evaporation on the steam-bath. Crystallization of the residue from methanol afforded long needles of the hydroxy methyl ester IX, m.p. 198–200°. The mixture melting point with the original purified hydroxy methyl ester IX was 199–200°.

Further methylation was attempted by adding 7 ml. of ethereal diazomethane (excess) to a solution of 40 mg. of hydroxy methyl ester IX in 8 ml. of methylene chloride. The reaction mixture was allowed to stand at 5° for 18 hours. Removal of volatile material left 40 mg. of unchanged starting material melting at 200–203° and, after admixture with starting material, at 201–203°.

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF BOSTON UNIVERSITY, BOSTON 15, MASS.]

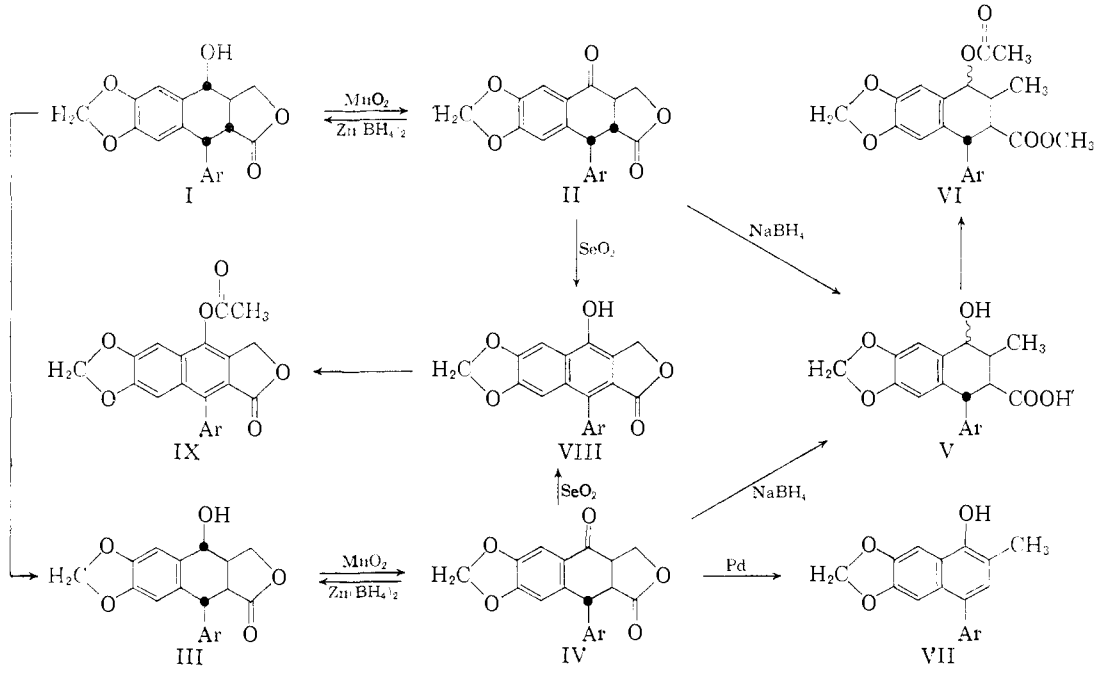
Compounds Related to Podophyllotoxin. XII. Podophyllotoxone, Picropodophyllone and Dehydropodophyllotoxin[1]

BY WALTER J. GENSLER, FRANCIS JOHNSON AND A. DAVID B. SLOAN

RECEIVED JUNE 2, 1960

Manganese dioxide converts podophyllotoxin and picropodophyllin to the corresponding ketones, podophyllotoxone and picropodophyllone. The ketones with sodium borohydride give the same product, a hydroxy acid. In contrast, zinc borohydride regenerates podophyllotoxin from podophyllotoxone, and regenerates picropodophyllin from picropodophyllone. Both podophyllotoxone and picropodophyllone on treatment with selenium dioxide give the naphthol, dehydropodophyllotoxin, identical with the material isolated by Kofod and Jørgensen from podophyllin resin. The ultraviolet and infrared absorption data, the fact that manganese dioxide readily oxidizes podophyllotoxin and picropodophyllin to the ketones, and the fact that an oxidative process converts the ketones to a naphthol all point to the α-tetralol structures of podophyllotoxin and picropodophyllin. Accordingly, the present work provides independent evidence for the presence of a secondary hydroxyl group, and, when the known carbon skeleton is taken into account, provides independent evidence for the position of the hydroxyl group. Methylmagnesium bromide adds to podophyllotoxone to give two stereoisomeric methylpodophyllotoxins. Phenylhydrazine reacts with podophyllotoxone to give a pyrazoline acid instead of the phenylhydrazone.

Continued work on podophyllotoxin compounds has developed a method of converting podophyllotoxin (I)[2] to the corresponding ketone, podophyllotoxone (II). This report describes the preparation and reactions of podophyllotoxone (II), as well as of its stereoisomer, picropodophyllone (IV), and its dehydrogenation product, dehydropodophyllotoxin (VIII).[3]



Podophyllotoxone (II) and Picropodophyllone (IV).—Finely divided manganese dioxide in an inert solvent[4] converted podophyllotoxin (I) to podophyllotoxone (II), and similarly converted picropodophyllin (III) to picropodophyllone (IV). Thus manganese dioxide proved effective where other reagents–*e.g.*, potassium dichromate,[5] chromic anhydride,[5] ethylene with copper chromite catalyst at 280°,[6] acetone with aluminum isopropoxide,[7]

(1) This work was supported by grants from the American Cancer Society (CBC-6), from the National Cancer Institute, United States Public Health Service (CY-2891), and from the Massachusettes Division of American Cancer Society.

(2) In all the structural formulas, Ar stands for 3,4,5-trimethoxyphenyl.

(3) A short description of some of this work has already appeared; *cf.* W. J. Gensler and F. Johnson, THIS JOURNAL, **77**, 3674 (1955).

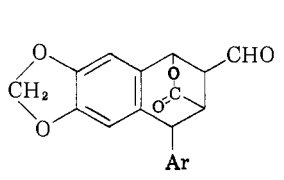
(4) Compare J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jansen and T. Walker, *J. Chem. Soc.*, 1094 (1952).

(5) E. H. Price, Doctoral Thesis, University of Maryland, College Park, Md., 1949.

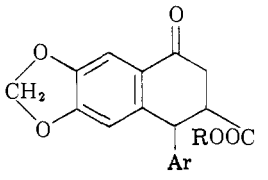
(6) H. Myers, Doctoral Thesis, University of Maryland, College Park, Md., 1951.

cyclohexanone with aluminum *t*-butoxide,[7] benzophenone with potassium *t*-butoxide, silver carbonate[8] and potassium permanganate[5,9]—failed.

Several lines of evidence place the ketonic carbonyl group in podophyllotoxone (II) and picropodophyllone (IV) directly attached to the methylenedioxyphenyl ring, and consequently rule out such a structure as X. Podophyllotoxone (II),



X

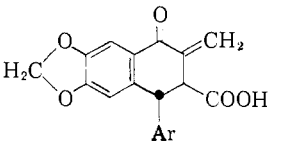


XI

picropodophyllone (IV) and synthetic tetralone XI[10] furnish ultraviolet absorption spectra, which although differing slightly in the position and extinction of corresponding maxima, resemble one another closely in general contour. The parent compounds, podophyllotoxin (I) and picropodophyllin (III) as well as other related derivatives with no tetralone carbonyl, show ultraviolet absorption maxima at significantly lower wave lengths.[11] The 5.95–5.99μ peaks in the infrared absorption curves of the three tetralones, II, IV and XI, are normal for α,β-unsaturated ketones, but not for non-conjugated ketones or aldehydes, which absorb at 5.75–5.87μ.[12] Further, the smooth oxidation of alcohol to carbonyl (as in I to II, or III to IV), if not proof of,[13] is at least consistent with, the benzylic nature of the alcohol, and accordingly with the α-tetralone nature of the carbonyl compounds. Lastly, formation of the naphthol lactone VIII by selenium dioxide oxidation of the carbonyl compounds is consistent with α-tetralone formulations II and IV, but not with formulation X.

The levorotation ($[\alpha]^{25}D$, $-142°$) of picropodophyllone (IV) seemed out of line with the weakly dextrorotations of other picropodophyllin derivatives.[11] Therefore, some check on the stereochemistry was desirable. Attempted reconversion of picropodophyllone (IV) to picropodophyllin (III) with sodium borohydride failed to give assurance on this point. Instead of picropodophyllin, the reduction product was an acidic material, for which structure V is suggested. The same material was isolated as the only pure product when podophyllotoxone (II) reacted with sodium borohydride. The carboxyl group of this reduction product V could be methylated. The presence of hydroxyl was shown by an infrared absorption peak at 3.01μ, and by acetylation to the acetate ester VI.

These results must be related to the sensitivity of podophyllotoxone and picropodophyllone to the hydroxide or ethoxide ions present in alcoholic sodium borohydride.[16] Under basic catalysis, the carbon alpha to the lactone carbonyl group in podophyllotoxone (as indeed in all podophyllotoxin analogs) should be readily inverted. Base-catalyzed β-elimination in the resulting picropodophyllone (IV) to give α,β-unsaturated ketone XII,[17] followed by reduction would lead to the observed product V.[18]



XII

In order to avoid conditions promoting enolate formation—and so to preclude epimerization and elimination—another reducing reagent was sought. Ethereal lithium aluminum hydride was a reasonable possibility, though the product would be a non-lactonic triol. The identity of the triol from the lithium aluminum hydride reduction of picropodophyllone (IV) with the triol derived from picropodophyllin (III)[19] would remove all question about the stereochemical relation between III and IV. Yet, because preservation of the lactone function was also desirable, lithium aluminum

(7) W. J. Gensler and C. M. Samour, unpublished work.

(8) Silver carbonate has been used to convert the allylic hydroxyl groups of codeine and methoxymethylmorphine to the α,β-unsaturated ketones; *cf.* H. Rapoport and H. N. Reist, THIS JOURNAL, **77**, 490 (1955); H. Rapoport, D. R. Baker and H. N. Reist, *J. Org. Chem.*, **22**, 1489 (1957).

(9) Earlier work with permanganate may also have been directed to formation of the ketones; *cf.* W. Borsche and J. Niemann, *Ann.*, **499**, 59 (1932); E. Späth, F. Wessely and L. Kornfeld, *Ber.*, **65**, 1536 (1932); E. Späth, F. Wessely and E. Nadler, *ibid.*, **66**, 125 (1933).

(10) W. J. Gensler, C. M. Samour, S. Y. Wang and F. Johnson, THIS JOURNAL, **82**, 1714 (1960).

(11) A comprehensive summary and review is given by J. L. Hartwell and A. W. Schrecker, *Fortschr. Chem. org. Naturstoffe*, **15**, 83 (1958).

(12) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1958, p. 132.

(13) Manganese dioxide was originally developed and introduced as a reagent to oxidize allyl (also propargyl and benzyl) alcohols—but not ordinary alcohols—to the corresponding α,β-unsaturated carbonyl compound.[4,14,15] However, manganese dioxide in hydroxylic solvents [M. Z. Barakat, M. F. Abdel-Wahab and M. M. El-Sadr, *J. Chem. Soc.*, 4685 (1956)] and even in inert solvents [ref.[15]; also *cf.* K. Schaffner, L. Caglioti, D. Arigoni and O. Jeger, *Helv. Chim. Acta*, **41**, 152 (1958); H. Bruderer, D. Arigoni and O. Jeger, *ibid.*, **39**, 858 (1956)] *can* oxidize saturated alcohols. Therefore, the fact that manganese dioxide is effective in oxidizing podophyllotoxin and picropodophyllin cannot be taken as unequivocal proof that these compounds have activated (*i.e.*, α-tetralol) hydroxyl groups.

(14) S. Ball, T. W. Goodwin and R. A. Morton, *Biochem. J.*, **42**, 516 (1948); F. Sondheimer, C. Amendolla and G. Rosenkranz, THIS JOURNAL, **75**, 5930 (1953); D. L. Turner, *ibid.*, **76**, 5175 (1954); K. R. Bharucha, *J. Chem. Soc.*, 2446 (1956). A review is given by R. M. Evans, *Quart. Revs. (London)*, **13**, 61 (1959).

(15) M. Harfenist, A. Bavley and W. A. Lazier, *J. Org. Chem.*, **19**, 1608 (1954).

(16) The hydrogen-liberating reaction between alcohol and sodium borohydride gives tetraethoxyborate ion, which dissociates only very slightly to triethyl borate and ethoxide ion. However, even small amounts of ethoxide ion would make the solution alkaline. Also, if water finds its way into the system, sodium borohydride could give sodium hydroxide plus the practically non-ionizing metaboric acid [*cf.* H. I. Schlesinger, H. C. Brown, A. E. Finholt, J. R. Gilbreath, H. R. Hoekstra and E. K. Hyde, THIS JOURNAL, **75**, 215 (1953)].

(17) A similar structure is reported by R. D. Haworth and G. Sheldrick, *J. Chem. Soc.*, 289 (1941).

(18) F. Sondheimer, M. Velasco, E. Batres and G. Rosenkranz [*Chemistry & Industry*, 1482 (1954)] have reported sodium borohydride reactions with 1,4-androstadiene-3,17-dione and with 1,4,6-androstatriene-3,17-dione analogous to the conversion of α,β-unsaturated ketone XII to saturated alcohol V. There are only few other reports of the sodium borohydride saturated of double bonds conjugated to carbonyl [*cf.* S. W. Chaikin and W. G. Brown, THIS JOURNAL, **71**, 122 (1949); R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler and W. M. McLamore, *ibid.*, **74**, 4223 (1952)].

(19) N. L. Drake and E. H. Price, *ibid.*, **73**, 201 (1951).

hydride was not used. A satisfactory reagent was found in ether-soluble zinc borohydride, conveniently prepared from sodium borohydride and zinc chloride.[20] With this reagent, picropodophyllone (IV) gave picropodophyllin (III), and podophyllotoxone (II) gave podophyllotoxin (I). Accordingly the stereochemistry in picropodophyllone is the same in corresponding centers of asymmetry as in picropodophyllin; the same is true for the podophyllotoxone–podophyllotoxin pair.[21]

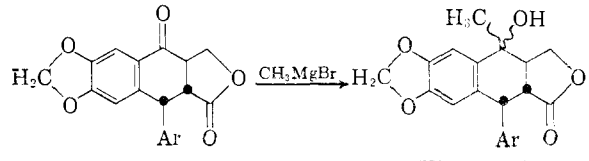
The assignment of a secondary alcohol grouping in podophyllotoxin and in picropodophyllin at the positions indicated in formulations I and III has rested mainly on the possibility of stereoisomerism, and on the relative ease of displacement reactions, at the hydroxyl-bearing carbon atom.[24] The argument against a tertiary alcohol has rested mainly on successful acetylations of podophyllotoxin and picropodophyllin, and on the failure of podophyllic acid to give formaldehyde with periodate.[24] Now, conversion of podophyllotoxin and picropodophyllin to the corresponding ketones confirms and independently establishes the secondary alcohol assignment. Furthermore, the carbon skeleton of picropodophyllin, which has been established by degradation[11] and by synthesis,[10] affords only a single position at which an α-tetralone carbonyl such as in II or IV can be accommodated. Accordingly, both the position and the secondary nature of the hydroxyl groups in podophyllotoxin (I) and picropodophyllin (III) are directly and independently proved.

Dehydropodophyllotoxin (VIII).—During the course of this work, Kofod and Jørgensen[25] described a new material, dehydropodophyllotoxin, which they isolated from podophyllin resin, and which they formulated as α-naphthol VIII. Confirmation of this proposed structure was attempted by relating tetralones II and IV to dehydropodophyllotoxin (VIII). Dehydrogenation of picropodophyllone (IV) over palladium-on-carbon catalyst failed to give dehydropodophyllotoxin. Instead a product was obtained whose infrared absorption spectrum, although indicating the presence of hydroxyl, showed the absence of carbonyl. Structure VII for this material is compatible with the available data.

Several chemical dehydrogenating agents were tried without success before selenium dioxide was found to be effective. With this reagent, both podophyllotoxone (II) and picropodophyllone (IV) were converted to the same naphthol derivative VIII.[26] The naphthol did not dissolve in aqueous alkali, nor did it give a red color with ferric chloride. However the presence of hydroxyl was indicated by an infrared absorption peak at 2.91μ, and by formation of the acetate IX.

The identity of dehydropodophyllotoxin isolated from podophyllin with that obtained as dehydrogenation product VIII was established by Dr. Kofod, to whom we are indebted. Direct comparisons of the two materials and their acetates by melting points, mixture melting points, paper chromatography, and ultraviolet absorption spectra showed that they are the same.[27] Since picropodophyllin has been synthesized,[10] its conversion to picropodophyllone (IV) and then to dehydropodophyllotoxin (VIII) completes a total synthesis of dehydropodophyllotoxin. The identity of dehydropodophyllotoxin acetate (IX) with the same material synthesized in another way has been fully documented.[28]

Methylpodophyllotoxin (XIII).—The ketone function in podophyllotoxone (II) offered the possibility of forming new podophyllotoxin derivatives, of potential value as antitumor agents. Reaction with methylmagnesium bromide, although complicated by the presence of the lactone carbonyl, furnished two adducts, methylpodophyllotoxin-A



II XIII (A and B)

and B (XIII). Both showed infrared and ultraviolet absorption curves consistent with those expected of XIII. Preservation of "toxin" stereochemistry was suggested by the levorotations, and by the location (5.61–5.63μ) of the lactone-carbonyl infrared absorption peaks.[11] Approach of the methyl Grignard reagent to the ketonic carbonyl

(20) Zinc borohydride has been obtained before from lithium borohydride and zinc chloride [E. Wiberg and W. Henle, *Z. Naturforsch.*, **6**, 579 (1952); E. Wiberg, *Angew. Chem.*, **65**, 16 (1953)] and from zinc hydride and diborane [G. D. Barbaras, C. Dillard, A. E. Finholt, T. Wartik, K. E. Wilzbach and H. I. Schlesinger, THIS JOURNAL, **73**, 4585 (1951)].

(21) Formation of podophyllotoxin instead of epipodophyllotoxin[11] in the zinc borohydride reduction of podophyllotoxone might have stereochemical implications. *If* the ketone carbonyl group in podophyllotoxone is unhindered in the sense that Barton and Dauben[22] use the term, and *if* zinc borohydride reduction follows the generalizations claimed for lithium aluminum hydride and sodium borohydride reductions,[22] the hydroxyl group formed in the reduction of podophyllotoxone to podophyllotoxin should be (pseudo) equatorial. The *trans*-lactone ring[23] in the inflexible podophyllotoxin molecule[11] is geometrically possible only with equatorial groups at both the 2- and 3-positions. Therefore, fixing the hydroxyl group in the equatorial conformation unequivocally establishes its *trans* relation (ee) to the lactone methylene group (*cf.* I). This assignment is the same as that reached before on other grounds.[24]

(22) D. H. R. Barton, *J. Chem. Soc.*, 1027 (1953); W. G. Dauben, G. J. Fonken and D. S. Noyce, THIS JOURNAL, **78**, 2579 (1956); W. G. Dauben, E. J. Blanz, J. Jiu and R. A. Micheli, *ibid.*, **78**, 3752 (1956).

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

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

(25) H. Kofod and C. Jørgensen, *Acta Chem. Scand.*, **8**, 1296 (1954).

(26) Study of the selenium dioxide dehydrogenation of several pairs of stereoisomeric 1,4-diketones to the corresponding $\Delta^{2,4}$-unsaturated-1,4-diketone has shown that the action is faster when the hydrogen atoms removed are *cis* to each other, and slower when the hydrogen atoms are *trans* [J. C. Banerji, D. H. R. Barton and R. C. Cookson, *J. Chem. Soc.*, 5041 (1957)]. This behavior, advanced as a generalization, has been interpreted in terms of two different mechanisms for the two stereoisomers [C. S. Barnes and D. H. R. Barton, *ibid.*, 1419 (1953); but note the dissenting opinion of E. J. Corey and J. P. Schaefer, THIS JOURNAL, **82**, 918 (1960)]. In the present work, the yields of dehydrogenation product VIII from podophyllotoxone (II) and from picropodophyllone (IV)—which are functionally analogous to the 1,4-diketones—were about the same. However, our experiments were designed neither to test nor to apply the generalization. Whether selenium dioxide oxidation of picropodophyllone *cis*-hydrogens between the carbonyl groups is faster than the oxidation of podophyllotoxone (*trans*-hydrogens between the carbonyl groups) remains to be determined.

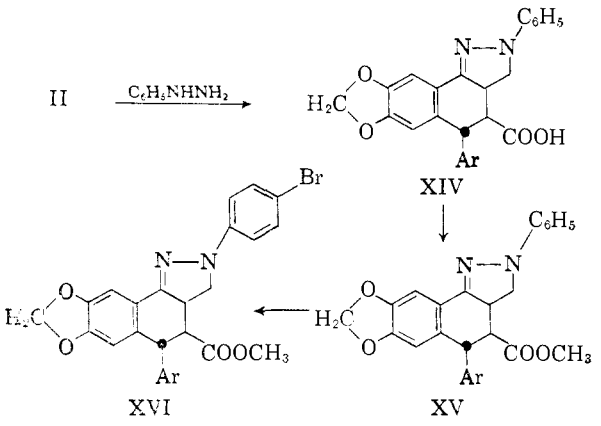
(27) Private communication from Dr. H. Kofod, April 21, 1955.

(28) G. N. Walker, THIS JOURNAL, **78**, 2316 (1956).

group of podophyllotoxone (II) is a little more open from the side of the (axial) hydrogen next to the carbonyl group than from the other side. Therefore, on the assumption that the somewhat larger yield of methylpodophyllotoxin-A (XIII) compared to methylpodophyllotoxin-B (XIII) reflects the ease of approach, the A-isomer has its hydroxyl group axial (*trans* to the adjacent axial hydrogen atom) and the B-isomer has its hydroxyl group equatorial (*cis* to the adjacent axial hydrogen).

Other products from the reation of podophyllotoxone (II) with methylmagnesium bromide are described in the Experimental Section.

Podophyllotoxone (II) with Phenylhydrazine.—Treatment of podophyllotoxone with phenylhydrazine in acetic acid solvent gave a yellow crystalline solid, for which pyrazoline structure XIV is suggested. The acid nature of product XIV was shown by its solubility in bicarbonate



solution, by its infrared absorption at 5.84μ, and by its reaction with diazomethane to form the methyl ester XV. Development of a blue-violet color on addition of sodium nitrite to the green-yellow solution of acid XIV (also of ester XV) in concentrated sulfuric acid constituted a positive Knorr test for a 1-phenylpyrazoline.[29-32] The pyrazoline structure was further supported by the characteristic brilliant blue fluorescence under ultraviolet light,[30,33] and by the absence of an infrared absorption peak corresponding to an N–H grouping.

Bromination of pyrazoline methyl ester XV replaced an atom of hydrogen with one of bromine. The analytical results did not distinguish between structure XVI (bromine is placed arbitrarily *para* to nitrogen) and one with two atoms of hydrogen less, as in the corresponding pyrazole. However, because the Knorr test was still positive and the fluorescence under ultraviolet light persisted, bromination was assumed to give the substituted pyrazoline XVI without concomitant oxidation to the pyrazole.[31,32]

Formation of pyrazoline XIV can be understood by keeping in mind the fact that podophyllotoxone (II) is a β-acyloxy ketone, related to other β-negatively substituted ketones that give pyrazolines with phenylhydrazine.[34] The intermediate here could be α,β-unsaturated ketone XII or its phenylhydrazone. Under the conditions used, epimerization at the position alpha to the carboxyl group is reasonably expected, so that the pyrazoline product XIV is formulated with *trans*-trimethoxyphenyl and carboxyl groups.

Activity.—Three of the compounds described above were submitted to the Chemical–Biological Coördination Center and to the Cancer Chemotherapy National Service Center for anticancer screening. Podophyllotoxone, II (CBC No. 109,446), was tested against Sarcoma-180 implanted in the mouse. Fourteen injections (each of 15 mg./kg.) spread equally over 7 days failed to significantly retard tumor growth.

Picropodophyllone, IV (CBC No. 109,445), was tested with mice in which C_3H-SX, 6C_3H-ED, S-180 and Ehrlich ascites tumors were active. The dosage was 100 mg./kg./day over a period of 7–9 days. The effect on the tumors was negative.

Methylpodophyllotoxin, XIII-A (m.p. 208°; NSC No. 21,624), was tested in mice against sarcoma (S-180), adenocarcinoma (Ca-755) and leukemia (L-1210). The compound was too toxic at 100 mg./kg./day. At a level of 25 mg./kg./day over an 8-day test period, the response with S-180—expressed as the ratio of tumor weight (t) in treated animals to tumor weight (c_1) in control animals—was $t/c_1 = 0.61$. Against Ca-755, at a dosage of 11.25 mg./kg./day for 12 days, $t/c_1 = 1.01$. At a dosage of 22.5 mg./kg./day, the effect of extending the life of animals infected with the L-1210 tumor was practically nil.

Experimental[35,36]

Podophyllotoxone (II) from Podophyllotoxin (I).—A mixture of 25 g. (0.053 mole) of podophyllotoxin hemibenzenate monohydrate, 140 g. of freshly prepared finely divided manganese dioxide[4] and 1500 ml. of chloroform was boiled for 2 hours. Solids were removed by filtration, with a filter aid if necessary, and were rinsed with 500 ml. of hot chloroform. All solvent was removed from the combined chloroform solutions by distillation first at atmospheric pressure and then under reduced pressures at steam-bath temperature. One crystallization of the residue from ethanol gave 17.1 g. (78%) of faintly yellow, nicely crystalline podophyllotoxone (II), m.p. 190–191.5°. A sample prepared for analysis by crystallization from benzene appeared as thick rods, m.p. 191–192° (soften 187°).

Anal. Calcd. for $C_{22}H_{20}O_8$: C, 64.07; H, 4.89. Found: C, 64.0; H, 5.0.

A 1.35×10^{-5} *M* alcoholic solution of podophyllotoxone (II) showed absorption maxima at 207 mμ (log ϵ 4.88), 235 mμ (log ϵ 4.64), 277 mμ (log ϵ 4.09) and 316 mμ (log ϵ 3.96). A mull with mineral oil showed infrared absorption peaks at 5.62 and 5.99 μ, but none in the hydroxyl region. The optical

(29) L. Knorr, *Ber.*, **26**, 100 (1893); K. Auwers and H. Voss, *Ber.*, **42**, 4411 (1909); L. C. Raiford and W. J. Peterson, *J. Org. Chem.*, **1**, 544 (1937).

(30) O. Neunhoeffer and H. Ulrich, *Chem. Ber.*, **88**, 1123 (1955).

(31) K. v. Auwers and P. Heimke, *Ann.*, **458**, 186 (1927).

(32) E. Fischer and O. Knoevenagel, *ibid.*, **239**, 194 (1887).

(33) F. Straus, *Ber.*, **51**, 1457 (1918); O. Neunhoeffer and D. Rosahl, *Chem. Ber.*, **86**, 226 (1953); D. Rosahl, *Ann. Physik*, [6] **12**, 35 (1953).

(34) *Cf.* T. L. Jacobs in "Heterocyclic Compounds," Vol. 5, R. C. Elderfield, editor, John Wiley and Sons, Inc., New York, N. Y., 1957, p. 57; W. L. Chambers and M. L. Willard, THIS JOURNAL, **82**, 3373 (1960).

(35) Elementary analyses were performed by Carol K. Fitz, 115 Lexington Ave., Needham Heights, Mass., and by S. M. Nagy, Microchemical Laboratory, Massachusetts Institute of Technology, Cambridge, Mass. Melting points are uncorrected.

(36) We wish to acknowledge the able technical assistance of Mr. William F. Sullivan with several of these experiments.

rotation at room temperature was $[\alpha]_D$ −125° (*c* 1.06 in chloroform).

Use of commercial "Active Manganese Dioxide" gave the same product although in much lower yield. Attempted oxidation of podophyllotoxin with silver carbonate in boiling benzene solution[8] afforded only unchanged (92%) starting material.

Three tests[37] for the aldehyde grouping (as in formula X) were applied[38] to each of the four compounds, podophyllotoxin (I), picropodophyllin (III), podophyllotoxone (II) and picropodophyllone (IV). Tests with Benedict reagent in water containing a minimum of alcohol, either after 20 minutes at room temperature or after 5 minutes at the boiling point, were uniformly negative. Tests with methone using alcohol or aqueous alcohol solvent either with or without heating were also uniformly negative.

Tollens' silver nitrate test was inconclusive. With a minimum of acetone to bring the compounds into solution, the following results were obtained. Podophyllotoxin (I) at room temperature for 20 minutes gave no blackening, but did show a positive test after 4 minutes at 40°. Picropodophyllin (III) showed the most rapid reaction, blackening being apparent after 1 minute at room temperature. Podophyllotoxone (II) gave blackening after 7 minutes at room temperature. Picropodophyllone (IV) gave blackening as well as signs of a silver mirror after 5 minutes at room temperature. With a minimum of absolute alcohol and the tests performed at room temperature, the following results were obtained. After 15 minutes, podophyllotoxin (I) darkened only very faintly. Picropodophyllin (III) did not darken over the same period, and darkened only slightly after 1 hour. Podophyllotoxone (II) produced definite blackening after 15 minutes and a mirror after 20 hours. Picropodophyllone (IV) produced a slight blackening—more than with podophyllotoxone—after 15 minutes, and a mirror after 1 hour. All solvent blanks were negative.

Schiff's test[37,38] was performed at room temperature by mixing 2 ml. of Schiff reagent with 1.5 ml. of a 2.4×10^{-5} molar methanolic solution of piperonal, podophyllotoxone (II), picropodophyllone (IV) and keto ethyl ester XI.[10] Piperonal gave a definite pink after 1 minute, a dark pink after 2 minutes, and a dark violet after 5 minutes. None of the other compounds could be distinguished from the blank (which gradually acquired a faint pink hue) even after 15 minutes.

Picropodophyllone (IV) from Picropodophyllin (III).—A mixture of picropodophyllin (8.0 g. or 0.019 mole), 44 g. of finely divided manganese dioxide[4] and 300 ml. of chloroform was boiled for 75 minutes. Removal of inorganic solids and then of solvent left a residue, which was crystallized twice from methanol (Darco decolorizing carbon was used) to give 4.5 g. (56%) of picropodophyllone (IV) as colorless needles, m.p. 150°. Another crystallization brought the melting point to 153–154°.

Anal. Calcd. for $C_{22}H_{20}O_8$: C, 64.07; H, 4.89. Found: C, 64.1; H, 5.0.

The picropodophyllone showed optical rotation at room temperature of $[\alpha]_D$ −142° (*c* 0.83 in chloroform), and ultraviolet absorption maxima in a 3.94×10^{-5} *M* alcoholic solution at 209 mμ (log ε 4.67), 240 mμ (log ε 4.42), 279 mμ (log ε 3.95) and 32 mμ (log ε 3.91). No infrared absorption peaks appeared in the hydroxyl region (mineral oil mull); carbonyl peaks were present at 5.66 and 5.98 μ.

When the reflux period was extended to 2.5 hours, the yield of picropodophyllone was unchanged. When the oxidation was carried out by stirring the mixture at room temperature for 9 days, the yield was 66%. Attempts to oxidize picropodophyllin to picropodophyllone with aluminum isopropoxide in benzene–acetone,[7] or with potassium *t*-butoxide–benzophenone gave only unchanged starting material.

Zinc Borohydride.—A mixture of "Reagent" grade anhydrous zinc chloride (4.0 g. or 0.029 mole) with 50 ml. of ether (distilled from lithium aluminum hydride) was boiled until most of the solid had dissolved. The mixture was allowed to stand, and the supernatant liquid was carefully decanted from 0.4 g. of insoluble material. The ethereal zinc chloride solution was added dropwise at room temperature to a stirred suspension of 2.7 g. of 96% sodium borohydride (0.069 mole) in 150 ml. of absolute ether. Stirring was continued overnight. The solids were allowed to settle, and the liquid was removed by decantation and if necessary clarified by centrifugation. This ethereal solution of zinc borohydride was stored in a stoppered bottle at 5°. An aliquot treated first with dilute nitric acid and then with silver nitrate solution gave no precipitate of silver chloride.

Podophyllotoxin (I) from the Zinc Borohydride Reduction of Podophyllotoxone (II).—A solution of 230 mg. (2.4 millimoles) of zinc borohydride in 15 ml. of ether was added to a solution of podophyllotoxone (100 mg. or 0.24 millimole) in 5 ml. of dry benzene and 20 ml. of dry ether. After 24 hours at room temperature, the mixture was treated with 4 ml. of water and, when effervescence had abated, with 1.5 ml. of glacial acetic acid in 4 ml. of water. The ether layer was separated, and the aqueous layer, diluted with water to a volume of 30 ml., further extracted with chloroform. The combined chloroform and ether solutions were washed twice with water, were dried with magnesium sulfate, and were taken to dryness in a stream of dry air at room temperature. The residue was dissolved in 3 ml. of benzene, and 1 ml. of ether saturated with water was added. The resulting white, needle-like crystals (94 mg. or 82%), m.p. 109–116° with effervescence, were collected and crystallized as before to give the water–benzene complex of podophyllotoxin (I), m.p. 110–113° with effervescence and with softening at 108°, and $[\alpha]_D$ −115.5° (*c* 0.91 in chloroform).

To confirm the identity of the reduction product, its acetate was prepared as follows. Acetic anhydride (2 ml.) containing 41 mg. of the above product was warmed on the steam-bath for 3 hours and then allowed to stand at room temperature overnight. The mixture was poured into water, and the crystalline precipitate was collected, and dried *in vacuo* over phosphorus pentoxide. The podophyllotoxin acetate on crystallization from methanol was obtained as colorless rods, m.p. 203–204° and $[\alpha]_D$ −138.5° (*c* 0.8 in chloroform).

Anal. Calcd. for $C_{24}H_{24}O_9$: C, 63.15; H, 5.30. Found: C, 62.9; H, 5.4.

The mixture melting point with authentic podophyllotoxin acetate (m.p. 204–205°, $[\alpha]_D$ −137°) was 203–204°.

Picropodophyllin (III) from the Zinc Borohydride Reduction of Picropodophyllone (IV).—Picropodophyllone (200 mg. or 0.49 millimole) was dissolved in 10 ml. of dry benzene. To this was added 20 ml. of dry ether followed by a solution of zinc borohydride (approximately 600 mg. of 6.3 millimoles) in 40 ml. of ether. More benzene (10 ml.) was added to dissolve some of the picropodophyllone that had deposited, and the mixture was allowed to stand at 20° for 48 hours.

Excess reagent was destroyed by dropwise addition of acetic acid until the evolution of hydrogen ceased. The ether solution was then washed several times with water, with saturated aqueous sodium bicarbonate solution, and finally with water. After drying with magnesium sulfate, the solution was stripped of solvent by warming on the steam-bath. Crystallization of the white solid residue from methanol gave 35 mg. (17%) of picropodophyllin, m.p. 215–220°. Recrystallization from the same solvent gave fine needles, which melted alone or admixed with authentic picropodophyllin (m.p. 220–222°, $[\alpha]_D$ 7° (*c* 1.0 in chloroform)) at 221–223°. The optical rotation was $[\alpha]_D$ 8.7° (*c* = 0.75 in chloroform). The infrared absorption curves of the product and of authentic picropodophyllin were identical.

In a second similar experiment, the zinc borohydride reduction of picropodophyllone (IV) gave recrystallized picropodophyllin, m.p. 219–221°, in 28% yield.

Confirmation of the identity of the reduction product was obtained by preparing its acetyl derivative. A mixture of 0.8 ml. of acetic anhydride, 1 ml. of pyridine and 13 mg. of picropodophyllin (III) from picropodophyllone was allowed to stand for 2 days at room temperature. The mixture was hydrolyzed and the product was isolated in the usual way. The acetate of picropodophyllin was obtained after crystallization from methanol; m.p. 211–212° and $[\alpha]_D$ 21.4° (*c* 0.51 in chloroform).

Anal. Calcd. for $C_{24}H_{24}O_9$: C, 63.15; H, 5.30. Found: C, 63.0; H, 5.5.

When the acetate was mixed with authentic picropodo-

(37) *Cf.* A. I. Vogel, "Elementary Practical Organic Chemistry, Part II, Qualitative Organic Analysis," Longmans, Green and Co., Inc., New York, N. Y., 1957.

(38) We are indebted to Dr. B. M. Tursch and to Mr. R. G. McInnis for their kindness in performing these tests.

phyllin acetate (m.p. 212–213°), the melting point was 212–213°.

Action of Podophyllotoxone (II) with Sodium Borohydride.—A solution of 0.5 g. of sodium borohydride (0.0132 mole) in 10 ml. of absolute ethanol at 0° was added to a solution of 0.2 g. (0.0048 mole) of podophyllotoxone (II) in 8 ml. of absolute ethanol at 35°. The mixture was allowed to stand at room temperature for 18 hours.

The reaction mixture was poured into ice (20 g.) and water (50 ml.), and 30% acetic acid was added dropwise until no more gas was evolved. The flocculent precipitate was extracted with ether and with chloroform, and the combined extracts were washed twice with water. Removal of solvent left a solid residue, which could be crystallized from alcohol–water, from methanol–water or from methanol. The product V was obtained in the form of fine long needles (86 mg.), m.p. 202–204° (softens 198°, effervescence). Further crystallization gave material with a melting point of 216–217° (softens 212°) and a rotation of $[\alpha]_D$ −19.8° (*c* 1.0 in chloroform).

Action of Picropodophyllone (IV) with Sodium Borohydride.—When picropodophyllone (IV) was treated with sodium borohydride in a manner essentially the same as that described above for podophyllotoxone (II), the results were similar. Product V was obtained with m.p. 218–219° (soften 213°) and $[\alpha]_D$ −19.2° (*c* 0.68 in chloroform). In one experiment, 0.12 g. of this material was obtained from 0.8 g. of picropodophyllone.

Anal. Calcd. for $C_{22}H_{24}O_8$: C, 63.45; H, 5.81; neut. equiv., 416.4. Found: C, 63.8, 63.4; H, 5.8, 5.8; neut. equiv., 412.5.

The mixture melting point with the same product derived from podophyllotoxone was 215–217°. In absolute ethanol solution, ultraviolet absorption maxima were evident at 210 mμ (log ε 4.64) and 292 mμ (log ε 3.61) and an inflection point at 245 mμ (log ε 3.96). The infrared absorption curve had peaks at 3.01, 5.82 and 6.06 μ (mineral oil mull). Product V dissolved in sodium bicarbonate solution, with release of carbon dioxide.

Methylation and acetylation to VI was effected as follows. Sixty milligrams of the compound was added to a solution of diazomethane (excess) in ether. The crude methylated product was freed of volatile material and was allowed to stand at room temperature for 2 days in a mixture of 3 ml. of dry pyridine and 1.5 ml. of acetic anhydride. Water (18 ml.) was added, and the flocculent precipitate was collected, washed with water, and dried *in vacuo* over phosphorus pentoxide. Two crystallizations from methanol afforded methylated acetylated derivative VI in the form of long white needles, m.p. 187°, $[\alpha]_D$ −98.6° (*c* 0.61 in chloroform). The infrared absorption curve taken with a mineral oil mull showed a peak at 5.78 μ, but none attributable to ketone carbonyl or to hydroxyl.

Anal. Calcd. for $C_{25}H_{28}O_9$: C, 63.55; H, 5.97. Found: C, 63.74; H, 6.14.

Structures V and VI are consistent with, but are not uniquely determined by, these data. For example, the hydroxyl group in V *a priori* could be shifted from the ring to the methyl group. The presence of an ethylenic bond, while not excluded by the carbon and hydrogen analyses, receives no support from the infrared or the ultraviolet absorption curves.

Dehydropodophyllotoxin (VIII) by Selenium Dioxide Aromatization of Podophyllotoxone (II) or Picropodophyllone (IV).—Selenium dioxide (28 mg. or 0.25 millimole of 99.5% purity) was dissolved in 6 ml. of boiling acetic acid that previously had been boiled with and distilled from selenium dioxide. Podophyllotoxone (202 mg. or 0.48 millimole) was added, and the mixture was boiled for 90 minutes. The reaction mixture, after standing at room temperature for a day, was poured into 30 ml. of water and the precipitated solids collected. Two crystallizations from ethanol including a treatment with decolorizing carbon (Darco) furnished 70 mg. of dehydropodophyllotoxin (VIII), m.p. 283–285° dec. The mixture melting point with the same material derived from picropodophyllone (see below) was 284–285° dec.

Dehydropodophyllotoxin could also be prepared by treating picropodophyllone (288 mg. or 0.70 millimole) in acetic acid with selenium dioxide (40 mg. or 0.36 millimole) in a manner similar to that described above. The crystallized product (115 mg.) appeared as plates melting at 283–287° dec. after preliminary softening and discoloration. A sample for analysis, crystallized from ethanol and dried *in vacuo* at 100° for 14 hours, melted in an evacuated capillary at 286–288° (softening at 275°, dec.).

Anal. Calcd. for $C_{22}H_{18}O_8$: C, 64.39; H, 4.42. Found: C, 64.2; H, 4.4.

Dehydropodophyllotoxin (VIII) did not dissolve in 10% aqueous sodium hydroxide solution, nor did it give color with ferric chloride solution. Infrared absorption peaks (mull) were noted at 2.91 and 5.69 μ. A series of ultraviolet absorption maxima for dehydropodophyllotoxin in 4×10^{-5} *M* alcohol solution was: 210 mμ (log ε 4.55), 226 mμ (log ε 4.49), 263 mμ (log ε 4.62), 269 mμ (shoulder, log ε 4.61), 312 mμ (shoulder, log ε 3.98), 323 mμ (log ε 4.02) and 356 mμ (log ε 3.72).

Dehydropodophyllotoxin did not melt cleanly, and the melting point behavior depended on the manner in which the melting point was taken. For illustration, a detailed description of a melting point determination follows. The sample in an open capillary tube was immersed in a bath at 260°, and the temperature of the bath was raised 5° per minute. The first sign of discoloration and shrinking was noted at 268°. Dark spots appeared in the sample at 273°. The material became brown at 278°, brown-black at 280°, and melted finally to a dark, very viscous liquid at 280–282°.

Other dehydrogenating agents tried unsuccessfully include lead tetraacetate in acetic acid, chloranil in boiling xylene, and bromine followed by dimethylaniline. Picropodophyllone was inert to chloranil, and could be recovered. No pure product was isolated from the lead tetraacetate reaction or from the bromination–dehydrobromination sequence.

Dehydropodophyllotoxin Acetate (IX).—A mixture of dehydropodophyllotoxin (80 mg.), pyridine (4 ml.) and acetic anhydride (3 ml.) was heated on the steam-bath for 12 hours, and then poured in water (40 ml.). The hydrolysis mixture was extracted with two 50-ml. portions of chloroform. The chloroform extracts were washed with 100 ml. of 10% hydrochloric acid, with water, and with saturated aqueous sodium bicarbonate solution, and were finally dried with sodium sulfate. The solvent was removed, and the residue recrystallized three times from chloroform–alcohol. Dehydropodophyllotoxin acetate (IX) was obtained in this way with m.p. 264–265° (softens 263°) with discoloration. The infrared absorption curve (mull) showed a broad, evidently unresolved, band at 5.65–5.69 μ, but no peak in the hydroxyl region. A 3.1×10^{-5} *M* solution of the acetate in 95% alcohol showed ultraviolet absorption maxima at 260 mμ (log ε 4.72), 313 mμ (log ε 3.99) and 347 mμ (log ε 3.62).

Anal. Calcd. for $C_{24}H_{20}O_9$: C, 63.71; H, 4.46. Found: C, 63.70; H, 4.6.

Comparison of Synthetic Dehydropodophyllotoxin (VIII) with the Same Material from Podophyllin.[25]—Direct comparisons of dehydropodophyllotoxin (VIII) and its acetate IX with the corresponding compounds derived from podophyllin were made by Dr. Helmer Kofod, who furnished the following information.[27]

Dehydropodophyllotoxin from podophyllin, synthetic material VIII, and a mixture of the two all behaved identically on the Kofler hot-stage. When the samples were brought quickly to 250° and the temperature then raised at a rate of 2° per minute, they melted at 275–280° dec. after becoming yellow-brown at 275° (all temperatures corrected).

A second set of the same three materials behaved identically when the melting points were determined in an open capillary tube. The samples were introduced into the bath at 260°, and the temperature was then raised 2° per minute. Melting was noted at 280–285° dec. with preliminary softening at 270°.

Synthetic dehydropodophyllin acetate (IX) when heated quickly to 230° on the hot-stage, and then heated further so that the temperature rose at a rate of 2° per minute, showed m.p. 255–258° dec. with softening at 250°. Under the same conditions, the acetylated material from podophyllin showed m.p. 250–258° with discoloration beginning at 240°.

Paper chromatography comparisons were made using benzene as the mobile phase and Whatman paper (No. 1) impregnated with formamide as the stationary phase. Fluorescence under ultraviolet light served to locate the spots. Natural and synthetic dehydropodophyllotoxin had the same R_f values. The corresponding acetyl derivatives,

which traveled much faster than dehydropodophyllotoxin, also showed identical R_f values.

Ultraviolet absorption data for natural dehydropodophyllotoxin in 96% alcohol were taken from a small-scale curve supplied by Dr. Kofod. In good agreement with synthetic material VIII, the natural material showed maxima at 225 mμ (log ε 4.4), 262 mμ (log ε 4.6), 311 mμ (shoulder, log ε 3.95), 321 mμ (log ε 4.0) and 358 mμ (log ε 3.7).

Natural dehydropodophyllotoxin is almost insoluble in base, and gives no color with ferric chloride.

The Catalytic Dehydrogenation of Picropodophyllone (IV).—Biphenyl (4.5 g.) containing 0.60 g. of picropodophyllone (IV) and 0.39 g. of 10% palladium-on-carbon was boiled for 2 hours. Acetone (30 ml.) was added, the mixture was filtered and the filtrate was distilled first at atmospheric and then at reduced pressure (*ca.* 1 mm.) to remove acetone and biphenyl. The residual gum was sublimed at 100° (1 mm.) and then at 210° (5×10^{-4} mm.). The material (224 mg.) collected at the higher temperature was recrystallized twice from methanol (decolorizing carbon was used) to give 120 mg. of shiny, faintly pink plates, m.p. 184°. A solution of this material in 20 ml. of benzene was allowed to flow through a column of acid-washed alumina (5 g.). Elution with 10 ml. of benzene failed to remove any solid material. Elution with 75 ml. of ether–benzene (3:7), however, did bring down product, which when isolated and crystallized from methanol appeared as white plates or needles, m.p. 178–179°. A second crystallization did not change the melting point. Drying this product, tentatively considered to be 2-methyl-4-(trimethoxyphenyl)-6,7-methylenedioxy-1-naphthol (VII), at 100° over phosphorus pentoxide for 3 hours brought the melting point to 182–184°.

Anal. Calcd. for $C_{21}H_{20}O_6$: C, 68.47; H, 5.47. Found: C, 68.5; H, 5.5.

Naphthol VII was insoluble in saturated sodium bicarbonate solution. Although aqueous ferric chloride gave no color, the presence of hydroxyl was indicated by an infrared absorption peak (mineral oil mull) at 2.87 μ. No infrared absorption was evident in the carbonyl region. A 2.46×10^{-5} *M* solution in 95% alcohol showed ultraviolet absorption maxima at 210 mμ (log ε 4.66), 256 mμ (log ε 4.64) and 305 mμ (log ε 3.97).

Addition of Methyl Grignard Reagent to Podophyllotoxone (II).—Podophyllotoxone (1.0 g. or 2.4 millimoles) was dissolved in a boiling mixture of 30 ml. of benzene and 20 ml. of ether. Both solvents were pure and dry; the benzene had been distilled from lithium aluminum hydride just before use. An atmosphere of dry oxygen-free nitrogen was maintained over the reaction mixture throughout the experiment. An ethereal solution of excess methylmagnesium bromide (5.19 millimoles in 66 ml.) was added dropwise during the course of 30 minutes to the boiling, magnetically stirred podophyllotoxone solution. Deposition of a yellow crystalline solid was noted. After stirring and heating the mixture for 5 hours, it was allowed to stand at room temperature for 12 hours.

Acetic acid (2 ml.) in water (8 ml.) was added. The upper organic layer was separated, diluted with 20 ml. of ether, and washed successively with 20-ml. portions of water, saturated aqueous sodium bicarbonate solution and (twice) water. The organic layer was dried with sodium sulfate, and then freed of solvent by warming on the steam-bath under reduced pressures. The residual pale-yellow gum was dissolved in 100 ml. of absolute ether, and the solution after concentration to a volume of 20 ml. was allowed to stand at room temperature. The precipitated crystals (0.29 g., m.p. 198–205° with softening at 191°) were recrystallized from ether to give 0.22 g. (21%) of methylpodophyllotoxin-A (XIII), m.p. 208–209°. The analytical sample melted at 210–212° and showed $[\alpha]_D$ −114° (*c* 1.22 in chloroform).

Anal. Calcd. for $C_{23}H_{24}O_8$: C, 64.48; H, 5.65. Found: C, 64.4; H, 5.5.

An ultraviolet absorption maximum in 3.5×10^{-5} *M* solution in 95% alcohol was noted at 290 mμ (log ε 3.65); an inflection point appeared at 242 mμ (log ε 4.01). A mineral oil mull of the product showed infrared absorption peaks at 2.85 and 5.65 μ, but none corresponding to tetralone carbonyl. The lactonic absorption peak shifted to 5.61 μ when the product was dissolved in methylene chloride.

The ethereal mother liquors from methylpodophyllotoxin-A were combined, and were taken to dryness on the steam-bath. The residual gum (0.79 g.) dissolved in 50 ml. of benzene was passed through a chromatography column (26 × 1.5 cm.) made up of 30 g. of Merck acid-washed alumina. The column was eluted with the following solvents, each 25 ml. of eluate being collected separately and examined: 150 ml. of benzene, 150 ml. of 9:1 benzene–ether, 50 ml. of 4:1 benzene–ether, 300 ml. of 1:1 benzene–ether, and finally benzene–ether and benzene containing increasing proportions of methanol. Some starting material was recovered from the 9:1 benzene–ether fractions. The combined crystalline fractions (0.23 g.) recovered from the 1:1 benzene–ether eluates were recrystallized from ether to give 0.12 g. of a new product, m.p. 129–131° (effervescence). Two further recrystallizations from ether gave 0.05 g. of methylpodophyllotoxin-B (XIII), m.p. 130–131° (effervescence), and $[\alpha]_D$ −103° (*c* 1.06 in chloroform). A 3.5×10^{-5} *M* solution in 95% alcohol showed $\lambda_{infl.}$ 245 mμ (log ε 4.01) and λ_{max} 290 mμ (log ε 3.69). A mineral oil mull had an infrared absorption peak at 2.85 μ and twin peaks at 5.67 and 5.71 μ. The compound in chloroform solution showed the *trans*-lactone absorption peak at 5.63 μ.

Anal. Calcd. for $C_{23}H_{24}O_8$: C, 64.48; H, 5.65. Found: C, 64.1; H, 5.7.

Non-hydroxylic Products from the Reaction of Methylmagnesium Bromide with Podophyllotoxone (II).—In another experiment, using 2 g. of podophyllotoxone, the molar ratio of Grignard reagent to ketone was taken at 5:4 instead of 2:1. The total crude oily products, from which all acidic material had been removed by washing with aqueous bicarbonate, was dissolved in acetonitrile, and the solution was diluted with ether. Substance-a deposited as a crystalline solid (36 mg.), m.p. 234–237°.

After concentration of the mother liquor under reduced pressure, the supernatant liquid containing a suspended solid was decanted from the lower heavy layer of oil. Filtration of the decanted portion gave 0.39 g. of material melting close to 100°. This solid, dissolved in benzene, was placed on a 30 × 2.2 cm. column of neutral alumina (Merck). More benzene was used for elution. Removal of all solvent from the first eluate fraction left a residue, which on slow crystallization from alcohol yielded 31 mg. of substance-b, m.p. 164–168°. A further quantity of substance-b (18 mg.), m.p. 161–168°, could be isolated from the second eluate fraction.

The chromatography column was rinsed with methanol, all methanol was removed from the eluate, and the residue was combined with the above-mentioned solvent-free heavy oil. This mixture in benzene solution was placed on a column of neutral alumina (Merck). Benzene, then benzene containing increasing proportions (up to 75%) of ether, and finally benzene containing increasing proportions (up to 10%) of methanol, was passed through the column. The only crystalline material isolated in significant amounts came through in the first fractions with pure benzene. The properties of the white crystals (0.33 g.), which melted at 155–159° and which showed infrared absorption peaks at 5.69 and 5.93 μ (in mineral oil mull), corresponded to those of picropodophyllone (IV).

Substance-a, after crystallization in acetonitrile (decolorizing carbon was used), showed m.p. 242–243.5°.

Anal. Calcd. for $C_{23}H_{20}O_7$ (*cf.* XVII): C, 67.64; H, 4.94. Found: C, 66.7; H, 5.7.

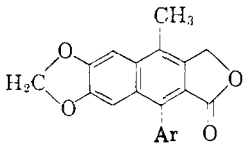
Substance-a in a mineral oil mull revealed no infrared hydroxyl group absorption, but did show a lactone peak at 5.63 μ. An isopropyl alcohol solution furnished an ultraviolet absorption curve—with maxima at 254 mμ (log ε 4.2), 259 mμ (log ε 4.2), 293 mμ (log ε 3.7), 314 mμ (shoulder, log ε 3.5) and 353 mμ (log ε 3.1)—that was reminiscent of the curve for dehydroanhydropicropodophyllin.[11]

Accordingly, the structure of methyldehydroanhydropicropodophyllin (XVII) was assigned provisionally to substance-a. This naphthalene compound could be formed by disproportionation of methyl-α-apopicropodophyllin (XVIII), as suggested by the behavior of related compounds,[39] or by autoxidation.

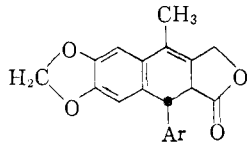
Substance-b was obtained as white needles or as prisms. Attempts to purify the material further by crystallization failed. In mineral oil mull, substance-b gave no infrared absorption band corresponding to hydroxyl group, but did show a lactonic absorption at 5.68 μ. The ultraviolet absorption spectrum taken in isopropyl alcohol solution showed

(39) J. Jacques and H. B. Kagan, *Bull. soc. chim. France*, 128 (1956)

λ_{max} 264.5 mμ (log ε 4.1) and 308.5 mμ (log ε 3.78). A tentative formulation of substance-b as methyl-α-apopicropodophyllin (XVIII) is suggested.



XVII



XVIII

Pyrazoline XIV from the Reaction of Podophyllotoxone (II) and Phenylhydrazine.—Phenylhydrazine (1.52 g. or 0.014 mole) was added to a solution of podophyllotoxone (4.0 g. or 0.0097 mole) in 8 ml. of glacial acetic acid. The mixture was warmed on the steam-bath for 10 minutes and then set aside for 15 hours. Addition of water precipitated a yellow solid, which was collected, washed with aqueous sodium bisulfite solution and with water, and then dried in the air for a day. Crystallization of this product (5.07 g.) from methanol afforded a total of 4.6 g. (91%) of pyrazoline XIV in two crops, both of which were dried at 100° *in vacuo*. The first crop (4.2 g.) showed m.p. 148–161°, the second, m.p. 151–163°. Different samples of this material in carbon tetrachloride solutions showed practically identical infrared absorption spectra, though the samples melted differently, and over a range.

A 1:1 hydrate was formed from the above-mentioned first crop by placing the solid in a vial next to a beaker of water, and covering both vial and beaker with an inverted beaker. Since the dry or partially dry product is bright yellow and the hydrate is pale yellow, the course of hydration could be followed conveniently by observing the progress of a zone of pale yellow color moving steadily downward into the vial. After one day the solid was entirely pale yellow, and the hydration was considered to be complete. The monohydrate, m.p. 153–156° with complete liquefaction at 176°, showed $[\alpha]^{24}D$ −263° (*c* 1.0 in 95% alcohol).

Anal. Calcd. for $C_{28}H_{26}N_2O_7 \cdot H_2O$: C, 64.60; H, 5.42; N, 5.38; H_2O, 3.46; neut. equiv., 520.5. Found: C, 64.9; H, 5.5; N, 5.6; weight loss on drying *in vacuo* for several hours at 110–130°, 3.5%; neut. equiv., 526.5, 526.5.

The monohydrate, as well as various non-stoichiometric hydrates of pyrazoline XIV encountered, was readily soluble in aqueous sodium bicarbonate solution. The pyrazoline, either in the crystalline state or in dilute benzene solution, fluoresced a brilliant blue under ultraviolet light. The methanol solution did not fluoresce. Addition of a few crystals of sodium nitrate to the green-yellow solution of pyrazoline XIV in cold concentrated sulfuric acid resulted in gradual deepening of the color to blue-violet. The pyrazoline in carbon tetrachloride solution did not show the infrared absorption peaks at 5.63 and 5.98 μ characteristic of the lactone and tetralone carbonyl groups of podophyllotoxone, but did show a new peak at 5.84 μ.

Methyl Ester XV of Pyrazoline XIV.—An ethereal solution (*ca.* 15 ml.) of diazomethane prepared from 1 g. of nitrosomethylurea and dried over solid potassium hydroxide was slowly added to a swirled, cold solution of 1.5 g. of pyrazoline XIV in 15 ml. of ether. The mixture was allowed to stand cold for 15 hours. The crystalline solids were collected and washed with a little cold ether. This product (1.5 g., m.p. 199.5–202.5°) was crystallized from benzene–methanol to give cream-colored crystals (1.46 g., 94%) of the methyl ester XV, m.p. 202–204°.

Anal. Calcd. for $C_{29}H_{28}N_2O_7$: C, 67.43; H, 5.46; N, 5.42. Found: C, 67.3; H, 5.5; N, 5.6.

The fluorescence behavior of the methyl ester under ultraviolet light was the same as that described above for the precursor pyrazoline-acid XIV. The methyl ester gave the same color test with sodium nitrite in concentrated sulfuric acid as the acid (see above). The infrared absorption spectrum taken with the methyl ester in carbon tetrachloride solution showed a strong peak at 5.73 μ.

Bromination of Pyrazoline Methyl Ester XV.—A solution of 0.53 millimole of bromine in 1.7 ml. of chloroform was added dropwise over a period of 10–15 minutes to a cold (−20°) solution of 0.258 g. of pyrazoline methyl ester (0.50 millimole) in 3–4 ml. of chloroform. The dark blue solution became yellow-brown after 1 hour at −20°. After 2.5 hours at −20°, the reaction mixture was allowed to come gradually to room temperature. The solvent was removed, and the oily residue was crystallized from benzene containing a little methanol. The heavy, pale green crystals weighed 0.21 g., and melted at 193–195° (gas evolution) with softening at 189°. A second crop (0.05 g.) showed m.p. 185–188° dec.

The material in the first crop was dissolved in 30 ml. of benzene and placed on a chromatography column (45 × 10 mm.) containing 4 g. of neutral alumina, Brockmann Grade III. Elution with benzene removed most of the material; a strongly adsorbed yellow zone was left at the top of the column. The benzene eluate showed a brilliant blue fluorescence under ultraviolet light. The bromo derivative XVI in the eluate, after crystallization from benzene–methanol, weighed 0.19 g. (60% yield) and showed m.p. 194–196° dec.

Anal. Calcd. for $C_{29}H_{27}N_2O_7Br$: C, 58.49; H, 4.57; N, 4.71; Br, 13.42. Found: C, 58.6; H, 4.3; N, 4.5; Br, 12.7.

The green-yellow color of a solution of this compound XVI in sulfuric acid at 0° changed on addition of a little sodium nitrite to deep violet and then to deep blue-violet. A carbon tetrachloride solution showed an intense infrared absorption peak at 5.74 μ, but none in the 14–14.5 μ region.

The above experiment arbitrarily combined equimolar amounts of bromine and pyrazoline. Other proportions would undoubtedly give different products; preliminary titration showed that pyrazoline XV rapidly consumed more than 2 moles of bromine. Podophyllotoxin (I) under the same conditions reacted only slowly with bromine.