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A Secoiridoid Dilactone from *Fraxinus ornus* Bark

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Summary. The isolation and structure elucidation of the secoiridoid hydroxyornoside is described. The behaviour of secoiridoid dilactones on acid methanolysis is discussed.

Keywords. Secoiridoids; *Fraxinus ornus* L.; Secoiridoid dilactones; Hydroxyornoside; Insularoside; Acid methanolysis of secoiridoid dilactones.

Ein Secoiridoiddilacton aus der Rinde von *Fraxinus ornus*

Zusammenfassung. Die Isolierung und Strukturaufklärung des Secoiridoids Hydroxyornosid wird beschrieben. Das Verhalten von Secoiridoiddilactonen bezüglich saurer Methanolyse wird diskutiert.

Introduction

In a preliminary short communication we announced the presence of 2″-hydroxyornoside (**1**) in the ethanolic extract of *F. ornus* bark [1]. Now, we present details on its isolation and structure elucidation, as well as investigations on the acid methanolysis of oleoside-type secoiridoid dilactones.

Results and Discussion

2″-Hydroxyornoside (**1**) was obtained as a powder, $[\alpha]_D^{20} = -79.47°$ (MeOH). A molecular formula of $C_{32}H_{36}O_{14}$ was established by its NMR (^1H and ^{13}C) and FAB-MS spectra ($[M + Na]^+$ at $m/z = 667$). The UV absorption (273 nm) and the IR bands (3423, 1713, 1620, 1507, 1439 cm^{-1}) resembled those of insularoside (**2**) which was recently isolated by us from the same plant and described under the name ornoside [2], and from *F. insularis* by *T. Tanahashi* and coworkers [3]. The presence of the symmetrical A_2X_2 pattern ($\delta = 7.26$, d, $J = 8.5$ Hz; $\delta = 6.87$, d, $J = 8.5$ Hz) and the AMX spin system ($\delta = 6.76$, d, $J = 8.0$ Hz; $\delta = 6.68$, dd, $J = 8.0$, 1.8 Hz; $\delta = 6.47$, d, $J = 1.8$ Hz) as well as the allylic acetal proton at $\delta = 5.74$ (br s, H-1) and the olefinic protons at $\delta = 7.45$ (s, H-3) and $\delta = 5.95$ (br q, $J = 7.0$ Hz, H-8) in its ^1H NMR spectrum clearly indicated that it is an analogue of insularoside (**2**). However, instead of the two protons at 2″ position as in **2**, only one proton was observed at $\delta = 4.80$. The ^{13}C NMR spectrum (Table 1) of **1** was in full agreement with that of **2** for all carbon atoms except for C-1″, C-2″, C-4″, and C-8″. The most striking difference

Table 1. ^{13}C chemical shifts of hydroxyornoside (**1**) and insularoside (**2**) in CD_3OD (ppm)

	1	2
C-1	95.2 d	95.1 d
C-3	155.6 d	155.3 d
C-4	109.5 s	109.7 s
C-5	31.3 d	31.3 d
C-6	40.8 t	40.8 t
C-7	172.6 s	172.6 s
C-8	125.2 d	124.9 d
C-9	129.9 s	129.9 s
C-10	13.6 q	13.7 q
C-11	167.6 s	167.9 s
C-1′	100.9 d	100.9 d
C-2′	74.7 d	74.2 d
C-3′	78.3 d	78.3 d
C-4′	71.4 d	71.4 d
C-5′	77.9 d	77.9 d
C-6′	62.7 t	62.7 t
C-1″	68.7 t	65.9 t
C-2″	71.6 d	35.8 t
C-3″	132.3 s	132.2 s
C-4″	129.2 d	131.5 d
C-5″	120.5 d	120.9 d
C-6″	158.5 s	157.4 s
C-7″	120.5 d	120.9 d
C-8″	129.2 d	131.5 d
C-1‴	66.3 t	66.3 t
C-2‴	34.7 t	34.7 t
C-3‴	138.3 s	135.4 s
C-4‴	120.3 d	120.0 d
C-5‴	147.7 s	147.6 s
C-6‴	146.7 s	147.0 s
C-7‴	117.8 d	117.7 d
C-8‴	125.2 d	125.1 d

was observed for C-2″ – a doublet at $\delta = 71.5$ for **1** instead of the triplet at $\delta = 35.8$ observed for **2**. Upon acetylation, **1** provided a hexaacetate (**1a**) which showed six acetyl singlets in its ^1H NMR spectrum instead of five for insularoside. It was suggested that **1** is a hydroxy derivative of **2** with an additional OH group located at position 2″. Extensive NOE studies (Fig. 1) on **1a** revealed the close relationships in space between the acetate group at C-2″ and H-3 as well as that of H-1″, H-2″, H-4″, and H-8″. On alkaline hydrolysis, **1** afforded a new phenolic compound whose structure was unambiguously shown to be 2″-hydroxyornosol (**3**) by its spectral data (^1H NMR and 70 eV mass spectra). These spectral and chemical evidences supported structure **1** for 2″-hydroxyornoside.

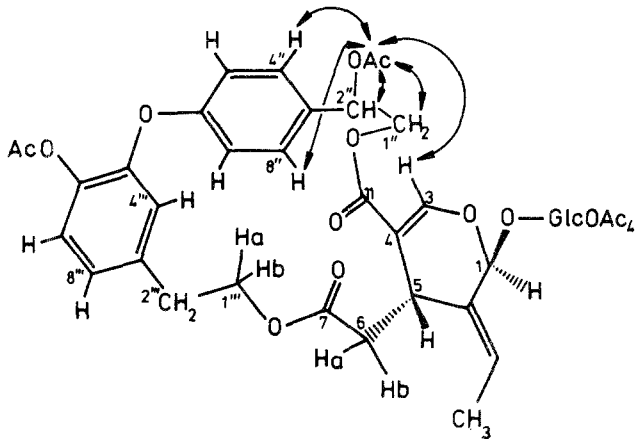


Fig. 1. Most important enhancements of the signals in homonuclear ^{1}H NOE spectra of **1a**; arrows (↔) designate mutual effects between the indicated protons

Acid hydrolysis of **1** using 16.0% methanolic H_2SO_4 resulted in a complex reaction mixture from which **4a, b** could be isolated as a minor product and **5a, b** as a major one. Ligstroside (**6**) afforded only **4a, b** under the same reaction conditions.

The ^{1}H NMR spectrum (Table 2) of the less polar compound **4a, b** showed no presence of aromatic and sugar protons. The doubling of all signals indicated the presence of two diastereomers in a nearly 1:1 ratio. The singlets characteristic of H-3 for oleoside type secoiridoids were observed at $\delta = 7.58$ and $\delta = 7.54$. ^{1}H NMR analysis showed that **4a, b** contained two methyl groups on oxygen-bearing secondary carbons ($\delta = 1.43$, d; $\delta = 1.41$, d), four carbomethoxy groups ($\delta = 3.71$, 3.69, 3.68, and 3.67), and two CH_2–CH fragments. Spindecoupling experiments established the carbon sequence as shown in **4a, b**. These NMR data were in agreement with those described for the natural compounds **7a** and **7b** [4]. However, no CHO signal was visible and the presence of two CH protons ($\delta = 4.39$, d, $J = 3.4$ Hz; $\delta = 4.26$, d, $J = 7.7$ Hz) and four OCH_3 signals was observed at $\delta = 3.36$, 3.34, and 3.31 (2x OMe). This suggested that compound **4a, b** is a derivative of **7a** and **7b** in which the CHO group at position 1 has been acetalized. The 70 eV mass spectrum of **4a, b** revealed a molecular ion at $m/z = 302$ and a base peak at $m/z = 75$ typical of dimethylacetals. To account for the presence of two diastereomers we accept that under the applied reaction conditions a rearrangement of the secoiridoid nucleus takes place *via* the mechanism already proposed by *Gariboldi et al.* for the formation of **7a** and **7b** from oleuropein (**8**) in plants [4]. This is followed by acetalization of the CHO group. The stereochemistry at C-8 and C-9 in **4a, b** could not be assigned with the help of NMR experiments because of the overlapping ^{1}H NMR signals.

The ^{1}H NMR spectrum of the more polar compound **5a, b** showed the absence of the sugar unit and revealed all protons of the hydroxyornosol moiety and the rearranged secoiridoid moiety. The doubling of all signals in the spectrum suggested the existence of two diastereomers in a nearly 1:1 ratio. The presence of the two COOMe signals at $\delta = 3.68$ and $\delta = 3.66$ in addition to the four OMe singlets ($\delta = 3.35$, 3.33, 3.31, and 3.29) from the acetalized CHO group indicated that after

Table 2. ^{1}H NMR data for compounds **4a, b**, **5a, b**, **9a, b**, and **10a, b** in $CDCl_3$ (ppm); coupling constants (Hz) are given in parentheses

	4a, b	**5a, b**	**9a, b**	**10a, b**
H-1 d	4.41 (3.4)	4.40–4.20 2H	4.39 (3.4)	4.41 (3.4)
	4.29 (7.8)		4.26 (7.7)	4.22 (7.0)
H-3 s	7.58	7.56	7.54	7.55
	7.54	7.52	7.50	7.50
H-5 m	3.30–3.10 2H	3.30–3.10 2H	3.30–3.10 2H	3.30–3.10 2H
H_a-6 dd	2.83 (15.3, 3.5)[a]	2.68 (16.0, 3.5)[a]	2.66 (16.0, 3.5)[a]	2.76 (16.0, 3.4)[a]
	2.39 (16.0, 4.4)[b]	2.27 (16.0, 4.0)[b]	2.29 (16.0, 4.2)[b]	2.34 (16.0, 4.4)[b]
H_b-6 dd	2.23 (15.5, 11.0)[a]	2.17 (16.0, 11.0)[a]	2.17 (16.0, 11.0)[a]	2.23 (16.0, 11.0)[a]
	2.57 (16.0, 8.0)[b]	2.58 (16.0, 8.0)[b]	2.57 (16.0, 7.9)[b]	2.60 (16.0, 7.8)[b]
H-8 m	4.20–4.10 2H	4.40–4.20 2H	4.20–4.10 2H	4.40–4.20 2H
H-9 m	2.00–1.90 2H	2.00–1.90 2H	2.00–1.90 2H	2.00–1.90 2H
H-10 d	1.43 (7.0)	1.41 (7.0)	1.41 (7.0)	1.42 (7.8)
	1.41 (7.0)	1.39 (7.0)	1.39 (7.0)	1.39 (6.5)
CH_2-1″ td		4.40–4.20 4H	4.32 (7.0, 2.0) 4H	4.30 t (6.0) 4H
CH_2-2″ td		4.40–4.20 2H	2.93 (7.0, 2.0) 4H	2.92 t (6.0) 4H
H-4″, H-8″ d		7.30 (8.5) 4H	7.18 (7.0) 4H	7.17 (8.5) 4H
H-5″, H-7″ d		6.98 (8.5) 4H	6.92 (7.0) 4H	6.92 (8.5) 4H
CH_2-1‴ t		3.78 (6.5) 4H	3.76 (6.5) 4H	4.22 (7.0) 4H
CH_2-2‴ t		2.75 (6.5) 4H	2.71 (6.5) 4H	2.85 (7.0) 4H
H-4‴ d		6.79 (1.8) 2H	6.75 (1.9) 2H	6.82 (1.8) 2H
H-7‴ d		6.99 (8.0) 2H	6.97 (7.0) 2H	7.07 (8.0) 2H
H-8‴ dd		6.92 (8.0, 1.8) 2H	6. 89 (7.9, 1.8) 2H	6.96 (8.0, 1.8) 2H
$COOCH_3$	3.71 3H, 3.69 3H	3.68 3H, 3.66 3H	3.67 3H, 3.66 3H	3.67 3H, 3.66 3H
	3.68 3H, 3.67 3H			
OCH_3	3.36 3H, 3.34 3H,	3.35 3H, 3.33 3H,	3.35 3H, 3.33 3H,	3.34 3H, 3.32 3H
	3.31 6H	3.31 3H, 3.29 3H	3.29 3H, 3.30 3H	3.30 3H, 3.29 3H
AcO	–			2.16 6H, 1.99 6H

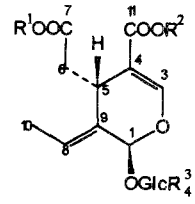
[a,b] Signals with identical indexes belong to the same isomer; the other signals are not assigned;
[c] CH(OH)-2″

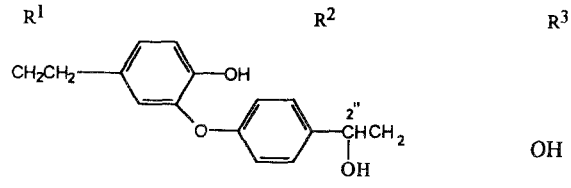
hydrolysis of one of the ester linkages in **1** a transesterification with MeOH has taken place. However, the place of opening of the macrocyclic ring could not be determined because of the unsufficient amount of **5a, b** for NOE studies.

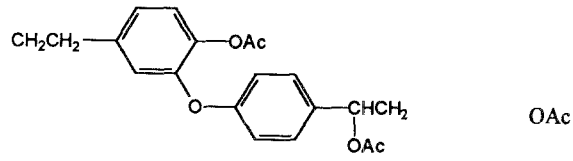
As compound **2** was present in larger quantity in the plant extract under investigation and its isolation was easier to be achieved compared to compound **1**, acid methanolysis of **2** has been performed and the expected products **4a, b** and **9a, b** have been isolated from the reaction mixture. The ^{1}H NMR spectra of **9a, b** and its acetate **10a, b** (Table 2) showed very close similarity to **5a, b**. As expected, two OAc signals (one aliphatic and one aromatic) were observed in **10a, b**. The NOE experiments performed on **10a, b** resulted in an enhancement of H-3, H-8″, H-4″, and CH_2-2″ upon irradiation of CH_2-1″ ($\delta = 4.32$ ppm) which means that the *p*-substituted phenetoxy unit is attached to C-11 of the secoiridoid nucleus. Therefore, under the applied conditions the acid methanolysis of **1** and **2** leads to

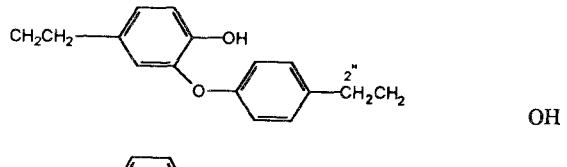
opening of the macrocyclic ring at C-7. This is in accordance with the results of *Tanahashi* and coworkers [3] for the mild alkaline hydrolysis of **2** and the previous observations of *LaLonde* [5] that saturated esters undergo easier solvolysis compared to α,β-unsaturated ones.

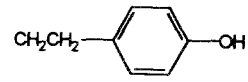
These results unambiguously confirm the proposed structures **1** and **2** and present a contribution to the chemistry of secoiridoid dilactones.

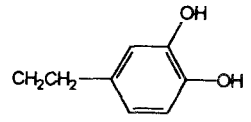


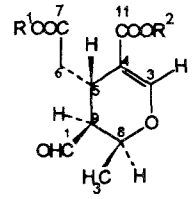


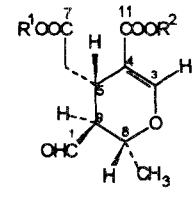


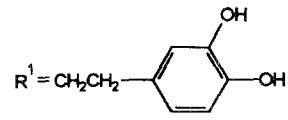


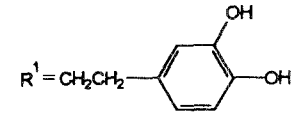


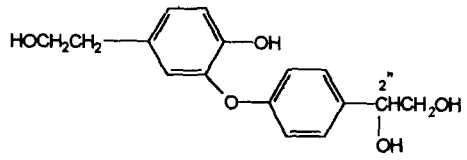












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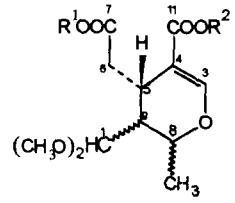
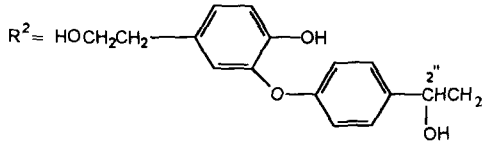
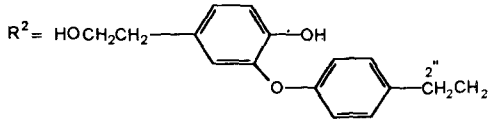


4 a,b $R^1 = R^2 = CH_3$

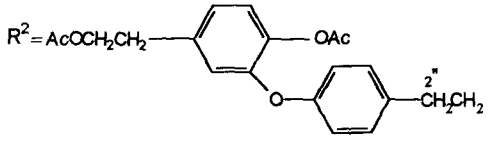
5 a,b $R^1 = CH_3$



9 a,b $R^1 = CH_3$



10 a,b $R^1 = CH_3$



Experimental

^1H and ^{13}C spectra (δ (ppm), J (Hz)) were obtained at 250 MHz (^1H NMR) and at 63 MHz (^{13}C NMR) using *TMS* as internal standard. NOE experiments were performed using standard Bruker software. MS: 70 eV; FAB-MS: monothioglycerol as matrix. TLC: aluminium sheets, silica gel 60 F_{254} (Merck), spots detected under UV light, after exposure to I_2 vapour, or by sparying with H_2SO_4 and heating. Prep. TLC: 20 × 20 cm plates coated with 1 mm of silica gel PF_{254} (Merck); CC: silica gel 60, Merck. Liquid vacuum chromatography (LVC): silica gel LS 5–40 μ (Chemapol). TLC solvent system: Et_2O-toluene (2:1), saturated with 10% HOAc (**A**).

Plant material

A commercial sample of *F. ornus* L. bark collected 1991 from the region of Dragoman, Bulgaria, was investigated. A voucher specimen is deposited in the Herbarium of the Institute of Botany, BAS, Sofia.

Isolation of glucosides

Dried and well-ground bark (1 kg) was extracted with hot EtOH (3 × 7 l). The insoluble material was removed by filtration and the extract was concentrated under reduced pressure to a small volume. After filtration of the deposited esculin (30.00 g), the mother liquor was concentrated under reduced pressure and subjected to solvent–solvent partition using *PE* and EtOAc to afford R-1 (20.01 g) and R-2 (50.02 g), respectively. R-2 (6.20 g) was further worked up by LVC over 70 g silica gel using *DXE* and *DXE*-MeOH with increasing polarity (10:1, 5:1, 3:1). Fractions eluted with *DXE*:MeOH (5:1) were combined and concentrated under reduced pressure to give residues R-3 (0.84 g), R-4 (0.94 g), and R-5 (0.32 g). R-3 was subjected to CC over 160 g silica gel. Elution with $CHCl_3$:MeOH (9:1) afforded compound **1** (0.13 g).

Hydroxyornoside (**1**)

Powder; $[\alpha]_D^{20} = -79.47°$ (EtOH, $c = 0.0083$); IR (KBr): $\nu = 3423$, 1713, 1620, 1507, 1439 cm^{-1}; UV (EtOH): λ_{max} (lgε) = 273 (3.55) nm; ^{1}H NMR (CD_3OD): $\delta = 7.45$ (1H, s, H-3), 7.26 (2H, d, $J = 8.5$ Hz, H-4″, H-8″), 6.87 (2H, d, $J = 8.5$ Hz, H-5″, H-7″), 6.76 (1H, d, $J = 8.0$ Hz, H-7‴), 6.68 (1H, dd, $J = 8.0$ and 1.8 Hz, H-8‴), 6.47 (1H, d, $J = 1.8$ Hz, H-4‴), 5.95 (1H, br q, $J = 7.0$ Hz, H-8), 5.74 (1H, br s, H-1), 4.80 (1H, obscured by the signal of the solvent, H-2″), 4.71 (1H, d, $J = 8.0$ Hz, H-1′), 4.44 (1H, dd, $J = 10.8$ and 3.8 Hz, H_a-1″), 4.20–4.00 (2H, m, H_a-1‴, H_b-1″), 3.94 (1H, dt, $J = 11.5$ and 5.0 Hz, H_b-1‴), 3.70 (1H, m, H_a-6′), 3.69 (1H, dd, $J = 10.5$ and 4.0 Hz, H-5), 3.55–3.50 (1H, m, H_b-6′), 3.40–3.20 (4H, m, H-2′, H-3′, H-4′, H-5′), 2.66 (2H, t-like, $J = 5.0$ Hz, CH_2-2‴), 2.14 (1H, dd, $J = 15.0$ and 4.0 Hz, H_a-6), 2.01 (1H, dd, $J = 15.0$ and 10.5 Hz, H_b-6), 1.49 (3H, br d, $J = 7.0$ Hz, CH_3-10) ppm. ^{13}C NMR: see Table 1; FAB-MS: $m/z = 667$ $[M + Na]^+$.

Acetylation of hydroxyornoside

Compound **1** (5.0 mg) was acetylated with Py–Ac_2O at room temperature for 24 h. The product (6.0 mg) was purified using solvent system **A** to give hydroxyornoside hexaacetate (**1a**, 3.3 mg).

^{1}H NMR ($CDCl_3$): 7.53 (1H, br s, H-3), 7.32 (2H, d, $J = 8.5$ Hz, H-4″, H-8″), 7.05 (1H, d, $J = 8.0$ Hz, H-7‴), 7.03 (2H, d, $J = 8.5$ Hz, H-5″, H-7″), 6.87 (1H, dd, $J = 8.0$ and 1.8 Hz, H-8‴), 6.62 (1H, d, $J = 1.8$ Hz, H-4‴), 5.98 (1H, dd, $J = 7.7$ and 3.8 Hz, H-2″), 5.95 (1H, br q, $J = 7.0$ Hz, H-8), 5.63 (1H, br s, H-1), 5.25 (1H, d, $J = 9.3$ Hz, H-3′), 5.11 (2H, br t, $J = 9.3$ Hz, H-2′, H-4′),5.01 (1H, d, $J = 8.0$ Hz, H-1′), 4.61 (1H, dd, $J = 11.0$ and 4.0 Hz, H_a-1″), 4.38 (1H, dd, $J = 11.0$ and 8.0 Hz, H_b-1″), 4.35–4.20 (2H, H_a-1‴, H_a-6′), 4.10–4.00 (2H, H_b-1‴, H_b-6′), 3.75 (1H, dd, $J = 10.0$ and 4.0 Hz, H-5), 3.70 (1H, m, H-5′), 2.81 (2H, t-like, $J = 5.0$ Hz, 2H-2‴), 2.30–2.25 (1H, H-6_a, obscured by the signal of OAc), 2.29 (3H, s, OAc), 2.15–2.10 (1H, H-6_b, obscured by the signal of OAc), 2.12 (3H, s, OAc), 2.03 (12H, s, OAc), 1.62 (3H, d, $J = 7.0$ Hz, CH_3-10) ppm.

Alkaline hydrolysis of hydroxyornoside

Compound **1** (10.0 mg) was hydrolyzed using the reaction conditions described in Ref. [2] to obtain pure hydroxyornosol (**3**, 3.0 mg). Amorphous powder; IR (KBr): $\nu = 3438$, 3296, 1598, 1505 cm^{-1}; UV (EtOH): λ_{max} (lgε) = 255 (3.27), 275 (3.56) nm; ^{1}H NMR ($CDCl_3$): 7.31 (2H, d, $J = 8.5$ Hz, H-4′, H-8′), 6.96 (2H, d, $J = 8.5$ Hz, H-5′, H-7′), 6.93 (1H, d, $J = 7.5$ Hz, H-7), 6.88 (1H, dd, 7.5 and 1.8 Hz, H-8), 6.75 (1H, d, $J = 1.8$ Hz, H-4), 4.82 (1H, dd, 8.0 and 3.5 Hz, H-2′), 3.78 (2H, t, $J = 6.5$ Hz, H-1), 3.69 (2H, m, H-1′), 2.74 (2H, t, $J = 6.5$ Hz, H-2); EIMS (70 eV): m/z (%) = 290 (21) $[M]^{+\cdot}$, 259 (67) $[M–CH_2OH]^+$, 241 (100) $[M–CH_2OH–H_2O]^+$.

Acid methanolysis of hydroxyornoside

Compound **1** (11.5 mg) was refluxed in 3.5 ml (16.0% H_2SO_4 in MeOH for 4 h. The reaction mixture was diluted with H_2O and extracted with EtOAc. The combined extracts were dried (Na_2SO_4) and after concentration subjected to prep. TLC (solvent system *A*) to obtain **4a, b** (0.8 mg) and **5a, b** (3.0 mg).

Compounds **4a, b**

^{1}H NMR: see Table 2; EIMS (70 eV): m/z (%) = 302 (1) $[M]^{+\cdot}$, 210 (57), 165 (75), 75 (100).

Compounds **5a, b**

Powder; IR (KBr): ν = 2930, 1737, 1703, 1631, 1505, 1438, 1254, 1180 cm^{-1}; UV (EtOH): λ_{max} (lgε) = 274 (3.61), 279 (3.60) nm; ^{1}H NMR: see Table 2.

Acid methanolysis of ligstroside

Ligstroside (**6**, 10.0 mg) was hydrolyzed under the reaction conditions described for hydroxyornoside (**1**) to obtain **4a, b** (3.0 mg).

Acid methanolysis of insularoside

Insularoside (**2**, 20.0 mg) was subjected to acid methanolysis as described above to obtain **4a, b** (1.8 mg) and **9a, b** (7.0 mg).

Compounds **9a, b**

Powder; IR (KBr): ν = 2923, 1736, 1703, 1631, 1506, 1454, 1254, 1180 cm^{-1}; UV (EtOH): λ_{max} (lgε) = 276 (3.43) nm; ^{1}H NMR: see Table 2; FAB-MS: m/z = 459 $[M + H]^+$.

Acetylation of compound **9a, b**

Compound **9a, b** (5.0 mg) was acetylated with Py/Ac_2O using the usual procedure to obtain the acetate **10a, b** (6.5 mg). Powder; ^{1}H NMR: see Table 2; FAB-MS: m/z = 629 $[M + H]^+$.

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