[Contribution from the Laboratory of Chemical Pharmacology, National Cancer Institute[1]]

Components of Podophyllin. XIX.[2] Isomorphism in the Podophyllotoxin Series

ANTHONY W. SCHRECKER, MARY M. TRAIL, and JONATHAN L. HARTWELL

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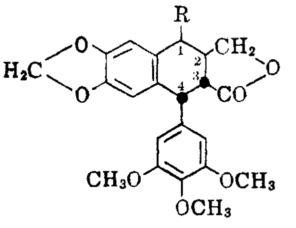
The binary mixtures of podophyllotoxin (PT), desoxypodophyllotoxin (DPT), and epipodophyllotoxin (EPT) form continuous series of mixed crystals, as shown by the respective phase diagrams. Admixture of PT raises the melting points of both DPT and EPT.

In a preliminary attempt to hydrogenate podophyllotoxin (PT) [I, R = OH, *trans*-(1:2)][2,3,4] to desoxypodophyllotoxin (DPT) [I, R = H],[4,5,6,7] reduction was incomplete. The product, which by its analysis was a mixture or an addition complex of PT (m.p. 183–184°[8]) and DPT (m.p. 167–168°), was found to melt at 192–194°. The surprisingly high melting point was confirmed with samples prepared by mixing the two components or by crystallizing a mixture from certain solvents. A similar elevation, rather than depression, of melting points was observed with mixtures of PT and epipodophyllotoxin (EPT) [I, R = OH, *cis*-(1:2)][3,4] (m.p. 157–158°), but not with mixtures of DPT and EPT. These observations made it desirable to study the melting point diagrams of the binary mixtures of PT, DPT, and EPT.



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The technique employed was the "thaw-melt" method of Rheinboldt,[9,10] *i.e.*, determination in capillaries of the temperatures of initial and final melting. Mixtures of DPT and PT were melted in test tubes, induced to crystallize by scratching, and then powdered in a mortar.[9,10] The melts obtained with mixtures of EPT and PT did not crystallize readily, especially when the proportion of PT was low. In this case, it was found preferable to crystallize a series of mixtures from ethyl acetate–hexane and to calculate the compositions of the resulting products from their specific rotations.[11] The latter procedure was the only one applicable

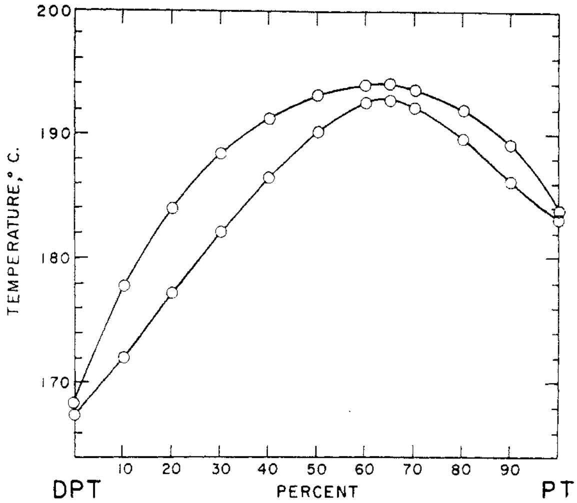

Fig. 1.—Initial and Final Melting Temperatures of Mixtures of Desoxypodophyllotoxin (DPT) and Podophyllotoxin (PT).

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(1) National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

(2) Paper XVIII, Schrecker, Hartwell, and Alford, *J. Org. Chem.*, **21**, 290 (1956).

(3) Hartwell and Schrecker, *J. Am. Chem. Soc.*, **73**, 2909 (1951).

(4) Schrecker and Hartwell, *J. Am. Chem. Soc.*, **75**, 5916 (1953).

(5) Hartwell, Schrecker, and Johnson, *J. Am. Chem. Soc.*, **75**, 2138 (1953).

(6) Hartwell and Schrecker, *J. Am. Chem. Soc.*, **76**, 4034 (1954).

(7) Kofod and Jørgensen, *Acta Chem. Scand.*, **9**, 346 (1955).

(8) The data reported in this paper apply only to the unsolvated modification "B," m.p. 183–184°,[2] of PT.

(9) Rheinboldt, *J. prakt. Chem.*, **111**, 242 (1925); Rheinboldt and Kircheisen, *J. prakt. Chem.*, **113**, 199 (1926).

(10) Rheinboldt in Houben-Weyl, *Methoden der organischen Chemie*, 4th ed., Georg Thieme Verlag, Stuttgart, 1953, vol. II, p. 857.

(11) The compositions of EPT-PT mixtures, and of EPT-DPT mixtures, are calculated with a standard error[12] of about 1 to 1.5%. Since the specific rotations of DPT and PT differ only by about 17°, the accuracy of determining the compositions of their mixtures was much lower (standard error about 4 to 5%), but the initial and final melting temperatures of mixtures crystallized from ethyl acetate–hexane were essentially the same as those of the melted and resolidified mixtures. With EPT and PT, the resolidified melts, as compared to the recrystallized mixtures, had low initial and final melting points, very likely because of incomplete crystallization.

(12) We are greatly indebted to Mr. Nathan Mantel for calculating the standard errors and deviations.

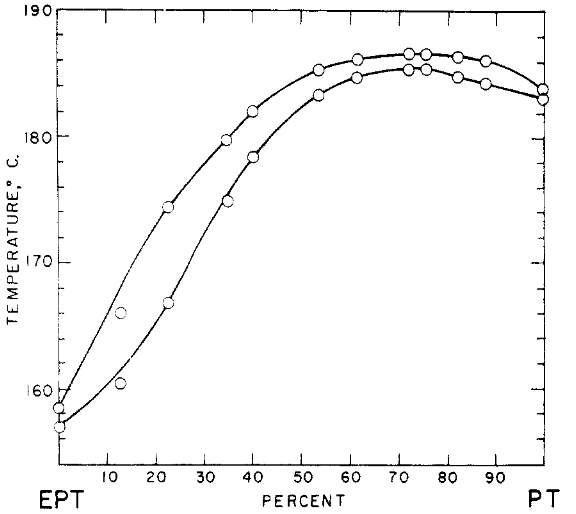

FIG. 2.—INITIAL AND FINAL MELTING TEMPERATURES OF MIXTURES OF EPIPODOPHYLLOTOXIN (EPT) AND PODOPHYLLOTOXIN (PT).

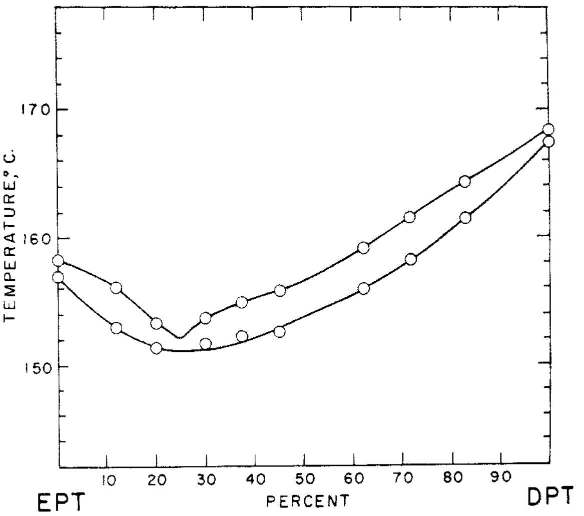

FIG. 3.—INITIAL AND FINAL MELTING TEMPERATURES OF MIXTURES OF EPIPODOPHYLLOTOXIN (EPT) AND DESOXYPODOPHYLLOTOXIN (DPT).

to mixtures of EPT and DPT.[11] The diagrams thus obtained are shown in Figures 1, 2, and 3.[13]

It appears from the diagrams that PT,[8] DPT, and EPT are isomorphous and that their binary mixtures form solid solutions in all proportions. Fig. 3 corresponds to Roozeboom's type III diagram,[15] with the solidus and liquidus curves passing through a minimum, while Figures 1 and 2 correspond to Roozeboom's type II diagram. The latter can be interpreted by the hypothesis that the individual components form a compound or molecular complex, which in turn forms a series of mixed crystals with either component,[16] or by assuming that the components form a continuous series of mixed crystals with one another, without compound formation.[17] If the former assumption were correct, the corresponding compounds could be 2 PT·DPT (maximum at *ca.* 65% PT) and 2 PT·EPT or 3 PT·EPT (maximum between 70 and 75% PT). The known ability of PT to form addition complexes with benzene[2] and other aromatic hydrocarbons[18] might seem to favor this hypothesis; however, no definite choice is possible on the basis of the available evidence.

Since PT can exist in two unsolvated modifications (B, m.p. 183–184°; D, m.p. 188–189°),[2,19] it could be possible, *a priori*, that DPT and EPT are isomorphous with form D rather than B. The shape of the curves seems to exclude this; moreover, the infrared spectrum in Nujol of a resolidified mixture of 10% DPT and 90% PT resembles that[2] of modification B rather than D.

The present findings render a re-examination of some previously published data desirable. Since the melting points of DPT and of EPT are raised by admixture of small amounts of PT, they are not necessarily the best criteria of purity. The preparation of DPT by hydrogenolysis of PT chloride (I, R = Cl) gave originally[5] a product, m.p. 167.8–168.8°. Repeating this procedure (with careful exclusion of moisture, which might hydrolyze part of the chloride) yielded, after several recrystallizations to constant melting point, a sample, m.p. 167.4–168.3°. Hydrogenolysis of EPT also led to material with a constant m.p. of 167.4–168.3°, while PT similarly gave a product, m.p. 168.4–169.4°. The lower melting point seems to denote a higher degree of purity, since traces of EPT are removed more readily by recrystallization from methanol than traces of PT. High melting points of DPT isolated from some natural sources may be explained by the presence of PT. Thus DPT from *Podophyllum peltatum* melted at 170–172° (cor.).[7] DPT (silicicolin) isolated from *Juniperus silicicola*[5,20] had melting points varying from 171–172°[5] to 173.9–175.5°.[20] PT is known to be present in other *Juniperus* species.[21] Both PT[21] and DPT[22] have been isolated from *J. sabina* var. *tamariscifolia*, the former from the male, the latter from the female plant.

EPT, prepared by hydrolysis of PT chloride or

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[13] The experimental points shown in the Figures are subject to the known inaccuracies[14] of determining initial and final melting temperatures by the "thaw-melt" method.

[14] Skau and Wakeham in Weissberger, *Physical Methods of Organic Chemistry*, 2nd ed., Interscience Publishers, Inc., New York, N. Y., 1949, vol. I, p. 87.

[15] Roozeboom, *Z. physik. Chem.*, **30**, 385 (1899).

[16] Campbell, *Nature*, **153**, 530 (1944); *cf.* ref. 10, p. 845.

[17] Timmermans, *Nature*, **154**, 23 (1944).

[18] F. Wessely, personal communication.

[19] Kofod and Jørgensen, *Acta Chem. Scand.*, **9**, 347 (1955).

[20] Hartwell, Johnson, Fitzgerald, and Belkin, *J. Am. Chem. Soc.*, **74**, 4470 (1952).

[21] Hartwell, Johnson, Fitzgerald, and Belkin, *J. Am. Chem. Soc.*, **75**, 235 (1953).

[22] Hartwell and Fitzgerald, unpublished.

bromide, was reported to have m.p. 159.4–161.2°.[3] This melting point was the highest obtained in a series of runs. Repeating the procedure, starting with freshly prepared and highly purified PT bromide, gave EPT with a constant m.p. of 157.0–158.3°, reproduced in several experiments. In view of the present findings, this lower m.p. probably represents a purer sample.

Isomorphism similar to that described in this paper has also been observed with other lignans, but their melting point diagrams have not been studied. Examples of compounds which do not depress the melting points of one another are α- and β-peltatin,[23] and also guaiaretic acid dimethyl ether and its dihydro derivative.[24]

EXPERIMENTAL[25,26]

Podophyllotoxin (PT) *and epipodophyllotoxin* (EPT) (I, R = OH, *trans*-(1:2) and *cis*-(1:2), respectively). Previously isolated PT was rechromatographed,[2] crystallized from ethanol-benzene-water, dried at 110° *in vacuo*, recrystallized from benzene-hexane, and redried; m.p. 183.3–184.0°, $[\alpha]^{20}_{D}$ −132.7°.[26] EPT, prepared from PT bromide[3] and recrystallized from 30% ethanol, then from 50% methanol to constant melting point, had m.p. 157.0–158.3° and $[\alpha]^{20}_{D}$ −73.8°.[26]

Desoxypodophyllotoxin (DPT) (I, R = H). Prepared by hydrogenolysis of PT chloride, using twice the reported[5] amount of catalyst and with special care to eliminate moisture, it had m.p. 167.4–168.3° and $[\alpha]^{21}_{D}$ −115.7°.[26] It was also obtained by hydrogenating 2 g. of EPT with 2 g. of 10% palladium-on-charcoal in 20 ml. of glacial acetic acid at 75–80° and atmospheric pressure for 5.5 hours, at which time uptake had ceased. The catalyst was removed (Celite), washed with hot acetic acid, and the filtrate was evaporated *in vacuo*. The residue was treated with water, extracted with chloroform, and the resulting solution was dried over magnesium sulfate, concentrated, and chromatographed on alumina. Elution with chloroform and crystallization from 50% ethanol gave 1.12 g. (58%) of colorless prismatic needles, m.p. 166.6–168.0°. Recrystallized twice from methanol, the product had m.p. 167.4–168.3° and $[\alpha]^{20}_{D}$ −116.1°.[26] PT gave analogously 67% DPT, m.p. 166–168.5° and 168.4–169.4° before and after recrystallizations, respectively, $[\alpha]^{20}_{D}$ −116.4°.[26]

Preparation of mixtures. Mixtures of DPT and PT (Fig. 1) were melted in small Pyrex test tubes (oil-bath) and the solidified (scratching) melt was powdered in an agate mortar.[9 10] Mixtures of EPT and PT (Fig. 2), and of EPT and DPT (Fig. 3), were crystallized from ethyl acetate-hexane (2:3 and 1:3, respectively, recoveries 70–85%) and the products were powdered and intimately mixed. The percentage compositions were calculated from the specific rotations.[11]

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(23) Hartwell and Detty, *J. Am. Chem. Soc.*, **72**, 246 (1950).

(24) Schroeter, Lichtenstadt, and Ireneu, *Ber.*, **51**, 1587 (1918).

(25) "Thaw points"[9,10] and final melting points were observed in 1.5–2.0 mm. Pyrex capillaries, using the Hershberg apparatus and calibrated Anschütz thermometers, with a heating rate of 0.2–0.3°/min.

(26) Optical rotations were determined on 1% solutions in chloroform using a 4-dm. tube, with a standard deviation[12] of about 0 004 × [α].