

TRITERPENOID SAPONINS FROM THE LEAVES OF *ILEX*
LATIFOLIA

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(Received in revised form 20 January 1997)

Key Word Index—*Ilex latifolia*; Aquifoliaceae; triterpene; saponins; latifolioside A–E; pomolic acid; siaresinolic acid; ilexgenin B.

Abstract—Five new triterpenoid saponins latifoliosides A–E were isolated from the leaves of *Ilex latifolia*, along with a known compound. Their chemical structures have been elucidated on the basis of the chemical and spectral methods. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Ilex latifolia Thunb, one of the species in the *Ilex* genus used in the tea Ku-Ding Cha [1], has been used in China as a diuretic and remedy for sore throat, weight loss and hypertension [2, 3]. In a previous paper [4, 5], we reported the identity of the triterpenoid glycosides from *I. kudincha*. As a part of our continuing phytochemical research on plants in the genus of *Ilex*, this paper deals with the isolation and structural elucidation of five new triterpenoid saponins, latifolioside A(1), B(2), C(3), D(4) and E(5), along with a known compound (6) from the leaves of *I. latifolia*.

RESULTS AND DISCUSSION

The butanol soluble fraction of the methanol extract of the leaves of *I. latifolia* was repeatedly chromatographed on silica gel to yield six saponins 1–6. By comparing of the ¹H and ¹³C NMR signals with reported data, compound (6) was identified as kudinoside G, isolated from *I. kudincha* [4]. Three compounds 1, 2, 4 and the other three compounds 3, 5, 6 were isomers, respectively. Their IR spectra showed ester group absorption (1730 cm⁻¹) together with strong hydroxyl absorption (3650–3100 cm⁻¹) and a C=C double bond (1640 cm⁻¹).

Compound 1 was a colourless powder and its molecular formula was determined as C₄₇H₇₆O₁₇ by the negative FAB-mass spectrum together with its NMR spectrum (DEPT). Cellulase treatment of 1

gave an aglycone (7), which was identified as pomolic acid by comparison of its spectral properties with reference data [6]. The ¹H, ¹³C NMR spectra of 1 indicated the presence of three anomeric signals; one α-arabinopyranosyl unit [H-1: δ 4.88 (*d*, *J* = 5.2 Hz), C-1: δ 104.8], one α-rhamnopyranosyl unit [H-1: δ 6.11, *br s*, C-1: δ 101.9], and one β-glucopyranosyl unit [H-1: δ 6.28 (*d*, *J* = 8.0 Hz), C-1: δ 96.0]. Compound 1 afforded L-arabinose, D-glucose, and L-rhamnose (1:1:1) (by HPLC) on acid hydrolysis. For the aglycone moiety, a glycosylation shift was observed for C-3 signal (+10.7 ppm, from δ 78.4 to δ 89.1). In the ¹³C NMR spectrum, the anomeric carbon of the glucose group at δ 96.0 suggested that 1 had a 28-*O*-glycosidic linkage, which was proved by the ¹³C NMR signal at δ 177.2 (C-28), alkaline hydrolysis and the HMBC spectrum (see Fig. 1). Alkaline hydrolysis of 1 gave compound (8) and D-glucose. The ¹H NMR and ¹³C NMR spectra of 8 indicated the presence of one α-arabinopyranosyl unit [H-1: δ 4.87 (*d*, *J* = 5.3 Hz), C-1: δ 104.9] and one α-rhamnopyranosyl unit [H-1: δ 6.20, *br s*, C-1: δ 102.0]. Comparison of the ¹³C NMR spectrum of 8 with that of ziyuglucoside 9 [7] showed a glycosylating shift for the C-2 signal of the arabinopyranosyl moiety, demonstrating that an α-rhamnopyranosyl group is located at the C-2 hydroxyl of arabinose. In the HMBC spectrum of 1, there were three characteristic cross-peaks between C-3 of the aglycone and the anomeric proton of arabinose, between the C-2 of arabinose and the anomeric proton of rhamnose, and between the quaternary carbon C-28 and the anomeric proton of glucose. Thus, compound 1 was identified as 3-*O*-[α-L-rhamnopyranosyl (1-2)]-α-L-arabinopyranosyl pomolic acid 28-*O*-β-D-glucopyranoside, and named as latifolioside A.

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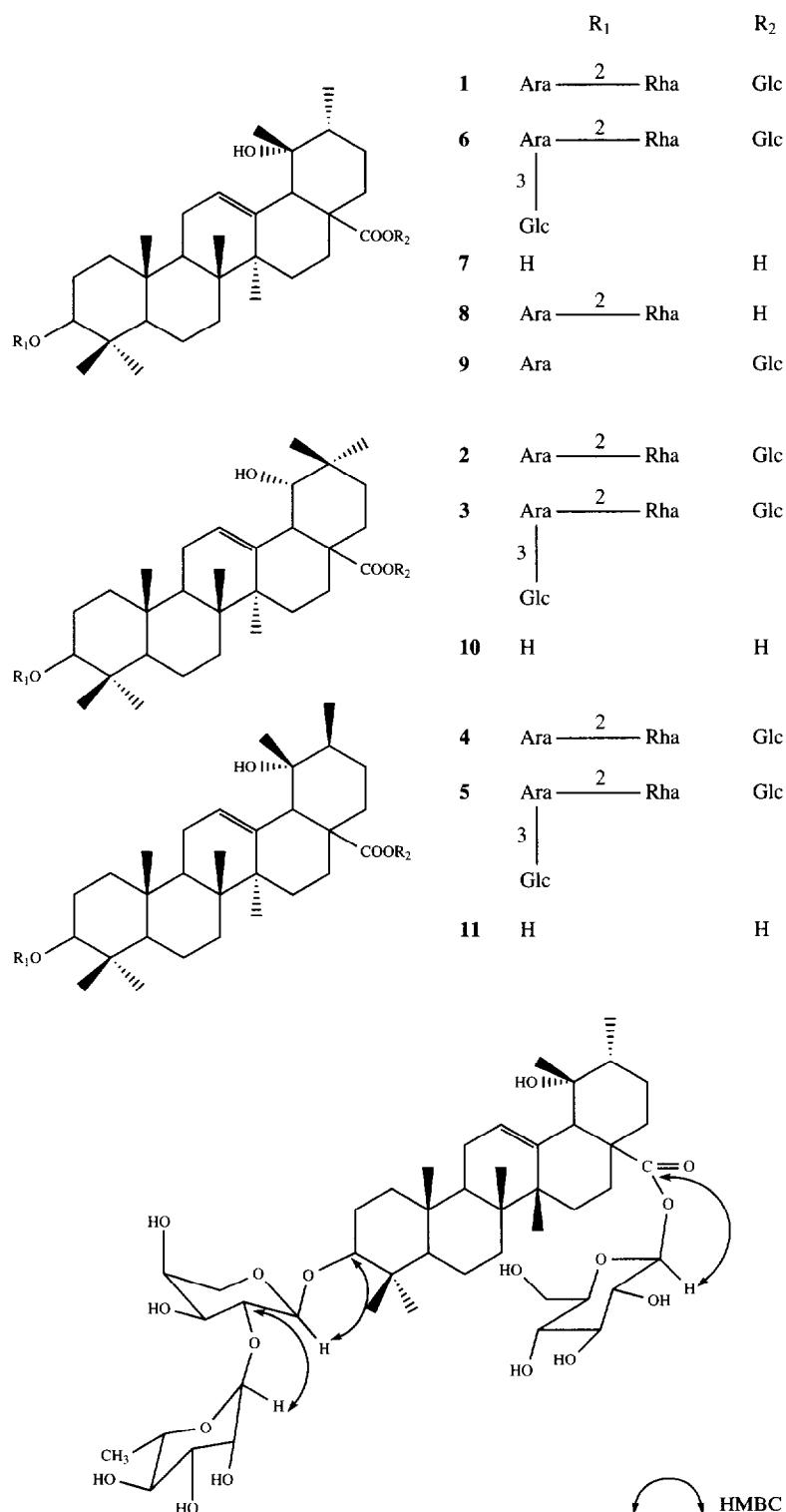


Fig. 1.

Compound **2** gave a quasi-molecular ion peak $[M-H]^-$ at m/z 911, which corresponded to $C_{47}H_{76}O_{17}$ in the negative FAB-mass spectrum and ^{13}C NMR (DEPT). Cellulase treatment of **2** provided an aglycone (**10**), which was identical with siaresinolic acid,

and a mixture of L-arabinose, D-glucose and L-rhamnose (1:1:1) as determined by HPLC analysis. The IR spectrum of **2** showed a similar absorption pattern to that of latifoloside A. Comparison of the ^{13}C NMR data for **2** with that of the sugar moiety of **1** showed

that **2** and **1** were the same sugar chain. Thus, **B(2)** was concluded to the 3-*O*-[α -L-rhamnopyranosyl(1-2)]- α -L-arabinopyranosyl siaresinolic acid 28-*O*- β -D-glucopyranoside (^{13}C NMR Table 1).

The element composition of compound **3** was proved to be $\text{C}_{53}\text{H}_{86}\text{O}_{22}$ by the negative FAB-mass spectrum and ^{13}C NMR (DEPT). Treatment of **3** with cellulase afforded siaresinolic acid by comparing the ^{13}C NMR data, and a mixture of L-arabinose, D-glucose and L-rhamnose (1:2:1). Comparison of the NMR data for **3** with that for **6** showed that **3** contained the same oligosaccharic sequence as **6** (Table 1). Consequently, latifoloside C (**3**) was identified as 3-*O*-[α -L-rhamnopyranosyl(1-2)]- β -D-glucopyranosyl (1-3)]- α -L-arabinopyranosyl siaresinolic acid 28-*O*- β -D-glucopyranoside.

The negative fast atom bombardment mass spectrum of compound **4** gave a quasi-molecular ion peak m/z 911 $[\text{M}-1]^+$, corresponding to $\text{C}_{47}\text{H}_{76}\text{O}_{17}$. Cellulase treatment of **4** furnished an aglycone (**11**) and a mixture of L-arabinose, D-glucose and L-rhamnose (1:1:1) by HPLC. In the ^1H NMR spectrum of **4** and **11**, one methyl δ 0.98 (3H, *d*, $J = 6.6$ Hz) was observed, which showed that the methyl connected with the carbon of methine. The NOESY experiment on the aglycone (**11**) exhibited two characteristic cross peaks between the signal assignable to the methine proton at C-18 (δ 3.16) and the signal assignable to the methyl proton at C-29 (δ 1.40), and the signal of the methyl proton at C-30 (δ 0.96). Comparison of the NMR data for **11** with that for **7** showed that resonances of the D- and E-ring carbons were significantly different (Table 1). The chemical shifts (**7** vs **11**) of signals due to C-18 (-7.2 ppm), C-22 (-4.2 ppm) and C-29 ($+2.7$ ppm) revealed the C-30 methyl group to be β (axial) in place of the α (equatorial) methyl in **7**. Hence, the aglycone of **4** was formulated as 30(*s*)-3 β ,19 α -dihydroxyurs-12-en-28-oic acid, which has the same aglycone as the triterpene ilexgenin B [6, 8]. As latifoliosides A, B, D were isomers, latifoloside D (**4**) was formulated as 3-*O*-[α -L-rhamnopyranosyl (1-2)]- α -L-arabinopyranosyl ilexgenin B 28-*O*- β -glucopyranoside.

By the same deduction, compounds **3**, **5**, **6** were isomers and **5** and **4** have the same aglycone (ilexgenin B). Thus, **5** was determined to be 3-*O*-[α -L-rhamnopyranosyl(1-2)]- β -D-glucopyranosyl(1-3)]- α -L-arabinopyranosyl ilexgenin B 28-*O*- β -D-glucopyranoside (^{13}C NMR Table 1).

EXPERIMENTAL

All mps were determined on a Beijing Micromelting apparatus and are uncorr. IR were run with a Perkin-Elmer 683 spectrometer. ^1H , ^{13}C NMR and 2D NMR were measured by Bruker AM 400 Hz spectrometers with pyridine- d_5 as a solvent and TMS as an int. standard. FAB-MS were taken on a ZAM-HB system spectrometer. CC and TLC were performed on silica

Table 1. ^{13}C NMR spectral data for latifoloside A-E (400 MHz, pyridine- d_5)

Carbon	A	(6)	B	C	D	E
1	39.1	39.3	39.0	39.0	39.2	39.3
2	26.8	26.8	26.7	26.7	27.0	27.0
3	89.1	88.4	89.2	88.3	89.1	88.4
4	39.6	39.7	39.7	39.7	39.7	39.8
5	56.1	56.2	56.2	56.2	56.2	56.2
6	18.9	18.8	18.9	18.8	18.9	18.9
7	33.6	33.6	33.1	33.1	32.1	32.0
8	40.7	40.7	40.2	40.3	40.6	40.6
9	47.9	47.9	48.4	48.4	48.0	48.0
10	37.1	37.1	37.3	37.3	37.2	37.2
11	24.2	24.2	24.2	24.2	24.2	24.2
12	128.5	128.6	123.7	123.6	127.8	127.8
13	139.4	139.4	144.4	144.4	139.0	138.9
14	42.2	42.2	42.2	42.2	42.3	42.3
15	29.4	29.4	29.2	29.2	29.4	29.4
16	26.2	26.3	28.1	28.1	24.9	24.8
17	48.8	48.8	46.6	46.6	48.6	48.5
18	54.6	54.6	44.7	44.7	47.4	47.4
19	72.8	72.8	81.1	81.1	73.6	73.6
20	42.2	42.2	35.7	35.7	42.9	42.9
21	26.7	26.8	29.0	29.0	26.8	26.8
22	37.9	37.9	33.3	33.3	33.7	33.6
23	28.3	28.3	28.2	28.2	28.3	28.3
24	16.8	16.9	17.0	17.1	17.2	17.2
25	15.8	15.9	15.7	15.7	15.9	15.9
26	17.5	17.5	17.6	17.7	17.7	17.6
27	24.7	24.7	24.8	24.2	24.5	24.5
28	177.2	177.3	177.5	177.5	177.5	177.3
29	27.2	27.2	28.7	28.8	29.9	29.8
30	17.1	17.2	25.0	25.0	16.3	16.2
Sugar						
3- <i>O</i> -Ara						
1	104.8	104.8	104.9	104.9	104.9	104.8
2	76.1	74.7	76.1	74.7	76.2	74.7
3	74.2	82.1	74.2	82.3	74.2	82.1
4	68.6	68.2	68.7	68.3	68.8	68.2
5	64.5	64.9	64.7	64.6	64.7	64.8
Rha						
1	101.9	102.0	101.9	102.0	101.9	102.0
2	72.7	72.5	72.4	72.6	72.5	72.5
3	72.8	72.6	72.6	72.5	72.5	72.6
4	74.1	74.2	74.1	74.2	74.1	74.2
5	70.0	70.2	70.1	70.1	70.1	70.2
6	18.7	18.7	18.7	18.7	18.8	18.7
Glc						
1		104.7		104.7		104.7
2		75.1		75.1		75.1
3		78.3		78.3		78.3
4		71.3		71.5		71.6
5		78.8		78.6		78.7
6		62.6		62.6		62.6
28- <i>O</i> -Glc						
1	96.0	96.0	96.0	96.0	96.0	96.0
2	73.7	74.0	73.7	74.0	73.8	74.0
3	79.0	79.0	79.0	79.0	79.0	79.0
4	71.4	71.5	71.3	71.2	71.2	71.2
5	79.3	79.4	79.3	79.4	79.4	79.4
6	62.5	62.4	62.2	62.2	62.3	62.3

gel, RP-8 and RP-18 using the following solvent systems: a) CHCl₃-MeOH-H₂O (7:3:0.5), CHCl₃-MeOH-H₂O (65:35:9) and MeOH-H₂O (6:4-7:3) for saponins; b) CHCl₃-MeOH-H₂O (7:3:1) lower-layer 9 ml+1 ml HOAc for sugars. Detection: for saponins, spraying with 5% H₂SO₄ following by heating for 5 min at 105°, for sugar, aniline-phthalate reagent.

Plant material. *Ilex latifolia* plants were collected in the Hunan Province of China in the Summer of 1993 and identified by Prof. Chong-Ren Yang. A voucher specimen 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 x 3 with MeOH at 50° for 8 hr, and the solvent was removed under red. pres. The combined extract (100 g) was suspended in H₂O and the aq. suspension was extracted with CHCl₃ and *n*-BuOH, respectively. The *n*-BuOH layer was evapd to dryness to give a residue (50 g) which was chromatographed on silica gel (1.5 kg, 200-300 mesh) with CHCl₃-MeOH-H₂O (7:3:0.5) to give 20 fr. Fr. 10 and fr. 14 were sepd on HPLC [ODS, eluting with MeOH-H₂O (8:2-6:4). Flow rate: 5 ml min⁻¹; Injection: 0.4 ml (10 mg ml⁻¹)] to afford latifolioside A (1, 80 mg), B (2, 50 mg), C (3, 75 mg), D (4, 80 mg), E (5, 140 mg) and F (6, 125 mg).

Latifolioside A (1). Colourless powder, mp (207-210)°, C₄₇H₇₆O₁₇, IR ν_{max}^{KBr} cm⁻¹: 3433 (OH), 2933 (C-H), 1734 (C=O), 1647 (C=C), 1458, 1386, 1074, 1028. FAB-MS *m/z*: 911 [M-H]⁻, 749 [M-H-162]⁻, 603 [M-H-162-146]⁻, 471 [M-H-162-146-132]⁻, 453 [M-H-162-146-132-H₂O]⁻. ¹H NMR: δ 0.89 (s, 3-H), 1.05 (*d*, *J* = 6.4 Hz, 3H), 1.07 (s, 3H), 1.39 (s, 3H), 1.75 (s, 3H), 2.92 (1H, *br s*, 18β-H), 3.23 (1H, *dd*, *J* = 11.3, 4.2 Hz, 3α-H), 5.55 (1H, *br s*, 12-H), 4.88 (1H, *d*, *J* = 5.2 Hz, C-1-H of Ara), 6.11 (1H, *br s*, C-1-H of Rha), 1.61 (3H, *d*, *J* = 6.2 Hz, C-6-H of Rha), 6.28 (1H, *d*, *J* = 8.0 Hz, C-1-H of Glc). ¹³C NMR data see Table 1.

Latifolioside B (2). Colourless powder, mp 225-228°, C₄₇H₇₆O₁₇; IR ν_{max}^{KBr} cm⁻¹: 3404 (OH), 2935 (C-H), 1730 (C=O), 1643 (C=C), 1450, 1380, 1072, 1030. FAB-MS *m/z*: 911 [M-H]⁻, 749 [M-H-162]⁻, 603 [M-H-162-146]⁻, 453 [M-H-162-146-132]⁻. ¹H NMR: δ 0.87 (s, 3H), 0.97 (s, 3H), 1.10 (s, 3H), 1.12 (s, 3H), 1.14 (s, 3H), 1.19 (s, 3H), 1.64 (s, 3H), 3.29 (*dd*, *J* = 11.2, 4.5 Hz, 3α-H), 5.50 (*br s*, 12-H), 3.54 (*t*-like), 3.50 (*br s*, 18α-H), 4.86 (1H, *d*, *J* = 5.5 Hz, C-1-H of Ara), 6.11 (1H, *br s*, C-1-H of Rha), 1.61 (3H, *d*, *J* = 6.2 Hz, C-6-H of Rha), 6.30 (1H, *d*, *J* = 7.9 Hz, C-1-H of Glc); ¹³C NMR data see Table 1.

Latifolioside C (3). Colourless powder, mp 231-234°, C₅₃H₈₆O₂₂, IR ν_{max}^{KBr} cm⁻¹: 3420 (OH), 2930 (C-H), 1731 (C=O), 1637 (C=C), 1454, 1386, 1070, 1026. FAB-MS *m/z*: 1073 [M-1]⁻, 911 [M-1-162]⁻, 749 [M-1-2-162]⁻, 765 [M-1-162-146]⁻, 603 [M-1-146-2×162]⁻, 453 [M-1-146-2×162-132-H₂O]⁻. ¹H NMR: δ 0.87 (s, 3H), 0.97 (s, 3H),

1.10 (s, 3H), 1.12 (s, 3H), 1.14 (s, 3H), 1.19 (s, 3H), 1.64 (s, 3H), 3.30 (1H, *dd*, *J* = 11.3, 4.3 Hz, 3α-H), 3.51 (*br s*, 18α-H), 5.50 (*br s*, 12-H), 4.86 (1H, *d*, *J* = 5.7 Hz, C-1-H of Ara), 5.09 (1H, *d*, *J* = 8.1 Hz, C-1-H of Glc), 6.16 (1H, *br s*, C-1-H of Rha), 1.62 (*d*, *J* = 6.8 Hz, C-6-H of Rha), 6.36 (1H, *d*, *J* = 7.5 Hz, C-1-H of Glc); ¹³C NMR data see Table 1.

Latifolioside D (4). Colourless powder, mp 212-215°, C₄₇H₇₆O₁₇, IR ν_{max}^{KBr} cm⁻¹: 3420 (OH), 2930 (C-H), 1733 (C=O), 1641 (C=C), 1450, 1387, 1074, 1026. FAB-MS *m/z*: 911 [M-1]⁻, 749 [M-1-162]⁻, 603 [M-1-162-146]⁻, 471 [M-1-162-146-132]⁻, 453 [M-1-162-146-132-H₂O]⁻. ¹H NMR: δ 0.88 (s, 3H), 0.96 (*dd*, *J* = 6.6 Hz, 3H), 1.06 (s, 3H), 1.13 (s, 3H), 1.16 (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), 5.27 (s, 19β-OH), 4.88 (1H, *J* = 5.1 Hz, C-1-H of Ara), 6.30 (1H, *d*, *J* = 7.9 Hz, C-1-H of Glc), 6.11 (1H, *br s*, C-1-H of Rha), 1.60 (3H, *d*, *J* = 5.7 Hz, C-6-H of Rha). ¹³C NMR data: see Table 1.

Latifolioside E (5). Colourless powder, mp 228-230°, C₅₃H₈₆O₂₂, IR ν_{max}^{KBr} cm⁻¹: 3430 (OH), 2932 (C-H), 1730 (C=O), 1454, 1388, 1072. FAB-MS *m/z*: 1073 [M-1]⁻, 911 [M-1-162]⁻, 765 [M-1-162-146]⁻, 749 [M-1-2×162]⁻, 603 [M-1-146-2×162]⁻, 471 [M-1-146-2×162-132]⁻, 453 [M-1-146-2×162-132-H₂O]⁻. ¹H NMR: δ 0.87 (s, 3H), 0.96 (*dd*, *J* = 7.0 Hz, 3H), 1.12 (s, 3H), 1.16 (s, 3H), 1.17 (s, 3H), 1.39 (s, 3H), 1.71 (s, 3H), 3.27 (*dd*, *J* = 11.2 Hz, 4.3 Hz, 3α-H), 3.16 (s, 18α-H), 5.22 (s, 19β-OH), 5.50 (*br s*, 12-H), 4.85 (1H, *d*, *J* = 5.3 Hz, C-1-H of Ara), 5.08 (1H, *d*, *J* = 7.6 Hz, C-1-H of Glc), 6.13 (1H, *br s*, C-1-H of Rha), 1.61 (3H, *d*, *J* = 6.1 Hz, C-6-H of Rha), 6.31 (1H, *d*, *J* = 8.0 Hz, C-1-H of Glc). ¹³C NMR data: see Table 1.

Compound (6). Colourless powder, mp 228-230°, C₅₃H₈₆O₂₂, IR ν_{max}^{KBr} cm⁻¹: 3428 (OH), 2932 (C-H), 1734 (C=O), 1638 (C=C), 1454, 1389, 1073, 1026. FAB-MS *m/z*: 1073 [M-1]⁻, 911 [M-1-162]⁻, 765 [M-1-162-146]⁻, 749 [M-1-2×162]⁻, 603 [M-1-146-2×162]⁻, 453 [M-1-146-2×162-132-H₂O]⁻. ¹H NMR: δ 0.87 (s, 3H), 1.06 (*d*, *J* = 6.4 Hz, 3H), 1.12 (s, 3H), 1.16 (s, 3H), 1.18 (s, 3H), 1.39 (s, 3H), 1.70 (s, 3H), 1.61 (3H, *d*, *J* = 6.0 Hz, C-6-H of Rha), 4.85 (1H, *d*, *J* = 5.4 Hz, C-1-H of Ara), 3.27 (1H, *dd*, *J* = 11.4, 4.5 Hz, 3α-H), 5.54 (1H, *br s*, 12-H), 5.08 (1H, *d*, *J* = 7.7 Hz, C-1-H of Glc), 6.14 (1H, *br s*, C-1-H of Rha), 6.28 (1H, *d*, *J* = 8.0 Hz, C-1-H of Glc).

Alkaline hydrolysis of latifolioside A (1). LiOH (6 mg) was added to a soln of latifolioside A (1, 24 mg) in H₂O (3.0 ml). The reaction mixt. was heated with stirring at 40° for 10 hr, then cooled to ambient temp. and the solvent was removed on a rotary evaporator to give **8** (18 mg). Compound **8** was purified by CC (silica gel, 3 g, CH₂Cl₂-MeOH, 3:1) to afford a hydrolysate (15 mg) and D-glucose.

Acid hydrolysis of latifolioside A-E. A soln of each compound (10 mg) in 5% H₂SO₄ in 50% EtOH was

heated at 100° for 10 hr. The reaction mixt. was diluted with H₂O, neutralized with 2% NaOH and evapd *in vacuo* to dryness. The mole ratio and D/L of each sugar was determined using RI detection (Waters 410) and chiral detection (Shodex OR-1), respectively, in HPLC (Shodex RS pak DC-613, MeCN-H₂O, 3:1, 1 ml min⁻¹, 70°) by comparison with authentic sugars (10 mM each of L-Ara, D-Glc and L-Rha). Each sugar gave a peak as follows: L-Ara, 6.0 min; D-Glc, 7.4 min and L-Rha, 4.8 min.

Enzymatic hydrolysis of latifoloside A (1). Latifoloside A (**1**) (35 mg) was taken in EtOH-H₂O (1:9) and 0.01 M NaH₂PO₄ buffer (pH 4.0), 5 ml of each, incubated with crude cellulase (50 mg, Sigma) for two weeks at 37° and worked-up as usual. The crude genin was chromatographed on a silica gel column with CHCl₃-MeOH-H₂O (250:40:1) giving pomolic acid (**7**, 12 mg) which was identical with an authentic sample of pomolic acid based on comparison of ¹H and ¹³C NMR data.

Enzymatic hydrolysis of latifoloside B (2) and C (3). Enzymatic hydrolysis of latifoloside B (**2**) (25 mg) or C (**3**) (30 mg) was carried out in the same way as for **1** to give siaresindic acid (**9**, 8 mg), which had the same NMR data as an authentic sample.

Enzymatic hydrolysis of latifoloside D (4) and E (5). Latifoloside D (**4**) (20 mg), or E (**5**) (30 mg), was hydrolysed in the same way as for **1** to give ilexgenin

B (**10**, 8 mg), which had the same NMR data as an authentic sample.

Acknowledgements—The authors are grateful to the staff of the Laboratory of Kunming Institute of Botany, Chinese Academy of Sciences for financial support and their valuable advice.

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