot.

After 9.25 hours, all the solvent was removed, and the weighed residues were dissolved in ethanol to give solutions of *ca.* 1 mg./ml. From the observed specific rotations of the residues from acid VI and acid XII ($[\alpha]^{23}D$ −48 and +81°, respectively) and from the optical rotations of pure compounds (−95° for VI, −45° for XII, and +81° for lactone VII), the approximate composition of the residues could be calculated. The yield of lactone VII from 3-(O-methyl)picropodophyllic acid (VI) was 27 ± 5%; the yield from 3-(O-methyl)epipicropodophyllic acid (XII) was 100 ± 5%.

Hydrogenolysis of 3-(O-Methyl)picropodophyllic Acid (VI) to 3-(O-Methyl)-4-desoxypicropodophyllic Acid (VIII).—Hydrogen was slowly bubbled through a mixture of 3-(O-methyl)picropodophyllic acid (VI, 113 mg., 0.25 mmole), 10% palladium on charcoal (127 mg., American Platinum Co.), and pure toluene (3 ml.) on the steam bath. After 1.5 hr., thin layer chromatography with system B showed that the starting material (R_f 0.25) was absent and that methoxy lactone VII and hydrogenolysis product VIII were both present (R_f 0.58 and 0.53, respectively). After 5 hr., the spot corresponding to the product was more intense while that corresponding to the lactone was less intense.

The hot solution was filtered, and the catalyst was rinsed with hot toluene. Cooling the combined filtrate and washings deposited white needles, m.p. 197–199° dec., which were homogeneous by thin layer chromatography. Crystallization from toluene–pentane gave 55 mg. (51%) of 3-(O-methyl)-4-desoxypicropodophyllic acid (VIII), m.p. 196–198.5°, and $[\alpha]^{22}D$ −68° (*c* 0.11, chloroform). A chloroform solution showed infrared absorption peaks at 3500, 1740, and 1707 (s) cm.$^{-1}$. An absolute alcohol solution (2×10^{-4} *M*) showed λ_{max} 295.5 mμ (log ε 3.69).

Anal. Calcd. for $C_{23}H_{26}O_8$: C, 64.17; H, 6.09; $4CH_3O$, 28.84. Found: C, 64.34; H, 6.06; CH_3O, 29.68.

No complications attributable to the solvent or catalyst were likely, since the only change that occurred on exposing acid VI to acetic acid containing catalyst was lactonization to VII. Thus, glacial acetic acid (0.1 ml.) containing 5 mg. of 3-(O-methyl)picropodophyllic acid (VI) and 6 mg. of 10% palladium on carbon was heated on the steam bath under nitrogen for 5 hr. Aliquots removed at hourly intervals and examined by thin layer chromatography (system B) showed only one spot whose R_f value (0.58) corresponded to that of lactone VII (0.59) but was far removed from that of the starting material VI (0.25). The ultraviolet absorption spectra of other aliquots dissolved in alcohol showed no sign of the 311-mμ maximum expected for an "α-apo" dehydration product but only the 293-mμ peak associated with the nonconjugated systems.

Storing the reaction mixture overnight in a vacuum desiccator over potassium hydroxide pellets removed all the acetic acid. The residual solids were warmed with chloroform and filtered. The infrared absorption curve of the clear filtrate showed no hydroxyl peak in the 3500-cm.$^{-1}$ region and proved to be identical with that of methoxy lactone VII.

3-(O-Methyl)desoxypicropodophyllic Acid (VIII) from Desoxypodophyllotoxin.—A 1.62-g. sample of desoxypodophyllotoxin (from podophyllotoxin, III[11]) was saponified and methylated essentially as described above for the conversion of podophyllotoxin (III) to 3-(O-methyl)picropodophyllic acid (VI). The acidic products, desoxypicropodophyllic acid plus 3-(O-methyl)-desoxypicropodophyllic acid (VIII), were dissolved in methylene chloride (*ca.* 100 ml.) and chromatographed on a column of silica gel (60 g.). The following solvents were passed through the column: 400 ml. of methylene chloride, 200 ml. of ethyl acetate–methylene chloride (1:9), 200 ml. of ethyl acetate–methylene chloride (1:4), and finally 300 ml. of ethyl acetate–methylene chloride (1:1). Removal of solvent from the last 300 ml. of eluate left a residue, which on recrystallization from methylene chloride–pentane afforded 0.43 g. of 3-(O-methyl)-desoxypicropodophyllic acid (VIII), m.p. 205.5–206.5°.

The mixture melting point with the same material from 3-(O-methyl)picropodophyllic acid (VI) was 202–204°. The two samples of VIII showed identical infrared absorption curves, and by thin layer chromatography with either solvent system were both homogeneous and indistinguishable.

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