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CYTOTOXIC LIGNANS FROM *PODOPHYLLUM*, AND THE NOMENCLATURE OF ARYLTETRALIN LIGNANS

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Key Word Index—*Podophyllum hexandrum*; *P. peltatum*; Podophyllaceae; aryltetralin; lignans; desoxypodophyllotoxin; podophyllotoxone; cytotoxicity; nomenclature.

Abstract—Roots of *Podophyllum hexandrum* and *P. peltatum* both contain (1*R*,2*R*,3*R*)-desoxypodophyllotoxin [(1α,2α,3β)-desoxypodophyllotoxin] and the previously unreported (1*R*,2*R*,3*R*)-podophyllotoxone [(1α,2α,3β)-podophyllotoxone]. Thermal isomerization of (1α,2α,3β)-podophyllotoxone readily occurs to yield (1α,2α,3α)-podophyllotoxone (isopicropodophyllone) with traces of (1α,2β,3β)-podophyllotoxone (picropodophyllone). Small amounts of (1α,2α,3α)-podophyllotoxone were also present in dried roots of *P. hexandrum* and *P. peltatum*. A more systematic nomenclature for podophyllotoxin derivatives and other aryltetralin lignans using α,β conventions is proposed.

INTRODUCTION

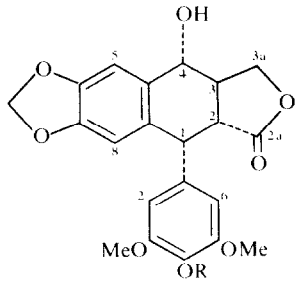
A wide range of aryltetralin lignans and glycosides having cytotoxic and/or antitumour activity has been isolated from Indian Podophyllum (*Podophyllum hexandrum* [1]), American Podophyllum (*P. peltatum*) and other species [2, 3]. TLC analysis (Si gel, $CHCl_3$–MeOH, 9:1 followed by $CHCl_3$–Me_2CO, 63:35) of *P. hexandrum* extracts [4, 5] shows three main zones, reported as 4′-demethylpodophyllotoxin (**1**), podophyllotoxin (**2**) and desoxypodophyllotoxin (**3**). However, although desoxypodophyllotoxin has been isolated from *P. peltatum* [6] and *P. pleianthum* [7], there are no reports of its presence in *P. hexandrum*, other than the above TLC data. Investigation of these chromatographic zones has confirmed the identification of 4′-demethylpodophyllotoxin and podophyllotoxin, but the third zone has now been identified as a mixture of desoxypodophyllotoxin (**3**) and a previously unreported natural product, podophyllotoxone (**4**). The corresponding chromatographic zone from *P. peltatum* extracts is also a mixture of **3** and **4**.

RESULTS AND DISCUSSION

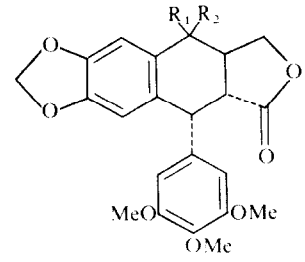
Lignans of P. hexandrum *and* P. peltatum

The non-glycosidic fraction of *P. hexandrum* root extract was further fractionated by TLC (Si gel, $CHCl_3$–MeOH, 25:1), and the three major bands at R_f 0.9, 0.3 and 0.2 were eluted. Material from the two lower bands when purified further and crystallized proved to be identical to 4′-demethylpodophyllotoxin (**1**) (R_f 0.2) and podophyllotoxin (**2**) (R_f 0.3), in agreement with published data [2]. The upper zone had a UV spectrum (EtOH) with two main absorbances at 278 and 315 nm, and minor bands/shoulders at 290–300 nm. Synthetic desoxypodophyllotoxin produced by catalytic hydrogenolysis of podophyllotoxin [8] has λ_{max} 289 and 293 nm, and chromatographically behaved as the isolated material in several solvent systems. The UV spectrum of the isolated material was thus consistent with desoxypodophyllotoxin (**3**) together with another compound with UV characteristics of an aryl ketone. Mass spectral analysis showed the presence of (**3**) (M^+ 398) and another compound (M^+ 412), which was identified as podophyllotoxone (**4**) by comparison with synthetic material obtained by MnO_2 [9] or CrO_3–pyridine [10] oxidation of podophyllotoxin. **3** and **4** could only be partially separated by TLC, but HPLC (Partisil-10 ODS2, MeOH–H_2O, 7:3) and gel filtration (Sephadex LH-20, EtOH) were successful. The two compounds were identical with the synthetic material in all respects (co-TLC, UV, IR, 250 MHz NMR, $[\alpha]_D$, MS) thus confirming both their structure and chirality (1*R*,2*R*,3*R*). Examination of the corresponding chromatographic zone from *P. peltatum* roots showed this also to be a mixture of **3** and **4**, but with a greater proportion of desoxypodophyllotoxin than in *P. hexandrum*. Podophyllotoxone is also present in fresh samples of *P. hexandrum* root.

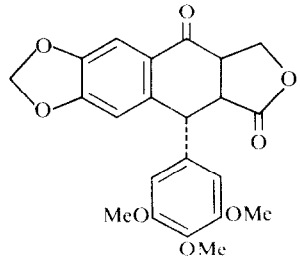
Podophyllotoxone has been known synthetically for several years, but has not previously been reported as a natural product. Although all other physical data were as reported for the synthetic material, mps of our natural and synthetic samples were somewhat lower (174–188° cf. 191–192° [9]) despite repeated purifications. This was especially marked when the rate of heating was slow. Analysis of the isolated material showed that a chemical change had occurred, and that three compounds were now present. These were unchanged podophyllotoxone, traces of its 1*R*,2*S*,3*R*-isomer (picropodophyllone) (**5**) confirmed by comparison with authentic material, and as major product the 1*R*,2*R*,3*S*-isomer (isopicropodophyllone) (**6**). The 2,3-*cis* stereochemistry was apparent from the more complex coupling pattern observed for the alicyclic protons in the NMR spectrum. The *trans*-fused lactones podophyllotoxone and isopodophyllotoxone [11] give highly resolved first-order spectra. Since both products contain a *cis*-fused lactone ring as opposed to the severely strained *trans*-fused system of podophyllo-



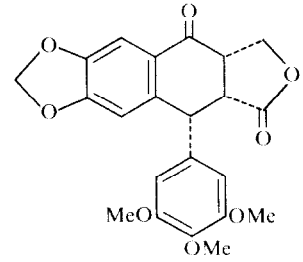
1 R = H
2 R = Me



3 $R_1 = R_2 = H$
4 $R_1R_2 = O$



5



6

toxone, the driving force for isomerization is presumably to relieve this ring strain. A similar mixture of products was obtained by heating **4** in diglyme at 160°, but base-treatment (NaOAc in EtOH, reflux) gave initially **5** which was broken down on further heating.

Isopicropodophyllone (**6**) has been reported as a constituent of *P. pleianthum* [12]. Our material had identical spectral characteristics to those reported for the natural products except for UV data. The isomerization of podophyllotoxone to **6** has previously been carried out [13, 14], but no experimental details are available. The ready isomerization reported here implies that traces of **6** may be found in dried plant material containing podophyllotoxone, depending on drying and extraction procedures. Indeed, traces of **6** (co-TLC, UV) were found in commercial samples of *P. hexandrum* and *P. peltatum* root even when all extractions were carried out in the cold. This isomer may or may not be an artefact in these plant materials.

The cytotoxicity of podophyllotoxone, desoxypodophyllotoxin and isopicropodophyllone has been reported in earlier studies [2, 12].

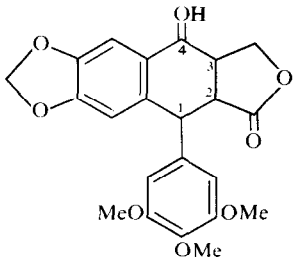
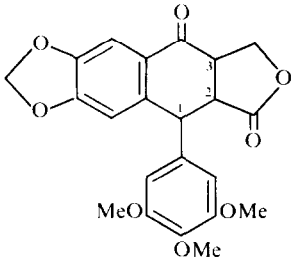
Nomenclature of Podophyllum *lignans*

Podophyllotoxin contains four chiral centres, and although most natural derivatives, e.g. 4′-demethylpodophyllotoxin, desoxypodophyllotoxin and podophyllotoxone, have the same absolute configuration at these centres (where appropriate), synthetic studies in this group of compounds have led to the adoption of a variety of trivial names to indicate configurations. The eight possible diastereoisomers of podophyllotoxin resulting from changes in chirality at positions 2, 3 and 4 have all been synthesized, and the currently used nomenclature is shown in Scheme 1 (cf. [14]).

The prefixes appear to have been adopted for historical reasons rather than to indicate chirality in a systematic manner [2]. Thus, inversion at position 2 of podophyllotoxin produces the *picro* series, but the *-tox-* syllable is omitted when the prefix *picro* is used. The prefix *epi* indicates a change in configuration at position 4, but because of an early misassignment of configuration, epiisopodophyllotoxin and epiisopicropodophyllin have the same chirality at C-4 as podophyllotoxin. The prefix *iso* has been reported to imply a change in configuration at C-1 [15], but usage of the term is ambiguous. (+) and (−) have been used to assign enantiomers, and the C-1 epimer of desoxypodophyllotoxin has been termed (−)-isodesoxypodophyllotoxin, its enantiomer, differing from desoxypodophyllotoxin at C-2 and C-3 being (+)-isodesoxypodophyllotoxin [16]. In general, therefore, current usage of *iso* without (+) or (−) implies inversion at three centres, C-2, C-3 and C-4. This leads to the clumsy *epiisopicro* prefix to represent inversion at C-3. Chirality at C-1 has also been assigned as D- or L-, the configuration of the natural podophyllotoxin series being taken as L [14, 17]. Numbering is also not consistent, and although most authors now number from the point of attachment of the aryl ring, older publications may use a system numbering from the hydroxyl of podophyllotoxin. In our opinion, this trivial nomenclature is too confusing and inconsistent for general use and should be revised.

Systematic nomenclature using the *R,S* conventions is not really satisfactory in the podophyllotoxin series because priorities can only be assigned by consideration of β- or even γ-substituents, and general usage becomes inappropriate. We propose that one name per structure is used throughout, and that absolute configuration is assigned by an α,β convention for substituents. This convention has already been employed by a number of

Scheme 1. Nomenclature of podophyllotoxin derivatives

Trivial name	Substituents	Chirality	Proposed nomenclature
			
L-Podophyllotoxin	1α,2α,3β,4α	1*R*,2*R*,3*R*,4*R*	(1α,2α,3β,4α)-podophyllotoxin
L-Picropodophyllin	1α,2β,3β,4α	1*R*,2*S*,3*R*,4*R*	(1α,2β,3β,4α)-podophyllotoxin
L-*Epipodophyllotoxin*	1α,2α,3β,4β	1*R*,2*R*,3*R*,4*S*	(1α,2α,3β,4β)-podophyllotoxin
L-Epipicropodophyllin	1α,2β,3β,4β	1*R*,2*S*,3*R*,4*S*	(1α,2β,3β,4β)-podophyllotoxin
L-Isopodophyllotoxin	1α,2β,3α,4β	1*R*,2*S*,3*S*,4*S*	(1α,2β,3α,4β)-podophyllotoxin
L-Isopicropodophyllin	1α,2α,3α,4β	1*R*,2*R*,3*S*,4*S*	(1α,2α,3α,4β)-podophyllotoxin
L-Epi-isopodophyllotoxin	1α,2β,3α,4α	1*R*,2*S*,3*S*,4*R*	(1α,2β,3α,4α)-podophyllotoxin
L-Epi-isopicropodophyllin	1α,2α,3α,4α	1*R*,2*R*,3*S*,4*R*	(1α,2α,3α,4α)-podophyllotoxin
D-Podophyllotoxin, etc.	1β,2α,3β,4α	1*S*,2*R*,3*R*,4*R*	(1β,2α,3β,4α)-podophyllotoxin
			
L-Podophyllotoxone	1α,2α,3β	1*R*,2*R*,3*R*	(1α,2α,3β)-podophyllotoxone
L-Picropodophyllone	1α,2β,3β	1*R*,2*S*,3*R*	(1α,2β,3β)-podophyllotoxone
L-Isopodophyllotoxone	1α,2β,3α	1*R*,2*S*,3*S*	(1α,2β,3α)-podophyllotoxone
L-Isopicropodophyllone	1α,2α,3α	1*R*,2*R*,3*S*	(1α,2α,3α)-podophyllotoxone
D-Podophyllotoxone, etc.	1β,2α,3β	1*S*,2*R*,3*R*	(1β,2α,3β)-podophyllotoxone
etc.			

authors [10–12, 14] to aid identification of trivial names. The diastereoisomers in Scheme 1 should thus be renamed as shown. This convention will, of course, require presentation of structural formulae in the above manner, i.e. aromatic ring of the tetrahydronaphthalene on the left, with pendent aryl substituent, but this is generally followed in almost all publications. Numbering must also be standardized, priority being given to the point of attachment of the pendent ring. Such a system will remove the confusion associated with nomenclature in the podophyllotoxin series, and can also be applied to all other aryltetralin lignans.

Thus, the two lignans reported in this paper should be named (1α,2α,3β)-desoxypodophyllotoxin and (1α,2α,3β)-podophyllotoxone. Thermal isomerization of (1α,2α,3β)-podophyllotoxone yields (1α,2α,3α)-podophyllotoxone with traces of (1α,2β,3β)-podophyllotoxone.

EXPERIMENTAL

General. Dried *P. hexandrum* (United Chemical and Allied Products, Calcutta) and *P. peltatum* (Joseph Flach, London) roots, and *P. hexandrum* plants (Jack Drake, Aviemore) were obtained commercially. TLC was carried out using 0.5-mm layers of Si gel (Merck TLC-Kiesel gel 60 GF_{254}). Me_2CO (Analar) was used for elution of TLC zones. ^1H NMR spectra were run in $CDCl_3$ soln with TMS as standard.

Isolation of **3** *and* **4** *from* P. hexandrum *and* P. peltatum. Powdered dried root material (30 g) was stirred with hot EtOH (4 × 100 ml) for 10 min. The filtered extracts were combined, evapd, treated with H_2O (100 ml), then extracted with EtOAc (4 × 100 ml). The combined extracts were evapd and separated by TLC ($CHCl_3$–MeOH, 25:1). Three major bands (R_fs 0.2, 0.3 and 0.9) were eluted. The two lower bands were identified as 4′-demethylpodophyllotoxin and podophyllotoxin, respectively. The upper zone was purified further by TLC (Me_2CO–petrol (bp 60–80°), 1:1, Et_2O–CH_2Cl_2, 6:1) to give a mixture of **3** and **4**. These were separated by gel filtration (Sephadex LH-20, column 25 cm × 1.5 cm, EtOH, flow rate 12 ml/hr, loading 6–7 mg), collecting 1-ml fractions. Fractions 45–50 contained desoxypopophyllotoxin (**3**), fractions 54–60 podophyllotoxone (**4**), whereas fractions 51–53 contained an unresolved mixture of **3** and **4** and were thus rechromatographed. Yields of recrystallized **3** and **4** were: *P. hexandrum* **3** 5 mg, **4** 17 mg; *P. peltatum* **3** 7 mg, **4** 6 mg.

(1α,2α,3β)-*Desoxypodophyllotoxin* (**3**). Recrystallized aq. MeOH; mp 167–169° (lit. [8] 166–168°); $[\alpha]_D$ −116° (c = 0.606,

$CHCl_3$) (lit. [8] −117°); UV λ_{max}^{EtOH} nm: 289 (log ε 3.68), 293 (3.68); IR ν_{max}^{KBr} cm^{-1}: 1768, 1590, 1508, 1488; ^1H NMR (250 MHz): δ 6.67 (1 H, *s*, H-5), 6.53 (1 H, *s*, H-8), 6.35 (2 H, *s*, H-2′,6′), 5.96 (1 H, *d*, *J* = 1.2 Hz, O—CH_2—O), 5.94 (1 H, *d*, *J* = 1.2 Hz, O—CH_2—O), 4.61 (1 H, *br s*, H-1), 4.47 (1 H, *dd* (*ca*), *J* = 8.5, 6.4 Hz, H-3aα), 3.93 (1 H, *dd*, *J* = 10.4, 8.5 Hz, H-3aβ), 3.81 (3 H, *s*, 4′-OMe), 3.75 (6 H, *s*, 3′,5′-OMe), 3.10 (1 H, *m*, H-4α), 2.76 (3 H, *m*, H-2,3,4β); MS: 398 (M^+, 100%), 230 (6), 185 (10), 181 (6), 173 (13), 168 (5). The natural product was identical to material synthesized by hydrogenolysis of (1α,2α,3β,4α)-podophyllotoxin [8].

(1α,2α,3β)-*Podophyllotoxone* (**4**). Recrystallized EtOH; mp 174–178°(slow heating), 189–192° (rapid heating) (lit [9] 191–192°); $[\alpha]_D$ −128° (*c* = 1.393, $CHCl_3$) (lit [9] −125°); UV λ_{max}^{EtOH} nm: 235 (log ε 4.19), 276 (3.87), 317 (3.80); IR ν_{max}^{KBr} cm^{-1}: 1780, 1765, 1665, 1615, 1587, 1505; ^1H NMR (250 MHz): δ 7.56 (1 H, *s*, H-5), 6.71 (1 H, *s*, H-8), 6.39 (2 H, *s*, H-2′,6′), 6.11 (1 H, *d*, *J* = 1.2 Hz, O—CH_2—O), 6.09 (1 H, *d*, *J* = 1.2 Hz, O—CH_2—O), 4.85 (1 H, *d*, *J* = 4.3 Hz, H-1), 4.57 (1 H, *dd*, *J* = 9.2, 7.6 Hz, H-3aα), 4.36 (1 H, *dd*, *J* = 10.4, 9.2 Hz, H-3aβ), 3.83 (3 H, *s*, 4′-OMe) 3.76 (6 H, *s*, 3′,5′-OMe), 3.53 (1 H, *ddd*, *J* = 15.6, 10.4, 7.6 Hz, H-3), 3.28 (1 H, *dd*, *J* = 15.6, 4.3 Hz, H-2); MS: 412 (M^+, 100%), 368 (4), 367 (18), 353 (4), 337 (6), 336 (4), 200 (4), 168 (32), 153 (8), 115 (4). The natural product was identical to material synthesized by MnO_2 oxidation [9] or CrO_3–pyridine oxidation [10] of (1α,2α,3β,4α)-podophyllotoxin.

Thermal isomerization of (1α,2α,3β)-*podophyllotoxone* (**4**) *to* (1α,2α,3α)-*podophyllotoxone* (**5**) (*isopicropodophyllone*). (1α,2α,3β)-Podophyllotoxone (70 mg) in diglyme (1 ml) was heated in a sealed vial at 160° for 20 hr. The reaction mixture was poured into H_2O (50 ml), then extracted with Et_2O (3 × 30 ml). The combined and evapd extracts were purified by TLC (Et_2O–CH_2Cl_2, 6:1). A band at R_f 0.85 was unchanged starting material, and a minor zone (R_f 0.7) was eluted and identified as (1α,2β,3β)-podophyllotoxone (**5**) (picropodophyllone) [identical to material synthesized by MnO_2 oxidation [9] of (1α,2β,3β,4α)-podophyllotoxin (picropodophyllin)]. Elution of the major band (R_f 0.6) and recrystallization from EtOH gave (1α,2α,3α)-podophyllotoxone (**6**) (isopicropodophyllone) (28 mg), mp 171–174° (lit. [12] 170–172°); $[\alpha]_D$ −288° (*c* = 1.106, $CHCl_3$) (lit. [12] −273°); UV λ_{max}^{EtOH} nm: 237 (log ε 4.42), 274 (3.87), 320 (3.85); IR ν_{max}^{KBr} cm^{-1}: 1765 1668, 1620, 1585, 1500; ^1H NMR (250 MHz): δ 7.43 (1 H, *s*, H-5), 6.69 (1 H, *s*, H-8), 6.28 (2 H, *s*, H-2′,6′), 6.09 (1 H, *d*, *J* = 1.2 Hz, O—CH_2—O), 6.07 (1 H, *d*, *J* = 1.2 Hz, O—CH_2—O), 4.57 (1 H, *d*, *J* = 5.5 Hz, H-1), *ca* 4.53 (1 H, *m*, H-3aα), *ca* 3.84 (1 H, *m*, H-3aβ), 3.80 (3 H, *s*, 4′-OMe), 3.73 (6 H, *s*, 3′,5′-OMe), *ca* 3.7–3.5 (2 H, *m*, H-2, 3); MS: 412 (M^+, 100%), 408 (5), 367 (24), 353 (5), 337 (8), 313 (7), 297 (24), 227 (7), 199 (6), 188 (21), 181 (5), 168 (10), 153 (6). A similar product mixture was obtained by melting **4** in a sealed vial at 190° for 15 min.

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