[Chem. Pharm. Bull. 20(6)1305—1311(1972)]

UDC 547.596' 918.04

Studies on Monoterpene Glucosides and Related Natural Products. XVIII.[1] Formation Sequences of Iridoid Glucosides in highly Oxidized Levels[2]

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(Received December 11, 1971)

The oxidative sequences for the formation of several iridoid glucosides from 7-desoxyloganic acid *via* loganic acid have been established.

In the preceding paper we reported that 7-desoxyloganic acid (**1**) is a precursor of several iridoid glucosides. Assuming that loganic acid (**2**) (or loganin (**3**)) is located between 7-desoxyloganic acid (**1**) and highly oxidized iridoid glucosides in the biosynthetic pathway, further administration experiments have been carried out, which proved that this assumption in the case of the biosynthesis of asperuloside (**10**) is correct. From the results of the administration of **1** to *Gardenia jasminoides* forma *grandiflora* (Lour.) Makino, it was further confirmed that gardenoside (**9**) is formed *via* geniposide (**5**).

By taking into consideration of the above results together with the structure of several iridoid glucosides the biosynthetic pathways shown in Chart 1 have been suggested.

As previously reported, hydroxylation of **1** leads to **2** or **3**.[4] Dehydration of **2** could give 10-desoxygeniposidic acid (**4**). Oxidation of the methyl group at C-8 of **4**, that is, the allylic position to the C-7 double bond, would give geniposidic acid (the acid corresponding to **5**), while oxidation of C-6, another allylic position of **4**, would give deacetylasperulosidic acid (**6**) or its C-6 epimer, scandoside (**7**). Allylic rearrangement between C-6 and C-8 of compound **6** or **7** could afford monotropein (**8**) or gardenoside (**9**). On the other hand, **6** would be converted to asperuloside (**10**) and paederoside (**11**).[5] Hydroxylation at C-5 of geniposide (**5**) could yield theviridoside (**12**). Aucubin (**13**) lacking the carboxyl group at C-4 is deduced from its absolute configuration to be formed through the decarboxylation of scandoside (**7**) as its direct precursor. Aucubin (**13**) could be further converted to catalpol (**14**) by epoxidation and to catalposide (**15**).

This paper describes administration experiments with several labelled iridoid glucosides regarded as intermediates in order to confirm the supposed pathway.

Among the labelled compounds employed in these experiments, [10-^{3}H]-10-desoxygeniposidic acid (**4**) and [10-^{3}H]-geniposide (**5**) were prepared according to routes outlined below.

Asperuloside tetraacetate (**16**) was subjected to catalytic hydrogenation over Pd-C with tritium in ethyl acetate and the product was chromatographed on silica gel impregnated with silver nitrate to give the desired [10-^{3}H]-10-desoxygeniposidic acid tetraacetate (**17**) which was completely free from 7-desoxyloganic acid tetraacetate (the 7,8-dihydro compound of

1) Part XVII: H. Inouye, S. Ueda, Y. Aoki, and Y. Takeda, *Chem. Pharm. Bull.* (Tokyo), **20**, (1287) (1972).
2) A part of this work has been published in a preliminary form. H. Inouye, S. Ueda, and Y. Takeda, *Tetrahedron Letters*, **1970**, 3351.
3) Location: *Yoshida-shimoadachi-cho, Sakyo-ku, Kyoto.*
4) Throughout the discussion of this work, free acids and the corresponding methyl esters are tentatively regarded to be equivalent. As Coscia's recent work also suggested, whether or not methylation and demethylation occurs at each step is a rather delicate problem which has to be studied further. However, as far as the compounds treated in this work are concerned, we may regard them as equivalent. cf. K.M. Madyastha, R. Guarnaccia, and C.J. Coscia, *Febs Letters*, **14**, 175 (1971).
5) The lactone ring of **10** could also be formed by a direct attack of the carboxyl group of geniposidic acid on the C-6 position.

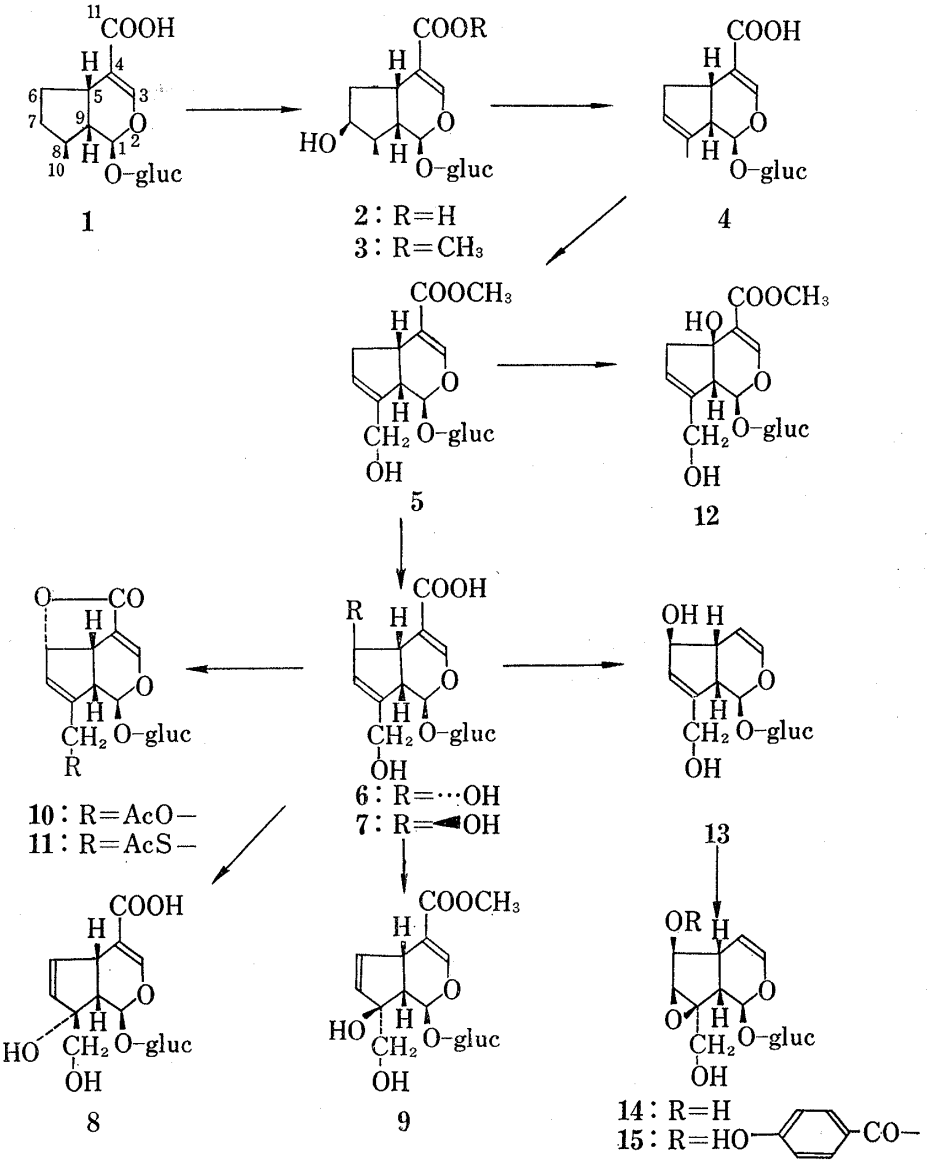
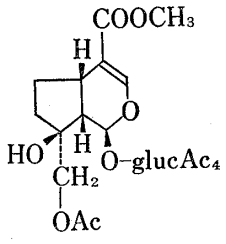


Chart 1



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17). Compound (**17**) was deacetylated with a saturated methanolic solution of $Ba(OH)_2$ to yield [10-^{3}H]-10-desoxygeniposidic acid (**4**).

On the other hand, [10-^{3}H]-**9** obtained biosynthetically by administration of [10-^{3}H]-**1** to *Gardenia* plant was subjected to a catalytic hydrogenation over Pd-C to give radioactive dihydrogardenoside, which was then acetylated to yield its pentaacetate (**18**). Dehydration with $POCl_3$-pyridine followed by deacetylation gave [10-^{3}H]-**5**.[6]

Both labelled compounds, [10-^{3}H]-desoxygeniposidic acid (**4**) and [10-^{3}H]-geniposide (**5**) were administered respectively to *Daphniphyllum macropodum* Miq. and asperuloside (**10**) was isolated and purified as its acetate. As shown in Table I, the incorporations of **4** and **5** into **10** were 0.24 and 1.6%, respectively. Taking into consideration of the results of this

6) H. Inouye and S. Saito, *Tetrahedron Letters*, **1969**, 2347.

Table I. Administration Experiments with [10-^{3}H]-10-Desoxygeniposidic Acid (**4**), [10-^{3}H]-Geniposide (**5**), [10-^{3}H]-Scandoside (**7**) and [10-^{3}H]-Aucubin (**13**)

Plant	Glucoside fed amt. & spec. activity, dpm/mmole	Glucoside isolated spec. activity, dpm/mmole	Incorporation %
Daphniphyllum macropodum	[10-^{3}H]-10-desoxygeniposidic acid (**4**) 9.97 mg, 1.12×10^{10}	asperuloside (**10**) 8.10×10^5	0.24
Daphniphyllum macropodum	[10-^{3}H]-geniposide (**5**) 20.4 mg, 5.40×10^7	asperuloside (**10**) 6.80×10^4	1.6
Cerbera manghus	[10-^{3}H]-geniposide (**5**) 28.3 mg, 2.44×10^7	theviridoside (**12**) 2.68×10^4	0.42
Gardenia jasminoides	[10-^{3}H]-scandoside (**7**) 2.0 mg, 1.74×10^{10}	gardenoside (**9**) 1.02×10^6	0.47
Aucuba japonica	[10-^{3}H]-scandoside (**7**) 2.0 mg, 1.74×10^{10}	aucubin (**13**) 2.41×10^4	0.04
Catalpa ovata	[10-^{3}H]-aucubin (**13**) 7.52 mg, 6.60×10^7	catalposide (**15**) 8.24×10^3	0.29

experiment together with those of the incorporation of **3** into **10**, reported in the preceding paper,[1] the following biosynthetic pathway could be established: 7-desoxyloganic acid (**1**)→loganic acid (**2**)→10-desoxygeniposidic acid (**4**)→geniposidic acid (acid corresponding to **5**)—asperuloside (**10**).

[10-^{3}H]-Geniposide (**5**) was then administered to *Cerbera manghus* L. and radioactive **12**[7] was isolated, which was further purified as its pentaacetate. Thus, as demonstrated in Table I, this experiment established the incorporation of **5** into **12**. It also supports the absolute configuration of **12** proposed by Sticher and Schmid.

As described previously, besides **12** both **6** and **7** are compounds in the next oxidation stage higher than that of **5** (and/or the corresponding acid). They are also assumed precursors for **8** or **9**.

Therefore, radioactive **7** was administered to *Gardenia jasminoides* and its incorporation into **9** was examined. [10-^{3}H]-Labelled **7** had been obtained biosynthetically by feeding of [10-^{3}H]-7-desoxyloganic acid to *Paederia scandens* (Lour.) Merrill var. *mairei* (Léveillé) Hara. Administration of [10-^{3}H]-scandoside (**7**) to *G. jasminoides* gave radioactive **9** which was subjected to catalytic hydrogenation followed by acetylation giving dihydrogardenoside pentaacetate (**18**). The results are presented in Table I, which reveal that 0.47% of the compound (**7**) was incorporated into **9** establishing the following biosynthetic sequence: geniposide(**5**)→scandoside (**7**)→gardenoside (**9**).

Between both C-6 epimers, deacetylasperulosidic acid (**6**) and scandoside (**7**), and both C-8 epimers, monotropein (**8**) and gardenoside (**9**), the correlations **6**—**8** and **7**—**9** are thus envisaged. Recent isolation of asperuloside (**10**) and monotropein (**8**) from *Asperula odorata* L. reported by Sticher[8] suggests the formation of **8** *via* **10**. However, any convincing conclusion on the relationship could not be drawn without experimental proof as epimerization of the alcoholic compound could also take place as is the case at the incorporation of 7-epiloganin into asperuloside **10**.[1]

We will now describe experimental results on the biosynthesis of a series of glucosides, *e.g.* aucubin (**13**) and catalposide (**15**). Based on the considerations so far described, [10-^{3}H]-

7) Theviridoside (**12**) has been isolated from *Thevetia peruviana* along with theveside. *cf.* O. Sticher and H. Schmid, *Helv. Chim. Acta*, **52**, 478 (1969); O. Sticher, *Tetrahedron Letters*, **1970**, 3195. Recently, we also have isolated both glucosides form *Cerbera manghus*. H. Inouye and T. Nishimura, *Phytochem.*, **11**, 1852 (1972).

8) O. Sticher, *Pharmaceutica Acta Helv.*, **46**, 121 (1971).

scandoside (**7**) was administered to *Aucuba japonica* Thunb. and radioactive aucubin (**13**) was isolated, which was further purified as its hexaacetate. The incorporation of **7** into **13** was 0.04% (Table I). As the value was too low compared with those obtained in feeding experiments with other glucosides, we further administered [10-^{3}H]-7-desoxyloganic acid (**1**) to *Aucuba* plant and tried to trap radioactive **7** by the dilution analysis in order to confirm this compound (**7**) as the precursor. Thereby, for further purification, **7** was converted to the crystalline hexaacetate methyl ester. This experiment made it apparent that at least 0.11% of the radioactive **1** was present as **7** in the *Aucuba* plant. [10-^{3}H]-Aucubin (**13**) was subsequently administered to *Catalpa ovata* G. Don and the isolated catalposide (**15**) was purified as its acetate. The incorporation was 0.29% (*cf.* Table I). The following biosynthetic sequence was established as the results of the administration experiments: scandoside (**7**)→ aucubin (**13**)→catalpol (**14**), catalposide (**15**).

In this way, the biosynthetic pathway shown in Chart 1 has almost been proved. In plant organisms, however, there might be many other variational pathways, the clarification of which will be one of the remaining subjects.

Experimental[9]

Preparation of [10-^{3}H]-10-Desoxygeniposidic Acid (4)——An aliquot of the mixture[1] consisting of [10-^{3}H]-10-desoxygeniposidic acid tetraacetate (**17**) and [10-^{3}H]-7-desoxyloganic acid tetraacetate (about 8 mCi) obtained by the catalytic hydrogenation of asperuloside tetraacetate (**16**) with $^3H_2$ over Pd-C in AcOEt was diluted with carrier (**17**) including one fifth of 7-desoxyloganic acid tetraacetate (212 mg) and subjected to chromatography on silica gel impregnated with 10% $AgNO_3$ (40 g, 2.5×15 cm) eluted with benzene: acetone (9:1). Fractions of 25 ml were collected. Each fraction was monitored by thin-lyer chromatography (TLC) on silica gel impregnated with 17% $AgNO_3$, and $CHCl_3$:Me_2CO:acetone (90:9:1). The spot was visualized by spraying ethanolic 50% H_2SO_4 followed by heating. Fractions No. 14—17 gave a single spot corresponding to that of **17**. They were combined and the solvent was removed *in vacuo* to give [10-^{3}H]-10-desoxygeniposidic acid tetraacetate (**17**) (19.5 mg) as a colorless syrup. This was diluted with carrier (20.2 mg) and recrystallized twice from aq. EtOH The resulting crystals were diluted again with carrier (17.0 mg) and recrystallized twice from aq. EtOH to give [10-^{3}H]-10-desoxygeniposidic acid tetraacetate (**17**) (45.0 mg) as colorless needles. This substance was dissolved in MeOH (5 ml) and the pH of the solution was adjusted to 10 with a saturated methanolic solution of $Ba(OH)_2$. After standing for 3 hr, the solution was neutralized with Amberlite IR-120 (H-form) and the ion exchange resin was filtered off. The filtrate was concentrated *in vacuo* to give a colorless syrup (33.2 mg). This substance was identified with an authentic sample of 10-desoxygeniposidic acid (**4**) by TLC ($CHCl_3$:MeOH 7:3).

Preparation of [10-^{3}H]-Geniposide (5)——[10-^{3}H]-Dihydrogardenoside pentaacetate (**18**) obtained by the catalytic hydrogenation of [10-^{3}H]-gardenoside (**9**) over Pd-C followed by the acetylation was dissolved in anhyd. pyridine (1.5 ml) and $POCl_3$ (0.3 ml) was added. After standing overnight in a refrigerator, the reaction mixture was poured into ice-water. The resulting white precipitate was extracted with three 10 ml portions of $CHCl_3$. The $CHCl_3$ extracts were combined and dried over anhyd. $MgSO_4$. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (30 g, 2.5×17 cm) with ether as eluent collecting 20 ml each of the fraction. Fractions No. 5—7 were combined and the solvent was evaporated *in vacuo*. The residue was repeatedly recrystallized from a mixture of CH_2Cl_2, ether and petr. ether to give [10-^{3}H]-geniposide pentaacetate as colorless needles (119 mg), mp 134—135°. This substance was identified with an authentic sample by a mixed melting point and TLC (ether). To a solution of the acetate (24 mg) in anhyd. MeOH (4 ml) was added anhyd. methanolic solution of 0.1N CH_3ONa (0.15 ml) and heated under reflux for 8 min. The solution was then cooled immediately 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 (20.6 mg). This substance was identified with an authentic sample of **5** by TLC ($CHCl_3$: MeOH 7:3).

Administration of [10-^{3}H]-10-Desoxygeniposidic Acid (4) to *Daphniphyllum macropodum* and Isolation of Asperuloside (10)——[10-^{3}H]-10-Desoxygeniposidic acid (**4**) (9.97 mg, spec. activity 1.12×10^{10} dpm/mmole) was dissolved in water (3 ml) and administered hydroponically and also through cotton wicks to a twig (about 25 cm in length with 9 leaves) of a *D. macropodum* plant in February. Eight days after the beginning of the administration, the plant (wet weight 33 g) was cut into pieces and extracted with four 250 ml portions of MeOH under reflux for 20 min. The MeOH extracts were combined and the solvent was removed *in vacuo*.

9) See footnote 18 in the preceding paper.[1]

The residue was extracted with water (total 100 ml) and the aqueous extracts was concentrated to about 30 ml under reduced pressure. It was then extracted with four 50 ml portions of *n*-BuOH. The *n*-BuOH extracts were combined and evaporated to dryness *in vacuo*. The residue was chromatographed on a charcoal column (7 g each of charcoal and celite, 2.5×13 cm), eluted successively with water (fr. No. 1—4), water: MeOH (9:1) (fr. No. 5), water:MeOH (4:1) (fr. No. 6), water:MeOH (1:1) (fr. No. 7) and MeOH (fr. No. 8—13) collecting 100 ml fractions. Fractions No. 8—13, which were positive to the mineral acid reaction[1] were combined and the solvent was removed *in vacuo* to give a somewhat crude **10** (416 mg) which was acetylated with Ac_2O and pyridine by the usual method. The resulting acetates was chromatographed on silica gel (40 g, 2.5×20 cm) with ether as eluent and 25 ml fractions were collected. Fractions No. 7—14 were combined and the solvent was removed *in vacuo*. The residue was recrystallized from EtOH to give 534 mg of colorless needles, mp 154—155°, which were identified with an authentic sample of asperuloside tetraacetate (**16**) by a mixed melting point and TLC (ether). This compound was repeatedly recrystallized from EtOH to constant activity.

Administration of [10-^{3}H]-Geniposide (5) to *Daphniphyllum macropodum* and Isolation of Asperuloside (10)——[10-^{3}H]-Geniposide (**5**) (20.4 mg, spec. activity 5.40×10^7 dpm/mmole) was dissolved in water (1 ml) and administered to a twig (about 20 cm in length with 10 leaves) of a *D. macropodum* plant in July. Eight days after the beginning of the administration, the plant (wet weight 55 g) was cut into pieces. Extraction and isolation were carried out as described before yielding somewhat crude **10** (505 mg) as colorless oil. Acetylation of this substance gave asperuloside tetraacetate (**16**) (387 mg) as colorless needles, mp 154—155°. This compound was repeatedly recrystallized to constant activity.

Administration of [10-^{3}H]-Geniposide (5) to Cerbera manghus and Isolation of Theviridoside (12)——[10-^{3}H]-Geniposide (**5**) (28.3 mg, spec. activity 2.44×10^7 dpm/mmole) was dissolved in water (2 ml) and fed hydroponically to a twig (about 20 cm in length with 11 leaves) of a *C. manghus* plant. Eight days after the beginning of the administration, the plant (wet weight 21 g) was cut into pieces and extracted with four 200 ml portions of MeOH under reflux for 30 min. The MeOH extracts were combined and the solvent was evaporated *in vacuo*. The residue was extracted five times with water (total 60 ml). The aq. extracts were combined and washed with three 25 ml portions of AcOEt. The aq. layer was concentrated *in vacuo*. The residue was chromatographed on silica gel (40 g, 2.5×20 cm), eluted successively with $CHCl_3$ (fr. No. 1—6), $CHCl_3$: MeOH (95:5) (fr. No. 7—12), $CHCl_3$:MeOH (9:1) (fr. No. 13—20) and $CHCl_3$:MeOH (85:15) (fr. No. 21—39) and fractions of 25 ml were collected. Fractions No. 21—37 were combined and the solvent was removed *in vacuo* to give crude theviridoside (**12**) as a brownish syrup. Yield 146 mg. The crude glucoside was acetylated by the usual method with Ac_2O and pyridine. The reaction product was chromatographed on silica gel (20 g, 1.5×20 cm) with ether as eluent and fractions of 15 ml were collected. Fractions No. 11—22 were combined and the solvent was removed *in vacuo*. The residue was recrystallized from aq. EtOH to give 139 mg of colorless needles, mp 123—124°. This substance was identified with an authentic sample of theviridoside pentaacetate by a mixed melting point and TLC (ether). The radioactive theviridoside pentaacetate was repeatedly recrystallized from aq. EtOH to constant activity.

Administration of [10-^{3}H]-Scandoside (7) to *Gardenia jasminoides*; Isolation of Gardenoside (9) and Its Conversion to Dihydrogardenoside Pentaacetate (18)——[10-^{3}H]-Scandoside (**7**) (2.0 mg, spec. activity 1.74×10^{10} dpm/mmole) was dissolved in water (1.5 ml) and administered hydroponically and also by the cotton wick method to a twig (about 15 cm in length, 12 leaves) of *G. jasminoides* plant. After 5 days administration, the plant (wet weight 8 g) was cut into pieces and extracted with four 150 ml portions of MeOH under reflux for 20 min. The MeOH extracts were combined and evaporated to dryness *in vacuo*. The residue was dissolved in water (40 ml) and the insoluble material was removed by filtration through a layer of celite. The celite layer was washed with water (total 20 ml), the filtrate and the washings were combined and then washed with three 30 ml portions of AcOEt. The aq. layer was evaporated to dryness *in vacuo* and the residue was chromatographed on silica gel (30 g, 2.5×15 cm), eluted successively with CH_2Cl_2 (fr. No. 1—6), CH_2Cl_2:MeOH (95:5) (fr. No. 7—10), CH_2Cl_2:MeOH (9:1) (fr. 11—15), CH_2Cl_2:MeOH (85:15) (fr. No. 16—21) and finally CH_2Cl_2:MeOH (4:1) (fr. No. 22—33) and fractions of 25 ml were collected. Fractions No. 20—32 were combined and concentrated *in vacuo*. The residue was chromatographed on a charcoal column (5 g each of charcoal and celite, 2.3×9 cm). The column was eluted successively with water (fr. No. 1), water: MeOH (9:1) (fr. No. 2), water:MeOH (4:1) (fr. No. 3), water:MeOH (1:1) (fr. No. 4—6) and finally with MeOH (fr. No. 7) collecting 200 ml fractions. Fractions No. 4—7 were combined and concentrated *in vacuo* to give a colorless syrup (199 mg). This substance was identified with an authentic sample of gardenoside (**9**) by TLC ($CHCl_3$:MeOH 7:3). The substance **9** was dissolved in MeOH and subjected to catalytic hydrogenation over Pd-C in the usual way. The product was chromatographed on silica gel (15 g, 2.5×8 cm) eluted successively with CH_2Cl_2 (fr. No. 1—6), CH_2Cl_2:MeOH (95:5) (fr. No. 7—10), CH_2Cl_2:MeOH (9:1) (fr. No. 11—18), CH_2Cl_2:MeOH (85:15) (fr. No. 19—24) and CH_2Cl_2:MeOH (4:1) (fr. No. 25—31) and fractions of 25 ml were collected. Fractions No. 18—30 were combined and the solvent was removed *in vacuo* to give a colorless syrup (131 mg). This substance was acetylated by the usual method. The reaction product was subjected to chromatography on silica gel (15 g, 1.5×15 cm) with ether as eluent and 10 ml fractions were collected. Fractions No. 5—19 were combined and the solvent was removed *in vacuo*. The residue was crystallized from a mixture of CH_2Cl_2–ether–petr. ether to give colorless needles (149 mg), mp

143—145°. This substance was identified with an authentic sample of dihydrogardenoside pentaacetate (**18**) by a mixed melting point and TLC (ether). It was repeatedly recrystallized from the same solvent mixture to constant activity.

Administration of [10-^{3}H]-Scandoside (7) to *Aucuba japonica* and Isolation of Aucubin (13)——[10-^{3}H]-Scandoside (**7**) (2.0 mg, spec. activity 1.74×10^{10} dpm/mmole) was dissolved in water (1.5 ml) and administered hydroponically and also through cotton wicks to a twig (about 25 cm in length with 11 leaves) of an *A. japonica* plant. After 8 days administration, the plant (wet weight 20.5 g) was cut into pieces and extracted with four 250 ml portions of MeOH under reflux for 20 min. The MeOH extracts were combined and the solvent was removed *in vacuo*. The residue was extracted four times with water (total 70 ml). After washing with three 40 ml portions of AcOEt, the aq. extract was evaporated *in vacuo* to dryness. The residue was chromatographed on a charcoal column (10 g each of charcoal and celite, 3×11.5 cm) and fractions of 250 ml were collected. The column was eluted successively with water (fr. No. 1—2), water:MeOH (9:1) (fr. No. 3), water:MeOH (4:1) (fr. No. 4—5) and water:MeOH (1:1) (fr. No. 6—7). Fractions No. 4—6, which were positive to the mineral acid reaction, were combined and concentrated *in vacuo* to give a colorless oil (671 mg). This substance was acetylated in the usual manner and the reaction product was chromatographed on silica gel (40 g, 2.3×18 cm) with ether as eluent and 25 ml fractions were collected. Fractions No. 3—5 were combined and recrystallized from the mixture of ether–petr. ether to give colorless needles (908 mg), mp 127—128°, which was identical with an authentic sample of aucubin hexaacetate (mixed melting point and TLC (ether)). It was repeatedly recrystallized from EtOH to constant activity.

Administration of [10-^{3}H]-7-Desoxyloganic Acid (1) to *Aucuba japonica*; Detection of Scandoside (7) by the Dilution Analysis——[10-^{3}H]-7-Desoxyloganic acid (**1**) (23.2 mg, spec. activity 2.97×10^{11} dpm/mmole) was dissolved in water (3 ml) and administered hydroponically and also by the cotton wick method to a twig (about 25 cm in length with 14 leaves) of an *A. japonica* plant in June. After 10 days administration, the plant (wet weight 30.8 g) was cut into pieces and extracted with four 250 ml portions of MeOH under reflux for 20 min. The MeOH extracts were combined and the solvent was evaporated *in vacuo*. The residue was dissolved in water (100 ml) and extracted three times with three 70 ml portions of *n*-BuOH. The *n*-BuOH layer and the aq. layer were worked up respectively.

i) Treatment of *n*-BuOH layer gave aucubin hexaacetate as colorless needles (28.1 mg), mp 127—128°, which were recrystallized to constant specific activity of 2.10×10^8 dpm/mmole. To a solution of the[10-^{3}H]-aucubin hexaacetate (9.0 mg) in anhyd. MeOH (1 ml) was added anhyd. methanolic 0.1 N CH_3ONa (0.1 ml) and the mixture was refluxed for 8 min. The reaction mixture was cooled immediately in ice-water and neutralized with Amberlite IRC-50 (H-form). After removal of the ion exchange resin, the solution was concentrated *in vacuo* to give a colorless syrup (7.6 mg) which was identified with an authentic sample of aucubin (**13**) by TLC ($CHCl_3$:MeOH 7:3).

ii) Treatment of the aq. layer: To the aq. layer described above was added non-radioactive **7** (499 mg) and the solvent was removed *in vacuo*. The residue was chromatographed on a charcoal column (15 g each of charcoal and celite, 3×16 cm) and eluted successively with water (300 ml), water:MeOH (9:1) (150 ml), water:MeOH (4:1) (200 ml), water:MeOH (1:1) (100 ml) and MeOH (1 liter). The fractions eluted with MeOH were combined and the solvent was removed *in vacuo* to give 986 mg of the residue, which was acetylated by the usual method. The reaction product was dissolved in MeOH (5 ml) and methylated with CH_2N_2–ether. After removal of the solvent *in vacuo*, the residue was chromatographed on silica gel (50 g, 2.5×25 cm) with ether as eluent and 30 ml fractions were collected. TLC (ether) of fractions No. 1—6 showed a spot corresponding to that of aucubin hexaacetate. Fractions No. 7—17 were combined and the solvent was removed *in vacuo*. The residue was rechromatographed on silica gel (30 g, 2×25 cm) with ether as eluent and 30 ml of fractions were collected. Fractions No. 6—10 were combined and the solvent was removed *in vacuo*. The residue was recrystallized from ether to give colorless needles (184 mg), mp 132—134°. This substance was identified with an authentic sample of scandoside hexaacetate methyl ester by a mixed melting point and TLC (ether). An aliquot of the radioactive scandoside hexaacetate methyl ester (15.5 mg) was diluted with carrier (299 mg) and repeatedly recrystallized from EtOH to constant specific activity of 2.83×10^7 dpm/mmole.

Administration of [10-^{3}H]-Aucubin (13) to *Catalpa ovata* and Isolation of Catalposide (15)——[10-^{3}H]-**13** (7.52 mg, spec. activity 6.60×10^7 dpm/mmole) was dissolved in water (2 ml) and administered hydroponically to a twig (about 25 cm in length with 8 leaves) of a *C. ovata* plant. After 5 days administration, the plant (wet weight 50 g) was cut into pieces and extracted with four 200 ml portions of MeOH under reflux for 25 min. The MeOH extracts were combined and the solvent was removed *in vacuo*. The residue was extracted with three 50 ml portions of water. The aq. extracts were combined and the solvent was evaporated *in vacuo*. The residue was dissolved in water (2 ml), subjected to chromatography on polyamide (15 g, 2×20 cm), and eluted successively with water (fr. No. 1, 100 ml), water:MeOH (9:1) (fr. No. 2, 80 ml), water:MeOH (4:1) (fr. No. 3, 60 ml), water:MeOH (7:3) (fr. No. 4, 80 ml), water:MeOH (1:1) fr. No. 5, 80 ml), and finally MeOH (fr. No. 6, 100 ml). Fractions No. 2—5 were combined and the solvent was removed *in vacuo*. The residue was chromatographed on silica gel (15 g, 2×13 cm), eluted successively with $CHCl_3$ (fr. No. 1—5), $CHCl_3$:MeOH (97:3) (fr. No. 6—10), $CHCl_3$:MeOH (93:7) (fr. No. 11—16) and $CHCl_3$:MeOH (9:1) (fr. No. 17—25) and 30 ml of fractions were collected. Fractions No. 17—

22 were combined and concentrated *in vacuo* to give a colorless syrup (259 mg). This substance was identified with an authentic sample of **15** by TLC ($CHCl_3$:MeOH 7:3). The catalposide(**15**)(253 mg) was acetylated by the usual method and the reaction product was recrystallized from EtOH to give colorless needles, mp 141—142°, which were identified with an authentic sample of catalposide hexaacetate by a mixed melting point and TLC (ether). They were repeatedly recrystallized to constant activity.

Acknowledgement This work was partly supported by a grant for scientific research from the Ministry of Education. The authors are grateful to Dr. O. Suzuka, Research and Developement Laboratory of Nippon Shinyaku Co., Ltd. for his kind gift of the plant materials. Thanks are also due to Professor H. Schmid of the University of Zurich and Dr. O. Sticher of E.T.H. for the generous supply of theviridoside.