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Studies on Monoterpene Glucosides and Related Natural Products. XVII.[1] The Intermediacy of 7-Desoxyloganic Acid and Loganin in the Biosynthesis of Several Iridoid Glucosides[2]

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Administration experiments of labelled compounds into several plants revealed that 7-desoxyloganic acid is the intermediate in the biosynthesis of iridoid glucosides such as verbenalin (**2**), loganin (**4**), geniposide (**5**), asperuloside (**6**), and aucubin (**10**).

Tracer experiments on asperuloside (**6**) proved that loganin (**4**) (or loganic acid (**3**)) locates biosynthetically between 7-desoxyloganic acid (**1**) and the glucosides formed after geniposide (**5**).

We previously reported that secoiridoid glucosides such as sweroside, swertiamarin and gentiopicroside are formed *via* mevalonic acid[4] and that sweroside was incorporated into gentiopicroside, vindoline, reserpinine, and quinine.[5]

At the start of this investigation, it had already been reported that mevalonic acid is incorporated into glucosides such as plumieride[6] and several indole alkaloids[7] and that geraniol[8] and loganin[9] are also incorporated into indole alkaloids. However, the process of the conversion into several glucosides after formation of the iridoid skeleton had still been ambiguous.[10]

In comparing the then known several iridoid glucosides, we presumed that desoxyloganic acid (**1**) could be the precursor of many glucosides having a carboxyl, a lactone or ester car-

1) Part XVI: H. Inouye and Y. Nakamura, *Yakugaku Zasshi*, **91**, 755 (1971).
2) A preliminary report of part of this work has been published. *a*) H. Inouye, S. Ueda, Y. Aoki, and Y. Takeda, *Tetrahedron Letters*, **1969**, 2351; *b*) H. Inouye, S. Ueda, and Y. Takeda, *ibid.*, **1970**, 3351; *c*) *idem*, *Z. Naturforsch.*, **24b**, 1666 (1969).
3) Location: *Yoshidashimoadachi-cho, Sakyo-ku, Kyoto.*
4) H. Inouye, S. Ueda, and Y. Nakamura, *Chem. Pharm. Bull.* (Tokyo), **18**, 2043 (1970).
5) H. Inouye, S. Ueda, and Y. Takeda, *Chem. Pharm. Bull.* (Tokyo), **19**, 587 (1971).
6) D.A. Yeowell and H. Schmid, *Experientia*, **20**, 250 (1964); J.E.S. Hüni, H. Hiltebrand, H. Schmid, D. Gröger, S. Johne, and K. Mothes, *ibid.*, **22**, 656 (1966).
7) T. Money, I.G. Wright, F. McCapra, and A.I. Scott, *Proc. Natl. Acad. Sci. U.S.*, **53**, 901 (1965); F. McCapra, T. Money, A.I. Scott, and I.G. Wright, *Chem. Commun.*, **1965**, 537; H. Goeggel and D. Arigoni, *ibid.*, **1965**, 538; A.R. Battersby, R.T. Brown, R.S. Kapil, A.O. Plunkett, and J.B. Taylor, *ibid.*, **1965**, 538.
8) E. Leete and S. Ueda, *Tetrahedron Letters*, **1966**, 4915; A.R. Battersby, R.T. Brown, J.A. Knight, J.A. Martin, and A.O. Plunkett, *Chem. Commun.*, **1966**, 346; P. Loew, H. Goeggel, and D. Arigoni, *ibid.*, **1966**, 347; E.S. Hall, F. McCapra, T. Money, K. Fukumoto, J.R. Hanson, R.S. Mootoo, G.T. Philips, and A.I. Scott, *ibid.*, **1966**, 348; T. Money, I.G. Wright, F. McCapra, E.S. Hall, and A.I. Scott, *J. Am. Chem. Soc.*, **90**, 4144 (1968).
9) A.R. Battersby, R.T. Brown, R.S. Kapil, J.A. Martin, and A.O. Plunkett, *Chem. Commun.*, **1966**, 890; A.R. Battersby, R.S. Kapil, J.A. Martin, and L. Mo, *ibid.*, **1968**, 133; P. Loew and D. Arigoni, *ibid.*, **1968**, 137.
10) *a*) After we had started this series of works, it was reported that mevalonic acid labelled stereospecifically with tritium in several positions had been incorporated into loganic acid (**3**). R. Guarnaccia, L. Botta, and C.J. Coscia, *J. Am. Chem. Soc.*, **91**, 204 (1969); C.J. Coscia, R. Guarnaccia, and L. Botta, *Biochemistry*, **8**, 5036 (1969); C.J. Coscia, L. Botta, and R. Guarnaccia, *Arch. Biochem. Biophys.*, **136**, 498 (1970); *b*) The formation of the iridoid skeleton by direct ring closure of 10-oxygeraniol was also reported. S. Escher, P. Loew, and D. Arigoni, *Chem. Commun.*, **1970**, 823; A.R. Battersby, S.H. Brown, and T.G. Payne, *ibid.*, **1970**, 827.

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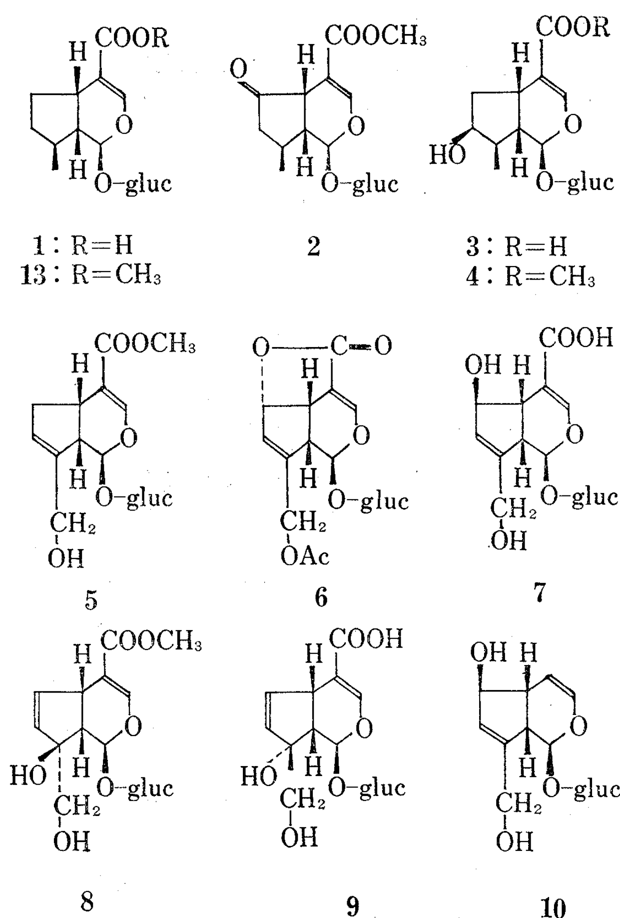

Chart 1

bonyl group at C-4 or also of several glucosides lacking C-4 substituent probably due to decarboxylation.

Based on this assumption, the oxidation at C-6 of desoxyloganic acid (**1**) or hydroxylation at C-7 of **1**, for example, would lead to verbenalin(**2**) or loganic acid (**3**) (or loganin (**4**)), respectively. Compound (**3**) (or **4**) was considered on the one hand to be the precursor of secoiridoid glucosides,[11] on the other hand, it was thought to be the precursor of the more oxidized iridoid glucosides such as geniposide (**5**), asperuloside (**6**), scandoside (**7**), gardenoside (**8**), monotropein (**9**), aucubin (**10**), and so on.

This paper describes feeding experiments of radioactive 7-desoxyloganic acid (**1**) and loganin (**4**), which confirmed the precursorship of both glucosides in the biosynthesis of the iridoid glucosides described above.

The radioactive desoxyloganic acid (**1**) employed in these experiments was prepared as follows: Catalytic hydrogenation of asperuloside tetraacetate (**11**) with tritium over Pd-C gave [^{3}H]-7-desoxyloganic acid tetraacetate (**12**), which was deacetylated with methanolic $Ba(OH)_2$ to give [^{3}H]-7-desoxyloganic acid (**1**). Kuhn-Roth oxidation of this compound (**1**) yielded acetic acid, whose radioactivity indicated, as shown in Table I, that about 90% of the radioactivity of [^{3}H]-7-desoxyloganic acid (**1**)[12] was located at C-10. Therefore, this acid (**1**) is assumed to be [10-^{3}H]-compound.

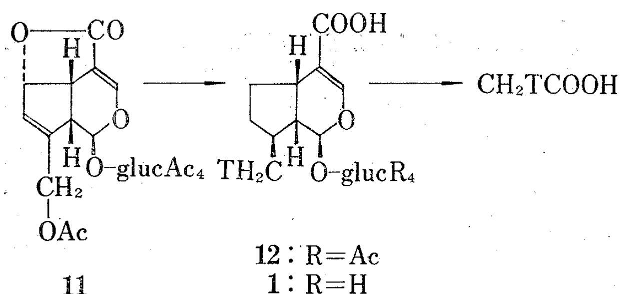

Chart 2

After administration of this [10-^{3}H]-7-desoxyloganic acid (**1**) to the plants shown in Table II, iridoid glucosides were isolated by the conventional method and the amount of the incorporation was examined. In this experiment, the isolated glucosides except scandoside (**7**) and gardenoside (**8**) were converted to the acetates and recrystallized to constant activity. Scandoside (**7**) was purified by the combination of repeated preparative

11) It had already been established that **4** is the precursor of the non-tryptophan portion of the indole alkaloids (*cf.* footnote 9).

12) During the catalytic hydrogenation, asperuloside tetraacetate (**11**) was enforced initial hydrogenolysis at C-10 by contact with a small amount of tritium gas. The tritium gas was then diluted with a large amount of hydrogen and hydrogenation was continued. This treatment mainly gives [10-^{3}H]-7-desoxyloganic acid (**1**).

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Table I. Kuhn–Roth Oxidation of [10-^{3}H]-7-Desoxyloganic Acid (**1**)

	Spec. activity of **1**, dpm/mmole	Spec. activity of AcOH, dpm/mmole	Percentage of radioactivity at C-10
Exp. 1	4.03×10^7	3.51×10^7	87
Exp. 2	1.23×10^7	1.12×10^7	92

thin-layer chromatography (TLC) on silica gel and Avicel until the product showed a single radioactive spot on a paper chromatogram. Gardenoside (**8**) was subjected to catalytic hydrogenation over Pd-C, followed by acetylation to give crystalline dihydrogardenoside pentaacetate, which was repeatedly recrystallized to constant activity. The results of these feeding experiments are shown in Table II.

Table II. Administration Experiments of [10-^{3}H]-7-Desoxyloganic Acid (**1**)

Plant	Amt. of **1** fed Spec. activity dpm/mmole	Glucoside isolated	Spec. activity dpm/mmole	Incorporation %
Verbena officinalis L.	42.8 mg 6.12×10^9	verbenalin (**2**)	2.93×10^8	11
Lonicera japonica Thunb.	71.6 mg 6.12×10^9	loganin (**4**)	2.14×10^7	0.27
Paederia scandens (Lour.) *Merrill* var. *mairei* (Lèveillè) Hara	15.9 mg 3.86×10^{11}	scandoside (**7**)	1.74×10^{10}	1.6[a]
Gardenia jasminoides Ellis forma *grandiflora* (Lour.) Makino	43.5 mg 2.79×10^{11}	geniposide (**5**)	1.63×10^9	0.05[a]
		gardenoside (**8**)	1.58×10^8	0.43[a]
Daphniphyllum macropodum Miq.	2.32 mg 1.29×10^9	asperuloside (**6**)	1.90×10^5	0.60[a]
Aucuba japonica Thunb.	1.99 mg 1.29×10^9	aucubin (**10**)	3.62×10^5	0.51[a]

a) The data of incorporation (%) were corrected by assuming that two thirds of the radioactivty of **1** remained in the glucosides.

An aliquot of radioactive asperuloside tetraacetate (**11**) isolated from the plant was subjected to catalytic hydrogenation over Pd-C. Methylation followed by the Zemplén reaction of the resulting 7-desoxyloganin tetraacetate gave radioactive 7-desoxyloganin (**13**). On the other hand, radioactive loganin pentaacetate isolated from *Lonicera japonica* was subjected to the Zemplén reaction to regenerate radioactive loganin (**4**). Both radioactive glucosides obtained here were subjected respectively to the Kuhn-Roth oxidation and the radioactivities of the resulting acetic acid were measured. As shown in Table III, the isotope is located only on C-10 in both glucosides. Thus it was proved that **1** must have had also been incorporated *in toto* into the glucosides described above.

Table III. Kuhn–Roth Oxidation of 7-Desoxyloganin (**13**) prepared from[10-^{3}H]-7-Desoxyloganic Acid derived Asperuloside (**6**), and Loganin (**4**) biologically converted from [10-^{3}H]-7-Desoxyloganic Acid

	Spec. activity dpm/mmole	Spec. activity of AcOH formed, dpm/mmole
7-desoxyloganin (**13**)	2.91×10^3	2.85×10^3
loganin (**4**)	2.10×10^4	2.50×10^4

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After the publication of these results in a preliminary form,[2] Rimpler[13] isolated 7-desoxyloganic acid (**1**) from *Physostegia virginiana*. Battersby and his coworkers[14] isolated its methyl ester (**13**) from *Vinca rosea*. They also prepared [carbo-^{14}C-methoxy]-7-desoxy-loganin (**13**) from loganin (**4**) and proved its incorporation into loganin (**4**) and indole alkaloids. All these facts support our conclusion.[2]

The fact that the specific activity of geniposide (**5**) isolated from *Gardenia jasminoides* forma *glandiflora* is about ten times as large as that of the coexisting gardenoside (**8**) suggests the formation of **8** *via* **5**.

We describe now the experimental results which proved loganin (**4**) or loganic acid (**3**)[15] to be the precursor of the highly oxidized iridoid glucosides (**5**—**10**). In an attempt to clarify this problem, we administered radioactive loganin (**4**) and 7-epiloganin (**18**) labelled in different positions to *Daphniphyllum macropodum* and examined its incorporation into asperuloside (**6**). [10-^{3}H]-loganin (**4**) employed here was obtained biosynthetically by the administration of **1** into *Lonicera japonica*. [10-^{3}H]-7-Epiloganin (**18**), [7-^{3}H]-7-epiloganin (**18**), and [7-^{3}H]-loganin (**4**) were prepared according to the route shown in Chart 3, which we had followed previously in the chemical conversion of asperuloside (**6**) into loganin (**4**).[16]

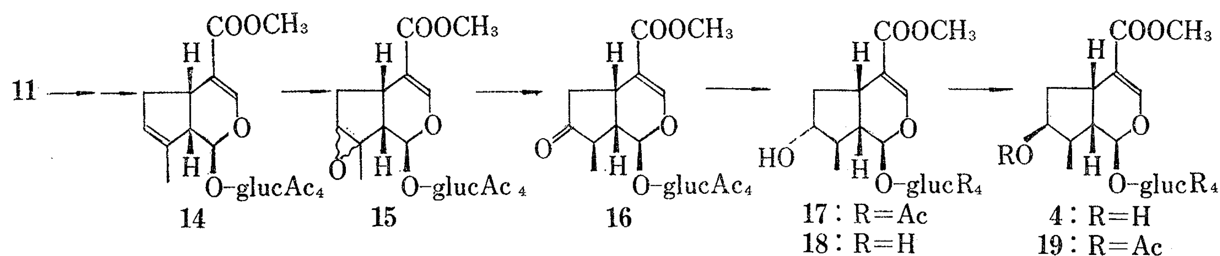

Chart 3

Asperuloside tetraacetate (**11**) was subjected to catalytic hydrogenation with tritium over Pd-C followed by methylation to yield [10-^{3}H]-10-desoxygeniposide tetraacetate (**14**), which reacted with perbenzoic acid to give the 7,8-epoxy derivative (**15**). This compound was then converted to [10-^{3}H]-7-epiloganin tetraacetate (**17**) by way of [10-^{3}H]-7-dehydro-loganin tetraacetate (**16**). **17** was finally deacetylated to [10-^{3}H]-7-epiloganin (**18**). 7-Dehydrologanin tetraacetate (**16**) prepared *via* the same route was reduced with NaB^3H_4 to give [7-^{3}H]-7-epiloganin (**18**). On the other hand, [7-^{3}H]-**17** was subjected to the Walden inversion after tosylation and the resulting [7-^{3}H]-loganin pentaacetate (**19**) was deacetylated to give [7-^{3}H]-loganin (**4**).

Table IV shows the results obtained by the examination of the incorporation of [10-^{3}H]-loganin (**4**), [10-^{3}H]-7-epiloganin (**18**), [7-^{3}H]-loganin (**4**), and [7-^{3}H]-7-epiloganin (**18**) into asperuloside (**6**) in *Daphniphyllum macropodum*. As shown in this table, both [10-^{3}H]- and [7-^{3}H]-loganin (**4**) were incorporated into asperuloside (**6**).[17] In the feeding experiments of both radioactive 7-epiloganins (**18**), however, radioactivity was observed in asperuloside

13) H. Rimpler and B. von Lehman, *Phytochem.*, **9**, 641 (1970).
14) A.R. Battersby, A.R. Burnett, and P.G. Parsons, *Chem. Commun.*, **1970**, 826.
15) We assumed throughout these experiments that the biological interconversions between **3** and **4** are rather arbitrary, that is, both are equivalent and the results coincide with the assumption. Recently, however, Coscia and his coworkers isolated methyltransferase from *Vinca rosea*. *cf.* K.M. Madyastha, R. Guarnaccia, and C.J. Coscia, *Febs Letters*, **14**, 175 (1971). This enzyme was reported to catalyze the transformation of **3** into **4**, while it was inactive in the conversion of **1** into **14**. This seems to be incompatible with the results obtained by Battersby, *et al.*[14] This problem remains to be examined in the future.
16) H. Inouye, T. Yoshida, S. Tobita, and M. Okigawa, *Tetrahedron*, **26**, 3905 (1970).
17) The poor incorporation of [7-^{3}H] compound (4) seems to be due to the seasonal influences. [10-^{3}H] Experiments were performed in August, while those with [7-^{3}H]compounds in March.

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Table IV. Administration of [10-^{3}H]-, [7-^{3}H]-Loganin (4), [10-^{3}H]-and [7-^{3}H]-7-Epiloganin (18) into *Daphniphyllum macropodum*

Glucoside fed	Amt. & spec. activity dpm/mmole	Spec. activity of asperuloside dpm/mmole	Incorporation %
[10-^{3}H]-loganin (**4**)	12.6 mg 1.95×10^7	7.87×10^3	0.45
[10-^{3}H]-7-epiloganin (**18**)	10.3 mg 3.02×10^7	1.15×10^4	0.44
[7-^{3}H]-loganin (**4**)	3.3 mg 3.41×10^9	3.32×10^4	0.05
[7-^{3}H]-7-epiloganin (**18**)	5.65 mg 3.16×10^9	0	0

(**6**) obtained in the experiment with [10-^{3}H]-7-epiloganin (**18**), while it could not be found in the [7-^{3}H] experiment. These experimental results indicate that loganin (**4**) or loganic acid (**3**) is the true intermediate in the biosynthesis of asperuloside (**6**). Although 7-epiloganin (**18**) was also incorporated into **6**, this phenomenon is reasonably explained by assuming that oxidation of **18** gave the 7-dehydro compound which was reduced to loganin (**4**) or loganic acid (**3**) and then led to **6**.

Coscia, *et al.*[10a] administered [2 R-2-^{3}H]-mevalonic acid to *Swertia caroliniensis* and obtained radioactive loganic acid (**3**) with tritium at C-7. Thus **1** is thought to be hydroxylated in the plant maintaining the original configuration at C-7 to give **3** (or **4**), which is further dehydrated.

Experimental[18]

Preparation of [10-^{3}H]-7-Desoxyloganic Acid Tetraacetate (12)——To a solution of asperuloside tetraacetate (**11**) (100 mg) in AcOEt (3 ml) and MeOH (0.1 ml) was added Pd-C (5%) (100 mg) and the mixture was stirred under $^3H_2$ (0.25 Ci) for 2 hr, H_2 (10 ml) was then introduced into the reaction mixture with stirring. After 2 hr further H_2 was added in large excess and stirring was continued for another 2 hr. The reaction mixture was then filtered through a layer of filter paper powder and the filtrate was evaporated *in vacuo*. The residue was dissolved in MeOH and the solvent was removed *in vacuo*. After repeating this procedure three times, colorless needles were obtained. This substance was identified with an authentic sample of 7-desoxyloganic acid tetraacetate (**12**) by TLC ($CHCl_3$: MeOH 95:5).

Preparation of [10-^{3}H]-7-Desoxyloganic Acid (1)——An aliquot (1/50) of the above radioactive compound and carrier (**12**) (211 mg) were dissolved in MeOH (5 ml) and a saturated methanolic solution of Ba-$(OH)_2$ was added dropwise to adjust the pH of the solution to 10. After standing overnight, the pH of the reaction mixture was adjusted to 4 by addition of Amberlite IR-120 (H-form) ion exchange resin. After removal of the ion exchange resin, the solution was evaporated *in vacuo* and the residue was recrystallized from EtOH to give colorless needles (78 mg), mp 113—114°. Specific activity 1.29×10^9 dpm/mmole. This substance was identified with an authentic sample of 7-desoxyloganic acid (**1**) by admixture and TLC ($CHCl_3$: MeOH 7:3).

18) Melting points were determined by a Yanagimoto Micromelting Point Apparatus and were not corrected. Unless otherwise specified, paper chromatography (PPC) was carried out on Toyo Roshi No. 50 filter paper with 10% NaOH: 95% EtOH (1:10 v/v) as solvent. The spots were detected by spraying the paper with a solution of bromocresol blue (50 mg) and citric acid (200 mg) in 100 ml of water. Unless otherwise noted, silica gel G acc. to Stahl (E. Merck) was used for the TLC and silica gel plates F_{254} (E. Merck) were employed for the radio TLC. The spots were detected by exposing the plates to iodine vapour. Silica gel (Mallinckrodt), polyamide C-200 (Wako), and charcoal (Shirasagi, Takeda) were used for column chromatography. The solvent ratio was expressed in volume. The radioactivity was measured by a Beckman Liquid Scintillation Counter Model LS-233 with samples dissolved in a scintillation mixture consisting of toluene (10 ml), 2,5-diphenyloxazole (PPO) (40 mg), 2,2′-*p*-phenylenebis(5-phenyloxazole) (POPOP) (0.5 mg) of dioxane (10 ml), naphthalene (1 g), PPO (70 mg), POPOP (5 mg). The specific activities are expressed as values before dilution.

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Kuhn-Roth Oxidation of [10-^{3}H]-7-Desoxyloganic Acid (1)——Radioactive 7-desoxyloganic acid (1) (124 mg) mixed with 2N H_2SO_4 (10 ml) containing CrO_3 (3 g) was heated for 3 hr and the resulting acetic acid was distilled with water. During the distillation, water (50 ml) was dropped into the reaction mixture to maintain its volume. The distillate (50 ml) was neutralized with aq. 0.1N NaOH (indicator: phenolphthalein). The solvent was removed *in vacuo* and an aliquot of the residue was monitored by PPC which showed a single spot corresponding to that of acetic acid (*Rf* 0.3). To the aq. solution (1 ml) of the rest of the residue were added α-naphthylamine-HCl (10 mg), N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide-HCl (80 mg) and conc. HCl (1 drop). The reaction mixture was extracted with benzene, dried over anhyd. $MgSO_4$, and the solvent was removed. The resulting α-N-acetylaminonaphthalene was distilled *in vacuo* to give colorless needles, mp 155—156°.

Administration of [10-^{3}H]-7-Desoxyloganic Acid (1) to *Verbena officinalis* and Isolation of [10-^{3}H]-Verbenalin (2)——[10-^{3}H]-7-Desoxyloganic acid (1) (42.8 mg, specific activity 6.12×10^9 dpm/mmole) was dissolved in water (3 ml) and the aq. solution was placed in glass tubes and administered by the cotton wick method to seven *V. officinalis* plants (about 30 cm in height) at their flowering stage in May. During the administration, a total of 40 ml water was added to the glass tubes to obtain maximal tracer absorption into the plants.[19] Seven days after the beginning of the administration, the shoots (wet weight 30 g) were harvested, cut into pieces and extracted under reflux with four 200 ml portions of MeOH. The combined extract was concentrated *in vacuo*, dissolved in water (20 ml) and the insoluble material was removed by filtration through a celite layer. The celite layer was washed with water (25 ml) and the washings were combined with the filtrate. The combined solution was washed with AcOEt (4×40 ml) and the aq. layer was concentrated *in vacuo* to about 20 ml, which was then extracted with *n*-BuOH (4×50 ml). The *n*-BuOH layer was washed with a small amount of water and evaporated *in vacuo*. The residue was chromatographed on polyamide (15 g, 2×10 cm), eluted successively with water (fractions (fr.) No. 1—6, water: MeOH 7:3) and MeOH (fr. No. 7) collecting 100 ml fractions. Fractions No. 1—6 were combined and evaporated *in vacuo*. The residue was chromatographed on silica gel (20 g, 1.5×20 cm), eluted successively with $CHCl_3$ (fr. No. 1—4), $CHCl_3$:MeOH 95:5 (fr. No. 5—8), $CHCl_3$:MeOH 93:7 (fr. No. 9—13) and $CHCl_3$: MeOH 9:1 collecting 25 ml fractions. Fractions No. 11—18 were combined and evaporated *in vacuo* to give verbenalin (2) (98.7 mg) as a colorless syrup, which was acetylated with 1 ml each of Ac_2O and pyridine and chromatographed on silica gel (30 g, 2×24 cm) with ether as eluent collecting 20 ml fractions. Fractions No. 3—5 were combined and the solvent was removed *in vacuo* to give 107.5 mg of verbenalin tetraacetate as a colorless syrup. An aliquot of this compound (1.07 mg) was diluted with carrier (101 mg) and repeatedly recrystallized from aq. EtOH to give colorless needles having a constant specific activity, mp 133—134°.

Administration of [10-^{3}H]-7-Desoxyloganic Acid (1) to *Lonicera japonica* and Isolation of [10-^{3}H]-Loganin (4)——[10-^{3}H]-7-Desoxyloganic acid (1) (71.6 mg, spec. activity 6.12×10^9 dpm/mmole) was dissolved in water (5 ml) and administered hydroponically and also through cotton wicks to five vines of *L. japonica* plants (about 20 cm in length) in May. Seven days after the beginning of the administration, the plants (wet weight 75.5 g) were cut into pieces and extracted under reflux with four 400 ml portions of MeOH. The extracts were combined and the solvent was removed *in vacuo*. The residue was extracted with water (4×70 ml) and the aq. extracts were combined, washed with AcOEt (4×60 ml) and extracted with *n*-BuOH (4×60 ml). The *n*-BuOH layer was concentrated *in vacuo* and the residue was chromatographed on polyamide (15 g, 2.5×18 cm) with water as eluent. The first 900 ml of the eluate was concentrated *in vacuo* and the residue was chromatographed on a charcoal column (10 g each of charcoal and celite, 2.5×14 cm) eluted with water (fr. No. 1—3, 50 ml each), water: MeOH 95:5 (fr. No. 4, 60 ml), water: MeOH 9:1 (fr. No. 5—9, 50 ml each) water: MeOH 8:2 (fr. No. 10—19, 40 ml each), water: MeOH 1:1 (fr. No. 20—25, 40 ml each) and MeOH (fr. No. 26—53, 50 ml each). Fractions No, 27—45 were combined and concentrated *in vacuo* to give a colorless syrup (152 mg). This was acetylated in the usual manner and chromatographed on silica gel (10 g, 1.5×10 cm) with ether as eluent collecting 25 ml fractions. Fractions No. 4—5 were combined and the solvent was removed to give a colorless syrup (98 mg). This substance was identified with an authentic sample of loganin pentaacetate (19) by TLC (ether.) An aliquot of this substance (19) (32.7 mg) was diluted with carrier (92.1 mg) and repeatedly recrystallized from EtOH to constant activity. Radioactive loganin pentaacetate (19) (80.3 mg) was dissolved in MeOH (3 ml) and the pH of the solution was adjusted to 10 with a saturated methanolic solution of $Ba(OH)_2$ and the mixture was allowed to stand overnight. After neutralization of the solution with Amberlite IR-120 (H-form), the ion exchange resin was filtered off and the filtrate was concentrated *in vacuo*. The residue was dissolved in a small amount of MeOH and treated with CH_2N_2-ether. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (5 g, 1×5 cm) eluted successively with $CHCl_3$ (fr. No. 1—3,30 ml each), $CHCl_3$:MeOH 96:4 (fr. No. 4—14, 20 ml each), and $CHCl_3$:MeOH 9:1 (fr. No. 15—26, 20 ml each). Fractions No. 22—25 were combined and the solvent was removed *in vacuo* to give 33.7 mg of loganin (4) as a colorless syrup. This substance was identified with an authentic sample of loganin (4) by TLC ($CHCl_3$:MeOH 7:3). The specific activity

19) This procedure was applied to all the administration experiments.

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of this compound (4) was the same as that of its pentaacetate. Radioactive loganin (4) (165.9 mg, spec. activity 2.10×10^4 dpm/mmole) was subjected to the Kuhn–Roth oxidation as in the case of 1 and the resulting acetic acid was converted to α-N-acetylaminonaphthalene.

Administration of [10-^{3}H]-7-Desoxyloganic Acid (1) to *Paederia scandens* var. *Mairei* and Isolation of [10-^{3}H]-Scandoside (7)——[10-^{3}H]-7-Desoxyloganic acid (1) (15.9 mg, spec. activity 3.86×10^{11} dpm/mmole) was dissolved in water (3 ml) and administered hydroponically to six vines of *P. scandens* plants (20—25 cm in length, 4—5 leaves). Six days after the beginning of the administration, the plants (wet weight 31 g) were cut into pieces and extracted with four 200 ml portions of hot MeOH. The extracts were combined and the solvent was removed *in vacuo*. The residue was dissolved in water (50 ml) and the insoluble material was removed by filtration through a celite layer. The celite layer was repeatedly washed with water (total 50 ml). The filtrate and the washings were combined and washed with AcOEt (3×40 ml). The aqueous layer was concentrated *in vacuo* and the residue was chromatographed on a charcoal column (10 g each of charcoal and celite, 3×12.5 cm), eluted successively with water (fr. No. 1—10), water:MeOH 9:1 (fr. No. 11—16), water:MeOH 4:1 (fr. No. 17—24), water:MeOH 1:1 (fr. No. 25—30) and MeOH (fr. No. 31—41) collecting 50 ml fractions. Fractions No. 25—41, which were positive to the mineral acid reaction,[20] were combined and the solvent was removed *in vacuo* to give a mixture of glucosides (16.5 mg). The mixture was then subjected to a preparative TLC on Avicel (0.37 mm in thickness, 20×20 cm, the upper layer of *n*-BuOH:EtOH:water 4:1:5), silica gel (0.5 mm in thickness, $CHCl_3$:MeOH 7:3) and finally on Avicel (as above) to give [10-^{3}H]-scandoside (7) (6.0 mg). On PPC (the upper layer of *n*-BuOH:EtOH:water 4:1:5), this substance showed the same *Rf* value as that of an authentic sample of scandoside (7). The paper strip on which the radioactive scandoside (7) had been developed was cut to 1 cm pieces and the radioactivity of each piece was monitored by a liquid scintillation counter. As shown in Fig. 1, this radiochromatogram showed a single peak corresponding to the spot of 7.

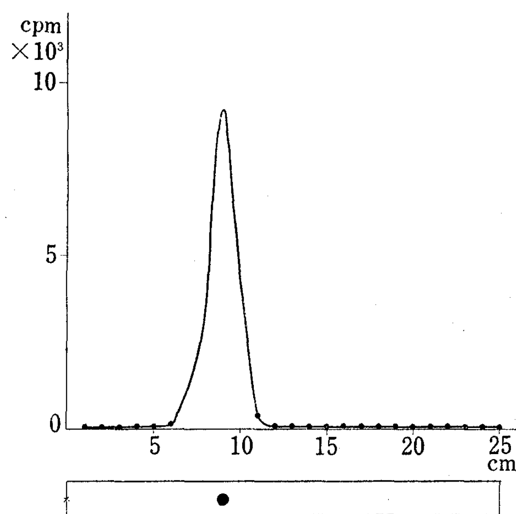

Fig. 1. Radiochromatogram of [10-^{3}H]-Scandoside biologically converted from [10-^{3}H]-7-Desoxyloganic Acid

Administration of [10-^{3}H]-7-Desoxyloganic Acid (1) to *Gardenia jasminoides* forma *grandiflora* and Isolation of [10-^{3}H]-Geniposide (5) and [10-^{3}H]-Gardenoside (8)——[10-^{3}H]-7-Desoxyloganic acid (1) (43.5 mg, spec. activity 2.79×10^{11} dpm/mmole) was dissolved in water (7 ml) and administered by the cotton wick method to two twigs (about 15 cm in length with 6 and 12 leaves) of *G. jasminoides* plants. Part of the radioactive material was also fed hydroponically. Nine days after the beginning of the administration, the plants (wet weight 15.3 g) were cut into pieces and extracted under reflux with four 200 ml portions of MeOH. The extracts were combined and the solvent was removed *in vacuo*. The residue was extracted four times with water (total 150 ml). Insoluble material was filtered off through a layer of celite. After washing the filtrate with three 50 ml portions of $CHCl_3$, the aq. layer was evaporated *in vacuo*. The residue was extracted five times with MeOH (total 40 ml). The extracts were combined and the solvent was removed *in vacuo* to give 1.85g of the residue. The residue was chromatographed on silica gel (40 g, 2.5×20 cm), eluted successively with CH_2Cl_2 (fr. No. 1—2, 150 ml each), CH_2Cl_2:MeOH 95:5 (fr. No. 3—4, 100 ml each), CH_2Cl_2:MeOH 93:7 (fr. No. 5—7, 50 ml each), CH_2Cl_2MeOH 9:1 (fr. No. 8—23, 20 ml each) and CH_2Cl_2: MeOH 85:15 (fr. No. 24—40, 25 ml each) (Chromatography No. 1). Fractions No. 11—15 were combined and the solvent was removed *in vacuo*. The residue was chromatographed on a charcoal column (3 g each of charcoal and celite, 2×5 cm), eluted successively with water (fr. No. 1—2), water: MeOH 9:1 (fr. No. 3), water:MeOH 8:2 (fr. No. 4), water: MeOH 1:1 (fr. No. 5), water: MeOH 3:7 (fr. No. 6—7) and MeOH (fr. No. 8—12) collecting 100 ml fractions. Fractions No. 6—12 were combined and the solvent was removed *in vacuo* to give 20.7 mg of the residue. On TLC ($CHCl_3$:MeOH 7:3), the residue was found to be mainly geniposide (5) contaminated by 7-desoxyloganic acid (1) that was fed and a compound which seemed to be a sugar. The crude geniposide (5) was acetylated in the usual manner and the reaction product was chromatographed on silica gel (15 g, 1.5×17 cm) with ether as eluent and 10 ml each of the eluate was collected. Fractions

20) Heating of a solution of iridoid glucosides such as asperuloside (6), gardenoside (8), or monotropein (9), whose cyclopentane ring is in a high degree of oxidation and unsaturation, with a few drops of mineral acids gives a blue color, which is remarkably effective for the detection of this series of glucosides. The color test reported by Trim and Hill (*cf.* A.R. Trim and R. Hill, *Biochem. J.*, **50**, 310 (1952)) could be considered to be a variation of this reaction.

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No. 6—11 were combined and the solvent was removed *in vacuo*. The residue was rechromatographed on silica gel (10 g, 1.2×18 cm) under the same conditions. Fractions No. 4—5 gave 4.1 mg of colorless needles, mp 134—135°, which were identical with geniposide pentaacetate (mixed mp, TLC ether)). All the radioactive geniposide pentaacetate was mixed with carrier (64.6 mg) and repeatedly recrystallized from a mixture of $CHCl_3$, ether and petr. ether. The resulting crystals (8.7 mg) were diluted again with carrier (127 mg) and recrystallized from $CHCl_3$–hexane to constant activity.

Fractions No. 25—35 obtained by the chromatography No. 1 (gardenoside fraction) were combined and the solvent was removed *in vacuo*. The residue was chromatographed on a charcoal column (5 g each of charcoal and celite, 2.5×7 cm), eluted successively with water (fr. No. 1—2, 150 ml each), water:MeOH 9:1 (fr. No. 3, 250 ml), water: MeOH 8:2 (fr. No. 4, 300 ml), and water–MeOH 1:1 (fr. No. 5—9, 140 ml each). Fractions No. 5—9 were combined and the solvent was removed *in vacuo* to give a colorless syrup (247 mg), which was identified with an authentic sample of gardenoside (**8**) by TLC ($CHCl_3$:MeOH 7:3). This substance was subjected to catalytic hydrogenation over Pd-C until the solution had absorbed 15.5 ml of H_2. The reaction product was chromatographed on silica gel (20 g, 2.5×20 cm), eluted successively with $CHCl_3$ (fr. No. 1—4, 50 ml each), $CHCl_3$:MeOH 95:5 (fr. No. 5—8, 25 ml each), $CHCl_3$:MeOH 93:7 (fr. No. 9—13, 25 ml each), $CHCl_3$:MeOH 9:1 (fr. No. 14—21, 20 ml each), $CHCl_3$:MeOH 85:15 (fr. No. 22—30, 20 ml each) and finally $CHCl_3$:MeOH 8:2 (fr. No. 31—42, 20 ml each). Fractions No. 9—15 gave 7-desoxyloganin (**13**) as colorless needles (57.0 mg). Although this substance (**13**) was obtained by catalytic hydrogenation of **8**, the possibility of a conta mination with trace of **13** formed by the *in vivo* methylation of [10-^{3}H]-7-desoxyloganic acid (**1**) fed to the plant could not entirely be excluded. Therefore, further examinations of this substance were hampered. Fractions No. 27—42 were combined and the solvent was removed *in vacuo* to give a colorless syrup (120 mg). This was identified with an authentic sample of dihydrogardenoside by TLC ($CHCl_3$:MeOH 7:3). The dihydrogardenoside (120 mg) was acetylated by the conventional method to give 171 mg of colorless needles, mp 143—145°, which were identical with dihydrogardenoside pentaacetate (mixed mp, TLC ether). An aliquot of the radioactive dihydrogardenoside pentaacetate (8.53 mg) was diluted with carrier (68.5 mg) and repeatedly recrystallized from a mixture of CH_2Cl_2, ether and petr. ether to constant activity.

Administration of [10-^{3}H]-7-Desoxyloganic Acid (1) to *Daphniphyllum macropodum* and Isolation of [10-^{3}H]-Asperuloside (6)——[10-^{3}H]-7-Desoxyloganic acid (**1**) (2.32 mg, spec. activity 1.29×10^9 dpm/mmole) was dissolved in water (10 ml) and administered hydroponically and also through cotton wicks to two twigs (about 15 cm in length, having 8 and 10 leaves) of *D. macropodum* plants. Eight days after the beginning of the administration, the plants (wet weight 40 g) were cut into pieces and extracted with hot MeOH (4×200 ml). The extracts were combined and the solvent was removed *in vacuo*. The residue was dissolved in water (50 ml) and the insoluble material was filtered off. The filtrate was extracted with *n*-BuOH (4×30 ml). The *n*-BuOH extracts were combined and the solvent was removed *in vacuo*. The residue was chromatographed on a column of charcoal (2 g each of charcoal and celite) with water as eluent. Fractions positive to the mineral acid reaction were combined and the solvent was removed *in vacuo*. The residue was dissolved in EtOH, treated with active charcoal and filtered. One half of the filtrate was concentrated *in vacuo* to give 211 mg of the crude glucoside, which was acetylated by the usual method and the resulting acetate was recrystallized from EtOH to give colorless needles (75 mg), mp 154—155°. This substance was identified with an authentic sample of asperuloside tetraacetate (**11**) by a mixed melting point and TLC (ether). It was repeatedly recrystallized from EtOH to constant activity.

Chemical Conversion of [10-^{3}H]-Asperuloside Tetraacetate (11) to [10-^{3}H]-7-Desoxyloganin (13)——[10-^{3}H]-Asperuloside tetraacetate (**11**) (173 mg) was subjected to catalytic hydrogenation over Pd-C. The reduction product was methylated with CH_2N_2–ether. After removal of the solvent *in vacuo*, the resulting residue was mixed with carrier 7-desoxyloganin tetraacetate (103 mg) and recrystallized from aq. EtOH to give colorless needles (181 mg), mp 116—117°. These were identified with an authentic sample of 7-desoxyloganin tetraacetate by a mixed melting point and TLC (ether). The [10-^{3}H]-7-desoxyloganin tetraacetate (161 mg) was dissolved in anhyd. MeOH (5 ml) and anhyd. methanolic 0.1 N CH_3ONa (0.5 ml) was added. After refluxing for 15 min, the solution was cooled immediately and neutralized with Amberlite IRC-50 (H-form). After removal of the resin, the solvent was removed *in vacuo* and the residue was recrystallized form AcOEt to give 7-desoxyloganin (**13**) as colorless needles (115 mg), mp 156—157°. This substance was identified with an authentic sample by a mixed melting point and TLC ($CHCl_3$:MeOH 7:3). Kuhn–Roth oxidation of the 7-desoxyloganin (**13**) (115 mg) revealed that the resulting acetic acid contained 98% of the radioactivity of the intact 7-desoxyloganin tetraacetate.

Administration of [10-^{3}H]-7-Desoxyloganic Acid (1) to *Aucuba japonica* and Isolation of [10-^{3}H]-Aucubin (10)——[10-^{3}H]-7-Desoxyloganic acid (**1**) (1.99 mg, spec. activity 1.29×10^9 dpm/mmole) was dissolved in water (4.5 ml) and administered hydroponically and also through cotton wicks to a twig (15 cm in length, 12 leaves) of an *A. japonica* plant. Eight days after the beginning of the administration, the plant (wet weight 50 g) was cut into pieces and extracted with MeOH (3×250 ml). The extracts were combined and the solvent was removed *in vacuo*. Water (100 ml) was added to the residue and the insoluble material was filtered off. The filtrate was washed three times with AcOEt (total 150 ml) and the aq. layer was concentrated *in vacuo*. The residue was subjected to chromatography on charcoal (2 g each of charcoal and celite)

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with water as eluent collecting 100 ml fractions. Fractions No. 2—4 were combined and the solvent was removed *in vacuo*. The residue was extracted five times with EtOH (total 50 ml). One half of the ethanolic extract was concentrated *in vacuo* to give 98.5 mg of a syrupy residue, which showed on TLC ($CHCl_3$:MeOH 7:3) a single spot corresponding to that of an authentic sample of aucubin (**10**). This substance was acetylated by the usual method and the reaction product was recrystallized twice from the mixture of ether–petr. ether to give colorless needles (40.1 mg), mp 127—128°, which were identified with an authentic sample of aucubin hexaacetate by a mixed melting point and TLC (ether). An aliquot of the radioactive aucubin hexaacetate (21.5 mg) was mixed with carrier (64.5 mg) and repeatedly recrystallized from the same solvent mixture to constant activity.

Administration of [10-^{3}H]-Loganin (4) to *Daphniphyllum macropodum* and Isolation of [10-^{3}H]-Asperuloside (6)——Biosynthetically derived [10-^{3}H]-loganin (**4**) (12.6 mg, spec. activity 1.95×10^7 dpm/mmole) was administered hydroponically and also by the cotton wick method to a twig (about 25 cm in length, 10 leaves) of a *D. macropodum* plant. Seven days after the beginning of the administration, the plant (wet weight 25 g) was cut into pieces. Working up on the same way as in the case of the administration of **1** to this plant gave radioactive asperuloside (**6**) (101 mg). Asperuloside tetraacetate (**11**), mp 154—155°, obtained by the conventional acetylation of this substance was identical with an authentic sample of **11** (mixed melting point, TLC (ether). This compound was recrystallized from EtOH to constant activity.

Catalytic Hydrogenation of Asperuloside Tetraacetate (11) with Tritium (Formation of [10-^{3}H]-10-desoxygeniposidic Acid Tetraacetate)——To a solution of asperuloside tetraacetate (**11**) in AcOEt (3 ml) was added 5% Pd-C (100 mg) and the mixture was stirred in a $^3H_2$ atmosphere (*ca.* 0.25 Ci). After stirring overnight, H_2 (20 ml) was introduced and the stirring was continued for another 1 hr. Working up in a similar way as in the preparation of the [10-^{3}H]compound (**12**) gave a syrupy reaction product, which was found to be a mixture of 7-desoxyloganic acid tetraacetate (**12**) and 10-desoxygeniposidic acid tetraacetate by TLC (silica gel impregnated with 17% $AgNO_3$, $CHCl_3$:acetone:MeOH 90:9:1). An aliquot of the mixture was diluted with carrier consisting of the mixture of the tetraacetates of both 7-desoxyloganic acid (**1**) and 10-desoxygeniposidic acid (3:1, 40.7 mg), dissolved in MeOH (5 ml) and methylated with CH_2N_2–ether. The solvent was removed and the residue was diluted again with the same carrier (500 mg) and recrystallized from aq. MeOH to give colorless needles (392 mg).

Epoxidation of [10-^{3}H]-10-Desoxygeniposide Tetraacetate (14)——[10-^{3}H]-10-Desoxygeniposide tetraacetate (**14**) containing about one fourth of 7-desoxyloganin tetraacetate (195 mg) was dissolved in anhyd. benzene (5 ml), a benzene solution of perbenzoic acid (34 mg perbenzoic acid/ml, 2.6 ml) was added and the mixture was allowed to stand overnight at room temperature. The mixture was poured into aq. 2N NaOH (15 ml) and the aq. layer was washed with benzene. The benzene layer was washed with water and dried over anhyd. $MgSO_4$. After removal of the solvent *in vacuo*, the resulting colorless syrup was subjected to chromatography on silica gel (20 g, 2.5×11 cm) with ether as eluent and 20 ml fractions were collected. Fractions No. 3—7 gave a mixture of α- and β-epoxide (**15**) (120 mg).

Preparation of [10-^{3}H]-7-Dehydrologanin Tetraacetate (16)——The mixture of both [10-^{3}H]-epoxides (**15**) (24 mg) was dissolved in anhyd. benzene (5 ml) without further purification and mixed with 47% BF_3–etherate (0.05 ml). The solution was allowed to stand for 1 hr at room temperature, washed with water (5 ml) and dried over anhyd. $MgSO_4$. After removal of the solvent *in vacuo*, the residue was recrystallized from EtOH to give colorless needles (17.7 mg), mp 103—105° (lit.[16] mp 108°). This substance was identified with an authentic sample of 7-dehydrologanin tetraacetate (**16**) by TLC (ether).

Preparation of [10-^{3}H]-7-Epiloganin (18)——To a solution of [10-^{3}H]-7-dehydrologanin tetraacetate (**16**) (45.7 mg) in dioxane (5 ml) was added $NaBH_4$ (11 mg) and then water (4 drops). After stirring for 1 hr, AcOH (3 drops) was added to the mixture and the solvent was removed *in vacuo*. The resulting residue was extracted with five 3 ml portions of $CHCl_3$ and the extracts were combined, washed with water, and dried over anhyd. $MgSO_4$. Removal of the solvent *in vacuo* gave a colorless oil (40 mg), which was chromatographed on silica gel (10 g, 1.5×16 cm) with ether as eluent collecting 10 ml fractions. Fractions No. 11—12 gave colorless needles (4.1 mg), which were identified with an authentic sample of 7-epiloganin tetraacetate (**17**) by TLC (ether). To a solution of this substance (**17**) (17.9 mg) in anhyd. MeOH (3 ml) was added an anhyd. methanolic solution of 0.1 N NaOMe (0.07 ml). Immediately after refluxing for 15 min, the reaction mixture was cooled in ice-water and neutralized with Amberlite IRC-50 (H-form). The ion exchange resin was filtered off, the filtrate was evaporated to dryness *in vacuo* and the resulting residue was dissolved in water (2 ml). After washing with three 3 ml portions of ether, the aq. layer was concentrated *in vacuo* to give a colorless syrup (10.3 mg), which was identified with an authentic sample of 7-epiloganin (**18**) by TLC ($CHCl_3$:MeOH 7:3). Radio TLC of **18** showed a single peak corresponding to a spot of authentic **18**.

Administration of [10-^{3}H]-7-Epiloganin (18) to Daphniphyllum macropodum and Isolation of [10-^{3}H]-Asperuloside (6)——[10-^{3}H]-7-Epiloganin (**18**) (10.3 mg, spec. activity 3.02×10^7 dpm/mmole) was dissolved in water (2 ml) and administered hydroponically and also through cotton wicks as described previously to a twig (about 35 cm in length, 12 leaves) of *D. macropodum* plant in September. Nine days after the beginning of the administration, the plant (wet weight 16 g) was treated as shown in the administration experiment of [10-^{3}H]-loganin (**4**) with the same plant giving crude radioactive asperuloside (**6**). Acetylation of this substance gave asperuloside tetraacetate (**11**) as colorless needles (39.0 mg), mp 154—155°. The radio-

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active asperuloside tetraacetate (**11**) was repeatedly recrystallized from EtOH to constant activity.

Preparation of [7-^{3}H]-7-Epiloganin (18)——To a solution of 7-dehydrologanin tetraacetate (**16**) (147 mg) in dioxane (18 ml) were added NaB^3H_4 (5 mCi, spec. activity 102 mCi/mmole), $NaBH_4$ (38.3 mg) and water (0.5 ml) and the mixture was stirred for 1 hr at room temperature. After addition of AcOH (3 drops) the solvent was removed *in vacuo*. The residue was dissolved in MeOH (5 ml) and the solvent was again removed under reduced pressure. This procedure was repeated three times. The resulting residue was extracted with five 5 ml portions of $CHCl_3$. The $CHCl_3$ extracts were combined, washed with water, dried over anhyd. $MgSO_4$ and the solvent was removed *in vacuo* to give colorless needles (138 mg), which were identified with an authentic sample of 7-epiloganin tetraacetate (**17**) by TLC (ether). Radio TLC of this substance showed a single peak corresponding to a spot of **17**. The combined solution of radioactive **17** (29.6 mg) in anhyd. MeOH (6 ml) and an anhyd. methanolic solution of 0.1 N CH_3ONa (0.12 ml) was refluxed for 10 min. Immediately after that, the reaction mixture was cooled in ice-water and neutralized with Amberlite IRC-50 (H-form). The ion exchange resin was filtered off and the filtrate was concentrated *in vacuo* to give a colorless syrup (22.6 mg), which was identified with an authentic sample of 7-epiloganin (**18**) by TLC ($CHCl_3$:MeOH 7:3).

Preparation of [7-^{3}H]-Loganin (4)——To a solution of [7-^{3}H]-7-epiloganin tetraacetate (**17**) (65.6 mg) in anhyd. pyridine (1.5 ml) was added *p*-toluenesulfonyl chloride (176 mg) and the solution was allowed to stand overnight at room temperature. Addition of ice-water (30 ml) to the reaction mixture resulted in the formation of a white precipitate, which was collected by centrifugation. Recrystallization of the precipitate from EtOH gave colorless needles (56.0 mg), mp 116—117°. This substance was identified with an authentic sample of the tosylate of 7-epiloganin tetraacetate by a mixed melting point and TLC (ether). To a solution of the tosylate (50.9 mg) in anhyd. acetone (8 ml) was added tetraethylammonium acetate (120 mg) and the mixture was refluxed for 22 hr. After removal of the solvent *in vacuo*, the residue was dissolved in $CHCl_3$ (10 ml), washed successively with water (3×10 ml), saturated aq. NaCl (3×10 ml) and water (3×10 ml), dried over anhyd. $MgSO_4$ and the solvent was removed *in vacuo*. The residue was chromatographed on silica gel (40 g, 2.5×22 cm) with ether as eluent collecting 15 ml fractions. Fractions No. 10—12 were combined and the solvent was removed *in vacuo*. Three recrystallizations of the residue from EtOH gave colorless needles (16.4 mg), mp 139—139.5°, which were identified with an authentic sample of loganin pentaacetate (**19**) by a mixed melting point and TLC (ether). To a solution of this radioactive substance (**19**) (14.0 mg) and carrier (**19**) (19.0 mg) in anhyd. MeOH (7 ml) was added anhyd. methanolic 0.1 N CH_3ONa (0.15 ml) and the solution was refluxed for 15 min, cooled in ice-water and neutralized with Amberlite IRC-50 (H-form). The ion exchange resin was filtered off and the filtrate was concentrated *in vacuo* to give a colorless syrup (13.2 mg), which was identified with an authentic sample of loganin (**4**) by TLC ($CHCl_3$:MeOH 7:3).

Administration of [7-^{3}H]-Loganin (4) to Daphniphyllum macropodum and Isolation of [7-^{3}H]-Asperuloside (6)——[7-^{3}H]-Loganin (**4**) (3.3 mg, spec. activity 3.41×10^9 dpm/mmole) was dissolved in water (2 ml) and administered hydroponically and also through cotton wicks to a twig (about 30 cm in length, 15 leaves) of a *D. macropodum* plant in March. This administration experiment was carried out simultaneously with that of [7-^{3}H]-7-epiloganin (**18**) described later. Eleven days after the beginning of the administration, the plant (wet weight 37 g) was subjected to the conventional isolation procedure and asperuloside tetraacetate (**11**) was obtained as colorless needles (269 mg), mp 154—155°, which were repeatedly recrystallized from EtOH to constant activity.

Administration of [7-^{3}H]-7-Epiloganin (18) to *Daphniphyllum macropodum* and Isolation of Asperuloside (6)——[7-^{3}H]-7-Epiloganin (**18**) (5.65 mg, spec. activity 3.16×10^9 dpm/mmole) was administered to a twig (about 25 cm in length, with 13 leaves) of a *D. macropodum* plant. The plant (wet weight 23 g) was treated in the usual way and asperuloside tetraacetate (**11**) was obtained as colorless needles (244 mg), mp 154—155°. Repeated recrystallizations of this substance from EtOH gave non-radioactive **11**.

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