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# INTERMEDIACY OF IRIDODIAL IN THE BIOSYNTHESIS OF SOME IRIDOID GLUCOSIDES*

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**Key Word Index**—*Lamium amplexicaule*; Labiatae; *Deutzia crenata*; Saxifragaceae; *Galium spurium* var. *echinospermon*; Rubiaceae; biosynthesis; iridoid glucosides.

**Abstract**—Administration of MVA-[2-$^{14}$C] to *Lamium amplexicaule* and *Deutzia crenata* as well as of 11-hydroxyiridodial glucoside-[10-$^{3}$H] and 7-deoxyloganic acid-[10-$^{3}$H] to the former plant suggested that, in contrast to secoiridoid-indole alkaloids, iridoid glucosides such as ipolamiide, lamiide, lamioside, deutzioside and scabroside in these plants are biosynthesized via iridodial. Iridodial as a precursor for the biosynthesis of asperuloside was also suggested from the results of the administration of MVA-[2-$^{14}$C] to *Galium spurium* var. *echinospermon*.

## INTRODUCTION

It was suggested that the cyclization of an acyclic monoterpene to the iridane skeleton may involve a Michael-type addition of 10-oxocitronellal to give rise to iridodial (**15**) which is assumed to be a key intermediate in the biosynthesis of various iridoids [1, 2]. However, Arigoni *et al.* [3] and Battersby *et al.* [4] showed that 10-hydroxynerol (**1**) (or 10-hydroxygeraniol **2**) can be a biosynthetic intermediate of loganin (**3**) or indole alkaloids, while citronellol and 10-hydroxycitronellol are not intermediates. Arigoni *et al.* [3] also observed the incorporation of C-9 of 10-hydroxynerol (**1**) (or 10-hydroxygeraniol **2**) into C-3 and C-11 of loganin (**3**) or the corresponding positions of indole alkaloids with extensive randomization.

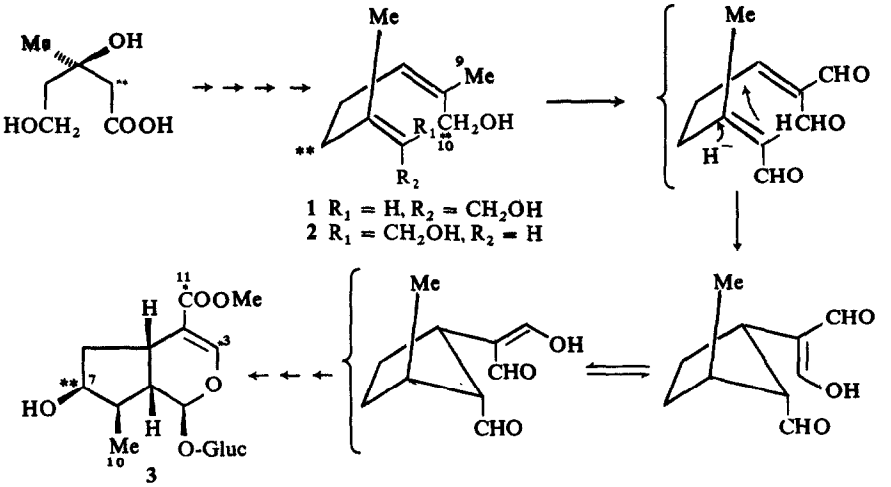
Although the equal distribution of radioactivity from MVA-[2-$^{14}$C] between C-3 and C-11 of loganin (**3**), secoiridoid glucosides or the corresponding positions of indole alkaloids has already been reported [5–7], the above results clearly demonstrated that randomization occurred because of randomization of C-9 and C-10 of **1** or **2** at a stage during cyclization to form iridane skeleton.

On the basis of these findings, Arigoni *et al.* [3] proposed the cyclization mechanism outlined in Scheme 1.

An equal distribution of $^{14}$C label from MVA-[2-$^{14}$C] in C-3 and C-11 was reported [2] also in the case of plumieride (**4**), a highly oxygenated iridoid glucoside. Similar randomization of varying degrees (16.8 to 44.6%) depending upon the age of the plants was observed [8]

* Part 36 of the series 'Studies on Monoterpene Glucosides and Related Natural Products'. For Part 35 see: Takeda, Y., Nishimura, H. and Inouye, H. (1977) *Phytochemistry* **16**, 1401.

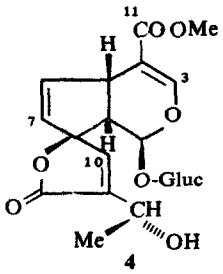
Abbreviation used: MVA, mevalonic acid.




Scheme 1. Proposed mechanism for iridane skeleton formation.

in verbenalin (**5**) and β-skytanthine **6** [9] (10 to 51.5%). Partial randomization between C-3 and C-11 as well as C-7 and C-10 was further observed in the case of nepetalactone **7** [10].

In addition to the usual iridoid glucosides bearing a C-11 carbomethoxy group such as plumieride (**4**) and verbenalin (**5**), there are several types of glucosides having other C-11 functional groups such as ixoroside (**8**) [11] and tarennoside (**9**) [12] having an aldehyde group, valerosidate (**10**) [13], villoside (**11**) [14] and patrinoside (**12**) [15] having a glucosyloxymethyl group, and lamioside (**26**) [16], deutzioside (**23**) [17] and scabroside (**24**) [18] having a methyl group.

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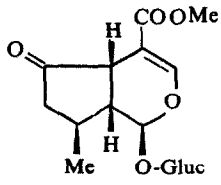


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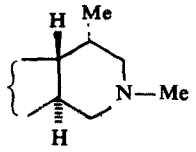


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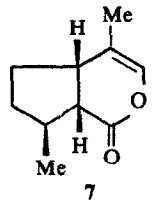


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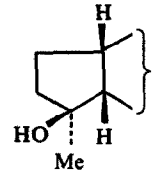


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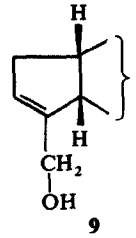


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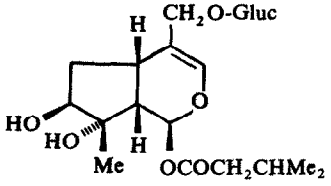


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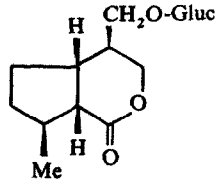


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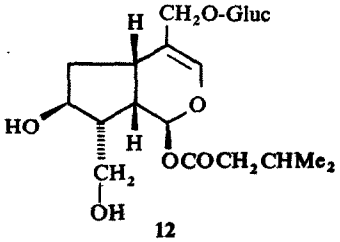


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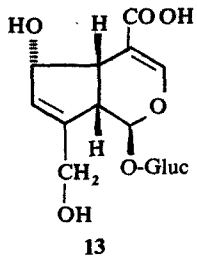


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A detailed investigation on the origin of C-3 and C-11 of these glucosides, especially of compounds having a C-11 methyl group would possibly clarify the mechanism of iridane skeleton formation since the reports on verbenalin (**5**), β-skytanthine (**6**) and nepetalactone (**7**) have left unresolved questions.

Therefore, we administered MVA-[2-$^{14}$C] to *Lamium amplexicaule*, which contains lamioside (**26**), and to *Deutzia crenata* which also contains deutzioside (**23**) and scabroside (**24**). The former plant was considered to be a particularly interesting species for examining the biosynthetic relationship between the C-11 methyl-bearing and C-11 carbomethoxy-bearing glucosides because it contains glucosides such as ipolamiide (**20**) [19] and lamiide (**21**) [19] of the latter type in addition to lamioside (**26**).

On the basis of the results of the above experiments we also administered MVA-[2-$^{14}$C] to *Galium spurium* var. *echinospermon* [20] which contains asperuloside (**22**), a C-11 lactone carbonyl-bearing glucoside.

## RESULTS AND DISCUSSION

We first examined the occurrence of the known iridoid glucosides [16–19] in domestic *Lamium* and *Deutzia* plants. It was revealed that *L. amplexicaule* contains, besides the three known glucosides **20**, **21** and **26**, a small amount of deacetylasperulosidic acid (**13**) [21] which has not been detected previously in this plant, but not lamiol (**25**) as reported in the literature [16]. Although the occurrence of both deutzioside (**23**) and scabroside (**24**) in *D. crenata* was confirmed, we noticed that both glucosides although found in the leaves and stems in spring were mostly localized in the stems in autumn with a remarkable decrease of the glucoside content in the leaves.

DL-MVA-[2-$^{14}$C] was administered to *L. amplexicaule*, from which ipolamiide (**20**), lamiide (**21**) and lamioside (**26**) were isolated and purified in the form of their acetates. The acetates of lamioside (**26**) and lamiide (**21**) after deacetylation, were submitted to Kuhn–Roth oxidation. The resulting acetic acid which originated from C-4, C-8, C-10 and C-11 of compound **26** and from C-8 and C-10 of compound **21** was converted to α-naphthylamide. The deacetylation product lamiol (**25**) obtained from the pentaacetate of **26**, was subjected to ozonolysis [2] yielding formic acid originating from C-3. This acid was also converted to the α-naphthylamide. On the other hand, the tetraacetate of ipolamiide (**20**) and the pentaacetate of lamiide (**21**) were subjected to alkali hydrolysis followed by pyrolysis [2] and the resulting $CO_2$ derived from C-11 was collected as $BaCO_3$.

The incorporation ratios of labelled MVA into these glucosides and the radioactivities in the resulting acetic acid, formic acid and $CO_2$, i.e., the distribution of the radioactivity between C-7 and C-10 as well as C-3 and C-11, are shown in Table 1. A significant amount of radioactivity was detected only in formic acid, but a negligible amount was found in acetic acid and $CO_2$ demonstrating that the incorporation of the labelling from MVA-[2-$^{14}$C] into these glucosides is restricted to C-3 and C-7, that is, randomization of the labelling between C-3 and C-11 as well as C-7 and C-10 does not occur.

On administration of DL-MVA-[2-$^{14}$C] to *D. crenata*, radioactive deutzioside (**23**) and scabroside (**24**) were isolated. The former was purified in the form of the pentaacetate and the latter as such. The acetate of **23** was

Table 1. Results of administration of MVA-[2-$^{14}$C]* to *Lamium amplexicaule* and *Deutzia crenata*

| | Sp. act. dpm/mM (amount mg)† | Incorp. (%) | Radioactivity (% of total) in | | |
|---|---|---|---|---|---|
| | | | HOAc | $CO_2$ from C-11 | $HCO_2H$ from C-3 |
| Lamioside (**26**) | $3.20 \times 10^5$ (42.43) | 0.004 | 3.1 (from C-4, C-11 and C-8, C-10) | — | 40.3 |
| Ipolamiide (**20**) | $7.72 \times 10^7$ (8.05) | 0.024 | — | 0.01 | — |
| Lamiide (**21**) | $4.80 \times 10^5$ (44.18) | 0.006 | 2.4 (from C-8, C-10) | 0.3 | — |
| Deutzioside (**23**) | $2.51 \times 10^5$ (174.16) | 0.026 | 1.8 (from C-4, C-11) | — | 41.4 |
| Scabroside (**24**) | $1.89 \times 10^5$ (106.00) | 0.017 | — | — | — |

*MVA (0.5 mCi, sp. act. 5.85 mCi/mM) was administered to *Lamium* plants and MVA (0.3 mCi, Sp. act. 6.33 mCi/mM) to *Deutzia* plants.

†As the glucosides **20**, **21**, **23** and **26** were purified in the form of acetate, the values for them correspond to their acetates, while that for **24** to the free glucoside.

subjected to Zemplén reaction and a portion of the resulting deutzioside (**23**) was submitted to Kuhn–Roth oxidation giving acetic acid, while another portion was subjected to ozonolysis [2] yielding formic acid. The results for both glucosides **23** and **24** are shown in Table 1 and demonstrate additional examples of the incorporation of MVA-[2-$^{14}$C] with retention of the radioactivity only at C-3 and C-7.

These findings suggest that the cyclization retains the non-equivalency of the carbons corresponding to C-3 and C-11 of the iridoids, namely, with retention of the methyl group corresponding to C-9 of geraniol. Therefore, we administered 7-deoxyloganic acid (**18**) and the corresponding 11-alcohol (**17**) to the *Lamium* plant in order to examine the incorporation of these substances into lamioside (**26**) and lamiide (**21**) which was a test of the assumed oxidation process at the 11-position (Me→ $CH_2OH$→COOH) in the course of the biosynthesis of these glucosides.

The labelled precursor, 7-deoxyloganic acid-[10-$^3$H] (**18**), was prepared by the catalytic reduction of asperuloside (**22**) over Pd/C in an atmosphere of $^3H_2$ [22], while 11-hydroxyiridodial glucoside-[10-3H] (**17**) was prepared by $LiAlH_4$ reduction of the tertraacetate of 7-deoxyloganin-[10-$^3$H] (**19**) [23] at low temperature.

* This substance was assumed in accordance with the proposal by Arigoni *et al.* [3]. Further administration experiments of several acyclic monoterpenes are required for final establishment of the precursor role of this substance.

The results of the administration experiments (Table 2) show that these labelled compounds were incorporated into lamiide (**21**), but not into lamioside (**26**). These findings suggest that after formation of the methylcyclopentane ring in the biosynthetic pathway of the iridoid glucosides oxidation proceeds at the 11 position, while the reverse reductive process does not exist.

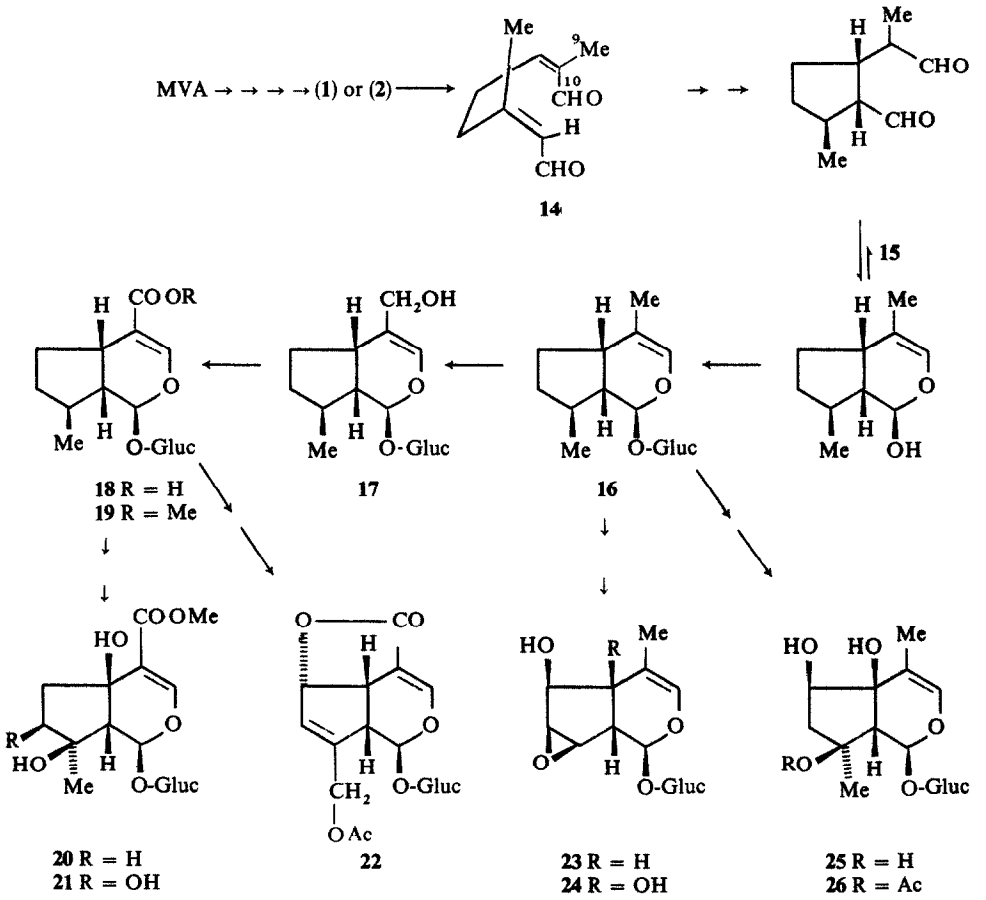
We thus assumed for the biosynthesis of the iridoid glucosides of these plants a pathway including cyclization of an acyclic monoterpene, possibly 10-oxoneral (**14**)*, to iridodial (**15**) followed by glucosidation, which is different from the so far established biosynthetic route to loganin **3**, secoiridoids and indole alkaloids. According to this assumption, the above results could be explained (Scheme 2) by a route passing through key intermediates iridodial (**15**) and its glucoside (**16**), successive modifications of the cyclopentane ring of the latter without accompanying oxidation of the C-11 methyl group leading to lamiol (**25**), lamioside (**26**), deutzioside (**23**) and scabroside (**24**) and those accompanying oxidation of the C-11 methyl group leading to ipolamiide (**20**) and lamiide (**21**).

The inference that iridane skeleton formation of the C-11 carbomethoxy-bearing iridoid glucosides such as ipolamiide (**20**) and lamiide (**21**) proceeds via the same cyclization process as for the coexisting C-11 methyl-bearing glucosides lamioside (**26**) etc. prompted us to administer DL-MVA-[2-$^{14}$C] to *G. spurium* var. *echinospermon* which contains asperuloside (**22**) bearing a C-11 lactone carbonyl group. Radioactive asperuloside (**22**) was isolated, purified as its tetraacetate and subjected to

Table 2. Results of administration of labelled compounds to *L. amplexicaule*

| | Sp. act. (mCi/mM) (total activity, mCi) of glucosides fed | Sp. act. (dpm/mM) of lamiide (**21**)* (incorp. %) | Sp. act. of lamioside (**26**)* |
|---|---|---|---|
| 11-Hydroxyiridodial glucoside-[10-$^3$H] (**17**) | 1.92 (0.040) | $2.07 \times 10^5$ (0.0037) | 0 |
| 7-Deoxyloganic acid-[10-$^3$H] (**18**) | 1.92 (0.080) | $1.30 \times 10^6$ (0.025) | 0 |

*Both glucosides **21** and **26** were purified as their acetates.




Scheme 2. Biosynthetic pathway of some iridoid glucosides.

catalytic hydrogenation over Pd/C. The product after deacetylation gave 7-deoxyloganic acid (**18**), a portion of which was then methylated to 7-deoxyloganin (**19**). Both glucosides **18** and **19** were submitted to decarboxylation. Glucoside **18** was subjected to pyrolysis in a $N_2$ stream [2], while **19** was refluxed with 6N HCl under $N_2$ [24]. Furthermore, **18** was subjected to Kuhn–Roth oxidation yielding acetic acid and **19** was subjected to ozonolysis yielding formic acid. The results of these experiments (Table 3) demonstrate the incorporation of MVA-[2-$^{14}$C] into asperuloside (**22**). About half of the radioactivity of **22** was recovered in formic acid originating from C-3 and there was a virtual lack of radioactivity in the resulting $CO_2$ from C-11 and acetic acid from C-8 and C-10. These results strongly suggest that asperuloside (**22**) is formed as shown in Scheme 2 via the same cyclization process passing through a key intermediate, iridodial (**15**) as in the case of glucosides described above.

Interesting problems remaining in the biosynthesis of iridoid glucosides are, above all, the elucidation of a more detailed mechanism for the cyclization of an acyclic monoterpene to the iridane skeleton and examination of the possibility that the pathway described above is ubiquitous for glucosides represented by asperuloside (**22**) having a highly oxidized cyclopentane ring. If so, another intriguing problem to be solved is whether or not the

Table 3. Results of administration of MVA-[2-$^{14}$C]* to *Galium spurium* var. *echinospermon*

| | Sp. act. (dpm/mM) (amount mg) | Incorp. (%) | Radioactivity (% of total) in | | |
|---|---|---|---|---|---|
| | | | HOAc from C-8, C-10 | $CO_2$ from C-11 | $HCO_2H$ from C-3 |
| Asperuloside (**22**)† | $3.11 \times 10^5$ (135.58) | 0.029 | — | — | — |
| 7-Deoxyloganic acid (**18**) | $3.15 \times 10^5$ | — | 1.1 | 0.9 | — |
| 7-Deoxyloganin (**19**) | $3.15 \times 10^5$ | — | — | 1.0 | 47.7 |

* MVA (0.3 mCi, sp. act. 7.16 mCi/mM) was administered.
† The glucoside (**22**) was purified as the acetate.

exclusive occurrence* of the iridoid glucosides having a highly oxidized cyclopentane ring or secoiridoid series substances including indole alkaloids in a given genus or type of plants could ultimately be dependent on the above-mentioned difference in the cyclization process.

## EXPERIMENTAL

*General procedures.* Mp's were uncorr. NMR spectra were measured in $CDCl_3$ or $CD_3OD$ with TMS or in $D_2O$ with DSS as internal standards. Si gel G employed for TLC of non-radioactive materials and Si gel $F_{254}$ for that of radioactive ones. Si gel $GF_{254}$ was used for PLC. Spots on TLC were visualized by $I_2$ exposure. Toyo Roshi No. 50 filter paper was used for PC and spots were detected by spraying with a mixture of vanillin (1 g), conc HCl (3 ml) and MeOH (150 ml) followed by heating. Carbon (Wako) and Si gel (Mallinckrodt) were employed for column chromatography. Radioactivity was measured by liquid scintillation counting in toluene (10 ml), PPO (50 mg) and POPOP (3 mg). A scanner was used for monitoring radioactive spots on TLC. Specific activities are expressed as values before dilution.

*Plant material. Lamium amplexicaule* L., *Deutzia crenata* Sieb. et Zucc. and *Galium spurium* L. var. *echinospermon* (Wallr.) Hayek. were collected on the campus of Kyoto University.

*Isolation of deacetylasperulosidic acid* (**13**), *ipolamiide* (**20**), *lamiide* (**21**) *and lamioside* (**26**) *from* Lamium amplexicaule. The whole herbs of *L. amplexicaule* (fr. wt 16 kg) collected in April were extracted with boiling MeOH (20 l. × 3). The combined MeOH extracts were concd *in vacuo* and the residue dissolved in $H_2O$. After being washed with EtOAc, the aq. soln was concd *in vacuo* to about 1.5 l., transferred to a charcoal column and eluted with MeOH – $H_2O$ with increasing MeOH content to give fractions A (3.1 g), B (5.8 g) and C (3.4 g). Fraction A was mainly composed of deacetylasperulosidic acid (**13**), which was identified with an authentic sample through PC [16] (*n*-BuOH–HOAc–$H_2O$ (63:10:27) $R_f$ 0.32) and TLC ($CHCl_3$–MeOH (6:4) $R_f$ 0.18). An aliquot (519 mg) after acetylation ($Ac_2O$–Py), was chromatographed on Si gel with $CHCl_3$ as eluent affording the tetraacetate (112 mg) of asperuloside (**22**), mp 148–150°. $[\alpha]_D^{18}$ −134.7° (EtOH; *c* 1.02). (Found: C, 53.48; H. 5.25. Calc. for $C_{26}H_{30}O_{15}$. C, 53.61; H, 5.19%). This substance was identified with an authentic sample by mmp and comparisons of TLC, IR and NMR spectra. Fraction B afforded ipolamiide (**20**) and lamiide (**21**) and fraction C gave lamioside (**26**). The physical data of these glucosides and their acetates were found to be in agreement with the reported values [16, 19].

*Administration of MVA-*[2-$^{14}$C] *to* Lamium amplexicaule. A soln of DL-MVA-[2-$^{14}$C] dibenzylethylenediamine (DBED) salt (0.5 mCi, sp. act. 5.85 mCi/mM) in $H_2O$ (30 ml) was administered hydroponically to the terrestrial parts of 36 *Lamium* plants in April. After 7 days administration, the plants (fr. wt 90 g) were extracted with MeOH (250 ml × 4) under reflux. The combined MeOH extracts were concd *in vacuo*. The residue was digested with $H_2O$ (100 ml) and the insoluble material filtered off. The filtrate, after concn *in vacuo* to *ca* 15 ml, was applied to a charcoal column (6 g) and eluted successively with $H_2O$, 10, 20, 50 and 70% MeOH. The 50–70% MeOH eluates containing iridoid glucosides were combined and concd *in vacuo*. The residue (255.6 mg) was acetylated and the product was purified by chromatography on Si gel (20 g) with $CHCl_3$–MeOH (99:1) as eluent. On concn *in vacuo*, the first eluate gave the pentaacetate (42.43 mg) of lamioside (**26**) as colourless needles, while the second one afforded an oily residue (101.70 mg) consisting mainly of the tetraacetate of ipolamiide (**20**) and the pentaacetate of lamiide (**21**). This residue was rechromatographed on Si gel (17 g) with $CHCl_3$–MeOH (99:5:0.5) as eluent. The faster eluate furnished the pentaacetate (44.18 mg) of **21** and the slower one the tetraacetate (8.05 mg) of **20**. The pentaacetate (42.43 mg) of **26**, the pentaacetate (44.18) of **21** and the tetraacetate (8.05 mg) of **20** were diluted with the corresponding carriers (122.58 mg, 206.06 mg and 114.94 mg) and recrystallized from EtOH to constant sp. act.

*Deacetylation of the pentaacetate of radioactive lamioside* (**26**) *followed by Kuhn–Roth oxidation and ozonolysis.* The pentaacetate (60.83 mg, sp. act. $3.20 \times 10^5$ dpm/mM) of radioactive lamioside (**26**) was dissolved in 0.4 N methanolic KOH (6 ml) and the mixture allowed to stand for 10 min at room temp. The soln was neutralized with Amberlite IR-120 ($H^+$-form) and concd *in vacuo*. The soln of the residue in MeOH (2 ml) was treated with a small amount of $CH_2N_2$–$Et_2O$ for 3 min† and concd *in vacuo* to give lamiol (**25**) (46.00 mg). This substance was dissolved in 2N aq. $H_2SO_4$ (10 ml) containing $CrO_3$ (5 g) and heated to steam distill the resulting HOAc over a period of 5 hr with dropwise addition of $H_2O$ (total 50 ml). The distillate (*ca* 50 ml) was neutralized with 0.1 N aq. NaOH and the solvent was removed *in vacuo*. To the soln of the resulting residue in $H_2O$ (4 ml) were added *N*-ethyl-*N'*(3-dimethylaminopropyl)-carbodiimide (200 mg) and α-naphthylamine (40 mg). After adjusting the pH to 3 with 1 N aq. HCl, the soln was stirred for 10 min and extracted with $Et_2O$. The $Et_2O$ layer was washed with $H_2O$, dried over $MgSO_4$ and concd *in vacuo*. The residue was recrystallized from $C_6H_6$–petrol and sublimed twice under red. pres. to give α-acetylnaphthylamide as colourless needles. Radioactive lamiol (**25**) (113.64 mg) obtained from the pentaacetate in the same way as above was dissolved in $H_2O$ (20 ml) and a stream of oxygen containing ozone was passed through the soln at 0° for 6 hr. The reaction mixture was left standing overnight and then heated to steam distill the resulting HCOOH in a similar manner as described above. The distillate (*ca* 50 ml) was neutralized with 0.1 N aq. NaOH and concd *in vacuo*. On treatment with α-naphthylamine (20 mg) and *N*-ethyl-*N'*(3-dimethylaminopropyl)carbodiimide (100 mg), the residue gave α-formylnaphthylamide. This substance was chromatographed on Si gel (5 g) and eluted successively with $C_6H_6$, 5 and 10% EtOAc–$C_6H_6$. The 10% EtOAc–$C_6H_6$ eluate was concd *in vacuo* and the residue was purified by recrystallization from $C_6H_6$–petrol followed by repeated sublimations under red. pres. to constant sp. act.

*Deacetylation of the pentaacetate of radioactive lamiide* (**21**) *followed by Kuhn–Roth oxidation.* The pentaacetate (117.95 mg, sp. act. $4.80 \times 10^5$ dpm/mM) of radioactive lamiide (**21**) was hydrolyzed with 0.4 N methanolic KOH in a similar manner as described above. The soln of deacetylation product in MeOH (2 ml) was treated with a small amount of $CH_2N_2$–$Et_2O$ for 3 min†. After concn of the reaction mixture *in vacuo*, the residue (92.00 mg) was subjected to Kuhn–Roth oxidation and the resulting HOAc converted in the usual manner to α-naphthylamide. The latter was purified by repeated sublimations under red. pres. to constant sp. act.

*Hydrolysis of the tetraacetate of radioactive ipolamiide* (**20**) *followed by decarboxylation.* A suspension of the tetraacetate (50.00 mg, sp. act. $7.72 \times 10^7$ dpm/mM) of radioactive ipolamiide (**20**) in 0.5 N aq. MeOH (10 ml) was stirred for 1 hr at room temp. The resulting soln was adjusted to pH 4 with Amberlite IR-120 ($H^+$-form) and concd *in vacuo*. The residue was chromatographed on Si gel (20 g) and eluted successively with $CHCl_3$, 1% MeOH–$CHCl_3$ and MeOH. After concn of the MeOH eluate *in vacuo*, the residue (36.30 mg) was heated at 180–190° for 1 hr under a stream of $N_2$. The $CO_2$ generated was captured in a satd aq. $Ba(OH)_2$ as $BaCO_3$, which was then collected by centrifugation, washed successively with $H_2O$, MeOH and $Et_2O$ and dried over $P_2O_5$ under red. pres., yield 14.50 mg. An aliquot (5.67 mg) of $BaCO_3$ was mixed with conc. $H_2SO_4$ (1 ml) and the liberated $CO_2$ was determined by liquid scintillation counting.

* Although, surprisingly, coexistence of both groups of substances was recognized in the genus *Cornus* and in the species *Galium mollugo* L. (*G. album*), various interpretations may be made about this phenomenon. cf. Jensen, S. R., Nielsen, B. J. and Dahlgren, R. (1975) *Bot. Notiser* **128**, 148. Private communication of Drs Jensen, S. R. and Nielsen, B. J.

† This procedure was employed to remove HOAc in the form of volatile MeOAc. The carbomethoxy group of **21** was retained under this hydrolysis condition.

*Hydrolysis of the pentaacetate of radioactive lamiide* (**21**) *followed by decarboxylation.* The pentaacetate (50.47 mg, sp. act. $4.80 \times 10^5$ dpm/mM) of radioactive lamiide (**21**) was hydrolyzed in the same manner as described above and the resulting syrupy residue (35.10 mg) was decarboxylated by heating at 180–190° for 1 hr under a stream of $N_2$. The resultant $CO_2$, after the same treatment as above, was subjected to liquid scintillation counting.

*Administration of MVA-[2-$^{14}C$] to* Deutzia crenata. A soln of DL-MVA-[2-$^{14}C$] DEBD salt (0.3 mCi, sp. act. 6.33 mCi/mM) in $H_2O$ (40 ml) was administered hydroponically to 9 stems of the *Deutzia* plants (*ca* 70 cm in height with many leaves) in September. After 7 days administration, the plants (fr. wt 103 g) were extracted with MeOH (300 ml × 4) under reflux. The combined MeOH extracts were concd *in vacuo*. The residue was digested with $H_2O$ (100 ml) and the insoluble material filtered off. The filtrate, after concn *in vacuo* to *ca* 15 ml, was transferred to a charcoal column (6 g) and eluted successively with $H_2O$, 10, 20, 50 and 70% MeOH. On concn, the faster eluate and the slower one gave residues, 333 mg and 272 mg, respectively. The former was subjected to chromatography on Si gel (40 g) with $CHCl_3$–MeOH as eluent with increasing MeOH content affording scabroside (**24**) (106 mg) as colourless needles, which were diluted with the carrier (62 mg) and recrystallized from EtOH to constant sp. act. The latter, on washing with EtOH (3 ml × 5), furnished crude deutzioside (**23**) (156 mg). This substance was acetylated and the product was chromatographed on Si gel (30 g) with $CHCl_3$ as eluent, giving the pentaacetate (174 mg) of deutzioside (**23**) as colourless needles, which were purified by recrystallization from MeOH to constant sp. act.

*Deacetylation of the pentaacetate of radioactive deutzioside* (**23**) *followed by Kuhn–Roth oxidation and ozonolysis.* To a soln of the pentaacetate (99.92 mg, sp. act. $2.51 \times 10^5$ dpm/mM) of radioactive deutzioside (**23**) in dry MeOH (5 ml) was added 0.2 N methanolic NaOMe (0.2 ml). After being refluxed for 5 min, the soln was neutralized with Amberlite IR-120 ($H^+$-form) and concd *in vacuo*. The residue (72.00 mg) was subjected to Kuhn–Roth oxidation and the resulting HOAc was converted to α-naphthylamide in the same way as in the case of lamioside (**26**) and lamiide (**21**). The amide was finally purified by repeated sublimations to constant sp. act. On the other hand, the glucoside (**23**) (125.38 mg) regenerated from the pentaacetate in the same way as above was subjected to ozonolysis by passing a stream of $O_2$ containing ozone through its soln in $H_2O$ (20 ml) at 0° for 6 hr in the same manner as in the case of **26**. After standing overnight, the reaction mixture was heated to steam distill the resulting $HCO_2H$, which was then converted to α-naphthylamide. The latter was purified by repeated sublimations to constant sp. act.

*Conversion of the tetraacetate of 7-deoxyloganin-[10-$^3H$]* (**19**) *into 11-hydroxyiridodial glucoside-[10-$^3H$]* (**17**). To a stirred suspension of $LiAlH_4$ (85 mg) in dry THF (15 ml) precooled to −20° was added a soln of the tetraacetate (81 mg) of 7-deoxyloganin-[10-$^3$H] (**19**) in dry THF (5 ml) for 10 min keeping the reaction mixture below −10°. The mixture was stirred at −10° for a further 2 hr and at −10 ~ 3° for 14 hr. The excess reagent was decomposed by addition of EtOAc. The inorganic materials precipitated by the successive addition of satd aq. $Na_2SO_4$ were filtered off. The filtrate was neutralized with Amberlite IR-120 ($H^+$-form) and concd *in vacuo*. The residue was chromatographed on a charcoal column (2 g) eluted with $H_2O$ and then with MeOH. The MeOH eluate, on conc *in vacuo*, yielded a syrupy residue (30 mg), which was subjected to PLC with $CHCl_3$–MeOH (7:3) as eluent. The band around $R_f$ 0.48 was scraped off and extracted with MeOH. After concn of the MeOH extract *in vacuo*, the residue was purified by rechromatography on a charcoal column (1 g) affording 11-hydroxyiridodial glucoside-[10-$^3$H] (**17**) (0.040 mCi, sp. act. 1.92 mCi/mM) as a hygroscopic white powder. The properties of **17** prepared in parallel experiment using unlabelled material are as follows: TLC ($CHCl_3$–MeOH 7:3, $R_f$ 0.48), $[\alpha]_D^{28}$ −106.7° (MeOH, *c* 0.71), IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3350, 2930, 1665, 1370; NMR (60 M Hz, $CD_3OD$): δ 1·07 (3 H, *d*, *J* = 5.6 Hz, 10-$H_3$), 3.81 and 4.10 (each 1 H, *d*, *J* = 11.5 Hz, 11-H), 5.14 (1 H, *d*, *J* = 3.5 Hz, 1-H), 6.23 (1 H, *m*, 3-H). (Found: C, 54.64; H, 7.52. $C_{16}H_{26}O_8$. 1/4 $H_2O$ requires: C, 54.77; H, 7.61%).

*Administration of 11-hydroxyiridodial glucoside-[10-$^3H$]* (**17**) *to* Lamium amplexicaule. A soln of radioactive 11-hydroxyiridodial glucoside-[10-$^3$H] (**17**) (0.040 mCi, sp. act. 1.92 mCi/mM) in $H_2O$ (20 ml) was administered hydroponically to the terrestrial parts of 46 *Lamium* plants in June. After 5 days administration, the plants (fr. wt 30.5 g) were extracted with MeOH (300 ml × 4) under reflux. The combined MeOH extracts were concd *in vacuo*. The residue was digested with $H_2O$ (100 ml) and the insol materials were filtered off. The filtrate, after concn *in vacuo* to *ca* 15 ml, was transferred to a charcoal column (6 g) and eluted with MeOH–$H_2O$ with increasing MeOH content. The $H_2O$–50% MeOH and the successive 50–70% MeOH eluates were combined and concd *in vacuo* to afford residues, 69.30 mg and 64.07 mg, respectively. The former was acetylated and the product was chromatographed on Si gel (17 g) with $CHCl_3$–MeOH (99.5:0.5) as eluent yielding the pentaacetate (10.00 mg) of lamiide (**21**) as colourless needles. The latter was worked up in the same way as above furnishing the pentaacetate (16.03 mg) of lamioside (**26**) as colourless needles. The pentaacetate (10.00 mg) of **21** and that (16.03 mg) of **26** were diluted with the corresponding carriers (6.85 mg and 12.18 mg) and recrystallized from EtOH to constant sp. act.

*Administration of 7-deoxyloganic acid-[10-$^3H$]* (**18**) to Lamium amplexicaule. A soln of 7-deoxyloganic acid-[10-$^3$H] (**18**) (0.080 mCi, sp. act. 1.92 mCi/mM) in $H_2O$ (20 ml) was administered hydroponically to the terrestrial parts of 37 *Lamium* plants in June. Isolation was carried out as described above yielding the pentaacetate (21.08 mg) of lamiide (**21**) and that (12.57 mg) of lamioside (**26**). Each acetate was diluted with the corresponding carrier (12.81 mg and 10.50 mg respectively) and recrystallized from EtOH to constant sp. act.

*Administration of* DL-*MVA-[2-$^{14}C$] to* Galium spurium *var.* echinospermon. A soln of DL-MVA-[2-$^{14}$C] DEBD salt (0.3 mCi, sp. act. 7.16 mCi/mM) in $H_2O$ (15 ml) was administered hydroponically to the terrestrial parts of 30 *Galium* plants in April. After 7 day administration, the plants (fr. wt 16 g) were extracted with MeOH (200 ml × 4) under reflux. The combined MeOH extracts were concd *in vacuo*. The residue was digested with $H_2O$ (50 ml) and the insol materials were filtered off. The filtrate, after concn *in vacuo* to *ca* 10 ml, was applied to a charcoal column (8 g) and eluted with MeOH–$H_2O$ with increasing MeOH content. The 70% MeOH eluate was concd *in vacuo* and the residue (143.61 mg) acetylated. The product was subjected to chromatography on Si gel (15 g) with $Et_2O$ as eluent yielding the tetraacetate (135.58 mg) of asperuloside (**22**) as colourless needles, which were purified by recrystallization from EtOH to constant sp. act.

*Catalytic hydrogenation of the tetraacetate of asperuloside* (**22**). A soln of the tetraacetate (135.58 mg, sp. act. $3.11 \times 10^5$ dpm/mM) of asperuloside (**22**) in MeOH (17 ml) was hydrogenated over Pd-C (prepared from 5% $PdCl_2$ soln (0.5 ml) and charcoal (Darco, 70 mg)). After an uptake of *ca* 3 mol of $H_2$, the catalyst was filtered off and the filtrate was concd *in vacuo*. The residue was recrystallized from EtOH–$H_2O$ to give the tetraacetate (63.10 mg) of 7-deoxyloganic acid (**18**) as colourless needles. After dilution with carrier (177.30 mg) the acetate was recrystallized from EtOH to constant sp. act. and then deacetylated [22] to afford 7-deoxyloganic acid (**18**) (120.61 mg). An aliquot of this substance was methylated in the usual manner yielding 7-deoxyloganin (**19**) (58.05 mg) [23].

*Decarboxylation of 7-deoxyloganic acid* (**18**). 7-Deoxyloganic acid (**18**) (23.56 mg, sp. act. $3.15 \times 10^5$ dpm/mM) was decarboxylated in the same way as for ipolamiide (**20**) and lamiide (**21**) and the radioactivity of the $CO_2$ generated was determined.

*Decarboxylation of 7-deoxyloganin* (**19**). A soln of 7-deoxyloganin (**19**) (5.00 mg, sp. act. $3.15 \times 10^5$ dpm/mM) in 6N aq. HCl (2 ml) was refluxed for 1.5 hr with a bubbling stream of $N_2$ and the radioactivity of the $CO_2$ generated was deter-

*Kuhn–Roth oxidation of 7-deoxyloganic acid* (**18**). 7-Deoxy-

loganic acid (**18**) (38.00 mg, sp. act. $3.15 \times 10^5$ dpm/mM) was subjected to Kuhn–Roth oxidation in the usual manner. The resulting HOAc was converted to α-naphthylamide and its radioactivity was determined.

*Ozonolysis of 7-deoxyloganin* (**19**). Radioactive 7-deoxyloganin (**19**) (53.05 mg, sp. act. $3.15 \times 10^5$ dpm/mM) was submitted to ozonolysis in the usual manner. The resulting $HCO_2H$ was converted to α-naphthylamide and its radioactivity was determined.

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