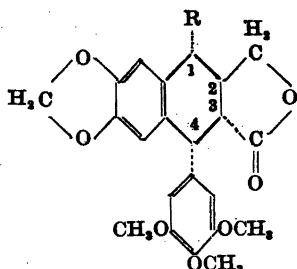


the commercial resin from the plant material.



- I R = H desoxypodophyllotoxin $C_{22}H_{22}O_7$
(398.4) *trans*-2:3-*cis*-3:4
II R = OH podophyllotoxin $C_{22}H_{22}O_8$
(414.4) *trans*-1:2-*trans*-2:3 *cis*-3:4
III R = Cl podophyllotoxin chloride
(Hartwell and co-workers)

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A Note on the Melting Point of Podophyllotoxin

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The melting points previously reported in the literature for podophyllotoxin vary within the limits *ca.* 100–189° C, the majority falling in the ranges 110–

118, 158–162 and 179–184° C. The explanation of this divergency is (1) that the substance is polymorphic, and occasionally (2) that investigators have overlooked ^{7, 8} the fact that podophyllotoxin retains liquid of crystallization very strongly ^{5, 6}, so that some melting points actually refer to solvated products. In either case the experimental melting point will be dependent upon the type of apparatus, the rate of heating *etc.*

The melting point 188–189° C, recently communicated by us ¹ is the highest ever obtained and it may need a few comments.

The general experience accumulated in this laboratory concerning the melting points of various podophyllotoxin preparations is summarized below. Unless otherwise stated the melting points — or rather melting intervals — were determined with the hot-stage microscope, essentially according to Kofler ². The apparatus was carefully calibrated against a series of pure standard substances. All podophyllotoxin samples were obtained in this laboratory by chromatographic fractionation of Podophyllin, *Pharmacopoeia Danica Ed. IX*, on either alumina or formamide-impregnated silica-gel, benzene being the eluent.

Several preparations of podophyllotoxin, recrystallized from commercial wet benzene and dried in the atmosphere under an infra-lamp (*i. e.* exposed to a maximum temperature of 50° C) had a melting point of 110–115°. The same melting point was normally obtained in a closed capillary heated in a silicone bath. Elementary analysis and the percentage loss upon drying to constant weight at 0.01 mm Hg (continuous pumping) and 100° C (up to 18 hours) almost invariably indicated the composition $2 C_{22}H_{22}O_8$, $2 H_2O$, C_6H_6 of the original air-dried solvate, *cf.* Ref. ^{4, 5}.

The pure, unsolvated podophyllotoxin obtained by the above drying procedure in the first period of our work had a m. p. 183–84°. Later preparations, however, obtained without any deliberate alteration of procedure, melted completely at 111–118°. When heated at 150° for 1–2 hours on the microscope slide the substance resolidified and the crystals eventually melted at 183–184°. In a few cases, particularly with excessively slow heating it was possible to pass through the temperature range 111–118° without any visible phase-transition. Then the substance finally melted at 188–189°. Such was the case with the sample mentioned in our first paper ¹.

Recently we have obtained a product, which, recrystallized from benzene and dried to constant weight under the conditions specified above, had a single melting point at 188–189°, both with the Kofler apparatus and as determined by the capillary method (rate of heating in the melting region 2°/min). Only a very slight temporary moistening was noticeable at about 115° C, even when heating rapidly. When, beyond the melting point, the heating was discontinued and the hot-stage allowed to cool to 180° the substance crystallized, and repeated melting point determination now gave 187–188°.

The molecular identity of the new modification with podophyllotoxin m. p. 183–184° was proved by elementary analysis (Found: C 63.29; H 5.55). Calc. for $C_{22}H_{22}O_8$: C 63.75; H 5.35), by paper chromatography (*vide* Ref.¹), by its ultraviolet absorption spectrum and by the optical rotation. Finally on epimerization with sodium acetate⁵ it yielded picropodophyllin, m. p. 228–231° (lit.⁶ 231.5–232.5°) which did not depress the melting point of an authentic sample.

Besides the modifications already mentioned a fourth one is known, melting at 158° (Ref.⁶). This modification has never been encountered in this laboratory. Its existence has, however, recently been confirmed by American investigators⁸ who report a melting point of 161–162° C.

It appears, therefore, that according to circumstances, dried and unsolvated podophyllotoxin may come out in four different crystal modifications:

- I m. p. 114–118 (without foaming, Ref.⁶); 111–118 (this laboratory)
- II m. p. 161–162 (Ref.⁶); 158 (without foaming, Ref.⁶); 157 (Ref.¹⁰)
- III m. p. 183–184 (Ref.⁶, p. 2913 and the present authors); 179 (Ref.¹¹)
- IV m. p. 188–189 (the present paper)

Various air-dried solvates have been described in the literature,

- e. g.*, 2 PT, 2 H₂O, C₆H₅OH, m. p. 106–108 (Ref.⁴)
 2 PT, 2 H₂O, C₆H₆, m. p. 114–116, foaming (Ref.⁶ and Ref.⁶, footnote 32); m. p. 114–117 (Ref.⁷, *cf.* Ref.⁶); 110–115 (this laboratory).

Since these solvates melt in much the same region as modification I, the postulated existence of I is open to the criticism that the experimental observations (*vide infra*) might as well be explained by assuming that the "modification" is a partially solvated podophyllotoxin melting around 115°, then losing solvent of crystallization, the last trace of which disappears at about 150°, subsequently resolidifying as modification III or IV, which finally melts at the appropriate temperature. Evidence against this explanation is, however, that both Borsche and Niemann's and our own preparation of modification I was dried under carefully controlled conditions and analyzed as unsolvated podophyllotoxin. No loss of weight was observed on further drying at 125°, 0.01 mm Hg for two hours. It should also be mentioned that the melting process of I was not accompanied by the foaming, characteristic of the solvates.

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