Two New 2-Phenylethyl Alcohol Derivatives and One New Lignan Derivative from the Root of *Ilex pubescens*

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The phytochemical investigation of the methanolic extract of the root of *Ilex pubescens* HOOK. et ARN. furnished six compounds including three new compounds, ilexin A (**1**), ilexin B (**2**), and ilexin C (**3**), besides vanillic acid, vanillin, and caffeic acid. Compounds **1** and **2** were identified as 2-phenylethyl alcohol derivatives, while compound **3** is a lignan derivative. The structures of these compounds have been elucidated by the combination of the analysis of NMR and MS data, CD spectra, and chemical evidences.

Introduction. – 'Mao-Dong-Qing', the dried root of *Ilex pubescens* HOOK. et ARN., which belongs to the family Aquifoliaceae, is a Chinese herbal medicine commonly used in Southern China for the treatment of cardiovascular diseases and hypercholestaemia. Previous chemical investigations have indicated the presence of triterpene saponins [1–4], lignan glucosides [5], and hemiterpene glycosides [6]. Pharmacological investigation demonstrated that extracts of 'Mao-Dong-Qing' not only enlarge blood vessels but also improve microcirculation, lower blood pressure, and inhibit platelet aggregation [6]. As our current interest lies in the study of medicinal uses of *Ilex pubescens* HOOK. *et* ARN., we carried out a phytochemical investigation on the root of the plant, which resulted in the isolation of three new compounds, ilexin A (**1**), ilexin B (**2**), and ilexin C (**3**) (see *Fig. 1*)[1]), as well as three known compounds.

Results and Discussion. – Compound **1** was obtained as colorless solid and showed a positive reaction by spraying the thin-layer chromatography (TLC) plate with a $FeCl_3$/EtOH reagent. Acid hydrolysis yielded D-glucose, which was identified by GC analysis. The HR-ESI-MS of **1** suggested the molecular formula of $C_{25}H_{34}O_{10}$. Its NMR (*Table 1*) data together with HSQC spectra suggested the presence of a 4-hydroxyphenyl moiety [7], a β-glucose moiety (anomeric H-atom at δ(H) 4.30 (d, J = 7.8, 1 H) and corresponding C-atom at δ(C) 103.4), an EtO group (δ(H) 3.97 (q, J = 7.2, 2 H), 1.12 (t, J = 7.2, 3 H), δ(C) 59.9, 14.2), a CHO group (δ(H) 9.29 (s, 1 H), δ(C) 195.8), a Me group connected with an olefinic CH group (δ(H) 1.94 (d, J = 6.9, 3 H), 6.80 (q,

[1]) Arbitrary numbering. For systematic names, see *Exper. Part.*

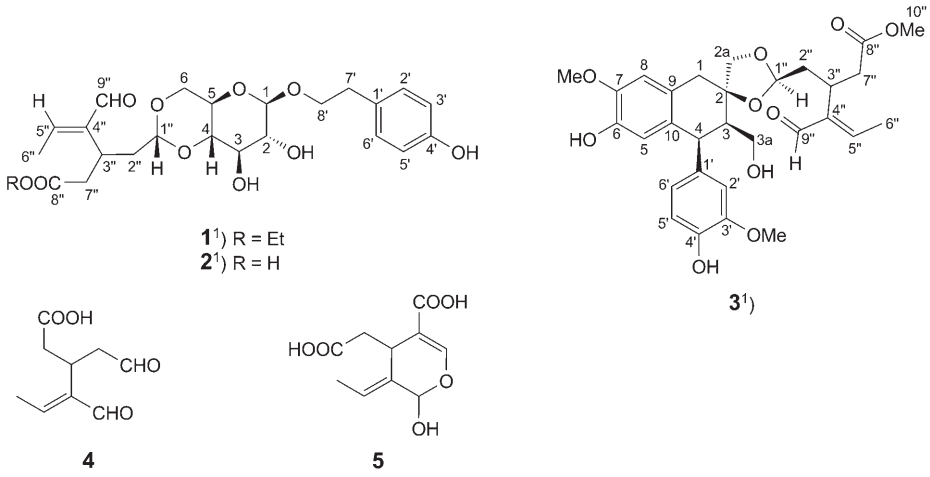
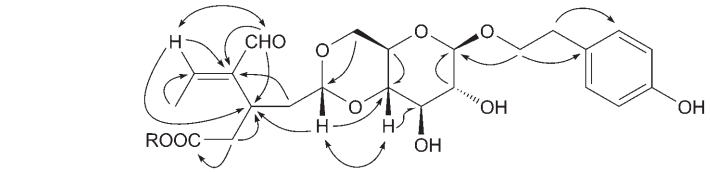


Fig. 1. *Structures of compounds* **1**–**3**, **4**, *and* **5**

$J = 6.9$, 1 H), δ(C) 14.8, 154.3) and a CO group (δ(C) 171.6). The ^{1}H- and ^{13}C-NMR signals (*Table 1*) were assigned with the aid of HSQC and HMBC spectra. The ^{1}H,^{13}C long-range correlations (*Fig. 2*) of H–C(8′)[1]) with C(1) and C(1′), and H–C(7′) with C(2′) led to the elucidation of a salidroside moiety [7]. The presence of a 4-formylhex-4-enediol moiety was confirmed by HMBC correlations (H–C(9″), H–C(5″)/C(4″); Me(6″), H–C(9″)/C(5″); H–C(5″), H–C(9″), H–C(1″)/C(3″); H–C(2″)/C(4″)). In the HMBC spectrum, the cross-peaks *Me*CH_2O/C(8″) and H–C(7″)/C(8″) showed the presence of an acetic acid ethyl ester moiety, the cross-peaks H–C(7″)/C(3″) and H–C(7″)/C(4″) indicated that C(7″) was connected with C(3″), and the cross-peaks H–C(6)/C(1″) and H–C(1″)/C(4) revealed that C(4) and C(6) were substituted by the ethyl 3-(2,2-dihydroxyethyl)-4-formylhex-4-enoate moiety (*Fig. 1*)[1]). With the aid of the NOESY correlation of H–C(1″) with H–C(4) of the D-glucose residue, the absolute configuration of **1** was established to be (1″*R*)[1]). In the NOESY spectrum, the cross-peak between H–C(5″) and H–C(9″) confirmed the (*E*)-configured C=C bond. Thus the structure of **1** was determined as 2-(4-hydroxyphenyl)ethyl 4,6-*O*-[(1*R*,4*E*)-3-(2-ethoxy-2-oxoethyl)-4-formylhex-4-en-1-ylidene]-β-D-glucopyranoside, as shown in *Fig. 1*, and named ilexin A.

Compound **2** was obtained as a colorless solid and responded positively to the $FeCl_3$ spraying reagent for TLC. Acid hydrolysis yielded D-glucose, which was identified by GC analysis. The molecular formula of $C_{23}H_{30}O_{10}$ was deduced from the HR-ESI-MS spectrum, which suggested that **2** is a homolog of **1**. By comparing the NMR spectral data with those of **1** (*Table 1*), the disappearance of the EtO signals were observed, suggesting that **2** is a carboxylic acid congener of **1**. The HMBC and NOESY experiments provided further evidence for this conclusion. With the NOESY data, the structure of **2** was confirmed as 2-(4-hydroxyphenyl)ethyl 4,6-*O*-[(1*R*,4*E*)-3-(carboxymethyl)-4-formylhex-4-en-1-ylidene]-β-D-glucopyranoside as shown in *Fig. 1* and named ilexin B.



1 R = Et
2 R = H

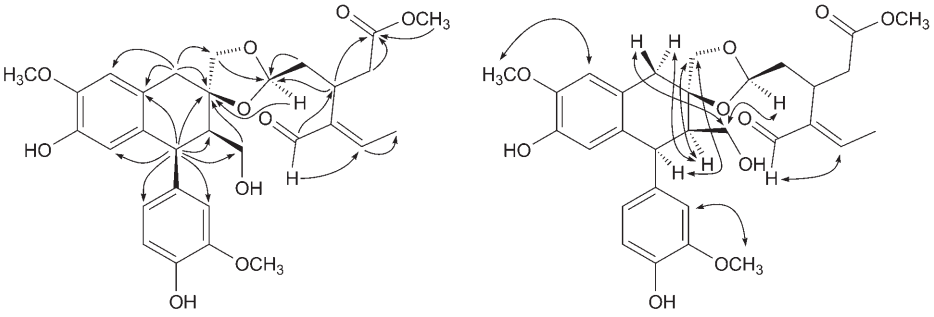


Fig. 2. *Key correlations of compounds* **1**–**3**

Compound **3** was obtained as a white amorphous solid. The HR-ESI-MS indicated the molecular formula of **3** to be $C_{30}H_{36}O_{10}$. The NMR spectra (*Table 2*) of **3** showed the presence of a 1,3,4-trisubstituted benzene moiety [3], a 1,2,4,5-tetrasubstituted benzene moiety [8], three MeO groups (δ(H) 3.72 (*s*), 3.68 (*s*), 3.50 (*s*), δ(C) 55.5, 55.7, 51.3), a CHO group (δ(H) 9.24 (*s*), δ(C) 195.6), a Me group connected with an olefinic CH group (δ(H) 1.85 (*d*, $J = 6.9$, 3 H), 6.75 (*q*, $J = 6.9$, 1 H), δ(C) 14.7, 153.7), and a CO group (δ(C) 172.1). The ^{1}H- and ^{13}C-NMR signals (*Table 2*) were assigned with the aid of HSQC and HMBC spectra. From the cross-peaks observed in the HMBC spectrum (H–C(4)/C(9), C(10), C(5), C(1′), C(2′), C(6′), C(2), C(3), and C(3a); H–C(1)/C(9), C(8), C(10), C(2), C(2a), and C(3); H–C(3)/C(10), C(1′), C(2a), C(2), and C(3a)) (*Fig. 2*), it was inferred that **3** possessed a structure derived of cycloolivil [8]. In the NOESY spectrum, the cross-peak of MeO–C(7) with H–C(8) as well as MeO–C(3′) with H–C(2′) indicated the location of two MeO groups. In the HMBC experiment, long-range correlations were observed between H–C(2a) and C(1″); H–C(3″) and C(4″), C(7″), C(8″), C(5″), and C(1″); H–C(1″) and C(2″), C(3″), and C(2); COO*Me* and C(8″), which revealed that C(2) and C(2a) were substituted by a methyl 3-(2,2-dihydroxyethyl)-4-formylhex-4-enoate moiety, which is similar to **1**. The absolute configuration at C(4) could be determined as (*S*) from the CD spectrum [9]

Table 1. *^1H-* (600 MHz in (D_6)DMSO) *and ^{13}C-NMR* (150 MHz in (D_6)DMSO) *Data for* **1** *and* **2**[1]). δ in ppm, J in Hz.

	1		**2**	
	δ(C)	δ(H)	δ(C)	δ(H)
1	103.4	4.30 (d, $J=7.8$)	103.4	4.30 (d, $J=7.8$)
2	74.4	2.97–3.00 (m)	74.4	2.98–3.01 (m)
3	73.0	3.27–3.30 (m)	73.0	3.29–3.32 (m)
4	80.3	3.04–3.06 (m)	80.3	3.03–3.05 (m)
5	65.9	3.13–3.16 (m)	65.9	3.15–3.18 (m)
6	67.5	3.98–4.02 (m, H_a), 3.30–3.36 (m, H_b)	67.5	4.00–4.04 (m, H_a), 3.32–3.38 (m, H_b)
1′	128.5		128.5	
2′, 6′	129.8	7.01 (d, $J=8.4$)	129.8	7.01 (d, $J=8.4$)
3′, 5′	115.1	6.65 (d, $J=8.4$)	115.1	6.65 (d, $J=8.4$)
4′	155.8		155.8	
7′	34.9	2.68–2.72 (m)	34.9	2.68–2.72 (m)
8′	70.4	3.76–3.83 (m, H_a), 3.55–3.61 (m, H_b)	70.3	3.75–3.82 (m, H_a), 3.53–3.59 (m, H_b)
1″	101.1	4.26–4.28 (m)	100.4	4.25–4.27 (m)
2″	36.0	1.94–1.96 (m, H_a), 1.67–1.72 (m, H_b)	36.1	1.92–1.94 (m, H_a), 1.69–1.74 (m, H_b)
3″	28.9	3.16–3.21 (m)	29.0	3.15–3.20 (m)
4″	143.0		143.8	
5″	154.3	6.80 (q, $J=6.9$)	153.8	6.75 (q, $J=6.6$)
6″	14.8	1.94 (d, $J=6.9$)	14.8	1.94 (d, $J=6.6$)
7″	37.1	2.48–2.65 (m)	38.1	2.40–2.47 (m)
8″	171.6		172.7	
9″	195.8	9.29 (s)	195.8	9.29 (s)
MeCH_2O	59.9	3.97 (q, $J=7.2$)		
MeCH$_2$O	14.2	1.12 (t, $J=7.2$)		

(*Fig. 3*). The *cis*-orientation of H–C(3) and H–C(4) to each other was established from the coupling constant of H–C(4) ($J=6.5$ Hz) by comparison with the values

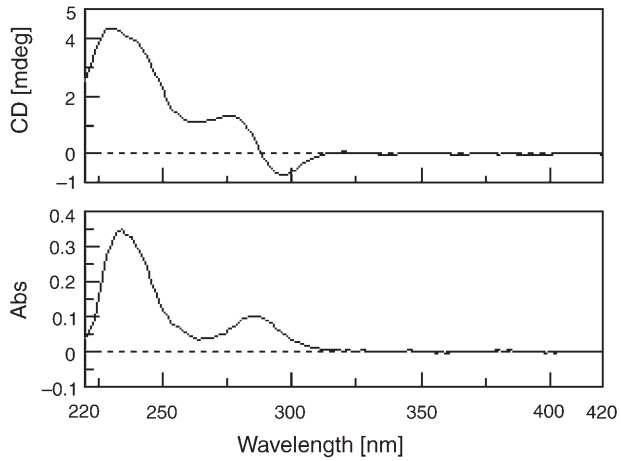


Fig. 3. *CD and UV Curves of* **3**

Table 2. *^1H-* (600 MHz in (D_6)DMSO) *and ^{13}C-NMR* (150 MHz in (D_6)DMSO) *Data for* **3**[1]). δ in ppm, J in Hz.

	3	
	δ(C)	δ(H)
1	39.5	2.83 (*d*, $J = 16.2$, H_α), 2.64 (*d*, $J = 16.2$, H_β)
2	80.5	
3	50.7	2.08–2.10 (*m*)
4	44.9	3.93 (*d*, $J = 6.5$)
5	116.7	6.18 (*s*)
6	144.7	
7	146.2	
8	112.1	6.60 (*s*)
9	125.0	
10	129.3	
2a	73.7	3.51–3.54 (*m*)
3a	60.1	3.62–3.65 (*m*), 3.23–3.25 (*m*)
1′	137.1	
2′	113.0	6.61 (br. *s*)
3′	147.5	
4′	144.9	
5′	115.1	6.64 (*d*, $J = 8.0$)
6′	121.3	6.33 (*d*, $J = 8.0$)
1″	102.8	4.74–4.75 (*m*)
2″	37.0	1.73–1.75 (*m*), 1.86–1.88 (*m*)
3″	29.0	3.14–3.16 (*m*)
4″	143.5	
5″	153.7	6.73 (*q*, $J = 6.9$)
6″	14.7	1.85 (*d*, $J = 6.9$)
7″	37.0	2.60–2.61 (*m*), 2.55–2.56 (*m*)
8″	172.1	
9″	195.6	9.24 (*s*)
*Me*O–CO	51.3	3.50 (*s*)
*Me*O–C(7)	55.5	3.72 (*s*)
*Me*O–C(3′)	55.7	3.68 (*s*)

reported for cycloolivil (*trans*-orientation, $J = 10.0$ Hz) [8] and di-*O*-methyl epicycloolivil (*cis*-orientation, $J = 5.5$ Hz) [10]. In addition, the observed NOE correlations (*Fig. 2*) of H–C(5″) with H–C(9″), H–C(4) with H–C(2a), and H–C(1″) with H–C(3a) confirmed the (*E*)-configuration of the C=C bond and established the absolute configuration of compound **3** as (1″*R*,2*R*,3*R*,4*S*)[1]). Based on the above results, the complete structure of compound **3** was determined as methyl (4*E*)-3-{[(2*R*,3′*R*,4*R*,4′*S*)-3′,4′-dihydro-6′-hydroxy-4′-(4-hydroxy-3-methoxyphenyl)-3′-(hydroxymethyl)-7′-methoxyspiro[1,3-dioxolane-4,2′(1′*H*)-naphthalen]-2-yl]methyl}-4-formylhex-4-enoate, as shown in *Fig. 1*, and named ilexin C.

The known compounds were determined to be vanillic acid [11], vanillin [12], and caffeic acid [13] by physical and spectroscopic evidence, and confirmed by comparison with the literature data.

The new compounds consist of two moieties each which are connected by an acetal linkage. Compounds containing a similar acetal structure have been isolated from other plants before, such as protosappanin E 2 [14], korolkoside [15], and fritillebinide D [16]. The (4*E*)-4-formyl-3-(2-oxoethyl)hex-4-enoic acid (**4**) moiety is included in all the new compounds isolated in this work. This acid may be biogenetically derived from the secoiridoid **5** [17] by opening of the dihydropyrane ring, conversion to the dialdehyde and decarboxylation of the β-oxo acid group.

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Experimental Part

General. GC: *FL-9790* Apparatus, *OV-17* (30 m × 0.32 mm) column. Column chromatography (CC): Silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Group, Co.*), or *ODS* (30–50 μm; *YMC CO. Ltd. Japan*). Prep. HPLC: *Hitachi Pump L-7110*, with a *Hitachi L-7420 UV* spectrophotometric detector at 210 nm, and a *TEDAchrom YWG C_{18}* reversed phase column (10 μm, 20 × 250 mm, flow rate: 4 ml/min). Optical rotation: *Perkin-Elmer 24 IMC* polarimeter. UV Spectra: *Shimadzu UV-260* spectrometer; in λ_{max} (log ε). CD Spectra: *JASCO CD-2095-plus.* NMR Spectra: *Bruker AV-600* spectrometer and *Bruker ARX-300* spectrometer, TMS as internal standard, δ in ppm, *J* in Hz. ESI-MS: *Finnigan LCQ* mass spectrometer. HR-ESI-MS: *QSTAR LCQ* mass spectrometer.

Plant Material. The roots of *Ilex pubescens* HOOK. et ARN. were collected in *Weikang Pharmaceutical Company* (Shenyang, P. R. China) in October 2006 and identified by Prof. *Qishi Sun*, Shenyang Pharmaceutical University. A voucher specimen (No. 20061009) was deposited with the Research Department of Natural Medicine, Shenyang Pharmaceutical University.

Extraction and Isolation. The air-dried roots (5 kg) of *Ilex pubescens* were extracted with MeOH (25 l) at r.t. for 7 d. The extract was concentrated under reduced pressure to yield a MeOH extract (150 g). The extract was partitioned between AcOEt (3 × 3 l) and H_2O (3 l) to give an AcOEt extract (100 g). A portion of the AcOEt extract (80 g) was chromatographed over a SiO_2 column (400 g), gradiently eluting with $CHCl_3$/acetone to afford *Fr. A* ($CHCl_3$), *Fr. B* ($CHCl_3$/acetone 100:10), and *Fr. C* ($CHCl_3$/acetone 100:15). *Fr. A* was rechromatographed over a SiO_2 column by eluting with petroleum ether (PE)/acetone (100:2) to yield vanillin (20 mg), and with PE/acetone (100:6) to yield vanillic acid (25 mg). The solid, which precipitated from *Fr. B* was filtered off and then purified by recrystallization from MeOH to give caffeic acid (15 mg). *Fr. C* was rechromatographed over an *ODS* column, eluting with 20% MeOH to give *Fr. C1*, and 40% MeOH to give *Fr. C2*. *Fr. C1* was then subjected to prep. RP-HPLC (38% MeOH) to yield **2** (13 mg, t_R = 50 min). *Fr. C2* was also subjected to prep. RP-HPLC (45% MeOH) to yield **1** (10 mg, t_R = 65 min) and **3** (13 mg, t_R = 58 min).

Acid Hydrolysis and GC Analysis of Compounds **1** *and* **2**. The procedure of acid hydrolysis and GC analysis was as reported by *Shuuji Hara et al.* [18]. The diastereoisomers, which were prepared by converting D-glucose and L-glucose to the trimethylsilyl (TMS) ethers of the corresponding methyl 2-(polyhydroxyalkyl)thiazolidine-(4*R*)-carboxylates, were clearly separated, and t_R was 17.26 min for the D-glucose derivative and 18.65 min for the L-glucose derivative. In the acid hydrolysate of **1** and **2**, D-glucoses were confirmed by the retention times of their trimethylsilylated thiazolidine derivatives, which had t_R of 17.32 and 17.35 min, resp.

Ilexin A (=*2-(4-Hydroxyphenyl)ethyl 4,6*-O-*[(1*R,*4*E*)*-3-(2-Ethoxy-2-oxoethyl)-4-formylhex-4-en-1-ylidene]-β-*D*-glucopyranoside*; **1**). Colorless solid. $[\alpha]_D^{20} = -31.89$ (c = 0.013, MeOH). UV (MeOH): 225 (4.32), 278 (3.33). IR (KBr): 3409, 2875, 1728, 1678, 1516, 1444, 1383, 1231, 1089, 1027, 831. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS: 517.4 ($[M+Na]^+$), 493.5 ($[M-H]^-$). HR-ESI-MS: 517.2047 ($[M+Na]^+$, $C_{25}H_{34}NaO_{10}^+$; calc. 517.2051).

Ilexin B (=*2-(4-Hydroxyphenyl)ethyl 4,6-O-[(1*R,*4*E*)-3-(Carboxymethyl)-4-formylhex-4-en-1-ylidene]-β-*D*-glucopyranoside*; **2**). Colorless solid. $[\alpha]_D^{20} = -18.42$ ($c = 0.006$, MeOH). UV (MeOH): 226 (4.13), 279 (3.22). IR (KBr): 3421, 2876, 1717, 1678, 1516, 1442, 1384, 1231, 1085, 1025, 828. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS: 489.5 ($[M+Na]^+$), 465.4 ($[M-H]^-$). HR-ESI-MS: 489.1698 ($[M+Na]^+$, $C_{23}H_{30}NaO_{10}^+$; calc. 489.1735).

Ilexin C (=*Methyl (4*E*)-3-[[(2*R,*3′*R,*4*R,*4′*S*)-3′,4′-Dihydro-6′-hydroxy-4′-(4-hydroxy-3-methoxyphenyl)-3′-(hydroxymethyl)-7′-methoxyspiro[1,3-dioxolane-4,2′(1′*H*)-naphthalen]-2-yl]methyl]-4-formylhex-4-enoate*; **3**). White amorphous solid. $[\alpha]_D^{20} = +3.61$ ($c = 0.008$, MeOH). UV (MeOH): 284 (3.68), 231 (4.23). CD ($c = 1.56 \cdot 10^{-5}$, MeOH): 223 (+4.35), 277 (+1.25), 298 (−0.76). IR (KBr): 3424, 2938, 1732, 1678, 1513, 1436, 1391, 1273, 1140, 1028, 824, 764. ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS: 579.5 ($[M+Na]^+$), 555.4 ($[M-H]^-$). HR-ESI-MS: 579.2215 ($[M+Na]^+$, $C_{30}H_{36}NaO_{10}^+$; calc. 579.2205).

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