Saponin and Sapogenol. L.[1] On the Constituents of the Roots of *Glycyrrhiza uralensis* FISCHER from Xinjiang, China. Chemical Structures of Licorice-Saponin L3 and Isoliquiritin Apioside

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From the air-dried roots of *Glycyrrhiza uralensis* FISCHER collected in Xinjiang province, China ("Shinkyo-Kanzo" in Japanese), a new oleanene-type triterpene oligoglycoside named licorice-saponin L3 and a new chalcone oligoglycoside named isoliquiritin apioside were isolated together with glycyrrhizin, 18α-glycyrrhizin, apioglycyrrhizin, araboglycyrrhizin, licorice-saponins A3, E2, G2, and H2, and six known flavonoid glycosides. On the basis of chemical and physicochemical evidence, the structures of licorice-saponin L3 and isoliquiritin apioside were elucidated as 3β-[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl(1→2)-β-D-glucuronopyranosyloxy]-22β-acetoxy-24-hydroxyolean-12-en-30-oic acid (1) and 4-*O*-[β-D-apiofuranosyl(1→2)-β-D-glucopyranosyl]isoliquiritigenin (6), respectively.

Keywords *Glycyrrhiza uralensis*; licorice-saponin L3; isoliquiritin apioside; licorice root; oleanene-type triterpene oligoglycoside; chalcone oligoglycoside

During the course of our chemical investigations on the constituents of botanically identified licorice-roots of Chinese origin, we have so far isolated ten oleanene-type triterpene oligoglycosides named licorice-saponins A3, B2, C2, D3 (**4**), E2, F3, G2, H2, J2, and K2 from the air-dried roots of *Glycyrrhiza uralensis* FISCHER, which were collected in the northeastern part of China (corresponding to one of so-called "Tohoku-Kanzo" in Japan), and elucidated their chemical structures.[2,3] Among these licorice-saponins, licorice-saponin B2 has been noted to exhibit hepatocyte function restoration-promoting activity against carbon tetrachloride-induced liver cell injury in rat (both *in vitro* and *in vivo*).[4] We have also investigated the chemical constituents of several licorice roots which were collected in Xinjiang province of China.[1,3] Licorice roots imported from China to Japan, claimed as originating from the Xinjiang area, are usually called "Shinkyo-Kanzo" in the market in Japan; however, these licorice roots are derived from at least five *Glycyrrhiza* species, *i.e.*, *G. inflata* BATALIN, *G. uralensis* FISCHER, *G. glabra* L., *G. aspera* PALL., and *G. korshinsky* G. GRIG.[5]

From one of those Xinjiang licorice-roots, of which the botanical origin was identified as *Glycyrrhiza inflata* BATALIN, we have isolated two sweet oleanene-type triterpene oligoglycosides designated apioglycyrrhizin and araboglycyrrhizin.[1,6] In our continuing studies of Xinjiang licorice-roots, we have isolated a new oleanene-type triterpene oligoglycoside named licorice-saponin L3 (**1**) and

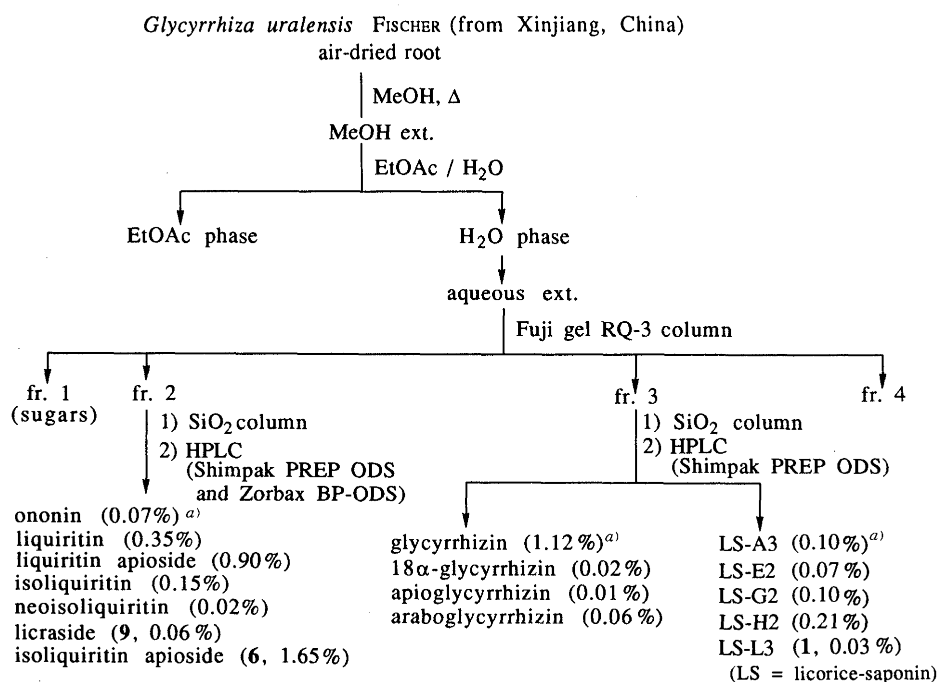

Fig. 1

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a new chalcone oligoglycoside named isoliquiritin apioside (**6**) from the air-dried roots of *Glycyrrhiza uralensis* FISCHER, which were collected in Xinjiang province of China.[7]

In this paper, we present a full account of the structure elucidation of licorice-saponin L3 (**1**) and isoliquiritin apioside (**6**).

The isolation of the chemical constituents from the air-dried roots of *Glycyrrhiza uralensis* FISCHER was carried out through the procedure shown in Fig. 1. The methanolic extract of the roots was partitioned into an ethyl acetate and water mixture, and the water-soluble portion (aqueous ext.) was subjected to reversed-phase silica gel column chromatography to provide four fractions. Ordinary silica gel column chromatography and subsequent preparative high-performance liquid chromatography (preparative HPLC) of fractions 2 and 3 furnished flavonoid glycosides, ononin,[8] liquiritin,[8] liquiritin-apioside,[8] isoliquiritin,[9] neoisoliquiritin,[9] licraside (**9**),[10] and isoliquiritin-apioside (**6**), from fraction 2, and triterpene glycosides, glycyrrhizin, 18α-glycyrrhizin,[11] apioglycyrrhizin,[1,6] araboglycyrrhizin,[1,6] licorice-saponins A3,[2a] E2,[2b] G2,[2b] H2,[2b] and L3 (**1**), from fraction 3.

Licorice-Saponin L3 (1) Licorice-saponin L3 (**1**) was obtained as colorless fine prisms of mp 233—234 °C. It showed no absorption maximum above 210 nm in its ultraviolet (UV) spectrum. The infrared (IR) spectrum of **1** showed a broad absorption band centered at 1704 cm^{-1} ascribable to acetyl and carboxyl functions and prominent broad absorption bands at 3650—3100 and at 1048 cm^{-1} which suggested the presence of the glycosidic structure. The positive fast atom bombardment MS (positive FAB-MS) of **1** showed the quasimolecular ion peaks at m/z 1007 $(M+Na)^+$ and m/z 985 $(M+H)^+$. The high-resolution MS (high MS) analysis of the quasimolecular ion $(M+Na)^+$ revealed the molecular formula of **1** to be $C_{49}H_{76}O_{20}$. The negative FAB-MS of **1** showed the quasimolecular ion peak at m/z 983 $(M-H)^-$ and fragment ion peaks at m/z 837 [**i**, $(M-C_6H_{11}O_4)^-$], m/z 705 [**ii**, $(M-C_{11}H_{19}O_8)^-$], and m/z 529 [**iii**, $(M-C_{17}H_{27}O_{14})^-$], which were presumably formed through fragmentations occurring at individual glycosidic linkages in the linear trisaccharide moiety of **1**.

Methanolysis of licorice-saponin L3 (**1**) with 9% hydrogen chloride in methanol afforded a triterpenoid lactone **2** derived from the aglycone part and three methyl glycosides, *i.e.*, methyl D-glucuronide, methyl L-arabinoside, and methyl L-rhamnoside, which were liberated from the carbohydrate portion. Among these methyl glycosides, methyl D-glucuronide was subjected to sodium borohydride ($NaBH_4$) reduction and subsequent acidic hydrolysis to provide D-glucose. Similarly, methyl L-arabinoside and methyl L-rhamnoside thus obtained were identified by acidic hydrolysis, providing L-arabinose and L-rhamnose, respectively.

The IR spectrum of the triterpenoid **2** showed absorption bands due to lactone and hydroxyl moieties. The MS and high MS of **2** showed the molecular ion peak at m/z 470 of $C_{30}H_{46}O_4$ and fragment ion peaks, one at m/z 224 (**iv**) of $C_{14}H_{24}O_2$ derived from the A/B ring and another at m/z 246 (**v**) of $C_{16}H_{22}O_2$ derived from the D/E ring, which were presumably formed through the characteristic retro-Diels Alder type fragmentation at the C ring of the olean-12-ene skeleton of **2**. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **2** showed signals at δ 3.45 (dd, $J=3.8$, 11.8 Hz) assignable to 3α-H geminal to an equatorial hydroxyl group, at δ 4.18 (d, $J=5.8$ Hz) assignable to 22α-H of a lactone methine proton, and at δ 4.19 and 4.23 (ABq, $J=8.8$ Hz) due to axial hydroxymethylene protons (24-H_2). Treatment of **2** with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid monohydrate furnished the 3,24-acetonide **2a**. The MS of **2a** showed fragment ion peaks at m/z 495 [$(M-CH_3)^+$, $C_{32}H_{47}O_4$], m/z 264 (**vi**, $C_{17}H_{28}O_2$), and m/z 246 (**v**).

Based on the above-mentioned evidence and a detailed comparison of the physicochemical data for **2** and **2a** with those for 11-deoxoglabrolide (**5**),[2b] the structure of **2** has been elucidated as 24-hydroxy-11-deoxoglabrolide. Fur-

TABLE I. ^{13}C-NMR Data for Licorice-Saponin L3 (**1**), **2**, Soyasaponin II (**3**), and Licorice-Saponin D3 Trimethyl Ester (**4a**, Sapogenol Moiety) (Pyridine-d_5 + D_2O, δ_C)

		1[a]	**2**[a]	**3**[b]	**4a**[c]
Sapogenol moiety	C-1	38.7	38.5	38.6	39.3
	C-2	26.3[d]	28.2	25.5	26.3[f]
	C-3	91.0	80.8	90.9	89.9
	C-4	44.0	43.2	43.8	44.0
	C-5	56.1	56.4	56.0	55.7
	C-6	18.8	19.1	18.7	20.7
	C-7	32.9	33.6	33.2	32.5
	C-8	40.1	39.8	39.8	40.0
	C-9	47.7	48.0	47.7	47.6
	C-10	36.2	36.1	36.3	35.9
	C-11	24.0	23.6	24.0	23.6
	C-12	124.7	125.2	122.3	122.2
	C-13	144.1	144.2	144.7	143.6
	C-14	41.9	42.8	42.3	41.6
	C-15	26.6[d]	26.5	26.3	26.1[f]
	C-16	27.8	27.5	28.6	28.1
	C-17	37.5	37.1	37.9	37.6
	C-18	44.2	45.2	44.8	44.0
	C-19	40.8	42.8	46.6	40.7
	C-20	29.8	32.1	30.8	29.8
	C-21	41.7	42.7	42.3	41.6
	C-22	78.1	80.2	75.5	77.9
	C-23	22.9	23.6	22.8	22.9
	C-24	63.4	64.0	63.3	16.4
	C-25	15.7	15.8	15.6	15.3
	C-26	16.8	16.9	16.9	16.7
	C-27	26.3[e]	26.5	25.5	26.3
	C-28	26.6[e]	26.5	28.1	26.3
	C-29	29.8	28.4	33.0	29.1
	C-30	179.4	172.4	21.0	177.2
	Acetyls	170.3, 21.4			170.1, 21.0
3-*O*-β-D-Glucuronopyranosyl	C-1′	105.4		105.1	
	C-2′	78.1		77.8	
	C-3′	75.8		75.5	
	C-4′	72.6		72.3	
	C-5′	77.6		77.3	
	C-6′	172.2		172.2	
2′-*O*-α-L-Arabinopyranosyl	C-1″	102.2		101.7	
	C-2″	78.1		77.2	
	C-3″	76.7		76.8	
	C-4″	70.7		70.0	
	C-5″	66.8		66.5	
2″-*O*-α-L-Rhamnopyranosyl	C-1‴	101.8		101.7	
	C-2‴	72.3		71.9	
	C-3‴	73.9		73.4	
	C-4‴	74.4		74.1	
	C-5‴	69.4		69.0	
	C-6‴	18.5		18.5	

Measured at *a*) 125 MHz, *b*) 67.8 MHz, *c*) 22.5 MHz. *d*—*f*) Assignments may be interchangeable within the same column.

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thermore, the absolute configuration of **2** has been clarified by means of the modified Horeau's method.[12)] Thus, **2** was first treated with *dl*-phenylbutyric anhydride and subsequently with (+)-*R*-phenylethylamine to convert residual phenylbutyric acid to the amide mixture. GLC analysis of the amide mixture revealed that *d*-phenylbutylamide was predominant over *l*-phenylbutylamide, so that the 3*S* configuration of **2** has been confirmed.

The ^{1}H-NMR spectrum of licorice-saponin L3 (**1**) showed a three-proton singlet at δ 2.02 due to an acetoxyl methyl group and three anomeric proton signals at δ 4.95 (d, J=7.6 Hz), 5.52 (d, J=7.3 Hz), and 6.15 (br s). Methylation of **1** with ethereal diazomethane furnished the dimethyl ester **1a**, the ^{1}H-NMR spectrum of which showed signals due to an acetoxyl methyl (δ 2.03) and two methoxycarbonyl groups (δ 3.70, 3.74). It was found in our previous work that the 22β-acetoxyl-20-carboxyl moiety of licorice-saponin D3 (**4**) is readily converted to the 30,22β-lactone structure under acidic conditions, and **4** liberates 11-deoxoglabrolide (**5**) as the sapogenol upon acidic hydrolysis.[2b)] In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1**, the carbon signals assignable to the aglycone part were very similar to the carbon signals of the aglycone part of licorice-saponin D3 trimethyl ester (**4a**)[2b)] except for some signals due to the 24-hydroxyl moiety.

Based on these results, the structure of the sapogenol part of licorice-saponin L3 (**1**) has been elucidated as 22β-acetoxy-3,24-dihydroxyolean-11-en-30-oic acid. On the other hand, the carbon signals assignable to the

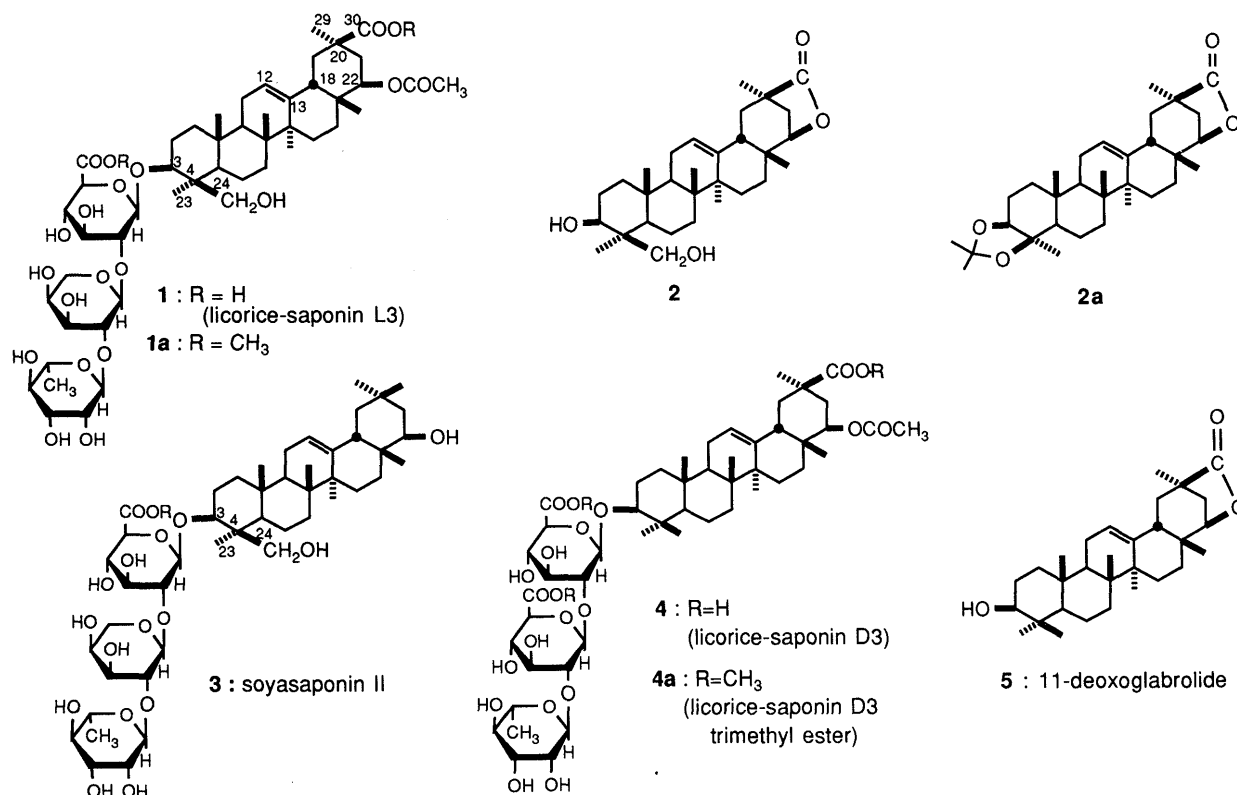

Fig. 2

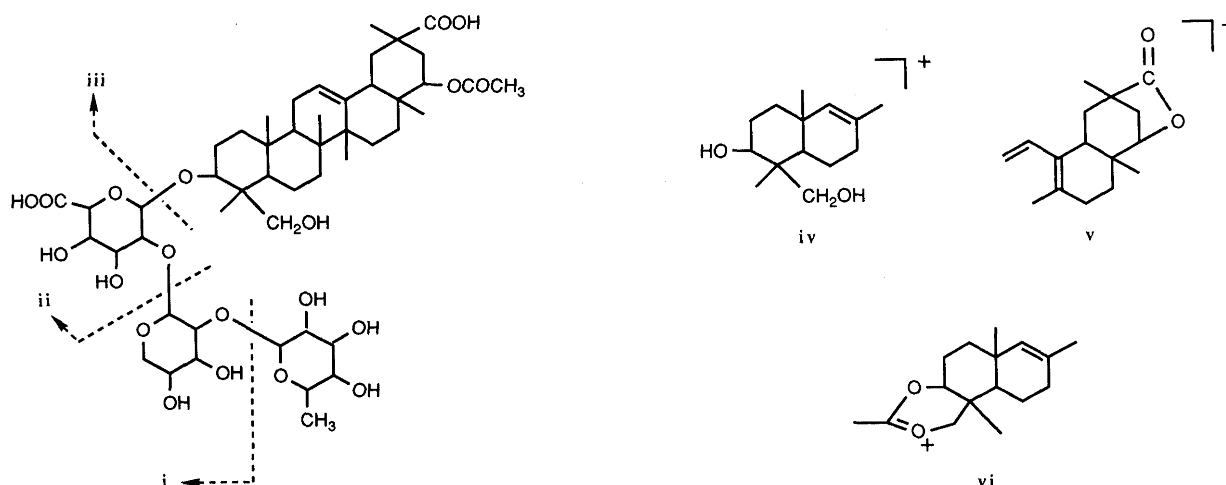

Fig. 3

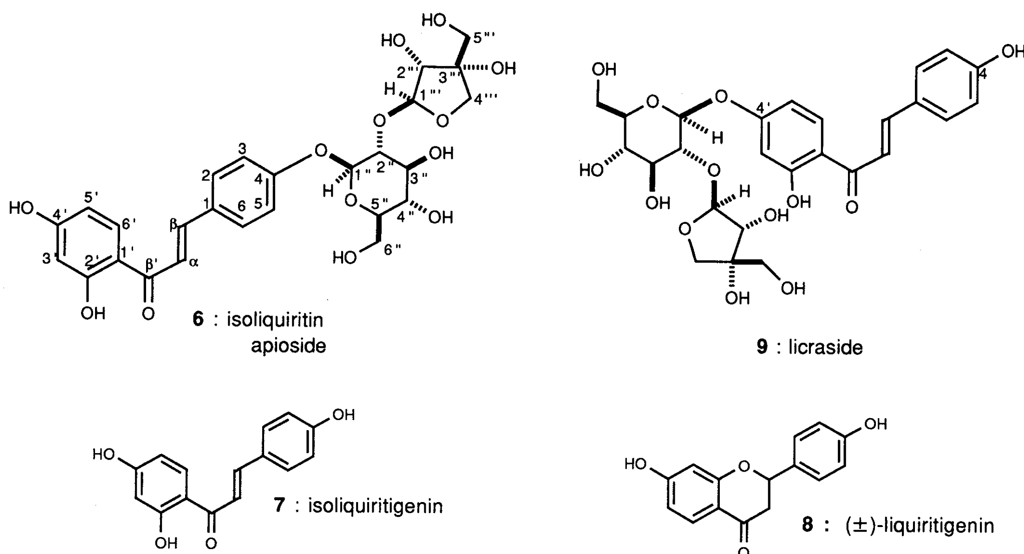

Fig. 4

oligosaccharide moiety of **1** were shown to be superimposable on those of soyasaponin II (**3**).[13] In order to determine the monosaccharide sequence of the oligosaccharide moiety of licorice-saponin L3 (**1**), the dimethyl ester **1a** was subjected to complete methylation.[14] Subsequent methanolysis of the product liberated methyl 3,4,6-tri-*O*-methylglucuronopyranoside (**a**), methyl 3,4-di-*O*-methylarabinopyranoside (**b**), and methyl 2,3,4-tri-*O*-methylrhamnopyranoside (**c**) in a 1 : 1 : 1 ratio. Finally, the $^{13}C-^{1}H$ coupling constants observed for the anomeric carbon signals, *i.e.*, 171 Hz (α-L-rhamnopyranosyl moiety),[2b,c,15] 161 Hz (α-L-arabinopyranosyl moiety),[15] and 160 Hz (β-D-glucuronopyranosyl moiety),[15] in the ^{13}C-NMR spectrum of **1** have corroborated the oligosaccharide moiety of licorice-saponin L3 (**1**). Thus, the structure of licorice-saponin L3 (**1**) has been determined as 3β-[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl(1→2)-β-D-glucuronopyranosyloxy)-22β-acetoxy-24-hydroxyolean-11-en-30-oic acid.

Isoliquiritin Apioside (6) Isoliquiritin apioside (**6**) was obtained as yellow plates of mp 140 °C. The molecular formula $C_{26}H_{30}O_{13}$ was determined from the molecular ion peak in the MS and by high-resolution MS measurement. Methanolysis of **6** with 9% hydrogen chloride in methanol yielded isoliquiritigenin (**7**) and (±)-liquiritigenin (**8**), together with methyl D-glucoside and methyl D-apioside. The latter two glycosides were further subjected to acidic hydrolysis to furnish D-glucose and D-apiose, respectively. The UV spectrum of **6** showed an absorption maximum at 360 nm (ε = 28000) ascribable to the 2′,4′,4-trihydroxychalcone structure.[16] The proton signals due to the aglycone moiety observed in the ^{1}H-NMR spectrum of **6** were superimposable on those of isoliquiritigenin (**7**). The ^{13}C-NMR spectrum of **6** showed carbon signals due to the isoliquiritigenin moiety and the β-D-apiofuranosyl(1→2)-β-D-glucopyranosyl moiety which closely resembled those of licraside (**9**), except for some signals shifted by glycosylation[17] at the C-4 position of **6**. Furthermore, the location of the glycosidic linkage in **6** has been shown to be the C-4 hydroxyl by an nuclear Overhauser effect (NOE) experiment. Namely, NOE was observed

Table II. ^{13}C-NMR Data for Isoliquiritin Apioside (**6**), Isoliquiritigenin (**7**) and Licraside (**9**) (22.5 MHz, DMSO-d_6, δ_C)

		6	7	9
Chalcone moiety	C-1′	113.0	113.2	115.1
	C-2′	165.9	164.6	163.5
	C-3′	102.8	102.6	103.6
	C-4′	166.0	165.4	165.2
	C-5′	108.6	107.9	108.3
	C-6′	133.1	132.3	132.6
	C-β	143.3	143.8	145.4
	C-α	119.5	117.8	117.5
	C-β′	191.5	191.4	192.2
	C-1	128.7	125.8	125.8
	C-2,6	130.8	130.6	131.5
	C-3,5	116.6	115.8	116.1
	C-4	159.4	159.9	160.7
β-Glucopyranosyl moiety	C-1″	98.7		98.2
	C-2″	77.1		76.9
	C-3″	76.3		76.3
	C-4″	70.2		69.7
	C-5″	76.3		76.3
	C-6″	59.7		60.1
β-Apiofuranosyl moiety	C-1‴	108.6		109.1
	C-2‴	77.1		76.9
	C-3‴	79.4		79.4
	C-4‴	74.1		74.1
	C-5‴	64.5		64.4

between the anomeric proton (1″-H) of the D-glucopyranosyl moiety at δ 5.04 (d, J = 7.3 Hz) and the aromatic protons at C-3 and C-5 (δ 7.08, 2H, d, J = 8.9 Hz). Finally, complete methylation of **6** followed by methanolysis furnished methyl 2,3,5-tri-*O*-methylapiofuranoside (**d**) and methyl 3,4,6-tri-*O*-methylglucopyranoside (**e**) in a 1 : 1 ratio. Consequently, the structure of isoliquiritin apioside has been clarified as 4-*O*-β-D-apiofuranosyl(1→2)-β-D-glucopyranosylisoliquiritigenin (**6**).

We are engaged in comparative analysis of the flavonoid constituents in various licorice-roots of different origins.[2,18] It has been found so far that isoliquiritin apioside (**6**) is commonly distributed in Chinese licorice-roots as the major flavonoid constituent.[19]

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Experimental

The instruments used for obtaining physical data and the experimental conditions for chromatography were the same as described in our previous paper.[1]

Isolation of Nine Oleanene-Type Triterpene Oligoglycosides and Seven Flavonoid Glycosides The air-dried roots of *Glycyrrhiza uralensis* FISCHER (from Xinjiang province, China, cut, 3 kg) were extracted with MeOH (10 l each) under reflux three times. Evaporation of the solvent under reduced pressure from the combined extract gave the MeOH extract (484 g). The extract (273 g) was then partitioned into an ethyl acetate–water (1 : 1) mixture (3 l). Removal of the solvent from the water phase under reduced pressure below 40 °C, provided the aqueous extract (200 g), of which 100 g was subjected to reversed-phase silica gel column chromatography [Fuji gel RQ-3, 75 Å, 30/50 μ, 800 g, gradient elution with H_2O–MeOH (7 : 1→1 : 3)] to furnish four fractions: fraction 1 (fr. 1) [eluted with H_2O–MeOH (7 : 1→5 : 1); mainly sugar constituents, 35 g], fr. 2 [H_2O–MeOH (5 : 1→3 : 1); mainly sugar and flavonoid glycosides, 30 g], fr. 3 [H_2O–MeOH (2 : 1→1 : 2); mainly saponins, 23 g] and fraction 4 [H_2O–MeOH (1 : 2→1 : 3); others, 8 g]. Silica gel column chromatography [SiO_2 300 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower phase and 6 : 4 : 1)] of fr. 2 (30 g) followed by preparative HPLC [Shimpak PREP ODS, 20 mm × 25 cm, MeOH–H_2O (3 : 2) and Zorbax BP-ODS, 4 mm × 25 cm, MeOH–H_2O (2 : 1)] afforded ononin, liquiritin, liquiritin apioside, isoliquiritin, neoisoliquiritin, licraside (**9**), and isoliquiritin apioside (**6**) in 0.07, 0.35, 0.90, 0.15, 0.02, 0.06, and 1.65% yields from the root, respectively. Silica gel column chromatography [SiO_2 350 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower phase and 6 : 4 : 1)] of fraction 3 (23 g) and subsequent preparative HPLC [Shimpak PREP ODS, 20 mm × 25 cm, CH_3CN–1% aqueous AcOH (65 : 35)] provided glycyrrhizin, 18α-glycyrrhizin, apioglycyrrhizin, araboglycyrrhizin, licorice-saponins A3, E2, G2, H2, and L3 (**1**) in 1.12, 0.02, 0.01, 0.06, 0.10, 0.07, 0.10, 0.21 and 0.03% yields from the root, respectively. Known saponins and flavonoid glycosides were identified by comparing the ^{1}H-NMR, IR, and $[\alpha]_D$ data with those of authentic samples and by mixed melting point determination.

Licorice-Saponin L3 (**1**): mp 233—234 °C (colorless fine prisms from MeOH), $[\alpha]_D^{25}$ +3.7° (c=0.3, MeOH). High-resolution FAB-MS: Found 1007.4840, Calcd for $C_{49}H_{76}O_{20}Na$ [(M+Na)$^+$] 1007.4828. IR ν_{max}^{KBr} cm^{-1}: 3650—3100 (br), 2912, 1704 (br), 1658, 1622, 1382, 1048. ^{1}H-NMR (500 MHz, pyridine-d_5+D_2O) δ: 0.92, 0.93, 0.99, 1.32, 1.33, 1.44 (all 3H, s), 1.74 (3H, d, J=6.1 Hz, rhamnose methyl), 2.02 (3H, s, acetoxyl methyl), 3.42 (1H, dd, J=3.6, 12.0 Hz, 3α-H), 4.95 (1H, d, J=7.6 Hz, 1′-H), 5.52 (1H, d, J=7.3 Hz, 1″-H), 5.54 (1H, br s, 12-H), 6.15 (1H, br s, 1‴-H). ^{13}C-NMR: as given in Table I. FAB-MS m/z (positive): 1007 [(M+Na)$^+$], 985 [(M+H)$^+$], (+LiCl): 993 [(M+Li$^+$]; (negative): 983 [(M−H)$^-$], 837 (**i**), 705 (**ii**), 529 (**iii**).

Isoliquiritin Apioside (**6**): mp 140 °C (yellow plates from $CHCl_3$–MeOH), $[\alpha]_D^{23}$ −91.3° (c=1.0, MeOH), −92.9° (c=0.6, EtOH). High-resolution FAB-MS: Found 551.1768, Calcd for $C_{26}H_{31}O_{13}$ [(M+H)$^+$] 551.1765. UV λ_{max}^{MeOH} nm (ε): 360 (28600); 395 [+NaOMe]; 415 [+$AlCl_3$, +$AlCl_3$/HCl]. IR ν_{max}^{KBr} cm^{-1}: 3550—3250 (br), 2926, 1633, 1599, 1509, 1369, 1229, 1068. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 320 (1H, dd, J=9.2, 9.2 Hz, 3″-H), 3.32, 3.35 (1H each, ABq, J=14.3 Hz, 5‴-H_2), 3.40—3.65 (5H, m, sugar protons), 3.66, 3.95 (1H eqch, ABq, J=9.5 Hz, 4‴-H), 3.76 (1H, d, J=1.2 Hz, 2‴-H), 5.04 (1H, d, J=7.3 Hz, 1″-H), 5.36 (1H, d, J=1.2 Hz, 1‴-H), 6.28 (1H, d, J=2.1 Hz, 3′-H), 6.40 (1H, dd, J=2.1, 8.7 Hz, 5′-H), 7.08 (2H, d, J=8.9 Hz, 3-H, 5-H), 7.76 (1H, d, J=15.3 Hz, α-H), 7.83 (1H, d, J=15.3 Hz, β-H), 7.84 (2H, d, J=8.9 Hz, 2-H, 6-H), 8.15 (1H, d, J=8.7 Hz, 6′-H). ^{13}C-NMR: as given in Table III (22.5 MHz, in DMSO-d_6); chemical shifts of sugar moiety [in pyridine-d_5, 67.8 MHz (JEOL JNM EX-270), δ_C]: glucose; 99.7 (C-1″), 80.6 (C-2″), 78.3, 78.4 (C-3″, C-5″), 71.0 (C-4″), 61.8 (C-6″), apiose; 110.2 (C-1‴), 77.6 (C-2‴), 80.6 (C-3‴), 75.4 (C-4‴), 65.9 (C-5‴). FAB-MS m/z (positive): 551 [(M+H)$^+$], 419, 257.

Methanolysis of Licorice-Saponin L3 (1) A solution of **1** (40 mg) in 9% HCl–MeOH (4 ml) was heated under reflux for 3 h. After cooling, the reaction mixture was neutralized with Amberlite IRA 400 (OH^- form) and the resin was removed by filtration. A part (*ca.* 1 mg) of the product, obtained by evaporation of the solvent from the filtrate under reduced pressure, was dissolved in pyridine (0.1 ml) and the solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.1 ml) to afford the trimethylsilyl (TMS) derivatives of methyl glucuronide, methyl arabinoside, and methyl rhamnoside. These TMS derivatives of methyl glycosides were identified by GLC comparison with authentic samples, which were prepared by methanolysis of D-glucuronic acid, L-arabinose, and L-rhamnose and subsequent silylation. GLC analysis: i) 3% SE-30 on Uniport B (80—100 mesh), 3 mm (i.d.) × 1 m, glass column, column temperature 120 °C; N_2 flow rate 35 ml/min; t_R: the TMS derivative of methyl arabinoside 14 min 42 s, 17 min 12 s and 18 min 34 s, the TMS derivative of methyl rhamnoside 15 min 38 s, 20 min 48 s. ii) under the same conditions as for i) except for column temperature (160 °C); t_R: the TMS derivative of methyl glucuronide, 7 min 0 s, 15 min 7 s. The methanolysis product (40 mg) was subjected to reversed-phase silica gel column chromatography [Bondapak C_{18} 5 g, H_2O–MeOH (2 : 3)] to afford **2** (11 mg) and a methyl glycoside fraction (20 mg). Silica gel column chromatography [SiO_2 3.5 g, $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower phase)] of the methyl glycoside fraction furnished methyl D-glucuronide (4.4 mg), methyl D-arabinoside (3.3 mg), and methyl L-rhamnoside (3.8 mg). Methyl D-glucuronide (4.4 mg), obtained from **1**, was dissolved in MeOH (1 ml) and the solution was treated with $NaBH_4$ (2 mg). The whole mixture was stirred at room temperature (24 °C) for 1 h and then neutralized with Dowex 50 W × 8 (H^+ form). After removal of the solvent from the filtrate under reduced pressure, the product was dissolved in 3% HCl (0.5 ml) and the solution was heated under reflux for 2 h. The reaction mixture was neutralized with Amberlite IRA-2 (OH^- form) and evaporation of the solvent from the filtrate provided D-glucose [$[\alpha]_D^{24}$ +42° (c=0.19, H_2O), measured after standing for 24 h following dissolution in H_2O]. Methyl L-arabinoside (3.3 mg), obtained from **1**, was dissolved in 3% HCl (0.5 ml) and the solution was heated under reflux for 2 h. The reaction mixture was worked up as described above to furnish L-arabinose [$[\alpha]_D^{24}$ +58° (c=0.22, H_2O, 24 h after dissolving in H_2O]. A solution of methyl L-rhamnoside (3.8 mg) in 3% HCl (0.5 ml) was also heated under reflux for 2 h. The reaction mixture was worked up as described above to yield L-rhamnose [$[\alpha]_D^{24}$ +9.0° (c=0.26, 24 h after dissolving in H_2O)].

2: mp 269—270 °C (colorless fine prisms from MeOH), $[\alpha]_D^{25}$ +20.4° (c=0.20, MeOH). High-resolution EI-MS: Found 470.3402, 246.1629, 224.1783, Calcd for $C_{30}H_{46}O_4$ (M^+) 470.3396; $C_{16}H_{22}O_2$ (**v**) 246.1619; $C_{14}H_{24}O_2$ (**iv**) 224.1777. IR ν_{max}^{KBr} cm^{-1}: 3432, 2932, 2858, 1740, 1658, 1460, 1381, 1083, 1038. ^{1}H-NMR (500 MHz, pyridine-d_5+D_2O) δ: 0.90, 0.93, 0.97, 1.26 (all 3H, s), 1.17 (6H, s), 3.45 (1H, dd, J=3.8, 11.6 Hz, 3α-H), 4.18 (1H, d, J=5.8 Hz, 22α-H), 4.19, 4.23 (1H both, ABq, J=8.8 Hz, 24-H_2), 5.31 (1H, dd, J=3.7, 3.7 Hz, 12-H). ^{13}C-NMR: as given in Table I. EI-MS m/z (%): 470 (M^+, 4.1), 246 (**v**, 100), 224 (**iv**, 11.9).

Preparation of 2a A solution of **2** (3 mg) in 2,2-dimethoxypropane (1 ml) was treated with *p*-TsOH·H_2O (2 mg) and the whole mixture was stirred at room temperature (23 °C) under an N_2 atmosphere for 20 min. The reaction mixture was poured into ice-water (300 ml) and the whole was extracted with AcOEt. The AcOEt extract was washed with saturated saline, dried over $MgSO_4$, then filtered. Work-up of the filtrate in the usual manner gaver **2a** (2 mg).

2a: mp 162—163 °C (colorless fine prisms from MeOH), $[\alpha]_D^{25}$ +16.2° (c=0.20, $CHCl_3$). High-resolution EI-MS. Found 495.3464, 264.2089, 246.1578, Calcd for $C_{32}H_{47}O_4$ [(M−CH_3)$^+$] 495.3471; $C_{17}H_{28}O_2$ (**vi**) 264.2089; $C_{16}H_{22}O_2$ (**v**) 246.1619. IR ν_{max}^{KBr} cm^{-1}: 2930, 2855, 1763, 1720, 1601, 1460, 1375, 1090, 1008. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.87, 0.88, 0.89, 1.16, 1.21, 1.37 (all 3H, s), 1.39, 1.44 (3H, both, s, acetonide methyls), 3.46 (1H, dd, J=4.0, 11.9 Hz, 3α-H), 4.17 (1H, d, J=5.8 Hz, 22α-H), 4.04, 4.22 (1H, both, ABq, J=9.1 Hz, 24-H_2), 5.34 (1H, dd, J=3.7, 3.7 Hz, 12-H). EI-MS m/z (%): 495 [(M−CH_3)$^+$, 10], 264 (**vi**, 11), 246 (**v**, 100).

Application of Modified Horeau's Method to 2 A solution of **2** (2.5 mg, 4 μmol) in pyridine (50 μl) was treated with *dl*-phenylbutyric anhydride (6.1 mg, 20 μmol) and the whole mixture was stirred at 45 °C under an N_2 atmosphere for 4 h. After cooling of the reaction mixture, (+)-*R*-phenylethylamine (3 ml) was added and the whole was left standing at 45 °C for 15 min. After cooling, the reaction mixture was dissolved in AcOEt (2 ml) and the solution was subjected to GLC analysis. GLC analysis: iii) FS-WSCOT silicone OV-101 capillary column, 0.25 mm (i.d.) × 50 m, column temperature 180 °C, injection temperature 200 °C; N_2 flow rate 35 ml/min with split method, split rate 45 : 1; t_R: the amide derived from *d*-phenylbutyric acid, 22 min 18 s and from *l*-phenylbutyric acid, 26 min 6 s. *P* value (peak area from *d*-phenylbutyric acid/peak area from *l*-phenylbutyric acid) of **2** was 1.2232 and cyclohexanol (*rac.*) was 1.0732 (*P* value of each entry was the average of five results). The peak increment of *d*-phenylbutylamide was +7.0%.

Diazomethane Methylation of Licorice-Saponin L3 (1) A solution of **1** (18 mg) in MeOH (2 ml) was treated with ethereal diazomethane (*ca.* 10 ml) until the yellow color persisted. The reaction mixture was left standing at room temperature (23 °C) for 1 h and evaporated under reduced pressure to give a product (18 mg), which was purified by silica gel column chromatography [SiO_2 2 g, $CHCl_3$–MeOH–H_2O (6 : 4 : 1)] to furnish **1a** (7 mg).

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1a: mp 199—200 °C (colorless fine prisms from MeOH), $[\alpha]_D^{25}$ +1.2° (c=0.3, MeOH). High-resolution SIMS: Found 1035.5087, Calcd for $C_{51}H_{80}O_{20}Na$ [(M+Na)$^+$] 1035.5140. High-resolution FAB-MS: Found 1035.5200, Calcd for $C_{51}H_{80}O_{20}Na$ [(M+Na)$^+$] 1035.5140. IR ν_{max}^{KBr} cm^{-1}: 3600—3250, 2929, 1729, 1680, 1386, 1255, 1051. ^{1}H-NMR (500 MHz, pyridine-d_5+D_2O) δ: 0.74, 0.91, 0.91, 1.13, 1.27, 1.42 (all 3H, s), 1.70 (3H, d, J=6.5 Hz, rhamnose methyl), 2.03 (3H, s, acetoxyl methyl), 3.38 (1H, dd, J=3.4, 11.6 Hz, 3α-H), 3.70, 3.74 (each 3H, both s, OCH_3), 4.92 (1H, d, J=7.6 Hz, 1′-H), 5.45 (1H, br s, 12-H), 5.52 (1H, d, J=7.2 Hz, 1″-H), 6.15 (1H, br s, 1‴-H). FAB-MS m/z (positive): 1035 [(M+Na)$^+$], (+LiCl): 1019 [(M+Li)$^+$]; (negative): 997 [(M−H)$^-$].

Complete Methylation of 1a Followed by Methanolysis A solution of **1a** (8 mg) in DMSO (0.5 ml) was treated with a dimsylcarbanion solution [1 ml, prepared with NaH (100 mg) and DMSO (2 ml)] and the mixture was stirred at room temperature (24 °C) in the dark for 2 h. The reaction mixture was treated with CH_3I (1 ml) and the whole was stirred for a further 10 h. The reaction mixture was poured into ice-water, then the whole was extracted with AcOEt. The AcOEt extract was worked up as described above to afford a product (4.8 mg). Without further purification, the product was dissolved in 9% HCl–MeOH (0.5 ml) and the solution was heated under reflux for 3 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 powder and the products were identified by TLC [benzene–acetone (2:1)] and GLC with methyl 3,4,6-tri-*O*-methylglucuronopyranoside (**a**), methyl 2,3-di-*O*-methylarabinopyranoside (**b**), and methyl 2,3,4-tri-*O*-methylrhamnopyranoside (**c**). The composition of these three methyl glycosides was determined from the GLC peak areas. GLC analysis: iv) 3% PEGS (polyethylene glycol succinate) on Uniport B (80—100 mesh); 3 mm (i.d.)×2 m, glass column; column temperature 170 °C; N_2 flow rate 35 ml/min; t_R, **a** 35 min 36 s, **b** 30 min 6 s, 35 min 36 s, **c** 11 min 24 s, 12 min 42 s, v) 15% DEGS (diethylene glycol succinate) on Uniport B (80—100 mesh); 3 mm (i.d.)×2 m, glass column; column temperature 170 °C; N_2 flow rate 35 ml/min; t_R, **a** 26 min 18 s, **b** 22 min 54 s, 28 min 18 s, **c** 8 min 54 s, 10 min 6 s.

Methanolysis of Isoliquiritin Apioside (6) A solution of **6** (25 mg) in 9% HCl–MeOH (5 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 powder and the whole was filtered to remove the inorganic material. After removal of the solvent from the filtrate, the product (22 mg) was purified by silica gel column chromatography [SiO_2 8 g, $CHCl_3$–MeOH (20:1—3:2)] to give isoliquiritigenin (**7**, 4.5 mg), (±)-liquiritigenin (**8**, 5.5 mg) and a methyl glycoside fraction (8.3 mg). Silylation of the methyl glycoside fraction (0.5 mg) with BSTFA–pyridine (1:1, 0.6 ml) gave the trimethylsily derivatives of methyl D-glucoside and methyl D-apioside which were identified by GLC comparison with authentic samples. GLC analysis: iv) 3% SE-30 on Uniport B (80—100 mesh); 3 mm (i.d.)×1 m, glass column, column temperature. 140 °C; N_2 flow rate 40 ml/min; t_R: TMS derivative of methyl apioside, 8 min 25 s, 9 min 3 s; TMS derivative of methyl glucoside, 32 min 24 s 36 min 54 s. Isoliquiritigenin (**7**) and (±)-liquiritigenin (**8**) were identified by comparisons of IR, UV, and ^{1}H-NMR data with those of authentic samples, and by mixed melting point determination, respectively.

The methyl glycoside fraction (7 mg) was subjected to silica gel column chromatography [SiO_2 2 g, $CHCl_3$–MeOH (10:1)] to afford methyl D-apioside (2.9 mg) and methyl D-glucoside (3.3 mg). Methyl D-apioside (2.9 mg), obtained from **6**, was dissolved in 2% H_2SO_4 and the whole mixture was heated under reflux for 2 h. The reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate provided D-apiose [$[\alpha]_D^{23}$ +10° (c=0.10, H_2O), 24 h after dissolving in H_2O]. A solution of methyl D-glucoside (3.3 mg), obtained from **6**, was also heated under reflux for 2 h. The reaction mixture was worked up as described above to afford D-glucose [$[\alpha]_D^{23}$ +45° (c=0.12, H_2O), 24 h after dissolving in H_2O].

Complete Methylation of 6 Followed by Methanolysis A solution of **6** (40 mg) in DMSO (3 ml) was treated with dimsyl carbanion [3 ml, prepared from NaH (300 mg) and DMSO (6 ml)] and the mixture was stirred at room temperature (24 °C) in the dark for 1.5 h. The reaction mixture was then treated with CH_3I (3 ml) and the whole was stirred for a further 10 h. The reaction mixture was poured into ice-water and the whole was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with saturated saline, dried over $MgSO_4$ powder, then filtered. After removal of the solvent from the filtrate, the residue was purified by silica gel column chromatography [SiO_2 5 g, $CHCl_3$–MeOH (5:2)] to give the methylated product (45 mg). The product was dissolved in 9% HCl–MeOH (5 ml) and the solution was heated under reflux for 3 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 powder. The methanolysis products were identified by TLC [benzene–acetone (2:1)] and GLC as methyl 2,3,5-tri-*O*-methylapiofuranoside (**d**) and methyl 3,4,6-tri-*O*-methylglucopyranoside (**e**). GLC analysis: vii) 5% DBU (butane-1,4-diol succinate) on Uniport B (80—100 mesh); 3 mm (i.d.)×2 m, glass column; column temperature 160 °C; N_2 flow rate 35 ml/min; t_R: **d** 1 mm 14 s, 2 min 27 s, **e** 5 min 27 s, 7 min 43 s.

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