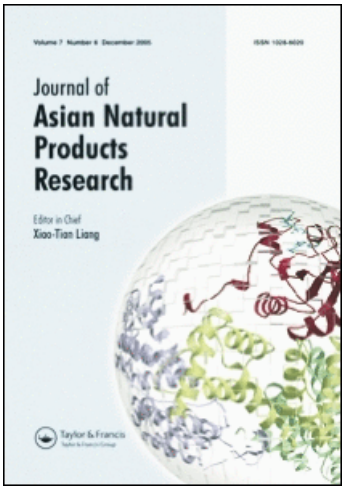

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Studies on the chemical constituents of *Ilex pubescens*

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Three new compounds, ilexisochromane (**1**), ilex acid A (**2**), and ilex acid B (**3**), were isolated from the roots of *Ilex pubescens*. Their structures were elucidated using the combination of 1- and 2-D NMR and mass spectrometry analyses.

Keywords: *Ilex pubescens*; ilexisochromane; ilex acid A; ilex acid B

1. Introduction

In China, 'Mao-Dong-Qing', the dried roots of *Ilex pubescens* Hook. *et* Arn., is widely used for the treatment of cardiovascular diseases and hypercholesterolemia. Previous chemical investigations indicated the presence of triterpene saponins [1–3] and simple phenolics [4] in Mao-Dong-Qing. Pharmacological investigation demonstrated that the extracts of Mao-Dong-Qing not only dilate blood vessels but also improve minicirculation, lower blood pressure, inhibit platelet aggregation, prevent thrombosis, reduce cardiac ischemia, decrease the excitation of the cardiac conduction system, and enhance anoxia resistance [5]. As our current interest is in the medicinal uses of *I. pubescens* Hook. *et* Arn., we also carried out a phytochemical investigation on the roots of *I. pubescens* Hook. *et* Arn., which resulted in three new compounds: ilexisochromane (**1**), ilex acid A (**2**), and ilex acid B (**3**). This paper deals with the isolation and structural elucidation of the new constituents on the basis of extensive studies of their MS and 1D and 2D NMR spectral data.

2. Results and discussion

Compound **1** was obtained as a colorless solid, $[\alpha]_D^{20} + 11.2$ ($c = 0.015$, MeOH), that responded positively to $FeCl_3$ reagent. The HRESIMS indicated the molecular formula of **1** to be $C_{18}H_{22}O_6$. The NMR spectra (Table 1) of **1** suggested the presence of a 1,2,4,5-tetrasubstituted benzene moiety [6], a methoxyl group [δ_H 3.51 (3H, s), δ_C 51.2], an aldehyde group [δ_H 9.20 (1H, s), δ_C 195.6], a methyl group connected with an olefinic methine group [δ_H 1.87 (3H, d, $J = 6.9$ Hz), 6.63 (1H, q, $J = 6.9$ Hz), δ_C 14.6, 152.3], and a carbonyl group (δ_C 172.4). The $^1H-^{13}C$ long-range correlations (Figure 2) of H-1 with C-9, C-10, and C-3, H-3a, 3b with C-10, and H-4a, 4b with C-3, C-5, C-9, and C-10 led to the elucidation of an isochromane structure [6]. In the $^1H-^1H$ COSY spectrum, H-11a and H-11b showed correlations with H-1 and H-12, and H-14 showed correlation with H-15. Together

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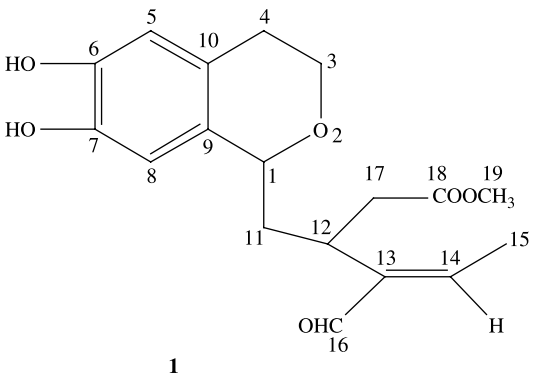
with $^{1}H-^{13}C$ long-range correlations between H-12 and C-13 and C-14, H-16, and C-13, C-14, it was possible to confirm that C-1 was substituted by the 3-formyl-3-pentenyl moiety. The presence of methoxycarbonylmethyl moiety was also showed by HMBC correlation: H-17 and H-19 were correlated with C-18. The observed $^{1}H-^{1}H$ COSY correlation between H-12 and H-17 indicated that C-17 was connected with C-12. The double bond was confirmed to be of E-configuration with the aid of NOE correlation between H-14 and H-16. Thus, the structure of compound **1**, named ilexisochromane, was determined as shown in Figure 1.

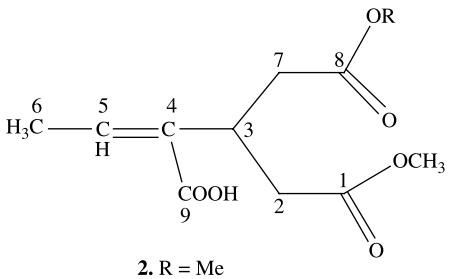
Compound **2** was obtained as a colorless solid, $[\alpha]_D^{20} - 7.5$ ($c = 0.013$, MeOH). The HRESIMS indicated the molecular formula of **2** to be $C_{11}H_{16}O_6$. The ^{13}C NMR and DEPT spectra showed eight signals for two methyls, one methylene, two methines, and three quaternary carbons, implying that three signals overlapped due to symmetry. The ^{1}H

Table 1. ^{1}H and ^{13}C NMR (600 MHz for ^{1}H and 150 MHz for ^{13}C in DMSO-d_6) spectral data for compound **1**.

	1	
Position	δ_C	δ_H (J in Hz)
1	73.2	4.56 brd (6.9)
2		
3	60.5	3.90 m, 3.55 m
4	27.0	2.63 m, 2.44 m
5	111.8	6.35 s
6	143.9	
7	143.7	
8	115.5	6.42 s
9	128.1	
10	124.0	
11	38.2	2.10 m, 1.72 m
12	29.4	3.28 m
13	145.1	
14	152.3	6.63 q (7.2)
15	14.6	1.87 d (7.2)
16	195.6	9.22 s
17	36.4	2.70 m
18	172.4	
19	51.2	3.51 s



1



2. R = Me
3. R = H

Figure 1. Structures of compounds **1**–**3**.

Table 2. ^{1}H and ^{13}C NMR (600 MHz for ^{1}H and 150 MHz for ^{13}C in DMSO-d_6) spectral data for compounds **2** and **3**.

	2		**3**	
Position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	172.1		172.2	
2	37.3	a: 2.71 dd (15.3, 8.7) b: 2.60 dd (15.3,6.0)	37.6	2.60 m
3	30.8	3.52 m	31.0	3.45 m
4	133.1		134.7	
5	139.4	6.80 q (6.9)	137.3	6.63 q (7.2)
6	13.9	1.79 d (6.9)	13.8	1.76 d (7.2)
7	37.3	a: 2.71 dd (15.3, 8.7) b: 2.60 dd (15.3,6.0)	38.4	2.60 m
8	172.1		173.5	
9	167.7		168.5	
1-OCH_3	51.3	3.54 s	51.3	3.54 s
8-OCH_3	51.3	3.54 s		

NMR spectrum of **2** showed the presence of two methoxyls [δ_H 3.54 (6H, s, 1- and 8-OCH_3)] and a methyl connected with an olefinic methine group [δ_H 1.79 (3H, d, $J = 6.9$ Hz, 6-H), 6.80 (1H, q, $J = 6.9$ Hz, 5-H)]. The ^{1}H and ^{13}C NMR signals (Table 2) were assigned with the aid of HSQC and HMBC spectra. In the $^{1}H-^{1}H$ COSY spectrum, H-5 showed correlation with H-6, and H-2 showed correlation with H-3. The presence of a 4-carboxyl-4-hexenoic acid methyl ester was confirmed by the $^{1}H-^{13}C$ long-range correlations between H-5 and C-3, C-9, H-3 and C-1, C-9, and 1-OCH_3 and C-1. There was another methoxycarbonylmethyl moiety connected with C-3, which was determined by HMBC correlations: H-7a, 7b were associated with C-2, C-3, C-4, and C-8, and 8-OCH_3 was correlated with C-8. Thus, the structure of compound **2**, named ilex acid A, was determined as shown in Figure 1.

Compound **3** was also obtained as a colorless solid, $[\alpha]_D^{20} - 8.3$ ($c = 0.011$, MeOH). Its molecular formula was deduced using HRESIMS to be $C_{10}H_{14}O_6$, which demonstrated that it is a homolog of **2**. The ^{13}C NMR spectra showed 10 signals, implying the disappearance of symmetry. By comparing the NMR spectral data of **3** with those of **2** (Table 2), the disappearance of methoxyl proton and carbon signals was confirmed. The HMBC experiment provided further evidence for this conclusion. The structure of compound **3**, named ilex acid B, was determined as shown in Figure 1.

3. Experimental

3.1 General experimental procedures

NMR spectra were recorded on a Bruker ARX 300 or an AV600 spectrometer, using TMS as an internal standard. ESIMS were performed on a Finnigan LCQ spectrometer. HRESIMS were performed on a QSTAR–LCQ mass spectrometer. Preparative HPLC was carried out on a Hitachi L-7420 UV–vis spectrophotometric detector at 210 nm and TEDA-chrom YWG C_{18} reversed phase column (250 mm × 20 mm, i.d. 10 μm). Silica gel for chromatography was supplied by the Qingdao Ocean Chemical Group Co. Ltd, Qingdao, China, and ODS (50 μm) for chromatography was purchased from YMC Co. Ltd, Kyoto, Japan.

3.2 Plant material

The plant material was collected in Weikang Pharmaceutical Co. (Shenyang, China)

in October 2006 and was identified by Professor Qishi Sun (Shenyang Pharmaceutical University). A voucher specimen (No. 20061009) has been deposited in the School of Chinese Medicine, Shenyang Pharmaceutical University in China.

3.3 Extraction and isolation

The air-dried roots (5 kg) of *I. pubescens* were extracted with MeOH (25 l) at room temperature for 7 days and then concentrated under reduced pressure to yield a MeOH extract (150 g). The extract was partitioned between EtOAc and water to give an EtOAc extract (100 g). The EtOAc extract (80 g) was chromatographed over a silica gel column (400 g) to yield fraction A ($CHCl_3$—CH_3COCH_3 100:10) and fraction B ($CHCl_3$—CH_3COCH_3 100:15). Fraction A was rechromatographed over a polyamide column eluting with $CHCl_3$ to give fraction A-1, and fraction A-1 was then subjected to preparative RP-HPLC (45% MeOH) to yield **1** (15 mg, $t_R = 70$ min). Fraction B was rechromatographed over an ODS column eluting with 10 and 20% MeOH to give fraction B-1 and fraction B-2, respectively. Fraction B-1 was then subjected to preparative RP-HPLC (22% MeOH) to yield **3** (13 mg, $t_R = 65$ min). Fraction B-2 was also subjected to preparative RP-HPLC (43% MeOH) to yield **2** (10 mg, $t_R = 50$ min).

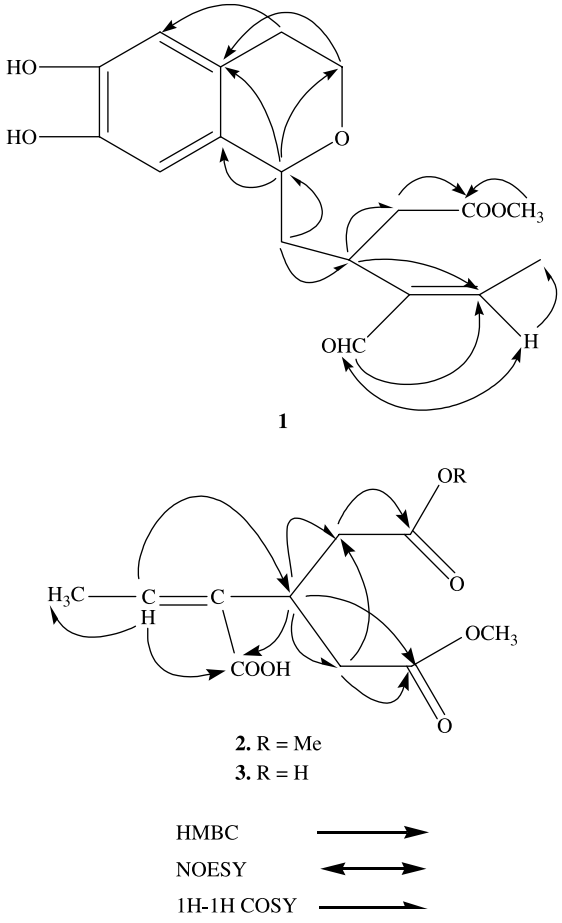


Figure 2. Important correlations of compounds **1**–**3**.

*3.3.1 Compound **1***

White amorphous solid, $[\alpha]_D^{20} + 11.2$ ($c = 0.015$, MeOH); ESIMS: *m/z* 357.4 $[M + Na]^+$ and 333.4 $[M - H]^-$; HRESIMS: *m/z* 334.1504 $[M]^+$ (calcd for $C_{18}H_{22}O_6$, 334.1416); for NMR spectra, see Table 1.

*3.3.2 Compound **2***

Colorless solid, $[\alpha]_D^{20} - 7.5$ ($c = 0.013$, MeOH); ESIMS: *m/z* 267.3 $[M + Na]^+$ and 243.3 $[M - H]^-$; HRESIMS: *m/z* 267.0853 $[M + Na]^+$ (calcd for $C_{11}H_{16}O_6Na$, 267.0845); for NMR spectra, see Table 2.

*3.3.3 Compound **3***

Colorless solid, $[\alpha]_D^{20} - 8.3$ ($c = 0.011$, MeOH); ESIMS: *m/z* 253.4 $[M + Na]^+$ and 229.2 $[M - H]^-$; HRESIMS: *m/z* 253.0692 $[M + Na]^+$ (calcd for $C_{10}H_{14}O_6$ Na, 253.0688); for NMR spectra, see Table 2.

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