[Chem. Pharm. Bull. 26(11)3384—3394(1978)]

UDC 547.918.04.057 : 581.192

Studies on Monoterpene Glucosides and Related Natural Products. XXXVII.[1,2] Biosynthesis of the Iridoid Glucosides in *Lamium amplexicaule*, *Deutzia crenata* and *Galium spurium* var. *echinospermon*

Hiroyuki Inouye, Shin-ichi Ueda, Shin-ichi Uesato, and Koji Kobayashi

Faculty of Pharmaceutical Sciences, Kyoto University[3]

(Received April 17, 1978)

Administration of labeled compounds such as [10-^{3}H]-10-hydroxygeraniol (**8**), [10-^{3}H]- and [11-$^3H_2$]-iridodial (**7**), [10-^{3}H]- and [11-$^3H_2$]-iridodial glucoside (**9**) to *Lamium amplexicaule*, *Deutzia crenata* and *Galium spurium* var. *echinospermon* established that lamioside (**1**), deutzioside (**2**), scabroside (**3**), ipolamiide (**4**), lamiide (**5**) and asperuloside (**6**) are biosynthesized in these plants *via* 10-hydroxygeraniol (**8**) and iridodial (**7**).

Keywords——*Lamium amplexicaule*; Labiatae; *Deutzia crenata*; Saxifragaceae; *Galium spurium* var. *echinospermon*; Rubiaceae; biosynthesis; iridoid glucosides; iridodial

In the previous paper,[1] we reported that the carbon atom 2 of [2-^{14}C]-mevalonic acid (MVA) is incorporated into C-3 and C-7, but not into C-11 and C-10 of the glucosides in *Lamium amplexicaule* L. and *Deutzia crenata* Sieb. *et* Zucc. such as lamioside (**1**), deutzioside (**2**) and scabroside (**3**) having an 11-methyl group, and ipolamiide (**4**) and lamiide (**5**) having an 11-carbomethoxy group. We also clarified that only oxidations ($CH_3 \rightarrow CH_2OH \rightarrow COOH$) take place at the 11 position in the biosynthesis of the iridoid glucosides of the *Lamium* plant. These findings suggested that the cyclization of acyclic monoterpene to iridane skeleton would occur with retention of the divergency of the carbons corresponding to C-3 and C-11 of iridoids, that is, without further oxidation of the methyl group corresponding to C-9 of geraniol. The results of the feeding experiment of [2-^{14}C]-MVA to *Galium spurium* L. var. *echinospermon* (Wallr.) Hayek also led us to assume that asperuloside (**6**) having an 11-lactone carbonyl group would be formed in this plant *via* the same cyclization process as described above. Thus, we suggested that the iridoid glucosides of these plants, in contrast to seco-iridoids and indole alkaloids, would be formed *via* iridodial (**7**) which formerly had been postulated as a key intermediate in the biosynthesis of iridoids.

This paper deals with the feeding experiments of 10-hydroxygeraniol (**8**), iridodial (**7**) and its glucoside (**9**) all labeled with tritium to the above-mentioned three plants to clarify the biosynthetic pathway of the iridoid glucosides in further detail. The role of the first substance (**8**) as an intermediate after MVA and geraniol for the biosynthesis of secoiridoids and indole alkaloids has been established by both groups of Arigoni[4] and Battersby.[5]

Prior to the preparation of the labeled compounds required for administration, nonlabeled 10-hydroxygeraniol (**8**), iridodial (**7**) and iridodial glucoside (**9**) were prepared by the route shown in Chart 1 and their various properties were examined in order to establish the labeling method.

1) Part XXXVI: H. Inouye, S. Ueda, and S. Uesato, *Phytochemistry*, **16**, 1689 (1977).
2) A part of this work has been published in a preliminary form: H. Inouye, S. Ueda, and S. Uesato, *Tetrahedron Lett.*, **1977**, 713.
3) Location: *Yoshida-shimoadachi-cho, Sakyo-ku, Kyoto.*
4) S. Escher, P. Loew, and D. Arigoni, *Chem. Commun.*, **1970**, 823.
5) A.R. Battersby, S.H. Brown, and T.G. Payne, *Chem. Commun.*, **1970**, 827.

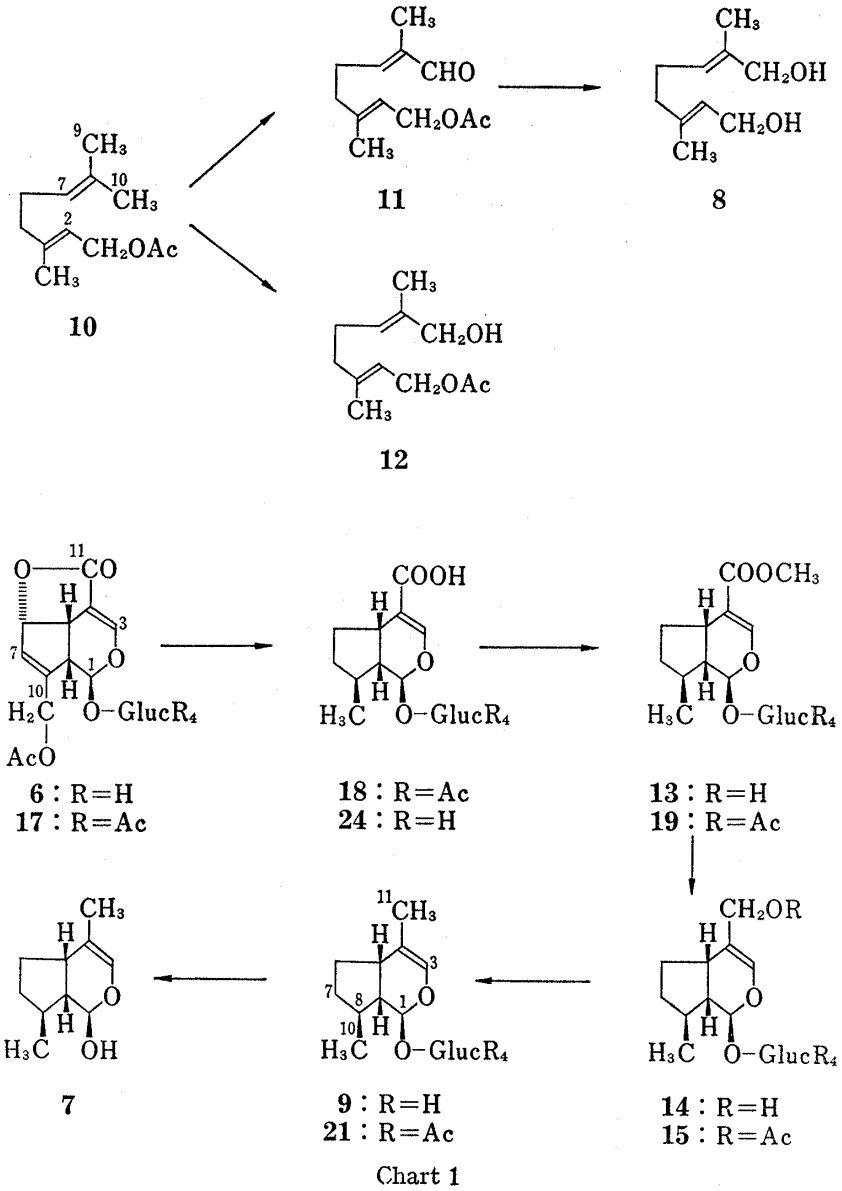


Chart 1

1) 10-Hydroxygeraniol (8)

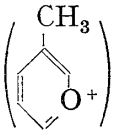
SeO_2 oxidation of geranyl acetate (**10**) gave 10-oxogeranyl acetate (**11**)[6] and 10-hydroxygeranyl acetate (**12**). Reduction of the former compound (**11**) with $LiAlH_4$ in the presence of $AlCl_3$ yielded 10-hydroxygeraniol (**8**) as a colorless syrup, bp 110° (0.3 mmHg), which shows infrared (IR) bands at 3300 (OH), 1660 (C=C), 835 cm^{-1} (trisubstituted C=C) as well as nuclear magnetic resonance (NMR) signals at δ 2.33 (singlet) assignable to two hydroxy groups and at δ 3.96 (singlet) attributable to methylene protons. The configuration of Δ^7-double bond in **8** is assumed to be E as in the case of 10-oxogeranyl acetate (**11**), since it has already been known that α,β-unsaturated aldehyde is reduced with $LiAlH_4$–$AlCl_3$ to allyl alcohol with complete retention of configuration of the double bond.[7] This assumption was confirmed by the NOE experiment. Namely, the intensity of the C-7 vinyl proton signal which was overlapped with the C-2 vinyl proton signal at δ 5.25—5.49 of **8** was increased by 6.6% by irradiation of methylene protons at δ 3.96 indicating unequivocally the NOE between the 10-methylene and the C-7 vinyl proton.

6) J. Meinwald, W.R. Thompson, T. Eisner, and D.F. Owen, *Tetrahedron Lett.*, **1971**, 3485.
7) U.T. Bhalerao, J.J. Plattner, and H. Rapoport, *J. Am. Chem. Soc.*, **92**, 3429 (1970).

2) **Iridodial Glucoside (9)**

Reduction of 7-deoxyloganin (**13**) with $LiAlH_4$ under cooling gave 11-hydroxyiridodial glucoside (**14**), which was converted into the pentaacetate (**15**) in the usual manner. The acetate (**15**) was subjected to hydrogenolysis over Pd-C followed by the Zemplén reaction yielding iridodial glucoside (**9**) as colorless needles, mp 168—169°. This compound (**9**) showed IR bands at 3400 (OH), 1670 (enol ether) and 830 cm^{-1} (trisubstitued C=C). The NMR spectrum of **9** exhibited a doublet (J=5.8 Hz) at δ 1.06 assignable to the 10-methyl group, a doublet (J=1.0 Hz) at δ 1.53 attributable to the vinyl methyl group and a multiplet at δ 5.98 owing to the C-3 proton. These spectral data as well as the above-mentioned preparation procedure clearly indicate that this compound should be represented as the structure (**9**) which has an 11-methyl group instead of an 11-carbomethoxy group in 7-deoxyloganin (**13**).

3) **Iridodial (7)**

Hydrolysis of the glucoside (**9**) with β-glucosidase followed by vacuum distillation gave iridodial (**7**) (designated as "synthetic iridodial" for convenience). It showed IR bands at 3400 (OH), 1665 (enol ether) and 845 cm^{-1} (trisubstituted C=C) in CCl_4. Similar bands were also observed in a film. These data led us to assume that it exists only in the enol-hemiacetal form. On the other hand, it has been reported that iridodial (**7**) isolated from ants[8] (designated as "natural iridodial" for convenience) displayed, besides the above-mentioned IR bands (CCl_4), extra absorptions at 2710 and 1725 cm^{-1} (CHO) suggesting that natural iridodial would exist as an equibrium mixture of the tautomers such as the enol-hemiacetal and the dialdehyde form at least in CCl_4 solution. Regarding the NMR spectrum of natural iridodial, no detailed work except only one report[9] describing the presence of the signal assignable to the aldehyde group has appeared in the literature. NMR spectrum of synthetic iridoidal displayed a doublet (J=6.2 Hz) assignable to the 10-methyl group at δ 1.07 and a multiplet attributable to the C-1 proton at δ 4.82,[10] but any signal characteristic of the aldehyde proton was not observed. The mass spectrum (MS) of natural iridodial[11] showed the parent peak at m/e 168 (M^+) and the following characteristic peaks at 153 (M^+–CH_3), 139 (M^+–CHO) and 111 (M^+–CH_3CHCHO) suggesting the structure of the dialdehyde form, whereas that of synthetic iridodial displayed, besides the above peaks, extra ones at 150 (M^+–H_2O) and 95  implying the enol-hemiacetal structure. These remarkable differences between the physical properties of both natural and synthetic iridodial could be explained by assuming that the former consists mainly of the dialdehyde form, whereas the latter is entirely composed of the enol-hemiacetal form.

dialdehyde form — iridodial (7) — enol-hemiacetal form

Chart 2

8) G.W.K. Cavill and D.L. Ford, *Aust. J. Chem.*, **13**, 296 (1960).
9) S.A. Abou-Donia, L.J. Fish, and G. Pattenden, *Tetrahedron Lett.*, **1971**, 4037.
10) This fact suggests that this iridodial would exist as a mixture of the C-1 epimers. On the other hand, the NMR-spectrum of synthetic iridodial which was not purified by vacuum distillation displayed a doublet (J=5.0 Hz) at δ 4.85, implying that it predominantly consists of the epimer with C-1 (R) configuration. The vacuum distillation seems to cause the epimerization at C-1.
11) G. Vidari, M. De Bernardi, M. Pavan, and L. Ragozzino, *Tetrahedron Lett.*, **1973**, 4065.

Therefore, we attempted to convert synthetic iridodial into the dialdehyde form by refluxing a solution of the former in 50% aqueous HCOOH[12] under N_2 streams to examine whether the product accords with the natural iridodial or not. The IR spectrum (CCl_4) of the conversion product showed bands at 2700 and 1720 cm^{-1} (CHO), but no band characteristic of the enol-hemiacetal form of synthetic iridodial was observed. Its NMR spectrum (CCl_4) displayed signals assignable to the 10- and 11-methyls at δ 0.97—1.22 and signals attributable to two aldehyde groups at δ 9.58—9.87. All these findings suggested that this conversion product almost entirely consists of the dialdehyde form.

Finally, each iridodial of the enol-hemiacetal form and the dialdehyde form thus derived from the former was converted into the bis-2,4-dinitrophenylhydrazone, whose mp and IR data were identical with those of natural iridodial bis-2,4-dinitrophenylhydrazone (**16**).[13]

On the basis of these preliminary experiments, we attempted to prepare the postulated intermediates **7**, **8** and **9** labeled with tritium by the above-mentioned route (*cf.* Chart 1).

1) $LiAl^3H_4$-$AlCl_3$ reduction of 10-oxogeranyl acetate (**11**) gave [10-^{3}H]-10-hydroxygeraniol (**8**).

2) $LiAl^3H_4$ reduction of 7-deoxyloganin (**13**) followed by acetylation gave [11-$^3H_2$]-11-hydroxyiridodial glucoside pentaacetate (**15**), which was worked up as in the case of the cold run to give [11-$^3H_2$]-iridodial glucoside (**9**) and [11-$^3H_2$]-iridodial (**7**).

3) Catalytic hydrogenation of asperuloside tetraacetate (**17**) over Pd-C with $^3H_2$ provided [10-^{3}H]-7-deoxyloganic acid tetraacetate (**18**),[14,15] which was converted into the methyl ester (**19**). Conventional work up of this substance gave [10-^{3}H]-iridodial glucoside (**9**) and [10-^{3}H]-iridodial (**7**).

The labeled compounds thus obtained were administered to the three plants described above, respectively, to examine the incorporation into the glucosides. [10-^{3}H]-10-hydroxygeraniol (**8**), [11-$^3H_2$]-iridodial (**7**) and [11-$^3H_2$]-iridodial glucoside (**9**) were administered to *D. crenata*, respectively and radioactive deutzioside (**2**) obtained was purified as its acetate. Deutzioside pentaacetate (**20**) was subjected to ths Zemplén reaction followed by the Kuhn–Roth oxidation and the resulting acetic acid was converted into the α-naphthylamide. The

Table I. Administration Experiments of Labeled Compounds to *Deutzia crenata*

Spec. act. (mCi/mM) and (amount (mg)) of substance fed	Spec. act. (dpm/mM) and (amount (mg)) of isolated deutzioside pentaacetate (**20**)	Incorporation (spec. incorp.) %	Radioactivity (% of total) in CH_3CO_2H from C-4 and C-11	Radioactivity (% of total) in HCO_2H from C-3
[10-^{3}H]-10-Hydroxygeraniol (**8**) 2.74 (32.14)	3.67×10^6 (205.25)	0.045[a] (0.061)	0.1	109
[11-$^3H_2$]-Iridodial (**7**) 0.85 (19.30)	8.32×10^6 (108.36)	0.76 (0.44)	81.9	—
[11-$^3H_2$]-Iridodial glucoside (**9**) 0.85 (41.03)	1.20×10^7 (117.28)	1.09 (0.64)	87.1	—

a) This value is based on the assumption that only half of the tritium labeling of **8** is incorporated into deutzioside (**2**).

12) R.M. Bowman and E. Leete, *Phytochemistry*, **8**, 1003 (1969).
13) K.J. Clark, G.I. Fray, R.H. Jaeger, and R. Robinson, *Tetrahedron*, **6**, 217 (1959).
14) H. Inouye, S. Ueda, Y. Aoki, and Y. Takeda, *Chem. Pharm. Bull.* (Tokyo), **20**, 1287 (1972).
15) Acetic acid obtained by the Kuhn–Roth oxidation of 7-deoxyloganic acid (**24**) derived from **18** was found to contain only 56% of the radioactivity of the original glucoside (**24**), whereas 90% of the radioactivity was found at the 10 position in the previous experiment.[14] Taking into consideration the mechanism of hydrogenolysis, it can be deduced that the residual radioactivity of **24** amounting to 44% is covered by tritium on C-6, C-7 and C-8. This dispersion of labeling occurred probably by an uptake of more than 1 mol equivalent of $^3H_2$. Strictly speaking, the $^3H_2$ labeled **18** mentioned here and all of its derivatives should not be designated as [10-^{3}H]-labeled compound. However, they were provisionally named [10-^{3}H]-7-deoxyloganic acid tetraacetate (**18**) *etc.*

results of these administration experiments shown in Table I clearly indicated the specific incorporation of the labeled compounds into deutzioside (**2**). In particular, chemical degradation of **2** demonstrated the incorporations of the carbon atom 10 of 10-hydroxygeraniol (**8**) into C-3 of deutzioside (**2**) without randomization and of the 11-methyl group of iridodial (**7**) or iridodial glucoside (**9**) into 11-methyl group of **2**. On the feeding experiment of [10-^{3}H]-10-hydroxygeraniol (**8**), we also attempted to detect iridodial glucoside (**9**) by a trapping experiment in order to verify the intermediacy of **9** in the biosynthesis of duetzioside (**2**). Namely, the carrier glucoside (**9**) was added to an extract of the *Deutzia* plant to which the labeled compound was administered and iridodial glucoside (**9**) isolated was converetd into the tetraacetate (**21**), which had the radioactivity of 2.20×10^5 dpm/mM corresponding to 0.00062% incorporation. Accordingly, it was shown that deutzioside (**2**) is formed *via* 10-hydroxygeraniol (**8**), iridodial (**7**) and its glucoside (**9**) as indicated in Chart 3.

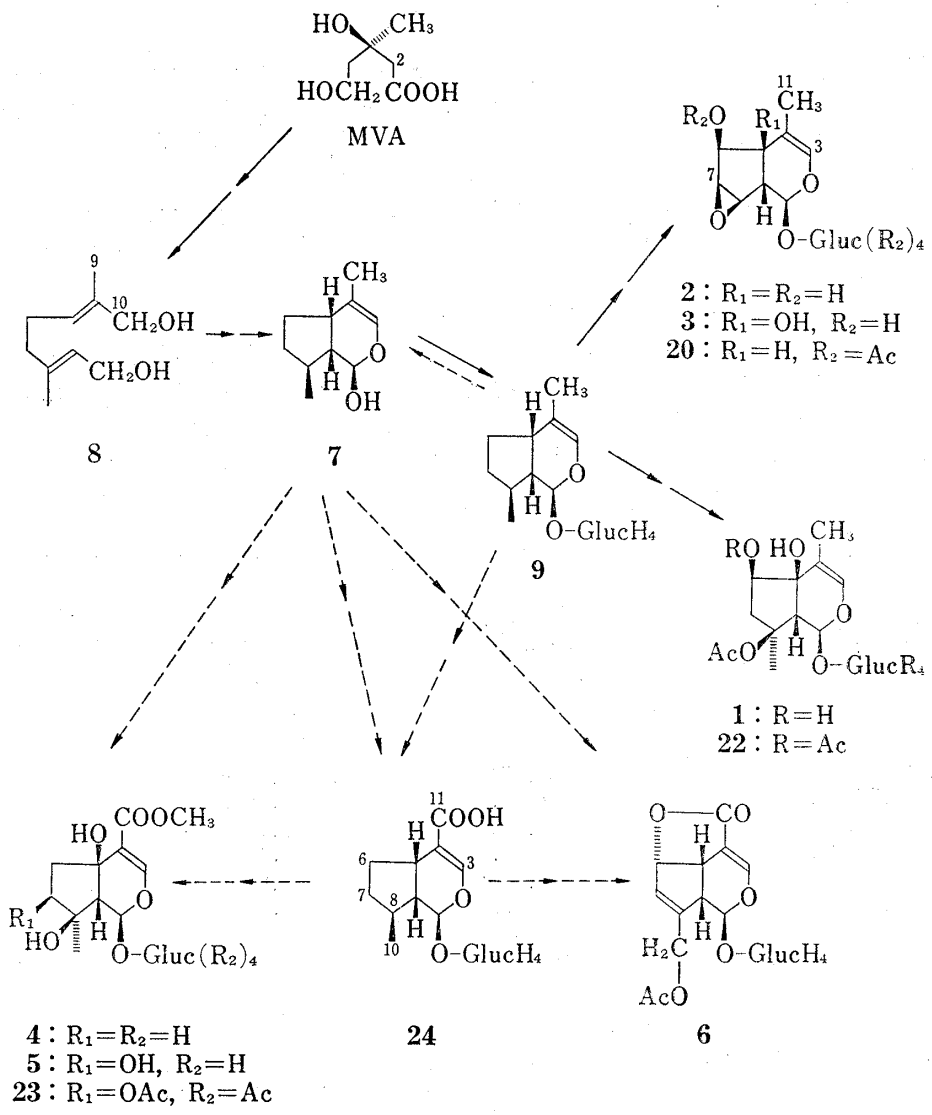


Chart 3

On administration of [10-^{3}H]-iridodial (**7**) and [10-^{3}H]-iridodial glucoside (**9**) to *L. amplexicaule* and *G. spurium* var. *echinospermon*, lamioside (**1**) and lamiide (**5**) were isolated from the former plant and asperuloside (**6**) from the latter. These glucosides **1**, **5** and **6** were purified in the form of their acetates **22**, **23** and **17**, respectively. Asperuloside tetraacetate (**17**) was

subjected to catalytic hydrogenation over Pd-C followed by alkali hydrolysis to give 7-deoxyloganic acid (**24**), which was subjected to the Kuhn–Roth oxidation and the resulting acetic acid was converted into the α-naphthylamide. The results of the administration experiments shown in Table II indicate the incorporation of iridodial (**7**) and iridodial glucoside (**9**) into these glucosides. However, it is noteworthy that the specific incorporations of [10-^{3}H]-iridodial glucoside (**9**) into the 11-carbonyl-bearing glucosides such as lamiide (**5**) and asperuloside (**6**) were lower as compared to those of [10-^{3}H]-iridodial into both glucosides and this tendency is especially remarkable in the case of **6**. Thus, we are dubious to conclude hastily that iridodial glucoside (**9**) is a biosynthetic intermediate of lamiide (**5**) and asperuloside (**6**). For example, the present experimental results do not exclude the possibility that iridodial (**7**) of the enol-hemiacetal-type which is formed from acyclic monoterpene is oxidized at the 11 position prior to the glucosylation at the 1 position, since this process could also be compatible with the fact that the carbons at C-3 and C-11 of the glucosides formed subsequently are not equivalent. Anyway, it is indisputable from our point of view that the iridoid glucosides of *L. amplexicaule* and *G. spurium* var. *echinospermon* are also formed *via* iridodial (**7**) as shown in Chart 3, where the pathways which are still questionable such as described above are shown in dotted lines.

Table II. Administration Experiments of Labeled Compounds to *Lamium amplexicaule* and *Galium spurium* var. *echinospermon*

Spec. act. (mCi/mM) and (amount(mg)) of substance administered	Spec. act. (dpm/mM) and (amount (mg)) of glucoside acetate	Incorporation (spec. incorp.) %
[10-^{3}H]-Iridodial (**7**) 3.77 (6.25)	Lamioside pentaacetate (**22**) 4.18×10^5 (9.82)	0.0021[a] (0.0050)
	Lamiide pentaacetate (**23**) 1.68×10^6 (10.41)	0.0078[a] (0.020)
[10-^{3}H]-Iridodial glucoside (**9**) 3.77(14.06)	Lamioside pentaacetate (**22**) 9.68×10^5 (17.57)	0.0094[a] (0.012)
	Lamiide pentaacetate (**23**) 2.78×10^5 (18.79)	0.0029[a] (0.0034)
[10-^{3}H]-Iridodial (**7**) 3.77 (8.03)	Asperuloside tetraacetate (**17**)[b] 1.12×10^7(333.36)	1.6[a] (0.14)
[10-^{3}H]-Iridodial glucoside (**9**)[c] 0.83(46.59)	Asperuloside tetraacetate (**17**) 1.44×10^5(180.72)	0.0026 (0.0079)

a) Incorporation and specific incorporation were calculated without taking into account a tritium-loss owing to the elimination of tritiums from C-6, C-7, C-8 and/or C-10, which would take place during the biosynthesis of **1**, **5** and **6** *via* **7** (See footnote 14). Accordingly, the actual values should be higher than these.
b) Degradation of this acetate (**17**) in the conventional method proved that the acetic acid originated from C-8 and C-10 of the glucoside contained 80% of the radioactivity of the original acetate (**17**).
c) Compound (**9**) having the tritium labeling only at the C-10 position was prepared by using 10-oxogeniposide tetraacetate as a starting material.[d] The incorporation and specific incorporation are uncorrected values.
d) H. Inouye, S. Uesato and K. Kobayashi, unpublished data, which will be reported elsewhere.

Though iridodial (**7**) was excluded from the member of the biosynthetic intermediate for secoiridoid glucosides and indole alkaloids,[12] the substance was confirmed from the results of this experiment to be a precursor of at least some iridoid glucosides.

Important subject matters remain unsolved to elucidate the stage at which the above-mentioned glucosylation actually takes place and to examine if the above-described biosynthetic route passing through iridodial (**7**) as a key intermediate is fairly common throughout the dicotyledons. Another interesting problem requiring further examination is the elucidation of the chemotaxonomic relationship between the plants having a biosynthetic pathway characterized by the iridane skeleton formation accompanying the randomization of the

carbons corresponding to C-3 and C-11 as was observed in the biosynthesis of secoiridoid glucosides as well as indole alkaloids and the plants in which iridoids are formed without the above-mentioned randomization.

Experimental[16]

SeO_2 Oxidation of Geranyl Acetate (10)——SeO_2 (1.1 g) freshly purified by sublimation was added to a solution of geranyl acetate (10) (1.0 g) in EtOH (20 ml) and the mixture was refluxed for 1 hr. After being cooled, the resulting black precipitate of Se was filtered off and washed with EtOH. The combined filtrate and washings were diluted with ice-water (200 ml) and extracted with ether. After washing with H_2O, the ether extract was concentrated *in vacuo*. The resulting reddish yellow syrup was chromatographed on silica gel (50 g) eluted successively with benzene–AcOEt (95: 5) (fractions Nos. 1—30) and (90: 10) (fr. Nos. 31—53) collecting 30 ml fractions. Fr. Nos. 9—15 and 35—44 were combined and concentrated *in vacuo*, respectively. The residue (286 mg) from the former fractions was subjected to vacuum distillation giving 10-oxogeranyl acetate (11) (163 mg) as a colorless syrup, bp 136° (1.6 mmHg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2910, 2900, 2850, 1718, 1670, 1420, 1360, 1220; NMR ($CDCl_3$) δ: 1.75 (m, 4-H_3, 9-H_3), 2.05 (s, $-OCOCH_3$), 2.16—1.75 (m, 5-H, 6-H), 4.60 (d, J=7.0 Hz, 1-H), 5.22—5.58 (m, 2-H), 6.32—6.35 (m, 7-H), 9.47 (s, -CHO). *Anal.* Calcd. for $C_{12}H_{18}O_3$: C, 68.25; H, 8.72. Found: C, 68.54; H, 8.63.

The residue (191 mg) from the latter fractions was subjected to preparative TLC with $CHCl_3$–MeOH (9: 1) as eluent and the band around *Rf* 0.30 was scraped off and extracted with MeOH. After concentration of the MeOH extract *in vacuo*, the residue was extracted with $CHCl_3$ and the extract was washed with H_2O, dried over MgSO and concentrated *in vacuo*. The resulting pale yellow syrup (151 mg) was further purified by vacuum distillation giving 10-hydroxygeranyl acetate (12) (91 mg) as a colorless syrup, bp 115° (1.0 mmHg). IR ν_{max}^{film} cm^{-1}: 3400, 2930, 1735, 1370, 1235, 1020, 840; NMR ($CDCl_3$) δ: 1.68 (m, 4-H_3, 9-H_3), 2.05 (s, $-OCOCH_3$), 3.98 (broad s, 10-H_2), 4.85 (d, J=7.0 Hz, 1-H), 5.17—5.52 (m, 2-H, 7-H). *Anal.* Calcd. for $C_{12}H_{20}O_3$: C, 67.89; H, 9.50. Found: C, 67.30; H, 9.46.

Conversion of 10-Oxogeranyl Acetate (11) into 10-Hydroxygeraniol (8)——A solution of 10-oxogeranyl acetate (11) (150 mg) in anhyd. ether (10 ml) was added to a stirred slurry of AlH_3 (prepared by adding anhyd. $AlCl_3$ (72.9 mg) to a suspension of $LiAlH_4$ (61.5 mg) in anhyd. ether (30 ml)) over a period of 10 min keeping the reaction mixture below −10°. The mixture was stirred further at −10° for 20 min and at 0° for 1.5 hr. The excess reagent was decomposed by the addition of H_2O. The inorganic materials precipitated were filtered off through a celite layer. The precipitate and the celite layer were washed with ether. The combined filtrate and washings were concentrated *in vacuo*. The residue was subjected to preparative TLC (2 plates) with $CHCl_3$–MeOH (9: 1) as eluent. The band around *Rf* 0.18 was scraped off and extracted with MeOH. After concentration of the MeOH extract, the residue (115 mg) was further purified by vacuum distillation yielding 10-hydroxygeraniol (8) (80 mg) as a colorless syrup, bp 110° (0.3 mmHg). IR ν_{max}^{film} cm^{-1}: 3300, 2900, 1660, 1435, 1380, 1000, 835; NMR ($CDCl_3$) δ: 1.64 (m, 4-H_3, 9-H_3), 1.93—2.33 (m, 5-H, 6-H), 3.96 (s, 10-H_2), 4.12 (d, J=7.0 Hz, 1-H_2), 5.25—5.49 (m, 2-H, 7-H). *Anal.* Calcd. for $C_{10}H_{18}O_2$: C, 70.55; H, 10.66. Found: C, 70.65; H, 10.46.

Conversion of 7-Deoxyloganin (13) into 11-Hydroxyiridodial Glucoside Pentaacetate (15)——To a stirred suspension of $LiAlH_4$ (1.8 g) in anhyd. THF (300 ml) precooled below −20° was added a solution of 7-deoxyloganin (13) (2.0 g) in anhyd. THF (120 ml) over a period of 30 min keeping the reaction mixture below −15°. The mixture was stirred further at −15° for 2 hr and at −15—0° for 14 hr. The excess reagent was decomposed by the addition of AcOEt. The inorganic materials precipitated by the successive addition of saturated aq. Na_2SO_4 were removed by filtration through a celite layer. The precipitate and the celite layer were washed with EtOH. The combined filtrate and washings were neutralized with Amberlite IR-120 (H^+-form) and the resin was filtered off. After concentration of the filtrate, the residue was chromatographed

16) All melting points are uncorrected. Optical rotations were measured with a Rex photoelectric polarimeter NEP-2 or a Hitachi automatic digital polarimeter PM-201. IR spectra were recorded on a Hitachi Model 215 grating infrared spectrophotometer and NMR spectra on a Varian A-60 spectrometer in D_2O with DSS and in other solvents with TMS as the internal standards. MS were recorded on a Hitachi RMU 6D spectrometer. Silica gel G acc. to Stahl (Merck) was employed for TLC of non-radioactive materials and silica gel F_{254} (Merck) for that of radioactive ones. Spots were visualized by exposure to I_2 vapor or spraying with a mixture of anisaldehyde (0.5 ml), conc. H_2SO_4 (0.5 ml), AcOH (a few drops) and 95% EtOH (9 ml) followed by heating. Silica gel G F_{254} (Merck) (20×20 cm, 0.5 mm thick) was used for preparative TLC and bands were visualized by I_2 exposure. Active charcoal (Wako) and silica gel (Mallinckrodt) were employed for column chromatography. Radioactivity was measured in a Beckman liquid scintillation counter, Model LS-233 or LS-230, with samples dissolved in a scintillation mixture consisting of toluene (10 ml), 2,5-diphenyloxazole (PPO) (40 mg) and 2,2′-*p*-phenylenebis(5-phenyloxazole) (POPOP) (0.5 mg). Specific activities are expressed as values before dilution.

on a charcoal column (8 g) eluting first with H_2O (400 ml) to remove inorganic materials and then with MeOH (500 ml). The MeOH eluate was concentrated *in vacuo* to furnish a syrupy product (1.3 g), which was acetylated with 10 ml each of Ac_2O and pyridine in the usual manner. The reaction product was recrystallized from EtOH to give **15** (644 mg) as colorless needles, mp 116.5—117°. $[\alpha]_D^{26}$ −119.6° (c=0.46, MeOH). IR ν_{max}^{KBr} cm^{-1}: 2950, 2860, 1730, 1665, 1375, 1240, 835; NMR ($CDCl_3$) δ: 1.05 (d, J=5.5 Hz, 10-H_3), 1.95—2.12 (5×-$OCOCH_3$), 6.25 (m, 3-H). *Anal.* Calcd. for $C_{26}H_{36}O_{13}$: C, 56.11; H, 6.52. Found: C, 56.35; H, 6.79.

Preparation of Iridodial Glucoside Tetraacetate (21) by Catalytic Hydrogenation of 11-Hydroxyiridodial Glucoside Pentaacetate (15)——A solution of **15** (205 mg) in MeOH (6 ml) was hydrogenated over Pd-C (prepared from 5% $PdCl_2$–HCl solution (0.2 ml) and activated charcoal (Darco G-60, 100 mg)). After an uptake of about 1 mol equivalent of H_2, the catalyst was filtered off and washed with $CHCl_3$. The combined filtrate and washings were concentrated *in vacuo*. The residue was recrystallized from EtOH to give **21** (120 mg) as colorless needles, mp 142—144.5°. $[\alpha]_D^{20}$ −177,3° (c=0.52, MeOH). IR ν_{max}^{KBr} cm^{-1}: 2950, 1755, 1670, 1370, 1230, 1050, 900, 850; NMR ($CDCl_3$) δ: 1.02 (d, J=5.0 Hz, 10-H_3), 1.48 (m, 11-H_3), 1.95—2.12 (4×-$OCOCH_3$), 5.87 (m, 3-H). *Anal.* Calcd. for $C_{24}H_{34}O_{11}$: C, 57.82; H, 6.87. Found: C, 57.66; H, 7.09.

Zemplén Reaction of Iridodial Glucoside Tetraacetate (21)——Anhyd. methanolic 0.26 N $NaOCH_3$ (0.1 ml) was added to a solution of **21** (99.3 mg) in anhyd. MeOH (5 ml). After refluxing for 5 min, the solution was cooled and neutralized with Amberlite IR-120 (H^+—form) and the resin was filtered off. Concentration of the filtrate *in vacuo* gave colorless needles (72.0 mg), which were recrystallized from acetone to furnish iridodial glucoside (**9**) (43.5 mg) as colorless needles, mp 168—169°. $[\alpha]_D^{25}$ −110.3° (c=0.46, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 2940, 1670, 1080, 1020, 900, 830; NMR ($CDCl_3$) δ: 1.06 (d, J=5.8 Hz, 10-H_3), 1.53 (d, J=1.0 Hz, 11-H_3), 5.06 (d, J=4.2 Hz, 1-H), 5.98 (m, 3-H). *Anal.* Calcd. for $C_{16}H_{26}O_7$: C, 58.17; H, 7.93. Found: C, 57.98; H, 8.10.

Enzymic Hydrolysis of Iridodial Glucoside (9)——β-Glucosidase (emulsin prepared from sweet almond, 33 mg) was added to a solution of **9** (470 mg) in acetate buffer (0.1 M, pH 4.8, 66 ml) and the mixture was allowed to stand at 33° for 15 hr. The reaction mixture was extracted with ether and the combined ether extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by vacuum distillation to give iridodial (**7**) (128 mg) as a colorless syrup, bp 87° (1.0 mmHg). $[\alpha]_D^{20}$ −47.7° (c=0.39, MeOH). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3400, 3050, 1665, 845; NMR ($CDCl_3$) δ: 1.07 (d, J=6.2 Hz, 10-H_3), 1.54 (d, J=1.0 Hz, 11-H_3), 3.29 (m, 1-OH), 4.83 (m, 1-H), 6.00 (m, 3-H). MS *m/e*: 168 (M^+), 153, 150, 139, 111, 95. *Anal.* Calcd. for $C_{10}H_{16}O_2$: C, 71.39; H, 9.59. Found: C, 71.69; H, 9.57.

Preparation of Bis-2,4-dinitrophenylhydrazone (16) of Iridodial (7)——Iridodial of the enol-hemiacetal form (**7**) (58 mg) was dissolved in the 2,4-dinitrophenylhydrazine–H_2SO_4 reagent (prepared by dropwise addition of a solution of 2,4-dinitrophenylhydrazine. HCl (1 g) in conc. H_2SO_4 (5.0 ml) to a mixture of H_2O (6.5 ml) and 95% EtOH (23.0 ml)) and the mixture was allowed to stand at room temperature for 45 min. The reddish yellow precipitate which gradually appeared was filtered, washed successively with 2 N aq. H_2SO_4 and H_2O and then submitted to preparative TLC (3 plates, 2 developments) with benzene–AcOEt (10: 1) as eluent. Of the two major yellow bands, the more polar one was scraped off and extracted with $CHCl_3$–MeOH (9: 1) (200 ml). After concentration of the extract, the residue was recrystallized from benzene–AcOEt to afford the bis-2,4-dinitrophenylhydrazone (**16**) (13 mg) as yellow needles, mp 230°. IR ν_{max}^{Nujol} cm^{-1}: 3270, 1615, 1590, 1510, 1330; *Anal.* Calcd. for $C_{22}H_{24}N_8O_8$: C, 50.00; H, 4.58; N, 21.20. Found: C, 50.22; H, 4.66; N, 21.27. This substance was found to be identical with the bis-2,4-dinitrophenylhydrazone of natural iridodial in mp and IR.

Treatment of Iridodial (7) with 50% aq. HCOOH followed by the Conversion of the Product into Its Bis-2,4-dinitrophenylhydrazone (16)——A solution of iridodial of the enol-hemiacetal form (**7**) (70 mg) in 50% aq. HCOOH (2 ml) was refluxed for 50 min under N_2 stream. After being cooled, the mixture was poured into saturated aq. NaCl and extracted with ether. The extract was washed successively with 5% aq. $NaHCO_3$ and saturated aq. NaCl, dried over $MgSO_4$ and concentrated *in vacuo*. The vacuum distillation of the residue gave iridodial of the dialdehyde form (**7**) (35 mg) as a colorless oil, bp 84° (1.0 mmHg). $[\alpha]_D^{17}$ +44.5° (c=1.37, MeOH). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3400, 2700, 1720; NMR ($CDCl_3$) δ: 0.97—1.22 (m, 10-H_3, 11-H_3), 9.58—9.87 (m, 2×-CHO).

On treatment of an aliquot (17 mg) of this iridodial (**7**) with the bis-2,4-dinitrophenylhydrazine–H_2SO_4 reagent, yellowish orange precipitate was formed immediately in contrast to the case of the iridodial of the enol-hemiacetal form. The product was worked up in the same way as mentioned above yielding yellow needles (3.8 mg), mp 232°, which were identical with the bis-2,4-dinitrophenylhydrazone of iridodial (**7**) of the enol-hemiacetal form in mp and IR.

Preparation of [10-^{3}H]-10-Hydroxygeraniol (8)——A solution of 10-oxogeranyl acetate (**11**) (150 mg) in anhyd. ether (10 ml) was added to a stirred slurry of Al^3H_3 (prepared by the addition of anhyd. $AlCl_3$ (72.9 mg) to a suspension of $LiAl^3H_4$ (8.6 mg) and carrier $LiAlH_4$ (61.5 mg) in anhyd. ether (30 ml) over a period of 30 min keeping the mixture below −10°. After being stirred further at −10° for 20 min and at 0° for 1.5 hr, the mixture was worked up in the same manner as in the case of the cold run to give [10-^{3}H]-10-hydroxygeraniol (**8**) (96.4 mg) (spec. activity 14.50 mCi/mM) as a colorless syrup.

Conversion of 7-Deoxyloganin (13) into [11-$^3H_2$]-11-Hydroxyiridodial Glucoside Pentaacetate (15)——To a stirred suspension of $LiAl^3H_4$ (3.0 mg) (spec. activity 132.98 mCi/mM) and the carrier $LiAlH_4$ (80.0 mg)

in anhyd. THF (15 ml) precooled below −15° was added a solution of 7-deoxyloganin (**13**) (100 mg) in anhyd. THF (6 ml) over a period of 30 min keeping the reaction mixture below −15°. The mixture was worked up in the same manner as in the case of the cold run to give [11-$^3H_2$]-11-hydroxyiridodial glucoside pentaacetate (**15**) (46.2 mg) (spec. activity 2.67 mCi/mM) as colorless needles.

Conversion of [11-$^3H_2$]-11-Hydroxyiridodial Glucoside Pentaacetate (15) into [11-$^3H_2$]-Iridodial Glucoside (9) and [11-$^3H_2$]-Iridodial (7)——A solution of [11-$^3H_2$]-11-hydroxyiridodial glucoside pentaacetate (**15**) (46.2 mg) and the carrier (99.2 mg) in MeOH (6 ml) was subjected to hydrogenation over Pd-C prepared from 5% $PdCl_2$–HCl solution (0.2 ml) and activated charcoal (Darco G-60) (70 mg). After an uptake of about 1 mol equivalent of H_2, the catalyst was filtered off and washed with $CHCl_3$. The combined filtrate and washings were concentrated *in vacuo* and the residue was recrystallized from EtOH to give colorless needles of [11-$^3H_2$]-iridodial glucoside tetraacetate (**21**) (110 mg) (spec. activity 0.85 mCi/mM), which were converted into [11-$^3H_2$]-iridodial glucoside (**9**) (74.3 mg) (spec. activity 0.85 mCi/mM) by the same treatment as in the case of the cold run. An aliquot (33.22 mg) of this compound (**9**) was hydrolysed with β-glucosidase yielding [11-$^3H_2$]-iridodial (**7**) (19.3 mg) (spec. activity 0.85 mCi/mM).

Kuhn-Roth Oxidation of [11-$^3H_2$]-Iridodial Glucoside (9)——[11-$^3H_2$]-Iridodial glucoside (**9**) (44.7 mg) (spec. activity 0.85 mCi/mM) was dissolved in 2 N aq. H_2SO_4 (10 ml) containing CrO_3 (5 g) and heated to steam distil the resulting AcOH 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 a solution of the residue in H_2O (2 ml) were added N-ethyl-N′-(3-dimethylaminopropyl)carbodiimide (100 mg) and α-naphthylamine (20 mg). After adjusting the pH to 3 with 1 N aq. HCl, the solution was stirred for 10 min and extracted with ether. The ether layer was washed with H_2O, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was recrystallized from benzene–petr. benzine and sublimed twice *in vacuo* to give α-acetylnaphthylamide, which showed a radioactivity of 1.60×10^9 dpm/mM, corresponding to 85.4% of the radioactivity of the original iridodial glucoside (**9**).

Preparation of [10-^{3}H]-Iridodial Glucoside (9) and [10-^{3}H]-Iridodial (7)——To a stirred suspension of $LiAlH_4$ (90 mg) in anhyd. THF (14 ml) precooled below −15° was added a solution of [10-^{3}H]-7-deoxyloganin tetraacetate (**19**)[14] (100 mg) (spec. activity 35.78 mCi/mM) in anhyd. THF (7 ml) over a period of 30 min keeping the mixture below −15°. The reaction mixture was treated in the same way as in the case of the cold run to give [10-^{3}H]-iridodial glucoside (**9**) (69.40 mg) (spec. activity 3.77 mCi/mM). An aliquot (45.07 mg) of this compound was hydrolysed with β-glucosidase to furnish [10-^{3}H]-iridodial (**7**) (20.31 mg) (spec. activity 3.77 mCi/mM).

Administration of [10-^{3}H]-10-Hydroxygeraniol (8) to *D. crenata*, Isolation of [3-^{3}H]-Deutzioside (2) and Detection of Iridodial Glucoside (9) by the Isotope Dilution Method——[10-^{3}H]-10-Hydroxygeraniol (**8**) was emulsified in H_2O (10 ml) with the aid of Tween 80 (two drops) and administered hydroponically to 11 stems of the *Deutzia* plants (*ca.* 80 cm in height with many leaves) in August. After 5 days of administration, the plants (180 g) were chopped and extracted with MeOH (400 ml × 4) under reflux. The MeOH extracts were combined and concentrated *in vacuo*. The residue mixed with the carrier glucoside (**9**) (28 mg) was digested with H_2O (100 ml). The insoluble materials were filtered off through a celite layer and washed with a small amount of H_2O. The combined filtrate and washings, after concentration *in vacuo* to about 15 ml, were transferred to a charcoal column (10 g) and eluted successively with H_2O (fr. Nos. 1—5), H_2O–MeOH (9: 1) (fr. Nos. 6—7), (8: 2) (fr. No. 8), (7: 3) (fr. Nos. 9—10), (6: 4) (fr. Nos. 11—13), (5: 5) (fr. Nos. 14—16) and (3: 7) (fr. Nos. 17—18) collecting 100 ml fractions. Fr. Nos. 9—11 (abbreviated as Chrom 1-1) and fr. Nos. 12—17 (Chrom 1-2) were combined and concentrated *in vacuo*, respectively. The residue (290 mg) from Chrom 1-1 was acetylated with 3 ml each of Ac_2O and pyridine and the product was subjected to chromatography on silica gel (30 g) with $CHCl_3$ as eluent collecting 10 ml fractions. Fr. Nos. 14—17 (Chrom 2-1) and fr. Nos. 21—24 (Chrom 2-2) were combined and concentrated *in vacuo* to give colorless needles of iridodial glucoside tetraacetate (**21**) (5.28 mg) (TLC, $CHCl_3$–MeOH, 99: 1, *Rf* 0.65) and deutzioside pentaacetate (**20**) (205.25 mg) (TLC, $CHCl_3$–MeOH 99: 1, *Rf* 0.55), respectively. The latter (**20**) was diluted with the carrier (**20**) (166.21 mg) and recrystallized from MeOH to constant activity. The residue (315 mg) from Chrom 1-2 was acetylated with 3 ml each of Ac_2O and pyridine and the product was chromatographed on silica gel (30 g) with $CHCl_3$ as eluent collecting 10 ml fractions. Fr. Nos. 5—8 were combined and concentrated *in vacuo* to afford colorless needles of **21** (11.10 mg), which, after addition of **21** (5.28 mg) from Chrom 2-1 and the carrier (**21**) (48.03 mg), were recrystallized from EtOH to constant activity.

Deacetylation of [3-^{3}H]-Deutzioside Pentaacetate (20) followed by the Kuhn-Roth Oxidation and Ozonolysis——To a solution of [3-^{3}H]-deutzioside pentaacetate (**20**) (104.4 mg) (spec. activity 3.67×10^6 dpm/mM) in anhyd. MeOH (6 ml) was added anhyd. methanolic 0.2 N $NaOCH_3$ (0.2 ml). After refluxing for 5 min, the solution was cooled and neutralized with Amberlite IR-120 (H^+—form) and the resin was filtered off. Concentration of the filtrate gave [3-^{3}H]-deutzioside (**2**) (76.3 mg) as colorless needles, which were subjected to the Kuhn–Roth oxidation and the resulting CH_3COOH was converted into α-acetylnaphthylamide. This compound was submitted to repeated sublimations under reduced pressure to constant activity. [3-^{3}H]-Deutzioside (**2**) (69.0 mg) regenerated from an aliquot of **20** was dissolved in H_2O (20 ml) and a stream of O_2 containing ozone was passed through the solution at 0° for 6 hr. The reaction mixture was left standing overnight and then heated to steam distil the resulting HCOOH in a similar manner as described above.

The distillate (*ca.* 50 ml) was neutralized with 0.1 N NaOH and concentrated *in vacuo*. The residue was worked up in the usual way to give the α-naphthylamide, which was purified by chromatography on silica gel (5 g) eluting successively with benzene, benzene–AcOEt (95: 5) (fr. Nos. 4—10) and (99: 10) (fr. Nos. 11—30) and 6 ml each of fractions were collected. After concentration of the combined fractions Nos. 12—17 *in vacuo*, the residue was recrystallized from benzene–petr. ether and then sublimed repeatedly under reduced pressure to give α-formylnaphthylamide as colorless crystals having constant activity.

Administration of [11-$^3H_2$]-Iridodial (7) to *D. crenata* and Isolation of [11-$^3H_2$]-Deutzioside (2)——[11-$^3H_2$]-Iridodial (7) (19.30 mg) (spec. activity 0.85 mCi/mM) was emulsified in H_2O (10 ml) with Tween 80 (two drops) and administered hydroponically to 6 stems of the *Deutzia* plants (80 cm in height with many leaves) in November. After 5 days of administration, the plants (65 g) were cut in pieces and worked up in a similar manner as above giving radioactive deutzioside pentaacetate (**20**) (108.36 mg), which was diluted with the carrier (**20**) (53.27 mg) and recrystallized from MeOH to constant activity.

Deacetylation of [11-$^3H_2$]-Deutzioside Pentaacetate (20) followed by the Kuhn-Roth Oxidation——[11-$^3H_2$]-Deutzioside (**2**) (49.46 mg) regenerated by the Zemplén reaction of the pentaacetate (**20**) (102.84 mg) (spec. activity 8.32×10^6 dpm/mM) was subjected to the Kuhn–Roth oxidation in the usual way and the resulting CH_3COOH was converted into α-naphthylamide, which was purified by repeated sublimations under reduced pressure to constant activity.

Administration of [11-$^3H_2$]-Iridodial Glucoside (9) to *D. crenata* and the Kuhn-Roth Oxidation of [11$_2$-H_2]-Deutzioside (2)——A solution of [11-$^3H_2$]-iridodial glucoside (**9**) (41.03 mg) (spec. activity 0.85 mCi/mM) in H_2O (15 ml) was administered hydroponically to 11 stems of the *Deutzia* plants (80 cm in height with many leaves) in November. After 5 days of administration, the plants (55 g) were chopped and worked up in the usual way yielding radioactive deutzioside pentaacetate (**20**) (117.28 mg). This substance, after dilution with the carrier (**20**) (32.61 mg), was recrystallized from MeOH to constant activity. [11-$^3H_2$]-Deutzioside (**2**) (32.74 mg) regenerated from an aliquot (61.41 mg) of the pentaacetate (**20**) was subjected to the Kuhn–Roth oxidation in the usual manner and the resulting CH_3COOH was converted into the α-naphthylamide, which was recrystallized to constant activity.

Administration of [10-^{3}H]-Iridodial (7) to *Lamium amplexicaule* and Isolation of [10-^{3}H]-Lamioside (1) and [10-^{3}H]-Lamiide (5)——[10-^{3}H]-Iridodial (**7**) (6.25 mg) (spec. activity 3.77 mCi/mM) was emulsified in H_2O (5 ml) with Tween 80 (1 drop) and administered hydroponically to 18 terrestrial parts of *L. amplexicaule* in February. After 5 days of administration, the plants (31 g) were extracted with MeOH (300 ml × 4) under reflux. The combined extracts were concentrated *in vacuo* and the residue was digested with H_2O (100 ml). The insoluble materials were removed by filtration through a celite layer and washed with a small amount of H_2O. The filtrate and washings were combined and concentrated to about 15 ml, which was applied to a charcoal column (10 g) and eluted successively with H_2O (fr. Nos. 1—3), H_2O–MeOH (9: 1) (fr. Nos. 4—5), (8: 2) (fr. Nos. 6—7), (7: 3) (fr. Nos. 8—9), (6: 4) (fr. Nos. 10—11), (5: 5) (fr. Nos. 12—14), (4: 6) (fr. Nos. 15—16) collecting 100 ml fractions. Fr. Nos. 10—11 (Chrom 1-1) and fr. Nos. 12—15 (Chrom 1-2) were combined and concentrated *in vacuo*, respectively. The residue (37.34 mg) from Chrom 1-1, after acetylation with 0.5 ml each of Ac_2O and pyridine, was subjected to chromatography on silica gel (17 g) with $CHCl_3$–MeOH (99.5: 0.5) as eluent collecting 15 ml fractions. Fr. Nos. 9—14 were combined and concentrated *in vacuo* giving radioactive lamiide pentaacetate (**23**) (10.41 mg) as colorless needles (TLC, $CHCl_3$–MeOH (96: 4), *Rf* 0.67). An analogous treatment of the residue (41.75 mg) from Chrom 1-2 gave radioactive lamioside pentaacetate (**22**) (9.82 mg) as colorless needles (TLC, $CHCl_3$–MeOH (96: 4), *Rf* 0.78). Each lamiide pentaacetate (**23**) (10.41 mg) and lamioside pentaacetate (**22**) (9.82 mg) was diluted with the corresponding carrier ((**23**) (33.36 mg) and (**22**) (38.68 mg)) and recrystallized from EtOH to constant activity.

Administration of [10-^{3}H]-Iridodial Glucoside (9) to *L. amplexicaule* and Isolation of Radioactive Lamioside (1) and Lamiide (5)——A solution of [10-^{3}H]-iridodial glucoside (**9**) (14.06 mg) spec. activity 3.77 mCi/mM) in H_2O (8 ml) was administered hydroponically to 30 terrestrial parts of *L. amplexicaule*. After 5 days of administration, the plants (43 g) were chopped and worked up in the same manner as stated above yielding radioactive lamiide pentaacetate (**23**) (18.79 mg) and lamioside pentaacetate (**22**) (17.57 mg) as colorless needles. Each acetate was diluted with the corresponding carrier (45.86 mg and 29.36 mg) and recrystallized from EtOH to constant activity.

Administration of [10-^{3}H]-Iridodial (7) to *Galium spurium* var. *echinospermon* and Isolation of Radioactive Asperuloside (6)——[10-^{3}H]-Iridodial (**7**) (8.03 mg) (spec. activity 3.77 mCi/mM) was emulsified in H_2O (8 ml) with Tween 80 (2 drops) and administered hydroponically to 36 terrestrial parts of the *Galium* plants in March. After 5 days of administration, the plants (49 mg) were extracted with MeOH (300 ml × 4) under reflux. The combined MeOH extracts were concentrated *in vacuo*. The residue was digested with H_2O (100 ml). The insoluble materials were filtered off through a celite layer and washed with a small amount of H_2O. The filtrate and washings were combined and concentrated *in vacuo* to about 15 ml, which was transferred to a charcoal column (8 g) and eluted successively with H_2O (fr. Nos. 1—2), H_2O–MeOH (9: 1) (fr. Nos. 3—4), (8: 2) (fr. Nos. 5—6), (7: 3) (fr. Nos. 7—8), (6: 4) (fr. Nos. 9—10), (5: 5) (fr. Nos. 11—17), (3: 7) (fr. Nos. 18—24) and MeOH (fr. No. 25) collecting 100 ml fractions. Fr. Nos. 18—22 were combined and concentrated *in vacuo* to afford a residue (385 mg), which was acetylated with 1 ml each of Ac_2O and pyridine. The product was chromatographed on silica gel (30 g) with ether as eluent collecting 20 ml fractions. Concentra-

tion of the combined fr. Nos. 9—15 gave asperuloside tetraacetate (**17**) (333.36 mg) as colorless needles (TLC, ether, *Rf* 0.27), which were recrystallized from EtOH to constant activity.

Catalytic Hydrogenation of Radioactive Asperuloside Tetraacetate (17)——Asperuloside tetraacetate (**17**) (90 mg) (spec. activity 1.12×10^7 dpm/mM) dissolved in MeOH (15 ml) was hydrogenated over Pd-C (prepared from 5% $PdCl_2$ solution (0.2 ml) and activated charcoal (Darco G-60) (70 mg)). After an uptake of H_2 ceased spontaneously, the catalyst was filtered off and washed with $CHCl_3$. The combined filtrate and washings were concentrated *in vacuo* and the residue was recrystallized from EtOH–H_2O yielding 7-deoxyloganic acid tetraacetate (**18**) (74.51 mg) as colorless needles. This acetate (**18**), after dilution with the carrier (**18**) (144.33 mg), was recrystallized from EtOH to constant activity.

Hydrolysis of Radioactive 7-Deoxyloganic Acid Tetraacetate (18) followed by the Kuhn-Roth Oxidation——Asperuloside tetraacetate (**17**) (87.00 mg) (spec. activity 1.12×10^7 dpm/mM) was dissolved in MeOH (15 ml) and the pH of the solution was adjusted to 10 with saturated methanolic $Ba(OH)_2$. After standing overnight at 5°, the solution was adjusted to pH 4 with Amberlite IR 120 (H^+—form) and the resin was filtered off. After concentration of the filtrate, the residue was methylated with CH_2N_2–ether giving rise to radioactive 7-deoxyloganin (**10**) (48.73 mg) as colorless needles. This substance was subjected to the Kuhn–Roth oxidation in the usual manner. The resulting CH_3COOH was converted into the α-naphthylamide of constant radioactivity.

Acknowledgement This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, to which we express our gratitude. We are grateful to Professor F. Murai of Aichi Medical College for his gift of an IR spectrum of iridodial-bis-2,4-dinitrophenylhydrazone. Thanks are also due to the members of the Microanalytical Center of this University for elemental analyses and to Dr. A. Kato, Mrs. M. Uobe and Miss R. Yamaguchi of this Faculty for measuring MS and NMR spectra.