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TRITERPENOID SAPONINS FROM *ILEX LATIFOLIA*

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Key Word Index—*Ilex latifolia*; Aquifoliaceae; triterpene; saponins; latifoloside F, G and H.

Abstract—Three new triterpenoid saponins, latifolosides F, G, H were isolated from the leaves of *Ilex latifolia*. Their structures were elucidated on the basis of chemical and spectral evidence. Latifoloside F was determined to be 3-O-[α-L-rhamnopyranosyl(1–2)]-[β-D-glucopyranosyl(1–3)-]-α-L-arabinopyranosyl ilexgenin B 28-O-[α-L-rhamnopyranosyl(1–2)]-β-D-glucopyranoside. Latifoloside G was 3-O-[α-L-rhamnopyranosyl(1-2)]-[β-D-glucopyranosyl(1–3)-]-α-L-arabinopyranosyl pomolic acid 28-O-[α-L-rhamnopyranosyl(1–2)]-β-D-glucopyranoside. Latifolioside H(**3**) was 3-O-[α-L-rhamnopyranosyl(1–2)]-[β-D-glucopyranosyl(1–3)-]-α-L-arabinopyranosyl siaresinolic acid 28-O-[α-L-rhamnopyranosyl(1–2)]-β-D-glucopyranoside.

INTRODUCTION

In previous papers [1–3], we reported nine new triterpenoid saponins from *Ilex kudincha* and five new triterpenoid saponins, latifolosides A–E from *I. latifolia*, two plants which are used as traditional tea (Ku-Ding-Cha) [4]. As a part of our continuing phytochemical research on *Ilex* species, we now report the isolation and structural determination of three new saponins from the leaves of *I. latifolia*.

RESULTS AND DISCUSSION

The butanol soluble fraction of a methanol extract of the leaves of *I. latifolia* gave a saponin fraction by separation with a Diaion column. Repeated separation of saponins by ordinary phase silica gel column chromatography and reversed phase HPLC furnished three new saponins, latifolosides F(**1**), G(**2**) and H(**3**). Saponins **2** and **3** were isomers of **1**.

Saponin **1** showed in the negative FAB-mass spectrum a $[M-H]^-$ ion at m/z 1219. Negative fragment ions at m/z 1073, 911, and 765 were attributed to the losses of deoxyhexose [M-146], deoxyhexose-hexose [M-146–162], and deoxyhexose-hexose-deoxyhexose [M-146–162–146], respectively. The saponin **1** of the aglycone, ilexgenin B(**4**), was identified by enzymic hydrolysis and comparison of its ^{1}H NMR and ^{13}C NMR data with reference values [5].

The presence of five sugar residues was deduced from the observation of five anomeric carbons at δ 105.0, 104.7, 102.0, 101.7, and 95.2 attached to protons at δ 4.83 (*d*, J = 6.0 Hz, Ara), 5.19 (*d*, J = 7.9 Hz, Glc), 6.19 (*brs*, Rha), 6.69 (*brs*, Rha), and 6.21 (*d*, J = 7.7 Hz, Glc) (HMQC). The anomeric carbon signal δ 95.2 of the sugar and the carboxyl group signal at δ 177.3 and the position of the C-3 signal of the genin at δ 88.7 indicated that saponin **1** was a bisdesmoside. Identification and assignment of the sugars were based on acid hydrolysis, HMQC, and TOCSY experiments. The sugars were one arabinose, two glucose and two rhamose moieties (Table 1).

Sequencing of the order of the sugars and their linkage in ilexgenin B(**4**) were achieved by a HMBC experiment. Observation of strong correlations between H-3 of the genin and C-1 arabinose, between the anomeric proton of rhamnose at δ 6.19 and C-2 of arabinose, between the anomeric proton of glucose at δ 5.19 and C-3 of arabinose, confirmed the sequence of sugars was rhamnose linking to position C-2 of the arabinose, glucose linking to position C-3 of the arabinose and this arabinose linking to C-3 of ilexgenin B (Fig. 1).

Other correlations in the HMBC spectrum between the carbonyl C-28 of ilexgenin B at δ 177.3 and the anomeric proton of glucose at δ 6.21, between the anomeric proton of the rhamnose at δ

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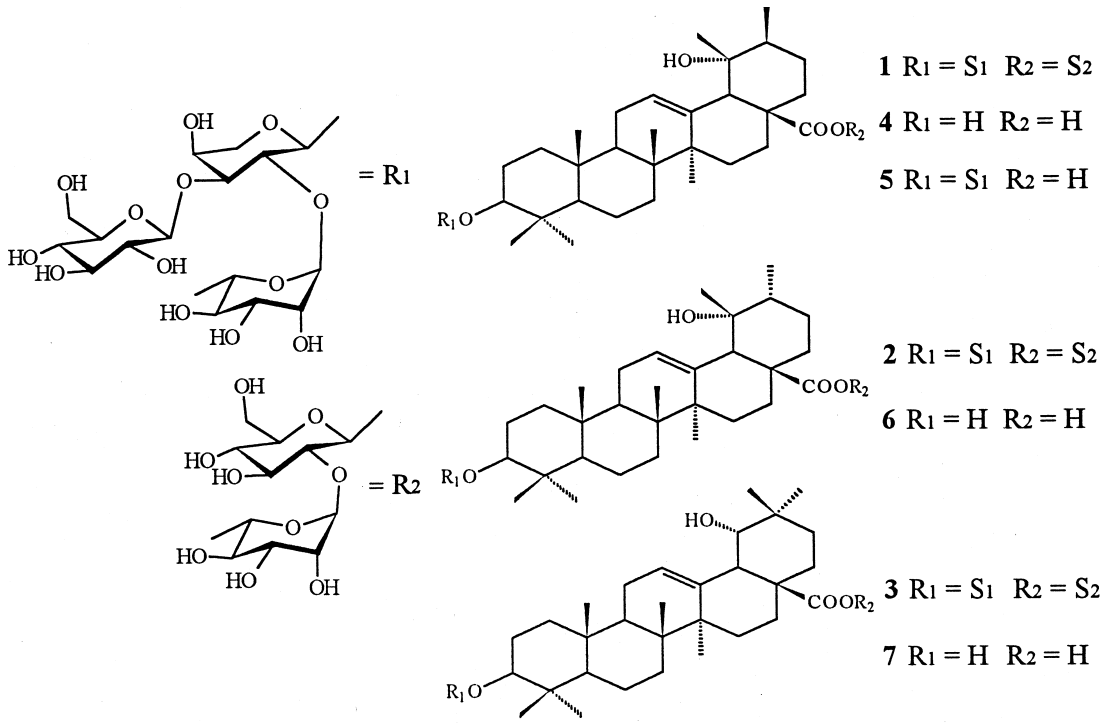

6.69 and C-2 of glucose, revealed the rhamnose linking to C-2 of the glucose and this glucose linking C-28 of the genin.

Alkaline hydrolysis of **1** gave saponin **5** and a mixture of glucose and rhamnose. The ^{1}H NMR and ^{13}C NMR spectra of saponin **5** indicated the

Table 1. ^{13}C NMR spectral data of latifoloside F–H (125 MHz, pyridine-d_5)

C	F(**1**)	G(**2**)	H(**3**)		(**1**)	(**2**)	(**3**)
				3-O-Ara			
1	39.5	39.2	39.0	1	105.0	104.8	104.9
2	26.9	26.8	26.7	2	74.7	74.7	74.7
3	88.7	89.1	88.3	3	82.5	82.1	82.3
4	39.8	39.6	39.7	4	68.4	68.2	68.3
5	54.5	56.1	56.2	5	65.0	64.9	64.6
6	18.9	18.9	18.8	Rha			
7	34.0	33.6	33.1	1	102.0	101.9	102.0
8	40.7	40.7	40.3	2	72.8	72.5	72.6
9	48.7	47.9	48.4	3	72.6	72.6	72.5
10	37.3	37.1	37.3	4	74.1	74.2	74.2
11	24.3	24.2	24.2	5	70.2	70.2	70.1
12	127.7	128.5	123.6	6	18.8	18.7	18.7
13	139.1	139.4	144.4	Glc			
14	42.6	42.2	42.2	1	104.7	104.6	104.7
15	29.8	29.4	29.2	2	75.0	75.1	75.1
16	26.9	26.2	28.1	3	78.6	78.3	78.3
17	48.7	48.8	46.6	4	71.6	71.3	71.5
18	47.8	54.6	44.7	5	78.3	78.8	78.6
19	73.8	72.8	81.1	6	62.7	62.6	62.6
20	42.9	42.2	35.7	28-O-Glc			
21	24.9	26.7	29.0	1	95.2	95.7	95.5
22	31.8	37.9	33.3	2	76.0	76.3	76.2
23	28.4	28.3	28.2	3	79.9	79.8	79.8
24	17.4	16.8	17.1	4	71.6	71.7	71.7
25	16.1	15.8	15.7	5	79.2	79.4	79.2
26	17.7	17.5	17.7	6	62.4	62.5	62.4
27	24.4	24.7	24.8	Rha			
28	177.3	177.2	177.5	1	101.7	101.7	101.6
29	30.0	27.2	28.8	2	72.5	72.4	72.5
30	16.3	17.1	25.0	3	72.4	72.4	72.4
				4	74.0	73.9	74.1
				5	70.1	70.0	70.1
				6	18.9	18.8	18.9

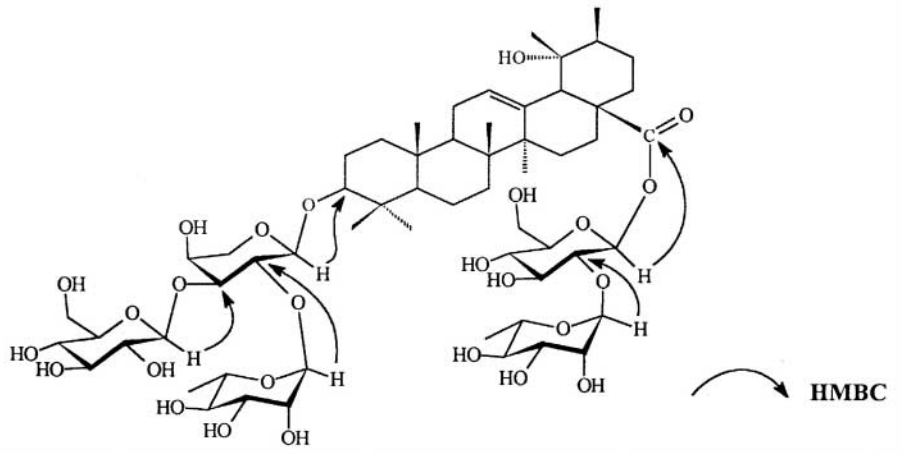

Fig. 1. The HMBC correlation of compound **1**.

presence of one arabinose [H-1: δ 4.58 (*d*, J = 5.3 Hz), C-1: δ 104.8], one rhamnose [H-1: δ 6.13, C-1: δ 102.0] and one glucose [H-1: δ 5.16 (*d*, J = 7.7 Hz, C-1: δ 104.7]. This sugar sequence is the same as that of kudinoside D [2]. These observations confirm the identity of saponin **1** as 3-O-[β-D-glucopyranosyl(1–3)]-[α-L-rhamnopyranosyl(1–2)-]-α-L-arabinopyranosyl ilexgenin B 28-O-[α-L-rhamnopyranosyl(1–2)]-β-D-glucopyranoside, and is named latifoloside F.

The structures of the related saponins **2**–**3** were elucidated by NMR spectroscopy, and in particular, by comparison of their data with those of saponin **1**. Saponin **2** has the same molecular weight as **1**. The FAB-mass spectrum of **2** showed a quasi-molecular ion peak at m/z 1219 $[M\text{-}H]^-$ and fragment ions at m/z 1073 $[M\text{-}H\text{-}146]^-$, 911 $[M\text{-}H\text{-}146\text{–}162]^-$, 749 $[M\text{-}H\text{-}146\text{–}2\times162]^-$, 765 $[M\text{-}H\text{-}2\times146\text{–}162]^-$, and 603 $[M\text{-}H\text{-}2\times146\text{–}2\times162]^-$. A crude cellulase treatment of **2** gave pomolic acid(**6**) [5], $C_{30}H_{46}O_4$, as the aglycone which showed a quasi-molecular ion peak at m/z 473 $[M+1]^+$, and 495 $[M+Na]^+$ in the mass spectrum. Comparison of the ^1H NMR and ^{13}C NMR spectra of **2** with those of **1** showed the same sugar moiety signals indicating the same sugar sequence. On the basis of the above findings, the structure of **2** was concluded to be 3-O-[α-L-rhamnopyranosyl(1–2)]-[β-D-glucopyranosyl(1–3)]-α-L-arabinopyranosyl pomolic acid 28-O-[α-L-rhamnopyranosyl(1–2)]-β-D-glucopyranoside, and is named latifoloside G.

Saponin **3**, an isomer of **1** and **2**, was shown to possess the same sugar chain as latifoliosides **1** and **2** by comparison of its ^1H NMR and ^{13}C NMR spectra with those of **1** (Table 1). The major differences concerned the genin moiety. On enzymatic hydrolysis, **3** afforded siaresinolic acid (**7**) [6] as the aglycone and a mixture of arabinose, glucose and rhamnose. Compound **3** showed the same sugar sequence as **1** and **2**. Therefore, compound **3** was formulated as 3-O-[α-L-rhamnopyranosyl(1–2)]-[β-D-glucopyranosyl(1–3)-]-α-L-arabinopyranosyl siaresinolic acid 28-O-[α-L-rhamnopyranosyl(1–2)]-β-D-glucopyranoside, and is named latifoloside H.

EXPERIMENTAL

All mps were determined on a Beijing Micromelting Apparatus and were uncorr. IR spectra run with a Perkin-Elmer 683 spectrometer. ^1H NMR (500 MHz), ^{13}C NMR (125 MHz) and 2D NMR performed with a Bruker spectrometer using pyridine-d_5 as a solvent and TMS as an internal standard. FAB-MS were taken on VG Autospec 3000 system spectrometer, for HPLC (Beckman gold system), YMC-Pack A312 ODS column (259 × 16 mm) was used. Column chromatography and TLC was on silica gel, RP-8, and RP-18 using the following solvent systems: a.) $CHCl_3$-MeOH-H_2O (7:3:0.5), $CHCl_3$-MeOH-H_2O (65:35:9) and MeOH-H_2O (6:4 ~ 7:3) for the saponins; and b.) $CHCl_3$-MeOH-H_2O (7:3:1) (lower-layer) 9 ml + 1 ml HOAc for sugars. Saponins were detected by spraying with 5% H_2SO_4, followed by heating for 5 min at 105°C. Sugars were detected by spraying with aniline-phthalate reagent.

Plant material Ilex latifolia

Thunb was collected in the Hunan Province of China in the Summer of 1993 and identified by Prof. Chong-Ren Yang. A voucher specimen (No. 643227) is deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Science.

Extraction and isolation of saponins

The dry leaves (800 g) were extracted 3× with MeOH (each time 15 l.) at 50° for 8 hr. The MeOH extract was concentrated under vacuum and the

extract (100 g) suspended in H_2O. The aq. suspension was extracted with $CHCl_3$ and *n*-BuOH. The *n*-BuOH layer was evaporated to dryness to give a residue (50 g). Crude saponin were treated with Diaion column first eluate 30% MeOH 1000 ml, then with 100% MeOH 1000 ml to give two fractions and MeOH fraction was chromatographed on silica gel (1.5 kg, 200–300 mesh) with 7000 ml, $CHCl_3$-MeOH-H_2O (7:3:0.5) to give twenty fractions. No. 16 and No. 20 were separated on HPLC [ODS, eluting with MeOH-H_2O (8:2–6:4), flow rate: 5 ml min^{-1}, injection: 0.4 ml (10 mg ml^{-1})] to afford latifolosides F(**1**, 180 mg), G(**2**, 80 mg), H(**3**, 75 mg).Latifoloside F(**1**). m.p. 235–238°, $C_{59}H_{96}O_{26}$, IR $\nu_{max}^{KBr}cm^{-1}$: 3430 (OH), 2927 (C-H), 1730 (C=O), 1640 (C=C). FAB-MS *m*/*z*: 1219 $[M\text{-}H]^-$, 1073 $[M\text{-}H\text{-}146]^-$, 911 $[M\text{-}H\text{-}146\text{–}162]^-$, 765 $[M\text{-}H\text{-}146\times2\text{–}162]^-$, 749 $[M\text{-}H\text{-}146\text{–}162\times2]^-$, 603 $[M\text{-}H\text{-}146\times2\text{–}162\times2]^-$. ^1H NMR: δ 0.85, 1.08, 1.10, 1.11, 1.33, 1.73 (3H × 6, *s*, Me-23, Me-24, Me-25, Me-26, Me-27 and Me-29), 0.87(3H, *d*, *J* = 6.5 Hz, Me-30), 3.22 (1H, *dd*, *J* = 4.5, 11.5 Hz, H-3), 4.83 (1H, *d*, *J* = 6.0 Hz, H-1 of Ara), 5.19 (1H, *d*, *J* = 7.9 Hz, H-1 of Glc), 6.19 (1H, *br.s*, H-1 of Rha), 6.21 (1H, *d*, *J* = 7.8 Hz, H-1 of 28-Glc), 6.69 (1H, *br.s*, H-1 of 28-Rha), ^{13}C NMR data see Table 1.Latifoloside G(**2**). m.p. 215–218°, $C_{59}H_{96}O_{26}$, IR $\nu_{max}^{KBr}cm^{-1}$: 3400–3100 (OH), 2932 (C-H), 1734 (C=O), 1640 (C=C). FAB-MS *m*/*z*: 1219 $[M\text{-}H]^-$, 1073 $[M\text{-}H\text{-}146]^-$, 911 $[M\text{-}H\text{-}146\text{–}162]^-$, 765 $[M\text{-}H\text{-}146\times2\text{–}162]^-$, 749 $[M\text{-}H\text{-}146\text{–}162\times2]^-$, 603 $[M\text{-}H\text{-}146\times2\text{–}162\times2]^-$. ^1H, ^{13}C NMR: δ 0.89, 1.12, 1.16, 1.18, 1.39, 1.70, (3H × 6, *s*, Me-23, Me-24, Me-25, Me-26, Me-27 and Me-29), 1.05 (3H, *d*, *J* = 6.4 Hz, Me-30), 3.27 (1H, *dd*, *J* = 4.5, 11.5 Hz, H-3), 5.54 (1H, *br.s*, H-12), 4.85 (1H, *d*, *J* = 5.4 Hz, H-1 of Ara), 5.80 (1H, *d*, *J* = 7.7 Hz, H-1 of Glc), 6.14 (1H, *br.s*, H-1 of Rha), 6.28 (1H, *d*, *J* = 8.0 Hz, H-1 of 28-Glc), 6.65 (1H, *br.s*, H-1 of 28-Rha). ^{13}C NMR data see Table 1.Latifoloside H(**3**). m.p. 227–231°, $C_{59}H_{96}O_{26}$, IR $\nu_{max}^{KBr}cm^{-1}$: 3430 (OH), 2928 (C-H), 1730 (C=O), 1642 (C=C). FAB-MS *m*/*z*: 1219 $[M\text{-}H]^-$, 1073 $[M\text{-}H\text{-}146]^-$, 911 $[M\text{-}H\text{-}146\text{–}162]^-$, 765 $[M\text{-}H\text{-}146\times2\text{–}162]^-$, 749 $[M\text{-}H\text{-}146\text{–}162\times2]^-$, 603 $[M\text{-}H\text{-}146\times2\text{–}162\times2]^-$. ^1H NMR: δ 0.87, 0.97, 1.10, 1.12, 1.14, 1.19, 1.64 (3H × 7, *s*), 3.30 (1H, *dd*, *J* = 4.3, 11.3 Hz, H-3), 4.86 (1H, *d*, *J* = 5.7 Hz, H-1 of Ara), 5.09 (1H, *d*, *J* = 8.1 Hz, H-1 of Glc), 6.16 (1H, *br.s*, H-1 of Rha), 6.36 (1H, *d*, *J* = 7.5 Hz, H-1 of 28-Glc), 6.65 (1H, *br.s*, H-1 of 28-Rha), ^{13}C NMR data see Table 1.

Alkaline hydrolysis of latifoloside F(**1**)

LiOH (6 mg) was added to a soln of latifoloside F(**1**, 24 mg) in H_2O (3.0 ml). The reaction mixture was heated with stirring at 40° for 10 hr, then cooled to ambient temperature, and the solvent removed *in vacuo* to give a product (**5**, 18 mg). The product was purified by CC (silica gel 3 g, CH_2Cl_2:MeOH, 3:1) to afford a hydrolysate (15 mg), and a mixture of rhamnose and glucose (1:1) that were detected by HPLC.

Acid hydrolysis of latifolosides F-H

A soln of each compound (10 mg) was heated at 100° in 5% H_2SO_4 and 50% EtOH for 10 hr. The reaction mixture was diluted with water, neutralized with 2% NaOH and evaporated *in vacuo* to dryness. The reaction product was a mixture of arabinose, glucose, and rhamnose (1:2:2). The mole ratio of each sugar was determined by using RI detection in HPLC (Shodex RS pak DC-613, 75% MeCN, 1 ml min^{-1}, 70°) by comparison with authentic sugars (10 mM each of Ara, Glc and Rha). The retention time of each sugar was as follows: Ara, 6.0 min; Glc, 7.4 min and Rha, 4.8 min.

Enzymatic hydrolysis of latifoloside F(**1**)

Latifoloside F (**1**) (35 mg) was taken up in 5 ml each of EtOH-H_2O (1:9) and 0.01 M NaH_2PO_4 buffer (pH 4.0), and incubated with crude cellulase (50 mg, Sigma) for two weeks at 37°. The soln was diluted with H_2O and then filtered. The resulting residue was subjected to MCI gel (CHP20P). Elution with water and MeOH afforded crude genin. The crude genin was chromatographed on a silica gel column with $CHCl_3$-MeOH-H_2O (250:40:1) giving ilexgenin B (**4**, 12 mg). Identification was by ^1H and ^{13}C NMR data.

Enzymatic hydrolysis of latifolosides G(**2**) *and H*(**3**)

Latifoloside G(**2**) (25 mg) and H(**3**) (30 mg) were hydrolyzed in the same way as for **1** to give pomolic acid (**6**, 8 mg), and siaresinolic acid (**7**, 9 mg), which had the same NMR data as an authentic sample.

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