Phytochemistry, Vol. 25, No. 11, pp. 2515–2521, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

BIOSYNTHESIS OF IRIDOID GLUCOSIDES IN *GALIUM MOLLUGO*, *G. SPURIUM* VAR. *ECHINOSPERMON* AND *DEUTZIA CRENATA*. INTERMEDIACY OF DEOXYLOGANIC ACID, LOGANIN AND IRIDODIAL GLUCOSIDE*

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(*Received* 19 *November* 1985)

Key Word Index—*Galium mollugo*; *G. spurium* var. *echinospermon*; Rubiaceae; *Deutzia crenata*; Saxifragaceae; Philadelphaceae; iridoid glucoside; biosynthesis; ^{2}H NMR.

Abstract—Administration of ^{2}H-labelled compounds to *Galium mollugo*, *G. spurium* var. *echinospermon* and *Deutzia crenata* established that deoxyloganic acid is a precursor of asperuloside, geniposidic acid and secogalioside in *G. mollugo* as well as asperuloside in *G. spurium*, while iridodial glucoside is a precursor of deutzioside in *D. crenata*. Additionally, the intermediacy of loganic acid in the biosynthesis of the iridoid and secoiridoid glucosides in the *Galium* plants was reconfirmed.

INTRODUCTION

By administration of various ^{3}H-labelled compounds to *Daphniphyllum macropodum*, *Aucuba japonica* etc., we provided evidence that iridoid glucosides including geniposide (**1**), asperuroside (**2**) and aucubin (**3**), all of which have a highly oxidized cyclopentane ring, are biosynthesized via deoxyloganic acid (**4**), loganic acid (**5**) and 10-deoxygeniposidic acid (**6**) [1, 2]. Subsequently, we found that iridodial (**7**) functions as a key intermediate for the biosynthesis of lamiide (**8**) and lamioside (**9**) in *Lamium amplexicaule*, deutzioside (**10**) in *Deutzia crenata* and asperuloside (**2**) in *Galium spurium* var. *echinospermon* [3] as well as patrinoside (**11**) in *Patrinia gibbosa* [4]. Very recently, we demonstrated the precursorship of iridodial (**7**) for the biosynthesis of secologanin (**12**) (in *Lonicera morrowii* [5, 6]), vindoline (**13**) (in *Catharanthus roseus* [5–7]) and ajmaline (**14**) and vomilenine (**15**) (in *Rauwolfia serpentina* cell cultures [7, 8]); these compounds had already been shown to be formed via deoxyloganin (**16**) and loganin (**17**). In contrast to these findings, Damtoft reported recently that 8-epideoxyloganin (**18**) with 8*R*-configuration is a precursor of lamiide (**8**) and ipolamiide (**19**) in *Hebenstreitia dentata* and 8-epideoxyloganic acid (**20**) is a precursor of aucubin (**3**) in *Scrophularia racemosa* and *Plantago major* and antirrhinnoside (**21**) in *Antirrhinium majus*, while the 8*S*-isomers, deoxyloganin (**16**) and deoxyloganic acid (**4**), are not precursors of these glucosides [9, 10]. The involvement of 8*R*-isomers, 8-epiiridodial (**22**) and 8-epiiridotrial glucoside (boschnaloside) (**23**), was also demonstrated by us for the biosynthesis of tarennoside (**24**) and gardenoside (**25**) in *Gardenia jasminoides* cell cultures [11, 12].

Thus, the biosynthetic pathways of **8**, **19**, **3**, **21**, **24** and **25**, which were originally considered to be biosynthesized by the same route, seem to differ depending on the kind of plant. However, account must be taken of the fact that the ^{3}H-labelled deoxyloganic acid (**4**), deoxyloganin (**16**) and iridodial (**7**) used for our feeding experiments were each recently found to contain *ca* 10% of the 8*R*-isomer. Thus the high resolution ^{1}H NMR spectrum of deoxyloganin tetraacetate (**27**) obtained by Pd/C-catalyzed hydrogenation of geniposide pentaacetate (**26**) showed a doublet (J = 6.8 Hz) at δ 1.03 due to the C-10 methyl protons and a doublet (J = 1.0 Hz) at δ 7.30 due to the C-3 vinyl proton as well as signals at δ 1.01 (*d*, J = 6.8 Hz) and 7.35 (*d*, J = 1.0 Hz) due to the corresponding protons of 8-epideoxyloganin tetraacetate (**28**). Judging from these signal intensities, the ratio of the two compounds was about 9 to 1. The same ratio of 8*S*- to 8*R*-isomer was also obtained in the hydrogenation of asperuloside tetraacetate (**29**) to deoxyloganic acid tetraacetate (**30**). In the present work, we therefore examined whether asperuloside (**2**), deutzioside (**10**) and the related glucosides are biosynthesized through 8*S*-compounds such as **4**, **7** and **16** or through the corresponding 8*R*-isomers.

RESULTS AND DISCUSSION

In order to examine the above problem the following ^{2}H-labelled putative precursors were synthesized and administered to *G. mollugo*, *G. spurium* var. *echinospermon* and *D. crenata*: deoxyloganic acid (**4**), 8-epideoxyloganic acid (**20**), iridotrial glucoside (**31**), iridodial glucoside (**32**) and 8-epiiridodial glucoside (**33**). Among the above plants, *G. mollugo* is unusual in that it contains not only the highly oxidized iridoid glucosides 10-hydroxyloganin (**34**), asperuloside (**2**) and geniposidic acid (**35**), but also the secoiridoid glucosides secogalioside (**36**) and 10-hydroxymorroniside (**37**) [13, 14]. Therefore, [7-^{2}H]loganic acid (**5**) and, as a reference, [7-^{2}H]-7-epiloganic acid (**38**) were also synthesized and adminis-

* Part 58 in the series "Studies on monoterpene glucosides and related natural products". For Part 57 see ref. [12].

tered to this plant in order to examine the pathway after deoxyloganic acid (**4**).

^{2}H-Labelled precursors required for the feeding experiments were prepared in the following way.

[7,8,10-$^2H_5$]- *and* [10-$^2H_3$]-8-*Epideoxyloganic acid* (**20**). [10-$^2H_2$] Geniposide pentaacetate (**26**) [15] was reduced with NaB^2H_4 in the presence of $Pd(PPh_3)_4$ and PPh_3 to give a mixture of [10-$^2H_3$]-10-deoxygeniposide tetraacetate (**39**) and [7,10-$^2H_3$]-7-deoxygardoside methyl ester tetraacetate (**40**) in a ratio of 4 to 1 [12]. This mixture was hydrogenated with deuterium over 5% Rh-C [9] to give [7,8,10-$^2H_5$]-8-epideoxyloganin tetraacetate (**28**). In the ^{1}H NMR spectrum of **28**, the signals due to the C-3 vinyl proton were observed at δ7.35 and δ7.30 in an intensity ratio of 97 to 3. Moreover, the signal intensity due to the C-10 methyl proton at δ1.01 was reduced by 88% relative to that of the unlabelled counterpart. Thus, this compound contained 3% of the 8*S*-isomer, deoxyloganin tetraacetate (**27**), and about 2.6 protons of its C-10 methyl group were replaced by deuterium. Alkaline hydrolysis gave [7,8,10-$^2H_5$]-8-epideoxyloganic acid (**20**).

[10-$^2H_3$]-10-Deoxygeniposide tetraacetate (**39**) [15] was hydrogenated over 5% Rh-C to yield [10-$^2H_3$]-**28**. Its ^{1}H NMR spectrum indicated contamination with 10% [10-$^2H_3$]-**27** as well as the replacement of 71% (corresponding to 2.1 protons) of the C-10 methyl protons with deuterium. Alkaline hydrolysis yielded [10-$^2H_3$]-8-epi-deoxyloganic acid (**20**).

[7,8,10-$^2H_5$]*Deoxyloganic acid* (**4**). [10-$^2H_2$]Geniposide pentaacetate (**26**) [15] was hydrogenated with deuterium over 5% Pd-C leading to [7,8,10-$^2H_5$]deoxyloganin tetraacetate (**27**). Its ^{1}H NMR spectrum indicated contamination with 7% of the 8*R*-isomer as well as replacement of 76% (corresponding to 2.3 protons) of the C-10 methyl protons by deuterium. Alkaline hydrolysis yielded [7,8,10-$^2H_5$]deoxyloganic acid (**4**).

[7,8,10-$^2H_5$]*Iridotrial glucoside* (**31**). [7,8,10-$^2H_5$]Deoxyloganin tetraacetate (**27**) was reduced with $LiAlH_2(OMe)_2$ followed by Pt-catalyzed oxidation to furnish [7,8,10-$^2H_5$]iridotrial glucoside (**31**).

[11-^{2}H]*Iridodial glucoside* (**32**). 11-Hydroxyiridodial glucoside pentaacetate (**41**) derived by $LiAlH_2(OMe)_2$ reduction of deoxyloganin tetraacetate (**27**) (containing 7% of the corresponding 8*R*-isomer) was subjected to hydrogenolysis with deuterium over 5% Pd-C leading to [11-^{2}H]iridodial glucoside tetraacetate (**42**), which on Zemplén reaction yielded [11-^{2}H]iridodial glucoside (**32**). Its ^{1}H NMR spectrum showed a 28% reduction of the signal intensity of the C-11 methyl protons indicating the introduction of about 0.84 deuterium into the C-11 position.

[11-^{2}H]-8-*Epiiridodial glucoside* (**33**). 8-Epi-11-hydroxyiridodial glucoside pentaacetate (**43**) derived from 8-epideoxyloganin tetraacetate (**28**) (containing 2% of the corresponding 8*S*-isomer) was subjected to hydrogenolysis with deuterium over 5% Pd-C to give [11-^{2}H]-8-epiiridodial glucoside tetraacetate (**44**) [12], which was deacetylated to [11-^{2}H]-8-epiiridodial glucoside (**33**). Its ^{1}H NMR spectrum indicated the replacement of about 0.87 proton at the C-11 position by deuterium.

[7-^{2}H]*Loganic acid* (**5**) *and* [7-^{2}H]-7-*epiloganic acid* (**38**). Reduction of 7-dehydrologanin tetraacetate (**45**) [16] with NaB^2H_4 gave [7-^{2}H]-7-epiloganin tetraacetate (**46**). Subsequent inversion [16, 17] of the 7-hydroxy group gave [7-^{2}H]loganin pentaacetate (**47**). The ^{1}H NMR spectra of both **46** and **47** lacked the signals due to the C-7 protons (around δ3.80 and δ5.15, respectively). Zemplén reaction and alkaline hydrolysis of the **47** yielded [7-^{2}H]loganic acid (**5**), whereas alkaline hydrolysis of **46** gave [7-^{2}H]-7-epiloganic acid (**38**).

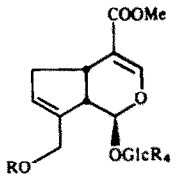
Feeding experiments

Initially, [7,8,10-$^2H_5$]deoxyloganic acid (**4**), [7,8,10-$^2H_5$]-8-epideoxyloganic acid (**20**) and [7,8,10-$^2H_5$]iridotrial glucoside (**31**) were administered separately to *G. mollugo* plants. After 6 days, asperuloside (**2**), secogalioside (**36**) and geniposidic acid (**35**) were isolated. The latter was further purified after methylation to geniposide (**1**). In the ^{2}H NMR spectra (measured in MeCN), only the glucosides isolated from the plants fed with [7,8,10-$^2H_5$]deoxyloganic acid (**4**) showed signals, i.e. in asperuloside (**2**) the ^{2}H-peaks appeared at δ5.79 and δ4.65 due to the C-7 vinyl and C-10 methylene deuterium, in geniposide (**1**) at δ5.82 and δ4.24 due to the C-7 vinyl and C-10 methylene deuterium, and in secogalioside (**36**) at around δ5.60 due to the C-10 deuterium. The incorporation ratios of ^{2}H-labelled **4** into the above glucosides were calculated using the respective percentage ^{2}H enrichments of these C-10-^{2}H signals, which were normalized relative to the ^{2}H-signal due to the natural abundance of deuterium in the MeCN (δ1.95) used as the solvent. The incorporation ratios (Table 1) of [7,8,10-$^2H_5$]-**4** into **2**, **35** and **36** were fairly high. In addition, the higher specific incorporation of **4** into **35** than into **2** supported the precursorship of **35** for the biosynthesis of **2**.

Subsequently, [7,8,10-$^2H_5$]deoxyloganic acid (**4**) and [10-$^2H_3$]-8-epideoxyloganic acid (**20**) were fed separately to the congeneric plant, *G. spurium* var. *echinospermon* which contains only asperuloside (**2**) as the iridoid. The incorporation ratios (Table 2) of these compounds into **2** calculated in the same way from the ^{2}H NMR spectra indicated that **4** was also the precursor of **2** in *G. spurium*. Although **20** was incorporated into **2** to a significant extent, the possibility of **20** serving as a biosynthetic precursor of **2** seems doubtful, since the differences between the total and specific incorporations of **4** and **20** into **2** would become much higher than the values obtained, in consideration of the fact that **4** was contaminated with 7% of **20**, while **20** was contaminated with 10% of **4**.

From the results mentioned so far, it was concluded that the iridoid and secoiridoid glucosides in the *Galium* plants were biosynthesized via deoxyloganic acid (**4**) with 8*S*-configuration in accordance with our previous proposal [2]. This conclusion coincides with the recent report of Damtoft *et al.* [18] that administration of deoxyloganic acid (**4**) and 8-epideoxyloganic acid (**20**) to *Theligonum cynocrambe* (Rubiaceae) gave a 2% incorporation of **4** into asperuloside (**2**) while **20** was not incorporated. The non-incorporation of iridotrial glucoside (**31**) into **2**, **35** and **36** in *G. mollugo* suggests that glucosidation may be at the level of the aglucone of deoxyloganic acid (**4**).

Asperuloside (**2**) was previously demonstrated by us to be biosynthesized via loganic acid (**5**), 10-deoxygeniposidic acid (**6**) and geniposidic acid (**35**) [2]. Furthermore, secogalioside (**36**), belonging to the secoiridoid glucosides, is naturally presumed to be formed via loganic acid (**5**) or loganin (**17**). In order to prove this presumption, administration of [7-^{2}H]loganic acid (**5**) and [7-^{2}H]-7-epiloganic acid (**38**) to *G. mollugo* was carried out. The incorporation of the above glucosides into **2**, **35** and **36** were calculated



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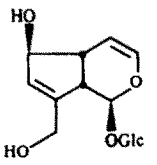

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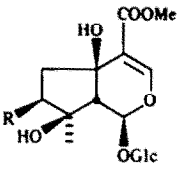
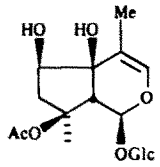


8 R = OH
19 R = H



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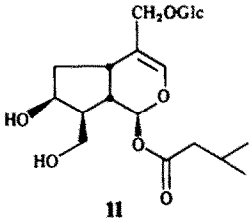

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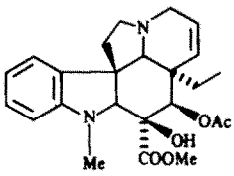

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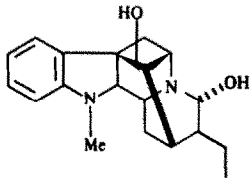

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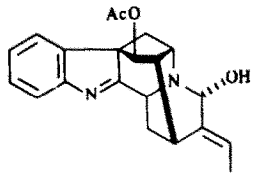

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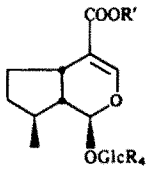

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27 R = Ac, R' = Me
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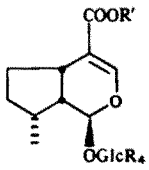

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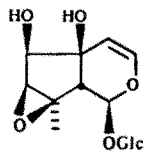
20 R = R' = H
28 R = Ac, R' = Me



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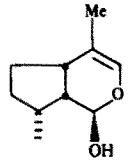

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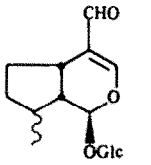
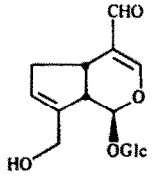


23 (8*R*)
31 (8*S*)



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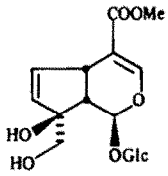

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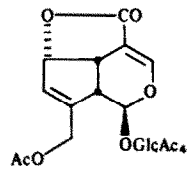

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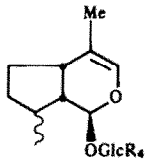
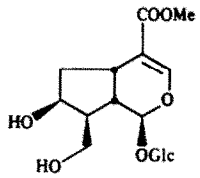


33 (8*R*) R = H
42 (8*S*) R = Ac
44 (8*R*) R = Ac



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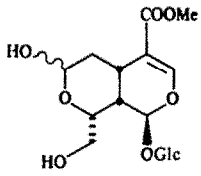

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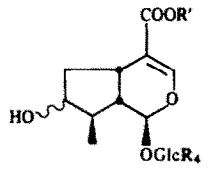
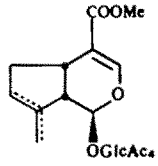
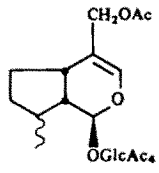


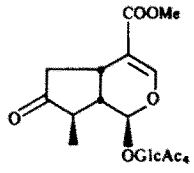
38 (7*R*) R = R' = H
46 (7*R*) R = Ac, R' = Me
47 (7*S*) R = Ac, R' = Me



39 7, 8 - dehydro
40 8, 10 - dehydro



41 (8*S*)
43 (8*R*)



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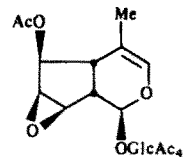


48

Table 1. Administration of ^2H-labelled putative precursors to *G. mollugo*

Compounds fed	Amount (mg)	Amount of plant material (g)	Metabolic period (days)	Amount (mg) of glucosides isolated and (second line) total incorporation and sp. incorporation (%)		
				2	**35**	**36**
[7,8,10-$^2H_5$]-**4**	9.2	17.7	6	43.2	9.2	26.5
				30.3 (6.9)	19.6 (19.7)	9.4 (3.5)
[7,8,10-$^2H_5$]-**20**	11.7	16.2	6	33.8	10.3	24.6
				0 (0)	0 (0)	0 (0)
[7,8,10-$^2H_5$]-**31**	11.9	17.1	6	30.4	6.7	31.5
				0 (0)	0 (0)	0 (0)
[7-^2H]-**5**	12.9	18.3	4	54.8	6.8	13.5
				37.9 (9.8)	29.3 (57.3)	10.0 (10.7)
[7-^2H]-**38**	11.5	15.9	4	44.7	—	12.4
				0 (0)	—	0 (0)

Table 2. Administration of ^2H-labelled deoxyloganic acid (**4**) and 8-epideoxyloganic acid (**20**) to *G. spurium* var. *echinospermon*

Compounds fed and amount (mg)	Amount of plant material (g)	Metabolic period (days)	Asperuloside (**2**) isolated		
			Amount (mg)	Total incorporation (%)	Sp. incorporation (%)
[7,8,10-$^2H_5$]-**4** 16.3	23.6	4	65.0	14.9	3.4
[10-$^2H_3$]-**20** 14.1	22.3	4	75.0	2.7	0.6

in the usual manner from the ^2H NMR spectra. As shown in Table 1, loganic acid (**5**) was incorporated into all these glucosides in high ratios, while 7-epiloganic acid (**38**) was not incorporated into any glucoside. The marked difference between the specific incorporation ratios of **5** into **35** and **2** corroborated the above-mentioned route, i.e. loganic acid (**5**) → 10-deoxygeniposidic acid (**6**) → geniposidic acid (**35**) → asperuloside (**2**).

Finally, [11-^2H]iridodial glucoside (**32**) and [11-^2H]-8-epiiridodial glucoside (**33**) were administered separately to *D. crenata*. The ^2H NMR spectra ($CHCl_3$) of the pentaacetate (**48**) of isolated deutzioside (**10**) showed signals at δ1.48 (11-^2H) originating from the ^2H-labelled **32** and **33**. Total and specific incorporation ratios (Table 3) were calculated by comparing the intensities of these signals with that of the natural abundance of deuterium in $CHCl_3$ (at δ7.25) used as the solvent.

It was found that the incorporation of iridodial glucoside (**32**) with 8*S*-configuration into **10** was much higher than that of 8-epiiridodial glucoside (**33**) with 8*R*-configuration. Considering the fact that **32** was contaminated with 7% **33**, while **33** was contaminated with 2% **32**, the differences between the specific and total incorporation ratios of **32** and **33** would become much larger. Therefore, it was also confirmed that deutzioside (**10**) was biosynthesized via iridodial (**7**) and iridodial glucoside (**32**) in accord with our previous result [3].

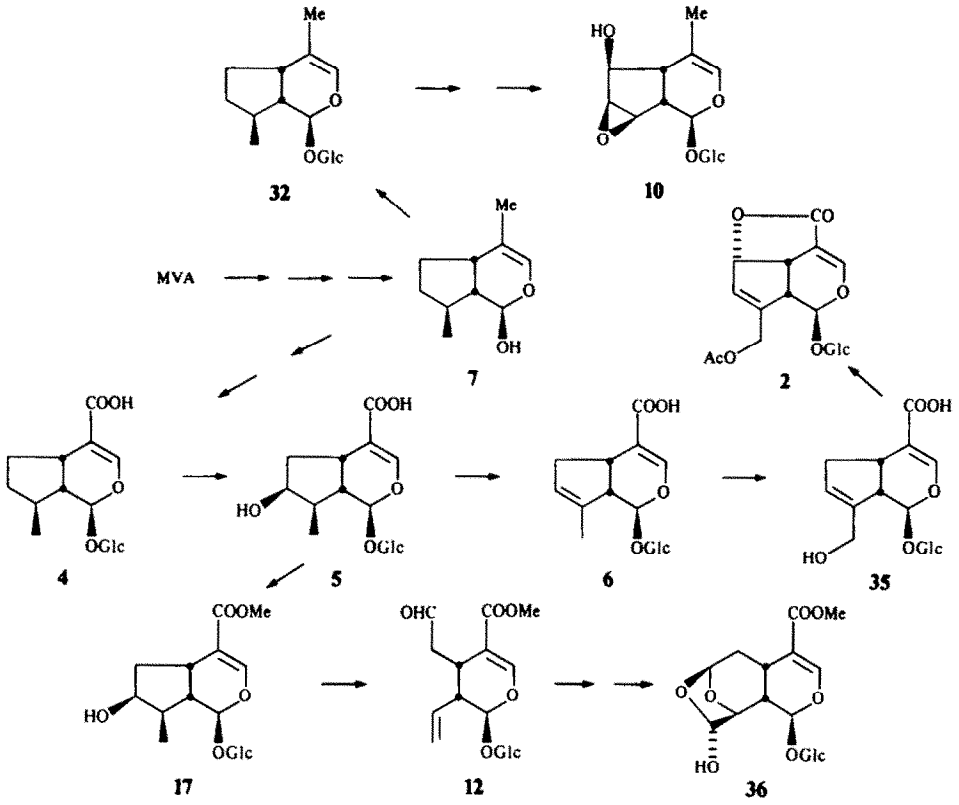
The present work corroborated our proposals that the iridoid and secoiridoid glucosides in *Galium* are biosynthesized via deoxyloganic acid (**4**), while the iridoid glucosides in *Deutzia* are formed via iridodial glucoside (**32**). This result is in contrast to those obtained in the feeding experiments with *Gardenia jasminoides* suspension cultures where tarennoside (**24**) [and hence, the subsequently formed geniposidic acid (**35**)] is biosynthesized via (8*R*)-precursors such as boschnaloside (**23**) [11, 12]. In *Galium*, **35** is formed via (8*S*)-compounds such as deoxyloganic acid (**4**). This is an example of the biosynthesis of identical iridoid glucosides by different pathways depending on the kind of plant.

It remains to be clarified whether aucubin (**3**) in *Aucuba* and lamiide (**8**) and lamioside (**9**) in *Lamium* are formed via deoxyloganic acid (**4**) or via 8-epideoxyloganic acid (**20**); this problem is intriguing since **20** was shown by Damtoft to be a precursor of the above iridoid glucosides in plants of other genera [9, 10].

EXPERIMENTAL

Mps: uncorr.; ^1H NMR: 199.50 MHz, $CDCl_3$ (acetates) or CD_3OD (free glucosides), TMS as internal standard; ^2H NMR: 30.63 MHz, MeCN (free glucosides) or $CHCl_3$ (acetates), natural abundant deuterium as internal standard. CC: silica gel 60 (70–230 mesh) (Merck) and activated charcoal (Wako); TLC and prep. TLC: silica gel 60 GF_{254} (Merck) (0.3 mm) and PF_{254} (Merck) (1.0 mm). Spots and bands were visualized by UV (254 mm) or by exposure to I_2 vapour. The main bands on prep. TLC plates were scraped off and extracted with $CHCl_3$–MeOH (9:1) and extracts were concd *in vacuo*.

Plant materials for feeding experiments. G. mollugo plants cultivated at the Medical Plant Garden, Faculty of Pharmaceutical Sciences, Kyoto University [14] were collected in



Scheme 1. Biosynthetic pathway of asperuloside (2), geniposidic acid (35), secogalioside (36) and deutzioside (10).

May, 1983; *G. spurium* var. *echinospermon* plants were collected on the campus of this faculty in May, 1983; *D. crenata* twigs were collected on the campus of the Faculty of Medicine, in Oct., 1983.

Conversion of [10-$^2H_2$]*geniposide pentaacetate* (**26**) *into* [7,8,10-$^2H_5$]-8-*epideoxyloganin tetraacetate* (**28**). ^{2}H-Labelled **26** [15] (584 mg) was reduced with NaB^2H_4 (97% ^{2}H) (102 mg) in the presence of $Pd(PPh_3)_4$ (111 mg) and PPh_3 (182 mg) according to the procedure described in the preceding paper [12] to yield a 4:1 mixture (367 mg) of [10-$^2H_3$]-10-deoxygeniposide tetraacetate (**39**) and [7,10-$^2H_3$]-7-deoxygardoside methyl ester tetraacetate (**40**) as colourless needles, mp 105–106°. A soln of this mixture in MeOH (5 ml) was hydrogenated with $^2H_2$ gas over 5% Rh-C until the uptake of $^2H_2$ had ceased. The usual work-up gave a solid (384 mg), which was purified by prep. TLC (Et_2O) and recrystallization from EtOH to yield [7,8,10-$^2H_5$]-8-epideoxyloganin tetraacetate (**28**) (214 mg) as colourless needles, mp 108–109°. ^{1}H NMR: δ1.01 (*d*, J = 6.8 Hz, 10-H_3 × 0.12), 1.94–2.09 (OAc × 4), 2.83–2.89 (*m*, 5-H), 3.70 ($COOCH_3$), 7.30 [*d*, J = 1.0 Hz, 3-H × 0.03 (**27**)], 7.35 (*d*, J = 1.0 Hz, 3-H × 0.97).

Alkaline hydrolysis of [7,8,10-$^2H_5$]-**28**. A suspension of [7,8,10-$^2H_5$]-**28** (42 mg) in 0.5 M NaOH (20 ml) was stirred vigorously at room temp. for 1.5 hr. The resultant soln was neutralized with Amberlite IR-120 (H^+-form) and concd *in vacuo*. The residue (25 mg) was subjected to prep. TLC ($CHCl_3$–MeOH 7:3, two developments) to yield [7,8,10-$^2H_5$]-8-epideoxyloganic acid (**20**) as a white powder (12.0 mg): ^{1}H NMR: δ1.06 (*m*, 10-H_3 × 0.12), 2.85–2.96 (*m*, 9-H), 3.59–3.94 (*m*, 6'-H_2), 4.67 [*d*, J = 7.8 Hz, 1'-H × 0.03 (**4**)], 4.69 (*d*, J = 7.6 Hz, 1'-H × 0.97), 5.20 [*d*, J = 5.9 Hz, 1-H × 0.03 (**4**)], 5.45 (*d*, J = 5.4 Hz, 1-H × 0.97), 7.42 (*d*, J = 1.2 Hz, 3-H).

Conversion of [10-$^2H_3$]-10-*deoxygeniposide tetraacetate* (**39**) *into* [10-$^2H_3$]-8-*epideoxyloganic acid* (**20**). A soln of [10-$^2H_3$]-**39**

Table 3. Administration of ^{2}H-labelled iridodial glucoside (**32**) and 8-epiiridodial glucoside (**33**) to *D. crenata*

Compounds fed and amount (mg)	Amount of plant material (g)	Metabolic period (days)	Deutzioside pentaacetate (**48**)		
			Amount (mg)	Total incorporation (%)	Sp. incorporation (%)
[11-^{2}H]-**32** 7.0	4.9	1	18.2	17.9	10.4
[11-^{2}H]-**33** 6.7	4.7	1	18.8	4.3	2.4

[15] (305 mg) in MeOH (4 ml) was hydrogenated with H_2 gas over 5% Rh-C (80 mg) in the same way as for the preparation of [7,8,10-$^2H_5$]-**28** to yield a colourless solid (271 mg). Recrystallization from EtOH gave [10-$^2H_3$]-8-epideoxyloganin tetraacetate (**28**) (202 mg) as colourless needles, mp 107–108°. Its ^{1}H NMR spectrum indicated contamination with 10% [10-$^2H_3$]-**27** as well as replacement of 71% of the C-10 methyl protons [δ1.01 (*s, br*)] by ^{2}H. An aliquot (45 mg) of this compound was suspended in 0.5 M NaOH (30 ml) and the mixture was stirred at room temp. for 4 hr. The resultant soln was neutralized with Amberlite IR-120 (H^+-form) and concd *in vacuo* to give a powdery residue (30 mg), which on prep. TLC ($CHCl_3$–MeOH 7:3, two developments) gave [10-$^2H_3$]-8-epideoxyloganic acid (**20**) (24.5 mg) as a white powder. Its ^{1}H NMR spectrum indicated contamination with 10% [10-$^2H_3$]-**4** as well as replacement of 71% of the C-10 methyl protons [δ1.06 (*s, br*)] by ^{2}H.

[7,8,10-$^2H_5$]*Deoxyloganic acid* (**4**). A soln of [10-$^2H_2$]-geniposide pentaacetate (**26**) (162 mg) in MeOH (4 ml) was hydrogenated with $^2H_2$ gas over 5% Pd-C (131 mg) until the uptake of H_2 had ceased. The mixture was filtered and concd *in vacuo* to give a residue (164 mg), whose prep. TLC (Et_2O–*n*-hexane–C_6H_6, 6:1:1) gave a colourless solid (119 mg). Recrystallization from EtOH furnished [7,8,10-$^2H_5$]deoxyloganin tetraacetate (**27**) as colourless needles (62 mg), mp 115°. ^{1}H NMR: δ1.03 [*s* (*br*), 10-H_3 × 0.24], 1.93–2.12 (OAc × 4), 3.67 ($COOCH_3$), 4.13–4.32 (*m*, 6′-H_2), 4.84 (*d*, *J* = 8.1 Hz, 1′-H), 7.30 (*d*, *J* = 1.0 Hz, 3-H × 0.93), 7.35 [*d*, *J* = 1.0 Hz, 3-H × 0.07 (**28**)]. An aliquot (24 mg) of this compound was hydrolyzed by stirring in 0.5 M NaOH (30 ml). The usual work-up gave [7,8,10-$^2H_5$]deoxyloganic acid (**4**) (containing 7% [7,8,10-$^2H_5$]-**20**) (9.2 mg) as a white powder. ^{1}H NMR: δ1.04 [*s* (*br*), 10-H_3 × 0.29], 2.81–2.92 (*m*, 9-H), 3.65–3.92 (*m*, 6′-H_2), 4.67 (*d*, *J* = 7.8 Hz, 1′-H × 0.93), 4.69 [*d*, *J* = 7.6 Hz, 1′-H × 0.07 (**20**)], 5.20 (*d*, *J* = 5.9 Hz, 1-H × 0.93), 5.45 [*d*, *J* = 5.4 Hz, 1-H × 0.07 (**20**)], 7.36 (*d*, *J* = 1.0 Hz, 3-H × 0.93), 7.41 [*d*, *J* = 1.2 Hz, 3-H × 0.07 (**20**)].

[7,8,10-$^2H_5$]*Iridotrial glucoside* (**31**). [7,8,10-$^2H_5$]Deoxyloganin tetraacetate (**27**) (151 mg) (5 ml) was reduced in dry THF (20 ml) with $LiAlH_2(OMe)_2$ [prepared from $LiAlH_4$ (420 mg) and dry MeOH (0.85 ml)] according to the method reported earlier [15]. A soln of the resultant reduction product (62 mg) in H_2O (1.5 ml) was added to a stirred suspension of Pt [prepared from PtO_2 (39 mg)] in H_2O (3.0 ml); the stirring was continued under an O_2 atmosphere at room temp. for 28 hr. After removal of the catalyst, the soln was concd *in vacuo* to give a residue (63 mg), which on prep. TLC ($CHCl_3$–MeOH, 4:1, two developments) gave [7,8,10-$^2H_5$]iridotrial glucoside (**31**) (41 mg) as a white powder. ^{1}H NMR: δ1.07 [*s* (*br*), 10-H_3 × 0.29], 3.62–3.93 (*m*, 6′-H_2), 4.69 (*d*, *J* = 7.8 Hz, 1′-H), 5.41 (*d*, *J* = 4.6 Hz, 1-H × 0.93), 5.62 [*d*, *J* = 3.9 Hz, 1-H × 0.07 (**23**)], 7.35 (*d*, *J* = 0.7 Hz, 3-H), 9.20 (*s*, 11-CHO).

[11-^{2}H]*Iridodial glucoside* (**32**). A soln of 11-hydroxyiridodial glucoside pentaacetate (**41**) [15] (138 mg) in MeOH (5 ml) was subjected to hydrogenolysis with $^2H_2$ gas over 5% Pd-C (96 mg). The mixture was filtered and the soln concd *in vacuo* to give a residue (120 mg), which on prep. TLC (Et_2O) gave a solid (92 mg). On recrystallization from EtOH, [11-^{2}H]iridodial glucoside tetraacetate (**42**) (57 mg) was obtained as colourless needles, mp 141–143°. ^{1}H NMR: δ1.03 (*d*, *J* = 5.6 Hz, 10-H_3), 1.47 [*s* (*br*), 11-H_3 × 0.72], 1.98–2.09 (OAc × 4), 3.68–3.76 (*m*, 5-H), 4.07–4.36 (*m*, 6′-H_2), 4.87 (*d*, *J* = 8.1 Hz, 1′-H), 5.86 [*s* (*br*), 3-H × 0.93], 5.92 [*s* (*br*), 3-H × 0.07 (**44**)]. 0.1 M methanolic NaOMe (0.2 ml) was added to a soln of [11-^{2}H]-**42** (57 mg) in dry MeOH (3 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 (32 mg) was subjected to prep. TLC ($CHCl_3$–MeOH, 4:1 two developments) to yield [11-^{2}H]iridodial glucoside (**32**) (29.6 mg) as a white powder. ^{1}H NMR: δ1.06 (*d*, *J* = 6.3 Hz, 10-H_3), 1.52 [*s* (*br*), 11-H_3 × 0.72], 3.62–3.91 (*m*, 6′-H_2), 4.64 (*d*, *J* = 7.8 Hz, 1′-H), 5.05 (*d*, *J* = 4.9 Hz, 1-H × 0.93), 5.28 [*d*, *J* = 2.9 Hz, 1-H × 0.07 (**33**)], 5.92 [*s* (*br*), 3-H × 0.07 (**33**)], 5.97 (*d*, *J* = 1.2 Hz, 3-H × 0.93).

[11-^{2}H]-8-*Epiiridodial glucoside* (**33**). A soln of 8-epi-11-hydroxyiridodial glucoside pentaacetate (**43**) [12] (141 mg) in MeOH (5 ml) was subjected to hydrogenolysis with $^2H_2$ gas over 5% Pd-C (102 mg). After removal of the catalyst, the soln was concd *in vacuo* to give a residue (108 mg). Purification by prep. TLC (Et_2O) and recrystallization from EtOH furnished [11-^{2}H]-8-epiiridodial glucoside tetraacetate (**44**) as colourless needles (68 mg), mp 120°. This compound was deacetylated in a similar manner to that described above and the product (34 mg) was subjected to prep. TLC ($CHCl_3$–MeOH, 4:1, two developments) to yield [11-^{2}H]-8-epiiridodial glucoside (**33**) (28.7 mg) as a white powder. It was shown to contain 2% iridodial glucoside (**32**) by ^{1}H NMR. ^{1}H NMR: δ1.05 (*d*, *J* = 7.1 Hz, 10-H_3), 1.15–1.83 (*m*, 6-H_2 and 7-H_2), 1.52 [*s* (*br*), 11-H_3 × 0.71], 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 × 0.02 (**32**)], 5.28 (*d*, *J* = 2.9 Hz, 1-H × 0.98), 5.96 [*s* (*br*), 3-H].

[7-^{2}H]*Loganic acid* (**5**). NaB^2H_4 (145 mg) was added to a stirred soln of 7-dehydrologanin tetraacetate (**45**) (1580 mg) in MeOH (30 ml) under ice cooling, and the stirring was continued for 10 min under the same conditions. The mixture was diluted with H_2O (300 ml) and extracted with $CHCl_3$ (100 ml × 3). The $CHCl_3$ layer was washed with H_2O (200 ml × 2), dried and concd *in vacuo* to give a solid. Recrystallization from Et_2O gave [7-^{2}H]-7-epi-loganin tetraacetate (**46**) (1140 mg) as colourless needles, mp 150–151°. ^{1}H NMR: δ1.11 (*d*, *J* = 6.4 Hz, 10-H_3), 1.94–2.10 (OAc × 4), 3.70 ($COOCH_3$), 4.13–4.35 (*m*, 6′-H_2), 4.86 (*d*, *J* = 8.1 Hz, 1′-H), 7.32 (*d*, *J* = 1.2 Hz, 3-H). The 7-H signal around at δ 3.80 was not observed.

The above compound [7-^{2}H]-**46** was tosylated with C_5H_5N (2 ml) and *p*-toluenesulfonyl chloride (295 mg) and the product was recrystallized from EtOH to give [7-^{2}H]-7-epi-7-tosylloganin tetraacetate (793 mg) as colourless needles, mp 113–115°. This compound (489 mg) was added to a soln of tetraethylammonium acetate (765 mg) in dry Me_2CO (10 ml) and the whole was refluxed at 56–60° for 24 hr under N_2 and then the solvent was removed *in vacuo*. The residue was taken up in $CHCl_3$ (50 ml) and the $CHCl_3$ soln was washed with H_2O (100 ml × 2), dried and concd *in vacuo*. The resulting brownish residue (866 mg) was chromatographed on silica gel (35 g) with $CHCl_3$ as an eluent to give [7-^{2}H]loganin pentaacetate (**47**) (176 mg) as colourless needles, mp 140–141°, the ^{1}H NMR spectrum of which did not show the 7-proton signal at around δ5.15. Subsequently, [7-^{2}H]-**47** (73 mg) was deacetylated with NaOMe in the usual way to give a solid. Purification by prep. TLC ($CHCl_3$–MeOH, 4:1, two developments) and recrystallization from EtOH-Me_2CO gave [7-^{2}H]loganin (**17**) (35 mg) as colourless needles, mp 218–220°.

This substance was dissolved in 0.5 M NaOH (23 ml) and the whole was stirred at room temp for 15 min. After neutralization with Amberlite IR-120 (H^+-form), the mixture was concd *in vacuo* to give a white powder (33 mg), which on prep. TLC ($CHCl_3$–MeOH, 7:3, two developments) furnished [7-^{2}H]-loganic acid (**5**) (22 mg) as a white powder. ^{1}H NMR: δ1.09 (*d*, *J* = 6.8 Hz, 10-H_3), 3.61–3.93 (*m*, 6′-H_2), 4.65 (*d*, *J* = 7.5 Hz, 1′-H), 5.27 (*d*, *J* = 4.4 Hz, 1-H), 7.36 (*d*, *J* = 1.0 Hz, 3-H).

[7-^{2}H]-7-*Epiloganic acid* (**38**). [7-^{2}H]-7-epiloganin tetraacetate (**46**) (43 mg) was suspended in 0.5 M NaOH (30 ml) and the whole was stirred vigorously at room temp. for 3 hr. The resulting soln was neutralized with Amberlite IR-120 (H^+-form) and concd *in vacuo* to give a residue (27 mg), which on prep. TLC

($CHCl_3$–MeOH, 7:3, two developments) yielded [7-^{2}H]-7-epiloganic acid (**38**) (18.2 mg) as a white powder. ^{1}H NMR: δ1.13 (*d*, J = 6.3 Hz, 10-H_3), 3.61–3.92 (*m*, 6'-H_2), 4.66 (*d*, J = 7.8 Hz, 1'-H), 5.32 (*d*, J = 5.1 Hz, 1-H), 7.41 (*d*, J = 1.2 Hz, 3-H).

Administration of ^{2}H-*labelled* **4**, **5**, **20**, **31** *and* **38** *to* G. mollugo. Each ^{2}H-labelled compound was dissolved in H_2O (4 ml) and administered hydroponically to 15–20 terrestrial parts (each *ca* 15–20 cm in length) of the plants in May. After 4 or 6 days, the plants were extracted with MeOH (200 ml × 4) for 20 min under reflux. The MeOH extracts were concd *in vacuo*. The residue was taken up in H_2O (100 ml), and insoluble materials were removed by filtration. The filtrate was transferred to an activated charcoal (6 g) column and eluted successively with H_2O (400 ml) and MeOH (500 ml). Concn of the MeOH eluate gave a glucoside fraction, which was subjected to prep. TLC (four plates, $CHCl_3$–MeOH 17:3, three developments). The most mobile band gave asperuloside (**2**) as colourless needles. The middle band afforded secogalioside (**36**) as a white powder. The least mobile band furnished a carboxylic acid fraction, which was treated with CH_2N_2. The reaction mixture was concd *in vacuo* and then subjected to prep. TLC ($CHCl_3$–MeOH, 4:1, two developments) to yield geniposide (**1**) as colourless needles. ^{2}H NMR (**2**): δ5.79 (7-^{2}H), 4.65 (10-^{2}H) (both originating from [7,8,10-$^2H_5$]-**4**); δ5.79 (7-^{2}H) (originating from [7-^{2}H]-**5**).

Administration of ^{2}H-*labelled* **4** *and* **20** *to* G. spurium *var.* echinospermon. Each ^{2}H-labelled compound was dissolved in H_2O (4 ml) and administered hydroponically to 17–18 terrestrial parts (each *ca* 15–20 cm in length) of *G. spurium* var. *echinospermon* plants in May. After 4 days, the plants were extracted with MeOH (100 ml × 4) under reflux for 20 min. The combined extracts were concd *in vacuo*. The residue was taken up in H_2O (50 ml) and insoluble matrials were filtered off. The filtrate was transferred to an activated charcoal (8 g) column and eluted successively with H_2O (400 ml), 5%, 10%, 20%, 30% MeOH–H_2O (each 200 ml). The MeOH eluate gave a glucoside fraction, which on prep. TLC ($CHCl_3$–MeOH, 17:3, four developments) yielded asperuloside (**2**) as colourless needles. ^{2}H NMR (**2**): δ5.79 (7-^{2}H), 4.64 (10-^{2}H) (both originating from [7,8,10-$^2H_5$]-**4**); δ4.64 (10-^{2}H) (originating from [10-$^2H_3$]-**20**).

Administration of ^{2}H-*labelled* **32** *and* **33** *to* D. crenata. Each ^{2}H-labelled compound was dissolved in H_2O (1.5 ml) and administered hydroponically to three young twigs (each 10–12 cm in length with many leaves) of *D. crenata* in Oct. After 24 hr, the twigs were cut into pieces and extracted with MeOH (50 ml × 4) under reflux for 20 min. The combined MeOH extracts were concd *in vacuo*. The residue was taken up in H_2O (50 ml) and insoluble materials were removed by filtration. The filtrate was transferred to an activated charcoal (3 g) column and eluted with H_2O (200 ml) and MeOH (300 ml). The MeOH eluate gave a glucoside fraction which was acetylated. The product was subjected to prep. TLC (C_6H_6–Et_2O, 5:2, two developments). Of two major bands, the more mobile one afforded deutzioside pentaacetate (**48**) as colourless needles. ^{2}H NMR: δ1.48 (11-^{2}H) (originating from both [11-^{2}H]-**32** and -**33**).

Acknowledgement—This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, to whom we express our gratitude.

REFERENCES

1. Inouye, H., Ueda, S., Aoki, Y. and Takeda, Y. (1972) *Chem. Pharm. Bull.* **20**, 1287.
2. Inouye, H., Ueda, S. and Takeda, Y. (1972) *Chem. Pharm. Bull.* **20**, 1305.
3. Inouye, H., Ueda, S., Uesato, S. and Kobayshi, K. (1978) *Chem. Pharm. Bull.* **26**, 3384.
4. Inouye, H. (1978) *Planta Medica* **33**, 193.
5. Uesato, S., Matsuda, S. and Inouye, H. (1984) *Chem. Pharm. Bull.* **32**, 1671.
6. Uesato, S., Matsuda, S. and Inouye, H. (1984) *Yakugaku Zasshi* **104**, 1232.
7. Uesato, S., Kanomi (née Matsuda), S., Iida, A., Inouye, H. and Zenk, M. H. (1986) *Phytochemistry* **25**, 839.
8. Uesato, S., Matsuda, S., Iida, A. and Inouye, H. (1984) *Chem. Pharm. Bull.* **32**, 3764.
9. Damtoft, S. (1981) *J. Chem. Soc. Chem. Comm.* 228.
10. Damtoft, S. (1983) *Phytochemistry* **22**, 1929.
11. Uesato, S., Ueda, S., Kobayashi, K., Miyauchi, M. and Inouye, H. (1984) *Tetrahedron Lett.* **25**, 573.
12. Uesato, S., Ueda, S., Kobayashi, K., Miyauchi, M., Itoh, H. and Inouye, H. (1986) *Phytochemistry* **25**, 2309.
13. Bock, K., Jensen, S. R. and Nielsen, B. J. (1976) *Acta Chem. Scand.* **B30**, 743.
14. Uesato, S., Ueda, M., Inouye, H., Kuwajima, H., Yatsuzuka, M. and Takaishi, K. (1984) *Phytochemistry* **23**, 2535.
15. Uesato, S., Kobayashi, K. and Inouye, H. (1982) *Chem. Pharm. Bull.* **30**, 3942.
16. Inouye, H., Yoshida, T., Tobita, S. and Okigawa, M. (1970) *Tetrahedron* **26**, 3905.
17. Battersby, A. R., Hall, E. S. and Southgate, R. (1969) *J. Chem. Soc. C* 721.
18. Damtoft, S., Jensen S. R. and Nielsen, B. J. (1983) *Biochem. Soc. Trans.* **11**, 593.