The Podophyllotoxin–Picropodophyllin Equilibrium[1,2]

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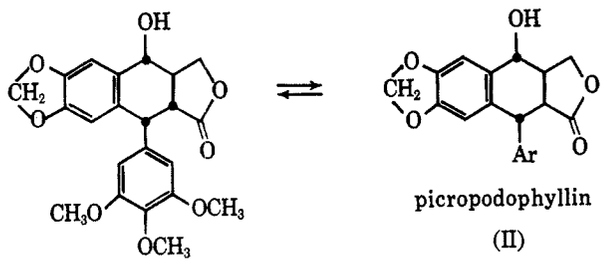
Received April 28, 1966

Podophyllotoxin and picropodophyllin dissolved in *t*-butyl alcohol containing 0.1 *M* piperidine are interconvertible. The equilibrium constants and standard free-energy changes in this system are, respectively, 37.0 (−2.18 kcal/mole) at 31.0°, 32.1 (−2.22 kcal/mole) at 49.5°, and 28.8 (−2.30 kcal/mole) at 71.5°. The standard enthalpy and entropy changes are, respectively, −1.3 kcal/mole and +2.9 eu. These quantitative results are compared with qualitative predictions drawn from scale models.

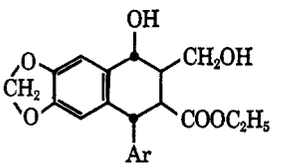
Podophyllotoxin (I), a lignan isolated from several species of *Podophyllum*, has been used clinically and experimentally as a potent cytotoxic agent.[3,4] In the presence of mild basic catalysts, podophyllotoxin epimerizes smoothly to picropodophyllin (II)[5,6] which shows little or no cytotoxic activity.[4,7] Although, *a priori*, this kind of epimerization might be expected to occur in both directions, the picropodophyllin-to-podophyllotoxin conversion has never been observed. Interest in the chemistry of podophyllotoxin compounds prompted us to study this system, which in fact is reversible and for which equilibrium constants and derived thermodynamics parameters have now been obtained.



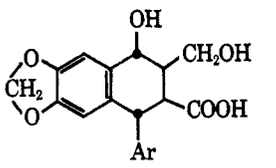
podophyllotoxin (I) ⇌ picropodophyllin (II)

Procedure and Results

Finding suitable equilibration conditions was complicated by the appearance of two extraneous materials. We believe these two compounds to be ethyl picropodophyllate (III), formed whenever ethanol was the solvent, and picropodophyllic acid (IV), formed whenever moisture was not rigorously excluded. A combination of dry *t*-butyl alcohol as solvent and 0.1 *M* piperidine as catalyst finally proved satisfactory in that isomerization proceeded at a reasonable rate and without side reactions. Another troublesome aspect was encountered in determining the equilibrium composition especially in regard to podophyllotoxin, which was always present in very low concentrations.



III



IV

The analytical problem was solved by working with radioactive substrates[8] and applying isotope dilution techniques.

The podophyllotoxin–picropodophyllin equilibrium constant, determined by approaching equilibrium from either direction, has a value of 37.0 at 31°. Accordingly, the equilibrium mixture is composed almost entirely (97.5%) of picropodophyllin (II). At 49.5 and 71.5° the equilibrium constants are 32.1 and 28.8, respectively. Figure 1 presents a plot of $\log K_{equil}$ *vs* $1/T$, from which enthalpy and entropy values were obtained. Table I summarizes the results and shows, among other things, that the standard free-energy change at 31° is −2.18 kcal/mole and that the standard enthalpy and entropy changes are, respectively, $\Delta H^\circ = -1.3$ kcal/mole and $\Delta S^\circ = +2.9$ eu. Interestingly and significantly, a large fraction—something between one-third and one-half—of the free-energy difference favoring picropodophyllin (II) is contributed by $T\Delta S$ energy.

Discussion

A scale model (Dreiding) of podophyllotoxin (I)[9] can be built, but only with some difficulty. Coplanarity of the methylenedioxybenzene ring with carbon atoms 1 and 4 coupled with the geometrical demands of the *trans*-locked lactone ring produces a strained and inflexible molecule.[10] So far as the mobility of the tri-

(1) This is paper XVI in the series entitled "Compounds Related to Podophyllotoxin." The preceding paper is by W. J. Gensler, M. V. Leeding, and A. S. Rao, *J. Org. Chem.*, **29**, 1594 (1964).

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

(3) M. G. Kelly and J. L. Hartwell, *J. Natl. Cancer Inst.*, **14**, 967 (1954); "Some New Applications of Podophyllin and Its Derivatives," S. B. Penick and Co., New York, N. Y., 1953. Recently, several derivatives of podophyllotoxin have attracted attention as anticancer agents, notably, podophyllinic acid ethylhydrazide and the benzylidene derivative of podophyllotoxin-β-D-glucoside [See, *inter alia*, K. A. Newton, G. Westbury, and W. F. White, *Cancer Chemotherapy Rept*, **43**, 33 (1964); G. Falkson, A. G. Sandison, and J. van Zyl, *S. African J. Radiology*, **2**, 1 (1964); O. Stamm, *Muench. Med. Wochschr.*, **106**, 41 (1964); *Cancer Chemotherapy Abstr.*, **5**, 84, 362 (1964)].

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

(5) W. Borsche and J. Niemann, *Ann.*, **494**, 126 (1932); E. Späth, F. Wessely, and L. Kornfeld, *Ber.*, **65**, 1536 (1932); N. L. Drake and E. H. Price, *J. Am. Chem. Soc.*, **73**, 201 (1951); J. L. Hartwell and W. F. Detty, *ibid.*, **72**, 246 (1950).

(6) A. Robertson and R. B. Waters, *J. Chem. Soc.*, 83 (1933).

(7) M. G. Kelly, E. N. Ligon, Jr., C. Davison, and P. K. Smith, *Cancer Res.*, **9**, 555 (1948); J. Leiter, V. Downing, J. L. Hartwell, and M. J. Shear, *ibid.*, **9**, 597 (1949); M. G. Kelly, A. P. Truant, and P. K. Smith, *Federation Proc.*, **8**, 306 (1949). Also see V. S. Waravdekar and J. Leiter, *Cancer Res.*, **9**, 625 (1949); J. Leiter, V. Downing, J. L. Hartwell, and M. J. Shear, *J. Natl. Cancer Inst.*, **10**, 1273 (1950).

(8) *Cf.* A. Stoll, J. Rutschmann, A. von Wartburg, and J. Renz, *Helv. Chim. Acta*, **39**, 993 (1956), for the preparation of the corresponding glucoside.

(9) The stereochemistry has been fixed by N. L. Drake and E. H. Price, *J. Am. Chem. Soc.*, **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).

(10) A. W. Schrecker and J. L. Hartwell, *J. Am. Chem. Soc.*, **76**, 752 (1954).

TABLE I

THERMODYNAMIC QUANTITIES FOR THE PODOPHYLLOTOXIN–PICROPODOPHYLLIN EQUILIBRIUM[a]

Temp, °C	K_{equil}	$\Delta F°$, kcal/mole[b]	$\Delta H°$, kcal/mole[c]	$\Delta S°$, eu[c]
31.0 ± 0.1	37.0 ± 0.5	−2.18 ± 0.01		
49.5 ± 0.3	32.1 ± 0.5	−2.225 ± 0.01	−1.3 ± 0.1	+2.9 ± 0.3
71.5 ± 0.6	28.8 ± 0.3	−2.30 ± 0.01		

[a] Uncertainties in the temperature are maximum deviations; all other uncertainties are standard deviations. [b] From $\Delta F° = -RT \ln K_{equil}$. [c] See Figure 1.

methoxyphenyl ring is concerned, a model of podophyllotoxin built with space-filling atoms (Courtauld) reveals that, although this ring can manage to rotate around its single bond to position 4, the rotation involves contact with other parts of the molecule and is not therefore entirely free. In contrast, models show that the fused-ring system in picropodophyllin (II) is considerably less rigid and less strained. The molecule can adopt four easily interconvertible, limiting conformations, in which the tetralin ring is either in a pseudo-boat or a pseudo-chair form. Intermediate forms could also be significantly populated. In most of these conformations, the trimethoxyphenyl ring can rotate reasonably freely.

Accordingly, models point to a strained podophyllotoxin (I) with considerable rigidity, and to a less strained picropodophyllin (II) with much more freedom of internal motion. These features predict that the podophyllotoxin–picropodophyllin equilibrium will show a negative enthalpy and a positive entropy change and, therefore, that the equilibrium will favor picropodophyllin. The present work clearly bears out these predictions and gives quantitative expression to them.

The isomerization of cytotoxically active podophyllotoxin (I) to the relatively inactive picropodophyllin (II) occurs under physiological conditions,[4,11] so that the conversion constitutes a mode of biological detoxication. The present work cautions that this kind of detoxication may not go to completion; *i.e.*, it might be limited by the equilibrium concentration of podophyllotoxin. The reversibility now demonstrated also serves notice that the low level of activity sometimes observed for picropodophyllin (and its analogs)[4,7] could be illusory—that is, that it might be attributable not so much to picropodophyllin as to the podophyllotoxin generated by equilibration.

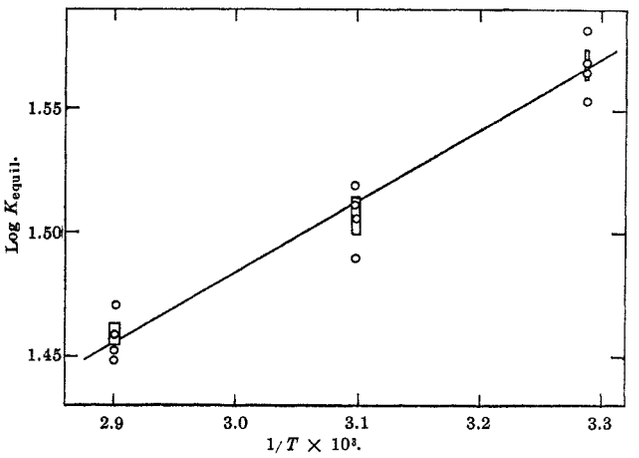


Figure 1.—Plot of log K_{equil} *vs.* reciprocal of absolute temperature. The rectangles refer to mean equilibrium constants and their standard deviations (see Table I); the width of the rectangle reflects the variation in bath temperature. The circles correspond to the experimental equilibrium constants given in Table IV. A least-squares treatment of these data furnished the slope, the ordinate intercept at $1/T = 0$, and their standard deviations (*cf.* W. J. Youden, "Statistical Methods for Chemists," John Wiley and Sons, Inc., New York, N. Y., 1951, Chapter 5). A rough calculation showed that the effect of the temperature variation on the standard deviations of slope and intercept is small in comparison with the effect of the ordinate errors. Accordingly, the uncertainty introduced by the temperature fluctuations was disregarded. The standard enthalpy and entropy values entered in Table I was calculated from $\Delta H° = (-2.303) \times R \times$ (slope) and from $\Delta S_0 = (2.303) \times R \times$ (intercept).

Experimental Section

General.—All infrared absorption curves were taken with double-beam recording instruments with the compounds dissolved in chloroform. For determination of specific rotation, the compounds were examined in chloroform solution at a concentration of approximately 1 g/100 ml of solution. Melting points (uncorrected) were taken in open capillaries, with the rate of heating of 1–2°/min. Elementary analyses were reported by the Microanalytical Laboratory, Massachusetts Institute of Technology, Cambridge, Mass., and by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Radioactivity determinations were performed by New England Nuclear Corporation, Boston, Mass.

For thin layer chromatography, layers of silica gel (Camag D-5), 0.3 mm thick and 20 cm long, were heated at 125–130° for 2–3 hr. The developing solvents were A, methylene chloride plus acetone (4:1 v/v); B, methylene chloride plus acetone (7:1 v/v); C, chloroform plus ethyl acetate (4:1 v/v); and D, ether plus methylene chloride (6:1 v/v). A spray of ethyl alcohol–sulfuric acid (1:1 v/v) made the chromatogram spots visible. Tests showed that either podophyllotoxin or picropodophyllin in amounts of 1 μg or less can be detected easily. Tests of mixtures with solvent A showed that 3% of podophyllotoxin in picropodophyllin gives rise to two distinct spots. At lower concentrations, the two spots tend to overlap. A mixture of 2% of picropodophyllin in podophyllotoxin gives two spots.

On the basis of elementary analysis and melting point (see below), the podophyllotoxin in this work was regarded as the half-hydrate. Unless otherwise specified, all weights and yields of podophyllotoxin refer to this composition.

Purification of Podophyllotoxin (I).—Commercial podophyllotoxin on thin layer chromatographic analysis (solvent A) showed three spots with the following R_f values: 0.87 (minor; presumed to be either α- or β-apopicropodophyllin, whose R_f values agree closely), 0.44 (large; podophyllotoxin), and *ca.* 0.3 (minor; not identified). The material was purified as follows. A solution of 2.0 g of podophyllotoxin in 25 ml of methylene chloride was filtered, and the clear filtrate, held at a gentle boil, was treated with petroleum ether (bp 30–60°) until cloudiness developed. With the mixture at room temperature, more petroleum ether (total, 10 ml) was added gradually over a 4-hr period. Solvent was removed from the needlelike solids with a pipet, and the crystallization procedure was repeated. The resulting fine white needles of podophyllotoxin (1.6 g), after drying *in vacuo* over phosphorus pentoxide at room temperature for 5 hr, showed mp 160–161.5°. The material was homogeneous according to thin layer chromatography.

An alternate procedure, making use of column chromatography, was also effective. Thus, a solution of podophyllotoxin (2.0 g) in a small volume of methylene chloride was placed on a 20 × 1.3 cm column of powdered silica gel (Baker Analyzed grade). Elution with methylene chloride (150 ml) removed no

(11) J. J. Kocsis, E. J. Walaszek, and E. M. K. Geiling, *Arch. Intern. Pharmacodyn.*, **111**, 134 (1957). Also see M. G. Kelly, J. Leiter, A. R. Bourke, and P. K. Smith, *Cancer Res.*, **11**, 263 (1951).

material. Elution with 210 ml of methylene chloride–acetone (4:1 v/v) removed a fast-running impurity, next the podophyllotoxin, and lastly a slow-moving material. The composition of the eluate fractions was followed by thin layer chromatography (solvent A). Those middle fractions showing the podophyllotoxin spot alone or accompanied only by faint satellite spots were combined and were passed through a sintered-glass disk. The solids, recovered from the filtrate, were crystallized from methylene chloride–petroleum ether to give white needles of podophyllotoxin (1.7 g), mp 160–162°, $[\alpha]_D$ −130.5°. This material gave only one spot on a thin layer chromatographic plate with solvent A. The purified podophyllotoxin was dried at room temperature (0.1 mm) for a day.

Anal. Calcd for $C_{22}H_{22}O_8$: C, 63.76; H, 5.35. Calcd for $C_{22}H_{22}O_8 \cdot 0.25H_2O$: C, 63.08; H, 5.42; H_2O, 1.08. Calcd for $C_{22}H_{22}O_8 \cdot 0.5H_2O$: C, 62.41; H, 5.48; H_2O, 2.13. Found: C, 63.18, 62.68, 62.47; H, 5.63, 5.55, 5.51.

Methylene chloride was not retained, since analysis showed Cl, 0.00.

On the assumption that the purified podophyllotixin is a definite compound, the formula with one-half molecule of water of hydration was taken as correct. Accordingly, the material contained 97.87% of anhydrous podophyllotoxin. It was stored in a desiccator over phosphorus pentoxide in the dark.

From the melting point and the analytical figures, the podophyllotoxin obtained here corresponds to the previously reported modification A.[12]

Preparation of Picropodophyllin (II).—A mixture of podophyllotoxin (10 g), absolute ethanol (150 ml), and 10% aqueous sodium acetate (100 ml) was boiled and stirred for 15 hr. A precipitate was noted in the initially homogeneous system after about 3 hr. The mixture, cooled to 0°, was filtered, and the solids were washed thoroughly with water and then dried (8.1 g). Additional material was obtained by concentrating and cooling the filtrate. Four recrystallizations from absolute ethanol gave cottonlike needles of picropodophyllin. Dried at 100° (0.1 mm) for 12 hr, this material showed mp 222–224° dec with sintering at 212°, $[\alpha]_D$ +5° (0.092 in chloroform), and a carbonyl absorption peak at 1765–1768 cm^{-1}. The picropodophyllin was homogeneous according to thin layer chromatography and showed R_F 0.48 (solvent B).

Purification and Radioactivity of Labeled Materials.—Commercial (4′-methoxy-C-14)-labeled podophyllotoxin[8] showed five spots on thin layer chromatography with solvent C. Chromatography of the material (100 mg) through a 12 × 2 cm column of silica gel (see above) gave fractions that were monitored with the help of thin layer chromatography (solvent A). Those fractions showing only the podophyllotoxin spot were combined and recrystallized. The crystalline product was dried at room temperature (0.1 mm) for 1 day. The radioactive podophyllotoxin (76 mg), mp 160–161°, showed a single spot on a thin layer chromatography plate with an R_F value the same as that of authentic podophyllotoxin. Table II summarizes the data on which we base an activity of 2.83 ± 0.05 μcurie/mg of anhydrous material for this starting material.

The radioactive picropodophyllin used here was material recovered from various equilibration experiments. The material was crystallized two or three times from alcohol (see above) until thin layer chromatography indicated homogeneity and several times thereafter. At each stage, samples were retained for radioactivity assay.

Preliminary Equilibration Experiments.—Redistilled absolute alcohol and absolute *t*-butyl alcohol fractionated over lithium aluminum hydride were the two solvents investigated. The catalysts include freshly distilled anhydrous piperidine, N-methylpiperidine, anhydrous sodium acetate, a freshly prepared dilute solution of clean sodium in ethanol, and dilute solutions of sodium or of potassium in *t*-butyl alcohol. Interconversions were followed by thin layer chromatography (solvent B). In addition to podophyllotoxin (R_F 0.43) and picropodophyllin (R_F 0.48), two other materials were encountered, *i.e.*, one with R_F 0.3 tentatively regarded as ethyl picropodophyllate (III) and one with R_F 0.0 tentatively regarded as picropodophyllic acid (IV). The ethyl ester III was observed with ethyl alcohol as solvent but not with *t*-butyl alcohol; the acid IV was observed with both solvents. In one experiment, a *t*-butyl alcohol solution containing appreciable amounts of picropodophyllic acid (IV) as well as picropodophyllin (II) was acidified to pH 4 with 0.2 *N* sulfuric acid. After 1 day at room temperature, the spot corresponding to acid IV was no longer present, although the picropodophyllin spot persisted. Presumably, the picropodophyllic acid (IV) had cyclized to picropodophyllin in the acid solution.

(12) A. W. Schrecker, J. L. Hartwell, and W. C. Alford, *J. Org. Chem.*, **21**, 288 (1956).

TABLE II

RADIOACTIVITY OF PODOPHYLLOTOXIN STARTING MATERIAL

Sample	Mp, °C	Dilution factor	Specific activity,[a] μcuries/mg: Measured	Specific activity,[a] μcuries/mg: Corrected for dilution
A[b]	160–161	1.000	2.953	2.95
B[b]	160–161	97.18	0.03045	2.96
B[b]	160–161	174.9	0.01522	2.66
B[b]	160–161.5	485.9	0.00582	2.83
C[b]	222–224	1.000	2.85	2.85
C[b]	222–224	485.9	0.0056	2.72
				av 2.83 ± 0.05[c]

[a] Microcurie per milligram of anhydrous material. [b] A. Purified undilated starting material. B. This sample was prepared by diluting an aliquot from a solution of purified radioactive podophyllotoxin in methylene chloride with unlabeled podophyllotoxin. The mixed podophyllotoxin was recovered, recrystallized, and checked for homogeneity by thin layer chromatography before assay. C. This sample refers to picropodophyllin obtained by epimerizing the corresponding podophyllotoxin sample with piperidine in *t*-butyl alcohol and then recrystallizing the isolated picropodophyllin until it was homogeneous. [c] Standard deviation of the mean.

Potassium *t*-butoxide in *t*-butyl alcohol rapidly converted podophyllotoxin to picropodophyllin and, possibly, at a lower concentration and with more careful exclusion of moisture, could serve as an equilibration catalyst. N-methylpiperidine was not a particularly effective catalyst. No tendency for formation of either side product III or IV was noted in *t*-butyl alcohol containing 1.5% piperidine for 2 days at 50°. Some of the acid IV was observed with 5% piperidine after 1 day at 50°, but this is long after equilibrium was reached. A combination of *t*-butyl alcohol solvent with piperidine (*ca.* 1% by volume) was chosen as the system giving the best results.

Equilibration Procedure.—A carefully weighed sample of pure radioactive podophyllotoxin or picropodophyllin was transferred quantitatively to a dry 50-ml flask with *t*-butyl alcohol that had been purified by fractional distillation from lithium aluminum hydride. Redistilled piperidine corresponding to $^1/_{100}$ the volume of *t*-butyl alcohol was added, and the flask was shaken in a thermostatted bath. The concentration of picropodophyllin or podophyllotoxin was 0.002–0.004 *M*; that of piperidine was approximately 0.1 *M*. At 71.5°, the reaction flask, sealed to a water-cooled condenser and protected from atmospheric moisture with a tube of anhydrous calcium sulfate, was shaken for 4 days. Shaking prevented formation of a crust that otherwise tended to appear on the wall of the flask. All equilibration mixtures were homogeneous throughout. In the 49.5° experiments, the same kind of reaction flask was shaken for 5 days. At 31.0°, the reaction flask was sealed before it was allowed to remain in the bath for 35 days.

Each equilibrium experiment was run side by side with a replicate run in which nonradioactive material was used. Thin layer chromatographic monitoring showed spots corresponding only to podophyllotoxin and picropodophyllin. At the end of the equilibration period, the volume of 0.2 *N* sulfuric acid necessary to bring the mixtures to pH 2 (approximate) was determined with the replicate run and used as described below with the hot run.

After equilibrium had been reached, the reaction flask was immersed in a mixture of acetone and solid carbon dioxide. Sulfuric acid (0.2 *N*) was added to the frozen *t*-butyl alcohol mixture which, when brought to room temperature, showed pH 2. The solution was transferred quantitatively to a 250-ml round-bottom flask with the help of chloroform. Accurately weighed samples of pure nonradioactive podophyllotoxin and picropodophyllin were added, and the mixture was warmed slightly to ensure complete solution, more chloroform being added if necessary. At this stage, a thin layer chromatography plate showed only the two spots expected for podophyllotoxin and picropodophyllin.

Distilled water was added, and the turbid mixture was concentrated *in vacuo* at temperatures below 50° until considerable solid was evident. Extraction with four 40-ml portions of chloro-

TABLE III

DATA FOR EQUILIBRATION EXPERIMENTS[a]

Temp, °C	Starting material: Compd[c]	Wt, mg	Mean specific activity,[b] ncuries/mg	t-Butyl alcohol solvent, ml	Wt of added diluents, mg: Podo	Picro	Mean specific activity of recovered material,[b] ncuries/mg: Podo	Picro
31.0 ± 0.1	Podo	20.97	246	30	109.14	150.36	1.20	27.8
	Picro	23.85	206.5	30	76.24	109.90	1.692	36.29
49.5 ± 0.3	Podo	22.74	246	21	105.31	135.46	1.60	33.4
	Picro	26.804	272	25	95.49	109.25	2.22	51.5
71.5 ± 0.6	Podo	11.05	2830	7	99.70	97.85	10.2	272
	Picro	37.19	206.5	35	97.99	132.85	2.65	44.2

[a] The weights and activities for podophyllotoxin have been adjusted to the dry basis. [b] The mean specific activity of material obtained from two, more often three, consecutive crystallizations. [c] Abbreviations are podo, podophyllotoxin; picro, picropodophyllin.

form removed both podophyllotoxin and picropodophyllin. The extracts were washed several times with water, dried with sodium sulfate, and warmed at 100° under reduced pressures to remove all solvent. One crystallization of the residual solids from absolute alcohol (8–15 ml) separated the less soluble picropodophyllin from the more soluble podophyllotoxin.

The picropodophyllin solids were crystallized several times from absolute alcohol (*ca.* 10 ml) until a thin layer chromatograhic plate showed only one spot with the proper R_F value. This material was recrystallized further, samples from the several crystallization stages being dried at 100° (0.01 mm) for 20 hr, accurately weighed, and then submitted for radioactivity assay. The melting points of the picropodophyllin sent for assay fell in the 222–226° range, with the exception of one sample that melted at 217°.

All volatile material was removed from the alcoholic mother liquor containing the podophyllotoxin. The residue was crystallized several times from methylene chloride–petroleum ether (bp 30–40°) until the crystals were homogeneous according to thin layer chromatography. This pure podophyllotoxin was recrystallized further, with samples at each stage being sent for assay after they had been dried overnight at room temperature (0.01 mm) over phosphorus pentoxide. The melting point in all cases was no lower than 159° and no higher than 162°. Tables III and IV summarize the experimental numbers.

For the radioactivity determinations, 1-mg samples, weighed to the nearest microgram, were submitted for analysis, which was performed by liquid scintillation. A background correction in the order of 30 cpm was routinely applied, although this correction generally was far less than 0.1% of the net count. The measured radioactivities of podophyllotoxin (I) and of picropodophyllin (II) from at least two crystallization stages—more often from three—were averaged to get the final value (Table III). The minor differences in radioactivity between samples from consecutive crystallizations, the thin layer chromatography results, and the correct melting points attest to the homogeneity and radiochemical purity of all these samples.

To show that only negligible isomerization occurs during the isolation procedure, a check was performed as follows. A solution of 35.0 mg of picropodophyllin in 30 ml of *t*-butyl alcohol containing 0.3 ml of piperidine was treated in the cold with 20 ml of 0.2 *N* sulfuric acid. The homogeneous solution showed pH 4 (approximate). Radioactive podophyllotoxin (2.0 mg; 249 ncuries/mg) was added followed by 10 ml of chloroform, and the mixture was warmed on the steam. Thereafter the procedure was essentially the same as above. The recovered picropodophyllin, after three crystallizations, showed a single spot on thin layer chromatography and melted at 222–224° dec with preliminary sintering. This material as well as that obtained from one additional crystallization were assayed for radioactivity. The activity observed (0.0052 and 0.0053 ncurie/mg) indicated a 0.04% conversion of podophyllotoxin to picropodophyllin during the isolation manipulation. Check of the starting picropodophyllin used here revealed the presence of trace activity (0.001 ncurie/mg), which if taken into account brings the conversion figure to 0.03%. Accordingly, whatever interconversion occurred during purification of the equilibrated materials could be neglected.

TABLE IV

EQUILIBRIUM CONSTANTS FOR THE PODOPHYLLOTOXIN–PICROPODOPHYLLIN SYSTEM

Temp, °C	Equilibrium approached from	Wt. at equilibrium,[a] mg.: Podo	Picro	Equilibrium constants: b	c	Mean[d]
31.0 ± 0·1	Podo	0.535	19.16	35.8	38.2	37.0 ± 0.5
	Picro	0.632	23.43	37.1	36.8	
49.5 ± 0·3	Podo	0.689	21.28	30.9	32.0	32.1 ± 0.5
	Picro	0.785	25.52	32.5	33.1	
71.5 ± 0·6	Podo	0.361	10.40	28.8	29.6	28.8 ± 0.3
	Picro	1.274	36.18	28.4	28.2	

[a] As calculated from the figures in Table III. The podophyllotoxin weights refer to anhydrous material. Note that the maximum difference between the weight of starting material and the sum of the equilibrium weights of podophyllotoxin and picropodophyllin is 6.1%; the average difference is 2.6%. [b] Equilibrium constants calculated as $(\text{picro})_{equil}/(\text{podo})_{equil}$. [c] Equilibrium constants calculated as [starting material − $(\text{podo})_{equil}$/$(\text{podo})_{equil}$. [d] The uncertainties are standard deviations.

Acknowledgment.—We wish to thank Sandoz Ltd., Basle, Switzerland, for generous gifts of unlabeled as well as of 4′-(methoxy-C-14)-labeled podophyllotoxin.