Phytochemistry, Vol. 25, No. 9, pp. 2093–2102, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

BIOSYNTHETIC RELATIONSHIP OF ARYLTETRALIN LACTONE LIGNANS TO DIBENZYLBUTYROLACTONE LIGNANS*

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(*Received* 9 *January* 1986)

Key Word Index—*Podophyllum hexandrum*: Podophyllaceae: lignans: aryltetralin: dibenzylbutyrolactone: biosynthesis: podophyllotoxin.

Abstract—Feeding experiments in *Podophyllum hexandrum* plants have shown that the dibenzylbutyrolactone lignan yatein is a satisfactory precursor of the aryltetralin lignan podophyllotoxin. The corresponding *cis* isomer of yatein was also incorporated, but to a lesser extent, and probably *via* yatein. Podorhizol, epipodorhizol and anhydropodorhizol were not incorporated into podophyllotoxin, despite the demonstrated presence of both podorhizol and anhydropodorhizol in *P. hexandrum* plants. A biosynthetic sequence from yatein to podophyllotoxin *via* a key quinonemethide intermediate is proposed. This intermediate is probably also the precursor of both podorhizol and anhydropodorhizol. Matairesinol is proposed as the branchpoint compound to the trimethoxy and hydroxydimethoxy series of aryltetralin lignans in *Podophyllum*. Labelling patterns in podophyllotoxin and 4′-demethylpodophyllotoxin derived from [*S*-methyl-^{14}C]methionine support this suggestion.

INTRODUCTION

Indian podophyllum (*Podophyllum hexandrum*) and American podophyllum (*P. peltatum*) contain a range of tumour-inhibitory lignans based on an aryltetralin lactone skeleton [1, 2]. Podophyllum is now economically important as a source of podophyllotoxin (**4**), a major constituent of the root, and the starting material for production of the semi-synthetic anticancer drugs etoposide and teniposide [3]. Feeding experiments [4, 5] in *Podophyllum hexandrum* plants have indicated that podophyllotoxin (**4**) is derived by hydroxylation of desoxypodophyllotoxin (**2**), and further oxidation yields podophyllotoxone (**6**) (Scheme 1). Podophyllotoxone may also be reduced *in vivo* to podophyllotoxin. β-Peltatin (**8**) is formed by an alternative 5-hydroxylation of desoxypodophyllotoxin [6]. A similar sequence of reactions holds for the related 4′-demethyl series of compounds **1**, **3**, **5** and **7** (Scheme 1), but these compounds do not appear to be methylated to the corresponding 3,4,5-trimethoxyphenyl series. Thus, 4′-demethylpodophyllotoxin (**3**) is not incorporated into podophyllotoxin (**4**). Instead, these two series of aryltetralin lactone lignans are formed separately from some common precursor, which has yet to be identified. In the earlier part of the biosynthetic pathway, two phenylpropane precursors with the 4-hydroxy-3-methoxy substitution pattern of ferulic acid couple *via* phenol oxidation to give a dimer as a common precursor. However, no intermediates between ferulic acid and desoxypodophyllotoxin (**2**) or 4′-demethyldesoxypodophyllotoxin (**1**) have been established.

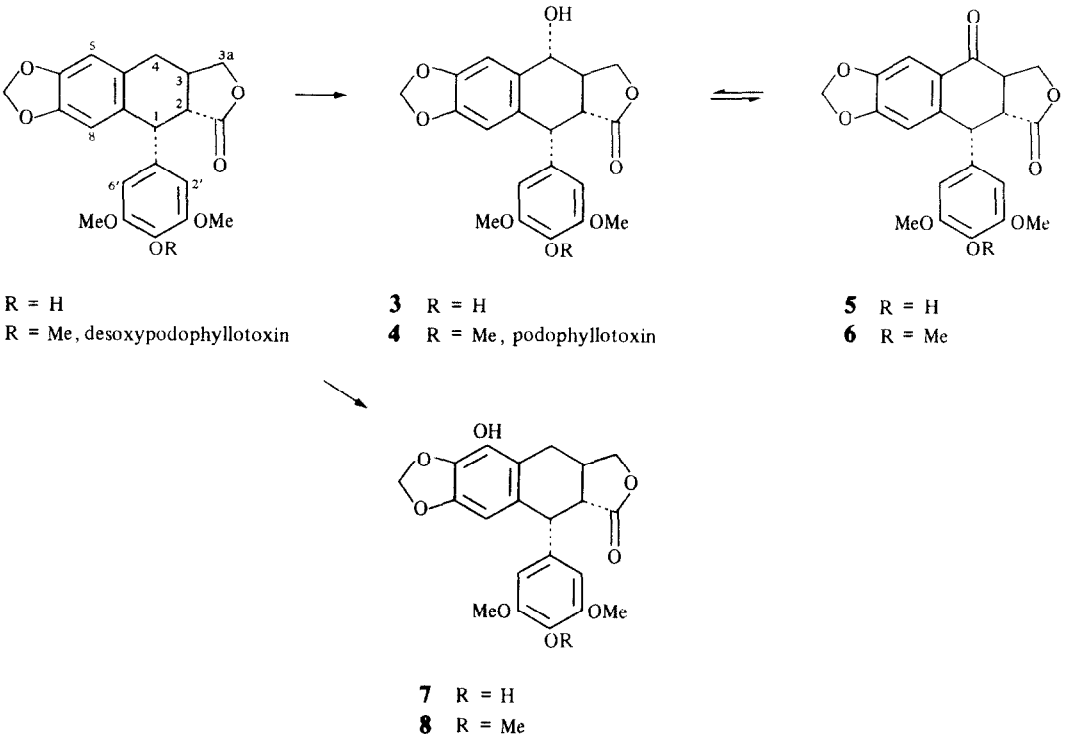
The widespread co-occurrence of aryltetralin lactone lignans with similarly substituted dibenzylbutyrolactone lignans [7–9] suggests compounds in the latter group could well function as biosynthetic precursors of lignans such as desoxypodophyllotoxin and podophyllotoxin. Specific examples of reported co-occurrences which lend strong support for such hypotheses include the isolations of desoxypodophyllotoxin, yatein (**15**), anhydropodorhizol (nemorosin, **11**) and podorhizol (**12**) from *Anthriscus sylvestris* [10–12], of **2**, **12** and **15** from *Hernandia ovigera* [13, 14] and *H. cordigera* [15], and of **2**, **3** and **15** from *Juniperus communis* [16]. Indeed, podorhizol glucoside (**16**) has been isolated from both *Podophyllum hexandrum* and *P. peltatum* [17], and on acid hydrolysis, cyclization to an aryltetralin structure has been observed [17]. In the light of these data, it became highly desirable to test podorhizol, anhydropodorhizol and yatein as potential precursors of podophyllotoxin in *Podophyllum* plants, to clarify that part of the biosynthetic pathway between ferulic acid and desoxypodophyllotoxin. These experiments are described here.

RESULTS AND DISCUSSION

Synthesis of precursors

A number of synthetic routes to dibenzylbutyrolactone lignans like podorhizol, yatein and anhydropodorhizol are available in the literature [18]. Thus, podorhizol (**12**), along with its epimer epipodorhizol (**13**), may be produced in good yield by treating the butyrolactone **9** with lithium hexamethyldisilazide, and reacting the anion produced with trimethoxybenzaldehyde (**10**) [19]. Podorhizol (**12**) and epipodorhizol (**13**) are readily separated by TLC. In theory, hydrogenolysis of either **12** or **13** should then yield yatein (**15**). However, both isomers proved extremely inert towards catalytic hydrogenation under a variety of conditions. Yatein has also been

*Part 4 in the series "Biosynthesis of *Podophyllum* Lignans". For Part 3 see ref. [6].



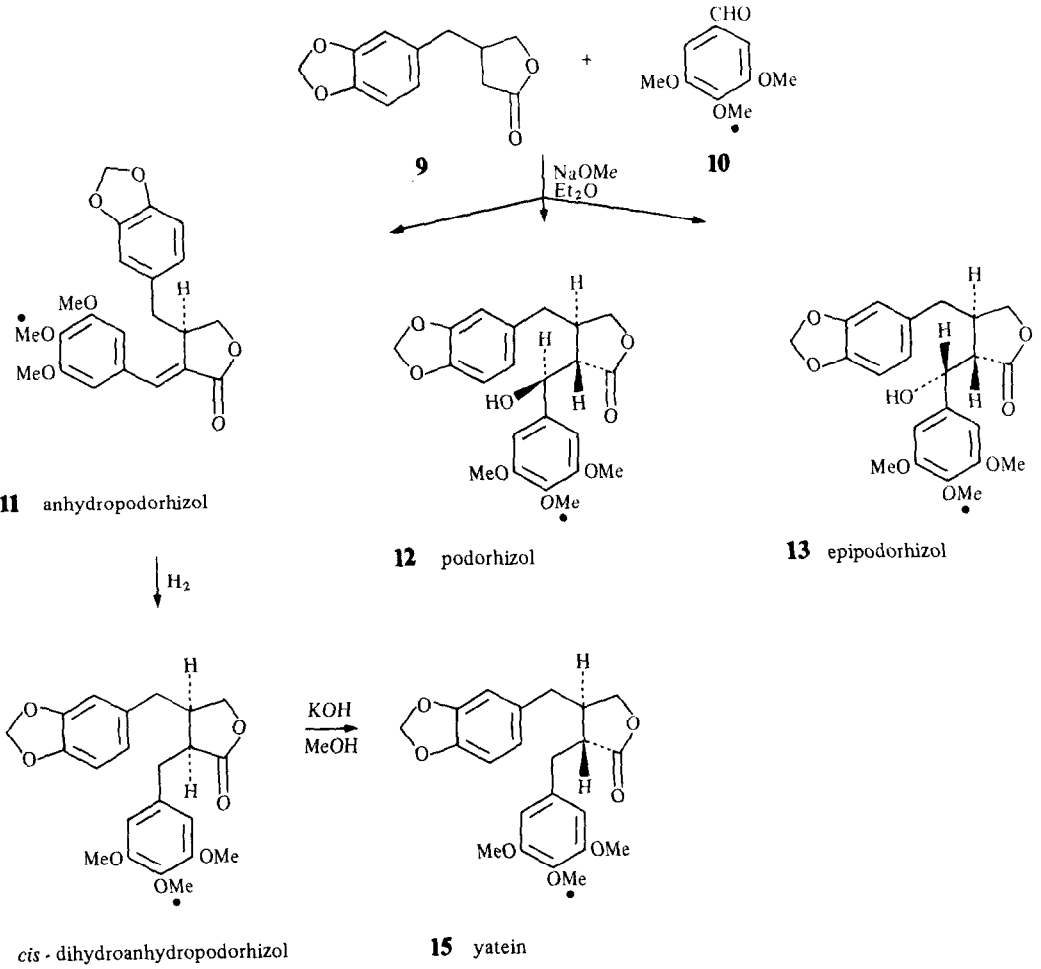
Scheme 1. Biosynthetic interrelationships for *Podophyllum* lignans [5, 6].

synthesized *via* the reaction of the enolate of butyrolactone **9** with 3,4,5-trimethoxybenzyl bromide [20]. In our hands, this reaction was far from satisfactory, and unsuitable for the small-scale synthesis of labelled compounds. Attempted dehydration of podorhizol or epipodorhizol also failed. Tosylation or mesylation gave minimal yields, suggesting this hydroxyl is hindered towards esterification. Base treatment of the alcohols inevitably yielded **9** and **10**, *via* a reverse aldol reaction.

Eventually, a satisfactory sequence to the required compounds was effected by utilizing a sodium methoxide catalysed aldol reaction [21] between **9** and **10**. This reaction is highly susceptible to changes in solvent, temperature and proportions of base used. By altering these variables, the relative proportions of the three major products podorhizol, epipodorhizol and anhydropodorhizol may be markedly influenced. Lower temperatures ($-10°$) favoured production of the condensation product **11**, whereas slightly higher temperatures (3–4°) resulted in formation of mainly the addition products **12** and **13**. Total yields were increased if excesses ($2\times$) of either sodium methoxide or the lactone were used. Dry ether was the preferred solvent to benzene. By employing [4-*O*-methyl-^{14}C]-3,4,5-trimethoxybenzaldehyde in the reaction, labelled anhydropodorhizol (**11**), podorhizol (**12**) and epipodorhizol (**13**) were obtained in a ratio of approximately 3:1.3:1, and in overall yield of about 50% (Scheme 2). NMR assignments (Table 1) confirmed their identity [12, 17, 19], and showed that the condensation product had the *E*-configuration (H-6 at δ7.52, *d*, J = 1.8 Hz) [12, 17]. No trace of the corresponding *Z*-isomer was observed in the reaction mixture. The *E*-isomer is favoured on purely steric grounds, but in addition there is evidence that a *Z*-isomer may isomerize to the *E*-isomer under acid conditions [22]. Since acid treatment is an essential part of the work-up (to recyclize the lactone ring opened by base), the non-appearance of the *Z*-isomer may be due to this.

Anhydropodorhizol (**11**) was smoothly hydrogenated to give the *cis*-dihydroanhydropodorhizol (**14**), which was easily epimerized by treatment with base to yield the thermodynamically more stable *trans*-dihydroanhydropodorhizol (yatein, **15**) (Scheme 2). These isomers were readily identified by their NMR spectra (Table 1) and comparison with literature data [23, 24]. All compounds synthesized were, of course, racemic materials.

The five [methyl-^{14}C]-labelled lignans **11**–**15** were subsequently tested as precursors of the aryltetralin lignans in feeding experiments. However, prior to this, the chemical cyclization of labelled podorhizol was investigated. Initial studies on the acid treatment of natural podorhizol glucoside had suggested that a mixture of the two compounds desoxypodophyllotoxin (**2**) and isodesoxypodophyllotoxin (**17**) was obtained [17]. Subsequent studies with podorhizol and structural analogues [19, 25–27] now show that only the *iso*-compound results from acid-catalysed cyclization (Scheme 3). Cyclization is probably a consequence of Friedel–Crafts reaction of the resultant carbocation onto the aromatic ring, and results in exclusive formation of the thermodynamically more stable 1β or *iso*-configuration with a quasi-equatorial aryl substituent rather than the natural 1α configuration which has the aryl substituent quasi-axial. However, since desoxypodophyllotoxin is a good biosynthetic precursor of podophyllotoxin and other *Podophyllum* lignans [5, 6], it is vital to know the precise outcome of chemical (acid-catalysed) cyclization of podorhizol (or epipodorhizol). In practice, neither podorhizol



Scheme 2. Synthesis of labelled dibenzylbutyrolactone lignans.

nor epipodorhizol is particularly acid-sensitive. Treatment with 1 M sulphuric acid at room temperature or at 60° gave unchanged starting material. Stronger acid treatment (CF_3CO_2H) of either material produced only the one compound, identified as isodesoxypodophyllotoxin (**17**). By repeating this reaction using labelled podorhizol, the presence/absence of desoxypodophyllotoxin was checked by dilution analysis. After repeated recrystallization (× 5), the desoxypodophyllotoxin carrier contained only trace amounts of activity (< 1 %). Thus, although traces of desoxypodophyllotoxin may in fact be present, the levels produced by chemical cyclization are too low to seriously distort data from feeding experiments.

Feeding experiments with lignan precursors

Labelled dibenzylbutyrolactone lignans podorhizol, epipodorhizol, anhydropodorhizol, yatein and *cis*-dihydroanhydropodorhizol (*ca* 1 mg samples) were fed to the root systems of *Podophyllum hexandrum* plants using techniques as described previously [4, 5]. Desoxypodophyllotoxin was also fed in a further experiment as a control. After feeding and subsequent work-up of the plants, podophyllotoxin was isolated in each case. This was diluted with carrier material as necessary, acetylated, then purified to constant specific activity by TLC and repeated recrystallizations. Incorporation data calculated are shown in Table 2. A duplicate set of experiments was performed.

From the results obtained, podorhizol, epipodorhizol and anhydropodorhizol are not precursors of podophyllotoxin. Satisfactory incorporations of yatein and its epimer *cis*-dihydroanhydropodorhizol were observed, but the lower value obtained for the *cis*-epimer suggests it is probably incorporated *via* the *trans*-epimer yatein. The *cis* to *trans* conversion occurs very readily, and it is usually possible to detect small amounts of the more favoured *trans*-isomer in NMR spectra of the *cis*-isomer. Yatein was not as well incorporated as desoxypodophyllotoxin, which is to be expected if it is a precursor of the latter. The incorporation data for the five dibenzylbutyrolactone lignans are not corrected for possible utilization of only one enantiomer from the (±)-precursors fed. It is almost certainly appropriate to correct the percentage incorporation figures by a factor of 2. The desoxypodophyllotoxin fed was optically pure.

Dibenzylbutyrolactone lignans in Podophyllum hexandrum

Although a wide range of aryltetralin lignans and their glycosides has been isolated [2, 7] from *P. hexandrum* and

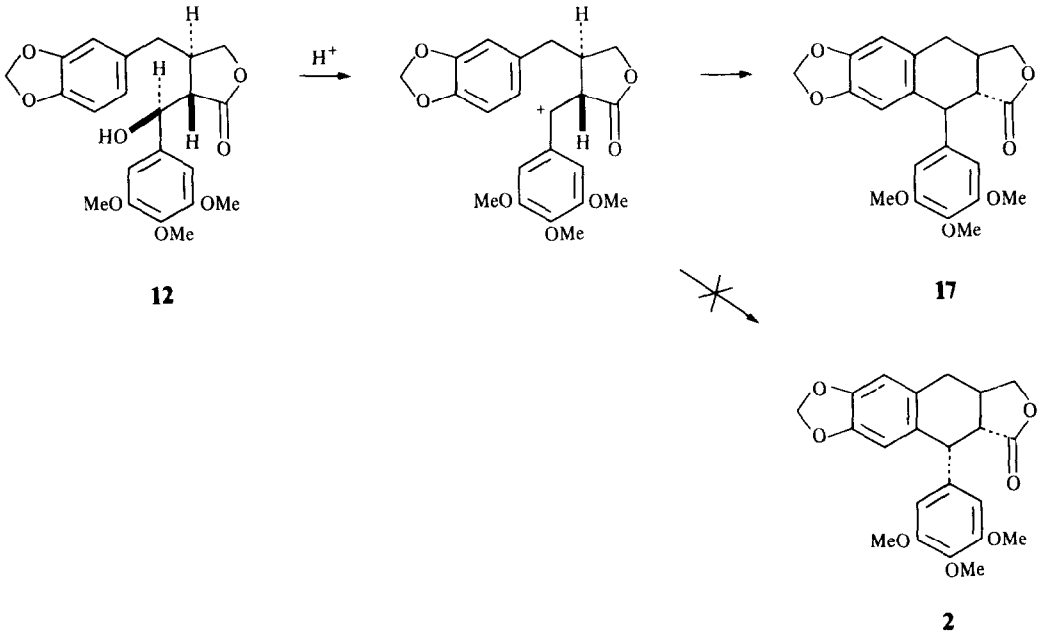
Table 1. 250 MHz ^{1}H NMR data* for dibenzylbutyrolactone lignans

Lignan	H-2	H-3	H-4α	H-4β	H-5		H-6	
Anhydropodorhizol (**11**)	—	*ca* 3.85 *m*	4.30 *dd* *J* = 8.9, 6.0	4.26 *dd* *J* = 8.9, 2.0	3.03 *dd* *J* = 14.4, 4.9	2.66 *dd* *J* = 14.4, 10.0	7.52 *d* *J* = 1.8	
Podorhizol (**12**)†	2.61 *dd* *J* = 5.8, 2.8	*ca* 2.80 *m*	4.39 *t* *J* = 8.6	3.97 *dd* *J* = 8.9, 5.4	2.47 *dd* *J* = 13.8, 7.7	2.25 *dd* *J* = 13.7, 8.1	5.25 *d* *J* = 2.6	
Epipodorhizol (**13**)†	2.62 *dd* *J* = 9.1, 8.0	*ca* 2.50 *m*	4.17 *dd* *J* = 9.3, 8.0	3.91 *dd* *J* = 9.3, 8.5	2.22 *dd* *J* = 13.9, 8.9	2.13 *dd* *J* = 13.8, 5.3	4.79 *d* *J* = 8.0	
cis-Dihydroanhydropodorhizol (**14**)	3.08 *ddd* *J* = 10.3, 6.9, 4.7	*ca* 2.63 *m*	*ca* 4.05 *m*		2.94 *dd* J = 13.4, 3.4	2.32 *dd* *J* = 13.4, 12.9	3.25 *dd* *J* = 14.7, 4.7	2.75 *dd* *J* = 14.6, 10.2
Yatein (**15**)	*ca* 2.5–2.6 *m*		4.18 *dd* *J* = 9.3, 7.1	3.90 *dd* *J* = 9.3, 7.5	*ca* 2.5–2.6 *m*		*ca* 2.91 *m*	

Lignan	H-2′, H-6′	H-2″	H-5″	H-6″	3′,5′-OMe	4′-OMe	OCH_2O
11	6.77 *s*	6.61 *br s*	6.71 *d* *J* = 8.4	6.60 *dd* *J* = 9.1, 1.6	3.89	3.90	5.94 *d*, *J* = 1.4 5.93 *d*, *J* = 1.4
12	6.47 *s*	6.22 *d* *J* = 1.5	6.59 *d* *J* = 7.8	6.30 *dd* *J* = 7.9, 1.5	3.82	3.82	5.94 *d*, *J* = 1.4 5.90 *d*, *J* = 1.4
13	6.65 *s*	6.33 *d* *J* = 1.7	6.66 *d* *J* = 8.2	6.34 *dd* *J* = 7.2, 1.7	3.89	3.84	5.92 *d*, *J* = 1.4 5.91 *d*, *J* = 1.4
14	6.50 *s*	6.53 *m*	6.73 *d* *J* = 8.3	6.53 *m*	3.87	3.86	5.93 *s*
15	6.35 *s*	6.46 *d* *J* = 1.5	6.70 *d* *J* = 8.5	6.47 *dd* *J* = 6.9, 1.7	3.83	3.83	5.95 *d*, *J* = 1.3 5.94 *d*, *J* = 1.3

*Chemical shifts in ppm (δ) relative to internal standard TMS in $CDCl_3$ solution, except as indicated. Coupling constants (*J*) in Hz.

†$CDCl_3$–D_2O.



Scheme 3. Acid-catalysed cyclization of podorhizol.

P. peltatum, the only reported dibenzylbutyrolactone structure from these plants is that of podorhizol glucoside [17]. This was confirmed in the present studies by β-glucosidase hydrolysis of the glycoside fraction from these plants, and the subsequent isolation of podorhizol. Small amounts (0.4 mg from 100 g root) of podorhizol itself were isolated from the aglycone fraction of *P. hexandrum*.

In view of the biogenetic relationships postulated, and now demonstrated by the incorporation of yatein into podophyllotoxin, it became desirable to establish if other dibenzylbutyrolactone lignans are also present in *Podophyllum*. Accordingly, extracts of *P. hexandrum* root were screened for the presence of the five lignans synthesized for the above feeding experiments. Material chromatographically similar to the authentic standards was obtained only in the cases of podorhizol and yatein/anhydropodorhizol. The latter two compounds were not resolved by TLC in any solvent system tried. However, NMR and UV analysis of this band showed it was entirely anhydropodorhizol (**11**), yield approximately 0.4 mg from 100 g root material, with no detectable amounts of yatein. Anhydropodorhizol has been isolated previously from the plant *Anthriscus sylvestris* [12], although it was first reported as a degradation product from podorhizol glucoside [17].

Biosynthetic relationships

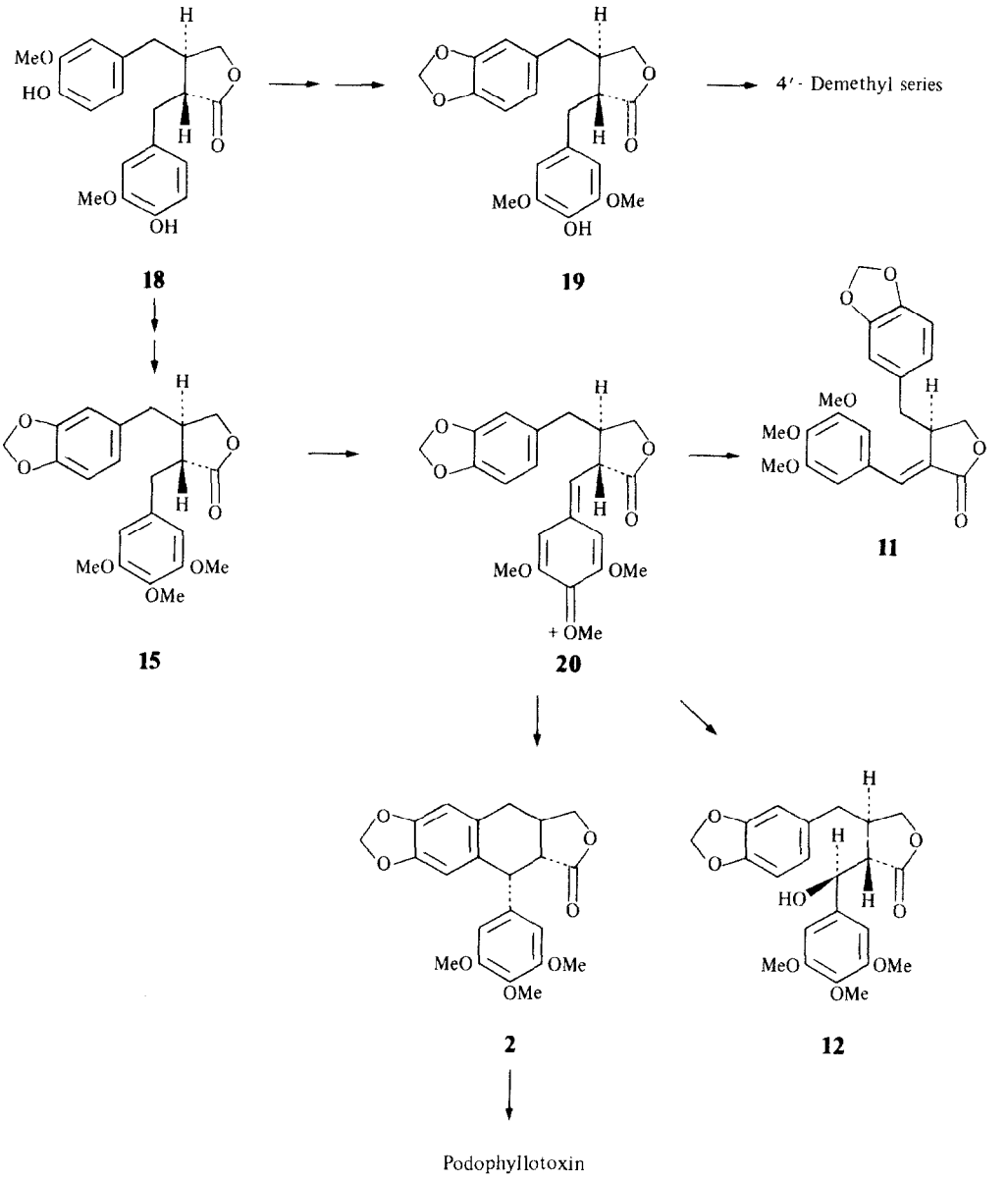
The incorporation of yatein, but neither of the functionalized compounds podorhizol or anhydropodorhizol, into podophyllotoxin suggests an oxidative cyclization proceeding through an intermediate other than these two compounds. A reasonable sequence could be oxidation to the quinone methide (**20**), which could than cyclize to desoxypodophyllotoxin (**2**), retaining the 4′-*O*-methyl label (Scheme 4). There is ample chemical evidence to support this type of cyclization [28–30], although chemical cyclization again produces the thermodynamically more stable unnatural configuration in the product [29]. Furthermore, a key central role for this quinone methide

Table 2. Incorporations* of labelled lignans into podophyllotoxin in *Podophyllum hexandrum*

Lignan fed†	Experiment (i)			Experiment (ii)		
	mg	% Incorp	Dilution	mg	% Incorp	Dilution
Podorhizol (**12**)	17.1	0.004	3.88×10^5	5.5	0.003	1.93×10^6
Epipodorhizol (**13**)	30.1	0.003	9.60×10^5	8.7	0.003	2.59×10^5
Anhydropodorhizol (**11**)	27.2	0.001	2.20×10^6	8.8	0.004	3.89×10^5
cis-Dihydroanhydropodorhizol (**14**)	31.7	0.070	3.77×10^4	7.8	0.076	8.28×10^3
Yatein (**15**)	49.4	0.11	3.57×10^4	7.2	0.19	6.59×10^3
Desoxypodophyllotoxin (**2**)	59.9	0.53	9.17×10^3	8.3	0.32	6.18×10^3

*Incorporations for lignans **11–15** are not corrected for utilization of one enantiomer from racemic mixtures fed.

†Lignans **11–15**: [4′-*O*-methyl-^{14}C]; lignan **2**: [4′-*O*-methyl-^{3}H].



Scheme 4. Proposed biosynthetic pathway to *Podophyllum* lignans.

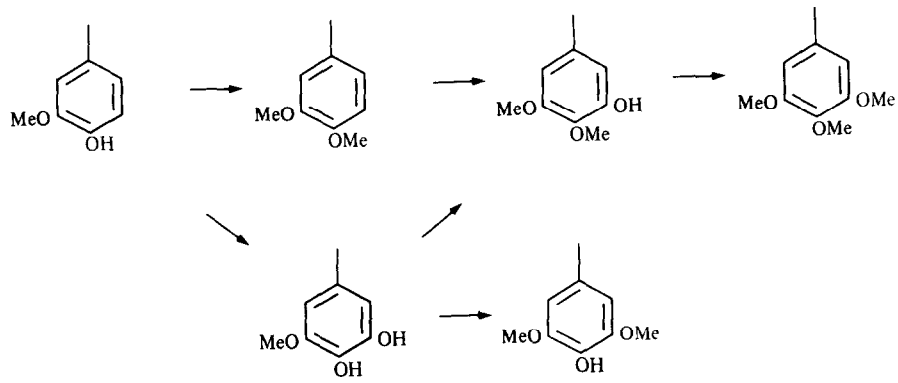
would explain the presence of podorhizol (**12**) and anhydropodorhizol (**11**) in *P. hexandrum*, and yet their non-incorporation into podophyllotoxin. Thus, these two compounds could be derived from **20** by the addition of water or loss of a proton respectively (Scheme 4). Our inability to detect the presence of yatein (**15**) in *P. hexandrum* suggests the pool size of this compound must be very small indeed.

The sequence of events leading to yatein has yet to be established. A logical precursor might be the widely-distributed matairesinol (**18**). This lignan would arise *via* coupling of two phenylpropane units having the ferulic substitution pattern, and subsequent elaboration could lead to yatein. Alternatively, elaboration to demethylyatein (**19**), the probable precursor of the 4′-demethyl series of aryltetralin lignans, could mean that matairesinol may be the branchpoint to these two groups of *Podophyllum* lignans. Further experimental evidence is required to test these hypotheses.

Feeding experiment with [S-*methyl*-^{14}C]*methionine*

Although no experiments have been reported, it is virtually certain that the methylenedioxy carbon and the three methoxyl carbons of podophyllotoxin are derived from the *S*-methyl of methionine. Indirect incorporation of the methylenedioxy carbon of 3,4-methylenedioxycinnamic acid supports this assumption [4]. Since the trimethoxy series and dimethoxyhydroxy series of aryltetralin lignans in *Podophyllum* appear to be derived from a common intermediate (matairesinol?), the pattern of labelling in the two series resultant from the feeding of [*S*-methyl-^{14}C]methionine might reflect how these different substitution patterns are built up. Accordingly, [*S*-

methyl-^{14}C]methionine was fed *via* the roots to a *P. hexandrum* plant, and the two major lignans podophyllotoxin aad 4'-demethylpodophyllotoxin (**3**) were isolated, diluted as appropriate, and purified rigorously. The incorporations were 0.48% and 0.11% respectively. The two compounds were partially degraded by sequences described earlier [4, 6]. Both compounds upon nitric acid oxidation yielded the quinone **21**, which was then reduced to the more easily purified quinol **22**, thus removing methyl groups at positions 3' and 4'. In addition, podophyllotoxin was degraded using BCl_3 giving the quinol **23** *via* loss of the methylenedioxy carbon. Relative specific activities of these degradation products are given in Table 3, and the corresponding relative activities of the methyl/methylene groups are then calculated as in Table 4. This assumes no labelling is present in the C_{18} skeleton of the lignans, in accord with earlier studies [4]. However, we may also assume that since two ferulic C_6C_3 units couple initially, the labelling in the methylene group will be of the same magnitude as one of the 3'/5'-methyls (arbitrarily taken as 3' here), and a revised pattern of labelling can be calculated as in Table 4. This shows significant differences exist in the labelling patterns for the pendent rings of podophyllotoxin and 4'-demethylpodophyllotoxin, and indicates that the 3',4',5'-trimethoxy pattern does not arise *via* the 3',5'-dimethoxy-4'-hydroxy pattern. If one considers alternative sequences for building up these substitution patterns (Scheme 5), one must conclude that the branch-point compound to the two series of lignans will have either 4'-hydroxy-3'-methoxy



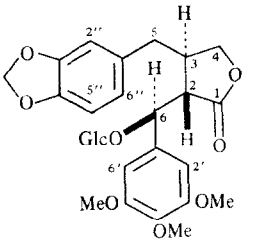
Scheme 5. Possible sequences for elaborating substitution patterns of the pendent aryl rings in *Podophyllum* lignans.

Table 3. Degradation of podophyllotoxin and 4'-demethylpodophyllotoxin derived from [*S*-methyl-^{14}C]methionine

	Podophyllotoxin		4'-Demethylpodophyllotoxin	
Compound	Specific activity (dpm/mM)	Relative specific activity	Specific activity (dpm/mM)	Relative specific activity
Podophyllotoxin (**4**)	5.15×10^5	1.00		
4'-Demethylpodophyllotoxin (**2**)			2.15×10^5	1.00
3',4'-Didemethylpodophyllotoxin (**22**)	2.32×10^5	0.45	1.32×10^5	0.61
6,7-Demethylenepodophyllotoxin (**23**)	3.78×10^5	0.73		

Table 4. Distribution of label from [*S*-methyl-^{14}C]methionine in podophyllotoxin and 4'-demethylpodophyllotoxin

	Podophyllotoxin		4'-Demethylpodophyllotoxin	
Group	Relative specific activity	Relative specific activity assuming $OCH_2O \equiv$ 3'-OMe	Relative specific activity	Relative specific activity assuming $OCH_2O \equiv$ 3'-OMe
OCH_2O	0.27	0.27	0.22	0.22
3'-OMe	0.18	0.27	0.39	0.22
4'-OMe	0.37	0.37	—	—
5'-OMe	0.18	0.09	0.39	0.56



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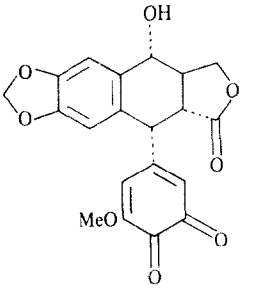

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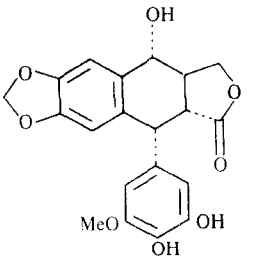

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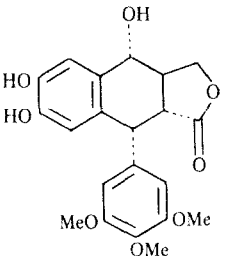

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substitution in the aromatic ring that ultimately becomes the pendent aryl. Thus, matairesinol (**18**) still figures as a likely branch-point compound.

EXPERIMENTAL

General. Techniques were as previously described [4].

Plant material, feeding techniques and isolation of lignans. The procedures utilized were as described in the earlier papers [4, 6].

Radiochemicals. L-[*S*-methyl-^{14}C]Methionine (60 mCi/mM) and [^{14}C]methyl iodide (58.5 mCi/mM) were purchased (Amersham). [4′-*O*-methyl-^{3}H]Desoxypodophyllotoxin (0.80 mCi/mM) was available from earlier studies [5].

[4-*O*-*methyl*-^{14}C]-3,4,5-*Trimethoxybenzaldehyde.* Syringaldehyde (390 mg) in dry DMF (8 ml) was stirred for 2 hr at 60° with dry K_2CO_3 (1 g), [^{14}C]methyl iodide (1 mCi, 58.5 mCi/mM) and MeI (120 μl, 270 mg). After this time, further MeI (20 μl, 45 mg) was added and the reaction was continued for a further 1 hr. The reaction mixture was then diluted with H_2O (30 ml) and extracted with $CHCl_3$ (3 × 50 ml). The combined extracts were washed with H_2O (3 × 100 ml), dried over $MgSO_4$ and evapd to dryness. The product was purified by TLC [Me_2CO–petrol (bp 60–80°), 1:1] to give [4-*O*-methyl-^{14}C]-3,4,5-trimethoxybenzaldehyde (390 mg).

(±)-[4′-O-*methyl*-^{14}C]*Podorhizol* (**12**), (±)-[4′-O-*methyl*-^{14}C]*epipodorhizol* (**13**) *and* (±)-[4′-O-*methyl*-^{14}C]*anhydropodorhizol* (**11**). A soln of [4-*O*-methyl-^{14}C]-3,4,5-trimethoxybenzaldehyde (390 mg) and freshly distilled 3-(3,4-methylenedioxybenzyl-γ-butyrolactone [31] (880 mg) in dry Et_2O (40 ml) was added dropwise to a cooled (−10°, ice-salt), stirred suspension of freshly-prepared NaOMe (116 mg) in dry Et_2O (40 ml). The reaction was allowed to proceed in a cold room (3°) for 24 hr, during which the reaction mixture gradually warmed to this temp. The reaction mixture was then quenched with MeOH–HOAc (9:1, 30 ml) and evapd to dryness. Purification by TLC ($CHCl_3$–MeOH, 25:1) gave (±)-[4′-*O*-methyl-^{14}C]podorhizol (80 mg), (±)-[4′-*O*-methyl-^{14}C]epipodorhizol (60 mg) and (±)-[4′-*O*-methyl-^{14}C]anhydropodorhizol (180 mg). Portions were further purified to constant specific activities as follows.

(±)-*Podorhizol* (**12**). TLC [$CHCl_3$–MeOH, 25:1; Me_2CO–petrol (60–80°): 1:1, Me_2CO–hexane, 1:2; Me_2CO–hexane, 2:1]. Specific activity 5.90×10^8 dpm/mM. Unlabelled material, recrystallized from Et_2O, had mp 124–126°, lit. [19] 126–128°: UV λ_{max}^{EtOH} nm: 284–286 (log ε 3.59); IR ν_{max}^{KBr} cm^{-1}: 3510, 1770, 1600, 1500, 1220; EIMS (probe) 70 eV, *m/z* (rel. int.): 416 [M]$^+$ (8%), 220 (47), 197 (43), 196 (77), 181 (25), 135 (100); ^{1}H NMR: see Table 1.

(±)-*Epipodorhizol* (**13**). TLC [$CHCl_3$–MeOH, 25:1; Me_2CO–petrol (60–80°), 1:1; Me_2CO–hexane, 2:1]. Specific activity 6.27×10^8 dpm/mM. Unlabelled material, recrystallized from EtOH had mp 132–134°, lit. [19] 133–134°: UV λ_{max}^{EtOH} nm: 284–286 (log ε 3.63); IR ν_{max}^{KBr} cm^{-1}: 3500, 1770, 1600, 1220; EIMS (probe) 70 eV, *m/z* (rel. int.): 416 [M]$^+$ (12%), 220 (43), 197 (52), 196 (76), 181 (29), 135 (100); ^{1}H NMR: see Table 1.

(±)*Anhydropodorhizol* (**11**). Separation from unreacted butyrolactone and further purification by TLC [Me_2CO–hexane, 1:3; hexane–EtOAc, 3:2; Me_2CO–petrol (60–80°), 1:1]. Specific activity 6.19×10^8 dpm/mM. Unlabelled material recrystallized from MeOH had mp 113–115°: UV λ_{max}^{EtOH} nm: 313 (log ε 4.26), 298 sh (4.24), 234 (4.30); IR ν_{max}^{KBr} cm^{-1}: 1750, 1650, 1580–1500; EIMS (probe) 70 eV, *m/z* (rel. int): 398 [M]$^+$ (21%), 264 (15), 263 (100), 135 (48); ^{1}H NMR: see Table 1.

(±)-[4′-O-*methyl*-^{14}C]-cis-*Dihydroanhydropodorhizol* (**14**). (±)-[4′-*O*-methyl-^{14}C]Anhydropodorhizol (25 mg) was dissolved in MeOH (10 ml) and hydrogenated over Pd/C catalyst (10%, 20 mg) for 3 hr at room temp. and 3 psi pressure (Petric 7300

gas-controlled hydrogenator). The catalyst was filtered off and the filtrate was evapd to dryness. The product was purified by TLC [Me_2CO–petrol (60–80°), 1:1] to give (±)-[4′-*O*-methyl-^{14}C]-*cis*-dihydroanhydropodorhizol (**14**, 20 mg). A portion of this material was purified to constant sp. act. (6.79×10^8 dpm/mM) by further TLC (Me_2CO–hexane, 1:2; hexane–EtOAc, 3:2). Unlabelled material, recrystallized from MeOH had mp 124–126°; UV λ_{max}^{EtOH} nm: 284–286 (log ε 3.65); IR ν_{max}^{KBr} cm^{-1}: 1770, 1595, 1500, 1250; EIMS (probe) 70 eV, *m/z* (rel. int.): 400 $[M]^+$ (30%), 220 (22), 181 (37), 135 (100); ^{1}H NMR: see Table 1.

(±)-[4′-O-*methyl*-^{14}C]*Yatein* (**15**). (±)-[4′-*O*-methyl-^{14}C]-*cis*-Dihydroanhydropodorhizol (10 mg) was dissolved in methanolic KOH (3%, 2 ml) and left to stand at room temp. for 72 hr. The mixture was then treated with MeOH–HOAc (9:1, 20 ml) and the soln was evaporated to dryness at 40°. The product was purified by TLC [Me_2CO–petrol (60–80°), 1:1] to give (±)-[4′-*O*-methyl-^{14}C]yatein (8 mg). A portion was purified to constant specific activity (6.00×10^8 dpm/mM) by further TLC (Me_2CO–hexane, 1:2; hexane–EtOAc, 3:2). Unlabelled material (oil) had UV λ_{max}^{EtOH} nm: 284–286 (log ε 3.65); IR ν_{max}^{KBr} cm^{-1}: 1770, 1600, 1510–1470, 1250; EIMS (probe) 70 eV, *m/z* (rel. int.): 400 $[M]^+$ (98%), 181 (100), 135 (67); ^{1}H NMR, see Table 1.

Cyclization of podorhizol. (±)-Podorhizol (20 mg) in CF_3CO_2H (2 ml) was kept at room temp. for 3 hr, then diluted with H_2O (10 ml) and extracted with EtOAc (3 × 50 ml). The combined extracts were washed with dilute $NaHCO_3$ (5%, 50 ml), H_2O (2 × 50 ml) and evapd to dryness. The products were then separated by TLC ($CHCl_3$–MeOH, 25:1) to give unreacted podorhizol (3 mg) and (±)-isodesoxypodophyllotoxin (**17**, 14 mg), recrystallized from EtOH, mp 255–257°, lit. [19] 256–258°: ^{1}H NMR (250 MHz, Me_2CO-d_6, TMS): δ6.67 (1H, *s*, H-8), 6.58 (2H, *s*, H-2′, H-6′), 6.29 (1H, *s*, H-5), 5.90 (2H, *s*, OCH_2O), 4.50 (1H, approx. *t*, *J* = 8 Hz, H-3aα), 4.05 (1H, *d*, *J* = 10 Hz, H-1), 4.03 (1H, *dd*, *J* = 10, 8 Hz, H-3aβ) 3.77 (6H, *s*, 3′,5′-OMe), 3.71 (3H, *s*, 4′-OMe), 3.0–2.5 (4H, *m*, H-2, H-3, H-4α, H-4β).

Degradation of podophyllotoxin to 6,7-demethylenepodophyllotoxin (**23**). The procedure described earlier [4] was employed.

Degradation of podophyllotoxin to 3′,4′-didemethylpodophyllotoxin (**22**). A soln of podophyllotoxin (100 mg) in glacial HOAc (AR, 10 ml) was added dropwise to a stirred mixture of HNO_3 (AR, 3 ml) and HOAc (AR, 10 ml) in an ice-salt bath at −2°. The reaction was allowed to proceed for 15 min and was then diluted with H_2O (50 ml) and extracted with $CHCl_3$ (4 × 50 ml). The combined extracts were washed with dilute aq. $NaHCO_3$, then H_2O (2 × 100 ml). The $CHCl_3$ extract was dried over $MgSO_4$ and evapd to dryness. Without further purification, the red quinone 3′,4′-dioxo-3′,4′-dihydro-3′,4′-didemethoxypodophyllotoxin (**21**) was dissolved in EtOH (10 ml) and sodium dithionite soln (5%) was added dropwise until the red colour had disappeared completely. Inorganic salts were filtered off, and the filtrate was concd under red. pres., diluted with H_2O (50 ml) and extracted with EtOAc (3 × 80 ml). The combined extracts were washed with H_2O (2 × 100 ml), dried over $MgSO_4$ and evapd to dryness. The product was purified by TLC [Me_2CO–petrol (60–80°), 1:1], crystallized from MeOH and recrystallized twice from aq. MeOH to give 3′,4′-didemethylpodophyllotoxin (**22**, 4 mg), mp 120–122°: ^{1}H NMR (60 MHz, Me_2CO-d_6, TMS): δ7.3–7.05 (4H, *m*, 3 × OH and H-5), 6.5 (1H, *d*, *J* = 1.5 Hz, H-6′), 6.35 (1H, *s*, H-8), 6.05 (1H, *d*, *J* = 1.5 Hz, H-2′), 5.90 (2H, *s*, OCH_2O), 4.9–4.1 (4H, *m*, H-1, H-3aα, H-3aβ, H-4), 3.75 (3H, *s*, 5′-OMe), 2.9–2.4 (2H, *m*, H-2, H-3).

Degradation of 4′-demethylpodophyllotoxin to 3′,4′-didemethylpodophyllotoxin (**22**). 4′-Demethylpodophyllotoxin (70 mg) was oxidized to quinone **21** and then reduced to quinol **22** (4 mg) in the same manner as above.

Isolation of podorhizol (**12**) *and anhydropodorhizol* (**11**) *from* Podophyllum hexandrum. Powdered *P. hexandrum* root (100 g) was extracted with hot EtOH (4 × 150 ml). After evapn of the solvent, H_2O (200 ml) was added and the lignans were extracted with EtOAc (3 × 200 ml). The residue remaining after evapn of EtOAc was dissolved in the minimum amount of warm EtOH (about 30 ml), and C_6H_6 (*ca* 30 ml) was added. After standing at 3° for 24 hr, the ppt (5 g), consisting mainly of a mixture of podophyllotoxin and 4′-demethylpodophyllotoxin, was filtered off. The thick resinous material obtained after evapn of the mother liquors was mixed with silica gel (100 g) until a powdered mass formed. This was packed into a column and eluted with $CHCl_3$–MeOH (25:1). Fractions (*ca* 50 ml) were analysed by TLC ($CHCl_3$–MeOH, 25:1) for lignan content. Fractions containing material chromatographically similar to podorhizol, epipodorhizol and anhydropodorhizol/*cis*-dihydroanhydropodorhizol/yatein markers were separately bulked and purified further by TLC.

A podorhizol band was purified via TLC ($CHCl_3$–*iso*-PrOH, 10:1; hexane–Me_2CO, 1:1). Repeated chromatography in the latter solvent system removed last traces of β-peltatin and gave a fraction chromatographically and spectrally (UV, ^{1}H NMR) identical to synthetic podorhizol. No epipodorhizol was detected. A band corresponding to the other three lignans (not resolved by TLC in the solvents used) was purified by TLC using solvents Me_2CO–petrol (bp 60–80°) (1:1); $CHCl_3$–petrol (60–80°) (10:1). UV and ^{1}H NMR spectra of this band showed it contained only anhydropodorhizol, spectrally identical to synthetic material. Recorded yields of podorhizol and anhydropodorhizol from 100 g root were 0.38 mg and 0.36 mg respectively.

Acknowledgements—We thank Dr. D. E. Jackson (Nottingham) for advice and assistance, Dr. J.-P. Robin (Le Mans, France) for synthetic data, and the Iraqi Government for financial support (to W.M.K.).

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