

[CONTRIBUTION FROM THE LABORATORY OF CHEMICAL PHARMACOLOGY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE]

Components of Podophyllin. XIV.<sup>1</sup> Acid-catalyzed Reactions of Podophyllotoxin and its Stereoisomers

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RECEIVED AUGUST 19, 1953

The acid-catalyzed dehydration of picropodophyllin and epipicropodophyllin and the conversion of the latter to the former are explained by a common carbonium ion mechanism. The failure of podophyllotoxin and of epipodophyllotoxin to undergo ionic elimination may be related to rigidity conferred by the *trans*-lactone fusion or to steric hindrance of the *cis* (3:4)-substituents.

While picropodophyllin (PP) (I, R = OH) and epipicropodophyllin (EPP)<sup>2</sup> are readily dehydrated to  $\alpha$ -apopodophyllin ( $\alpha$ -apoPP) (II) by such reagents as sulfuric acid in acetic anhydride,<sup>3,4</sup> phosphorus trichloride in benzene,<sup>2</sup> iodine in benzene or hydrochloric acid in acetic acid, podophyllotoxin (PT) and epipodophyllotoxin (EPT) fail to undergo similar elimination reactions. Explanations based on structural differences<sup>3,5,6</sup> were invalidated by the finding<sup>2</sup> that PT, PP, EPT and EPP are diastereoisomers of structure I (R = OH).

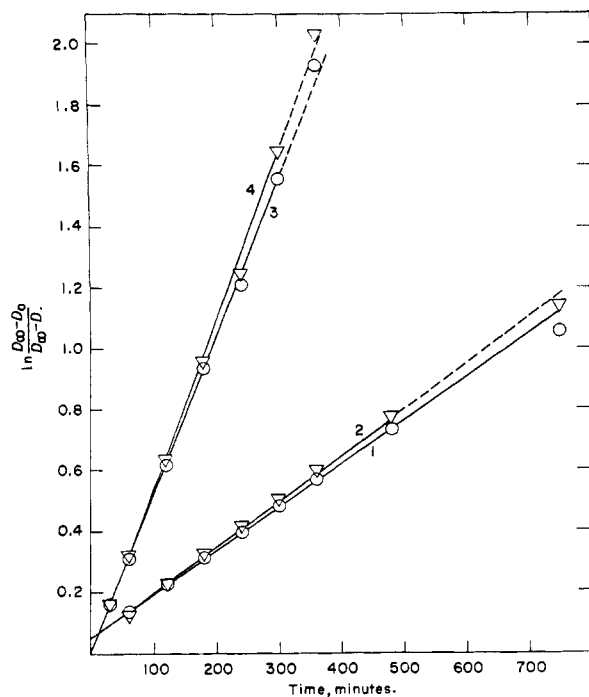
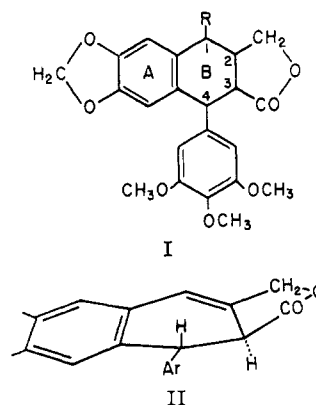
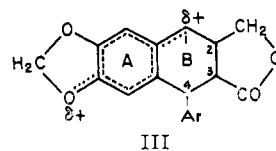


Fig. 1.—Determination of the dehydration rate of picropodophyllin (PP, circles) and epipicropodophyllin (EPP, triangles) in acetic-hydrochloric acid at 25°; curve 1,  $10^{-4}$  M PP and 0.04 M HCl; curve 2,  $10^{-4}$  M EPP and 0.04 M HCl; curve 3,  $10^{-4}$  M PP and 1.24 M HCl; curve 4,  $10^{-4}$  M EPP and 1.24 M HCl/liter of  $\text{CH}_3\text{CO}_2\text{H}$ . The optical densities  $D$  were measured at 311 m $\mu$ .

Similarly, preferential *trans*-elimination<sup>7</sup> is not involved since the hydroxyl group at C<sub>1</sub> and the hydrogen at C<sub>2</sub> are *cis* in both PT and PP, and *trans* in both EPT and EPP.<sup>8</sup> Hence it seems that either the *trans*-lactone fusion or the *cis*-relation of the substituents at C<sub>3</sub> and C<sub>4</sub><sup>8</sup> must render PT and EPT resistant to ionic elimination reactions.



The acid-catalyzed dehydration of PP and EPP appears to proceed through a common carbonium ion, which would be expected to be stabilized by resonance with an oxonium ion structure (transition state III). Such resonance stabilization requires essential coplanarity of C<sub>1</sub>, C<sub>2</sub> and C<sub>4</sub> with ring A. A kinetic study of the reaction of PP and EPP with hydrochloric acid in acetic acid at room temperature (Fig. 1) showed that the two compounds were dehydrated at essentially equal rates. Loss of a proton from the common intermediate III appears, therefore, to be the rate-determining step. Supporting evidence for the easy formation of III is afforded by the ready conversion of EPP to PP in the presence of dilute mineral acid.<sup>2</sup> Similarly, PP ethyl ether (I, R =  $\text{OC}_2\text{H}_5$ ) was obtained in comparable yields (68–80%) when either PP, EPP or EPP ethyl ether was refluxed with sulfuric acid in ethanol for a short



(1) Paper XIII: A. W. Schrecker and J. L. Hartwell, *THIS JOURNAL*, **75**, 5924 (1953).

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

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

(4) A. W. Schrecker and J. L. Hartwell, *THIS JOURNAL*, **74**, 5676 (1952).

(5) W. Borsche and J. Niemann, *Ann.*, **494**, 126 (1932).

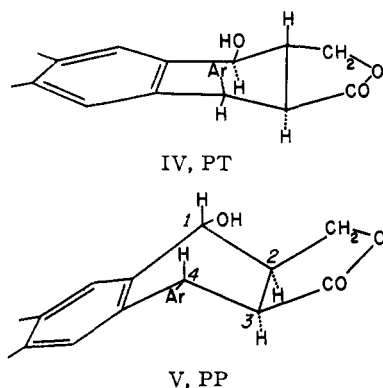
(6) E. Späth, F. Wessely and L. Kornfeld, *Ber.*, **65**, 1536 (1932).

(7) D. H. R. Barton, *Experientia*, **6**, 316 (1950), D. H. R. Barton and W. J. Rosenfelder, *J. Chem. Soc.*, 1048 (1951).

(8) A. W. Schrecker and J. L. Hartwell, *THIS JOURNAL*, **75**, 5916 (1953).

time, pointing to addition of an ethoxide ion to C<sub>1</sub> in III.

Study of molecular models indicates that the *trans*-lactone ring fusion fixes PT and EPT rigidly in a chair-type conformation<sup>9</sup> (IV), in which the substituents at C<sub>2</sub> and C<sub>3</sub> must be equatorial.<sup>10</sup> On the other hand, PP and EPP are flexible molecules which could assume either chair or boat conformations. The boat form (V) may be favored<sup>11</sup> because it places both the *cis*-lactone ring and the bulky aryl group in the preferred<sup>7</sup> equatorial position. However, there is evidence that the chair form may be thermodynamically more stable in molecules of this type.<sup>10,12,13</sup> In any event it appears reasonable that the *cis*-lactones can undergo deformation much more readily than the *trans*-lactones.<sup>10</sup>



The failure of EPT and PT to undergo ionic elimination reactions could then be explained on the basis of their *trans*-lactone fusion. Too large an activation energy might be needed to form an ionic transition state (III) with its coplanarity requirement from the rigid conformation possessed by these molecules.<sup>14</sup> However, acid-catalyzed conversions from the EPT to the PT series do occur, although less readily than with the *cis*-lactones. Hence the possible formation of an ionic intermediate similar to III cannot be excluded. Thus the acetate of PT rather than that of EPT was isolated from the reaction of EPT with acetic anhydride and sulfuric acid.<sup>2</sup> When EPT was refluxed with dilute hydrochloric acid in acetone, it was partly converted to PT. This was demonstrated by the optical rotation of the reaction product and the isolation of the sparingly soluble PP after treatment with piperidine in ethanol. The isomerization proceeded, however, to a much smaller extent than in the case of EPP,<sup>2</sup> as shown in Table I.

As an alternative explanation of the failure of PT and EPT to undergo acid-catalyzed dehydration, it

(9) W. H. Mills and I. G. Nixon, *J. Chem. Soc.*, 2510 (1930); K. Ganapathi, *Ber.*, **72**, 1381 (1939).

(10) S. J. Angyal and C. G. Macdonald, *J. Chem. Soc.*, 686 (1952).

(11) W. S. Johnson, *Experientia*, **7**, 315 (1951).

(12) C. W. Beckett, N. K. Freeman and K. S. Pitzer, *THIS JOURNAL*, **70**, 4227 (1948).

(13) Two chair forms can be written for PP or EPP. One of them has the aryl group in the hindered<sup>7</sup> polar position. In the other, the hydroxyl at C<sub>1</sub> would be polar in PP and equatorial in EPP, which is inconsistent<sup>7</sup> with the irreversible acid-catalyzed conversion of EPP to PP and EPP ethyl ether to PP ethyl ether.

(14) Somewhat related arguments have been presented by Angyal and Macdonald (ref. 10) and by E. L. Eliel, *Experientia*, **9**, 91 (1953).

TABLE I

CONVERSION FROM THE "EPI" TO THE "NORMAL" SERIES

Starting material	Medium	Time of heating, hr.	Conversion, %	
			From optical rotation of product <sup>a</sup>	From weight of picropodophyllin
Epipodophyllotoxin	1 vol. acetone 1 vol. <i>N</i> HCl	1	18	14
Epipodophyllotoxin	1 vol. acetone 1 vol. 2 <i>N</i> HCl	1	33	25
Epipicropodophyllin	1 vol. acetone 2 vol. <i>N</i> HCl	0.5	..	80 <sup>b</sup>

<sup>a</sup> Calculated by using the values for PT and EPT previously obtained (ref. 2). <sup>b</sup> Cf. ref. 2.

should be pointed out that such a reaction could not yield  $\alpha$ -apoPP (II), but would lead to its hypothetical diastereoisomer,  $\alpha$ -apoPT, in which the substituents of C<sub>3</sub> and C<sub>4</sub> would be *cis*.<sup>8</sup> It is quite conceivable that the steric hindrance of these *cis*-substituents might prevent the formation of such a compound.<sup>15</sup>

While both factors (rigidity of the *trans*-lactone structure and steric hindrance of the *cis*-substituents at C<sub>3</sub> and C<sub>4</sub>) may be operative in preventing PT and EPT from undergoing acid-catalyzed dehydration, the relative contribution of each will await studies on appropriate model compounds.

#### Experimental<sup>16</sup>

**Dehydration of Epipicropodophyllin with Acetic Anhydride and Sulfuric Acid.**—Treatment of EPP (I, R = OH) by the method used for PP<sup>3,4</sup> produced a 54% yield of  $\alpha$ -apoPP (II), m.p. 238–243°, [ $\alpha$ ]<sub>D</sub><sup>20</sup> –17° (c 0.55, chloroform).

**Reaction of the 1-Hydroxylactones (I, R = OH) with Iodine.**—Suspensions of PT, PP, EPT or EPP in 30 ml. of benzene were refluxed with 0.03 g. of iodine for 6.5 hours, and the solutions allowed to stand at room temperature overnight. In the case of PP and EPP (0.72 g. each),  $\alpha$ -apoPP was collected and washed with benzene. The yields were 68% and 50% and the melting points 239–240° and 233–235°, respectively. Starting material (1.0 g.) was recovered from PT (1.6 g.). EPT (1.0 g.) yielded what appeared to be a mixture of starting material and PT, which could not be separated into its components.

**Dehydration of Picropodophyllin and Epipicropodophyllin with Acetic and Hydrochloric Acids.** (a) **Isolation of  $\alpha$ -Apopodophyllin (II).**—Solutions of 500 mg. of the compounds in 5 ml. of glacial acetic acid were treated with 0.016 ml. of concd. hydrochloric acid and allowed to stand at room temperature for 44 hours. The small colorless needles of  $\alpha$ -apoPP were collected, washed with ethanol and dried; m.p. 245–246°. The yields were 420 mg. (88%) from PP and 444 mg. (93%) from EPP. When PT and EPT were treated similarly, the solutions remained clear. Dilution with water precipitated colorless flocculent material, which after drying melted in both cases between 110° and 125°. The infrared absorption spectra showed bands characteristic for PT or EPT and their respective acetates, but none of the maxima specific<sup>4</sup> for  $\alpha$ - or  $\beta$ -apoPP.

(b) **Kinetic Study (Fig. 1).**—Solutions of PP or EPP in glacial acetic and hydrochloric acids were prepared by adding measured amounts of concd. hydrochloric acid (diluted first with acetic acid for the 0.04 *N* solutions) to aliquots of freshly prepared stock solutions of the compound in acetic

(15) It appears from models that the aryl group is essentially equatorial in  $\alpha$ -apoPP (II), while it would be essentially polar, thus more hindered sterically,<sup>7</sup> in  $\alpha$ -apoPT. The partial double-bond character of the single bond connecting C<sub>1</sub> to ring A should render ring B more planar than models would indicate. However, this factor would tend to accentuate rather than decrease the greater steric hindrance of *cis*-as contrasted with *trans*-substituents.

(16) Melting points are corrected. Ultraviolet absorptions were measured with a Beckman model DU spectrophotometer.

acid placed in volumetric flasks, which were then filled to the mark with acetic acid. They were immediately transferred to 1-cm. stoppered quartz cells, which were placed in a cell compartment kept at 25° by circulating water from a constant temperature bath. The blank cells contained acetic and hydrochloric acids alone. The optical densities ( $D$ ) at 311  $m\mu$ , the absorption maximum of the end-product, were measured at regular intervals. Assuming first-order kinetics, the following equation holds for the rate constant  $k$

$$kt = \ln(D_{\infty} - D_0/D_{\infty} - D)$$

where  $D_{\infty} = 7530c$ ;  $D_0 = 37c$  (for PP) and  $56c$  (for EPP), with  $c$  being the initial molarities of the reactants and with the numerical values corresponding to the molar extinction coefficients of  $\alpha$ -apoPP, PP and EPP, respectively, in glacial acetic acid. The following first-order rate constants were evaluated from the slopes of the straight-line portions of the graphs: PP,  $k = 1.4 \times 10^{-3}/\text{min.}$  and EPP,  $k = 1.5 \times 10^{-3}/\text{min.}$  (solutions 0.04  $N$  in hydrochloric acid); PP,  $k = 5.2 \times 10^{-3}/\text{min.}$  and EPP,  $k = 5.5 \times 10^{-3}/\text{min.}$  (solutions 1.24  $N$  in hydrochloric acid).

**Picropodophyllin Ethyl Ether** ( $I$ ,  $R = OC_2H_5$ ).—A solution of 0.20 g. of EPP in 2 ml. of absolute ethanol containing 0.06 ml. of concd. sulfuric acid was refluxed for 15 minutes. The felt-like colorless needles, which separated almost immediately, were collected, after diluting with another 6 ml. of absolute ethanol and chilling; they were washed with ethanol, water, and again ethanol, then dried. The yield was 0.17 g. (80%); in analogous experiments with PP and with EPP ethyl ether<sup>2</sup> it was 68% and 77%, respectively. The crude material melted at 243–245° with sintering at 196°, when immersed at room temperature;

when it was immersed at 210°, it melted with foaming, resolidified and melted again at 243–245°;  $[\alpha]^{21}_D + 58^\circ$  ( $c$  0.50, chloroform). Two recrystallizations from chloroform-ethanol provided long colorless needles, m.p. 227–229°,  $[\alpha]^{21}_D + 58^\circ$  ( $c$  0.52, chloroform).

*Anal.* Calcd. for  $C_{24}H_{26}O_8$ : C, 65.15; H, 5.92; 4 alkoxy calcd. as  $OCH_3$ , 28.06. Found: C, 65.08; H, 5.97;  $OCH_3$ , 27.80.

The behavior of the crude material upon heating was apparently caused by the presence of residual sulfuric acid. Pyrolysis of the crude product at 225° gave  $\alpha$ -apoPP, while the purified substance was recovered unchanged.

**Conversion of Epipodophyllotoxin to Podophyllotoxin** (Table I).—A solution of 1.0 g. of EPT in 6 ml. of acetone and 6 ml. of 2  $N$  hydrochloric acid was refluxed for one hour, then diluted with water. The resulting oil (0.82 g.) solidified partly on chilling and completely after washing with water and drying at 60°; m.p. 167–178°,  $[\alpha]^{21}_D - 94^\circ$  ( $c$  1, chloroform). Epimerization with piperidine in aqueous ethanol<sup>2</sup> gave PP, m.p. 224–227.5° (acetate<sup>5</sup> m.p. and mixed m.p. 214.5–216°). With  $N$  hydrochloric acid, the product (0.93 g.) had m.p. 164–175°,  $[\alpha]^{21}_D - 85^\circ$  and was converted to impure PP, m.p. 207–222°.

**Acknowledgments.**—The authors are grateful to Dr. D. H. R. Barton and to several staff members of the National Institutes of Health for stimulating criticism and discussions. They wish to thank Dr. W. C. Alford and co-workers for microanalyses.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CARNEGIE INSTITUTE OF TECHNOLOGY]

## The Role of Chloroacetylpolyglycolic Acids in the Potassium Bromide Catalyzed Conversion of Chloroacetic Acid and Naphthalene into $\alpha$ -Naphthylacetic Acid<sup>1</sup>

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RECEIVED AUGUST 3, 1953

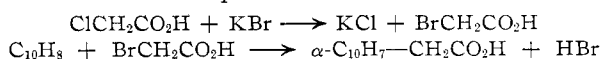
When heated at the reflux temperature in the presence of small amounts of potassium bromide, chloroacetic acid is converted into a mixture of chloroacetylpolyglycolic acids. This type of polyester will react with boiling naphthalene to yield  $\alpha$ -naphthylacetic acid or products saponifiable to  $\alpha$ -naphthylacetic acid. This sequence of two reactions is evidently involved in the conversion of naphthalene into  $\alpha$ -naphthylacetic acid when naphthalene is heated with chloroacetic acid in the presence of potassium bromide. Mixtures of glycolide and chloroacetic acid also react with naphthalene to give  $\alpha$ -naphthylacetic acid.

The reaction of chloroacetic acid with naphthalene to yield  $\alpha$ -naphthylacetic acid was first reported in patents issued to Wolfram, Schörnig and Hausdörfer.<sup>3</sup> The reaction was described as taking place when the reactants were kept for 48 hours at the reflux temperature of the mixture, and the use of catalysts was not mentioned. The reaction seemed remarkable in that alkylation of an aromatic hydrocarbon was achieved in the absence of any acid catalyst of the type used in Friedel-Crafts reactions.

When we investigated the utility of this reaction as a preparative method, we found that even when refluxing was continued for as long as 80 hours the yield of  $\alpha$ -naphthylacetic acid reached only about 3%, and that large portions of both starting ma-

terials remained unchanged. Acids such as hydrogen chloride, hydrogen bromide and sulfuric acid, and typical Friedel-Crafts catalysts such as aluminum chloride and zinc chloride (which led to tar formation) did not improve the yield. Ferric chloride did appear to have a beneficial effect, but only to a minor degree.

On the other hand, we found that bromoacetic acid, mentioned in the patents as reacting in analogous fashion, gave higher yields. Experiments were then performed to test the possibility that by adding a mole of potassium bromide to a mole each of chloroacetic acid and naphthalene, bromoacetic acid might be formed *in situ* and might then react with naphthalene



Much improved yields (ca. 15%) were, in fact, obtained in this way, but when it was found that 0.1 mole of potassium bromide<sup>4</sup> produced at least as favorable an effect on the yield as 1 mole, and

(1) The initial phase of this investigation was supported by a research grant from the Niagara Chemical Division, Food Machinery and Chemical Corporation. The major portion of this report was abstracted from a thesis submitted by William L. Walsh in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Carnegie Institute of Technology, June, 1953.

(2) Institute Fellow in Organic Chemistry, 1952–1953.

(3) A. Wolfram, L. Schörnig and E. Hausdörfer (to I. G. Farbenind. A.-G.), German Patent 562,391 (Feb. 1, 1929), U. S. Patent 1,951,686 (March 20, 1934).

(4) Other bromides, such as sodium bromide and lithium bromide were ineffective, as were potassium chloride, lithium chloride and calcium chloride.