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TWO TRITERPENOID GLYCOSIDES FROM LEAVES OF *ILEX CORNUTA*

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Key Word Index—*Ilex cornuta*; Aquifoliaceae; two triterpene glycosides; pomolic acid; ilexside I methyl ester; ilexside II.

Abstract—Two new triterpene glycosides, a diglycoside (as the corresponding methyl ester) and a bis-desmoside were isolated from leaves of *Ilex cornuta*. Their structures, termed as ilexside I methyl ester and ilexside II, have been established to be 3β-*O*-[β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl]-pomolic acid methyl ester and 3β-*O*-[β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl]-pomolic acid-(28→1)-β-D-glucopyranosyl ester, respectively, based on chemical and spectral evidence, but notably on novel and efficient application of high resolution ^{1}H NMR spectroscopy.

INTRODUCTION

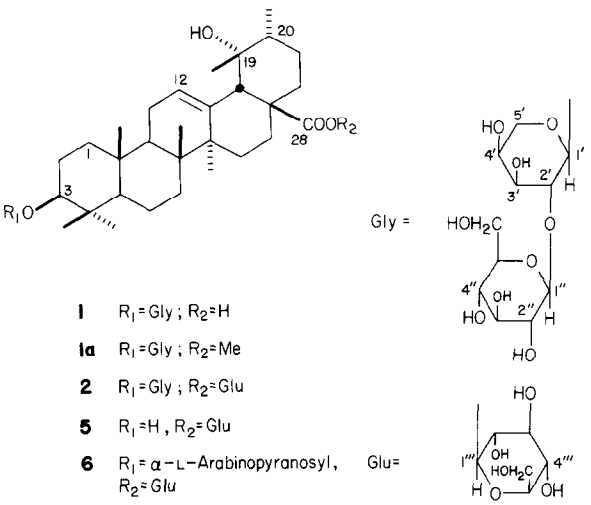
Dried leaves of *Ilex cornuta* Lindl. (Aquifoliaceae) have been used as a crude drug for medicinal purposes in some southern districts of China. Since no previous work seemed to have been done on the chemical constituents of *I. cornuta*, we examined leaves of this plant and isolated two new and major glycosides (one of them as the corresponding methyl ester). We have named them ilexside I methyl ester and ilexside II, and established their structures as **1a** and **2**.

RESULTS AND DISCUSSION

The total glycoside mixture from the methanol extracts was chromatographed on Si gel to give two main glycoside fractions. The less polar fraction consisted of a major acidic glycoside and a small amount of a neutral glycosidic contaminant. The fraction was therefore methylated with CH_2N_2 and then subjected to prep. TLC. Ilexside I methyl ester (**1a**) was separable from the unreacted contaminant and was isolated in a pure form. The more polar fraction was recrystallized from methanol to yield another pure glycoside, ilexside II (**2**).

The methyl ester (**1a**), $C_{42}H_{68}O_{13}$, exhibited an intense molecular ion peak $[M + Na]^+$ at *m/z* 803 (the base peak) on field desorption mass spectroscopy (FD-MS). Two significant fragment peaks at *m/z* 641 and 509 are respectively due to the loss of one hexose unit (162) and of both hexose and aldopentose units (total 294) [1]. The IR spectrum showed a methyl ester carbonyl at 1718 cm^{-1} and the ^{1}H NMR spectrum (Table 1) gave seven methyl signals and a carbomethoxy singlet. These spectral data indicate that **1a** is a diglycoside of a triterpene carboxy acid methyl ester.

By analogy, the other glycoside (**2**), $C_{47}H_{76}O_{18}$, gave a molecular ion $[M + Na]^+$ at *m/z* 951 (33%) on FD-MS. Three abundant and moderately abundant fragments with *m/z* 789 (the base peak), 657, and 627, correspond to $[(M + Na) - 162]^+$, $[(M + Na) - 294]^+$, and $[(M + Na) - 324]^+$, respectively. The IR [1725 cm^{-1} (ester C = O)] and ^{1}H NMR (Table 2) spectra were investigated in a similar manner as for **1a**. The results presume **2** to be a triglycoside, the aglycone triterpene carboxy acid of which is linked with one of the sugar components by an ester bond.



1 R_1=Gly; R_2=H

1a R_1=Gly; R_2=Me

2 R_1=Gly; R_2=Glu

5 R_1=H, R_2=Glu

6 R_1=α-L-Arabinopyranosyl, R_2=Glu

7 R_1=H, R_2=Me

Acidic hydrolysis of the triglycoside (**2**) gave two artefact aglycones as a dienic mixture, and D-glucose and L-arabinose (assumed to be of D- and L-configurations respectively; molar ratio 2 : 1, judged by GC) as sugar components. The aglycone mixture

Table 1. ^{1}H NMR spectral data for **1a** and its per-trifluoroacetate [δ relative to TMS; 400 MHz, C_5D_5N; multiplicity and coupling constant (Hz) in parentheses]

		1a	Trifluoroacetate
Inner sugar protons	1′-H	4.97 (*d*, 5.9)	5.14 (*d*, 5.9)
	2′-H	4.61 (*dd*, 5.9; 7.0)	4.70 (*dd*, 5.9; 8.1)
	3′-H	4.36 (*ddd*, 7.0; 2.0; 4.3)	6.08 (*dd*, 8.1; 3.5)
	4′-H	4.38 (*dddd*, 2.0; 4.1; *ca* 1.0; 4.3)	6.03 (*ddd*, 3.5; 4.3; 1.9)
	5′α-H	3.80 (*dd*, 11.9; *ca* 1.0)	4.17 (*dd*, 12.0; 1.9)
	5′β-H	4.31 (*dd*, 11.9; 4.1)	4.43 (*dd*, 12.0; 4.3)
	3′-OH	6.67 (*d*, 4.3)	—
	4′-OH	6.38 (*d*, 4.3)	—
Terminal sugar protons	1″-H	5.20 (*d*, 7.6)	5.89 (*d*, 7.6)
	2″-H	4.11 (*ddd*, 7.6; 8.9; 3.5)	6.15 (*dd*, 7.6; 9.5)
	3″-H	4.21 (*ddd*, 8.9; 9.2; 4.1)	6.67 (*dd*, 9.5; 9.5)
	4″-H	4.36 (*ddd*, 9.2; 8.9; 4.3)	6.34 (*dd*, 9.5; 9.5)
	5″-H	3.84 (*dt*, 8.9; 3.8)	5.15 (*ddd*, 9.5; 4.3; 2.4)
	6″-H_2	4.46 (*dd*, 3.8; 5.9)	5.06 (*dd*, 12.4; 4.3) 4.99 (*dd*, 12.4; 2.4)
	2″-OH	7.68 (*d*, 3.5)	—
	3″-OH	7.24 (*d*, 4.1)	—
	4″-OH	7.16 (*d*, 4.3)	—
	6″-OH	5.77 (*t*, 5.9)	—
Aglycone region protons	3α-H	3.22 (*dd*, 11.9; 4.3)	3.31 (*dd*, 11.9; 4.3)
	12-H	5.49 (*br t*, 3.5)	5.49 (*br t*, 3.5)
	19α-OH	5.14 (*s*)	*
	COOMe	3.73 (*s*)	3.74 (*s*)
	20α-Me	1.09 (*d*, 7.0)	1.09 (*d*, 7.0)
	Me × 6	0.88, 0.91, 1.08, 1.25, 1.37, 1.67 (3H each, all *s*)	0.88, 0.96, 1.07, 1.23, 1.40, 1.67 (3H each, all *s*)

*Unclear.

was modified by methylation and acetylation, and then subjected to fractional recrystallization to afford two pure methyl ester acetates, respectively identified with the authentic samples of methyl tomentosolate acetate (**3**) and vanguerolate acetate (**4**) [2]. The sugar constituents of the diglycoside (**1a**) were examined in the same manner and the presence of 1 mol each of D-glucose and L-arabinose was confirmed as described in the Experimental.

Compound **2** was hydrolysed with alkali, treated with a cationic resin, and methylated with diazomethane to yield quantitatively a methyl ester (also a diglycoside), consistent with **1a**. Based on this evidence, ilexside II (**2**) is structurally correlated to ilexside I methyl ester (**1a**), i.e. **2** possesses a glucosyl ester moiety in place of the methyl ester of **1a**.

In order to clarify the structure of the genuine aglycone, enzymic hydrolysis was undertaken. Ilexside II (**2**) was incubated with mixed glycosidases from *Turbo cornutus* for conversion into two prosapogenols, a monoglucoside (**5**) and a bisdesmosyl diglycoside which was identical with the authentic specimen of natural ziyuglycoside (**6**) [3]. The other ester (**5**) was hydrolysed under alkaline conditions to afford a carboxylic acid (the genuine aglycone) which was converted without prior isolation, to the corresponding methyl ester, which was identical to authentic methyl pomolate (**7**) [4]. The accumulated evidence provides the gross features for both glycosides as follows: **1a**, 3β-*O*-(D-glucosyl-α-L-arabinopyranosyl)-pomolic acid methyl ester and **2**, 3β-*O*-(D-glucosyl-α-L-arabinopyranosyl)-pomolic acid-(28 → 1)-D-glucosyl ester.

The final structure for **1a** was established as follows. Information concerning the pyranose form of the sugars, the configuration of the glycosidic linkages, and the position (on the inner arabinosyl moiety) where terminal glucose was connected through an ether bond, was obtained by detailed studies of the high resolution ^{1}H NMR spectrum. The assignments were confirmed by double resonance experiments (Table 1). The anomeric (C-1′) proton

Table 2. ^{1}H NMR spectral data for **2** and its per-trifluoroacetate [δ relative to TMS; 400 MHz, C_5D_5N; multiplicity and coupling constant (Hz) in parentheses]

		2	Trifluoroacetate
Inner sugar protons	1′-H	4.97 (*d*, 5.9)	5.15 (*d*, 5.7)
	2′-H	4.61 (*dd*, 5.9; 7.8)	4.79 (*dd*, 5.7; 7.8)
	3′-H	4.36 (*)	6.08 (*dd*, 7.8; 3.2)
	4′-H	4.37 (*)	6.04 (*ddd*, 3.2; 4.6; 2.2)
	5′α-H	3.80 (*dd*, 12.4; 2.4)	4.19 (*dd*, 13.2; 2.2)
	5′β-H	†	4.45 (*dd*, 13.2; 4.6)
	3′-OH	6.65 (*d*, 4.3)	—
	4′-OH	6.40 (*)	—
Terminal sugar protons	1″-H	5.20 (*d*, 7.6)	5.92 (*d*, 7.8)
	2″-H	4.11 (*ddd*, 7.6; 8.6; 3.2)	6.13 (*dd*, 7.8; 9.5)
	3″-H	4.21 (*ddd*, 8.6; 9.2; 3.8)	6.67 (*dd*, 9.5; 9.5)
	4″-H	†	6.32 (*dd*, 9.5; 9.5)
	5″-H	3.83 (*dt*, 9.5; 3.5)	5.18 (*ddd*, 9.5; 3.0; 3.0)
	6″-H_2	4.45 (*)	5.07 (*dd*, 3.0; *)
			5.00 (*dd*, 3.0; *)
	2″-OH	7.68 (*d*, 3.2)	—
	3″-OH	7.23 (*d*, 3.8)	—
	4″-OH	7.18 (*d*, 3.2)	—
	6″-OH	5.79 (*t*, 5.9)	—
Ester sugar protons	1‴-H	6.34 (*d*, 8.1)	6.75 (*d*, 8.1)
	2‴-H	4.27 (*)	6.43 (*dd*, 8.1; 9.5)
	3‴-H	4.30 (*)	6.94 (*dd*, 9.5; 9.2)
	4‴-H	4.35 (*)	6.40 (*dd*, 9.2; 9.7)
	5‴-H	4.07 (*ddd*, 9.2; 4.1; 3.0)	5.23 (*dt*, 9.7; 3.0)
	6‴-H_2	4.47 (*)	5.07 (*)
		4.37 (*)	
	2‴-OH	7.53 (*d*, 5.7)	—
	3‴-OH	7.43 (*br s*)	—
	4‴-OH	7.27 (*d*, 3.8)	—
	6‴-OH	6.37 (*t*, 5.4)	—
Aglycone region protons	3α-H	3.20 (*dd*, 11.6; 4.6)	3.32 (*dd*, 11.6; 4.6)
	12-H	5.57 (*br t*, 3.5)	5.56 (*br t*, 3.5)
	20α-Me	1.08 (*d*, 6.8)	1.10 (*d*, 7.3)
	Me × 6	0.92, 1.07, 1.21, 1.22, 1.41, 1.70 (3H each, all *s*)	0.94, 1.01, 1.08, 1.25, 1.40, 1.68 (3H each, all *s*)

*Multiplicity and/or coupling constant obscure, due to partial overlap.
†Chemical shift was unclear.

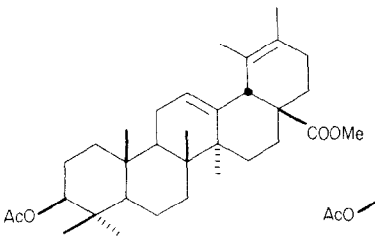
doublet (J = 5.9 Hz) at δ 4.97 and the other proton signals, due to the inner sugar, confirm the presence of α-L-arabinopyranoside ($^1C_4$ conformation). Similarly, the anomeric (C-1″) proton signal (δ 5.20) with a large coupling constant (*d*, J = 7.6 Hz) and the other proton signals due to the terminal sugar are in agreement with β-D-glucopyranoside ($^4C_1$ conformation).

All hydroxy proton signals attributable to the disaccharide part, as well as a singlet arising from the 19α-OH proton, were observed (Table 1) and reveal that the 2′α-OH group on L-arabinopyranoside was linked with D-glucopyranose by an ether bond via the anomeric hydroxyl of the glucose. This was further corroborated by the following alternative investigation. The chemical shift of each proton on the arabinosyl moiety was compared with that of the corresponding per-trifluoroacetate (see Experimental), the assignments of which were also made with the aid of the double resonance technique (Table 1). The 3′- and 4′-protons of the derivative (δ 6.08 and 6.03) both exhibited downfield shifts by *ca* 1.7 ppm

compared with those (δ 4.36 and 4.38) of the original alcohol (**1a**). By contrast, the 2'-porton of the derivative (δ 4.70) resonated at almost the same field as that (δ 4.61) of **1a**. The combined evidence allows the assignment of 3β-*O*-[β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl]-pomolic acid methyl ester (**1a**) for ilexside I methyl ester.

The full structure for ilexside II (**2**) was also determined by an analogous ^{1}H NMR investigation. The assignments for **2** and the corresponding per-trifluoroacetate (see Experimental) were achieved with the aid of double resonance studies and by comparison with those for **1a** and its derivative (Table 1) and they are summarized in Table 2. In **2**, all proton signals attributable to the third sugar, the ester glucoside, including the anomeric (C-1‴) proton doublet (δ 6.34) with a large *J*-value (8.1 Hz), were in agreement with a β-D-glucopyranoside ($^4C_1$ conformation) structure. This, coupled with the other evidence, defines ilexside II as 3β-*O*-[β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl]-pomolic acid-(28→1)-β-D-glucopyranosyl ester (**2**). Furthermore, the data for **2** and its derivative (Table 2) confirm this structure (**2**). Both glycosides, ilexside I (**1**) (assumed to be the natural form of **1a**) and ilexside II (**2**), are the second example of the natural occurrence of pomolic acid glycosides.

The hemolytic activity of the four related glycosides, **1** (see Experimental), **1a**, **2**, and **4** was examined. Both of the mono-desmosides (**1**) and (**1a**) were consistently hemolytic at a concentration of *ca* 2 μg/ml. In contrast, the bis-desmosides (**2**) and (**4**) exhibited no activity at a concentration of 100 μg/ml. This may indicate a correlation of glycoside structure with its hemolytic activity.



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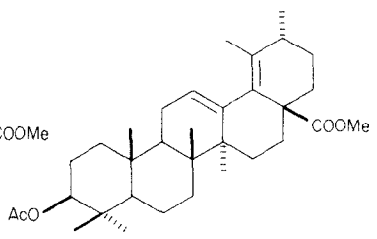

4

EXPERIMENTAL

General remarks. Mps are uncorr. FDMS using silicon emitters: accelerating *V*, 2–3 kV; emitter current, 23–29 mA; chamber temp., room temp. MS and accurate MS: 75 eV. GLC with FID were performed on a stainless steel column (3 mm × 2 m) packed with 1.5% SE-52. Si gel for CC: Kieselgel 60 (Merck). TLC: pre-coated Si-gel plates (Merck HF-254 and PF_{254}). Mixed gylcosidases from *Turbo cornutus* (Lot. No. ET9401) was commercially available.

Plant material. Leaves of *Ilex cornuta* were collected in July at Kosobe Conservatory Experimental Farm of Kyoto University, Takatsuki, Japan and identified by one of us (K.Y.).

Isolation of ilexside I *methyl ester* (**1a**) *and ilexside* II (**2**). The air-dried leaves (9 kg) were crushed and extracted with MeOH (80 l.) at room temp. for 20 days. Evaporation of the solvent under red. pres. yielded a syrup (806 g), part (628 g) of which was partitioned between *n*-BuOH–H_2O (1 : 1). The organic layer was evaporated to give a residue (510 g), a portion (478 g) of which was dissolved in a minimal amount of MeOH and poured into a large quantity of EtOAc with stirring. The ppt (total glycoside mixture) (69 g) was collected by filtration, and a part (40 g) of it was chromatographed on Si gel (1.1 kg), eluting gradually with each lower phase of $CHCl_3$–MeOH–H_2O (11 : 3 : 1) and (7 : 3 : 1). The less polar fraction (3.3 g) obtained with the former eluent was found to consist of a major acidic glycoside and a small amount of a neutral glycosidic contaminant (no isolation) from the ^{1}H NMR spectrum (400 MHz; C_5D_5N). The fraction (300 mg) in MeOH was treated with Dowex 50 W × 8 (*ca* 2 g) and reacted with excess CH_2N_2. TLC ($CHCl_3$–MeOH–H_2O, 7 : 3 : 1, lower phase) showed two spots due to the methyl ester derived from the original acidic glycoside and the unreacted contaminant. Prep. TLC separation (2 and 0.5 mm thickness; developed with the same solvent system as for TLC; eluted with MeOH) gave the pure methyl ester (**1a**) (220 mg) as colourless crystals, mp 240–243° (from MeOH), $[\alpha]_D^{20}$ + 15.6° (MeOH; *c* 0.80). IR ν_{max}^{KBr} cm^{-1}: 3310 (OH), 1718 (COOMe), 1635 (C=C), 1065 (Et_2O); ^{1}H NMR: Table 1; FDMS *m/z* (rel. int.): 803 $[M+Na]^+$ (100), 641 $[(M+Na)-162]^+$ (13.7), 509 $[(M+Na)-294]^+$ (5.9) (Found: C, 61.75; H, 8.80. $C_{42}H_{68}O_{13}$. $2H_2O$ requires C, 61.74; H, 8.88%.) The polar fraction obtained with the latter eluent afforded pure ilexside II (**2**) (9.4 g) as a white powder, mp 268–270° (from MeOH), $[\alpha]_D^{20}$ − 15.0° (MeOH; *c* 0.71). IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1725 (ester C=O), 1630 (C=C), 1060 (Et_2O); ^{1}H NMR: Table 2; FDMS *m/z* (rel. int.): 951 $[M+Na]^+$ (33), 789 $[(M+Na)-162]^+$ (100), 657 $[(M+Na)-294]^+$ (5.9), 627 $[(M+Na)-324]^+$ (11.8) (Found: C, 58.69; H, 8.37. $C_{47}H_{76}O_{18}$. $2H_2O$ requires C, 58.49; H, 8.36%.)

Preparation of the per-trifluoroacetates of **1a** *and* **2**. To **1a** (30 mg) in C_5D_5N (0.5 ml), trifluoroacetic anhydride (50 μl) was added and the soln allowed to stand at room temp. After the reaction was complete (giving one product as checked by TLC), the soln was subjected to ^{1}H NMR analysis (Table 1). The product, judged by the data, corresponds to the hexatrifluoroacetate, in which the 19α-*tert* hydroxyl group remained unreacted. The per-trifluoroacetate of **2** was also prepared in a similar manner and the ^{1}H NMR data are shown in Table 2. Based on the data, the product seems to be the nonatrifluoroacetate with a 19α-*tert* hydroxyl moiety.

Acidic hydrolysis of **2**. Compound **2** (500 mg) was dissolved in 2 N H_2SO_4–30% aq. MeOH (50 ml) and refluxed for 3 hr. The mixture was poured into ice–H_2O and extracted

with *n*-BuOH–EtOAc (1 : 1). The organic phase was washed with H_2O, evaporated under red. pres. and dried *in vacuo*. The product was purified by prep. TLC [0.5 mm thickness; developed with $CHCl_3$–EtOAc (4 : 1); eluted with $CHCl_3$–MeOH (1 : 1)] and re-crystallization from MeOH to afford an aglycone (200 mg), colourless plates, which was shown to be a dienic mixture of two triterpene acids by the ^1H NMR spectrum (400 MHz; $CDCl_3$). Hence, the mixture was methylated with CH_2N_2, acetylated with Ac_2O (1 ml)–pyridine (2 ml) (at 37° for 2 days), worked-up in the usual manner, and purified by prep. TLC to yield a mixture of two methyl ester acetates (70 mg). Crystallization of the mixture from EtOAc–MeOH (1 : 2) gave colourless needles (less soluble component in MeOH) (29.3 mg) of mp 233–235.5°, identical with authentic methyl tomentosolate acetate (**3**) [2] of mp 233–235°, by mmp 233–235°, and by comparison of IR (KBr), MS, and TLC. The mother liquor on crystallization from MeOH gave colourless prisms (more soluble component in MeOH) (24.1 mg) of mp 183–185°, which had mp (mmp 183–186°), IR (KBr), UV (EtOH), MS data, and TLC behaviour, consistent with authentic methyl vanguerolate acetate (**4**) [2] of mp 184–186°. The aq. layer of the hydrolysate was neutralized with ion-exchange resin (IRA-45) (*ca* 10 g) and evaporated to dryness. The residue was trimethylsilylated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA)–pyridine, and subjected to GLC to demonstrate the presence of glucose and arabinose in a molar ratio of 2 : 1.

Methanolysis of **1a**. Compound **1a** (30 mg) was dissolved in 6% HCl–dry MeOH (4 ml) (prepared from acetyl chloride and dry MeOH [5]) and refluxed for 3 hr. The mixture was neutralized with Ag_2CO_3, filtered, and evaporated to dryness. The residue was, after trimethylsilylation (BSTFA–pyridine), examined by GLC. Methyl glucoside and arabinoside (molar ratio 1 : 1) was characterized.

Alkaline hydrolysis of **2** *followed by methylation giving* **1a**. Compound **2** (1 g) in 20% KOH–MeOH (60 ml) was refluxed for 3 hr. The soln was poured into a large quantity of a 5% aq. HCl–ice mixture and extracted with *n*-BuOH–EtOAc (1 : 1). The organic layer was washed with H_2O, evaporated under red. pres. and dried *in vacuo*. The residue was dissolved in MeOH, treated with Dowex 50 W × 8 (*ca* 2 g), and methylated with CH_2N_2. The product was purified by prep. TLC [2 mm thickness; developed with $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower layer)] and recrystallized from MeOH to yield a methyl ester of a monodesmosyl diglycoside (802 mg) as colourless crystals of mp 240–243° (found: C, 61.90; H, 8.75. $C_{42}H_{68}O_{13}$. $2H_2O$ requires C, 61.74; H, 8.88%) which was identical with ilexside I methyl ester (**1a**) by mmp 240–243° and by comparison of $[\alpha]_D$ (MeOH), IR (KBr), FDMS, ^1H NMR (400 MHz; C_5D_5N), and TLC.

Ilexside I (**1**). Alkaline hydrolysis of ilexside II (**2**) followed by treatment with Dowex 50 W × 8 and trituration from MeOH–Et_2O gave an acidic diglycoside (**1**) as an amorphous ppt. IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1690 (COOH), 1636 (sh) (C=C), 1070 (Et_2O); FDMS *m/z* (rel. int.): 789 $[M+Na]^+$ (100). Several attempts at crystallization failed so that satisfactory data for the elemental analysis could not be obtained and prep. TLC purification resulted in formation of the corresponding carboxylate, IR ν_{max}^{KBr} cm^{-1}: 3400, 1636 (C=C), 1542 (COO^-), 1070; FDMS *m/z* (rel. int.): 789 $[M+Na]^+$ (100). Natural ilexside I has not been isolated but the specimen obtained here seems to be equivalent to it (identified by TLC). This specimen was the ilexside I (**1**) used for biological examination.

Enzymic hydrolysis of **2**. A mixture of **2** (500 mg) in acetate (Na^+) buffer (pH 4.0; 30 ml) was treated with mixed glycosidases from *Turbo cornutus* and incubated with stirring at 37° for 17 days. After addition of each 100 ml cold H_2O and *n*-BuOH–EtOAc (2 : 1), the resulting mixture was filtered. The aq. layer of the filtrate was further extracted with *n*-BuOH–EtOAc (2 : 1). The organic extracts were combined with the organic layer of the filtrate, and the soln was washed with H_2O, evaporated under red. pres. and dried *in vacuo*. TLC ($CHCl_3$–MeOH–H_2O, 12 : 3 : 1, lower phase) showed the presence of two prosapogenols. The product (290 mg) was subjected to prep. TLC (developed with the same solvent system as for TLC) and gave, from the less polar band (eluted with MeOH), a monoglucoside (**5**) (111 mg), colourless crystals, mp 279–280° (from EtOH), $[\alpha]_D^{20}$ + 17.8° (MeOH; *c* 0.11). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1723 (ester C=O), 1630 (C=C), 1070; FDMS *m/z* (rel. int.): 657 $[M+Na]^+$ (100), 639 $[(M+Na)-H_2O]^+$ (9.8), 495 $[(M+Na)-162]^+$ (11.8), 472 $[M-162]^+$ (2.9); MS *m/z* (rel. int.): 472.355 $[M-C_6H_{10}O_5]^+$, $C_{30}H_{48}O_4$ requires 472.355 (2.2), 454 (4.9), 429 (8), 264 (46.4), 246 (76.5), 201 (100) (found: C, 67.92; H 9.42. $C_{36}H_{58}O_9$ requires C, 68.11; H, 9.21%) and from the more polar band (eluted with MeOH), a bisdesmosyl diglycoside (129.5 mg), colourless needles, mp 255–258°, $[\alpha]_D^{20}$ + 16.7 (pyridine; *c* 2.3). FDMS *m/z* (rel. int.): 789 $[M+Na]^+$ (100), 657 $[(M+Na)-132]^+$ (13.7), 627 $[(M+Na)-162]^+$ (48), identified with the authentic sample of ziyuglycoside I (**6**) [3] by mmp 255–258°, $[\alpha]_D$ (pyridine), IR (KBr), and TLC.

Alkaline hydrolysis of **5** *followed by methylation to yield* **7**. A soln of **5** (29 mg) in 20% KOH–MeOH (7 ml) was heated under reflux for 1 hr. The soln was poured into 2% aq. HCl–ice, and extracted with Et_2O. The Et_2O was washed with H_2O, dried over $MgSO_4$ and methylated with excess CH_2N_2 to give colourless needles (20 mg), mp 126–128° (from MeOH), $[\alpha]_D^{20}$ + 40.5° ($CHCl_3$; *c* 0.36), agreed with authentic methyl pomolate (**7**) [4], mp 126–128°, by mmp and by comparison of $[\alpha]_D$ ($CHCl_3$), IR (KBr), MS, and TLC.

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