Isolation of Oleayunnanoside from *Fraxinus insularis* and Revision of Its Structure to Insularoside-6‴-*O*-β-D-glucoside[1)]

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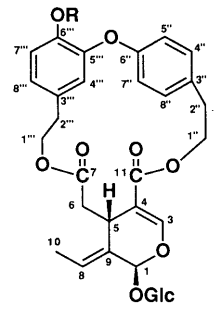
A secoiridoid glucoside, insularoside-6‴-*O*-β-D-glucoside, was isolated along with 9 known compounds from the leaves of *Fraxinus insularis* and its structure was determined to be 2 on the basis of spectroscopic and chemical studies. Direct comparison of this glucoside with a known secoiridoid glucoside, oleayunnanoside, led to the conclusion that the two were identical, and that the proposed structure 2a for oleayunnanoside should be revised to 2.

Keywords *Fraxinus insularis*; structure revision; oleayunnanoside; insularoside-6‴-*O*-β-D-glucoside; secoiridoid glucoside; oleaceae

In a previous paper,[2)] we reported the isolation of an unusual secoiridoid glucoside, insularoside (**1**) from the $CHCl_3$-soluble fraction of a methanolic extract of the leaves of *Fraxinus insularis* HEMSL., which grows in Taiwan. In the course of further studies on the glycosidal constituents of the same plant, we isolated and characterized a second secoiridoid glucoside, insularoside-6‴-*O*-β-D-glucoside from the polar fraction. This paper describes the structure elucidation of the glucoside.

The fresh leaves of *F. insularis* were extracted with hot MeOH. The *n*-BuOH-soluble portion of the extract was separated by a combination of chromatographic procedures to yield ten compounds. Of these compounds, nine were identified as follows: three coumarin derivatives, cichoriin,[3)] esculetin[4)] and esculin,[5)] two phenylpropanoid glycosides, desrhamnosylacteoside[6)] and 2-(3,4-dihydroxyphenyl)ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside,[7)] two flavonoid glycosides, hyperin[8)] and quercitrin,[8a,b)] and two secoiridoid glucosides, insularoside (**1**) and oleuropein,[3)] by comparison of their physical and spectral data with those described in the literature or by direct comparison with authentic samples.



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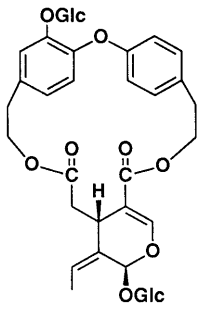
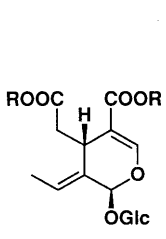
2: R = Glc



2a



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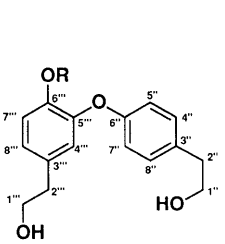
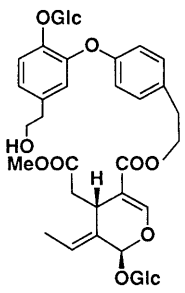


5: R = Glc
6: R = H



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Compound **2** was obtained as an amorphous powder with the molecular formula $C_{38}H_{46}O_{18}$. Its UV spectrum, besides the typical absorption (232 nm) of iridoidic enol ether systems conjugated with a carbonyl group, presented additional absorptions at 272 sh and 282 sh nm due to aromatic chromophore(s). It showed IR bands at 3404 (OH), 1710 (ester), 1626 (C=C), and 1508 cm^{-1} (aromatic ring). The ^{1}H-NMR spectrum of **2** exhibited a singlet characteristic of H-3 of an oleoside (**3**)-type secoiridoid glucoside at δ 7.54 (s), and signals due to a vinyl methyl group at δ 1.61 (dd), an anomeric proton at δ 4.77 (d), an allylic acetal proton at δ 5.86 (br s), an olefinic proton at δ 6.05 (qd), and an aromatic AA′BB′ and an AMX spin systems at δ 6.57—7.21. These signals, together with the ^{13}C-NMR spectrum (Table I), showed close similarity to those of insularoside (**1**) except for residual signals due to another glycosyl unit. Attachment of the glucose unit at C-6‴ of the insularoside (**1**) moiety was suggested by the ^{13}C-NMR spectrum of **2**, which showed downfield shifts of C-3‴, C-5‴ and C-7‴ by 3.3, 2.2 and 1.4 ppm, respectively, when compared with those of **1**. The anomeric configuration of the glucosyl linkage was determined to be β from the coupling constant (d, J = 7.5 Hz) of the anomeric proton. All these findings allowed us to propose a plausible structure **2** for the isolated compound.

To confirm this assumption, the following series of reactions was conducted. Alkaline hydrolysis of **2** followed by methylation with CH_2N_2–Et_2O gave two compounds **4** and **5**. The spectral and physical data of **4** were in good accordance with those of oleoside 7,11-dimethyl ester,[9)] confirming the absolute stereostructure of the secoiridoid moiety in **2**. Compound **5** was comparable with the diphenyl ether **6**, which was prepared from insularoside (**1**).[2)] The ^{1}H- and ^{13}C-NMR spectral features of **2** demonstrated clearly the presence of a glucosyl moiety in addition to the

TABLE I. ^{13}C-NMR Data for Insularoside (**1**), **2**, **5** and **7** in CD_3OD

C	1		2		7		5	
1	95.2		95.2		95.2			
3	155.4		155.4		155.2			
4	109.8		109.8		109.5			
5	31.4		31.4		31.8			
6	40.9		40.9		41.0			
7	172.7		172.6		173.6			
8	125.2[a]		125.2		124.9			
9	130.1		130.0		130.5			
10	13.7		13.7		13.7			
11	168.0		168.0		168.1			
1′, 1‴	101.0		101.0,	103.0	100.9,	102.8		102.8
2′, 2‴	74.8		74.8,[c]	74.9[c]	74.8,[h]	74.9[h]		74.9
3′, 3‴	78.0		78.0,	78.0[g]	78.0,[i]	77.9[i,m]		77.9[o]
4′, 4‴	71.5		71.5,[d]	71.4[d]	71.5,[j]	71.3[j]		71.3
5′, 5‴	78.4		78.4,[e]	78.3[e,g]	78.4,[k]	78.2[k,m]		78.3[o]
6′, 6‴	62.8		62.8,[f]	62.6[f]	62.8,[l]	62.6[l]		62.6
1″, 1‴	66.0,[b]	66.4[b]	66.0,	66.2	66.1,	64.1	64.1,	64.3
2″, 2‴	35.9,	34.8	35.9,	34.9	35.4,	39.4	39.5,[n]	39.4[n]
3″, 3‴	135.5,	132.3	136.0,	135.6	134.1,	135.8	134.9,	135.7
4″, 4‴	131.6,	120.1	131.7,	120.3	131.3,	122.7	131.2,	122.5
5″, 5‴	121.0,	147.1	121.4,	149.3	118.7,	147.1	118.7,	147.4
6″, 6‴	157.5,	147.7	157.1,	147.9	158.1,	148.7	157.8,	148.6
7″, 7‴	121.0,	117.8	121.4,	119.2	118.7,	119.0	118.7,	119.0
8″, 8‴	131.6,	125.0[a]	131.7,	124.8	131.3,	126.3	131.2,	126.1
OMe					52.2			

a—*o*) Assignments with the same superscript may be interchanged.

same diphenyl ether system as in **6**. Enzymic hydrolysis of **5** with β-glucosidase liberated **6**, indicating the linkage of a β-D-glucose unit to **6** in the molecule of **5**. On the other hand, mild alkaline hydrolysis of **2**, followed by methylation, furnished **7**, an ester derivative of oleoside methyl ester with the diphenyl ether **5**. From the chemical shift of the methoxyl signal in the ^{1}H-NMR spectrum of **7**, the carbomethoxyl group could be located at the α,β-saturated position, *i.e.* at C-7.[10] This was further confirmed by heteronuclear maltiple bond connectivity (HMBC) experiments with **7**, which showed a ^3J interaction between $COOC\underline{H}_3$ and C-7 (δ 173.6), that in turn correlated with C-6 methylene proton signals at δ 2.40 and 2.66. Therefore, the diphenyl ether moiety should be connected to the oleoside skeleton at C-11. The HMBC and ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) spectra of **7**, furthermore, allowed the assignments of all proton and carbon signals of the diphenyl ether (**5**) moiety, indicating that the *p*-substituted phenethoxy moiety was attached to C-11 of the oleoside (**3**) skeleton as in insularoside (**1**). Accordingly, the structure of the glucoside **2** was characterized as insularoside-6‴-*O*-β-D-glucoside.

Oleayunnanoside, a unique secoiridoid glucoside with a diphenyl ether linkage, has previously been isolated from *Olea yunnanensis*[11] and formulated as **2a**. However, the close similarity of the spectral data of our glucoside **2** to those reported for oleayunnanoside prompted us to reexamine the structure of the latter. As a result of direct comparison, we found that the two glucosides were identical and that the reported NMR data for oleayunnanoside were partially erroneous. Therefore, the structure of oleayunnanoside was revised to insularoside-6‴-*O*-β-D-glucoside (**2**) with corrected assignments of the ^{1}H- and ^{13}C-NMR signals.

Experimental

UV and IR spectra were obtained on a Shimadzu UV-240 spectrophotometer and a Hitachi 270-30 infrared spectrophotometer, respectively. Optical rotations were measured on a JASCO DIP-180 digital polarimeter. Secondary ion mass spectrometry (SIMS) spectra were recorded on a Hitachi M-4100 mass spectrometer using glycerol as the matrix. All NMR experiments were performed with a Varian VXR-500 spectrometer operating at 499.8 (^{1}H) and 125.7 MHz (^{13}C), with tetramethylsilane as an internal standard.

Isolation The leaves of *F. insularis* were collected in Heng-Chun Tropical Botanical Garden, Taiwan in August 1990. A voucher specimen (IT-9001) has been deposited in the Herbarium of Gifu Pharmaceutical University, Gifu 502, Japan. Fresh leaves of *F. insularis* (950 g) were extracted with hot MeOH. After concentration, the extract (176.2 g) was triturated in H_2O and filtered through a Celite layer. The filtrate and washings were combined and extracted with $CHCl_3$ and *n*-BuOH successively. The *n*-BuOH layer was concentrated to give a foamy residue (68.0 g). An aliquot (20.0 g) of the residue was resuspended in MeOH, and after removal of the precipitated crude cichoriin (926 mg) by filtration, the mother liquor was chromatographed on a silica gel column with $CHCl_3$–MeOH. Combined fractions eluted with $CHCl_3$–MeOH (95 : 5), (95 : 5 to 92 : 8), (92 : 8) and (9 : 1 to 85 : 15) were concentrated *in vacuo* to afford fractions I (284 mg), II (660 mg), III (1.49 g) and IV (3.73 g), respectively. Fractions I and II were further purified by preparative TLC (AcOEt–C_6H_6–EtOH, 4 : 1 : 1) to give esculetin (252 mg) and insularoside (**1**) (278 mg), respectively. An aliquot (47 mg) of III was submitted to preparative TLC (AcOEt–C_6H_6–EtOH, 4 : 1 : 1), yielding esculin (4 mg) and desrhamnosylacteoside (4 mg). Fraction IV was further submitted to column chromatography on silica gel with AcOEt–C_6H_6–EtOH (4 : 1 : 0 to 80 : 20 : 1), providing IV/1 (992 mg), IV/2 (247 mg), IV/3 (579 mg), IV/4 (478 mg), IV/5 (834 mg) and IV/6 (641 mg). Fraction IV/1 was subjected to preparative HPLC (μBondasphere 5μ C8-100 Å, MeOH–H_2O, 4 : 6), giving desrhamnosylacteoside (177 mg) and 2-(3,4-dihydroxyphenyl)ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside (52 mg). In the same way, the following fractions were purified by a combination of preparative TLC (AcOEt–C_6H_6–EtOH, 4 : 1 : 1) and preparative HPLC (μBondasphere 5μ C8-100 Å, MeOH–H_2O, 12 : 13). Fraction IV/2 gave 2-(3,4-dihydroxyphenyl)ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside (43 mg), quercitrin (14 mg) and hyperin (8 mg); IV/3: cichoriin (59 mg); IV/4: esculin (48 mg), **2** (179 mg); IV/5: **2** (428 mg); IV/6: oleuropein (4 mg), **2** (52 mg).

Insularoside-6‴-*O*-β-D-glucoside (2) Powder, $[\alpha]_D^{22}$ −81.5° (*c* = 1.2, MeOH). UV λ_{max}^{MeOH} nm (log ε): 232 (4.24), 272 sh (3.36), 282 sh (3.18). IR

ν_{max}^{KBr} cm^{-1}: 3404, 1710, 1626, 1508. ^{1}H-NMR (CD_3OD) δ: 1.61 (3H, dd, J=7.0, 1.5 Hz, H_3-10), 2.16 (1H, dd, J=15.0, 11.0 Hz, H-6), 2.31 (1H, dd, J=15.0, 3.5 Hz, H-6), 2.79 (2H, t-like, J=5.0 Hz, H_2-2‴), 2.91 (1H, ddd, J=14.5, 5.5, 3.5 Hz, H-2″), 3.02 (1H, ddd, J=14.5, 9.0, 4.0 Hz, H-2″), 3.28 (1H, dd, J=9.0, 8.0 Hz, H-2′), 3.31 (1H, m, H-5′ or H-5⁗), 3.39 (1H, t, J=9.0 Hz, H-3′), 3.40 (1H, m, H-3⁗), 3.45 (1H, m, H-5⁗ or H-5′), 3.49 (1H, dd, J=9.0, 7.5 Hz, H-2⁗), 3.64 (1H, dd, J=12.0, 6.0 Hz, H-6′ or H-6⁗), 3.69 (1H, dd, J=12.0, 6.0 Hz, H-6⁗ or H-6′), 3.79 (1H, dd, J=11.0, 3.5 Hz, H-5), 3.85 (1H, dd, J=12.0, 2.0 Hz, H-6⁗ or H-6′), 3.86 (1H, dd, J=12.0, 2.0 Hz, H-6′ or H-6⁗), 4.03 (1H, dt, J=11.0, 5.5 Hz, H-1‴), 4.27 (1H, dt, J=11.0, 5.5 Hz, H-1‴), 4.46 (1H, ddd, J=11.0, 9.0, 3.5 Hz, H-1″), 4.54 (1H, ddd, J=11.0, 5.5, 4.0 Hz, H-1″), 4.77 (1H, d, J=8.0 Hz, H-1′), 4.99 (1H, d, J=7.5 Hz, H-1⁗), 5.86 (1H, br s, H-1), 6.05 (1H, qd, J=7.0, 1.0 Hz, H-8), 6.57 (1H, d, J=2.0 Hz, H-4‴), 6.87 (1H, dd, J=8.0, 2.0 Hz, H-8‴), 6.95 (2H, AA′BB′ pattern, J=8.5 Hz, H-5″, H-7″), 7.20 (1H, d, J=8.0 Hz, H-7‴), 7.21 (2H, AA′BB′ pattern, J=8.5 Hz, H-4″, H-8″), 7.54 (1H, s, H-3). ^{13}C-NMR: see Table I. HR-SIMS m/z: 813.2595 $(M+Na)^+$. Calcd for $C_{38}H_{46}NaO_{18}$: 813.2579. *Anal.* Calcd for $C_{38}H_{46}O_{18}\cdot H_2O$: C, 56.43; H, 5.98. Found: C, 56.53; H, 6.04.

Hydrolysis of Insularoside-6‴-*O*-β-D-glucoside (2) Followed by Methylation A solution of **2** (30.5 mg) in 0.5 M NaOH (5 ml) was stirred for 6 h at room temperature, neutralized with Amberlite IR-120 (H^+ form) and concentrated *in vacuo*. A solution of the resulting residue (30.4 mg) in MeOH was treated with CH_2N_2–Et_2O under ice-cooling. The reaction mixture was evaporated *in vacuo* and the residue (31.6 mg) was subjected to preparative HPLC (μBondasphere 5μ C8-100 Å, MeOH–H_2O, 1 : 1) to give **4** (8.1 mg) and **5** (9.8 mg). Compound **4** was identified as oleoside dimethyl ester (^{1}H-NMR, UV, IR, $[\alpha]_D$).[9]

Compound **5**: Powder, $[\alpha]_D^{23}$ −36.6° (c=0.8, MeOH). UV λ_{max}^{MeOH} nm (log ε): 220 sh (4.24), 274 (3.43), 282 sh (3.34). IR ν_{max}^{KBr} cm^{-1}: 3388, 1508, 1274, 1220. ^{1}H-NMR (CD_3OD) δ: 2.71 (2H, t, J=7.0 Hz, H_2-2‴), 2.78 (2H, t, J=7.0 Hz, H_2-2″), 3.32—3.42 (4H, m, H-2⁗, H-3⁗, H-4⁗, H-5⁗), 3.66 (1H, dd, J=12.0, 5.0 Hz, H-6⁗), 3.67 (2H, t, J=7.0 Hz, H_2-1‴), 3.73 (2H, t, J=7.0 Hz, H_2-1″), 3.84 (1H, dd, J=12.0, 2.0 Hz, H-6⁗), 4.91 (1H, d, J=7.5 Hz, H-1⁗), 6.81 (1H, d, J=2.0 Hz, H-4‴), 6.88 (2H, AA′BB′ pattern, J=9.0 Hz, H-5″, H-7″), 6.97 (1H, dd, J=8.5, 2.0 Hz, H-8‴), 7.17 (2H, AA′BB′ pattern, J=9.0 Hz, H-4″, H-8″), 7.21 (1H, d, J=8.5 Hz, H-7‴). ^{13}C-NMR: see Table I. HR-SIMS m/z: 459.1646 $(M+Na)^+$. Calcd for $C_{22}H_{28}NaO_9$: 459.1630.

Enzymic Hydrolysis of 5 β-D-Glucosidase (Sigma) (6.3 mg) was added to an acetate buffer solution (0.2 M, pH 5.0) (5 ml) of **5** (7.7 mg) and the mixture was incubated at 37 °C overnight. The solution was extracted with AcOEt and concentration of the AcOEt layers afforded an aglucone (3.7 mg). The ^{1}H-NMR and electron impact (EI) mass spectral data for the compound were identical with those of **6** derived from insularoside (**1**).

Partial Hydrolysis of Insularoside-6‴-*O*-β-D-glucoside (2) Followed by Methylation A solution of **2** (47.9 mg) in 0.2 M NaOH (10 ml) was stirred for 55 min at room temperature, and worked up in the same way as described above. The resulting residue (46.6 mg) was dissolved in MeOH and treated with excess CH_2N_2–Et_2O. After evaporation of the solvent, the residue (44.1 mg) was subjected to preparative HPLC (μBondasphere 5μ C8-100 Å, MeOH–H_2O, 1 : 1), giving **4** (1.8 mg), **5** (3.9 mg) and **7** (12.6 mg).

Compound **7**: Powder, $[\alpha]_D^{23}$ −116.2° (c=0.9, MeOH). UV λ_{max}^{MeOH} nm (log ε): 220 sh (4.39), 235 sh (4.31), 271 sh (3.48), 280 sh (3.39). IR ν_{max}^{KBr} cm^{-1}: 3432, 1704, 1636, 1508. ^{1}H-NMR (CD_3OD) δ: 1.71 (3H, dd, J=7.0, 1.5 Hz, H_3-10), 2.40 (1H, dd, J=14.0, 9.5 Hz, H-6), 2.66 (1H, dd, J=14.0, 4.5 Hz, H-6), 2.71 (2H, t, J=7.0 Hz, H_2-2‴), 2.93 (2H, t, J=7.0 Hz, H_2-2″), 3.28—3.43 (8H, m, H-2′, H-2⁗, H-3′, H-3⁗, H-4′, H-4⁗, H-5′, H-5⁗), 3.62 (3H, s, COOMe), 3.66 (2H, dd, J=12.0, 6.0 Hz, H-6′ and H-6⁗), 3.68 (2H, t, J=7.0 Hz, H_2-1‴), 3.84 (1H, dd, J=12.0, 2.0 Hz, H-6⁗ or H-6′), 3.88 (1H, dd, J=12.0, 2.0 Hz, H-6′ or H-6⁗), 3.96 (1H, dd, J=9.5, 4.5 Hz, H-5), 4.30 (1H, dt, J=11.5, 7.0 Hz, H-1″), 4.34 (1H, dt, J=11.5, 7.0 Hz, H-1″), 4.80 (1H, d, J=7.5 Hz, H-1′), 4.90 (1H, d, J=7.5 Hz, H-1⁗), 5.90 (1H, br s, H-1), 6.09 (1H, qd, J=7.0, 1.0 Hz, H-8), 6.82 (1H, d, J=2.0 Hz, H-4‴), 6.88 (2H, AA′BB′ pattern, J=8.5 Hz, H-5″, H-7″), 6.98 (1H, dd, J=8.5, 2.0 Hz, H-8‴), 7.21 (2H, AA′BB′ pattern, J=8.5 Hz, H-4″, H-8″), 7.21 (1H, d, J=8.5 Hz, H-7‴), 7.47 (1H, s, H-3). ^{13}C-NMR: see Table I. HR-SIMS m/z: 845.2862 $(M+Na)^+$. Calcd for $C_{39}H_{50}NaO_{19}$: 845.2841.

Acknowledgement The excellent technical assistance of Misses N. Suzuki and N. Takinami is acknowledged. Thanks are also due to Dr. M. Sugiura (Kobe Women's College of Pharmacy) for ^{1}H- and ^{13}C-NMR spectra, to Dr. K. Saiki (Kobe Women's College of Pharmacy) for mass spectra measurements and to the staff of the Microanalytical Centre of Kyoto University for microanalysis.

References and Notes

1) A part of this study was presented at the 39th Annual Meeting of the Japanese Society of Pharmacognosy, Tokyo, September, 1992.
2) T. Tanahashi, A. Shimada, N. Nagakura, K. Inoue, H. Kuwajima, K. Takaishi, C.-C. Chen, *Phytochemistry*, **33**, 397 (1993).
3) H. Kuwajima, M. Morita, K. Takaishi, K. Inoue, T. Fujita, Z.-D. He, C.-R. Yang, *Phytochemistry*, **31**, 1277 (1992).
4) S. Nishibe, H. Tsukamoto, I. Agata, S. Hisada, K. Shima, T. Kakemoto, *Shoyakugaku Zasshi*, **35**, 251 (1981).
5) H. Tsukamoto, S. Hisada, S. Nishibe, *Chem. Pharm. Bull.*, **33**, 396 (1985).
6) H. Shimomura, Y. Sashida, K. Ogawa, *Phytochemistry*, **26**, 1981 (1987).
7) H. Shimomura, Y. Sashida, T. Adachi, *Phytochemistry*, **26**, 249 (1987).
8) *a*) S. Takagi, M. Yamaki, K. Ishiguro, S.-T. Lu, *Yakugaku Zasshi*, **102**, 593 (1982); *b*) K. R. Karkham, B. Ternai, R. Stanley, H. Geiger, T. J. Mabry, *Tetrahedron*, **34**, 1389 (1978); *c*) B. Bennini, A. J. Chulia, M. Kaouadji, F. Thomasson, *Phytochemistry*, **31**, 2483 (1992).
9) H. Tsukamoto, S. Hisada, S. Nishibe, *Shoyakugaku Zasshi*, **39**, 90 (1985).
10) T. Kamikawa, K. Inoue, T. Kubota, M. C. Woods, *Tetrahedron*, **26**, 4561 (1970).
11) Z.-D. He, Z.-M. Shi, C.-R. Yang, *Acta Botanica Sinica*, **32**, 544 (1990).