Triterpene Saponins from the Leaves of *Ilex pernyi*

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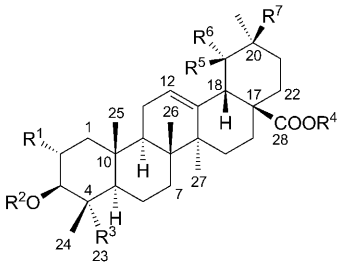
Eight new triterpene saponins, ilexpernosides C–J (**1**–**8**, resp.), and eight known triterpene saponins were isolated from the 70% EtOH extract of the leaves of *Ilex pernyi*. The structures of the new compounds were elucidated by spectroscopic data and chemical degradation.

Introduction. – *Ilex pernyi* FRANCH. (Aquifoliaceae), an evergreen shrub, is distributed mainly in the Yangtze River valley and south region of Qinling Mountains in the P. R. China [1]. Its leaves are used as folk medicine to treat tussis and febris in China [2]. Several compounds isolated from the genus *Ilex* have been reported to possess interesting bioactivities. Triterpenes from *I. kudincha* [3][4], *I. cornuta*, and *I. latifolia* [5] exhibit inhibitory activity on acyl CoA cholesteryl acyl transferase (ACAT). Adenosine, isolated from *I. cornuta*, enhances coronary blood flow [6]. Triterpene saponins from *I. oblonga* inhibit tobacco mosaic virus (TMV) [7].

As a part of the systematic chemical investigation on this genus [8][9], we recently investigated the saponin constituents of *I. pernyi* [10]. Herein, we report the structural elucidation of the eight new compounds, Ilexpernosides C–J (**1**–**8**, resp.), isolated from the leaves of *I. pernyi*, together with eight known compounds **9**–**16**.

Results and Discussion. – Compound **1** was obtained as a colorless gum. The HR-ESI-MS showed an $[M+Na]^+$ ion at 673.3944, in accordance with a molecular formula of $C_{36}H_{58}O_{10}$. Positive results for both *Liebermann–Burchard* and *Molish* reactions indicated a triterpene saponin. The ^{13}C-NMR spectrum (*Tables 1* and *2*) of **1** revealed 36 C-atom signals, of which 6 were assigned to a hexosyl unit, and the remaining 30 to the aglycon.

The ^{1}H-NMR spectrum (*Table 3*) indicated the presence of five Me *singlet*s at δ(H) 0.88, 0.91, 1.04, 1.38, and 1.63, one Me *doublet* at δ(H) 1.06 ($J = 6.5$), one O-bearing CH H-atom at δ(H) 4.21 (*dd*, $J = 12.0, 4.5$, H–C(3)), one oxygenated CH_2 group at δ(H) 3.66 and 4.28 (*AB*, $J = 11.0$, CH_2(23)), and one olefinic H-atom at δ(H) 5.54 (br. *s*, H–C(12)) in the aglycon moiety. The ^{13}C-NMR spectrum for aglycon moiety of **1** exhibited the signals due to one CO group at δ(C) 180.5 (*s*), one oxygenated quaternary C-atom at δ(C) 72.4 (*s*), one oxygenated CH group at δ(C) 82.0 (*d*), one oxygenated CH_2 group at δ(C) 64.4 (*t*), and two olefinic C-atoms at δ(C) 127.8 (*d*), 139.7 (*s*). The ^{1}H- and ^{13}C-NMR spectra of **1** were almost superimposable with those of



	R^1	R^2	R^3	R^4	R^5	R^6	R^7
1	H	Glc	CH_2OH	H	Me	OH	H
2	H	GlcA	CH_2OH	H	Me	OH	H
3	OH	(6'-BuO)GlcA	CH_2OH	Glc	Me	H	H
4	H	$Glc^1\rightarrow{}^4$(6'-BuO)GlcA	Me	Glc	H	H	Me
5	H	$Rha^1\rightarrow{}^2Glc$	CH_2OH	H	Me	OH	H
6	H	$Ara^3\leftarrow{}^1Glc$	CH_2OH	Glc	Me	H	H
7	H	$Glc^2\leftarrow{}^1Glc$	CH_2OH	H	Me	OH	H
8	H	$Glc^2\leftarrow{}^1Glc$	CH_2OH	Glc	Me	OH	H
9	H	Glc	CH_2OH	H	H	H	Me
10	H	$Ara^3\leftarrow{}^1Glc$	CH_2OH	H	Me	OH	H
11	H	GlcA(6'-OMe)	CH_2OH	Glc	Me	H	H
12	H	GlcA	Me	Glc	Me	H	H
13	H	GlcA(6'-OMe)	Me	Glc	Me	H	H
14	H	$Ara^2\leftarrow{}^1Rha$	CH_2OH	Glc	H	H	Me
15	H	$Ara^3\leftarrow{}^1Glc$	CH_2OH	Glc	H	H	Me
16	H	GlcA	CH_2OH	Glc	Me	H	H

randiasaponin II (**10**) for the aglycon moiety [11], corresponding to rotundic acid, and the main difference was observed for the signals of the sugar moiety. The NMR spectra of the sugar unit indicated it to be a D-glucose, which was confirmed by acid hydrolysis and GC analysis of the thiazolidine derivative. The correlation between the anomeric H-atom signal of the D-glucose at δ(H) 5.08 (d, $J = 8.0$) and the C-atom signal at δ(C) 82.0 (C(3) of aglycon) in the HMBC spectrum suggested that the glucose was attached to C(3) of the aglycon. Meanwhile, the anomeric configuration of D-glucose was deduced to be β from the coupling constant value observed for the H–C(1′) ($J = 8.0$). Hence, the structure of **1** was established as (3β)-3-(β-D-glucopyranosyloxy)-19,23-dihydroxyurs-12-en-28-oic acid, and named ilexpernoside C.

Compound **2**, a colorless gum, exhibited a $[M + Na]^+$ peak at m/z 687.3716 by HR-ESI-MS, which was consistent with the molecular formula $C_{36}H_{56}O_{11}$, indicating 9 degrees of unsaturation. The ^{1}H- and ^{13}C-NMR spectra of **2** displayed many similarities with those of **1**, except for the sugar moiety, indicating that **1** and **2** have the same aglycon (rotundic acid). The sugar moiety was elucidated to be a D-glucuronic acid (GlcA) by comparison of the NMR signals for the sugar unit of **2** with the corresponding signals of cynarasaponin C (**12**) [12], a saponin isolated from *I. pernyi* as well. Acid hydrolysis, together with GC analysis, confirmed this elucidation. The β-linkage of the glucuronic acid moiety was suggested by the J value of the anomeric H-atom ($J = 7.5$). Thus, the structure of **2** was established as (3β)-19,23,28-trihydroxy-28-

Table 1. *^{13}C-NMR Data for the Aglycons of* **1**–**8** (125 MHz, C_5D_5N). δ in ppm.

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	38.4	38.5	47.5	38.6	39.1	39.0	38.6	38.7
2	25.6	25.9	66.7	26.1	26.3	26.1	25.8	25.8
3	82.0	82.0	88.1	89.2	81.4	81.7	82.7	82.7
4	43.2	43.3	44.6	39.5	43.4	43.4	43.3	43.4
5	47.4	47.4	47.0	55.7	47.7	47.3	48.0	48.0
6	18.1	18.2	18.0	18.4	18.2	18.0	18.3	18.3
7	32.9	33.1	33.0	33.1	33.1	33.0	33.1	33.1
8	40.1	40.2	40.1	39.9	40.2	40.0	40.2	40.4
9	47.5	47.6	48.0	48.0	47.8	48.0	47.6	47.7
10	36.6	36.7	37.6	36.9	36.7	36.7	36.7	36.7
11	23.8	23.9	23.6	23.4	23.9	23.6	23.9	24.0
12	127.8	127.9	126.0	122.8	127.9	126.0	127.9	128.3
13	139.7	139.8	138.3	144.1	139.8	138.3	139.9	139.2
14	41.8	42.0	42.4	42.1	42.0	42.3	42.0	42.0
15	29.1	29.2	28.5	28.2	29.2	28.6	29.2	29.1
16	26.1	26.2	24.5	23.7	26.3	24.5	26.2	26.0
17	48.0	48.2	48.2	47.0	48.2	48.2	48.1	48.5
18	54.3	54.5	53.2	41.7	54.5	53.2	54.5	54.3
19	72.4	72.5	39.2	46.2	72.5	38.8	72.5	72.5
20	42.1	42.2	39.0	30.8	42.2	39.2	42.2	42.0
21	26.7	26.8	30.7	34.0	27.0	30.6	26.8	26.6
22	38.3	38.4	36.7	32.5	38.4	36.7	38.4	37.6
23	64.4	64.3	63.4	28.0	63.8	64.1	65.0	65.0
24	13.4	13.5	14.6	16.9	14.0	13.5	13.3	13.3
25	15.7	16.0	17.3	15.5	16.0	21.1	15.9	16.1
26	17.3	17.1	17.5	17.4	17.1	16.2	17.1	17.3
27	24.6	24.6	23.6	26.5	24.6	23.6	24.6	24.5
28	180.5	180.6	176.1	176.4	180.5	176.1	180.5	176.9
29	26.8	27.0	17.6	33.1	26.8	17.2	27.0	26.9
30	16.5	16.7	21.2	23.6	16.7	17.6	16.7	16.6

oxours-12-en-3-yl β-D-glucopyranosiduronic acid, for which the trivial name ilexpernoside D was proposed.

Compound **3** was obtained as a white, amorphous powder. The positive HR-ESI-MS spectrum exhibited a $[M+Na]^+$ peak at m/z 905.4835, corresponding to the molecular formula $C_{46}H_{74}O_{16}$. The ^{1}H-NMR spectrum (*Table 3*) indicated the presence of four Me *singlet*s at δ(H) 0.96, 1.00, 1.06, 1.12, two Me *doublet*s at δ(H) 0.84 ($J = 5.5$) and 0.88 ($J = 6.0$), two oxygenated CH groups at δ(H) 4.19–4.21 (*m*, H–C(2)) and 4.22–4.25 (*m*, H–C(3)), an oxygenated CH_2 group at δ(H) 3.66, 4.46 (*AB*, $J = 11.0$, CH_2(23)), and one olefinic H-atom at δ(H) 5.38 (*t*, $J = 3.0$, H–C(12)) in the aglycon moiety. The ^{13}C-NMR spectrum for the aglycon moiety of **3** (*Table 1*) exhibited the signals due to one ester CO group at δ(C) 176.1 (*s*), one oxygenated CH_2 group at δ(C) 63.4 (*t*), two oxygenated CH groups at δ(C) 66.7 (*d*), 88.1 (*d*), and two olefinic C-atoms at δ(C) 126.0 (*d*) and 138.3 (*s*). The data above indicated that compound **3** may have the same aglycon as ilekudinoside C [4]. Comparison of the NMR signals of the sugar moiety of **3** with those of 1-*O*-{(3β)-3-[(6-butyl-β-D-glucopyranuronosyl)oxy]-28-

Table 2. *^{13}C-NMR Data for the Sugar Moieties of* **1**–**8** (125 MHz, C_5D_5N). δ in ppm.

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
3-*O*-Sugar	Glc[a])	GlcA[a])	GlcA	GlcA	Glc	Ara[a])	Glc	Glc
1	105.6	106.1	105.8	107.0	104.5	106.4	103.7	103.7
2	75.6	75.3	75.1	74.6	77.2	71.8	83.9	84.0
3	78.4	78.0	77.8	76.1	79.8	84.0	78.3	78.4
4	71.4	73.3	72.9	82.4	72.0	69.1	71.1	71.1
5	78.0	77.7	76.9	75.3	77.9	66.9	77.9	78.0
6	62.6	173.0	169.7	169.5	62.7		62.6	62.6
Ester moiety COOBu								
1			65.1	65.5				
2			30.7	30.7				
3			19.1	19.3				
4			13.6	13.8				
Terminal sugar				Glc	Rha[a])	Glc	Glc	Glc
1				104.9	101.4	106.2	105.8	105.9
2				74.8	72.3	75.6	76.7	76.7
3				78.2	72.4	78.2	77.9	78.0
4				71.6	74.0	71.4	71.2	71.2
5				78.5	69.4	78.6	78.2	78.2
6				62.1	18.5	62.5	62.4	62.4
28-*O*-Sugar			Glc	Glc		Glc		Glc
1			95.6	95.7		95.6		95.7
2			74.0	74.1		73.9		74.0
3			78.8	78.9		78.7		78.8
4			71.1	71.0		70.9		71.1
5			79.1	79.4		79.1		79.1
6			62.2	62.5		62.1		62.2

[a]) Glc = β-D-glucopyranose; GlcA = β-D-glucuronopyranose; Ara = α-L-arabinopyranose; Rha = α-L-rhamnopyranose.

oxoolean-12-en-28-yl}-β-D-glucopyranose permitted the assignment of the sugar moiety of **3** as a D-glucuronyl moiety esterified at C(6′) with BuOH, and a D-glucose [13]. This assignment was confirmed by acid hydrolysis and GC analysis. Furthermore, the sites of glycosylation and the location of the *O*-Bu group were also established by HMBC experiments showing long-range correlations between H–C(1′) of the D-glucuronyl moiety at δ(H) 5.23 (*d*, $J = 7.5$) and C(3) of aglycon at δ(C) 88.1, H–C(1″) of the D-glucose (δ(H) 6.24 (*d*, $J = 8.0$) and C(28) (δ(C) 176.1) of the aglycon, and CH_2(1‴) of the butyl (δ(H) 4.14–4.17 (*m*) and 4.17–4.21 (*m*)) and C(6′) (δ(C) 169.7) of the D-glucuronyl moiety. Thus, the structure of **3** was assigned as 1-*O*-{(2α,3β)-3-[(6-butyl-β-D-glucopyranuronosyl)oxy]-2,23-dihydroxy-28-oxours-12-en-28-yl}-β-D-glucopyranose, and named ilexpernoside E.

Compound **4**, a white and amorphous powder, was assigned the molecular formula $C_{52}H_{84}O_{19}$ (HR-ESI-MS *m*/*z* 1035.5496, $[M + Na]^+$). The ^{1}H- and ^{13}C-NMR spectra of **4** were almost superimposable with those of 1-*O*-(3β)-3-{[4-*O*-(β-D-glucopyranosyl)-β-D-glucopyranuronosyl]oxy}-28-oxoolean-12-en-28-yl]-β-D-glucopyranose except that **4**

Table 3. *^{1}H-NMR Data of* **1**–**4** (500 MHz, C_5D_5N). δ in ppm, J in Hz.

Position	**1**	**2**	**3**	**4**
Triterpene moiety				
2	1.82–1.86 (*m*), 2.20–2.24 (*m*)	1.95–1.98 (*m*), 2.23–2.26 (*m*)	4.19–4.21 (*m*)	1.82–1.85 (*m*), 2.04–2.07 (*m*)
3	4.21 (*dd*, *J* = 12.0, 4.5)	4.30–4.33 (*m*)	4.22–4.25 (*m*)	3.27 (*dd*, *J* = 11.7, 3.9)
12	5.54 (br. *s*)	5.55 (br. *s*)	5.38 (*t*, *J* = 3.0)	5.40 (br. *s*)
18	2.98 (*s*)	3.00 (*s*)	2.47 (*d*, *J* = 11.5)	3.18 (*dd*, *J* = 13.3, 3.8)
23	3.66, 4.28 (*AB*, *J* = 11.0)	3.68, 4.32 (*AB*, *J* = 10.0)	3.66, 4.46 (*AB*, *J* = 11.0)	1.25 (*s*)
24	0.91 (*s*)	0.92 (*s*)	0.96 (*s*)	0.94 (*s*)
25	0.88 (*s*)	0.88 (*s*)	1.00 (*s*)	0.80 (*s*)
26	1.04 (*s*)	1.06 (*s*)	1.12 (*s*)	1.07 (*s*)
27	1.63 (*s*)	1.67 (*s*)	1.06 (*s*)	1.25 (*s*)
29	1.38 (*s*)	1.39 (*s*)	0.88 (*d*, *J* = 6.0)	0.89 (*s*)
30	1.06 (*d*, *J* = 6.5)	1.08 (*d*, *J* = 6.5)	0.84 (*d*, *J* = 5.5)	0.86 (*s*)
3-*O*-Sugar	Glc[a])	GlcA[a])	GlcA	GlcA
1	5.08 (*d*, *J* = 8.0)	5.23 (*d*, *J* = 7.5)	5.23 (*d*, *J* = 7.5)	4.97 (*d*, *J* = 7.7)
2	3.98 (*t*, *J* = 8.0)	4.11 (*t*, *J* = 8.0)	4.10–4.13 (*m*)	4.09 (*t*, *J* = 8.5)
3	4.13 (*t*, *J* = 9.0)	4.21 (*t*, *J* = 9.0)	4.08–4.11 (*m*)	4.29–4.32 (*m*)
4	4.15 (*t*, *J* = 9.5)	4.54 (*t*, *J* = 10.0)	4.37–4.40 (*m*)	4.52 (*t*, *J* = 9.6)
5	3.85–3.88 (*m*)	4.56 (*t*, *J* = 9.5)	4.42–4.45 (*m*)	4.67 (*d*, *J* = 9.8)
6	4.32 (*dd*, *J* = 12.0, 5.0), 4.46 (br. *d*, *J* = 10.5)			
Ester moiety COOBu				
1			4.14–4.17 (*m*), 4.17–4.21 (*m*)	4.28–4.31 (*m*), 4.40–4.43 (*m*)
2			1.51–1.54 (*m*)	1.59–1.62 (*m*)
3			1.24–1.27 (*m*)	1.27–1.31 (*m*)
4			0.71 (*t*, *J* = 7.5)	0.74 (*t*, *J* = 7.3)
Terminal sugar				Glc
1				5.10 (*d*, *J* = 7.9)
2				3.98–4.02 (*m*)
3				4.16–4.19 (*m*)
4				4.13–4.16 (*m*)
5				3.96–3.99 (*m*)
6				4.25–4.29 (*m*), 4.44–4.47 (*m*)
28-*O*-Sugar			Glc	Glc
1			6.24 (*d*, *J* = 8.0)	6.33 (*d*, *J* = 8.0)
2			4.16–4.19 (*m*)	4.20 (*t*, *J* = 8.5)
3			4.25 (*t*, *J* = 8.5)	4.26–4.29 (*m*)
4			4.33 (*t*, *J* = 9.0)	4.37 (*t*, *J* = 9.1)
5			3.97–4.01 (*m*)	4.00–4.03 (*m*)
6			4.35–4.38 (*m*), 4.40–4.43 (*m*)	4.37–4.41 (*m*), 4.53–4.56 (*m*)

[a]) Glc = β-D-glucopyranose; GlcA = β-D-glucuronopyranose.

showed additional *O*-butyl signals (δ(H) 0.74 (*t*, $J = 7.3$); δ(C) 65.5 (*t*), 30.7 (*t*), 19.3 (*t*) and 13.8 (*q*)) [14], suggesting that **4** is a butyl ester derivative of the latter compound. The sugar moieties were confirmed by acid hydrolysis and GC analysis. Location of the *O*-Bu group and the sites of glycosylation were also confirmed by HMBC experiments, showing long-range correlations between CH_2(1‴′) of the butyl group (δ(H) 4.28–4.31 (*m*) and 4.40–4.43 (*m*)) and C(6′) (δ(C) 169.5) of the D-glucuronyl moiety, H–C(1′) of the D-glucuronyl moiety (δ(H) 4.97 (*d*, $J = 7.7$)) and C(3) (δ(C) 89.2) of aglycon, H–C(1″) of the D-glucose (δ(H) 5.10 (*d*, $J = 7.9$)) and C(4′) (δ(C) 82.4) of D-glucuronyl moiety, H–C(1‴) of the D-glucose (δ(H) 6.33 (*d*, $J = 8.0$)) and C(28) (δ(C)176.4) of the aglycon. Therefore, the structure of **4** was determined as 1-*O*-[(3β)-3-{[6-butyl-4-*O*-(β-D-glucopyranosyl)-β-D-glucopyranuronosyl]oxy}-28-oxoolean-12-en-28-yl]-β-D-glucopyranose, for which the trivial name ilexpernoside F was given.

Compound **5**, obtained as a colorless gum, showed a quasimolecular-ion peak ($[M+H]^+$) at *m*/*z* 797.4675 in the HR-ESI-MS, corresponding to the molecular formula $C_{42}H_{68}O_{14}$. Analysis of the ^{1}H- and ^{13}C-NMR spectroscopic data indicated that **5** possessed the same aglycon as that of **1** and **2**, but differed in a sugar moiety. The ^{13}C-NMR chemical shift of C(3) (δ(C) 81.4) indicated that a sugar moiety was attached to C(3) of the aglycon. Acid hydrolysis of **5** afforded D-glucose and L-rhamnose. The presence of two anomeric H-atom signals (*Table 4*) at δ(H) 5.14 (*d*, $J = 8.0$) and 6.54 (*d*, $J = 1.5$), together with the two corresponding C-atom signals at δ(C) 104.5 and 101.4, indicated that **5** is a saponin with a disaccharide of D-glucose and L-rhamnose. The linkage and the anomeric configuration of the sugar units agreed with the disaccharide found in ilekudinoside K [8]. Hence, compound **5** was elucidated as (3β)-3-{[2-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy}-19,23-dihydroxyurs-12-en-28-oic acid, named ilexpernoside G.

Compound **6**, a colorless gum, showed the molecular formula $C_{47}H_{76}O_{18}$ (HR-ESI-MS *m*/*z* 929.5082 $[M+H]^+$). Acid hydrolysis afforded D-glucose and L-arabinose. The ^{1}H-NMR spectrum (*Table 4*) indicated the presence of four Me *singlet*s at δ(H) 0.84, 0.89, 0.93, and 1.10, two Me *doublet*s at δ(H) 0.89 ($J = 6.5$) and 1.12 ($J = 6.0$), one oxygenated CH group at δ(H) 4.21–4.24 (*m*, H–C(3)), one oxygenated CH_2 group at δ(H) 3.67 and 4.28 (*AB*, $J = 10.5$, CH_2(23)), and one olefinic H-atom at δ(H) 5.41 (br. *s*, H–C(12)) in the aglycon moiety. The ^{13}C-NMR spectrum for the aglycon moiety of **6** (*Table 1*) exhibited signals due to one ester CO group at δ(C) 176.1 (*s*), one oxygenated CH_2 group at δ(C) 64.1 (*t*), one oxygenated CH group at δ(C) 81.7 (*d*), and two olefinic C-atoms at δ(C) 126.0 (*d*) and 138.3 (*s*). The data above indicated that **6** has the same aglycon as 1-*O*-{(3β)-23-hydroxy-3-[(6-methyl-β-D-glucopyranuronosyl)oxy]-28-oxo-urs-12-en-28-yl}-β-D-glucopyranose (**11**) [12]. Detailed ^{1}H- and ^{13}C-NMR data analysis suggested that **6** possessed the same sugar moiety as 1-*O*-[(3β)-3-{[3-*O*-(β-D-glucopyranosyl)-α-L-arabinopyranosyl]oxy}-23-hydroxy-28-oxoolean-12-en-28-yl]-β-D-glucopyranose (**15**), which was also isolated from *I. pernyi* [15]. Confirmation was given from the HMBC correlation signals between H–C(1′) (δ(H) 4.93 (*d*, $J = 7.5$)) of arabinose and C(3) (δ(C) 81.7) of the aglycon, H–C(1″) (δ(H) 5.27 (*d*, $J = 8.0$)) of the terminal glucose and C(3′) (δ(C) 84.0) of the arabinose, and H–C(1‴) (δ(H) 6.23 (*d*, $J = 8.0$)) of glucose and C(28) (δ(C) 176.1) of the aglycon. Thus, compound **6** was elucidated as 1-*O*-[(3β)-3-{[3-*O*-(β-D-glucopyranosyl)-α-L-arabinopyranosyl]oxy}-23-hydroxy-28-oxours-12-en-28-yl]-β-D-glucopyranose, named ilexpernoside H.

Table 4. *^{1}H-NMR Data of* **5**–**8** (500 MHz, C_5D_5N). δ in ppm, J in Hz.

Position	**5**	**6**	**7**	**8**
Triterpene moiety				
2	1.95–1.98 (*m*), 2.31–2.34 (*m*)	1.94–1.97 (*m*), 2.17–2.21 (*m*)	1.88–1.92 (*m*), 2.20–2.23 (*m*)	1.92–1.95 (*m*), 2.14–2.17 (*m*)
3	4.25–4.28 (*m*)	4.21–4.24 (*m*)	4.11–4.15 (*m*)	4.10–4.13 (*m*)
12	5.55 (*t*, *J* = 3.5)	5.41 (br. *s*)	5.55 (br. *s*)	5.51 (*t*, *J* = 3.5)
18	3.00 (*s*)	2.47 (*d*, *J* = 11.5)	3.00 (*s*)	2.89 (*s*)
23	3.71, 4.24 (*AB*, *J* = 10.5)	3.67, 4.28 (*AB*, *J* = 10.5)	3.72, 4.33 (*AB*, *J* = 11.0)	3.73 (*d*, *J* = 11.0), 4.32–4.35 (*m*)
24	1.11 (*s*)	0.89 (*s*)	1.06 (*s*)	1.08 (*s*)
25	0.91 (*s*)	0.84 (*s*)	0.89 (*s*)	0.94 (*s*)
26	1.07 (*s*)	0.93 (*s*)	1.06 (*s*)	1.18 (*s*)
27	1.65 (*s*)	1.10 (*s*)	1.64 (*s*)	1.60 (*s*)
29	1.40 (*s*)	0.89 (*d*, *J* = 6.5)	1.39 (*s*)	1.35 (*s*)
30	1.08 (*d*, *J* = 6.5)	1.12 (*d*, *J* = 6.0)	1.08 (*d*, *J* = 6.5)	1.03 (*d*, *J* = 6.5)
3-*O*-Sugar	Glc[a])	Ara[a])	Glc	Glc
1	5.14 (*d*, *J* = 8.0)	4.93 (*d*, *J* = 7.5)	5.05 (*d*, *J* = 6.5)	5.06 (*d*, *J* = 7.5)
2	4.24–4.27 (*m*)	4.52–4.55 (*m*)	4.12–4.15 (*m*)	4.12–4.15 (*m*)
3	4.08 (*t*, *J* = 8.5)	4.04–4.07 (*m*)	4.17–4.21 (*m*)	4.15–4.18 (*m*)
4	4.13 (*t*, *J* = 9.0)	4.33–4.36 (*m*)	4.14–4.17 (*m*)	4.31–4.35 (*m*)
5	3.77–3.80 (*m*)	3.58–3.61 (*m*), 4.10–4.13 (*m*)	3.75–3.78 (*m*)	3.76–3.79 (*m*)
6	4.31–4.34 (*m*), 4.44–4.47 (*m*)		4.27–4.30 (*m*), 4.45 (br. *d*, *J* = 10.0)	4.30–4.48 (*m*)
Terminal sugar	Rha[a])	Glc	Glc	Glc
1	6.54 (*d*, *J* = 1.5)	5.27 (*d*, *J* = 8.0)	5.35 (*d*, *J* = 7.5)	5.36 (*d*, *J* = 7.5)
2	4.75–4.78 (*m*)	3.97–4.01 (*m*)	4.07–4.10 (*m*)	4.08–4.11 (*m*)
3	4.64–4.67 (*m*)	4.22–4.25 (*m*)	4.17–4.20 (*m*)	4.17–4.21 (*m*)
4	4.28–4.32 (*m*)	4.17–4.21 (*m*)	4.27 (*t*, *J* = 9.0)	4.11–4.13 (*m*)
5	4.77–4.80 (*m*)	3.93–3.96 (*m*)	3.85–3.89 (*m*)	3.87–3.91 (*m*)
6	1.67 (*d*, *J* = 6.0)	4.30–4.33 (*m*), 4.47–4.50 (*m*)	4.40 (*dd*, *J* = 11.0, 3.5), 4.45 (br. *d*, *J* = 10.0)	4.30–4.48 (*m*)
28-*O*-Sugar		Glc		Glc
1		6.23 (*d*, *J* = 8.0)		6.27 (*d*, *J* = 8.0)
2		4.16 (*t*, *J* = 8.5)		4.20 (*t*, *J* = 8.5)
3		4.23–4.26 (*m*)		4.25–4.29 (*m*)
4		4.34 (*t*, *J* = 9.1)		4.28 (*t*, *J* = 9.1)
5		3.95–3.98 (*m*)		4.01–4.04 (*m*)
6		4.35–4.39 (*m*), 4.39–4.42 (*m*)		4.30–4.48 (*m*)

[a]) Glc = β-D-glucopyranose; Ara = α-L-arabinopyranose; Rha = α-L-rhamnopyranose.

Compound **7**, obtained as a white and amorphous powder, was assigned the molecular formula as $C_{42}H_{68}O_{15}$, determined from the quasimolecular-ion peak ($[M + H]^+$) at *m*/*z* 813.4630 in the HR-ESI-MS. Its spectroscopic features indicated **7** to be another rotundic acid disaccharide, differing in the sugar moiety from **1**, **2**, and **5**. Acid hydrolysis of **7** afforded D-glucose only. Careful NMR data analysis indicated that **7** had the same disaccharide chain as ilekudinoside N [8]. The sites of glycosylation were

confirmed by the HMBC correlation signals between H–C(1′) (δ(H) 5.05 (d, J = 6.5)) of inner glucose and C(3) (δ(C) 82.7) of the aglycon, and H–C(1″) (δ(H) 5.35 (d, J = 7.5)) of terminal glucose and C(2′) (δ(C) 83.9) of the inner glucose. Therefore, the structure of **7** was determined as (3β)-3-{[2-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy}-19,23-dihydroxyurs-12-en-28-oic acid, and named ilexpernoside I.

Compound **8** was obtained as a white, amorphous powder. The HR-ESI-MS showed a $[M+H]^+$ ion at m/z 975.5157, in accordance with an empirical molecular formula of $C_{48}H_{78}O_{20}$. The spectroscopic data indicated that **8** was a further rotundic acid trisaccharide, differing in the sugar units from those of **1**, **2**, and **5**. Acid hydrolysis of **8** afforded only D-glucose. The ^{1}H- and ^{13}C-NMR spectra of **8** were superimposable with those of **7**, except for an additional D-glucose. The long-range correlation between H–C(1‴) (δ(H) 6.27 (d, J = 8.0)) of the additional glucose and C(28) (δ(C) 176.9) of the aglycon in HMBC indicated that the additional D-glucose was attached to C(28) of the aglycon. Hence, compound **8** was elucidated as 1-*O*-[(3β)-3-{[2-*O*-(β-D-glucopyranosyl)-α-D-glucopyranosyl]oxy}-23-hydroxy-28-oxours-12-en-28-yl]-β-D-glucopyranose, for which the name ilexpernoside J was given.

The natural occurrence of triterpene saponins possessing butyl-esterified glucuronic acid in the sugar portion is extremely rare [13][16]. The new compounds **3** and **4** are regarded as genuine natural products and not artifacts formed during extraction by BuOH. Such statement was confirmed by an additional experiment in which cynarasaponin C, a known compound isolated from *I. pernyi*, was treated with a slightly acidic (0.001 % (w/v) H_2SO_4) BuOH solution (80° for 2 h) [16], resulting in the detection of only the unreacted cynarasaponin C.

The eight known compounds were identified as (3β)-3-(β-D-glucopyranosyloxy)-hederagenin (**9**) [17], randiasaponin II (**10**) [11], 1-*O*-{(3β)-23-hydroxy-3-[(6-methyl-β-D-glucopyranuronosyl)oxy]-28-oxours-12-en-28-yl}-β-D-glucopyranose (**11**) [12], cynarasaponin C (**12**) [12], 1-*O*-{(3β)-3-[(6-methyl-β-D-glucopyranuronosyl)oxy]-28-oxours-12-en-28-yl}-β-D-glucopyranose (**13**) [12], 1-*O*-[(3β)-3-{[2-*O*-(6-deoxy-α-L-mannopyranosyl)-α-L-arabinopyranosyl]oxy}-23-hydroxy-28-oxoolean-12-en-28-yl]-β-D-glucopyranose (**14**) [18], 1-*O*-[(3β)-3-{[3-*O*-(β-D-glucopyranosyl)-α-L-arabinopyranosyl]oxy}-23-hydroxy-28-oxoolean-12-en-28-yl]-β-D-glucopyranose (**15**) [15], and cynarasaponin E (**16**) [12], on the basis of their NMR and MS data, and by comparison with the literature data. All the eight known compounds were found for the first time in this plant species.

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Experimental Part

General. Column chromatography (CC): silica gel *H* (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Industry*), *ODS* gel (25–40 μm; *Merck*), and *D101* porous polymer resin (*Tianjin Chemical Industry*). Semi-prep. HPLC: *Waters 600* Pump with *600* controller, *Waters C18 Nova-Pak* column (300 × 7.8 mm, 5 μm), with *ELSD* detector (*Alltech)*; flow rate, 2.5 ml/min. GC: *Agilent 6890N* gas chromatograph; capillary column (28 m × 0.32 mm i.d.; *HP-5*); *FID* detector, operated at 260° (column temp. 180°); N_2 as carrier gas (40 ml/min). Optical rotations: *Perkin-Elmer 243B* digital polarimeter. IR spectra: *NEXUS-470 FTIR* (*Nicolet*) spectrometer; KBr pellets; in cm^{-1}. NMR Spectra: *Varian Inova-*

500 and *Varian Unity-500* instruments; at 500 (^{1}H) or 125 MHz (^{13}C) in C_5D_5N at r.t.; δ in ppm rel. to TMS, *J* in Hz. ESI-MS (positive): *QSTAR* (*ABI*, *USA*) mass spectrometer; in *m*/*z*. HR-ESI-MS (positive): *Bruker APEX II FT-ICR-MS* mass spectrometer; in *m*/*z*.

Plant Material. The leaves of *Ilex pernyi* were collected by Dr. *S.-X. Zhou* in April 2005 at the Nature Protect Area of Shennongjia, Hubei Province, China. The identification of the plant was performed by Prof. *P.-F. Tu*, Peking University. A voucher specimen was deposited with the herbarium of Peking University Modern Research Center for Traditional Chinese Medicine (MEC0504).

Extraction and Isolation. The air-dried and powdered leaves (15 kg) of *I. pernyi* were extracted with 70% EtOH (3 × 80 l) at 60° for 2 h. After removal of the solvent under vacuum, the residue was suspended in H_2O (12 l) and partitioned successively with AcOEt (3 × 15 l) and BuOH (3 × 15 l) after being defatted with petroleum ether (PE; 2 × 10 l). 400 g BuOH extract (490 g in total) was subjected to CC (*D101* porous polymer resin) and eluted with H_2O and 10, 30, 50, 70, 95% aq. EtOH, resp. The fractions eluted with 50% and 70% aq. EtOH (97 g) were subjected to CC (SiO_2, $CHCl_3$/MeOH/H_2O 10:1:0 → 1:1:0.1) to afford 6 subfractions (*Fr. 1–6*). *Fr. 2* was subjected to CC (*ODS*, 70% aq. MeOH) to afford hederagenin 3-*O*-*β*-D-glucopyranoside (**9**, 14 mg) and *Fr. 2-1*. *Fr. 2-1* was purified by semi-prep. HPLC (MeOH/0.05% aq. TFA 3:2) to afford **1** (25 mg, t_R: 13.2 min). *Fr. 3* was subjected to CC (*ODS*, MeOH/H_2O, 2:3 → 4:1) to afford *Fr. 3-1–3-5*. Each fraction was separated by semi-prep. HPLC (MeOH/H_2O 3:2 for *Fr. 3-1*; MeOH/0.05% aq. TFA 67:33 for *Fr. 3-2*; MeOH/0.05% aq. TFA 29:21 for *Fr. 3-3*; MeOH/0.05% aq. TFA 13:7 for *Fr. 3-4* and *3-5*) to yield randiasaponin II (**10**, 10 mg, t_R: 22.4 min), 1-*O*-{(3*β*)-23-hydroxy-[(6-methyl-*β*-D-glucopyranuronosyl)oxy]-28-oxours-12-en-28-yl}-*β*-D-glucopyranose (**11**, 11 mg, t_R: 17.6 min), **2** (6 mg, t_R: 19.7 min), cynarasaponin C (**12**, 13 mg, t_R: 30.8 min) and **3** (10 mg, t_R: 27.4 min), resp. *Fr. 4* was subjected to CC (*ODS*, 80% aq. MeOH) to afford **4** (8 mg) and *Fr. 4-1*, which was further purified by CC (SiO_2, $CHCl_3$/MeOH/H_2O 10:1:0.1) to give ursolic acid 1-*O*-{(3*β*)-3-[(6-methyl-*β*-D-glucopyranuronosyl)oxy]-28-oxours-12-en-28-yl}-*β*-D-glucopyranose (**13**, 12 mg). *Fr. 5* was subjected to CC (*ODS*, MeOH/H_2O 3:2 → 4:1) to afford *Fr. 5-1–5-4*. Each fraction was separated by semi-prep. HPLC (MeOH/0.05% aq. TFA 3:2 for *Fr. 5-1*, *5-2*, and *5-3*; MeOH/0.05% aq. TFA 53:47 for *Fr. 5-4*) to yield **5** (70 mg, t_R: 17.0 min), 1-*O*-[(3*β*)-3-{[2-*O*-(6-deoxy-*α*-L-mannopyranosyl)-*α*-L-arabinopyranosyloxy}-23-hydroxy-28-oxoolean-12-en-28-yl]-*β*-D-glucopyranose (**14**, 20 mg, t_R: 43.0 min), **6** (15 mg, t_R: 35.2 min), and 1-*O*-[(3*β*)-3-{[3-*O*-*β*-D-glucopyranosyl-*α*-L-arabinopyranosyl]oxy}-23-hydroxy-28-oxoolean-12-en-28-yl]-*β*-D-glucopyranose (**15**, 20 mg, t_R: 42.4 min), **7** (25 mg, t_R: 26.2 min), and cynarasaponin E (**16**, 15 mg, t_R: 30.0 min), resp. *Fr. 6* was subjected to CC (*ODS*, 40% aq. MeOH) to give *Fr. 6-1*, which was further purified by semi-prep. HPLC (MeOH/0.05% aq. TFA 23:27) to afford **8** (87 mg, t_R: 28.0 min).

Acid Hydrolysis. Each saponin (4 mg) was heated in 4 ml of 10% HCl/dioxane (1:1) at 80° for 4 h. After the dioxane was removed, H_2O (5 ml) was added and the soln. was extracted with AcOEt (5 ml × 3). The aq. fractions were evaporated and the residues were prepared as thiazolidine derivatives for GC analysis according to the methods described in the literature [8].

Ilexpernoside C (*=(3β)-3-(β-D-Glucopyranosyloxy)-19,23-dihydroxyurs-12-en-28-oic Acid*; **1**). Colorless gum. $[\alpha]_D^{25} = +9.6$ (*c* = 2.5, MeOH). IR (KBr): 3420, 2934, 1691, 1453, 1076, 1037. ^{1}H- and ^{13}C-NMR: *Tables 1–3*. ESI-MS (pos.): 673 ($[M+Na]^+$). HR-ESI-MS (pos.): 673.3944 ($[M+Na]^+$, $C_{36}H_{58}NaO_{10}^+$; calc. 673.3928).

Ilexpernoside D (*=(3β)-19,23,28-Trihydroxy-28-oxours-12-en-3-yl β-D-Glucopyranosiduronic Acid*; **2**). Colorless gum. $[\alpha]_D^{25} = +5.15$ (*c* = 0.7, MeOH). IR (KBr): 3415, 2931, 1683, 1202, 1046. ^{1}H- and ^{13}C-NMR: *Tables 1–3*. ESI-MS (pos.): 687 ($[M+Na]^+$). HR-ESI-MS (pos.): 687.3716 ($[M+Na]^+$, $C_{36}H_{56}NaO_{11}^+$; calc. 687.3720).

Ilexpernoside E (*=1*-O-*{(2α,3β)-3-[(6-Butyl-β-D-glucopyranuronosyl)oxy]-2,23-dihydroxy-28-oxo-urs-12-en-28-yl}-β-D-glucopyranose*; **3**). White, amorphous powder. $[\alpha]_D^{25} = -3.33$ (*c* = 0.9, MeOH). IR (KBr): 3421, 2925, 1739, 1587, 1066. ^{1}H- and ^{13}C-NMR: *Tables 1–3*. ESI-MS (pos.): 905 ($[M+Na]^+$). HR-ESI-MS (pos.): 905.4835 ($[M+Na]^+$, $C_{46}H_{74}NaO_{16}^+$; calc. 905.4875).

Ilexpernoside F (*=1*-O-*{(3β)-3-{[6-Butyl-4*-O-*(β-D-glucopyranosyl)-β-D-glucopyranuronosyl]oxy}-28-oxoolean-12-en-28-yl}-β-D-glucopyranose*; **4**). White, amorphous powder. $[\alpha]_D^{25} = +5.71$ (*c* = 0.7, MeOH). IR (KBr): 3421, 2929, 1742, 1632, 1463, 1074. ^{1}H- and ^{13}C-NMR: *Tables 1–3*. ESI-MS (pos.): 1035 ($[M+Na]^+$). HR-ESI-MS (pos.): 1035.5496 ($[M+Na]^+$, $C_{52}H_{84}NaO_{19}^+$; calc. 1035.5505).

Ilexpernoside G (=*(3β)-3-{[2-*O*-(6-Deoxy-α-*L*-mannopyranosyl)-β-*D*-glucopyranosyl]oxy}-19,23-dihydroxyurs-12-en-28-oic Acid*; **5**). Colorless gum. $[\alpha]_D^{25} = -9.0$ ($c = 3.0$, MeOH). IR (KBr): 3422, 2931, 1691, 1628, 1385, 1048. ^{1}H- and ^{13}C-NMR: *Tables 1*, *2*, and *4*. ESI-MS (pos.): 797 ($[M+H]^+$). HR-ESI-MS (pos.): 797.4675 ($[M+H]^+$, $C_{42}H_{69}O_{14}^+$; calc. 797.4687).

Ilexpernoside H (=*1-*O*-[(3β)-3-{[3-*O*-(β-*D*-Glucopyranosyl)-α-*L*-arabinopyranosyl]oxy}-23-hydroxy-28-oxours-12-en-28-yl]-β-*D*-glucopyranose*; **6**). Colorless gum. $[\alpha]_D^{25} = +13.3$ ($c = 1.5$, MeOH). IR (KBr): 3421, 2925, 1727, 1676, 1456, 1075, 1030. ^{1}H- and ^{13}C-NMR: *Tables 1*, *2*, and *4*. ESI-MS (pos.): 929 ($[M+H]^+$). HR-ESI-MS (pos.): 929.5082 ($[M+H]^+$, $C_{47}H_{77}O_{18}^+$; calc. 929.5100).

Ilexpernoside I (=*(3β)-3-{[2-*O*-(β-*D*-Glucopyranosyl)-β-*D*-glucopyranosyl]oxy}-19,23-dihydroxyurs-12-en-28-oic Acid*; **7**). White, amorphous powder. $[\alpha]_D^{25} = +0.029$ ($c = 2.5$, MeOH). IR (KBr): 3451, 2925, 1696, 1457, 1077, 1030. ^{1}H- and ^{13}C-NMR: *Tables 1*, *2*, and *4*. ESI-MS (pos.): 813 ($[M+H]^+$). HR-ESI-MS (pos.): 813.4630 ($[M+H]^+$, $C_{42}H_{69}O_{15}^+$; calc. 813.4636).

Ilexpernoside J (=*1-*O*-[(3β)-3-{[2-*O*-(β-*D*-Glucopyranosyl)-α-*D*-glucopyranosyl]oxy}-23-hydroxy-28-oxours-12-en-28-yl]-β-*D*-glucopyranose*; **8**). White, amorphous powder. $[\alpha]_D^{25} = +13.01$ ($c = 4.6$, MeOH). IR (KBr): 3428, 2928, 1669, 1632, 1074. ^{1}H- and ^{13}C-NMR: *Tables 1*, *2*, and *4*. ESI-MS (pos.): 975 ($[M+H]^+$). HR-ESI-MS (pos.): 975.5157 ($[M+H]^+$, $C_{48}H_{79}O_{20}^+$; calc. 975.5165).

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