Triterpene Saponins from the Leaves of *Ilex kudingcha*

Li Tang,[†] Yong Jiang,[†] Hai-Tao Chang,[†] Ming-Bo Zhao,[†] Peng-Fei Tu,*[,†] Jing-Rong Cui,[‡] and Rui-Qing Wang[‡]

School of Pharmaceutical Sciences, Peking University Health Science Center, Beijing 100083, People's Republic of China, and The State Key Laboratory of Natural and Biomimetic Drugs, Beijing 100083, People's Republic of China

Received February 4, 2005

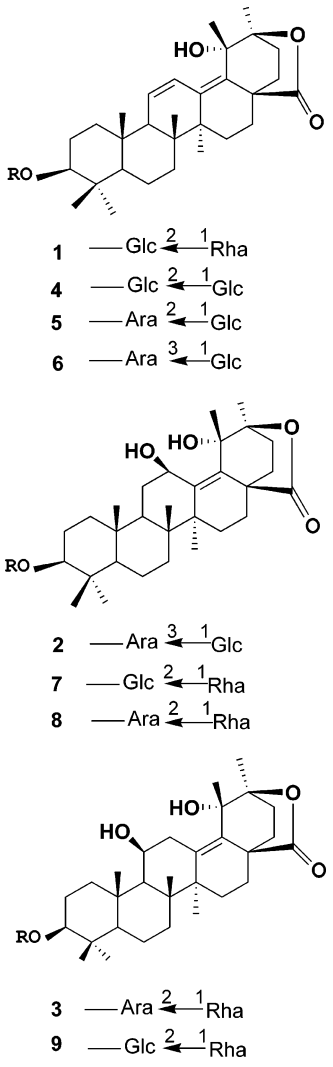
Nine new triterpene saponins, ilekudinosides K−S (**1**−**9**), and eight known triterpene saponins were isolated from the 70% ethanol extract of the leaves of *Ilex kudingcha*. The new saponins were characterized as 3-*O*-α-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranosyl-α-kudinlactone (**1**), 3-*O*-*β*-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl-*β*-kudinlactone (**2**), 3-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl-*γ*-kudinlactone (**3**), 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl-α-kudinlactone (**4**), 3-*O*-*β*-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-α-kudinlactone (**5**), 3-*O*-*β*-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl-α-kudinlactone (**6**), 3-*O*-α-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranosyl-*β*-kudinlactone (**7**), 3-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl-*β*-kudinlactone (**8**), and 3-*O*-α-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranosyl-*γ*-kudinlactone (**9**), respectively. The structures and stereochemistry of compounds **1**−**9** were elucidated by spectroscopic data interpretation and chemical degradation.

Ilex kudingcha C. J. Tseng (Aquifoliaceae) is distributed widely in the southern region of the People's Republic of China. The leaf of this plant is a traditional tea product, known as "Ku-Ding-Cha".[1] It is also used in the form of a commercial herbal product as a central nervous system stimulant, a diuretic and antihypertensive agent, a treatment for sore throats, and an aid to losing weight.[1] In a previous report, Chen et al. reported on the hypertensive and antiobesity activities of *I. kudingcha*.[2] Triterpenes and triterpene saponins have been isolated from an extract of the leaves of *I. kudingcha*.[3−5] A methanol extract of *I. kudingcha* has shown inhibitory activity against acyl CoA cholesteryl acyl transferase (ACAT).[6,7] This paper reports the isolation and structural elucidation of nine new triterpene saponins (**1**−**9**) from *I. kudingcha*, along with eight known triterpenoids.

Results and Discussion

The crude saponin mixture, obtained from the ethanolic extract of the leaves of *I. kudingcha*, was dissolved in water and passed through a porous polymer resin (AB-8) column. The 60% methanolic eluate was chromatographed on octadecylsilyl silica gel (ODS) and separated using preparative HPLC to afford 17 saponins, including nine new (**1**−**9**) and eight known compounds. The NMR data of the new saponins are presented in Tables 1−3.

Ilekudinoside K (**1**) was assigned a molecular formula of $C_{42}H_{64}O_{13}$ determined on the basis of its positive FABMS $[M + Na]^+$ ion peak at *m/z* 799 and its ^{13}C and DEPT NMR spectra. The UV spectrum showed an absorption maximum at 260 nm. The IR spectrum exhibited absorptions at 3421 cm^{-1} (hydroxyl) and 1729 cm^{-1} (*γ*-lactone). The spectroscopic features and physicochemical properties suggested that the compound **1** is a triterpene saponin. Of the 42 carbons (Table 1), 30 were assigned to the aglycon part and 12 to the sugar moiety. The ^1H NMR spectrum (Table 2) showed seven singlets for tertiary methyls at *δ* 0.78, 0.84, 1.01, 1.13, 1.21, 1.50, and 1.66, two low-field signals for two olefinic protons at *δ* 5.70 (d, *J* = 10.5 Hz) and 7.46 (d, *J* = 10.5 Hz), and a typical signal of axial H-3 at *δ* 3.32 (dd, *J* = 11.5, 4.5 Hz).[3] The ^{13}C and DEPT NMR spectra indicated seven sp^3 carbons at *δ* 16.4, 16.5, 18.3, 18.6, 19.4, 23.6, and 27.6 and two coupled sp^2 olefinic carbons at *δ* 127.0 (CH), 128.4 (CH), 134.9 (C), and 140.7 (C). These data were used to assign the aglycon moiety of **1** as having an ursane-11,13(18)-diene unit.[3] The ^{13}C NMR signals at *δ*



* To whom correspondence should be addressed. Tel/Fax: +86-10-82802750. E-mail: pengfeitu@bjmu.edu.cn.
[†] Peking University Health Science Center.
[‡] The State Key Laboratory of Natural and Biomimetic Drugs.

10.1021/np050043z CCC: $30.25
Published on Web 07/20/2005

Table 1. ^{13}C NMR Spectroscopic Data (δ) of **1**−**9** (125 MHz in pyridine-d_5)[a]

position	1	2	3	4	5	6	7	8	9
1	38.3	38.9	39.3	38.2	38.5	38.5	38.8	39.4	38.8
2	26.6	28.8	27.2	26.8	26.7	26.9	26.4	29.2	26.6
3	88.8	88.7	88,9	88.9	89.1	88.9	88.5	89.2	88,5
4	39.5	39.6	39.8	39.6	39.9	40.0	39.1	39.9	39.1
5	55.3	56.0	56.1	55.1	55.5	55.5	55.8	56.5	55.6
6	18.2	18.5	18.8	18.3	18.9	18.7	18.0	18.9	18.1
7	32.8	35.5	35.5	32.8	33.2	33.3	35.0	35.8	34.8
8	42.1	41.7	43.1	42.1	42.5	42.5	41.3	42.1	42.4
9	54.4	44.9	50.4	54.4	54.8	54.9	44.3	44.3	49.7
10	36.4	37.0	37.4	36.5	36.9	36.9	36.5	37.3	36.7
11	127.0	26.8	71.8	127.1	127.5	127.5	26.4	26.6	71.8
12	128.4	66.0	33.8	128.5	128.8	128.8	65.8	66.4	32.9
13	140.7	146.3	143.9	140.7	141.1	141.1	145.9	146.7	143.5
14	42.0	44.1	46.5	42.1	42.5	42.5	43.8	44.4	45.9
15	25.8	26.2	29.5	25.8	26.2	26.2	28.5	29.2	28.8
16	26.2	26.7	27.2	26.4	26.7	26.7	25.8	26.9	26.6
17	43.8	43.9	45.8	43.8	44.2	44.2	43.5	45.2	45.2
18	134.9	137.5	135.8	135.3	135.3	135.4	136.9	137.8	135.1
19	74.0	74.3	74.1	74.1	73.7	74.4	73.5	74.2	72.9
20	85.9	85.6	85.7	85.9	86.3	86.2	85.4	86.0	85.1
21	28.5	28.3	29.6	28.5	28.9	28.9	27.8	28.7	28.9
22	32.8	32.8	32.8	32.8	33.2	33.3	32.2	33.2	32.1
23	27.6	28.1	28.3	27.8	28.2	28.1	27.6	28.4	27.7
24	16.4	16.9	17.1	16.5	16.5	16.7	16.6	17.3	16.6
25	18.3	16.6	17.1	18.3	18.7	18.7	16.2	17.0	16.4
26	16.5	18.2	17.4	16.2	16.8	16.9	17.6	18.5	16.7
27	18.6	23.4	21.9	18.6	18.7	19.0	23.0	23.8	21.2
28	175.2	175.4	175.8	175.2	175.6	175.6	175.3	175.7	175.3
29	23.6	25.2	26.9	23.7	24.0	24.0	24.8	25.6	26.2
30	19.4	19.4	20.5	19.5	19.8	19.8	19.0	19.8	19.8
3-*O*-sugar									
Glc or Ara	Glc	Ara	Ara	Glc	Ara	Ara	Glc	Ara	Glc
1	105.1	107.4	105.1	105.2	105.2	107.7	104.9	105.2	105.1
2	79.4	71.9	76.3	83.5	81.4	72.3	79.2	76.3	79.4
3	77.9	84.1	74.3	78.0	74.5	84.4	77.5	74.7	77.8
4	71.8	69.3	69.0	71.6	68.6	69.7	71.6	69.1	71.2
5	77.4	67.0	64.9	78.0	65.3	67.4	77.4	65.1	77.4
6	62.5			62.7			62.2		62.5
terminal sugar									
Rha or Glc	Rha	Glc	Rha	Glc	Glc	Glc	Rha	Rha	Rha
1	101.3	106.4	102.0	106.1	106.3	106.7	101.2	102.1	101.3
2	72.5	75.7	72.8	77.1	76.7	76.0	71.8	72.8	72.1
3	72.1	78.4	72.7	78.3	78.5	79.0	71.6	72.8	72.0
4	73.7	71.5	73.6	71.6	71.9	71.9	73.8	74.4	73.7
5	69.3	78.6	70.2	78.3	78.5	78.7	69.2	70.2	69.3
6	18.3	62.6	18.7	62.7	62.9	63.0	18.2	18.9	18.3

[a] Assignments were based on DQF-COSY, $^1H-^1H$ COSY, DEPT, HSQC, NOESY, and HMBC experiments. Ara = α-L-arabinopyranose; Rha = α-L-rhamnopyranose; Glc = β-D-glucopyranose.

85.9 (C-20) and 175.2 (C-28) and the carbon resonances of the E ring indicated the presence of a hexacyclic lactone ring. A 19-*O*-substituent on the ursane skeleton was proved by the signal at δ 74.0 (C-19) in the ^{13}C and DEPT NMR spectra.[3,4] With additional 2D NMR experiments, the aglycon of **1** was identified as 3β,19α-dihydroxyurs-11(12),-13(18)-diene-28,20β-lactone, also known as α-kudinlactone.[3] The ^{13}C NMR chemical shift of C-3 (δ 88.5) suggested that **1** has a glycosyl linkage at C-3. Acid hydrolysis of **1** afforded sugar components identified by TLC and GC analysis as D-glucose and L-rhamnose. The presence of two anomeric proton signals at δ 4.95 (d, J = 8.0 Hz) and 6.55 (brs) and two corresponding carbon signals at δ 105.1 and 101.3 indicated that **1** is a disaccharide of D-glucose and L-rhamnose. The other sugar proton signals were overlapped in the region between δ 3.90 and 4.86. The identities of the monosaccharides and the disaccharide sequence were determined by a combination of $^1H-^1H$ COSY, DQF-COSY, HSQC, HMBC, and NOESY NMR experiments. The $^1H-^1H$ COSY and DQF-COSY experiments allowed analyses of the disaccharide spin systems and assignments of their proton resonances. The assignments of the proton and the carbon resonances revealed the presence of a inner β-glucose unit and a terminal α-rhamnose unit. The large $^3J_{H1-H2}$ coupling constant (8.0 Hz) suggested a β-anomeric configuration for the glucose unit. The anomeric proton of the rhamnose unit was observed as a broad singlet. The ^1H NMR splitting pattern and the three-bond strong HMBC correlations from the anomeric proton to C-3 and C-5 indicated the approximate 180° dihedral angles between H-1 and C-3 and H-1 and C-5, which suggested an equatorial anomeric proton and its α-configuration in the $^1C_4$ form.[8] The positions and sequence of the disaccharide moiety were defined unambiguously from the HMBC correlations between signals of H-1 (δ 4.95) of the β-glucose and C-3 (δ 88.8) of the aglycon, and H-1 (δ 6.55) of the terminal α-rhamnose and C-2 (δ 79.4) of the inner β-glucose. The same sugar sequence was supported by the NOESY experiment. Therefore, ilekudinoside K (**1**) was elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-α-kudinlactone.

Ilekudinoside L (**2**) exhibited a quasi-molecular ion peak at *m/z* 803 $[M + Na]^+$ in its positive FABMS, consistent with a molecular formula of $C_{41}H_{64}O_{14}$. The UV spectrum showed an absorption maximum at 209 nm. The IR spectrum exhibited absorptions at 3424 cm^{-1} (hydroxyl)

Table 2. ^{1}H NMR Spectroscopic Data (δ) of **1**−**5** (500 MHz in pyridine-d_5)[a]

	1	**2**	**3**	**4**	**5**
Triterpene moiety					
3	3.32 dd (11.5, 4.5)	3.33 dd (11.5, 4.0)	3.17 dd (11.5, 4.0)	3.28 dd (12.0, 4.0)	3.20 dd (11.5, 4.5)
11	7.46 d (10.5)		4.91 dd (12.0,5.0)	7.49 d (10.5)	7.47 d (10.5)
12	5.70 d (10.5)	5.91 brs		5.73 d (10.5)	5.73 d (10.5)
23	1.21 s	1.31 s	1.11 s	1.28 s	1.20 s
24	1.13 s	0.97 s	0.97 s	1.06 s	0.99 s
25	0.84 s	0.86 s	0.76 s	0.84 s	0.84 s
26	0.78 s	0.90 s	0.84 s	0.81 s	0.80 s
27	1.01 s	1.61 s	1.21 s	1.02 s	1.00 s
29	1.66 s	1.64 s	1.75 s	1.67 s	1.66 s
30	1.50 s	1.48 s	1.50 s	1.51 s	1.50 s
3-*O*-sugar					
Glc or Ara	Glc	Ara	Ara	Glc	Ara
1	4.95 d (8.0)	4.72 d (5.5)	4.86 d (5.0)	4.91 d (8.0)	4.95 d (5.0)
2	4.29 d (8.7, 8.0)	4.57 d (7.5)	4.52 dd (9.5, 5.0)	4.20 dd (9.4, 8.0)	4.58 t (6.7)
3	4.30 d m	4.22 m	4.27 dd (9.0, 4.5)	4.30 dd (9.0, 9.4)	4.35 m
4	4.14 t (9.4, 3.0)	442 brs	4.28 brs	4.14 m	4.38 m
5	3.97 m	3.73 d (11.7)	3.78 d (9.6)	3.98 m	3.75 brs
		4.18 dd (11.7, 4.6)	4.29		4.26 dd (11.0, 4.0)
6	4.58 d (9.5)			4.39 dd (11.0, 5.0)	
	4.37 m			4.51 m	
Terminal sugar					
Rha or Glc	Rha	Glc	Rha	Glc	Glc
1	6.55 brs	5.37 d (7.5)	6.07 brs	5.35 d (8.0)	5.15 d (7.5)
2	4.86 brs	4.02 dd (8.0, 7.1)	4.57 dd (9.6, 3.0)	4.09 dd (9.0, 8.0)	4.08 dd (8.4, 7.6)
3	4.69 dd (9.5, 3.7)	4.25 t (8.5)	4.71 dd (9.5, 2.7)	4.23 d (8.5)	4.17 dd (9.0, 3.0)
4	4.35 brs	4.20 m	4.27 m	4.37 m	4.31 m
5	4.80 dq (9.6, 6.5)	3.98 m	4.49 dd (9.4, 6.2)	3.97 m	3.81 m
6	1.68 d (6.5)	4.36 m	1.58 d (6.5)	4.36 dd (11.4, 2.0)	4.42 d (9.0)
		4.52 d (9.5)		4.47 m	4.44 m

[a] Assignments were based on DQF-COSY, ^{1}H−^{1}H COSY, DEPT, HSQC, NOESY, and HMBC experiments. Ara = α-L-arabinopyranose; Rha = α-L-rhamnopyranose; Glc = β-D-glucopyranose

Table 3. ^{1}H NMR Spectroscopic Data (δ) of **6**−**9** (500 MHz in pyridine-d_5)[a]

	6	**7**	**8**	**9**
Triterpene moiety				
3	3.34 dd (12.0,5.0)	3.26 dd (11.5, 4.0)	3.23 dd (11.5,4.0)	3.27 dd (11.5,4.0)
11	7.49 dd (11.0, 3.0)			4.87 d (12.0)
12	5.76 d (11.0)	5.87 brs	5.91 brs	
23	1.28 s	1.18 s	1.17 s	1.15 s
24	0.93 s	1.10 s	1.06 s	1.06 s
25	0.87 s	0.77 s	0.86 s	0.71 s
26	0.82 s	0.81 s	0.89 s	0.79 s
27	1.03 s	1.53 s	1.60 s	1.17 s
29	1.67 s	1.56 s	1.64 s	1.71 s
30	1.50 s	1.43 s	1.48 s	1.47 s
3-*O*-sugar				
Glc or Ara	Ara	Glc	Ara	Glc
1	4.73 d (5.0)	4.82 d (7.0)	4.89 d (5.5)	4.85 d (7.5)
2	4.58 t (9.0)	4.19 dd (9.6, 8.0)	4.53 m	4.18 d (9.2)
3	4.23 d (8.1)	4.21 d (9.6)	4.28 m	4.21 m
4	4.42 brs	4.08 t (8.7)	4.30 m	4.06 d (7.0)
5	3.71 d (11.7)	3.89 m	3.81 dd (11.5, 2.0)	3.90 m
	4.18 dd (11.7, 4.5)		4.31 d (11.5)	
6		4.45 d (9.5)		4.50 dd (9.6, 2.5)
		4.30 d (9.5)		4.31 m
Terminal sugar				
Rha or Glc	Glc	Rha	Rha	Rha
1	5.38 d (7.5)	6.48 brs	6.12 brs	6.46 brs
2	4.02 d (7.5)	4.72 brs	4.60 dd (9.4, 3.5)	4.79 brs
3	4.27 d (11.5)	4.62 dd (9.5, 3.0)	4.71 brs	4.62 dd (9.0, 3.2)
4	4.21 m	4.28 d (9.6)	4.26 m	4.27 d (6.1)
5	3.98 m	4.70 dq (9.4, 6.2)	4.55 d (6.2)	4.70 m
6	4.53 d (11.0)	1.63 d (6.0)	1.61 d (5.5)	1.63 d (6.5)
	4.37 dd (11.0, 5.0)			

[a] Assignments were based on DQF-COSY, ^{1}H−^{1}H COSY, DEPT, HSQC, NOESY, and HMBC experiments. Ara = α-L-arabinopyranose; Rha = α-L-rhamnopyranose; Glc = β-D-glucopyranose.

and 1722 cm^{-1} (γ-lactone). The NMR spectra suggested that compound **2** is a triterpene saponin. The ^{13}C NMR spectrum (Table 1) showed 41 carbon signals, 30 from the triterpene moiety and 11 from the saccharide portion. The ^{1}H NMR spectrum (Table 2) displayed signals characteristic for a triterpene of the ursan-18-ene type: seven singlets for tertiary methyls at δ 0.86, 0.90, 0.97, 1.31, 1.48, 1.61, and 1.64, a low-field H-12 signal at δ 5.91 (brs), which suggested a 12-*O*-substituted ursane triterpenoid proton,[4] and a typical signal at δ 3.33 (dd, J = 11.5, 4.0 Hz)

ascribable to an axial H-3. Further features in the ^{13}C NMR spectrum were seven sp^3 carbon signals at δ 16. 6, 16.9, 18.2, 19.4, 23.4, 25.2, and 28.1 and two olefinic carbons at δ 137.5 (C) and 146.3 (C), assignable to C-13 and C-18, respectively. The signals at δ 74.3 (C-19), 85.6 (C-20), and 175.4 (C-28) and the carbon resonances of the E ring indicated the presence of a 19-*O*-substituent and a δ-lactone ring.[3,4] Therefore, the aglycon of **2** was identified as 3β,12β,19α-trihydroxyurs-13(18)-ene-28,20β-lactone, reported before as β-kudinlactone.[4] The ^{13}C NMR chemical shift of C-3 (δ 88.7) revealed that the sugar moiety was attached to C-3 of the aglycon. Acid hydrolysis of **2** yielded L-arabinose and D-glucose. The 1D and 2D NMR spectra showed the presence of two anomeric signals at δ 4.72 (d, J = 5.5 Hz), 107.4 and δ 5.37 (d, J = 7.5 Hz), 106.4. The overall structural assignments were determined following the same procedure for elucidation as used in compound **1**. The results of 2D NMR spectroscopic methods indicated the presence of a terminal β-glucose unit and an inner 3-substituted α-arabinose unit. The $^3J_{H1-H2}$ coupling constant (5.5 Hz) indicated the α-anomeric configuration of the arabinose unit. The large $^3J_{H1-H2}$ value (7.5 Hz) confirmed the β-anomeric configuration of the glucose. The linkage position and sequence of the sugar chain were established by HMBC correlations between signals of H-1 (δ 4.72) of the inner α-arabinose and C-3 (δ 88.7) of the aglycon, and H-1 (δ 5.37) of the terminal β-glucose and C-3 (δ 84.1) of the inner α-arabinose. The elucidation of the disaccharide chain connected at C-3 of the aglycon was verified by a NOESY experiment. Therefore, ilekudinoside L (**2**) was elucidated as 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-β-kudinlatone.

Ilekudinoside M (**3**) showed a quasi-molecular ion peak at *m/z* 787 $[M + Na]^+$ in its FABMS, suggesting a molecular formula of $C_{41}H_{64}O_{13}$. The UV spectrum showed an absorption maximum at 211 nm. The IR spectrum exhibited absorptions at 3389 cm^{-1} (hydroxyl) and 1719 cm^{-1} (γ-lactone). The ^{13}C NMR spectrum (Table 1) showed 41 carbons, of which 30 were assigned to a triterpene moiety and 11 to the saccharide portion. The ^1H, ^{13}C, and DEPT NMR (Tables 1 and 2) indicated that **3** has an aglycon similar to **2** except that the hydroxyl substitution position was changed from C-12 to C-11.[3] Multiple 2D NMR experiments were used to elucidate the aglycon as 3β,11β,-19α-trihydroxyurs-13(18)-ene-28,20β-lactone, also known as γ-kudinlactone.[3] The chemical shift of C-3 (δ 88.9) indicated that the sugar chain was attached to C-3 of the aglycon. Acid hydrolysis yielded L-arabinose and L-rhamnose. The 1D and 2D NMR spectra showed two anomeric signals at δ 4.86 (d, J = 5.0 Hz), 105.1 and δ 6.07 (brs), 102.0, assigned to arabinose and rhamnose units, respectively. Further NMR spectroscopic analysis indicated the presence of terminal α-rhamnose and 2-substituted α-arabinose moieties. The $^3J_{H1-H2}$ coupling constant (5.0 Hz) suggested an α-anomeric configuration of the arabinose unit. Comparison of the NMR data of **3** with those of α-rhamnose in **1** suggested an α-anomeric configuration of rhamnose. The linkage position and sequence of the disaccharide moiety was established by HMBC correlations between signals of H-1 (δ 4.86) of the α-arabinose and C-3 (δ 88.9) of the aglycon, and H-1 (δ 6.07) of the terminal α-rhamnose and C-2 (δ 76.3) of the inner α-arabinose. Further support was obtained from the observed NOESY experiment. Therefore, ilekudinoside M (**3**) was elucidated as 3-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl-γ-kudinlatone.

Ilekudinoside N (**4**) was assigned a molecular formula of $C_{42}H_{64}O_{14}$, determined on the basis of its ion peak at *m/z* 815 $[M + Na]^+$ in the positive FABMS and by ^{13}C NMR data analysis. Its ^1H and ^{13}C NMR spectra (Tables 1 and 2) indicated that saponin **4** possessed the same aglycon as that of **1** but differed in the sugar moiety. Acid hydrolysis of **4** afforded only D-glucose. The presence of two anomeric proton and carbon signals at δ 4.91 (d, J = 8.0 Hz), 105.2 and δ 5.35 (d, J = 8.0 Hz), 106.1 in the ^1H and ^{13}C NMR spectra indicated that **4** is a disaccharide with two glucose units. Assignments of the proton and the carbon NMR resonance indicated the presence of a terminal β-glucose and an inner 2-substituted β-glucose. The large $^3J_{H1-H2}$ coupling constant (8.0 Hz) confirmed the β-anomeric configuration for the two glucose units. The exact position and sequence of the disaccharide moiety were established by HMBC correlations between signals of H-1 (δ 4.91) of the inner β-glucose and C-3 (δ 88.9) of the aglycon, and H-1 (δ 5.35) of the terminal β-glucose and C-2 (δ 83.5) of the inner β-glucose. Therefore, ilekudinoside N (**4**) was elucidated as 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-α-kudinlactone.

Ilekudinoside O (**5**) displayed a quasi-molecular ion peak at *m/z* 785 $[M + Na]^+$ in its FABMS, suggesting a molecular formula of $C_{41}H_{62}O_{13}$. Analysis of the ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) indicated that **5** possesses the same aglycon as that of **1** and **4**, but differed in the sugar moiety. The ^{13}C NMR chemical shift of C-3 (δ 89.1) revealed that the sugar moiety was attached to C-3 of the aglycon. Acid hydrolysis of **5** yielded L-arabinose and D-glucose. Detailed NMR analysis indicated two anomeric signals at δ 4.95 (d, J = 5.0 Hz), 105.2 and δ 5.15 (d, J = 7.5 Hz), 106.3. The comparison of ^1H and ^{13}C NMR data of the sugar portion of **5** with those of **2** suggested that **5** possesses the same monosaccharide units, but the only structural difference between the sugar chain of **5** and **2** was due to the sugar arrangement. The linkage position and sequence of the disaccharide moiety was established by HMBC correlations between H-1 (δ4.95) of the inner α-arabinose and C-3 (δ 89.1) of the aglycon, and H-1 (δ 5.15) of the terminal β-glucose and C-2 (δ 81.4) of the inner α-arabinose. The anomeric configuration of the sugar units were similar to the corresponding ones in **2**. Thus, ilekudinoside O (**5**) was elucidated as 3-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-α-kudinlactone.

Ilekudinoside P (**6**) possesses the molecular formula $C_{41}H_{62}O_{13}$, as determined from the quasi-molecular ion peak $[M + Na]^+$ at *m/z* 785 in the FABMS and the ^{13}C NMR data, which is the same as that of **5**. Its spectroscopic features suggested **6** to be a further α-kudinlactone disaccharide differing in its sugar unit from those of **4** and **5**. The ^{13}C NMR chemical shift of C-3 (δ 88.9) revealed that the disaccharide moiety was attached to C-3 of the aglycon. Acid hydrolysis of **6** yielded L-arabinose and D-glucose. Detailed NMR analysis (Tables 1 and 3) suggested that **6** possesses an identical disaccharide chain at the C-3 position of the aglycon as **2**. Confirmation was obtained from the HMBC correlation signals between H-1 (δ 4.73) of arabinose and C-3 (δ 88.9) of the aglycon, and H-1 (δ 5.38) of glucose and C-3 (δ 84.4) of the arabinose. Therefore, ilekudinoside P (**6**) was elucidated as 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-α-kudinlactone.

Ilekudinoside Q (**7**) gave a molecular formula of $C_{42}H_{66}O_{14}$, on the basis of the quasi-molecular ion peak $[M + Na]^+$ at *m/z* 817 in its FABMS and its ^{13}C NMR spectroscopic data. NMR analysis indicated that **7** is another β-kudinlactone disaccharide differing in its sugar portion from **2**. Acid

hydrolysis of **7** yielded D-glucose and L-rhamnose. The ^{13}C NMR chemical shift of C-3 (δ 88.5) revealed that the sugar moiety was attached to C-3 of the aglycon. The disaccharide chain attached to C-3 of the aglycon of **7** has the same monosaccharide units and sugar arrangement as that of **1**, and further support was obtained from the HMBC correlation signals between H-1 (δ 4.82) of the glucose and C-3 (δ 88.5) of the aglycon, and H-1 (δ 6.48) of the rhamnose and C-2 (δ 79.2) of the glucose. Thus, ilekudinoside Q (**7**) was elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-β-kudinlactone.

Ilekudinoside R (**8**) exhibited a molecular formula of $C_{41}H_{64}O_{13}$, identical to that of **3**, as determined on the basis of its quasi-molecular ion peak $[M + Na]^+$ at *m/z* 787 in the FABMS and its ^{13}C NMR data. Acid hydrolysis of **8** yielded L-arabinose and L-rhamnose. The ^{13}C NMR chemical shift of C-3 (δ 89.2) revealed that the sugar moiety was attached to C-3 of the aglycon. Detailed NMR analysis (Tables 1 and 3) indicated that **8** also possesses the same aglycon as that **2** and **7**, and the disaccharide chain attached to C-3 of the aglycon has the same monosaccharide units and sugar arrangement as that of **3**. HMBC correlations verified this conclusion. Thus, ilekudinoside R (**8**) was elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-β-kudinlatone.

Ilekudinoside S (**9**) gave a molecular formula of $C_{42}H_{64}O_{14}$, identical to that of **7**, determined from the quasi-molecular ion peak $[M + Na]^+$ at *m/z* 817 in the FABMS and its ^{13}C NMR spectrum. Acid hydrolysis of **9** afforded D-glucose and L-rhamnose. The ^{13}C NMR chemical shift of C-3 (δ 88.5) revealed that the sugar moiety is attached to C-3 of the aglycon. Detailed NMR analysis indicated that **9** is a further γ-kudinlatone glycoside partly differing in its sugar moiety from **3**. Comparison of the ^1H and ^{13}C NMR data of **9** (Tables 1 and 3) with those of **1** and **7** suggested that **9** possesses the same disaccharide chain at the C-3 position of the γ-kudinlactone aglycon unit. The sequence of the sugar units was verified from the observed HMBC correlations. Thus ilekudinoside S (**9**) was elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-γ-kudinlactone.

Additionally, the previously reported compounds kudinosides A, C,[3] D, E, and F,[4] ilekudinoside I,[7] 3-*O*-α-L-arabinopyranosyl-α-kudinlatone, and 3-*O*-α-L-arabinopyranosyl-β-kudinlatone[4] were also isolated, and these compounds were identified by spectroscopic data comparison with literature values.

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a Perkin-Elmer 243B digital polarimeter. UV spectra were measured with a Shimadzu spectrometer. IR spectra were determined using a Nicolet Avatar 360 FT-IR spectrometer. NMR spectra were measured with an Inova 500 NMR spectrometer. Saponins were analyzed in pyridine-d_5 with TMS as an internal standard. FABMS were measured on a Jmx-sx mass spectrometer. HRFABMS were measured on an AutoSpec Ultima-TOF mass spectrometer. Preparative HPLC was performed on a Waters model 2487 instrument (Waters ODS, 7.8 i.d. × 300 mm, detected at UV 260 and 210 nm). GC analysis was carried out on an Agilent 6890N gas chromatograph using a HP-5 capillary column (28 m × 0.32 mm i.d.); detection, FID; detector temperature, 260 °C; column temperature, 180 °C; carrier gas, N_2; flow rate, 40 mL/min. AB-8 porous polymer resin (Tianjin University Chemical Corporation, Tianjin, People's Republic of China), silica gel (Qingdao Ocean Chemical Corporation, Qingdao, People's Republic of China), and ODS (100−200 mesh, Fuji Sylisia Chemical, Ltd., Aichi, Japan) were used for column chromatography.

Plant Material. Leaves of *Ilex kudingcha* C. J. Tseng were collected from Daxin Prefecture, Guangxi Province, People's Republic of China, in June 2003. The plant material was identified by one of the authors (P.-F.T.). A voucher specimen (20030601) has been deposited at the Department of Natural Medicine, Peking University.

Extraction and Isolation. The powdered dried leaves of *I. kudingcha* (10 kg) were extracted twice with 70% ethanol under reflux, each for 2 h. Evaporation of the solvent under reduced pressure provided 1200 g of the 70% ethanol extract. This was suspended in H_2O and partitioned sequentially with petroleum, $CHCl_3$, and *n*-BuOH. Evaporation of the solvent under reduced pressure from the petroleum-, $CHCl_3$-, and *n*-BuOH-soluble fractions yielded 126, 89, and 630 g of each residue, respectively. The *n*-BuOH-soluble fraction (240 g) was applied to a column of porous polymer resin (AB-8) and eluted with a gradient H_2O−MeOH to give the crude saponins. The crude saponin mixture (100 g) was first separated on a silica gel column gradient eluting with $CHCl_3$−MeOH. Five fractions (1−5) were obtained. Fractions 2, 3, and 4 were further purified using RP-18 column chromatography using a step gradient of H_2O−MeOH (70:30 → 20:80) to yield five subfractions designated 2-3, 2-4, 2-5, 3-2, and 4-3, respectively. Each subfraction was repeatedly subjected to HPLC {ODS column 2.5 mm × 30 cm [H_2O−MeOH (30:70) for fraction 2-3, (35:65) for fractions 2-4 and 2-5, (40:60) for fractions 3-2 and 4-3] + 0.05% TFA, flow rate 3.0 mL/min, UV 210 and 254 nm} to yield **1** (24 mg), **2** (32 mg), **3** (18 mg), **4** (8 mg), **5** (11 mg), **6** (20 mg), **7** (16 mg), **8** (31 mg), **9** (11 mg), ilekudinoside I (15 mg), kudinoside A (102 mg), kudinoside C (124 mg), kudinoside D (146 mg), kudinoside E (185 mg), kudinoside F (10 mg), 3-*O*-α-L-arabinopyranosyl-α-kudinlatone (37 mg), and 3-*O*-α-L-arabinopyranosyl-β-kudinlatone (18 mg), respectively.

Ilekudinoside K (1): amorphous powder; $[\alpha]^{20}_D$ −16.9° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 260 nm (log ϵ 3.95); IR (KBr) ν_{max} 3421, 2939, 1729, 1079 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz), see Table 1; ^{13}C NMR (pyridine-d_5, 125 MHz), see Table 3; FABMS (positive ion mode) *m/z* 799 $[M + Na]^+$; HRFABMS *m/z* 799.4248 (calcd for $C_{42}H_{64}O_{13}$, 799.4245).

Ilekudinoside L (2): amorphous powder; $[\alpha]^{20}_D$ −23.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 209 nm (log ϵ 3.84); IR (KBr) ν_{max} 3424, 2942, 1722, 1075 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz), see Table 1; ^{13}C NMR (pyridine-d_5, 125 MHz), see Table 3; FABMS (positive ion mode) *m/z* 803 $[M + Na]^+$; HRFABMS *m/z* 803.4239 (calcd for $C_{41}H_{64}O_{14}$, 803.4252).

Ilekudinoside M (3): amorphous powder; $[\alpha]^{20}_D$ −20.3° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 211 nm (log ϵ 3.67); IR (KBr) ν_{max} 3389, 2941, 1719, 1069 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz), see Table 1; ^{13}C NMR (pyridine-d_5, 125 MHz), see Table 3; FABMS (positive ion mode) *m/z* 787 $[M + Na]^+$; HRFABMS *m/z* 787.4233 (calcd for $C_{41}H_{64}O_{13}$, 787.4244).

Ilekudinoside N (4): amorphous powder; $[\alpha]^{20}_D$ −18.6° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 260 nm (log ϵ 4.01); IR (KBr) ν_{max} 3430, 2941, 1728, 1072 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz), see Table 1; ^{13}C NMR (pyridine-d_5, 125 MHz), see Table 3; FABMS (positive ion mode) *m/z* 815 $[M + Na]^+$; HRFABMS *m/z* 815.4184 (calcd for $C_{42}H_{64}O_{14}$, 815.4193).

Ilekudinoside O (5): amorphous powder; $[\alpha]^{20}_D$ −17.1° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 260 nm (log ϵ 4.00); IR (KBr) ν_{max} 3430, 2939, 1723, 1079 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz), see Table 1; ^{13}C NMR (pyridine-d_5, 125 MHz), see Table 3; FABMS (positive ion mode) *m/z* 785 $[M + Na]^+$; HRFABMS *m/z* 785.4094 (calcd for $C_{41}H_{62}O_{13}$, 785.4088).

Ilekudinoside P (6): amorphous powder; $[\alpha]^{20}_D$ −17.6° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 261 nm(log ϵ 3.97); IR (KBr) ν_{max} 3434, 2938, 1723, 1075 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz), see Table 2; ^{13}C NMR (pyridine-d_5, 125 MHz), see Table 3; FABMS (positive ion mode) *m/z* 785 $[M + Na]^+$; HRFABMS *m/z* 785.4118 (calcd for $C_{41}H_{62}O_{13}$, 785.4146).

Ilekudinoside Q (7): amorphous powder; $[\alpha]^{20}_D$ −20.5° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 212 nm(log ϵ 3.81); IR (KBr)

ν_{max} 3413, 2940, 1729, 1072 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz), see Table 2; ^{13}C NMR (pyridine-d_5, 125 MHz), see Table 3; FABMS (positive ion mode) *m/z* 817 [M + Na]$^+$; HRFABMS *m/z* 817.4361 (calcd for $C_{42}H_{66}O_{14}$, 817.4350).

Ilekudinoside R (8): amorphous powder; $[\alpha]^{20}_D$ −17.9° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 212 nm (log ϵ 3.85); IR (KBr) ν_{max} 3429, 2943, 1733, 1075 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz), see Table 2; ^{13}C NMR (pyridine-d_5, 125 MHz), see Table 3; FABMS (positive ion mode) *m/z* 787 [M + Na]$^+$; HRFABMS *m/z* 787.4282 (calcd for $C_{41}H_{64}O_{13}$, 787.4303).

Ilekudinoside S (9): amorphous powder; $[\alpha]^{20}_D$ −24.6° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 215 nm(log ϵ 3.71); IR (KBr) ν_{max} 3390, 2937, 1727, 1071 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz), see Table 2; ^{13}C NMR (pyridine-d_5, 125 MHz), see Table 3; FABMS (positive ion mode) *m/z* 817 [M + Na]$^+$; HRFABMS *m/z* 817.4291 (calcd for $C_{42}H_{66}O_{14}$, 817.4277).

Acid Hydrolysis of Saponins. Each saponin (5 mg) was heated in 3 mL of 10% HCl−dioxane (1:1) at 80 °C for 4 h. After the dioxane was removed, the solution was extracted with EtOAc (3 mL × 3) to yield the aglycon and the sugar, respectively.

Sugar Analysis by TLC and GC. The sugar components in the aqueous layer left after acid hydrolysis of **1**−**9** were analyzed by silica gel TLC by comparison with standard sugars. The solvent system was $CHCl_3$−MeOH−H_2O (8:5:1), and spots were visualized by spraying with 95% EtOH−H_2SO_4−anisaldehyde (9:0.5:0.5, v/v), then heated at 120 °C for 10 min. For sugars of **1**−**9**, the R_f of glucose, arabinose, and rhamnose by TLC was 0.30, 0.56, and 0.50, respectively. The results were confirmed by GC analysis of the methyl sugar peracetates. The aqueous layer was evaporated and dissolved in anhydrous pyridine (100 μL), 0.1 M L-cysteine methyl ester hydrochloride (200 μL) was added, and the mixture was warmed at 60 °C for 1 h. The trimethysilylation reagent HMDS-TMCS (hexamethyldisilazane−trimethylchlorosilane−pyridine, 2:1:10) (Acros Organics, Geel Belgium) was added and warmed at 60 °C for 30 min. The thiazolidine derivatives were analyzed by GC for sugar identification. The retention times of L-arabinose (t_R, 5.29 min), D-glucose (t_R, 12.45 min), and L-rhamnose (t_R, 5.36 min) were confirmed by comparison with those of authentic standards.[9]

References and Notes

(1) Jiangsu New Medical College. *Dictionary of Chinese Materia Medica*; Shanghai Science and Technology Publisher: Shanghai, 1997; p 1288.
(2) Chen, Y.; Li, K. S.; Xie, T. G. *Chin. Trad. Herb. Drugs* **1995**, *26*, 250−252.
(3) Ouyang, M. A.; Yang, C. R.; Chen, Z. L.; Wang, H. Q. *Phytochemistry* **1996**, *41*, 871−877.
(4) Ouyang, M. A.; Yang, C. R.; Chen, Z. L.; Yang, C. R. *Phytochemistry* **1996**, *43*, 441−443.
(5) Ouyang, M. A.; Wang, H. Q.; Su, J. H.; Liu, Y. Q.; Yang, C. R. *Nat. Prod. Res. Dev.* **1997**, *9*, 19−23.
(6) Nishimura, K.; Toshiyuki, F.; Miyase, T.; Noguchi, H.; Chen, X. M. *J. Nat. Prod*. **1999**, *62*, 1061−1064.
(7) Nishimura, K.; Miyase, T.; Noguchi, H. *J. Nat. Prod.* **1999**, *62*, 1128−1133.
(8) Melek F. R.; Miyase T.; Abdel-Khalik S. M.; Hetta M. H.; Mahmoud I. I. *Phytochemistry* **2002**, *60*, 185−195.
(9) Hara, S.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **1986**, *34*, 1843−1844.

NP050043Z