[CONTRIBUTION FROM THE NATIONAL CANCER INSTITUTE AND THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES[1]]

Components of Podophyllin. XVIII.[2] Polymorphic Modifications of Podophyllotoxin

ANTHONY W. SCHRECKER, JONATHAN L. HARTWELL, AND WILLIAM C. ALFORD

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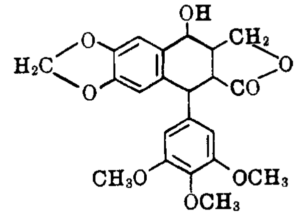
Formula $C_{22}H_{22}O_8$, corresponding to structure I, for podophyllotoxin (PT) is confirmed. PT can crystallize in at least four modifications: A, with water of crystallization, m.p. 161–162°; B, unsolvated, m.p. 183–184°; C, with water and benzene of crystallization, m.p. 114–118° (foaming); D, unsolvated, m.p. 188–189°.

The existence of several polymorphic modifications and solvates of podophyllotoxin[3,4] (PT), $C_{22}H_{22}O_8$ (I), has been known for some time.[4] Failure to recognize the presence of solvent of crystallization in analytical samples has led to erroneous conclusions[5] concerning the formula of the compound. While the formula $C_{22}H_{22}O_8$ for PT has been reconfirmed recently[6,7,8] the suggestion has been made[5] that the compound possessing structure I may be an artifact produced by decomposition during isolation or when "native" PT is heated. In order to settle the argument definitely, it was decided to study again the identity of PT, isolated by a method at least as gentle as the one employed recently.[5] In addition, reinvestigation of the various modifications and solvates[4,9,10,11] appeared desirable, since this question has been discussed again[11] in connection with the isolation of a new modification,[7] m.p. 188–189°.



I

In the isolation of PT, "enzymatic hydrolysis"[5] was not employed, since it was desired to isolate only originally free PT and not that bound in the form of the glucoside.[6] Extraction of dried *Podophyllum peltatum* roots and rhizomes at room temperature, chromatography on neutral alumina, and crystallization from dichloromethane-pentane yielded colorless prismatic needles melting at 160.4–161.6° (*modification A*). The method of isolation (see "Experimental") makes it quite certain that the product is "native" PT. Modification A is identical (as shown by comparison of its infrared spectrum in Nujol with that of a sample kindly supplied by Prof. Wessely) with the product, m.p. 158°, obtained by Späth, Wessely, and Kornfeld[9] from *P. emodi* after crystallization from aqueous ethanol. It is noteworthy that this modification could never be isolated in our Laboratory until Prof. Wessely's sample was received and used for seeding purposes. Subsequently, it crystallized readily without seeding from various solvent mixtures (see Table I). The highest melting point

TABLE I

MODIFICATIONS OBTAINED FROM VARIOUS SOLVENTS

Solvents[a]	Modification	M.p., °C.
Ethanol-water	A	160–162
Dichloromethane-pentane	A	161–162
Dichloromethane-hexane	A	160–162
Dichloromethane-benzene-hexane[b]	A	160–161
Ethylene chloride-hexane	A	160–161
Ethyl acetate-hexane	B	183–184[c]
Benzene-hexane	B	183–184[c]
Toluene-hexane	B	183–184[c]

[a] Sample B-3 (see "Experimental") was dissolved in the first-named solvent, the solution filtered, boiled, diluted with the second solvent, and crystallization induced by scratching. The products were air-dried at 60°. [b] The same product was obtained when 5 ml. of benzene, then 10 ml. of hexane were added to 200 mg. of PT in 5 ml. of dichloromethane, and when the order dichloromethane-benzene was reversed. [c] The m.p. was the same when the sample was immersed at 175°.

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

(2) Paper XVII, Schrecker, Maury, Hartwell, and Leiter, *J. Am. Chem. Soc.*, **77**, 6565 (1955).

(3) Späth, Wessely, and Nadler, *Ber.*, **65**, 1773 (1932).

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

(5) Press and Brun, *Helv. Chim. Acta*, **37**, 190, 1543 (1954); *cf.* Schrecker and Hartwell, *Helv. Chim. Acta*, **37**, 1541 (1954).

(6) Stoll, Renz, and von Wartburg, *Helv. Chim. Acta*, **37**, 1747 (1954).

(7) Jørgensen and Kofod, *Acta Chem. Scand.*, **8**, 941 (1954).

(8) Bartek, Potěšilová, Mašínová, and Šantavý, *Chem. Listy*, **49**, 1550 (1955).

(9) Späth, Wessely, and Kornfeld, *Ber.*, **65**, 1536 (1932).

(10) Borsche and Niemann, *Ann.*, **494**, 126 (1932).

(11) Kofod and Jørgensen, *Acta Chem. Scand.*, **9**, 347 (1955).

TABLE II

ANALYSES OF PODOPHYLLOTOXIN

Sample	Analysis C	H	OCH_3	Solvent
$C_{22}H_{22}O_8$[a]	63.76	5.35	22.47	—
B-1	63.70	5.50	22.59	—
B-2	63.76	5.56	22.62	—
B-4	63.89	5.49	22.53	—
$C_{22}H_{22}O_8 \cdot 0.25H_2O$[a]	63.07	5.41	22.23	1.08
A-1	63.01	5.43	—	0.98[c]
A-2[b]	63.06	5.37	—	—
A-3	62.80	5.39	—	—
$C_{22}H_{22}O_8 \cdot 0.5H_2O$[a]	62.40	5.48	21.99	2.13
A-2	62.42	5.47	21.90	1.20[c]
$C_{22}H_{22}O_8 \cdot 0.5C_6H_6 \cdot 1.5H_2O$[a]	62.49	5.87	19.38	13.75
C-1	62.67	6.00	19.89	13.68[c]
$C_{22}H_{22}O_8 \cdot 0.5C_6H_6 \cdot 1H_2O$[a]	63.68	5.77	19.75	12.10
C-2	63.79	5.96	20.31	12.58[c]
C-3	63.73	6.19	19.36	11.96[c]

[a] Theoretical composition. [b] Sample dried in the combustion boat at 110°/0.01 mm. for 6 hrs., then analyzed. [c] Weight loss at 110°/0.01 mm. (5–8 hrs.).

recorded by us for modification A was 161.4–162.0° (sample isolated from *P. emodi*). It crystallized with 0.25 or 0.5 mole of water, which is retained tenaciously, even after drying at 110° *in vacuo*. The presence of residual water in a sample thus dried was proved by heating it at 120° *in vacuo* in a microdistillation assembly and collecting the distillate over anhydrous copper sulfate.

Drying modification A at 137° *in vacuo* yielded the unsolvated form[4] (*modification B*), $C_{22}H_{22}O_8$, m.p. 183.3–184.0°. This modification also separated as rod-shaped prisms when modification A (or B itself) was crystallized from benzene-hexane and other solvent mixtures (Table I). The same product was obtained from *P. peltatum* and from *P. emodi*.[12] It was first believed that the hydrated modification A would preferentially crystallize in the presence, the anhydrous form B in the absence, of moisture. However, it appears that the type of solvent rather is the determining factor. Thus dissolving PT in boiling ethylene chloride (which has a boiling point above that of benzene) and adding hexane gave modification A, which was also obtained when dichloromethane, then hexane was added to a benzene solution of the compound. Cross-seeding experiments showed that the solvent rather than the type of seed crystals determined the resulting modification.

The solvate, m.p. 114–118° (foaming)[4,9,10,11] (*modification C*), which was obtained as prismatic needles from aqueous ethanol containing some benzene, was always found to contain 0.5 mole of benzene, but the water content was variable. One sample, dried in air for 5 hours, had the composition $C_{22}H_{22}O_8 \cdot 0.5C_6H_6 \cdot 1.5H_2O$. Most air-dried samples, however, contained only 1 mole of water.[13] At 110°, both water and benzene were lost and the unsolvated modification B was obtained. Upon microdistillation, the presence of water was confirmed by the copper sulfate test and that of benzene (previously identified[9] as the dinitro derivative) by its characteristic ultraviolet spectrum. It is interesting that the solvated form C (m.p. 112–119°) was also obtained by crystallizing PT from benzene alone (sample C-2, *cf.* "Experimental"), while modification B was formed in the presence of hexane.[14]

Kofod and Jørgensen's unsolvated modification,[7,11] m.p. 188–189°, (*modification D*) has not yet been obtained in our Laboratory, but its melting point was confirmed with a sample kindly provided by Dr. Kofod.[15]

The infrared absorption spectra of chloroform solutions of all the samples prepared in this investigation (including the one isolated under mild conditions from *P. peltatum*) or received from Prof. Wessely or Dr. Kofod were completely identical. This demonstrates the molecular identity (except for solvent of crystallization) of the four modifications, confirms the identity of PT from *P. peltatum*[4] and from *P. emodi*,[12] and proves definitely that "native" PT is not altered when isolated by the usual procedures[4,9,12] or during heat-drying. On the other hand, the different modifications showed different spectra (Fig. 1) in Nujol mulls, as would be expected in the case of polymorphs that possess different crystal structures.[17] This confirms the view[11] that the unsolvated forms B and D are actually different polymorphic modifications and that B (m.p. 183–184°) is not simply less pure D (m.p. 188–189°), in which case the spectra of their chloroform solutions would have shown differences comparable to those observed with the Nujol mulls.

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[12] Nadkarni, Hartwell, Maury, and Leiter, *J. Am. Chem. Soc.*, **75**, 1308 (1953).

[13] The analysis from which the recent erroneous formula[5] of PT was deduced agrees well with the composition $C_{22}H_{22}O_8 \cdot 0.5C_6H_6 \cdot 0.5H_2O$ (Calc'd: C, 64.92; H, 5.67; OCH_3, 20.13. Found:[5] C, 64.89, 64.81; H, 5.53, 5.33; OCH_3, 20.01).

[14] Prof. Wessely informed us that several other aromatic hydrocarbons can replace benzene in modification C.

[15] In addition, Kofod and Jørgensen[11] describe an unsolvated modification, m.p. 111–118°, which they obtained by drying modification C at 100°/0.01 mm. and which may be identical with the product, m.p. 116.8–119.5° (sintering at 114.5°), obtained by Drake and Price[16] on vacuum-sublimation of PT.

[16] Drake and Price, *J. Am. Chem. Soc.*, **73**, 201 (1951).

[17] The assignment of modification A, B, C, or D to a given sample, prepared by us or received from abroad, on the basis of its melting point was always confirmed by recording the Nujol spectrum. In one instance, an apparent discrepancy (sample melting at 183–184°, but spectrum of form A) was resolved when it was found that on slow heating transition from modification A to B, promoted by the presence of a trace of B, occurred; then the sample was immersed at 162°, it melted immediately.

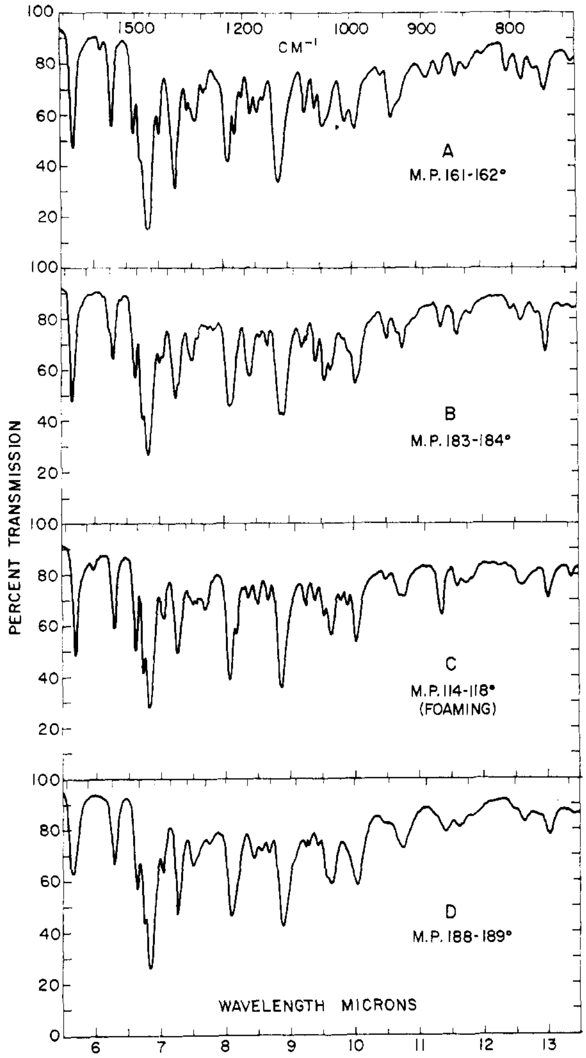

FIG. 1.—INFRARED ABSORPTION SPECTRA IN NUJOL MULLS OF THE VARIOUS POLYMORPHIC MODIFICATIONS AND SOLVATES OF PODOPHYLLOTOXIN.

EXPERIMENTAL[18]

Isolation of podophyllotoxin from Podophyllum peltatum. Ground roots and rhizomes (S. B. Penick and Co., New York) were stirred at room temperature with U.S.P. chloroform for 1 hr., the mixture was filtered with suction, and the residue was washed with chloroform. The yellow filtrate was concentrated in an air-current, filtered from flocculent material, and diluted with ligroin ("purified benzin," Merck, b.p. 30–75°). The precipitate was collected, redissolved in cold chloroform, and reprecipitated with ligroin, then dissolved again in chloroform, chromatographed on 17 parts of neutral alumina (Woelm), and eluted with chloroform. The yellow eluate was evaporated in an air-current, the oil was dissolved in dichloromethane, and the solution was diluted with pentane to incipient turbidity and seeded with a trace of previously obtained PT, m.p. 161.4–162.0°. The product, m.p. 154–156°, was recrystallized three times from dichloromethane-pentane to yield rosettes of colorless prismatic needles, which, when dried at room temperature and 0.03 mm. for 24 hrs. (*sample A-1*), melted at 160.4–161.6°. The melt resolidified on further slow heating, to melt again at 183–184°. Dried at 137°/0.01 mm. for 24 hrs., the material (*sample B-1*) melted at 183.3–184.0°. Recrystallization of A-1 from benzene-hexane gave rosettes of small rod-shaped prisms, which after drying at 137°/0.01 mm. for 24 hrs. also melted at 183.3–184.0° (*sample B-2*).

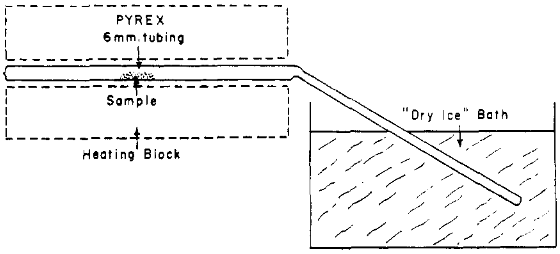

FIG. 2.—APPARATUS USED IN DETECTING SOLVENT OF CRYSTALLIZATION.

Purification of podophyllotoxin from P. emodi. PT, isolated previously[12] and melting at 181.6–183.0°, was chromatographed as above and recrystallized twice from 50% ethanol containing some benzene to yield colorless prismatic needles which, after drying in air for 5 hrs., had m.p. 114–117° (foaming at 118°) (*sample C-1*). Drying at 110°/0.01 mm. for 6 hrs. gave a product melting at 182.6–184.0° (*sample B-3*). A portion of B-3 was dissolved in boiling benzene. Addition of hexane and scratching precipitated rosettes of rod-shaped prisms, m.p. 182.6–183.5°, which were dried in a non-evacuated desiccator over magnesium perchlorate and paraffin for 1 week (*sample B-4*). However, when the benzene solution of B-3 was cooled and scratched without adding hexane, small needles, melting at 112–119° after similar drying in a non-evacuated desiccator, were obtained (*sample C-2*). Another portion of B-3, recrystallized from benzene-containing 50% ethanol, furnished prismatic needles, which, dried in air for 16 hrs., melted at 114–118° (foaming at 117°) (*sample C-3*). Recrystallizing B-3 twice from aqueous ethanol according to the reported procedure[9] yielded prismatic needles, which melted at 160.2–162.0°, both when dried in air for 16 hrs. (*sample A-2*) and at 110°/0.01 mm. for 8 hrs. (*sample A-3*). Recrystallized from dichloromethane-pentane, B-3 also gave prismatic needles, which melted at 161.4–162.0° when dried at room temperature and 0.03 mm. for 16 hrs.

Detection of solvent of crystallization. The apparatus employed is shown in Fig. 2. The sample was placed in the wide portion of the well-dried tube, which then was evacuated and sealed off. For detecting water, a small amount of anhydrous copper sulfate was placed in the capillary. The tube was heated in the heating block, which was thermostatically controlled at 120°. Solvent condensed in the capillary side-arm, which then was sealed off and centrifuged to collect the solvent at the tip. An anhydrous sample (B-4, m.p. 182.6–183.5°) gave no evidence of distillate, and the copper sulfate remained white. With a hydrated sample (A-3, m.p. 160.2–162.0°), droplets condensed, which turned copper sulfate blue. A sample containing both water and benzene (C-3, m.p. 114–118°) gave a distillate which also turned copper sulfate blue. In the absence of copper sulfate, two layers were present after centrifugation, in a volume ratio of about 2 C_6H_6 (upper):1 H_2O (lower), in approximate agreement with the molar ratio 0.5:1, derived from the analysis. The capillary then was broken under ethanol. The ultraviolet spectrum of the solution (obtained with a Cary recording spectrophotometer, blank cell containing ethanol alone) was indistinguishable from that of benzene in ethanol.

Cross-seeding experiments. A solution of 200 mg. of sample B-3 (m.p. 182.6–184.0°) in 5 ml. of dichloromethane

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[18] All melting intervals were determined in Pyrex capillaries with the Hershberg apparatus and calibrated Anschütz thermometers. Infrared spectra were recorded with the Perkin-Elmer model 21 spectrometer, using 4% chloroform solutions in 0.1-mm. sodium chloride cells or Nujol mulls.

was filtered, diluted with 5 ml. of pentane, seeded with a trace of sample B-2 (m.p. 183.3–184.0°), and allowed to crystallize undisturbed in a stoppered flask. Prismatic needles separated rather rapidly, melting between 161 and 184° when heated slowly, but melting at once when immersed at 162°. Similarly, adding 3 ml. of hexane to a filtered solution of 200 mg. of B-3 in 6 ml. of boiling benzene, then seeding with sample A-1 (m.p. 160.4–161.6°) gave rod-shaped prisms, m.p. 183.2–184.0°. This indicates that the kind of modification obtained depends primarily on the solvent mixture employed.

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BETHESDA 14, MARYLAND