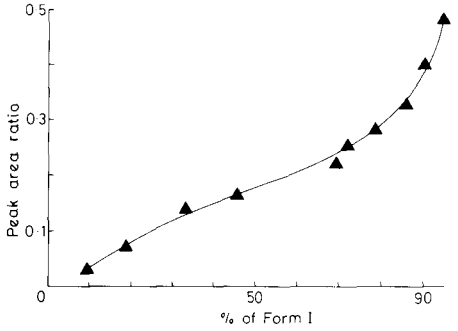

FIG. 3. Graph of peak area ratio (see Table 1) against the percentage weight of Form I in the mixture.

were prepared and subjected to DSC analyses. From the scans of these mixtures, peak areas corresponding to endothermic transitions were measured and a tabulation of peak area ratios obtained for the mixtures are given in Table 1. The relative peak area of Form I was plotted as a function of the percent Form I in the mixture (Fig. 3). The shape of the curve suggests that in the presence of Form II crystals there is some premelt solid-solid conversion of Form I to Form II. This premelt conversion and the closeness of the two endotherms inhibits the estimation of composition of mixtures of polymorphs of this drug. It may however, be feasible to apply this DSC method of analyses to polymorphic mixtures of other drugs.

Conclusions. The drug studied was found to exist in two polymorphic states, Form I (m.p. 193 °C) and Form II (m.p. 196 °C). These polymorphs are interconvertible by solid-melt, melt-solid transitions. Cyclic DSC heat-cool studies were employed to characterize these polymorphic transitions. The study demonstrates application of the DSC method to examine reversible crystalline phase transitions of polymorphs.

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Determination of the aryltetralin lignan content of podophyllum resins and roots/rhizomes

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A simple, quantitatively reproducible method for determining the aryltetralin lignan content of the resins and roots/rhizomes of *Podophyllum hexandrum* and *P. peltatum* is described. The method confirms that *P. hexandrum* resins and roots/rhizomes contain approximately four times the quantity of lignans as do those of *P. peltatum* and also that there is a significant variation in the lignan content of *P. hexandrum* resins.

Although the resin from *Podophyllum hexandrum* Royle and *P. peltatum* L. is the subject of an official monograph in many pharmacopoeias, none standardize the resin on lignan content. Methods for isolating and determining *individual* lignans have been published (Treppendahl & Jakobsen 1980; Cairnes et al 1981) but, as yet, none has tackled the problem of pharmacopoeial resins. This is particularly important because, whereas most pharmacopoeias only allow the resin to be manufactured from *P. peltatum*, the British Pharmacopoeia 1980 allows the resin from both *P. hexandrum* and *P. peltatum*. Previous reports have indicated that both the root/rhizome and the resin of *P. hexandrum* contain approximately four times the quantity of lignans found in those from *P. peltatum* (Kelly & Hartwell 1954 (crystallization or derivitization methods); Jackson & Dewick 1984 (preparative TLC)). A procedure is now described which allows the determination of the total lignan content in both podophyllum resins and podophyllum roots/rhizomes.

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Method

Total lignan content of resin. Approximately 0·5 g resin, accurately weighed, was dissolved in a small volume of ethanol (96% v/v) and sufficient ethanol (96% v/v) was added to produce 100 mL. To 10·0 mL of this solution, in a separator, 190 mL water was added and this mixture was extracted with 6 × 30 mL dichloromethane: the combined dichloromethane layers were extracted with 10 mL 0·2 M NaOH followed by 5 × 10 mL water. Each of the six aqueous layers was washed with the same 20 mL dichloromethane. The dichloromethane fractions were combined, filtered through a plug of cotton wool and evaporated to dryness. The residue was dissolved in ethanol (96% v/v) and sufficient ethanol

Table 1. Total lignan content, determined as podophyllotoxin, in roots/rhizomes and resins of *P. hexandrum* and *P. peltatum*.

	P. hexandrum						*P. peltatum*				
	Root/Rhizome			Resin			Root/Rhizome			Resin	
Sample	(i)	(ii)	(iii)	(iv)	(v)	(vi)	(vii)	(viii)*	(ix)*	(x)	(xi)
Mean total lignan content (% w/w)	5·02	4·52	4·72	55·0	66·2	50·1	0·98	1·23	0·89	16·3	16·8
Range total lignan content (% w/w)	4·79–5·23	4·30–4·83	4·53–4·85	54·2–55·9	65·3–67·5	49·0–50·9	0·92–1·05	1·17–1·27	0·87–0·90	15·9–16·8	16·3–17·1
Replicate assays	5	4	5	6	5	4	4	4	2	5	3

* Museum samples of *P.* peltatum *root/rhizome.*

(96% v/v) added to produce 100 mL. 10·0 mL of this solution was diluted to 100 mL with ethanol (96% v/v). The absorbance of a 1 cm layer of the resulting solution was measured at the maximum at about 292 nm using ethanol (96% v/v) as the blank solution. The content of total lignans, determined as podophyllotoxin, was calculated taking 107·8 as the value of A (1 per cent, 1 cm) at the maximum at about 292 nm (Hartwell & Schrecker 1958).

Total lignan content of roots/rhizomes. Approximately 5 g root/rhizome, accurately weighed, was extracted in a soxhlet apparatus with 75 mL ethanol (96% v/v) for 4 h. After cooling, the extraction liquid was transferred to a 100 mL volumetric flask with sufficient ethanol (96% v/v) to produce 100 mL. The method was continued as for 'Total lignan content of resin' commencing at the words '. . . To 10·0 mL of this solution, in a separator, . . .'.

Results and discussion

Several resin and root/rhizome samples were assayed by the stated method. The *P. hexandrum* resins were taken from our own production batches and complied with the British Pharmacopoeia 1980; the *P. peltatum* resins were purchased as complying with the United States Pharmacopeia 1980 and authenticated. The *P. hexandrum* roots/rhizomes were obtained from botanical drug wholesalers in Europe and the Far East as type samples of material which was commercially available; these, too, were authenticated. One sample of *P. peltatum* root/rhizome (authenticated) was obtained from a European botanical drug wholesaler as being typical of material commercially available, the remaining two samples were from our own botanical drug museum. Results for the total lignan content of these samples are given in Table 1; replicate determinations were performed on all samples.

A sample of technical grade podophyllotoxin was purchased and assayed by measuring the absorbance of solutions in ethanol (96% v/v) at the maximum at about 292 nm using an A (1 per cent, 1 cm) of 107·8. When subsequently assayed by the procedure described under 'Total lignan content of resin', recoveries of 97·2–99·5% were achieved. Similar recoveries were obtained from a laboratory prepared sample of podophyllotoxin.

The method described is simple, quantitatively reproducible (Table 1), and may be used to determine the total aryltetralin lignan content, expressed as podophyllotoxin, of podophyllum resins and the roots/rhizomes from which they are obtained. This work confirms the difference in total lignan content between the resins of *P. hexandrum* and *P. peltatum* and also demonstrates that there can be a significant variation in the lignan content of *P. hexandrum* resins. The production of pharmacopoeial resins standardized on total lignan content would, therefore, seem desirable.

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