Latifolosides I and J, Two New Triterpenoid Saponins from the Bark of *Ilex latifolia*

Jing HUANG,[a] Xu WANG,[b] Yukio OGIHARA,[a] Noriko SHIMIZU,[b] Tadahiro TAKEDA,*[,b] and Toshiyuki AKIYAMA[c]

Faculty of Pharmaceutical Sciences, Nagoya City University,[a] Tanabe-dori, Mizuho-ku, Nagoya 467–8603, Japan, Kyoritsu College of Pharmacy,[b] 1–5–30 Shibakoen, Minato-ku, Tokyo 105–8512, Japan, and Research Planning Department, Sankyo Co. Ltd.,[c] 1–2–58 Hiromachi, Shinagawa-ku, Tokyo 140–8710, Japan.
Received August 14, 2000; accepted October 17, 2000

Two new triterpenoid saponins, latifoloside I (1), 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl 3β,21α,28-trihydroxy-urs-12-ene 21-*O*-β-D-glucopyranoside; latifoloside J (2), 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-3β,21α-dihydroxy-ursolic acid 21-*O*-β-D-glucopyranoside, along with two known compounds, latifoloside C (3) and latifoloside E (4), were isolated from the bark of *Ilex latifolia* THUNB. Structure assignments were established on the basis of spectroscopic data and chemical evidence.

Key words *Ilex latifolia*; Aquifoliaceae; triterpenoid saponin; latifoloside I; latifoloside J

Ilex latifolia THUNB., one of the species in the *Ilex* genus used in the tea Ku-Ding-Cha, has been used in China as a diuretic, remedy for sore throats, weight loss and for a relief of hypertension.[1] From the leaves, Ouyang *et al.*[2] have isolated eight new triterpenoid saponins. In a previous paper,[3] we reported the identity of the triterpenes 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 I (**1**) and J (**2**), along with two known compounds, latifoloside C (**3**) and latifoloside E (**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, and then after repeated HPLC purification over octadecyl silica (ODS) gel, furnished two new saponins (**1**, **2**), and two known ones (**3**, **4**).

Latifoloside I (**1**) was obtained as a colorless powder, and had the molecular formula $C_{48}H_{80}O_{17}$ based on the high resolution (HR)-FAB-MS spectrum. The ^{13}C-NMR spectral data of **1** showed 48 carbon signals, 30 of which were assigned to the aglycone part, while 18 were assigned to the carbohydrate moiety. The seven methyl carbon signals at δ 16.0, 17.0, 17.2, 17.8, 17.8, 23.0, and 28.1, and two olefinic carbons at δ 125.1 and 139.5, indicated that the aglycone of **1** had an uvaol-type skeleton. In comparing with the ^{1}H-, ^{13}C-NMR spectral data of **1** with those of uvaol,[4] they were very similar, except for a signal at δ 76.8 (CH by distortionless enhancement by polarization transfer, DEPT). From a heteronuclear multiple bond coherence spectroscopy (HMBC) experiment of **1**, the long-range coupling of H-30 (δ 1.27) with δ 76.8 was observed. Therefore, there should be a hydroxyl group at C-21. This assignment was confirmed by the downfield shifts of C-20 (+3.7 ppm), C-21 (+43.1 ppm), and C-22 (+8.4 ppm), and the upfield shifts of C-30 (−3.5 ppm). The signal at δ 88.8 on the ^{13}C-NMR spectrum of **1** revealed there should be a hydroxyl group at C-3. The relative configuration of the hydroxyl at C-3 could be determined using the rotating frame Overhouser enhancement spectroscopy (ROESY) experiment. The correlations of H_{ax}-3 (δ 3.22, dd, J=11.3, 4.0 Hz) and H-5 (δ 0.71, dd, J=11.3, 4.6 Hz) indicated that the hydroxyl at C-3 should have a β-configuration. The relatively small coupling constants (2.9 Hz) of H-21 (δ 4.32) indicated that the hydroxyl at C-21 should have an α-configuration. Based on these findings, the structure of the aglycone part of **1** was established to be 3β,21α,28-trihydroxy-urs-12-ene.

The ^{1}H- and ^{13}C-NMR data of **1** showed three anomeric signals at δ 4.96 (d, J=7.5 Hz), 5.08 (d, J=7.6 Hz), and 6.56 (br s), and δ 105.5, 101.8, and 101.6, respectively. Acid hydrolysis of **1** gave two monosaccharides: glucose and rhamnose (2 : 1), which were analyzed by GC as their alditol acetate derivatives. The absolute configurations of the sugars were shown to be D-glucose, and L-rhamnose according to the method reported by Hara and co-workers.[5] 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 ($^3J_{HH}$, $^1J_{CH}$), observing their H-1 chemical shifts, and comparing their ^{13}C-NMR spectral data with those of methyl glycosides.[6] From the relatively large H-1 coupling constants (7.5, 7.6 Hz), the anomeric configuration for both glucose moieties should be β. In the insensitive nuclei enhanced by polarization transfer (INEPT) spectrum, the CH coupling constant of the C-1 (δ 101.8) signal was 178 Hz, indicating that the glycosidic bond of rhamnose was linked in an α-configuration. Based on these results, the three sugars and their anomeric configurations in **1** were determined to be 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 a glucose was attached to the 3-OH of the aglycone, as indicated by the C-3 chemical shift (δ 88.8) of **1** as well as by the correlations of H-1 (δ 4.96) of an inner glu-

* To whom correspondence should be addressed. e-mail: takeda-td@kyoritsu-ph.ac.jp

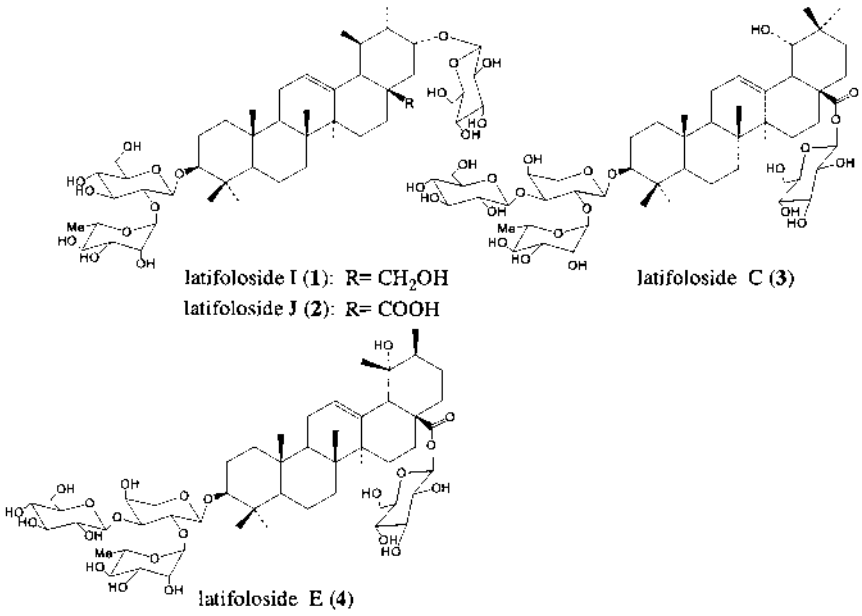

Chart 1

cose and C-3 of the aglycone in HMBC, and H-1 of the inner glucose and H-3 (δ 3.22) of the aglycone in ROESY. From the HMBC experiment of **1**, the following correlations were observed: H-1 (δ 5.08) of the terminal glucose and C-21 (δ 76.8) of the aglycone; H-1 (δ 6.56) of a rhamnose and C-2 (δ 77.9) of the inner glucose. Based on the above findings, the structure of **1** was elucidated to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl 3β,21α,28-trihydroxy-urs-12-ene 21-*O*-β-D-glucopyranoside. This is a new triterpenoid saponin, trivially named latifoloside I.

Latifoloside J (**2**) was also obtained as a colorless powder, and had a molecular formula $C_{48}H_{78}O_{18}$ based on its HR-FAB-MS spectrum. The ^{13}C-NMR spectral data of **2** showed 48 carbon signals, 30 of which were assigned to the aglycone part, while 18 were assigned to the carbohydrate moiety. The seven methyl carbon signals at δ 15.8, 17.2, 17.5, 17.6, 17.8, 23.3, and 28.1, and three sp^2 carbons at δ 125.2 and 139.6, and 180.6 indicated that the aglycone of **2** had an ursolic acid skeleton. In comparing with the ^{1}H-, ^{13}C-NMR spectra data of **2** with those of literature,[5] the aglycone part of **2** was identified as 3β,21α-dihydroxy-urs-12-en-28-oic acid.

The ^{1}H- and ^{13}C-NMR data of **2** displayed three anomeric signals at δ 4.94 (d, J=7.3 Hz), 5.01 (d, J=7.6 Hz), and 6.55 (br s), and δ 105.4, 101.7, and 101.6, respectively. Acid hydrolysis gave two monosaccharides, glucose and rhamnose in a ratio of 2 : 1, which were analyzed using the same method as the identification in **1**. Using the same methods in **1**, glucose was determined to have a D-configuration, while rhamnose was 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 of an inner glucose was attached to the 3-OH of the aglycone, as indicated by the C-3 chemical shift (δ 88.9) of **2**, and the correlations of H-1 (δ 4.93) of the inner glucose and C-3 of the aglycone in HMBC, as well as of H-1 of the inner glucose with H-3 (δ 3.37, dd, J=11.2, 4.6 Hz) in ROESY. The rhamnose attached to C-2 of the inner glucose was determinated by the correlation between H-1 (δ 6.55) of rhamnose and C-2 (δ 77.8) of the inner glucose in HMBC experiment of **2**. The other glucose attached to 21-OH of the aglycone had a correlation between H-1 (δ 5.01) of glucose and C-21 (δ 76.9) of the aglycone; and the down-field shift (+5.1 ppm) of C-21 in **2** was different from the literature.[6] Based on the above findings, the structure of **2** was elucidated to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl 3β,21α-dihydroxy-urs-12-en-28-oic acid 21-*O*-β-D-glucopyranoside. This is a new triterpenoid saponin, trivially named latifoloside J.

Latifolosides C (**3**) and E (**4**) were obtained as colorless powders. Their molecular peaks were both at *m*/*z* 1074 $[M]^-$ in FAB-MS. Comparing the ^{1}H-, ^{13}C-NMR data of **3** and **4** with those of literature,[2a] **3** and **4** were identified as 3-*O*-β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)-]-α-L-arabinopyranosyl siaresinolic acid 28-*O*-β-D-glucopyranoside and 3-*O*-β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)-]-α-L-arabinopyranosyl ilexgenin B 28-*O*-β-D-glucopyranoside, respectively.

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 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).

Isolation of Saponins The bark of *Ilex latifolia* THNUB. was obtained in Tokyo, Japan in 1998. The voucher specimens were identified by Dr. Toshiyuki Akiyama and deposited in Research Planning Department, Sankyo Co. Ltd. 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, respec-

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

Carbons	I (**1**)	J (**2**)	DEPT	Sugars	I (**1**)	J (**2**)	DEPT
C-1	39.4	39.3	CH_2	3-*O*-Glucose			
C-2	26.9	26.9	CH_2	G-1	105.5	105.4	CH
C-3	88.8	88.9	CH	G-2	77.9	77.8	CH
C-4	39.5	39.5	C	G-3	78.2	78.2	CH
C-5	56.0	56.2	CH	G-4	72.2	72.1	CH
C-6	18.5	18.5	CH_2	G-5	78.3	78.3	CH
C-7	33.3	33.6	CH_2	G-6	63.2	63.2	CH_2
C-8	42.6	42.9	C	Rhamnose			
C-9	48.0	48.0	CH	R-1	101.6	101.6	CH
C-10	36.8	36.9	C	R-2	72.5	72.4	CH
C-11	23.6	23.6	CH_2	R-3	72.6	72.5	CH
C-12	125.1	125.2	CH	R-4	74.2	74.1	CH
C-13	139.5	139.6	C	R-5	69.6	69.6	CH
C-14	40.1	39.8	C	R-6	18.5	18.5	CH_3
C-15	26.6	29.2	CH_2	21-*O*-Glucose			
C-16	26.5	27.7	CH_2	G-1	101.8	101.7	CH
C-17	37.3	48.5	C	G-2	75.5	75.4	CH
C-18	54.8	54.3	CH	G-3	79.0	78.9	CH
C-19	33.7	33.6	CH	G-4	72.3	72.2	CH
C-20	43.1	42.9	CH	G-5	79.9	79.8	CH
C-21	76.8	76.9	CH	G-6	63.0	62.9	CH_2
C-22	39.0	39.1	CH_2				
C-23	28.1	28.1	CH_3				
C-24	17.2	17.2	CH_3				
C-25	16.0	15.8	CH_3				
C-26	17.0	17.6	CH_3				
C-27	23.0	23.3	CH_3				
C-28	68.8	180.6	CH_2(C)				
C-29	17.8	17.5	CH_3				
C-30	17.8	17.8	CH_3				

tively. 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 get a precipitate (40 g) and ether-soluble part (8 g). Thirty grams of the ether precipitate was 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 get six fractions according to their TLC behavior. Fraction 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; 7 : 4 : 1) to get parts 1—7. Part 6 (830 mg) was chromatographed on a Lobar RP-8 column (solvent: CH_3OH : H_2O=5 : 5) to get p6-1—3. P6-1 (400 mg) was chromatographed on a Lobar RP-18 column with CH_3CN : H_2O (3 : 7) as an eluent to get latifoloside I (**1**, 24 mg). P6-2 (360 mg) was chromatographed on a silica gel column with a solvent system of $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2), to get p6-2-1—2. P6-2-1 (190 mg) was further purified on a Lobar RP-18 column with CH_3CN : H_2O (3 : 7) as an eluent to get latifoloside J (**2**, 14.2 mg). P6-2-2 (150 mg) was isolated on HPLC with CH_3OH : H_2O (5 : 5) as an eluent to get latifoloside C (**3**, 6.8 mg) and latifoloside E (**4**, 6.8 mg).

Latifoloside I (**1**): $[\alpha]_D^{25}$: +36.7° (*c*=0.21, MeOH); HR-FAB-MS (negative): *m/z*: 927.5375 $[M-1]^-$ (Calcd for $C_{48}H_{79}O_{17}$; 927.5317). ^{1}H-NMR (500 MHz, pyridine-d_5): δ (ppm) 0.71 (1H, dd, *J*=11.3, 4.6 Hz, H-5), 0.86 (3H, s, H-25), 0.94 (3H, s, H-26), 0.96 (3H, d, *J*=6.7 Hz, H-29), 1.12 (3H, s, H-27), 1.19 (3H, s, H-24), 1.23 (3H, s, H-23), 1.27 (3H, d, *J*=6.4 Hz, H-30), 1.72 (3H, d, *J*=6.1 Hz, H-6 of rhamnose), 2.54 (1H, d, *J*=11.6 Hz, H-18), 3.22 (1H, dd, *J*=11.3, 4.0 Hz, H-3), 3.58 (1H, d, *J*=10.4 Hz, H-28a), 3.88 (1H, d, *J*=10.4 Hz, H-28b), 4.32 (1H, m, H-21), 4.96 (1H, d, *J*=7.5 Hz, H-1 of inner glucose), 5.08 (1H, d, *J*=7.6 Hz, H-1 of terminal glucose), 6.56 (1H, br s, H-1 of rhamnose). ^{13}C-NMR spectral data are given in Table 1.

Latifoloside J (**2**): $[\alpha]_D^{25}$: +7.4° (*c*=0.67, MeOH); HR-FAB-MS (negative): *m/z*: 941.5110 $[M-1]^-$ (Calcd for $C_{48}H_{77}O_{18}$; 941.5037). ^{1}H-NMR (500 MHz, pyridine-d_5): δ (ppm) 0.71 (1H, dd, *J*=11.6, 4.4 Hz, H-5), 0.83 (3H, s, H-25), 1.01 (3H, d, *J*=6.7 Hz, H-29), 1.02 (3H, s, H-26), 1.11 (3H, s, H-27), 1.17 (3H, s, H-24), 1.22 (3H, s, H-23), 1.26 (3H, d, *J*=6.4 Hz, H-30), 1.70 (3H, d, *J*=6.4 Hz, H-6 of rhamnose), 2.78 (1H, d, *J*=11.3 Hz, H-18), 3.37 (1H, dd, *J*=11.2, 4.6 Hz, H-3), 4.28 (1H, m, H-21), 4.94 (1H, d, *J*=7.3 Hz, H-1 of inner glucose), 5.01 (1H, d, *J*=7.6 Hz, H-1 of terminal glucose), 6.55 (1H, br s, H-1 of rhamnose). ^{13}C-NMR spectral data are given in Table 1.

Latifoloside C (**3**): $[\alpha]_D^{25}$: +10.2° (*c*=0.69, MeOH); FAB-MS: *m/z*: 1074 $[M]^-$.

Latifoloside E (**4**): $[\alpha]_D^{25}$: +16.4° (*c*=0.65, MeOH); FAB-MS: *m/z*: 1074 $[M]^-$.

Compounds **1** and **2** (1 mg each) were hydrolyzed and acetylated. The glucitol and rhamnitol acetates from compounds **1** and **2** were detected by GC analysis in a ratio of 2 : 1. (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 method reported by Hara and co-workers[5] using GC. GC conditions: column: 3% ECNSS-M (2 m×0.3 mm) (Alltech Assos, Inc.); column temp.: 190 °C; injection temp.: 210 °C; retention times (min): L-rhamnose (8.6), D-glucose (49.2).

Acknowledgements We are grateful to Mrs. J. Hada, Dr. A. Nagatsu, Miss S. Kato, Miss T. Nakano, Miss. K. Iwasawa, Miss. T. Naito and Miss. H. Suzuki for providing NMR and MS data, respectively.

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