0031-9422(94)00683-0

Phytochemistry, Vol. 38, No. 3, pp. 615–621, 1995

0031-9422/95 $9.50 + 0.00

BIOSYNTHESIS OF SECOIRIDOIDS IN *FONTANESIA*

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(*Received* 7 *July* 1994)

Key Word Index—*Fontanesia fortunei*, *F. phillyreoides*; Oleaceae; secoiridoid glucosides; secologanic acid; loganic acid; biosynthesis; ^{2}H labelled precursors; 7-(*p*-coumaroyl)-5-hydroxysecologanol; verbascoside; chlorogenic acid.

Abstract—*Fontanesia fortunei* was found to contain the new secoiridoid glucoside 7-*E*/*Z*-(*p*-coumaroyl)-5-hydroxysecologanol together with the known compounds secologanic acid, secologanol, secologanoside, fontanesioside, verbascoside and chlorogenic acid. This secoiridoid profile is different from that found in other known genera of the Oleaceae, since no oleoside-derived compounds were present. Deuterium labelled deoxyloganic acid, 7-*epi*-loganic acid and loganic acid were administered to *F. fortunei* and *F. phillyreoides* and the incorporations into secologanic acid in both plants measured by ^{2}H NMR. Feeding experiments with specifically labelled compounds showed that the hydroxylation of deoxyloganic acid to give loganic acid takes place with retention of the 7α-hydrogen and that the latter also is retained during the ring cleavage to secologanic acid.

INTRODUCTION

The genus *Fontanesia* was originally placed within the tribe Fraxineae by Knowblauch [1], but Taylor found its chromosome number to be $n = 13$ compared with $n = 23$ in *Fraxinus* spp., suggesting a more definite separation of these genera [2]. In addition, the basic morphological features of fruits and flowers showed little resemblance with those of *Fraxinus* and other genera of the subfamily Oleoideae, thus *Fontanesia* and *Abeliophyllum* were segregated as the tribe Fontanesieae [2]. However, Johnson later transferred *Abeliophyllum* to Forsythieae as indicated by their common chromosome number ($n = 14$) leaving Fontanesieae as a monotypic tribe in the subfamily Jasminoideae [3].

In a preceding paper [4], we reported on the iridoid content of one of the two recognized species of *Fontanesia*, namely *F. phillyreoides*. Loganic acid (**1**), secologanic acid (**2**), secologanol (**3**), secologanoside (**4**) and the 5-hydroxylated derivatives 5-hydroxysecologanol (**5**), swertiamarin (**6**) and fontanesioside (**7**) were found as the only iridoid constituents. Thus, the oleoside-type secoiridoids (**8**) often present in oleaceous plants were absent, but the species did contain verbascoside which is almost ubiquitous in the Oleaceae.

The biosynthesis of oleosides in *Fraxinus excelsior* and *Syringa josikaea* has been investigated [5]. The route leading to oleoside derivatives (**8**) involves 7-ketologanic acid (**9**), since 7-*epi*-loganic acid (**10**) and loganic acid (**1**) were found to be equally efficient precursors. However, the isolated metabolites from *F. phillyreoides* point to a different biosynthetic pathway leading directly from **1** to **2** with subsequent elaboration of the latter (Scheme 1). Thus, **9** is probably not involved in the biogenesis of iridoids in this genus. Intrigued by the unexpected occurrence of secologanin-type secoiridoids (and the concomitant absence of oleosides, **8**) in *Fontanesia*, an investigation of the iridoid biosynthesis in the two recognized species, *F. fortunei* and *F. phillyreoides*, was undertaken to test the hypothetical pathway outlined above. The present paper reports on the compound obtained from work-up of *F. fortunei* and the results from the biosynthetic experiments performed on the genus *Fontanesia*.

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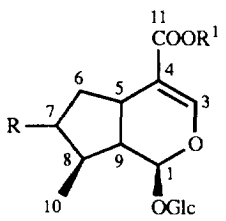
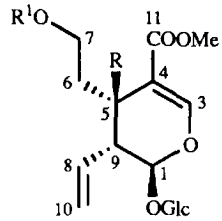
RESULTS AND DISCUSSION

Investigation of plant material

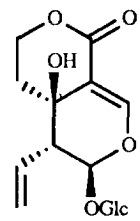
The water-soluble extract of the aerial parts of *F. fortunei* was fractionated by reverse phase chromatography giving successive fractions of secologanic acid (**2**), secologanol (**3**), verbascoside and fontanesioside (**7**). In addition, a less polar fraction was obtained, apparently containing a mixture of secoiridoids with a 5-hydroxysecologanol nucleus. In order to isolate these compounds in a pure state, a larger batch was worked-up in a similar way. On chromatography only the least polar fraction was collected. But attempts to separate the components of this mixture by TLC failed, since the two developed bands both yielded a mixture of two components [**11** and **12**, i.e. a *cis*–*trans* mixture of 7(*p*-coumaroyl)-5-hydroxysecologanol] upon work-up. This led to the



	R	R^1
1	β - OH	H
10	α - OH	H
14	H	H
18	α - OH	Me
20	β - OH	Me



	R	R^1
3	H	H
5	OH	H
7	OH	p - OH - benzoyl
11	OH	cis - p - coumaroyl
12	OH	trans - p - coumaroyl



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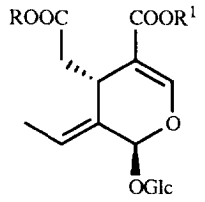

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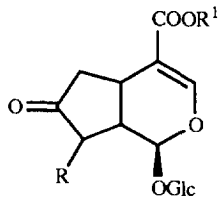
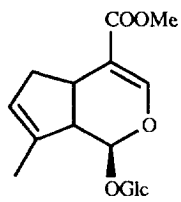


	R	R^1
9	β - Me	H
17	β - Me	Me
19	α - Me	Me



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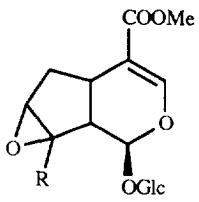
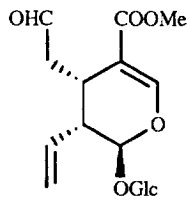


	R
15	β - Me
16	α - Me



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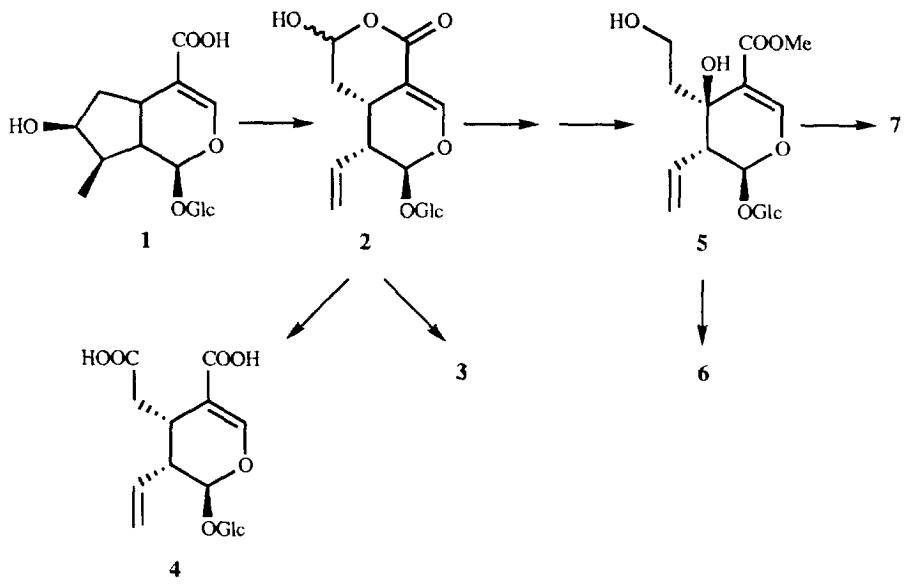
conclusion that the preparation consisted of an interconvertible isomer pair. Confirmation was reached by a thorough examination of the ^{1}H NMR spectrum where two sets of signals were assignable to a *cis*- and a *trans*-*p*-coumaroyl moiety, respectively. Even when daylight was excluded during development of the TLC plates and the subsequent work-up of the bands, no significant separation was obtained, indicating the *cis–trans* equilibrium to be extremely sensitive to light or to the weakly acidic silica gel. Likewise, the ^{13}C NMR spectra of **7** and the pair **11/12** exhibited almost identical signals corresponding to the secoiridoid part of the molecules (Table 1). Also, two sets each of nine carbons present in the ^{13}C NMR spectrum were attributable to the *cis–trans* isomers of a *p*-coumaroyl ester. Owing to their rapid isomerization, the glucosides **11/12** were characterized as a mixture. In an attempt to obtain more stable derivatives, and thus facilitate their separation, methylation with diazomethane was attempted. However, the reaction proved to be sluggish, and the resultant long reaction time led to considerable degradation of the secoiridoids. Finally, acetylation afforded the mixture **11b/12b** which was separable by TLC, but still proved to be too unstable to keep in isomerically pure state, and characterization was thus limited to NMR.

Synthesis of labelled precursors

Starting from deoxy[6,10-^{2}H]geniposide (**13**), deoxy[6,7,8,10-^{2}H]loganic acid (**14**) was prepared as described previously [16]. Epoxidation of the tetraacetate, [6,10-^{2}H]-**13a**, yielded both possible isomers, [6,10-^{2}H]-**15a** and [6,10-^{2}H]-**16a**, where the latter was rearranged (with loss of most of the labelling at C-6) to 7-keto[10-^{2}H]loganic tetraacetate (*d*-**17a**) [5].



Scheme 1.

Table 1. ^{13}C NMR spectral data of compounds **7**, **11** and **12** (CD_3OD, 62.5 MHz)

C	7	11	12
1	97.6	97.5	97.6
3	153.2	153.1	153.1
4	111.5	111.3	111.5
5	68.7	68.6	68.6
6	35.5	35.2	35.4
7	60.6	60.1	60.4
8	132.3	132.4	132.4
9	51.9	51.7	51.7
10	120.0	120.0	120.0
11	167.1	167.5	168.1
OMe-11	50.7	50.8	50.8
1′	98.8	98.8	98.8
2′	73.3	73.3	73.3
3′	76.7	76.7	76.7
4′	70.3	70.3	70.3
5′	77.3	77.2	77.2
6′	61.5	61.5	61.5
1″	121.6	126.5	125.9
2″/6″	132.5*	130.0*	130.0*
3″/5″	116.1*	114.7*	115.7*
4″	163.8	158.8	160.1
α		115.6	114.0
β		143.8	145.4
C=O	168.4	167.1	167.4

*Doubled intensity.

Three selectively labelled samples of loganic acid (**1**) were needed for the feeding experiments. Synthesis of [10-^{2}H]loganic acid (**1**) was performed as follows: First, reduction of [10-^{2}H]-**17a** with sodium borohydride gave 7-epi-[10-^{2}H]loganin tetraacetate (**18a**), which in turn was subjected to a Mitsunobu inversion followed by hydrolysis to give [10-^{2}H]-**1**. Likewise, [7-^{2}H]-**1** was prepared from unlabelled **17a** using sodium borodeuteride in the reduction step. Finally, [6,7,8-^{2}H]loganic acid (**1**) was prepared from the α-epoxide **15a**: rearrangement gave 8-epi-7-ketologanin tetraacetate (**19a**), which on treatment with sodium methoxide in mono-deutero methanol underwent concomitant deacetylation, epimerization (at C-8) and ^{2}H exchange to give [6,8-$^2H_2$]-**17**. Subsequent acetylation to [6,8-$^2H_2$]-**17**, reduction with sodium borodeuteride, and further elaboration as above gave [6,7,8-^{2}H]loganic acid (**1**). Deacetylation and saponification of 7-*epi*-[10-^{2}H]loganin tetraacetate (**18a**) gave [10-^{2}H]-**10**.

Biosynthetic experiments

A biosynthetic route (briefly reviewed in [5]) involving the direct conversion of either loganin (**20**) or loganic acid (**1**) to secologanin (**21**) or secologanic acid (**2**) with retention of both H-7 and H-8 has been established in species from the secoiridoid-containing families of the Gentianales and Cornanae [7, 8]. As stated in the introduction, the compounds found in *Fontanesia* seem to arise from a similar biosynthetic route, contrary to the secoiridoids in the other investigated species of the Oleaceae, where 7-*epi*-loganic acid (**10**) and loganic acid (**1**) are equally efficient precursors [5].

In order to study this, deoxyloganic acid (**14**), 7-*epi*-loganic acid (**10**) and loganic acid (**1**) were tested as precursors in *F. fortunei*. Thus, hydroponic feeding experiments on freshly cut leaves using [6,7,8,10-^{2}H]-**14**, [10^{2}H]-**1** and [10-^{2}H]-**10** were performed (experiments nos 1–3 in Table 2). Work-up followed by reverse phase chromatography yielded fractions containing **1**–**3**, **3**, **7** and **11**/**12**. Although the two first fractions were not entirely pure, they were also analysed by ^{2}H NMR spectrometry.

Table 2. Results from biosynthetic experiments with *F. fortunei* and *F. phillyreoides*

Experiment	1	2	3	4	5	6	7	8
	d_4-**14***	[10-^{2}H]-**10**	[10-^{2}H]-**1**	[7-^{2}H]-**1**	d_4-**14***	d_3-**1**†	d_4-**14***	d_3-**1**†
Precursor (mg)	19	15	16	14	17	25	15	23
Epimeric purity (%)	> 98	> 99	> 99	> 99	92	> 99	92	> 99
Plant (g)	18	17	17	14	14	16	9.5	8
Metabolic period (days)	6	6	6	4	6	6	6	6
Loganic acid‡ (**1**; mg)	9	—	8	7	—	18	—	6
Incorporation (%)	14	—	—	(3)	8.5	(12.5)	—	(11.5)
Secologanic acid§ (**3**; mg)	152	122	127	102	119	191	48	62
Incorporation (%)‖	19	—	16	—	20	nm	8	14
2a/b (mg)	—	—	—	124	133	232	—	—
Incorporation(%) ‖	—	—	—	8	15	15	—	—

*d_4 = [6,7,8,10-^{2}H].

†d_3 = [6,7,8-^{2}H].

‡Impure and in experiment 5 not separated from **2** (see Experimental for purification).

§Impure; in experiment 6 collected in admixture with **6.**

‖The given value is the calculated incorporation when no isotope effects are taken into consideration. If there was total preference for ^{1}H over ^{2}H in the abstraction process the incorporations would only be 75% of the above stated values.

nm: Not measured as the signal from H-7 in **2** was obscured by the HOD signal.

No incorporations could be found in **3**, **7** or **11/12**, but deoxy[6,7,8,10-^{2}H]loganic acid (**14**) gave high incorporations into both **1** and **2**, 14 and 19%, respectively. In the latter case this was a maximum value, however, since conversion to **2** requires a hydrogen abstraction at C-10 in the formation of the 8,10-double bond, and it is not known whether any isotope effect is operative (see footnote to Table 2). Likewise, [10-^{2}H]loganic acid (**1**) gave 16% incorporation into **2**, while 7-epi-[10-^{2}H]loganic acid (**10**) was not incorporated; instead it was re-isolated in undiluted form in 39% yield. This excludes a pathway (via **10**) similar to that leading to the oleoside derivatives (**8**) found in *Syringa* and *Fraxinus* [5]. Hence, the above route established in the Gentianales seems also to be operative in *Fontanesia.*

To investigate whether the ring cleavage proceeds with retention of H-7 or not, we next administered [7-^{2}H]-**1** (experiment 4). The isolated secologanic acid (**2**) was acetylated yielding an epimeric mixture of lactol pentaacetates (**2a/b**). The H-7 signal (δ6.5) showed that there had been an incorporation of 8%.

The purpose of the next experiment was to demonstrate the so far assumed conservation of H-8 during the ring cleavages of **1** (to **2**). Here, it was necessary to use as a precursor, loganic acid (**1**), labelled at C-8 but not at C-10 in order to avoid overlap of the signals of the vinyl group in the metabolite (**2**). Hence, the precursor [6,7,8-^{2}H]-**1** was used in this experiment (No. 6), the label at C-7 serving as a reference. After feeding and biotransformation, labelled **2** was obtained. Upon acetylation (to the anomeric mixture **2a/b**) the incorporation was found to be 15% when calculated from the ^{2}H-8 signal (δ5.5). The ratio of deuterium integrals $I(7):I(8) = 0.8$ for the isolated **2a/b** indicated that only a small loss of labelling from C-7 had occurred, as the precursor employed was fully labelled at C-6, C-7 and C-8 [i.e. $I(7):I(8) = 1.0$].

It also proved possible to obtain more information about the 7-hydroxylation. The experiment with deoxy[6,7,8,10-^{2}H]loganic acid (**14**) with the following approximate ^{2}H labelling-ratio: $I(7\alpha):I(7\beta):I(8\alpha):I(10) = 0.75:0.25:0.8:1.9$ was repeated (experiment 5). The isolated loganic acid (**1**) showed 8.5% incorporation at the 10-methyl group at δ1.0 as measured by the ^{2}H integrals. After acetylation, the purified pentaacetate (**1a**) according to the ^{2}H integrals and a ^{2}H labelling ratio of $I(7):(8\alpha):I(10) = 0.6:0.7:1.9$. In view of the amount of labelling found in the 7-position, and allowing for the expected experimental error in the measurement of the ^{2}H integrals of *ca* 10%, we conclude that the 7α-proton in **1a** (and **1**) must be derived from the 7α-proton in **14**. Thus, the hydroxylation of deoxyloganic acid (**14**) proceeds with retention of configuration. Similar results were obtained with *F. phillyreoides* (experiment 7 and 8) showing the pathway leading from **14** to **2** via **1** to be general in this genus.

The lack of incorporation beyond compound **2** (Scheme 1) may either be caused by a slow biosynthetic turnover of the relatively large pool of **2** (i.e. a dilution phenomenon) or a regulatory inhibition of the steps after **2** because of the introduction of abnormally high levels of early intermediates (e.g. **1** and **14**) in these feeding experiments.

CONCLUSIONS

We have demonstrated that the biosynthesis of secoiridoids in the genus *Fontanesia* is different from that found in the remaining part of the Oleceae, where 7-ketologanin (**17**) or 7-ketologanic acid (**9**) have been shown to be the intermediates leading to the secoiridoids of the oleoside-type [5]. As earlier noted [4], *Fontanesia* might, therefore, chemotaxonomically be considered to be a link

to the Gentianales where secologanin-derived compounds are almost ubiquitous [8].

EXPERIMENTAL

General. Mps: uncorr.; ^{1}H NMR (250 MHz): D_2O using HOD-signal at 4.75 ppm as int. standard, acetates were recorded in $CDCl_3$ ($CHCl_3$ signal at 7.27 ppm); ^{13}C NMR (62.5 MHz): C-6′ was set to 61.5 ppm as standard [9]; ^{2}H NMR: 77 MHz, glucosides in H_2O with 0.0156% ^{2}H of natural abundance, and acetates in $CHCl_3$ with 0.017% ^{2}H of natural abundance; prep. TLC: 20 × 40 cm plates coated with 1 mm layers of silica gel PF_{254} (Merck), bands were detected in UV-light (254 nm); reversed phase MPLC: Merck Lobar C-18 columns size B and C, H_2O–MeOH mixts were used as eluents and peaks were detected by UV (240 nm).

Plant material. Foliage of *F. phillyreoides* and *F. fortunei* was obtained from The Botanical Garden of Copenhagen and voucher specimens (no. IOK-15-93 and IOK-16-93) are deposited at The Botanical Museum, Copenhagen.

Work-up of plant material. Cut shoots and single leaves (17 g) of *F. fortunei* were kept in a beaker with H_2O for 4 days. Then they were blended with EtOH (350 ml). The filtrate was concd *in vacuo* yielding a syrup, which was partitioned in H_2O–Et_2O (2:3; 250 ml). The aq. layer was evapd *in vacuo* and the residue dissolved in MeOH (50 ml) and passed through act. C (1.5 g) over Celite. Evapn *in vacuo* of the filtrate afforded a foam (1.02 g), which was dissolved in 10% HOAc and subjected to MPLC (B-column; 25:1 to 1:1). First a mixt. (5:1, 5 mg) of unidentified iridoids was obtained. Elution with 3:1 gave almost pure secologanic acid (**2**; 119 mg; 0.7%), whereas continued elution with 2:1 afforded secologanol (**3**; 11 mg). Verbascoside (105 mg; 0.6%) was collected when eluting with 3:2. Then came fontanesioside (1:1, **7**; 97 mg; 0.6%) followed by a fr. (63 mg) apparently containing a mixt. of secoiridoids.

Work-up of a large batch. Fresh foliage (200 g) of *F. fortunei* was similarly worked-up giving a syrup (11 g), which was submitted MPLC (C-column; 2 portions; 5:1 to 2:3). Only the apolar frs were collected and evapd *in vacuo* to give crude **7** (1:1; 1.42 g) and fr. A (2:3; 0.62 g). Fr. A was sepd into apparently two components (according to ^{1}H NMR) by repeated prep. TLC ($CHCl_3$–MeOH 5:1; R_f 0.56 and 0.63 after two developments), but upon work-up (EtOAc) both bands yield *cis–trans* mixts that were combined (200 mg). Further attempts to carry out the purification in the dark, again yielded an amorphous *cis–trans* mixt. of 7-(*p*-coumaroyl)-5-hydroxysecologanol (**11/12**; 149 mg; *cis/trans ca* 2:3). $C_{26}H_{32}O_{13}\cdot 2H_2O$ requires H 6.17, C 53.06, found H 6.03, C 53.23%.

trans-*Isomer* (**12**). ^{1}H NMR (250 MHz; CD_3OD): δ7.61 (*s*, H-3), 5.77–5.67 (1H, obsc. by H-1's and H_{cis}-α, H-8), 5.71 (*d*, *J* = 5.5 Hz, H-1), 5.42 (*dd*, *J* = 17.5 and 2 Hz, H_a-10), 5.34 (*dd*, *J* = 10.5 and 2 Hz, H_b-10), 4.38 (*t*, 2H, *J* = 7.5 Hz, 2 × H-7), 3.74 (*s*, 3H, 11-OMe), 2.87 (*dd*, *J* = 9 and 5.5 Hz, H-9), 2.60 (*m*, H_a-6), 2.22 (*m*, H_b-6), 4.70 (*d*, *J* = 8 Hz, H-1′), 3.94 (*dd*, *J* = 12 and 2 Hz, H_a-6′), 3.75-3.30 (*m*, 4H, H_b-6′, H-3′, H-5′ and H-4′), 3.24 (*dd*, *J* = 9 and 8 Hz, H-2′), 7.62 (*d*, *J* = 16 Hz, H-β″), 7.49 (*d*, 2H, *J* = 8.5 Hz, H-2″ and H-6″), 6.85 (*d*, 2H, *J* = 8.5 Hz, H-3″ and H-5″), 6.31 (*d*, *J* = 16Hz, H-α″); ^{13}C NMR: Table1.

cis-*Isomer* (**11**). ^{1}H NMR (250 MHz; CD_3OD): δ7.62 (*s*, H-3), 5.67 (*d*, *J* = 5.5 Hz, H-1), 5.70–5.60 (1H, obsc. by H-1′ s, H-8), 5.40 (*dd*, partly obsc. by the *trans*-isomer, *J* = 17.5 and 2 Hz, H_a-10), 5.32 (*dd*, *J* = 10.5 and 2 Hz, H_b-10), 4.32 (*t*, 2H, *J* = 7,5 Hz, 2 × H-7), 3.74 (*s*, 3H, 11-OMe), 2.83 (*dd*, partly obsc. by the *trans*-isomer, *J* = 9 and 5.5 Hz, H-9), 2.54 (*m*, H_a-6), 1.97 (*m*, H_b-6), 4.70 (*d*, *J* = 8 Hz, H-1′), 3.94 (*dd*, *J* = 12 and 2 Hz, H_a-6′), 3.75-3.30 (*m*, 4H, H_b-6′, H-3′, H-5′ and H-4′), 3.24 (*dd*, *J* = 9 and 8 Hz, H-2′), 7.62 (*d*, 2H, *J* = 8.5 Hz, H-2″ and H-6″), 6.89 (*d*, partly obsc. by H-3″/H-5″ of the *trans*-isomer, *J* = 13 Hz, H-β″), 6.79 (*d*, 2H, *J* = 8.5 Hz, H-3″ and H-5″), 5.76 (*d*, *J* = 13 Hz, H-α″); ^{13}C NMR: Table 1.

Methylation and acetylation of mixture **11/12**. The mixture **11/12** (125 mg) was methylated (excess CH_2N_2–Et_2O in MeOH) overnight as methylation for 2 hr twice did not give a 100% conversion. MPLC (B-column; 1:1 and 1:2) afforded a mixt. of methylated derivatives **11a/12a** (63 mg) upon elution with 1:2. Acetylation (pyridine–Ac_2O 1:1; 2 ml; 2 hr at room temp.) yielded a crude mixt. of tetraacetates **11b/12b** (101 mg). Purification by prep. TLC (Et_2O) gave almost pure **12b** (R_f 0.46; 28 mg) and **11b/12b** in a 1:1 mixt. (R_f 0.54; 29 mg).

trans-*Isomer* (**12b**). ^{1}H NMR (250 MHz; $CDCl_3$): δ7.44 (*s*, H-3), 5.56 (*dt*, *J* = 17.5 and 2 × 9.5 Hz, H-8), 5.42 (*d*, *J* = 4.5 Hz, H-1), 5.41 (*dd*, *J* = 17.5 and 2 Hz, H_a-10), 5.34 (*dd*, *J* = 10 and 2 Hz, H_b-10), 4.37 (*t*, 2H, *J* = 7.5 Hz, 2 × H-7), 3.83 (*s*, 3H, phenolic OMe), 3.75 (*s*, 3H, 11-OMe), 2.89 (*dd*, *J* = 9.5 and 4.5 Hz, H-9), 2.63 (*dt*, *J* = 14.5 and 2 × 7.5 Hz, H_a-6), 1.90 (*dt*, *J* = 14.5 and 2 × 7.5 Hz, H_b-6), 2.09, 2.03, 2.01 and 1.98 (*s*, each 3H, 4 × Ac-Me), 5.25 (*t*, *J* = 9.5 Hz, H-3′), 5.10 (*t*, *J* = 10 Hz, H-4′), 5.02 (*dd*, *J* = 9.5 and 8 Hz, H-2′), 4.86 (*d*, *J* = 8 Hz, H-1′), 4.28 (*dd*, *J* = 12.5 and 4.5 Hz, H_a-6′), 4.15 (*dd*, *J* = 12.5 and 2.5 Hz, H_b-6′), 3.78–3.70 (1H, obsc. by 11-OMe, H-5′), 7.60 (*d*, *J* = 16 Hz, H-β″), 7.45 (*d*, 2H, *J* = 9 Hz, H-2″ and H-6″), 6.89 (*d*, 2H, *J* = 9 Hz, H-3″ and H-5″), 6.25 (*d*, *J* = 16 Hz, H-α″).

cis-Isomer (**11b**). ^{1}H NMR (250 MHz; $CDCl_3$): δ7.40 (*s*, H-3), 4.30 (*t*, 2H, *J* = 7.5 Hz, 2 × H-7), 2.85 (*dd*, *J* = 9.5 and 4.5 Hz, H-9), 2.55 (*dt*, *J* = 14.5 and 2 × 7.5 Hz, H_a-6), 1.87 (*dt*, *J* = 14.5 and 2 × 7.5 Hz, H_b-6), 7.67 (*d*, 2H, *J* = 9 Hz, H-2″ and H-6″), 6.87 (*d*, 2H, *J* = 9 Hz, H-3″ and H-5″), 6.81 (*d*, *J* = 13 Hz, H-β″), 5.76 (*d*, *J* = 13 Hz, H-α″), the remaining signals were identical to those of the *trans*-isomer.

[6,10-^{2}H]-**13** *and* [6,7,8,10-$^2H_4$]-**14**. These compounds were prepared as described previously [6]. The ^{2}H content was determined by ^{1}H NMR, thus, in [6,10-^{2}H]-**13** 0.9 ^{2}H was present at C-10 while less than 0.2 ^{2}H was seen at C-6, and [6,7,8,10-^{2}H]-**14** was found to have the following labelling pattern: C-6 (1.0 ^{2}H; α:β *ca* 1:1), C-7 (*ca* 1.0 ^{2}H; α:β *ca* 3:1), C-8 (*ca* 0.8 ^{2}H), C-10 (1.9 ^{2}H).

[10-^{2}H]*Loganic acid* (**1**). The tetraacetate [10-^{2}H]-**18a** (247 mg; [5]), Ph_3P (408 mg) and HOAc (86 μl) were mixed in THF (10 ml). Diethyl azodicarboxylate (DEAD;

240 μl) was added dropwise to the above soln, which was allowed to stand at room temp. for 16 hr (50% conversion) when the same amounts of all reagents were added once more. After an additional 8 hr, the soln was evapd *in vacuo* and the residue purified by prep. TLC (Et_2O) giving the pentaacetate [10-^{2}H]-**20a** (R_f 0.63; 228 mg; 86%) upon work-up (EtOAc).

[10-^{2}H]-**20a** (287 mg) was deacetylated in 0.1 M methanolic NaOMe (10 ml) for 1 hr, at which time H_2O (10 ml) was added and most of the MeOH removed by evapn *in vacuo*. Upon standing at room temp. for 6 hr (only partial hydrolysis took place according to analytical HPLC) HOAc was added to pH 6. MPLC (B-column; 6:1 to 3:2) gave [10-^{2}H]-**1** (3:1; 84 mg; 47%) and [10-^{2}H]-**20** (2:1; 55 mg; 30%). ^{1}H NMR showed the presence of 0.9 ^{2}H at C-10 in both compounds.

7-epi-[10-^{2}H]*Loganic acid* (**10**). Preparation as in ref. [5] gave [10-^{2}H]-**10** containing 0.9 ^{2}H at C-10.

[7-^{2}H]*Loganin pentaacetate* [7-^{2}H]-**20a**). 7-Ketologanin tetraacetate (**17a**; 158 mg; 0.28 mmol) was evapd twice with EtOD (2 × 2 ml) and then dissolved in EtOD (7 ml). After addition of $NaBD_4$ (12 mg; 0.28 mmol) the soln was stirred for 1.5 hr. Excess $NaBD_4$ was quenched by HOAc. Evapn *in vacuo* gave a residue, which was redissolved in $CHCl_3$ (15 ml) and washed with H_2O (2 × 7 ml). The org. layer was passed through act. C (0.2 g) and concd *in vacuo* to give crude [7-^{2}H]-**18a** (139 mg; 87%). Mitsunobu-inversion as above afforded [7-^{2}H]-**20a** (132 mg; 88%; 1.0 ^{2}H at C-7) which was converted into [7-^{2}H]-**1** as above.

[6,7,8-$^2H_3$]*Loganic acid* (**1**). The α-epoxide, **15a**, (0.43 g; [5]) in dry toluene (35 ml) was treated with BF_3–Et_2O (2.0 ml) for 3 min. Additional toluene (30 ml) and ice-water (50 ml) were added to the reaction mixt. and the aq. layer was re-extracted with toluene (2 × 25 ml). The combined organic layers were washed with H_2O (75 ml) and dried (Na_2SO_4). Evapn *in vacuo* yielded crude **19a** (393 mg), which was deacetylated (0.1 M NaOMe in MeOD; 4 ml) for 15 min. Addition of HOAc (to pH 6) and evapn *in vacuo* followed by acetylation pyridine–Ac_2O 1:1; 2 hr at room temp.) afforded crude [6,8-$^2H_2$]-**17a** (412 mg). A dioxane soln (25 ml) of this product was mixed with $NaBD_4$ (60 mg in 2 ml D_2O). Stirring was continued for 25 min when 10% HOAc was added. Concn *in vacuo* gave a residue, which was partitioned in $CHCl_3$–H_2O (2:1; 75 ml). The org. layer was washed with H_2O (25 ml), dried ($MgSO_4$) and evapd *in vacuo*. The residue (mainly [6,7,8-$^2H_3$]-**18a**) was directly subjected to Mitsunobu-inversion (1.35 g PH_3P; 0.3 ml HOAc; 0.8 ml DEAD; 10 ml THF). After 2.5 days the reaction mixt. was purified by prep. TLC (Et_2O) and the band at R_f 0.68 was deacetylated (NAOMe in MeOH; 1 hr at room temp. and 10 min at 50°). Addition of HOAc and evapn *in vacuo* followed by MPLC (column size C; 4:1 to 2:1) yielded [6,7,8,-$^2H_3$]-**20** (60 mg). Hydrolysis (3 ml of 1 M NaOH) for 6 hr and subsequent MPLC (B-column; H_2O, 5:1 to 3:1) of the neutralized mixt. afforded pure [6,7,8-$^2H_3$]-**1** (3:1; 46 mg), ^{1}H NMR (250 MHz; D_2O): δ7.37 (*br s*, H-3), 5.35 (*d*, *J* = 4 Hz, H-1), 4.07 (*br s*, < 0.05 ^{1}H, H-7), 2.96 (*d*, *J* = 9 Hz, H-5), 2.05 (*dd*, *J* = 9 and 4 Hz, H-9), 1.9–1.65 (*br m s*, each < 0.05 ^{1}H, H-8 and H_b-6), 1.99 (*s*, 3H, 10-Me), i.e. > 95% ^{2}H labelling at C-6, C-7 and C-8.

Feeding experiments. ^{2}H labelled precursors were dissolved in H_2O (3 ml) and the stems of the leaves (14–18 g of *F. fortunei* and 8–10 g of *F. phillyreoides*) were immersed into these solns. Over the next 2–6 hr the absorption was almost completed, and a further 1–2 ml were added and absorbed within a few hr. The water uptake was still substantial (10–15 ml) the following night, and a similar amount was absorbed per day for the remaining part of the metabolic period.

Work-up of feeding experiments. The plant batches of *F. fortunei* were worked up as described above for the small portion. Frs of **1** (slightly impure), **2**, **3**, **7** and **11**/**12** were collected. Similar work-up of the batches of *F. phillyreoides* gave a residue which was submitted to MPLC (B-column; in 10% HOAc; 10:1 to 1:1) giving frs of **1**, a 3:1-mxst. of **2** and **6**, **3** and **7**.

Purification of ^{2}H*-labelled iridoid frs.* The fr. of **2** isolated from *F. fortunei* fed with d_4-**14** showed additional incorporation possibly corresponding to **1** (10-Me at δ1.0), and upon methylation (CH_2N_2–Et_2O in MeOH) and addition of carrier (**20**; 37 mg), the mixt. was chromatographed (B-column; 4:1 to 1:1) yielding pure **20** (2:1; 40 mg) with a similar incorporation. Likewise, the only fr. with incorporation obtained from the experiment with *d*-**10** (*F. fortunei*) was methylated and subjected to MPLC (B-column; 6:1 to 4:1). Elution with 4:1 gave pure undiluted (according to ^{1}H NMR) 7-epi-[10-^{2}H]loganin (*d*-**18**; 6 mg; *ca* 40%). The fr. of **2** (102 mg) isolated from *F. fortunei* fed with [7-^{2}H]-**1** was acetylated (pyridine–Ac_2O 1:1; 2 hr at room temp.) and purified by prep. TLC (Et_2O). Work-up (EtOAc) of the major band (R_f 0.41) afforded a mixt. of 7-epimeric pentaacetates (**2a**/**b**; 124 mg). From the experiments in which *F. fortunei* were fed with [6,7,8-$^2H_3$]-**1** and d_4-**14** impure mixts of **1** and **2** were collected (119 and 191 mg, respectively). The fr. from the former experiment was acetylated and purified by prep. TLC as above giving **2a**/**b** (232 mg) and **6** (R_f 0.30; 13 mg). The fr. from the latter experiment was mixed with loganin (**20**; 25 mg) and then successively acetylated and methylated with CH_2N_2–Et_2O in Et_2O); prep. TLC (Et_2O) gave **2a**/**b** (133 mg) and **20a** (R_f 0.60; 36 mg).

Acknowledgements—We thank the Danish Natural Science Research Council for access to NMR facilities, Brdr. Hartmann's Foundation for the ^{2}H NMR probe and Bertel Hansen (The Botanical Museum of the University of Copenhagen) for verifying the plant material.

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