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BIOSYNTHESIS OF IRIDOIDS IN *SYRINGA* AND *FRAXINUS*: SECOIRIDOID PRECURSORS*

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

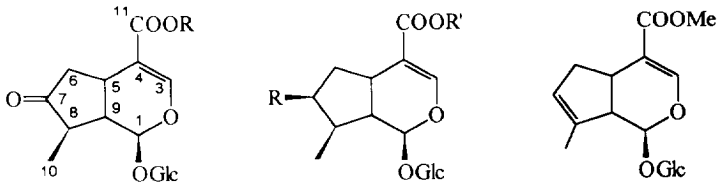
Abstract—Several deuterium-labelled secoiridoids have been prepared and tested as possible precursors for the iridoids in *Fraxinus excelsior*, *Syringa josikaea* and *S. vulgaris*. Oleoside 11-methyl ester was an efficient precursor for the oleosides, whereas secologanin-type iridoids gave only significant incorporation in *S. josikaea*. In this plant low incorporations into the oleosides were also seen for kingiside and 8-epi-kingiside. The major pathway to the oleosides therefore seems to proceed via a direct ring fission of ketologanin to oleoside 11-methyl ester. A Baeyer–Villiger-like mechanism which explains the different compounds found in the plants is proposed, and the taxonomy of the Oleaceae is discussed. Due to the unique presence of the usual pathway leading to secologanin and its congeners in *Fontanesia*, the Oleaceae is considered to be a member of the Gentiananae rather than Scrophulariales/Lamianae.

INTRODUCTION

In the preceding papers [1, 2] we have shown that the biosynthesis of oleosides (i.e. secoiridoids with an 8,9-double bond) proceeds via 7-ketologanic acid (**1**) or ketologanin (**2**), and that it thus differs from the biosynthesis of the secologanin-type iridoids, where neither **1** nor **2** are intermediates. Possible secoiridoid precursors for the oleosides were considered some time ago and two routes were discussed [3]. The first route proceeds via secologanin (**3**), which might undergo rearrangement of the 8,10-double bond to compound **4** and subsequent oxidation at C-7 to oleoside 11-methyl ester (**5**). The second route was through 8-epi-kingiside (**6**), which by anti-elimination could give **5**. To discriminate between these alternatives, feedings of [11-$O^{14}CH_3$]-**3**, [11-$O^{14}CH_3$]-**6** and [11-$O^{14}CH_3$]kingiside (**7**) to *Olea europaea* were carried out. Both lactone epimers (**6** and **7**) gave the same incorporations (0.13%) into oleuropein (**8**), whereas secologanin (**3**) gave a slightly higher incorporation (0.34%). The two kingisides (**6** and **7**) gave even lower incorporations (0.02%) into jasminine (**9**) in *Jasminum primulinum* [3, 4]. We have noted that iridoid-producing plants seem to be rather effective in converting externally supplied precursors [1, 2 and refs therein], and therefore significant results with late-stage iridoid precursors require at least 1% incorporation. Consequently, we consider all these reported incorporations to be of doubtful significance and a pathway through the kingisides thus seems unlikely. The next study [5] was based on the assumption that secologanin (**3**) was a common intermediate in the biosynthesis of the oleosides as well as of the ligustalosides (secoiridoids with a 10-aldehyde functionality, e.g. **10** and **11**). Assuming that the next step is epoxidation of the 8,10-vinylic bond of **3**, the oleosides might be formed by a reductive opening of the epoxide (8*S*-**12**) with subsequent elimination of water, and the formation of 10-hydroxy-oleosides and ligustalosides could thus be rationalized. Nevertheless, when feeding the epoxy-secologanins 8*R*-**12** and 8*S*-**12** as well as both epimers of epoxy-secoxyloganin (8*S*-**13** and 8*R*-**13**) to three oleaceous plants: *Olea europaea*, *Osmanthus fragrans* and *Ligustrum japonicum*, the largest incorporation into the oleosides was 0.14% while 8*S*-**12** gave 0.51% incorporation into **10**. Again, the incorporations were not convincing, and as a continuation of our work with carbocyclic precursors [1, 2] we decided to reinvestigate potential secoiridoid precursors for the oleosides in plants with a known, high biosynthetic capacity.

In this work we have thus synthesized deuterated analogues of 8-epi-kingisidic acid (**14**) and kingisidic acid (**15**) as well as the methyl esters **6** and **7**. Additionally, secologanin (**3**), secoxyloganin (**16**) and secologanoside (**17**) have been prepared in labelled form together with oleoside 11-methyl ester (**5**) and the oleosidic secologanin analogue **4**. They have been tested as precursors for the iridoids found in *Fraxinus* and *Syringa*. These compounds were discussed in the preceding paper [2].

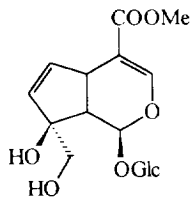
*Part 3 in the series 'Biosynthesis of iridoids in *Syringa* and *Fraxinus*' for part 2, see ref. [2]



1 R = H
2 R = Me

38 R = H; R' = H
39 R = OH; R' = Me

18



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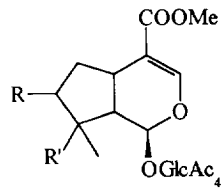
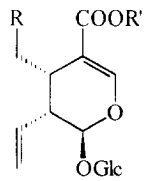
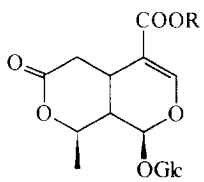


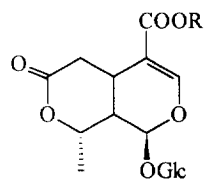
20a R = α-OH, R' = β-OH
22a R = β-OH, R' = α-OH

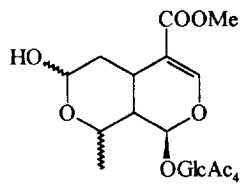
Fig. 1. Carbocyclic iridoids.



3 R = CHO, R' = Me
16 R = COOH, R' = Me
17 R = COOH, R' = H
25 R = $CH(OMe)_2$, R' = Me
38 R = CHO, R' = COOH



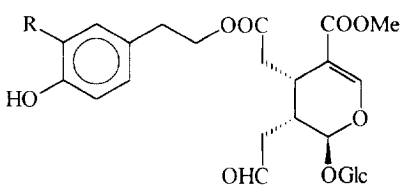


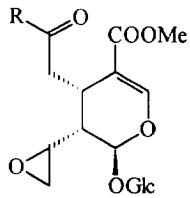


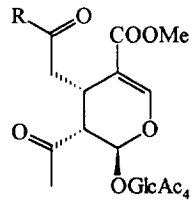
6 R = Me
14 R = H
36 R = CH_2CH_2-(4-OH-Ph)
37 R = CH_2CH_2-(3,4-di-OH-Ph)

7 R = Me
15 R = H

24a





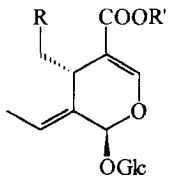


10 R = H
11 R = OH

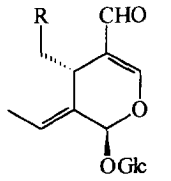
12 R = H
13 R = OH

21a R = H
23a R = OMe
31a R = OH

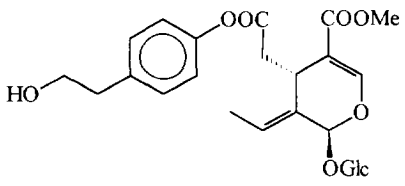
Fig. 2. Other secoiridoids.



4 R = CHO, R' = Me
5 R = COOH, R' = Me
26 R = COOMe, R' = Me
27 R = COOH, R' = H
29 R = COOGlc, R' = Me
32 R = $CH(OMe)_2$, R' = Me



28 R = COOMe
33 R = $CH(OMe)_2$



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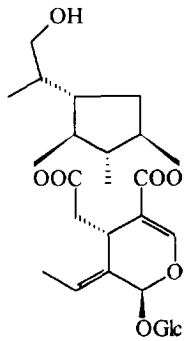

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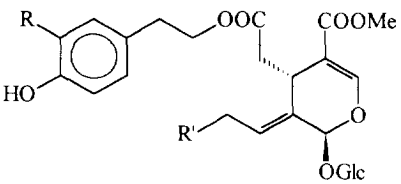

8 R = OH, R' = H
30 R = R' = H
34 R = H, R' = OH

Fig. 3. Oleoside-type secoiridoids.

RESULTS AND DISCUSSION

Synthesis of labelled kingisides

Three different approaches have earlier been reported for the synthesis of derivatives of 8-epi-kingiside (**6**), namely (i) a Baeyer–Villiger oxidation of 7-ketologanin tetraacetate (**2a**) [6], (ii) oxidative cleavage of carbocyclic 7,8-diols [7,8] and (iii) modification of secologanin (**3**) [9]. In our choice of a synthetic route to 8-epi-kingisidic acid (**14**) and its 8-epimer (**15**), the availability of the starting material [1, 7, 8] played an important role. Compound **2a**, and the *trans*-diols **20a**/**22a**, can be obtained from deoxygeniposide tetraacetate (**18a**), which in turn can be readily prepared from gardenoside hexaacetate (**19a**, >1% from dry *Gardenia jasminoides*). On the contrary, secologanin (**3**) was not available in large quantities at the time of year when the synthesis was undertaken. Moreover, setting out from secologanin (**3**) would only allow the preparation of 8-epi-kingiside (**6**) and not its 8-epimer (**7**), which would be needed as a reference in the feeding experiments [9].

Route (i) was attempted first: Baeyer–Villiger oxidation of 7-ketologanin tetraacetate (**2a**) with perbenzoic acid in chloroform containing a catalytic amount of boron trifluoride has been reported to give **6a** in 11% yield [6]. Using *m*-chloroperbenzoic acid (MCPA) as the oxidant we were able to carry out the reaction in low yield (9%), but even by preparative TLC the product **6a** could not be purified completely. Instead, the oxidative cleavage of 7,8-diols was investigated. Both *cis*-diols [7] and *trans*-diols [8] have been employed. Our main reason for choosing the latter strategy was our recent optimization of the epoxidation of deoxygeniposide tetraacetate (**18a**) to give a 96% yield of an α- and β-epoxide mixture (*ca* 1:2) [1]. By opening the epoxide mixture with perchloric acid in acetone as described [6], the *trans*-diol tetraacetate mixture could thus be obtained in 53% overall yield from **18a**.

The following oxidative cleavage had previously been effected by lead tetraacetate [6], but to avoid this reagent, sodium periodate was tested. This turned out to be a slow and selective reagent. The 7α,8β-compound (**20a**)

was cleaved to the expected 8-keto-7-oxo compound (**21a**), whereas the 7β,8α-isomer (**22a**) was left unchanged; probably because the 8α-hydroxy group is too hindered to participate in the formation of a cyclic intermediate in the cleavage reaction. However, Jones' oxidation of **22a** followed by methylation with diazomethane gave **23a** in 56% yield. The 7α,8β-epimer **20a** proved to react similarly by Jones' oxidation followed by methylation; **23a** was also obtained. Reduction of the 8-keto-group of **23a** with sodium borohydride proved difficult to carry out as this resulted in a mixture of the two kingiside tetraacetate epimers (**6a** and **7a**), unconverted starting material and a mixture of hemi-acetals, **24a**. Apparently the reaction proceeds by a relatively slow initial reduction of the 8-keto group followed by a fast lactone formation. The resulting lactones are then further reduced to their hemiacetalic counterparts. Therefore, it proved necessary to employ a large excess of sodium borohydride and extended reaction time and a second Jones' oxidation step to reconvert the hemiacetals into the desired lactones. The synthesis thus developed allowed introduction of deuterium at C-8 by reduction of **23a** with sodium borodeuteride, and the products [8-^{2}H]kingiside tetraacetate (**7a**) and [8-^{2}H]epi-kingiside tetraacetate (**6a**) could be separated and isolated in 30% and 32% yield, respectively. Deacetylation with methoxide gave the corresponding glucosides which were saponified with sodium hydroxide. Finally, the kingisides *d*-**6** and *d*-**7**, labelled in the methyl ester group, were prepared by treatment of the corresponding acids with deuterodiazomethane.

Synthesis of labelled secologanin derivatives

Synthesis of [^{2}H-OMe]secologanin (**3**) is not as straightforward as it may seem, since treatment of secologanic acid (**38**) with diazomethane primarily gives the methyl ketone [10, 11] resulting from insertion at C-7. Consequently, [11-OC^{2}H$_3$]secologanin (*d*-**3**) was prepared by treating secologanin dimethyl acetal (**25**) with sodium deutero-methoxide in deutero-methanol until a reasonable exchange had been achieved (1.7 ^{2}H in the 11-methoxy group). Deacetalization was performed with 6% perchloric acid in acetone.

Secologanoside (**17**) and secoxyloganin (**16**) labelled in the 6-position were readily obtained from secologanin (**3**). Thus, secologanin tetraacetate (**3a**) was partially exchanged with deuterium at the carbon next to the aldehyde (i.e. C-6) by treatment with triethylamine in deutero-ethanol. The crude product was more than 90% pure and subsequent Jones' oxidation yielded almost exclusively the expected **16a**. Upon deacetylation this one-pot synthesis yielded *d*-**16** in an overall yield of 48%. Alkaline hydrolysis afforded the diacid *d*-**17** in high yield (96%).

Synthesis of labelled oleosides

During hydrolysis of oleoside dimethyl ester (**26**) the ester group at C-7 proved much more labile than that at C-11. Thus, treatment of **26** with two moles of aqueous base for two days at ambient temperature gave oleoside 11-methyl ester (**5**) in reasonable yield (56%). This selectivity allowed synthesis of [11-OC^{2}H$_3$]-**5** by performing a complete hydrolysis of **26** (or of another oleoside derivative). Methylation of the resulting diacid **27** with deutero-diazomethane and subsequent selective hydrolysis yielded the desired 11-deutero-methyl ester (*d*-**5**).

Labelled **4** could be obtained, although in poor yield, in the following way. Silylation of oleoside dimethyl ester (**26**) [12] afforded the tetrasilyl derivative in quantitative yield. When testing the procedure, reduction of this compound was effected with DIBAL (0.93 equivalents) in toluene for two hours, keeping the temperature below − 60°. Desilylation of the product mixture was accomplished with a mixture of methanol and an aqueous solution of a weak acid (5% ammonium chloride, acetic acid or citric acid served equally well). Fractionation of the crude product gave 8% of **4**, masked as its dimethyl acetal (**32**, formed during desilylation) and 47% of starting material. For the synthesis of labelled material, silylated 7,11-[(OC^{2}H$_3$)$_2$]-**26** was reduced with an excess of DIBAL (1.25 equivalents) at a slightly higher temperature in an attempt to improve the yield of aldehyde. After desilylation, this gave 15% of the labelled 7-aldehyde (*d*-**4**) contaminated with *d*-**28**. Additionally, 43% of **26** was recovered. To separate *d*-**4** and *d*-**28**, *d*-**4** was converted to the dimethyl acetal (*d*-**32**) which was separated from *d*-**28** by MPLC. Deacetalization of *d*-**32** was accomplished by perchloric acid in acetone to give the pure *d*-**4**.

Biosynthetic experiments with Fraxinus excelsior

The results of the biosynthetic experiments are summarized in Table 1. Neither of the kingiside derivatives (*d*-**6**/*d*-**7** and *d*-**14**/*d*-**15**) gave significant incorporations (experiments 1–4, Table 1) and in all cases a large percentage of the precursor was re-isolated. Secologanin (**3**) gave a small incorporation (0.5%) into 7-glucosyl 11-methyl oleoside (**29**), but neither secologanoside (**17**) nor secoxyloganin (**16**) showed reasonable incorporations. The only compound that gave significant incorporations was oleoside 11-methyl ester (**5**, experiment 9, Table 1) which showed incorporation into all the iridoids except secologanoside (**17**) and 8-epi-kingiside (**6**). The accumulated incorporation was 19%, indicating that **5** is a major participant in oleoside biosynthesis. The last compound tested (**4**) was not incorporated at all.

Biosynthetic experiments with Syringa *spp.*

The results with *S. josikaea* are summarized in Table 2. No incorporations were seen for the kingisidic acids (**14** and **15**) in this plant. Kingiside (**7**) showed a very low incorporation into oleuropein (**8**) while 8-epi-kingiside (**6**) gave low incorporations into oleoside 11-methyl ester (**5**), oleuropein (**8**) and ligstroside (**30**). On the other hand, secologanin (**3**) gave a quite high (4%) incorporation into 7-glucosyl 11-methyl oleoside (**29**) and lower incorporations into the more complex oleosides. Again sec-

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

Experiment	1	2	3	4	5	6	7	8	9
Precursor	*d*-**15**	*d*-**14**	*d*-**7**	*d*-**6**	*d*-**3**	*d*-**17**	*d*-**16**	*d*-**4**	*d*-**5**
Labelling site(s)	8	8	11-OMe	11-OMe	11-OMe	6	6	11-OMe	11-OMe
(mg)	17	19	18	15	18	20	20	17	20
Epimeric purity (%)	> 98	> 98	> 98	> 98	—	—	—	—	—
Plant (g)	20	19	23	23	23	19	18	32	19
Metabolic period (days)	4	4	4	4	4	4	4	6	4
Reisolated precursor (%)	> 25*	> 25*	50	26	36†	11	19	19	1
29 (incorp. %)	—	—	0.5		0.5	1.5‡	1‡	12§	5
6 (incorp. %)	—	—	—	26			—	—	—
Amount (mg; ratio)	21(1:1)	14(3:2)	16(1:1)	26(1:2)	21(3:2)	9(3:2)	11 (1:1)	(22 (3:2)	12 (2:1)
5 (incorp. %)		—	1	—		—	—	7§	1
17 (incorp. %)	—			—	—	11	3‡	—	—
Amount (mg, ratio)		—	11			15(1:3)	7(1:2)	6	5
34 (incorp. %, mg)	– (32)	– (**)	– (51)	(39)	– (44)	– (23)	– (32)	– (24)	0.5¶ (33)
8 (incorp. %, mg)	– (27)	– (9)	– (32)	– (18)	– (23)	– (13)	– (18)	– (50)	0.5 (23)
35 (incorp. %, mg)	– (84)	– (66)	– (50)	– (36)	– (79)	(47)	– (24)	– (50)	7 (51)
30 (incorp. %, mg)	– (48)	– (27)	– (62)	– (51)	– (73)	– (29)	– (43)	– (75)	4 (47)

* As judged by ^{1}H NMR from the purity and intensity of the H-8 peak which was partially overlapping the HOD-signal.

† A fr., eluted with H_2O–MeOH 2:1, contained 32% of a secologanin-type compound which gave rise to ^{2}H NMR peaks at δ3.65 and δ3.45; the successive fr. of **26** also contained labelling at these positions, corresponding to 4% incorp.

‡ May be ionized precursor.

§ As the precursor was detectable in these frs by ^{1}H NMR, the incorp. was ascribed to this compound.

¶ Verified for the pure pentaacetate.

** Not collected.

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

Experiment	10	11	12	13	14	15	16	17	18	19
Precursor	*d*-**15**	*d*-**14**	*d*-**7**	*d*-**6**	*d*-**3**	*d*-**17**	*d*-**16**	*d*-**5**	*d*-**5**	*d*-**5**
Labelling site(s)	8	8	11-OMe	11-OMe	11-OMe	6	6	11-OMe	"	"
(mg)	20	19	18	16	18	20	21	13	14	15
Epimeric purity (%)	> 98	> 98	> 98	> 98	—	...	...	...	...	...
Plant (g)	13	14	16	16	16	15	14	16	16	16
Metabolic period (days)	5	5	5	5	5	5	5	1	2	3
29 (incorp. %)			(0.5)*		4†			2.2	3.6	1.5
6 (incorp. %)	...	...	—	37	0	...	...	...	...	...
Amount (mg, ratio)	12	9	6 (1:2)	14 (1:2)	14 (1:1)	13 (1:1)	5	14	19	12
5 (incorp. %)	—		(3)‡	0.5	0.1		—	2.4	2.6	4.1
17 (incorp. %)	...	...	...	...	...	28	(16)§	...	...	—
Amount (mg, ratio)	28	37	43 (1:1)	36 (1:1)	45 (1:1)	25 (1:3)	29 (1:4)	23 (1:1)	47 (1:1)	36 (1:1)
8 (incorp. %, mg)	– (129)	– (140)	0.2 (165)	0.2 (167)	0.4 (236)	– (125)	– (152)	0.4 (64)	0.8 (97)	3 (119)
30 (incorp. %, mg)	– (28)	– (35)	– (54)	0.4 (38)	0.7 (57)	– (26)	– (50)	2 (22)	2 (38)	3 (30)

* Probably ring opened precursor.

† After addition of carriers (**5** and **16**) and [Me] the mixt. was purified by MPLC, but only the fr. of **29** still showed incorporation.

‡ Probably native precursor.

§ More likely to be reisolated precursor.

ologanoside (**17**) and secoxyloganin (**16**) gave no incorporations. The incorporation rate of oleoside 11-methyl ester (**5**) was tested in a series with three different metabolic periods. After one day significant incorporations into **29** and **30** were seen but only a small incorporation into **8** was observed. After two days the same pattern prevailed but the incorporation into oleuropein (**8**) had increased and was the highest observed after three days. This points to a rather slow biosynthetic conversion which may be limited by transport of the precursors within the plant. It also seems likely that **8** is formed with hydroxylation of ligstroside (**30**) as the final step.

In *Syringa vulgaris* (experiments 20–22, Table 3) no significant incorporations were seen for kingiside (**7**), 8-epi-kingiside (**6**) or secologanin (**3**).

Biosynthesis in the Oleaceae

In the preceding [2] paper, we have lined up the different biosynthetic pathways that we have observed in some different genera within the family. However, a pathway via iridodial, up to and including deoxyloganic acid (**38**), appears to be a common feature within the family, and indeed within the entire order Gentianales [13–15].

In the present work, we have tested the probable secoiridoid intermediates between ketologanin (**2**) and oleoside methyl ester (**5**), the compound from which oleuropein (**8**) and similar iridoids in the Oleaceae are derived. However, even in plant species tested for their high biosynthetic ability, neither of the likely intermediates of kingiside-type nor secologanin-type were incorporated to a degree expected for a true intermediate between **2** and **5**. Furthermore, the oleoside-like compound **4**, with an aldehyde functionality at C-7, was not incorporated to any significant degree. In this respect, we find it noteworthy that all the compounds found in these plants are oxidized to the acid stage at C-7. We therefore conclude that **2** is the immediate precursor for **5** and this indicates that the conversion most probably is a single-step reaction through a Baeyer–Villiger-type intermediate (Fig. 4). A reaction initiated by rupture of the peroxide bond followed by cleavage of the 7,8-bond and simultaneous abstraction of H-9 (route i) would give rise to oleoside 11-methyl ester (**5**). A similar reaction with abstraction of a proton from C-10 (route ii) would produce secoxyloganin (**16**). Finally, 8-epi-kingiside (**6**) could be formed via an alkyl shift of C-8 (route iii). Such a mechanism could fully explain the presence of the other iridoid products found co-occurring with the oleosides.

In the sense that no discrete compound apparently is formed between **2** and **5**, we have a situation where the biosynthesis of the oleosides is analogous to that presently accepted for the conversion of loganin (**39**) to secologanin (**3**) and its congeners, the 'ordinary' secoiridoids [13, 14]. Both reactions include an oxidative ring fission, but the pathway to the oleosides starts at a higher oxidation level and consequently the products are also more highly oxidized.

Taxonomy

The systematic position of the Oleaceae is disputed by taxonomists and has been so far a long time. Thus, Takhtajan [16] and Dahlgren [17] favour a relationship

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

Experiment	20	21	22
Precursor	*d*-**7**	*d*-**6**	*d*-**3**
Labelling site(s)	11	11	11
(mg)	18	15	17
Epimeric purity (%)	> 98	> 98	—
Plant (g)	25	25	25
Metabolic period (days)	5	5	5
14 (incorp. % mg)	1.3*(17)	– (22)	– (22)
5 (incorp. %, mg)	—	– (13)	– (6)
29 (incorp. %)	2*	—	—
6 (incorp. %, mg)	—	34 (22)	– (21)
5 (incorp. %)	—	—	—
26 (incorp. %, mg)	—	– (36)	– (28)
Total amount (mg)	77	71	55
26 (incorp. %, mg)	– (32)	– (35)	25†(39)
36 (incorp. %, mg)	– (113)	– (124)	2†(140)
37 (incorp. %, mg)	– (109)	– (170)	– (149)
8 (incorp. %, mg)	– (359)	– (399)	– (414)
Mixt. of secoiridoids (incorp. %, mg)	– (100)	– (128)	0.5(124)

*May be reisolated precursor, partially opened (contaminating the fr. of **14**).

†Two signals at δ3.65 and 3.4 indicating the fr. to be impure, possibly containing secologanin-type impurities/compounds.

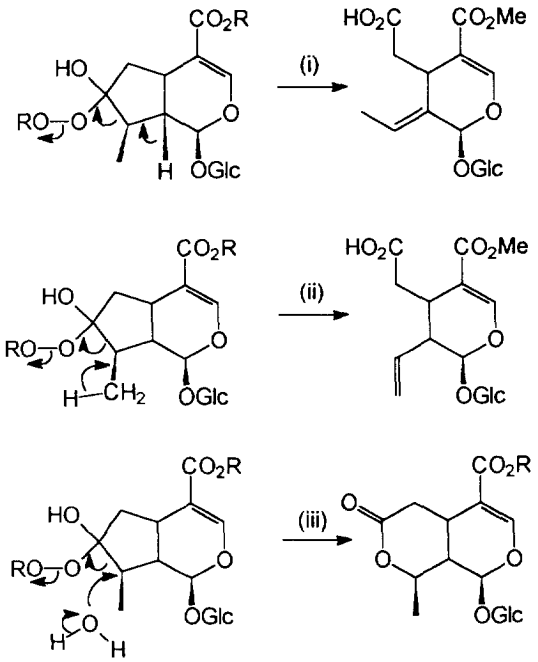

Fig. 4. Proposed ring-fission mechanism.

with Gentianales, while Cronquist [18] and Thorne [19] include the Oleaceae in their definition of Scrophulariales. Moreover, recent data from molecular biology infer that the Oleaceae might have profound affinities to the Scrophulariaceae. Sequencing of the chloroplast gene *rbc*L, coding for a subunit of the ubiquitous enzyme rubisco [20], suggested an inclusion of the Oleaceae (represented by *Ligustrum vulgare*) in Scrophulariales. A similar result was obtained in a phylogenetic study based on restriction-site mapping of the inverted repeat region of the chloroplast genome. Herein, the Oleaceae (represented by the genera *Forsythia*, *Ligustrum* and *Syringa*) exhibited a closer resemblance to Scrophulariales than to Gentianales [21].

With regard to phytochemistry, two groups of compounds with a systematic potential are present in the Oleaceae, namely iridoids on one hand and verbascoside and its analogues on the other. The secoiridoids, namely secologanin (**3**) and its congeners, have a delimited distribution [14] in the super orders Cornanae, Loasanae and Gentiananae (*sensu* Dahlgren [17]). They have never been reported from Lamianae or Ericanae, the remaining iridoid-containing super orders. Conversely, verbascoside and its analogues are widespread in Lamianae, where most families contain it [15]. Besides in the Oleaceae, there are only a few, scattered reports of these compounds outside Lamianae.

The iridoids found in the Oleaceae are, at least formally, secoiridoids. Even though the biosynthesis of the oleosides has now been shown to be different from that of the 'ordinary' secoiridoids, we have also shown that the wide spectrum of compounds present in the family are apparently biosynthesized from deoxyloganic acid (**38**), like the 'ordinary' secoiridoids. Furthermore, we have demonstrated [22] that the biosynthesis of the secoiridoids in the genus *Fontanesia* follows the route via loganin (**38**) and secologanin (**3**).

Verbascoside and similar compounds have been reported from a number of genera within the Oleaceae [15] and we have found such compounds to be present in all the species of Oleaceae we have investigated, including *Fontanesia*. They appear to be ubiquitous in the family.

The phytochemical evidence is therefore conflicting. However, in view of the coincidence of the unique biosynthetic pathway to the secoiridoids found in *Fontanesia* and Gentianales proper, we consider that the Oleaceae should be included in Gentiananae. Consequently, the ability to produce oleoside-type iridoids may be seen as a derived character within the family. This is consistent with the fact that the genera considered by Taylor [23] to be the most primitive (*Forsythia* [24], *Abeliophyllum* [25] and *Fontanesia* [22, 26]) do not contain oleosides, while the more advanced genera investigated (*Chionanthus* [27], *Fraxinus* [1], *Ligustrum* [1], *Olea* [3], *Osmanthus* [28], *Phillyrea* [1] and *Syringa* [1]) all produce them. The genus *Jasminum* also contains the oleosides [3], and even though Taylor [23] lists it as a primitive taxon this genus seems to be chemically the most advanced of all, due to the large number of complex compounds that have been reported from it [cf 29].

EXPERIMENTAL

The general procedures and the experimental procedure for the biosynthetic experiments are given in the previous paper [2].

Baeyer–Villiger oxidation of **2a**. 7-Ketologanin tetraacetate (**2a**, 144 mg, 0.259 mmole) in $CHCl_3$ (4 ml) was mixed with a soln of MCPA (195 mg, 1.06 mmol) in $CHCl_3$ (3 ml), and BF_3–Et_2O (10 μl) was added. After stirring for 4.5 days at room temp. in the dark, $CHCl_3$ (10 ml) was added and the soln washed with satd Na_2SO_3 (10 ml). The organic layer was dried (Na_2SO_4) and concd *in vacuo*. The residue was dissolved in Et_2O (15 ml) and the soln washed with satd $NaHCO_3$ and H_2O (10 ml each). Drying (Na_2SO_4) of the organic layer and subsequent evapn *in vacuo* gave a residue, which was fractionated by prep. TLC (Et_2O) to give only a poor yield of impure 8-epi-kingiside tetraacetate (**6a**, 13 mg).

Synthesis of trans*-diols* (**20a** *and* **22a**) *from deoxygeniposide tetraacetate* (**18a**). To a soln of **18a** (6.40 g, 11.9 mmol) in dry benzene (50 ml) was added MCPA (2.8 g, 80% active, 16.2 mmol). After standing overnight at room temp. more MCPA (0.4 g, 2.3 mmol) was added and the soln stirred for an additional 45 min (TLC showed completion of the reaction). The reaction mixt. was washed with 1M NaOH (2 × 100 ml) and H_2O (100 ml). Subsequently, the organic layer was dried (Na_2SO_4 and $MgSO_4$) and evapd to give a syrup (6.92 g) consisting of a 1:2 mixt. of α- and β-epoxide tetraacetates. This was dissolved in Me_2CO (200 ml) and 6% $HClO_4$ (15 ml) and heated (45–50°) for 8 hr. The soln was allowed to cool slowly overnight. The soln was diluted with Me_2CO (100 ml) and dried (Na_2SO_4 and

K_2CO_3) and the solvent removed to give a foam (7.14 g) which was fractionated by flash chromatography (400 g silica gel, $CHCl_3$–EtOH 10:1, frs of 20 ml were collected at 30 ml min^{-1}) giving a syrup (frs 35–51, 6.10 g) containing polar impurities, in addition to **20a** and **22a**. On rechromatography (400 g silica gel), successively eluting with Et_2O–EtOAc (10:1 1000 ml), $CHCl_3$–EtOH (15:1 1200 ml) and EtOAc (700 ml), 115 frs (20 ml) were collected. Frs 86–101 afforded a 1:2 mixt. of 8-epi-caryoptoside tetraacetate (**22a**) and 7-epi-caryoptoside tetraacetate (**20a**, 3.54 g, 52% overall). Later frs (102–105) contained almost pure **20a** (55 mg, 1%).

7-epi-Caryoptoside tetraacetate (**20a**). ^{1}H NMR (250 MHz, $CDCl_3$): δ7.35 (*d*, J = 1 Hz, H-3), 5.54 (*d*, J = 2 Hz, H-1), 5.30–4.95 (3H, H-3′, H-4′ and H-2′), 4.89 (*d*, J = 8 Hz, H-1′), 4.26 (*br s*, 2H, 2 × H-6′), 4.00 (*t*, J = 9 Hz, H-7), 3.75 (*s*, 3H, 11-OMe), 2.96 (*q*, J = 9.5 Hz, H-5), 2.68 (*m*, H_a-6), 2.38 (*dd*, J = 10 and 2 Hz, H-9), 2.17–1.93 (1H, obsc. by Ac-Me's, H_b-6), 1.23 (*s*, 3H, 10-Me), 2.14, 2.08, 2.04, 1.95 (*s*, each 3H, 4 × Ac-Me). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ149.1 (C-3), 113.4 (C-4), 93.1 (C-1), 79.4 and 79.3 (C-7 and C-8), 51.2 (11-OMe), 47.0 (C-9), 36.8 (C-6), 24.5 (C-5), 17.2 (C-10), 95.8 (C-1′), 72.5 (C-5′), 72.1 (C-3′), 70.7 (C-2′), 68.1 (C-4′), 61.5 (C-6′).

8-epi-Caryoptoside tetraacetate (**22a**). ^{1}H NMR (250 MHz, $CDCl_3$): δ7.40 (*br s*, H-3), 5.38 (*d*, J = 5 Hz, H-1), 3.87 (*br s*, H-7), 3.77 (*s*, 3H, 11-OMe), 3.13 (*q*, J = 9.5 Hz, H-5), 1.45 (*s*, 3H, 10-Me).

$NaIO_4$ *oxidation of* trans-7,8-*diol tetraacetates* (**20a** and **22a**). To a mixt. of **20a** and **22a** (3.54 g, 6.17 mmol) in Me_2CO (100 ml), $NaIO_4$ (1.45 g in 30 ml H_2O, 6.78 mmol) was added. Stirring was continued for 5 days at room temp. (including 7 hr at 45–50°). The solvent was removed and the residue dissolved in H_2O. The aq. layer was extracted with EtOAc (2 × 200 ml) and the combined organic layers washed with H_2O (200 ml) and dried (Na_2SO_4). The soln was filtered through activated C (1 g). Evapn of the filtrate yielded a syrup (3.26 g, 92%) which was fractionated by flash chromatography (400 g silica gel). Elution with Et_2O–EtOAc (10:1, 2200 ml) and Et_2O–EtOAc (5:1, 1200 ml) gave 140 frs of 20–25 ml. Frs 66–90 afforded **21a** (1.14 g, 32%). Elution with EtOAc (1000 ml) yielded almost pure **22a** (1.28 g, 36% of the total educt mixt.).

8-Keto-7-oxo compound (**21a**). ^{1}H NMR (250 MHz, $CDCl_3$): δ9.65 (*s*, H-7), 7.52 (*s*, H-3), 5.49 (*d*, J = 8.8 Hz, H-1), 3.76 (*s*, 3H, 11-OMe), 3.71 (*m*, H-5), 3.08 (*dd*, J = 8.8 and 5.5 Hz, H-9), 2.89 (*dd*, J = 18.6 and 8.7 Hz, H_a-6), 2.62 (*dd*, J = 18.6 and 3.2 Hz, H_b-6), 2.31 (*s*, 3H, 10-Me), 5.26 (*t*, J = 9.0 Hz, H-3′), 5.14 (*t*, J = 9.5 Hz, H-4′), 5.03 (*t*, J = 8.5 Hz, H-2′), 4.95 (*d*, J = 8.0 Hz, H-1′), 4.36 (*dd*, J = 12.3 and 4.7 Hz, H_a-6′), 4.19 (*dd*, J = 12.3 and 2.5 Hz, H_b-6′), 3.79 (*m*, H-5′), 2.17, 2.10, 2.09 and 2.07 (*s*, each 3H, 4 × Ac-Me) [6]. ^{13}C NMR (62.5 MHz, $CDCl_3$): δ206.3 (C-8), 199.2 (C-7), 166.2 (C-11), 152.0 (C-3), 108.9 (C-4), 94.8 (C-1), 51.4 (11-OMe), 50.6 (C-9), 44.9 (C-6), 32.1 (C-5), 25.9 (C-10), 96.9 (C-1′), 72.3 (C-5′), 71.9 (C-3′), 70.7 (C-2′), 68.0 (C-4′), 61.5 (C-6′), 170.0, 169.2, 169.0 and 167.1 (4 × Ac-CO), 20.6 and 20.4* (4 × Ac-Me); * denotes higher intensity. A DEPT spectrum was used to assign C-6 and C-9.

Jones' oxidation of 8-epi-caryoptoside tetraacetate (**22a**). To **22a** (1.28 g, 2.23 mmoles) in Me_2CO (40 ml) was added, dropwise, Jones' reagent (1.25 ml, 3.35 mmoles CrO_3) under ice-cooling. The mixt. was stirred for 30 min at 0° when the reaction was stopped by addition of $Na_2S_2O_5$ and worked up by addition of H_2O (50 ml) and extraction with EtOAc (2 × 75 ml). The combined organic layers were washed with H_2O (100 ml). Evapn gave crude **31a** (1.16 g) which, on methylation (CH_2N_2–Et_2O), afforded a syrup (1.19 g), purified by prep. TLC (Et_2O–EtOAc, 10:1) to give **23a** (0.75 g, 56%).

8-Keto-7-carboxylic acid tetraacetate (**31a**). ^{1}H NMR (250 MHz, $CDCl_3$): δ7.49 (*s*, H-3), 5.53 (*d*, J = 9 Hz, H-1), 3.77 (*s*, 3H, 11-OMe), 3.55 (*m*, H-5), 3.08 (*dd*, J = 9 and 5.5 Hz, H-9), 2.76–2.55 (*m*, 2H, 2 × H-6), 2.32 (*s*, 3H, 10-Me), 5.30–4.92 (4H, H-3′, H-4′, H-2′ and H-1′), 4.40–4.15 (2H, 2 × H-6′), 3.79 (*m*, H-5′), 2.16, 2.08, 2.06 and 2.04 (*s*, each 3H, 4 × Ac-Me). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ206.5 (C-8), 175.6 (C-7), 166.2 (C-11), 152.0 (C-3), 108.6 (C-4), 95.0 (C-1), 51.5 (11-OMe), 50.4 (C-9), 34.8 (C-6), 32.2 (C-5), 28.1 (C-10), 97.1 (C-1′), 72.4 (C-5′), 71.9 (C-3′), 70.7 (C-2′), 68.1 (C-4′), 61.5 (C-6′), 170.7, 170.1, 169.3 and 169.1 (4 × Ac-CO), 20.6 and 20.4* (4 × Ac-Me).

The NMR spectra of the dimethyl ester (**23a**) is given below.

Jones' oxidation of 7-epi-caryoptoside tetraacetate (**20a**). Compound **20a** (55 mg, 0.096 mmol) in Me_2CO (85 ml) was treated with Jones' reagent as above (100 µl, 0.267 mmol) and worked up to give crude **31a** (56 mg, 97%).

Jones' oxidation of **21a**. A soln of **21a** (470 mg, 0.822 mmol) in Me_2CO (4 ml, reagent grade) was cooled below −5° and Jones' reagent (460 µl, 1.23 mmol CrO_3) was added. A green ppt was formed during 30 min when work-up ($Na_2S_2O_5$, 10 ml 2M H_2SO_4 and 15 ml EtOAc) was performed. The aq. phase was extracted with EtOAc (15 ml) and the combined organic layers washed with H_2O (10 ml) and dried (Na_2SO_4). Filtration through activated C (0.2 g) followed by concn gave a residue, which was methylated (CH_2N_2–Et_2O) yielding crude methyl ester **23a** (460 mg, 93% overall). An aliquot (125 mg) was subjected to prep. TLC (Et_2O–EtOAc, 10:1). The central part of the band around R_f 0.63 afforded the pure methyl ester **23a** (78 mg), $[\alpha]_D^{21}$ −85.1° ($CHCl_3$; *c* 1.6). ^{1}H NMR (250 MHz, $CDCl_3$): δ7.47 (*br s*, H-3), 5.51 (*d*, J = 8.8 Hz, H-1), 3.74 (*s*, 3H, 11-OMe), 3.65 (*s*, 3H, 7-OMe), 3.54 (*dt*, J = 8.3 and 2 × 4.8 Hz, H-5), 3.03 (*dd*, J = 8.8 and 5.5 Hz, H-9), 2.57 (*dd*, J = 17.2 and 8.3 Hz, H_a-6), 2.49 (*dd*, J = 17.2 and 4.5 Hz, H_b-6), 2.28 (*s*, 3H, 10-Me), 5.23 (*t*, J = 9.0 Hz, H-3′), 5.10 (*t*, J = 9.5 Hz, H-4′), 5.00 (*t*, J = 8.8 Hz, H-2′), 4.92 (*d*, J = 7.6 Hz, H-1′), 4.34 (*dd*, J = 12.4 and 4.8 Hz, H_a-6′), 4.14 (*dd*, J = 12.4 and 2.7 Hz, H_b-6′), 3.76 (*m*, obsc. by 11-OMe, H-5′), 2.13, 2.05, 2.04 and 2.02 (*s*, each 3H, 4 × Ac-Me). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ205.7 (C-8), 171.9 (C-7), 166.1 (C-

*Denotes higher intensity.

11), 151.9 (C-3), 108.7 (C-4), 95.0 (C-1), 51.4 and 51.3 (7-OMe and 11-OMe), 50.5 (C-9), 34.8 (C-5), 28.2 (C-10), 97.1 (C-1'), 72.3 (C-5'), 71.8 (C-3'), 70.6 (C-2'), 68.0 (C-4'), 61.5 (C-6'), 170.4, 169.9, 169.1, 168.9 (4 × Ac-CO), 20.5* and 20.3 (4 × Ac-Me); * denotes double intensity. (Found C, 51.46; H, 5.77. $C_{26}H_{34}O_{16}$ requires C, 51.83; H, 5.69%).

$NaBH_4$-*reduction of* **23a**. The methyl ester **23a** (98 mg) was dissolved in dioxane (5 ml) and $NaBH_4$ (9 mg) in H_2O (0.5 ml) was added. Stirring was continued for 80 min. Addition of 2 M H_2SO_4 (10 ml) was followed by extraction of the aq. layer with EtOAc (2 × 15 ml). The organic layers were combined and dried (Na_2SO_4). Evapn yielded a syrup (95 mg) consisting of epimeric morroniside tetraacetates (**24a**), kingiside tetraacetate and its 8-epimer (**7a** and **6a**), and **23a** in a 1:1:1 ratio.

Jones' oxidation of the hemiacetalic by-products (**24a**). The above mixt. was dissolved in Me_2CO (4 ml) and cooled below 0°. Jones' reagent (100 μl, 0.267 mmole) was added and oxidation allowed to proceed for 15 min. After addition of $Na_2S_2O_5$, H_2O (10 ml) was added and the mixt. extracted with EtOAc (2 × 15 ml). Evapn *in vacuo* gave a syrup (87 mg), which was a *ca* 2:1 mixt. of **6a**/**7a** and **23a**.

Kingiside (**7**) *and 8-epi-kingiside* (**6**). Frs mainly containing lactone tetraacetates (**6a** and **7a**, 163 mg) were deacetylated (7 ml, 0.1 M NaOMe, 30 min at room temp. and 10 min at 50°). Upon addition of HOAc and concn *in vacuo*, the residue was subjected to MPLC (B-column, 25:1 to 5:1). Kingiside (**7**, 16 mg, 25%) and 8-epi-kingiside (**6**, 9 mg, 14%) were obtained when eluting with 5:1. ^{1}H NMR as reported [30, 31].

[8-^{2}H]-8-*Kingisidic acid* (**15**) *and* [8-^{2}H]-8-*epi-kingisidic acid* (**14**). A dioxane soln (30 ml) of **23a** (923 mg, 156 mmole) was mixed with $NaBD_4$ (116 mg, 2.99 mmoles) in D_2O (2 ml). After stirring for 3 hrs at room temp., the reaction was completed and the mixt. worked up as above yielding a syrup (0.86 g, 98%) containing both hemiacetals (**24a**) and lactones (**6a** and **7a**), but no **23a**. The mixt. was subjected to Jones' oxidation (25 ml Me_2CO, 1.18 ml reagent, 3.13 mmol CrO_3) at 0° for 45 min. Work-up yielded a crude mixt. of [8-^{2}H]-**6a** and [8-^{2}H]-**7a** (0.70 g, 80%). Prep. TLC (Et_2O and Et_2O–EtOAc, 10:1, 1 development with each eluent) gave two bands, which yielded almost epimer-free (>98%) [8-^{2}H]kingiside tetraacetate (*d*-**7a**, R_f 0.4, 210 mg, 30%) and [8-^{2}H]-8-epi-kingiside tetraacetate (*d*-**6a**, R_f 0.5, 226 mg, 32%). Deacetylation of *d*-**7a** (210 mg) with 0.1M NaOMe (MeOH) at room temp. for 30 min gave, after neutralization (HOAc) and subsequent MPLC (B-column, H_2O and 4:1), [8-^{2}H]kingiside (*d*-**7**, 111 mg, 76%). Deacetylation, as above, of *d*-**6a** (226 mg) yielded, upon MPLC (B-column, H_2O and 3:1), [8-^{2}H]-8-epi-kingiside (*d*-**6**, 135 mg, 85%). Hydrolysis (2 ml 1M NaOH in 3 ml H_2O) of *d*-**7** (130 mg) for 4 hr at room temp. was followed by evapn with 10% HOAc (2 × 5 ml). Chromatography of the product dissolved in 10% HOAc (B-column, H_2O to 4:1) gave [8-^{2}H]kingisidic acid (*d*-**15**, 41 mg, 33%, 1.0 ^{2}H at C-8) and a partially ring-opened product (21 mg, 17%). Hydrolysis and chromatography as above of *d*-**6** (101 mg) afforded [8-^{2}H]-8-epi-kingisidic acid (*d*-**14**, 70 mg, 72%, 1.0 ^{2}H at C-8) and recovered *d*-**6** (9 mg, 9%).

[11-OC^{2}H$_3$]-8-*epi-Kingiside* (d_3-**6**). Impure **14** (*ca* 100 mg from various plant sources) was evapd with HOAc (2–3 drops) and EtOD. The residue was redissolved in EtOD (5 ml) and an excess of CD_2N_2–Et_2O added. Upon addition of HOAc and evapn, the residue was remethylated. MPLC (B-column, 7:1 to 3:1) of the product gave d_3-**6** (3:1, 44 mg, 2.2 ^{2}H in the 11-OMe group).

[11-OC^2-H$_3$]*Kingiside* (d_3-**7**). Impure **15** (*ca* 100 mg) was evapd with HOAc and EtOD. Subsequent methylation (CD_2N_2–Et_2O in EtOD) and MPLC (B-column, 15:1 to 4:1) gave recovered **15** (7:1, 40 mg, 5–10% ring-opened) and d_3-**7** (4:1, 28 mg, 15–20% ring opened). Treatment of the former was repeated to give a further amount of d_3-**7** (26 mg), ^{1}H NMR showed the presence of 2.0 ^{2}H in the 11-OMe group.

[11-OC^{2}H$_3$]*Secologanin* (d_3-**3**). Secologanin (**3**, 349 mg from *Cornus officinalis*) in MeOH (10 ml) with conc. H_2SO_4 (50 μl) added was allowed to stand at 0° for 1 hr [9]. Addition of NaOMe to pH 8 and HOAc to pH 6 was followed by filtration through activated C (0.2 g). The filtrate was taken to dryness and evapd with CD_3OD (2 ml). Then 0.1 M $NaOCD_3$ (10 ml) was added to the residue and the soln stirred on a water bath (35–40°) for 75 min; subsequently the soln was left overnight at 0°C. After addition of HOAc and evapn, the residue was subjected to MPLC (C-column, 3:1 to 1:1). The isolated [11-OC^{2}H$_3$]secologanin dimethyl acetal (*d*-**25**, 299 mg) showed 1.7 ^{2}H in the 11-OMe group (^{1}H NMR [9]). To a Me_2CO solution (20 ml) of *d*-**25**, 299 mg) was added 6% $HClO_4$ (5 ml). After stirring overnight at 5°, work-up and purification by MPLC (B-column, 5:1 to 2:1) gave [11-OC^{2}H$_3$]-**3** (96 mg, 1.7 ^{2}H in the 11-OMe group).

[6-^{2}H]*Secologanoside* (*d*-**17**) *and* [6-^{2}H]*secoxyloganin* (*d*-**16**). Acetylation (5 ml Ac_2O and 5 ml pyridine) of secologanin (**3**, 318 mg) for 1 hr gave **3a** (0.35 g, 77%) as a syrup. This was dissolved in EtOD (10 ml) and Et_3N (0.1 ml) was added and the soln stirred for 3 hr at room temp. Addition of HOAc (50 ml) and evapn *in vacuo* yielded a syrup, which according to ^{1}H NMR had *ca* 60% ^{2}H at C-6. The crude [6-^{2}H]secologanin tetraacetate (*d*-**3a**) was dissolved in Me_2CO (15 ml) and Jones' reagent (1 ml, 2.67 mmol CrO_3) was added. After 30 min the soln was worked up with H_2O and EtOAc (30 ml each). The aq. layer was re-extracted with EtOAc (30 ml). The combined organic layers were washed with H_2O (30 ml) and dried. Evapn gave a syrup (0.32 g), which according to ^{1}H NMR was a 8:1 mixt. of *d*-**16a** and *d*-**3a**. Repeated oxidation with Jones' reagent (0.2 ml, 2.67 mmol ml^{-1}), as above, for 15 min yielded crude *d*-**16a** (0.32 g). Deacetylation was performed with 0.05 M NaOMe in MeOH (10 ml) for 30 min. MPLC (C-column, H_2O, 3:1 to 1:1) furnished pure *d*-**16** (157 mg, 48% overall from **3**, 1.2 ^{2}H at C-6) when eluting with 3:2. Hydrolysis of *d*-**16** (83 mg) in 40% NaOD–D_2O (150 μl and 4 ml, respectively) for 6 hr at room temp. was followed by addition of HOAc (to pH < 6). Chromatogra-

phy (B-column, H_2O to 3:2) gave *d*-**17** (77 mg, 96%) when eluting with 3:2. ^1H NMR showed 1.2 ^2H at C-6 (equally distributed in the *pro-R* and *pro-S* positions) by comparison with an authentic sample.

7-Oxo-oleoside 11-methyl ester dimethyl acetal (**32**). Oleoside dimethyl ester (**26**, 0.36 g, 0.86 mmol) was silylated in pyridine (3 ml) with hexamethyldisilazane (HMDS, 0.6 ml, 2.86 mmol) and TMSiCl (0.3 ml, 2.38 mmol) for 30 min. Addition of Et_2O (40 ml) and 5% HOAc (20 ml) gave, after stirring, two clear layers. The Et_2O layer was washed with satd $NaHCO_3$ (20 ml) and H_2O (10 ml). After drying, toluene was added and the soln evapd *in vacuo* giving the silylated derivative (**26b**) as a syrup (0.62 g) in quantitative yield. ^1H NMR (250 MHz, $CDCl_3$): δ7.55 (*s*, H-3), 6.10 (*q*, J = 6.5 Hz, H-8), 5.83 (*br s*, H-1), 4.76 (*d*, J = 7.5 Hz, H-1'), 4.07 (*dd*, J = 9.5 Hz and 5 Hz, H-5), 3.76 (*s*, 3H, 11-OMe), 3.67 (*s*, 3H, 7-OMe), 3.80–3.70 (1H obsc. by OMe's, H_a-6'), 3.60–3.40 (*m*, 4H, H_b-6', H-3', H-4' and H-2'), 3.27 (*m*, H-5'), 2.76 (*dd*, J = 15 and 5 Hz, H_a-6), 2.39 (*dd*, J = 15 and 9.5 Hz, H_b-6), 1.80 (*dd*, J = 6.5 and 1.5 Hz, 10-Me), 0.21 (*s*, 18H, 2 × O-TMSi), 0.17 (*s*, 9H, O-TMSi), 0.10 (*s*, 9H, O-TMSi). **26b** (0.62 g; 0.86 mmol) in toluene (50 ml) was placed in a three-necked, round-bottomed flask (250 ml) fitted with a T-tube, a stopper and an adapter with a rubber membrane. From a syringe, 1M DIBAL (0.8 ml) was added dropwise during 5 min at − 65°. The soln was stirred for 2 hr at − 65°. Then the temp. was allowed to rise to − 30°C, after which MeOH (2 ml) and 5% Na–K-tartrate (25 ml) were added. The organic layer was washed with 5% Na–K-tartrate (25 ml) and H_2O (25 ml) and dried. Filtration of the soln on activated C over Celite and evapn *in vacuo* gave a residue, which was desilylated in MeOH (60 ml) with 5% HOAc (15 ml) for 3 hr. The pH was adjusted to *ca* 7 by addition of satd aq. $NaHCO_3$. Pptd salts were filtered off and the filtrate concd *in vacuo*. The residue was fractionated by MPLC (C-column, 2:1 to 1:1). Two frs were collected. Recovered **26** (0.17 g, 47%) and **32** (31 mg, 8%). ^1H NMR (250 MHz, D_2O): δ7.50 (*s*, H-3), 6.07 (*q*, J = 6.5 Hz, H-8), 5.90 (*s*, H-1), 4.90 (*d*, J = 8 Hz, H-1'), 4.41 (*dd*, J = 9 and 3.5 Hz, H-7), 3.86 (*dd*, J = 12 and 2 Hz, H_a-6'), 3.70 (*s*, 3H, 11-OMe), 3.72–3.58 (*m*, 2H, obsc. by 11-OMe, H_b-6' and H-5), 3.54–3.30 (*m*, 4H, H-3', H-5', H-4' and H-2'), 3.31 and 3.26 (*s*, each 3H, 2 × acetal-OMe), 2.05 (*m*, H_a-6), 1.69 (*d*, J = 6.5 Hz, 10-Me), 1.64 (*m*, H_b-6).

[11-(CO^{2}H$_3$)]-7-Oxo-oleoside 11-methyl ester (*d*-**4**). Oleoside (**27**, 314 mg) was evapd with D_2O and HOAc (2 ml and 5 drops, respectively). Methylation with CD_2N_2–Et_2O in EtOD–THF (1:1, 10 ml) and chromatography (B-column, 6:1, 3:1 and 2:1) gave [7,11-(OC^{2}H$_3$)$_2$]oleoside dimethyl ester (*d*-**26**, 248 mg, 74%). Silylation as above gave *d*-**26b** (0.85 g, 120 mmol) which was dissolved in toluene (80 ml) and cooled to − 75° while DIBAL (1M, 1.5 ml) was added dropwise during 15 min. Over the next 30 min, the temp. rose to − 55°, and upon addition of MeOH (2 ml), the reaction mixt. was worked up as above yielding a syrup (0.73 g), which was desilylated overnight at room temp. Evapn *in vacuo* followed by MPLC (C-column, 4:1 to 1:1) gave a mixt. of monoaldehydes (*d*-**4** and *d*-**28**, 74 mg, 14%) when eluting with 3:1, whereas recovered **26** (233 mg, 43%) was obtained with 3:2. Finally, **32** and **33** (13 mg, 2%) were eluted with 1:1. The above mixt. of monoaldehydes (74 mg) was dissolved in MeOH (10 ml) and conc. H_2SO_4 (0.1 ml) was added. After 1 hr at 0°, 1 M NaOMe was added to pH 7, the pptd salts were filtered off and the filtrate evapd. The residue was fractionated by MPLC (B-column, H_2O, 2:1 and 3:2), and the 7,7-dimethyl acetal (*d*-**32**, 32 mg) was eluted by 3:2. Compound *d*-**32** was treated with 6% $HClO_4$ (3 ml) in Me_2CO (10 ml) at 5° for 24 hrs under stirring. After addition of $NaHCO_3$ (to pH > 7), the mixt. was taken to dryness. The residue was chromatographed (B-column, 5:1 to 1:1). Pure [11-(OC^{2}H$_3$)]-7-oxo-oleoside 11-methyl ester (*d*-**4**, 20 mg) was eluted with 2:1. ^1H NMR (250 MHz, D_2O; mainly (75%) hydrate form): δ7.47 (*s*, H-3), 6.04 (*q*, J = 6.5 Hz, H-8), 5.88 (*br s*, H-1), 4.87 (*d*, J = 8 Hz, H-1'), 1.90 (*ddd*, J = 14, 8.5 and 4 Hz, H_a-6), 1.66 (*d*, J = 7 Hz, 10-Me), 1.65–1.55 (*m*, obsc. by 10-Me's, H_b-6). Additional signals were seen for the aldehyde form: δ9.58 (*t*, J = 2.5 Hz, H-7), 7.52 (*s*, H-3), 6.03 (*q*, obsc. by H-8 of the hydrate form, H-8), 5.86 (*br s*, H-1), 4.88 (*d*, J = 8 Hz, H-1'), 4.04 (*dd*, J = 8.5 and 5.5 Hz, H-5), 2.78 (*ddd*, J = 15.5 and 2.5 Hz, H_a-6), 2.58 (*ddd*, J = 15.5, 8.5 and 3 Hz, H_b-6), 1.65 (*d*, J = 6.5 Hz, H-8). ^{13}C NMR (62.5 MHz, D_2O): δ170.7 (C-11), 154.9 (C-3), 129.5 (C-9), 125.1 (C-8), 110.4 (C-4), 95.7 (C-1), 89.8 (C-7), 43.1 (C-6), 30.7 (C-5), 13.8 (C-10), 100.5 (C-1'), 77.3 (C-5'), 76.6 (C-3'), 73.6 (C-2'), 70.4 (C-4'), 61.5 (C-6'); additional signals were seen for the free aldehyde (*ca* 25%): 49.7 (C-6), 29.0 (C-5), whereas C-7 was not seen without relaxation delay.

Selective hydrolysis of oleoside dimethyl ester (**26**). Hydrolysis (1.2 ml 1 M NaOH in 5 ml H_2O) of **26** (252 mg, 0.60 mmol) was done for 2 days at room temp. After addition of 10% HOAc to pH 6, the soln was subjected to MPLC (B-column, 6:1 to 1:1) to give oleoside (**27**, 68 mg; 29%) and oleoside 11-methyl ester (**5**, 137 mg, 56%).

Oleoside (**27**). ^1H NMR (250 MHz, D_2O): δ7.56 (*s*, H-3), 6.10 (*q*, J = 7.1 Hz, H-8), 5.91 (*br s*, H-1), 4.91 (*d*, J = 8.0 Hz, H-1'), 3.96 (*dd*, J = 9.7 and 4.6 Hz, H-5), 3.87 (*dd*, J = 12.4 and 4.6 Hz, H_a-6'), 3.67 (*dd*, J = 12.4 and 5.7 Hz, H_b-6'), 3.55–3.33 (*m*, 4H, H-3', H-5', H-4' and H-2'), 2.77 (*dd*, J = 13.7 and 4.6 Hz, H_a-6), 2.39 (*dd*, J = 13.7 and 9.7 Hz, H_b-6), 1.69 (*dd*, 3H, J = 7.1 and 1.2 Hz, 10-Me). ^{13}C NMR (62.5 MHz, D_2O): δ176.8 (C-7), 170.9 (C-11), 155.8 (C-3), 129.0 (C-9), 126.0 (C-8), 95.7 (C-1), 49.8 (C-6), 31.5 (C-5), 13.8 (C-10), 100.5 (C-1'), 77.2 (C-5'), 76.5 (C-3'), 73.5 (C-2'), 70.3 (C-4'), 61.5 (C-6').

Oleoside [11-OC^{2}H$_3$]*methyl ester* (**d-5**). Oleoside (**27**, 314 mg) was evapd with D_2O and HOAc (2 ml and 5 drops, respectively) and methylated with CD_2N_2–Et_2O in EtOD–THF (1:1, 10 ml). Chromatography (B-column, 3:1 and 2:1) gave [7,11-(OC^{2}H$_3$)$_2$]-**26** (248 mg, 74%). Hydrolysis of *d*-**26** (170 mg, 0.41 mmol) was performed overnight at 0° (0.42 ml 1 M NaOH in 3 ml H_2O) followed by addition of HOAc (pH 6) and MPLC (B-column, H_2O, 5:1 to 3:1). Elution with 3:1 afforded *d*-**5** (137 mg, 2.8 ^2H in the 11-OMe group).

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