Glycosides from the Leaves of *Ilex latifolia*

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Nine new triterpenoid saponins: latifolosides I—Q and three known compounds: Kudinoside A, *cis*-roseoside, and kaempferol-3-*O*-α-*L*-rhamnopyranosyl (1 → 6)-*O*-β-*D*-glucopyanoside were isolated from the leaves of *Ilex latifolia*. Their structures were elucidated by spectroscopic and chemical methods.

Keywords Ku-ding-cha, *Ilex latifolia*, aquifoliaceae, triterpene, glycosides

The leaves of *Ilex latifolia* Thunb are one of the original materials of Ku-ding-cha, a local herb tea in southern China. The taxonomic and morphological aspects in *Ilex* have caused confusions. Especially, the morphology and constituents of two plants (*Ilex latifolia* Thunb and *I. kudincha* C. J. Tseng) are very similar. All of them were known as *Ilex latifolia* Thunb before 1991 and were used as Ku-ding-cha, a potential cardiovascular agent. From the two plants, recently, more than fifty compounds were isolated. These basic chemical data offered important chemical taxonomic evidence. Previously, we reported the isolation and structural elucidation of eight new triterpenoid saponins called latifolosides A—H[1,2] from the leaves of *I. latifolia*, and, now, continued to discuss the isolation and structure elucidation of nine new triterpenoid saponins, latifolosides I—Q (**1**—**9**) and three known compounds, kudinoside A (**10**),[3] *cis*-roseoside (**11**),[4] and kaempferol-3-*O*-α-*L*-rhamnopyanosyl(1→6)-*O*-β-*D*-glucopyranoside (**12**)[5,7] from the title plant.

Results and discussion

The water soluble fraction from the methanol extract of the leaves of *I. latifolia* yields glycosides **1**—**12** using silica and RP-8 column chromatography to repeat chromatographic purification. Saponins **1**—**6** are bisdesmosides and saponin **10** is monodesmoside, which contain aglycones of ursane type. Saponins **7**—**9** possess bisdesmosides with oleanolic acid as the aglycone. Compound **11** is a thirteen-carbon glycoside and compound **12** is a flavonoid glycoside.

Latifoloside I (**1**) was obtained as a white amorphous powder. The negative FAB-MS displayed a quasi-molecular ion peak $[M-H]^-$ at *m/z* 927, whereupon, a molecular formula $C_{47}H_{76}O_{18}$ of **1** was deduced by its DEPT experiment of ^{13}C NMR and the negative FAB-MS. Other fragment ion peaks at *m/z* 765 $[M-H-162]^-$, 603 $[M-H-2\times162]^-$, indicating the respective elimination of one terminal hexosyl and the other terminal hexosyl unit. The ^1H, ^{13}C and HMQC NMR spectra revealed seven methyl signals [δ_H 0.88 (s), 0.96 (d, J = 6.0 Hz), 1.13 (s), 1.16 (s), 1.17 (s), 1.40 (s)] and 1.71 (s), a tri-substituted olefinic proton signal at δ 5.49 in the aglycone moiety, and three anomeric proton and carbon signals {δ_H 4.88 [(d, J = 5.1 Hz), δ_C 107.3], δ_H 5.25 [(d, J = 7.3 Hz), δ_C 106.2] and δ_H 6.30 (d, J = 7.9 Hz), δ_C 95.9]} in the sugar moieties. On acid hydrolysis, **1** yielded glucose and arabinose and an aglycone of ilexgenin B. On alkaline hydrolysis, **1** yielded a prosapogenin and glucose. The hydrolysate of sugar indicated that C-28 position was glycosidation. The presence of signals at δ_C 88.9 and δ_C 177.1 in the ^{13}C NMR spectrum also agreed with the glycosidation at C-3 and C-28. The common *D*-configuration for glucose and *L*-configuration for arabinose were compared with the authentic samples by

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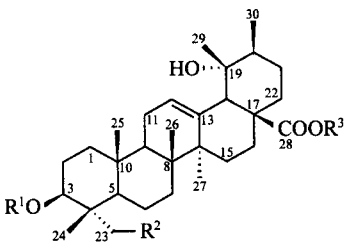
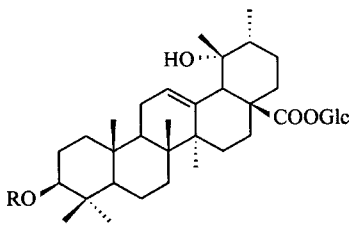
HPLC. Evaluation of spin-spin couplings and chemical shifts showed α-arabinopyranose, β-glucopyranose, respectively. In the HMBC spectrum, the key important correlations were obtained. The correlations were between signals at δ_H 4.88 (Ara-1) and δ_C 88.9 (Agly-C-3), between δ_H 5.25 (Glc-1) and δ_C 84.1 (Ara-3), between δ_H 6.30 (Glc-1′) and δ_C 177.1 (Agly-C-28). These correlations showed that the Ara was bound to the C-3 of aglycone, the Glc was linked at the C-3 of Ara, and another Glc′ was linked at the C-28 of the aglycone. Based on the above results, the structure of latifoloside I (**1**) was elucidated as 3-*O*-β-*D*-glucopyranosyl(1→3)-α-*L*-arabinopyranosyl ilexgenin B-28-*O*-β-*D*-glucopyranosyl ester.



1: R^1=S^1 R^2=H R^3=Glc
2: R^1=S^2 R^2=H R^3=S^4
3: R^1=S^1 R^2=OH R^3=Glc



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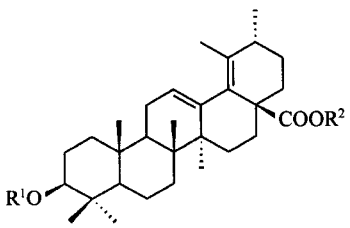
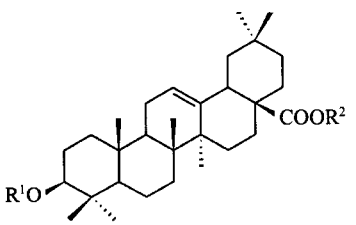
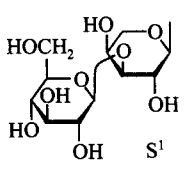


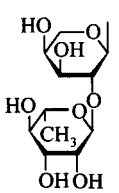
5: R^1=S^2 R^2=Glc
6: R^1=S^3 R^2=S^4



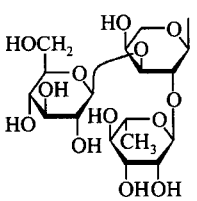
7: R^1=S^3 R^2=Glc
8: R^1=S^2 R^2=S^4
9: R^1=S^3 R^2=S^4



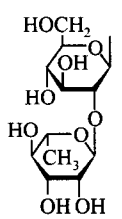
S^1



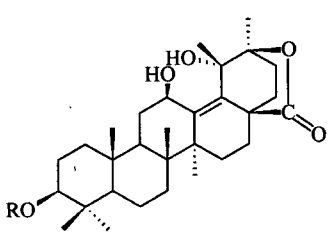
S^2



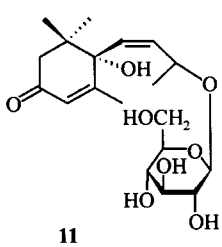
S^3



S^4



10: R=S^3



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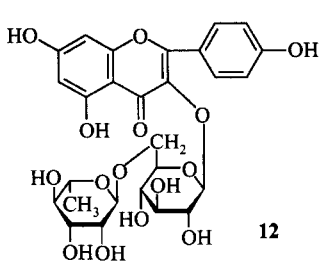

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Latifoloside J (**2**) showed one $[M-H]^-$ ion peak at *m/z* 1057 in the negative FAB-MS. Its molecular formula was deduced to be $C_{53}H_{86}O_{21}$ by its DEPT spectrum of ^{13}C NMR and the negative FABMS. The ^{13}C NMR spectrum of **2** gave four anomeric carbon signals at δ_C 95.1, 101.5, 101.8 and 104.7 and the same aglycone (ilexgenin B) as latifoloside I (**1**). Upon acid hydrolysis, **2** afforded a mixture of glucose, arabinose and rhamnose as sugar moieties. At the same time, the ^1H NMR spectrum showed four anomeric proton signals at

δ_H 4.86 (d, J = 4.8 Hz), 6.14 (br.s), 6.21 (d, J = 7.2 Hz) and 6.67 (br.s). The sugar sequences and linkages were determined by the HMBC spectrum, which afforded the important cross peaks between the anomeric proton signal at δ_H 4.86 (H-1 of Ara) and the carbon signal at δ_C 89.1 (C-3), between the anomeric proton δ_H 6.14 (H-1 of Rha) and the carbon signal at δ_C 76.1 (C-2 of Ara), between the anomeric proton signal at δ_H 6.21 (H-1′ of Glc′) and the carbon signal at δ_C 176.9 (C-28), between the anomeric proton signal at δ_H 6.67 (H-1′ of Rha′) and carbon signal at δ_C 76.2 [C-2′ of Glc′ (linked at C-28 position)]. These evidences indicated that the Ara linked at C-3 position of the aglycone, the Rha linked at C-2 of Ara, the Glc′ linked at C-28 position of the aglycone, and the Rha linked at C-2′ of the Glc′. Thus, latifoloside J (**2**) was identified as 3-*O*-α-*L*-rhamnopyranosyl(1→2)-α-*L*-arabinopyranosyl ilexgein B-28-*O*-α-*L*-rhamnopyranosyl (1→2)-β-*D*-glucopyranosyl ester.

The ^{13}C NMR spectrum of latifoloside K (**3**) showed the same sugar sequences and linkages as the sugar moieties of latifoloside I (**1**). **3** afforded one $[M-H]^-$ ion peak at m/z 943 in the negative FAB-MS, consistent with a molecular formula of $C_{47}H_{76}O_{19}$ deduced by its DEPT experiment of ^{13}C NMR and the negative FAB-MS. The ^{1}H NMR spectrum displayed the presence of six methyl signals at δ_H 0.89 (s), 0.95 (d, J = 6.5 Hz), 0.97 (s), 1.15 (s), 1.36 (s), and 1.53 (s) in an aglycone moiety. Comparison of aglycones of **3** and **1** (ilexgenin B)[2] exhibited that most carbon signals were similar except for the carbon signals of the A- and B-ring. The chemical shifts (the aglycone of **1** vs. one of **3**) of carbon signals due to δ_C 64.6 (C-23, +36.3) and δ_C 47.8 (C-5; -8.3) revealed that the C-23 methyl group was oxidized. Herein, the methyl signals in the NMR spectra exhibited six methyl signals, which also demonstrated that the methyl group was oxidized. On the HMBC spectrum, the aglycone moiety revealed important correlations such as the proton signal (δ_H 0.89, s, H-24) and the carbon signal (δ_C 64.6, C-23), the proton signal (δ_H 0.89, s, H-24) and the carbon signal (δ_C 82.1, C-3) (see Fig. 1). Hence, the aglycone of **3** was formulated as 20(*S*)-3β,19α,23-trihydroxyurs-12-en-28-oic acid, which was a new triterpene and named latifolic acid. The HMBC spectrum also showed the correlations of the sugar moieties: between the anomeric proton signal at δ_H 4.91 (d, J = 7.5 Hz, Ara) and the carbon signal at δ_C 82.1 (C-3), between the anomeric proton signal at δ_H 5.15 (d, J = 7.2 Hz, Glc) and the carbon signal at δ_C 84.3 (C-3 of Ara), and between the anomeric proton signal at δ_H 6.22 (d, J = 7.8 Hz, Glc′) and the carbon signal at δ_C 177.7 (C-28) (see Fig. 1). Thus, latifoloside K was determined as 3-*O*-β-*D*-glucopyranosyl(1→3)-α-*L*-arabinopycanosyl latifolic acid 28-*O*-β-*D*-glucopyranosyl ester.

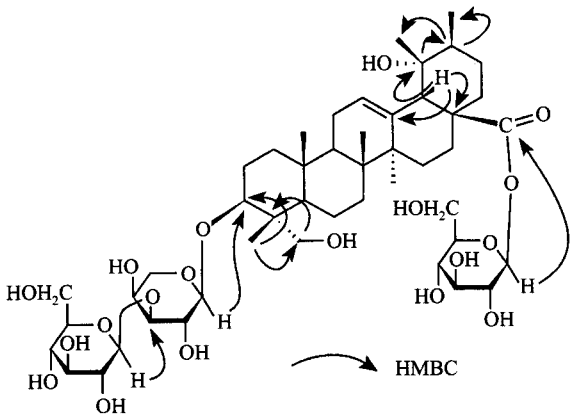

Fig. 1 Key correlation of latifoloside K in HMBC spectrum.

Latifoloside L (**4**) also displayed one $[M-H]^-$ ion peak at m/z 927 and a molecular formula of $C_{47}H_{76}O_{18}$ deduced by its DEPT experiment of ^{13}C NMR and the negative FAB-MS. Saponin **4** exhibited the same sugar sequences and linkages as the portion of saponin **1** or **3** in the NMR spectra, but their aglycones were different. Comparison of the ^{13}C NMR spectra of the aglycone moiety of **4** with one of ilexside II[9] showed that the two saponins had the same molecular formula, pomolic acid of the aglycone, and sequences of sugars (such as arabinose and glucose), but their sugar linkages were different. Ilexside II was Glc(1→2)Ara, and **4** was Glc(1→3)Ara. Thus, **4** could be represented as 3-*O*-β-*D*-glucopyranosyl (1→3)-α-*L*-arabinopyranosyl pomolic acid 28-*O*-β-*D*-glucopyranosyl ester.

Latifoloside M (**5**) showed FAB-MS at m/z 1055 $[M-H]^-$, 893 $[M-H-162]^-$, 747 $[M-H-146-162]^-$, 585 $[M-H-2\times162-146]^-$, and 453 $[M-H-2\times162-146-132]^-$, consistent with $C_{53}H_{84}O_{21}$ by its DEPT spectrum of ^{13}C NMR and the negative FAB-MS. The ^{13}C NMR spectrum exhibited the same sequences and linkages of sugar moieties as those of kudinoside G.[3] The main difference was the aglycone

moieties. The ^{13}C NMR spectrum also revealed a trisubstitued olefinic bond [a quaternary carbon at δ_C 139.0 (C-13) and a methine at δ_C 127.3 (C-12) which attached a proton at δ_H 5.60] and a quaternary substituted double bond [a quaternary carbon at δ_C 134.9, which was from a methine at δ_H 54.3 (C-18) (pomolic acid) to the quaternary carbon δ_C 134.9]. Two methyl groups (C-29, C-30) showed at δ_C 20.5 and 20.3 in **5** instead of δ_C 29.8 and 16.2 in pomolic acid. These evidences supported the formation of the olefinic group at position C-18 and C-19. Other carbons of the aglycone in **5** were assigned by comparison of chemical shifts with those described for kudinoside G and pomolic acid, thus the aglycone of **5** was confirmed as 3β-hydroxyurs-12 (13), 18(19)-diene-28-oic acid, which was a known triterpene (vanguerolic acid).[8] Accordingly, latifoloside M was formulated as 3-*O*-β-*D*-glucopyranosyl(1→3)-[α-*L*-rhamnopyranosyl(1→2)]-α-*L*-arabinopyranosyl-vanguerolic acid 28-*O*-β-*D*-glucopyranosyl ester.

Latifoloside N (**6**) has a molecular formular $C_{53}H_{84}O_{20}$ deduced from the negative FAB- MS and the DEPT experiment of ^{13}C NMR spectrum. The NMR spectra displayed four anomeric signals [δ_C 104.7/4.93 (d, J = 7.4 Hz, 1H, C-1-H of Ara), 101.9/6.10 (br.s, 1H, C-1-H of Rha), 95.0/6.15 (d, J = 8.0 Hz, 1H, C-1-H of Glc) and 101.6/6.46 (br.s, 1H, C-1-H of Rha)], and its sugar moieties had the same sequences and linkages as latifoloside J (**2**) and the aglycone was the same as latifoloside M (**5**). So, latifoloside N (**6**) was determined as 3-*O*-α-*L*-rhamnopyranosyl (1→2)-α-*L*-arabinopyranosyl vanguerolic acid 28-*O*-α-*L*-rhamnopyranosyl(1→2)-β-*D*-glucopyranosyl ester.

Saponin **7** exhibited a quasi-molecular weight (m/z 1057 [M - H]$^-$) in the negative FAB-MS. On acid hydrolysis, saponin **7** afforded glucose, arabinose and rhamnose. In the ^1H and ^{13}C NMR spectra, they showed four anomeric carbon signals at δ_C 104.8, 104.7, 102.0 and 95.8, and its proton signals at δ_H 4.85 (d, J = 5.2 Hz), 5.12 (d, J = 7.8 Hz), 6.15 (br.s) and 6.27 (d, J = 8.0 Hz), respectively. The sugar sequences and linkages were the same as the ones of kudinoside G[3] and latifoloside C[2] by comparison of their ^1H and ^{13}C NMR data. A genin part of **7** was identified as oleanolic acid by comparison of reference data.[6] Saponin **7** contains the C-3, C-28 of the aglycone resonating at δ_C 88.3, 176.5 instead of δ_C 78.7, 181.0 in oleanolic acid and only glucose was detected in the hydrolysate on alkaline hydrolysis. These evidences indicated the glycosylations of the C-3 position and the C-28 position of the aglycone. Thus the saponin **7** was determined as 3-*O*-β-*D*-glucopyranosyl (1→3)-[α-*L*-rhamnopyranosyl(1→2)]-α-*L*-arabinopyranosyl oleanolic acid 28-*O*-β-*D*-glucopyranosyl ester, and named as latifoloside O (Table 1).

Latifoloside P (**8**) showed the same sugar chain as latifoloside J (**2**) by comparison of its ^1H and ^{13}C NMR with those of **2**. The main differences concerned the genin parts. Latifoloside P on acid hydrolysis afforded oleanolic acid by comparing the ^{13}C NMR data[6] and mixture sugars of arabinose, glucose and rhamnose. Its negative ion FAB-MS displayed an ion at m/z 1041 [M - H]$^-$ (a molecular formula deduced as $C_{53}H_{86}O_{20}$ combined with DEPT spectrum) and main fragment ions at m/z 895, 733, 587, 455, which were attributed to the losses of a rhamnose, a glucose, a rhamnose and an arabinose, successively. The signal of C-3 resonatesd at δ_C 89.0 and C-28 resonated at δ_C 176.5 in **8** instead of δ_C 78.7 and 181.0 in oleanolic acid, manifesting that the glycosidating positions and a bisdesmosic saponin. Thus, **8** was 3-*O*-α-*L*-rhamnopyranosyl (1→2)-α-*L*-arabinopyranosyl oleanolic acid 28-*O*-α-*L*-rhamnopyranosyl(1→2)-β-*D*-glucopyranosyl ester.

Latifoloside Q (**9**) on acid hydrolysis afforded glucose, arabinose, and rhamnose. It showed a quasi-molecular peak at m/z 1203 [M - H]$^-$ and main fragment ions at m/z 1057,1041, 895, 733, 587 and 455 in the negative FAB-MS. These are attributed to the losses of two terminal rhamnoses, a terminal glucose and a disaccharide component, respectively. The structure of the aglycone (oleanolic acid[6]) was determined by comparing ^1H and ^{13}C NMR data. The ^1H, ^{13}C and HMQC spectra showed five anomeric carbon signals at δ_C 104.8, 104.6, 102.0, 101.5 and 95.0 attached to protons at δ_H 4.78 (d, J = 5.8 Hz), 5.08 (d, J = 7.7 Hz), 6.14 (br.s), 6.64 (br.s) and 6.17 (d, J = 7.9 Hz), respectively. The sequences of the sugar chain and linkages on oleanolic acid were established by HMBC and ROESY experiments. Observation of the Overhauser effects between H-3 of the genin and H-1 of arabinose in the ROESY experiment confirmed the sugar chain linking at position C-3 of oleanolic acid. On the HMBC spectrum, strong correlations were observed between the anomeric proton at δ_H 6.17 (H-1′ of Glc′)

Table 1 ^{13}C NMR spectral data for compounds **1—10** (pyridine-d_5)

Aglycone	1	2	3	4	5	6	7	8	9	10
1	39.1 CH_2	39.2 CH_2	39.0 CH_2	39.1 CH_2	39.7 CH_2	39.6 CH_2	39.2 CH_2	39.1 CH_2	39.2 CH_2	39.4 CH_2
2	27.1 CH_2	26.9 CH_2	26.9 CH_2	27.1 CH_2	27.0 CH_2	26.9 CH_2	29.1 CH_2	28.8 CH_2	28.9 CH_2	28.5 CH_2
3	88.9 CH	89.1 CH	82.1 CH	89.1 CH	88.3 CH	89.1 CH	88.3 CH	89.0 CH	88.4 CH	88.4 CH
4	39.7 C	39.6 C	40.6 C	39.7 C	39.6 C	39.6 C	39.7 C	39.5 C	39.7 C	39.8 C
5	56.1 CH	56.2 CH	47.8 CH	56.1 CH	56.4 CH	56.3 CH	56.2 CH	56.1 CH	56.3 CH	56.4 CH
6	18.9 CH_2	18.9 CH_2	18.6 CH_2	18.8 CH_2	18.4 CH_2	18.2 CH_2	18.6 CH_2	18.8 CH_2	18.7 CH_2	18.8 CH_2
7	33.6 CH_2	31.7 CH_2	31.9 CH_2	33.8 CH_2	34.9 CH_2	34.8 CH_2	32.6 CH_2	32.4 CH_2	32.4 CH_2	35.6 CH_2
8	40.7 C	40.7 C	40.6 C	40.6 C	40.0 C	40.1 C	40.0 C	40.1 C	40.1 C	41.9 C
9	48.0 CH	47.9 CH	48.0 CH	48.5 CH	48.4 CH	48.5 CH	48.2 CH	48.2 CH	48.3 CH	45.0 CH
10	37.2 C	37.2 C	37.1 C	37.7 C	37.4 C	37.4 C	37.2 C	37.2 C	37.2 C	37.2 C
11	24.1 CH_2	24.2 CH_2	24.2 CH_2	24.4 CH_2	24.1 CH_2	24.2 CH_2	23.9 CH_2	23.9 CH_2	23.9 CH_2	28.9 CH_2
12	127.8 CH	127.6 CH	127.8 CH	128.6 CH	127.3 CH	127.0 CH	122.7 CH	122.7 CH	122.8 CH	66.3 CH
13	138.8 C	138.9 C	138.9 C	139.3 C	139.0 C	138.3 C	144.2 C	144.3 C	144.4 C	146.4 C
14	42.2 C	42.4 C	42.3 C	42.3 C	44.8 C	44.9 C	42.3 C	42.3 C	42.4 C	44.1 C
15	29.3 CH_2	29.8 CH_2	29.3 CH_2	29.7 CH_2	28.9 CH_2	28.7 CH_2	28.3 CH_2	28.3 CH_2	28.3 CH_2	29.0 CH_2
16	24.8 CH_2	24.8 CH_2	24.8 CH_2	26.7 CH_2	26.7 CH_2	26.7 CH_2	23.8 CH_2	23.8 CH_2	23.6 CH_2	26.6 CH_2
17	48.5 C	48.6 C	48.4 C	48.7 C	48.3 C	48.2 C	47.1 C	47.3 C	47.3 C	44.3 C
18	47.8 CH	47.7 CH	47.3 CH	54.3 CH	134.9 C	134.9 C	42.3 CH	42.7 CH	42.2 CH	137.7 C
19	73.6 C	73.5 C	73.6 C	72.8 C	~ C	~ C	46.3 CH_2	46.5 CH_2	46.5 CH_2	74.5 C
20	42.9 CH	42.9 CH	42.9 CH	42.2 CH	37.0 CH	37.0 CH	30.9 C	30.8 C	30.8 C	85.8 C
21	26.8 CH_2	26.6 CH_2	26.3 CH_2	26.8 CH_2	33.9 CH_2	33.9 CH_2	34.1 CH_2	34.2 CH_2	34.2 CH_2	26.4 CH_2
22	33.7 CH_2	33.9 CH_2	33.3 CH_2	37.8 CH_2	34.6 CH_2	34.5 CH_2	32.7 CH_2	33.2 CH_2	33.3 CH_2	32.9 CH_2
23	28.3 CH_3	28.3 CH_3	64.6 CH_2	28.3 CH_3	28.3 CH_3	28.2 CH_3	28.3 CH_3	28.2 CH_3	28.3 CH_3	28.2 CH_3
24	17.1 CH_3	17.0 CH_3	13.7 CH_3	16.7 CH_3	16.4 CH_3	16.4 CH_3	17.1 CH_3	17.1 CH_3	17.2 CH_3	17.0 CH_3
25	15.7 CH_3	16.1 CH_3	16.3 CH_3	15.7 CH_3	14.3 CH_3	14.3 CH_3	15.9 CH_3	15.9 CH_3	15.9 CH_3	16.9 CH_3
26	17.6 CH_3	17.5 CH_3	17.7 CH_3	17.6 CH_3	17.2 CH_3	17.1 CH_3	17.7 CH_3	17.5 CH_3	17.6 CH_3	18.3 CH_3
27	24.4 CH_3	24.1 CH_3	24.4 CH_3	24.6 CH_3	2.4 CH_3	22.4 CH_3	26.2 CH_3	26.0 CH_3	26.1 CH_3	23.6 CH_3
28	177.1 C	176.9 C	177.1 C	177.1 C	175.1 C	175.2 C	176.5 C	176.5 C	176.6 C	175.6 C
29	29.8 CH_3	29.8 CH_3	29.7 CH_3	27.2 CH_3	20.5 CH_3	20.4 CH_3	33.2 CH_3	33.4 CH_3	33.3 CH_3	25.3 CH_3
30	16.2 CH_3	15.8 CH_3	16.1 CH_3	16.7 CH_3	20.3 CH_3	20.3 CH_3	23.5 CH_3	23.5 CH_3	23.5 CH_3	18.7 CH_3
Sugar C-3										
Ara-1	107.3 CH	104.7 CH	106.4 CH	107.3 CH	104.7 CH	104.7 CH	104.7 CH	104.8 CH	104.8 CH	104.8 CH
2	71.9 CH	76.1 CH	72.4 CH	71.8 CH	74.3 CH	76.0 CH	74.8 CH	76.9 CH	75.0 CH	74.8 CH
3	84.1 CH	74.1 CH	84.3 CH	84.0 CH	82.1 CH	74.2 CH	82.1 CH	74.1 CH	82.6 CH	82.1 CH
4	69.3 CH	68.4 CH	69.3 CH	69.3 CH	68.1 CH	69.0 CH	68.2 CH	68.5 CH	68.7 CH	68.1 CH
5	64.7 CH_2	64.3 CH_2	67.0 CH_2	64.7 CH_2	64.8 CH_2	64.8 CH_2	64.8 CH_2	64.5 CH_2	64.8 CH_2	64.8 CH_2
Glc-1	106.2 CH		106.2 CH	106.1 CH	104.6 CH		104.8 CH		104.6 CH	104.7 CH
2	75.7 CH		74.2 CH	75.7 CH	75.0 CH		75.0 CH		75.4 CH	75.0 CH
3	78.6 CH		78.7 CH	78.5 CH	78.6 CH		78.7 CH		78.9 CH	78.6 CH
4	71.6 CH		71.3 CH	71.7 CH	71.6 CH		71.6 CH		71.8 CH	71.6 CH
5	78.4 CH		78.4 CH	78.4 CH	78.3 CH		78.3 CH		78.6 CH	78.3 CH
6	62.8 CH_2		62.4 CH_2	62.7 CH_2	62.6 CH_2		62.6 CH_2		62.7 CH_2	62.6 CH_2
Rha-1		101.8 CH			102.0 CH	101.9 CH	102.0 CH	101.8 CH	102.0 CH	101.9 CH
2		72.4 CH			72.4 CH	72.4 CH	72.5 CH	72.4 CH	72.4 CH	72.4 CH
3		72.7 CH			72.6 CH	72.6 CH	72.6 CH	72.6 CH	72.6 CH	72.6 CH
4		74.0 CH			74.0 CH	74.1 CH	74.2 CH	74.0 CH	74.0 CH	74.0 CH
5		70.0 CH			70.1 CH	70.1 CH	70.2 CH	70.0 CH	70.2 CH	70.1 CH
6		18.8 CH_3			18.6 CH_3	18.7 CH_3	18.7 CH_3	18.8 CH_3	18.8 CH_3	18.6 CH_3
C-28										
Glc-1′	95.9 CH	95.1 CH	95.9 CH	96.0 CH	95.8 CH	95.0 CH	95.8 CH	95.0 CH	95.0 CH	
2′	74.2 CH	76.2 CH	75.7 CH	74.2 CH	75.2 CH	76.1 CH	74.9 CH	76.1 CH	76.0 CH	
3′	79.2 CH	79.8 CH	79.2 CH	79.1 CH	79.0 CH	79.7 CH	79.3 CH	79.8 CH	79.8 CH	
4′	71.4 CH	71.7 CH	71.7 CH	71.4 CH	71.6 CH	71.6 CH	71.3 CH	71.5 CH	71.8 CH	
5′	79.0 CH	78.9 CH	79.0 CH	78.9 CH	78.8 CH	79.2 CH	79.0 CH	78.9 CH	79.0 CH	
6′	62.5 CH_2	62.4 CH_2	62.8 CH_2	62.4 CH_2	62.5 CH_2	62.6 CH_2	62.2 CH_2	62.3 CH_2	62.3 CH_2	
Rha-1′		101.5 CH				101.6 CH		101.5 CH	101.5 CH	
2′		72.4 CH				72.3 CH		72.4 CH	72.2 CH	
3′		72.7 CH				72.6 CH		72.6 CH	72.3 CH	
4′		73.7 CH				73.9 CH		74.0 CH	74.2 CH	
5′		69.8 CH				70.0 CH		69.9 CH	70.2 CH	
6′		18.6 CH_3				18.7 CH_3		18.6 CH_3	18.6 CH_3	

~ : the signal is overlapped with the solvent signal.

inner glucose and C-28, between the anomeric proton at δ_H 6.64 (H-1′ of Rha′) and C-2′ of Glc′, between the anomeric proton at δ_H 4.78 (H-1 of Ara) and C-3 (Aglycone), between the anomeric proton at δ_H 5.08 (H-1 of Glc) and C-3 (Ara), between the anomeric proton at δ_H 6.14 (H-1 of Rha) and C-2 (Ara). All these observations confirmed **9** as 3-*O*-β-*D*-glucopyranosyl(1→3)-[α-*L*-rhamnopyranosyl(1→2)]-α-*L*-arabinopyranosyl oleanolic acid 28-*O*-α-*L*-rhamnopyranosyl (1→2)-β-*D*-glucopyranosyl ester.

Experimental

General procedure

^1H and ^{13}C NMR spectra were obtained with a Bruker AM-400 spectrometer and a DRX-500 spectrometer and the solvents are pyridine-d_5 for **1**—**10** and methanol-d_4 for **11**—**12**. FAB-MS was taken on a VG Autospec 3000 system spectrometer. Optical rotations were taken on a JASCO-20C digital polarimeter and the IR spectrum was recorded with a Perkin-Elmer 1750 FTIR spectrometer. Chromatographic stationary phase used RP-8 (40—60 μm, Merck), silica gel (160—200 mesh), Sephadex LH-20 (25—100 μm, Pharmacia Fine Chemical Co., Ltd.) and MCI-gel CHP20P (75—150 μm, Mitsubish Chemical Industries, Ltd.). The following solvent systems were used: a.) $CHCl_3$-MeOH-H_2O (7:3:0.5), $CHCl_3$-MeOH-H_2O (65:35:9) and MeOH-H_2O (0%—100%) for the saponins; and b.) $CHCl_3$-MeOH-H_2O (7:3:1) lower-layer (9 mL) + HOAc (1 mL) for sugars. Spot of TLC was detected by spraying with 5% H_2SO_4 followed by heating. Sugars were detected by spraying with aniline-phthalate reagent.

Plant material

The leaves of *Ilex latifolia* Thunb were collected in the Hunan Province of China, in August 1993 and identified by Yang, Chong-Ren. A voucher specimen (No. 643227) is deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The dry leaves (800 g) were extracted three times with MeOH (15 L) at 50℃ for 8 h. The methanol extract was concentrated under vacuum and the extract (100 g) suspended in H_2O. The aqueous suspension was extracted with $CHCl_3$ and *n*-BuOH. The *n*-BuOH layer was evaporated to dryness to give a residue (50 g). Crude saponins were treated with Diaion column first eluated with 30% MeOH (1 L), then with 100% MeOH (1 L) to give two fractions, and MeOH fraction was chromatographed on silica gel (1.5 kg, 200—300 mesh) with 7 L, $CHCl_3$-MeOH-H_2O (7:3:0.5) to give twenty fractions. The fractions of 1—15 were separated on RP-8 gel (40—60 μm) and silica gel (10—40 μm) columns to give **1** (50 mg), **2** (70 mg), **3** (53 mg), **4** (40 mg), **5** (37 mg), **6** (22 mg), **7** (45 mg), **8** (20 mg), **9** (35 mg), **10** (25 mg), **11** (15 mg), and **12** (40 mg).

Latifoloside I (**1**), $C_{47}H_{76}O_{18}$, $[\alpha]_D^{27}$ + 73.2 (*c* 0.012, MeOH), ^1H NMR δ_H: 0.88 (s, 3H), 0.96 (d, J = 6.0 Hz, 3H), 1.13 (s, 3H), 1.16 (s, 3H), 1.17 (s, 3H), 1.40 (s, 3H), 1.71 (s, 3H), 3.18 (dd, J = 11.4, 4.4 Hz, 3α-H), 3.16 (s, 18α-H), 5.49 (br.s, 12-H), 4.88 (d, J = 5.1 Hz, 1H, C-1-H of Ara), 5.25 (d, J = 7.3 Hz, 1H, C-1-H of Glc), 6.30 (d, J = 7.9 Hz, 1H, C-1-H of Glc); FAB-MS *m/z* 927 $[M-H]^-$, 765 $[M-H-162]^-$, 603 $[M-H-2\times162]^-$; IR ν_{max}^{KBr}: 3400 (OH), 2920 (C-H), 1730 (C=O), 1640 (C=C), 1451, 1380, 1075 cm^{-1}.

Latifoloside J (**2**), $C_{53}H_{86}O_{21}$, $[\alpha]_D^{27}$ − 21.5 (*c* 0.012, MeOH); ^1H NMR δ_H: 0.89, 1.02, 1.08, 1.13, 1.29, 1.75 (s, 3H × 6), 0.89 (d, J = 6.8 Hz, 3H), 1.62 (d, J = 5.6 Hz, 3H, C-6-H of Rha), 1.77 (d, J = 5.6 Hz, 3H, C-6-H of Rha), 3.09 (s, 1H, 18α-H), 3.19 (dd, J = 8.4, 4.1 Hz, 1H, 3α-H), 5.15 (1H, s, 19α-OH), 5.54 (br.s, 1H, 12-H), 4.86 (d, J = 4.8 Hz, 1H, C-1-H of Ara), 6.14 (br.s, 1H, C-1-H of Rha), 6.21 (d, J = 7.2 Hz, 1H, C-1-H of Glc), 6.67 (br.s, 1H, H-1 of Rha); FAB-MS *m/z*: 1057 $[M-H]^-$, 911 $[M-H-146]^-$, 749 $[M-H-2\times146]^-$, 603 $[M-H-2\times146-162]^-$; IR: 3420 (OH), 2930 (C—H), 1733 (C=O), 1641 (C=C), 1450, 1384, 1072 cm^{-1}.

Latifoloside K (**3**), $C_{47}H_{76}O_{19}$, $[\alpha]_D^{27}$ + 4.89 (*c* 0.022, MeOH); ^1H NMR δ_H: 0.89 (s, 3H, CH_3), 0.95 (d, 3H, J = 6.5 Hz, CH_3-30), 0.97 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 3.12 (br.s, 1H, H-18), 3.56

(dd, J = 11.5, 4.5 Hz, 1H, H-3), 4.91 (d, J = 7.5 Hz, 1H, C-1-H of Ara), 5.15 (d, J = 7.2 Hz, 1H, C-1-H of Glc), 6.22 (d, J = 7.8 Hz, 1H, C-1-H of Glc); FAB-MS m/z: 943 $[M-H]^-$, 781 $[M-H-162]^-$, 619 $[M-H-2\times162]^-$.

Latifoloside L (**4**), $C_{47}H_{76}O_{18}$, $[\alpha]_D^{27}$ + 46.6 (c 0.012, MeOH); ^1H NMR δ_H: 0.89 (s, 3H), 1.05 (d, J = 6.4 Hz, 3H), 1.07 (s, 3H), 1.12 (s, 3H), 1.18 (s, 3H), 1.39 (s, 3H), 1.75 (s, 3H), 2.92 (br.s, 1H, 12-H), 4.88 (d, J = 11.3, 4.2 Hz, 1H, 3α-H), 5.55 (d, J = 7.2 Hz, 1H, C-1-H of 3-Glc), 6.28 (d, J = 7.9 Hz, 1H, C-1-H of 28-Glc); FAB-MS m/z: 927 $[M-H]^-$, 765 $[M-H-162]^-$, 603$[M-H-2\times162]^-$.

Latifoloside M (**5**), $C_{53}H_{84}O_{21}$, $[\alpha]_D^{27}$ + 22.5 (c 0.022, MeOH); ^1H NMR δ_H: 0.85 (s, 3H), 1.00 (d, J = 6.8 Hz, 3H), 1.09 (s, 3H), 1.11 (s, 3H), 1.20 (s, 3H), 1.27 (s, 3H), 1.71 (s, 3H), 3.29 (dd, J = 11.0, 4.0 Hz, 1H, H-3), 5.60 (br.s, 1H, H-12), 4.85 (d, J = 5.2 Hz, 1H, H-1 of Ara), 5.07 (d, J = 7.7 Hz, 1H, H-1 of Glc), 6.13 (br.s, 1H, H-1 of Rha), 6.31(d, J = 7.9 Hz, 1H, H-1 of Glc); IR ν_{max}^{KBr}: 3428 (OH), 2932 (C—H), 1734 (C = O), 1638 (C = C), 1454, 1387, 1071 cm^{-1}; FAB-MS m/z: 1055 $[M-H]^-$, 893 $[M-H-162]^-$, 731 $[M-H-2\times162]^-$, 747 $[M-H-146-162]^-$, 585 $[M-H-2\times162-146]^-$, 453 $[M-H-2\times162-146-132]^-$.

Latifoloside N (**6**), $C_{53}H_{84}O_{20}$, $[\alpha]_D^{27}$ - 99.2 (c 0.010, MeOH); ^1H NMR δ_H: 0.86, 0.98 (d, J = 6.8 Hz, 3H), 1.04, 1.12, 1.15, 1.16, 1.82 (s, 3H × 6), 1.63 (d, J = 6.0 Hz, 3H, H-6 of Rha), 1.75 (d, J = 6.2 Hz, 3H, H-6 of Rha), 3.30 (dd, J = 11.2, 1H, 4.3 Hz, H-3), 5.66 (br.s, 1H, H-12), 4.93 (d, J = 7.4 Hz, 1H, C-1-H of Ara); 6.10 (br.s, 1H, C-1-H of Rha), 6.15 (d, J = 8.0 Hz, 1H, C-1-H of Glc), 6.46 (br.s, 1H, C-1-H of Rha); FAB-MS m/z: 1039 $[M-H]^-$, 893 $[M-H-146]^-$, 747 $[M-H-146-146]^-$, 731 $[M-H-146-162]^-$, 585 $[M-H-146-162-146]^-$.

Latifoloside O (**7**), $C_{53}H_{86}O_{21}$, $[\alpha]_D^{27}$ + 55.1 (c 0.012, MeOH); ^1H NMR δ_H: 0.80, 0.83, 1.07, 1.11, 1.18, 1.24 (s, 3H × 7), 1.62 (d, J = 6.1 Hz, 3H, C-6 of Rha), 3.21 (dd, J = 11.2, 4.0 Hz, 1H, H-3), 5.16 (br.s, H-12), 4.85 (d, J = 5.2 Hz, 1H, C-1-H of Ara), 5.12 (d, J = 7.8 Hz, 1H, C-1-H of Glc), 6.15 (br.s, 1H, C-1-H of Rha), 6.27 (d, J = 8.0 Hz, 1H, C-1-H of Glc); FAB-MS m/z: 1057 $[M-H]^-$, 895 $[M-H-162]^-$, 733 $[M-H-2\times162]^-$, 749 $[M-H-146-162]^-$, 587 $[M-H-2\times162-146]^-$.

Latifoloside P (**8**), $C_{53}H_{86}O_{20}$, $[\alpha]_D^{27}$ - 128.8 (c 0.005, MeOH); ^1H NMR δ_H: 0.79, 0.84, 0.87, 1.02, 1.06, 1.10, 1.25 (s, 3H × 7), 1.60(d, J = 6.1 Hz, 3H, H-6 of Rha), 1.74(d, J = 5.8 Hz, 3H, H-6′ of Rha), 0.96 (br.s, 1H, H-19), 1.89 (br.s, 1H, H-9), 3.18 (dd, J = 14.0, 2.0 Hz, 1H, H-3), 5.35 (br.s, 1H, H-12), 4.85 (d, J = 5.4 Hz, 1H, C-1-H of Ara), 6.16 (br.s, 1H, C-1-H of Rha), 6.20 (d, J = 7.3 Hz, 1H, C-1-H of Glc), 6.66 (br.s, 1H, C-1-H of Rha); FAB-MS m/z: 1041$[M-H]^-$, 895 $[M-H-146]^-$, 733 $[M-H-162-146]^-$, 587 $[M-H-162-2\times146]^-$; IR ν_{max}^{KBr}: 3417 (OH), 2922 (C-H), 1730 (C = O), 1641 (C = C) cm^{-1}.

Latifoloside Q (**9**), $C_{59}H_{96}O_{25}$, $[\alpha]_D^{27}$ - 120 (c 0.005, MeOH); ^1H NMR δ_H: 0.77, 0.83, 0.88, 1.04. 1.07, 1.10, 1.28 (s, 3H × 7, CH_3), 3.17 (dd, J = 11.2, 4.0 Hz, 1H, H-3), 4.78 (d, J = 5.8 Hz, 1H, C-1-H of Ara), 5.08 (d, J = 7.7 Hz, 1H, C-1-H of Glc), 5.25 (d, J = 6.0 Hz, 1H, H-12), 6.14 (br.s, 1H, C-1-H of Rha), 6.17 (d, J = 7.9 Hz, 1H, C-1-H of Glc), 6.64 (br.s, 1H, C-1-H of Rha); IR ν_{max}^{KBr}: 3425 (OH), 2920 (C—H), 1732 (C = O), 1641 (C = C) cm^{-1}; FAB-MS m/z: 1203 $[M-H]^-$, 1057 $[M-H-146]^-$, 1041 $[M-H-162]^-$, 895 $[M-H-146-162]^-$, 733 $[M-H-146-2\times162]^-$, 587 $[M-H-2\times146-2\times162]^-$, 455 $[M-H-2\times146-2\times162-132]^-$.

Acid hydrolysis

A solution of each compound (10 mg) was heated at 100℃ in 5 % H_2SO_4 and 50% EtOH for 10 h. The reaction mixture diluted with water, neutralized with 2% NaOH and evaporated in vacuum to dryness. The reaction product was a mixture of sugar. The molar ratio of each sugar was determined by using RI detection in HPLC (Shodex RS pak DC-613, 75% MeCN, mL/min, 70℃) by comparison with authentic sugars (10 mM each of *L*-Ara, *D*-Glc and *L*-Rha). The retention time of each sugar was as follows: 6.0 min (Ara), 7.4 min (Glc) and 4.8 min (Rha).

Alkaline hydrolysis

Each saponin (3 mg) was refluxed in 0.5 M KOH (2 mL) for 2 h. The mixture was adjusted to pH 6 with 1 M HCl and then the extract was concentrated to dryness, which was extracted with pyridine from the residue and was analyzed by HPTLC to detect sugars.

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