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SHORT REPORTS

A SECOIRIDOID GLUCOSIDE FROM *FRAXINUS FORMOSANA*

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Key Word Index—*Fraxinus formosana*; Oleaceae; leaves; secoiridoid glucosides; fraxiformoside; ligstroside; isoligustroside.

Abstract—A new secoiridoid glucoside, fraxiformoside, was isolated from *Fraxinus formosana*, together with the known secoiridoid glucosides, ligstroside and isoligustroside. The structural elucidation of fraxiformoside by spectroscopic and chemical studies is described.

INTRODUCTION

In a continuation of our previous investigations of the secoiridoid glucosides from the family Oleaceae [1], we examined the leaves of *Fraxinus formosana* Hay., which grows in Taiwan. This study has resulted in the isolation of a new secoiridoid glucoside. We report here the structural elucidation of this compound.

RESULTS AND DISCUSSION

The methanolic extract of the fresh leaves of *F. formosana* was fractionated as described in the Experimental section to give three secoiridoid glucosides, ligstroside (**1**) [2], **2** and **3** along with three phenolic compounds, which were identified as 2-(4-hydroxyphenyl)ethanol (tyrosol), 2-(4-hydroxyphenyl)ethyl β-D-glucopyranoside (salidroside) [3] and acteoside [4] from their spectroscopic data.

Glucoside **2**, $C_{25}H_{32}O_{12}$, was recognized as an isomer of compound **1**. It showed UV, IR and ^1H and ^{13}C NMR (Table 1) spectral features closely similar to those of compound **1**. A significant difference in their ^1H NMR spectra was a chemical shift of the carbomethoxyl group (δ3.71 in **1** and 3.63 in **2**), suggesting compound **2** to be isoligustroside, previously isolated as its acetate from *Syringa vulgaris* [5]. A comparison of the spectral data of the acetate **2a** with those reported in the literature confirmed that compound **2** was isoligustroside. This is the first report on the characterization of compound **2** as a glucoside.

Compound **3**, named fraxiformoside, was assigned the molecular formula $C_{32}H_{38}O_{13}$. It showed UV maxima at 204.5, 225.5, 240.5 (sh), 270 (sh), 275 (sh) and 286 (sh) nm and IR bands at 3420, 1756, 1704, 1634 and 1518 cm^{-1}. Its ^1H NMR spectrum exhibited a singlet characteristic of H-3 of a secoiridoid glucoside at δ7.51 (*s*), signals for a vinyl methyl group at δ1.75 (*dd*), an anomeric proton at δ4.80 (*d*), an allylic acetal proton at δ6.01 (*br s*) and an olefinic proton at δ6.17 (*qd*), indicating the presence of an oleoside (**4**) moiety in the molecule. Furthermore, its ^1H NMR spectrum showed duplicated aromatic AA'BB' spin systems centred at δ6.88 and 7.12, along with signals of two sets of OCH_2CH_2Ar moieties, which appeared as an ABX_2 spin system at δ4.31 (*dt*), 4.28 (*dt*) and 2.87 (*t*) and as an A_2X_2 spin system at δ3.75 (*t*) and 2.82 (*t*). Acetylation of compound **3** yielded the acetate **3a**, which exhibited signals of five alcoholic and one phenolic acetyl groups in its ^1H NMR spectrum. These findings reflected the presence of two non-equivalent tyrosol moieties attached to the oleoside (**4**) moiety, obviously at the C-7 and C-11 positions through different types of ester linkages.

The nature of the ester linkage at C-7 in compound **3** was deduced from comparative studies of ^1H and ^{13}C NMR spectra. The downfield shifts of H_2-6 as well as the upfield shift of the C-7 signal of compound **3**, when compared with the corresponding signals of compounds **1**§ and **2**, strongly suggested that the C-7 carbonyl group should be linked to a phenolic oxygen rather than an alcoholic one [3]. The HMBC experiment with compound **3** revealed a ^3J interaction between H-3 and C-11 (δ168.2), that was in turn correlated to methylene proton signals at δ4.31 and 4.28. Hence the carbonyl group at C-11 was concluded to be esterified with a primary alcohol, and fraxiformoside was characterized as compound **3**.

Finally, as an additional structural confirmation, compound **3** underwent methanolysis to yield isoligustroside (**2**) and tyrosol. The preferential methanolysis of C-7 could be attributed to the better leaving ability of phenoxide as opposed to alkoxide and the greater ease in solvolysis with saturated esters relative to α,β-unsaturated esters. Therefore, compound **2** could conceivably be an artifact formed from compound **3** in the extraction process.

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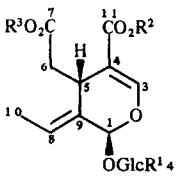
§The signals for H_2-6 appeared at δ2.44 and 2.70.



	R^1	R^2	R^3
1	H	Me	CH_2CH_2–C$_6$H$_4$–OH
2	H	CH_2CH_2–C$_6$H$_4$–OH	Me
2a	Ac	CH_2CH_2–C$_6$H$_4$–OAc	Me
3	H	CH_2CH_2–C$_6$H$_3$(OH)–C$_6$H$_3$(OH)–CH_2CH_2OH	
3a	Ac	CH_2CH_2–C$_6$H$_4$–OAc	CH_2CH_2OAc
4	H	H	H

Table 1. ^{13}C NMR data of ligstroside (**1**), isoligustroside (**2**) and fraxiformoside (**3**) in CD_3OD

C	1	2	3
1	95.3	95.2	95.4
3	155.2	155.1	155.3
4	109.5	109.6	109.5
5	31.9	31.8	31.8
6	41.3	40.9	41.1
7	173.3	173.6	171.8
8	125.0	124.9	125.2
9	130.6	130.5	130.7
10	13.6	13.5	13.8
11	168.7	168.2	168.2
OMe	51.9	52.1	—
1′	101.0	100.9	101.1
2′	74.9	74.8	74.8
3′	78.0	78.0	78.0
4′	71.6	71.5	71.5
5′	78.5	78.4	78.4
6′	62.9	62.7	62.7
1″,1‴	66.9	66.4	66.5, 64.1
2″,2‴	35.2	35.3	35.3, 39.6
3″,3‴	130.2	130.3	130.3, 138.3
4″,4‴	131.1	131.0	131.0, 131.0
5″,5‴	116.4	116.3	116.4, 122.6
6″,6‴	157.2	157.1	157.2, 150.6
7″,7‴	116.4	116.3	116.4, 122.6
8″,8‴	131.1	131.0	131.0, 131.0

EXPERIMENTAL

^{1}H (500 MHz) and ^{13}C (125 MHz) NMR: TMS as int. standard; FAB-MS: glycerol or 3-nitrobenzylalcohol as the matrix; CC and TLC: silica gel.

Plant material. The leaves of *F. formosana* were collected in Heng-Chun Tropical Botanical Garden, Taiwan, in November 1986. A voucher specimen is deposited in the Herbarium of the Institute of Botany, Faculty of Science, Kyoto University, Sakyo-ku, Kyoto 606, Japan.

Isolation of glucosides. Fresh leaves of *F. formosana* (63.5 g) were extracted with hot MeOH. After concn, the extract (8.4 g) was dissolved 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, after evapn of the solvent, was subjected to CC with $CHCl_3$–MeOH of increasing MeOH content. Combined fractions eluted with 3–5%, 7–10% and 10–20% MeOH–$CHCl_3$ were concd *in vacuo* to afford residues R-1 (151.7 mg), R-2 (669.2 mg) and R-3 (734.3 mg), respectively. Recrystallization of R-1 from Et_2O–C_6H_6 gave tyrosol (82.9 mg). R-2 was further purified by prep. HPLC (μBondasphere 5μ C18–100 Å, MeOH–H_2O, 1:1) yielded salidroside (166.7 mg), **1** (7.9 mg), **2** (6.0 mg) and **3** (212.1 mg) in order of elution. R-3 was repeatedly subjected to CC with EtOAc–C_6H_6–EtOH (79:20:1 to 76:19:5), giving rise to acteoside (131.0 mg).

Isoligustroside (**2**). Powder, $[\alpha]_D^{30}$ −152° (MeOH; *c* 0.27). UV λ_{max}^{MeOH} nm (log ε): 203 (4.02), 227.5 (4.21), 240sh (4.12), 278 (3.26), 286sh (3.12). IR ν_{max}^{KBr} cm^{-1}: 3432, 1712, 1634, 1520. ^{1}H NMR (CD_3OD): δ1.72 (3H, *dd*, *J* = 7.0 and 1.5 Hz, H_3-10), 2.39 (1H, *dd*, *J* = 14.5 and 9.5 Hz, H-6), 2.66 (1H, *dd*, *J* = 14.5 and 4.5 Hz, H-6), 2.86 (2H, *t*, *J* = 6.5 Hz, H_2-2″), 3.40 (1H, *t*, *J* = 8.5 Hz, H-3′), 3.63 (3H, *s*, CO_2Me), 3.66 (1H, *dd*, *J* = 12.0 and 5.5 Hz, H-6′), 3.88 (1H, *dd*, *J* = 12.0 and 1.5 Hz, H-6′), 3.95 (1H, *dd*, *J* = 9.5 and 4.5 Hz, H-5), 4.26 (1H, *dt*, *J* = 11.0 and 6.5 Hz, H-1″), 4.29 (1H, *dt*, *J* = 11.0 and 6.5 Hz, H-1″), 4.79 (1H, *d*, *J* = 7.5 Hz, H-1′), 5.90 (1H, *br s*, H-1), 6.09 (1H, *qd*, *J* = 7.0 and 1.0 Hz, H-8), 6.70 and 7.06 (4H, AA′BB′ pattern, *J* = 8.5 Hz, H-5″, 7″ and H-4″, 8″), 7.46 (1H, *s*, H-3). Negative ion FAB-MS, *m/z*: 523 [M−H]$^-$. (Found: C, 55.25; H, 6.03. $C_{25}H_{32}O_{12}\cdot H_2O$ requires: C, 55.35; H, 6.32%.) Acetylation of this compound gave a product which had identical spectral data to those of isoligustroside pentaacetate (**2a**) [5].

Fraxiformoside (**3**). Powder, $[\alpha]_D^{28}$ −118° (MeOH, *c* 1.1). UV λ_{max}^{MeOH} nm (log ε): 204.5 (4.26), 225.5 (4.32), 240.5sh (4.16), 270sh (3.31), 275sh (3.28), 286sh (3.13). IR ν_{max}^{KBr} cm^{-1}: 3420, 1756, 1704, 1634, 1518. ^{1}H NMR (CD_3OD): δ1.75 (3H, *dd*, *J* = 7.0 and 1.2 Hz, H_3-10), 2.67 (1H, *dd*, *J* = 15.0 and 9.5 Hz, H-6), 2.82 (2H, *t*, *J* = 7.0 Hz, H_2-2″), 2.86 (1H, *dd*, *J* = 15.0 and 4.5 Hz, H-6), 2.87 (2H, *t*, *J* = 6.5 Hz, H_2-2″), 3.41 (1H, *t*, *J* = 9.0 Hz, H-3′), 3.63 (1H, *dd*, *J* = 12.0 and 5.5 Hz, H-6′), 3.75 (2H, *t*, *J* = 7.0 Hz, H_2-1‴), 3.82 (1H, *dd*, *J* = 12.0 and 1.0 Hz, H-6′), 4.07 (1H, *dd*, *J* = 9.5 and 4.5 Hz, H-5), 4.28 (1H, *dt*, *J* = 11.0 and 6.5 Hz, H-1″), 4.31 (1H, *dt*, *J* = 11.0 and 6.5 Hz, H-1″), 4.80 (1H, *d*, *J* = 7.5 Hz, H-1′), 6.01 (1H, *br s*, H-1), 6.17 (1H, *qd*, *J* = 7.0 and 1.0 Hz, H-8), 6.70 and 7.06 (4H, AA′BB′ pattern, *J* = 8.5 Hz, H-5″, 7″ and H-4″, 8″), 6.99 and 7.25 (4H, AA′BB′ pattern, *J* = 8.5 Hz, H-5‴, 7‴ and H-4‴, 8‴), 7.51 (1H, *s*, H-3). Negative ion FAB-MS, *m/z*: 629 [M−H]$^-$. (Found: C, 58.87; H, 6.11. $C_{32}H_{38}O_{13}\ H_2O$ requires: C, 59.25; H, 6.22%.)

Acetylation of compound **3**. Glucoside **3** (17.4 mg) was acetylated in the usual way and the crude acetate (25.3 mg) was purified by prep. TLC with $CHCl_3$–MeOH (97:3) to yield **3a** (16.3 mg) as an amorphous powder. $[\alpha]_D^{23}$ −85° ($CHCl_3$; *c* 0.98). UV λ_{max}^{MeOH} nm (log ε): 205 (4.32), 217 (4.28), 240sh (4.11), 270sh (3.01). IR ν_{max}^{KBr} cm^{-1}: 1758, 1750, 1708, 1636, 1512. ^{1}H NMR ($CDCl_3$): δ1.76 (3H, *dd*, *J* = 7.5 and 1.2 Hz, H_3-10), 1.99, 2.028, 2.029, 2.039, 2.043 (15H, each *s*, 5 × Ac), 2.29 (3H, *s*, Ac). 2.63 (1H, *dd*, *J* = 15.0 and 9.0 Hz, H-6), 2.91 (1H, *dd*, *J* = 15.0 and 4.5 Hz, H-6), 2.92 (2H, *t*, *J* = 7.0 Hz, H_2-2‴), 2.97 (2H, *t*, *J* = 7.0 Hz, H_2-2″), 3.75 (1H, *ddd*, *J* = 9.5, 5.0 and 2.5 Hz, H-5′), 4.057 (1H, *dd*, *J* = 12.0 and 2.5 Hz, H-6′), 4.062 (1H, *dd*, *J* = 9.0 and 4.5 Hz, H-5), 4.24 (1H, *dd*, *J* = 12.0 and 5.0 Hz, H-6′), 4.26 (2H, *t*, *J* = 7.0 Hz, H_2-1‴), 4.34

(1H, *dt*, $J = 11.0$ and 7.0 Hz, H-1″), 4.38 (1H, *dt*, $J = 11.0$ and 7.0 Hz, H-1″), 5.03 (1H, *d*, $J = 8.0$ Hz, H-1′), 5.12 (1H, *t*, $J = 9.5$ Hz, H-4′), 5.14 (1H, *dd*, $J = 9.5$ and 8.0 Hz, H-2′), 5.27 (1H, *t*, $J = 9.5$ Hz, H-3′), 5.80 (1H, *br s*, H-1), 6.08 (1H, *br q*, $J = 7.0$ Hz, H-8), 6.98 and 7.20 (4H, AA′BB′ pattern, $J = 8.5$ Hz, H-5‴, 7‴ and H-4‴, 8‴), 7.02 and 7.24 (4H, AA′BB′ pattern, $J = 8.5$ Hz, H-5″, 7″ and H-4″, 8″), 7.46 (1H, *s*, H-3). Positive ion FAB-MS, *m/z*: 883 $[M+H]^+$.

Methanolysis of compound **3**. A soln of **3** (91.3 mg) in MeOH (10 ml) was heated for 40 hr under reflux. After concn *in vacuo*, the residue was purified by prep. HPLC (μBondasphere 5μ C18–100 Å, MeOH–H_2O, 1:1) to give tyrosol (13.8 mg), **2** (53.2 mg) and **3** (1.7 mg).

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