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TUMOUR-INHIBITORY ARYLTETRALIN LIGNANS FROM *PODOPHYLLUM PLEIANTHUM*

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Key Word Index—*Podophyllum pleianthum*; Podophyllaceae; aryltetralin; lignan; podophyllotoxin.

Abstract—Roots of *Podophyllum pleianthum* contain eight aryltetralin lignans: podophyllotoxin, desoxypodophyllotoxin, podophyllotoxone, isopicropodophyllone and the four corresponding 4′-demethyl derivatives. The lignan pattern is very similar to that of *P. hexandrum*. A useful TLC spray reagent for *Podophyllum* lignans is described.

INTRODUCTION

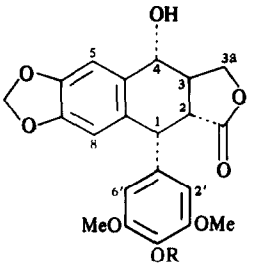
A number of plant-derived lignans have potentially useful anticancer activity, and those present in *Podophyllum* species have now been chemically modified to produce the clinically useful drugs etoposide and teniposide [1]. Etoposide is of particular value in the treatment of testicular cancer and small-cell lung cancer, and is now produced commercially from podophyllotoxin (**1**), a major lignan of *Podophyllum hexandrum* and *P. peltatum*. Analysis of root material from these two species has shown that they contain the same range of at least ten aryltetralin lignans (**1–10**), although the proportions vary markedly [2]. Thus, α- and β-peltatins (**6** and **5**), major lignans in *P. peltatum*, are present as trace constituents only in *P. hexandrum*. Glucosides of several of these lignans have been identified in both species [3]. Although several other species of *Podophyllum* have been identified, these are rarely encountered and chemical examination has consequently been very limited [4–7]. We were fortunate to be supplied with a sample of root and rhizome material of *Podophyllum pleianthum* from Taiwan, where it is much valued as a medicinal plant. Earlier investigations of *P. pleianthum* had indicated the presence of podophyllotoxin (**1**) [4], desoxypodophyllotoxin (**3**) [4, 5] and isopicropodophyllone (**9**) [5] in this plant.

RESULTS AND DISCUSSION

Analysis of the non-glycosidic lignans present in an EtOH extract of the dried root/rhizome by TLC (silica gel, $CHCl_3$–MeOH, 25:1) as previously [2] showed the presence of six clearly-defined chromatographic zones. These were eluted, purified further by TLC, then identified by 250 MHz ^{1}H NMR [2] and comparison with authentic samples derived from *P. hexandrum* and *P. peltatum*. The eight lignans listed in Table 1 were characterised, the content of each being assessed from UV absorbance measurements. Included in Table 1 for comparison are data obtained for *P. hexandrum* and *P. peltatum* from the earlier studies [2].

The range of lignans observed in *P. pleianthum* was the same as that recorded for *P. hexandrum* and *P. peltatum*, except that the peltatins, if present, were at too low a level for satisfactory identification. The relative proportions of the individual lignans present resemble those of *P. hexandrum* rather than *P. peltatum*. The identification of podophyllotoxone (**7**) contrasts with the reported absence of this compound in a sample of *P. pleianthum* analysed by Chang and coworkers [5]. They isolated the isomeric ketone isopicropodophyllone (**9**). However, since **7** is readily isomerised to **9** by heat [8], the latter compound may well be an artefact from podophyllotoxone in *Podophyllum* extracts, the amount isolated varying according to conditions of drying, extraction, etc. Similar comments apply to the 4′-demethyl ketones (**8** and **10**). Chang also indicated that desoxypodophyllotoxin (**3**) predominated over isopicropodophyllone (**9**), whereas in our sample, this compound appears to be present at quite low levels relative to the ketones **7** and **9** (*cf. P. hexandrum* also). Since desoxypodophyllotoxin is a biosynthetic precursor of podophyllotoxin and podophyllotoxone [9], minor regulation of the biosynthetic enzymes could account for this variation between individual plants. Similarly, variations in proportions of trimethoxyphenyl derivatives relative to hydroxydimethoxyphenyl lignans in individual plants also appear to depend on regulation of entry of biosynthetic precursors into each of the two groups [9].

The location of aryltetralin lignans on thin layer chromatograms is readily achieved by the use of UV light together with fluorescent indicators in the adsorbent. However, this method gives no information about the lignan, nor indeed assists in identifying a particular quenching zone as a lignan rather than any other class of unsaturated/aromatic metabolite. Chromogenic reagents specific for lignans are also not available. During systematic degradation of podophyllotoxin in our biosynthetic studies [10], we utilised the nitric acid oxidation of this lignan to the bright red quinone (**11**) [11]. This reaction is believed to progress *via* acid-catalysed demethylation to 4′-demethylpodophyllotoxin followed by further oxidation/demethylation [11]. We have found that the reagent used, conc nitric acid in acetic acid (3:10), also makes an extremely valuable chromogenic spray reagent in the TLC of *Podophyllum* lignans. All ten lignans identified in *Podophyllum* (Table 1) react to give red



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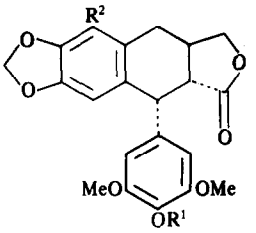
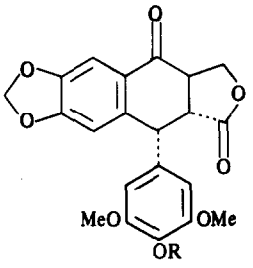
2 R = H



3 R^1 = Me, R^2 = H
4 R^1 = H, R^2 = H
5 R^1 = Me, R^2 = OH
6 R^1 = H, R^2 = OH



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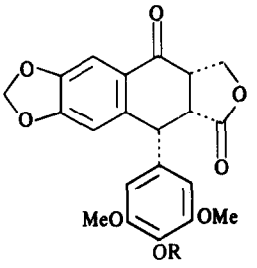

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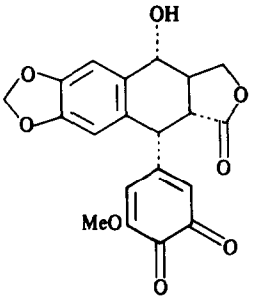

11

Table 1. Lignan content* of *Podophyllum* species

Lignan	*P. pleianthum*	*P. hexandrum*†	*P. peltatum*†
Podophyllotoxin (**1**)	13.5	128	7.5
4′-Demethylpodophyllotoxin (**2**)	4.1	13.5	0.2
Desoxypodophyllotoxin (**3**)	1.0	0.5	0.7
4′-Demethyldesoxypodophyllotoxin (**4**)	0.3	0.3	0.2
β-Peltatin (**5**)	—	0.3	10.0
α-Peltatin (**6**)	—	0.2	7.5
Podophyllotoxone (**7**)	3.7	1.7	0.6
4′-Demethylpodophyllotoxone (**8**)	1.3	0.4	0.2
Isopicropodophyllone (**9**)	2.0	1.0	0.2
4′-Demethylisopicropodophyllone (**10**)	0.5	0.2	0.1

*Lignan content expressed as mg/10 g dried root/rhizome material.
†Data from ref. [2].

colourations, or brown in the case of the peltatins. Furthermore, the 4'-demethyl derivatives react almost immediately, whereas the trimethoxy group of lignans react more slowly and are best visualised by subsequently warming the sprayed plate with a hot-air dryer. This presumably reflects the additional demethylation step necessary for quinone formation. Although we have not explored the applicability of this reaction with other lignans, the reagent is potentially very useful for analysis of *Podophyllum* lignans, and preliminary division into trimethoxy or hydroxydimethoxy derivatives.

EXPERIMENTAL

Podophyllum pleianthum root/rhizome was supplied and authenticated by Dr. Ta-Wei Hu, Taiwan Forestry Research Institute, Tapei, Taiwan. The root/rhizome was dried at 30°, then coarsely powdered. A sample of the root (10 g) was extracted by stirring with hot EtOH (3 × 100 ml) for 10 min, the extracts combined and concentrated to about 30 ml under red. press. The extract was poured into H_2O (100 ml), then extracted with EtOAc (3 × 100 ml). The combined extracts were evaporated and separated by TLC (Merck TLC-Kiesel gel 60 GF_{254}; $CHCl_3$–MeOH, 25:1), zones being located by UV, then separately eluted with Me_2CO (Analar). The zones were purified further by TLC using solvent systems A (Et_2O–CH_2Cl_2, 6:1), B ($CHCl_3$–*iso*-PrOH, 10:1) or C ($CHCl_3$–MeOH, 6:1) as indicated. The zones, listed in order of decreasing R_fs, were identified by NMR, MS and comparison with authentic samples, as follows: zone 1 (solvent A) = mixture of podophyllotoxone (**7**) and desoxypodophyllotoxin (**3**), separated by HPLC as described earlier [8]; zone 2 (solvent B) = isopicropodophyllone (**9**); zone 3 (solvent B) = mixture of 4'-demethylpodophyllotoxone (**8**) and 4'-demethyldesoxpodophyllotoxin (**4**), separated by HPLC as described earlier [2]; zone 4 (solvent B) = 4'-demethylisopicropodophyllone (**10**); zone 5 (solvent C) = podophyllotoxin (**1**); and zone 6 (solvent C) = 4'-demethylpodophyllotoxin (**2**).

Spray reagent. TLC plates were sprayed with a soln of conc HNO_3 and HOAc (3:10), then left at room temp. for approx 1 min for colouration from **2**, **4**, **6**, **8** and **10** to develop. The plate was then warmed gently with a hair-dryer for a further 1 min to develop colours from **1**, **3**, **5**, **7** and **9**.

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