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INTERMEDIACY OF 8-EPIIRIDODIAL IN THE BIOSYNTHESIS OF IRIDOID GLUCOSIDES BY *GARDENIA JASMINOIDES* CELL CULTURES*

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Key Word Index—*Gardenia jasminoides*; Rubiaceae; cell suspension cultures; biosynthesis; iridoid glucosides; tarennoside; gardenoside; iridodial cation; 8-epiiridodial; boschnaloside.

Abstract—By administration of ^{2}H-labelled iridodial, its congeners and ^{13}C-labelled 10-hydroxygeraniol to *Gardenia jasminoides* cell suspension cultures it was demonstrated that tarennoside and gardenoside were biosynthesized, after iridodial cation formation, via 8-epiiridodial, 8-epiiridotrial, 8-epiiridotrial glucoside and 7,8-dehydroiridotrial glucoside. However, the coexistence of a pathway via the iridodial cation, 7,8-dehydroiridodial, 7,8-dehydroiridotrial and 7,8-dehydroiridotrial glucoside could not be excluded.

INTRODUCTION

In a preceding paper, we demonstrated that tarennoside (**1**) and gardenoside (**2**) of *Gardenia jasminoides* cell cultures were biosynthesized via cyclization of 10-oxogeranial (**3a**) [or 10-oxoneral (**3b**)] to the iridodial cation **4**, followed by the complete randomization of the carbon atoms 3 and 11 [2]. The present paper concerns the biosynthetic process after iridodial cation (**4**) formation.

RESULTS AND DISCUSSION

There seemed to be three possible routes (Scheme 1) from the iridodial cation **4**† to tarennoside (**1**): (a) via iridodial (**5**), (b) via 8-epiiridodial (**6**) and (c) via 7,8-dehydroiridodial (**7**). Compounds **5** and **6** would be formed by hydride attack on C-8 of the cation **4** from the α- and the β-side, respectively, whereas **7** would be formed by the elimination of H-7 of **4**. In view of the intermediary role of geniposide (**8**) in the biosynthesis of asperuloside (**9**) from iridodial (**5**) [3] in another plant [4] it seemed possible that tarennoside (**1**), a probable precursor of **8**, would be biosynthesized via route (a). However, due to the structural relationship of **1** with **7**, route (c) was also a possibility.

In order to examine the above possibilities, we administered a mixture of [10-$^{2}H_3$]iridodial (**5**) and [11-$^{2}H_3$]-7,8-dehydroiridodial (**7**) each with the enol-hemiacetal (E-H) form [5] to *G. jasminoides* cell cultures 2 weeks after inoculation (Table 1, Expt 1). After 7 days of incubation, labelled tarennoside (**1**) was isolated, together with a mixture of [10-$^{2}H_3$]iridotrial glucoside (**10**) formed from [10-$^{2}H_3$]-**5** and [11-^{2}H]-7,8-dehydroiridotrial glucoside (**11**) formed from [11-$^{2}H_3$]-**7**. The ^{2}H NMR spectrum of the pentaacetate (**12**) of **1** showed signals at δ 7.34 (3-^{2}H) and 9.24 (11-^{2}H) in a 2:3 ratio, both originating from [11-$^{2}H_3$]-**7**, but no signals originating from [10-^{2}H]-**5**. These results suggested that 7,8-dehydroiridodial was a precursor (**7**). Iridodial (**5**) was converted to **10**, but not to tarennoside (**1**). Incubation with a large amount of unlabelled iridodial (**5**) prior to that of [11-$^{2}H_3$]-**7** (Expt 2) did not reduce the specific incorporation ratio of **7** into **1**; this fact supported the above evidence.

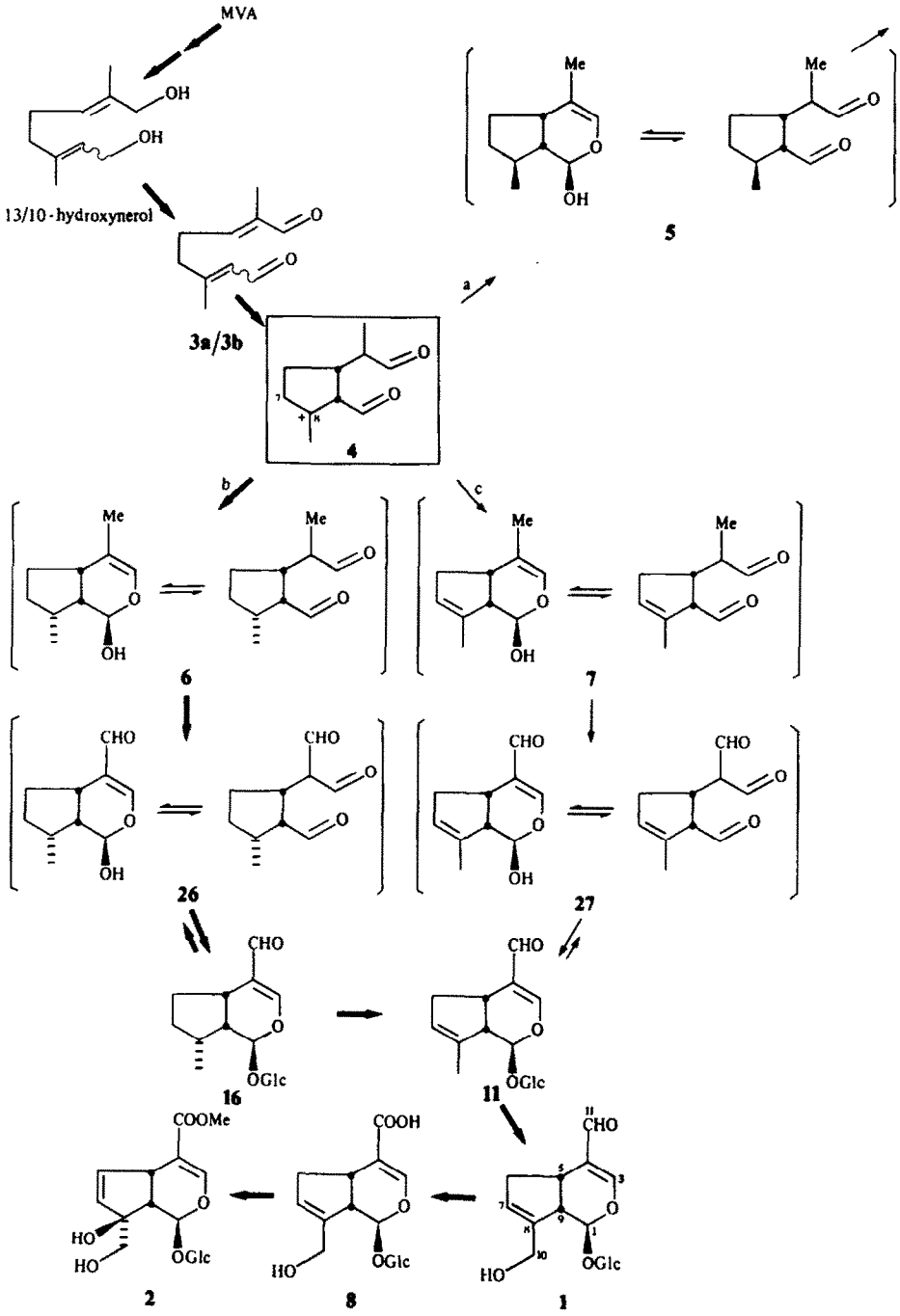
Dilution analysis of 7,8-dehydroiridotrial glucoside (**11**) was attempted in order to confirm its intermediacy: the cell cultures were incubated with [4-^{13}C]-10-hydroxygeraniol (**13**) [6] for 5 days, and then extracted with MeOH. Unlabelled **11** and, for comparison, iridotrial glucoside (**10**) were added to the extract (Expt 3). The ^{13}C NMR spectrum of the acetate mixture (**14** and **15**) of the reisolated **10** and **11** did not show any ^{13}C NMR enrichment at the 10-methyl carbons, but showed an unexpected ^{13}C signal at δ16.10, which was attributed to the C-10 methyl group of the acetate (**17**) of contaminating [10-^{13}C]boschnaloside (8-epiiridotrial glucoside) (**16**) [7], formed from the [4-^{13}C]**13** fed. In keeping with this finding, a trace of boschnaloside (**16**) was isolated, together with tarennoside (**1**) and gardenoside (**2**), from the cell cultures grown for 3 weeks.

This evidence clearly suggested the possibility of the 8-epiiridodial (**6**) series of compounds functioning as intermediates. Therefore, the following precursors were tested: [10-$^{2}H_3$]-8-epiiridodial (**6**), [11-^{2}H]boschnaloside (**16**) and [11-^{2}H]-7,8-dehydroiridotrial glucoside (**11**). These ^{2}H-labelled compounds were synthesized as mentioned below.

[10-$^{2}H_3$]-8-*Epiiridodial* (**6**). [10-$^{2}H_3$]-10-Deoxygeniposide tetraacetate (**18**) (containing *ca* 7% of [10-$^{2}H_3$]deoxyloganin tetraacetate, **19**) prepared by a previously reported method [5] was subjected to Rh–C-catalysed hydrogenation [8] to give [10-$^{2}H_3$]-8-

*Part 57 in the series "Studies on monoterpene glucosides and related natural products". Part of this work was reported in a preliminary communication [1]. For part 56, see Uesato, S., Kanomi (née Matsuda), S., Iida, A., Inouye, H. and Zenk, M. H. (1986) *Phytochemistry* **25**, 839.

†As mentioned in the preceding paper, the iridodial cation (**4**) may not exist as a cation if the cyclization proceeds by a concerted mechanism, or if a cyclic intermediate is bound to enzymes.



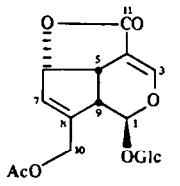
Scheme 1. Proposed pathway (→) for the biosynthesis of tarennoside (1) and gardenoside (2).

epideoxyloganin tetraacetate (20). Its ^{1}H NMR spectrum showed a signal due to the C-10 methyl protons at δ1.01 with an intensity of 0.87 proton, revealing that 71% of the C-10 methyl protons were substituted by deuterium. Furthermore, since the C-3 vinyl protons were observed at δ7.35 and 7.30 with a 9:1 ratio of intensities, respectively, 20 was contaminated with 10% of its (8*S*)-isomer (19). Reduction of [10-$^2H_3$]-20 with $LiAlH_2(OMe)_2$ followed by acetylation [5] yielded [10-$^2H_3$]-8-epi-11-hydroxy-iridodial glucoside pentaacetate (21), which was then subjected to hydrogenolysis over Pd–C to form [10-$^2H_3$]-8-epiiridodial glucoside tetraacetate (22). This compound was further converted, via [10-$^2H_3$]-8-epiiridodial glucoside (23), to [10-$^2H_3$]-8-epiiridodial (6) through deacetylation and β-glucosidase-catalysed hydrolysis. The ^{1}H NMR spectrum of 6 showed signals at δ1.07 (*s* (*br*), 10-H_3 × 0.36), 1.60 (*d*, J = 0.97 Hz, 11-H_3), 5.00 (*d*, J = 4.88 Hz, 1-H), etc. Therefore, 64% of the C-10 methyl

Table 1. Administration of labelled putative precursors to *G. jasminoides* cell cultures

Compounds fed	Labelled glucosides isolated or detected Total incorp. (Spec. incorp.) (%)*	Ratio of signal intensities between 3-^{2}H and 11-^{2}H in tarennoside (1)
Expt. 1 [10-$^2H_3$]Iridodial (5) +	[10-$^2H_3$]Iridotrial glucoside (10) — (—)	
[11-$^2H_3$]-7,8-Dehydroiridodial (7)	[11-^{2}H]-7,8-Dehydroiridotrial glucoside (11) — (—)	
	[3,11-$^2H_2$]Tarennoside (1) 3.3 (23.5)	2:3
Expt. 2 Unlabeled iridodial (5) +	[3,11-$^2H_2$]Tarennoside (1)	3:7
[11-$^2H_3$]-7,8-dehydroiridodial (7)	5.4 (17.2)	
Expt. 3 [4-^{13}C]-10-Hydroxygeraniol (13)	[10-^{13}C]Boschnaloside (16) — (—)	
Expt. 4 [10-$^2H_3$]-8-Epiiridodial (6) +	[10-$^2H_2$]Tarennoside (1)	
[11-$^2H_3$]-7,8-dehydroiridodial (7)	10.1 (13.2)	
	[3,11-$^2H_2$]Tarennoside (1) 21.0 (32.5)	1:2
Expt. 5 [11-^{2}H]Boschnaloside (16)	[3,11-$^2H_2$]Tarennoside (1) 13.1 (41.7)	1:3
Expt. 6 [11-^{2}H]-7,8-Dehydroiridotrial glucoside (11)	[3,11-$^2H_2$]Tarennoside (1) 29.5 (70.4)	1:3

*Calculated on the assumption that there was no deuterium isotope effect causing preferential removal of hydrogen during the biosynthetic process.



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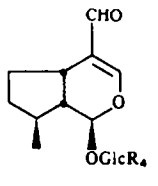
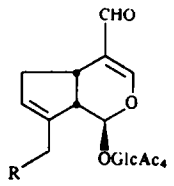
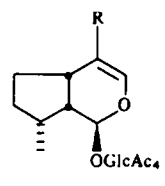


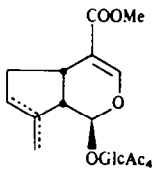
10 R = H
14 R = Ac



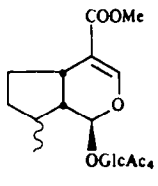
12 R = OAc
15 R = H



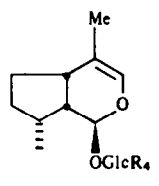
17 R = CHO
21 R = CH_2OAc



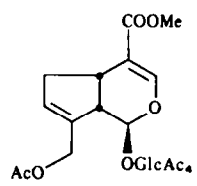
18 7,8-dehydro
25 8,10-dehydro



19 (8*S*)
20 (8*R*)



22 R = Ac
23 R = H



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protons were replaced by deuterium. Besides the above peaks, the spectrum contained weak aldehyde proton signals at δ9.63 (*d*, J = 2.20 Hz) and 9.84 (*d*, J = 5.13 Hz), indicating the presence of not only the E-H tautomer, but also the dialdehyde (D-A) tautomer though in a low ratio (*ca* 10%). Inspection of the Dreiding model showed that the C-10 methyl group and the C-1 proton in **6** of the E-H form are in close proximity, in contrast to those in iridodial (**5**) and dehydroiridodial (**7**) of the same form [3, 5]. This steric congestion may make **6** of the E-H form relatively less stable and cause the partial conversion of this form into the D-H form. Compound **6** is presumed to be contaminated with *ca* 7% of its (8*S*)-isomer, since [10-$^2H_3$]-**23** was found to contain 7% of the (8*S*)-isomer in the ^{1}H NMR spectrum.

[11-^{2}H]*Boschnaloside* (**16**). Geniposide pentaacetate

(24) was reduced with $NaBH_4$ in the presence of $Pd(PPh_3)_4$ and PPh_3 to give a mixture of 10-deoxygeniposide tetraacetate (18) and 7-deoxygardoside methyl ester tetraacetate (25). Its ^1H NMR spectrum showed a 0.8 proton signal (δ5.45) due to the C-7 vinyl proton of 18, as well as a 0.4 proton signal (δ5.35) due to the C-10 methylene of 25, thus indicating that the product was a 4:1 mixture of 18 and 25. Catalytic hydrogenation of this mixture over 5% Rh-C gave 8-epideoxyloganin tetraacetate (20). The ^1H NMR spectrum of this compound showed signals due to the C-3 vinyl proton at δ7.35 and 7.30 in a 97:3 ratio, respectively, indicating contamination with 3% of the (8*S*)-isomer 19. Reduction of 20 with $LiAl^2H_2(OMe)_2$ followed by Pt-catalysed oxidation gave rise to the desired [11-^{2}H]boschnaloside (16). Its ^1H NMR spectrum lacked the signal due to the C-11 aldehyde proton at δ9.17.

[11-^{2}H]-7,8-*Dehydroiridotrial glucoside* (11). Reduction of 10-deoxygeniposide tetraacetate (18) (containing 7% of 19) [5] with $LiAl^2H_2(OMe)_2$ followed by Pt-catalysed oxidation yielded the desired [11-^{2}H]-11. Its ^1H NMR spectrum contained signals due to the C-3 vinyl protons at δ7.41 and 7.35 in a 93:7 ratio, indicating a 7% contamination with 10. Furthermore, the spectrum did not show the C-11 aldehyde proton at δ9.14.

Initially, a mixture of [10-$^2H_3$]-8-epiiridodial (6) (with the E-H and D-A forms) and [11-$^2H_3$]-7,8-dehydroiridodial (7) (with the E-H form) was administered to the cell cultures and cultivation was continued for 7 days (Expt 4). The ^2H NMR spectrum of the acetate (12) of the isolated tarennoside (1) indicated that the label from [10-$^2H_3$]-6 was incorporated only into the 10-position (δ4.71), whereas that from [11-$^2H_3$]-7 was introduced into the 3 and 11 positions (δ7.31 and 9.28) in a 1:2 ratio.

Subsequently, [11-^{2}H]boschnaloside (16) and [11-^{2}H]-7,8-dehydroiridotrial glucoside (11) were administered separately to the cell cultures. After 5 days of incubation, tarennoside (1) was isolated (Expts 5 and 6). The ^2H NMR spectra of the pentaacetate (12) of 1 isolated in both runs showed that the ^{2}H-label of both 16 and 11 was incorporated into 1 with a 1:3 distribution ratio on C-3 and C-11. In Expts 4, 5 and 6, the total and specific incorporation ratios of ^{2}H-labelled compounds into 1 were always more than 10%; particularly, the specific incorporation values of boschnaloside (16) and 7,8-dehydroiridotrial glucoside (11) amounted to 41.7 and 70.4%, respectively.

In Expts 1, 2 and 4, differences were observed in the distribution ratios of ^{2}H label between the 3 and 11 positions of 1. This could be explained if some of the administered 7,8-dehydroiridodial (7) with the E-H form sequentially underwent opening of the dihydropyran ring, oxidation of the C-11 methyl group (or *vice versa*) and the randomization of the 3 and 11 carbons, whereas the remainder of 7 was metabolized without such ring opening and randomization. In Expts 5 and 6, even the 11-label of the fed glucosides 16 and 11 was incorporated into the 3 and 11 positions of tarennoside (1) with partial scrambling. This finding was rationalized by assuming that 8-epiiridotrial (26) and 7,8-dehydroiridotrial (27) formed by the hydrolysis of parts of 16 and 11 underwent ring opening and scrambling of the C-11 and C-3 followed by ring-closure and glucosidation to 16 and 11, which were further metabolized to 1. In Expt 6, the ^1H NMR spectrum of the acetate 15 of the [11-^{2}H]-7,8-dehydroiridotrial glucoside recovered (11) showed a weak doublet (J = 6.8 Hz) at δ1.02 corresponding to the C-10 methyl group of boschnaloside tetraacetate (17), suggesting contamination of 11 with endogeneous boschnaloside (16).

From the results mentioned so far, it was concluded that iridoid glucosides tarennoside (1) and gardenoside (2) in *G. jasminoides* cell cultures were biosynthesized by route (b) passing through 10-oxogeranial (3a)/10-oxoneral (3b), iridodial cation (4), 8-epiiridodial (6), 8-epiiridotrial (26), boschnaloside (16) and 7,8-dehydroiridotrial glucoside (11) as depicted with bold lines in Scheme 1.

However, the cultures were indiscriminately able to metabolize 5, 6 and 7 to the corresponding trials 10, 16 and 11, respectively in good yields. Thus, when 7 is fed, its product 11 is 'by accident' on pathway (b) to 1, and therefore pathway (c) cannot be excluded.

EXPERIMENTAL

Mps were uncorr. ^1H, ^{13}C and ^2H NMR were measured at 199.50, 50.23 and 30.63 MHz, respectively. For ^1H and ^{13}C NMR, free glucosides were dissolved in either CD_3OD or $CDCl_3$ with TMS as internal standard. For ^2H NMR, samples were dissolved in MeCN with CD_3CN as internal reference, δ1.95. Kieselgel 60 (Merck) and active charcoal (Wako) were used for CC. TLC was carried out using silica gel 60 GF_{254} (Merck). Spots were detected under $UV_{(254\ nm)}$, by I_2 exposure and also by spraying with anisaldehyde–H_2SO_4 followed by heating. Prep. TLC was carried out using silica gel 60 PF_{254} (Merck). The bands were visualized under $UV_{(254\ nm)}$, scraped off, extracted with $CHCl_3$–MeOH (9:1), and the extracts concd *in vacuo*. Unless otherwise stated, labelled compounds were administered to cell suspension cultures of *G. jasminoides* [2] (2 weeks after inoculation) for 5 days.

The physical data of compounds except for the ^1H NMR spectra are those for non-labelled compounds obtained in the foregoing model reactions.

Simultaneous administration of [10-$^2H_3$]*iridodial* (5) *and* [11-$^2H_3$]-7,8-*dehydroiridodial* (7) *to cell cultures.* A soln of [10-$^2H_3$]-5 (18.0 mg) and [11-$^2H_3$]-7 (19.2 mg) in EtOH–H_2O (1:1, 4 ml)–Tween 80 (1 drop) was administered to the cell cultures (200 ml × 4). After 7 days incubation, the cells (94 g) were extracted with MeOH (100 ml × 4) for 20 min under reflux. Concn of the combined extracts *in vacuo* gave a residue, which was chromatographed on a charcoal (5 g) column and eluted successively with H_2O (200 ml), H_2O–MeOH (9:1, 4:1, 200 ml each) and MeOH (500 ml). The residue (128.7 mg) of the MeOH eluate was subjected to prep. TLC ($CHCl_3$–MeOH, 4:1, three developments) to give fractions of tarennoside (1) (18.2 mg), gardenoside (2) (43.5 mg) and tri-al glucoside mixture (8.1 mg). Acetylation of the tarennoside (1) and tri-al glucoside fractions followed by purification by prep. TLC (each, Et_2O) gave tarennoside pentaacetate (12) (7.3 mg) and tr-ial glucoside tetraacetate (2.7 mg) both as colourless needles, respectively. The latter was found to be a 1:1 mixture of the acetates 14 and 15 of [10-$^2H_3$]-10 and [11-^{2}H]-11 by comparing the signal intensities of the C-3 proton of 14 and the C-10 methyl of 15 in ^1H NMR. ^2H NMR (12): δ7.34 (3-^{2}H), 9.24 (11-^{2}H); ^1H NMR (14): δ1.07 (*d*, J = 5.9 Hz, 10-H_3 × 0.33), 1.93–2.09 (OAc × 4), 7.06 (*s* (*br*), 3-H), 9.26 (*s*, 11-H); (15): δ1.77 (*s*, 10-H_3), 1.93–2.09 (OAC × 4), 5.45 (*s* (*br*), 7-H), 7.13 (*s* (*br*), 3-H), 9.26 (*s*, 11-H).

Administration of unlabelled iridodial (5) *and* [11-$^2H_3$]-*dehydroiridodial* (7) *to cell cultures.* A soln of unlabelled 5 (145 mg) in EtOH–H_2O (3:2, 5 ml)–Tween 80 (2 drops) was

administered to the cell cultures (200 ml × 4). After incubating for 4 hr, a soln of [11-$^2H_3$]-**7** (15.0 mg) in EtOH–H_2O (1:3, 4 ml)–Tween 80 (1 drop) was administered, and cultivation was continued for an additional 7 days. The cells (102 g) were extracted with MeOH (100 ml × 4) for 20 min under reflux. The residue of the extracts was subjected to prep. TLC to give fractions of tarennoside (**1**) (27.6 mg) and gardenoside (**2**) (70.7 mg). The former was acetylated and purified by prep. TLC affording tarennoside pentaacetate (**12**) (13.0 mg) as colourless needles. ^2H NMR: δ7.37 (3-^2H), 9.27 (11-^2H).

Administration of [4-^{13}C]-10-*hydroxygeraniol* (**13**) *to cell cultures and detection of endogenous boschnaloside* (**16**) *by dilution analysis.* A soln of [4-^{13}C]-**13** (16 mg) in EtOH–Tween 80 (3 ml–1 drop) was, administered to cell cultures (200 ml × 3). After incubating for 5 days, the cells (60 g) were extracted with MeOH (100 ml × 2) for 12 hr at room temp., and then a mixture of **10** and **11** (15 mg each) was added to the extracts prior to their concn *in vacuo*. The residue was subjected to charcoal (8 g) CC and eluted successively with H_2O (400 ml), H_2O–MeOH (9:1, 4:1, 200 ml each) and MeOH (300 ml). The residue (160 mg) of the MeOH eluate was purified by prep. TLC ($CHCl_3$–MeOH, 4:1, two developments) followed by acetylation to afford a mixture (28 mg) of tetraacetates (**14**, **15** and **17**). ^{13}C NMR: δ15.15 (*q*, C-10, **15**), 16.10 (*q*, C-10, **17**), 19.75 (*q*, C-10, **14**), 29.94 (*t*, C-6, **14**), 31.16 (*d*, C-5, **14**), 31.31 (*d*, C-5, **15**), 33.01 (*t*, C-7, **14**), 34.76 (*d*, C-8, **14**), 37.24 (*t*, C-6, **15**), 48.02 (*d*, C-9, **14**), 49.06 (*d*, C-9, **15**), 61.80 (*t*, C-6', **14** and **15**), 68.42 (*d*, C-4', **14** and **15**), 70.82 (*d*, C-2', **14** and **15**), 72.27 (*d*, C-3', **14** and **15**), 72.55 (*d*, C-5', **14** and **15**), 96.12 (*d*, C-1 or C-1', **14** and **15**), 96.34 (*d*, C-1' or C-1, **14** and **15**), 125.15 (*s*, C-4, **15**), 125.24 (*s*, C-4, **14**), 127.56 (*d*, C-7, **15**), 137.12 (*s*, C-8, **15**), 159.33 (*d*, C-3, **14**), 160.20 (*d*, C-3, **15**), 190.30 (*d*, C-11, **14** and **15**).

Isolation of boschnaloside (**16**) *from cell cultures.* Cell cultures (200 ml × 3) grown for 3 weeks in the dark were filtered and the cells (58 g) extracted with MeOH (30 ml × 5) for 10 min under reflux. The residue (2.63 g) of the MeOH extracts was dissolved in H_2O and the insoluble materials filtered off. The filtrate was chromatographed over a charcoal (8 g) column and eluted successively with H_2O (200 ml), H_2O–MeOH (9:1, 4:1, 200 ml each) and MeOH (400 ml). The residue (81 mg) of the MeOH eluate was subjected to prep. TLC ($CHCl_3$–MeOH, 17:3, four developments) to give tri-al glucoside (1.4 mg), geniposide (Me ester of **8**) (3.0 mg), tarennoside (**1**) (9.5 mg) and gardenoside (**2**) (47.2 mg) in order of increasing polarity. The tri-al glucoside was identified as boschnaloside (**16**) by ^{1}H NMR. ^{1}H NMR: δ1.07 (*d*, J = 7.1 Hz, 10-H_3), 5.62 (*d*, J = 4.2 Hz, 1-H), 9.17 (*s*, 11-H).

Conversion of [10-$^2H_3$]-10-*deoxygeniposide tetraacetate* (**18**) *into* [10-$^2H_3$]-8-*epideoxyloganin tetraacetate* (**20**). A soln of [10-$^2H_3$]-**18** (305 mg, contaminated with *ca* 7% of [10-$^2H_3$]deoxyloganin tetraacetate (**19**)) in MeOH (4 ml) was hydrogenated over 5% Rh-C (80 mg) until the uptake of H_2 had ceased. The catalyst was filtered off and the filtrate was concd *in vacuo* to give [10-$^2H_3$]-8-epideoxyloganin tetraacetate (**20**) (265 mg) (containing 10% of [10-$^2H_3$] deoxyloganin tetraacetate (**19**)), mp 109°; $[\alpha]_D$ −91.5° (MeOH, *c* 0.40); UV λ_{max}^{MeOH} nm (log ε): 236 (4.02); IR ν_{max}^{KBr} cm^{-1}: 2950, 1760, 1710, 1640, 1440, 1370, 1220, 1070, 1040, 900; ^{1}H NMR: δ1.01 (*d*, J = 6.8 Hz, 10-H_3 × 0.29), 1.24–1.30 (*m*, 6-H), 1.55–1.80 (*m*, 6-H, 7-H), 1.94–2.09 (OAc × 4), 2.24–2.33 (*m*, 7-H and 9-H), 2.83–2.89 (1H, *m*, 5-H), 5.26 (*d*, J = 3.2 Hz, 1-H), 7.30 (*d*, J = 1.0 Hz, 3-H × 0.10 (**19**)), 7.35 (*d*, J = 1.0 Hz, 3-H × 0.90). (Found: C, 55.31; H, 6.43. Calc. for $C_{25}H_{34}O_{13}$: C, 55.35; H, 6.32%.)

Conversion of [10-$^2H_3$]-8-*epideoxyloganin tetraacetate* (**20**) *into* [10-$^2H_3$]-8-*epi*-11-*hydroxyiridodial glucoside pentaacetate* (**21**). [10-$^2H_3$]-**20** (192 mg) was reduced in dry THF (10 ml) with $LiAlH_2(OMe)_2$ [prepared from $LiAlH_4$ (230 mg) and dry MeOH (0.49 ml)] at −20° according to our previous report [5] to yield a white powder (123 mg). This compound was acetylated and the product was purified by recrystallization from EtOH to give [10-$^2H_3$]-**21** (131 mg) [containing *ca* 10% of the (8*S*)-isomer] as colourless needles, mp 119°. $[\alpha]_D$ −133.8° (MeOH, *c* 0.53); IR ν_{max}^{KBr} cm^{-1}: 2960, 2880, 1745, 1730, 1670, 1380, 1240, 1060, 1040, 910; ^{1}H NMR: δ0.99 (*d*, J = 6.8 Hz, 10-H_3 × 0.30), 1.13–1.34 (*m*, 7-H), 1.62–1.85 (*m*, 6-H and 7-H), 1.86–2.42 (*m*, 8-H and 9-H), 2.00–2.09 (OAc × 4), 2.55–2.70 (*m*, 5-H), 4.24 (*d*, J = 11.5 Hz, 11-H), 4.67 (*d*, J = 11.5 Hz, 11-H), 5.23 (*d*, J = 1.7 Hz, 1-H), 6.26 (*s* (*br*), 3-H × 0.10 [(8*S*)-isomer]), 6.28 (*s* (*br*), 3-H × 0.90). (Found: C, 56.17; H, 6.57. Calc. for $C_{26}H_{36}O_{13}$: C, 56.11; H, 6.52%.)

Hydrogenolysis of [10-$^2H_3$]-8-*epi*-11-*hydroxyiridodial glucoside pentaacetate* (**21**) *to form* [10-$^2H_3$]-8-*epiiridodial glucoside tetraacetate* (**22**). [10-$^2H_3$]-**21** (131 mg) was hydrogenated in MeOH (5 ml) over 5% Pd–C (90 mg) until 1 mole H_2 had been absorbed. The usual work-up gave a solid (129 mg), which was recrystallized from EtOH to give [10-$^2H_3$]-**22** (85 mg) [containing *ca* 10% of the (8*S*)-isomer] as colourless needles, mp 120°. $[\alpha]_D$ −147.2° (MeOH, *c* 0.54); IR ν_{max}^{KBr} cm^{-1}: 2960, 2870, 1760, 1670, 1370, 1230, 1060, 1040, 910; ^{1}H NMR: δ0.99 (*d*, J = 7.1 Hz, 10-H_3 × 0.34), 1.48 (*d*, J = 1.0 Hz, 11-H_3), 1.98–2.03 (OAc × 4), 3.67–3.74 (*m*, 5-H), 4.09–4.33 (*m*, 6'-H_2), 4.86 (*d*, J = 8.1 Hz, 1'-H), 5.86 (*s* (*br*), 3-H × 0.10 [(8*S*)-isomer]), 5.92 (*s* (*br*), 3-H × 0.90). (Found: C, 57.42; H, 6.84. Calc. for $C_{24}H_{34}O_{11}$: C, 57.82; H, 6.87%.)

Zemplén reaction of [10-$^2H_3$]-8-*epiiridodial glucoside tetraacetate* (**22**). 0.1 N methanolic NaOMe (0.4 ml) was added to a soln of [10-$^2H_3$]-**22** (71 mg) in dry MeOH (5 ml) and the mixture was refluxed for 5 min. After cooling, the mixture was neutralized with Amberlite IR 120 (H^+-form) and concd *in vacuo*. The residue was purified by prep. TLC ($CHCl_3$–MeOH, 4:1, two developments) to give [10-$^2H_3$]-8-epiiridodial glucoside (**23**) (44 mg) [containing *ca* 7% of the (8*S*)-isomer] as a white powder. $[\alpha]_D$ −146.8° (MeOH, *c* 0.76); IR ν_{max}^{KBr} cm^{-1}: 3400, 2930, 2880, 1680, 1380, 1070, 1010, 910; ^{1}H NMR: δ1.05 (*d*, J = 7.1 Hz, 10-H_3 × 0.36), 1.15–1.83 (*m*, 6-H_2 and 7-H_2), 1.52 (*s* (*br*), 11-H_3), 2.09–2.36 (*m*, 8-H and 9-H), 2.45–2.64 (*m*, 5-H), 3.60–3.91 (*m*, 6'-H_2), 4.62 (*d*, J = 7.8 Hz, 1'-H), 5.05 (*d*, J = 4.9 Hz, 1-H × 0.07 [(8*S*)-isomer]), 5.28 (*d*, J = 2.9 Hz, 1-H × 0.90), 5.96 (*s* (*br*), 3-H). (Found: C, 56.84; H, 7.96. Calc. for $C_{16}H_{26}O_7 \cdot 1/2H_2O$: C, 56.62; H, 8.02%.) The ^{1}H NMR spectrum showed that 64% of the C-10 methyl protons were replaced by deuterium.

Hydrolysis of [10-$^2H_3$]-8-*epiiridodial glucoside* (**23**) *with* β-*glucosidase.* [10-$^2H_3$]-**23** (44 mg) was hydrolysed with β-glucosidase (5 mg) (emulsin prepared from almond) to give [10-$^2H_3$]-8-epiiridodial (**6**) (14.2 mg) (containing *ca* 7% of [10-$^2H_3$]-**5**) as a colourless oil, bp 90° [1 mm Hg (Kugelrohr apparatus)]. This compound was found to be a 9:1 mixture of the E-H and D-A form by comparing the signal intensities of the C-3 protons of these forms in the ^{1}H NMR spectrum. $[\alpha]_D$ −7.9° (MeOH, *c* 2.05); IR ν_{max}^{neat} cm^{-1}: 3400, 2950, 2920, 2850, 1740, 1720, 1680, 1380, 850; ^{1}H NMR (E-H form): δ1.07 (*s* (*br*), 10-H_3 × 0.36), 1.60 (*d*, J = 0.97 Hz, 11-H_3), 5.00 (*d*, J = 4.9 Hz, 1-H), 6.01 (*s* (*br*), 3-H); (D-A form): δ1.08 (*d*, J = 6.6 Hz, 11-H_3), 1.09 (*d*, J = 7.0 Hz, 10-H_3 × 0.36), 9.63 (*d*, J = 2.2 Hz, 3-H), 9.84 (*d*, J = 5.1 Hz, 1-H); EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 168.11506 $[M]^+$ (76) ($C_{10}H_{16}O_2$), 150 $[M-H_2O]^+$ (34), 135 $[M-(H_2O+CH_3)]^+$ (85), 122 (32), 97 (66), 81 (86), 71 (70), 43 (100).

Reduction of geniposide pentaacetate (**24**) *to a mixture of* 10-*deoxygeniposide tetraacetate* (**18**) *and* 7-*deoxygardoside methyl ester tetraacetate* (**25**). $Pd(PPh_3)_4$ (620 mg) and PPh_3 (998 mg) were added together with $NaBH_4$ (524 mg) to a soln of **24** (3.24 g) in dry THF (90 ml). The mixture was stirred for 2.5 hr at 80° under N_2, poured into iced H_2O (500 ml) and extracted

with $CHCl_3$ (100 ml × 4). The $CHCl_3$ layer was washed with H_2O (500 ml × 2), dried and concd *in vacuo*. CC of the residue (5.44 g) over silica gel (250 g) with $CHCl_3$–Me_2CO as eluent followed by recrystallization from EtOH afforded colourless needles, mp 105–106°. This substance was found to be a 4:1 mixture (2.19 g) of **18** and **25** by comparing the signal intensities of the C-7 proton of **18** and the C-10 protons of **25** in the ^1H NMR spectrum. IR ν_{max}^{KBr} cm^{-1}: 2940, 1750, 1710, 1640, 1440, 1370, 1220, 1080, 1040, 900; ^1H NMR (**18**): δ1.76 (*s* (*br*), 10-H_3), 1.93–2.10 (OAc × 4), 3.70 (COOMe), 4.87 (*d*, *J* = 8.1 Hz, 1′-H), 5.45 (*s* (*br*), 7-H), 7.40 (*d*, *J* = 1.2 Hz, 3-H); (**25**): δ1.93–2.10 (OAc × 4), 3.70 (*s*, COOMe) 4.87 (*d*, *J* = 8.1 Hz, 1′-H), 5.35 (*d*, *J* = 3.7 Hz, 10-H_2), 7.39 (*d*, *J* = 1.5 Hz, 3-H). (Found: C, 55.59; H, 5.96. Calc. for $C_{25}H_{32}O_{13}$: C, 55.55; H, 5.97%.)

Hydrogenation of a mixture of 10-*deoxygeniposide tetraacetate* (**18**) *and* 7-*deoxygardoside methyl ester tetraacetate* (**25**) *to form* 8-*epideoxyloganin tetraacetate* (**20**). 5% Rh–C (205 mg) was added to a soln of the above mixture of **18** and **25** (2.19 g) in MeOH (35 ml), and the whole was vigorously stirred under H_2 until absorption of H_2 had ceased. After filtration, the filtrate was concd *in vacuo* to give a residue (2.20 g) which was recrystallized from EtOH to give **20** (1.86 g) as colourless needles, mp 109°. Its ^1H NMR spectrum showed contamination with *ca* 3% of the (8*S*)-isomer (**19**).

Conversion of 8-*epideoxyloganin tetraacetate* (**20**) *to form* [11-^2H]*boschnaloside* (**16**). Compound **20** (395 mg) was reduced in dry THF (20 ml) with $LiAl^2H_2(OMe)_2$ [prepared from $LiAl^2H_4$ (460 mg) and dry MeOH (0.88 ml)] in the same way as for the preparation of [10-$^2H_3$]-**21** to yield a white powder (255 mg), which was dissolved in D_2O (4 ml) and oxidized with Pt [prepared from PtO_2 (30 mg)]. The mixture was stirred for 76 hr under O_2 and then filtered. The soln was concd *in vacuo* to yield a white powder, which was purified by prep. TLC ($CHCl_3$–MeOH, 17:3, three developments) to give [11-^2H]-**16** (77 mg) (containing *ca* 3% of [1-^2H]-**10**) as a white powder. $[\alpha]_D$ −123.2° (MeOH, *c* 1.33); UV λ_{max}^{EtOH} nm (log ε): 249 (4.16); IR ν_{max}^{KBr} cm^{-1}: 3400, 2930, 2880, 1660, 1630, 1070, 900, 840; ^1H NMR: δ1.07 (*d*, *J* = 6.8 Hz, 10-H_3), 1.23–1.90 (*m*, 7-H_2), 1.47–2.14 (*m*, 6-H_2), 2.21–2.39 (*m*, 8-H and 9-H), 2.86–3.02 (*m*, 5-H), 3.59–3.95 (*m*, 6′-H_2), 4.70 (*d*, *J* = 7.8 Hz, 1′-H), 5.62 (*d*, *J* = 4.0 Hz, 1-H), 7.38 (*d*, *J* = 0.7 Hz, 3-H), (the signal at δ9.17 was not seen). (Found: C, 54.22; H, 7.11. Calc. for $C_{16}H_{24}O_8 \cdot 1/2H_2O$: C, 54.38; H, 7.13%.)

Conversion of 10-*deoxygeniposide tetraacetate* (**18**) *to form* [11-^2H]-7,8-*dehydroiridotrial glucoside* (**11**). Compound **18** (331 mg) (containing 7% of **19**) was subjected to $LiAl^2H_2(OMe)_2$ reduction followed by Pt-catalysed oxidation to yield [11-^2H]-**11** (117 mg). Its ^1H NMR spectrum indicated contamination with 7% of **10** and replacement of the C-11 aldehyde proton by deuterium.

Simultaneous administration of [10-$^2H_3$]-8-*epiiridodial* (**6**) *and* [11-$^2H_3$]-7,8-*dehydroiridodial* (**7**) *to cell cultures.* A soln of [10-$^2H_3$]-**6** (13.0 mg) and [11-$^2H_3$]-**7** (13.0 mg) in EtOH–H_2O (1:1, 4 ml) and Tween 80 (1 drop) was administered to cell cultures (200 ml × 4). After 7 days of incubation, the cells (87 g) were worked up in the usual manner giving tarennoside (**1**) (22.5 mg) and gardenoside (**2**) (62.2 mg). Acetylation of the former gave tarennoside pentaacetate (**12**) (23.1 mg). ^2H NMR: δ4.62 (10-^2H), 7.31 (3-^2H), 9.28 (11-^2H).

Administration of [11-^2H]*boschnaloside* (**16**) *to cell cultures.* A soln of [11-^2H]-**16** (33.4 mg) in H_2O (4 ml) was administered to cell cultures (200 ml × 4) in the usual way. Work-up of the cells (89 g) yielded tarennoside (**1**) (18.5 mg) and tri-al glucoside (1.0 mg). The former, on acetylation, gave tarennoside pentaacetate (**12**) (16.7 mg) as colourless needles. ^2H NMR: δ7.31 (3-^2H), 9.27 (11-^2H). The latter was identified as ^2H-labelled **16** diluted with endogenous **16** by ^1H NMR spectroscopy.

Administration of [11-^2H]-7,8-*dehydroiridotrial glucoside* (**11**) *to cell cultures.* A soln of [11-^2H]-**11** (33.5 mg) in H_2O (4 ml) was administered to the cell cultures (200 ml × 4). Work-up of the cells (87 g) gave tarennoside pentaacetate (**12**) (22.5 mg) and tri-al glucoside acetate (0.8 mg). ^2H NMR (**12**): δ7.29 (3-^2H), 9.29 (11-^2H). The latter was identified as [11-^2H]-**15**, contaminated with a trace of boschnaloside tetraacetate (**17**) by ^1H NMR spectroscopy. ^1H NMR: δ1.02 [*d*, *J* = 6.8 Hz, 10-H_3 (**17**)], 1.77 (*s* (*br*), 10-H_3), 1.96–2.09 (OAc × 4), 2.13–2.24 (*m*, 6-H), 2.65–2.96 (*m*, 6-H and 9-H), 3.09–3.22 (*m*, 5-H), 5.34 (*d*, *J* = 4.4 Hz, 1-H), 5.46 (*s* (*br*), 7-H), 7.13 (*s*, 3-H), 9.26 (*s*, 11-H).

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