Biosynthesis of Oleoside-, 10-Hydroxyoleoside- and Ligustaloside-Type Glucosides from Secologanin

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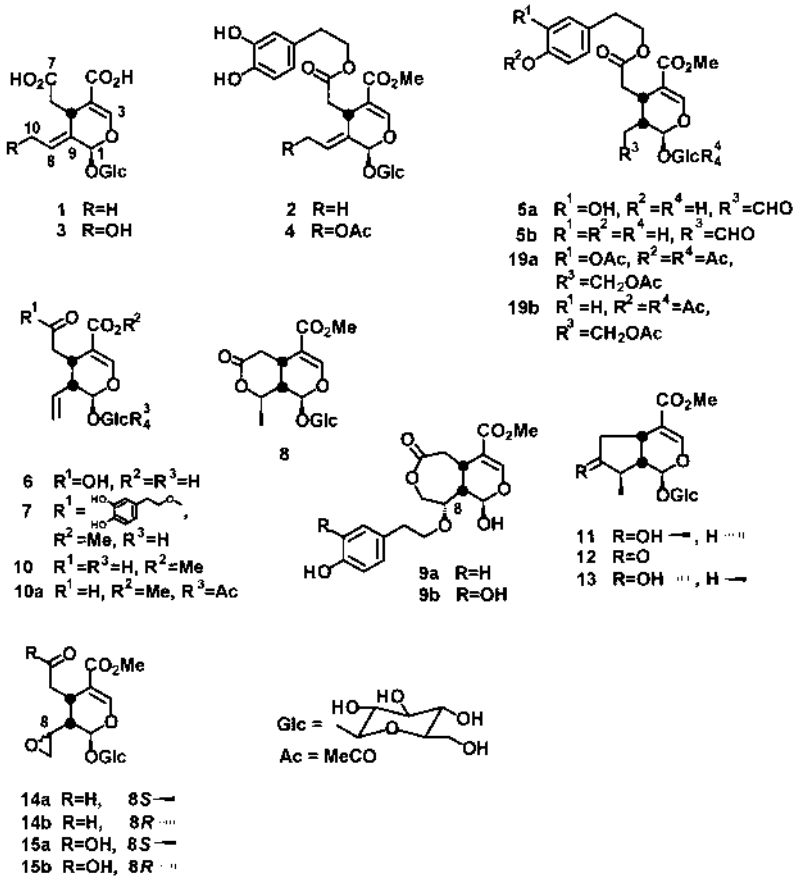
A biosynthetic pathway from secologanin to oleoside-, 10-hydroxyoleoside- and ligustaloside-type secoiridoid glucosides was examined by feeding experiments in which the respective C-8 stereoisomers of [8-^{3}H]-8,10-epoxyse cologanin and [8-^{3}H]-8,10-epoxysecoxyloganin as well as [carbomethoxy-$^2H_3$]secologanin were administered separately to three oleaceous plants. The results showed that (8*S*)-8,10-epoxysecologanin was an intermediate between the secologanin and secoiridoid glucosides of the three types.

Key words Oleaceae; secoiridoid glucoside; biosynthesis; feeding experiment; secologanin; 8,10- epoxysecologanin

Secoiridoid glucosides of three types, namely, oleoside (**1**)-type [*e.g.* oleuropein (**2**)], 10-hydroxyoleoside (**3**)-type [*e.g.* 10-acetoxyoleuropein (**4**)] and ligustaloside (**5**)-type secoiridoid glucosides [*e.g.* ligustalosides A, B (**5a**, **5b**)] were found in oleaceous plants[1] and their hypothetical biosynthetic pathway was proposed as depicted in Fig. 1.[2] Later, secologanoside (**6**)-type [*e.g.* oleuroside (**7**)][3] and 8-*epi*-kingiside (**8**)-type glucosides [*e.g.* 8-*epi*-kingiside (**8**) and jasmolactones A, B (**9a**, **9b**)][4,5] were isolated together with various glucosides of the three above types from oleaceous plants. The biosynthesis of the glucosides of the two types **6** and **8** was also presumed to proceed along the above-mentioned pathway (Fig. 1). In this pathway, in addition to the mechanism for the formation of structures around C-8, -9 and -10 in these glucosides, another problem is whether epoxidation of C-8—C-10 occurs before or after the oxidation of the aldehyde group at C-7 to the carboxy group. Regarding the biosynthesis of **1**-type glucosides, earlier experiments with radio-active precursors have shown that **1**-type glucosides were biosynthesized from loganin (**11**) *via* secologanin (**10**) and not *via* 7-ketologanin (**12**).[6] Recent experiments with deuterium-labeled precursors, on the other hand, have shown that these glucosides were formed from 7-*epi*-loganin (**13**) *via* **12**.[7] Further experiments were required to



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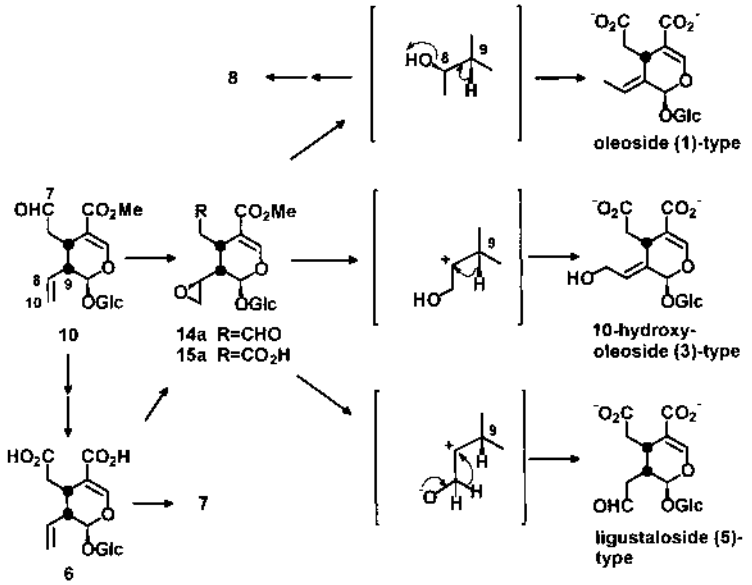

Fig. 1. Possible Biosynthetic Pathway of Oleaceous Secoiridoid Glucosides

clarify these conflicting results.

This paper presents results of an examination of the intermediacy of secologanin (8*S*)-8,10- and (8*R*)-8,10-epoxides (**14a**, **b**), and their C-7 carboxy derivatives (**15a**, **b**) in the biosynthesis of the oleaceous secoiridoid glucosides according to the hypothetical route shown in Fig. 1.[8)]

Preparation of Labeled Precursors Secologanin (**10**), the immediate precursor of epoxide (**14** or **15**), was incorporated into **1**- and **3**-type secoiridoids[6)] but was not examined as a precursor of **5**-type secoiridoid glucosides. [Carbomethoxy-$^2H_3$]-**10** used in this study was prepared through proton–deuterium exchange of the carboxymethyl group of secologanin tetraacetate (**10a**) *via* the Zemplén reaction (0.1 N $NaOCD_3$ in CD_3OD).

Next, the respective isomers of tritium-labelled epoxysecologanin (**14**) and -secoxyloganin (**15**) were synthesized according to our established method.[9)] After the introduction of tritium was carried out by the reduction of ketone **16**, prepared from secologanin tetraacetate (**10a**) through several steps (Fig. 2),[9)] the resulting ^{3}H-labeled hydroxy derivatives (**17**) were converted with 1,5-diazabicyclo [4.3.0] non-5-ene (DBN) to a mixture of epoxides (**18a**, **b**), which were separated by multiple preparative thin layer chromatography (PTLC). The stereochemistry and purity of these epoxides **18a** and **18b** were confirmed by comparison of their behavior on TLC with that of cold authentic samples. Deprotection of ^{3}H-labeled (8*S*) epoxide **18a** under UV irradiation gave [8-^{3}H]-(8*S*)-8,10-epoxysecologanin (**14a**) which was oxidized with Jones reagent to afford [8-^{3}H]-(8*S*)-8,10-epoxysecoxyloganin (**15a**). Likewise, (8*R*)-isomer **18b** was deprotected to aldehyde **14b** which in turn was oxidized to carboxylic acid **15b**.

Precursor Administration To confirm that **5**-type secoiridoid glucosides were biosynthesized from **10**, [carbomethoxy-$^2H_3$]-**10** was administered to *Ligustrum japonicum* by a hydroponic method. Ligustaloside B (**5b**) was then isolated from the plant and identified by comparing its ^{1}H-NMR spectrum to that of an authentic sample.[2)] The ^{2}H-NMR spectrum of **5b** showed that deuterium was retained

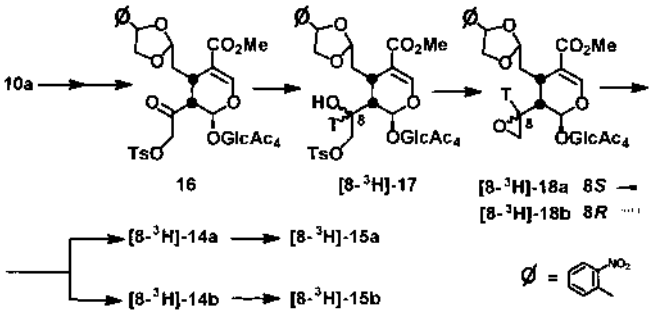

Fig. 2

with 2.74% of ^{2}H-enrichment at δ 3.69. Accordingly, as was expected, **5**-type glucosides were also formed from **10**. [8-^{3}H]-**14a**, **b**, **15a** and **b** were then administered separately to *Olea europaea*, *Osmanthus fragrans* and *Ligustrum japonicum* by the hydroponic method and glucosides **2**, **4**, **5a** and **b** were isolated. The results of these experiments are shown in Tables 1 and 2. (8*S*)-8,10-Epoxysecologanin (**14a**) was incorporated into four glucosides at the level of 0.14—0.51%, whereas the (8*R*)-epoxide (**14b**) showed lower incorporation (0.07—0.01%). Namely, **14a** was incorporated 3 times more highly into **2**, 10 times more highly into **4**, 5 times more highly into **5a** and 7.7 times more highly into **5b** than its (8*R*)-isomer (**14b**), respectively. The specific incorporation of **14a** and **14b** into **5a** were lower by one order of magnitude than that into **5b**, presumably due to the higher dilution by the large amount of endogenous **5a**. On the other hand, the incorporation of both stereoisomers (**15a**, **b**) of 8,10-epoxysecoxyloganin did not significantly differ and were lower than that of **14a**. It seems likely from these results that **14a** is on the biosynthetic pathway of glucosides **2**, **4**, **5a** and **b** but its (8*R*)-isomer (**14b**) and the two stereoisomers of **15** are not. Therefore, at the present stage, the above-mentioned three types of glucosides were assumed to be biosynthesized from **10** *via* **14a** by the mechanism depicted in Fig. 1 (R=CHO). The stereochemical course of the dehydration leading to **1**-type from the (8*S*)-hydroxy derivative formed

Table 1. Experiments on the Administration of [8-^{3}H]Epoxysecologanins and [8-^{3}H]Epoxysecoxyloganins to *Olea europaea* and *Osmanthus fragrans*

Substance fed [amount (mg) and spec. activity (dpm/mmol)]	Glucosides isolated [amount (mg), spec. activity (dpm/mmol) and spec. incorporation (%)]	
	2 (*O. europaea*)	**4** (*O. fragrans*)
[8-^{3}H]-**14a** (0.45 mg, 4.59×10^{11})	105.6, 6.62×10^8, 0.1442	28.4, 4.33×10^8, 0.0943
[8-^{3}H]-**14b** (0.17 mg, 6.9×10^{11})	78.8, 2.95×10^8, 0.0425	9.3, 6.53×10^7, 0.0094
[8-^{3}H]-**15a** (0.48 mg, 2.60×10^{11})	73.4, 1.22×10^8, 0.0469	28.7, 7.77×10^7, 0.0298
[8-^{3}H]-**15b** (0.65 mg, 1.87×10^{11})	60.3, 1.05×10^8, 0.0561	20.3, 4.66×10^6, 0.0025

Table 2. Experiments on the Administration of [8-^{3}H]Epoxysecologanins and [8-^{3}H]Epoxysecoxyloganins to *Ligustrum japonicum*

Substance fed [amount (mg) and spec. activity (dpm/mmol)]	Glucosides isolated [amount (mg), spec. activity (dpm/mmol) and spec. incorporation (%)[a)]]	
	5a	**5b**
[8-^{3}H]-**14a** (0.45 mg, 4.59×10^{11})	76.5, 2.10×10^8, 0.0458	22.9, 2.34×10^9, 0.5098
[8-^{3}H]-**14b** (0.17 mg, 6.93×10^{11})	52.9, 6.15×10^7, 0.0089	18.6, 4.59×10^8, 0.0662
[8-^{3}H]-**15a** (0.48 mg, 2.60×10^{11})	91.9, 7.46×10^7, 0.0287	30.3, 7.84×10^7, 0.0302
[8-^{3}H]-**15b** (0.65 mg, 1.87×10^{11})	74.8, 6.13×10^7, 0.0327	28.6, 8.28×10^7, 0.0443

a) Values were calculated on the basis of radioactivities of dihydro acetates **19a** and **b** derived from **5a** and **b**, respectively.

after the cleavage of **14a** was in agreement with that in the chemical reaction.[10] The formation of **7** [**6**-type glucosides] and **8**, not tested in the present study, could be also explained by this pathway. However, taking into account the less although still significant incorporation of the three compounds **14b**, **15a** and **b**, a satisfactory conclusion requires further biosynthetic studies on the enzymatic level.

Experimental

^{1}H-NMR spectra were taken on a JEOL JNM FX 200 with tetramethylsilane as the internal standard and ^{2}H-NMR spectra were recorded with deuterium signal (δ 4.82) of the solvent (H_2O) as the internal standard. Column chromatography was performed on Silica gel 60 (70—230 mesh, Merck) and on highly porous polymer Diaion HP-21 (Mitsubishi Chemical Industries). For TLC, Silica gel 60 GF_{254} (Merck) and for PTLC, Silica gel 60 PF_{254} (Merck), were used and spots or bands were detected under UV light. Radioactivity was measured in a Packard liquid scintillation spectrometer, model 3380, with samples dissolved in a solution of Insta-gel (Packard). Specific activities are those before dilution. Radioactive chromatograms were monitored with an Aloka thin-layer chromatogram scanner, type JTC-501.

Synthesis of [Carbomethoxy-$^2H_3$]secologanin (10) A solution of secologanin tetraacetate (**10a**) (1 g) in 0.1 N $NaOCD_3$–CD_3OD (50 ml, CD_3OD: ^{2}H-enrichment, 99.5%, CEA) was stirred for 45 min under ice-cooling. After neutralization with Amberlite IR 120B (H^+ form), the mixture was concentrated *in vacuo* and the crude product (653.1 mg) was purified by PTLC (1st, benzene : EtOAc : EtOH=1 : 4 : 1; 2nd, $CHCl_3$: MeOH=5 : 1) to yield [carbomethoxy-$^2H_3$]-**10** (320.4 mg). ^{1}H-NMR (D_2O) δ: 2.48 (1H, ddd, H-6a), 2.75 (1H, ddd, H-6b), 2.96 (1H, m, H-9), 3.70 (1H, CO_2CH_3), 5.21—5.31 (2H, m, H-10), 5.48 (1H, d, H-1), 5.61 (1H, ddd, H-8), 7.60 (1H, s, H-3), 9.70 (1H, t like s, H-7). ^{2}H-enrichment: 66.7% (calculated from the ratio of the signal intensities of the carbomethoxy group and H-3).

Preparation of (8*S*)- and (8*R*)-Epoxysecologanins (14a, 14b) and (8*S*)- and (8*R*)-Epoxysecoxyloganins (15a, 15b) ^{3}H-labeled compounds **14a**, **14b**, **15a** and **15b** were synthesized according to the procedure described in the previous paper.[9] A solution of **16** (25 mg) in dioxane–MeOH (3 : 2) (0.5 ml) was added to NaB^3H_4 (0.47 mg, 100 mCi, NEN) under ice-cooling and the mixture was stirred for 15 min. After further addition of $NaBH_4$ (2 mg), the mixture was stirred for another 20 min. After decomposition of the excess reagent by adding a drop of AcOH, the reaction mixture was concentrated *in vacuo* to give a residue which was diluted with H_2O (2 ml) and extracted with $CHCl_3$ (3 ml×4). The $CHCl_3$ layer was washed with brine, dried and concentrated *in vacuo*. The resulting residue (24.9 mg) was purified by PTLC ($CHCl_3$: MeOH=50 : 1, 2 developments) to give a white powder (21.7 mg) of [8-^{3}H]-hydroxy tosylate ([8-^{3}H]-**17**). This substance was identical with an authentic sample of **17** on TLC ($CHCl_3$: MeOH=50 : 1). Specific activity: 2.22×10^{12} dpm/mmol. This hydroxy tosylate ([8-^{3}H]-**17**, 21.7 mg) was treated with DBN to give to a mixture of epoxides (16.6 mg), which was purified by PTLC (benzene : EtOAc=5 : 1, 8 developments) to give [8-^{3}H]-epoxides **18a** (5.8 mg) and **-18b** (4.6 mg). Labeled compounds **18a** and **18b** obtained thus were identified as (8*S*)- and (8*R*)-8,10-epoxysecologanin protected with *O*-nitrophenylethylene glycol, respectively, by comparison of their behavior on TLC with that of authentic samples prepared in a cold run.[9] Labeled epoxides **18a** (5.6 mg) and **18b** (4.6 mg) were separately deprotected under UV irradiation to give epoxy-aldehydes [8-^{3}H]-**14a**, **14b**, which, after dilution with cold samples, were in turn oxidized with Jones reagent to epoxy-carboxylic acids ([8-^{3}H]-**15a** and **-15b**). [8-^{3}H]-(8*S*)-8,10-Epoxysecologanin ([8-^{3}H]-**14a**, 1.8 mg): Specific activity; 4.59×10^{11} dpm/mmol. [8-^{3}H]-(8*R*)-8,10-Epoxysecologanin ([8-^{3}H]-**14b**, 0.5 mg): Specific activity; 6.93×10^{11} dpm/mmol. [8-^{3}H]-(8*S*)-8,10-Epoxysecoxyloganin ([8-^{3}H]-**15a**, 1.9 mg): Specific activity; 2.60×10^{11} dpm/mmol. [8-^{3}H]-(8*R*)-8,10-Epoxysecoxyloganin ([8-^{3}H]-**15b**, 2.6 mg): Specific activity; 1.87×10^{11} dpm/mmol.

Administration of [Carbomethoxy-$^2H_3$]secologanin (10) to *Ligustrum japonicum* A solution of [carbomethoxy-$^2H_3$]-**10** (20.0 mg, ^{2}H-enrichment, 66.7%) in H_2O (1.0 ml) was given hydroponically to five twigs (*ca*. 15 cm long) of *L. japonicum* in June. After 3 d, the leaves (8.34 g) were cut into pieces and extracted with hot MeOH (50 ml×3). After concentration *in vacuo*, the combined extract was turbulated with H_2O (30 ml) and the insoluble material was filtered off through a Celite layer. The Celite layer was washed with H_2O (10 ml×3), and the filtrate and washings were combined and lyophilized to give 1.09 g of residue which was subjected to chromatography on Diaion HP-21 (5.0 ml) and eluted successively with H_2O (200 ml) and MeOH (200 ml). The residue (700.0 mg) of the MeOH eluate obtained through concentration *in vacuo* was subjected to PTLC ($CHCl_3$: MeOH, 4 : 1=3 developments) to give 4 bands (B-1—B-4 in order of increasing polarity). B-1 gave a white powder of ligustaloside A (**5a**) (393.2 mg), and B-2 afforded ligustaloside B (**5b**) (85.6 mg) as a white powder. **5b**: ^{2}H-NMR (H_2O) δ: 3.69 ($CO_2C^2H_3$, ^{2}H-enrichment: 2.74%, calculated from the intensity of ^{2}H signal), 4.82 (HDO). Incorporation: 4.11%. The deuterium signal due to the carboxymethyl group of isolated **5a** was not detected in the ^{2}H-NMR spectrum probably because of the higher dilution by the large amount of endogenous **5a**. The same phenomena were observed in the feeding experiments with tritium-labeled 8,10-epoxysecologanin (see Table 2).

Administration of [8-^{3}H]-(8*S*)-8,10-Epoxysecologanin (14a), [8-^{3}H]-(8*R*)-8,10-Epoxysecologanin (14b), [8-^{3}H]-(8*S*)-8,10-Epoxysecoxyloganin (15a) and [8-^{3}H]-(8*R*)-8,10-Epoxysecoxyloganin (15b) to *Olea europaea* In a solution of [8-^{3}H]-**14a** (0.45 mg, spec. activity: 4.59×10^{11} dpm/mmol) in H_2O (0.25 ml) was immersed a twig (*ca*. 15 cm long) of *O. europaea* in June. After 2 d, the leaves (2.3 g) were extracted with hot MeOH (30 ml×3). After concentration *in vacuo*, the extract was turbulated with H_2O (20 ml)

and the insoluble material was filtered off through a Celite layer. The Celite layer was washed with H_2O (10 ml), and the filtrate and washings were concentrated *in vacuo* to *ca*. 5 ml. The solution was subjected to chromatography on Diaion HP-21 (5.0 ml) and eluted successively with H_2O (50 ml) and MeOH (50 ml). The residue (159.6 mg) of the MeOH eluate was purified by PTLC ($CHCl_3$: MeOH=4 : 1, 2 developments) to give [8-^{3}H]oleuropein (**2**) (105.6 mg, spec. activity: 6.62×10^8 dpm/mmol).

[8-^{3}H]-**14b** (0.17 mg, spec. activity: 6.93×10^{11} dpm/mmol) was given to *O. europaea* in the same way as above and [8-^{3}H]-**2** (78.8 mg, spec. activity: 2.95×10^8 dpm/mmol) was obtained from the leaves (1.8 g).

Likewise, the leaves (1.8 g) given [8-^{3}H]-**15a** (0.48 mg, spec. activity: 2.60×10^{11} dpm/ mmol) afforded [8-^{3}H]-**2** (73.4 mg, spec. activity: 1.22×10^8 dpm/mmol). The final experiment with [8-^{3}H]-**15b** (0.65 mg, spec. activity: 1.87×10^{11} dpm/mmol) was carried out in the same way and afforded [8-^{3}H]-**2** (60.3 mg, spec. activity: 1.05×10^8 dpm/mmol) from the leaves (2.1 g).

Administration of [8-^{3}H]-(8*S*)-8,10-Epoxysecologanin (14a), [8-^{3}H]-(8*R*)-8,10-Epoxysecologanin (14b), [8-^{3}H]-(8*S*)-8,10-Epoxysecoxyloganin (15a) and [8-^{3}H]-(8*R*)-8,10-Epoxysecoxyloganin (15b) to *Osmanthus fragrans* A solution of [8-^{3}H]-**14a** (0.45 mg, spec. activity: 4.59×10^{11} dpm/mmol) in H_2O (0.25 ml) was given hydroponically to a twig (*ca*. 12 cm long) of *O. fragrans* in October. After 3 d, the leaves (6 g) were extracted with hot MeOH (75 ml×4). After concentration *in vacuo*, the extract was turbulated with H_2O (30 ml) and the insoluble material was filtered off through a Celite layer. It was washed with H_2O (10 ml) and the filtrate and washings were combined and concentrated *in vacuo* to *ca*. 5 ml. This solution was subjected to chromatography on Diaion HP-21 (11.6 ml) and eluted successively with H_2O (100 ml) and MeOH (70 ml). The MeOH eluate was concentrated *in vacuo* to give 298.5 mg of residue, which was subjected to PTLC ($CHCl_3$: MeOH=4 : 1, 2 developments) to give [8-^{3}H]-10-acetoxyoleuropein (**4**) (28.4 mg) as a white powder. This substance was purified by PTLC to constant activity. Specific activity: 4.33×10^8 dpm/mmol.

[8-^{3}H]-**14b** (0.17 mg, spec. activity: 6.93×10^{11} dpm/mmol) was given to *O. fragrans* in the same way as above and the extract of the leaves (3.0 g) was chromatographed on Diaion HP-21 (5.8 ml). The residue (120.0 mg) obtained through concentration *in vacuo* of the MeOH eluate was subjected to PTLC to give [8-^{3}H]-**4** (9.3 mg), which was purified by PTLC to constant activity. Specific activity: 6.53×10^7 dpm/mmol.

Likewise, [8-^{3}H]-**15a** (0.48 mg, spec. activity: 2.60×10^{11} dpm/mmol) was given to *O. fragrans* and the extract of the leaves (7.5 g) was chromatographed on Diaion HP-21 (14.5 ml). The residue (369.7 mg) obtained from the MeOH eluate was subjected to PTLC to give [8-^{3}H]-**4** (28.7 mg), which was purified by PTLC to constant activity. Specific activity: 7.77×10^7 dpm/mmol.

In the final experiment, [8-^{3}H]-**15b** (0.65 mg, spec. activity: 1.87×10^{11} dpm/mmol) was given to *O. fragrans* and the extract of the leaves (3.0 g) was chromatographed on Diaion HP-21 (5.8 ml). The MeOH eluate (170.6 mg) was subjected to PTLC to give [8-^{3}H]-**4** (20.3 mg), which was purified by PTLC to constant activity. Specific activity: 4.66×10^6 dpm/ mmol.

Administration of [8-^{3}H]-(8*S*)-8,10-Epoxysecologanin (14a), [8-^{3}H]-(8*R*)-8,10-Epoxysecologanin (14b), [8-^{3}H]-(8*S*)-8,10-Epoxysecoxyloganin (15a) and [8-^{3}H]-(8*R*)-8,10-Epoxysecoxyloganin (15b) to *Ligustrum japonicum* In a solution of [8-^{3}H]-**14a** (0.45 mg, spec. activity: 4.59×10^{11} dpm/mmol) in H_2O (0.25 ml) was immersed a twig (*ca*. 15 cm long) of *L. japonicum* in May. After 4 d, the leaves (1.5 g) were extracted with hot MeOH (30 ml×4). After concentration *in vacuo*, the extract was turbulated with H_2O (30 ml) and the insoluble material was filtered off through a Celite layer. After washing the Celite layer with H_2O (5 ml), the filtrate and washings were combined and concentrated *in vacuo* to *ca*. 5 ml. This solution was subjected to chromatography on Diaion HP-21 (6 ml) and eluted successively with H_2O (50 ml) and MeOH (50 ml). The residue (174.9 mg) obtained through concentration *in vacuo* of the MeOH eluate was subjected to PTLC (1st, $CHCl_3$: MeOH=4 : 1, 3 developments; 2nd, $CHCl_3$: MeOH=25 : 1) to give the two major bands. The more polar one gave [8-^{3}H]-ligustaloside A (**5a**) (76.5 mg, spec. activity: 2.71×10^8 dpm/ mmol) and the less polar one afforded [8-^{3}H]ligustaloside B (**5b**) (22.9 mg, spec. activity: 1.13×10^9 dpm/mmol) as a white powder, respectively.

To a solution of 25.5 mg of [8-^{3}H]-**5a** in EtOH (1 ml) was added $NaBH_4$ (2.6 mg) and the mixture was stirred for 40 min. After decomposition of the excess reagent by adding a drop of AcOH, the reaction mixture was diluted with H_2O (1 ml). This solution was subjected to chromatography on Diaion HP-21 (3 ml) and eluted successively with H_2O (5 ml) and MeOH (10 ml). The residue (24.8 mg) of the MeOH eluate, on concentration *in vacuo*, was acetylated with pyridine–Ac_2O (each 0.3 ml) in the usual manner and the product was subjected to PTLC ($CHCl_3$: MeOH=50 : 1) to give a crystalline substance (30.8 mg), which was identical with an authentic sample of dihydroligustaloside A heptaacetate (**19a**)[2] on TLC ($CHCl_3$: MeOH=25 : 1). This substance was recrystallized from EtOH to constant activity. Specific activity: 2.10×10^8 dpm/mmol.

[8-^{3}H]-**5b** (11.5 mg) was dissolved in EtOH (0.5 ml) and reduced with $NaBH_4$ (1.4 mg). The product was acetylated in the usual manner and subjected to PTLC as described above to give [8-^{3}H]dihydroligustaloside B hexaacetate (**19b**) (14.0 mg) as a white powder.[2] This substance was purified by PTLC to constant activity. Specific activity: 2.34×10^9 dpm/mmol.

[8-^{3}H]-**14b** (0.17 mg, spec. activity: 6.93×10^{11}) was given to *L. japonicum* and the leaves (1.3 g) were worked-up in the same way as above to give [8-^{3}H]-**5a** (52.9 mg, spec. activity: 7.53×10^7 dpm/mmol) and [8-^{3}H]-**5b** (18.6 mg, spec. activity: 7.26×10^8 dpm/mmol), respectively.

[8-^{3}H]-**5a** (26.5 mg) was reduced with $NaBH_4$ (2.5 mg) and acetylated to yield [8-^{3}H]-**19a** (32.4 mg, spec. activity: 6.15×10^7 dpm/mmol). [8-^{3}H]-**5b** (9.3 mg) was reduced with $NaBH_4$ (0.9 mg) and acetylated to yield [8-^{3}H]-**19b** (10.9 mg, spec. activity: 4.59×10^8 dpm/mmol).

[8-^{3}H]-**15a** (0.48 mg, spec. activity: 2.60×10^{11} dpm/mmol) was given to *L. japonicum* and the leaves (1.5 g) were worked-up in the same way to give [8-^{3}H]-**5a** (91.9 mg, spec. activity: 1.17×10^8 dpm/mmol) and [8-^{3}H]-**5b** (30.3 mg, 7.19×10^7 dpm/mmol), respectively.

[8-^{3}H]-**5a** (36.6 mg) was reduced with $NaBH_4$ (3.0 mg) and acetylated to give [8-^{3}H]-**19a** (26.4 mg). Specific activity: 7.46×10^7 dpm/mmol.

[8-^{3}H]-**5b** (15.2 mg) was reduced with $NaBH_4$ (1.5 mg) and acetylated to give [8-^{3}H]-**19b** (10.7 mg). Specific activity: 7.84×10^7 dpm/mmol.

In the final experiment, [8-^{3}H]-**15b** (0.65 mg, spec. activity: 1.87×10^{11} dpm/mmol) was given to *L. japonicum* and the leaves (1.7 g) was worked-up to give [8-^{3}H]-**5a** (74.8 mg, spec. activity: 8.61×10^7 dpm/mmol) and [8-^{3}H]-**5b** (28.6 mg, spec. activity: 1.74×10^8 dpm/mmol).

[8-^{3}H]-**5a** (24.9 mg) was reduced with $NaBH_4$ (2.5 mg) and acetylated to give [8-^{3}H]-**19a** (24.3 mg). Specific activity: 6.13×10^7 dpm/mmol.

[8-^{3}H]-**5b** (14.3 mg) was reduced with $NaBH_4$ (1.4 mg) and acetylated to give [8-^{3}H]-**19b** (18.2 mg). Specific activity: 8.28×10^7 dpm/mmol.

References and Notes

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8) Oleaceous plants used in this paper contain the following secoiridoid glucosides: *O. europaea* (leaves); oleuropein, ligstroside [oleoside (**1**)-type] and oleuroside [secologanoside (**6**)-type]; *L. japonicum* (leaves); oleuropein, ligstroside [oleoside (**1**)-type], 10-hydroxyoleuropein [10-hydroxyoleoside (**3**)-type] and ligustalosides A, B [ligustaloside (**5**)-type]; *O. fragrans* (leaves); 10-acetoxyligustroside, 10-acetoxy-oleuropein [10-hydroxyoleoside (**3**)-type].
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