Latifolosides K and L, Two New Triterpenoid Saponins from the Bark of *Ilex latifolia*

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Two new triterpenoid saponins, latifoloside K (1), 3-*O*-β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)-]-α-L-arabinopyranosyl 3β-hydroxy-urs-12,18-dien-28-oic acid 28-*O*-β-D-glucopyranosyl ester and latifoloside L (2), 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)-]-α-L-arabinopyranosyl 3β,19α-dihydroxyursolic acid, were isolated from the bark of *Ilex latifolia* THUNB. Also isolated were two known compounds, ilekudinoside A (3) and kudinoside G (4). Structural assignments were established on the basis of spectroscopic data and chemical evidence.

Key words *Ilex latifolia*; Aquifoliaceae; triterpenoid saponin; latifoloside K; latifoloside L

Ilex latifolia THUNB., one of the species in the *Ilex* genus used in the Chinese tea "Ku-Ding-Cha," has been used in China as a diuretic, remedy for sore throats, weight loss and for the relief of hypertension.[1)] From the leaves of *I. latifolia*, Ouyang *et al.*[2)] have isolated eight new triterpenoid saponins. In our previous papers,[3)] we reported the isolation and identification of triterpenes and two new saponins (latifoloside I, 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl 3β,21α,28-trihydroxy-urs-12-en 21-*O*-β-D-glucopyranoside, and latifoloside J, 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl 3β,21α-dihydroxy-urs-12-en-28-oic acid 21-*O*-β-D-glucopyranoside) from the bark of this species. As a part of our continuing study, this paper deals with the isolation and structural elucidation of two new triterpenoid saponins, latifoloside K (**1**) and L (**2**), along with two known compounds, ilekudinoside A (**3**) and kudinoside G (**4**), from the bark of *I. latifolia.*

Results and Discussion

The bark of *I. latifolia* was extracted with methanol and the methanol extract was partitioned between water with *n*-hexane and *n*-butanol, respectively. Chromatography of the *n*-butanol extract on silica gel, Lobar RP-18, and Sephadex LH-20, after repeated HPLC purification over an octadecylsilica (ODS)gel column, furnished two new saponins (**1**, **2**), and two known ones (**3**, **4**). By comparing the ^{1}H- and ^{13}C-NMR signals with reported data, compound **3** was identified as ilekudinoside A isolated from *I. kudincha*,[4)] while compound **4** was identified as kudinoside G isolated from *I. kudincha*[5)] and from the leaves of *I. latifolia.*[2a)]

Latifoloside K (**1**) was obtained as a colorless powder, and had a molecular formula of $C_{53}H_{84}O_{21}$ based on the high resolution (HR)-FAB-MS spectrum. The ^{13}C-NMR spectral data of **1** showed 53 carbon signals, 30 of which were assigned to the aglycone part, while 23 were assigned to the carbohydrate moiety. The seven methyl carbon signals at δ 16.4, 17.1, 18.3, 20.2, 20.5, 22.3 and 28.2, the four olefinic carbon signals at δ 127.2, 134.8, 135.2 and 138.9, and a carbonyl carbon signal at δ 175.0 were observed in the ^{13}C-NMR spectrum of **1**. Combining the proton signals in the ^{1}H-NMR spectrum, the aglycone of **1** was indicated as an urs-diene type triterpene. From the heteronuclear multiple bond coherence spectroscopy (HMBC) experiment of **1**, the long-range correlations were observed between protons and carbons: H-12 (δ 5.62)/C-18 (δ 134.8); H-29 (δ 1.84)/C-18 (δ 134.8), C-19 (δ 135.2). These correlations revealed two double bonds should be at C-12 (C-13) and C-18 (C-19), respectively. These assignments were confirmed from the downfield shifts of C-18 (+80.2 ppm), C-19 (+62.4 ppm), and C-30 (+3.7 ppm), and the up-field shifts of C-12 (−1.4 ppm), C-13 (−0.5 ppm), C-17 (− 22.1 ppm), C-20 (−5.0 ppm), and C-29 (−7.0 ppm) in comparing the ^{13}C-NMR spectral data of **1** with those of kudinoside G (**4**).[5)] The signal at δ 88.3 on the ^{13}C-NMR spectrum of **1** revealed a hydroxyl group should be substituted at C-3 of the aglycone. The configuration of the hydroxyl at C-3 can be determined using the rotating frame Overhauser enhancement spectroscopy (ROESY) experiment. The correlation of H_{ax}-3 (δ 3.35) and H-5 (δ 0.80) indicated that the hydroxyl at C-3 should be a β-configuration. Based on these findings, the structure of the sapogenin of **1** was established to be 3β-hydroxy-urs-12,18-dien-28-oic acid.

The ^{1}H- and ^{13}C-NMR data of **1** showed four anomeric signals at δ 4.90 (d, J=5.8 Hz), 5.11 (d, J=7.6 Hz), 6.16 (br s), and 6.32 (d, J=8.2 Hz), and δ 104.7, 104.8, 101.9, and 95.8, respectively. Acid hydrolysis of **1** gave three monosaccharides: arabinose, glucose and rhamnose (1 : 1 : 1), which were analyzed by GC as their alditol acetate derivatives. Alkaline hydrolysis of **1** gave glucose. The absolute configurations of the sugars were shown to be L-arabinose, D-glucose, and L-rhamnose according to the method reported by Hara and co-workers.[6)] The NMR techniques, ^{1}H–^{1}H shift correlation spectroscopy (COSY), heteronuclear multiple quantum coherence spectroscopy (HMQC), HMBC, and ROESY, were used to determine the nature of the monosaccharides and sequences of the oligosaccharide chain of **1**. The anomeric configurations and ring sizes of each sugar were obtained following analysis on the H-1 vicinal coupling constants ($^3J_{HH}$,

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$^1J_{CH}$), observing their H-1 chemical shifts, and comparing their ^{13}C-NMR spectral data with those of methyl glycosides.[7] From the relatively large H-1 coupling constants (7.5, 8.2 Hz), the anomeric hydroxyls of both glucose were determined as the β-configuration. In the insensitive nuclei enhanced by polarization transfer (INEPT) spectrum, the CH coupling constant of the C-1 (δ 101.9) signal was 178 Hz, indicating that the glycosidic bond of rhamnose was linked in an α-configuration. Based on these results, the four sugars and their anomeric configurations in **1** were determined to be an α-L-arabinopyranose, two β-D-glucopyranoses and an α-L-rhamnopyranose.

The sequence of the oligosaccharide chain was deduced from ^{13}C shift differences between individual sugar residues and model compounds, and from HMBC and ROESY experiments. The C-1 of arabinose was attached to 3-OH of the aglycone, as indicated by the C-3 chemical shift (δ 88.3) of **1**, the correlation between H-1 (δ 4.90) of arabinose and C-3 of the aglycone in the HMBC experiment, and H-1 of arabinose and H-3 (δ 3.35) in the ROESY experiment. From the HMBC experiment of **1**, the following correlations were observed: H-1 (δ 5.11) of a glucose and C-3 (δ 82.5) of arabinose; H-1 (δ 6.16) of rhamnose and C-2 (δ 74.7) of arabinose; H-1 (δ 6.32) of another glucose and C-28 (δ 175.0) of the aglycone. Based on the above findings, the structure of **1** was determined to be 3-*O*-β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)-]-α-L-arabinopyranosyl 3β-hydroxyurs-12,18-dien-28-oic acid 28-*O*-β-D-glucopyranosyl ester. This is a new triterpenoid saponin, named latifoloside K.

Latifoloside L (**2**) was also obtained as a colorless powder, and had a molecular formula of $C_{53}H_{86}O_{22}$ based on its HR-FAB-MS spectrum. The ^{13}C-NMR spectral data of **2** showed 53 carbon signals, 30 of which were assigned to the aglycone part, while 23 were assigned to the carbohydrate moiety. The seven methyl carbon signals at δ 15.6, 16.7, 17.1, 17.2, 24.7, 27.0 and 28.3, and two olefinic carbons at δ 128.0 and 139.8, and a carbonyl carbon signal at δ 180.9, indicated that the aglycone of **2** was very similar with pomolic acid. Comparing ^{1}H-, ^{13}C-NMR spectral data with those of pomolic acid,[8] the aglycone of **2** was identified as pomolic acid.

The ^{1}H- and ^{13}C-NMR spectral data of **2** displayed four anomeric signals at δ 4.90 (d, J=4.2 Hz), 5.12 (d, J=7.6 Hz), 5.38 (d, J=7.3 Hz), and 5.96 (br s), and δ 104.2, 103.5, 106.0 and 101.5, respectively. Acid hydrolysis of **2** gave three monosaccharides: arabinose, glucose, and rhamnose in a ratio of 1 : 2 : 1, which were analyzed by the same method as the identification in **1**. Likewise as in **1**, the glucose was determined to have a D-configuration, while the arabinose and the rhamnose were determined to have an L-configuration. The sequence of the oligosaccharide chain was deduced from ^{13}C shift differences between individual sugar residues and model compounds, and from HMBC and ROESY experiments. The C-1 (δ 104.2) of arabinose was attached to 3-OH of the aglycone, as indicated by the C-3 chemical shift (δ 88.6) of **2**, the correlation between H-1 (δ 4.90) of arabinose and C-3 (δ 88.6) of the aglycone in the HMBC experiment, and between H-1 of arabinose and H-3 (δ 3.20) in the ROESY experiment. Rhamnose attached to C-2 of arabinose was determinated by the correlation between H-1 (δ 5.96) of rhamnose and C-2 (δ 74.9) of arabinose in the HMBC experiment of **2**. A glucose (inner) attached to C-3 of arabinose, and another glucose (terminal) attached to C-2 of inner glucose were dependent on the correlations between H-1 (δ 5.12) of inner glucose and C-3 (δ 82.9) of arabinose, and between H-1 (δ 5.38) of terminal glucose and C-2 (δ 85.9) of inner glucose, respectively. Based on the above findings, the structure of **2** was shown to be 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)-]-α-L-arabinopyranosyl 3β,19α-dihydroxyursolic acid. This is a new triterpenoid saponin, named latifoloside L.

Experimental

General Optical rotations were measured using a DIP-140 digital polarimeter (JASCO corporation). HR-FAB-MS was conducted using a JMS-SX102A (JEOL) mass spectrometer. ^{1}H- and ^{13}C-NMR were recorded using a JEOL FT-NMR JNM A-500 and/or a Lambda 500 FT-NMR spectrometer (^{1}H at 500 MHz, ^{13}C at 125 MHz). Standard pulse sequences were used for

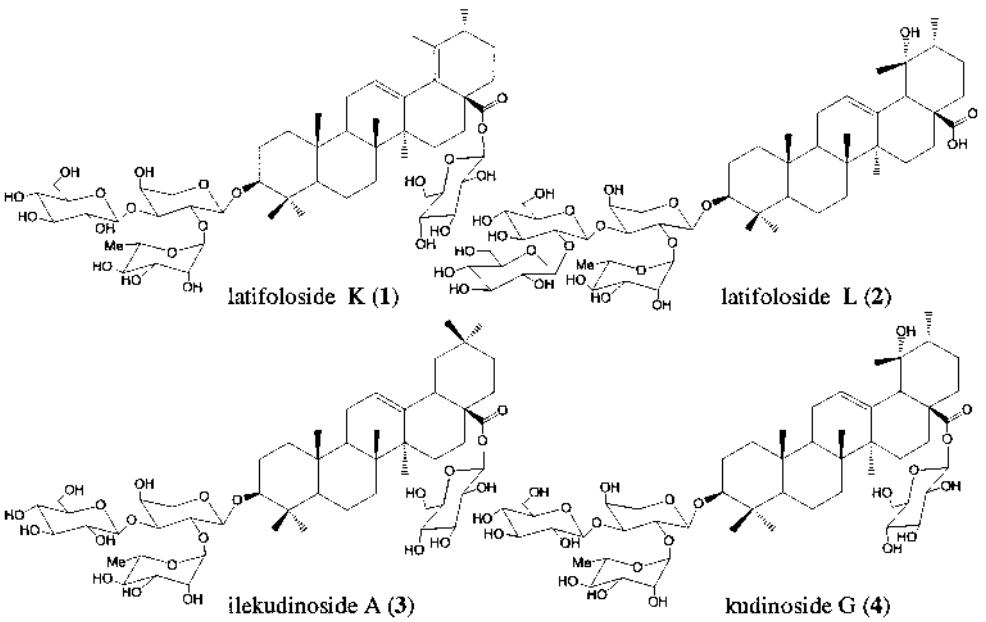

Chart 1

the two dimensions (2D)-NMR experiments. Chemical shifts were expressed in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard, and coupling constants (J) were reported in Hertz (Hz). TLC was carried out on Silica gel $60F_{254}$, and the spots were visualized by spraying with 10% H_2SO_4 and heating. Silica gel (Silica gel 60—70, 230 mesh, Merck), Lichroprep. RP-18 (Lobar, 40—63 μm, Merck) and Sephadex LH-20 were used for column chromatography. Preparative HPLC was performed using an ODS column (PEGASIL ODS, 250×10 mm, Senshu Pak; detector: refractive index and UV 210 nm). GC was run on a Shimadzu GC-14B gas chromatographer.

Isolation of Saponins The bark of *Ilex latifolia* THUNB. was obtained in Tokyo, Japan in 1998. The voucher specimens were identified by Dr. Toshiyuki Akiyama and deposited in the Research Planning Department, Sankyo Co., Ltd. The air dried powder (2 kg) of the bark of *I. latifolia* was extracted with MeOH (12 l×2) under reflux conditions. The MeOH extract (2 l) was partitioned successively between water with *n*-hexane and *n*-butanol, respectively. After removing the solvent, the *n*-butanol extract (58 g) was dissolved in methanol (350 ml) and the methanol solution was dropped into ether (6 l) to obtain a precipitate (40 g) and an ether-soluble part (8 g). Thirty grams of the ether precipitate were chromatographed on a silica gel column with a solvent system of $CHCl_3$–MeOH–H_2O (10 : 2 : 0.2; 7 : 2 : 0.2) to obtain six fractions (frs. 1—6) according to their TLC behavior. Fr. 6 (13 g) was chromatographed on a silica gel column with a solvent system of $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2; 8 : 4 : 0.2; and 7 : 4 : 1) to obtain seven parts (P-1—7). P-5 (3 g) was chromatographed on a Lobar RP-8 column (solvent: $CH_{3O}H : H_2O$=3 : 7; 5 : 5; 7 : 3) to obtain five parts (P-5-1—5). P-5-5 (290 mg) was chromatographed on a silica gel column with $CHCl_3$: MeOH : H_2O=8 : 4 : 0.2 as an eluent to obtain three parts (P-5-5-1—3). P-5-5-1 (100 mg) was chromatographed on a Lobar RP-18 column (solvent: MeOH : H_2O=6 : 4) to obtain latifoloside K (**1**, 22 mg), latifoloside L (**2**, 10.5 mg), and ilekudinoside A (**3**, 5 mg). P-6 (830 mg) was chromatographed on a Lobar RP-18 column with $CH_3CN : H_2O$=5 : 5 as an eluent to obtain three parts (P-6-1—3). P-6-2 (360 mg) was chromatographed on a silica gel column with $CHCl_3$: MeOH : H_2O=8 : 2 : 0.2 as an eluent to obtain two parts (P-6-2-1—2). P-6-2-2 (150 mg) was further purified by HPLC with CH_3CN : H_2O=5 : 5 as an eluent to obtain kudinoside G (**4**, 30 mg).

Latifoloside K (**1**) was obtained as a colorless powder. $[\alpha]_D^{25}$: +0.74° (MeOH, c=0.78). HR-FAB-MS (positive): m/z: 1079.5477 $[M+Na]^+$ (Calcd for $C_{53}H_{84}O_{21}Na$; 1079.5403). ^{1}H-NMR (500 MHz, pyridine-d_5): δ (ppm) 0.80 (1H, dd, J=11.6, 4.6 Hz, H-5), 0.88 (3H, s, H-25), 1.03 (3H, d, J=7.0 Hz, H-30), 1.12 (6H, s, H-26, H-27), 1.13 (3H, s, H-24), 1.84 (3H, s, H-29), 1.19 (3H, s, H-24), 1.23 (3H, s, H-23), 1.64 (3H, d, J=6.1 Hz, H-6 of rhamnose), 3.35 (1H, dd, J=11.6, 4.3 Hz, H-3), 4.90 (1H, d, J=5.8 Hz, H-1 of arabinose), 5.11 (1H, d, J=7.6 Hz, H-1 of glucose), 5.62 (1H, m, H-12), 6.16 (1H, br s, H-1 of rhamnose), 6.32 (1H, J=8.2 Hz, H-1 of 28-*O*-glucose). ^{13}C-NMR spectral data are given in Table 1.

Latifoloside L (**2**) was obtained as a colorless powder. $[\alpha]_D^{25}$: +7.46° (MeOH; c=0.35); HR-FAB-MS (positive): m/z: 1097.5530 $[M+Na]^+$ (Calcd for $C_{53}H_{86}O_{22}Na$; 1097.5508) ^{1}H-NMR (500 MHz, pyridine-d_5): δ (ppm) 0.80 (1H, dd, J=11.3, 4.0 Hz, H-5), 0.84 (3H, s, H-25), 1.07 (3H, s, H-24), 1.11 (3H, s, H-26), 1.13 (3H, d, J=6.4 Hz, H-30), 1.18 (3H, s, H-23), 1.46 (3H, s, H-29), 1.65 (3H, d, J=6.1 Hz, H-6 of rhamnose), 1.74 (3H, s, H-27), 3.04 (1H, s, H-18), 3.20 (1H, dd, J=11.6, 4.5 Hz, H-3), 4.90 (1H, d, J=4.2 Hz, H-1 of arabinose), 5.12 (1H, d, J=7.6 Hz, H-1 of glucose), 5.38 (1H, d, J=7.3 Hz, H-1 of 28-*O*-glucose), 5.60 (1H, m, H-12), 5.96 (1H, br s, H-1 of rhamnose). ^{13}C-NMR spectral data are given in Table 1.

Compounds **1** and **2** (1 mg each) were hydrolyzed and acetylated. The arabitol, glucitol and rhamnitol acetates, in a ratio of 1 : 1 : 1 from compound **1**, and in a ratio of 1 : 2 : 1 from compound **2**, were detected by GC analysis (Condition: Supelco SP-2380 fused silica capillary column; 0.53 mm i.d.×15 m, 0.2 μm film; column temperature: 140 °C→220 °C, 4 °C/min; injection temperature: 250 °C). The absolute configurations of the sugars were determined according to the same methods used in our previous paper.[3b]

Compound **1** (1 mg) was refluxed with 3% KOH for 30 min, and worked-up in the usual way. The aqueous layer was neutralized and lyophilized. The residue was chromatographed over a silica gel TLC using to test for sugar (solvent: *n*-BuOH : AcOH : H_2O=2 : 1 : 1).

Table 1. ^{13}C-NMR Spectral Data of Latifolosides K (**1**) and L (**2**) (125 MHz in Pyridine-d_5)

Aglycone	K (**1**)	**4**[a]	L (**2**)	Sugars	K (**1**)	**4**[a]	L (**2**)
C-1	39.4	39.2	37.0	3-*O*-Arabinose			
C-2	26.7	26.8	26.5	A-1	104.8	104.8	104.2
C-3	88.3	88.4	88.6	A-2	74.7	74.7	74.9
C-4	39.6	39.7	39.5	A-3	82.5	82.4	82.9
C-5	56.3	56.2	56.0	A-4	68.2	68.2	67.9
C-6	18.4	18.8	18.7	A-5	64.9	64.9	63.8
C-7	28.9	33.6	33.5	Rhamnose			
C-8	44.7	40.6	42.3	R-1	101.9	102.0	101.5
C-9	48.3	47.9	47.7	R-2	72.5	72.5	72.2
C-10	36.9	37.2	39.0	R-3	72.5	72.6	72.4
C-11	34.6	24.2	24.0	R-4	74.2	74.0	74.9
C-12	127.2	128.6	128.0	R-5	70.0	70.2	70.0
C-13	138.9	139.4	139.8	R-6	19.0	18.7	18.5
C-14	34.9	42.2	40.3	Glucose			
C-15	26.7	28.4	29.3	G-1	104.7	104.7	103.5
C-16	34.9	26.3	26.3	G-2	75.0	75.1	85.9
C-17	26.7	48.8	48.3	G-3	78.2	78.3	78.4
C-18	134.8	54.6	54.5	G-4	71.5	71.6	71.1
C-19	135.2	72.8	72.6	G-5	78.6	78.6	78.1
C-20	37.3	42.3	42.1	G-6	62.4	62.4	62.3
C-21	28.9	26.7	26.9	28-*O*-glucose			
C-22	34.8	38.0	38.5	G-1	95.8	96.0	106.0
C-23	28.2	28.3	28.3	G-2	73.9	73.7	75.8
C-24	17.1	17.2	17.1	G-3	78.9	79.0	78.4
C-25	16.4	15.9	15.6	G-4	71.3	71.4	70.7
C-26	18.3	17.5	17.2	G-5	79.2	79.3	78.1
C-27	22.3	24.7	24.7	G-6	62.4	62.6	62.3
C-28	175.0	177.3	180.9				
C-29	20.2	27.2	27.0				
C-30	20.5	16.8	16.7				

a) Data were listed from the literature.[4]

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