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

Compounds Related to Podophyllotoxin. XIV. Isopodophyllotoxin and Epiisopodophyllotoxin[1]

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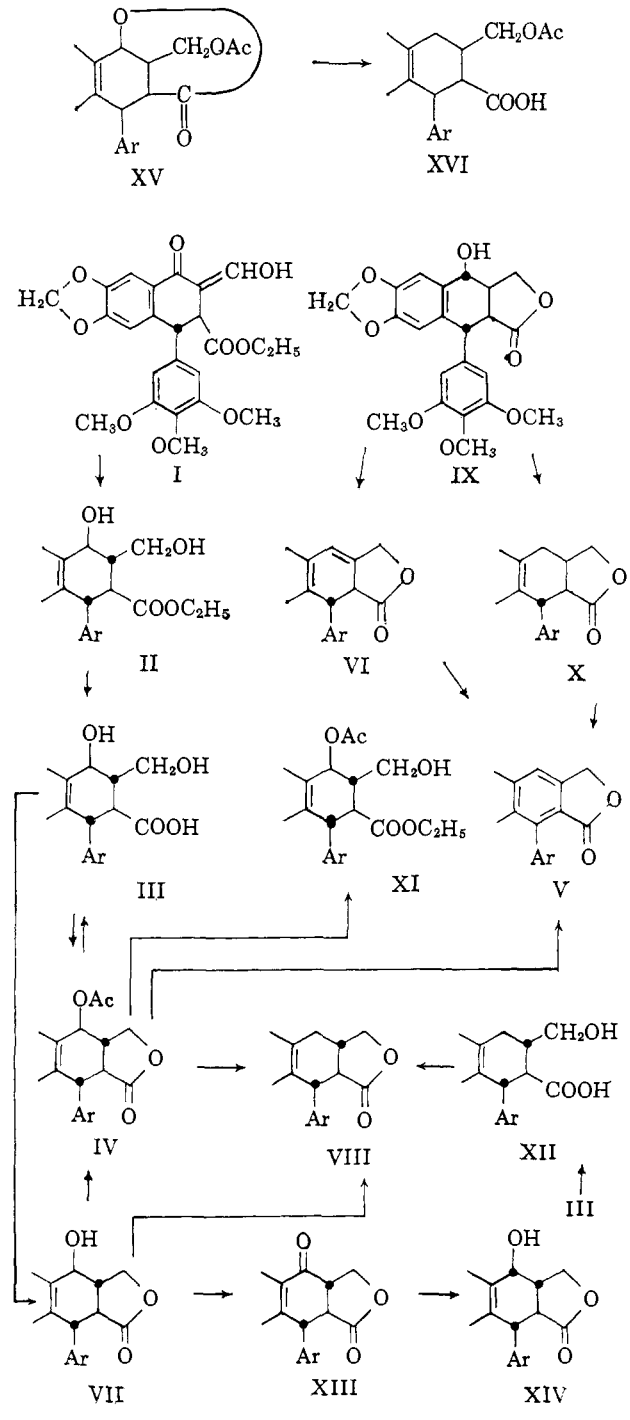
Lactonization of 1-(trimethoxyphenyl)-3-hydroxymethyl-4-hydroxy-6,7-methylenedioxy-1,2,3,4-tetrahydro-2-naphthoic acid with dicyclohexylcarbodiimide gave isopodophyllotoxin. Lactonization with acetic anhydride gave the corresponding acetate. Hydrolysis of this acetate lactone not only removed the acetate group but also opened the lactone ring to regenerate unchanged acidic starting material. Aromatization of the acetate with palladium at elevated temperatures produced dehydroanhydropicropodophyllin, which was prepared for comparison from α-apopicropodophyllin as well as from desoxypicropodophyllin. The nature of the lactone system and the hydroxyl group in isopodophyllotoxin is proved by its oxidation with manganese dioxide to isopodophyllotoxone, a tetralone lactone. The stereochemistry at three of the four centers of asymmetry is established by hydrogenolysis of the hydroxyl group and identification of the resulting desoxy compound as (*trans,trans*) desoxyisopodophyllotoxin. The configurations of the hydroxyl group in isopodophyllotoxin and in the stereoisomeric epiisopodophyllotoxin derived from isopodophyllotoxone by reduction have been assigned by Schreier on the basis of n.m.r. studies.

The dihydroxy ester II,[2] formed by sodium borohydride reduction of hydroxymethylene derivative I, not only provided a key intermediate in recent syntheses of picropodophyllin (IX)[3] and podophyllotoxin,[4] but also opened the way to two new stereoisomers.[5,6] The present paper discusses the formation, structure, and stereochemistry of these new compounds (*cf.* VII and XIV).

Preparation and Structure.—The acetate IV of one of the stereoisomers was obtained smoothly by lactonization of the dihydroxy acid III corresponding to ester II with acetic anhydride. The structure of acetate IV is consistent with its conversion over hot palladium to dehydroanhydropicropodophyllin (V), which was obtained for comparison by dehydrogenating α-apopicropodophyllin (VI)[7] with selenium dioxide as well as by aromatizing desoxypicropodophyllin (X) with sulfur.

Regeneration of dihydroxy acid III from acetate IV by mild hydrolysis showed that no isomerization had occurred. However, acetate IV proved not to be useful in preparing the desired hydroxy lactone VII, since the conditions that removed the acetyl group also opened the lactone ring. A direct and straightforward way of forming hydroxy lactone VII was found in the cyclization of dihydroxy acid III with dicyclohexylcarbodiimide.[8] The possibility of a change in stereochemistry during the carbodiimide reaction[9] could be dismissed since acetylation of hydroxy lactone VII gave acetate IV.

Evidence that lactones IV and VII have the oxygen of the primary alcohol in the ring and not that of the secondary alcohol (as for example in XV) was obtained by hydrogenolysis of acetate IV to an acetate-free lactone VIII. If lactonization had occurred on the sec-



(1) This work was supported by a PHS research grant from the National Cancer Institute, United States Public Health Service (CY-2891).

(2) With some obvious exceptions, the compounds referred to in this paper are racemic.

(3) W. J. Gensler, C. M. Samour, S. Y. Wang, and F. Johnson, *J. Am. Chem. Soc.*, **82**, 1714 (1960).

(4) *Cf.* W. J. Gensler and C. D. Gatsonis in paper No. XIII in this series [*J. Am. Chem. Soc.*, **84**, 1748 (1962)].

(5) J. L. Hartwell and A. W. Schrecker[6] have reviewed the chemistry of picropodopyllin and its epimer, podophyllotoxin.

(6) J. L. Hartwell and A. W. Schrecker, *Progr. Chem. Org. Nat. Prod.*, **15**, 83 (1958).

(7) A. W. Schrecker and J. L. Hartwell, *J. Am. Chem. Soc.*, **74**, 5676 (1952).

(8) *Cf.* R. B. Woodward, F. E. Bader, H. Bickel, A. J. Frey, and R. W. Kierstead, *Tetrahedron*, **2**, 1 (1958); W. S. Johnson, V. J. Bauer, J. L. Margrave, M. A. Frisch, L. H. Dreger, and W. N. Hubbard, *J. Am. Chem. Soc.*, **83**, 606 (1961).

(9) Epimerization at enolizable positions by dicyclohexylcarbodiimide has been reported by K. Hofmann, M. E. Woolner, G. Spühler, and E. T. Schwartz, *ibid.*, **80**, 1486 (1958); G. W. Anderson and F. W. Callahan, *ibid.*, **80**, 2902 (1958); H. Schwarz and F. M. Bumpus, *ibid.*, **81**, 890 (1959).

ondary alcohol, hydrogenolysis of the lactone acetate XV would be expected to cleave the lactone ring to give acetate acid XVI instead of VIII. Also directly pertinent to the lactone structures is the fact that manganese dioxide oxidized hydroxy lactone VII to keto lactone XIII showing ultraviolet and infrared absorptions characteristic of an α-tetralone but not of a tetralyl aldehyde.

Stereochemistry.—The stereochemistry at three of the four centers of asymmetry was determined by removing the hydroxyl group and examining the resulting desoxy lactone VIII. Hydrogenolysis of either hydroxy lactone VII or acetoxy lactone IV gave the desoxy compound VIII directly. Hydrogenolysis of dihydroxy acid III followed by cyclization of the resulting monohydroxy acid XII with acetic anhydride gave the same desoxy lactone. Comparison of lactone VIII with desoxyisopodophyllotoxin[10] showed that the two were the same. For example, the 256° melting point for racemic lactone VIII agreed well with the reported 251° melting point for desoxyisopodophyllotoxin. More important, the infrared solution spectrum of the racemic lactone was identical with that of desoxyisopodophyllotoxin and was significantly different from the spectra of the other isomers. With lactone VIII identified as the racemic form of desoxyisopodophyllotoxin, the *trans,trans* configuration could be assigned. Further evidence supporting the *trans*-lactone system in VIII was obtained on treating the precursor lactone IV with alcoholic sodium acetate. In a manner analogous to that observed with desoxyisopodophyllotoxin but not with the other stereoisomers,[10] the lactone ring in IV opened to give compound XI.

The possibility that elimination of water from hydroxy lactone VII (or of acetic acid from acetoxy lactone IV) occurred during the hydrogenolysis reaction to give α-apopicropodophyllin (VI), which then hydrogenated to desoxy compound VIII, could be ruled out. Hydroxy lactone VII on treatment with hot dilute hydrochloric acid showed no tendency to form olefinic unsaturation. Also pertinent is the resistance of acetoxy lactone IV to conversion to apopicropodophyllin under conditions (2 hr. in boiling acetic anhydride) harsher than those used for the hydrogenolyses (2 to 5 hr. in acetic acid at 60–75°). Even if the formation of apopicropodophyllin were possible here, the products to be expected would not be compatible with those actually observed. The hydrogenation of α-apopicropodophyllin is known to give twice as much (or more) *trans,cis*-lactone X as *trans,trans*-lactone VIII.[10] Yet our hydrogenolyses furnished *only* the *trans,trans* isomer VIII and this in yields as high as 52%.

A probable configurational assignment for the secondary hydroxyl position in XIV was arrived at originally[11] by drawing an analogy between the zinc borohydride reduction of keto lactone XIII to hydroxy lactone XIV and of podophyllotoxone to podophyllotoxin.[12] However, recent n.m.r. results[13] with the acetates of epimeric hydroxy lactones VII and XIV, by convincingly supporting the opposite configuration at the 4-position, raise serious doubts about the analogy. We see no grounds for questioning the n.m.r. conclusions and, therefore have adopted the more recent assignments for the 4-position—*viz.*, equatorial hydroxyl with *trans*-3,4-H's for VII and axial hydroxyl with *cis*-3,4-H's for XIV.

The above considerations serve to define two new podophyllotoxin stereoisomers which, according to the naming scheme used before,[6] may be called DL-isopodophyllotoxin (VII) and DL-epiisopodophyllotoxin (XIV). Keto lactone XIII may be called DL-isopodophyllotoxone.

Activity.—The acetate IV of DL-isopodophyllotoxin was submitted to the Cancer Chemotherapy National Service Center for anticancer screening. The material (NSC No. 15798) proved ineffective against sarcoma-180, adenocarcinoma-755, and leukemia-1210 in the mouse.

Experimental[14]

DL-Isopodophyllotoxin (VII) by Carbodiimide Cyclization of DL-Isopodophyllic Acid (III).—DL-Isopodophyllic acid (0.40 g. or 0.93 mmole) was dissolved in 80 ml. of boiling dioxane. To this solution at a temperature a little above room temperature was added 0.20 g. (0.97 mmole) of dicyclohexylcarbodiimide in 10 ml. of dioxane. The mixture was allowed to stand at room temperature for 48 hr. Solvent was removed from the heterogeneous mixture by distillation under, vacuum at 50°, and the dry white residue was boiled for 3–4 min. with 20 ml. of methanol. Insoluble material was collected by filtration, washed with cold methanol and with ether, and allowed to dry in the air. This solid (0.30 g. or 78%), m.p. 265°, was crystallized four times from dioxane–ethyl propionate to give analytically pure DL-isopodophyllotoxin (VII), m.p. 272°.

Anal. Calcd. for $C_{22}H_{22}O_8$: C, 63.76; H, 5.35. Found: C, 63.9; H, 5.6.

The observed melting point of DL-isopodophyllotoxin depends on the temperature of immersion and on the rate of heating. The melting points given here were determined by immersing the capillary sample at 200° and raising the temperature thereafter at a rate of 5° per minute. Slow decomposition is evident above 250°.

Since the product was insufficiently soluble in suitable solvents, the infrared absorption spectrum was taken with the material mulled with mineral oil. Two forms of the product, melting either separately or mixed at 272°, could be distinguished. One form with infrared absorption peaks at 5.63 and 2.85 μ was deposited from hot solutions of dioxane–propanol, dioxane–ethyl propionate, or dioxane. The second form, showing infrared peaks at 5.72 and 2.90 μ, crystallized from dioxane at room temperature or from methylene chloride–methanol. The two forms were interconvertible by employing the appropriate crystallization procedure. The temperature at which the crystals appeared may have been the factor determining the form. Occasionally, when a hot dioxane solution was cooled rapidly, both forms deposited together.

Attempts to lactonize dihydroxy acid III by heating it at 165° gave unchanged starting material. Higher temperatures led to mixtures containing the desired lactone VII plus either α- or β-apopicropodophyllin. Exposing the dihydroxy ester II to sodium hydride in boiling benzene gave only unchanged ester.[15] Long treatment with hot 10% sulfuric acid or with hot water removed two molecules of water from the hydroxy acid to form an unsaturated lactone.[3] Whether this last process occurred by lactonization followed by elimination or *vice versa* has not been determined.

Acetate (IV) of DL-Isopodophyllotoxin. A. By the Action of Acetic Anhydride on DL-Isopodophyllic Acid (III).—A mixture of DL-isopodophyllic acid (175 mg.) and 10 ml. of redistilled acetic anhydride was boiled for 2 hr. The solution was distilled to dryness *in vacuo* on the steam bath, and the white residue was dissolved in a relatively large volume of 6:1 methylene chloride–methanol. Concentration with progressive addition of methanol until the volume was about 20 ml. led to sudden crystallization. The mixture was allowed to stand. The needle-like crystals of the acetate IV of DL-isopodophyllotoxin, after collection and air drying, weighed 174 mg. and showed m.p. 264–265°. Recrystallization did not change the melting point. Infrared absorption peaks were noted at 5.77 and 5.62 μ.

Anal. Calcd. for $C_{24}H_{24}O_9$: C, 63.15; H, 5.30. Found: C, 62.90; H, 5.25.

(10) The four stereoisomers of lactone VIII, in their optically active forms, have been derived from podophyllotoxin and have been characterized and sterically defined. The reported melting points of these optically active desoxylactones are: 251–252°, 202.3–203.2°, 169.5–171°, and 168–169° [A. W. Schrecker and J. L. Hartwell, *J. Am. Chem. Soc.*, **75**, 5916 (1953)].

(11) Reference 6, p. 119.

(12) W. J. Gensler, F. Johnson, and A. D. B. Sloan, *J. Am. Chem. Soc.*, **82**, 6074 (1960).

(13) E. Schreier, *Helv. Chim. Acta*, **46**, 75 (1963).

(14) 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. Unless otherwise indicated, infrared spectra were taken with mineral oil mulls.

(15) Unpublished work by Shih Yi Wang.

In larger runs the product was recrystallized more conveniently by solution in boiling dioxane, addition of hot propanol, concentration to the point of crystallization, and cooling.

B. By Acetylation of DL-Isopodophyllotoxin (VII).—DL-Isopodophyllotoxin (30 mg.) was dissolved in 2 ml. of dry pyridine by heating, 1 ml. of acetic anhydride was added to the hot solution, and the mixture was allowed to stand at room temperature for 4 hr. Dropwise addition of the reaction mixture to 20 ml. of cold water deposited a white precipitate, which was collected, washed on the funnel with water, and dried. A solution of this solid in 10 ml. of chloroform, after dilution with 20 ml. of boiling methanol, was distilled until the appearance of crystals. The mixture was allowed to stand at room temperature. The collected needles (30 mg., m.p. 262–263°) on crystallization from chloroform–methanol furnished DL-isopodophyllotoxin acetate (IV) melting at 263–264° either alone or mixed with the product described in part A. The infrared absorption curves of the two acetates were identical.

Hydrolysis of DL-Isopodophyllotoxin Acetate (IV) to DL-Isopodophyllic Acid (III).—Acetate IV (15 mg.) was exposed to a boiling solution of potassium bicarbonate (30 mg.) in 3 ml. of methanol plus 1 ml. of water for 18 hr. After distillation until the volume was 1.5 ml., 3 ml. of water was added, and the mixture was filtered. The filtrate, to which a few pieces of ice were added, was treated with one drop of concentrated hydrochloric acid and allowed to stand at 5° for 2 hr. The collected solids were washed with three 2-ml. portions of water, and sucked dry on the funnel. Crystallization of the partially dry product from methanol furnished 10 mg. of DL-isopodophyllic acid (III), m.p. 231–232°, which had the same infrared absorption curve as that obtained before,[3] and which when mixed with DL-isopodophyllic acid melted at 230–231°.

Attempted deacetylation of acetate IV with aqueous dioxane containing some hydrochloric acid gave inconclusive results.

Dehydroanhydropicroporophyllin (V) by Selenium Dioxide Oxidation of α-Apopicropodophyllin (VI).—Selenium dioxide (0.019 g. or 0.17 mmole) was dissolved in boiling acetic acid (5 ml.) that had been distilled from selenium dioxide. α-Apopicropodophyllin (0.125 g. or 0.315 mmole) was added to the hot solution, and the resulting brown mixture was boiled for 45 min. and then allowed to stand at room temperature overnight.

Water (50 ml.) was added, the mixture was filtered, and the solids were washed with water, pressed on the funnel, and dried *in vacuo* over phosphorus pentoxide. Crystallization from methanol–chloroform, including treatment with decolorizing carbon (Darco), gave pale yellow needles (89 mg.) of dehydroanhydropicropodophyllin, m.p. 263–264° with softening at 255°. For further purification, this material in solution with 10 ml. of chloroform was placed on a 6 × 0.75 cm. column of 5 g. of Merck acid-washed alumina. Elution with 100 ml. of 1:1 chloroform–ether gave product V, which after two recrystallizations from chloroform–ethanol melted at 263–264°. A 2.03×10^{-5} *M* alcohol solution showed absorption maxima at 209 mμ (log ε 4.62), 258 (4.75), 311 (4.04), and 351 (3.77). The ultraviolet absorption spectrum was essentially the same as that determined before[16] for material with m.p. 270–271° (cor.).

Anal. Calcd. for $C_{22}H_{18}O_7$: C, 67.00; H, 4.60. Found: C, 66.7; H, 4.5.

Dehydroanhydropicropodophyllin (V) from Desoxypicropodophyllin (X) by Sulfur Aromatization.—Biphenyl (2.0 g.) containing 120 mg. (0.30 mmole) of desoxypicropodophyllin and 40 mg. (1.25 mmoles) of sulfur was boiled for 45 min. The cooled mixture was digested with petroleum ether (30–70°) and was filtered. Crystallization of the solids from methanol (decolorizing carbon was employed) gave 25 mg. of product, m.p. 252–256°. Another crystallization followed by sublimation at 240° (0.05 mm.) and finally crystallization from methanol–methylene chloride gave pale yellow needles of dehydroanhydropicropodophyllin (V), m.p. 264°. The mixture melting point with the same material prepared from α-apopicropodophyllin was 263–265°.

Dehydroanhydropicropodophyllin (V) from DL-Isopodophyllotoxin Acetate (IV).—The acetate (50 mg.) together with 25 mg. of 10% palladium-on-charcoal catalyst was heated at 275° in a stream of carbon dioxide. After the initial effervescence had ceased, materials sublimable at 275–285° (0.75 mm.) were collected. The sublimate was dissolved in methylene chloride, and the solution was concentrated with gradual addition of methanol until crystals appeared. The mixture was allowed to stand at room temperature until precipitation was complete. The crystals (13 mg., m.p. 255–258°) were collected and were recrystallized to give dehydroanhydropicropodophyllin, m.p. 262.5–263.5°. Mixed with the same product derived from α-apopicropodophyllin, the crystals melted at 263–264°. The two samples of dehydroanhydropicropodophyllin showed identical infrared absorption curves, including an absorption peak at 5.67 μ (lactone).

(16) A. W. Schrecker and J. H. Hartwell, *J. Am. Chem. Soc.*, **74**, 5672, 5676 (1952).

DL-Isopodophyllotoxone (XIII) from DL-Isopodophyllotoxin (VII).—Chloroform (15 ml.) was added to a hot solution of 300 mg. (0.725 mmole) of DL-isopodophyllotoxin in 30 ml. of dioxane followed by 3 g. (34 mmoles) of freshly prepared alkali-free manganese dioxide. The mixture was boiled for 4 hr. After removal of solids, the dark brown solution was evaporated to dryness. Crystallization of the brown gummy residue from methylene chloride–methanol gave 30 mg. of recovered starting material.

Solvent was removed from the mother liquors, and the gummy residue (75 mg.) was dissolved in 20 ml. of benzene. The solution was filtered, and the filtrate allowed to flow through a 3 × 0.75 cm. column of Merck acid-washed alumina (3 g.). The column was eluted with 50 ml. of benzene, with 75 ml. of benzene–ether (9:1), and finally with 75 ml. of benzene–ether (4:1). The red gum (50 mg.) obtained from the mixed solvents crystallized on trituration with methanol. Three crystallizations from methanol afforded 21 mg. of colorless square plates of DL-isopodophyllotoxone (XIII), m.p. 180.5–181°. Infrared absorption maxima were evident at 5.66 and 5.99 μ but not in the hydroxyl region. A 3.69×10^{-5} *M* solution in 95% alcohol showed ultraviolet absorption maxima at 241 mμ (log ε 4.45), 282 (3.96), and 328 (3.94).

Anal. Calcd. for $C_{22}H_{20}O_8$: C, 64.07; H, 4.89. Found: C, 63.9; H, 5.0.

DL-Desoxyisopodophyllic Acid (XII) by Hydrogenolysis of DL-Isopodophyllic Acid (III).—Shaking a mixture of 0.21 g. of DL-isopodophyllic acid (III), an equal weight of 10% palladium-on-carbon catalyst, and 30 ml. of glacial acetic acid at 60° for 2 hr. under an atmosphere of hydrogen resulted in absorption of approximately 115% of the equimolar amount of hydrogen (corrected for catalyst absorption). The catalyst was separated, and the solvent was removed by distillation under reduced pressure at steam temperatures. The white crystalline residue, dissolved in 5 ml. of methanol, was added to 60 ml. of benzene, and the solution was extracted with 30 ml. of saturated sodium bicarbonate solution followed with 30 ml. of water. (In one experiment it was shown that small quantities of DL-desoxyisopodophyllotoxin (VIII) were present in the organic layer after removal of acidic material with bicarbonate.) The combined aqueous layers were added dropwise to a mixture of 50 g. of ice, 10 ml. of water, and 10 ml. of concentrated hydrochloric acid. The precipitate was collected, washed thoroughly with water, and dried partially by pulling air through the filter cake. The damp solid was dissolved in 10 ml. of methanol, and the solution was filtered, concentrated to 3 ml., and cooled. The fine needle-like crystals of DL-desoxyisopodophyllic acid (XII), after drying, weighed 90 mg.; a capillary sample of this product when immersed in a bath at 200°, melted at 217–227°, resolidified, and melted again at 249–251°. Several recrystallizations from methanol and from methanol–methylene chloride brought the melting point to 231–233° (resolidification, then remelting at 252–254°). Desoxy acid XII in chloroform solution showed absorption maxima at 2.92 and 5.90 μ.

Anal. Calcd. for $C_{22}H_{24}O_8$: C, 63.45; H, 5.81. Found: C, 63.33; H, 5.95.

DL-Desoxyisopodophyllotoxin (VIII) by Hydrogenolysis of DL-Isopodophyllotoxin (VII).—A suspension of DL-isopodophyllotoxin (65 mg.), 140 mg. of 5% palladium-on-carbon catalyst, and 25 ml. of glacial acetic acid was hydrogenated at 75° for 3 hr. The organic solids gradually went into solution. The cooled mixture was filtered, and the catalyst was extracted with 5 ml. of boiling acetic acid. The combined acetic acid solutions were taken to dryness, and the white residue crystallized from methanol to give 34 mg. of fine needles, m.p. 248–250°. Two further crystallizations led to DL-desoxyisopodophyllotoxin (VIII), which melted alone at 256° and when mixed with the material prepared as above at 255–256°. The identity was further confirmed by comparison of infrared absorption spectra.

DL-Desoxyisopodophyllotoxin (VIII) by Hydrogenolysis of the Acetyl Derivative (IV) of Isopodophyllotoxin.—Acetate IV (200 mg.) in 30 ml. of glacial acetic acid containing 200 mg. of 10% palladium-on-carbon catalyst was shaken for 5 hr. at 60° under hydrogen at 40 lb. pressure. Removal of catalyst and solvent left a residue, which on crystallization from methanol gave 80 mg. of needles, m.p. 236–239°. Recrystallizations from methanol led to fine needles of DL-desoxyisopodophyllotoxin, m.p. 252–254°. The mixture melting point was 253–255°.

DL-Desoxyisopodophyllotoxin (VIII) by Acetic Anhydride Cyclization of DL-Desoxyisopodophyllic Acid (XII).—Acetic anhydride (3 ml.) containing 55 mg. of DL-desoxyisopodophyllic acid (XII) was boiled for 1.5 hr. Volatile material was removed by distillation *in vacuo* at steam temperature. The white crystalline residue was dissolved in 5 ml. of methylene chloride, and the solution was filtered, diluted with 10 ml. of hot methanol, and concentrated to 8 ml. Cooling the concentrated solution precipitated 45 mg. of DL-desoxyisopodophyllotoxin (VIII), m.p. 254–256°, with softening at 252°. Three recrystallizations of the

fine needles from methanol–methylene chloride brought the melting point to 256–256.5°.

Anal. Calcd. for $C_{22}H_{22}O_7$: C, 66.32; H, 5.57. Found: C, 66.29; H, 5.56.

Comparison of the infrared spectrum of this racemic material with that of optically active desoxyisopodophyllotoxin,[10] when both materials were taken as mulls with mineral oil, showed some differences. For example, the lactone absorption of the racemic material appeared at 5.67 μ, while that of the optically active form appeared at 5.61 μ. However, when the spectra were determined with the materials dissolved in methylene chloride, the two curves showed the same lactone absorption at 5.61 μ and were also superimposable at all other points.

The mixture melting point of the synthetic and the optically active material (m.p. 252°) was 246–248°, with softening at 240°.

Ethyl DL-Isopodophyllate Acetate (XI) by Reaction of DL-Isopodophyllotoxin Acetate (IV) with Alcoholic Sodium Acetate.—Absolute alcohol (10 ml.) containing 30 mg. of acetate IV and 200 mg. of anhydrous sodium acetate was boiled for 2.5 hr. The solid gradually dissolved. Most of the alcohol was removed by distillation under reduced pressures on the steam bath, 10 ml. of water was added, and the resulting precipitate collected by filtration. The solids, washed with water and air-dried, weighed 21 mg. and showed m.p. 159–163°. Three crystallizations from acetone–petroleum ether (30–60°) afforded the analytically pure acetyl derivative of ethyl DL-isopodophyllate (XI) in the form of fine needles, m.p. 166–167°. Infrared absorption peaks were noted at 2.99 and 5.80 μ.

Anal. Calcd. for $C_{26}H_{30}O_{10}$: C, 62.14; H, 6.02. Found: C, 62.2; H, 6.2.

Desoxypicropodophyllin (X) by Hydrogenolysis of Picropodophyllin (IX).—Picropodophyllin (0.5 g.) dissolved in 50 ml. of glacial acetic acid containing 0.5 g. of 10% palladium-on-charcoal catalyst was stirred under hydrogen for 1.5 hr. at 60°. No hydrogen absorption was noted after this time. The glassy material obtained after removal of catalyst and solvent was dissolved in 25 ml. of 9:1 benzene–ether, and the solution was allowed to percolate through a 7 × 1 cm. column of Merck acid-washed alumina (10 g.). The material was eluted with 210 ml. of 9:1 benzene–ether. Removal of solvent left a glass, which when triturated with methanol gave 0.3 g. (60%) of crystalline desoxypicropodophyllin (X), m.p. 167–168.5°. Sublimation at 156° (5×10^{-5} mm.) gave product with m.p. 169°. A sample for analysis, prepared by several recrystallizations from methanol, showed m.p. 170–171.5°. This product had a rotation of $[\alpha]^{25}D$ +34.4° (*c* 1.65 in chloroform), and gave an absorption peak at 5.68 μ but none in the hydroxyl region. The crystals tended to become electrically charged. These characteristics correspond well to those previously reported[10] for desoxypicropodophyllin prepared in a different way.

Anal. Calcd. for $C_{22}H_{22}O_7$: C, 66.32; H, 5.57. Found: C, 66.52; H, 5.52.

To show that picropodophyllin (IX) is not dehydrated under the hydrogenolysis conditions employed, a mixture of picropodophyllin (0.1 g.), glacial acetic acid (10 ml.), and 0.1 g. of 10% palladium-on-charcoal was stirred for 2 hr. at 60° under an atmosphere of nitrogen. Unchanged picropodophyllin could be recovered to an extent of 75%.

DL-Epiisopodophyllotoxin (XIV) by Zinc Borohydride Reduction of DL-Isopodophyllotoxone (XIII).—A solution of 0.10 g. (1.05 mmoles) of zinc borohydride in 4 ml. of ether was added to a solution of 28 mg. (0.68 mmole) of DL-isopodophyllotoxone (XIII) in benzene (2 ml.) plus ether (4 ml.). After 20 hr. at 5°, the mixture was hydrolyzed by cautious addition of 1 ml. of water. When no further effervescence was evident, 1 ml. of acetic acid was added. The ether layer was removed, and the aqueous layer was filtered. The collected solids, after thorough washing with water, were dissolved in 50 ml. of methylene chloride–methanol solvent. The ether layer was diluted with more ether, was washed twice with water, once with saturated sodium bicarbonate solution (20 ml.), and again with water. The combined ether and methylene chloride–methanol solutions were concentrated and cooled to give 21 mg. (79%) of needle-like crystals, m.p. 248–251°. Two further crystallizations from methanol furnished 12 mg. of DL-epiisopodophyllotoxin (XIV), m.p. 255°. The compound absorbed at 5.61 and 2.90 μ, but not in the tetralone-carbonyl position. The infrared absorption curve did not resemble that of either of the two forms of DL-isopodophyllotoxin (VII). When mixed with DL-isopodophyllotoxin (VII), compound XIV softened at 236°, melted at 238–244°, and was entirely liquid at 250°.

Anal. Calcd. for $C_{22}H_{22}O_8$: C, 63.76; H, 5.35. Found: C, 63.75; H, 5.28.

Acknowledgment.—We wish to thank Dr. Jonathan L. Hartwell, who provided a sample of desoxyisopodophyllotoxin, and Dr. E. Schreier, who very kindly made his collection of pertinent n.m.r. curves[13] available to us for study.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY 4, CALIF.]

The Total Synthesis of (±)-Thujopsene[1]

BY WILLIAM G. DAUBEN AND ARNOLD C. ASHCRAFT[2]

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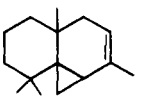
Thujopsene, a tricyclic sesquiterpene, has been synthesized and has been shown to possess the *cis* structure I.

Thujopsene, a tricyclic sesquiterpene, has been shown to be a constituent of the wood oil and of the heartwood of many genera belonging to the natural order Cupressales.[3] It has been suggested by Erdtman and Norin[4] that all conifers possessing the sweet odor of "pencil wood" apparently contain this odoriferous principle. In the past few years the chemistry of this widely occurring hydrocarbon has been extensively investigated and of the three structures for the material tentatively suggested,[5-7] it recently has been shown that the compound possesses the gross structure I.[3,4,8-11]



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The steric relationship between the angular methyl group and the cyclopropane ring, however, has been assigned both *cis*[3] and *trans*[8,10] orientations, but the evidence presented for each postulate does not permit an unequivocal conclusion to be reached. This problem has now been settled by a stereospecific synthesis of thujopsene.[12]

The synthetic approach was based on the recent finding[13] that when an alicyclic allyl alcohol, such as cyclohexenol (II), was allowed to react with methylene iodide in the presence of zinc–copper couple (the Simmons reagent), the exclusive product formed was the bicyclo[4.1.0]heptane-2-ol (III) with the cyclo-

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(2) Procter and Gamble Predoctoral Fellow, 1962–1963.

(3) For a summary of the numerous isolations of thujopsene, see T. Norin, *Acta Chem. Scand.*, **15**, 1676 (1961).

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