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Biosynthesis of iridoids lacking C-10 and the chemotaxonomic implications of their distribution

Lotte Boe Frederiksen, Søren Damtoft, Søren Rosendal Jensen*

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

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Abstract

Continuing earlier experiments in which deoxyloganic acid proved to be a precursor of the doubly decarboxylated iridoids in *Thunbergia alata*, we have now shown that deuterium labelled 6-deoxyretzioside was incorporated into stilbericoside in this plant. In the same experiment labelled retzioside was isolated, indicating that this is also a likely intermediate in the biosynthetic pathway. Feedings in two species of *Deutzia* were also performed with deuterium-labelled iridoids, namely iridodidal glucoside, 8-epiiridodial glucoside, 7-hydroxy-iridodial glucoside and 10-hydroxy-iridodial glucoside (=decapetaloside). Of these, only the first and the last were incorporated. Apparently, loss of C-10 in the biosynthesis of scabroside is analogous to that of stilbericoside and the sequence of events after formation of the 7,8-double bond appears in both cases to be the same as that found for the biosynthesis of antirrhinoside. The close relationship between *Nuxia*, *Retzia* and the Stilbaceae indicated by chloroplast DNA sequencing results is substantiated by the common occurrence of a special kind of iridoids in these taxa. A similar close relationship is found between Hydrangeaceae and Loasaceae.

Keywords: *Thunbergia*; Acanthaceae; *Deutzia*; Hydrangeaceae; Iridoid glucosides; Stilbericoside; Deutzioside; Biosynthesis; Chemotaxonomy; Loasaceae; *Nuxia*; *Retzia*; Stilbaceae

1. Introduction

Iridoid compounds lacking C-11, i.e. aucubin and congeners, are very common in genera from the families within the order Lamiales *sensu* Dahlgren (1989) and they have been shown to constitute a valuable taxonomic character to delineate these families from those belonging to Cornales and Gentianales which mainly contain secoiridoids (Jensen, 1991, 1992). Conversely, iridoids lacking C-10 have been found in only a limited number of families of the dicotyledons, namely in Stilbaceae (*Stilbe* and *Xeroplana*) (Dahlgren, Nielsen, Goldblatt & Rourke, 1979; Rimpler & Pistor, 1974), in Retziaceae (*Retzia capensis*) (Dahlgren et al., 1979; Damtoft, Franzyk, Jensen & Nielsen, 1993c), in Buddlejaceae (*Nuxia*) (Jensen, Ravnkilde & Schripsema, 1998), in Acanthaceae (*Thunbergia*) (Jensen & Nielsen, 1989; Jensen, Jensen & Nielsen, 1988), Ericaceae (*Arbutus* and *Arctostaphylos*) (Geissman, Knaack & Knight, 1966; Jahodár, Kolb & Leifertová, 1981), in Hydrangeaceae (*Deutzia*) (Bonadies, Esposito & Guiso, 1974; Esposito & Guiso, 1973), and in Loasaceae (*Mentzelia*) (Danielson & Hawes, 1973; El-Naggar, Beal & Doskotch, 1982; Jensen, Mikkelsen & Nielsen, 1981). The first four taxa all belong to Lamiales and the iridoids from these as well as those from Ericaceae are characterized by the lack of both C-10 and C-11, e.g. unedoside (**1**) and stilbericoside (**2**). In the following, these compounds are designated C-8 iridoids, as they only have eight carbon atoms in the aglucone. The iridoids from the genera *Deutzia* and *Mentzelia* are different since they always have C-11 present as a methyl or an oxymethylene substituent, examples of compounds found in *Deutzia* are deutzioside (**3**) and scabroside (**4**).

* Corresponding author. Tel.: +45-452-521-03; fax: +45-459-339-68.

E-mail address: oksrj@pop.dtu.dk (S.R. Jensen).

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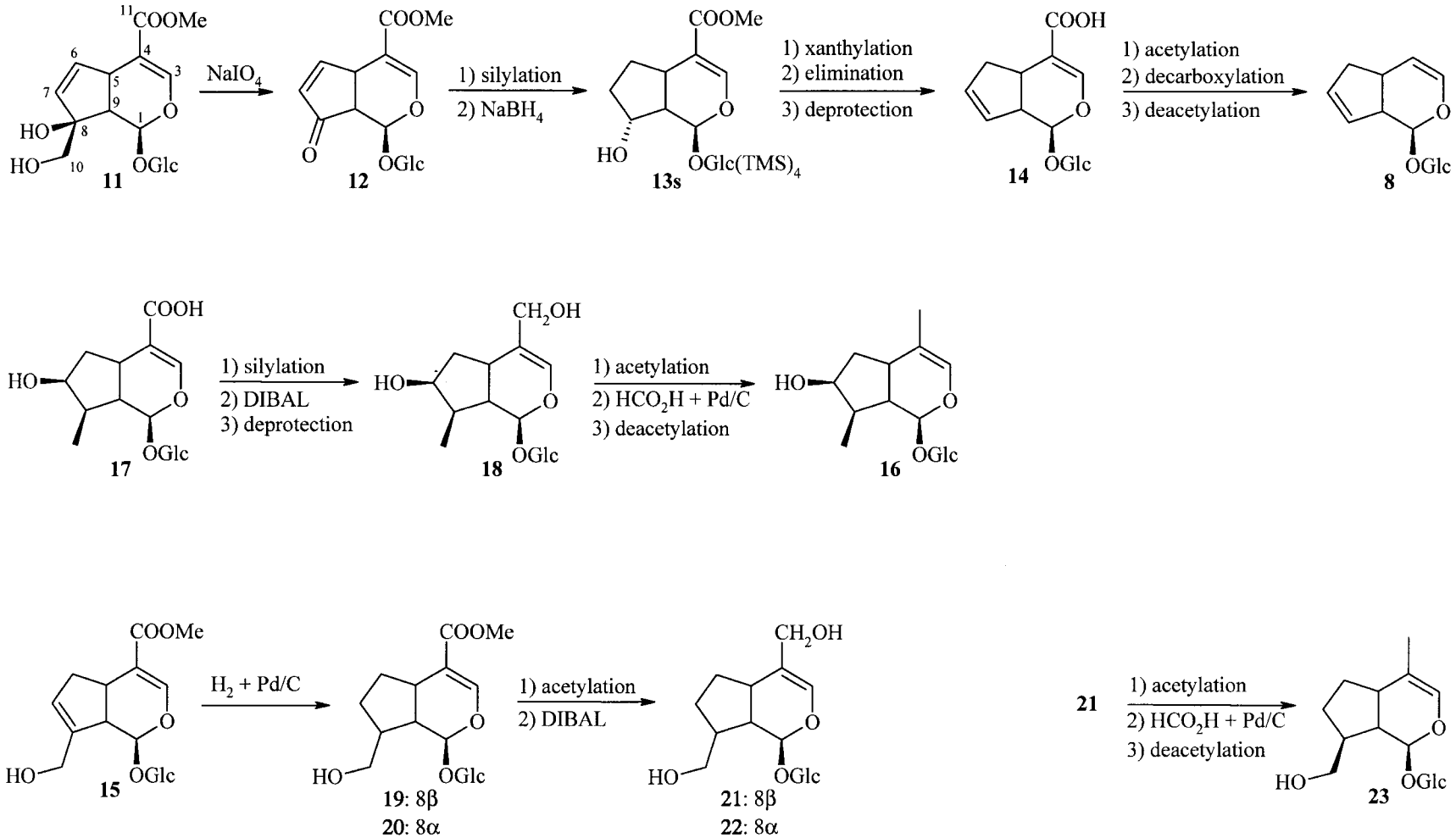


Scheme 1. Synthesis of labelled iridoid precursors.

The biosynthesis of the aucubin type of iridoid glucosides is well known (cf. Jensen, 1991), proceeding through a pathway including the key compounds 8-epiiridodial (**5**) and epideoxyloganic acid (**6**), with C-11 being lost by decarboxylation of an α,β-unsaturated acid (Damtoft, Jensen, Jessen & Knudsen, 1993b). Compounds derived from such a pathway including **5** and **6** are widespread in Lamiales (Jensen, 1991, 1992). Surprisingly, our initial results with *Thunbergia* (Damtoft, Frederiksen & Jensen, 1994), and more recently with *Nuxia* (Jensen et al., 1998) on the biosynthesis of unedoside (**1**) type iridoids which lack both C-10 and C-11, have shown that these compounds are biosynthesized through deoxyloganic acid (**7**), and not from epideoxyloganic acid (**6**). The latter had earlier been the surmised intermediate (Jensen, 1992) due to lack of specific evidence. Furthermore, administration of deuterium labelled **7** to *T. alata* indicated that the loss of C-10 does not involve decarboxylation of an α,β-unsaturated acid since labels at both H-7 and H-8 were retained in the isolated stilbericoside (**2**) (Damtoft et al., 1994). To further investigate the biosynthetic sequence after the decarboxylation of C-10 in stilbericoside (**2**), deuterium labelled 6-deoxy-retzioside (**8**) has been synthesized and tested as a precursor in *T. alata*.

Regarding the compounds found in *Deutzia*, some experiments have been performed with *D. crenata*. Thus, Inouye, Ueda and Uesato (1977) showed that [2-^{14}C]-MVA was incorporated into deutzioside (**3**) and scabroside (**4**) without scrambling. Also, tritium labelled 10-hydroxygeraniol, iridodial and iridodial glucoside (**9**) have been shown (Inouye, Ueda, Uesato & Kobayashi, 1978) to be precursors for **3**. Finally, parallel administrations (Uesato, Miyauchi, Itoh & Inouye, 1986) of iridodial glucoside (**9**) and 8-epiiridodial glucoside (**10**) indicated a lack of stereospecificity since both were incorporated into **3** although **9** gave a much better incorporation than **10**. However, both precursors were probably contaminated with their 8-epimer, but in different ratios, which somewhat obscured the results (Jensen, 1991). In order to fully clarify this and to gain further knowledge of the biosynthesis of the *Deutzia* iridoids, we have tested some possible precursors.

2. Results

2.1. Synthesis of labelled precursors

The synthesis (Scheme 1) of 6-deoxyretzioside (**8**) was accomplished starting from gardenoside (**11**) obtained from *Gardenia jasminoides*. Selective oxidation with sodium periodate cleaved only the C8–C10 bond (cf. Damtoft, Jensen & Nielsen, 1993a) of the aglucone to give randioside (**12**) which was protected as the trimethylsilyl ether (**12s**)[1] and then reduced with sodium borohydride to give, selectively, the 8α-hydroxy compound (**13s**). In order to avoid an E2 elimination which would presumably produce an 8,9-double bond, a Chugaev reaction (Depuy & King, 1960) was attempted. Conversion of the free hydroxy group to the methylxanthate was performed using the phase transfer procedure by Lee, Chan, Wong and Wong (1989). Surprisingly, the primary TMS group at C-6′ was unstable under these conditions since an additional methyl xanthate group was introduced also at this position in the product (**13sx**) as seen by NMR, mainly by the low field position of C-6′ (δ 72.5). Pyrolysis in quinoline using the procedure of Bordwell and Landis (1958) gave the 10-nor-geniposide derivative **14sx** methyl ester as a crude product. Desilylation and saponification provided 10-*nor*-geniposidic acid (**14**) in only 12% yield from **13sx**. Acetylation to **14a** followed by a copper-catalyzed decarboxylation in quinoline (Murai & Tagawa, 1980) yielded the tetraacetate **8a** in 21% yield. Finally, deacetylation provided **8** in an overall yield of 0.8% from **11**. Labelling was introduced selectively into the 6β- and 8β-positions by performing the reduction of randioside tetra-*O*-TMS ether (**12s**) with sodium borodeuteride. The resulting labelled 6,8-[$^{2}H_2$]-retzioside (d-**8**) contained more than 0.9 ^{2}H at both C-6 and C-8 as measured by ^{1}H NMR. The yields in this sequence were somewhat better than those above, namely 1.8% from **11**.

Our previous syntheses of iridodial glucoside (**9**) (Jensen, Kirk & Nielsen, 1989) and of 8-epiiridodial glucoside (**10**) (Breinholt, Damtoft, Demuth, Jensen & Nielsen, 1992) have shown that both these compounds are more or less contaminated (ca. 15 and 2–5%, respectively) with the 8-epimer, when synthesized from **11** or from geniposide (**15**). In our hands, these compounds co-crystallize and no significant purification is obtained by repeated recrystallisation. In order to secure an epimer-free preparation of deuterium labelled **9**, we in this case treated 5-deoxystansioside (iridotrial glucoside), readily obtained from *Chaenostomum foetidum* (Jensen et al., 1989), with sodium borodeuteride. Transformation of the resulting labelled 11-OH-iridodial glucoside to selectively labelled d-**9** as earlier described (Jensen et al., 1989) provided the desired epimer-free preparation. Conversely, we were able to purify the above previously prepared multiply labelled d-**10** by chromatography on an efficient RP-18 column (see Experimental).

Labelled 7-hydroxy-iridodial glucoside (d-**16**) was

[1] The following suffixes are used: **a**: acetate; **s**: trimethylsilylether; **x**: xanthate. Deuterated compounds have the prefix: d-.

Table 1
Results of administrations to *T. alata*

	Deoxyloganic acid (d-**7**)		6-Deoxyretzioside (d-**8**)	
	amount (mg)	incorp. (%)	amount (mg)	incorp. (%)
Precursor fed	43		20	
Crude extract	180		190	
Sugars	106	0	104	0
Epistilbericoside	10	0	8	0
Stilbericoside (**2**)	10	3	9	4
Thunaloside (**24**)[a]	2	1	trace	0
Retzioside (**25**)[a]	–	–	3.5	3
MeOH-eluate[b]	9	14	ca. 10	6

[a] The fraction was contaminated with tryptophan.
[b] Fraction containing recovered precursor.

prepared (Scheme 1) starting from loganin (**17**). Protection to give the trimethylsilyl ether, followed by reduction of the carboxyl ester with diisobutyl aluminium hydride and deprotection, gave 7,11-dihydroxy-iridodial glucoside (**18**). Deuterium label was introduced at C-11 by transfer hydrogenation of the hexaacetate (**18a**) using Pd/C and dideutero formic acid. Deacetylation gave selectively labelled d-**16**.

Palladium catalyzed reduction of geniposide (**15**) with deuterium gas and added triethylamine gave rise to seemingly unpredictable mixtures (5:1 to 1:3) of multiply labelled adoxoside (d-**19**) and the 8-epimer (d-**20**), which could be separated only with difficulty (Damtoft et al., 1994). However, since separation of the epimers proved possible at a later stage, such a mixture (2:3) was acetylated and reduced with diisobutyl aluminium hydride. From this experiment, both the labelled 11-hydroxy-decapetaloside (d-**21**) and its epimer d-**22** could be isolated. Finally, transfer hydrogenation of the hexa-acetate d-**21a** using Pd/C and formic acid gave the desired multiply labelled decapetaloside (d-**23**).

2.2. Feeding experiments

In the biosynthetic pathway to the compounds found in *Thunbergia* earlier proposed (Damtoft et al., 1994), we suggested 6-deoxy-retzioside (**8**) as a likely intermediate. The labelled analogue d-**8** was therefore fed to leaves of young *T. alata* plants. In addition, deuterium labelled deoxyloganic acid (d-**7**) was administered in a control experiment and the results are presented in Table 1. Consistent with the proposed pathway, both compounds were incorporated into stilbericoside (**2**) and the higher incorporation of d-**8** (4%) than of d-**7** (3%) is consistent with **8** being a probable later intermediate. A trace of unlabelled thunaloside (**24**) as well as labelled retzioside (**25**) was also isolated from the feeding experiment with 6-deoxyretzioside (d-**8**); in the ^{1}H NMR spectrum of **25**, a signal from H-8 (δ 6.0) was absent and it was obvious that no significant dilution with unlabelled material had taken place. This iridoid glucoside has so far not been isolated from *Thunbergia* species, but it has been found in *Retzia capensis* (Damtoft et al., 1993c) which also contains the 10,11-decarboxylated iridoids including **2**. No incorporation into 6-*epi*-stilbericoside was seen in either case, but when feeding with d-**7** a small fraction containing labelled thunaloside (**24**) in admixture with tryptophan (1:2) was isolated. The label (at C-10) in **24** was not diluted to any visible degree, but due to the small amount and to the presence of tryptophan in the fraction, it was not possible to determine this with certainty.

The earlier reported apparent lack of stereospecificity in the biosynthesis of the *Deutzia* iridoids (Uesato et al., 1986) was investigated by feeding labelled, epimerically pure iridodial glucoside (d-**9**) and 8-epiiridodial glucoside (d-**10**) to young shoots of *D.*

Table 2
Results of administrations to *Deutzia species*

Plant	*D. schneideriana*				*D. scabra*			
	d-**9**		d-**10**		d-**16**		d-**23**	
	mg	incorp. (%)	mg	incorp. (%)	mg	incorp. (%)	mg	incorp. (%)
Precursor fed	19		18		30		28	
Crude extract	1200		1600		1030		1070	
Sugars	640		800		550		450	
Scabroside (**4**)	300	3	320	0	31	0	33	1[a]
Deutzioside (**3**)	80	5	100	0	55	0	58	4[a]
MeOH-eluate[b]	100	33	150	16	160	10	165	6

[a] Incorporations calculated are minimum values assuming that all ^{2}H-7 and ^{2}H-8 from precursor are retained.
[b] Fraction containing recovered precursor.

schneideriana in June. The results (Table 2) clearly show that only iridodial glucoside (**9**) is a precursor for deutzioside (**3**) and scabroside (**4**).

The next experiments were performed with growing shoots of *D. scabra* in late August when the other species seemed too mature to exhibit full biosynthetic activity. When feeding 7-hydroxy-iridodial glucoside and decapetaloside (d-**16** and d-**23**, respectively), only the latter was incorporated into deutzioside (**3**) and scabroside (**4**) (4 and 1%, respectively; see Table 2). The incorporations are minimum values since they were calculated assuming that both ^{2}H-7 and ^{2}H-8 are fully retained.

3. Discussion

3.1. Biosynthesis

The good incorporation of labelled 6-deoxyretzioside (d-**8**) apparently supports our earlier (Damtoft et al., 1994) proposed route for the biosynthesis of the C_8 iridoid glucosides in *Thunbergia* (Scheme 2). The fact that when feeding with d-**8**, the produced retzioside (**25**) and the recovered precursor were fully labelled, might indicate that these two compounds are not natural intermediates on the biosynthetic pathway. However, also in the experiment where d-**7** was used as precursor, the produced thunaloside (**24**) was only little diluted, if at all. The explanation could be that the experiments were performed with a large amount of precursor compared to the total amount of iridoids present in the plant. Thus, de novo synthesis of iridoids may have been suppressed more or less completely and thus depleting the pool of natural intermediates by the massive flux of labelled compounds. As mentioned above, the unedoside (**1**) derivatives in *Nuxia* has also been shown to be derived from deoxyloganic acid (**7**) and not from **6**. This, together with the fact that *Retzia capensis* and members of the Stilbaceae have the same iridoid garniture as that found in *Thunbergia*, indicates that the same biosynthetic route to the C_8 iridoids may be present in all these taxa. It is notable that in the proposed biosynthetic pathway to stilbericoside (**2**), the last steps after formation of the 7,8-double bond are completely analogous to the last steps in the biosynthesis of antirrhinoside (**27**; Scheme 2) (Damtoft, Jensen & Schacht, 1995), the only difference being the presence or absence of C-10.

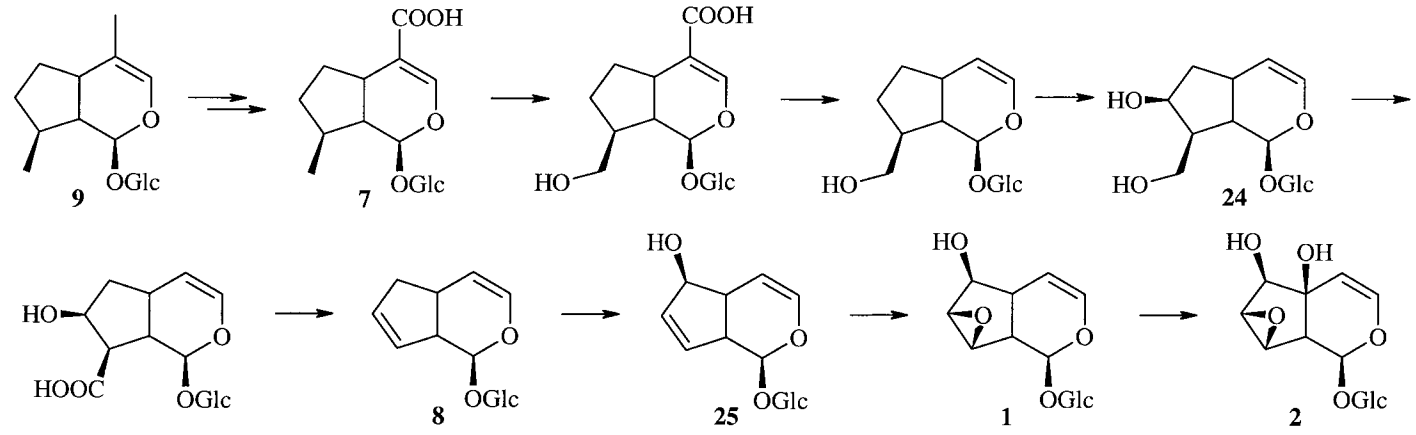
As might have been expected, only iridodial glucoside (**9**) was incorporated into the iridoid glucosides of *Deutzia* when epimer-free, labelled precursors were tested. The fact that 10-hydroxyiridodial glucoside (**23**) was incorporated while **16** was not, allows us to sketch a probable biosynthetic pathway to deutzioside (**3**) and scabroside (**4**), assuming it to be analogous to the pathways discussed above (Scheme 1) after the formation of the 7,8-double bond. Support for such a pathway in *Deutzia* and in *Mentzelia* (Loasaceae) is found in the actual isolation of the hypothetical intermediates decapetaloside (**23**) and decaloside (**28**) from *Mentzelia decapetala* (El-Naggar et al., 1982).

3.2. Chemotaxonomy

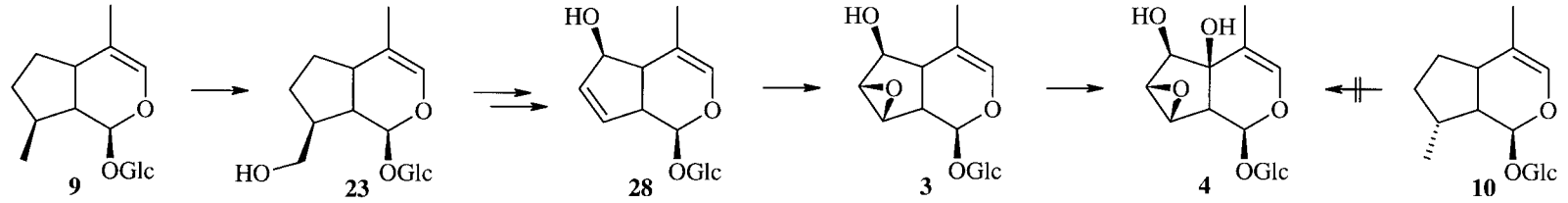
The common presence of C_8 iridoids in *Nuxia*, *Retzia*, Stilbaceae and *Thunbergia*, suggests a common biosynthetic pathway and thus indicates the possibility of a phylogenetic relationship which will be discussed in the following. *Nuxia* and *Retzia* have traditionally been included in Loganiaceae in the tribe Buddlejeae (Leeuwenberg & Leenhouts, 1980) while Stilbaceae was thought to be closely related to Verbenaceae (Cronquist, 1988). The presence of C_8 iridoids in both *Retzia* and Stilbaceae, however, led Dahlgren et al. (1979) to propose a close affinity between *Retzia* and Stilbaceae, not only because of the iridoids present, but also for morphological and other reasons. This view was later corroborated by the embryological investigation by Engell (1987). Most contemporary taxonomists have raised the tribe Buddlejeae (including *Nuxia*) to family rank and placed Buddlejaceae close to Scrophulariaceae (Cronquist, 1988; Dahlgren, 1989; Thorne, 1992). Very recent results based on chloroplast DNA sequencing (Oxelman, Backlund & Bremer, 1999) implicates that *Nuxia*, *Retzia* and Stilbaceae are more related to each other than to any other taxa. The sequencing results also indicate a much closer relationship between this assembly and Scrophulariaceae/Buddlejaceae than with Loganiaceae. This is completely in concert with the chemical profiles, where Loganiaceae are characterized by loganin and secoiridoids combined with lack of verbascoside and other caffeoyl phenylethanoid glucosides (CPG's), while Scrophulariaceae and Buddlejaceae usually contain decarboxylated iridoids and CPG's, a combination also found in *Nuxia*, *Retzia* and Stilbaceae (Jensen, 1992). The relationship between these three taxa and *Thunbergia* is less striking since the latter appears to be a true member of Acanthaceae (Oxelman et al., 1999; Scotland, Sweere, Reeves & Olmstead, 1995), a family placed in the same order. The significance of the C_8 iridoids reported from members of the Ericaceae is difficult to assess, since this family is taxonomically unrelated to the taxa discussed above.

Deutzia is the only genus in Hydrangeaceae reported to have iridoids lacking C-10, and likewise, *Mentzelia* is unique in Loasaceae with this feature. Until recently, most taxonomists considered the two taxa not to be particularly closely related. Thus, Cronquist (1988) places these two families in two different superorders

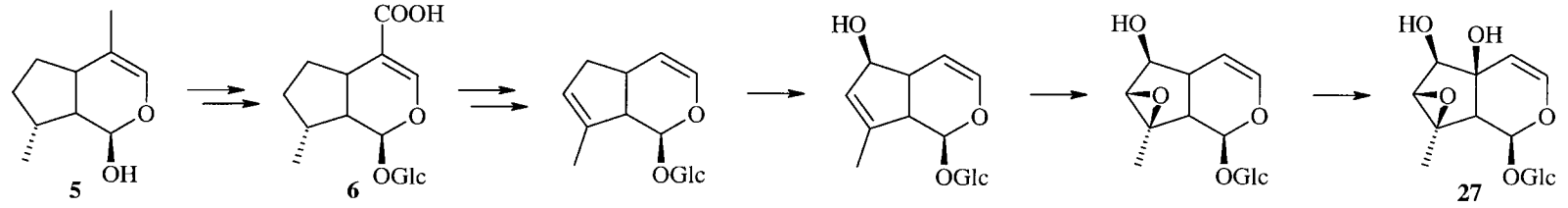
Biosynthesis of the C-8 iridoid glucosides in *Thunbergia alata*:



Biosynthesis of the iridoid glucosides in *Deutzia*:



The last steps in the biosynthesis of antirrhinoside:



Scheme 2. Biosynthetic pathways.

with Hydrangeaceae in Rosidae/Rosales and Loasaceae in Dilleniidae/Violales. In Dahlgren's system (1989), Hydrangeaceae are a member of the Cornales and Loasaceae have been assigned an order of its own. Thorne (1992) prefers a similar arrangement. However, a very close relationship between the two families was found in a cladistic analysis on morphological and chemical grounds by Hufford (1992). More recently, this result has been corroborated by chloroplast DNA sequencing analyses (Hempel, Reeves, Olmstead & Jansen, 1995; Xiang, Soltis & Soltis, 1998), indicating that Hydrangeaceae and Loasaceae make up a clade which should be regarded as a sister group to the remainder of a restricted Cornales. These findings are fully corroborated by the chemical data (Jensen, Nielsen & Dahlgren, 1975; Jensen et al., 1981) where most genera of the two families are characterized by the presence of loganin and secoiridoids, but each with a single genus producing the structurally unique iridoid glucosides lacking C-10 (but keeping C-11), compounds so far not reported from other plant taxa.

4. Experimental

4.1. General

Microanalyses were performed by Leo Micronalytical Laboratory, Ballerup, Denmark. M.p.'s: uncorr. ^{1}H and ^{13}C NMR spectra were recorded in D_2O, using the solvent peak (δ 4.75) and the C-6′ signal (δ 61.5) (Damtoft, Jensen & Nielsen, 1981), respectively, as standards or in $CDCl_3$ (standards δ 7.27 and 77.0). ^{2}H NMR (77 MHz) spectra were recorded in H_2O with 0.0156% ^{2}H of natural abundance. Preparative chromatography was performed at medium pressure (MPLC) on reverse phase SiGel (Polygoprep C_{18} 50–60 μm; 550 g) or on Merck Lobar C_{18}-columns (size B or C), eluting with H_2O–MeOH mixtures as specified and monitoring simultaneously at 206 and 254 nm with a UV detector. Plant material was either grown in a greenhouse (*T. alata*) or in the free (*D. species*). Both were provided by the Botanical Garden of Copenhagen.

4.2. Synthesis of 6-deoxyretzioside

4.2.1. Randioside (*12*)

Gardenoside (**11**, 6.0 g) in phosphate buffer (pH 6.0, 40 ml) was stirred at RT with $NaIO_4$ (4.31 g) for 15 min when the mixt. was filtered through act. C on Celite. The filtrate was evapd to a foam which was fractd by MPLC (C-column). Elution with H_2O–MeOH (3:1) gave pure **12** (4.40 g, 73%). ^{1}H NMR (250 MHz, D_2O): δ 5.60 (d, J = 4.2 Hz, H-1), 7.35 (s, H-3), 3.96 (br d, J = 6.9 Hz, H-5), 8.00 (dd, J = 6.9 and 2.8 Hz, H-6), 6.18 (dd, J = 6.9 and 1.4 Hz, H-7), 3.10 (dd, J = 6.9 and 4.2 Hz, H-9), 3.69 (s, 11-OMe), 4.68 (d, J = 8.3 Hz, H-1′), 3.22 (t, J = 8.3 Hz, H-2′), 3.43 (t, J = 8.3 Hz, H-3′), 3.34 (t, J = 8.3 Hz, H-4′), 3.40 (m, H-5′), 3.83 (dd, J = 12.5 and 1.4 Hz, H-6a′), 3.65 (dd, J = 12.5 and 5.6 Hz, H-6b′), essentially as reported by Uesato, Ali, Nishimura, Kawamura and Inouye (1989). ^{13}C NMR (62.5 MHz, D_2O): δ 95.1 (C-1), 153.2 (C-3), 110.1 (C-4), 36.8 (C-5), 132.3 (C-6), 169.0 (C-7), 209.6 (C-8), 50.4 (C-9), 169.2 (C-11), 53.0 (11-OMe), 99.3 (C-1′), 73.4 (C-2′), 76.4 (C-3′), 70.3 (C-4′), 77.1 (C-5′), 61.5 (C-6′).

4.2.2. Randioside tetra-O-trimethylsilylether (*12s*)

Randioside (**12**, 4.40 g) was dissolved in Py (30 ml) followed by HMDS (hexamethyldisilazane; 10 ml) and TMCS (trimethylchlorosilane; 10 ml), under stirring. After 15 min Et_2O (100 ml) was added followed by 5% AcOH (100 ml) and the phases were separated. The Et_2O layer was washed successively with satd aq. $NaHCO_3$ and H_2O and dried (Na_2SO_4). Evapn of the solvent gave crude **12s** (6.74 g, 88%). This preparation was used in the next step without further characterization.

4.2.3. Tetrahydrorandioside tetra-O-trimethylsilylether (*13s*)

The above TMS-ether (**12s**, 3.50 g) was dissolved in MeOH (30 ml) and $NaBH_4$ (217 mg) was added under stirring. After 5 min AcOH (0.5 ml) was added and the solvent removed. The residue was dissolved in PhMe, filtered and taken to dryness, leaving crude **13s** as a yellow syrup (3.27 g, 94%). The product was characterized solely by ^{1}H NMR (250 MHz, $CDCl_3$): δ 5.28 (d, J = 8.3 Hz, H-1), 7.44 (s, H-3), 2.72 (br q, J = 8.3 Hz, H-5), 1.74 (m, H-6a), 1.49 (m, H-6b), 2.20 (m, H-7a), 1.74 (m, H-7b), 4.52 (m, H-8), 2.31 (t, J = 8.3 Hz, H-9), 3.70 (s, 11-OMe), 4.72 (d, J = 8.3 Hz, H-1′), 3.45-3.21 (m, 4H, H-2′, H-3′, H-4′, H-5′), 3.79 (dd, J = 12.5 and 1.4 Hz, H-6a′), 3.58 (dd, J = 12.5 and 5.6 Hz, H-6b′), 0.20-0.08 (4 × s, 4 × Tms). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 95.4 (C-1), 151.9 (C-3), 111.5 (C-4), 33.7 (C-5), 30.3 (C-6), 34.5 (C-7), 74.7 (C-8), 43.5 (C-9), 167.5 (C-11), 51.1 (11-OMe), 99.8 (C-1′), 73.5 (C-2′), 77.2 (C-3′), 71.3 (C-4′), 77.6 (C-5′), 61.5 (C-6′), 1.3–0.4 (4 × Tms).

4.2.4. Tetrahydrorandioside tri-O-trimethylsilylether 6′,8-dixanthate (*13sx*)

To a solution of **13s** (3.27 g) and MeI (340 μl) in PhMe (25 ml), CS_2 (25 ml) and 50% aq. NaOH (25 ml) were added with $(n\text{-Bu})_4NHSO_4$ (171 mg). After vigorous stirring for 3 h at RT the phases were separated. The aq. phase was extracted with PhMe (2 × 25 ml), the combined org. layers were dried (Na_2SO_4) and

evapd to give a yellow syrup (2.17 g, 57%) which in NMR appeared sufficiently pure for the next step. ^{1}H NMR (250 MHz, $CDCl_3$): δ 5.60 (d, J = 4.2 Hz; H-1), 7.52 (s, H-3), 3.09 (q, J = 6.9 Hz, H-5), 1.52 (m, H-6), 1.77 (2H, H-6 and H-7), 2.06 (m, H-7), 6.24 (q, J = 5.6 Hz, H-8), 2.38 (m, H-9), 3.78 (s, OMe), 4.69 (d, J = 6.9 Hz, H-1′), 3.4–3.7 (3H, H-2′, H-3′, H-4′), 2.79 (m, H-5′), 4.57 (dd, J = 5.6 and 12.5 Hz, H-6a′), 4.97 (br. d, J = 12.5 Hz, H-6b′), 2.60 and 2.65 (2 × 3H, *s*'s, 2 × SMe). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 92.5 (C-1), 151.0 (C-3), 111.5 (C-4), 30.5 (C-5), 29.8 (C-6), 30.5 (C-7), 84.3 (C-8), 43.5 (C-9), 166.5 (C-11), 50.9 (11-OMe), 98.3 (C-1′), 74.2 (C-2′), 77.5 (C-3′), 71.6 (C-4′), 76.4 (C-5′), 72.5 (C-6′), 18.2 and 18.8 (2 × SMe), 214.4 and 215.3 (2 × C=S).

*4.2.5. 10-Nor-geniposidic acid tetra-acetate (**14a**)*

The above crude dixanthate (**13sx**, 2.17 g) was dissolved in quinoline (20 ml) in a 100 ml flask and thermolyzed in an oil bath under a stream of N_2 at 200°C for 90 min. The dark residue was partitioned between CH_2Cl_2 (100 ml) and 2 M H_2SO_4 (50 ml) and the organic phase washed with more 2 M H_2SO_4 (50 ml) and satd $NaHCO_3$ (50 ml), dried and taken to dryness. Desilylation was performed in MeOH (60 ml) with AcOH (20 ml 50%). After 17 h the solvents were removed and the crude product hydrolyzed with 1 M NaOH (10 ml, 3.5 hrs). After neutralisation with AcOH, fractionation by MPLC (C-column) with H_2O–MeOH (2:1) afforded impure 10-nor-geniposidic acid (**14**, 120 mg, 12%). Acetylation with Ac_2O/Py 1:2 (2 ml, 2 h) gave after work-up the tetra-acetate (**14a**, 160 mg, 92%) which crystallized. Recryst. from $CHCl_3$ gave the analytical sample, m.p. 181.5–182.5°C (found: C, 53.0; H, 5.7. $C_{23}H_{28}O_{13}\cdot 1/2H_2O$ requires: C 53.0; H, 5.6%); $[\alpha]_D^{20} = +29°$ ($CHCl_3$; *c* 0.5); ^{1}H NMR (250 MHz, $CDCl_3$): δ 5.14 (d, J = 4.5 Hz, H-1), 7.54 (s, H-3), 3.11 (m, H-5), 2.84 (br dd, J = 16.5 and 8.0 Hz, H-6β), 2.25 (br d, J = 16.5 Hz, H-6α), 5.91 (br d, J = 8.0 Hz, H-7), 5.66 (br d, J = 8.0 Hz, H-8), 2.97 (m, H-9), 4.88 (d, J = 8.0 Hz, H-1′), 5.10 (dd, J = 9.3 and 8.0 Hz, H-2′), 5.25 (t, J = 9.3 Hz, H-3′), 5.02 (t, J = 9,3 Hz, H-4′), 3.75 (m, H-5′), 4.29 (dd, J = 12.0 and 4.5 Hz, H-6a′), 4.12 (dd, J = 12.0 and 2.1 Hz, H-6b′); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 96.1 (C-1), 152.6 (C-3), 111.5 (C-4), 39.0 (C-5), 31.7 (C-6), 133.5 (C-7), 128.1 (C-8), 46.7 (C-9), 172.3 (C-11), 96.3 (C-1′), 70.4 (C-2′), 71.8 (C-3′), 68.0 (C-4′), 72.3 (C-5′), 61.5 (C-6′), 170.5–169.1 (4 × OAc), 20.5–20.1 (4 × Ac–CH_3).

*4.2.6. 6-Deoxyretzioside (**8**)*

The above tetra-acetate (**14a**, 104 mg) was dissolved in dry quinoline (5 ml) and $CuCO_3Cu(OH)_2$ (13 mg) was added. Under stirring, the mixt. was heated in an oil bath at 200°C for 2 h. After cooling in the air for 15 min, Ac_2O (0.4 ml) was added and stirring continued for 1 h when EtOH (5 ml) was added. The dark mixt. was filtered and the solvents evapd and the oily residue partitioned between 2 M H_2SO_4 (50 ml) and $CHCl_3$ (50 ml). The org. phase was washed with H_2O (50 ml) and satd aq. $NaHCO_3$ (2 × 50 ml) and dried ($MgSO_4$). Evapn of the solvent gave a brownish syrup (150 mg) which was deacetylated at RT with 0.1 M NaOMe in MeOH (4 ml, 2 h). Chromatography (B-column; 3:1) gave pure **8** as a syrup (13 mg, 21%). ^{1}H NMR (250 MHz, D_2O): δ 5.32 (d, J = 4.2 Hz, H-1), 6.19 (br d, J = 5.6 Hz, H-3), 4.88 (dd, J = 5.6 and 2.8 Hz, H-4), 2.87 (m, H-5), 2.63 (br dd, J = 13.9 and 6.9 Hz, H-6β), 2.11 (br d, J = 13.9 Hz, H-6α), 5.90 (m, H-7), 5.68 (m, H-8), 2.96 (m, H-9), 4.73 (d, obsc. by the HDO-signal, H-1′), 3.25 (t, J = 8.3 Hz, H-2′), 3.5-3.3 (3H, H-3′, H-4′ and H-5′), 3.87 (dd, J = 12.5 and 1.4 Hz, H-6a′), 3.66 (dd, J = 12.5 and 5.6 Hz, H-6b′); ^{13}C NMR (62.5 MHz, D_2O): δ 96.2 (C-1), 139.3 (C-3), 110.0 (C-4), 40.0 (C-5), 31.6 (C-6), 134.4 (C-7), 130.0 (C-8), 48.9 (C-9), 99.2 (C-1′), 73.6 (C-2′), 76.5 (C-3′), 70.4 (C-4′), 77.0 (C-5′), 61.5 (C-6′).

*4.3. Synthesis of deuterium labelled 6-deoxyretzioside (d-**8**)*

*4.3.1. [6β,8β-$^2H_2$]-Tetrahydrorandioside tetra-O-trimethylsilylether (d-**13s**)*

Reduction of **12s** (3.09 g) with $NaBD_4$ (240 mg) as above gave d-**13s** (3.12 g, quant), containing 0.95 ^{2}H in both the 6β- and 8β-positions according to the ^{1}H NMR spectrum.

*4.3.2. [6β,8β-$^2H_2$]-Tetrahydrorandioside tri-O-trimethylsilylether 6′,8-dixanthate (d-**13sx**)*

Treatment of (d-**13s**, 3.1 g) in PhMe (25 ml) with MeI (0.32 ml) as in Section 4.2.4 gave d-**13sx** (1.7 g, 47%).

*4.3.3. [6β,8β-$^2H_2$]-10-Nor-geniposidic acid tetraacetate (d-**14a**)*

Crude d-**13sx** (1.7 g) was thermolyzed as in Section 4.2.5 for 75 min, desilylated with AcOH and finally hydrolyzed with 1 M NaOH to give, after chromatography d-**14** (160 mg, 21%). Acetylation gave d-**14a** (200 mg, 92%) as a cryst. residue.

*4.3.4. [6β,8β-$^2H_2$]-6-Deoxyretzioside (d-**8**)*

Decarboxylation of d-**14a** (200 mg) was performed in quinoline (5 ml) with $CuCO_3Cu(OH)_2$ (34 mg) as in Section 4.2.6. The crude product (163 mg) was deacetylated and gave, after chromatography d-**8** (37 mg, 26%).The ^{1}H NMR spectrum was identical to the spectrum of the unlabelled compound, except that the compound contained 0.95 ^{2}H at the 6β-position (δ 2.63) and 0.92 ^{2}H at the 8-position (δ 5.68).

4.4. Synthesis of [11-^{2}H]-iridodial glucoside (d-**9**)

Iridotrial glucoside (800 mg), isolated from *Chaenostomum foetidum* (cf. Jensen et al., 1989), in H_2O (15 ml) was reduced with $NaBD_4$ (125 mg) under stirring for 1 h, when AcOH was added until neutral. Evapn and chromatography (C-column; 2:1) gave 11-OH-(11-^{2}H(-iridodial glucoside (721 mg, 90%). An aliquot (480 mg) was acetylated with Py (6 ml) and Ac_2O (3 ml) for 2 h to give the penta-acetate (770 mg). This was dissolved in dioxane (10 ml) and Pd/C (5%, 120 mg) and HCOOH (72 mg) was added under stirring and heating to reflux. After 1 h, TLC showed only 10% conversion and more Pd/C (140 mg) was added. The reaction still commenced sluggishly and after 10 h the reaction was stopped and the mixt. filtered, taken to dryness and evapd twice with EtOH (2 × 50 ml). Deacetylation was performed in MeOH (20 ml) and NaOMe was added to pH 14. After 10 min, the mixt. was neutralized with AcOH. Evapn and chromatography (C-column; 1.5:1 and 1:1) gave first unconverted 11-OH-iridotrial glucoside (140 mg, 29%), then d-**9** (260 mg, 56%); ^{1}H NMR (250 MHz, D_2O) was essentially as reported (Jensen et al., 1989) but the preparation was free of the 8-epimer as seen by the absence of the peak at δ 5.39 present in the earlier preparation. The deuterium content was determined to be 1.0 ^{2}H at C-11 by the integral of the peak at δ 1.48.

4.5. [6,7,8,10-$^2H_4$]-8-Epiiridodial glucoside (d-**10**)

The earlier prepared compound (Breinholt et al., 1992), had a ^{2}H content of ca. 0.8 at C-6, 0.8 at C-7, 0.9 at C-8 and 1.9 at C-10. Prep. HPLC using a Merck HiBar coloumn (250 × 25 mm) packed with LiChrosorb RP-18 (7 μm) and eluting with 1:1 gave virtually baseline separation of d-**10** from the small contamination of d-**9**.

4.6. Synthesis of 7-hydroxyiridodial glucoside (**16**)

4.6.1. Loganin penta-O-trimethylsilylether (**17s**)

Loganin (**17**, 1.60 g) in Py (15 ml) was treated with HMDS (3.5 ml) and TMCS (3.5 ml) under stirring for 20 min. Et_2O (75 ml) was added, followed by 5% AcOH (60 ml). The org. phase was washed successively with satd $NaHCO_3$ and H_2O (50 ml each) and dried ($MgSO_4$). Evapn of the solvent gave a residue which was evapd from PhMe (25 ml), to give **17s** as a yellowish syrup (2.76 g, 90%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 5.06 (d, J = 5.6 Hz, H-1), 7.43 (s, H-3), 3.20 (m, H-5), 2.26 (dd, J = 13.9 and 6.9 Hz, H-6a), 1.41 (m, H-6b), 4.01 (t, J = 1.4 Hz, H-7), 1.84 (m, H-8), 2.00 (q, J = 8.3 Hz, H-9), 1.04 (d, J = 6.5 Hz, 3H, H-10), 3.72 (s, 11-OMe), 4.62 (d, J = 8.3 Hz, H-1′), 3.20 (m, H-2′), 3.47 (t, J = 8.3 Hz, H-3′), 3.31 (*t*, J = 8,3 Hz, H-4′), 3.53 (m, H-5′), 3.75 (br s, 2H, H-6′), 0.27-0.03 (5 × s, 5 × Tms). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 96.7 (C-1), 151.2 (C-3), 112.2 (C-4), 41.5 (C-5), 31.6 (C-6), 74.7 (C-7), 42.7 (C-8), 44.5 (C-9), 13.7 (C-10), 167.4 (C-11), 50.8 (11-OMe), 98.6 (C-1′), 74.3 (C-2′), 76.6 (C-3′), 70.9 (C-4′), 77.9 (C-5′), 61.5 (C-6′).

4.6.2. 7,11-Dihydroxyiridodial glucoside (**18**)

The silylether **17s** (2.76 g) was dissolved in dry PhMe (25 ml) in a three-necked flask, fitted with a rubber septum, a stopper and a T-tube. The solution was stirred under dry N_2 while cooling to −60°C. DIBAL (20% in PhMe, 8.0 ml) was slowly added by syringe. Stirring was continued for an additional 10 min at −60°C and then AcOH (10 ml) was added. When the reaction mixt. had reached RT, more PhMe (50 ml) was added and it was washed with H_2O (25 ml). The PhMe soln was filtered through Celite and taken to dryness. Desilylation of the residue (2.51 g) was performed with 10% aq. AcOH (20 ml) in MeOH (100 ml) in 90 min when the solvents were removed to give a crude product (1.5 g). Chromatography on a C-column (2 runs), eluting with 4:1 gave slightly impure **18** (430 mg, 32%), characterized solely by NMR: ^{1}H NMR (250 MHz, D_2O): δ 5.20 (d, J = 2.8 Hz, H-1), 6.20 (s, H-3), 2.83 (br q, J = 6.9 Hz, H-5), 2.13-1.91 (4H, 2 × H-6, H-8 and H-9), 4.10 (m, H-7), 0.97 (d, J = 6.9 Hz, 3H, H-10), 4.04 (br d, J = 12.5 Hz, H-11a), 3.82 (d, J = 12.5 Hz, H-11b), 4.75 (obsc. by the HDO-signal, H-1′), 3.56–3.20 (4H, m, H-2′, H-3′, H-4′ and H-5′), 3.68 (dd, J = 12.5 and 5.6 Hz, H-6a′), 3.86 (partly obsc. by H-11; H-6b′); ^{13}C NMR (62.5 MHz, D_2O): δ 96.7 (C-1), 137.0 (C-3), 119.6 (C-4), 38.6 (C-5), 30.9 (C-6), 75.3 (C-7), 40.7 (C-8), 46.3 (C-9), 12.9 (C-10), 61.7 (C-11), 99.1 (C-1′), 73.5 (C-2′), 76.4 (C-3′), 70.4 (C-4′), 77.1 (C-5′), 61.5 (C-6′).

4.6.3. 7-Hydroxyiridodial glucoside (**16**)

The glucoside **18** (430 mg) was acetylated with Ac_2O/Py 1:1 (2 h) to give the hexaacetate (**18a**, 550 mg). This was dissolved in EtOH (25 ml) and Pd/C (5%, 213 mg) was added and the soln was stirred under gentle reflux. A mixture of NEt_3 (42 mg) and HCOOH (46 mg, 1.1 eq) in EtOH (10 ml) was added dropwise (5 min) and the reaction was followed by TLC (Et_2O). After 65 min the reaction mixt. was filtered through Celite and evapn of the filtrate gave a residue (498 mg). Deacetylation was performed in 0.1 M NaOMe (10 ml, 2.5 h) and chromatography (B-column, 3:1) gave recovered **18** (50 mg, 12%) and **16** (190 mg, 61%). An analytical sample was cryst. from EtOH, m.p. 214–215°C (found: C, 55.4; H, 7.6. $C_{16}H_{26}O_8$ requires: C, 55.5; H, 7.6%); $[\alpha]_D^{22}$ −96.2°(EtOH; *c* 0.2,); ^{1}H NMR (250 MHz, D_2O): δ 5.14 (d, J = 3.0 Hz, H-1), 5.92 (br s, H-3), 2.62 (q, J = 6.9 Hz, H-5), 1.95 (m, H-6a), 1.69 (m, H-6b), 4.08

(br s, H-7), 1.81 (m, H-8), 1.95 (m, H-9), 1.00 (d, J = 13.9 Hz, 3H, H-10), 1.48 (s, 3H, H-11), 4.68 (d, J = 8.3 Hz, H-1′), 3.23 (t, J = 8.3 Hz, H-2′), 3.44 (t, J = 8.3 Hz, H-3′), 3.35 (t, J = 8.3 Hz, H-4′), 3.40 (m, H-5′), 3.84 (dd, J = 12.5 and 2.8 Hz, H-6a′), 3.65 (dd, J = 12.5 and 5.6 Hz, H-6b′); ^{13}C NMR (62.5 MHz, D_2O): δ 96.6 (C-1), 132.4 (C-3), 117.8 (C-4), 39.2 (C-5), 34.9 (C-6), 75.4 (C-7), 41.1 (C-8), 46.8 (C-9), 12.0 (C-10), 16.1 (C-11), 99.0 (C-1′), 73.6 (C-2′), 76.5 (C-3′), 70.4 (C-4′), 77.0 (C-5′), 61.5 (C-6′).

*4.7. Synthesis of [11-^{2}H]-7-Hydroxyiridodial glucoside (d-**16**)*

The hexa-acetate **18a** (130 mg) in EtOD (5 ml) was reduced with Pd/C (5%, 63 mg), DCOOD (2 × 10 μl) and NEt_3 (10 μl) during 4 h. Deacetylation and chromatography gave **18** (10 mg) and d-**16** (40 mg, 47%). ^{1}H NMR showed that the signal at δ 1.48 (H-11) had an integral corresponding to 2.3 H.

*4.8. Synthesis of [6,7,8,10-^{2}H]-decapetaloside (d-**23**)*

*4.8.1. [6,7,8,10-^{2}H]-Adoxoside/epiadoxoside (d-**19**/d-**20**)*

Reduction of geniposide (**15**, 1.20 g) over Pd/C with D_2 and added Et_3N (0.25 ml) was performed as described (Damtoft et al., 1994), to give, after chromatography (C-column, 2 runs, 2:1), a 2:3 mixt. (880 mg, 73%) of labelled adoxoside (d-**19**) and 8-epiadoxoside (d-**20**).

*4.8.2. [6,7,8,10-^{2}H]-11-Hydroxydecapetaloside (d-**22**)*

The above mixt. (d-**19**/d-**20**; 410 mg) was acetylated with Ac_2O/Py 1:1 (7 ml, 2 h) to give the mixt. of pentaacetates (d-**19a**/d-**20a**, 560 mg, 83%). An aliquot (280 mg) in dry PhMe at −40°C (50 ml) was reduced with DIBAL (20% in PhMe,14.0 ml) for 10 min, then AcOH (10 ml) was added. The reaction mixt. was extracted with H_2O (2 × 25 ml) and the combined aq. extracts were filtered through celite and evapd to a white foam. Chromatography (C-column, 4:1) gave 2 frs with mainly d-**21** (96 mg) and mainly d-**22** (58 mg). Spectral data for d-**21**: ^{1}H NMR (250 MHz, D_2O): δ 5.22 (d, J = 4.2 Hz, H-1), 6.37 (br s, H-3), 2.74 (br t, J = 5.6 Hz, H-5), 1.74 (m, 0.4 H, 0.6 ^{2}H, H-6α), 1.32 (m, 0.7 H, 0.3 ^{2}H, H-6β), 1.87 (m, 0.2 H, 0.8 ^{2}H, H-7α), 1.59* (m, 0.5 H, 0.5 ^{2}H, H-7β), 2.02 (m, 0.3 H, 0.7 ^{2}H, H-8), 1.99 (m, H-9), 3.60-3.37 (m, H-10a, H-10b), 4.12 (br d, J = 13.9 Hz, H-11a), 3.90 (br d, J = 13.9 Hz, H-11b), 4.78 (obsc. by the HDO-signal, H-1′), 3.29 (t, J = 8.3 Hz, H-2′), 3.60–3.37 (m, H-3′, H-4′ and H-5′), 3.90 (br d, J = 12.5 Hz, H-6a′), 3.69 (dd, J = 12.5 and 5.6 Hz, H-6b′), similar to those reported (Jensen et al., 1985); ^{13}C NMR (62.5 MHz, D_2O; *low intensity signals): δ 97.3 (C-1), 138.6 (C-3), 117.7 (C-4), 34.8 (C-5), 27.4* (C-6), 29.5* (C-7), 42.5* (C-8), 44.6 (C-9), 66.0 (C-10), 61.6 (C-11), 99.5 (C-1′), 73.5 (C-2′), 76.4 (C-3′), 70.4 (C-4′), 77.0 (C-5′), 61.5 (C-6′), close to those reported (Damtoft et al., 1981).

*4.8.3. [6,7,8,10-^{2}H]-Decapetaloside (d-**23**)*

Impure d-**21** (145 mg) was acetylated to give the hexaacetate (d-**21a**, 143 mg) as a colourless syrup. It was hydrogenated as above in EtOH (20 ml) with 5% Pd/C (220 mg) and of NEt_3 (14 mg) and HCOOH (17 mg, 1.1 eq). The reaction was followed by TLC (Et_2O). After 75 min more HCOOH (17 mg, 1.1 eq) was added. The catalyst was filtered off after a total of 6 h and the filtrate was taken to dryness (133 mg) and deacetylated with NaOMe/MeOH. Chromatography (B-column, 4:1 to 2:1) gave first d-**21** (21 mg, 25%), and then d-**23** (28 mg, 34%) as a syrup; ^{1}H NMR showed a ^{2}H content of 0.6 ^{2}H in the 6α-position, 0.4 ^{2}H in the 6β-position, 0.8 ^{2}H in the 6α-position, 0.6 ^{2}H in the 7β-position, 0.8 ^{2}H at C-8 and 0.4 ^{2}H at C-10. ^{1}H NMR (500 MHz, D_2O): δ 5.19 (d, J = 5.6 Hz, H-1), 6.10 (s, H-3), 2.53 (t-like, J = 7.0 Hz, H-5), 1.88 (0.4H, m, H-6α), 1.59 (obsc. by the 11-Me-signal, ca. 0.5H, H-6β), 1.67 (0.2H, m, H-7α), 1.35 (0.4H, m, H-7β), 2.08 (0.2H, q-like, J = 7.0 Hz, H-8), 1.96 (m, H-9), 3.62 (d, J = 11.1 Hz, H-10a), 3.56 (d, J = 11.1 Hz, H-10b), 1.57 (s, 3H, H-11), 4.76 (obsc. by the HDO-signal, H-1′), 3.31 (t, J = 9.7 Hz, H-2′), 3.53 (t, J = 9.7 Hz, H-3′), 3.42 (t, J = 9.7 Hz, H-4′), 3.49 (m, H-5′), 3.94 (dd, J = 12.5 and 2.8 Hz, H-6a′), 3.74 (dd, J = 12.5 and 5.6 Hz, H-6b′), some assignments are uncertain (similar to that reported by Jensen et al. (1985)). ^{13}C NMR (125 MHz, D_2O; *low intensity peaks): δ 97.3 (C-1), 134.1 (C-3), 116.2 (C-4), 38.6 (C-5), 29.6* (C-6), 27.4* (C-7), 43.0* (C-8), 45.1 (C-9), 66.1* (C-10), 15.8 (C-11), 99.4 (C-1′), 73.6 (C-2′), 76.6 (C-3′), 70.4 (C-4′), 77.0 (C-5′), 61.5 (C-6′), almost identical to that reported by Jensen et al. (1981).

4.9. Feeding experiments

4.9.1. Thunbergia alata

The labelled precursors (d-**7** and d-**8**) were dissolved in H_2O (2 ml) in 10 ml beakers. Leaves (ca. 14 g) from young *T. alata* plants (with 8–10 leaves), were immersed into the above solns to absorb the precursor. In order to ensure complete transfer to the plant, more H_2O (2 × 1 ml) was added when almost all had been taken up. After 3–5 h the leaves were placed in a larger beaker to metabolize the precursor. Due to beginning withering, the plants were worked up after 3 days. The leaves were homogenized with EtOH (250 ml), filtered and concentrated. The residue was partitioned between H_2O and Et_2O. The aq. phase was evapd to a greenish syrup which was dissolved in MeOH (20 ml) and filtered through a layer of act. C

(400 mg) on Celite. The filtrate was taken to dryness and chromatographed (B-column; 1:0 to 1:1). The fractions thus obtained (Table 1) were analyzed by ^{2}H and ^{1}H NMR for incorporation and for identity, respectively. The compounds containing measurable amounts of ^{2}H incorporation were: **2** (δ 3.75, D-8), **24** (δ 3.7, D-10), **25** (δ 6.0, D-8) and **8** (δ 5.7, D-8). The ^{1}H NMR spectrum of the fraction with **25** showed that this compound was contaminated with an equimolar amount of tryptophan but also that it was almost devoid of ^{1}H at C-8 (250 MHz, D_2O): δ 5.26 (br d, J = 5.6 Hz, H-1), 6.18 (br d, J = 6.9 Hz, H-3), 5.0 (m, H-4), 2.64 (m, H-5), 4.50 (br. s, H-6), 5.94 (br s, H-7), 6.0 ($<$0.05H, *m*, H-8), 3.2 (m, H-9), 4.7 (obsc. by the HDO-signal, H-1′), almost identical to that reported (Damtoft et al., 1993c).

4.9.2. Deutzia species

Precursors were dissolved in H_2O (3 ml) in 10 ml beakers. Young shoots (ca. 50 g) from *D. Schneideriana* or *D. scabra* were immersed into the solns and after a metabolic period of 4 days the plants were worked up as in Section 4.9.1. The aq. extracts were treated with Al_2O_3 (neutral, 50 g). Chromatography was performed as above and the fractions were analyzed by ^{2}H NMR. The results are listed in Table 2.

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