[Chem. Pharm. Bull. 24(1) 79 — 84 (1976)] UDC 547.918.04 : 542.98 : 581.192

Studies on Monoterpene Glucosides and Related Natural Products. XXX.[1] The Fate of the C-8 Proton of 7-Deoxyloganic Acid in the Biosynthesis of Secoiridoid Glucosides

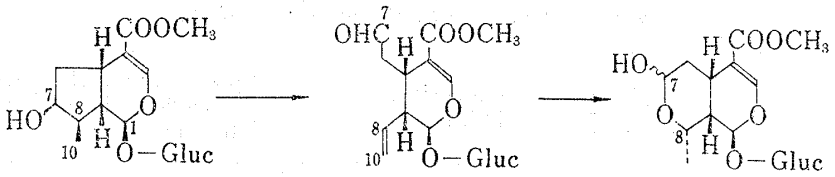
Yoshio Takeda and Hiroyuki Inouye

Faculty of Pharmaceutical Sciences, Kyoto University[2]

(Received May 14, 1975)

Administration experiments of (7,8-$^3H_2$)-7-deoxyloganic acid (**5**) into *Lonicera morrowii*, *Cornus officinalis* and *Gentiana thunbergii* plants revealed that the C-8 proton of (7,8-$^3H_2$)-**5** was retained in loganin (**2**), secologanin (**3**) and morroniside (**1**), respectively.

Previously, we have demonstrated that a secoiridoid glucoside morroniside (**1**) is biosynthesized *via* loganin (**2**) and secologanin (**3**), the C-7 proton of **2** being incorporated intact into the C-7 position of morroniside (**1**).[3]



2 **3** **1**

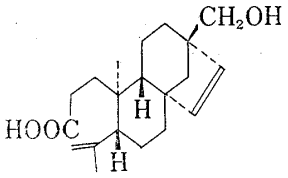
Chart 1. Biosynthetic Route leading to Morroniside (**1**)

Two mechanisms of the cyclopentane ring cleavage of loganin (**2**) has so far been proposed. The one involves the direct ring cleavage of loganin (**2**) and the other passes through 10-hydroxyloganin.[4] However, the conclusive biosynthetic experimental evidence for any route has not been obtained.

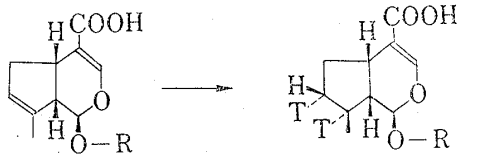
Regarding the chemical cleavage of the cyclopentane ring of the iridoid, research work on the reaction of all the four C-7 and C-8 stereoisomers of loganin aglucone 1-O-methyl ether with lead tetraacetate has been reported. In spite of the existing evidences which suggest that each of these compounds was once cleaved as a radical, the desired seco-type product was not obtained because of the recyclization of the radical.[5] Of the tosylates of 10-hydroxyloganin aglucone 1-O-methyl ether and the corresponding 7-epi derivative, only the latter was recently reported to be cleaved at the cyclopentane ring on treatment with alkali to yield the secologanin-type compound.[6]

As to the biosynthesis of the natural product of a seco-type structure, conversion of beyerene to 3,4-secobeyerene acid (**4**) through the cleavage of ring A of the 19-hydroxylated compound has been observed by the administration experiments using the tritium labeled compounds.[7]

1) Part XXIX: H. Inouye, K. Inoue, T. Nishioka, and M. Kaniwa, *Phytochemistry*, **14**, 2029 (1975).
2) Location: *Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto.*
3) H. Inouye, S. Ueda, and Y. Takeda, *Tetrahedron Letters*, **1971**, 4069; H, Inouye, S. Ueda, K. Inoue, and Y. Takeda, *Chem. Pharm. Bull.* (Tokyo), **22**, 676 (1974).
4) A.R. Battersby, *Pure and Applied Chem.*, **14**, 117 (1967).
5) J.J. Partridge, N.K. Chadha, S. Faber, and M.R. Uskoković, *Syn. Commun.*, **1**, 233 (1971).
6) L. F. Tietze, *J. Am. Chem. Soc.*, **96**, 946 (1974).
7) H.J. Bakker, E.L. Chisaberti, and P.R. Jefferies, *Phytochemistry*, **11**, 2221 (1972).



4



6 : R=GlucAc$_4$ 7 : R=GlucAc$_4$
15 : R=Gluc 5 : R=Gluc

Chart 2. Preparation of (7,8-$^3H_2$)-7-Deoxyloganic Acid (5)

In order to clarify the mechanism of the cyclopentane ring cleavage of loganin (2), it would also give some clue to examine whether or not the C-8 proton of 2 might be retained after the ring cleavage yielding secologanin (3).

We thus synthesized (7,8-$^3H_2$)-7-deoxyloganic acid (5) and administered it to plants to examine especially the fate of the C-8 proton in the course of the conversion to morroniside (1) *via* loganin (2) and secologanin (3). Namely, 10-deoxygeniposidic acid tetraacetate (6) obtained by the catalytic hydrogenation of asperuloside tetraacetate was hydrogenated over Pd-C with tritium giving rise to (7,8-$^3H_2$)-7-deoxyloganic acid tetraacetate (7), deacetylation of which gave (7,8-$^3H_2$)-7-deoxyloganic acid (5).

Table I. Feeding Experiments of (7,8-$^3H_2$)-7-Deoxyloganic Acid (5) to *Lonicera morrowii*, *Cornus officinalis* and *Gentiana thunbergii* Plants

Plant	Amt. and spec. activity of 5 dpm/mmole	Glucosides isolated	Spec. activity of glucoside isolated dpm/mmole	Incorporation ratio (%)
Lonicera morrowii	57.9 mg 4.58×10^{10}	secologanin (3)	5.08×10^{8}	1.10
Cornus officinalis	63.2 mg 8.77×10^{10}	loganin (2)	9.24×10^{7}	0.30
		morroniside (1)	2.40×10^{7}	0.03
Gentiana thunbergii	62.7 mg 1.51×10^{10}	morroniside (1)	5.95×10^{7}	0.21

(7,8-$^3H_2$)-5 thus obtained was administered to *Lonicera morrowii* A. Gray (Caprifoliaceae) containing secologanin (3), *Cornus officinalis* Sieb. et Zucc. (Cornaceae) containing loganin (2) and morroniside (1) and *Gentiana thunbergii* (G. Don) Grieseb. (Gentianaceae) containing morroniside (1), respectively, and examined the incorporation of this precursor into each glucoside, the results of which are shown in Table I.

Radioactive loganin (2) obtained from *C. officinalis* was purified as the acetate (8) and then regenerated by the Zemplén reaction. This substance (2) was subjected to Jones oxidation followed by acetylation to give 7-dehydrologanin tetraacetate (9), the radioactivity of which was about 50% of that of loganin (2). This result indicating the retention of both tritium labelings of (7,8-$^3H_2$)-5 in loganin (2) is in good accordance with the finding[8] that the hydrogen atom that originated in the 2S hydrogen of mevalonic acid was eliminated during the hydroxylation of 5.

Next, a part of the tetraacetate (10) of radioactive secologanin (3) obtained from *L. morrowii* was subjected to sodium borohydride reduction to give sweroside tetraacetate (11), mp 165—166°. The remainder of the acetate (10) was converted to the dimethyl ester (12) (secologanoside tetraacetate dimethyl ester[9,10]), mp 142—143° by Jones oxidation followed by methylation. The ratio of the specific activity of 11 to that of 12 was 100: 65.

8) C.J. Coscia, L. Botta, and R. Guarnaccia, *Archiv. Biochem. Biophys.*, **136**, 498 (1970).
9) R. Guarnaccia, L. Botta, and C.J. Coscia, *J. Am. Chem. Soc.*, **96**, 7079 (1974).
10) H. Inouye and K. Uobe, unpublished work.

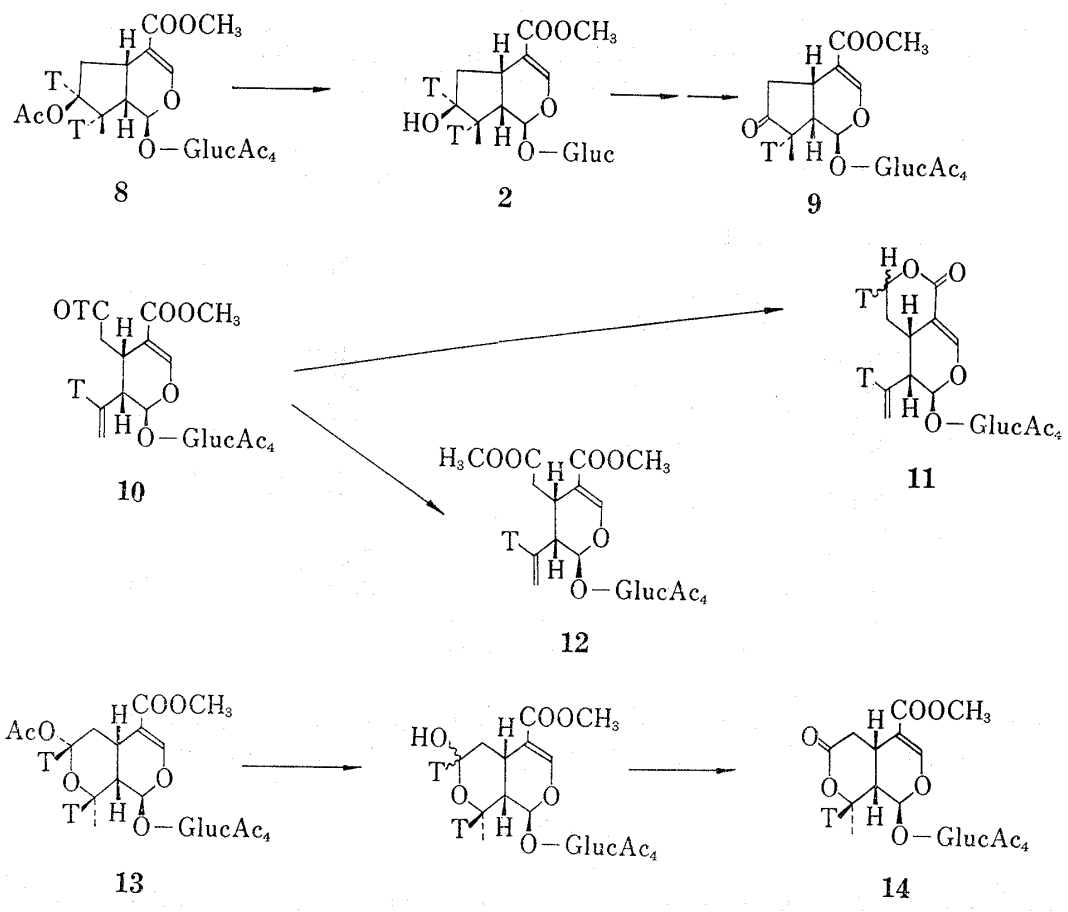


Chart 3. Degradation Reactions of Radioactive Loganin Pentaacetate (**8**), Secologanin Tetraacetate (**10**) and Morroniside Pentaacetate (**13**)

Finally, on partial hydrolysis with potassium hydrogen-carbonate followed by Jones oxidation, pentaacetate (**13**)[11] of radioactive morroniside (**1**) isolated from *C. officinalis* as well as *G. thunbergii* was converted to kingiside tetraacetate (**14**), mp 163—164°, respectively, the radioactivities of which were 60 and 53%[12] of that of intact **13**. These results summarized in Table II clearly indicate that the tritium labelings at both C-7 and C-8 positions of (7,8-$^3H_2$)-**5** are retained throughout loganin (**2**), secologanin (**3**) and morroniside (**1**). These findings are compatible with the previously obtained experimental results demonstrating that the C-7 proton of **2** is retained in morroniside (**1**).[3] Consequently, it could be supposed that the cleavage of the cyclopentane ring of loganin (**2**) might proceed by either of the mechanisms retaining C-7 and C-8 protons, namely, the ring cleavage initiated by the abstraction of a hydride from the C-8 methyl group of **2**, the fission through a radical reaction or that passing through 10-hydroxyloganin. At present, however, we cannot conclude which route actually operates.

11) Although morroniside (**1**) exists as an unseparable mixture of C-7 epimers, the glucoside mostly gave the 7-α-acetoxy derivative on acetylation. Judging also from the melting point, the morroniside pentaacetate (**13**) used for the degradation reactions was the 7-α-acetoxy derivative. *cf.* H. Inouye, S. Tobita, Y. Akiyama, K. Ito, and T. Shingu, *Chem. Pharm. Bull.* (Tokyo), **21**, 846 (1973).

12) The difference in the radioactivity between the whole molecule and the C-7 tritium in each case was rather greater than the activity of the C-7 tritium. This could be due to the randomization during the catalytic hydrogenation of 10-deoxygeniposidic acid tetraacetate (**6**) with tritium. Owing to the scarcity of the material, a further experiment to eliminate C-8 proton of **12** as well as **14** has not been carried out. However, to say the least, the results obtained here clearly demonstrate that the tritium was retained at C-8 position of **12** and **14**.

Table II. The Results of Degradation Reactions of Radioactive Loganin Pentaacetate (**8**), Secologanin Tetraacetate (**10**) and Morroniside Pentaacetate (**13**)

Compounds	Spec. activity dpm/mmole	%
Loganin pentaacetate (**8**)		
from *C.*	5.87×10^6	
7-Dehydrologanin tetraacetate (**9**)	2.90×10^6	49.4
Secologanin tetraacetate (**10**)		
from *L.*	4.10×10^7	
Sweroside tetraacetate(**11**)	4.37×10^7	100
Dimethyl ester (**12**)	2.85×10^7	65.2
Morroniside pentaacetate (**13**)		
from *C.*	1.55×10^6	
Kingiside tetraacetate (**14**)	9.27×10^5	59.8
Morroniside pentaacetate (**13**)		
from *G.*	5.30×10^6	
Kingiside tetracetate (**14**)	2.82×10^6	53.2

C.; *Cornus officinalis, L.*; *Lonicera morrowii, G.*; *Gentiana thunbergii*

It was recently reported that the C-8 tritium labeling of (6,8-$^3H_3$)-loganin (**2**) was retained in the biosynthesis of an indole alkaloid camptothecin,[13] which interlocks our results.

Experimental[14]

Preparation of (7,8-$^3H_2$)-7-Deoxyloganic Acid (**5**)——A mixture[15] (100 mg) consisting of tetraacetates of 10-deoxygeniposidic acid (**15**) and 7-deoxyloganic acid (**5**) in the ratio 4.5: 1 prepared by the catalytic hydrogenation of asperuloside tetraacetate over Pd-C was dissolved in MeOH (15 ml) and hydrogenated over Pd-C (5%) (50 mg) with $^3H_2$ gas (2.0 Ci).[16] After removal of the catalyst by filtration, the filtrate was concentrated *in vacuo*. The residue was dissolved in a small quantity of MeOH and the solvent was removed *in vacuo*. After repeating this procedure three times in order to remove labile tritium, colorless needles were obtained.

A mixture of an aliquot (1/50) of the above residue and nonradioactive 7-deoxyloganic acid tetraacetate (**7**) (106.6 mg) was chromatographed on a silica gel column (15 g, 1.5×15 cm) with $CHCl_3$–MeOH as eluent, eluted first with $CHCl_3$ (fr. No. 1—10) and then with $CHCl_3$–MeOH 99: 1 (fr. NO. 11—17) collecting 25 ml fractions. Fractions No. 14—17 were combined and evaporated *in vacuo* to leave a residue (75.4 mg). This was diluted with carrier (101.3 mg) and recrystallized twice from EtOH to give colorless needles (90.3 mg), which showed a single peak on a thin-layer chromatography (TLC) radiochromatogram ($CHCl_3$–MeOH 95: 5). All of this substance was dissolved in MeOH (5 ml) and a saturated methanolic $Ba(OH)_2$ was added to adjust the pH of the solution to 14. After standing for 2.5 hr at room temperature, the pH of the reaction mixture was adjusted to 4 with Amberlite IR120 ion exchange resin (H-form). After removal of the resin, the solution was concentrated *in vacuo* and the residue was recrystallized from EtOH to give colorless needles (54.5 mg), mp 113—114°, spec. activity 6.66×10^{10} dpm/mmole, which were identified with an authentic sample of 7-deoxyloganic acid (**5**) by TLC ($CHCl_3$–MeOH 7: 3).

Administration of (7,8-$^3H_2$)-7-Deoxyloganic Acid (**5**) **to *Lonicera morrowii* and Isolation of Secologanin** (**3**)——A solution of (7,8-$^3H_2$)-7-deoxyloganic acid (**5**) (57.9 mg, spec. activity 4.58×10^{10} dpm/mmole) in H_2O (2 ml) was administered hydroponically to seven twigs (about 20 cm in height) of *L. morrowii* plant in August. During the administration, a total of 52 ml of H_2O added was absorbed into the plants.[17] Five days after

13) C.R. Hutchinson, A.H. Heckendorf, and P.E. Daddona, *J. Am. Chem. Soc.*, **96**, 5609 (1974).
14) The experimental procedures were the same as described in the footnote 18 of the 17th paper of this series. *cf.* H. Inouye, S. Ueda, Y. Aoki, and Y. Takeda, *Chem. Pharm. Bull.* (Tokyo), **20**, 1287 (1972). However, charcoal activated for chromatography (Wako) was used for charcoal column chromatography.
15) The molar ratio of both substances was judged by nuclear magnetic resonance (NMR) spectrum. When this mixture was chromatographed on silica gel impregnated with silver nitrate, 10-deoxygeniposidic acid tetraacetate (**6**) could be isolated in a pure state.
16) See footnote 14.
17) This procedure was applied to all the administration experiments described in this paper.

beginning the administration, the plants (wet weight 20.5 g) were cut into pieces and extracted under reflux with four 300 ml portions of MeOH. After concentration of the combined extracts the residue was extracted four times with a total of 60 ml of H_2O and the solvent was removed *in vacuo*. The residue was chromatographed on a charcoal column (10 g, 2×13 cm) with H_2O–MeOH as eluent, eluted first with H_2O (fr. No. 1—2) and then successively with H_2O–MeOH 8: 2 (fr. No. 3—4), H_2O–MeOH 1: 1 (fr. No. 5—6) and MeOH (fr. No. 7—11) collecting 250 ml fractions. Fractions No. 5—8 were combined and evaporated *in vacuo* to furnish a residue (903.3 mg), which was chromatographed on a silica gel column (40 g, 2.2×26 cm), eluted successively with $CHCl_3$ (200 ml), $CHCl_3$–MeOH 97: 3 (200 ml), $CHCl_3$–MeOH 96: 4 (200 ml), $CHCl_3$–MeOH 95: 5 (50 ml), $CHCl_3$-MeOH 94: 6 (120 ml), $CHCl_3$—MeOH 93: 7 (150 ml). The eluates with $CHCl_3$–MeOH 95:5～93:7 were combined and evaporated *in vacuo*. The residue (128.8 mg) was acetylated with a mixture of Ac_2O and pyridine in the usual manner and the reaction product was chromatogrpahed on a silica gel column (15 g, 1.5×15 cm) with $CHCl_3$ as eluent collecting 5 ml fractions. Fractions No. 12—20 were combined and evaporated *in vacuo* and the residue was again chromatographed on a silica gel column (30 g, 2×14 cm) with $(C_2H_5)_2O$ as eluent collecting 5 ml fractions. Fractions No. 23—27 were evaporated to give the radioactive secologanin tetraacetate (**10**) (49.9 mg) as a colorless syrup.

$NaBH_4$ Reduction of (7,8-$^3H_2$)-Secologanin Tetraacetate (10)——To a solution of radioactive **10** (15.0 mg) diluted with carrier (158 mg) in dioxane (10 ml) was added $NaBH_4$ (170 mg) and H_2O (3 drops) and then stirred at room temperature for 1.5 hrs. After decomposition of the excess reagent with AcOH, H_2O (30 ml) was added to the reaction mixture and extracted with three 25 ml portions of $CHCl_3$. After washing with H_2O, the combined $CHCl_3$ extracts were dried over anhyd. $MgSO_4$. The solvent was removed *in vacuo* and the resulting residue was chromatographed on a silica gel column (15 g, 1.5×15 cm) with benzene–$(C_2H_5)_2O$ as eluent, eluted first with benzene (fr. No. 1—4) and then successively with benzene–$(C_2H_5)_2O$ 95: 5 (fr. No. 5—9), benzene–$(C_2H_5)_2O$ 9: 1 (fr. No. 10—12), benzene–$(C_2H_5)_2O$ 85: 15 (fr. No. 13—16), benzene–$(C_2H_5)_2O$ 8: 2 (fr. No. 17—20), benzene–$(C_2H_5)_2O$ 7: 3 (fr. No. 21—24) and benzene–$(C_2H_5)_2O$ 6: 4 (fr. No. 25—30) collecting 25 ml fractions. Fractions No. 25—28 were combined and evaporated *in vacuo*. Recrystallization of the residue from EtOH gave colorless needles, mp 165—166°, yield 66.6 mg, which were identified with an authentic sample of sweroside tetraacetate (**11**) by mixed melting point and TLC (benzene–$(C_2H_5)_2O$ 1: 1). This substance (**11**) was further purified to a constant activity by recrystallization from EtOH.

Jones Oxidation of (7,8-$^3H_2$)-Secologanin Tetraacetate (10) and Methylation of the Oxidation Product——Jones reagent was added to a solution of the radioactive secologanin tetraacetate (**10**) (15.0 mg) and carrier (158 mg) dissolved in $(CH_3)_2CO$ (10 ml) while stirring under ice cooling until the supernatant of the reaction mixture showed a pale orange color. After 1 hr, H_2O (30 ml) was added to the reaction mixture and extracted with three 25 ml portions of $CHCl_3$. The $CHCl_3$ extracts were rinsed with H_2O, dried over anhyd·$MgSO_4$ and evaporated *in vacuo*. The residue was chromatographed on a silica gel column (15 g, 1.5×15 cm) with $CHCl_3$–MeOH as eluent, eluted first with $CHCl_3$ (fr. No. 1—4) and then successively with $CHCl_3$–MeOH 99.5: 0.5 (fr. No. 5—18) and $CHCl_3$–MeOH 99: 1 (fr. No. 19—30) collecting 25 ml fractions. Fractions No. 25—29 were combined and evaporated *in vacuo*. The residue (105.7 mg) was methylated with ethereal CH_2N_2 solution in the usual manner and the reaction product was chromatographed on a silica gel column (5 g, 1.5×5 cm) with $CHCl_3$ as eluent collecting 5 ml fractions. Fractions No. 5—8 were combined and evaporated *in vacuo* to give a residue which was recrystallized from EtOH to furnish colorless needles, mp 142—143°, yield 83.4 mg. This substance was identified with an authentic sample of secologanoside tetraacetate dimethyl ester (**12**) by mixed melting point and TLC ($CHCl_3$–MeOH 98: 2). This substance was further recrystallized from EtOH to a constant activity.

Administration of (7,8-$^3H_2$)-7-Deoxyloganic Acid (5) to *Cornus officinalis* and Isolation of Morroniside (1) and Loganin (2)——(7,8-$^3H_2$)-7-Deoxyloganic acid (**5**) (63.2 mg, spec. activity 8.77×10^{10} dpm/mmole) was dissolved in H_2O (3 ml) and administered hydroponically to three twigs (about 15 cm in length with 3 leaves and a total of 22 fruits) of *C. officinalis* plant in August. Five days after beginning the administration, the fruits (wet weight 7.0 g) were extracted under reflux with four 40 ml portions of MeOH. The extracts were combined and the solvent was removed *in vacuo*. The residue was chromatographed on a charcoal column (10 g, 2.2×13 cm) with H_2O–MeOH as eluent, eluted first with H_2O (fr. No. 1—5) and then successively with H_2O–MeOH 8: 2 (fr. No. 6—10), H_2O–MeOH 1: 1 (fr. No. 11—15) and MeOH (fr. No. 16—18) and 100 ml fractions were collected. Fractions No. 16—18 were combined and the solvent was removed *in vacuo*. A mixture of this residue (74.4 mg) and nonradioactive loganin (**2**) (29.6 mg) was chromatographed on a silica gel column (20 g, 1.2×19 cm) with $CHCl_3$–MeOH as eluent, eluted first with $CHCl_3$ (fr. No. 1—7) and then successively with $CHCl_3$–MeOH 97: 3 (fr. No. 8—14), $CHCl_3$–MeOH 95: 5 (fr. No. 15—18), $CHCl_3$–MeOH 93: 7 (fr. No. 19—26), $CHCl_3$–MeOH 9: 1 (fr. No. 27—34), $CHCl_3$–MeOH 88: 12 (fr. No. 35—40) and $CHCl_3$–MeOH 85: 15 (fr. No. 41—45) collecting 30 ml fractions. Fractions No. 32—35 were combined and the solvent was removed *in vacuo* to give 16.5 mg of crude morroniside (**1**). Likewise, fractions No. 36—42 afforded 45.8 mg of crude loganin(**2**).

1) Purification of Morroniside (**1**): Crude morroniside (**1**) (16.5 mg) described above was acetylated with a mixture of Ac_2O and pyridine in the usual manner and the reaction product was chromatographed on a silica gel column (5 g, 1.2×8 cm) with $CHCl_3$ as eluent and 4 ml each of the eluates was collected. Fractions No. 14—16 were combined and the solvent was removed *in vacuo* to furnish a colorless syrup (12.8 mg). This

substance was identified with an authentic sample of morroniside pentaacetate (13) by TLC ($(C_2H_5)_2O$). All the radioactive morroniside pentaacetate (13) was mixed with carrier (73.3 mg) and recrystallized five times from a mixture of $CHCl_3$, $(C_2H_5)_2O$ and petr. ether. The radioactive morroniside pentaacetate (13) (47.4 mg) thus obtained was diluted again with carrier and repeatedly recrystallized from EtOH to give colorless needles having a constant activity, mp 147—148°.

2) Purification of Loganin (2): The above crude loganin (2) (45.8 mg) was acetylated with a mixture of Ac_2O and pyridine in the usual manner and the reaction product was chromatographed on a silica gel column (10 g, 1.5×10 cm) with $CHCl_3$ as eluent collecting 6 ml fractions. Fractions No. 6—16 were combined and the solvent was removed *in vacuo* to leave a colorless syrupy residue (43.3 mg). This substance was identified with an authentic sample of loganin pentaacetate (8) by TLC ($(C_2H_5)_2O$). All the radioactive loganin pentaacetate (8) was mixed with carrier (107.4 mg) and repeatedly recrystallized from EtOH to a constant activity.

Chemical Conversion of (7,8-$^3H_2$)-Loganin Pentaacetate (8) to (8-^3H)-7-Dehydrologanin Tetraacetate (9)——The mixture of radioactive loganin pentaacetate (8) (27.0 mg) and carrier (77.6 mg) was dissolved in anhyd. MeOH (10 ml) and anhyd. methanolic 0.1 N CH_3ONa (0.6 ml) was added. After refluxing 15 min, the solution was cooled immediately and neutralized with Amberlite IRC-50 ion exchange resin (H-form) and the solvent was removed *in vacuo* to give a colorless syrupy residue (77.0 mg), which was dissolved in a mixture of $(CH_3)_2CO$ (30 ml) and MeOH (0.5 ml). After the work up of this solution with Jones reagent (0.64 ml) at 0° for 10 min, $NaHSO_3$ (300 mg) was added while stirring and the insoluble material was filtered off. The filtrate was concentrated *in vacuo* to give a residue which was acetylated with a mixture of Ac_2O and pyridine in the usual manner and the reaction product was chromatographed on a silica gel column (15 g, 1.5×15 cm) with $(C_2H_5)_2O$ as eluent and 4 ml each of the eluates was collected. Fractions No. 14—16 were combined and concentrated *in vacuo*. The residue was recrystallized from EtOH to give 7-dehydrologanin tetraacetate (9) (10.5 mg) as colorless needles which were further recrystallized from EtOH to a constant activity.

Chemical Conversion of Pentaacetate (13) of (7,8-$^3H_2$)-Morroniside (1) obtained from *C. officinalis* to (8-^3H)-Kingiside Tetraacetate (14)——$KHCO_3$ (25 mg) dissolved in H_2O (3.5 ml) was added to a solution of pentaacetate (13) (39.2 mg) of radioactive morroniside (1) obtained from *C. officinalis* and carrier (70.9 mg) in EtOH (17 ml) and the mixture was left standing at 35° for 4 days. After concentration of the reaction mixture to about 5 ml *in vacuo*, H_2O (30 ml) was added and extracted with three 20 ml portions of $CHCl_3$. After washing the $CHCl_3$ extracts with H_2O, the combined $CHCl_3$ extracts were dried over anhyd. $MgSO_4$ and evaporated *in vacuo* to give a colorless syrupy residue (13.8 mg). Jones reagent (0.02 ml) was added to a solution of this syrup in $(CH_3)_2CO$ (3 ml) and stirred at 0° for 35 min. After an addition of H_2O (15 ml), the reaction mixture was extracted with three 10 ml portions of $CHCl_3$. The $CHCl_3$ extracts were washed with H_2O, dried over anhyd. $MgSO_4$ and evaporated *in vacuo*. The residue was chromatographed on a silica gel column (5 g, 1.5×5 cm) with $CHCl_3$–MeOH as eluent, eluted first with $CHCl_3$ (fr. No. 1—48) and then with $CHCl_3$–MeOH 99: 1 (fr. No. 49—53) collecting 5 ml fractions. Fractions No. 40—52 were combined and evaporated *in vacuo* to give a colorless syrupy residue (8.4 mg). This substance was identified with an authentic sample of kingiside tetraacetate (14) by TLC ($CHCl_3$–MeOH 95: 5) which was further recrystallized from EtOH to a constant activity.

Administration of (7,8-$^3H_2$)-7-Deoxyloganic Acid (5) to *Gentiana thunbergii* and Isolation of Morroniside (1)——(7,8-$^3H_2$)-7-Deoxyloganic acid (5) (62.7 mg, spec. activity 1.51×10^{10} dpm/mmole) was dissolved in H_2O (5 ml) and administered to eleven clumps of *G. thunbergii* plants (about 5 cm in height) in April. Five days after beginning the administration, all the plants (wet weight 15.5 g) were worked up in a similar manner as in the isolation of morroniside (1) from *C. officinalis* to give morroniside pentaacetate (13) (56.5 mg) as colorless needles, which were mixed with carrier (276 mg) and repeatedly recrystallized from a mixture of $CHCl_3$, $(C_2H_5)_2O$ and petr. ether to give a somewhat purified morroniside pentaacetate (13) (33.2 mg). All this substance was diluted again with carrier (43.2 mg) and repeatedly recrystallized from EtOH to a constant activity.

Chemical Conversion of Pentaacetate (13) of (7,8-$^3H_2$)-Morroniside (1) obtained from *G. thunbergii* to (8-^3H)-Kingiside Tetraacetate (14)——A mixture of the radioactive morroniside pentaacetate (13) (12.8 mg) and carrier (54.0 mg) was worked up as described above to give (8-^3H)-kingiside tetraacetate (14) (6.5 mg), which was repeatedly recrystallized from EtOH to a constant activity.

Acknowledgement The authors are grateful to professor I. Nishioka, Faculty of Pharmaceutical Sciences, Kyushu University for collecting *Gentiana thunbergii* plants.