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A SECOIRIDOID GLUCOSIDE FROM *LIGUSTRUM JAPONICUM**†

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Key Word Index—*Ligustrum japonicum*; Oleaceae; secoiridoid glucosides; methyl glucooleoside; 8-epikingiside; structure; biogenesis.

Abstract—Besides several secoiridoid glucosides including 8-epikingiside, a new secoiridoid glucoside, methyl glucooleoside, was isolated from the ripe berries of *Ligustrum japonicum* and its structure elucidated.

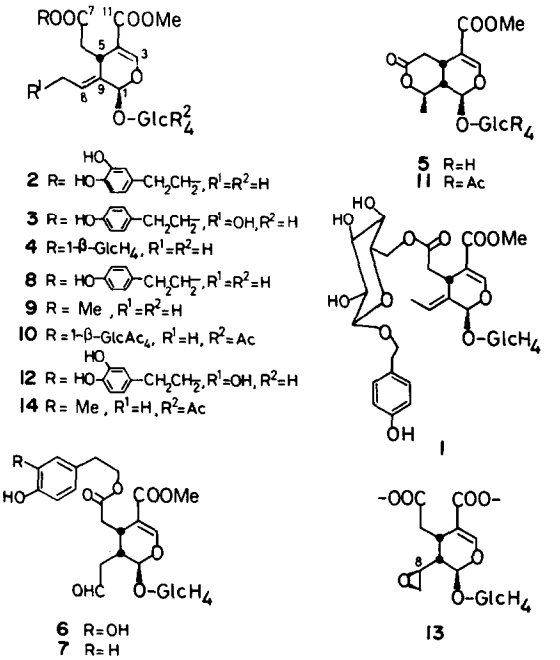
INTRODUCTION

Ripe berries of *Ligustrum japonicum* Thunb. (Japanese name, Nezumimochi) have been used together with those of *L. lucidum* Ait. (Japanese name, Tonezumimochi) as a tonic in traditional medicine in Japan. Thirteen secoiridoid glucosides including nuezhenide (**1**), oleuropein (**2**) and 10-hydroxyligustroside (**3**) have already been isolated from the berries of *L. japonicum* and of the congeneric plant, *L. lucidum* [1–5]. In the course of preliminary studies on the biosynthesis of oleoside type secoiridoid glucosides, we reinvestigated the secoiridoid glucosides of the ripe berries of *L. japonicum* and isolated a new glucoside, methyl glucooleoside (**4**), in addition to 8-epikingiside (**5**) which had hitherto only been obtained by chemical conversion of loganin [6] and some other glucosides such as ligustalosides A (**6**) and B (**7**). We describe here the structure of the glucoside **4**.

RESULTS AND DISCUSSION

The water soluble part of the methanolic extract of fresh ripe berries of *L. japonicum* gave on column chromatography on Diaion HP-21 and silica gel followed by further purification by preparative TLC and HPLC a new secoiridoid glucoside (**4**) along with the known glucosides, 8-epikingiside (**5**), nuezhenide (**1**), 10-hydroxyligustroside (**3**), ligustaloside A (**6**), ligustaloside B (**7**) and ligstroside (**8**).

The new glucoside (**4**), $C_{23}H_{34}O_{16}\cdot 2H_2O$, was obtained as a white powder, $[\alpha]_D^{16}$ −164.71° (MeOH). It showed UV absorption at 238 nm (log ε 4.08), IR bands at 3350, 1740, 1700 and 1620 cm^{-1} and a ^1H NMR signal due to H-3 at δ 7.54, all of which are features common to iridoid compounds. The ^1H NMR spectrum of **4** was very similar to that of oleoside dimethyl ester (**9**) [6] except it lacked a signal due to a carbomethoxy group and contained extra signals for an additional sugar moiety indicated by the signals of two anomeric protons, the usual one at δ 4.82 (1H, *d*, *J* = 8.0 Hz) and a second at δ 5.44 (1H, *d*, *J* = 7.6 Hz). The ^{13}C NMR spectrum of **4** also showed a signal arising from an extra anomeric carbon at δ 95.9. The high field position of this signal when compared to the glucosidic bound anomeric carbon at *ca* δ 100 suggested an ester linkage to this carbon [7]. Thus, it was concluded that **4** was a β-glucopyranosyl ester of oleoside monomethyl ester. Glucoside **4** gave on acetylation the octaacetate **10** and on Zemplén reaction the oleoside dimethyl ester **9** and D-glucose. The esterification position of the second glucose to the secoiridoid moiety was



*Part 62 in the series 'Studies on Monoterpene Glucosides and Related Natural Products'. For part 61 see Inouye, H., Takeda, Y., Nishimura, H., Kanomi, A., Okuda, T. and Puff, C. (1988) *Phytochemistry* **27**, 2591.

†A part of this work was presented at the 107th Annual Meeting of Pharmaceutical Society of Japan, Kyoto, April 1987.

concluded to be 7 from the chemical shift (δ 3.72) of the C-11 carbomethoxy group of **4**. The β-glucosidic linkage of the second sugar was inferred from the coupling constant ($J = 7.6$ Hz) of the anomeric proton. Thus, the structure of **4** was elucidated as 7-(1-β-D-glucosyl)-oleoside 11-methyl ester.

The glucoside (**5**), $C_{17}H_{24}O_{11}$, was obtained as a white powder of $[\alpha]_D^{25}$ $-45.87°$ (MeOH). It showed a UV maximum at 232 nm (log ε 4.15), IR bands at 3350, 1700 and 1620 cm^{-1} and a ^1H NMR signal due to H-3 at δ 7.58, again features found in iridoid compounds. The ^1H- and ^{13}C NMR spectra of **5** coupled with its molecular formula further suggested that it should be 8-epikingiside (**5**) [6]. This was confirmed through comparison of the spectral data of the acetate of **5** with those of 8-epikingiside acetate (**11**) chemically derived from loganin [6]. This is the second example of naturally occurring epikingiside.†

Based on the co-occurrence of **6**, **7** and **12** in *L. japonicum* and their stereochemistry on C-8, Inouye *et al.* [2] postulated that this series of glucosides would be biosynthesized via an epoxide with the 8*S*-configuration such as **13**. The occurrence of glucoside **5** together with several other oleoside type glucosides in this plant supports this proposal.

EXPERIMENTAL

General. Mps: uncorr; NMR: ^1H, 200 MHz; ^{13}C, 50.10 MHz, TMS as int. standard; TLC: silica gel GF_{254}, spots visualized by irradiation under UV light (254 nm) or by exposure to I_2 vapour; prep. TLC; silica gel PF_{254}, bands detected under UV light or by exposure to I_2 vapour; medium pressure CC: silica gel PF_{254}; CC: highly porous polymer Diaion HP-21 (Mitsubisi Kasei Co. Ltd.); prep. HPLC: column dimension: 300 × 22 mm; packing: ODS YMC-30 (Yamamura Chemical Laboratories Co. Ltd.); solvent: 30% MeOH–H_2O; flow rate: 5 ml/min; detect: UV 254 nm. Reagents of glucose measurement: System Gluc-DH (Merck).

Plant material. The berries of *Ligustrum japonicum* grown in the Herbal Garden, Faculty of Pharmaceutical Sciences, Kinki University were collected in December 1986. A voucher specimen (K. Inoue OL No. 1) was deposited in the Herbarium of the Institute of Botany, Faculty of Science, Kyoto University, Kitashirakawaoiwake-cho, Sakyo-ku, Kyoto 606, Japan.

Isolation of glucosides. The fresh ripe berries (1.15 kg) of *L. japonicum* were extracted with hot MeOH (2 l × 5) and the residue obtained by the removal of the solvent *in vacuo* was triturated with H_2O (800 ml). After separation of the insoluble material by filtration through a layer of Celite, it was washed with H_2O (300 ml × 2). The combined filtrate and washings were concd *in vacuo* to give a residue (75.8 g) which was chromatographed on Diaion HP-21 (1.52 l) eluting successively with H_2O (5 l) and MeOH (5 l). The residue (63.1 g) obtained from the MeOH eluate through concn *in vacuo* was further submitted to medium pressue CC on silica gel (200 g) with $CHCl_3$–MeOH (19:1, 500 ml), (9:1, 4 l), (17:3, 4 l) and MeOH (2 l) as eluant, collecting 300 ml fractions. Fractions 7–9, 100–104, 122–163 and 182–254 were concd *in vacuo* to give residues R-1 (3.51 g), R-2 (2.50 g), R-3 (8.24 g) and R-4 (5.14 g), respectively. R-1 was subjected to Diaion HP-21 (70.2 ml) CC eluting successively with 10% MeOH–H_2O (200 ml) and MeOH (200 ml). The residue (36.1 mg) obtained from the 10% MeOH–H_2O eluate was further subjected to prep. TLC with $CHCl_3$–MeOH (6:1, 8 developments) followed by prep. HPLC. The eluates with R_t = 30.2 min gave 8-epikingiside (**5**) (20.6 mg) as a white powder. The MeOH eluate yielded ligstroside (**8**) (1.84 g) as a white powder. Next, an aliquot of R-2 (70.2 mg) was purified by prep. TLC ($CHCl_3$–MeOH, 4:1), and the bands around R_f 0.52, 0.40 and 0.28 yielded ligustaloside B (**7**) (11.2 mg), 10-hydroxyligustroside (**3**) (9.1 mg) and ligustaloside A (**6**) (45.3 mg) as a white powder, respectively. Likewise, an aliquot of R-3 (123.7 mg) was subjected to prep. TLC (1st: $CHCl_3$–MeOH, 4:1, 3 developments; 2nd: C_6H_6–AcOEt–EtOH, 1:4:1, R_f 0.22) to give nuezhenide (**5**) (89.1 mg) as a white powder. Finally, an aliquot of R-4 (2.02 g) was purified by prep. TLC (1st: $CHCl_3$–MeOH, 7:3, R_f 0.13; 2nd: C_6H_6–AcOEt–EtOH, 1:4:1, 2 developments), giving rise to methyl glucooleoside (**4**) (27.8 mg) as a white powder.

Table 1. ^{13}C NMR signals of compounds **4**, **5** and **11**

C	4	5	11
1	95.5 *d*	96.3 *d*	93.9 *d*
3	155.4 *d*	154.4 *d*	151.4 *d*
4	109.2 *s*	109.6 *s*	110.3 *s*
5	31.4 *d*	28.1 *d*	25.3 *d*
6	40.6 *t*	34.6 *t*	33.4 *t*
7	171.9 *s*	174.7 *s*	170.5 *s*
8	125.5 *d*	75.8 *d*	73.2 *d*
9	130.3 *s*	41.9 *d*	40.8 *s*
10	13.8 *q*	21.7 *q*	20.4 *q*
11	168.8 *s*	168.3 *s*	166.1 *s*
OMe	52.0 *q*	52.0 *q*	51.6 *q*
1′	101.0 *d*	100.7 *d*	96.6 *d*
2′	74.8 *d*	74.7 *d*	70.6 *d*
3′	78.4 *d*[a]	78.5 *d*	72.4 *d*
4′	71.5 *d*[b]	71.7 *d*	68.2 *d*
5′	78.0 *d*	77.9 *d*	72.4 *d*
6′	62.8 *t*[c]	62.9 *t*	61.5 *t*
1″	95.9 *d*		
2″	73.9 *d*		
3″	78.7 *d*[a]	esterified glucose	
4″	71.1 *d*[b]	moiety	
5″	78.0 *d*		
6″	62.2 *t*[c]		

The spectra of **4** and **5** were measured in CD_3OD; **11** was measured in $CDCl_3$.

Assignments were made by gated decoupling mode.

Values with the same superscript may be interchangeable.

Methyl glucooleoside (**4**). $[\alpha]_D^{16}$ $-164.71°$ (MeOH; *c* 0.51). UV λ_{max}^{MeOH} nm (log ε): 238 (4.08); IR ν_{max}^{KBr} cm^{-1}: 3350, 1740, 1700, 1620; ^1H NMR (CD_3OD) δ: 1.77 (3H, *dd*, $J = 7.1$ and 1.2 Hz, H_3-10), 2.59 (1H, *dd*, $J = 15.4$ and 9.0 Hz, H_a-6), 2.80 (1H, *dd*, $J = 15.4$ and 3.9 Hz, H_b-6), 3.72 (3H, *s*, COOMe), 3.82 (1H, *dd*, $J = 12.5$ and 1.7 Hz, H_S-6′ or H_S-6″), 3.90 (1H, *dd*, $J = 12.5$ and 1.7 Hz, H_S-6′ or H_S-6″), 4.02 (1H, *dd*, $J = 9.0$ and 3.9 Hz, H-5), 4.82 (1H, *d*, $J = 8.0$ Hz, H-1′), 5.44 (1H, *d*, $J = 7.6$ Hz, H-1″), 5.95 (1H, *br s*, H-1),

†During the preparation of the manuscript of this paper, we noticed that 8-epikingiside had been isolated from another oleaceous plant, *Syringa vulgaris*, by Kikuchi, M., Yamauchi, Y., Takahashi, Y., Nagaoka, I. and Sugiyama, M. (1988) *Yakugaku Zasshi* **108**, 355.

6.12 (1H, *qd*, $J = 7.1$ and 1.0 Hz, H-8), 7.54 (1H, *s*, H-3). FAB-MS *m/z*: 567 $[M+H]^+$. Found: C, 45.79; H, 6.25. $C_{23}H_{34}O_{16} \cdot 2H_2O$ requires: C, 45.85; H, 6.36.

Acetylation of methyl glucooleoside (**4**). **4** (13.3 mg) was acetylated with pyridine–Ac_2O (each 0.13 ml) in the usual way. The product (15.3 mg) was purified by prep. TLC ($CHCl_3$–MeOH, 97:3) followed by recrystallization from EtOH to give methyl glucooleoside octaacetate (**10**) (11.1 mg) as colourless needles, mp 114°. $[\alpha]_D^{16}$ −94.74° ($CHCl_3$; *c* 0.38). UV λ_{max}^{EtOH} nm (log ε): 237 (4.07); IR ν_{max}^{KBr} cm^{-1}: 1740, 1700 sh, 1620; ^1H NMR ($CDCl_3$) δ 1.75 (3H, *dd*, $J = 7.1$ and 1.2 Hz, H_3-10), 2.01, 2.03, 2.04, 2.09 and 2.11 (24H, each *s*, OAc), 2.53 (1H, *dd*, $J = 15.9$ and 7.8 Hz, H_a-6), 2.72 (1H, *dd*, $J = 15.9$ and 3.9 Hz, H_b-6), 3.73 (3H, *s*, COOMe), 3.74–3.87 (2H, *m*, H-5′ and H-5″), 3.96 (1H, *dd*, $J = 7.8$ and 3.9 Hz, H-5), 4.11 (1H, *dd*, $J = 12.7$ and 2.2 Hz, H_S-6′ or H_S-6″), 4.15 (1H, *dd*, $J = 12.7$ and 2.2 Hz, H_S-6′ or H_S-6″), 4.27 (1H, *dd*, $J = 12.7$ and 4.6 Hz, H_R-6′ or H_R-6″), 4.37 (1H, *dd*, $J = 12.7$ and 4.6 Hz, H_R-6′ or H_R-6″), 5.04 (1H, *d*, $J = 7.8$ Hz, H-1′), 5.25 (1H, *t*, $J = 9.0$ Hz, H-3′ or H-3″), 5.28 (1H, *t*, $J = 9.0$ Hz, H-3′ or H-3″), 5.63 (1H, *br s*, H-1), 5.66 (1H, *d*, $J = 8.0$ Hz, H-1″), 6.02 (1H, *qd*, $J = 7.1$ and 0.9 Hz, H-8), 7.46 (1H, *s*, H-3). FAB-MS *m/z*: 903 $[M+H]^+$. Found: C, 51.61; H, 5.56. $C_{39}H_{50}O_{24}$ requires: C, 51.89; H, 5.58.

Zemplén reaction of methyl glucooleoside (**4**). Methanolic NaOMe (1 M, 1 drop) was added to a soln of methyl glucoleoside (**4**) (10.2 mg) in abs. MeOH (0.5 ml) at room temp. The reaction mixture was neutralized with Amberlite IR-120 B (H^+ form) and the resin was filtered off. The filtrate was concd *in vacuo* and the resulting residue (9.8 mg) was submitted to prep. TLC ($CHCl_3$–MeOH, 4:1). Of the two bands with R_f 0.43 and 0.97, the former gave on concn *in vacuo* a residue (3.2 mg) which was acetylated with pyridine–Ac_2O in the usual way. On recrystallization from EtOH, the product afforded 1.7 mg colourless needles, mp 115°, which were identified with oleoside dimethyl ester tetraacetate (**14**) derived from oleuropein (**2**). ^1H NMR ($CDCl_3$) δ 1.74 (3H, *dd*, $J = 7.0$ and 1.5 Hz, H_3-10), 2.02, 2.03 and 2.08 (12H, each *s*, OAc), 2.42 (1H, *dd*, $J = 14.5$ and 9.0 Hz, Ha-6), 2.76 (1H, *dd*, $J = 14.5$ and 4.5 Hz, Hb-6), 3.63 (3H, *s*, 7-OMe), 3.73 (3H, *s*, 11-OMe), 3.77 (1H, *ddd*, $J = 9.0$, 5.0 and 2.5 Hz, H-5′), 3.99 (1H, *dd*, $J = 9.0$ and 4.5 Hz, H-5), 4.12 (1H, *dd*, $J = 12.0$ and 2.5 Hz, H_S-6′), 4.32 (1H, *dd*, $J = 12.0$ and 5.0 Hz, H_R-6′), 5.04 (1H, *d*, $J = 7.5$ Hz, H-1′), 5.13 (2H, *dd*, $J = 9.0$ and 7.5 Hz, H-2′ and *t*, $J = 9.0$ Hz, H-4′), 5.29 (1H, *t*, $J = 9.0$ Hz, H-3′), 5.71 (1H, *br s*, H-1), 6.02 (1H, *qd*, $J = 7.0$ and 1.5 Hz, H-8), 7.48 (1H, *s*, H-3). The second band obtained on TLC gave a white residue (0.9 mg). To the aq. soln (0.1 ml) of this residue a phosphate buffered soln of mutarotase and NAD (1 ml, pH 7.6) and an aq. soln of glucose dehydrogenase were added. After standing for 3 hr at room temp., the amount of NADH was determined by measuring the absorbance at 340 nm to detect D-glucose.

8-Epikingiside (**5**). $[\alpha]_D^{25}$ −45.87° (MeOH; *c* 1.88). UV λ_{max}^{MeOH} nm (log ε): 232 (4.15); IR ν_{max}^{KBr} cm^{-1}: 3350, 1700, 1620; ^1H NMR (CD_3OD) δ: 1.51 (3H, *d*, $J = 6.4$ Hz, H_3-10), 2.14 (1H, *br q*, $J = \sim 7.3$ Hz, H-9), 2.50 (1H, *dd*, $J = 16.5$ and 11.4 Hz, Hax-6), 2.86 (1H, *dd*, $J = 16.5$ and 4.4 Hz, Heq-6), 3.08 (1H, *dddd*, $J = 11.4$, 7.3, 4.4 and 1.0 Hz, H-5), 3.63 (1H, *dd*, $J = 12.0$ and 6.1 Hz, H_R-6′), 3.73 (3H, *s*, COOMe), 3.92 (1H, *dd*, $J = 12.0$ and 2.0 Hz, H_S-6′), 4.49 (1H, *br quintet*, $J = \sim 6.8$ Hz, H-8), 4.70 (1H, *d*, $J = 7.8$ Hz, H-1′), 5.49 (1H, *d*, $J = 7.6$ Hz, H-1), 7.58 (1H, *d*, $J = 1.0$ Hz, H-3). FAB-MS *m/z*: 405 $[M+H]^+$ (M: $C_{17}H_{24}O_{11}$).

Acetylation of 8-epikingiside (**5**). **5** (8.7 mg) was acetylated with pyridine–Ac_2O (each 0.1 ml) in the usual way and the product (12.6 mg) was recrystallized from EtOH to give 8-epikingiside tetraacetate (**11**) (11.5 mg) as colourless needles, mp 114.5–115° (lit. mp 114.5–115.5° [6]), $[\alpha]_D^{26}$ −44.44° ($CHCl_3$; *c* 0.54) (lit. $[\alpha]_D^{19}$ −55.0° ($CHCl_3$; *c* 0.89) [6]). UV λ_{max}^{EtOH} nm (log ε): 231 (4.03); IR ν_{max}^{KBr} cm^{-1}: 1740, 1620; ^1H NMR ($CDCl_3$) δ 1.50 (3H, *d*, $J = 6.3$ Hz, H_3-10), 1.97, 2.01, 2.04 and 2.10 (12H, each *s*, $OCOCH_3$), 2.31–2.46 (2H, *m*, H_a-6 and H-9), 2.98–3.18 (2H, *m*, H-5 and H_b-6), 3.74 (4H, *s*, COOMe and *ddd*, $J = 11.6$, 4.6 and 2.4 Hz, H-5′), 4.16 (1H, *dd*, $J = 12.5$ and 2.4 Hz, H_S-6′), 4.29 (1H, *dd*, $J = 12.5$ and 4.4 Hz, H_R-6′), 4.37 (1H, *brqd*, $J = \sim 6.6$ and ~ 6.4 Hz, H-8), 4.88 (1H, *d*, $J = 8.1$ Hz, H-1′), 5.02 (1H, *dd*, $J = 9.3$ and 8.1 Hz, H-2′), 5.11 (1H, *dd*, $J = 9.5$ and 9.0 Hz, H-4′), 5.24 (1H, *t*, $J = 9.3$ Hz, H-3′), 5.28 (1H, *d*, $J = 5.6$ Hz, H-1), 7.47 (1H, *d*, $J = 1.0$ Hz, H-3). FAB-MS *m/z*: 573 $[M+H]^+$. Found: C, 52.22; H, 5.84. $C_{25}H_{32}O_{15}$ requires: C, 52.45; H, 5.63.

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