not applicable in this case, since neither ethoxymethylene malonate nor ethoxymethylene aniline was detectable in the reaction mixture.

Experimental Section

Bis(*m*-chlorophenyl)formamidine used in these investigations was prepared by the known reaction of *m*-chloroaniline and ethyl orthoformate. *m*-Chloroaniline (51.0 g) and ethyl orthoformate (29.6 g) were refluxed for 2 hr in a reaction vessel provided with a short column filled with Raschig rings to allow the alcohol formed to distil slowly. After the termination of the reaction the column was removed, the vessel was put under vacuum and the remaining base was used in the next step without any further treatment.

Method A. "Total-Conversion" Method. Ethyl (α-Carbethoxy-β-*m*-chloroanilino)acrylate.—The above reaction product, or crystalline bis(*m*-chlorophenyl)formamidine base (53 g, 0.2 mole), ethyl orthoformate (35.6 g, 0.2 mole +20% excess), and diethyl malonate (32 g, 0.2 mole) were stirred for 20 hr at 126°. The evolved alcohol was continuously removed by distillation.

To the half-converted reaction mixture a further portion (32 g, 0.2 mole) of diethyl malonate was added, and stirring was continued for 48 hr at 126°. From the product the small quantities of unchanged malonate, ethyl orthoformate, and alcohol were removed by distillation under vacuum.

The viscous residue was dissolved in benzene (120 ml) and shaken with 10% hydrochloric acid (120 ml) to remove traces of basic impurities. The benzene layer containing the acrylic ester was washed with water, filtered, and dried over sodium sulphate, and the solvent was evaporated under vacuum.

The distillation residue (112.5 g, 94%) a light colored substance, crystallized within a short time.

This crude product containing about 96% of acrylic ester may be used without further purification in the cyclization step of the 4-hydroxy-7-chloroquinoline synthesis. Recrystallization from petroleum ether (bp 40–60°) gave the pure acrylic ester (mp 57–58°) in 90% yield.

Method B. "Half-Conversion" Method.—Bis(*m*-chlorophenyl)formamidine (53 g, 0.2 mole), ethyl orthoformate (17 g, 0.1 mole +15% excess), and diethyl malonate (32 g, 0.2 mole) were stirred for 20 hr at 126°. The mixture was dissolved in benzene (400 ml) and diluted with 10% hydrochloric acid (300 ml) to precipitate the formed bis(*m*-chlorophenyl)formamidine hydrochloride. This was filtered off, washed with benzene, and dried (30 g). The benzene filtrate was washed with three 80-ml portions of water and dried over sodium sulfate; then the solvent was evaporated under reduced pressure. The residual oil was dissolved in petroleum ether (47 ml) and allowed to stand overnight. The substance which separated was recovered by filtration and dried to give 45.2 g of product, 76%, mp 56–57°.

Synthesis of Podophyllotoxin[1,2]

WALTER J. GENSLER AND CHRISTOS D. GATSONIS

Contribution from the Department of Chemistry, Boston University, Charles River Campus, Boston, Massachusetts

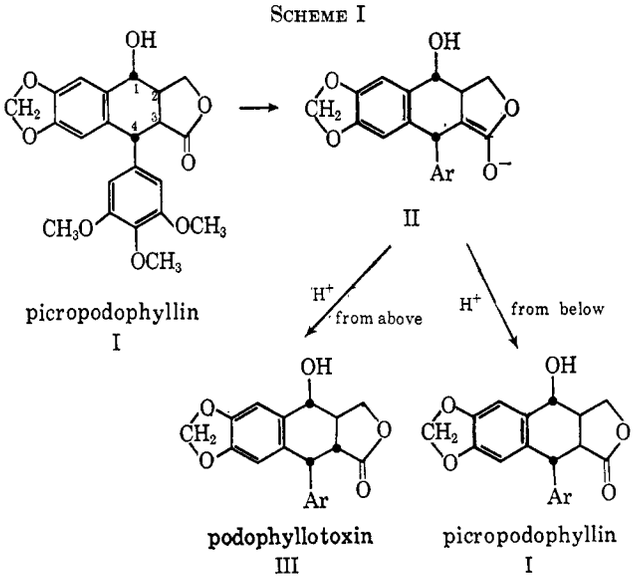
Received June 20, 1966

Picropodophyllin and podophyllotoxin can be converted to their O-tetrahydropyranyl derivatives. Treatment with aqueous acid regenerates the starting materials. The podophyllotoxin derivative epimerizes with mild base to the picropodophyllin derivative. The action of triphenylmethylsodium on either tetrahydropyranyl derivative produces the enolate common to both. Irreversible protonation of the enolate followed by removal of the protective group gives a 45:55 (approximate) mixture of podophyllotoxin and picropodophyllin. An interpretation of the results based on a model for the transition states is proposed. Since picropodophyllin has been synthesized, its conversion to podophyllotoxin completes a total synthesis of podophyllotoxin.

Podophyllotoxin (III) and other related lignan lactones from *Podophyllum* species have received considerable attention as cancer chemotherapeutic agents.[3,4] Although a number of podophyllotoxin derivatives have been synthesized,[4-7] podophyllotoxin itself has not. In this connection, since picropodophyllin (I), an epimer, has been synthesized,[8] its conversion to podophyllotoxin (III) would constitute the last step in a total synthesis of podophyllotoxin. The present paper is concerned with this conversion[9] (see Scheme I).

Podophyllotoxin (III) epimerizes readily under base catalysis to picropodophyllin (I).[10] The reverse reaction has also been demonstrated,[11] so that picropodophyllin can, in fact, be converted to podophyllotoxin by equilibration. The proportion of podophyllotoxin in the equilibrium mixture (*ca.* 3%) is too small, however, for this to be regarded as a practical—much less, an attractive—means of arriving at podophyllotoxin.

SCHEME I



(1) (a) This is paper XVII in the series entitled Compounds Related to Podophyllotoxin. (b) The preceding paper is by W. J. Gensler and C. D. Gatsonis, *J. Org. Chem.*, **31**, 3224 (1966).

(2) This investigation was supported by Public Health Service Research Grant No. CA-02891 from the National Cancer Institute.

(3) See, *inter alia*, J. L. Hartwell and M. Shear, *Cancer Res.*, **7**, 716 (1947); M. Belkin, *J. Pharmacol. Exptl. Therap.*, **93**, 18 (1948); M. G. Kelley and J. L. Hartwell, *J. Natl. Cancer Inst.*, **14**, 967 (1954).

(4) H. Emmenegger, H. Stähelin, J. Rutschmann, J. Renz, and A. von Wartburg, *Arzneimittel-Forsch.*, **11**, 327, 459 (1961).

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

(6) J. Renz, M. Kuhn, and A. von Wartburg, *Ann.*, **681**, 207 (1965).

(7) J. Rutschmannn and J. Renz, *Helv. Chim. Acta*, **42**, 890 (1959).

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

(9) A brief note appeared earlier: W. J. Gensler and C. D. Gatsonis, *ibid.*, **84**, 1748 (1962).

(10) W. Borsche and J. Niemann, *Ann.*, **494**, 126 (1932); E. Späth, F. Wessely, and L. Kornfeld, *Ber.*, **65**, 1536 (1932); A. Robertson and R. B. Waters, *J. Chem. Soc.*, 83 (1933).

(11) Paper XVI.[1b]

In order to break out of this thermodynamic limitation, advantage was taken of the fact that enolate II is almost certainly an intermediate[12] in the picropodophyllin-to-podophyllotoxin isomerization. If proton removal from picropodophyllin to give II could be carried to completion, and proton return then conducted as a separate and irreversible step, a mixture would result in which the ratio of podophyllotoxin to picropodophyllin is no longer controlled by the equilibrium constant but by the relative rates of proton delivery to the topside of the enolate (to give podophyllotoxin, III) and the bottomside (to give picropodophyllin, I).[13] Examination of the transition states for the two modes of protonation should give some idea of the utility of this scheme.

As our model of the transition states we took the enolate II of picropodophyllin with the proton carrier located either above or below the planar enolate grouping (sp^2 carbon) and close to a line perpendicular to this plane and passing through the enolate carbon atom (position 3).[14] Figure 1 shows a Dreiding model of enolate II, which, so far as the fused-ring system is concerned, turns out to be rigid.[15] Inspection of this model or, even more apropos, of the model built with space-filling atoms shows that there is appreciably more room above the enolate than below. Accordingly, since topside protonation might well compete effectively with bottomside protonation, the method appeared promising and worth trying.

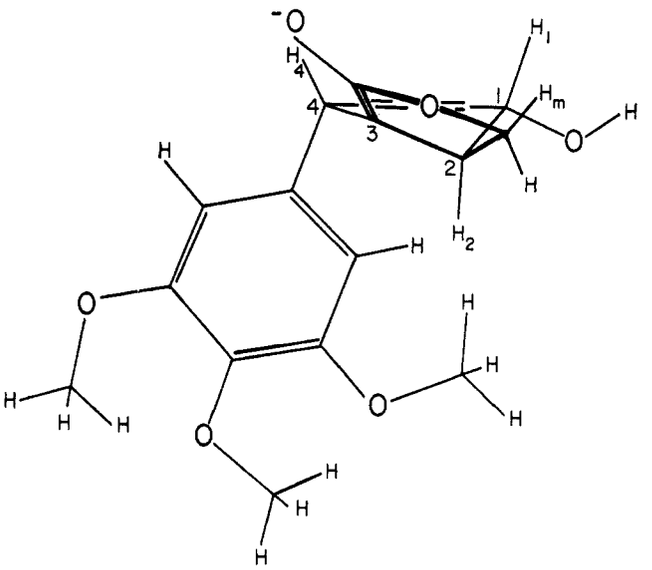


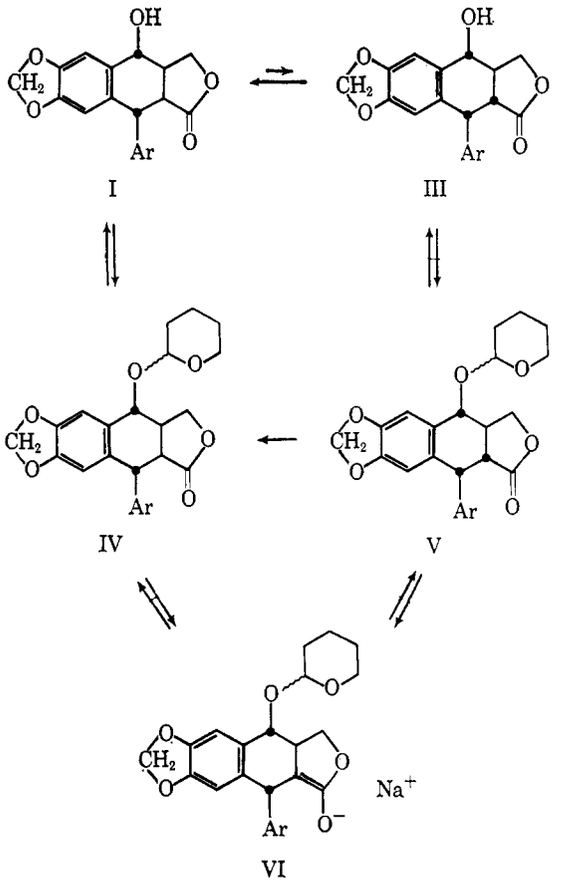
Figure 1.—Enolate II of picropodophyllin. The sketch is based on scale models (Dreiding). The enolate is seen with the lactone ring oxygen closest to the eye and with the methylenedioxybenzene ring farthest from the eye and viewed edgewise.

Results

Some explanatory work was done on generating enolate II directly from picropodophyllin (I). Since the hydroxyl hydrogen is more acidic than the hydrogen at position 3, the enolate, if formed at all, would be a dianion. Attempts to form this dianion by treating picropodophyllin with 2 equiv or more of triphenylmethylsodium failed to give satisfactory results. Blocking the hydroxyl function would get rid of this complication and, for this purpose, the tetrahydropyranyl group served admirably.

Treatment with dihydropyran in the presence of an acid catalyst converted picropodophyllin (I) to O-tetrahydropyranylpicropodophyllin (IV) in good yield. A similar process starting with podophyllotoxin (III) produced O-tetrahydropyranylpodophyllotoxin (V), which could be converted by mild base catalysis to the picropodophyllin derivative IV. No stereochemical change occurred on formation of derivatives IV and V, since treatment with aqueous acid removed the protecting groups and regenerated the unchanged hydroxy compounds, I and III, respectively. Attaching a tetrahydropyranyl group introduces a new center of asymmetry, so that compounds IV and V were obtained as mixtures. Several crystallizations produced two homogeneous stereoisomers from the O-tetrahydropyranylpicropodophyllin mixture IV, but the separation was tedious and inefficient and, fortunately, was unnecessary for our purpose. (See Scheme II.)

SCHEME II



(12) See, for example, J. Hine, "Physical Organic Chemistry," 2nd ed, McGraw-Hill Book Co., Inc., New York, N. Y., 1962, Chapter 10.

(13) Zimmerman and his co-workers have reported related rate-controlled protonations. A review is given by H. E. Zimmerman, "Molecular Rearrangements," Vol. 1, P. de Mayo, Ed., Interscience Publishers, Inc., New York, N. Y., 1963, pp 345–372.

(14) This model was suggested by the results of Zimmerman and coworkers.[13] In this connection, note S. K. Malhotra and F. Johnson [*J. Am. Chem. Soc.*, **87**, 5493 (1965)]. Note also the generalization of G. S. Hammond [*ibid.*, **77**, 334 (1955)], as well as the conclusions of E. J. Corey and R. A. Sneen [*ibid.*, **78**, 6269 (1956)], and of H. Shechter, M. J. Collis, R. Dessy, Y. Okuzumi, and A. Chen [*ibid.*, **84**, 2905 (1962)]. Although we have no proof, we lean toward the enol rather than the enolate as the form accepting the proton at the 3 position.

(15) N. L. Drake and E. H. Price, *ibid.*, **73**, 201 (1951); A. W. Schrecker and J. L. Hartwell, *ibid.*, **75**, 5916 (1953); **79**, 3827 (1957); *J. Org. Chem.*, **21**, 381 (1956); E. Schreier, *Helv. Chim. Acta*, **46**, 75 (1963); W. J. Gensler, M. V. Leeding, and A. S. Rao, *J. Org. Chem.*, **29**, 1594 (1964); J. Renz, M. Kuhn, and A. von Wartburg, *Ann.*, **681**, 207 (1965).

The key step called for the preparation of sodium enolate VI from tetrahydropyranylpicropodophyllin (IV) by treatment with triphenylmethylsodium. This was accomplished without difficulty. Quenching the enolate with excess acetic acid gave a mixture of tetrahydropyranyl derivatives V and IV, which was exposed directly to the action of aqueous acid to remove the protecting group. Crystallization effected a clean separation and gave the desired podophyllotoxin (III) in 38% conversion and picropodophyllin in 51% recovery. We believe the actual figures to be something in the order of 45 and 55%, respectively since according to thin layer chromatography, the material in the combined mother liquors (dry weight *ca.* 13%) consisted almost entirely of podophyllotoxin and picropodophyllin, with the former predominating. By these results, the conversion of picropodophyllin to podophyllotoxin was shown to be possible in modest though still useful yield, and thus the goal of a total synthesis of podophyllotoxin was reached. To complete the cycle of interconversions, tetrahydropyranylpodophyllotoxin (V) was transformed to enolate VI and quenched as before with acetic acid. Similar results were obtained.

Discussion

A 45:55 mixture of podophyllotoxin and picropodophyllotoxin is far richer in podophyllotoxin than the equilibrium mixture. In terms of free energy, approximately 2 kcal/mole has been injected into the equilibrium system[11] to bring it to the 45:55 mixture. Notwithstanding the improvement over the equilibrium ratio, these results are not so good as we had hoped for. Actually, instead of topside protonation being faster than bottomside protonation, it turned out to be slightly slower. However, a reasonable explanation becomes available as soon as the protonation transition states are taken not with the enolate carbon planar, but instead with this carbon beginning to show sp^3 geometry.[16] Such a change, while providing only a little more room in the already open region above the enolate in the podophyllotoxin transition state, leads to appreciably more room below the enolate in the picropodophyllin transition state. In the latter case, as the enolate carbon atom tends toward the tetrahedral (a downward displacement in Figure 1), the hindering trimethoxyphenyl group tends to swing away from, and so clear, the line of proton approach. Furthermore, change of the planar enolate transition state toward podophyllotoxin geometry introduces the unfavorable free energy of podophyllotoxin[11] as a factor in the activation energy, whereas change in the direction of picropodophyllin geometry introduces the smaller, more favorable free energy of picropodophyllin. Accordingly, the offplanar model provides effects that increase the activation energy for podophyllotoxin protonation relative to picropodophyllin protonation and that, therefore, could account for the experimental results.

Whether different protonating agents under different conditions can improve the yield of podophyllotoxin remains to be seen. Meanwhile, a method is now available that opens the way to base-sensitive podophyllotoxin analogs by way of their corresponding stereochemically stable, though physiologically inert, picropodophyllin epimers.

Cancer Activity.—The tetrahydropyranyl derivative V of podophyllotoxin was screened by Cancer Chemotherapy National Service Center (compound NSC-67162). *In vivo* trials with three tumor systems, sarcoma 180, carcinoma 755, and leukemia L1210, showed a level of activity insufficient to warrant further extensive testing.

Experimental Section

General.—On the basis of elementary analysis and melting point, the podophyllotoxin in this work is regarded as the half-hydrate,[11] and all weights and yields of podophyllotoxin refer to this composition. The general experimental features described in the preceding paper[1b] also apply here.

Tetrahydropyranyl Derivative IV of Picropodophyllin.—*p*-Toluenesulfonic acid (0.4 g) was added to a solution of 10.0 g (0.248 mole) of picropodophyllin (I) in 300 ml of ethanol-free chloroform plus 50 ml of dihydropyran (freshly distilled from sodium hydroxide pellets). An exothermic reaction brought the temperature to 50°. After standing for 4 hr at room temperature, the light yellow solution was washed with 80 ml of 2% sodium bicarbonate solution and then several times with water until the washings were neutral. The organic layer was dried with sodium sulfate, and all volatile material was removed *in vacuo*. Mixing the residue with a small amount of methanol–ether (1:1, v/v) afforded a solid, which on crystallization from absolute ethanol gave 10.3 g (85%) of crystalline tetrahydropyranyl derivative IV, mp 208–212°.

Thin layer chromatography with solvent C[11] resolved this product into two components with R_f 0.83 and 0.87, respectively. On the same plate, picropodophyllin (I) traveled more slowly, with R_f 0.47. Tetrahydropyranyl derivative IV showed no hydroxyl absorption at 3700–3125, but did show a lactone peak at 1765–1768, and a relatively intense peak at 1076 cm^{-1}, an absorption characteristic of COC.[17]

Although giving poor yields, crystallization did separate the two epimers of IV. Thus, four recrystallizations from chloroform–ethanol gave the less soluble, single-spot (R_f 0.83) epimer, with mp 219–220° and $[\alpha]D$ −20°. The absorption curve, taken with pure ethanol as solvent, showed a maximum at 290 mμ (log ε 3.55).

Anal. Calcd for $C_{27}H_{30}O_9$: C, 65.05; H, 6.07. Found: C, 64.70; H, 6.10.

The mother liquors were combined and taken to dryness *in vacuo* at room temperature. One crystallization of the residue from chloroform–methanol (1:9, v/v) followed by five crystallizations from methanol led to the constant-melting (203–204°), homogeneous (R_f 0.87) epimer IV, with $[\alpha]D$ +103° and λ_{max} 291 mμ (log ε 3.51) in ethanol solution.

The infrared absorption spectra of the two epimers are identical, with the exception of the carbonyl peak; the lower melting epimer absorbs at 1765; the higher melting epimer absorbs closer to 1768 cm^{-1}. Picropodophyllin (I) shows a peak at 1765–1768 cm^{-1}.

Picropodophyllin tetrahydropyranyl derivative IV could also be prepared with phosphorus oxychloride as catalyst by a procedure similar to that described below for the podophyllotoxin derivative.

Recovery of Picropodophyllin (I) from Its Tetrahydropyranyl Derivative (IV).—A solution of 0.50 g of the tetrahydropyranyl compound IV (both epimers), mp 207–211°, in 50 ml of water–tetrahydrofuran (2:3, v/v) containing 5 drops of concentrated sulfuric acid was boiled for 4 hr. Water was added to the homogeneous, slightly yellow solution, and the resulting precipitate was collected and dried over phosphorus pentoxide. Crystallization of the dry solid from absolute ethanol afforded 0.35 g (88%) of picropodophyllin (I), mp 222–225° and $[\alpha]D$ +5°. The mixture melting pont with authentic picropodophyllin (mp 224–226°) was 222–225°. The infrared absorption spectrum of the

(16) Zimmerman points out that a certain amount of hybridization to sp^3 must occur at the transition state.[13] See also J. Hine, J. G. Houston, J. H. Jensen, and J. Mulders, *J. Am. Chem. Soc.*, **87**, 5050 (1965); C. G. Swain and A. S. Rosenberg, *ibid.*, **83**, 2154 (1961); C. G. Swain and E. R. Thornton, *ibid.*, **84**, 817 (1962).

(17) L. J. Bellamy, "Infrared Spectra of Complex Molecules," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1958, p 114.

recovered picropodophyllin was identical with that of authentic material.

When the pure higher melting epimer of IV was hydrolyzed for 12 hr in hot, aqueous tetrahydrofuran plus acetic acid and some hydrochloric acid, picropodophyllin was isolated in 72% yield. Excellent results were also obtained at room temperature.

Tetrahydropyranyl Derivative (V) of Podophyllotoxin.—Two drops of phosphorus oxychloride was added to a magnetically stirred mixture of 10.0 g of podophyllotoxin (III) and 30 ml of dihydropyran freshly distilled from sodium hydroxide pellets. The temperature rose to 65°, and the mixture became homogeneous within 5 min. After 3 hr at room temperature, the clear solution was added with vigorous stirring to 600 ml of ice-cold petroleum ether (bp 30–60°, purified by distillation from sodium). The resulting solids were collected and dissolved in anhydrous ether (60 ml), and the ether solution, after filtration, was slowly added to 500 ml of cold petroleum ether as before. The precipitated solids were dried at room temperature (0.1 mm) for 2 days. This material, the desired tetrahydropyranyl derivative V, weighed 10.1 g (85%) and showed mp 74–97° and $[\alpha]D$ −103°. The product was pure white and had no odor. It showed infrared absorption peaks at 1770–1772 (lactone carbonyl) and at 1071 and 1149 (COC) but none at 3700–3125 cm^{-1} (hydroxyl). Note that, in podophyllotoxin, the lactonic carbonyl absorption appeared at 1777 cm^{-1}. In ethanol solution, tetrahydropyranyl derivative V showed an absorption maximum at 292–293 mμ (log ε 3.43).

Anal. Calcd for $C_{27}H_{30}O_9$: C, 65.05; H, 6.07. Found: C, 65.16; H, 6.24.

On a thin layer chromatographic plate with solvent C, podophyllotoxin (III) traveled about five times more slowly than its tetrahydropyranyl derivative V.

The same derivative V was also prepared with methylene chloride as solvent and *p*-toluenesulfonic acid as catalyst.

Recovery of Podophyllotoxin (III) from Its Tetrahydropyranyl Derivative V.—A clear solution of tetrahydropyranyl derivative V (0.50 g) in 25 ml of aqueous methanol (1:1, v/v) containing 2 ml of 10% hydrochloric acid was boiled for 2 hr. Cooling the reaction mixture (2 days) precipitated colorless needles, which were recrystallized from methanol–water. The resulting crystals, dried at 100° (0.1 mm) for 5 hr over phosphorus pentoxide, weighed 0.36 g (85%) and showed mp 156–158° (slight sinter at 115°). The mixture melting point with authentic podophyllotoxin (mp 156–167°) was 156–157.5°. The infrared absorption spectrum of the regenerated material was identical witd that of authentic podophyllotoxin. The specific rotation was −132°.

Presumably, the crystals obtained from aqueous methanol were solvated. The melting point was 119–122° (sinter 115°), and the drying procedure described above led to a product with a somewhat less crystalline appearance and a weight loss of about 12%.

The tetrahydropyranyl group could also be removed at lower temperatures. Thus, a homogeneous mixture of 1.00 g of podophyllotoxin tetrahydropyranyl derivative V in 40 ml of 95% alcohol, 32 ml of water, and 3 ml of concentrated hydrochloric acid was allowed to stand at 35–40° for 3.5 hr. The reaction mixture, diluted with *ca.* 500 ml of cold water, was cooled for 2 hr and then filtered. The solids, washed with water and dried over calcium chloride, furnished 0.77 g (91%) of podophyllotoxin (III), mp 156–158°. The mixture melting point with authentic podophyllotoxin (mp 155–157°) was 155–157°; the infrared absorption spectra were identical.

Epimerization of Podophyllotoxin Tetrahydropyranyl Derivatives V to IV.—A solution of tetrahydropyranyl derivative V (0.50 g) in 10 ml of absolute ethanol and 5 ml of 10% aqueous sodium acetate was boiled and stirred overnight. A precipitate was noted after 20 min. After cooling the mixture for 7 hr, the solids were collected and were washed on the funnel with a small volume of alcohol. This solid, when dried, weighed 0.44 g and showed mp 207–215°. It was separated into the two forms of picropodophyllin derivative IV as follows.

Two crystallizations of the solids from chloroform–ethanol gave white crystals, which were washed with some ethanol and then dried at 100° (0.1 mm) over phosphorus pentoxide. This material, the high-melting tetrahydropyranyl derivative IV of picropodophyllin, weighed 0.25 g and showed mp 218–220° and $[\alpha]D$ −18°. The identity was checked by mixture melting point (mp 218–220°) and by comparison of infrared absorption spectra.

The combined chloroform–ethanol mother liquors were evaporated to dryness under reduced pressure. The portion of the residual solids that refused to dissolve in a minimal volume of cold methanol was collected and recrystallized from methanol. The product, dried at 70° (0.1 mm) over phosphorus pentoxide, weighed 0.14 g, melted at 200–203°, and showed $[\alpha]D$ +101°. A mixture with the low-melting tetrahydropyranyl derivative IV of picropodophyllin (mp 203–204°) melted at 200–203°. The infrared absorption spectra of this material and of the same material obtained directly from picropodophyllin were the same.

In another experiment, the precipitate obtained on allowing the tetrahydropyranyl derivative V of podophyllotoxin to stand in absolute ethanol containing a trace of sodium ethoxide for 3 hr at room temperature was crystallized from chloroform–alcohol. The higher melting form of the picropodophyllin derivative IV was obtained in 50% yield.

Podophyllotoxin (III) from Tetrahydropyranyl Derivative IV of Picropodophyllin.—A solution of triphenylmethylsodium[18] in tetrahydrofuran was prepared by shaking a mixture of triphenylmethyl chloride (9.0 g, 0.030 mole) in 170 ml of tetrahydrofuran with 175 g of 1% sodium amalgam (0.076 g-atom of sodium) for 20 hr. The solvent used here was distilled once from lithium aluminum hydride and then redistilled from lithium aluminum hydride with the condensate collected directly in the 200 ml, long-necked, round-bottomed reaction flask. All apparatus was dried at 120°. The dark red mixture was allowed to stand undisturbed for 1 day to give insoluble material a chance to settle out. Low-pressure nitrogen was applied to force aliquots of this solution through a sintered-glass disk, which was sealed to the end of a tube dipping into the solution and leading to a graduated dropping funnel. The aliquots were used either for standardization or for enolate formation.

The triphenylmethylsodium solutions were standardized by adding a measured volume to water and then titrating the vigorously stirred, heterogeneous system with 0.2 *N* sulfuric acid to a methyl red end point. In another way, titration of pure benzoic acid in dry tetrahydrofuran with the red solution afforded a specific measure of the organometal content. A red color persisting for 10 min was taken as the end point. The two modes of standardization gave results differing by no more than 10%, with the benzoic acid procedure generally indicating the lower concentration. The total yield of triphenylmethylsodium was a little over 60%; the concentration was in the order of 0.13 *N*. Evidently, triphenylmethylsodium can react with tetrahydrofuran; consequently long storage was avoided. The equivalents specified below are based on aqueous titrations performed just before each run.

A mixture of dry tetrahydropyranyl derivative (IV) of picropodophyllin (1.78 g, 3.56 mmoles) plus 350 ml of tetrahydrofuran—collected directly in a 1-l., three-necked reaction flask by distillation from lithium aluminum hydride—was stirred at 50° to effect solution. With the solution at room temperature, small portions of triphenylmethylsodium (total 35 ml, 3.82 mmoles) were added with stirring until the red color persisted. This point was reached in 10 min, after which time more reagent (3 ml) was added, and the red, homogeneous solution was stirred further for 5 min. Then, with vigorous stirring, 30 ml of glacial acetic acid was added in one portion. The red color changed immediately to yellow. Aqueous, 0.07 *M* sulfuric acid (240 ml) was added, and the still-homogeneous solution was set aside for 5 days at room temperature. The solution was warmed to 40–50° several times during this period. After 5 days, thin layer chromatography (solvent A)[11] showed three components, with R_f values corresponding, respectively, to podophyllotoxin, picropodophyllin and triphenylmethane.

The mixture was distilled under vacuum in a rotatory evaporator at 40–50° while adding water to keep the volume approximately constant. The mixture, now containing much solid, was extracted with two 150-ml portions of chloroform. The combined chloroform extract, washed twice with water and then dried with sodium sulfate, was exposed to a water-pump vacuum at room temperature to remove all volatiles.

The residual gum, dissolved in a minimal amount of chloroform, was placed on a 22 × 2.5 cm silica gel column. Elution with 200 ml of methylene chloride followed by 100 ml of methylene chloride–acetone (9:1, v/v) removed triphenylmethane. Further elution with 200 ml of chloroform–ethanol (3:2, v/v) gave a fraction which, by thin layer chromatography, contained only podophyllotoxin and picropodophyllin. Stripping all sol-

(18) C. R. Hauser and B. E. Hudson, Jr., *Org. Reactions*, **1**, 286 (1942).

vent from this fraction by distillation *in vacuo* left a glassy solid.

Picropodophyllin (I) was isolated by one crystallization from absolute ethanol. The white needles, dried at 100° (0.1 mm) over phosphorus pentoxide, gave only a single spot corresponding to picropodophyllin on thin layer chromatography (solvent A), but this material (0.75 g) melted a little low (218–219°, sintering at 208°). A second crystallization afforded pure picropodophyllin (0.71 g, 51%), mp 222–224° and $[\alpha]D$ +3°. Admixture of authentic picropodophyllin (mp 220–223°) brought the melting point to 220–223°. The infrared absorption spectrum was identical with that of picropodophyllin.

The alcoholic mother liquor from the first crystallizaion of picropodophyllin was stripped of solvent. One crystallization of the residual glass from methylene chloride–petroleum ether (bp 30–60°) gave needle-like crystals, which were dried *in vacuo* at room temperature. This product (0.55 g, 38%) showed a single spot on thin layer chromatography with solvent A; the R_f value was the same as that of authentic podophyllotoxin (III) spotted on the same plate. The infrared absorption spectra of synthetic and authentic podophyllotoxin were identical. Checks showed that the optical rotation was $[\alpha]D$ −131° and that the melting point was 160–161°. In another experiment, a mixture of the synthetic podophyllotoxin (mp 160–161.5°) with authentic material melted at 160–161.5°.

The combined mother liquors were warmed under reduced pressure to remove solvent. The residue weighed 0.18 g and, according to thin layer chromatography (system A), consisted largely of podophyllotoxin (heavy spot) and picropodophyllin (a lighter spot), with other materials present in minor amounts.

Recovery of picropodophyllin as its tetrahydropyranyl derivative is not excluded. Thus, in experiments with ether as solvent, much if not most of the picropodophyllin tetrahydropyranyl derivative survived the protonation conditions and could be recovered by taking advantage of its relative insolubility in ether. Also, see the experiment below on protonation of the enolate derived from tetrahydropyranyl podophyllotoxin.

Ineffectiveness of Sodium Acetate–Acetic Acid in Epimerizing the Tetrahydropyranyl Derivative V of Podophyllotoxin.—The following experiment showed that podophyllotoxin derivative V, once formed by quenching enolate VI with acetic acid, is not epimerized to picropodophyllin during the subsequent processing. The solution obtained on dissolving 0.45 g (0.90 mmole) of podophyllotoxin tetrahydropyranyl derivative V in 100 ml of absolute ether was treated with 5 ml of glacial acetic acid containing 0.1 g of anhydrous sodium acetate. The stirred mixture deposited a white solid, but after 10 min of stirring only a small quantity remained undissolved.

After adding water (100 ml), the ether was removed in a current of air. The aqueous mixture was extracted with chloroform, and the extract, after drying with sodium sulfate, was warmed to remove all solvent. The tetrahydropyranyl group was removed by boiling a solution of the residual oil in 12 ml of methanol plus 10 ml of 5% hydrochloric acid for 2.5 hr. Cooling the hydrolysis mixture deposited a solid (6 mg, mp 185–203°), which was removed by filtration and discarded. The filtrate was extracted with chloroform, and the extract was washed with water, dried, and warmed *in vacuo* to remove solvent. The residual oil, crystallized from methylene chloride–cyclohexane, gave colorless needles (0.29 g, 80%), melting alone or mixed with authentic podophyllotoxin (III) at 161–162°. The optical rotation was determined as $[\alpha]D$ −132°.

The mother liquor, concentrated and diluted with pentane, afforded an additional 0.06 g (14%) of white, second-crop material with $[\alpha]D$ −114° and with an infrared absorption spectrum identical with that of podophyllotoxin.

Protonation of Enolate VI Obtained from Podophyllotoxin Derivative V.—A solution of tetrahydropyranyl derivative V (1.2 g, 2.4 mmoles), mp 81–94°, in 400 ml of ether distilled directly from lithium aluminum hydride was treated with an ethereal solution of triphenylmethylsodium (2.6 mmoles) in small portions. The general procedure was the same as the one described above. The red color was discharged almost immediately, and the addition was completed within 5 min. The red color from the last small portion persisted for at least 15 min. A precipitate was evident in the ether mixture. Adding glacial acetic acid (30 ml) in one portion led to a yellow, homogeneous solution.

The solution, diluted with 200 ml of chloroform, was washed twice with 250-ml portions of water and dried with sodium sulfate, and the dry solution was evaporated under vacuum at 100°. Adding 20 ml of ether to the gummy residue gave an insoluble solid, which was crystallized twice from chloroform–ethanol. The crystals, dried *in vacuo* over phosphorus pentoxide, weighed 0.29 g (24%), and melted either with or without admixture of the higher melting picropodophyllin tetrahydropyranyl derivative (mp 219–220°) at 218–220°. The infrared absorption curves of recovered and authentic derivative IV were identical.

The ether-soluble fraction (see above) was freed of ether, and a solution of the resulting gummy residue in 30 ml of methanol and 20 ml of 3% hydrochloric acid was boiled for 4 hr. Water (80 ml) was added to the cloudy mixture, which was then extracted with 100 ml of chloroform. The extract was rinsed with water, dried with sodium sulfate, and held *in vacuo* at room temperature until all solvent was removed.

The residue was placed on a 15 × 1.5 cm column of neutral alumina (Merck). Elution with 60 ml of benzene removed the triphenylmethane, which after crystallization from petroleum ether weighed 0.5 g, melted at 88–90°, and showed the same infrared absorption spectrum as authentic material. Elution next with 100 ml of chloroform–methanol (4:1, v/v) removed the podophyllotoxin (III), which, after crystallization from methylene chloride–petroleum ether, weighed 0.27 g (28%), melted at 160.5–162° (mmp 161–162°), and showed $[\alpha]D$ −131°. Continued elution with the same chloroform–methanol solvent removed no additional material. No further attempt was made to recover picropodophyllin.

In a repetition of the above experiment, enough concentrated sulfuric acid was introduced with the quenching acetic acid to react with the sodium acetate formed. No improvement in the yield of podophyllotoxin was observed, a further indication that the podophyllotoxin derivative V is not particularly sensitive to sodium acetate plus acetic acid.

Acknowledgment.—We wish to acknowledge the able assistance of Dr. Subodh C. Chakravarti and Mr. Raymond G. McInnis, who performed some of the early experiments with the tetrahydropyranyl derivatives. We are grateful also to Sandoz, Ltd., Basle, Switzerland, for generously providing the podophyllotoxin used in this work.