Ligustrinoside, a New Bisiridoid Glucoside from *Strychnos ligustrina*

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A new bisiridoid glucoside, ligustrinoside (1), along with three known iridoids, loganin, loganetin and loganic acid was isolated from the wood of *Strychnos ligustrina* BL. (Loganiaceae), collected in Indonesia. The structure of ligustrinoside (1) has been determined as an ester dimmer of loganin and loganic acid between C-7 and C-11.

Keywords *Strychnos ligustrina*; Loganiaceae; ligustrinoside; iridoid glucoside; loganin; loganetin; loganic acid

Strychnos ligustrina BL. is a widely distributed plant in tropical regions. The wood of this plant is called “kaju ular” and is used for the treatment of malaria in the Indonesian folkloric remedy “jamu”. Earlier work on the constituents of this plant described the isolation of seven strychnos alkaloids.¹⁾ This paper describes the characterization of a new bisiridoid glucoside, ligustrinoside (1), which was isolated from the wood of *S. ligustrina* collected on the Island of Java.

Ligustrinoside (1), a white powder, $[\alpha]_D -64.1^\circ$ (MeOH), showed pseudomolecular ions at m/z 771 $[M+Na]^+$, 749 $[M+H]^+$ in positive ion fast-atom bombardment mass spectrometry (FAB-MS). From the FAB-MS and elementary analysis, the molecular formula was concluded to be $C_{33}H_{48}O_{19}$. The infrared (IR) spectrum of 1 showed the presence of hydroxyl (ν_{max} 3400 cm^{-1}) and α,β -unsaturated ester carbonyl groups (ν_{max} 1700, 1640, 1290 cm^{-1}).²⁾ Its ultraviolet (UV) spectrum exhibited absorption maximum at 235 nm due to a carbonyl enol ether chromophore typical of the iridoid.²⁾ Acetylation of 1 afforded a nona-acetate (1a), which showed molecular ions at m/z 1149 $[M+Na]^+$, 1127 $[M+H]^+$ in the FAB-MS and the presence of signals due to nine acetyls (δ 1.98–2.10 ppm) in the proton nuclear magnetic resonance (1H -NMR) spectrum. The 1H -NMR spectrum of 1 showed characteristic signals for dimeric iridoid glycoside; a pair of signals due to the olefinic protons at δ 7.60, 7.67 (each 1H, d, $J=1$ Hz), methyl protons at δ 0.93, 1.23 (each 3H, d, $J=7$ Hz) and anomeric protons at δ 5.32, 5.39 (each 1H, d, $J=8$ Hz) and others as shown in Table I. Its carbon-13 (^{13}C)-NMR spectrum also showed a pair of signals due to the α,β -unsaturated ester groups at δ 167.0, 167.4, 151.1, 151.4, 112.5, 113.6, methyls at δ 13.3, 13.7 and anomeric carbons at δ 100.7, 100.8 suggesting that 1 was an ester dimmer of iridoid glycoside. Alkaline hydrolysis of 1 afforded loganic acid (4) which on subsequent methylation with diazomethane yielded loganin (2). These results suggested that the structure of 1 is an ester dimmer of loganin and loganic acid. The location of linkage

was determined by comparing the 1H - and ^{13}C -NMR chemical shifts of 1 with those of loganin (2). Marked downfield shifts for H-7 ($\Delta\delta=1.0$ ppm) and a C-7 ($\Delta\delta=3.2$ ppm) in 1 suggested that the structure of 1 was esterified between the hydroxyl group at C-7 of loganin and carboxylic acid group of loganic acid. On the basis of the above results, the structure of ligustrinoside was determined to be 1.

In addition to 1, the known iridoids loganin (2),²⁾

TABLE I. 1H - and ^{13}C -NMR Spectral Data for Compounds 1 and 2

Position	1		2	
	H	C	H	C
1	5.58 d (4)	96.7	5.69 d (4)	97.5
3	7.60 d (1)	151.4	7.70 s	151.3
4	—	112.5	—	113.3
5	3.24 br q (8)	31.6	3.53 br q (8)	31.8
6	1.81 m	39.8	1.74 m	41.7
	2.40 m		2.65 ddd (14, 8, 1)	
7	5.27 m	76.6	4.27 t (6)	73.4
8	2.14 m	39.8	2.04 m	42.9
9	2.22 m	46.6	2.45 m	46.0
10	0.93 d (7)	13.3	1.21 d (7)	13.7
11	—	167.4	—	167.7
1'	5.32 d (8)	100.7	5.40 d (8)	100.8
2'	4.02 m	74.7	4.05 m	74.8
3'	4.23 m	73.5	4.27 m	78.5
4'	4.23 m	71.7	4.27 m	71.5
5'	3.98 m	78.7	4.05 m	78.8
6'	4.30 dd (12, 4)	62.8	4.39 br dd (12, 4)	62.7
	4.47 dd (12, 2)		4.57 br d (12)	
1''	5.62 d (4)	97.8		
3''	7.67 d (1)	151.1		
4''	—	113.6		
5''	3.48 br q (8)	31.8		
6''	1.72 m	43.1		
	2.60 ddd (14, 8, 1)			
7''	4.23 m	73.5		
8''	2.05 m	41.7		
9''	2.43 m	46.0		
10''	1.23 d (7)	13.7		
11''	—	167.0		
1'''	5.39 d (8)	100.8		
2'''	4.02 m	74.7		
3'''	4.23 m	73.5		
4'''	4.23 m	71.7		
5'''	4.23 m	78.4		
6'''	4.30 dd (12, 4)	62.7		
	4.47 br q (12)			
COOCH ₃	3.56 s	51.0	3.58 s	50.9

The spectra were measured in C_5D_5N . Coupling constants (J in Hz) are given in parentheses. The signal assignments were based on spectral analysis of 1H - 1H shift correlation spectra. Abbreviations: s=singlet, d=doublet, dd=doublet of doublets, t=triplet, br d=broad doublet, br q=broad quartet, m=multiplet.

Chart 1

2 : $R_1 = \text{glc}$, $R_2 = \text{CH}_3$
 3 : $R_1 = \text{H}$, $R_2 = \text{CH}_3$
 4 : $R_1 = \text{glc}$, $R_2 = \text{H}$

loganetin (**3**)³⁾ and loganic acid (**4**)⁴⁾ were isolated and identified by direct comparison with authentic samples. This is the first report on the isolation of compounds (**2**–**4**) from this plant.

Experimental

Melting points were taken on a Yanagimoto micro-melting point apparatus and are uncorrected. UV and IR spectra were recorded on a Hitachi 340 and a Hitachi 260-30 spectrophotometers, respectively. Optical rotations were measured on a JASCO DIP-4 digital polarimeter. The ¹H- and ¹³C-NMR spectra were recorded with a JEOL JNM GX-400 (¹H, 400 MHz; ¹³C, 100 MHz) spectrometer. Chemical shifts are given on the δ -scale (ppm downfield from tetramethylsilane as an internal standard) and coupling constants (*J*) in Hz. FAB-MS was run on a JEOL JMS DX-303 mass spectrometer. Medium-pressure liquid chromatography (MPLC) was carried out on silica gel (CQ-3, Fuji gel, 24 mm i.d. $\times$ 360 mm, detector: 254 nm).

Extraction and Isolation Dried crushed wood (4.4 kg) of *S. ligustrina*, collected on the Island of Java in 1989, was extracted for 3 h with *n*-hexane (3 l $\times$ 3) and MeOH (3 l $\times$ 3). The MeOH extract was concentrated under reduced pressure to give a residue (150 g), to which an equal volume of water was added. The aqueous solution was extracted with CHCl₃ (3 l), EtOAc (3 l) and *n*-BuOH (3 l), successively. The *n*-BuOH soluble extract (74 g) was chromatographed on Diaion HP-20 (Mitsubishi Kasei) with H₂O and an increasing content with MeOH as eluents. The fraction eluted with MeOH–H₂O (1:1) was evaporated to give a residue, which was fractionated sequentially by silica gel column chromatography (CHCl₃–MeOH–H₂O = 10:5:1 and 6:4:1) and MPLC (CHCl₃–MeOH–H₂O = 30:10:1, 10:5:1 and 6:4:1) to give ligustrinoside (**1**, 130 mg) along with known compounds, loganin (**2**, 160 mg), loganetin (**3**, 15 mg), loganic acid (**4**, 76 mg).

Ligustrinoside (**1**): White powder. $[\alpha]_D^{25}$ -64.1° (*c* = 2.1, MeOH). FAB-MS (*m/z*): 771 [M+Na]⁺, 749 [M+H]⁺. Anal. Found: C, 49.85; H, 6.46. Calcd for C₃₃H₄₈O₁₉·5/2H₂O: C, 49.93; H, 6.73. IR $\nu_{\max}$ (KBr) cm⁻¹: 3400, 2900, 1700, 1640, 1290. UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 235 (3.51). ¹H- and ¹³C-NMR see Table I.

Acetylation of Ligustrinoside A mixture of ligustrinoside (**1**, 10 mg), acetic anhydride (1 ml), and pyridine (1 ml) was kept at room temperature for 30 min and treated as usual. Ligustrinoside nona-acetate (**1a**, 14 mg) was obtained as a white powder. FAB-MS *m/z*: 1149 [M+Na]⁺, 1127 [M+H]⁺. IR $\nu_{\max}$ (KBr) cm⁻¹: 3400, 2950, 1710, 1650, 1080. ¹H-NMR (δ in C₅D₅N): 0.95 (3H, d, *J* = 6 Hz), 1.03 (3H, d, *J* = 6 Hz), 1.98, 1.99, 2.02, 2.03, 2.03, 2.05, 2.06, 2.07, 2.10 (each 3H, s), 2.25 (2H, m), 2.36 (2H, m), 3.68 (3H, s), 4.43 (2H, dt, *J* = 12, 2 Hz), 4.65 (2H, dt, *J* = 12, 4 Hz), 5.29 (2H, t, *J* = 4 Hz), 7.56 (1H, d, *J* = 2 Hz), 7.57 (1H, d, *J* = 2 Hz).

Alkaline Hydrolysis of Ligustrinoside A solution of ligustrinoside (**1**, 20 mg) in 1 N KOH (1 ml) was reacted at 50 °C for 3.5 h. After cooling, the reaction mixture was neutralized with ion-exchange resin (Amberlite MB-3), evaporated to give a residue (**1c**, 8.6 mg). This compound was identified with loganic acid (from alkalysis of loganin), by direct comparison with authentic sample [thin layer chromatography (TLC), $[\alpha]_D$, IR, ¹H-NMR and MS spectra].

Methylation of Loganic Acid A solution of loganic acid (19.7 mg) in MeOH (0.5 ml) was treated with diazomethane for 1.5 h. The reaction mixture was evaporated and recrystallized with EtOAc–MeOH to give colorless needles (**2**, 18.4 mg). This compound was identified with loganin by direct comparison with authentic sample [TLC, $[\alpha]_D$, IR, ¹H-NMR and MS spectra].

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