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Note

Isolation of α- and β-peltatin and podophyllotoxin by liquid chromatography and analysis by high-performance liquid chromatography

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Podophyllum resin extracted from the roots and rhizomes of *Phodophyllum peltatum* is a commonly used caustic for genital warts[1]. The major active components in the drug are believed to be α- and β-peltatin and podophyllotoxin. Recent interest[2] in application of the pure components of Podophyllum resin have made their easy access desirable. Thus we have developed a simple preparative chromatographic method for α- and β-peltatin and podophyllotoxin. In order to analyse for their purity and presence in podophyllum preparations we have also developed a high-performance liquid chromatographic (HPLC) method which can be applied in qualitative and quantitative analysis.

EXPERIMENTAL

Chromatographic systems

Preparation. Silica gel 60 (70–230 mesh) in a glass column (75 × 2.5 cm) was eluted with chloroform containing 2.3% ethanol at a flow-rate of 123 ml/h. The detector system was a LKB 2089 Uvicord III operating at 206 and 280 nm.

Analysis. A Perkin-Elmer 604 high-performance liquid chromatograph was used, with a constant wavelength detector operating at 254 nm. Peak areas were determined by means of an autolab minigrator (Spectra-Physics). The column was a Perkin-Elmer silica A (55 × 0.25 cm). The eluent was analytical grade chloroform containing 1.8% ethanol. The flow-rate was 0.8 ml/min.

RESULTS AND DISCUSSION

α- and β-Peltatin and podophyllotoxin have previously been isolated in pure form by Hartwell and Detty[3]. They used alumina and benzene–ethanol or benzene–ethanol–water in their chromatographic system. Certain disadvantages are connected with that system. First, tailing from podophyllotoxin, leaving the column first, contaminates the following fractions of α- and β-peltatin. Secondly, alumina is very polar and retains a lot of material. To wash out α-peltatin, addition of water to the eluent is required; this results in equilibration problems when the next portion of podophyllum extract is chromatographed.

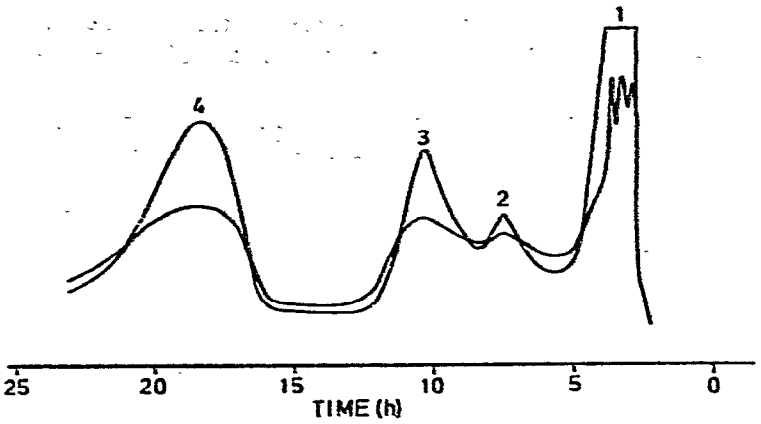

Fig. 1. Preparative chromatography of α- and β-peltatin and podophyllotoxin from podophyllum extract. Stationary phase: silica gel 60. Mobile phase: chloroform containing 2.3% ethanol; flow-rate, 123 ml/h. Peaks: 1 = unidentified; 2 = β-peltatin; 3 = α-peltatin; 4 = podophyllotoxin.

We have developed a simpler preparative procedure using silica gel and chloroform with a few percent ethanol. With this system the preparation can be carried out isocratically in one single run (see Fig. 1).

In a typical run, 10 g of podophyllin (Ph. Nord. 1963; E. Merck, Darmstadt, G.F.R.) was stirred overnight with 100 ml chloroform (Ph. Nord. 63). The filtrate was evaporated to *ca.* 10 ml and injected on the column. After elution (see Fig. 1),

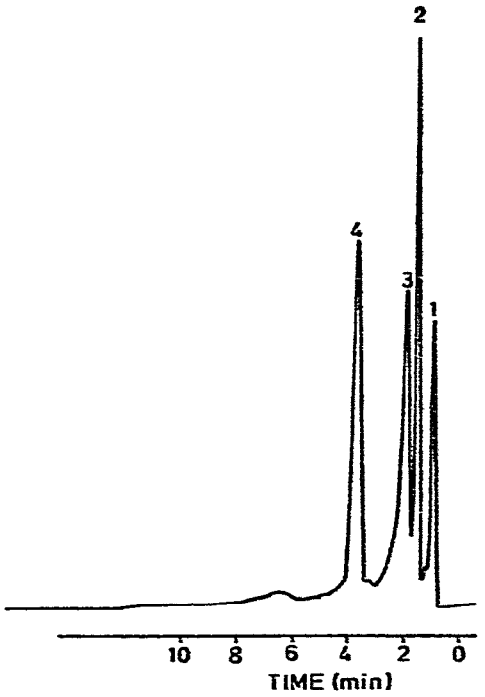

Fig. 2. HPLC of podophyllum extract on a silica A column. Mobile phase: chloroform containing 1.8% ethanol; flow-rate, 0.8 ml/min. Peaks numbered as in Fig. 1.

0.4 g β-peltatin, 0.7 g of α-peltatin and 0.9 g of podophyllotoxin were obtained. The compounds were identified by m.p., C,H,N analysis, mass spectra and ^{13}C nuclear magnetic resonance.

In the analytical method we again used a silica gel column and chloroform as eluent. The retention times for α- and β-peltatin and podophyllotoxin were 223, 179 and 434 sec, respectively (see Fig. 2).

For all three compounds, there was a rectilinear relationship between peak area and amount applied to the column (Figs. 3, 4 and 5), at least over the range 0.3–7.1 μg.

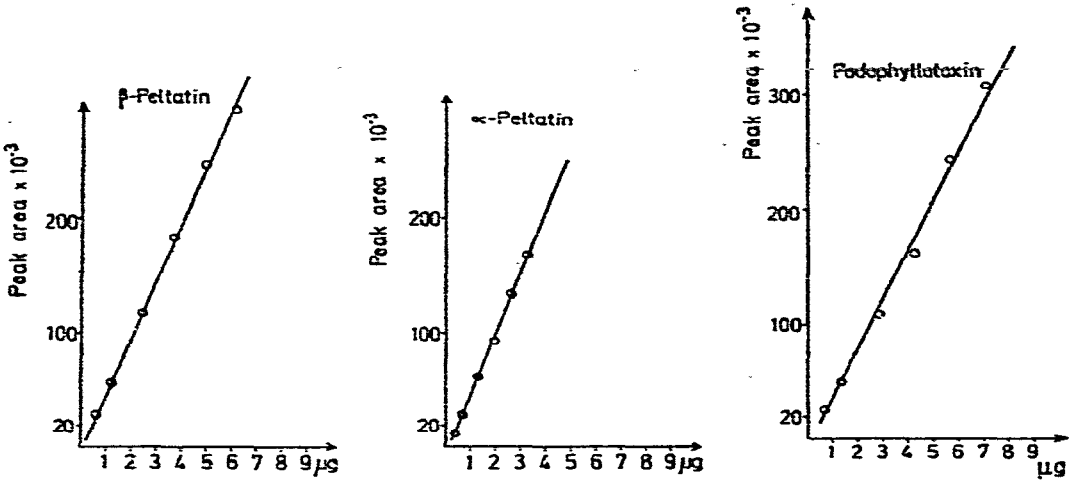

Fig. 3. Calibration graph for β-peltatin.

Fig. 4. Calibration graph for α-peltatin.

Fig. 5. Calibration graph for podophyllotoxin.

When the method was applied to a crude filtered extract of podophyllum resin the contents of β-peltatin, α-peltatin and podophyllotoxin were determined to be 10.7%, 13.0% and 15.2%, respectively.

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