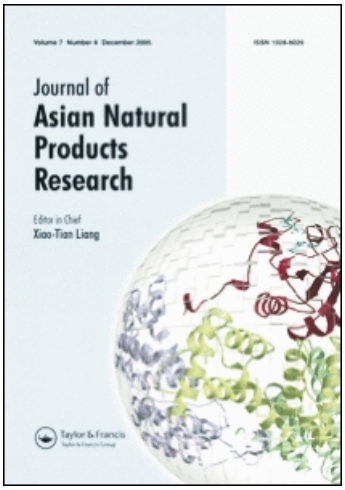

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Water-soluble constituents from the leaves of *Ilex oblonga*

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Water-soluble constituents from the leaves of *Ilex oblonga*

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Four new water-soluble constituents, oblongaroside A (**1**), oblongar ester A (**2**), oblongaroside B (**3**), and oblongaroside C (**4**), were isolated along with four known compounds: 4-*O*-β-D-glucopyranosyl-3-hydroxybenzalcohol (**5**), 7-methoxyl-4-*O*-β-D-glucopyranosyl-3-hydroxybenzalcohol (**6**), 4-*O*-β-D-glucopyranosyl-3-hydroxybenzoic acid (**7**), and 3,4-dihydroxybenzoic acid (**8**) from the leaves of *Ilex oblonga*. Identification of their structures was achieved by 1D and 2D NMR experiments, including $^{1}H-^{1}H$ COSY, NOESY, HMQC, and HMBC methods and FAB mass spectral data.

Keywords: *Ilex oblonga*; Aquifoliaceae; Hemiterpene; Hemiterpenoid glucoside; Phenolic glucosides

1. Introduction

In a continuation of our study on the constituents of the medicinal plants of the Aquifoliaceae family [1–8], we investigated the water-soluble fraction of *Ilex oblonga*. This plant is a well-known endemic herb to treat gumboils, eczema, rheumatism, scalds and bruises in Guangxi province of China [9]. No previous phytochemical investigation on *I. oblonga* has been reported. In genus *Ilex*, phytochemical studies mostly showed the presence of triterpenes, triterpenoid saponins, flavones and its glycosides in the leaves. Other components have rarely been isolated from it. In this paper, we describe the isolation and structure elucidation of a new hemiterpene, two new hemiterpenoid glucosides and a new phenolic ester glycoside designated as oblongaroside A (**1**), oblongar ester A (**2**), oblongaroside B (**3**), and oblongaroside C (**4**), along with four known phenolic acid and phenolic glycosides (**5**–**8**) from the leaves of *I. oblonga*.

2. Results and discussion

A water-soluble fraction of methanolic extract of the leaves from *I. oblonga* was subjected to Sephadex LH-20, RP-18, and silica gel column chromatography to afford eight water-soluble

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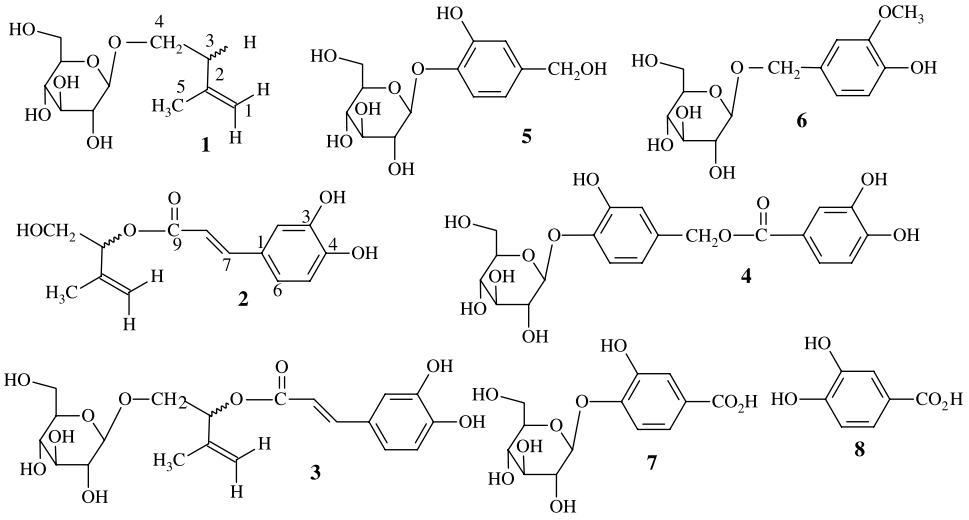

Figure 1. The structures of compounds **1**–**8**.

compounds (**1**–**8**) (see figure 1). The known 4-*O*-β-D-glucopyranosyl-3-hydroxy-benzalcohol (**5**) [10], 7-methoxyl-4-*O*-β-D-glucopyranosyl-3-hydroxy-benzalcohol (**6**) [11], 4-*O*-β-D-glucopyranosyl-3-hydroxybenzoic acid (**7**) [12], and 3,4-dihydroxy-benzoic acid (**8**) [13] were isolated. Their structures were identified by comparing their spectral data with the reported data.

Compound **1** was isolated as a colourless amorphous powder. Its negative FAB-MS showed a quasi-molecular ion peak at *m/z* 263 $[M - H]^-$. The molecular formula was established as $C_{11}H_{20}O_7$ by HRFAB-MS representing two unsaturations. The IR spectrum of **1** presented hydroxyl groups (3310 cm^{-1}) and double bond (1643, 3012 cm^{-1}). The ^1H NMR and ^{13}C NMR (see table 1) gave two olefinic proton signals (δ 5.02 and 4.88), an oxygenated methine signal (δ 4.24), two oxygenated methylene signals (δ3.97, 3.44), a methyl signal (δ 1.73), and an anomeric proton (δ 4.29). The ^{13}C NMR exhibited 11 carbon signals including six signals of a hexose, so the genin has only five carbon signals including one double bond (δ 145.5 and 112.6), one oxygenated methine (δ 75.4), one oxygenated methylene (δ 74.3), and one methyl (δ 19.0). These signals implied the presence of a hemiterpene moiety as the genin in compound **1**. Its ^1H NMR and ^{13}C NMR data (table 1) suggested the presence of a β-glucosyl moiety [anomeric proton at δ 4.29 ($J = 7.7$ Hz) and anomeric carbon at δ 104.6]. The acid hydrolysis of **1** afforded glucose, which was confirmed by HPTLC comparison with authentic samples. The HMBC spectra of **1** showed correlations between the two olefinic proton signals and the carbon signal at δ 75.4 (C-3), a methyl carbon signal at δ 19.0 (C-5), between the oxygenated methylene signals, the oxygenated methine signal, the methyl signal and the carbon signal at δ 145.5 (C-2), between the anomeric proton signal and the oxygenated methylene carbon signal at δ 74.3 (C-4) (see figure 2). These correlations led to the elucidation of a 3,4-dihydroxy-2-methyl-1-butene as the aglycone of **1** and the glucosylation position to be at C-4 of the aglycone. Thus the structure of **1** was established as 3-hydroxy-2-methyl-1-butene-4-*O*-β-D-glucopyranoside, named oblongaroside A.

Compound **2** exhibited absorptions of hydroxyl (3820 cm^{-1}), conjugated ester (1680 cm^{-1}), conjugated double bond (1617 cm^{-1}), and aromatic ring (1600 cm^{-1}) in the IR spectrum. Its negative FAB-MS showed a quasi-molecular ion peak at *m/z* 263 $[M - H]^-$.

Table 1. ^{1}H NMR and ^{13}C NMR data of compounds **1–3 (CD_3OD)**[†].

No.		***1***		***2***		***3***
1	112.6	5.02 (1H, br. S) 4.88 (1H, br. S)	112.9	5.05 (1H, br. s) 4.92 (1H, br. s)	113.7	5.03 (1H, br. s) 4.95 (1H, br. s)
2	145.5		145.7		142.6	
3	75.4	4.24 (1H, dd, $J = 9.1$, 6.0 Hz)	74.2	4.30 (1H, dd, $J = 7.3$, 4.0 Hz)	77.9	5.47 (1H, dd, $J = 7.7$, 3.0 Hz)
4	74.3	3.97 (1H, dd, $J = 10.0$, 6.0 Hz) 3.44 (1H, dd, $J = 10.0$, 9.1 Hz)	67.7	4.23 (1H, dd, $J = 11.3$, 4.0 Hz) 4.11 (1H, dd, $J = 11.3$, 7.3 Hz)	71.6	4.01(1H, dd, $J = 11.1$, 3.0 Hz) 3.80 (1H, dd, $J = 11.1$, 8.0 Hz)
5	19.0	1.73 (3H, s)	18.6	1.77 (3H, s)	19.4	1.79 (3H, s)
1′	104.6	4.29 (1H, d, $J = 7.7$ Hz)			104.9	4.34 (1H, d, $J = 7.7$ Hz)
2′	75.1	3.23 (1H, t-like, $J = 9.0$ Hz)			74.9	3.19 (1H, t-like, $J = 9.1$ Hz)
3′	77.7	3.38 (1H, t-like, $J = 9.0$ Hz)			77.7	3.35(1H, t-like, $J = 9.1$ Hz)
4′	71.4	3.30 (1H, t-like, $J = 9.0$ Hz)			71.5	3.29 (1H, t-like, $J = 9.0$ Hz)
5′	77.8	3.28 (1H, m)			77.9	3.27 (1H, m)
6′	62.6	3.85 (1H, br. d, $J = 11.8$ Hz) 3.66 (1H, dd, $J = 11.8$, 4.8 Hz)			62.8	3.87 (1H, br. d, $J = 11.5$ Hz) 3.66 (1H, dd, $J = 11.5$, 5.1 Hz)
1″			127.7		127.7	
2″			115.0	7.02 (1H, d, $J = 1.9$ Hz)	115.1	7.05 (1H, d, $J = 2.0$ Hz)
3″			146.8		146.8	
4″			149.6		149.6	
5″			116.5	6.76 (1H, d, $J = 8.2$ Hz)	116.6	6.70 (1H, d, $J = 8.1$ Hz)
6″			122.9	6.92 (1H, dd, $J = 8.2$, 1.9 Hz)	123.0	6.95 (1H, dd, $J = 8.1$, 2.0 Hz)
7″			115.2	6.26 (1H, d, $J = 15.8$ Hz)	115.1	6.30 (1H, d, $J = 15.9$ Hz)
8″			147.1	7.57 (1H, d, $J = 15.8$ Hz)	147.2	7.56 (1H, d, $J = 15.9$ Hz)
9″			169.1		168.6	

[†] Measured in CD_3OD at 400 MHz for ^{1}H and 100 MHz for ^{13}C.

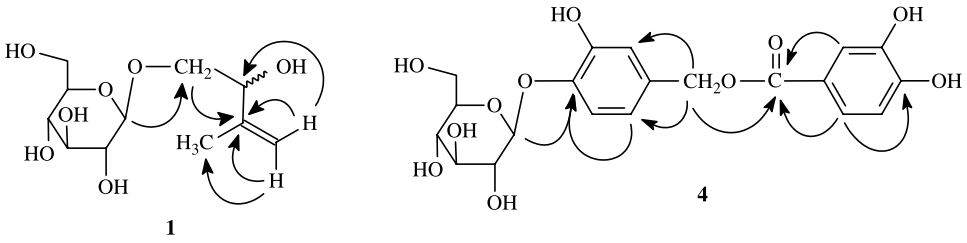

Figure 2. Selected HMBC correlations for compounds **1**, **4**.

The molecular formula was established as $C_{14}H_{16}O_5$ by HRFAB-MS representing seven unsaturations. Its ^{1}H NMR and ^{13}C NMR (see table 1) gave the typical signals of hemiterpene moiety (similar signals to **1**), an ABX spin system signals [δ7.02 (1H, d, $J = 1.9$ Hz), 6.92 (1H, dd, $J = 8.2$, 1.9 Hz), and 6.76 (1H, d, $J = 8.2$ Hz)] in the aromatic ring, and *trans*-conjugated double bond signals [δ 7.57 (1H, d, $J = 15.8$ Hz) and 6.26 (1H, d, $J = 15.8$ Hz)]. These signals indicated the presence of caffeoyl moiety. The HMBC spectra of **2** offered the key correlations between the carbon [δ 169.1 (C-9′)] and the oxygenated methine signal (δ 4.30), two olefinic proton signals (δ 7.57 and 6.26). These correlations indicated that the caffeoyl moiety should be linked at C-3 of the hemiterpene moiety. On the above spectroscopic evidence, **2** was concluded to be 3-*O*-caffeoyl-2-methyl-1-butene, named oblongar ester A.

Compound **3** was a derivative of **2**. The NMR signals of **3** were similar to those of **2** except for an additional sugar moiety (see table 1). The ^{1}H NMR and ^{13}C NMR data of **3** suggested the presence of a β-glucosyl moiety including the anomeric proton at δ 4.34 ($J = 7.7$ Hz) and anomeric carbon at δ 104.9. The HMBC spectra of **3** showed the key correlation between the anomeric proton (δ 4.34) and the methylene carbon (δ 71.6), this correlation indicated that the sugar should be linked at C-4 of the genin. So, compound **3** was identified as 3-*O*-caffeoyl-2-methyl-1-butene-4-*O*-β-D-glucopyranoside, named oblongaroside B.

Compound **4** was isolated as amorphous powder. Its negative FAB-MS showed a quasi-molecular ion peak at *m/z* 437 $[M - H]^-$. The molecular formula was established as $C_{20}H_{22}O_{11}$ by HRFAB-MS representing ten unsaturations. IR spectrum exhibited absorptions of hydroxyl (3338 cm^{-1}), conjugated carbonyl (1689 cm^{-1}) and aromatic ring (1602, 1595 cm^{-1}). The ^{1}H NMR gave two ABX spin system signals [δ 6.93 (1H, d, $J = 2.0$ Hz), 7.16 (1H, d, $J = 8.0$ Hz), 6.84 (1H, dd, $J = 8.0$, 2.0 Hz), and 7.44 (1H, d, $J = 2.0$ Hz), 6.79 (1H, d, $J = 8.1$ Hz), 7.42 (1H, dd, $J = 8.1$, 2.0 Hz)], two oxygenated methylene signals [δ 3.89 (1H, br. d, $J = 12.0$ Hz), 3.72 (1H, br. d, $J = 10.6$ Hz)], and anomeric proton signal [δ 4.78 (1H, d, $J = 7.3$ Hz)]. By detailed analysis of the ^{13}C NMR data, the signals attributed to 20 carbon signals including a carbonyl signal, 12 aromatic carbon signals, six signals of glucosyl moiety, and an oxygenated methylene signals (see table 2). Comparing the NMR data of **4** with those of **5** showed similar signals of 4-*O*-β-D-glucopyranosyl-3-hydroxybenzalcohol moiety, and additional signals of an aromatic ring and a carbonyl, indicating the presence of a 3,4-dihydroxy-benzoic acid. The HMBC spectra of **4** showed the key correlations between the H-1′ (δ 4.78) and C-4 (δ 148.3), between the H-7 (δ 5.15) and C-2 (δ 116.9), C-6 (δ 120.8), C-7″ (δ 168.1), between the H-6″ (δ 7.24) and C-7″ (δ 168.1), C-4″ (δ 151.7). These correlations indicated the sugar should be linked at C-4 and the ester was located at C-7. On the basis of the above results, the structure of **4** was

Table 2. ^{1}H NMR and ^{13}C NMR data of compounds **4–8 (CD_3OD)**.[†]

No.		***4***	***5** [10]*	***6** [11]*	***7** [12]*	***8** [13]*
1	133.3		138.4	134.9	128.5	123.2
2	116.9	6.93 (1H, d, $J = 2.0$ Hz)	115.9	116.8	116.5	115.8
3	146.6		146.0	146.3	147.0	146.0
4	148.3		148.3	148.3	150.7	151.5
5	118.6	7.16 (1H, d, $J = 8.0$ Hz)	118.8	118.7	118.1	117.7
6	120.8	6.84 (1H, dd, $J = 8.0$, 2.0 Hz)	119.6	120.6	125.7	123.9
7	67.0	5.15 (2H, s)	64.8	75.2	170.1	170.3
$-OCH_3$				58.0		
1′	104.1	4.78 (1H, d, $J = 7.3$ Hz)	104.4	104.3	104.4	
2′	74.8	3.30 (1H, t, $J = 8.9$ Hz)	74.8	74.8	74.9	
3′	77.5	3.53 (1H, t, $J = 8.9$ Hz)	77.5	77.6	77.7	
4′	71.2	3.51 (1H, overlap)	71.2	71.2	71.5	
5′	78.2	3.48 (1H, overlap)	78.2	78.2	78.4	
6′	62.3	3.89 (1H, br. d, $J = 12.0$ Hz)	62.4	62.4	62.5	
		3.72 (1H, br. d, $J = 10.6$ Hz)				
1″	122.6					
2″	118.6	7.44 (1H, d, $J = 2.0$ Hz)				
3″	146.1					
4″	151.7					
5″	115.9	6.79 (1H, d, $J = 8.1$ Hz)				
6″	123.7	7.42 (1H, dd, $J = 8.1$, 2.0 Hz)				
7″	168.1					

[†] Measured in CD_3OD at 400 MHz for ^{1}H and 100 MHz for ^{13}C.

established as 4-*O*-β-D-glucopyranosyl-3-hydroxy-benzalcohol-7-*O*-3,4-dihydroxy-benzoic ester, named oblongaroside C.

3. Experimental

3.1 General experimental procedures

IR spectra were recorded with a Perkin–Elmer 1750 FTIR spectrometer, the films of all the samples were measured on KBr disks. Optical rotations were measured with a Jasco DIP-180 digital polarimeter spectrophotometer. The ^{1}H, ^{13}C, DEPT, $^{1}H-^{1}H$ COSY, NOESY, HMQC and HMBC NMR spectra were performed using a Bruker AM-400 and a DRX-500 spectrometer. FAB mass spectra were recorded on a Jeol JMS-HX 110 instrument. Chromatographic stationary phase used RP-8 (40–60 μm, Merck), silica gel (160–200 mesh), Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemical Co. Ltd.) and MCI-gel CHP20P (75–150 μm, Mitsubishi Chemical Industries Ltd.). The following solvent systems were used: (a) $CHCl_3$/MeOH/H_2O (80:20:3), $CHCl_3$/MeOH/H_2O (70:30:5) and MeOH/H_2O (0–100%) for the glycosides; and (b) $CHCl_3$/MeOH/H_2O (7:3:1) lower-layer 9 ml + 1 ml HOAc for sugars. Compounds on TLC were detected by spraying with 5% H_2SO_4 followed by heating. Sugars were detected by spraying with aniline-phthalate reagent.

3.2 Plant material

The leaves of *Ilex oblonga* C.J. Tseng were collected at the Plant Garden of Guangxi Institute of Botany, Chinese Academy of Sciences in July 1999. A voucher specimen (No. 13523) is deposited in the Herbarium of Guangxi Institute of Botany. The plant was identified by Professor C.H. Li.

3.3 Extraction and isolation

The leaves of *I. oblonga* (690 g) were extracted (2 × 4 L) with MeOH at room temperature (7 days × 2). The extract was evaporated *in vacuo* to yield a residue, which was dissolved in water and filtered. The water-soluble fraction was passed through a Diaion column and eluted with water and methanol. Evaporation of the methanol eluate yielded 37 g of a brown fraction (A). The fraction A was subjected to dry column chromatography (DCC) on silica gel (1.0 kg), eluted with $CHCl_3$/MeOH/H_2O (10:2:0.2) to afford 13 fractions. Each fraction was purified by Sephadex LH-20, RP-8 gel column chromatography (solvent: MeOH/H_2O, 10–70%) and finally repeatedly purified by a silica gel column with $CHCl_3$/MeOH/H_2O (100:10:1–70:30:5) as solvent to yield **1** (18 mg), **2** (24 mg), **3** (41 mg), **4** (56 mg), **5** (44 mg), **6** (22 mg), **7** (75 mg), and **8** (35 mg).

3.3.1 Oblongaroside A (1). Colourless amorphous powder, FAB-MS *m/z* 263 $[M - H]^-$; HRFAB-MS *m/z* 263.1131 (calcd for $C_{11}H_{19}O_7$, 263.1131), $[\alpha]_D^{21} + 22$ (*c* 0.24, MeOH); IR ν_{max} (liquid) cm^{-1}: 3310, 3012, 2931, 1643, 1437, 1318, 1072, 895; ^1H NMR and ^{13}C NMR data: see table 1.

3.3.2 Oblongar ester A (2). Colourless amorphous powder, $[\alpha]_D^{21} + 11$ (*c* 0.12, MeOH); FAB-MS *m/z* 263 $[M - H]^-$; HRFAB-MS *m/z* 263.0919 $[M - H]^-$ (calcd for $C_{14}H_{15}O_5$, 263.0917); IR ν_{max} (liquid) cm^{-1}: 3280, 3012, 3004, 2927, 1680, 1641, 1617, 1600, 1470, 1410, 1348, 1002, 913; ^1H NMR and ^{13}C NMR: see table 1.

3.3.3 Oblongaroside B (3). Amorphous powder, $[\alpha]_D^{21} + 39$ (*c* 0.26, MeOH); FAB-MS *m/z* 425 $[M - H]^-$, 263 $[M - H - 162]^-$, 262 $[M - H - C_9H_7O_3]^-$; HRFAB-MS *m/z* 425.1445 (calcd for $C_{20}H_{25}O_{10}$, 425.1448); IR ν_{max} (liquid) cm^{-1}: 3320, 3010, 3005, 2921, 1682, 1640, 1615, 1605, 1510, 1430, 1378, 1032, 1000; ^1H NMR and ^{13}C NMR: see table 1.

3.3.4 Oblongaroside C (4). Amorphous powder, $[\alpha]_D^{21} - 12$ (*c* 0.63, MeOH); FAB-MS *m/z* 437 $[M - H]^-$, 301 $[M - H - C_7H_5O_3]^-$, and 275 $[M - H - 162]^-$; HRFAB-MS *m/z* 437.1082 (calcd for $C_{20}H_{21}O_{11}$, 437.1084); IR ν_{max} (liquid) cm^{-1}: 3338, 2910, 2856, 1689, 1602, 1595, 1470, 1380, 1078, 1036, 968; ^1H NMR and ^{13}C NMR: see table 2.

3.3.5 Acid hydrolysis. A solution of each compound (10 mg) was heated at reflux at 100°C in 2 M aqueous CF_3COOH (5 ml) in a water bath for 3 h. The reaction mixture was then diluted with H_2O (15 ml) and extracted with CH_2Cl_2 (3 × 5 ml). The combined CH_2Cl_2 extracts were washed with H_2O and then evaporated to dryness *in vacuo*. After evaporation to dryness of the aqueous layer then with MeOH until neutral, the sugars were analysed by comparison with an authentic sample (solvent system b) on silica gel HPTLC.

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