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Secoiridoid glucosides from *Fraxinus americana*

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Abstract

Investigation of the leaves of *Fraxinus americana* led to the isolation of five secoiridoid glucosides, demethylligstroside, (2″*R*)- and (2″*S*)-2″-hydroxyoleuropeins, fraxamoside and frameroside, together with 18 known compounds. Their structures were determined on the basis of spectroscopic studies and chemical evidence. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Fraxinus americana; Oleaceae; Secoiridoid glucosides; Cyclic monoterpene

1. Introduction

Our previous phytochemical work on the *Fraxinus* species of the family Oleaceae led to the isolation of various new secoiridoid glucosides esterified with various phenylethanoid units (Tanahashi et al., 1992, 1993a,b, 1998). In the course of our studies on the plants of the same genus, we examined the leaves of *Fraxinus americana* L., the dried barks of which have been used as "White Ash" in European folk medicine (Hansel et al., 1994) in a similar manner of those of *F. japonica* in Asia. Earlier phytochemical studies of *F. americana* reported the isolation of secoiridoid glucosides Gl-3, 5 and 6 from the seeds (Lalonde et al., 1976), and of acteoside, 10-hydroxyligstroside, ligstroside and syringin from the bark (Nishibe et al., 1997). We describe here the isolation and structural elucidation of five secoiridoid glucosides (**1–5**).

2. Results and discussion

The *n*-BuOH-soluble portion of the methanolic extract of dried leaves of *F. americana* was fractionated by ODS column chromatography and then purified by preparative HPLC, to afford five new compounds **1–5** along with four phenylethanoids, 3,4-dihydroxyphenethyl alcohol, *p*-hydroxyphenethyl alcohol, acteoside and campneoside I, seven secoiridoid glucosides, oleoside 11-methyl ester (**6**), oleoside dimethyl ester (**7**), oleuropein (**8**), ligstroside (**9**), nuezhenide and (2″*R*)- and (2″*S*)-2″-methoxyoleuropeins (**10** and **11**) and seven flavonoid glycosides.

Compound **1**, $C_{24}H_{30}O_{12}$, was isolated as a colourless amorphous powder, $[\alpha]_D$ -110° (MeOH). Its UV spectrum revealed, besides the typical absorption at 225 nm of an iridoidic enol ether system conjugated with a carbonyl group, additional absorptions at 277 and 284 nm due to a phenolic function. It showed IR bands at 3413 (OH), 1715 and 1638 (α,β-unsaturated ester), and 1518 (aromatic ring) cm^{-1}. Its ^{1}H NMR spectrum (Table 1) exhibited typical signals of an oleoside (**12**) unit [H-3 at δ 7.38 (*s*), an allylic acetal proton at δ 5.86 (*br s*), an anomeric proton at δ 4.80 (*d*) and an ethylidene group at δ 6.04 (1H, *qd*) and δ 1.66 (3H, *dd*)] together with an aromatic AA′BB′ spin system at δ 6.71 (2H, d, $J=8.5$ Hz) and δ 7.05 (2H, *d*, $J=8.5$ Hz). The ^{13}C NMR spectroscopic signals due to the sugar moiety of **1** coincided well with those ascribable to the same part of oleoside dimethyl ester (**7**), indicating the presence of a β-D-glucosyl moiety. These ^{1}H and ^{13}C NMR spectral features resembled those of ligstroside (**9**), except for the lack of signals due to a carbomethoxyl group. Furthermore, **1** was treated with CH_2N_2–Et_2O to give **9**. Thus, compound **1** was designated demethylligstroside.

Compounds **2** and **3**, $C_{25}H_{32}O_{14}$, were recognized as isomers. Their ^{1}H NMR (Table 1) spectral features were

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Table 1
^{1}H NMR spectral data for compounds **1–5** and **17** in CD_3OD

H	**1**			**2**			**3**			**4**		
1	5.86	*br s*		5.95	*br s*		5.95	*br s*		5.92	*br s*	
3	7.38	*s*		7.52	*s*		7.52	*s*		7.51	*s*	
5	4.02	*br d*	(9.5)	4.00	*dd*	(9.0, 4.5)	3.99	*dd*	(9.0, 4.5)	4.05	*dd*	(12.0, 4.5)
6	2.40	*dd*	(13.5, 9.5)	2.50	*dd*	(14.0, 9.0)	2.50	*dd*	(14.0, 9.0)	2.50	*dd*	(13.5, 12.0)
	2.81	*m*		2.76	*dd*	(14.0, 4.5)	2.75	*dd*	(14.0, 4.5)	2.93	*dd*	(13.5, 4.5)
8	6.04	*qd*	(7.0, 1.5)	6.10	*qd*	(7.0, 1.0)	6.09	*qd*	(7.0, 1.0)	6.15	*qd*	(7.0, 1.0)
10	1.67	*dd*	(7.0, 1.5)	1.70	*dd*	(7.0, 1.5)	1.70	*dd*	(7.0, 1.5)	1.79	*dd*	(7.0, 1.5)
OMe	–			3.72	*s*		3.72	*s*		3.73	*s*	
1′	4.80	*d*	(8.0)	4.81	*d*	(7.5)	4.81	*d*	(7.5)	4.66	*d*	(7.5)
2′	3.30–3.41	*m*		3.30–3.43	*m*		3.30–3.43	*m*		3.34	*dd*	(9.5, 7.5)
3′										3.37	*dd*	(9.5, 9.0)
4′										2.99	*dd*	(9.5, 9.0)
5′										3.53	*td*	(9.5, 2.0)
6′	3.68	*dd*	(12.0, 5.0)	3.67	*dd*	(12.0, 5.5)	3.66	*dd*	(12.0, 5.5)	3.03	*dd*	(12.0, 9.5)
	3.87	*dd*	(12.0, 1.0)	3.89	*dd*	(12.0, 2.0)	3.89	*dd*	(12.0, 2.0)	3.88	*dd*	(12.0, 2.0)
1″	4.08	*dt*	(10.5, 7.0)	3.99	*dd*	(11.5, 8.0)	4.03	*dd*	(11.0, 4.5)	3.62	*dd*	(12.0, 1.5)
	4.20	*dt*	(10.5, 7.0)	4.13	*dd*	(11.5, 4.0)	4.10	*dd*	(11.0, 8.0)	4.52	*dd*	(12.0, 9.0)
2″	2.81	t	(7.0)	4.71	*dd*	(8.0, 4.0)	4.72	*dd*	(8.0, 4.5)	4.29	*dd*	(9.0, 1.5)
4″	7.04	*d*	(8.5)	6.82	*d*	(2.0)	6.82	*d*	(2.0)	6.71	*d*	(2.0)
5″	6.71	*d*	(8.5)	–			–			–		
7″	6.71	*d*	(8.5)	6.74	*d*	(8.0)	6.74	*d*	(8.0)	6.76	*d*	(8.0)
8″	7.04	*d*	(8.5)	6.70	*dd*	(8.0, 2.0)	6.69	*dd*	(8.0, 2.0)	6.59	*dd*	(8.0, 2.0)

H	**5**			**17**		
1	5.92	*br s*		5.91	*br s*	
3	7.51	*s*		7.52	*s*	
5	3.88	*dd*	(9.0, 4.5)	3.97	*dd*	(9.0, 4.5)
6	2.47	*dd*	(14.5, 9.0)	2.47	*dd*	(14.0, 9.0)
	2.70	*dd*	(14.5, 4.5)	2.69	*dd*	(14.0, 4.5)
8	6.10	*qd*	(7.0, 1.0)	6.11	*qd*	(7.0, 1.0)
10	1.74	*dd*	(7.0, 1.0)	1.73	*dd*	(7.0, 1.5)
OMe	3.71	*s*		3.72	*s*	
1′	4.81	*d*	(8.0)	4.81	*d*	(7.5)
2′	3.32–3.33	*m*		3.32–3.34	*m*	
3′	3.41	*t*	(9.0)	3.41	*t*	(9.0)
4′	3.32–3.33	*m*		3.32–3.34	*m*	
5′	3.32–3.33	*m*		3.32–3.34	*m*	
6′	3.69	*dd*	(12.0, 5.0)	3.69	*dd*	(12.0, 5.5)
	3.89	*dd*	(12.0, 1.5)	3.90	*dd*	(12.0, 2.5)
1″	2.51	*br sextet*	(7.0)	2.24	*m*	
2″	2.60	*br d*	(7.0)	2.50	*m*	
3″	2.27	*brs*	(7.0)	2.50	*m*	
4″	1.38	*tt*	(12.0, 8.0)	1.54	*qd*	(11.5, 7.5)
	1.82	*br dt*	(12.0, 7.0)	1.87	*m*	
5″	1.18	*m*		1.24	*dddd*	(12.0, 11.5, 8.5, 7.0)
	2.12	*dtd*	(12.0, 8.0, 1.5)	2.02	*dtd*	(12.0, 7.5, 1.5)
6″	0.99	*d*	(7.0)	1.07	*d*	(7.0)
8″	2.70	*m*		2.79	*ddd*	(11.0, 6.5, 4.0)
9″	4.13	*dd*	(11.0, 4.0)	4.12	*dd*	(11.5, 6.5)
	4.32	*dd*	(11.0, 8.0)	4.27	*dd*	(11.5, 4.0)
OMe	–			3.61	*s*	
OMe	–			3.66	*s*	

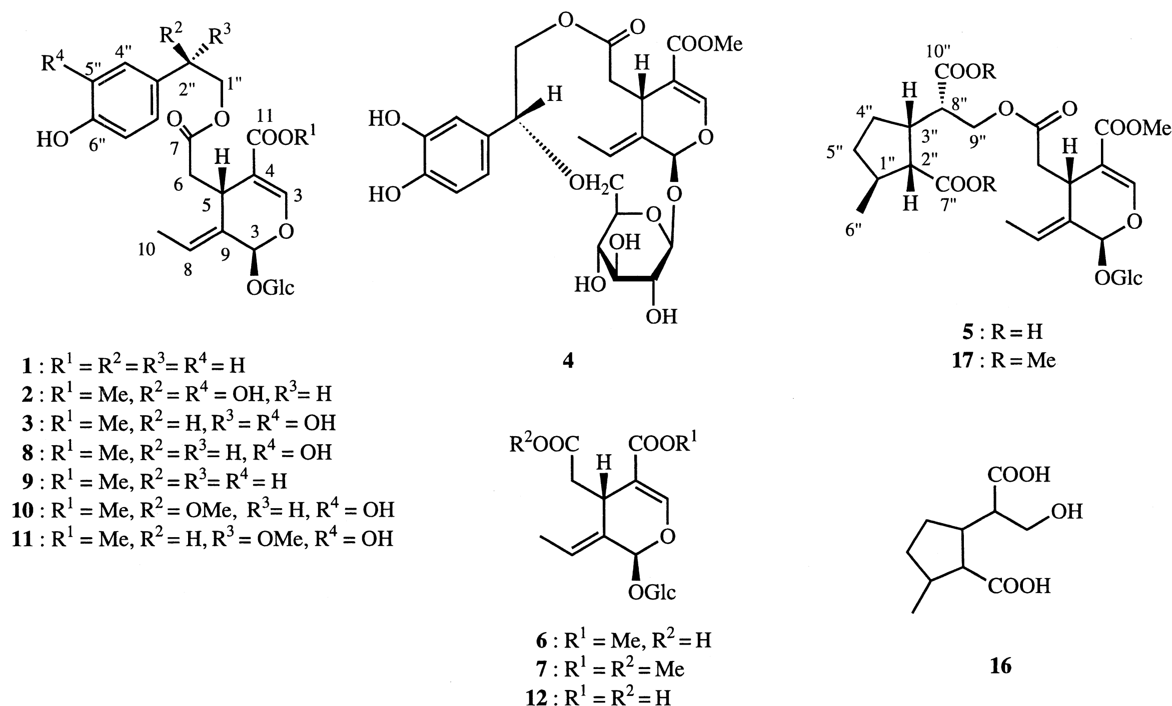
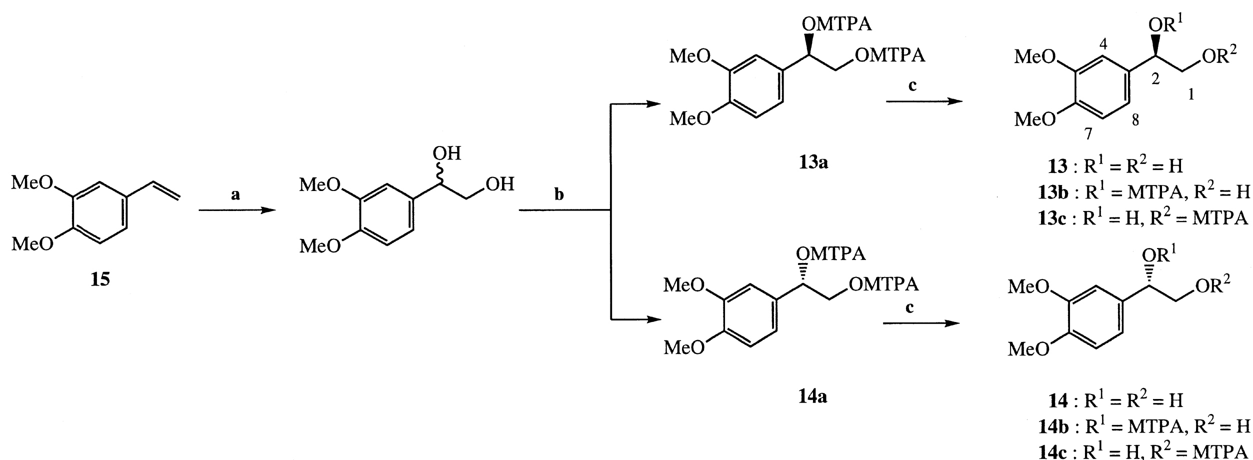


Table 2
^{13}C NMR spectral data for **1–5**, **9** and **17** in CD_3OD

C	**1**	**2**	**3**	**4**	**9**	C	**5**	**17**
1	94.8	95.3	95.3	100.2	95.1	1	95.1	94.9
3	157.1	155.2	155.3	155.6	155.1	3	155.2	155.2
4	n.d.[a]	109.4	109.4	109.7	109.4	4	109.5	109.4
5	32.6	31.9	31.8	32.6	31.8	5	31.8	31.9
6	41.4	41.1	41.2	40.4	41.3	6	41.2	41.0
7	173.6	173.1	173.1	172.7	173.2	7	173.2	172.7
8	124.1	124.9	125.0	125.5	124.9	8	124.8	124.8
9	131.5	130.5	130.5	130.5	130.5	9	130.7	130.7
10	13.7	13.6	13.6	13.9	13.5	10	13.7	13.7
11	n.d.[a]	168.8	168.8	168.5	168.7	11	168.7	168.6
OMe	–	52.0	52.0	51.9	51.9	OMe	52.0	51.9[c]
1′	100.9	101.0	101.0	105.0	100.9	1′	100.7	100.7
2′	74.9	74.8	74.8	73.0	74.7	2′	74.8	74.9
3′	78.0	78.0	78.0	77.8	77.9	3′	78.0	78.0
4′	71.5	71.5	71.5	73.0	71.4	4′	71.6	71.7
5′	78.4	78.5	78.5	77.5	78.4	5′	78.5	78.6
6′	62.8	62.7	62.7	70.7	62.7	6′	62.7	62.9
1″	66.8	70.5	70.5	68.7	66.9	1″	36.5	40.5
2″	35.2	72.7	72.6	85.1	35.4	2″	57.2	54.9
3″	130.1	133.8	133.8	131.0	130.7	3″	43.9	41.1
4″	131.0	114.6	114.6	114.6	117.1	4″	30.6	31.2
5″	116.3	146.4	146.4	146.8	146.2	5″	33.8	34.9
6″	152.8	146.2	146.2	146.7	144.9	6″	22.8	21.4
7″	116.3	116.3	116.3	116.5	116.4	7″	180.0[b]	175.1[d]
8″	131.0	119.0	119.0	119.3	121.3	8″	48.8	47.9
–						9″	66.6	65.7
–						10″	180.0[b]	177.3[d]
–						OMe	–	51.9[c]
–						OMe	–	52.5[c]

[a] n.d. Not detected.
[b] Not detected directly (localized through the HMBC spectrum).
[c,d] Assignments may be interchangeable.

similar to those of oleuropein (**8**), except that the protons of the phenylethanol unit in **2** and **3** appeared as an ABX spin system instead of an ABX_2 system as in **8**. In the ^{13}C NMR spectrum of each glucoside (Table 2), the signal of C-2″ which was observed as a methylene carbon at δ 35.4 in **8** was replaced by an oxymethine carbon (**2**: δ 72.7, **3**: δ 72.6). These findings, coupled with the downfield shifts of C-1″, C-3″, C-4″, C-6″ and C-8″, when compared with the corresponding signals of **8**, suggested that **2** and **3** possessed a hydroxyl group at C-2″. Moreover, there were only differences in the chemical shifts of the methylene protons at C-1″ (**2**: δ 3.99, 4.13; **3**: δ 4.03, 4.10), ascribing the structural difference between **2** and **3** to the absolute configuration at C-2″. This assumption was further supported by comparative studies of the NMR spectra of both compounds with those of (2″*R*)- and (2″*S*)-2″-methoxyoleuropeins (**10** and **11**), recently isolated from *Jasminum officinale* var. *grandiflorum* (Tanahashi et al., 1999). In order to establish the stereochemistry at C-2″ of 2-hydroxy-1-(3,4-dihydroxyphenyl)ethanol moieties of **2** and **3**, (*R*)- and (*S*)-2-hydroxy-1-(3,4-dimethoxyphenyl)ethanols (**13** and **14**) were prepared from 3,4-dimethoxystyrene (**15**) as illustrated in Scheme 1. Compound **15** was oxidized with OsO_4 to afford (±)-2-hydroxy-1-(3,4-dimethoxyphenyl)ethanol, which was esterified with (*R*)-MTPA acid. Partial methanolysis of the resulting MTPA esters **13a** and **14a** gave **13**, **13b**, and **13c**, and **14**, **14b**, and **14c**, respectively. The absolute stereochemistry of **13** and **14** was determined by comparing the chemical shifts of proton signals of their (*R*)-MTPA esters, **13b** and **14b** (Ohtani et al., 1991). Finally, compounds **2** and **3** were subjected to methylation followed by methanolysis with the respective products identified as (*R*)- and (*S*)-2-hydroxy-1-(3,4-dimethoxyphenyl)ethanols by chiral HPLC analysis with the authentic samples **13** and **14**. Accordingly, glucosides **2** and **3** were characterized as (2″*R*)-2″-hydroxyoleuropein and (2″*S*)-2″-hydroxyoleuropein, respectively.



Scheme 1. (a) (1) OsO_4/Et_2O-Py, (2) $NaHSO_3/H_2O$-Py, (b) (*R*)-MTPA, DMAP, DCC/CH_2Cl_2, (c) NaOMe /MeOH.

Compound **4**, named fraxamoside, displayed a HR-SIMS peak at *m/z* 537.1612 [M-H]$^-$ consistent with a molecular formula of $C_{25}H_{30}O_{13}$, suggesting a loss of H_2O by comparison to those of **2** and **3**. Its ^1H and ^{13}C NMR (Tables 1 and 2) spectra suggested a structural similarity to **2** and **3**, that is, the oxygenated substituent was situated at C-2″ of the 2-(3,4-dihydroxyphenyl) ethanol moiety of **4**. Its significant HMBC ^3J correlations between the oxymethine proton at δ 4.29 and the hydroxy methylene carbon (C-6′) and between H_2-6′ and C-2″ as well as correlations between H-2″ and C-8″ and between H_2-1″ and C-7, indicated that in the structure of **4**, C-2″ was linked with the hydroxyl group at C-6′ to form a 14 membered ether ring. The detailed

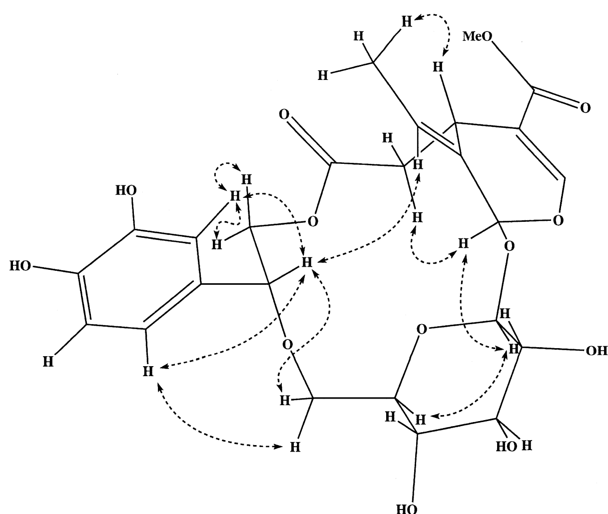


Fig. 1. Significant NOESY correlations observed for **4**.

NMR spectroscopic examinations suggested an absolute configuration at C-2″ of **4**. The coupling constants between H-5 and H_2-6, between H-5′ and H_2-6′ and between H_2-1″ and H-2″, together with NOESY interactions between H-1 and H-1′/H-6 (δ 2.50) and between H-1′ and H-5′, showed that **4** adopts the conformation as shown in Fig. 1. The *R*-configuration at C-2″ was deduced from the important NOEs between H-6′ (δ 3.03) and H-2″, H-6′ (δ 3.88) and H-8″, H_2-1″ and H-4″ and H-2″ and H-8 as observed in the NOESY spectrum. This was further supported by the fact that H-6 (δ 3.03) and H-4′ (δ 2.99) resonated at anomalously high fields due to the aromatic system in this conformation. Consequently, the structure of fraxamoside was formulated as **4**.

Compound **5** was obtained as a colourless amorphous powder. The HR-SIMS of **5** exhibited a strong $[M-H]^-$ at *m/z* 601.2113, indicating a molecular formula of $C_{27}H_{38}O_{15}$ for **5**. The UV absorption, IR bands and NMR spectroscopic signals showed common features to secoiridoid glucosides with an oleoside 11-methyl ester (**6**) unit. The ^{1}H NMR spectrum, moreover, displayed signals for a secondary methyl group, three pairs of methylene protons and four methine protons. Its ^{13}C NMR spectrum showed, besides the signals corresponding to **6**, resonances of ten carbons including two carbonyls. With the aid of ^{1}H-^{1}H COSY, HMQC and HMBC experiments, the remaining ten carbons were evaluated as a cyclopentanoid terpene unit with the planar structure **16**. The presence of two carboxyl groups was further confirmed by the fact that compound **5** was methylated with CH_2N_2-Et_2O to give its dimethylated compound **17**. The hydroxyl group at C-9″ was linked to the C-7 carboxyl group on the basis of the HMBC correlations between H_2-9″ and C-7. The relative configurations of the cyclopentane moiety in **5** was determined by NOESY experiments. The important NOE cross-peaks observed between H_3-6″ and H-2″, H-3″, H-5″ (δ 2.12) and between H-5″ (δ 2.12) and H-3″ demonstrated that H-2″ and H-3″ exist on the same β face as the methyl group (H_3-6″) and therefore the substituents at C-2″ and C-3″ have α orientations. Further correlations between H-9″ (δ 4.13) and H-3″, H-4″β (δ 1.82), and between H-4″α (δ 1.38) and H-8″ (Fig. 2) suggest the relative configuration at C-8″ as shown.

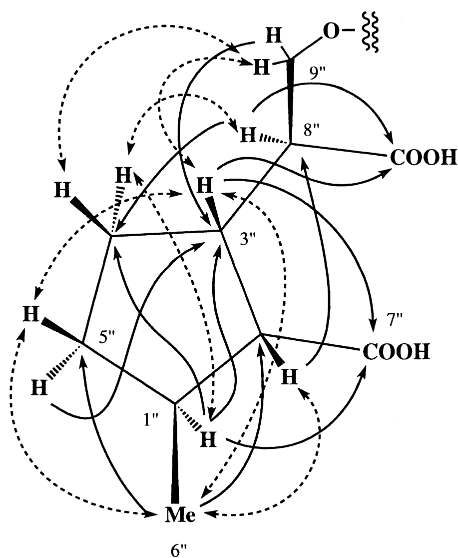
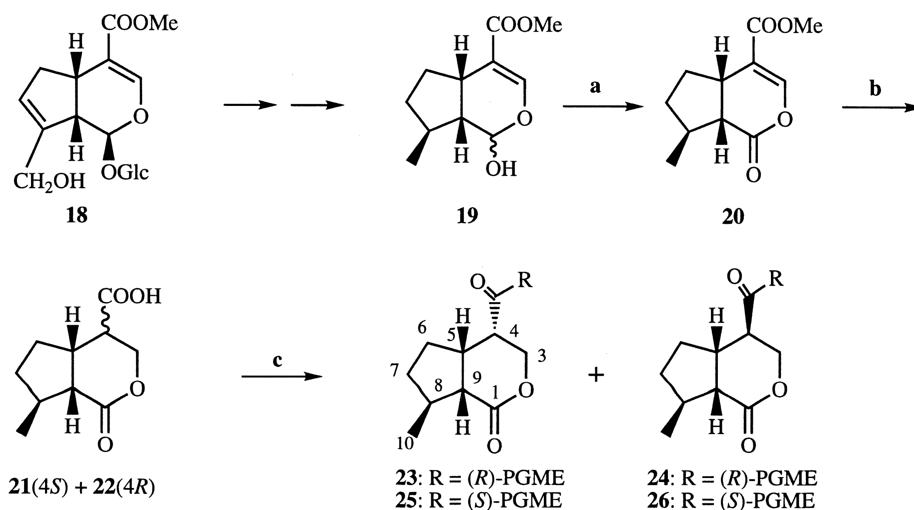


Fig. 2. Significant HMBC (bold arrows) and NOESY (dotted arrows) correlations observed for the monoterpene portion in **5**.

For the determination of the absolute configurations, the cyclic monoterpene unit (**16**) in **5** was chemically correlated with geniposide (**18**) as follows. Geniposide (**18**) followed the established route to deoxyloganin, which was subjected to enzymatic hydrolysis to give deoxyloganin aglycone (**19**) (Inouye and Nishioka, 1973; Inoue et al., 1992). Subsequent oxidation of **19** with PCC gave a lactone **20**. Reduction of **20** with $NaBH_4$ under alkaline conditions afforded a mixture of two 4-epimers, **21** and **22**, and then the mixture was treated with (*R*)-PGME in the presence of PyBOP, HBT and TEA to give a diastereomeric mixture of (*R*)-PGME amides, **23** and **24**, which were separated by preparative HPLC (Nagai and Kusumi, 1995). (*S*)-PGME amides, **25** and **26** were prepared from **20** in the same way (Scheme 2).

The configuration at C-4 of **23** and **24** was determined by detailed ^{1}H NMR spedroscopic analyses. The long-range W-coupling ($J=2.0$ Hz) between H-3β (δ 4.33) and H-5 as well as NOE interactions depicted in Fig. 3, indicated a boat-like conformation of the lactone ring in **23**, where H-4 must be oriented axially from the coupling constant $J_{4,5}$ (6.5 Hz). Accordingly, the configuration at C-4 of **23** was determined to be *S*. On the other hand, the coupling constants $J_{3\beta,4}$ and $J_{4,5}$ (each 10.5 Hz) in the ^{1}H NMR spectrum of **24** showed *trans* diaxial relationships between H-3β and H-4 and between H-4 and H-5, and NOE cross-peaks (Fig. 3) suggested that **24** also possessed a lactone ring with a boat-like form, in which the substituent at C-4 is equatorial and the configuration at C-4 is *R*. The absolute configuration of C-4 of **23** and **24** was further supported by differences in the chemical shifts of the corresponding proton signals between **23** and **25** and between **24** and **26**, respectively (Nagai and Kusumi, 1995).

Finally, compound **5** was subjected to alkaline hydrolysis and the resulting monoterpene was converted to a (*R*)-PGME amide, the ^{1}H NMR spectral data of which were identical to those of **23** but not to those of **24**, **25** and **26**, the latter two compounds of which must show theoretically identical NMR data of (*R*)-PGME amides derived from the enantiomers of **22** and **21**, respectively. Furthermore, the (*R*)-PGME amide derived from **5** was identified as **23** by HPLC analysis, indicating the absolute configurations in the amide compound to be 4*S*, 5*R*, 8*S* and 9*R*. Thus, the structure



Scheme 2. (a) PCC/CH_2Cl_2. (b) (1) $NaBH_4$/0.5N NaOH, (2) Amberlite IR-120. (c) (*R*)- or (*S*)-PGME•HCl, PyBOP, HOBT, TEA/DMF.

of the new secoiridoid glucoside was represented by **5** with the absolute stereochemistry, 1″*S*, 2″*R*, 3″*S* and 8″*S* and designated as frameroside.

The present work gives additional examples of oleuropein-type secoiridoid glucosides with an *O*-function at C-2″. Neither interconversion of the two glucosides **2** and **3** nor transformation of **2** and **3** to **4**, **10** and **11** were observed during the separation procedures. However, we could not completely rule out the possibility that the isolated compounds are artificially formed from a sole genuine natural product, 2″-hydroxyoleuropein, as previously proposed for related compounds (Tanahashi et al., 1999). Oleoside-type secoiridoid glucosides esterified with a cyclopentanoid monoterpene have so far been found only in plant species of the genus *Jasminum* and their cyclopentane unit showed *trans* relationship between H-2 and H-3 in all cases. Glucoside **5** having a cyclopentanoid monoterpene unit with H-2/H-3 *cis* relationship seems to be unique for *Fraxinus americana*.

3. Experimental

3.1. General

The UV spectra were recorded on a Shimadzu UV-240 spectrophotometer and the IR spectra on a Shimadzu FTIR-8200 spectrophotometer. The optical rotations were measured on a Jasco DIP-370 digital polarimeter. SIMS, EIMS, HR-SIMS and HR-EIMS were obtained with a Hitachi M-4100 mass spectrometer. Glycerol or 3-NOBA was used as the matrix for SIMS. The NMR experiments were performed with Varian VXR-500 and Varian Gemini-300 spectrometers, with tetramethyl silane as internal standard. HPLC was performed using a Waters system (510 HPLC Pump, 486 Tunable Absorbance Detector). Thin-layer chromatography was performed on precoated Kieselgel $60F_{254}$ plates (Merck) and spots were visualized under UV light.

3.2. Plant material

The leaves of *Fraxinus americana* were collected in Bole, Xinjiang, People's Republic of China. A voucher specimen (96001) is deposited in the laboratory of Xinjiang Medical College, Urumqi, People's Republic of China.

3.3. Isolation of glucosides

Dried leaves of *F. americana* (103 g) were extracted with hot MeOH. After concentration, the extract (17.8 g) was suspended in H_2O and filtered through a Celite layer. The filtrate and washings were combined and extracted successively with $CHCl_3$ and *n*-BuOH. The *n*-BuOH extract (4.07 g) was subjected to a Wakogel LP-$40C_{18}$ (Wako Pure Chemical Industries Ltd, Osaka, Japan) column chromatography. Elution with MeOH–H_2O mixtures of the indicated MeOH content gave 5 fractions. Fraction I (0–15% MeOH eluent, 318 mg) was further purified by preparative HPLC (μBondasphere 5μC18-100Å, H_2O–MeOH, 17:3 or 3:1), giving 3,4-dihydroxyphenethyl alcohol (15.1 mg), *p*-hydroxyphenethyl alcohol (14.8 mg), oleoside 11-methyl ester (**6**) (15.1 mg) and oleoside dimethyl ester (**7**) (7.1 mg) in order of elution. The following fractions were also purified by preparative HPLC (μBondasphere 5μC18-100Å, H_2O–MeOH, 31:19 or 3:2 or 11:9) or preparative TLC ($CHCl_3$–MeOH, 7:3 or *n*-BuOH-AcOH-H_2O, 4:1:0.5). Fr II (20% MeOH eluent, 306 mg) yielded quercetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl-β-D-galactopyranoside) (12.4 mg), quercetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside) (20.5 mg), acteoside (8.7 mg), **7** (16.8 mg), demethylligstroside (**1**) (4.9 mg), (2″*R*)-2″-hydroxyoleuropein (**2**) (3.9 mg) and

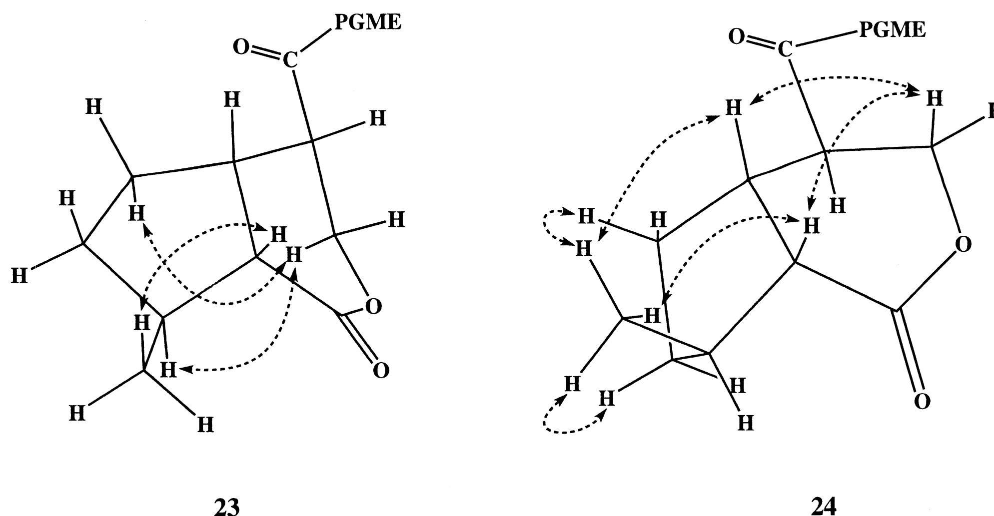


Fig. 3. Significant NOESY correlations observed for **23** and **24**.

(2″*S*)-2″-hydroxyoleuropein (**3**) (3.5 mg); fr III (30% MeOH eluent, 614 mg): kaempferol 3-*O*-(6-*O*-α-L-rhamnopyranosyl-β-D-galactopyranoside) (22.8 mg), kaempferol 3-*O*-(6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside) (25.6 mg), quercetin 3-*O*-(2,6-di-*O*-α-L-rhamnopyranosyl-β-D-galactopyranoside) (13.0 mg), quercetin 3-*O*-(2,6-di-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside) (36.6 mg), quercetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl-β-D-galactopyranoside) (18.9 mg), quercetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl-β-D-glucopyrano side) (63.0 mg), acteoside (66.6 mg), campneoside I (4.7 mg) and **1** (5.4 mg); fr IV (40% MeOH eluent, 880 mg): kaempferol 3-*O*-(6-*O*-α-L–rhamnopyranosyl-β-D-glucopyranoside) (25.6 mg), quercetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside) (63.0 mg), acteoside (38.9 mg), oleuropein (**8**) (243 mg), nuezhenide (28.3 mg), (2″*R*)-2″-methoxyoleuropein (**10**) (21.2 mg) and (2″*S*)-2″-methoxyoleuropein (**11**) (20.7 mg); fr V (40% MeOH eluent, 465 mg): kaempferol 7-*O*-(2-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside) (25.5 mg), **8** (12.5 mg), ligstroside (**9**) (127 mg), fraxamoside (**4**) (6.2 mg) and frameroside (**5**) (18.1 mg).

3.4. Demethylligstroside (**1**)

Colourless amorphous powder, $[\alpha]_D^{26}$ −110° (*c* 0.32, MeOH); UV λ_{max}^{MeOH} nm (log ε): 225 (4.08), 277 (3.25), 284 (3.19); IR ν_{max}^{KBr} cm^{-1}: 3413, 1715, 1638, 1518, 1076, 822; ^{1}H and ^{13}C NMR: see Tables 1 and 2; Significant HMBC correlations: H_2-6→C-7, H_2-1″→C-7, C-2″, C-3″, H-2″→C-1″, C-4″, C-8″, OMe→ C-11; HR-SIMS Found: 509.1684 [M−H]$^-$; $C_{24}H_{29}O_{12}$ requires 509.1660.

3.5. (2″R)-2″-Hydroxyoleuropein (**2**)

Colourless amorphous powder, $[\alpha]_D^{28}$ −152° (*c* 0.30, MeOH); UV λ_{max}^{MeOH} nm (log ε): 232.5 (4.16), 281.5 (3.47); IR ν_{max}^{KBr} cm^{-1}: 3391, 1733, 1717, 1628, 1541, 1076, 818; ^{1}H and ^{13}C NMR: see Tables 1 and 2; Significant HMBC correlations: H_2-6→C-7, H_2-1″→ C-7, C-2″, C-3″, H-2″→C-1″, C-4″, C-8″, OMe→C-11; HR-SIMS Found: 555.1733 [M−H]$^-$; $C_{25}H_{31}O_{14}$ requires 555.1715.

3.6. (2″S)-2″-Hydroxyoleuropein (**3**)

Colourless amorphous powder, $[\alpha]_D^{29}$ −140° (*c* 0.24, MeOH); UV λ_{max}^{MeOH} nm (log ε): 232.5 (4.20), 281.5 (3.50); IR ν_{max}^{KBr} cm^{-1}: 3369, 1734, 1717, 1636, 1522, 1076, 818; ^{1}H and ^{13}C NMR: see Tables 1 and 2; Significant HMBC correlations: H_2-6→C-7, H_2-1″→ C-7, C-2″, C-3″, H-2″→C-1″, C-4″, C-8″, OMe→C-11; HR-SIMS Found: 555.1718 [M−H]$^-$; $C_{25}H_{31}O_{14}$ requires 555.1715.

3.7. Fraxamoside (**4**)

Colourless amorphous powder, $[\alpha]_D^{22}$ −137° (*c* 0.12, MeOH); λ_{max}^{MeOH} nm (log ε): 233 (4.14), 282 (3.45); IR ν_{max}^{KBr} cm^{-1}: 3417, 1732, 1709, 1639, 1520, 1080, 820; ^{1}H and ^{13}C NMR: see Tables 1 and 2; Significant HMBC correlations: H_2-6→C-7, H_2-1″→C-7, C-2″, H-2″→C-1″, C-3″, C-4″, C-8″, C-6′, H_2−6′→ C−2″, OMe→C−11; HR-SIMS Found: 537.1612 [M−H]$^-$; $C_{25}H_{29}O_{13}$ requires 537.1607.

3.8. Frameroside (**5**)

Colourless amorphous powder, $[\alpha]_D^{27}$ −134° (*c* 1.09, MeOH); λ_{max}^{MeOH} nm (log ε): 236 (4.03); IR ν_{max}^{KBr} cm^{-1}: 3414, 1733, 1707, 1634, 1078, 818; ^{1}H and ^{13}C NMR: see Tables 1 and 2; Significant HMBC correlations: H_2-6→C-7, H_2-9″→C-7, OMe→C-11; HR-SIMS Found: 601.2113 [M−H]$^-$; $C_{27}H_{37}O_{15}$ requires 601.2134.

3.9. Methylation of 1

A solution of **1** (0.5 mg) in MeOH was treated with CH_2N_2–Et_2O to give **9** (0.5 mg). Compound **9** was identified as ligstroside (^{1}H NMR, HPLC).

3.10. Preparation of (R)- and (S)-2-hydroxy-1-(3,4-dimethoxyphenyl)ethanols (13 and 14)

3.10.1. OsO_4 oxidation of 15 followed by esterification with MTPA

A mixture of OsO_4 (0.5 g, 1.97 mmol), pyridine (0.7 ml) and dry Et_2O (15 ml) was added dropwise to a stirred solution of 3,4-dimethoxystyrene (**15**) (0.3 g, 1.83 mmol) in dry Et_2O (15 ml). After stirring at room temperature for 20 h, a mixture of $NaHSO_3$ (0.9 g, 8.65 mmol), pyridine (13 ml) and H_2O (15 ml) was added to the reaction mixture. The whole was stirred for a further 1 h. The reaction mixture was evaporated in vacuo and the residue was dissolved in H_2O and extracted with $CHCl_3$. The organic layer was concentrated in vacuo and the resulting residue (472 mg) was applied to a silica gel column. Elution with MeOH–$CHCl_3$ mixtures of increasing MeOH content (0–10%) gave **15** ($CHCl_3$ eluent, 36.4 mg 12 % yield) and 2-hydroxy-1-(3,4-dimethoxyphenyl)ethanol (10% MeOH, 310 mg 80 % yield). EIMS *m/z* 198 $[M]^+$, 167, 139, 124, 108. 2-Hydroxy-1-(3,4-dimethoxyphenyl)ethanol (50 mg) was dissolved in CH_2Cl_2 (2 ml), and (*R*)-MTPA acid (130 mg), DMAP (80 mg) and DCC (130 mg) were added. The whole was stirred at room temperature for 20 h, then more (*R*)-MTPA acid (30 mg) and DCC (50 mg) was added and stirred. After 5 h, the reaction mixture was poured into dil. HCl and extracted with $CHCl_3$. The $CHCl_3$ layer was dried and concentrated in vacuo. The residue was applied to a silica gel column with $CHCl_3$–MeOH (19:1) as eluent to yield a mixture of **13a** and **14a** (113 mg). The mixture (39.4 mg) was purified by prep. HPLC (μBondasphere 5μC18-100Å, H_2O-CH_3CN, 3:7) to yield **13a** (10.7 mg) and **14a** (23.0 mg). **13a**: ^{1}H NMR ($CDCl_3$): δ 3.36, 3.48 (each 3H, *br s*, MTPA-OMe × 2), 3.66, 3.88 (each 3H, *s*, 5-OMe, 6-OMe), 4.49 (1H, *dd*, *J* = 12.0, 8.5 Hz, H-1), 4.68 (1H, *dd*, *J* = 12.0, 3.0 Hz, H-1), 6.13 (1H, *dd*, *J* = 8.5, 3.0 Hz, H-2), 6.60–6.80 (3H, *m*, H-4, 7, 8), 7.20–7.50 (10H, *m*, MTPA-Ph × 2).* EIMS *m/z* 630 $[M]^+$, 167, 139, 124, 108. **14a**: ^{1}H NMR ($CDCl_3$): δ 3.37, 3.42 (each 3H, *br s*, MTPA-OMe × 2), 3.77, 3.89 (each 3H, *s*, 5-OMe, 6-OMe), 4.48 (1H, *dd*, *J* = 12.0, 7.5 Hz, H-1), 4.69 (1H, *dd*, *J* = 12.0, 4.0 Hz, H-1), 5.30 (1H, *dd*, *J* = 7.5, 4.0 Hz, H-2), 6.80–7.00 (3H, *m*, H-4, 7, 8), 7.20–7.50 (10H, *m*, MTPA-Ph × 2).* EIMS *m/z* 630 $[M]^+$, 167, 139, 124, 108. (*In order to avoid confusion, the numbering system of the phenylethyl moiety in oleuropein was also used for **13a**, **14a** and their derivatives.

3.10.2. Partial methanolysis of 13a

A solution of **13a** (10.7 mg, 0.017 mmol) in 0.1 M NaOMe (1 ml) and MeOH (1 ml) was stirred for 2 h at room temperature. After neutralization with Amberlite IR-120 (H^+ form), the reaction mixture was concentrated in vacuo and the residue was subjected to prep. TLC ($CHCl_3$-MeOH, 49:1) to give **13** (1.3 mg, 39% yield), **13b** (2.0 mg, 28% yield) and **13c** (2.1 mg, 30% yield). **13**: Colourless oil, $[\alpha]_D^{26}$ −40° ($CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 3.67 (1H, *dd*, *J* = 11.0, 8.0 Hz, H-1), 3.75 (1H, *dd*, *J* = 11.0, 3.5 Hz, H-1), 3.88, 3.90 (each 3H, *s*, 5-OMe, 6-OMe), 4.78 (1H, *dd*, *J* = 8.0, 3.5 Hz, H-2), 6.85 (1H, *d*, *J* = 8.0 Hz, H-7), 6.91 (1H, *dd*, *J* = 8.0, 2.0 Hz, H-8), 6.93 (1H, *d*, *J* = 2.0 Hz, H-4). EIMS *m/z* 198 $[M]^+$, 167. **13b**: ^{1}H NMR ($CDCl_3$): δ 3.60 (3H, *d*, *J* = 0.5 Hz, MTPA-OMe), 3.73, 3.88 (each 3H, *s*, 5-OMe, 6-OMe), 3.88 (1H, *dd*, *J* = 12.0, 8.0 Hz, H-1), 3.94 (1H, *dd*, *J* = 12.0, 4.0 Hz, H-1), 5.99 (1H, *dd*, *J* = 8.0, 4.0 Hz, H-2), 6.67–6.87 (3H, *m*, H-4, 7, 8), 7.30–7.50 (5H, *m*, MTPA-Ph). EIMS *m/z* 414 $[M]^+$, 189, 167, 139, 105. **13c**: ^{1}H NMR ($CDCl_3$): δ 3.55 (3H, *d*, *J* = 1.0 Hz, MTPA-OMe), 3.86, 3.88 (each 3H, *s*, 5-OMe, 6-OMe), 4.45 (2H, *d*, *J* = 5.8 Hz, H_2-1), 4.96 (1H, *t*, *J* = 5.8 Hz, H-2), 6.85 (1H, *d*, *J* = 6.6 Hz, H-7), 6.92 (1H, *dd*, *J* = 6.6, 2.0 Hz, H-8), 6.92 (1H, *d*, *J* = 2.0 Hz, H-4), 7.37–7.53 (5H, *m*, MTPA-Ph). EIMS *m/z* 414 $[M]^+$, 189, 167, 139, 105.

3.10.3. Partial methanolysis of 14a

Compound **14a** (10 mg, 0.016 mmol) was worked up in the same way as described for **13**, giving **13c** (2.2 mg, 70 %), **14b** (0.9 mg, 14 %) and **14c** (1.0 mg, 15 %). **14**: Colourless oil, $[\alpha]_D^{26}$ +36° ($CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 3.67 (1H, *dd*, *J* = 11.0, 8.0 Hz, H-1), 3.75 (1H, *dd*, *J* = 11.0, 3.5 Hz, H-1), 3.88, 3.90 (each 3H, *s*, 5-OMe, 6-OMe), 4.78 (1H, *dd*, *J* = 8.0, 3.5 Hz, H-2), 6.85 (1H, *d*, *J* = 8.0 Hz, H-7), 6.91 (1H, *dd*, *J* = 8.0, 2.0 Hz, H-8), 6.93 (1H, *d*, *J* = 2.0 Hz, H-4). EIMS *m/z* 198 $[M]^+$, 167. **14b**: ^{1}H NMR ($CDCl_3$): δ 3.49 (3H, *d*, *J* = 0.5 Hz, MTPA-OMe), 3.85, 3.89 (each 3H, *s*, 5-OMe, 6-OMe), 3.87 (1H, *dd*, *J* = 11.0, 8.0 Hz, H-1), 3.92 (1H, *dd*, *J* = 11.0, 4.0 Hz, H-1), 6.03 (1H, *dd*, *J* = 8.0, 4.0 Hz, H-2), 6.82–6.97 (3H, *m*, H-4, 7, 8), 7.35–7.55 (5H, *m*, MTPA-Ph). EIMS *m/z* 414 $[M]^+$, 189, 167, 139, 105. **14c**: ^{1}H NMR ($CDCl_3$): δ 3.54 (3H, *br s*, MTPA-OMe), 3.86, 3.88 (each 3H, *s*, 5-OMe, 6-OMe), 4.45 (2H, *br d*, *J* = 6.4 Hz, H_2-1), 4.97 (1H, *br t*, *J* = 5.2 Hz, H-2), 6.85 (1H, *d*, *J* = 8.0 Hz, H-7), 6.91 (1H, *dd*, *J* = 8.0, 2.0 Hz, H-8), 6.92 (1H, *d*, *J* = 2.0 Hz, H-4). EIMS *m/z* 414 $[M]^+$, 189, 167, 139, 105.

3.10.4. HPLC analysis of 13 and 14

Standard (*R*)- and (*S*)-2-hydroxy-1-(3,4-dimethoxy phenyl)ethanols were analyzed by chiral HPLC [column, CHIRALCEL OB-H (4.6 mm i.d. × 250 mm, Daicel Chemical Industries Ltd); mobile phase, *n*-hex-

Table 3
^{1}H NMR spectral data for **23–26** in $CDCl_3$

H	23			24			25			26		
3	4.33	*tdd*	(12.0, 3.5, 2.0)	4.26	*dd*	(11.0, 10.5)	4.37	*br d*	(7.0)	4.22	*dd*	(11.5, 10.0)
	4.39	*dd*	(12.0, 10.0)	4.40	*dd*	(11.0, 3.0)	4.37	*br d*	(7.0)	4.28	*dd*	(11.5, 4.0)
4	2.94	*ddd*	(10.0, 6.5, 3.5)	2.38	*td*	(10.5, 3.5)	2.99	*dt*	(7.0, 6.5)	2.37	*td*	(10.0, 4.0)
5	2.89	*m*		2.81	*dtd*	(10.5, 9.5, 7.0)	2.79	*m*		2.87	*dtd*	(11.5, 10.0, 7.0)
6	1.66	*tdd*	(12.0, 9.5, 6.5)	1.23	*tdd*	(11.5, 9.5, 6.0)	1.48	*m*		1.32	*tdd*	(11.5, 9.5, 6.5)
	1.96	*m*		1.94	*m*		1.51	*m*		2.16	*m*	
7	1.26	*td*	(12.0, 6.5)	1.15	*m*		1.12	*td*	(12.0, 7.0)	1.23	*m*	
	1.99	*m*		1.87	*m*		1.86	*m*		1.93	*dtd*	(12.5, 5.5, 1.5)
8	2.04	*m*		2.33	*m*		1.99	*m*		2.36	*m*	
9	2.49	*t*	(10.0)	2.46	*dd*	(10.5, 8.0)	2.46	*t*	(10.5)	2.47	*dd*	(10.0, 8.0)
10	1.20	*d*	(6.5)	1.20	*d*	(7.0)	1.17	*d*	(6.5)	1.21	*d*	(6.5)
PGME-NH	6.54	*br d*	(7.0)	6.73	*br d*	(7.0)	6.64	*br d*	(7.5)	6.65	*br d*	(7.0)
PGME-CH	5.52	*d*	(7.0)	5.55	*d*	(7.0)	5.55	*d*	(7.5)	5.55	*d*	(7.0)
PGME-OMe	3.73	*s*		3.74	*s*		3.74	*s*		3.74	*s*	
PGME-Ph	7.31–7.40	*m*		7.33–7.40	*m*		7.31–7.39	*m*		7.32–7.40	*m*	

ane-2-propanol (22:3); flow rate, 0.6 ml/min; detection, 270 nm]. Under these conditions compound **13** was eluted at rt 22 min and **14** at rt 26 min.

*3.11. Methylation of **2** and **3** followed by methanolysis*

A solution of **2** (0.7 mg, 0.0013 mmol) in MeOH was treated with CH_2N_2–Et_2O under ice-cooling. The reaction mixture was evaporated in vacuo, then the residue was dissolved in dry MeOH (1 ml) and 0.1 M NaOMe (1 ml) and the solution was stirred for 5 h at room temperature. After neutralization with Amberlite IR-120 (H^+ form), the reaction mixture was concentrated in vacuo, and the residue was distributed between H_2O and $CHCl_3$, with the organic layer yielding a residue (0.2 mg) upon evaporation. This was shown to be identical with **13** derived from **15** (chiral HPLC). A solution of **3** (0.7 mg, 0.0013 mmol) was treated as described above to give a $CHCl_3$-soluble product (0.2 mg), which was identified as **14** by chiral HPLC analysis.

*3.12. Methylation of **5***

A solution of 5 (4.0 mg, 0.0066 mmol) in MeOH was treated with CH_2N_2 and concentrated in vacuo. The residue was subjected to preparative HPLC (H_2O–MeOH, 2:3) to give **17** (4.1 mg, 98% yield). Colourless amorphous powder, $[\alpha]_D^{23}$-154° (*c* 0.21, MeOH); ^{1}H and ^{13}C NMR: see Tables 1 and 2; Significant NOESY correlations: H_3-6″↔H-2″, H-4″ (δ 1.54)↔H-1″, H-8″, H_2-9″, H-9″ (δ 4.12)↔10″-OMe; HR-SIMS Found: 629.2461 $[M-H]^-$; $C_{29}H_{41}O_{15}$ requires 629.2447.

*3.13. Preparation of PGME amides **23**, **24**, **25** and **26***

*3.13.1. Preparation of **20** from geniposide (**18**)*

Deoxyloganin was prepared from **18** by a modification of Inouye's methods (Inouye and Nishioka, 1973; Inoue et al., 1992). Separation of deoxyloganin tetraacetate from its 8-epimer was performed by preparative HPLC (μBondasphere 5μC18-100Å, H_2O–MeCN, 1:1). Deoxyloganin was hydrolysed with β-glucosidase to deoxyloganin aglucone (**19**). To a solution of **19** (218 mg, 1.0 mmol) in CH_2Cl_2 (3 ml) was added PCC (420 mg). After standing for 15 h at room temperature, the reaction mixture was applied to a silica gel column, eluted with $CHCl_3$. Evaporation of the eluate followed by preparative HPLC (μBondasphere 5μC18-100Å, H_2O–MeCN, 1:1) gave **20** (38.7 mg, 18% yield). ^{1}H NMR ($CDCl_3$): δ 1.21 (3H, *d*, $J=6.0$ Hz, H_3-10), 1.26, 1.42 (each 1H, *m*, H_2-6), 1.95, 2.30 (each 1H, *m*, H_2-7), 2.54 (2H, *m*, H-8, H-9), 3.16 (1H, *br q*, $J=8.5$ Hz, H-5), 3.77 (3H, *s*, OMe), 7.44 (1H, *s*, H-3). EIMS m/z 210 $[M]^+$.

*3.13.2. Preparation of (R)-PGME amides **23** and **24** from **20***

To a solution of **20** (18.3 mg, 0.087 mmol) in 0.5 M NaOH (2 ml), was added $NaBH_4$ (10 mg, 0.26 mmol). After standing at room temperature for 1 h, the solution was neutralized with Amberlite IR-120, and stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo to give a mixture (10.5 mg) of **21** and **22**. To a solution of the mixture in DMF (0.5 ml) were added (*R*)-PGME·HCl (D-(−)-α-phenylglycine methyl ester hydrochloride, 20 mg), PyBOP (benzotriazol-1-yl-oxy-tris(pyrolidino)phosphonium hexafluoro phosphate, 45 mg), HBT (1-hydroxybenzotriazole, 12 mg) and

TEA (30 mg), and the whole was stirred at room temperature for 1.5 h. The reaction mixture was poured into dil. HCl and extracted with $CHCl_3$. The $CHCl_3$ layer was dried and concentrated in vacuo. The residue was purified by preparative HPLC (μBondasphere 5μC18-100Å, H_2O–MeCN, 1:1), giving **23** (12.2 mg, 41%) and **24** (8.5 mg, 28%). **23**: ^{1}H NMR: see Table 3. Significant NOESY correlations: H-3α (δ 4.39)$\leftrightarrow$H-6α(δ 1.66), H-8, H-9 $\leftrightarrow$H$_3$-10; EIMS *m/z* 345 $[M]^+$. **24**: ^{1}H NMR: see Table 3. Significant NOESY correlations: H-3β (δ 4.26)$\leftrightarrow$H-5, H-9, H$_3$-10$\leftrightarrow$H-5, H-6β (δ 1.94), H-7β (δ 1.86), H-9; EIMS *m/z* 345 $[M]^+$.

3.13.3. Preparation of (S)-PGME amides ***25*** *and* ***26*** *from* ***20***

(*S*)-PGME was prepared from (L)-(+)-α-phenylglycine and $SOCl_2$ in MeOH according to Nagai and Kusumi. Compound **20** (20.8 mg, 0.099 mmol) was dissolved in 0.5 M NaOH (0.5 ml) following which $NaBH_4$ (10 mg, 0.26 mmol) was added, with the reaction mixture treated in the same way as described above. The reaction residue was dissolved in DMF (0.5 ml) and (*S*)-PGME·HCl (20 mg), PyBOP (45 mg), HBT (12 mg) and TEA (30 mg) were added, and the whole was stirred at room temperature for 1.5 h and worked up in the same way as described above. The resulting residue was subjected to prep. HPLC (μBondasphere 5μC18-100Å, H_2O–CH_3CN, 1:1), giving **25** (12.7 mg, 37%) and **26** (6.9 mg, 20%). **25**: ^{1}H NMR: see Table 3. EIMS *m/z* 345 $[M]^+$. **26**: ^{1}H NMR: see Table 3. EIMS *m/z* 345 $[M]^+$.

3.14. Alkaline hydrolysis of ***5*** *followed by preparation of the (R)-PGME amide*

A solution of **5** (5.7 mg 0.0095 mmol) in 0.5 M NaOH (1.5 ml) was stirred for 1 h at room temperature, and then neutralized with Amberlite IR-120 (H^+ form) and the whole was stirred for 2 h. The reaction mixture was extracted with $CHCl_3$ and the $CHCl_3$ layer was concentrated to give a residue (2.2 mg). To a solution of the residue in DMF (0.5 ml) were added (*R*)-PGME·HCl (3 mg), PyBOP (7 mg), HBT (2 mg) and TEA (6 mg), and the whole was stirred at room temperature for 1.5 h and worked up in the same way as described above. The resulting residue was subjected to preparative HPLC (μBondasphere 5μC18-100Å, H_2O–CH_3CN, 1:1) to give the amide compound (1.2 mg, 37%), which was identified with **23** (^{1}H NMR, HPLC).

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