

Desoxypodophyllotoxin, Isolated from Podophyllin

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In the course of a systematic re-investigation of the constituents of Podophyllin (the resin from *Podophyllum peltatum* L.) by means of chromatographic methods¹ a substance has been isolated as colourless needles, m. p. 170–172°C corr., yield approximately 0.1%. It analyzed as $C_{22}H_{22}O_7$. (Found: C 65.7; H 5.58; OCH_3 23.3. Calc. C 66.3; H 5.57; 3 OCH_3 23.4.) The optical rotation in chloroform solution ($c = 0.98$) was $[\alpha]_D^{25} = -123^\circ \pm 10^\circ$. These data are close to those reported for desoxypodophyllotoxin (I), a compound which was prepared artificially by Hartwell, Schrecker and Johnson² by hydrogenolysis of podophyllotoxin chloride (III). These authors reported a melting point of 168–69° and $[\alpha]_D^{20} = -115^\circ$ (chloroform, $c = 0.50$). The location of our natural substance on paper chromatograms (formamide-paper/benzene¹) close to the solvent front and thus with a much higher R_F -value than podophyllotoxin (II) is consistent with the absence of the hydroxy-group (cf. Ref.¹, p. 950). Further evidence is provided by the ultra-violet spectrum, Fig. 1. It shows the essential features of the spectrum reported by Schrecker and Hartwell² for the synthetic desoxypodophyllotoxin. Although the minimum is slightly flattened, this and the maximum are identical with those of the synthetic substance with respect to the wave lengths. Finally on base-catalyzed epimerization the natural product yielded a substance identical with desoxypicropodophyllin (I, *cis*-2:3-*trans*-3:4) by melting point and optical rotation (Found m. p. 171–173°C, $[\alpha]_D^{21} = +33^\circ \pm 10^\circ$ ($c = 0.25$, chloroform); lit. m. p. 170.7–172.0°, $[\alpha]_D^{21} = +32^\circ$ ($c = 0.50$, chloroform)²; m. p. 168–170°, $[\alpha]_D^{19} = +39^\circ$ ($c = 0.53$, chloroform)³, p. 5922; m. p. 169.5–171°, $[\alpha]_D^{20} = +37^\circ$ ($c = 1.0$, chloroform)³, p. 5922.

To our knowledge this is the first time desoxypodophyllotoxin has been isolated from a *Podophyllum* species. It has previously been obtained from *Juniperus*

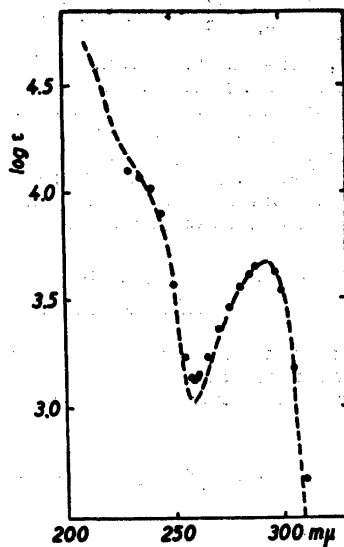
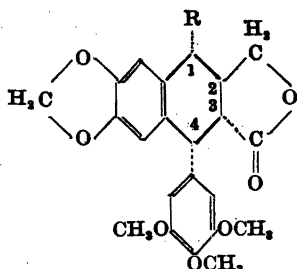


Fig. 1. Absorption in the ultra-violet of desoxypodophyllotoxin (I) in 95% ethanol. Curve given by Schrecker and Hartwell² for their synthetic product. O Natural product described in the present communication.

silicicola (Small) Bailey by Hartwell and co-workers^{2,4}, and was originally named silicicolin (m. p. 173.9–175.5° corr., $[\alpha]_D^{19} = -119$ (chloroform) (Ref.⁴, cf. Ref.², footnote 15); m. p. 171–172° corr.². The discoverers of silicicolin also demonstrated⁷ that anthricin, isolated in 1940 (Noguchi and Kawanami⁸) from *Anthriscus silvestris* Hoffm. and hernandion, isolated in 1942 (Hata⁹) from *Hernandia ovigera* L. were in fact identical with desoxypodophyllotoxin.

A direct detection of desoxypodophyllotoxin in extracts of fresh plant material is impracticable even with the highly sensitive paper chromatographic technique¹. It has not been definitely proved, therefore, whether the isolated desoxypodophyllotoxin is a genuine constituent in the plant or an artifact. However, a formation of the substance by dehydroxylation of podophyllotoxin, the principal constituent of podophyllin, or in fact of any other recorded constituent, must be regarded as extremely unlikely under the conditions normally applied during the preparation of

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.