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BIOSYNTHESIS OF IRIDOIDS IN *FORSYTHIA* SPP.

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IN HONOUR OF PROFESSOR ROBERT HEGNAUER'S SEVENTY-FIFTH BIRTHDAY

Key Word Index—*Forsythia europaea*; *F. viridissima*; Oleaceae; iridoid glucosides; biosynthesis; 11-glucosyl forsythide; 11-methyl forsythide.

Abstract—The biosynthesis of the carbocyclic iridoid glucosides present in the genus *Forsythia* has been investigated. Deuterium labelled deoxyloganic acid, 8-*epi*-deoxyloganic acid and adoxosidic acid were fed to *Forsythia viridissima* and *F. europaea*. These experiments showed that the biosynthesis presumably follows a pathway including both deoxyloganic acid and adoxosidic acid.

Two new compounds were isolated from *F. europaea*, i.e. 11-glucosyl forsythide and 11-methyl forsythide.

INTRODUCTION

In a previous paper [1] we have reported on the biosynthetic pathway leading to oleoside-type secoiridoids which apparently are limited to the Oleaceae. Deoxyloganic acid (**1**) [but not its 8-epimer] was shown to be a potent precursor of these secoiridoids in both *Fraxinus excelsior* and *Syringa josikaea*.

No biosynthetic work has been reported on the genus *Forsythia* (eight species), which along with the monotypic genus *Abeliophyllum* constitute the tribe, Forsythieae, usually referred to as the subfamily Jasminoideae of the Oleaceae [2]. The relationship between *Forsythia* and *Abeliophyllum* is substantiated by the general vegetative appearance and their common chromosome number ($n = 14$) [3]. However, in an earlier investigation of *F. viridissima*, the carbocyclic iridoid forsythide (**2**) was isolated as the only genuine iridoid constituent [4]. The 8β-configuration of the carboxyl group in **2** indicates its biosynthesis to proceed via the same early precursors (e.g. **1**) as the secoiridoids in Oleoideae.

Nevertheless, precursors of the 8-*epi*-series might in fact be involved, since a spontaneous (under weakly acidic or basic conditions) or enzymatic epimerization to the more stable 8β-configuration might take place at the 10-aldehyde stage. Also hypothetically, the origin of the 8β-carboxylic group in **2** might well be geniposidic acid (**3**), which could undergo further oxidation at C-10 and hydrogenation at the α-side of the 7,8-double bond. Thus, deoxyloganic acid (**1**) as well as its 8-epimer (**4**) could be intermediates in the biosynthesis of **2**.

This paper describes biosynthetic experiments designed to test these possibilities and the structure elucidation of two novel iridoid glucosides.

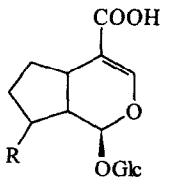
RESULTS AND DISCUSSION

Investigation of plant material

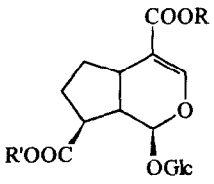
As mentioned above, forsythide (**2**) has earlier been identified as a constituent of *F. viridissima*, *F. europaea* and *F. japonica* (in the last two only by comparative TLC), whereas it appeared to be absent from *F. suspensa* and *F. koreana* according to TLC analyses [4]. The present reinvestigation of a large batch of *F. viridissima* allowed the isolation of a small quantity of adoxosidic acid (**5**) and a novel iridoid, in addition to a substantial amount of forsythide (**2**).

The presence of adoxosidic acid (**5**) agrees with the theory that **2** truly belongs to the 8β-series, possibly derived from deoxyloganic acid (**1**). Furthermore, chlorogenic acid as well as verbascoside were obtained from *F. viridissima*. Only a small amount of the novel compound (**6**) was obtained, and it was isolated as an apparently inseparable (by MPLC) mixture containing 5–10% of another unidentified iridoid. Identification and characterization of **6** were therefore performed solely by NMR spectroscopy.

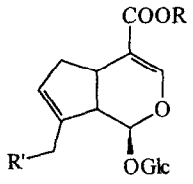
The ^{1}H NMR spectrum of **6** showed all the signals corresponding to a forsythide-type skeleton when compared with the spectrum of **2**. However, the integrals of the signals assignable to the usual glucosylic protons were doubled except for that of the anomeric proton (partly obscured by the HOD-signal at δ4.75). An additional doublet at δ5.6 was attributable to an anomeric proton of a 1β-glucosyl ester, thus accounting for the presence of two glucose units in **6**. Hence, **6** was assumed to be the 10→1β- or 11→1β-glucosyl ester of **2**. Methylation of **6**



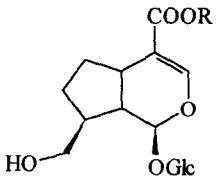
R
1 β-Me
4 α-Me



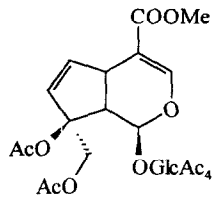
	R	R'
2	H	H
6	Glc	H
7	Glc	Me
8	Me	Me
9	H	Me
10	Me	H



	R	R'
3	H	OH
12	Me	H
13	Me	OH



R
5 H
14 Me



11a

with diazomethane afforded, as expected, a monomethyl ester (7).

The ^{13}C NMR assignments (Table 1) of 2 and of forsythide dimethyl ester (8) were confirmed by the multiplicities obtained in a DEPT spectrum and the correlations revealed by a ^{1}H–^{13}C COSY spectrum, which was interpretable due to the complete assignment of the ^{1}H NMR spectra performed by decoupling experiments.

Table 1. ^{13}C NMR data of forsythide-type glucosides (D_2O)

C	2	6	7	8	A (10)	10a
1	97.6	97.8	97.7	97.4	97.7	94.9
3	153.6	155.2	155.2	153.1	153.2	150.2
4	111.7	110.8	110.8	111.7	111.8	111.6
5	34.8	34.8	34.6	34.7	34.9	33.0
6	32.3	32.3	32.1	32.2	32.4	30.4
7	29.0	29.0	28.7	28.6	29.0	27.3
8	45.9	45.8	45.7	45.7	45.9	44.2
9	44.5	44.5	44.5	44.5	44.5	44.0
10	180.4	180.3	178.9	178.8	180.4	179.6
11	171.9	168.3	168.2	170.6	170.8	166.9
OMe-10			53.5	53.4		
OMe-11				52.7	52.7	51.1
1′	99.9	99.9	99.9	99.8	99.9	95.9
2′	73.5	73.5	73.5	73.5	73.5	70.5
3′	76.3	76.5	76.5	76.5	76.5	72.4
4′	70.4	70.4	70.4	70.4	70.4	68.4
5′	77.2	77.2	77.2	77.1	77.2	77.0
6′	61.5	61.5	61.5	61.5	61.5	61.5
1″		94.6	94.6			
2″		72.8	72.8			
3″		76.5	76.5			
4″		70.0	70.0			
5″		77.7	77.7			
6″		61.4	61.3			

In 8 the more upfield carbonyl signal was assigned to C-11, since this is a conjugated methyl ester. From inspection of Table 1, it is seen that 11-glucosylation most convincingly accommodates the observed shift differences for C-3 (+2.1 ppm), C-4 (−0.9 ppm) and C-11 (−2.4 ppm) between 7 and 8. Moreover, the chemical shift (δ53.5) of the carbomethoxy group in 7 indicates it to be part of a saturated ester, since a carbomethoxy group at the conjugated C-11 would be located in the narrow range δ52.8–52.5 (e.g. 52.7 in 8) when recorded in D_2O [5]. Also, an upfield shift (1.4 ppm) of C-10 was seen upon methylation of 6, whereas the chemical shift of C-11 was almost unchanged. Thus, the structure of 6 was established as 11-glucosyl forsythide.

Similarly, work-up of a small amount of *F. europaea* yielded forsythide (2), chlorogenic acid and verbascoside as major components, whereas the minor constituents were cornoside, 11-glucosyl forsythide (6) and adoxosidic acid (5). In addition, a monomethyl ester (A) of 2 was obtained in a substantial quantity.

The finding of a methylated derivative of 2, using an extraction procedure not involving boiling with methanol, allows it to be considered a natural metabolite. Forsythide 10-methyl ester (9) has earlier been isolated as an artifact from *F. viridissima*, and 9 has been shown to arise during the extraction of the plant with hot methanol [4].

Thus, to decide whether the present monomethyl ester (A) isolated from *F. europaea* had the structure 9 or 10, both ^{13}C NMR data and chemical evidence were employed. The ^{13}C NMR spectrum of A (Table 1) exhibited a carbomethoxy group at δ52.7 supporting a position at C-11 (i.e. 10) when compared to 8. Furthermore, a signific-

ant difference (1.6 ppm) between the C-10 of the methyl ester (A) and **8**, as well as the almost identical chemical shifts of C-11 in these compounds agree with the conjecture that A is the 11-methyl ester (**10**). A similar conclusion can be drawn from the relative positions of C-10 and C-11 in A and **2**. Final proof was obtained by the synthesis of **10** by selective alkaline hydrolysis [4] of **8** (available by methylation of **2**) and the observation that A and **10** had identical NMR spectra.

Synthesis of labelled precursors

Catalytic transfer hydrogenation of **11a** to deoxygeniposide (**12**) and selective catalytic deuteration of the latter allowed the preparation of ^{2}H-labelled **1** and **4** [6, 7].

Adoxosidic acid (**5**) was prepared by catalytic (Pd/C) deuteration under basic conditions (triethyl amine added) of geniposide (**13**) followed by alkaline hydrolysis [8].

Biosynthetic experiments

As already stated, a likely pathway to forsythide (**2**) might involve deoxyloganic acid (**1**) as well as adoxosidic acid (**5**) of which the latter was found in both species of *Forsythia* examined in this work (Table 2). However, in order to exclude a route via intermediates of the α-series, a negative experiment with such a likely precursor, together with positive results for precursors of the β-series was needed. Accordingly, both **1** and **4** were tested as precursors in *F. viridissima*; cut stems were fed with d_4-**1** (13% **4** present) and d_4-**4** (>98% epimeric purity) in early August 1991 (experiment nos 1 and 2). Usual work-up and reverse phase chromatography of the water-soluble part of the ethanolic extracts yielded fractions of **2** also containing a small amount of **5**. Without further purification these fractions were subjected to ^{2}H NMR, which revealed incorporations of **1** into both **2** and **5** (2 and 8%, respectively, see below). Conversely, no signals in the ^{2}H NMR spectrum of the corresponding iridoid acid fraction from the plant fed with d_4-**4** were attributable to **2** or **5**. However, a signal at δ 1.25 might be assignable to a 10-methyl group of an iridoid bearing an 8-hydroxy substituent, but detection by ^{1}H NMR was not possible as only a minute amount of this compound was present, indicating it to be an unnatural metabolite.

Further validation and a more thorough analysis of these results were undertaken in experiments 3 and 4 (performed in June 1993), where *F. europaea* was employed since its higher capability to absorb precursors was expected to result in even higher incorporations. Thus, d_4-**1** and d_3-**5** were applied hydroponically to cut stems, and upon fractionation of the extracts pure fractions of 11-methyl forsythide (**10**) were obtained. Likewise, methylation of the isolated impure mixtures of **2** and **5** (to which additional **5** was added) followed by rechromatography afforded pure fractions of the methyl esters **8** and **14**. Almost the same degree of incorporation (6.5–8.5%) of **1** and **5** into **2** and **10** was obtained; in addition **1** was efficiently transformed into **5** (5.5%) showing the latter to be a true intermediate. Further information was obtained by comparison of selected ratios of the observed ^{2}H integrals of the samples (in the case of the precursors these values were estimated by ^{1}H NMR). Only a slight decrease relative to d_4-**1** was seen for the ratio I(8)/I(6+7) in **2** and **10** (Table 3) which may reflect that the 10-aldehyde stage is short-lived, since a significant isotope exchange at C-8 would otherwise have been expected. Surprisingly, the adoxosidic acid (**5**) isolated from experiment 3 (purified as the methyl ester **14**) contained less label in the 10-position than expected: I(10)/I(6+7+8) should be 0.5 (against the measured value 0.3 if 2/3 of the original label was retained, or even higher when taking a possible isotope effect into account). However, an explanation

Table 2. Results from biosynthetic experiments

	F. viridissima		*F. europaea*	
	Expt 1	Expt 2	Expt 3	Expt 4
Precursor	d_4-**1**	d_4-**4**	d_4-**1**	d_3-**5**
Epimeric purity	87	>98	92	>98
(%) (mg)	19	16.5	22	11
Plant (g)	29	28	15	15
Metabolic period (days)	5	5	4	4
Adoxosidic acid (**5**)	*	*	14†	11†
Incorporation (%)	8	—	5.5	(24)
Forsythide (**2**)	218 mg	284 mg	45§	47§
Incorporation (%)	2	‡	7.5	8.5
11-Methyl forsythide (**10**)	—	—	60	79
Incorporation (%)	—	—	6.5	6.5

*Present as a small impurity in the fraction of **2**.

†Isolated after addition of carrier (9 mg) as the methyl ester (**14**).

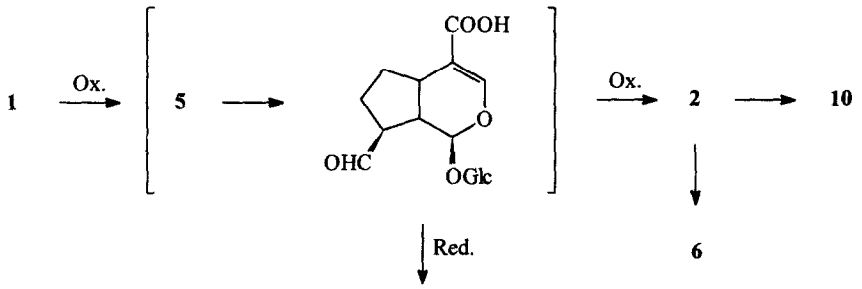
‡A 4% incorporation into an unidentified trace compound having a methyl group at δ 1.3–1.2.

§Isolated as the dimethyl ester (i.e. **5** cf. Experimental).

Table 3. Ratios of ^{2}H integrals at positions **6–8** and **10**

	Expt 3	Expt 4
d_4-**1**		
I(8)/I(6+7)	0.47	
I(10)/I(6+7+8)	0.76	
I(6β+7α+8)/I(6α+7β)	3.2	
d_3-**5**		
I(6β+7α+8)/I(6α+7β)		4.0
Adoxoside (**14**)*		
I(10)/I(6+7+8)	0.30	
I(6β+7α+8)/I(6α+7β)	3.2	4.4
Forsythide dimethyl ester (**8**)†		
I(8)/I(6+7)	0.42	
I(6β+7α+8)/I(6α+7β)		3.9
11-Methyl forsythide (**10**)		
I(8)/I(6+7)	0.41	

*†Represents the values for **5** and **2**, respectively.



Scheme 1. Proposed route for the biosynthesis from **1** of **2**, **6** and **10**.

might be that the isolated **5** was partly derived by a reductive pathway from the 10-aldehyde, which otherwise is usually oxidized to **2**. This leads to the assumption that **1** may in fact be converted to **2** by a multistep enzyme as depicted in Scheme 1. Such a route would require I(10)/I(6 + 7 + 8) = 0.25 (or somewhat higher if abstraction of ^1H is preferred over ^2H during oxidation) in **5**, which is close to the observed value of 0.3. Moreover, the ratio I(6β + 7α + 8)/I(6α + 7β) was seen to be retained during the conversion of **1** into **2** (and **10**) as well as by the oxidation of **5** to **2**. Thus, removal of protons at C-6, C-7 or C-8 has presumably not occurred in the biogenesis of forsythide-type iridoids, and the accumulated evidence points to a route involving oxidation of **1** to **2** via **5** and the 10-oxo intermediate (Scheme 1).

Chemotaxonomic implications

Earlier studies of the phenolic constituents in *Forsythia* species [9, 10] have shown rutin to be ubiquitous in this genus, and the isomeric phenylpropanoids, verbascoside (e.g. in *F. viridissima*) and forsythiaside (e.g. in *F. europaea*) were found in the seven species examined. However, in the present work, verbascoside was isolated as the sole compound of the latter type, both in *F. viridissima* and *F. europaea*. The verbascoside analogues are primarily concentrated in the Oleaceae and in the families of the superorder Lamianae [11], whereas chlorogenic acid is widely distributed among the sympetalous families. Thus, the finding of chlorogenic acid in *Forsythia* species was not surprising. By contrast, the co-occurrence of cornoside and iridoids in *F. europaea* is noteworthy, since these compounds are usually considered to be mutually exclusive [11]. The putative close relationship between *Forsythia* and *Abeliophyllum* [2, 3] is chemically supported by their common content of verbascoside, cornoside and rutin [12].

EXPERIMENTAL

General procedures. Mps: uncorr. ^1H NMR: 250 and 500 MHz, D_2O using the 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 a standard [5]. ^2H NMR: 77 MHz; glucosides in H_2O with 0.0156% ^2H of natural abundance, and acetates in $CHCl_3$ with 0.017% ^2H 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). Reverse 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).

^2H labelling in the precursors. Determination of the labelling in **1** was performed by ^1H NMR which showed 0.8 ^2H at C-6 (α:β 5:3), 0.9 ^2H at C-7 (α:β 8:1), 0.8 ^2H at C-8 and 1.9 ^2H at C-10. A similar labelling pattern was observed for **4**. According to ^1H NMR labelling was present at C-6 [*ca* 0.5 ^2H; (α:β 1:4)], C-7 [0.9 ^2H; (α:β 8:1)], and C-8 (0.8 ^2H) in **5**.

Plant material. Shoots of *F. viridissima* were obtained in August 1991 and twigs of *F. europaea* were collected in June 1993 from The Botanical Garden of Copenhagen.

Work-up of plant material. Frozen foliage (500 g) of *F. viridissima* was homogenized with EtOH (2.5 l). Filtration of insoluble material, concn *in vacuo* and subsequent partitioning in H_2O–Et_2O (1:2; 900 ml) gave an aq. phase which was passed through Celite (5 g). Evapn *in vacuo* of the filtrate yielded a foam (44.4 g), which was dissolved in H_2O (150 ml) and extracted with *n*-BuOH (3 × 250 ml). Evapn *in vacuo* of the aq. layer afforded A (12.4 g), which was redissolved in MeOH–H_2O (15:1; 160 ml) and filtered though activated C (10 g). The filtrate was taken to dryness yielding a syrup (11.3 g), which was applied to an alumina column (75 g). Elution with H_2O (750 ml) and concn *in vacuo* of the eluate gave a yellowish syrup (9.7 g) which was chromatographed (dissolved in 10% HOAc; 3 portions; size C) giving 3 frs when eluting with 3:1, A-1 (196 mg), A-2 (38 mg) and **2** (189 mg; 0.04%).

Rechromatography of A-1 dissolved in 10% HOAc (B-column; 7:1 to 4:1) afforded **6** in admixture with *ca* 10% of another unidentified iridoid (16 mg; 0.003%); methylation (CH_2N_2–Et_2O in MeOH) and MPLC (B-column; 10:1 to 1:1) gave upon elution with 5:2 almost pure **7** (8 mg). Similarly, A-2 (7:1 to 4:1) furnished **5** (19 mg; 0.004%).

The concd *n*-BuOH extracts were partitioned in H_2O–EtOAc (1:3; 400 ml); evapn *in vacuo* yielded B (21.7 g) and an EtOAc-soluble extract, C (10.0 g). An aliquot of B (8.7 g) was subjected to MPLC (C-column; 10:1 to 3:1) and upon elution with 3:1, **2** (0.62 g; 0.3%) was obtained.

Forsythide (**2**). ^1H NMR (250 MHz; D_2O): δ 7.49 (*br s*, H-3), 5.27 (*d*, J = 5.5 Hz, H-1), 4.75 (*d*, J = 8.5 Hz, H-1′), 2.91 (*q*, J = 7.5 Hz, H-5), 2.82 (*q*, J = 8 Hz, H-8), 2.53 (*q*, J

= 7 Hz, H-9), 2.14 (*m*, H_a-6), 2.02 (*m*, H_a-7), 1.78 (*m*, H_b-7), 1.53 (*m*, H_b-6); ^{13}C NMR: Table 1.

11-*Glucosyl forsythide* (**6**). ^{1}H NMR (250 MHz; D_2O): δ7.67 (*br s*, H-3), 5.33 (*d*, J = 5.5 Hz, H-1), 2.98 (*q*, J = 7.5 Hz, H-5), 2.82 (*m*, H-8), 2.57 (*m*, H-9), 2.20 (*m*, H_a-6), 2.05 (*m*, H_a-7), 1.79 (*m*, H_b-7), 1.55 (*m*, H_b-6), 5.59 (*d*, J = 7.5 Hz, H-1″), 3.88 (*dd*, 2H, J = 12 Hz and 2.5 Hz, H_a-6′ and H_a-6″), 3.69 (*dd*, 2H, J = 12 Hz and 5 Hz, H_b-6′ and H_b-6″), 3.60–3.22 (*m*, 6H, remaining glucosyl protons); ^{13}C NMR: Table 1.

10-*Methyl*-11-*glucosyl forsythide* (**7**). ^{1}H NMR (250 MHz; D_2O): δ7.67 (*br s*, H-3), 5.34 (*d*, J = 6 Hz, H-1), 3.68 (*s*, 3H, 10-OMe), 2.99 (*q*, J = 7.5 Hz, H-5), 2.89 (*q*, J = 8 Hz, H-8), 2.60 (*q*, J = 6.5 Hz, H-9), 2.18 (*m*, H_a-6), 2.02 (*m*, H_a-7), 1.82 (*m*, H_b-7), 1.57 (*m*, H_b-6), 5.58 (*d*, J = 8 Hz, H-1″), 4.76 (obscured by the HOD-signal, H-1′), 3.87 (*dd*, 2H, J = 12 Hz and 2.5 Hz, H_a-6′ and H_a-6″), 3.74–6.64 (*m*, 2H, H_b-6′ and H_b-6″), 3.61–3.31 (*m*, 6H, H-3′, H-3″, H-5′, H-5″, H-4′ and H-4″), 3.26 (*t*, 2H, J = 9 Hz, H-2′ and H-2″); ^{13}C NMR: Table 1.

Frozen foliage (8.3 g) of *F. europaea* was extracted with EtOH and the crude extract subsequently partitioned in H_2O–Et_2O to give a H_2O-soluble extract (0.91 g), which was dissolved in 10% HOAc and applied to a B-column (25:1 to 1:1). Elution with 10:1 afforded cornoside (5 mg; 0.06%), whereas an impure fr. (7 mg) containing 11-glucosyl forsythide (**6**) was obtained with 5:1. Impure **5** (6 mg) was followed by **2** (46 mg; 0.6%) and chlorogenic acid (11 mg; 0.1%) when eluting with 4:1. Lastly, 11-methyl-forsythide (3:1; **10**; 32 mg; 0.4%) and verbascoside (1:1; 183 mg; 2.2%) were eluted.

Selective alkaline hydrolysis of **8**. Methylation of **2** in MeOH with CH_2N_2–Et_2O gave **8**. Partial alkaline hydrolysis of **8** (325 mg) in dilute NaOH (0.25 M; 4 ml) for 2 days at 0° yielded upon MPLC (B-column; H_2O to 2:1) **2** (4:1; 102 mg; 34%) and **10** (2:1; 172 mg; 55%), which was obtained as a hygroscopic foam, $[\alpha]_D^{21}$ −56.9° (MeOH; *c* 0.9) $C_{17}H_2O_{11} \cdot H_2O$ requires H 6.20, C 48.34, found H 6.19, C 48.41%.

Forsythide dimethyl ester (**8**). ^{1}H NMR (500 MHz; D_2O): δ7.54 (*d*, J = 1.2 Hz, H-3), 5.34 (*d*, J = 5.8 Hz, H-1), 3.77 (*s*, 3H, 11-OMe), 3.76 (*s*, 3H, 10-OMe), 3.00 (*br q*, J = 7.7 Hz, H-5), 2.93 (*dt*, J = 2 × 7.3 Hz and 9.1 Hz, H-8), 2.61 (*dt*, J = 2 × 6.2 Hz and 8.0 Hz, H-9), 2.23 (*m*, H_a-6), 2.07 (*m*, H_a-7), 1.85 (*dq*, J = 3 × 8.0 Hz and 13.3 Hz, H_b-6), 1.56 (*dq*, J = 3 × 7.5 Hz and 13.5 Hz, H_b-6), 4.82 (*d*, J = 8.1 Hz, H-1′), 3.94 (*dd*, J = 12.3 Hz and 2.3 Hz, H_a-6′, 3.76 (obscured by OMes, H_b-6′) 3.54 (*t*, J = 9.2 Hz, H-3′), 3.50 (*ddd*, J = 9.8 Hz, 5.0 Hz and 2.3 Hz, H-5′), 3.44 (*br t*, J = 9.6 Hz, H-4′), 3.34 (*dd*, J = 8.1 Hz, H-2′); ^{13}C NMR: Table 1.

11-*Methyl forsythide* (**10**). ^{1}H NMR (250 MHz; D_2O): δ7.47 (*br s*, H-3), 5.26 (*d*, J = 5.5 Hz, H-1), 3.69 (*s*, 3H, 11-OMe), 2.93 (*q*, J = 7.5 Hz, H-5), 2.83 (*q*, J = 8 Hz, H-8), 2.53 (*q*, J = 7 Hz, H-9), 2.17 (*m*, H_a-6), 2.02 (*m*, H_a-7), 1.78 (*m*, H_b-7), 1.48 (*m*, H_b-6), 4.75 (*d*, J = 8.0 Hz, H-1′), 3.86 (*dd*, J = 12 Hz and 1.5 Hz, H_a-6′), 3.68 (*dd*, J = 12 Hz and 5 Hz, H_b-6′), 3.52–3.32 (*m*, 3H, H-3′, H-4′ and H-5′), 3.27 (*br t*, J = 9 Hz, H-2′); ^{13}C NMR: Table 1.

Acetylation of **10**. Acetylation of **10** (Ac_2O–pyridine 1:1; 2 hr at room temp.) and work-up gave a tetraacetate (**10a**) which could not be crystallized from EtOH (and Et_2O). ^{1}H NMR (250 MHz; $CDCl_3$): δ7.40 (*d*, J = 1 Hz, H-3), 5.35 (*d*, J = 3.5 Hz, H-1), 3.74 (*s*, 3H, 11-OMe), 3.02 (*dt*, J = 2 × 7 and 4 Hz, H-5), 2.76 (*q*, J = 8 Hz, H-8), 2.65 (*dt*, J = 2 × 8 and 3.5 Hz, H-9), 2.21–1.89 (3H, obscured by Ac-Mes, H_a-6 and 2 × H-7), 2.12, 2.06, 2.03 and 1.94 (*s*, each 3H, 4 × Ac-Me), 1.78 (*m*, H_b-6), 5.25 (*t*, J = 9.5 Hz, H-3′), 5.13 (*t*, J = 10 Hz, H-4′), 5.01 (*dd*, J = 9.5 and 8 Hz, H-2′), 4.88 (*d*, J = 8 Hz, H-1′), 4.33 (*dd*, J = 12.5 and 4.5 Hz, H_a-6′), 4.17 (*dd*, J = 12.5 and 2.5 Hz, H_b-6′), 3.80–3.70 (1H, obscured by 11-OMe, H-5′); ^{13}C NMR: Table 1.

Feeding experiments. The labelled glucosides were dissolved in H_2O (2–3 ml). Young, herbaceous shoots of *F. viridissima* (2–3, *ca* 25 cm, *ca* 30 g) and *F. europaea* (2–3, 20–25 cm, *ca* 15 g), freshly cut under H_2O, were immersed in the above solns. The precursors were absorbed within 4–6 hr and more H_2O (2–3 ml) was added to ensure complete absorption. In order to keep the plants fresh in the remaining part of the metabolic period, they were placed in a chamber with moist air. At the time of work-up the plants still looked fresh.

Work-up of feeding experiments. The ethanolic extract (using 400 ml EtOH) of the plant material was partitioned in H_2O–Et_2O (1:2; 90 ml). A methanolic soln of the aq. extract was filtered through activated C (0.5 g) and chromatographed (column size C; prewashed with 7:1 +0.25% HOAc) eluting with 7:1 to 1:1. *F. viridissima* gave first **2** in admixture with several minor unidentified iridoid compounds (*ca* 10 mg); continued elution with 5:1 afforded a fr. (200–300 mg) of **2** containing a small amount of **5**. Later frs consisted of chlorogenic acid (2:1; *ca* 80 mg) and verbascoside (3:2; 0.4–0.5 g). From *F. europaea*, cornoside (*ca* 35 mg) was first obtained (7:1) then a mixt. containing **2** and a minute amount of adoxosidic acid (5) was eluted with 3:1. Elution with 3:2 afforded **10** (see Table 2 for amounts). To the mixts of **2** and **5** in MeOH, **5** was added as carrier (9 mg) and treatment with CH_2N_2–Et_2O gave the methyl esters. Rechromatography (B-column; 25:1, 3:1 to 3:2) yielded frs of **14** (5:2; 10–15 mg) and **8** (3:2; *ca* 45 mg). Results are shown in Table 2.

Incorporation of ^{2}H into the isolated iridoid glucosides (Table 2) was measured by ^{2}H NMR (3 ml H_2O; 10 mm tubes) using the integrals of the peak from the natural abundance of $^1H^2HO$ and the largest peak corresponding to incorporated labelling.

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REFERENCES

1. Damtoft, S., Franzyk, H. and Jensen, S. R. (1993) *Phytochemistry* **34**, 1291.
2. Johnson, L. A. S. (1957) *Contr. N. S. W. Nat. Herb.* **2**, 395.
3. Taylor, H. (1945) *Brittonia* **5**, 337.

4. Inouye, H. and Nishioka, T. (1973) *Chem. Pharm. Bull.* **21**, 497.
5. Damtoft, S., Jensen, S. R. and Nielsen, B. J. (1981) *Phytochemistry* **20**, 2717.
6. Jensen, S. R., Kirk, O. and Nielsen, B. J. (1989) *Phytochemistry* **28**, 97.
7. Damtoft, S., Jensen, S. R. and Nielsen, B. J. (1992) *Phytochemistry* **31**, 135.
8. Damtoft, S., Frederiksen, L. B. and Jensen, S. R. (1994) *Phytochemistry* (in press).
9. Kitagawa, S., Hisida, S. and Nishibe, S. (1984) *Phytochemistry* **23**, 1635.
10. Kitagawa, S., Nishibe, S., Benecke, R. and Thieme, H. (1988) *Chem. Pharm. Bull.* **36**, 3667.
11. Jensen, S. R. (1992) *Ann. Missouri Bot. Gard.* **79**, 284.
12. Kuwajima, H., Takahashi, M., Ito, M., Wu, H., Takaishi, K. and Inoue, K. (1993) *Phytochemistry* **33**, 137.