Triterpenoid Saponins from *Ilex kudincha*

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Received March 29, 1999

Ten new triterpene saponins, ilekudinosides A−J (**2**, **6**−**8**, **10**, **13**−**17**), together with seven known triterpene saponins, ilexoside XLVIII (**1**); cynarasaponin C (**5**); latifolosides A (**9**), C (**3**), G (**12**), and H (**4**); and kudinoside G (**11**), were isolated from an aqueous extract of the leaves of *Ilex kudincha*. They possessed oleanane- and ursane-type triterpenoids as the aglycons. The structures were elucidated by 1D and 2D NMR experiments, including ROE difference, HOHAHA difference, ^{1}H−^{1}H COSY, and ^{1}H−^{13}C COSY (HMQC, HMBC) methods and sugar analysis. Compounds **1** and **5** exhibited acyl CoA cholesteryl acyl transferase (ACAT) inhibitory activity.

In the course of studies to search for acyl CoA cholesteryl acyl transferase (ACAT) inhibitors from natural sources, we have reported the isolation and structure elucidation of seven triterpenes from a methanolic extract of the leaves of *Ilex kudincha* C. J. Tseng (Aquifoliaceae) as ACAT inhibitors.[1] In the People's Republic of China, the leaves of *I. kudincha* are used as an herbal tea called "Ku-Ding-Cha" in Guangxi Province. Chen et al.[2] reported the hypotensive and anti-obesity activities of extracts of this plant, and Ouyang et al.[3] reported 12 ursane-type triterpenoids as constituents of *I. kudincha*. In the present paper, we describe the isolation, structure elucidation, and the ACAT inhibitory activity of 10 new [ilekudinosides A−J (**2**, **6**−**8**, **10**, **13**−**17**)] and seven known [ilexoside XLVIII (**1**);[4] cynarasaponin C (**5**);[5] latifolosides A (**9**),[6] C (**3**),[6] G (**12**),[7] and H (**7**);[7] and kudinoside G (**11**)[3a]] triterpene saponins from this plant. The known saponins were identified by comparison of their ^{1}H and ^{13}C NMR data with reported values. The structures of the new saponins were elucidated by 1D and 2D NMR experiments (ROE difference, HOHAHA difference, ^{1}H−^{1}H COSY, HMQC, HMBC) and by sugar analysis. Compounds **1** and **5** exhibited significant ACAT inhibitory activity.

Results and Discussion

The aqueous extract of the leaves of *I. kudincha* was passed through a porous polymer gel Diaion HP-20 column, and the adsorbed material was eluted with 40% aqueous methanol and methanol after washing with water. The methanol eluate was subjected to Si gel column chromatography using a chloroform−methanol−water system as solvent and then reversed-phase preparative HPLC, to give 17 pure compounds (**1**−**17**).

Ilekudinoside A (**2**) had a molecular formula $C_{53}H_{86}O_{21}$, as determined by positive-ion FABMS {*m*/*z* 1082 [M + Na]$^+$} and its ^{13}C NMR spectrum. The ^{13}C NMR spectrum showed 53 signals, of which 30 were assigned to a triterpenoid moiety and 23 to a saccharide moiety. Acid hydrolysis afforded L-arabinose, L-rhamnose, and D-glucose as the component sugar moiety.[8] The ^{1}H NMR spectrum of **2** showed signals for seven tertiary methyl groups (δ 0.88, 0.90, 0.92, 1.10, 1.12, 1.21, 1.27), a secondary methyl group [δ 1.64 (d, J = 6 Hz)], a trisubstituted olefinic proton of H-12 [δ 5.44 (dd, J = 3, 3 Hz)], a typical H-3ax proton [δ 3.31 (dd, J = 11.5, 4.5 Hz)] due to the presence of a β-*O*-function at C-3,[9] and four anomeric protons [δ 4.88 (d, J = 5 Hz), 5.10 (d, J = 8 Hz), 6.10 (br s), 6.32 (d, J = 8 Hz)]. In the ^{13}C NMR spectrum (Table 2), the signals at δ 122.8 and 144.1, ascribable to C-12 and C-13, confirmed the Δ^{12}-oleanene skeleton.[10] Signals at δ 88.2 and 176.4 suggested **2** was a bisdesmoside of oleanolic acid.[9] All carbon signals due to the aglycon moiety were assigned by comparison with reported data for elatoside E {3-*O*-β-D-xylopyranosyl-(1→2)-[β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester}[11] and araliasaponin I {3-*O*-β-D-xylopyranosyl(1→3)-β-D-glucopyranosyl(1→3)-[β-D-xylopyranosyl(1→2)]-α-L-arabinoyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester}.[9] Sugar proton signals were assigned with the aid of a HOHAHA difference spectrum on irradiating at each anomeric proton signal, and of HMQC and HMBC spectra. The anomeric proton signal (δ 6.32) of the D-glucosyl moiety was correlated to the carbon signal at δ 95.8 in the HMQC spectrum and to the C-28 carbon signal at δ 176.4 in the HMBC NMR spectrum. The anomeric proton signal (δ 4.88) of the L-arabinosyl moiety was correlated to the C-3 carbon signal at δ 88.2 in the HMBC spectrum. On irradiation of the anomeric proton signal (δ 4.88) of L-arabinose, a ROE was observed for H-3. ROEs were observed at H-3 and H-2 of L-arabinose on irradiation of the anomeric proton signals at δ 5.10 (glucose) and 6.10 (rhamnose). The anomeric configuration of the L-rhamnosyl moiety was determined as α from the ^{13}C NMR chemical shifts of C-3 and C-5 of the rhamnosyl moiety,[13] and those of the L-arabinosyl and D-glucosyl moieties were α and β, respectively, from the $^3J_{H1-H2}$ coupling constant. On the basis of this evidence, ilekudinoside A (**2**) was identified as 3-*O*-β-D-glucopyranosyl(1→3)-[α-L-rhamnoyranosyl(1→2)]-α-L-arabinoyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester.

Ilekudinoside B (**6**) showed a pseudo-molecular ion peak [M + Na]$^+$ at *m*/*z* 833 in the positive FABMS and gave ^{13}C NMR data consistent with the molecular formula $C_{42}H_{66}O_{15}$. Sugar analysis by GC after acid hydrolysis afforded D-glucose and D-glucuronic acid as component monosaccharides. The ^{1}H NMR spectrum (Table 1) revealed signals for six tertiary methyl groups (δ 0.88, 1.00, 1.16, 1.29, 1.39, 1.70), a secondary methyl group [δ 1.07 (d, J = 6.5 Hz)], an oxymethine proton signal [δ 3.39 (dd, J = 12, 4 Hz)], a trisubstituted olefinic proton [δ 5.58 (dd, J = 3, 3 Hz)], and two anomeric protons [δ 5.01 (d, J = 8 Hz) and 6.26 (d, J

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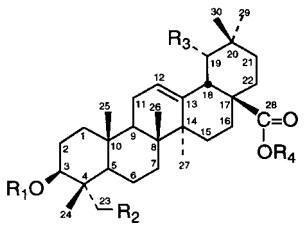
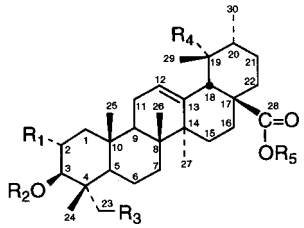
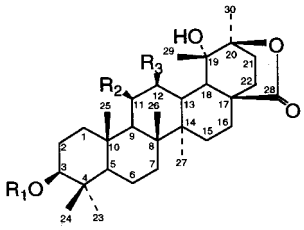
10.1021/np990128y CCC: $18.00
Published on Web 07/16/1999



	R_1	R_2	R_3	R_4
1	Glc A	OH	H	Glc
2	Ara ^{3}Glc (Rha ^{2})	H	H	Glc
3	Ara ^{3}Glc (Rha ^{2})	H	OH	Glc
4	Ara ^{3}Glc (Rha ^{2})	H	OH	Glc ^{2}Rha



	R_1	R_2	R_3	R_4	R_5
5	H	Glc A	OH	H	Glc
6	H	Glc A	H	OH	Glc
7	OH	Ara	OH	H	Glc
8	H	Ara	OH	OH	Glc
9	H	Ara ^{2}Rha	H	OH	Glc
10	H	Ara ^{3}Glc	H	OH	Glc
11	H	Ara ^{3}Glc (Rha ^{2})	H	OH	Glc
12	H	Ara ^{3}Glc (Rha ^{2})	H	OH	Glc ^{2}Rha
13	H	Ara ^{3}Glc^{2}Glc (Rha ^{2})	H	OH	Glc ^{2}Rha ($\Delta^{20(30)}$)



	R_1	R_2	R_3
14	Ara ^{3}Glc^{2}Glc (Rha ^{2})	H	H (Δ^{12}, 18β-H)
15	Ara ^{3}Glc ^{2}Glc (Rha ^{2})	OH	H ($\Delta^{13(18)}$)
16	Ara ^{2}Glc	H	OH ($\Delta^{13(18)}$)
17	Glc ^{2}Glc	H	OH ($\Delta^{13(18)}$)

= 8 Hz)]. A methine proton signal at δ 2.92 (br s) was correlated to carbon signals at δ 26.3, 42.2, 48.7, 72.8, 128.6, 139.3, and 177.0 in the HMBC spectrum, suggesting that this proton could be assigned to H-18 of a 19-oxygenated ursane-type triterpene.[12] All tertiary methyl proton signals were assigned with the aid of HMQC and HMBC experiments. The ^{1}H NMR signal for the C-27 methyl group (δ 1.70) was shifted downfield by 0.54 ppm compared with that of **5** (δ 1.16), indicating that the 19-*O*-function was α-OH.[12] The ^{13}C NMR signals at δ 128.6 and 139.3 due to C-12 and C-13, respectively, supported the identification of the aglycon as pomolic acid.[12] Sugar proton signals were assigned from the HOHAHA difference spectrum. The anomeric proton signals of glucuronic acid [δ 5.01 (d, J = 8 Hz)] and glucose [δ 6.26 (d, J = 8 Hz)] were correlated to the carbon signals of C-3 (δ 89.3) and C-28 (δ 177.0), respectively, in the HMBC spectrum. These results led us to assign the structure of ilekudinoside B (**6**) as 3-*O*-β-D-glucuronopyranosyl pomolic acid 28-*O*-β-D-glucopyranosyl ester.

The FABMS of ilekudinoside C (**7**) showed a pseudo-molecular ion peak $[M + Na]^+$ at *m/z* 805, and the ^{13}C NMR data were consistent with the molecular formula $C_{41}H_{66}O_{14}$. Acid hydrolysis afforded L-arabinose and D-glucose as sugar moieties. The ^{1}H NMR spectrum showed the presence of four tertiary methyl groups (δ 0.98, 1.07, 1.11, 1.17), two secondary methyl groups [δ 0.89 (d, J = 6 Hz), 0.93 (d, J = 7 Hz)], two oxymethylene protons [δ 3.66 (d, J = 12 Hz), 3.69 (d, J = 12 Hz)], a trisubstituted olefinic proton [δ 5.42 (dd, J = 3, 3 Hz)], a methine proton [δ 2.51 (d, J = 11 Hz)], and two anomeric protons [δ 4.95 (d, J = 8 Hz), 6.25 (d, J = 8 Hz)]. The two oxymethylene proton signals at δ 3.66 and 3.69 were correlated to a carbon signal at δ 63.8 in the HMQC spectrum and to carbon signals at δ 14.7, 44.7, 47.7, and 88.4 in the HMBC spectrum. The carbon signal at δ 14.7 was assigned to a methyl carbon signal at C-4 correlating to a methyl proton at δ 0.98 in the HMQC spectrum, which, in turn, was correlated to a carbon signal at δ 88.4 in the HMBC spectrum. Therefore, the orientation of the upfield-shifted carbon signal at δ 14.7 was determined as axial. The oxymethine carbon signal at δ 88.4 was shifted downfield by 6.2 ppm when compared with the analogous data for **5**, suggesting that C-2 was oxygenated. The ^{13}C NMR chemical shifts of C-1, C-2, C-3, and C-4 were similar to those of lucyoside B (a 2α, 23-dihydroxy-3-*O*-β-D-glucopyranosyl oleanane-type saponin).[14] HMBC correlations were observed between H-1 of arabinose (δ 4.95) and C-3 (δ 88.4) and between H-1 of glucose (δ 6.25) and C-28 (δ 176.2). Thus, the structure of ilekudinoside C (**7**) was assigned as 3-*O*-α-L-arabinoyranosyl 2α,23-dihydroxyursolic acid 28-*O*-β-D-glucopyranosyl ester.

The FABMS of ilekudinoside D (**8**) showed the same pseudo-molecular ion peak $[M + Na]^+$ as **7**, and sugar analysis after acid hydrolysis afforded L-arabinose and D-glucose. The ^{1}H NMR spectrum of **8** revealed signals for five tertiary methyl groups (δ 0.94, 1.02, 1.21, 1.38, 1.63), a secondary methyl group [δ 1.06 (d, J = 7 Hz)], a methine proton [δ 2.92 (br s)], two oxymethylene protons [δ 3.69 (d, J = 10 Hz), 4.25 (overlapped with other signal)], a trisubstituted olefinic proton [δ 5.55 (dd, J = 3, 3 Hz)], and two anomeric protons [δ 4.96 (d, J = 7.5 Hz), 6.28 (d, J = 8 Hz)]. On comparison of the ^{13}C NMR chemical shifts of C-23 and C-24 with those of **1** and of the E-ring carbons with those of **6**, the aglycon of **8** was deduced as 19α,23-dihydroxyursolic acid. An HMBC correlation between the anomeric proton signal and the glycosidic carbon led to the assignment of the structure of ilekudinoside D (**8**) as 3-*O*-α-L-arabinopyranosyl 19α,23-dihydroxyursolic acid 28-*O*-β-D-glucopyranosyl ester.

The ^{13}C NMR spectrum of ilekudinoside E (**10**) showed a bisdesmosidic triglycoside of pomolic acid. Acid hydrolysis gave L-arabinose and D-glucose as sugar components. After assignment of all protons of the sugar moiety and determination of HMBC correlations, and by conducting a ROE experiment, the structure of ilekudinoside E (**10**) was

Table 1. ^{1}H NMR Spectral Data for Ilekudinosides A−J (**2**, **6**−**8**, **10**, **13**−**17**) in Pyridine-d_5 at 35 °C

	2	**6**	**7**	**8**	**10**	**13**	**14**	**15**	**16**	**17**
aglycon-2			4.23[a]							
3	3.31 dd (11.5, 4.5)	3.39 dd (12, 4)	4.23[a]	4.25[a]	3.34 dd (12, 4.5)	3.27 dd (11, 4)	3.30 dd (12, 4.5)	3.27 dd (11.5, 4)	3.23 dd (11.5, 4.5)	3.30 dd (9.5, 3.5)
5	0.80 br d (11)	0.82 br d (11)	2.34 br d (11)	2.24 br d (12)	0.85 br d (12)	0.79 br d (11)	0.88[a]	0.74 br d (11)	0.84 br d (12)	0.83 br d (11.5)
11								4.93 m		
12	5.44 dd (3, 3)	5.58 dd (3, 3)	5.42 dd (3, 3)	5.55 dd (3, 3)	5.56 dd (3, 3)	5.60 dd (3,3)	6.16 m		5.92 m	5.91 m
18	3.20 dd (14, 4.5)	2.92 br s	2.51 d (11)	2.92 br s	2.94 br s	3.06 br s	2.57 br s			
23	1.21 s	1.29 s	3.66 d (12)	3.69 d (10)	1.30 s	1.17 s	1.23 s	1.22 s	1.25 s	1.32 s
		3.69 d (12)	4.25[a]							
24	1.12 s	1.00 s	0.98 s	0.94 s	1.00 s	1.12 s	1.18 s	1.11 s	1.05 s	1.13 s
25	0.88 s	0.88 s	1.07 s	1.02 s	0.93 s	0.92 s	0.88 s	0.84 s	0.89 s	0.88 s
26	1.10 s	1.16 s	1.17 s	1.21 s	1.19 s	1.14 s	0.80 s	0.90 s	0.92 s	0.91 s
27	1.27 s	1.70 s	1.11 s	1.63 s	1.70 s	1.75 s	1.22 s	1.27 s	1.62 s	1.61 s
29	0.92 s	1.39 s	0.93 d (7)	1.38 s	1.40 s	1.61 s	1.55 s	1.80 s	1.66 s	1.66 s
30	0.90 s	1.07 d (6.5)	0.89 d (6)	1.06 d (7)	1.07 d (7)	4.71 br s	1.47 s	1.56 s	1.51 s	1.50 s
						4.96 br s				
sugar at C-3										
GlcA-1		5.01 d (8)								
2		4.11 dd (9, 8)								
3		4.30 dd (9, 9)								
4		4.58 dd (9.5, 9)								
5		4.67 d (9.5)								
Ara-1	4.88 d (5)		4.95 d (8)	4.96 d (7.5)	4.73 d (8)	4.76 d (5)	4.80 d (6)	4.79 d (6)	4.97 d (5.5)	
2	4.64 dd (6, 5)		4.47[a]	4.40[a]	4.46[a]	4.71[a]	4.72[a]	4.71 dd (6, 6)	4.58 dd (7, 5.5)	
3	4.32[a]		3.98[a]	4.06 dd (8, 3)	4.21[a]	4.23[a]	4.26[a]	4.24[a]	4.36[a]	
4	4.51[a]		4.16[a]	4.24[a]	4.43[a]	4.57[a]	4.60 m	4.60 m	4.36[a]	
5	3.74 br d (11)		3.65 br d (12)	3.72 br d (11)	3.73 br d (11)	3.70 br d (11)	3.73 dd (11.5, 1.5)	3.71 br d (11)	3.79 dd (12, 2)	
	4.25[a]		4.21[a]	4.26 br d (11)	4.21[a]	4.22[a]	4.20[a]	4.20 dd (11, 4)	4.29[a]	
Rha-1	6.10 br s					6.33 br s	6.34 br s	6.33 br s		
2	4.71 m					4.64 m	4.65 m	4.63 m		
3	4.57[a]					4.49[a]	4.53 dd (9, 3)	4.51 dd (9, 3)		
4	4.21[a]					4.30[a]	4.26[a]	4.26[a]		
5	4.56[a]					4.70[a]	4.73[a]	4.69[a]		
6	1.64 d (6)					1.70 d (6)	1.71 d (6)	1.69 d (6)	(Glc at C-2 of Ara)	(Glc at C-3)
Glc (at C-3 of Ara)-1	5.10 d (8)				5.35 d (8)	5.16 d (8)	5.16 d (8)	5.16 d (8)	5.17 d (8)	4.91 d (8)
2	3.95 dd (8, 8)				4.01[a]	4.00[a]	3.98 dd (8, 7.5)	3.99 dd (8.5, 8)	4.08 dd (9, 8)	4.21 dd (9, 8)
3	4.15[a]				4.21[a]	4.27[a]	4.25[a]	4.26[a]	4.18 dd (9, 9)	4.30 dd (9, 9)
4	4.15[a]				4.23[a]	4.12[a]	4.12 dd (9, 9)	4.11 dd (9, 9)	4.30[a]	4.14[a]
5	3.91 m				3.97 m	3.89 m	3.89 m	3.90 m	3.82 m	3.91 m
6	4.31[a]				4.34[a]	4.28[a]	4.28[a]	4.29[a]	4.42 dd (11, 3.5)	4.45[a]
	4.47[a]				4.52 dd (11, 3)	4.48[a]	4.48 dd (12, 2)	4.49 dd (11, 2)	4.44 dd (11, 3)	4.53 dd (11.5, 2)
Glc (at C-2 of Glc)-1						5.28 d (8)	5.26 d (8)	5.27 d (8)		5.36 d (8)
2						4.24[a]	4.24[a]	4.29[a]		4.11 dd (9, 8)
3						4.14[a]	4.14 dd (9, 9)	4.13 dd (9, 9)		4.23 dd (9, 9)
4						4.30[a]	4.30[a]	4.22[a]		4.32[a]
5						3.82 m	3.81 m	3.81 m		3.91 m
6						4.35[a]	4.34 dd (12, 4.5)	4.33 dd (11, 4.5)		4.47[a]
						4.42[a]	4.42 dd (12, 2)	4.41 dd (11, 2)		4.47[a]
sugar at C-28										
Glc-1	6.32 d (8)	6.26 d (8)	6.25 d (8)	6.28 d (8)	6.29 d (8)	6.18 d (8)				
2	4.20[a]	4.20 dd (8.5, 8)	4.18[a]	4.20 dd (8, 8)	4.21[a]	4.50[a]				
3	4.29[a]	4.27 dd (8.5, 8.5)	4.27[a]	4.29[a]	4.30[a]	4.36[a]				
4	4.34[a]	4.30 dd (8.5, 8.5)	4.34[a]	4.34[a]	4.31[a]	4.30[a]				
5	4.03[a]	4.02 m	4.02[a]	4.03 m	4.04 m	4.02 m				
6	4.38[a]	4.37 dd (12, 4)	4.38[a]	4.39[a]	4.36[a]	4.36[a]				
	4.48[a]	4.45 dd (12, 2)	4.45[a]	4.46 dd (10, 2.5)	4.47 dd (11, 2.5)	4.46[a]				
Rha-1						6.66 br s				
2						4.80 m				
3						4.60[a]				
4						4.20[a]				
5						4.60[a]				
6						1.78 d (6)				

The assignment was based upon HOHAHA difference, COSY, HMQC, and HMBC experiments. Coupling constants (Hz) are in parentheses. [a] Overlapped with other signals.

Table 2. ^{13}C NMR Spectral Data for Ilekudinosides A−J (**2**, **6**−**8**, **10**, **13**−**17**) in Pyridine-d_5 at 35 °C[a]

	2	**6**	**7**	**8**	**10**	**13**	**14**	**15**	**16**	**17**
aglycon-1	39.1	38.9	47.3	39.0	39.0	39.3	38.8	39.2	39.0	39.0
2	26.6	26.7	66.9	26.2	26.2	26.7	26.7	26.8	28.5	28.4
3	88.2	89.3	88.4	82.1	88.9	88.6	88.3	88.2	89.0	89.1
4	39.6	39.6	44.7	43.5	39.7	39.6	39.6	39.6	39.6	39.6
5	56.1	56.0	47.7	47.7	56.0	56.4	56.3	56.1	56.1	56.1
6	18.6	18.8	18.2	18.4	18.8	18.8	18.8	18.6	18.6	18.6
7	32.6	33.6	33.2	33.3	33.6	33.9	33.4	35.3	35.5	35.6
8	40.0	40.6	40.8	40.7	40.6	40.4	40.8	42.9	41.8	41.7
9	48.1	47.8	48.2	47.9	47.8	47.8	47.7	50.2	44.9	44.9
10	37.1	37.0	37.8	37.0	37.1	37.1	37.3	37.2	37.0	37.0
11	23.5	24.1	23.9	24.2	24.1	24.1	24.3	71.6	26.3	28.8
12	122.8	128.6	126.2	128.5	128.5	128.6	125.8	33.6	66.1	66.2
13	144.1	139.3	138.4	139.3	139.3	139.0	136.1	143.6	146.4	146.5
14	42.2	42.2	42.6	42.2	42.2	42.4	42.2	45.6	43.9	43.9
15	28.3	29.3	28.7	29.3	29.3	29.7	26.8	29.4	28.9	26.3
16	23.9	26.3	24.7	26.8	26.8	26.5	26.5	27.0	26.6	26.8
17	47.1	48.7	48.4	48.7	48.7	48.8	40.2	46.3	44.1	44.1
18	41.8	54.5	53.4	54.5	54.5	55.6	50.0	135.8	137.6	137.6
19	46.3	72.8	39.2	72.3	72.8	73.0	73.0	73.4	74.4	74.4
20	30.8	42.2	39.4	42.2	42.2	156.0	85.5	85.4	85.7	85.7
21	34.1	26.2	30.8	26.8	26.8	28.6	31.3	29.3	28.9	28.8
22	33.1	37.8	36.8	37.8	37.8	38.5	25.4	32.6	32.9	32.9
23	28.2	28.3	63.8	64.7	28.2	28.2	28.1	28.2	28.3	28.2
24	17.2	17.0	14.7	13.6	17.0	17.2	17.1	17.2	16.8	16.8
25	15.7	15.7	17.4	16.3	15.7	15.9	15.8	17.0	16.6	16.6
26	17.5	17.5	17.6	17.5	17.5	17.5	16.2	17.0	18.2	18.2
27	26.1	24.7	23.8	24.6	24.6	23.8	23.5	21.7	23.5	23.5
28	176.4	177.0	176.2	177.0	177.0	176.3	178.7	175.4	175.4	175.4
29	33.1	27.1	17.8	17.5	27.1	27.6	26.4	26.6	25.2	25.2
30	23.7	16.7	21.3	16.7	16.7	105.5	20.3	20.3	19.5	19.5
sugar at C-3										
GlcA-1		107.3								
2		75.6								
3		78.2								
4		73.5								
5		77.9								
6		172.8								
Ara-1	104.6		106.6	106.6	107.3	105.1	105.1	105.1	104.8	
2	74.9		73.1	73.2	71.9	74.6	74.5	74.5	81.0	
3	81.8		74.9	74.7	84.2	82.8	82.6	82.7	73.4	
4	68.1		69.7	69.6	69.3	69.3	69.3	69.4	68.2	
5	64.7		67.8	66.9	66.9	65.6	65.6	65.6	64.9	
Rha-1	102.0					101.0	101.1	101.1		
2	72.4					72.4	72.4	72.4		
3	72.6					72.4	72.6	72.6		
4	74.0					73.9	74.0	74.0		
5	70.1					69.8	69.9	69.9		
6	18.6					18.3	18.3	18.3		
									Glc at C-2 of Ara	Glc at C-3
Glc (at C-3 of Ara)-1	104.6				106.3	103.1	103.0	103.1	106.0	105.1
2	75.0				75.8	84.6	84.7	84.6	76.4	83.5
3	78.3				78.4	78.3	78.4	78.4	78.2	78.0
4	71.5				71.7	71.0	71.0	71.0	71.7	71.8
5	78.6				78.7	78.4	78.5	78.5	78.2	78.0
6	62.3				62.8	62.5	62.5	62.5	62.7	62.9
Glc(at C-2 of Glc)-1						106.3	106.4	106.3	106.1	
2						76.2	76.2	76.2		77.1
3						78.2	78.3	78.3		78.4
4						70.6	70.6	70.7		71.7
5						78.8	78.9	78.9		78.2
6						61.9	62.0	62.1		62.8
sugar at C-28										
Glc-1	95.8	95.9	95.7	95.9	95.9	95.1				
2	74.2	74.1	74.1	74.1	74.1	75.5				
3	79.3	79.0	78.9	79.0	79.0	80.0				
4	71.3	71.4	71.4	71.4	71.4	71.6				
5	78.3	79.2	79.2	79.2	79.2	79.0				
6	62.6	62.5	62.5	62.5	62.5	62.3				
Rha-1						101.4				
2						72.5				
3						72.6				
4						74.0				
5						69.7				
6						18.7				

[a] Assignments were based on COSY, HMQC, and HMBC experiments.

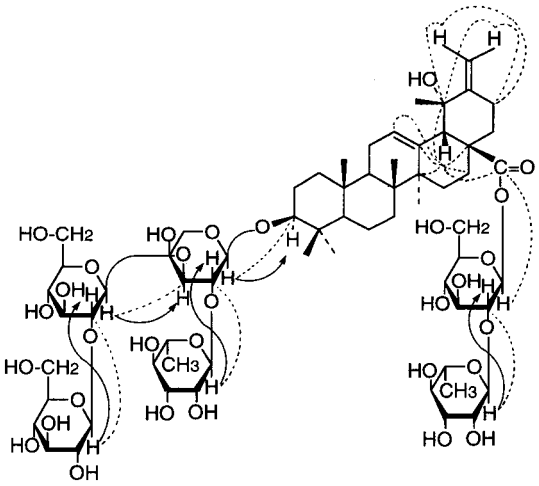


Figure 1. Key ROEs (→) and HMBC (···) correlations for compound **13**.

Table 3. ACAT Inhibitory Activity of Compounds **1**−**17**[a]

compound	1 mg/mL	0.2 mg/mL	0.05 mg/mL
1	64.3	36.5	15.7
2	25.4	negative	negative
3	20.0	negative	negative
4	negative	negative	negative
5	63.9	29.0	31.7
6	12.5	negative	NT[b]
7	35.5	negative	negative
8	18.9	negative	negative
9	72.8	negative	negative
10	negative	negative	NT[b]
11	31.8	10.3	10.9
12	negative	10.6	negative
13	60.0	negative	NT[b]
14	negative	negative	negative
15	negative	negative	negative
16	24.1	negative	NT[b]
17	43.0	negative	negative

[a] For protocols used, see Experimental Section. [b] NT = not tested.

elucidated as 3-*O*-*β*-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl pomolic acid 28-*O*-*β*-D-glucopyranosyl ester.

Ilekudinoside F (**13**) had a molecular formula $C_{65}H_{104}O_{31}$ from the observed positive FABMS *m*/*z* 1404 $[M + Na]^+$ and its ^{13}C NMR data. The ^1H NMR spectrum revealed the presence of six tertiary methyl groups (*δ* 0.92, 1.12, 1.14, 1.17, 1.61, 1.75), a methine proton [*δ* 3.06 (br s)], two exocyclic methylene protons [*δ* 4.71 (br s), 4.96 (br s)], a trisubstituted olefinic proton [*δ* 5.60 (dd, *J* = 3, 3 Hz)], and six anomeric protons [*δ* 4.76 (d, *J* = 5 Hz), 5.16 (d, *J* = 8 Hz), 5.28 (d, *J* = 8 Hz), 6.18 (d, *J* = 8 Hz), 6.33 (br s), 6.66 (br s)]. The methine proton at *δ* 3.06 was assigned to H-18 of a pomolic acid-type triterpene, having correlations with C-12 (*δ* 128.6), C-13 (*δ* 139.0), C-14 (*δ* 42.4), C-16 (*δ* 26.5), C-17 (*δ* 48.8), C-19 (*δ* 73.0), and C-28 (*δ* 176.3) in the HMBC spectrum. Moreover, long-range couplings were also observed between exocyclic methylene protons and C-19 (Figure 1). Thus, the aglycon of this compound was 20(30)-didehydropomolic acid. From the sugar analysis of **13** after hydrolysis, sugar chains were composed of L-arabinose, L-rhamnose, and D-glucose. Sugar sequences were determined using ROE difference and HMBC experiments. The anomeric configurations of the rhamnose moieties were deduced to be both α from the same evidence as **2**. Therefore, the structure of ilekudinoside F was determined as 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl(1→3)-[α-L-rhamnopyranosyl(1→2)]-α-L-arabinopyranosyl pomolic acid 28-*O*-α-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranosyl ester.

The ^{13}C NMR data for ilekudinoside G (**14**) suggested the sugar sequence at C-3 to be the same as **13**. The ^1H NMR spectrum revealed seven singlet methyls (*δ* 0.80, 0.88, 1.18, 1.22, 1.23, 1.47, 1.55), a methine proton [*δ* 2.57 (br s)], and a trisubstituted olefinic proton signal [*δ* 6.16 (m)] in the aglycon moiety. The methine proton was assigned to H-18, which correlated to C-17 (*δ* 40.2) and C-28 (*δ* 178.7) in the HMBC spectrum and to the carbon at *δ* 50.0 in the HMQC spectrum. HMBC correlations between H-29 (*δ* 1.55) and C-18 (*δ* 50.0), C-19 (*δ* 73.0), C-20 (*δ* 85.5) and between H-30 (*δ* 1.47) and C-19, C-20, C-21 (*δ* 31.3) led us to conclude that the structure of ilekudinoside G (**14**) is 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl-[α-L-rhamnopyranosyl(1→2)]-α-L-arabinopyranosyl 3*β*,19α-dihydroxy-urs-12-en-28,20*β*-olide.

The FABMS of ilekudinoside H (**15**) showed a pseudomolecular ion peak at *m*/*z* 1112 $[M + Na]^+$, 16 mass units higher than that of **14**, and the ^{13}C NMR data were consistent with the molecular formula $C_{53}H_{84}O_{23}$. The ^{13}C NMR spectrum was very similar to that of kudinoside F[3a] in the aglycon moiety and to that of **14** in the sugar moiety. Using ROE difference and HMBC experiments, the structure of ilekudinoside H (**15**) was elucidated as 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl-[α-L-rhamnopyranosyl(1→2)]-α-L-arabinopyranosyl *γ*-kudinlactone.[3a]

Ilekudinosides I (**16**) and J (**17**) had the same aglycon as kudinoside E[3a] and diglycoside unit at C-3 by comparing their ^{13}C NMR spectra with that of kudinoside E. On acid hydrolysis, compound **16** gave L-arabinose and D-glucose, and **17** gave D-glucose as the sugar moiety. The ROE difference and HMBC NMR spectra showed the sugar sequences to be 3-*O*-*β*-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl in **16** and 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl in **17**. Thus, the structures of **16** and **17** were assigned as 3-*O*-*β*-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl *β*-kudinlactone and 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl *β*-kudinlactone, respectively.

The ACAT inhibitory activity for the *I. kudincha* isolates is listed in Table 3. Compounds **1** and **5** exhibited inhibitory activity, but the activities of these compounds were weaker than those of several nonglycosidic triterpenoids isolated from the diethyl ether layer of a methanol extract of the same plant.[1]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 polarimeter. ^1H and ^{13}C NMR spectra were run on a JEOL α-400 NMR spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C) at 35 °C in pyridine-d_5 with tetramethylsilane as an internal standard. Inverse-detected heteronuclear correlations were measured using the HMQC (optimized for $^1J_{C-H} = 145$ Hz) and HMBC (optimized for $^nJ_{C-H} = 8$ Hz) pulse sequences with a pulsed-field gradient. FABMS were recorded on a JEOL JMS−SX102A mass spectrometer in a positive mode using *m*-nitrobenzyl alcohol as a matrix. HPLC was carried out with a JASCO system 800. Si gel 60 (Merck 70−230 mesh) was used for column chromatography. Precoated Si gel Kieselgel 60 F_{254} plates (0.25 mm thick) were used for TLC, and the spots were detected by spraying with 50% H_2SO_4, followed by heating. Radioactivities were measured on a Aloka LSC-700 liquid scintillation system.

Plant Material. The leaves of *Ilex kudincha* C. J. Tseng were collected at Daxin Prefecture, Guangxi Province, People's Republic of China, in December 1996. The plant material was authenticated by Prof. Zhaoguang Liu, Chengdu Institute of Biology, Academia Sinica, People's Republic of China, and a

voucher specimen has been deposited at the Herbarium of the School of Pharmaceutical Sciences, University of Shizuoka.

Extraction and Isolation. Air-dried and powdered leaves of *I. kudincha* (5 kg) were extracted with boiling H_2O for 4 h twice. The H_2O extract was passed through a porous polymer gel (Mitsubishi Diaion HP-20) column (9 × 80 cm), and the adsorbed material was eluted with 40% aqueous MeOH and MeOH after washing with H_2O. A part (100 g) of the MeOH eluate (315 g) was subjected to a Si gel column (9 × 37 cm) using $CHCl_3$−MeOH−H_2O (70:30:5) as an eluent to give fractions A−N. Fractions E (7.95 g), G (13.09 g), H (30.34 g), and K (7.48 g) were further separated by preparative HPLC using a reversed-phase column (ODS, PhA) with a CH_3CN−H_2O [or with a trifluoroacetic acid (TFA)] solvent system, to afford **1** (7 mg), **2** (16 mg), **3** (87 mg), **4** (181 mg), **5** (80 mg), **6** (228 mg), **7** (20 mg), **8** (22 mg), **9** (22 mg), **10** (24 mg), **11** (188 mg), **12** (264 mg), **13** (20 mg), **14** (9 mg), **15** (86 mg), **16** (11 mg), and **17** (21 mg).

Ilekudinoside A (2): amorphous powder, $[\alpha]^{23}_D$ −13.8° (*c* 0.78, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 1082 $[M + Na]^+$.

Ilekudinoside B (6): amorphous powder, $[\alpha]^{23}_D$ −15.3° (*c* 0.49, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 833 $[M + Na]^+$.

Ilekudinoside C (7): amorphous powder, $[\alpha]^{23}_D$ +8.6° (*c* 0.66, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 805 $[M + Na]^+$.

Ilekudinoside D (8): amorphous powder, $[\alpha]^{23}_D$ +11.5° (*c* 2.16, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 805 $[M + Na]^+$.

Ilekudinoside E (10): amorphous powder, $[\alpha]^{23}_D$ −0.7° (*c* 0.51, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 951 $[M + Na]^+$.

Ilekudinoside F (13): amorphous powder, $[\alpha]^{23}_D$ +6.4° (*c* 0.65, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 1404 $[M + Na]^+$.

Ilekudinoside G (14): amorphous powder, $[\alpha]^{23}_D$ −60.6° (*c* 0.45, pyridine); ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 1096 $[M + Na]^+$.

Ilekudinoside H (15): amorphous powder, $[\alpha]^{23}_D$ −99.3° (*c* 2.34, pyridine); ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 1112 $[M + Na]^+$.

Ilekudinoside I (16): amorphous powder, $[\alpha]^{23}_D$ −49.3° (*c* 0.35, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 803 $[M + Na]^+$.

Ilekudinoside J (17): amorphous powder, $[\alpha]^{23}_D$ −21.9° (*c* 2.11, MeOH); ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 833 $[M + Na]^+$.

Sugar Analysis of Saponins (2, 6, 7, 8, 10, 13−17). Each saponin (1 mg) was treated with dioxane (50 μL) and 2 M HCl (50 μL) for 1 h at 100 °C. The reaction mixture was diluted with H_2O and extracted with EtOAc three times. The H_2O layer of the reaction mixture for **6** was treated with Ag_2CO_3, and the supernatant was concentrated. The H_2O layer of the reaction mixture for each of **2**, **7**, **8**, **10**, **13**−**17** was passed through an Amberlite IRA-60E column (6 × 50 mm), and the eluate was concentrated. Each monosaccharide fraction was disolved in pyridine (25 μL) and stirred with D-cysteine methyl ester (3 mg) for 1.5 h at 60 °C. To the reaction mixture, hexamethyldisilazane (10 μL) and trimethylsilyl chloride (10 μL) were added, and the reaction mixture was stirred for 30 min at 60 °C. The supernatant was injected to gas chromatography, conditions: column, Supelco SPB-1® 0.25 mm × 27 m; column temperature, 230 °C; carrier gas, N_2; t_R, L-arabinose (8.3 min), D-arabinose (8.6 min), L-rhamnose (9.6 min), D-rhamnose (9.2 min),[16] D-glucose (13.8 min), L-glucose (13.3 min), D-glucuronic acid (10.8 min), L-glucuronic acid (10.6 min).[17] D-Glucose was detected in **17**. D-Glucose and D-glucuronic acid were detected in **6**. D-Glucose and L-arabinose were detected in **7**, **8**, **10**, and **16**. D-Glucose, L-arabinose, L-rhamnose were detected in **2**, **13**, **14**, and **15**.

Bioassay. Seventeen saponins were estimated for ACAT using the same procedure as reported before,[1] and the data obtained are listed in Table 3.

Acknowledgment. We thank the staff of the Central Analytical Laboratory of the University of Shizuoka for measurement of the mass spectra.

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NP990128Y