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Saponins from Chinese Folk Medicine, "Liang Wang Cha," Leaves and Stems of *Nothopanax delavayi*, Araliaceae

RYOJI KASAI,[a] TOSHIHIKO OINAKA,[a] CHONG-REN YANG,[b] JUN ZHOU,[b] and OSAMU TANAKA*,[a]

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,[a] Kasumi, Minami-ku, Hiroshima 734, Japan and Kunming Institute of Botany, Academia Sinica,[b] Kunming, China

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From the Chinese folk medicine "Liang Wang Cha" (leaves and leafstalks of *Nothopanax delavayi*, Araliaceae), two new oleanane type triterpene glycosides, named liangwanosides I (**1**) and II (**2**), were isolated. The structures of **1** and **2** were determined as the 28-β-D-glucopyranosyl and 28-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl esters of 3-*O*-α-L-arabinopyranosyl-3β-hydroxyolean-12-ene-28,29-dioic acid, respectively.

Keywords——*Nothopanax delavayi*; Araliaceae; liangwanoside I; liangwanoside II; Liang Wang Cha; saponin; serratagenic acid; oleanane type triterpene glycoside; Chinese folk medicine

Nothopanax delavayi (FR.) HARMS (Araliaceae) is a tall tree which grows in Yunnan province, China. Leaves and leafstalks of this plant are used as a well known folk medicine "Liang Wang Cha," as an anti-pyretic and anti-inflammatory. We now report the isolation and structural elucidation of two new saponins from this plant.

A methanolic extract of leaves and stems of the young plant was partitioned between water and *n*-hexane. The water layer was passed through a column of highly porous polymer resin (Diaion HP-20). The 80% MeOH eluate, a crude glycosides mixture was further chromatographed on silica gel, affording two new glycosides named liangwanosides I (**1**) and II (**2**) in yields of 0.6% and 1.0%, respectively.

On mineral acid hydrolysis, **1** and **2** gave a common aglycone (**3**). On treatment with diazomethane, **3** gave a dimethyl ester (**4**). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **3** exhibited signals due to six methyls on quaternary carbons, an olefinic proton, a carbinyl proton and an allylic proton at δ 2.62, a characteristic signal due to 18β-H of olean-12-ene type triterpenes having a carboxyl group at C-17. Comparison of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **4** with that of methyl oleanolate (**5**)[1] suggested the formulation of **3** as 3β-olean-12-ene-28,29-dioic acid, which has already been isolated as a sapogenin named serratagenic acid from *Clerodendron serratum* SPRENG (Verbenaceae).[2] The presence of an α-carbomethoxyl group at C-20 of **4** was confirmed by the difference of its carbon signals due to the *E*-ring carbons from those of phytolaccagenin (**6**),[3] which has a β-carbomethoxyl group at C-20. The identification of **3** was finally established by comparison of the physical constants and ^{1}H-NMR spectrum with reference data.[2]

Acid hydrolysis of **1** gave D-glucose and L-arabinose. Identification of the absolute configuration of these monosaccharides was carried out by the procedure reported by Oshima *et al.*[4] The electron impact mass (EI-MS) spectrum of peracetylated **3** exhibited fragment ions at *m/z* 331[(Glc)Ac$_4$, terminal glucose] and 259 [(Ara)Ac$_3$, terminal arabinose]. The ^{13}C-NMR spectrum of **1** as well as anomeric proton signals showed the presence of one β-D-glucopyranoside unit and one α-L-arabinopyranoside unit in **1**. The ^{13}C-NMR glycosylation

shifts[5,6] observed between **1** and **3** indicated that **1** is a bisdesmoside of **3** with glycosyl linkages at 3-OH and either 28- or 29-COOH. On selective cleavage of the ester glycosidic linkage with LiI and 2,6-lutidine in anhydrous methanol,[7] **1** gave a methyl glucoside and a prosapogenin (**7**) which was formulated as the 3-*O*-α-L-arabinopyranoside of **3** based on the ^{13}C-NMR spectrum. The location of a glucosyl linkage not at 29-COOH but at 28-COOH was revealed as follows. Formation of the characteristic bromolactone has been used as chemical evidence of the presence of a free carboxyl group at C-17 of olean-12-ene triterpenes.[8] On treatment with diazomethane, **1** afforded a monomethyl ester (**8**). On selective hydrolysis of the ester glycoside linkage with alkali, **8** gave a monodesmoside (**9**). Formation of a bromolactone (**10**) from **9** was observed on treatment of **9** with bromine in the presence of sodium acetate. It follows that **1** is formulated as the 28-β-D-glucopyranosyl ester of 3-*O*-α-L-arabinopyranosyl-serratagenic acid.

Acid hydrolysis of **2** gave D-glucose, L-arabinose and L-rhamnose. The EI-MS of peracetylated **2** exhibited fragment ions at *m/z* 259 [(Ara)Ac_3, terminal arabinose], 273[(Rha)Ac_3, terminal rhamnose], 849 [(Glc-Glc-Rha)Ac_9] and 561 [(Glc-Rha)Ac_6]. The ^{1}H- and ^{13}C-NMR spectra of **2** revealed the presence of four monosaccharide units. The

TABLE I. ^{13}C-NMR Chemical Shifts of Aglycone Moieties in C_5D_5N

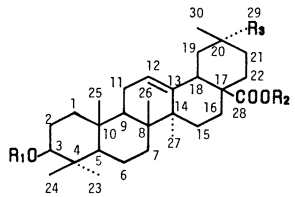
Carbon	1	2	3	4[d]	5[d,1]	6[3]	7
1	38.9	38.9	38.9	38.5	38.5	44.8	38.7
2	26.3	26.5	28.0	27.2	27.1	71.5	26.3
3	88.9	88.8	78.1	79.0	78.7	73.0	88.7
4	39.4	39.3	39.4	38.7	38.7	42.4	39.5
5	56.1	55.9	55.8	55.2	55.2	48.1	55.8
6	18.6	18.5	18.8	18.3	18.3	18.2	18.5
7	33.3	33.1	33.2	32.7	32.6	33.0	33.1
8	40.0	39.8	39.7	39.3	39.3	39.8	39.7
9	48.1	48.0	48.0	47.6	47.6	48.5	48.0
10	37.1	36.9	37.4	37.0	37.0	37.2	37.0
11	23.8	23.6	23.8	23.2	23.1	23.9	23.8
12	123.5	123.6	122.2	123.4	122.1	123.3	123.1
13	143.5	143.4	144.3	142.8	143.4	144.4	144.4
14	42.4	42.2	42.5	41.6	41.6	42.2	42.7
15	28.3	28.2	28.3	28.3	27.7	28.4	28.2
16	23.8	23.6	23.8	23.4	23.4	23.9	23.8
17	47.0	46.9	46.6	46.4	46.6	46.1	46.6
18	40.8	40.7	41.1	40.2	41.3	43.3	41.1
19	40.8[a]	40.7[a]	41.1[a]	39.7[a]	45.8	42.7	41.1[a]
20	42.2	42.0	42.2	42.2	30.6	44.1	42.2
21	29.1[a]	29.1[a]	29.4[a]	27.6[a]	33.8	30.8	29.3[a]
22	31.7[a]	31.6[a]	32.4[a]	31.2[a]	32.3	34.5	32.5[a]
23	28.3	28.2	28.3	28.1	28.1	67.7	28.2
24	16.8[b]	16.8[b]	16.6[b]	15.5[b]	15.6[a]	14.5	17.0[b]
25	15.5[b]	15.5[b]	15.5[b]	15.3[b]	15.3[a]	17.4	15.5[b]
26	17.5[b]	17.4[b]	17.4[b]	16.8	16.8	17.2	17.4[b]
27	25.9	25.9	26.1	25.9	26.0	26.2	26.1
28	176.0[c]	176.2[c]	180.0[c]	178.0[c]	177.9	179.7	180.2[c]
29	180.9[c]	180.8[c]	181.2[c]	177.2[c]	33.1	28.4	181.5[c]
30	19.9	19.8	20.0	19.3	23.6	177.1	20.1
COOMe				51.7 51.9	51.3	51.6	

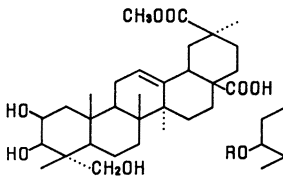
a—*c*) Assignments may be reversed in each column. *d*) In $CDCl_3$.

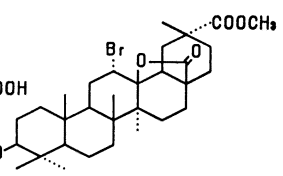
TABLE II. ^{13}C-NMR Chemical Shifts of Sugar Moieties in C_5D_5N

Sugar	1	2	Sugar	1	2
28-Sugar			Rha-1		102.4
Glc-1	95.5	95.5	Rha-2		72.4
Glc-2	74.0	73.6	Rha-3		72.4
Glc-3	78.4	78.6[a)]	Rha-4		73.6
Glc-4	71.4	70.7	Rha-5		70.1
Glc-5	78.4	76.7	Rha-6		18.1
Glc-6	62.5	69.2	3-Sugar		
Glc-1′		104.4	Ara-1	106.3	106.6
Glc-2′		74.9	Ara-2	72.6	72.1
Glc-3′		76.3	Ara-3	73.8	74.1
Glc-4′		78.3[a)]	Ara-4	68.6	68.0
Glc-5′		77.5	Ara-5	65.7	65.9
Glc-6′		61.4			

a) Assignments may be interchanged.



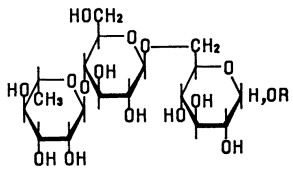




1: R_1=α-L-Ara, R_2=β-D-Glc, R_3=COOH
2: R_1=α-L-Ara, R_2=β-D-Glc^6—β-D-Glc^4—α-L-Rha, R_3=COOH
3: R_1=H, R_2=H, R_3=COOH
4: R_1=H, R_2=CH_3, R_3=$COOCH_3$
5: R_1=H, R_2=CH_3, R_3=CH_3
7: R_1=α-L-Ara, R_2=H, R_3=COOH
8: R_1=α-L-Ara, R_2=β-D-Glc, R_3=$COOCH_3$
9: R_1=α-L-Ara, R_2=H, R_3=$COOCH_3$

6

10: α-L-Ara



11: R=CH_3
12: R=H

Chart 1

selective cleavage of the ester glycosidic linkage of **2** with LiI and 2,6-lutidine in anhydrous methanol afforded the same prosapogenin **7** as that of **1**, together with a methyl glycoside (**11**) of the trisaccharide (**12**). This methyl glycoside (**11**) was identified as methyl α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucoside by comparison of the ^{13}C-NMR spectrum with that of an authentic sample which has already been obtained from huzhangoside B.[9)] A carbon signal at δ 95.5 indicated that the terminal glucose moiety of **12** is linked with one of the carboxyl groups of **2** in β-anomeric configuration. Location of this ester-glycosyl linkage at 28-COOH was established by the formation of the bromolactone (**10**), as in the case of **1**. Based on these results, the structure of **2** was established as illustrated in Chart 1.

Experimental

General Procedure——Melting points were determined on a Yanaco micro hot stage and are uncorrected. Optical rotations were measured with a Union PM-101 automatic digital polarimeter. Infrared (IR) spectra were

taken on a Shimadzu IR-408 spectrometer. NMR spectra were recorded on a JEOL FX-100 instrument using tetramethylsilane (TMS) as an internal standard. For gas liquid chromatography (GLC), a Shimadzu GC-8A apparatus was used. Mass spectra (MS) were taken on a JEOL JMS-01-SG-2 spectrometer by the direct inlet method; ionization voltage 75 eV. For column chromatography, Kieselgel 60 (70—230 mesh, Merck) and Diaion HP-20 (Mitsubishi Chem. Ind. Co., Ltd.) were used. All solvent systems for chromatography were homogeneous.

Plant Material——The plant was collected at Mt. Xi San, Kunming, Yunnan and identified by Kunming Institute of Botany. A specimen has been deposited in the Herbarium of this Institute.

Extraction and Separation of 1 and 2——Dried leaves and stems of the young plant (186 g) were extracted with MeOH. The MeOH extract was evaporated to dryness. The residue (25.4 g) was partitioned between H_2O and *n*-C_6H_{14}. The aqueous layer was chromatographed on a Diaion HP-20 column and eluted with H_2O, 10% MeOH, 50% MeOH, 80% MeOH, MeOH and then $CHCl_3$, successively. The 80% MeOH eluate was purified by column chromatography on silica gel (EtOAc–EtOH–H_2O, 7 : 2 : 1) to give **1** and **2** in yields of 0.6% and 1.0%, respectively.

Compound **1**: A white powder, $[\alpha]_D^{19}$ +19.1° (c=1.89, MeOH). *Anal.* Calcd for $C_{41}H_{64}O_{14}\cdot 5/2H_2O$: C, 59.61; H, 8.13. Found: C, 59.34; H, 8.13. IR (Nujol): 3400 (OH), 1720 (COOH), 1700 (COOH) cm^{-1}. ^{1}H-NMR (C_5D_5N) δ: 4.75 (1H, d, J=7 Hz, anomeric proton of α-Ara), 6.30 (1H, d, J=6 Hz, anomeric proton of β-Glc). The ^{13}C-NMR data are given in Tables I and II.

Compound **2**: A white powder, $[\alpha]_D^{20}$ −11.9° (c=1.59, MeOH). *Anal.* Calcd for $C_{53}H_{84}O_{23}\cdot 9/2H_2O$: C, 54.39; H, 8.01. Found: C, 54.48; H, 7.84. IR (Nujol): 3400 (OH), 1720 (COOR), 1690 (COOH) cm^{-1}. ^{1}H-NMR (C_5D_5N) δ: 4.66 (1H, d, J=6 Hz, anomeric proton of α-Ara), 4.80 (1H, d, J=7.5 Hz, anomeric proton of β-Glc), 5.50 (1H, s, anomeric proton of Rha), 6.01 (1H, d, J=6.5 Hz, anomeric proton of β-Glc′). The ^{13}C-NMR data are given in Tables I and II.

Acid Hydrolysis of 1 and 2——A solution of **1** (29.6 mg) in 7% HCl–dioxane (1 : 1, 5 ml) was refluxed for 4 h. The reaction mixture was diluted with H_2O and then extracted with Et_2O. The Et_2O layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was crystallized from MeOH to give **3** (7.6 mg): colorless needles, mp 300 °C, $[\alpha]_D^{25}$ +22.9° (c=0.85, C_5H_5N). IR (Nujol): 3450, 1710 cm^{-1}. ^{1}H-NMR (C_5D_5N) δ: 0.91, 1.03, 1.04, 1.24, 1.29, 1.60 (each 3H, s), 2.62 (1H, J=6.2, 6 Hz, H-18), 5.57 (1H, m, H-12). The ^{13}C-NMR data are given in Table I. **2** (33.4 mg) afforded **3** (16.7 mg). The H_2O layer was neutralized with Amberlite MB-3 ion exchange resin and evaporated to dryness. Identification of the resulting monosaccharides, including the absolute configuration, was carried out according to the method reported by Oshima *et al.*[4]

Methylation of 3——Compound **3** was methylated with CH_2N_2 in MeOH as usual to give **4**: colorless needles, mp 202—204 °C (from MeOH), $[\alpha]_D^{25}$ +31.5° (c=0.91, $CHCl_3$). IR ($CHCl_3$): 1710 ($COOCH_3$) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.72, 0.78, 0.91, 0.99, 1.14, 1.25, 3.46, 3.67 (each 3H, s), 2.85 (1H, dd, J=6.2, 6 Hz, H-18), 5.30 (1H, m, H-12). The ^{13}C-NMR data are given in Table I.

Selective Cleavage of the Ester Glycosyl Linkage[7] **of 1 and 2**——A solution of **1** (25 mg), anhydrous LiI (20 mg) and 2,6-lutidine (3 ml) in anhydrous MeOH was refluxed for 15 h under an N_2 stream. After cooling, the reaction mixture was diluted with 50% MeOH (5 ml), neutralized with Amberlite MB-3 resin and evaporated to dryness. The residue was chromatographed on a column of silica gel ($CHCl_3$–MeOH–H_2O, 80 : 10 : 1—6 : 4 : 1) to give **7** (7 mg) and an anomeric mixture of methyl glucoside (3 mg); the latter was trimethylsilylated with trimethylsilylimidazole and identified by GLC comparison with an authentic sample.

By the same method, **2** (106 mg) afforded **7** (16 mg) and **11** (34 mg); the latter was identified as methyl α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl(1→6)-D-glucopyranoside by comparison of the ^{13}C-NMR spectrum with that of an authentic sample obtained from huzangoside B[9] under the same conditions. Compound **7**: A white powder, $[\alpha]_D^{20}$ −8.9° (c=1.23, C_5H_5N). *Anal.* Calcd for $C_{35}H_{54}O_9\cdot 2H_2O$: C, 64.19; H, 8.93. Found: C, 64.37; H, 8.68. IR (Nujol): 3400 (OH), 1690 (COOH) cm^{-1}. ^{1}H-NMR (C_5D_5N) δ: 0.86, 0.95, 1.00, 1.27, 1.30, 1.58 (each, 3H, s), 4.78 (1H, d, J=6.6 Hz, anomeric proton of α-Ara), 5.54 (1H, m, H-12). The ^{13}C-NMR data are given in Table I.

Formation of the Bromolactone (10)[8]——**1** (7 mg) was methylated with CH_2N_2 in MeOH, and the product (**8**) was dissolved in 1 N NaOH (3 ml) and heated at 80 °C for 2 h. After cooling, the reaction mixture was neutralized with 2 N H_2SO_4 and passed through a SEP-PAK (C-18, Waters Assoc.) to remove the resulting sugar component. The solution was evaporated to dryness to give **9**. A solution of AcOH (2 ml) containing Br_2 (0.1 ml) was added to a solution of **9** and AcONa (10 mg) in 90% AcOH (1 ml) and the mixture was stirred at room temperature for 15 h under an N_2 stream. The reaction mixture was poured into an aqueous solution saturated with $NaHSO_3$ and extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated to dryness and the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH–H_2O, 80 : 10 : 1 and AcOEt–EtOH–H_2O, 8 : 2 : 1) to give **10** (4.1 mg) as a white powder, IR ($CHCl_3$): 3500 (OH), 1765 (five-membered ring lactone), 1720 ($COOCH_3$) cm^{-1}, ^{1}H-NMR (C_5D_5N) δ: 0.86, 0.90, 1.23, 1.46 (each 3H, s), 3.72 (3H, s, H-29), 4.12 (1H, br s, H-12), 4.34 (1H, m, H-3). When subjected to the same procedure, **2** (7 mg) also afforded **10** (2.2 mg).

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