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Biosynthesis of yatein in *Anthriscus sylvestris*†

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Little is known about the biosynthesis of yatein, in spite of its importance as a typical heartwood lignan and a key biosynthetic intermediate of the antitumor lignan podophyllotoxin. The present study, based on individual administration of [^{13}C]phenylalanine and deuterium labelled lignans and simultaneous administration of two distinct lignans labelled with deuterium atoms to *Anthriscus sylvestris*, established the two independent branch pathways from matairesinol, one to afford yatein *via* thujaplicatin, 5-methylthujaplicatin, and 4,5-dimethylthujaplicatin and the other to bursehernin *via* pluviatolide. The latter pathway did not lead to yatein, eliminating the presence of a metabolic grid from matairesinol to yatein.

Introduction

An antitumor lignan, podophyllotoxin, has been successfully used as the starting material for semi-synthetic anti-cancer drugs, etoposide and teniposide.[1–6] Podophyllotoxin is isolated from herbaceous perennial *Podophyllum* plants.[5,7,8] However, due to the limited supply of the plants, much attention has been focused on the availability and biosynthesis of the lignan.[1,3,5,8]

In the 1980s, Dewick and co-workers reported a series of detailed feeding experiments with radio-labelled precursors and revealed the pathways from yatein (**11**) to podophyllotoxin and its congeners *via* deoxypodophyllotoxin (=desoxypodophyllotoxin, anthricin).[9–12] Next, they showed that matairesinol (**2**) is metabolized to podophyllotoxin and proposed that (**11**) was the possible intermediate in the conversion of (**2**) to podophyllotoxin.[13] During the last decade, significant progress has been made in the biosynthetic studies of lignans, and the biosynthesis of matairesinol (**2**) from coniferyl alcohol has been established.[1,14] Recently, several enzymes involved in the transformation of deoxypodophyllotoxin to podophyllotoxin, β-peltatin, and 5-methoxypodophyllotoxin have been isolated.[15–17] These data allow us to draw the outline of biosynthetic pathways for podophyllotoxin derivatives as in Fig. 1. Briefly, the dimerization of coniferyl alcohol, followed by three metabolic steps gives rise to matairesinol (**2**). Then, matairesinol (**2**) is transformed to yatein (**11**) which is in turn converted to podophyllotoxin congeners. However, the pathway from matairesinol (**2**) to yatein (**11**) remains unknown.

Besides the role for the precursor of podophyllotoxin, yatein (**11**) is also of importance as a heartwood lignan.[18] Heartwood is the colored region of the inner part of tree trunks, and its formation is a metabolic event specific to woody plants, as it does not operate in herbaceous plants. Recently, metabolic engineering of trees has been developed significantly. For example, a transgenic aspen (*Populus tremuloides*) with less lignin and higher cellulose contents has been successfully produced by Chiang and co-workers.[19] These developments were achieved with the aid of the knowledge of herbaceous plant molecular biology. Now, it is necessary to exploit knowledge of molecular mechanisms for metabolic events specific to woody plants in order to accelerate metabolic engineering of trees. Although nothing is known about the molecular mechanisms, the facts that biosynthesis of lignans, norlignans and related compounds are involved in heartwood formation implies that the molecular mechanisms of their biosynthesis can be a clue to help us elucidate heartwood formation mechanisms.

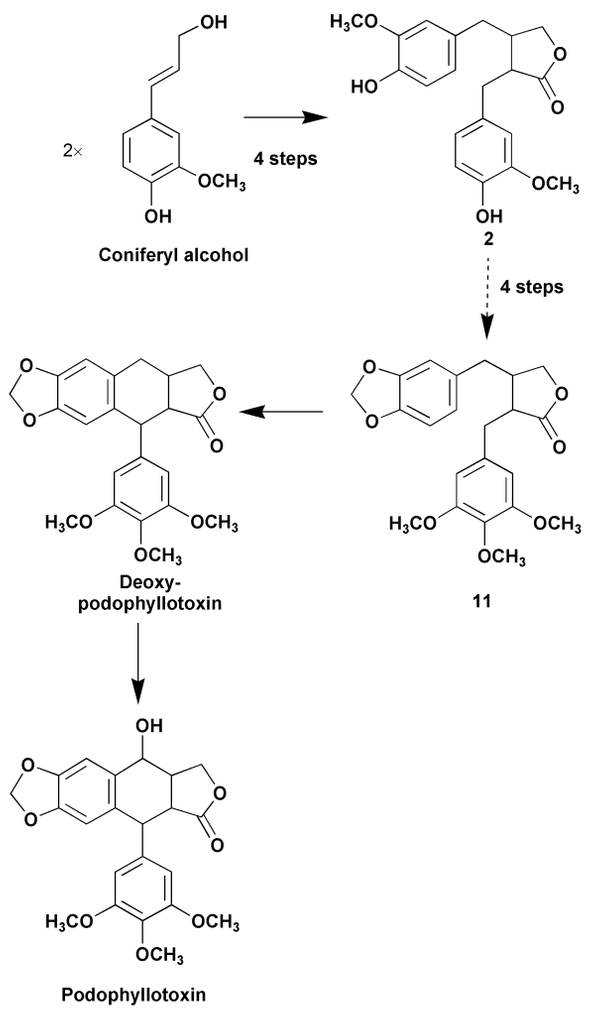

Fig. 1 A proposed biosynthetic pathway from coniferyl alcohol to podophyllotoxin. **2**: matairesinol, **11**: yatein. Solid arrow: established in previous reports. Broken arrow: established in this report.

Anthriscus sylvestris[20–26] is a good source of yatein (**11**) and deoxypodophyllotoxin, both of which are effective precursors of podophyllotoxin.[9–11] In addition, angeloyl podophyllotoxin has been isolated from this species.[27] The high content of yatein (**11**) and deoxypodophyllotoxin as well as the good growth behaviour stimulated us to employ *A. sylvestris* as a plant

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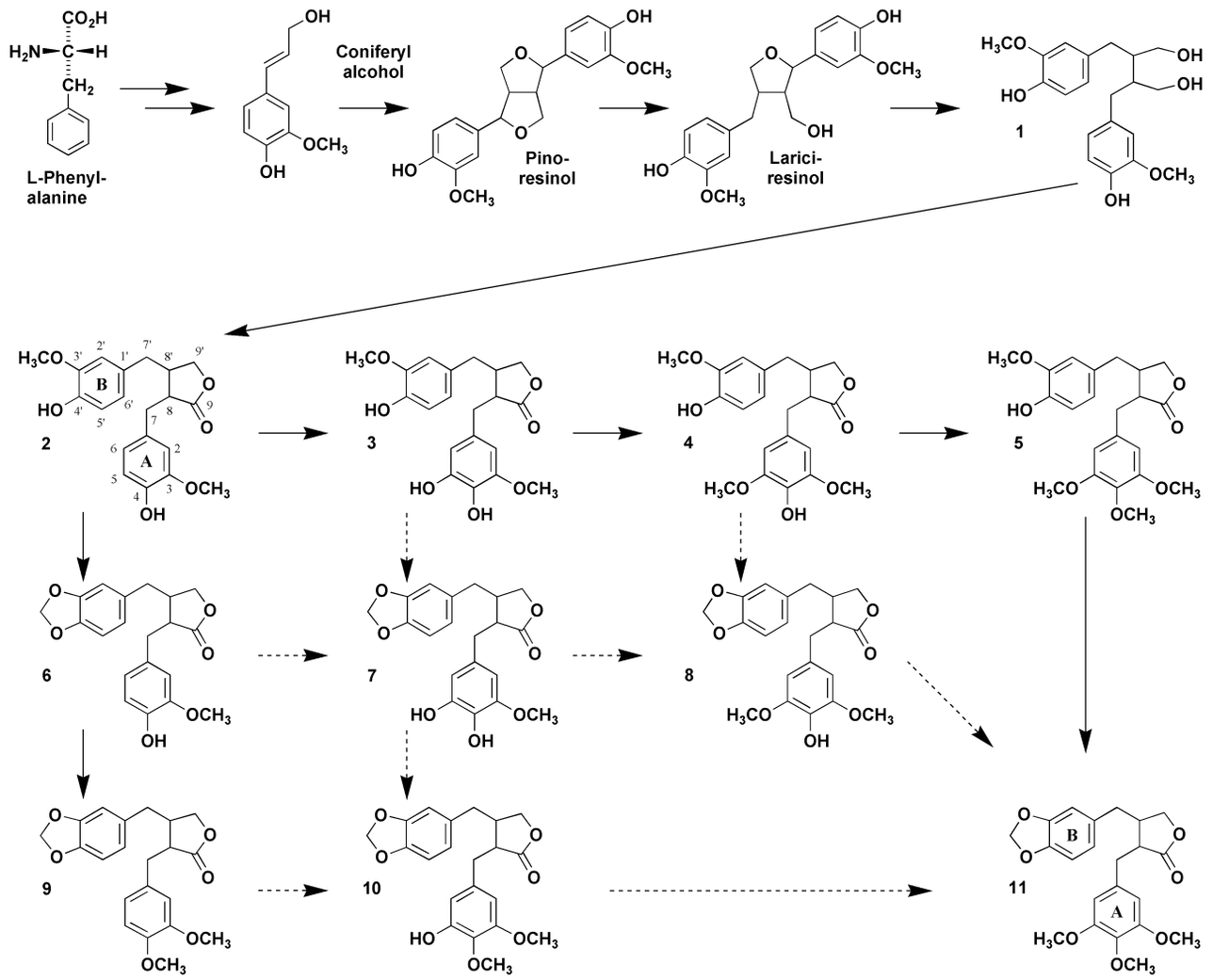


Fig. 2 A proposed biosynthetic pathway of yatein in *Anthriscus sylvestris*. **1**: secoisolariciresinol, **2**: matairesinol, **3**: thujaplicatin, **4**: 5-methylthujaplicatin, **5**: 4,5-dimethylthujaplicatin, **6**: pluviatolide, **7**: 5-hydroxypluviatolide, **8**: 4-demethylyatein, **9**: bursehernin, **10**: 5-demethylyatein, **11**: yatein. Solid arrow: proposed biosynthetic pathway in *Anthriscus sylvestris*. Broken arrow: physiologically insignificant pathway.

material for the study of yatein biosynthesis, and we have recently reported the survey of lignans in this species.[26]

The conversion of matairesinol (**2**) to yatein (**11**) involves four steps: 5-hydroxylation, dual methylation at C4OH and C5OH, and methylenedioxy bridge formation at C3′ and C4′ (Fig. 2). Many possible orders of their occurrence can be envisaged suggesting that a metabolic grid might be present in the transformation. The aim of the present study is to identify the physiologically important biosynthetic pathway from matairesinol (**2**) to yatein (**11**).

Herein, we report the administration of [^{13}C]phenylalanine and a series of deuterium labelled lignans to *A. sylvestris*, and discuss the biosynthetic pathway from matairesinol (**2**) to yatein (**11**) in this species.

Results

Lignans in *Anthriscus sylvestris*

Recently, we have surveyed lignans occurring in *Anthriscus sylvestris*:[26] GC-MS analysis of the MeOH extracts of the plant showed the presence of lignans, lariciresinol, secoisolariciresinol (**1**), matairesinol (**2**), pluviatolide (**6**), yatein (**11**), hinokinin, nemerosin, and deoxypodophyllotoxin. Additionally, the existence of small amounts of bursehernin (**9**) was suggested by mass chromatography.[26] Further GC-MS analysis in the present study indicated the presence of 5-methylthujaplicatin (**4**) and 4,5-dimethylthujaplicatin (**5**) in the MeOH extracts, which were identified by comparing their mass spectra [5-methylthujaplicatin (**4**) (TMS ether): *m*/*z* (EI) 532 (M^+, 100%), 239 (84.1), 209 (26.0); 4,5-dimethylthujaplicatin (**5**) (TMS ether): *m*/*z* (EI) 474 (M^+, 100%), 209 (71.0), 181 (56.0)] and retention times on GC with those of chemically synthesized authentic samples. In addition, yatein (**11**) and deoxypodophyllotoxin were isolated from the MeOH extracts and identified by comparison of the ^{1}H-NMR and mass spectrum (data not shown) with those of the literature data.[28]

Feeding experiments

Small scale feeding experiments. Nine lignans labelled with deuterium atoms at the methoxy groups, (±)-[3-OC^{2}H$_3$]matairesinols (**2**-d_3), (±)-[3-OC^{2}H$_3$]thujaplicatins (**3**-d_3), (±)-[3,5-(OC^{2}H$_3$)$_2$]-5-methylthujaplicatins (**4**-d_6), (±)-[3,5-(OC^{2}H$_3$)$_2$]-4,5-dimethylthujaplicatins (**5**-d_6), (±)-[3-OC^{2}H$_3$]pluviatolides (**6**-d_3), (±)-[3-OC^{2}H$_3$]-5-hydroxypluviatolides (**7**-d_3), (±)-[3,5-(OC^{2}H$_3$)$_2$]-4-demethylyateins (**8**-d_6), (±)-[3,4-(OC^{2}H$_3$)$_2$]-bursehernins (**9**-d_6), and (±)-[3,4-(OC^{2}H$_3$)$_2$]-5-demethylyateins (**10**-d_6), were fed individually to young shoots of *A. sylvestris* (0.4 mg of each lignan to one shoot). Results of GC-MS analysis of the MeOH extracts obtained following the administration are summarized in Tables 1–3. Table 1 shows the molecular ion and important daughter ion regions of unlabelled authentic yatein (**11**) and of this lignan obtained following the administration: unlabelled yatein (**11**) gives a molecular ion at *m*/*z* 400 and principal daughter ions at *m*/*z* 181 and 135 which are derived from benzylic cleavage of A- and B-rings (Fig. 2), respectively. When the lignans labelled

Table 1 Mass tables of molecular-ion and principal daughter-ion regions of lignan (**11**) obtained following administration of ^{13}C- or deuterium-labelled precursors into *A. sylvestris*.

Yatein (**11**)

	Relative intensity (%)													
		Administrated labelled compounds												
m/z	Unlabelled	[$^{13}C_6$]Phe	**1**-d_3	**2**-d_3	**3**-d_3	**4**-d_6	**5**-d_6	**6**-d_3	**7**-d_3	**8**-d_6	**9**-d_6	**10**-d_6	**1**-d_5 + **3**-d_3	**1**-d_5 + **6**-d_3
135	30.6[b]	37.2[b]	43.4[b]	38.2[b]	42.6[b]	47.0[b]	39.9[b]	33.5[b]	46.3[b]	42.9[b]	30.3[b]	36.6[b]	41.8[b]	41.1[b]
136	13.5	12.7	16.3	14.1	13.1	13.3	13.6	12.4	16.0	12.7	10.6	13.9	12.9	13.1
137	4.7	5.2	6.7	3.5	4.7	5.2	3.3	3.3	8.7	5.4	4.1	6.2	6.6	5.4
138	3.1	2.0	2.7	1.6	2.0	2.5	2.6	1.4	2.8	2.1	2.1	3.6	1.9	1.5
139	1.3	1.6	2.1	0.9	2.5	1.2	1.0	0.7	3.8	1.9	3.4	1.0	2.2	1.6
140	1.0	0.7	0.4	0.0	1.0	0.7	0.0	0.3	1.5	0.5	0.0	0.0	1.0	0.5
141	0.7	**4.5**[b]	0.4	0.0	1.1	1.3	0.0	0.6	1.4	0.2	0.0	0.0	1.5	0.5
142	1.1	1.4	0.3	0.2	0.1	1.0	0.3	0.0	0.1	0.2	2.4	0.0	0.4	0.3
143	0.4	1.0	0.9	0.7	0.9	0.4	0.9	0.4	0.5	0.6	1.2	0.0	0.8	0.8
181	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]
182	48.9	58.5	56.8	51.2	55.3	55.6	53.2	61.9	61.3	62.7	54.9	60.2	53.3	54.4
183	5.6	7.9	7.4	7.0	6.9	6.3	6.7	6.7	8.3	8.2	8.3	10.7	7.0	7.6
184	1.2	0.8	**10.3**[a]	0.0	**11.7**[a]	1.0	1.5	1.4	**19.3**[a]	2.3	1.8	1.2	**11.8**[a]	2.0
185	2.2	1.3	5.0	1.5	6.7	1.0	0.3	0.1	11.1	0.6	0.0	0.0	7.4	1.6
186	0.8	2.1	1.0	0.9	1.7	1.0	0.9	0.4	2.1	0.1	0.0	0.0	**8.2**[a]	**3.5**[a]
187	0.9	**17.7**[a]	0.9	0.9	0.5	**16.9**[a]	**4.2**[a]	0.1	1.3	**12.0**[a]	0.0	**12.3**[a]	3.7	2.3
188	1.8	7.9	1.1	1.0	2.1	9.9	2.4	1.9	2.0	8.6	0.0	8.8	1.2	0.6
189	2.2	2.3	2.9	2.1	1.8	2.5	2.7	2.7	3.1	2.8	2.9	4.6	1.8	3.2
400	70.1[c]	78.0[c]	74.2[c]	68.9[c]	71.7[c]	73.7[c]	67.0[c]	75.2[c]	93.7[c]	92.1[c]	76.6[c]	88.9[c]	75.2[c]	76.4[c]
401	19.8	20.2	21.7	19.1	19.8	21.4	20.6	18.6	22.2	23.0	16.6	21.9	21.9	21.2
402	3.7	4.8	6.7	5.5	4.2	4.6	3.8	5.1	4.5	4.6	4.3	6.3	4.6	5.3
403	0.0	1.4	**9.0**[c]	2.5	**7.8**[c]	1.9	1.8	1.6	**16.1**[c]	2.1	0.0	1.3	**10.3**[c]	1.6
404	0.0	0.0	2.4	1.3	3.2	1.6	0.8	0.6	4.6	1.7	0.0	0.0	4.4	2.0
405	0.0	1.1	0.7	0.4	0.6	1.6	1.0	0.5	1.8	0.0	0.0	0.0	**7.3**[c]	**3.4**[c]
406	0.0	**3.9**[c]	0.7	0.0	0.0	**12.9**[c]	**2.5**[c]	0.5	0.0	**13.3**[c]	0.0	**9.3**[c]	1.8	2.3
407	0.0	1.5	0.3	0.2	0.7	4.5	1.6	0.0	0.0	4.7	0.0	3.0	0.6	0.8
408	0.0	0.8	0.0	0.0	0.0	0.9	0.4	0.4	0.0	1.5	0.0	0.0	1.3	0.0
412	0.0	**13.0**[c]	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.4	0.0
413	0.0	1.6	0.0	0.0	0.0	0.4	0.1	0.0	0.0	0.0	0.0	0.0	0.5	0.0
414	0.0	0.3	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[$^{13}C_6$]Phe: [*ring*-$^{13}C_6$]phenylalanine, **1**-d_3: (±)-[3-OC^{2}H$_3$]secoisolariciresinols, **2**-d_3: (±)-[3-OC^{2}H$_3$]matairesinols, **3**-d_3: (±)-[3-OC^{2}H$_3$]thujaplicatins, **4**-d_6: (±)-[3,5-(OC^{2}H$_3$)$_2$]-5-methylthujaplicatins, **5**-d_6: (±)-[3,5-(OC^{2}H$_3$)$_2$]-4,5-dimethylthujaplicatins, **6**-d_3: (±)-[3-OC^{2}H$_3$]pluviatolides, **7**-d_3: (±)-[3-OC^{2}H$_3$]-5-hydroxypluviatolides, **8**-d_6: (±)-[3,5-(OC^{2}H$_3$)$_2$]-4-demethylyateins, **9**-d_6: (±)-[3,4-(OC^{2}H$_3$)$_2$]bursehemins, **10**-d_6: (±)-[3,4-(OC^{2}H$_3$)$_2$]-5-demethylyateins. [a] Ions formed after benzylic cleavages of A-ring. [b] Ions formed after benzylic cleavages of B-ring. [c] Molecular ions. Bold-faced: extra ions due to incorporation of ^{13}C or deuterium atoms.

with six deuterium atoms at the two methoxy groups on the A-ring (Fig. 2), (±)-[3,5-(OC^{2}H$_3$)$_2$]-5-methylthujaplicatins (**4**-d_6), (±)-[3,5-(OC^{2}H$_3$)$_2$]-4,5-dimethylthujaplicatins (**5**-d_6), (±)-[3,5-(OC^{2}H$_3$)$_2$]-4-demethylyateins (**8**-d_6), and (±)-[3,4-(OC^{2}H$_3$)$_2$]-5-demethylyateins (**10**-d_6), were administrated, the extra ions at *m/z* 406 and 187 were observed in the mass spectra of (**11**), while in the feeding of the lignans with one OC^{2}H$_3$ group and three deuterium atoms, (±)-[3-OC^{2}H$_3$]thujaplicatins (**3**-d_3) and (±)-[3-OC^{2}H$_3$]-5-hydroxypluviatolides (**7**-d_3), extra ions at *m/z* 403 and 184 were observed. These results unequivocally indicate the conversion of (**3**-d_3), (**4**-d_6), (**5**-d_6), (**7**-d_3), (**8**-d_6), and (**10**-d_6) to yatein labelled with three or six deuterium atoms (**11**-d_3 or **11**-d_6). However, no evidence of isotope incorporation into yatein (**11**) was observed when (±)-[3-OC^{2}H$_3$]matairesinols (**2**-d_3), (±)-[3-OC^{2}H$_3$]pluviatolides (**6**-d_3), and (±)-[3,4-(OC^{2}H$_3$)$_2$]bursehernins (**9**-d_6) were administrated (Table 1). Similarly, Tables 2 and 3 show mass spectral data of the other lignans obtained following the administration.

Thus, these data demonstrate the conversions of the labelled precursors into several lignans summarized as follows:

(±)-[3-OC^{2}H$_3$]matairesinols (**2**-d_3) ⟶ [^{2}H$_3$]pluviatolide (**6**-d_3) (Table 3), and [^{2}H$_3$]bursehernin (**9**-d_3) (Table 3)

(±)-[3-OC^{2}H$_3$]thujaplicatins (**3**-d_3) ⟶ [^{2}H$_3$]-5-methylthujaplicatin (**4**-d_3) (Table 2), [^{2}H$_3$]-4,5-dimethylthujaplicatin (**5**-d_3) (Table 2), and [^{2}H$_3$]yatein (**11**-d_3) (Table 1)

(±)-[3,5-(OC^{2}H$_3$)$_2$]-5-methylthujaplicatins (**4**-d_6) ⟶ [^{2}H$_6$]-4,5-dimethylthujaplicatin (**5**-d_6) (Table 2), and [^{2}H$_6$]yatein (**11**-d_6) (Table 1)

(±)-[3,5-(OC^{2}H$_3$)$_2$]-4,5-dimethylthujaplicatins (**5**-d_6) ⟶ [^{2}H$_6$]yatein (**11**-d_6) (Table 1)

(±)-[3-OC^{2}H$_3$]pluviatolides (**6**-d_3) ⟶ [^{2}H$_3$]bursehernin (**9**-d_3) (Table 3)

(±)-[3-OC^{2}H$_3$]-5-hydroxypluviatolides (**7**-d_3) ⟶ [^{2}H$_3$]-4-demethylyatein (**8**-d_3) (Table 3), [^{2}H$_3$]-5-demethylyatein (**10**-d_3) (Table 3), and [^{2}H$_3$]yatein (**11**-d_3) (Table 1)

(±)-[3,5-(OC^{2}H$_3$)$_2$]-4-demethylyateins (**8**-d_6) ⟶ [^{2}H$_6$]yatein (**11**-d_6) (Table 1)

(±)-[3,4-(OC^{2}H$_3$)$_2$]-5-demethylyateins (**10**-d_6) ⟶ [^{2}H$_6$]yatein (**11**-d_6) (Table 1)

Large scale feeding experiments. The following compounds were administered to *A. sylvestris* in the same manner as the small scale experiments, but scaled up ten times (4 mg of each compound to 10 shoots): [*ring*-$^{13}C_6$]phenylalanine, (±)-[3-OC^{2}H$_3$]secoisolariciresinols (**1**-d_3), (±)-[3-OC^{2}H$_3$]matairesinols (**2**-d_3), (±)-[3-OC^{2}H$_3$]pluviatolides (**6**-d_3), and (±)-[3,4-(OC^{2}H$_3$)$_2$]bursehernins (**9**-d_6). GC-MS analysis confirmed the transformation [(**2**-d_3) ⟶ (**6**-d_3) and (**9**-d_3); and (**6**-d_3) ⟶(**9**-d_3)] as in the small scale experiments (data not shown). In addition, the conversions listed below were detected by GC-MS analysis.

[*ring*-$^{13}C_6$]phenylalanine ⟶ [$^{13}C_{12}$] and [$^{13}C_6$]matairesinol (**2**-$^{13}C_{12}$ and $^{13}C_6$) (Table 2), [$^{13}C_{12}$] and [$^{13}C_6$]-5-methylthujaplicatin (**4**-$^{13}C_{12}$ and $^{13}C_6$) (Table 2), [$^{13}C_{12}$] and [$^{13}C_6$]-4,5-dimethylthujaplicatin (**5**-$^{13}C_{12}$ and $^{13}C_6$) (Table 2), [$^{13}C_{12}$] and [$^{13}C_6$]pluviatolide (**6**-$^{13}C_{12}$ and $^{13}C_6$) (Table 3), [$^{13}C_{12}$]

Table 2 Mass tables of molecular-ion and principal daughter-ion regions of lignans (**2**), (**4**) and (**5**) obtained following administration of ^{13}C- or deuterium-labelled precursors into *A. sylvestris*

Matairesinol (**2**) (TMS ether)				5-Methylthujaplicatin (**4**) (TMS ether)					4,5-Dimethylthujaplicatin (**5**) (TMS ether)					
	Relative intensity (%)				Relative intensity (%)					Relative intensity (%)				
		Administrated labelled compounds				Administrated labelled compounds					Administrated labelled compounds			
m/z	Unlabelled	[$^{13}C_6$]Phe	**1**-d_3	*m/z*	Unlabelled	[$^{13}C_6$]Phe	**1**-d_3	**3**-d_3	*m/z*	Unlabelled	[$^{13}C_6$]Phe	**1**-d_3	**3**-d_3	**4**-d_6
209	100.0[a,b]	21.9[a,b]	100.0[a,b]	209	54.4[b]	26.6[b]	16.3[b]	8.2[b]	209	46.4[b]	30.8[b]	27.1[b]	17.3[b]	23.3[b]
210	29.3	7.8	28.3	210	16.7	6.1	3.7	2.0	210	25.2	18.0	15.0	3.5	15.4
211	8.4	3.1	9.5	211	5.9	2.5	1.2	4.0	211	6.2	6.7	3.6	0.0	0.0
212	1.6	0.3	**71.4**[a]	212	0.0	7.3	8.2	2.0	212	1.3	4.3	33.1	15.8	12.6
213	0.4	1.9	18.3	213	0.0	5.4	3.4	7.4	213	0.0	0.0	22.7	9.9	10.4
214	0.0	1.7	5.3	214	0.0	4.5	3.5	2.2	214	1.5	6.2	3.2	0.0	0.5
215	0.3	**18.6**[a,b]	0.0	215	0.0	**11.4**[b]	0.2	0.0	215	0.0	**21.2**[b]	2.9	0.0	4.9
216	0.9	4.7	0.0	216	0.0	4.0	0.7	0.0	216	1.7	5.3	1.4	13.5	3.0
217	2.7	2.8	1.6	217	0.0	5.2	0.2	0.0	217	2.0	0.8	1.8	0.0	0.0
502	58.4[c]	100.0[c]	28.8[c]	239	83.0[a]	21.2[a]	31.3[a]	9.9[a]	181	100.0[a]	74.0[a]	100.0[a]	100.0[a]	88.5[a]
503	26.7	46.7	15.6	240	31.2	11.9	13.7	6.0	182	73.1	36.3	68.0	55.0	78.5
504	11.4	20.6	12.3	241	10.5	3.4	5.6	4.1	183	8.7	16.5	11.2	12.2	17.7
505	3.6	9.5	**84.0**[c]	242	3.4	1.3	**10.0**[a]	**4.5**[a]	184	1.1	0.0	**24.2**[a]	**42.6**[a]	3.5
506	1.4	3.2	33.4	243	11.4	0.2	3.7	3.7	185	0.0	2.8	13.3	24.7	0.0
507	0.5	4.8	13.0	244	1.4	0.0	1.5	1.7	186	0.5	0.0	4.8	8.8	0.0
508	0.0	**9.1**[c]	0.0	245	0.0	**10.0**[a]	1.1	0.0	187	0.0	**33.9**[a]	1.3	2.9	**30.0**[a]
509	0.4	9.4	0.0	246	0.0	9.0	1.3	7.0	188	0.6	21.7	2.4	9.3	13.0
510	0.2	5.6	0.0	247	2.9	2.9	4.9	0.0	189	2.0	0.0	3.0	14.7	2.0
514	0.1	**9.4**[c]	0.0	532	100.0[c]	100.0[c]	28.1[c]	14.2[c]	474	95.9[c]	100.0[c]	36.7[c]	24.1[c]	38.3[c]
515	0.0	4.2	0.0	533	46.5	46.4	14.1	12.5	475	35.8	38.3	14.2	16.7	27.0
516	0.0	2.6	0.0	534	21.5	8.4	14.9	4.9	476	11.3	0.0	10.3	0.0	8.6
				535	2.1	0.0	**35.1**[c]	**10.4**[c]	477	1.5	9.0	**99.1**[c]	**12.3**[c]	0.0
				536	0.0	0.0	17.8	4.0	478	0.0	0.0	33.6	0.0	0.0
				537	0.0	0.0	6.1	0.0	479	0.0	7.7	16.2	0.0	0.0
				538	0.0	**9.6**[c]	0.0	0.0	480	0.0	**20.5**[c]	5.8	0.0	**28.8**[c]
				539	0.0	8.3	0.0	0.0	481	0.0	9.3	1.9	0.0	15.1
				540	0.0	0.0	0.0	0.0	482	0.0	2.5	0.0	0.0	0.0
				544	0.0	**20.3**[c]	0.0	0.0	486	0.0	**30.8**[c]	1.4	0.0	0.0
				545	0.0	0.0	0.0	0.0	487	0.0	5.5	0.0	0.0	0.0
				546	0.0	0.0	0.0	0.0	488	0.0	3.1	0.0	0.0	0.0

[$^{13}C_6$]Phe: [*ring*-$^{13}C_6$]phenylalanine, **1**-d_3: (±)-[3-OC^{2}H$_3$]secoisolariciresinols, **3**-d_3: (±)-[3-OC^{2}H$_3$]thujaplicatins, **4**-d_6: (±)-[3,5-(OC^{2}H$_3$)$_2$]-5-methylthujaplicatins. [a] Ions formed after benzylic cleavages of A ring. [b] Ions formed after benzylic cleavages of B-ring. [c] Molecular ions. Bold-faced: extra ions due to incorporation of ^{13}C or deuterium atoms.

Table 3 Mass tables of molecular-ion and principal daughter-ion regions of lignans (**6**), (**8**)–(**10**) obtained following administration of ^{13}C- or deuterium-labelled precursors into *A. sylvestris*

Pluviatolide (**6**) (TMS ether)					4-Demethyl-yatein (**8**) (TMS ether)			Bursehernin (**9**)						5-Demethyl-yatein (**10**) (TMS ether		
	Relative intensity (%)					Relative intensity (%)			Relative intensity (%)						Relative intensity (%)	
		Administrated labelled compounds					Administrated labelled compounds			Administrated labelled compounds						Administrated labelled compounds
m/z	Unlabelled	[$^{13}C_6$]Phe	**1**-d_3	**2**-d_3	*m/z*	Unlabelled	**7**-d_3	*m/z*	Unlabelled	[$^{13}C_6$]Phe	**1**-d_3	**2**-d_3	**6**-d_3	*m/z*	Unlabelled	**7**-d_3
135	21.7[b]	9.5[b]	11.8[b]	8.1[b]	135	20.0[b]	48.7[b]	135	26.2[b]	28.5[b]	13.7[b]	23.6[b]	21.4[b]	135	40.9[b]	17.7[b]
136	4.1	5.6	1.6	3.2	136	4.9	15.0	136	10.4	14.2	11.2	10.5	6.8	136	7.2	6.2
137	3.4	0.2	2.4	0.5	137	3.9	6.2	137	3.0	7.0	2.5	2.6	3.1	137	2.4	4.7
138	0.0	3.7	0.0	0.0	138	0.7	3.8	138	1.4	2.9	0.7	0.6	0.0	138	0.5	0.0
139	0.8	0.0	2.9	0.0	139	0.8	2.3	139	0.4	0.9	1.3	0.7	0.2	139	0.6	0.0
140	0.6	0.1	0.8	0.3	140	0.0	4.1	140	0.1	0.4	3.3	1.3	0.0	140	0.4	0.0
141	0.0	**1.0**[b]	1.8	0.0	141	0.0	2.3	141	0.2	**9.8**[b]	1.1	1.5	0.2	141	0.4	0.0
142	1.3	0.1	0.3	0.0	142	0.3	2.9	142	0.2	3.9	0.0	0.2	1.0	142	0.0	0.0
143	0.6	0.1	0.0	0.0	143	0.3	0.0	143	0.5	3.5	0.0	0.3	0.4	143	0.7	0.2
209	100.0[a]	7.0[a]	100.0[a]	24.3[a]	239	100.0[a]	0.0	151	100.0[a]	47.4[a]	61.4[a]	5.5[a]	36.3[a]	239	87.7[a]	0.0
210	20.4	3.2	30.1	16.2	240	33.6	0.0	152	17.8	7.2	12.0	1.7	6.8	240	80.3	0.0
211	7.6	0.4	10.0	5.7	241	9.0	0.0	153	1.9	1.5	7.4	2.3	3.9	241	17.9	0.0
212	2.9	0.9	**57.0**[a]	**100.0**[a]	242	2.1	**19.6**[a]	154	0.4	2.6	**28.8**[a]	**100.0**[a]	**100.0**[a]	242	5.2	**59.5**[a]
213	0.8	1.0	14.5	19.0	243	1.1	11.9	155	0.0	2.3	7.0	16.6	14.8	243	0.9	54.9
214	0.0	0.0	2.9	5.1	244	0.3	0.0	156	0.4	2.3	1.6	2.1	1.6	244	0.0	20.2
215	2.5	**7.0**[a]	0.6	0.0	245	0.8	0.0	157	0.3	**26.0**[a]	0.0	0.4	0.0	245	0.4	0.0
216	0.7	1.1	2.1	1.4	246	0.9	0.0	158	0.5	6.7	0.1	0.3	0.0	246	0.7	0.0
217	2.9	0.9	9.0	5.1	247	2.8	2.9	159	0.6	4.6	2.6	0.5	0.1	247	2.5	0.0
428	57.5[c]	6.4[c]	3.9[c]	2.1[c]	458	88.8[c]	0.0	370	50.0[c]	26.1[c]	29.7[c]	3.8[c]	20.1[c]	458	100.0[c]	0.0
429	18.8	4.3	3.8	0.8	459	30.4	0.0	371	14.5	5.0	7.7	1.3	5.0	459	34.6	0.0
430	7.5	3.5	1.0	1.1	460	10.1	0.0	372	3.0	2.7	4.0	2.3	4.1	460	12.4	0.0
431	1.0	0.0	**7.3**[c]	**22.0**[c]	461	2.9	**25.4**[c]	373	1.0	1.7	**17.1**[c]	**42.4**[c]	**43.4**[c]	461	3.5	**100.0**[c]
432	0.0	1.4	0.9	6.9	462	1.5	8.8	374	0.3	0.0	7.3	12.1	11.3	462	1.1	24.4
433	0.0	0.0	0.0	3.0	463	1.0	0.0	375	0.3	0.0	1.8	3.0	2.0	463	0.4	28.2
434	0.0	**3.7**[c]	1.4	1.6	464	0.4	0.0	376	0.1	**4.4**[c]	0.0	0.9	1.5	464	0.3	0.0
435	0.0	1.1	0.0	0.7	465	0.4	0.0	377	0.0	2.4	0.0	0.4	0.0	465	0.0	0.0
436	0.0	1.4	0.0	0.3	466	0.0	0.0	378	0.0	0.0	0.0	0.1	0.5	466	0.0	0.0
440	0.0	**4.4**[c]	0.0	1.2	470	0.0	0.0	382	0.0	**11.0**[c]	0.0	0.0	0.0	470	0.0	0.0
441	0.0	2.6	1.5	0.6	471	0.0	0.0	383	0.0	4.0	0.4	0.0	0.0	471	0.0	0.0
442	0.0	0.9	1.3	0.0	472	0.0	0.0	384	0.0	0.7	0.0	0.0	0.0	472	0.0	0.0

[$^{13}C_6$]Phe: [*ring*-$^{13}C_6$]phenylalanine, **1**-d_3: (±)-[3-OC^{2}H$_3$]secoisolariciresinols, **2**-d_3: (±)-[3-OC^{2}H$_3$]matairesinols, **6**-d_3: (±)-[3-OC^{2}H$_3$]pluviatolides, **7**-d_3: (±)-[3-OC^{2}H$_3$]-5-hydroxypluviatolides. [a] Ions formed after benzylic cleavages of A-ring. [b] Ions formed after benzylic cleavages of B-ring. [c] Molecular ions. Bold-faced: extra ions due to incorporation of ^{13}C or deuterium atoms.

and [$^{13}C_6$]bursehernin (**9**-$^{13}C_{12}$ and $^{13}C_6$) (Table 3), and [$^{13}C_{12}$] and [$^{13}C_6$]yatein (**11**-$^{13}C_{12}$ and $^{13}C_6$) (Table 1)

(±)-[3-OC^2H_3]secoisolariciresinols (**1**-d_3) ⟶ [$^2H_3$]matairesinol (**2**-d_3) (Table 2), [$^2H_3$]thujaplicatin (**3**-d_3), [$^2H_3$]-5-methylthujaplicatin (**4**-d_3) (Table 2), [$^2H_3$]-4,5-dimethylthujaplicatin (**5**-d_3) (Table 2), [$^2H_3$]pluviatolide (**6**-d_3) (Table 2), [$^2H_3$]-bursehernin (**9**-d_3) (Table 3), and [$^2H_3$]yatein (**11**-d_3) (Table 1)

(±)-[3-OC^2H_3]matairesinols (**2**-d_3) ⟶ [$^2H_3$]thujaplicatin (**3**-d_3)

The deuterium incorporation into thujaplicatin (**3**) was further confirmed by selected ion monitoring (SIM). The SIM chromatograms of thujaplicatin (TMS ether) obtained following administration of (**1**-d_3) and (**2**-d_3) clearly indicate that the molecular ion of unlabelled (**3**) at *m*/*z* 590 and the fragment ion at *m*/*z* 297 due to the benzylic cleavage of A-ring of unlabelled (**3**) are accompanied by extra ions at *m*/*z* 593 and 300, respectively, indicating clearly the formation of [$^2H_3$]-thujaplicatin (**3**-d_3) (Figs. 4-B and -C).

On the other hand, no evidence was obtained for the formation of ^{13}C-labelled [$^{13}C_{12}$] or [$^{13}C_6$]-5-hydroxypluviatolide (**7**-$^{13}C_{12}$ or $^{13}C_6$), [$^{13}C_{12}$] or [$^{13}C_6$]-4-demethylyatein (**8**-$^{13}C_{12}$ or $^{13}C_6$), or [$^{13}C_{12}$] or [$^{13}C_6$]-5-demethylyatein (**10**-$^{13}C_{12}$ or $^{13}C_6$) from [*ring*-$^{13}C_6$]phenylalanine. Thus, as shown in Fig. 5, neither endogenous unlabelled (**7**), (**8**), and (**10**) which have molecular ions at *m*/*z* 516, 458 and 458 (TMS ether), respectively, nor the lignans labelled with twelve or six ^{13}C atoms were detected.

Similarly, no deuterium-labelled (**7**-d_3), (**8**-d_3), and (**10**-d_3 or d_6) from any of the deuterium labelled lignans, (**1**-d_3), (**2**-d_3), (**6**-d_3), and (**9**-d_6) were observed. For example, Fig. 6 demonstrates that neither unlabelled, endogenous 5-hydroxypluviatolide (**7**) nor deuterium-labelled (**7**) was detected following administration of (**6**-d_3). In addition, no deuterium incorporation into yatein (**11**) from (±)-[3-OC^2H_3]pluviatolides (**6**-d_3) and (±)-[3,4-$(OC^2H_3)_2$]bursehernins (**9**-d_6) was observed. The mass spectra of yatein (**11**) obtained following administration of (**6**-d_3) and (**9**-d_6) did not show any extra ions in comparison with unlabelled authentic sample (Table 1).

Moreover, administration of (±)-[3-OC^2H_3]thujaplicatin (**3**-d_3) and (±)-[3,5-$(OC^2H_3)_2$]-5-methylthujaplicatins (**4**-d_6) in the small scale experiments did not lead to the formation of any of deuterium-labelled (**7**-d_3), (**8**-d_3 or d_6), and (**10**-d_3) (data not shown).

Simultaneous administration of two distinctly labelled lignans. Simultaneous administration of (±)-[3-OC^2H_3]thujaplicatins (**3**-d_3) and (±)-[7,7-$^2H_2$, 3-OC^2H_3]secoisolariciresinols (**1**-d_5) gave both [$^2H_3$]yatein (**11**-d_3) (*m*/*z* 403, Table 1) and [$^2H_5$]yatein (**11**-d_5) (*m*/*z* 405, Table 1), whereas the administration of both (±)-[3-OC^2H_3]pluviatolides (**6**-d_3) and (±)-[7,7-$^2H_2$,3-OC^2H_3]secoisolariciresinols (**1**-d_5) resulted in [$^2H_5$]yatein (**11**-d_5) (*m*/*z* 405, Table 1); the intensity of *m*/*z* 403 ion corresponding to [$^2H_3$]yatein (**11**-d_3) formation was insignificant (Table 1).

Discussion

We first examined the formation of the 5-methyl ether of the A-ring and the methylenedioxy bridge of the B-ring of yatein (**11**) (Fig. 1). When the deuterium-labelled dibenzylbutyrolactone lignans with 3,3′,4,4′,5-pentaalkoxy groups {(±)-[3-OC^2H_3]thujaplicatins (**3**-d_3), (±)-[3,5-$(OC^2H_3)_2$]-5-methylthujaplicatins (**4**-d_6), (±)-[3,5-$(OC^2H_3)_2$]-4,5-dimethylthujaplicatins (**5**-d_6), (±)-[3-OC^2H_3]-5-hydroxypluviatolides (**7**-d_3), (±)-[3,5-$(OC^2H_3)_2$]-4-demethylyateins (**8**-d_6), and (±)-[3,4-$(OC^2H_3)_2$]-5-demethylyateins (**10**-d_6) (Fig. 2)} were administered individually to *A. sylvestris* young shoots, all of them were found to be transformed to deuterium labelled [$^2H_3$] or [$^2H_6$]yatein (**11**-d_3 or **11**-d_6) (Tables 1–3). The results indicate that parallel pathways on the possible metabolic grid can operate in *A. sylvestris*, provided that these dibenzylbutyrolactone lignans are supplied to an appropriate reaction site of yatein biosynthesis in *A. sylvestris* cells. However this does not indicate all of these dibenzylbutyrolactone lignans are physiologically significant endogenous precursors of yatein (**11**) in the species.

Thus, our attention was next focused on the physiological roles of the parallel conversions. First, feeding experiments with more upstream compounds were conducted. Phenylalanine is the entrance compound of phenylpropanoid biosynthesis and upon administration this compound is expected to move smoothly into a physiologically important pathway.[29] Hence we fed [*ring*-$^{13}C_6$]phenylalanine to *A. sylvestris*. GC-MS analysis indicated that ^{13}C was incorporated into matairesinol (**2**), 5-methylthujaplicatin (**4**), 4,5-dimethylthujaplicatin (**5**), pluviatolide (**6**), bursehernin (**9**), and yatein (**11**) (Tables 1–3), but no evidence was obtained for the formation of ^{13}C-labelled [$^{13}C_{12}$] or [$^{13}C_6$]-5-hydroxypluviatolide (**7**-$^{13}C_{12}$ or $^{13}C_6$), [$^{13}C_{12}$] or [$^{13}C_6$]-4-demethylyatein (**8**-$^{13}C_{12}$ or $^{13}C_6$), or [$^{13}C_{12}$] or [$^{13}C_6$]-5-demethylyatein (**10**-$^{13}C_{12}$ or $^{13}C_6$) from [*ring*-$^{13}C_6$]phenylalanine (Fig. 5).

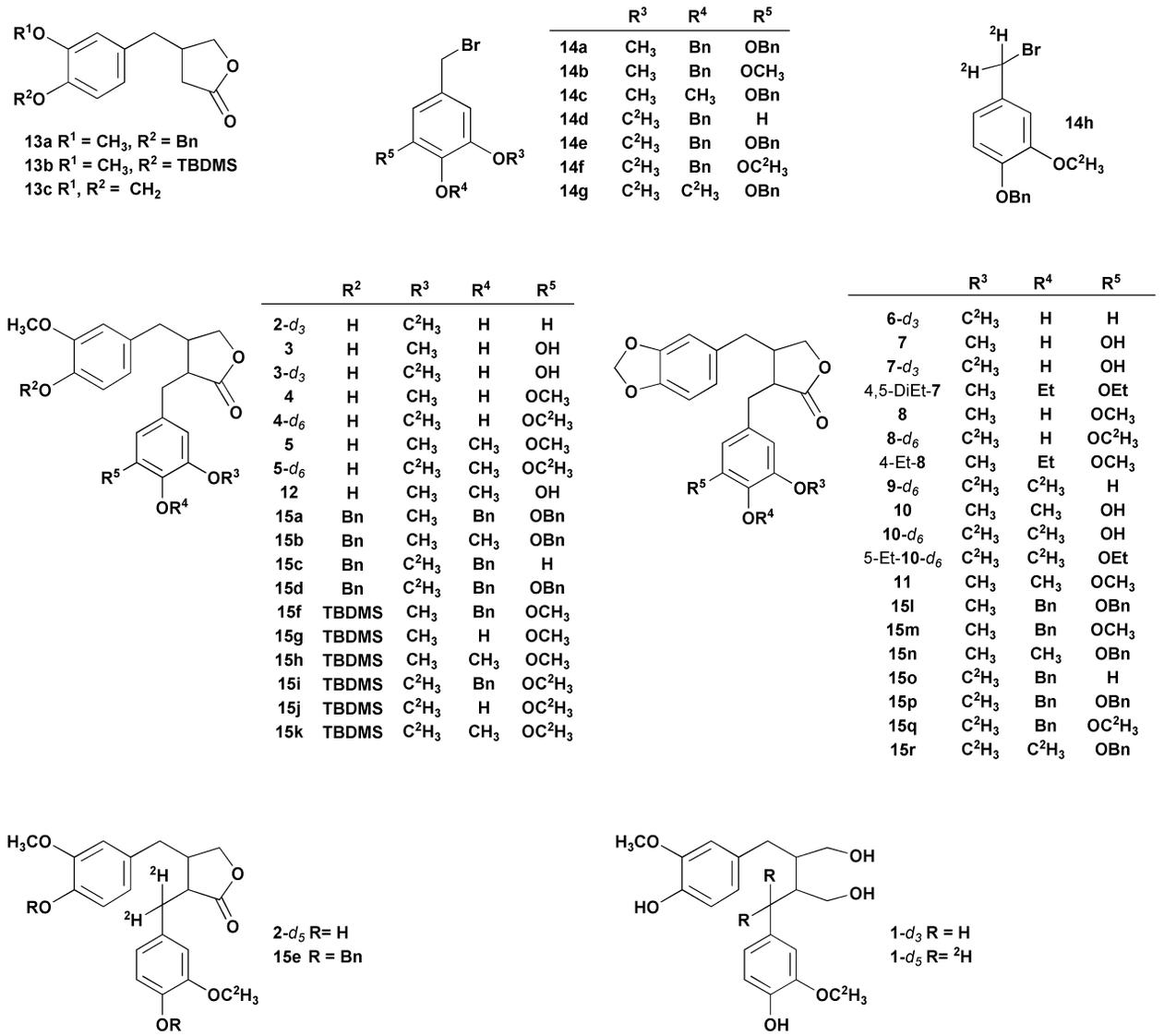
Second, (±)-[3-OC^2H_3]secoisolariciresinols (**1**-d_3) were administered, because secoisolariciresinol (**1**) is known to be converted to matairesinol (**2**) by the action of secoisolariciresinol dehydrogenase.[29–33] This resulted in deuterium incorporation into matairesinol (**2**), thujaplicatin (**3**), 5-methylthujaplicatin (**4**), 4,5-dimethylthujaplicatin (**5**), pluviatolide (**6**), bursehernin (**9**), and yatein (**11**) (Fig. 4 and Tables 1–3), but again no formation of deuterium-labelled (**7**-d_3), (**8**-d_3), or (**10**-d_3) was observed.

Third, in order to examine 5-hydroxylation step(s), the deuterium labelled dibenzylbutyrolactone lignans without the 5-hydroxy or 5-methoxy group were prepared. Administration of (±)-[3-OC^2H_3]matairesinols (**2**-d_3) resulted in deuterium incorporation into thujaplicatin (**3**) (Fig. 4) as well as pluviatolide (**6**) and bursehernin (**9**) (Tables 1–3). In contrast, formation of deuterium-labelled 5-hydroxy- or 5-methoxy-lignans, (**7**-d_3), (**10**-d_3 or d_6), and (**8**-d_3), was not observed when (±)-[3-OC^2H_3]matairesinols (**2**-d_3), (±)-[3-OC^2H_3]pluviatolides (**6**-d_3), and (±)-[3,4-$(OC^2H_3)_2$]bursehernins (**9**-d_6) were administered.

These results are in accord with the results of metabolic profiling, which detected the following lignans in the MeOH extracts of *A. sylvestris* young shoots: secoisolariciresinol (**1**), matairesinol (**2**), 5-methylthujaplicatin (**4**), 4,5-dimethylthujaplicatin (**5**), pluviatolide (**6**), bursehernin (**9**), and yatein (**11**). In addition, the presence of endogenous, unlabelled thujaplicatin (**3**) was detected by mass chromatography (data not shown) and selected ion monitoring (Figs. 4-B and 4-C). However, no evidence indicating the presence of any of 5-hydroxypluviatolide (**7**), 4-demethylyatein (**8**), and 5-demethylyatein (**10**) was obtained (Fig. 5).

Thus, although 5-hydroxypluviatolide (**7**), 4-demethylyatein (**8**), and 5-demethylyatein (**10**) were converted to yatein (**11**) effectively under the experimental conditions employed, their conversion to yatein (**11**) is probably negligible in *de novo* lignan biosynthesis in *A. sylvestris*. This conclusion is based on the above mentioned facts summarized as follows: First, the upstream precursors, phenylalanine and secoisolariciresinol (**1**) were not transformed to any of (**7**), (**8**), and (**10**). Second, the lignans, (**7**), (**8**), and (**10**), could not be detected in the MeOH extracts from the plant. Third, conversion of pluviatolide (**6**), bursehernin (**9**), thujaplicatin (**3**), and 5-methylthujaplicatin (**4**) into (**7**), (**8**), and (**10**) was not observed.

Taken together, these results indicate two independent branch metabolic pathways (solid arrows in Fig. 2) operating to affect the fate of matairesinol (**2**). One pathway leads to yatein (**11**) *via* thujaplicatin (**3**), 5-methylthujaplicatin (**4**), and 4,5-dimethylthujaplicatin (**5**); and the other gives bursehernin (**9**) through pluviatolide (**6**). The involvement of 4-methylthujaplicatin (**12**) (Fig. 3), a regioisomer of 5-methylthujaplicatin (**4**), also seemed possible, but this is unlikely, because isotope incorporation into this lignan was not observed from any of labelled precursors tested (data not shown). The operation of two independent pathways was further reinforced



	R3	R4	R5
14a	CH_3	Bn	OBn
14b	CH_3	Bn	OCH_3
14c	CH_3	CH_3	OBn
14d	C^2H_3	Bn	H
14e	C^2H_3	Bn	OBn
14f	C^2H_3	Bn	OC^2H_3
14g	C^2H_3	C^2H_3	OBn

	R2	R3	R4	R5
2-d_3	H	C^2H_3	H	H
3	H	CH_3	H	OH
3-d_3	H	C^2H_3	H	OH
4	H	CH_3	H	OCH_3
4-d_6	H	C^2H_3	H	OC^2H_3
5	H	CH_3	CH_3	OCH_3
5-d_6	H	C^2H_3	CH_3	OC^2H_3
12	H	CH_3	CH_3	OH
15a	Bn	CH_3	Bn	OBn
15b	Bn	CH_3	CH_3	OBn
15c	Bn	C^2H_3	Bn	H
15d	Bn	C^2H_3	Bn	OBn
15f	TBDMS	CH_3	Bn	OCH_3
15g	TBDMS	CH_3	H	OCH_3
15h	TBDMS	CH_3	CH_3	OCH_3
15i	TBDMS	C^2H_3	Bn	OC^2H_3
15j	TBDMS	C^2H_3	H	OC^2H_3
15k	TBDMS	C^2H_3	CH_3	OC^2H_3

	R3	R4	R5
6-d_3	C^2H_3	H	H
7	CH_3	H	OH
7-d_3	C^2H_3	H	OH
4,5-DiEt-7	CH_3	Et	OEt
8	CH_3	H	OCH_3
8-d_6	C^2H_3	H	OC^2H_3
4-Et-8	CH_3	Et	OCH_3
9-d_6	C^2H_3	C^2H_3	H
10	CH_3	CH_3	OH
10-d_6	C^2H_3	C^2H_3	OH
5-Et-10-d_6	C^2H_3	C^2H_3	OEt
11	CH_3	CH_3	OCH_3
15l	CH_3	Bn	OBn
15m	CH_3	Bn	OCH_3
15n	CH_3	CH_3	OBn
15o	C^2H_3	Bn	H
15p	C^2H_3	Bn	OBn
15q	C^2H_3	Bn	OC^2H_3
15r	C^2H_3	C^2H_3	OBn

Fig. 3 Structures of benzyl-γ-butyrolactones, benzyl bromides and lignans.

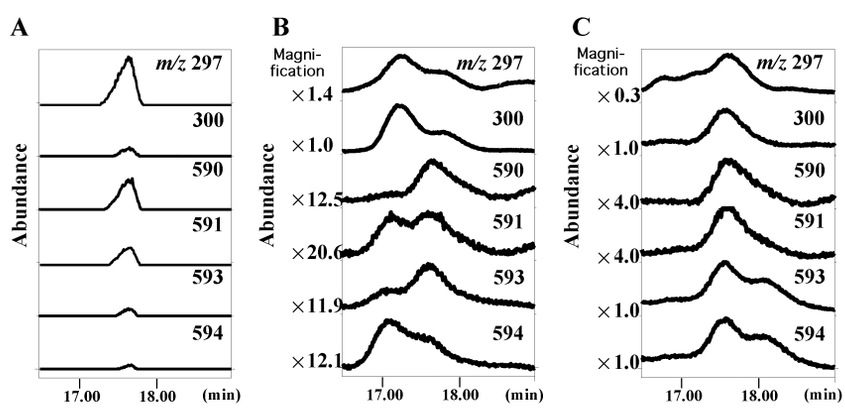


Fig. 4 Selected ion monitoring chromatograms of thujaplicatin (TMS ether). *A*: unlabelled, synthesized authentic sample; *B* and *C*: thujaplicatins obtained following administration of (±)-[3-OC^2H_3]secoisolariciresinols (**1**-d_3) and (±)-[3-OC^2H_3]mataireinols (**2**-d_3), respectively. Shown are the spectra for the molecular ion *m/z* 590 and the ion formed after benzylic cleavages of the A-ring of unlabelled thujaplicatin *m/z* 297. The peaks at 17.14 min (**B**) and 18.12 min (**C**) are impurities.

by two sets of simultaneous feeding experiments. When (±)-[3-OC^2H_3]thujaplicatins (**3**-d_3) and (±)-[7,7-$^2H_2$, 3-OC^2H_3]secoisolariciresinols (**1**-d_5) were fed at the same time, both [$^2H_3$]yatein (**11**-d_3) and [$^2H_5$]yatein (**11**-d_5) were formed, whereas the simultaneous administration of both (±)-[3-OC^2H_3]pluviatolides (**6**-d_3) and (±)-[7,7-$^2H_2$,3-OC^2H_3]secoisolariciresinols (**1**-d_5) resulted in [$^2H_5$]yatein (**11**-d_5) formation, but [$^2H_3$]yatein (**11**-d_3) formation was insignificant (Table 1). If pluviatolide (**6**) is converted to yatein (**11**), both [$^2H_3$]yatein (**11**-d_3) and [$^2H_5$]yatein (**11**-d_5) would be formed in both simultaneous feeding experiments. However, the results indicate that this was not the case, confirming that two independent pathways from mataireinol (**2**) are operating. Thus, the presence of a metabolic grid is eliminated. It should be noted that (±)-[3-OC^2H_3]pluviatolides (**6**-d_3) were transformed to [$^2H_3$]bursehernin (**9**-d_3) in the simultaneous feeding, suggesting strongly that the administered (±)-

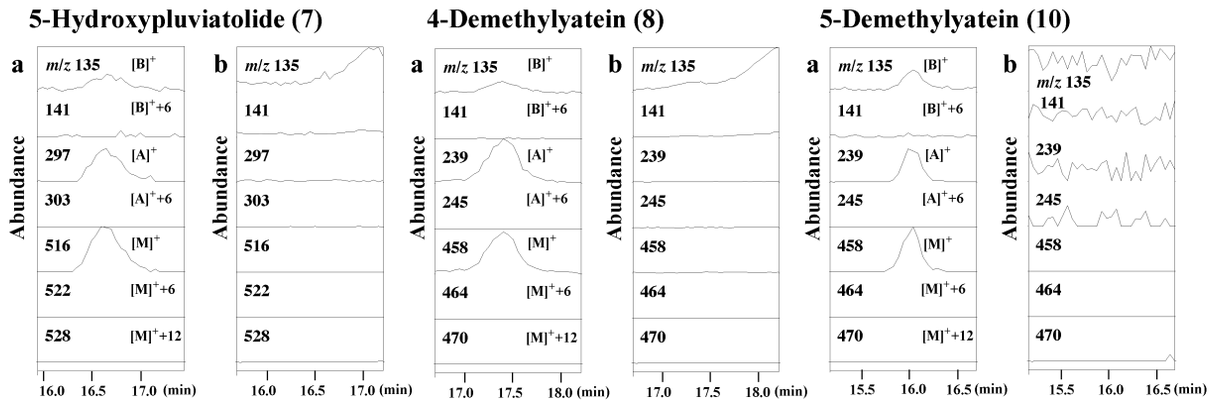


Fig. 5 Mass chromatograms of molecular ions and fragment ions formed after benzylic cleavages of A- and B-rings of 5-hydroxypluviatolide (**7**), 4-demethylyatein (**8**), and 5-demethylyatein (**10**) (TMS ether). *a*: unlabelled, synthesized authentic sample; *b*: the fraction corresponding to (**7**), (**8**), or (**10**) following administration of [*ring*-$^{13}C_6$]phenylalanine. M^+: molecular ion of unlabelled, synthesized authentic sample; A^+ and B^+: fragment ions formed after benzylic cleavages of A- and B-rings of unlabelled, synthesized authentic sample, respectively.

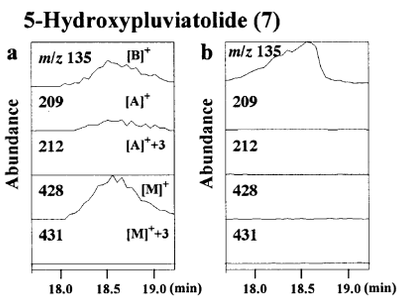


Fig. 6 Mass chromatograms of molecular ions and fragment ions formed after benzylic cleavages of A- and B-rings of 5-hydroxypluviatolide (**7**) (ethyl ether). *a*: unlabelled, synthesized authentic sample; *b*: the fraction corresponding to (**7**) following administration of (±)-[3-OC^2H_3]pluviatolides (**6**-d_3). M^+: molecular ion of unlabelled, synthesized authentic sample; A^+ and B^+: fragment ions formed after benzylic cleavages of A- and B-rings of unlabelled, synthesized authentic sample.

[3-OC^2H_3]pluviatolides (**6**-d_3) were transported to the lignan synthesis site under the conditions employed.

Kawai *et al*. administrated [7,7-$^2H_2$, 3-OC^2H_3]matairesinol (**2**-d_5) to *Thuja occidentalis* shoots, and detected deuterium incorporation into thujaplicatin (**3**), which indicates that the conversion of matairesinol (**2**) to thujaplicatin (**3**) is not specific to *A. sylvestris*, but also occurs in another species, *T. occidentalis*.[34]

Recently, we have detected pinoresinol/lariciresinol reductase (PLR) activity in *A. sylvestris*,[26] catalyzing the reduction of pinoresinol to secoisolariciresinol (**1**) *via* lariciresinol (Fig. 2). This suggests the intermediacy of pinoresinol and laricirsinol in lignan biosynthesis in this species. Taken together, we propose a possible biosynthetic pathway of yatein (**11**) from coniferyl alcohol, concomitant with a branch leading to bursehernin (**9**) from matairesinol (**2**) (Fig. 2).

The biosynthetic pathway for yatein (**11**) in *Anthriscus sylvestris* (Fig. 2) is in line with the previous reports outlined in Fig. 1. However, the pathway differs from two other recently proposed pathways in relation to the biosynthesis of podophyllotoxin derivatives in *Linum* spp..[35,36] One of these proposed pathways had the intact incorporation of 3,4-methylenedioxycinnamic acid into podophyllotoxin,[35] and the other had the benzylic hydroxylation occurring at an earlier stage (C7′ of matairesinol was hydroxylated to afford 7′-hydroxymatairesinol, which was then metabolized to 5-methoxypodophyllotoxin[36]).

In conclusion, the biosynthetic pathway from matairesinol (**2**) to yatein (**11**) has been presented for the first time based on concrete experimental evidence. The pathway involves 5-hydroxylation of matairesinol (**2**) to give thujaplicatin (**3**), followed by dual methylation and finally 3′,4′-methylenedioxy bridge formation to afford yatein (**11**). Additionally, the conversion of matairesinol (**2**) to bursehernin (**9**) was also found to occur, but does not lead to yatein (**11**).

Experimental

Plant materials

Anthriscus sylvestris Hoffm. (Umbelliferae) plants were collected in October 2001 at Kyoto University Forest in Ashiu, Kyoto. The plants were maintained in the experimental garden of Wood Research Institute, Kyoto University.

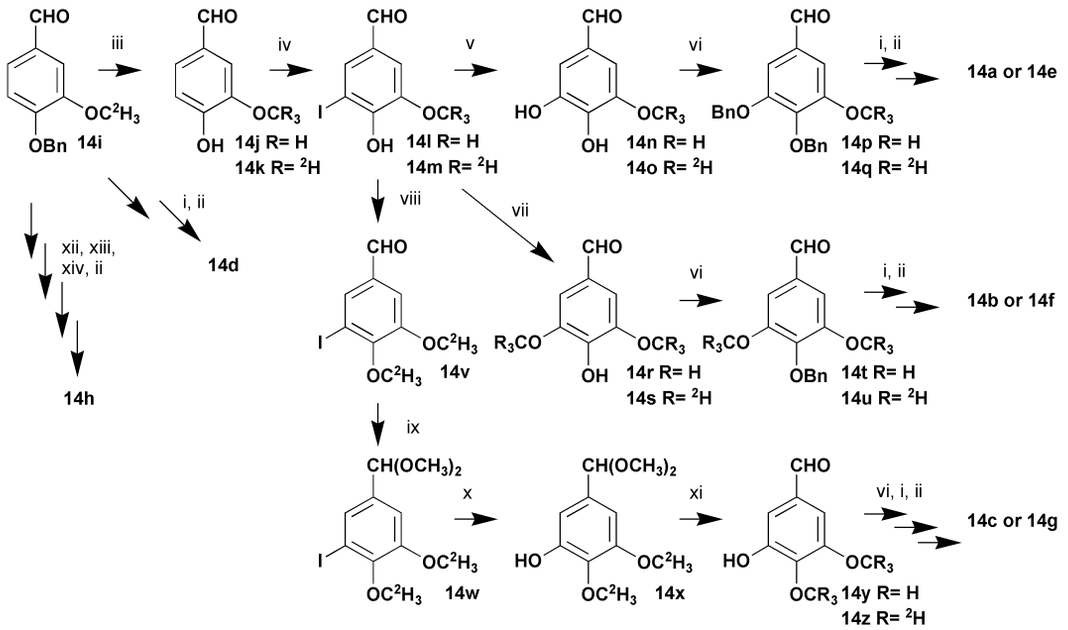
Instrumentation

^{1}H NMR and ^{13}C NMR spectra were taken with a JAM-LA400MK FT NMR System (JEOL). Chemical shifts and coupling constants (*J*) were given in δ and Hz, respectively. GC-MS was performed on a JMS-DX303HF mass spectrometer (JEOL) equipped with a Hewlett-Packard 5890J gas chromatograph and a JMA-DA5000 mass data system. GC-MS measurement conditions were as follows: electron-impact mode, 70 eV; gas-chromatographic column, Shimadzu HiCup CBP-10 M25–025 (5 m × 0.2 mm); temperature, 40 °C at t = 0–2 min, then to 230 °C at 30 °C min^{-1}; carrier gas, He; splitless injection. Samples dissolved in *N,O*-bis(trimethylsilyl)-acetamide were subjected to GC-MS measurement after heating at 60 °C for 45 min.

Synthesis of lignans and chemicals

Synthesis of benzyl-γ-butyrolactones. (±)-3-(4-Benzyloxy-3-methyloxybenzyl)-γ-butyrolactones (**13a**) and (±)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactones (**13c**) were prepared exactly as previously described[30,40] (**13a**): δ_H ($CDCl_3$) 7.24–7.44 (5 H, m), 6.81 (1 H, d, *J* 8.0), 6.67 (1 H, d, *J* 2.0), 6.61 (1 H, dd, *J* 8.2 and 1.8), 5.12 (2 H, s), 4.32 (1 H, dd, *J* 9.1 and 7.0), 4.02 (1 H, dd, *J* 9.3 and 6.1), 3.87 (3 H, s), 2.75–2.86 (1 H, m), 2.67–2.72 (2 H, m), 2.59 (1 H, dd, *J* 17.6 and 8.1), 2.27 (1 H, dd, *J* 17.4 and 7.0). (**13c**): δ_H($CDCl_3$) 6.74 (1 H, d, *J* 7.8), 6.63 (1 H, s), 6.59 (1 H, d, *J* 7.8), 5.94 (2 H, s), 4.32 (1 H, dd, *J* 7.3 and 6.8), 4.01 (1 H, dd, *J* 9.3 and 6.1), 2.74–2.84 (1 H, m), 2.66–2.72 (2 H, m), 2.59 (1 H, dd, *J* 17.6 and 8.0), 2.26 (1 H, dd, *J* 17.3 and 6.8).

(±)-3-(4-*tert*-Butyldimethylsilyloxy-3-methyloxybenzyl)-γ-butyrolactones (**13b**) were prepared by catalytic (Pd–C) hydrogenation of (**13a**) followed by *tert*-butyldimethylsilylation (*tert*-butyldimethylsilyl chloride, imidazole, DMF, rt) (**13b**): δ_H ($CDCl_3$) 6.77 (1 H, d, *J* 8.0), 6.61 (1 H, d, *J* 2.0), 6.58 (1 H, dd, *J* 7.8 and 1.9), 4.31 (1 H, dd, *J* 9.0 and 7.1), 4.02 (1 H, dd, *J* 9.1 and 6.2), 3.78 (3 H, s), 2.76–2.86 (1 H, m), 2.66–2.72 (2 H, m), 2.56 (1 H, dd, *J* 17.4 and 8.2), 2.27 (1 H, dd, *J* 17.4 and 7.0), 0.98 (9 H, s), 0.13 (6 H, s).



Scheme 1 Synthetic routes for deuterium-labelled benzyl bromide derivatives (**14a**–**14h**). *Reagents and conditions*: i, $NaBH_4$, MeOH, rt, 10 min; ii, PBr_3, Et_2O, rt, 1 h; iii, *c*. HCl, AcOH, reflux, 1 h; iv, I_2, KI, NaOH (aq), rt, 2 h; v, NaOH (aq), $CuSO_4$, 125 °C, 6 h; vi, BnBr, K_2CO_3, DMF, 70 °C, 4 h; vii, $C^2H_3O^2H$, Na, 128 °C, 3 h; viii, C^2H_3I, K_2CO_3, DMF, rt, 2 h; ix, $CH(OMe)_3$, *p*-TsOH, MeOH, 0 °C, 10 min; x, O_2, BuLi, Et_2O, −35 °C, 40 min; xi, 1 M HCl, acetone, 0 °C, 5 min; xii, $KMnO_4$, acetone, rt, 1 h; xiii, CH_3CHN_2, Et_2O, 0 °C, 3 min; xiv, $LiAl^2H_4$, Et_2O, −35 °C, 40 min.

Synthesis of benzyl bromides. Unlabelled and deuterium-labelled benzyl bromides (**14a**–**14h**) were synthesized from the vanillin derivatives [vanillin (**14j**), syringaldehyde (**14r**), 3,4-dimethoxy-5-hydroxybenzaldehyde (**14y**) (Aldrich), and [3-OC^2H_3]benzylvanillin (**14i**)[30]], respectively, by the methods described previously,[30,40–43] which are outlined in Scheme 1.

4,5-Dibenzyloxy-3-methoxybenzyl bromide (**14a**): $\delta_H(CDCl_3)$ 7.24–7.50 (10 H, m), 6.68 (1 H, d, *J* 2.0), 6.63 (1 H, d, *J* 2.0), 5.10 (2 H, s), 5.03 (2 H, s), 4.44 (2 H, s), 3.85 (3 H, s).

4-Benzyloxy-3,5-dimethoxybenzyl bromide (**14b**): $\delta_H(CDCl_3)$ 7.23–7.52 (5 H, m), 6.60 (2 H, s), 4.99 (2 H, s), 4.42 (2 H, s), 3.83 (6 H, s).

5-Benzyloxy-3,4-dimethoxybenzyl bromide (**14c**): $\delta_H(CDCl_3)$ 7.17–7.42 (5 H, m), 6.60 (1 H, d, *J* 1.9), 6.56 (1 H, d, *J* 2.0), 5.02 (2 H, s), 4.35 (2 H, s), 3.80 (3 H, s), 3.80 (3 H, s).

[3-OC^2H_3]-4-Benzyloxy-3-methoxybenzyl bromide (**14d**): $\delta_H(CDCl_3)$ 7.24–7.46 (5 H, m), 6.92 (1 H, d, *J* 1.9), 6.87 (1 H, dd, *J* 8.3 and 2.0), 6.81 (1 H, d, *J* 8.0), 5.15 (2 H, s), 4.48 (2 H, s).

[3-OC^2H_3]-4,5-Dibenzyloxy-3-methoxybenzyl bromide (**14e**): $\delta_H(CDCl_3)$ 7.24–7.47 (10 H, m), 6.68 (1 H, d, *J* 2.0), 6.63 (1 H, d, *J* 2.0), 5.09 (2 H, s), 5.02 (2 H, s), 4.44 (2 H, s).

[3,5-$(OC^2H_3)_2$]-4-Benzyloxy-3,5-dimethoxybenzyl bromide (**14f**): $\delta_H(CDCl_3)$ 7.24–7.50 (5 H, m), 6.59 (2 H, s), 4.99 (2 H, s), 4.45 (2 H, s).

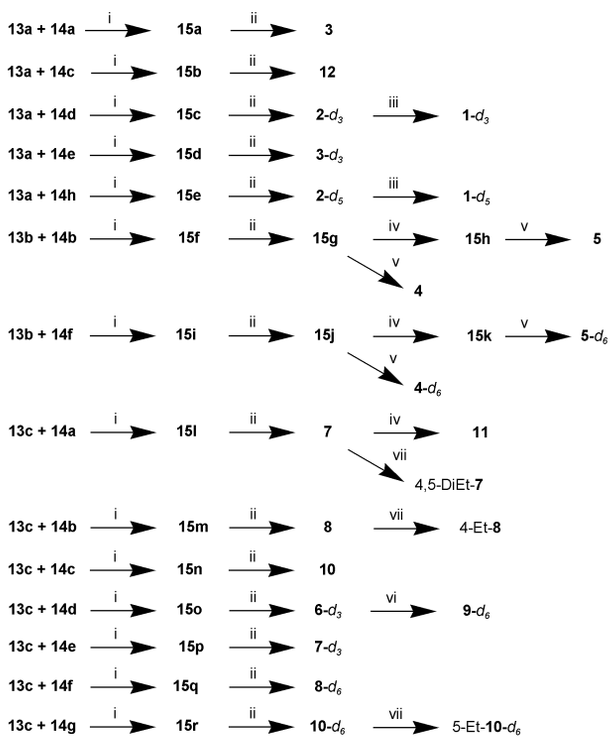
[3,4-$(OC^2H_3)_2$]-5-Benzyloxy-3,4-dimethoxybenzyl bromide (**14g**): $\delta_H(CDCl_3)$ 7.16–7.47 (5 H, m), 6.59 (1 H, s), 6.55 (1 H, s), 5.06 (2 H, s), 4.36 (2 H, s).

[α,α-$^2H_2$, 3-OC^2H_3]-4-Benzyloxy-3-methoxybenzyl bromide (**14h**): $\delta_H(CDCl_3)$ 7.21–7.40 (5 H, m), 6.87 (1 H, d, *J* 2.2), 6.82 (1 H, dd, *J* 8.2 and 2.1), 6.74 (1 H, d, *J* 8.1), 5.09 (2 H, s).

Synthesis of dibenzylbutyrolactone lignans. (±)-Matairesinols (**2**),[37] (±)-pluviatolides (**6**),[38] and (±)-bursehernins (**9**)[39] were prepared previously.

The dibenzylbutyrolactone lignans, (±)-[3-OC^2H_3]matairesinols (**2**-d_3), (±)-[7,7-$^2H_2$, 3-OC^2H_3]matairesinols (**2**-d_5), (±)-thujaplicatins (**3**), (±)-[3-OC^2H_3]thujaplicatins (**3**-d_3), (±)-5-methylthujaplicatins (**4**), (±)-[3,5-$(OC^2H_3)_2$]-5-methylthujaplicatins (**4**-d_6), (±)-[3-OC^2H_3]pluviatolides (**6**-d_3), (±)-5-hydroxypluviatolides (**7**), (±)-[3-OC^2H_3]-5-hydroxypluviatolides (**7**-d_3), (±)-4-demethylyateins (**8**), (±)-[3,5-$(OC^2H_3)_2$]-4-demethylyateins (**8**-d_6), (±)-5-demethylyateins (**10**), (±)-[3,4-$(OC^2H_3)_2$]-5-demethylyateins (**10**-d_6), and (±)-4-methylthujaplicatins (**12**) were prepared by lithium hexamethyldisilylamide-catalyzed condensation of appropriate combinations of γ-butyrolactones (**13a**–**13c**) and benzyl bromides (**14a**–**14h**) followed by deprotection as previously reported,[30,40–43] which is outlined in Scheme 2.

(±)-[3-OC^2H_3]Matairesinols (**2**-d_3): $\delta_H(CDCl_3)$ 6.80 (1 H, d, *J* 8.6), 6.78 (1 H, d, *J* 7.8), 6.56–6.62 (2 H, m), 6.50 (1 H, d, *J* 8.0), 6.34 (1 H, s), 4.13 (1 H, dd, *J* 8.6 and 7.6), 3.90 (1 H, dd, *J* 8.8 and 8.8), 3.80 (3 H, s), 2.94 (1 H, dd, *J* 13.7 and 4.9), 2.87 (1 H, dd, *J* 13.7 and 7.3), 2.40–2.64 (4 H, m); *m*/*z* (EI) 361.1578



Scheme 2 Synthetic routes for unlabelled and deuterium-labelled lignans. *Reagents and conditions*: i, Lithium hexamethyldisilylamide, HMPA, THF, −35 °C, 45 min; ii, 10% Pd-C, H_2, THF, MeOH, rt, 1 h; iii, $LiAlH_4$, Et_2O, −35 °C, 40 min; iv, CH_3I, K_2CO_3, DMF, rt, 2 h; v, *n*-Bu_4NF, THF, 0 °C, 20 min; vi, C^2H_3I, K_2CO_3, DMF, rt, 2 h; vii, CH_3CHN_2, Et_2O, rt, 10 h.

(M^+, 42.8%. $C_{20}H_{19}{}^2H_3O_6$ requires 361.1604), 360 (1.1), 359 (1.1), 358 (0.5), 149 (10.5), 140 (100), 137 (42.1), 131 (12.4), 122 (12.5).

(±)-[7,7-$^2H_2$, 3-OC^2H_3]Matairesinols (**2**-d_5): δ_H ($CDCl_3$) 6.70–6.79 (2 H, m), 6.50–6.57 (2 H, m), 6.44 (1 H, dd, *J* 6.4 and 2.0), 6.34 (1 H, d, *J* 2.0), 5.45 (2 H, d, *J* 8.6), 4.08 (1 H, dd, *J* 9.2 and 7.2), 3.82 (1 H, dd, *J* 8.3 and 8.0), 3.74 (3 H, s), 2.58–2.35 (4 H, m); *m*/*z* (EI) 363.1734 (M^+, 48.2%. $C_{20}H_{17}{}^2H_5O_6$ requires 363.1730), 362 (3.5), 361 (1.5), 360 (0.6), 359 (0.2), 358 (0.0), 149 (11.5), 142 (100), 137 (47.5), 131 (10.9), 122 (7.8).

(±)-Thujaplicatins (**3**): δ_H($CDCl_3$) 6.72 (1 H, d, *J* 8.1), 6.44 (1 H, dd, *J* 7.9 and 1.8), 6.38 (1 H, d, *J* 1.7), 6.30 (1 H, d, *J* 2.0), 6.17 (1 H, d, *J* 2.0), 4.09 (1 H, dd, *J* 9.1 and 6.9), 3.82 (1 H, dd, *J* 9.1 and 6.9), 3.76 (3 H, s), 3.73 (3 H, s), 2.85 (1 H, dd, *J* 13.9 and 4.9), 2.75 (1 H, dd, *J* 14.0 and 6.9), 2.36–2.58 (4 H, m); *m*/*z* (EI) 374.1380 (M^+, 73.9%. $C_{20}H_{22}O_7$ requires 374.1366), 237 (15.0), 223 (9.7), 163 (11.9), 153 (100), 137 (92.8), 131 (24.7), 122 (20.3).

(±)-[3-OC^2H_3]Thujaplicatins (**3**-d_3): δ_H($CDCl_3$) 6.78 (1 H, d, *J* 7.8), 6.50 (1 H, dd, *J* 8.0 and 2.0), 6.44 (1 H, d, *J* 1.7), 6.36 (1 H, d, *J* 2.0), 6.23 (1 H, d, *J* 1.7), 4.15 (1 H, dd, *J* 9.2 and 7.0), 3.88 (1 H, dd, *J* 9.2 and 7.2), 3.82 (3 H, s), 2.92 (1 H, dd, *J* 14.0 and 5.0), 2.81 (1 H, dd, *J* 14.0 and 6.9), 2.42–2.64 (4 H, m); *m*/*z* (EI) 377.1557 (M^+, 76.8%. $C_{20}H_{19}{}^2H_3O_7$ requires 377.1554), 376 (4.1), 375 (4.1), 374 (1.4), 240 (14.2), 226 (10.6), 163 (14.1), 156 (100), 137 (98.5), 131 (25.8), 122 (20.5).

(±)-5-Methylthujaplicatins (**4**): δ_H($CDCl_3$) 6.75 (1 H, d, *J* 7.8), 6.45 (1 H, dd, *J* 7.9 and 1.8), 6.34 (1 H, d, *J* 1.7), 6.25 (2 H, s), 4.11 (1 H, dd, *J* 9.0 and 7.3), 3.82 (1 H, dd, *J* 9.2 and 7.4), 3.76 (6 H, s), 3.73 (3 H, s), 2.86 (1 H, dd, *J* 13.8 and 5.4), 2.80 (1 H, dd, *J* 13.9 and 6.6), 2.33–2.60 (4 H, m); *m*/*z* (EI) 388.1512 (M^+, 73.5%. $C_{21}H_{24}O_7$ requires 388.1522), 173 (11.7), 167 (100), 149 (13.7), 137 (46.1), 131 (9.9), 122 (11.9).

(±)-[3,5-(O$C^2H_3)_2$]-5-Methylthujaplicatins (**4**-d_6): δ_H($CDCl_3$) 6.79 (1 H, d, *J* 8.0), 6.51 (1 H, dd, *J* 8.1 and 1.9), 6.40 (1 H, d, *J* 1.9), 6.30 (2 H, s), 4.17 (1 H, dd, *J* 9.1 and 7.2), 3.88 (1 H, dd, *J* 9.1 and 7.4), 3.79 (3 H, s), 2.92 (1 H, dd, *J* 14.2 and 5.4), 2.87 (1 H, dd, *J* 14.2 and 6.8), 2.40–2.65 (4 H, m); *m*/*z* (EI) 394.1897 (M^+, 57.9%. $C_{21}H_{18}{}^2H_6O_7$ requires 394.1898), 393 (0.0), 392 (2.3), 391 (0.8), 390 (0.6), 389 (0.4), 388 (0.0), 173 (100), 149 (4.0), 137 (35.1), 131 (10.6), 122 (6.8).

(±)-[3-OC^2H_3]Pluviatolides (**6**-d_3): δ_H ($CDCl_3$) 6.76 (1 H, d, *J* 8.1), 6.52–6.65 (3 H, m), 6.34–6.42 (2 H, m), 5.83–5.87 (2 H, m), 4.03 (1 H, dd, *J* 9.0 and 7.1), 3.77 (1 H, dd, *J* 10.4 and 7.1), 2.88 (1 H, dd, *J* 14.1 and 5.1), 2.80 (1 H, dd, *J* 14.2 and 6.8), 2.23–2.56 (4 H, m); *m*/*z* (EI) 359.1429 (M^+, 42.2%. $C_{20}H_{17}{}^2H_3O_6$ requires 359.1448), 358 (2.6), 357 (1.5), 356 (0.6), 223 (11.4), 162 (10.1), 140 (100), 135 (38.2), 131 (9.5), 122 (9.6).

(±)-5-Hydroxypluviatolides (**7**): δ_H ($CDCl_3$) 6.68 (1 H, d, *J* 8.3), 6.45 (1 H, m), 6.44 (1 H, s), 6.37 (1 H, d, *J* 1.7), 6.26 (1 H, d, *J* 1.4), 5.83–5.87 (2 H, m), 4.11 (1 H, dd, *J* 9.3 and 7.3), 3.84 (1 H, dd, *J* 9.0 and 6.8), 3.81 (3 H, s), 2.91 (1 H, dd, *J* 13.9 and 4.9), 2.80 (1 H, dd, *J* 13.9 and 6.8), 2.40–2.63 (4 H, m); *m*/*z* (EI) 372.1205 (M^+, 89.5%. $C_{20}H_{20}O_7$ requires 372.1209), 236 (31.4), 191 (16.3), 161 (16.7), 153 (100), 149 (15.8), 135 (86.1), 131 (34.0), 122 (13.0).

(±)-[3-OC^2H_3]-5-Hydroxypluviatolides (**7**-d_3): δ_H ($CDCl_3$) 6.68 (1 H, d, *J* 8.3), 6.44–6.48 (1 H, m), 6.45 (1 H, s), 6.37 (1 H, d, *J* 1.7), 6.27 (1 H, d, *J* 1.7), 5.91–5.94 (2 H, m), 4.11 (1 H, dd, *J* 9.3 and 6.8), 3.85 (1 H, dd, *J* 9.2 and 7.0), 2.92 (1 H, dd, *J* 14.0 and 5.0), 2.81 (1 H, dd, J 13.9 and 6.8), 2.42–2.64 (4 H, m); *m*/*z* (EI) 375.1375 (M^+, 89.1%. $C_{20}H_{17}{}^2H_3O_7$ requires 375.1397), 374 (5.7), 373 (7.3), 372 (2.5), 239 (28.8), 194 (13.9), 161 (19.0), 156 (100), 149 (34.0), 135 (73.1), 173 (23.1), 122 (11.3).

(±)-4-Demethylyateins (**8**): δ_H ($CDCl_3$) 6.69 (1 H, d, *J* 7.6), 6.43–6.48 (2 H, m), 6.35 (2 H, s), 5.92–5.93 (2 H, m), 4.15 (1 H, dd, *J* 9.3 and 7.3), about 3.87 (1 H), 3.85 (6 H, s), 2.89 (2 H, d, *J* 6.1), 2.42–2.64 (4 H, m); *m*/*z* (EI) 386.1362 (M^+, 55.3%. $C_{21}H_{22}O_7$ requires 386.1366), 250 (13.8), 167 (100), 161 (8.8), 135 (32.7), 131 (8.9).

(±)-[3,5-(O$C^2H_3)_2$]-4-Demethylyateins (**8**-d_6): δ_H ($CDCl_3$) 6.69 (1 H, d, *J* 7.8), 6.46 (1 H, d, *J* 7.6), 6.45 (1 H, s), 6.34 (2 H, s), 5.92–5.93 (2 H, m), 4.13 (1 H, dd, *J* 9.5 and 7.6), 3.86 (1 H, dd, *J* 9.0 and 7.6), 2.89 (2 H, d, *J* 6.1), 2.43–2.65 (4 H, m); *m*/*z* (EI) 392.1735 (M^+, 57.9%. $C_{21}H_{16}{}^2H_6O_7$ requires 392.1742), 391 (0.0), 390 (2.4), 389 (1.0), 388 (0.5), 387 (0.7), 386 (0.8), 256 (15.9), 173 (100), 161 (6.6), 149 (18.8), 135 (32.0), 131 (9.8).

(±)-5-Demethylyateins (**10**): δ_H ($CDCl_3$) 6.62 (1 H, d, *J* 8.3), 6.37–6.42 (2 H, m), 6.32 (1 H, d, *J* 2.0), 6.21 (1 H, d, *J* 1.7), 5.83–5.87 (2 H, m), 4.07 (1 H, dd, *J* 9.3 and 6.8), about 3.79 (1 H), 3.79 (3 H, s), 3.79 (3 H, s), 2.88 (1 H, dd, *J* 13.9 and 4.9), 2.73 (1 H, dd, *J* 13.9 and 7.1), 2.35–2.57 (4 H, m); *m*/*z* (EI) 386.1382 (M^+, 90.3%. $C_{21}H_{22}O_7$ requires 386.1365), 250 (24.8), 237 (12.9), 205 (14.6), 167 (100), 161 (16.9), 135 (70.8), 131 (18.3).

(±)-[3,4-(O$C^2H_3)_2$]-5-Demethylyateins (**10**-d_6): δ_H ($CDCl_3$) 6.68 (1 H, d, *J* 8.3), 6.43–6.49 (2 H, m), 6.38 (1 H, d, *J* 2.0), 6.26 (1 H, d, *J* 1.7), 5.87–5.93 (2 H, m), 4.13 (1 H, dd, *J* 9.3 and 6.6), 3.85 (1 H, dd, *J* 9.3 and 7.0), 2.94 (1 H, dd, *J* 14.0 and 4.8), 2.80 (1 H, dd, *J* 13.9 and 7.1), 2.46–2.65 (4 H, m); *m*/*z* (EI) 392.1740 (M^+, 75.1%. $C_{21}H_{16}{}^2H_6O_7$ requires 392.1742), 391 (0.0), 390 (2.3), 389 (1.4), 388 (1.1), 387 (0.0), 386 (0.0), 256 (30.2), 243 (15.3), 211 (14.6), 173 (100), 161 (12.6), 135 (56.0), 131 (17.5).

(±)-4-Methylthujaplicatins (**12**): δ_H($CDCl_3$) 6.79 (1 H, d, *J* 8.0), 6.50 (1 H, dd, *J* 8.1 and 1.9), 6.47 (1 H, d, *J* 2.0), 6.37 (1 H, d, *J* 2.0), 6.27 (1 H, d, *J* 1.9), 4.14 (1 H, dd, *J* 9.0 and 6.8), about 3.87 (1 H), 3.86 (3 H, s), 3.83 (3 H, s), 3.79 (3 H, s), 2.92 (1 H, dd, *J* 13.9 and 5.1), 2.83 (1 H, dd, *J* 13.9 and 6.8), 2.44–2.64 (4 H, m); *m*/*z* (EI) 388.1503 (M^+, 92.3%. $C_{21}H_{24}O_7$ requires 388.1522), 167 (100), 149 (39.7), 137 (55.8), 131 (27.2), 122 (20.3).

(±)-4,5-Dimethylthujaplicatins (**5**): δ_H ($CDCl_3$) 6.74 (1 H, d, *J* 8.0), 6.45 (1 H, dd, *J* 8.1 and 1.7), 6.37 (1 H, d, *J* 1.7), 6.26 (2 H, s), 4.12 (1 H, dd, *J* 9.0 and 7.3), 3.83 (1 H, dd, *J* 8.8 and 8.0), 3.75 (3 H, s), 3.74 (3 H, s), 3.74 (6 H, s), 2.88 (1 H, dd, *J* 13.9 and 5.4), 2.83 (1 H, dd, *J* 14.2 and 6.6), 2.36–2.62 (4 H, m); *m*/*z* (EI) 402.1696 (M^+, 100%. $C_{22}H_{26}O_7$ requires 402.1679), 251 (13.4), 181 (92.9), 137 (45.0), 131 (11.3), 122 (11.5).

(±)-[3,5-(O$C^2H_3)_2$]-4,5-Dimethylthujaplicatins (**5**-d_6): δ_H ($CDCl_3$) 6.80 (1 H, d, *J* 8.0), 6.52 (1 H, dd, *J* 8.0 and 2.0), 6.43 (1 H, d, *J* 2.0), 6.32 (2 H, s), 4.18 (1 H, dd, *J* 9.2 and 7.2), 3.88 (1 H, dd, *J* 9.0 and 7.6), 3.81 (3 H, s), 3.80 (3 H, s), 2.94 (1 H, dd, *J* 13.9 and 5.4), 2.88 (1 H, dd, *J* 14.2 and 6.6), 2.42–2.67 (4 H, m); *m*/*z* (EI) 408.2037 (M^+, 75.1%. $C_{22}H_{20}{}^2H_6O_7$ requires 408.2055), 407 (0.0), 406 (0.0), 405 (0.0), 404 (0.0), 403 (0.8), 402 (0.0), 257 (8.0), 187 (100), 137 (35.5), 131 (10.2), 122 (6.0).

(±)-[3,4-(O$C^2H_3)_2$]Bursehernins (**9**-d_6): δ_H($CDCl_3$) 6.76 (1 H, d, *J* 8.1), 6.62–6.78 (3 H, m), 6.44 (1 H, d, *J* 8.1), 6.42 (1 H, s), 5.91–5.95 (2 H, m), 4.10 (1 H, dd, *J* 9.2 and 6.7), 3.84 (1 H, dd, *J* 9.0 and 7.1), 2.95 (1 H, dd, *J* 14.0 and 5.1), 2.87 (1 H, dd, *J* 14.0 and 7.0), 2.40–2.70 (4 H, m); *m*/*z* (EI) 376.1779 (M^+, 47.1%. $C_{21}H_{16}{}^2H_6O_6$ requires 376.1793), 375 (0.0), 374 (1.3), 373 (0.4), 372 (0.7), 371 (0.0), 370 (0.0), 240 (13.2), 214 (5.4), 161 (5.6), 157 (100), 135 (22.4).

(±)-Yateins (**11**): δ_H($CDCl_3$) 6.68 (1 H, d, *J* 8.0), 6.45 (1 H, d, *J* 8.1), 6.44 (1 H, s), 6.34 (2 H, s), 5.90–5.94 (2 H, m), 4.16 (1 H, dd, *J* 9.1 and 7.2), 3.86 (1 H, dd, *J* 9.0 and 5.6), 3.81 (3 H, s), 3.81 (6H, s), 2.92 (1 H, dd, *J* 14.2 and 5.4), 2.87 (1 H, dd, *J* 13.9 and 6.4), 2.40–2.66 (4 H, m); *m*/*z* (EI) 400.1509 (M^+, 76.4%. $C_{22}H_{24}O_7$ requires 400.1522), 251 (11.2), 181 (100), 167 (10.5), 149 (12.4), 135 (28.6).

(±)-4,5-Diethyl-5-hydroxypluviatolides (4,5-DiEt-**7**): δ_H ($CDCl_3$) 6.62 (1 H, d, *J* 8.5), 6.36–6.42 (2 H, m), 6.28 (1 H, d, *J* 2.0), 6.27 (1 H, d, *J* 2.0), 5.85–5.88 (2 H, m), 4.08 (1 H, dd, *J* 9.2 and 7.2), 3.96 (2 H, q, *J* 7.1), 3.95 (2 H, q, *J* 7.1), 3.80 (1 H, dd, *J* 9.2 and 7.3), 3.74 (3 H, s), 2.87 (1 H, dd, *J* 13.9 and 5.1), 2.79 (1 H, dd, *J* 14.0 and 7.0), 2.46–2.58 (4 H, m), 1.35 (3 H, t, *J* 7.0), 1.27 (3 H, t, *J* 7.1); *m*/*z* (EI) 428.1833 (M^+, 100%. $C_{24}H_{28}O_7$ requires 428.1835), 399 (12.0), 210 (33.9), 181 (38.6), 153 (30.6), 135 (59.4).

(±)-4-Ethyl-4-demethylyateins (4-Et-**8**): $\delta_H(CDCl_3)$ 6.62 (1 H, d, *J* 8.3), 6.36–6.42 (2 H, m), 6.29 (2 H, s), 5.85–5.87 (2 H, m), 4.09 (1 H, dd, *J* 9.1 and 6.9), 3.95 (2 H, q, *J* 7.1), 3.80 (1 H, dd, *J* 9.3 and 7.6), 3.74 (6 H, s), 2.88 (1 H, dd, *J* 13.9 and 5.2), 2.80 (1 H, dd, *J* 13.9 and 6.8), 2.35–2.60 (4 H, m), 1.27 (3 H, t, *J* 7.1); *m/z* (EI) 414.1660 (M^+, 100%. $C_{23}H_{26}O_7$ requires 414.1678), 385 (16.8), 278 (7.1), 217 (6.2), 195 (34.8), 167 (86.5), 135 (66.0), 131 (10.5).

(±)-[3,4-($OC^2H_3)_2$]-5-Ethyl-5-demethylyateins (5-Et-**10**-d_6): $\delta_H(CDCl_3)$ 6.63 (1 H, d, *J* 8.3), 6.37–6.43 (2 H, m), 6.28 (1 H, d, *J* 2.0), 6.26 (1 H, d, *J* 2.0), 5.85–5.88 (2 H, m), 4.09 (1 H, dd, *J* 9.1 and 7.2), 3.97 (2 H, q, *J* 7.0), 3.80 (1 H, dd, *J* 9.0 and 7.6), 2.85 (1 H, dd, *J* 13.9 and 5.1), 2.80 (1 H, dd, *J* 13.9 and 6.3), 2.36–2.65 (4 H, m), 1.36 (3 H, t, *J* 7.1); *m/z* (EI) 420.2047 (M^+, 100%. $C_{23}H_{20}{}^2H_6O_7$ requires 420.2055), 419 (0.0), 418 (0.0), 417 (0.0), 416 (1.0), 415 (1.0), 414 (0.0), 284 (12.3), 201 (92.0), 173 (30.9), 135 (46.9) 131 (12.4).

Synthesis of dibenzylbutane lignans. (±)-[3-OC^2H_3]Secoisolariciresinols (**1**-d_3) and (±)-[7,7-$^2H_2$, 3-OC^2H_3]secoisolariciresinols (**1**-d_5) were prepared by reduction of (±)-[3-OC^2H_3]-matairesinols (**2**-d_3) and (±)-[7,7-$^2H_2$, 3-OC^2H_3]matairesinols (**2**-d_5), respectively, as previously.[44] (**1**-d_3): δ_H ($CDCl_3$) 6.79 (2 H, d, *J* 7.8), 6.55–6.65 (4 H, m), about 3.82 (2 H), 3.80 (3 H, s), 3.54 (2 H, dd, *J* 11.3 and 4.3), 2.73 (2 H, dd, *J* 13.7 and 8.1), 2.63 (2 H, dd, *J* 13.7 and 6.2), 1.85 (2 H, m); *m/z* (EI) 365.1935 (M^+, 18.0%. $C_{20}H_{23}{}^2H_3O_6$ requires 365.1917), 364 (2.9), 353 (3.3), 362 (2.7), 347 (11.8), 192 (8.6), 189 (9.2), 140 (100), 137 (98.7), 122 (19.4). (**1**-d_5): δ_H ($CDCl_3$) 6.73 (2 H, d, *J* 8.0), 6.50–6.58 (4 H, m), about 3.75 (2 H), 3.74 (3 H, s), 3.48 (2 H, dd, *J* 11.5 and 4.4), 2.67 (1 H, dd, *J* 13.8 and 8.2), 2.57 (1 H, dd, *J* 13.8 and 6.7), 1.78 (2 H, m); *m/z* (EI) 367.2031 (M^+, 27.1%. $C_{20}H_{21}{}^2H_5O_6$ requires 367.2043), 366 (3.6), 365 (6.1), 364 (3.1), 363 (8.7), 362 (1.3), 349 (14.8), 194 (7.6), 189 (6.8), 142 (99.2), 137 (100), 122 (11.2).

Chemicals

L-[*ring*-$^{13}C_6$]phenylalanine (99 atom % ^{13}C),C^2H_3I (99.5 atom% ^2H), and $LiAl^2H_4$ (98 atom% ^2H) were purchased from ICON, Aldrich, and Aldrich, respectively. All chemicals used were reagent grade unless otherwise stated.

Lignans in *Anthriscus sylvestris*

Young shoots of *Anthriscus sylvestris* were harvested and washed sequentially with tap and distilled water. The plant materials thus obtained (2.4 g) were frozen (liquid N_2), freeze-dried, disintegrated with scissors, and then extracted with hot MeOH (16 ml). The MeOH extract was treated with β-glucosidase (from almonds, SIGMA G-0395, 68 units in 5.0 ml of NaOAc buffer at pH 5.0) for 24 h at 37 °C. The reaction mixture was extracted with AcOEt (3.0 ml × 3), and the solvent was evaporated off. The AcOEt extracts (16.3 mg) thus obtained were submitted to TLC separation (solvents: appropriate mixtures of AcOEt–$CHCl_3$) to afford 3 fractions. An aliquot of each fraction was subjected to GC-MS analysis after heating with 14 μ l of *N,O*-bis(trimethylsilyl)acetamide (BSA) for 45 min at 60 °C[44] so that lignans present in the fractions were identified. In a separate experiment, freeze-dried, aerial parts (including leaves, petioles and racemes) of *A. sylvestris* (39.8 g) were pulverized and extracted with hot MeOH as above but scaled up proportionately. The MeOH extract (17.3 g) thus obtained was treated with β-glucosidase and the reaction products were extracted with AcOEt. The AcOEt extract (1.2 g) was subjected to repeated silica gel column and thin layer chromatography to afford pure yatein (**11**) (90.2 mg) and deoxypodophyllotoxin (17.9 mg).

Feeding experiments

Administration of lignans to *Anthriscus sylvestris*: deuterium labelled lignans (0.4 mg each), (±)-[3-OC^2H_3]matairesinols (**2**-d_3), (±)-[3-OC^2H_3]thujaplicatins (**3**-d_3), (±)-[3,5-($OC^2H_3)_2$]-5-methylthujaplicatins (**4**-d_6), (±)-[3,5-($OC^2H_3)_2$]-4,5-dimethylthujaplicatins (**5**-d_6), (±)-[3-OC^2H_3]pluviatolides (**6**-d_3), (±)-[3-OC^2H_3]-5-hydroxypluviatolides (**7**-d_3), (±)-[3,5-($OC^2H_3)_2$]-4-demethylyateins (**8**-d_6), (±)-[3,4-($OC^2H_3)_2$]bursehernins (**9**-d_6), and (±)-[3,4-($OC^2H_3)_2$]-5-demethylyateins (**10**-d_6), were dissolved individually in MeOH or DMSO (62 μl) and dispersed in distilled water (438 μl). Young shoots (about 3 cm long) of *A. sylvestris* were cut by means of scissors, and the cut end of each shoot was placed directly in each solution of the deuterium labelled compounds. Following uptake, each shoot was metabolized for 3 d and freeze-dried. The resulting dried material was hand-disintegrated using scissors and extracted with hot MeOH. The MeOH extract was treated with β-glucosidase and was submitted to trimethylsilylation followed by GC-MS analysis. The peaks on GC corresponding to the lignans (**1**) (t_R = 10.6 min), (**2**) (t_R = 15.3 min), (**3**) (t_R = 17.5 min), (**4**) (t_R = 18.4 min), (**5**) (t_R = 18.9 min), (**6**) (t_R = 14.6 min), (**7**) (t_R = 16.6 min), (**8**) (t_R = 17.4 min), (**9**) (t_R = 15.2 min), (**10**) (t_R = 16.1 min), (**11**) (t_R = 18.3 min), and (**12**) (t_R = 16.9 min) were analyzed for ^{13}C or deuterium incorporation.

In separate experiments, individual administration of (±)-[3-OC^2H_3]secoisolariciresinols (**1**-d_3), (±)-[3-OC^2H_3]matairesinols (**2**-d_3), (±)-[3-OC^2H_3]pluviatolides (**6**-d_3), and (±)-[3,4-($OC^2H_3)_2$]bursehernins (**9**-d_6) was conducted exactly as above but scaled up proportionately ten times using ten shoots and the same concentration of the solutions. Following uptake, the shoots were metabolized for 7 d and then freeze-dried and extracted with hot MeOH. The MeOH extracts were treated with β-glucosidase and the reaction products were extracted with AcOEt as above.

The AcOEt extracts obtained after the administration of (±)-[3-OC^2H_3]pluviatolides (**6**-d_3) and (±)-[3,4-($OC^2H_3)_2$]bursehernins (**9**-d_6) were treated with an excess of diazoethane followed by TLC separation (solvents: appropriate mixtures of AcOEt–$CHCl_3$) to give the following two fractions: Fr. 1 corresponding to 4-demethylyatein (**8**) and 5-demethylyatein (**10**), and Fr. 2 corresponding to 5-hydroxypluviatolide (**7**) and yatein (**11**). The whole of each fraction was then dissolved in 14 μl of BSA for 45 min at 60 °C[44] and analyzed by GC-MS. At the retention times corresponding to the authentic ethylated lignans (±)-4,5-diethyl-5-hydroxypluviatolides (4,5-DiEt-**7**) (t_R = 18.6 min), (±)-4-ethyl-4-demethylyateins (4-Et-**8**) (t_R = 18.5 min), (±)-[3,4-($OC^2H_3)_2$]-5-ethyl-5-demethylyateins (5-Et-**10**-d_6) (t_R = 18.3 min), and (±)-yateins (**11**) (t_R = 18.3 min), mass spectra were analyzed for deuterium incorporation.

The AcOEt extracts so obtained following administration of (±)-[3-OC^2H_3]secoisolariciresinols (**1**-d_3) and (±)-[3-OC^2H_3]-matairesinols (**2**-d_3) were submitted individually to TLC separation (solvents: appropriate mixtures of AcOEt–$CHCl_3$) to give the following three fractions: Fr. 1 corresponding to pluviatolide (**6**), 4-demethylyatein (**8**), bursehernin (**9**), 5-demethylyatein (**10**), and yatein (**11**); Fr. 2 corresponding to matairesinol (**2**), 5-methylthujaplicatin (**4**), 4,5-dimethylthujaplicatin (**5**), 5-hydroxypluviatolide (**7**), and 4-methylthujaplicatin (**12**); and Fr. 3 corresponding to secoisolariciresinol (**1**) and thujaplicatin (**3**). The whole of each fraction was then dissolved in 14 μl of BSA for 45 min at 60 °C[44] and subjected to GC-MS analysis to determine deuterium incorporation into lignans.

Administration of L-[*ring*-$^{13}C_6$]phenylalanine: L-[*ring*-$^{13}C_6$]-phenylalanine (1.0 mg) was dissolved in 0.1 M KPB buffer (pH 7.0, 217.0 μl), and administered to ten young shoots. Following uptake and metabolism for 7 d, the plant materials were submitted to GC-MS analysis after TLC purification exactly as above. Simultaneous administration of two distinct lignans: A solution of a mixture of (±)-[3-OC^2H_3]pluviatolides (**6**-d_3) (2.0

mg) and (±)-[7,7-$^2H_2$, 3-OC^2H_3]secoisolariciresinols (**1**-d_5) (2.0 mg) in a mixture of DMSO (620 µl) and distilled water (4380 µl) was administered to ten young shoots of *A. sylvestris*. Following uptake and metabolism for 7 d, the plant materials were treated with β-glucosidase and were submitted to GC-MS analysis after TLC purification exactly as above. Simultaneous administration of (±)-[3-OC^2H_3]thujaplicatins (**3**-d_3) and (±)-[7,7-$^2H_2$, 3-OC^2H_3]secoisolariciresinols (**1**-d_5) were conducted exactly as above.

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