Pergamon

0031-9422(95)00210-3

Phytochemistry, Vol. 40, No. 3, pp. 785–792, 1995

0031-9422/95 $9.50 + 0.00

BIOSYNTHESIS OF IRIDOIDS IN *SYRINGA* AND *FRAXINUS*: CARBOCYCLIC IRIDOID PRECURSORS*

SØREN DAMTOFT, HENRIK FRANZYK and SØREN ROSENDAL JENSEN

Department of Organic Chemistry, The Technical University of Denmark, DK-2800 Lyngby, Denmark

(*Received* 19 *December* 1994)

Key Word Index—*Fraxinus excelsior*; *Syringa josikaea*; *S. vulgaris*; Oleaceae; secoiridoid glucosides; biosynthesis; oleosides; oleuropein.

Abstract—Feeding experiments with deuterium-labelled precursors to *Fraxinus excelsior*, *Syringa josikaea* and *S. vulgaris* have shown that the biosynthesis of the oleoside-type secoiridoids (e.g. oleuropein) proceeds via iridodial, iridotrial, deoxy-loganic acid aglucon and deoxy-loganic acid. Hydroxylation of the 7α-position is followed by oxidation and methylation of C-11 to give 7-ketologanin, the last carbocyclic iridoid precursor of the oleosides. The sequences of the steps between deoxy-loganic acid and 7-ketologanin may differ with plant species and time of year. 8-Epi-kingisidic acid and 8-epi-kingiside can be formed from 8-ketologanic acid (and its methyl ester). The secoiridoids, fliederoside and lilacoside, from *S. vulgaris* have been characterized.

INTRODUCTION

We have previously shown that biosynthesis of oleosides (i.e. secoiridoids with an exocyclic 8,9-olefinic functionality) differs from the biosynthesis of other secoiridoids. Both deoxyloganic acid (**1**), 7-epi-loganic acid (**2**) and loganic acid (**3**) gave high incorporations into ligstroside (**4**) in *Fraxinus excelsior*. Based on these results we postulated that 7-ketologanic acid (**5**) was a likely intermediate in the biosynthesis of oleosides [1].

In the present work, deuterium-labelled analogues of 7-epi-loganic acid (**2**), loganic acid (**3**), 7-ketologanic acid (**5**) and their methyl esters, as well as some early putative precursors, have been prepared. They were administered to three oleoside-containing members of the Oleaceae, namely *Fraxinus excelsior*, *Syringa josikaea* and *Syringa vulgaris*. The iridoids found in these species are the oleosides, ligstroside (**4**), oleuropein (**22**), 7-glucosyl 11-methyl oleoside (**15**) and oleoside 11-methyl ester (**17**), as well as the secoiridoids 8-epi-kingiside (**6**) and secologanoside (**24**). (Fig. 1). Additionally, *Fraxinus excelsior* contains oleoside dimethyl ester (**25**), 10-hydroxy-ligstroside (**27**) and excelsioside (**16**). *Syringa vulgaris* also contains **25**, 8-epi-kingiside (**6**) and large amounts of its derivatives lilacoside (**7**) and fliederoside (**8**), which here are characterized for the first time as the free glucosides. The free acid 8-epi-kingisidic acid (**23**) is present in both *S. vulgaris* and *S. josikaea*.

*Part 2 in the series Biosynthesis of iridoids in *Syringa* and *Fraxinus*; Ref. [11] is the first paper in this series.

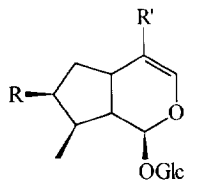
RESULTS AND DISCUSSION

Synthesis of labelled precursors

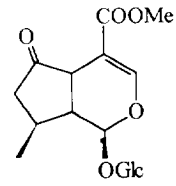
The preparation of [6,7,8,10-^{2}H]-labelled iridodial glucoside (**9**), iridotrial glucoside (**10**) and deoxy-loganic acid (**1**) was performed as previously described [2]. The aglucones of these glucosides were prepared by treatment with β-glucosidase. Deuterium-labelled 7-epi-loganic acid (**2**) and loganic acid (**3**) were prepared as described [1]. 7-Ketologanin (**11**), labelled at the 10-position, was prepared as reported earlier [1]. Most of the initial labelling at the 6-position was lost. Saponification gave labelled 7-ketologanic acid (**5**). Deuteromethyl esters were prepared by treatment of the acids in EtOD with deutero diazomethane [3].

Biosynthetic experiments with Fraxinus excelsior

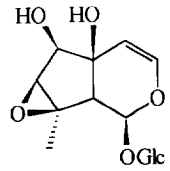
In the first set of experiments, labelled iridodial (**12**), iridotrial (**13**) and deoxyloganic acid aglucone (**14**) were administered (experiments 1–3 in Table 1). Iridodial gave a detectable incorporation (4.5%) into 7-ketologanin (**11**) only, whereas iridotrial, in addition to an incorporation into **11**, gave low incorporations (each 1%) into 7-glucosyl 11-methyl oleoside (**15**) and excelsioside (**16**). When feeding with deoxy-loganic acid aglucone (**14**), only an incorporation of 2% into excelsioside (**16**) was observed. Assuming that 7-ketologanin (**11**) is an intermediate in the biosynthesis of the oleosides, it was thus likely that the three administered compounds might be intermediates in the biosynthesis of oleosides as excelsioside (**16**). In the second set of experiments (4 and 5 in Table 1)



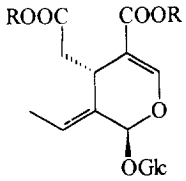
9 R = H, R' = CH_3
10 R = H, R' = CHO
19 R = OH, R' = COOMe



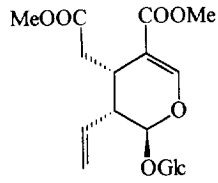
20



21

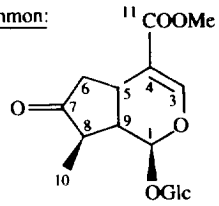


28 R = H

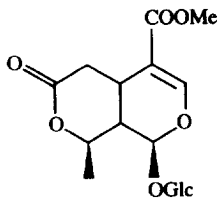


26

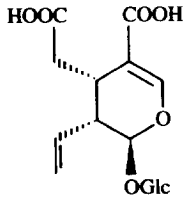
Iridoids in common:



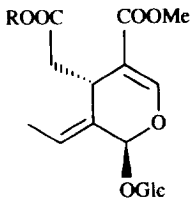
11



6

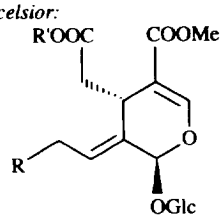


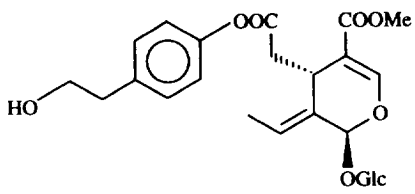
24



4 R = CH_2CH_2-(4-OH-Ph)
15 R = Glc
17 R = H
22 R = CH_2CH_2-(3,4-di-OH-Ph)

Fraxinus excelsior:

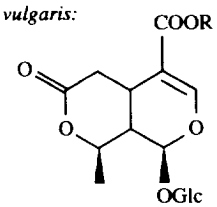




16

25 R = H; R' = Me
27 R = OH; R' = CH_2CH_2-(-OH-Ph)

Syringa vulgaris:



7 R = CH_2CH_2-(3,4-di-OH-Ph)
8 R = CH_2CH_2-(4-OH-Ph)
23 R = H (also in *S.josikaea*)

Fig. 1. Iridoids and secoiridoids in *Syringa* and *Fraxinus*.

Table 1. Results from biosynthetic experiments with *F. excelsior*

Experiment	1	2	3	4	5	6	7	8	9
Precursor	**d-12**	**d-13**	**d-14**	**d-1**	**d-5**	**d-1**	**d-19***	**d-18**	**d-11**
Labelling site(s)	6,7,8,10	6,7,8,10	6,7,8,10	6,7,8,10	10	6,7,8,10	10	10,11-OMe	10,11
(mg)	10	12	8	12	13	15	17	20	17
Epimeric purity (%)	88	83	92	92	> 99	92	> 99	> 99	> 99
Plant (g)	31	31	30	28	28	23	19	20	23
Metabolic period (days)	4	4	2.25	5	5	4	4	4	4
15 (incorp. %)		1							
6 (incorp. %)						5.5		2.5†	4.5
Amount (mg; *ca* 1 : 1)	23	39	33			21	15	18	30
17 (incorp. %)				0.5	5	3.5			3.5
24 (incorp. %)									
Amount (mg; *ca* 1 : 1)	12			5	12	11	9		15
Impure iridoid fr. (mg)	36	25	41						16
11 (incorp. %)	4.5	2.5							14
27 (incorp. %, mg)	– (71)	– (48)	(‡)			(50)	– (17)	(‡)	0.5§ (45)
Oleoside-type impurity (%)						1.5			0.5
22 (incorp. %, mg)				—	—	– (25)	(27)	0.5 (20)	– (35)
16 (incorp. %, mg)	– (68)	1(59)	2(75)	7(75)	8(82)	2.5(76)	– (57)	3(53)	3(83)
4 (incorp. %, mg)	- (47)	– (36)	(‡)	< 0.5 (30)	2.5(36)	1.5(67)	– (52)	2(56)	0.5(41)

*A fr. (14 mg) containing 42% of reisolated precursor was also obtained.
†In addition 14% reisolated precursor was present.
‡Not collected.
§Verified for the pure hexaacetate.

deoxy-loganic acid (**1**) and 7-ketologanic acid (**5**) were administered. Both compounds gave 7–8% incorporations into excelsioside (**16**), but only **5** gave significant incorporations into oleoside 11-methyl ester (**17**) and ligstroside (**4**). This result confirms our earlier assumption that 7-ketologanic acid (**5**) is indeed involved in the biosynthesis of the oleosides.

In the last series of experiments (6–9 in Table 1) in this plant, deoxy-loganic acid (**1**; as a reference) was administered together with 7-epi-loganin (**18**), loganin (**19**) and 7-ketologanin (**11**). Loganin (**19**) was not incorporated whereas the other three compounds gave significant incorporations into the oleosides. The absence of incorporation for loganin (**19**) may seem to be at variance with our previous findings, where loganic acid (**3**) was an efficient precursor for the oleosides [1]. An explanation may be that the plant is able to utilize the acid with the wrong stereochemistry at C-7 (i.e. **3**) whereas the corresponding methyl ester (**19**) does not fit into the active site of the enzyme oxidizing 7-epi-loganin (**18**) to 7-ketologanin (**11**). A less likely explanation is seasonal variations in the formation of iridoids in the plant. It appeared that the biosynthetic capacity of the plants used in this series of experiments was somewhat lower than in the plants used before [1], as judged by the accumulated incorporations. Nevertheless, the results show that the main biosynthetic pathway to the oleosides comprises a 7α-hydroxylation of deoxy-loganic acid followed by methylation and oxidation to 7-ketologanin (**11**). The sequence of the last steps (i.e. the point of methylation) is not evident, since both 7-epi-loganin (**18**) and 7-ketologanic acid (**5**) gave high incorporation.

Biosynthetic experiments with Syringa josikaea

Feeding this plant with iridodial glucoside (**9**) and iridotrial glucoside (**10**) (Table 2, experiments 10 and 11) gave only very small incorporations into oleoside 11-methyl ester (**17**). This indicates that the two compounds are not intermediates in the biosynthesis of the oleosides. The compounds are probably hydrolysed to the aglucones in the plant, as is also observed in the biosynthesis of cornin (**20**) in *Verbena* [2] and of antirrhinoside (**21**) in *Antirrhinum* [4]. The low, but measurable, incorporation thus further supports the biosynthesis of oleosides via iridodial (**12**) and iridotrial (**13**).

The subsequent experiments (12–18 in Table 2) again show that loganin (**19**) is not a precursor for the oleosides. On the contrary, 7-epi-loganin (**18**) and 7-ketologanin (**11**) are both efficiently incorporated into the oleosides. The corresponding acids, **2** and **5**, are not incorporated into the more elaborated oleosides (**22** and **4**), but only into **17**, which may well be an intermediate in the biosynthesis of **22** and **4**. Several explanations may account for this fact. The acids may not be intermediates but can nevertheless be used slowly (perhaps after methylation). Furthermore, the transport of the acids might be slow in the plant. However, the results are in keeping with those of *Fraxinus* where oleosides are formed via 7-epi-loganin (**18**) and 7-ketologanin (**11**) or their corresponding acids.

Biosynthetic experiments with Syringa vulgaris

Two set of experiments were performed at different times of year (Table 3). In the first experiments 19 and 20

Table 2. Results from biosyntehtic experiments with *S. josikaea*

Experiment	10	11	12	13	14	15	16	17	18
Precursor	**d-9**	**d-10**	**d-1**	**d-3**	**d-2**	**d-5**	**d-19**	**d-18**	**d-11**
Labelling site(s)	6,7,8,10	6,7,8,10	6,7,8,10	10	10	10	10	10,11	10,11
(mg)	14	14	16	15	13	13	17	19	14
Epimeric purity (%)	88	83	92	>99	>99	>99	>99	>99	>99
Plant (g)	23	23	16	23	23	23	14	16	16
Metabolic period (days)	5	5	5	5	5	5	5	5	5
Incorp. (%) in **15**			0.5					2.5*	1.5
Incorp. (%) in **6**			0.5						0.5
15 and **6** (mg, ratio)			13(2:1)				14	21(1:3)	18(1:1)
Incorp. (%) in **17**	0.1	0.5	5	1.5	3.5‡	2		13	3.5
Incorp. (%) in **11**†			1.5	0.5		2.5			3
Incorp. (%) in **24**									
Amount (mg; **17**:**24**)	14	15	42(3:1)	17	19	25	34	31(1:2)	36(1:1)
22 (mg)	79	109	168	72	111	114	137	256	195
Incorp. (%)			4.5					4	6
4 (mg)	14	16	42	12	20	20	29	56	26
Incorp. (%)			3					9	5

*Reisolated precursor (5%) was also present.

†Only present in very minute amount (i.e. the ratio given is between the two other compounds).

‡After methylation, addition of carriers and MPLC, oleoside dimethyl ester (**25**) containing 3.5% incorporation was isolated.

In experiment 16 an impure fr. (18 mg) containing reisolated precursor (48%) was obtained.

in Table 3) deoxy-loganic acid (**1**) and 7-ketologanic acid (**5**) were fed to the plant. Surprisingly, no incorporations into the oleosides were observed, whereas both compounds gave high incorporations (6 and 8%) into 8-epi-kingisidic acid (**23**). The fact that **5** is an efficient precursor for **23** indicates that the latter compound should be regarded as an oleoside-type iridoid rather than a secologanin-type iridoid. The possible intermediacy of **23** in the biosynthesis of oleosides is addressed in the following paper.

The last two experiments (21 and 22 in Table 3) gave quite different results. Deoxy-loganic acid (**1**) gave incorporation into all iridoids, except for secologanoside (**24**), with over 20% recovery of labelling, whereas 7-ketologanin (**11**) was mainly incorporated (> 16%) into the oleosides. A small incorporation (*ca* 1%) into 8-epi-kingiside (**6**) was, however, noticeable. The biosynthesis of the iridoids in *Syringa vulgaris* thus seems to differ quite extensively over the year.

Biosynthesis of iridoids in the Oleaceae

The results of the above experiments show that the biosynthesis of the oleosides found in *Fraxinus* and *Syringa* proceeds via iridodial (**12**), iridotrial (**13**), deoxy-loganic acid aglucon (**14**) and deoxy-loganic acid (**1**). Further hydroxylation at the 7-position gives 7-epi-loganic acid (**2**), which by methylation and oxidation yields 7-ketologanin (**11**) and further elaboration of this gives rise to the oleosides. The biosynthesis of the oleosides thus resembles that of the secologanin-type seco-iridoids up to the point of deoxy-loganic acid (**1**). This compound is apparently the key intermediate in the biosynthesis of all the iridoids found in the Oleaceae as shown in Fig. 2; 7α-hydroxylation leads not only to the oleosides, but also to the compounds derived from 8-epi-kingisidic acid (**23**). Hydroxylation of **1** to give loganic acid (**3**) leads to the formation of the secologanin derivative secologanic acid (**29**) in *Fontanesia* [5], whereas hydroxylation of **1** in the 10-position gives adoxosidic acid (**30**) which is subsequently oxidized to forsythide (**31**) in *Forsythia* [6].

EXPERIMENTAL

General. Mps: uncorr. ^{1}H NMR (250 MHz) spectra recorded in D_2O and acetates in $CDCl_3$ using solvent signals at δ4.75 and 7.27, respectively, as internal standards. In all ^{13}C NMR (62.5 MHz) spectra, C-6′ was set to δ61.5 as a standard [7]. The ^{2}H NMR spectra recorded at 77 MHz; glucosides in H_2O with 0.0156% ^{2}H of natural abundance, and acetates in $CHCl_3$ with 0.17% ^{2}H of natural abundance. Prep. TLC performed on 20 cm × 40 cm plates coated with 1 mm layers of silica gel PF_{254} (Merck); bands detected in UV light (254 nm). Reverse-phase MPLC performed on Merck Lobar C-18 columns size B and C. H_2O–MeOH mixts were used as eluents as specified in each case and peaks were detected by UV (250 nm).

Plant material. Leaves and top shoots were collected from specimens of *Fraxinus excelsior*, *Syringa josikaea* (both used earlier) [1] and from *Syringa vulgaris* (Voucher IOK 21-94) in June–July 1992–1993.

Table 3. Results from biosynthetic experiments with *S. vulgaris*

Experiment	19	20	21	22
Precursor	**d-1**	**d-5**	**d-1**	**d-11**
Labelling site(s)	6,7,8,10	10	6,7,8,10	10
(mg)	13	14	16	16
Epimeric purity (%)	92	>99	92	>99
Plant (g)	29	28	25	25
Metabolic period (days)	5	5	5	5
23 (incorp. %, mg)	6*(40)	8*(39)	0.5 (16)	– (6)
28 (incorp. %, mg)		–	0.5(7)	—
15 (incorp. %)		–	1.5	—
6 (incorp. %, mg)		–	0.5(10)	1
17 (incorp. %)		—	4.5	7.5‡
24 (incorp. %, mg)		– (32)	—	
Total amoung (mg)	66	66	49	89
25 (incorp. %, mg)	– (27)	– (32	2(29)	2(40)
7 (incorp. %, mg)	– (43)	– (43)	3.5†(126)	– (123)
Oleoside-type impurity (%)	–	–	—	1
8 (incorp. %, mg)	– (78)	– (90)	1(144)	– (129)
Oleoside-type impurity (%)		—	1.5	2
22 (incorp. %, mg)	– (132)	– (150)	4(419)	6.5(372)
Mixt. of secoiridoids (%, mg)	– (45)	– (52)	3(101)	1(31)

*The incorporations were verified for the purified methyl esters.

† Reisolated precursor (δ0.95).

‡And 1% at δ1.1 (i.e. reisolated precursor).

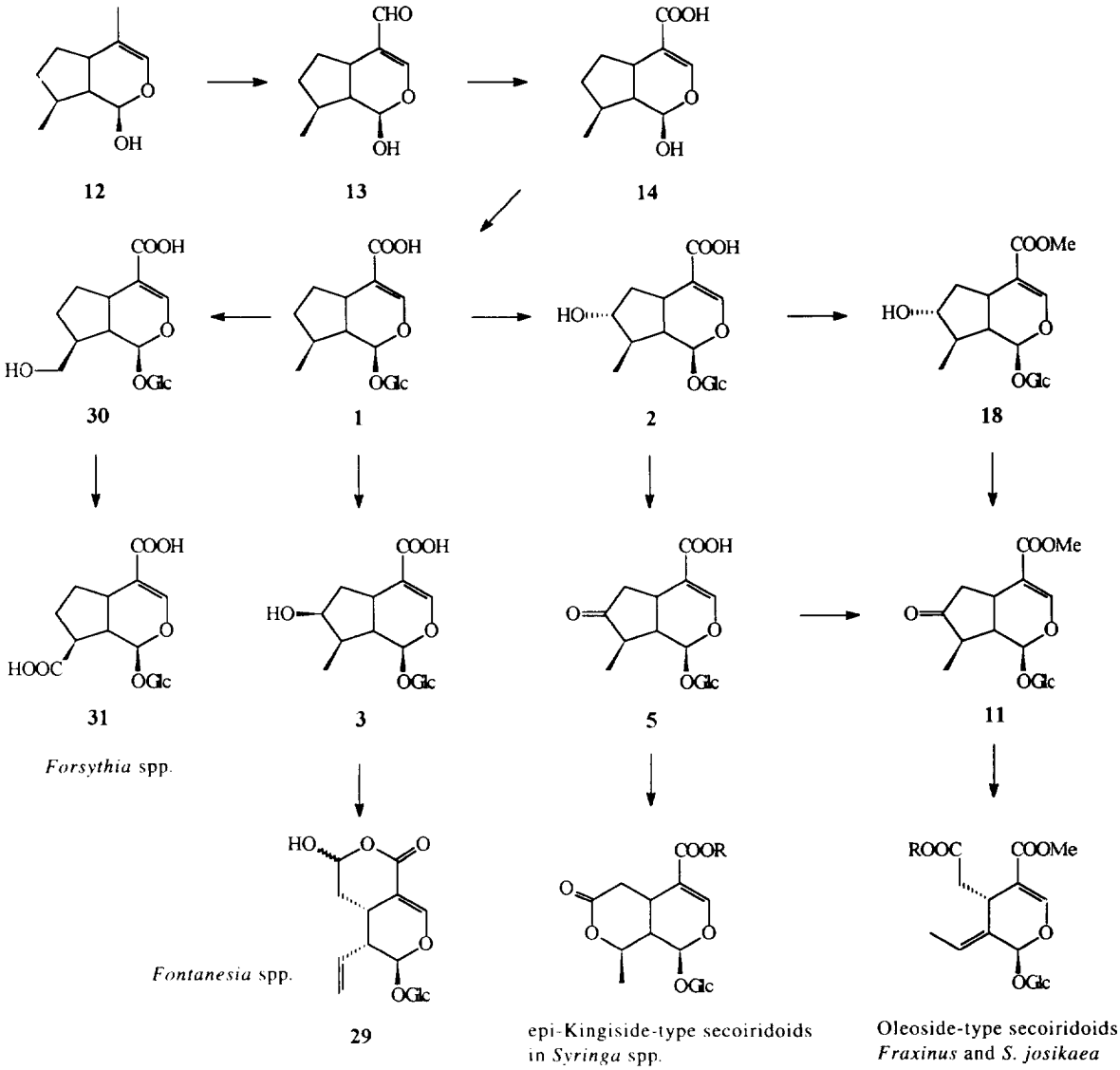


Fig. 2. Biosynthetic routes to iridoids in the Oleaceae.

[6,7,8,10-^{2}H]*Iridodial glucoside* (**d-9**). Prepn as in ref. [2] gave **d-9** of 88% epimeric purity; ^{2}H content as in **d-1** (see below).

[6,7,8,10-^{2}H]*Iridotrial glucoside* (**d-10**). Prepn as in ref. [2] gave **d-10** of 83% epimeric purity; ^{2}H content as in **d-1** (see below).

[6,7,8,10-^{2}H]*Deoxy-loganic acid* (**d-1**). Prepn as in ref. [2] afforded **d-1** of 92% epimeric purity after MPLC. ^{1}H NMR showed a ^{2}H content of 0.8 ^{2}H at C-6 (α:β, 5:3), 1.1 ^{2}H at C-7 (α:β, 8:3), 0.8 ^{2}H at C-8 and 2.0 ^{2}H at C-10.

Preparation of aglucones. Aglucones (**d-12**, **d-13** and **d-14**) of the above glucosides (25–30 mg) were prepd by treatment of the above glucosides with a β-glucosidase (5 mg, Sigma) in H_2O (6 ml) overnight. The mixt. was extracted with Et_2O (3 × 7 ml), the combined organic extracts were washed with brine (7 ml) and concd to a smaller volume (5 ml). The soln was dried (Na_2SO_4) and transferred to a small beaker (10 ml) where the soln was evapd by a flow of air. Before administration, the aglucones were further dried under an IR lamp.

[10-^{2}H, 11-OC^{2}H$_3$]-7-*Epi-loganin* (**d-18**). [10-^{2}H]-7-Epi-loganic acid (**d-2**) containing 0.9 ^{2}H at C-10 (and < 0.2 ^{2}H at C-6) was prepd as in [1]. Methylation with C^{2}H$_2$N$_2$–Et_2O in EtOD and subsequent MPLC (B column; H_2O and 4:1) gave **d-18** when eluting with 4:1. According to ^{1}H NMR, 2.7 ^{2}H was introduced in the 11-OMe group.

[10-^{2}H]*Loganin* (**d-19**). Prepn as in ref. [1] with 0.9 ^{2}H present at C-10 (and *ca* 0.1 ^{2}H at C-6).

7-Ketologanic acid (**5**). 7-Ketologanin (**11**, 160 mg, [1]) was hydrolysed (1 ml 1M NaOH in 5 ml H_2O) for 3 hrs. Upon addition of HOAc (0.5 ml) the mixt. was chromatographed on a RP-18 B column (H_2O, 4:1 to 2:1) to give **5** (100 mg) as a foam (unreacted **11** (37 mg)

was recovered with 2:1). $[\alpha]_D^{20}$ − 167° (MeOH; *c* 1.2). ^{1}H NMR (250 MHz, D_2O): δ7.49 (*br s*, H-3), 5.64 (*d*, *J* = 2.5 Hz, H-1), 4.78 (*d*, *J* = 8 Hz, H-1′), 3.88 (*dd*, *J* = 12 and 2 Hz, H_a-6′), 3.67 (*dd*, *J* = 12 and 5.5 Hz, H_b-6′), 3.50–3.30 (*m*, 3H, H-4′, H-5′ and H-3′), 3.30–3.18 (*m*, 2H, H-2′ and H-5), 2.70 (*dd*, *J* = 19.5 and 8.5 Hz, H_a-6), 2.54 (*br d*, *J* = 19.5 Hz, H_b-6), 2.44 (*ddd*, *J* = 10.5, 7.5 and 2.5 Hz, H-9), 2.22 (*m*, H-8), 1.09 (*d*, 3H, *J* = 7 Hz, 10-Me). ^{13}C NMR (62.5 MHz, D_2O): δ215.9 (C-6), 171.4 (C-11), 153.3 (C-3), 110.8 (C-4), 95.4 (C-1), 45.3 (C-9), 44.5 (C-6), 42.9 (C-8), 27.1 (C-5), 13.0 (C-10), 99.4 (C-1′), 77.2 (C-5′), 76.3 (C-3′), 73.4 (C-2′), 70.4 (C-4′), 61.5 (C-6′). (Found: C, 49.0; H, 6.4, $C_{16}H_{22}O_{10}$. H_2O requires C, 49.0; H, 6.2%.)

[10-^{2}H, 11-OC^2H_3]-7-*Ketologanin* (**d_4-11**). Crystalline [10-^{2}H]-7-ketologanin (**d_1-11**, 148 mg) was hydrolysed in 1M NaOH (5 ml) for 6 hrs followed by work-up and chromatography as above. Elution with 3:1 afforded pure [10-^{2}H]-7-ketologanic acid (**d-5**, 101 mg, 71%, 1.0 ^{2}H at C-10) as a white foam. Methylation with $C^2H_2N_2$–Et_2O in EtOD (upon evapn with HOAc–EtOD) and subsequent MPLC (B column; H_2O, 4:1 to 2:1) gave **d_4-11** in almost quantitative yield. ^{1}H NMR showed the presence of 0.8 ^{2}H at C-10 and 2.7 ^{2}H in the 11-OMe group.

Work-up of Syringa vulgaris. Homogenization of fresh twigs (28 g) with EtOH (500 ml), filtration and concn of the filtrate gave a syrup which was partitioned in H_2O–Et_2O. The aq. layer was evapd to give a foam (1.15 g). Dissolution in MeOH (50 ml) and filtration through activated C (1 g) and subsequent evapn yielded an extract (1.15 g) which was dissolved in 10% HOAc (5 ml) and subjected to MPLC (B column, 10:1 to 1:1). Elution with 5:1 gave 8-epi-kingisidic acid (**23**, 34 mg) while continued elution with 4:1 yielded a fr. containing 8-epi-kingiside (**6**), secologanoside (**24**) and oleoside 11-methyl ester (**17**) (69 mg). Oleoside dimethyl ester (**25**, 30 mg) and lilacoside (**8**, 49 mg) were successively eluted by 2:1. Then came frs of fliederoside (**7**, 100 mg) and oleuropein (**22**, 190 mg) when eluting with 3:2, whereas a complex mixt. (56 mg) of unidentified minor compounds was obtained with 1:1.

Lilacoside (**8**). $[\alpha]_D^{20}$ −36° (MeOH; *c* 0.7). ^{1}H NMR 250 MHz, CD_3OD): δ7.52 (*s*, H-3), 6.68 (*d*, *J* = 8.5 Hz, H-5″), 6.65 (*d*, *J* = 1.5 Hz, H-2″), 6.53 (*dd*, *J* = 8.5 Hz and 1.5 Hz, H-6″), 5.46 (*d*, *J* = 7.5 Hz, H-1), 4.46 (*dq*, *J* = 6 Hz, H-8), 4.25 (*m*, 2H, 2 × H-α), 3.02 (*m*, H-5), 2.86–2.74 (obsc. by H-β's, H_a-6), 2.78 (*t*, 2H, *J* = 6.5 Hz, 2 × H-β), 2.43 (*dd*, *J* = 16.5 Hz and 11 Hz, H_b-6), 2.10 (*q*, *J* = 7 Hz, H-9), 1.49 (*d*, *J* = 6 Hz, 10-Me), 4.69 (*d*, *J* = 8 Hz, H-1′), 3.91 (*dd*, *J* = 12 Hz and 2 Hz,H_a-6′), 3.69–3.17 (*m*, 5H, H_b-6′, H-3′, H-5′, H-4′ and H-2′). ^{13}C NMR (125 MHz, CD_3OD: δ174.7 (C-7), 167.8 (C-11), 154.4 (C-3), 109-7 (C-4), 96.3 (C-1), 75.7 (C-8), 41.9 (C-9), 34.6 (C-6), 28.1 (C-5), 21.7 (C-10), 100.6 (C-1′), 78.5 (C-3′), 77.9 (C-5′), 74.7 (C-2′), 71.7 (C-4′), 62.9 (C-6′), 146.2 (C-3″), 144.9 (C-4″), 130.9 (C-1″), 121.3 (C-6″), 117.0 (C-2″), 116.4 (C-5″), 66.4 (C-α), 35.5 (C-β). (Found: C, 53.2, H, 6.0. $C_{24}H_{30}O_{13}$. 1.5 H_2O requires: C, 52.9; H, 5.9%.)

Fliederoside (**7**). $[\alpha]_D^{20}$ −28° (MeOH; *c* 0.4). ^{1}H NMR (250 MHz, D_2O): δ7.36 (*s*, H-3), 6.96 (*d*, 2H, *J* = 8.5 Hz, H-2″ and H-6″), 6.69 (*d*, 2H, *J* = 8.5 Hz, H-3″ and H-5″), 5.30 (*d*, *J* = 7 Hz, H-1), 4.45 (*dq*, *J* = 6 Hz, H-8), 4.15 (*br m*, 2H, 2 × H-α), 2.83 (*m*, H-5), 2.69 (*br t* like *s*, 2H, 2 × Hβ), 2.60 (*dd*, *J* = 17 Hz and 5 Hz, H_a-6), 2.12 (*dd*, *J* = 17 Hz and 10.5 Hz, H_b-6), 1.34 (*d*, *J* = 6 Hz, 10-Me). ^{13}C NMR (62.5 MHz, D_2O): δ175.9 (C-7), 168.8 (C-11), 155.2 (C-3), 109.6 (C-4), 95.9 (C-1), 76.0 (C-8), 40.3 (C-9), 33.8 (C-6), 25.9 (C-5), 21.0 (C-10), 99.9 (C-1′), 77.2 (C-5′), 76.6 (C-3′), 73.5 (C-2′), 70.4 (C-4′), 61.5 (C-6′), 154.4 (C-4″), 131.2 (C-2″ and C-6″), 131.0 (C-1″), 116.3 (C-3″ and C-5″), 66.6 (C-α), 34.4 (C-β). (Found: C, 53.9; H, 6.2. $C_{24}H_{30}O_{12}$. 1.5 H_2O requires: C, 53.6; H, 6.2%.)

Feeding experiments. The feeding experiments using glucosides with *F. excelsior* and *S. josikaea* were carried out as earlier described [1].

Administration of aglucones. The administration of the aglucones to *F. excelsior* was performed in late June 1992 using single leaves (8 per experiment, 15–25 cm, *ca* 30 g). The aglucones were emulsified with detergent (1%, 3 ml) [2] and were then absorbed by the freshly cut leaves. After the metabolic period the plant was worked up as in ref. [1].

Feeding experiments with S. vulgaris. Experiments 19 and 20 took place in the middle of May 1992. Fresh herbaceous shoots (15–20 cm) with leaves were employed. The precursors were dissolved in H_2O (3 ml) and the freshly cut shoots were immersed into these solns. Additional water was supplied in small portions ensuring complete absorption. Whenever necessary, the plants were placed in a tank satd with moisture to keep them fresh. After a total metabolic period of 5 days, the plants were worked up as above. In some of the later experiments (21 and 22) almost complete separation of 8-epi-kingiside (**6**), secologanoside (**24**) and oleoside 11-methyl ester (**17**) was achieved.

Purification of fractions showing incorporation

Experiments 1 *and* 2 (F. excelsior). Frs with iridoid mixts (23 and 39 mg, respectively) showing incorporation (signals at δ1.1–1.0) were obtained. After methylation and addition of carriers, they were rechromatographed (C column, 5:1 to 2:1). The methylated fr. from expt 1 yielded **18** (10 mg), almost pure **11** (23 mg) and **19** (10 mg); the methylated fr. from expt 2 gave similar frs (10 mg, 17 mg and 8 mg, respectively). Only the frs of 7-ketologanin (**11**) showed significant incorporations. Upon acetylation and subsequent prep. TLC (Et_2O), the incorporations were still measurable in the obtained 7-ketologanin tetraacetate (**11a**).

Experiment 9 (F. excelsior). To the impure fr. of **27** was added unlabelled **27** (32 mg) and the mixt. was then acetylated (Py–Ac_2O 1:1, 2 ml, 2 hr at room temp.). Usual work-up and prep. TLC (Et_2O–EtOAc, 10:1) afforded the pure hexaacetate (39 mg), which, according to ^{2}H NMR, exhibited 0.5% incorporation.

Experiment 14 (*S. josikaea*). To the fr. (19 mg) containing a mixt. of **17** and **24** was added an unlabelled sample

of secologanoside (10 mg), and the resulting mixt. was subsequently methylated (CH_2N_2–Et_2O in MeOH). After addition of unlabelled oleoside dimethyl ester (**25**, 17 mg) as a carrier, the mixt. was subjected to MPLC (C column, 2:1 to 1:1). The only frs isolated were **25** (2:1, 18 mg) and **26** (1:1, 11 mg). ^{2}H NMR showed only incorporation in **25** (3.5%).

Experiment 19 *and* 20 (S. vulgaris). The frs of **23** were methylated as above, and rechromatographed (B column, 4:1 to 2:1). The obtained frs of **6** were > 98% pure according to ^{1}H NMR and showed unchanged incorporations compared to those of the free acids (determined by ^{2}H NMR).

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 at the Botanical Garden of the University of Copenhagen for verifying the plant material.

REFERENCES

1. Damtoft, S., Franzyk, H. and Jensen, S. R. (1993) *Phytochemistry* **34**, 1291.
2. Kirk, O., Jensen, S. R. and Nielsen, B. J. (1989) *Phytochemistry* **28**, 97.
3. Markey, S. P. and Shaw, G. J. (1978) *J. Org. Chem.* **43**, 3414.
4. Breinholt, J., Damtoft, S., Demuth, H., Jensen, S. R. and Nielsen, B. J. (1992) *Phytochemistry* **31**, 795.
5. Damtoft, S., Franzyk, H. and Jensen, S. R. (1994) *Phytochemistry* **38**, 615.
6. Damtoft, S., Franzyk, H. and Jensen, S. R. (1994) *Phytochemistry* **37**, 173.
7. Damtoft, S., Jensen, S. R. and Nielsen, B. J. (1981) *Phytochemistry* **20**, 2717.