Saponin and Sapogenol. XLVII.[1] On the Constituents of the Roots of *Glycyrrhiza uralensis* FISCHER from Northeastern China. (1). Licorice-Saponins A3, B2, and C2

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From the air-dried root of *Glycyrrhiza uralensis*, collected in the northeastern part of China, ten new oleanane-type triterpene oligoglycosides were isolated together with glycyrrhizin (1) and several known flavonoids. Among the newly isolated triterpene oligoglycosides, the chemical structures of licorice-saponin A3 (2), licorice-saponin B2 (3), and licorice-saponin C2 (4) have been determined, on the basis of chemical and physicochemical evidence, to be expressed as 30-*O*-β-D-glucopyranosylglycyrrhizin, 11-deoxo-glycyrrhizin, and 3-*O*-[β-D-glucuronopyranosyl(1→2)-β-D-glucuronopyranosyl]oleana-11,13(18)-dien-30-oic acid, respectively. During the course of these studies, facile conversions from glycyrrhizin (1) to licorice-saponins A3 (2), B2 (3), and C2 (4) have been accomplished.

Keywords *Glycyrrhiza uralensis*; Glycyrrhizae Radix; licorice-saponin; Leguminosae; oleanane-type triterpene oligoglycoside; glycyrrhizin

Licorice root has been most frequently prescribed in Chinese traditional medicine. It has also been used as a sweetening agent and a flavor. In Japan, licorice root is a very widely used Chinese crude drug, so that the chemical constituents have been the subject of many investigations, and a number of flavonoids and their glycosides have been identified.[2] Among those flavonoids, retrochalcones were shown to be characteristic constituents of the licorice root from Xinjiang province in China and the biogenetic pathway of retrochalcones was clarified.[2a] As for the other major ingredients of licorice root, glycyrrhizin (**1**) is the only saponin constituent identified and several oleanane-type triterpenoids were simply but sapogenols isolated from the hydrolysate of the glycosidic mixture of European *Glycyrrhiza* spp.[2b,3]

In Chinese traditional medicine, licorice root has been used either as air-dried slices or as slices of roots baked beforehand with *e.g.* honey (this being a kind of processed licorice root). In addition, licorice root with the cortex removed (another example of processed licorice root) has been used in Japan. As a part of our chemical characterization studies of crude drug processing,[4a] we have begun to compare the chemical constituents of processed and unprocessed licorice roots.

In Japanese Pharmacopea XII, the botanical origins of licorice root (Glycyrrhizae Radix) are prescribed as the roots and stolons of *Glycyrrhiza uralensis* FISCHER, *G. glabra* L., and related *Glycyrrhiza* spp. of the Leguminosae family. However, most of the studies carried out so far in Japan were done on imported licorice roots which were simply indicated as "Tohoku-Kanzo" (東北甘草) (licorice roots from the northeastern part of China), "Seihoku-Kanzo" (西北甘草) (those from the northwestern part), or "Shinkyo-Kanzo" (新疆甘草) (from Xinjiang province) according to their regions of origin. In other words, the botanical origins of those licorice roots have seldom been identified. On the other hand, licorice roots medicinally used in China have been of defined botanical origin; *e.g.*

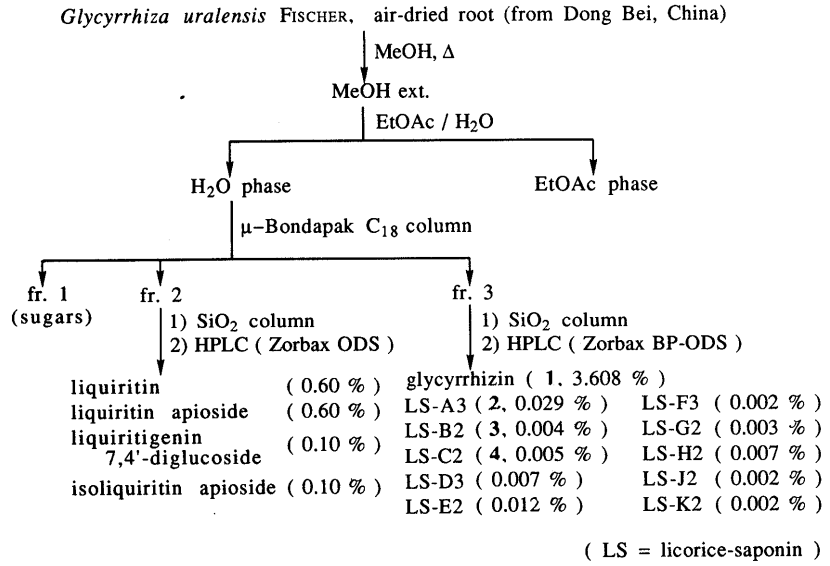

Fig. 1. Isolation Procedure for Flavonoids and Saponins from *Glycyrrhiza uralensis* Root

Glycyrrhiza uralensis FISCHER is "Uraru-Kanzo" (ウラル甘草), *G. inflata* BATAL is "Choka-Kanzo" (脹果甘草), and *G. glabra* L. is "Koka-Kanzo" (光果甘草).

Consequently, prior to a detailed comparative study of the processed and unprocessed licorice roots, precise chemical analysis of botanically identified licorice roots was essential. In this and the following papers, we present the details of our chemical investigations on the constituents of botanically identified licorice roots provided by Chinese Scientists.

The chemical constituents of the root of *Glycyrrhiza uralensis* FISCHER, from Dong Bei district, China,[4b] were separated through the procedure shown in Fig. 1. Thus, the methanolic extract of the root was first partitioned into an ethyl acetate–water mixture, and the reversed-phase silica gel column chromatography of the water-soluble portion provided three fractions. Silica gel column chromatography of fraction 2 gave three known flavonoid glycosides (liquiritin,[5] liquiritin apioside,[5] and liquiritigenin 7,4′-diglucoside[6]) and a new chalcone glycoside named isoliquiritin apioside,[7] whereas from fraction 3, ten new saponins designated licorice-saponins A3 (**2**), B2 (**3**), C2 (**4**), D3, E2, F3, G2, H2, J2, and K2[8] were isolated together with the major saponin glycyrrhizin (**1**). This paper presents a full account of the structure elucidation of licorice-saponins A3 (**2**), B2 (**3**), and C2 (**4**), including chemical conversions of glycyrrhizin (**1**) to these licorice-saponins (**2**, **3**, **4**).[9]

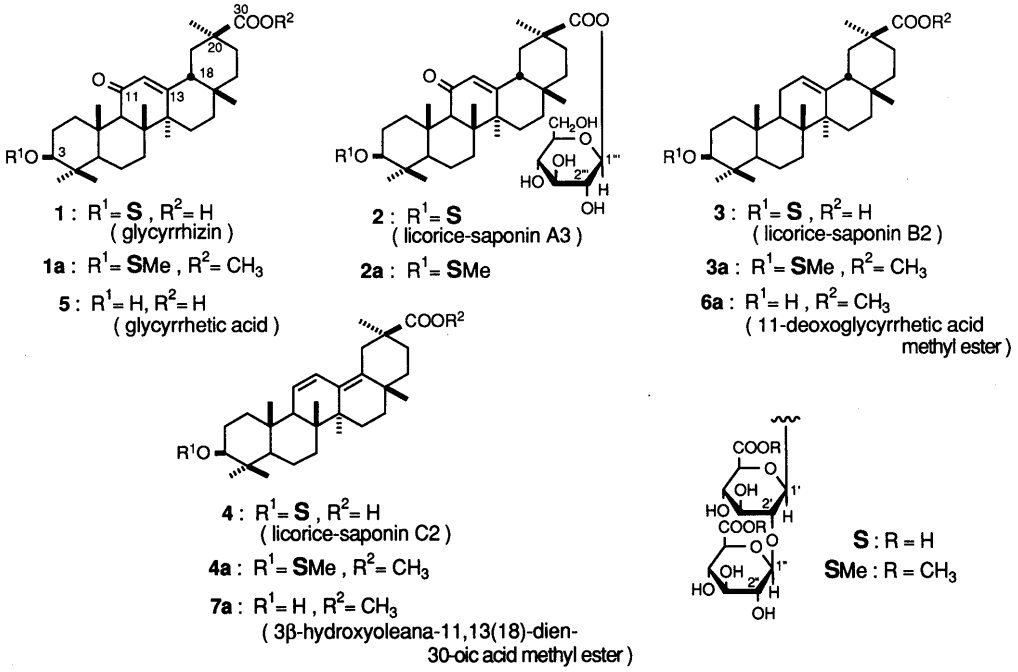

Chart 1

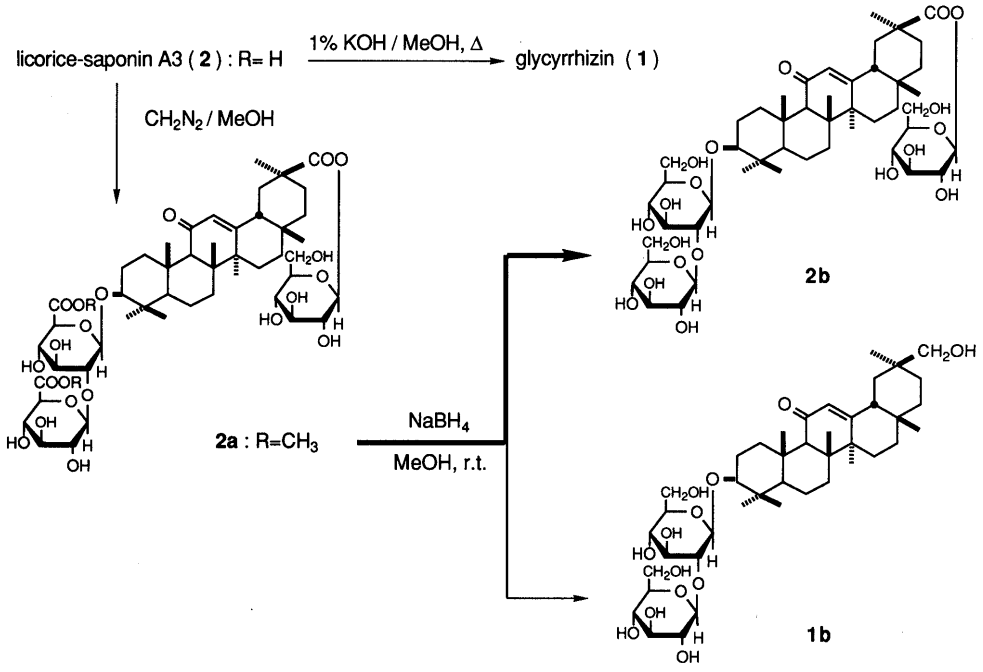

Chart 2

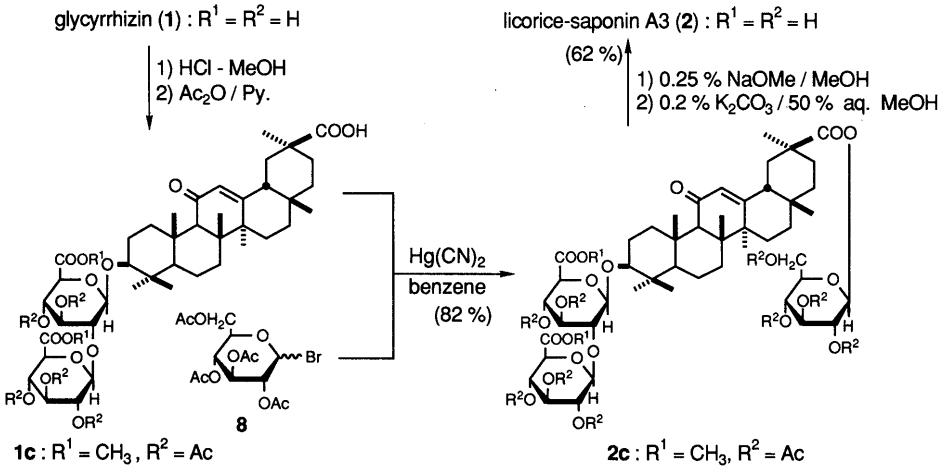

Chart 3

Licorice-Saponin A3 (2) Licorice-saponin A3 (**2**) was obtained as colorless prisms. The infrared (IR) spectrum of **2** showed absorption bands due to hydroxyl, ester, carboxyl, and enone moieties (3360, 1741, 1716, 1650 cm^{-1}), while the ultraviolet (UV) spectrum showed the presence of an enone chromophore by an absorption maximum at 248 nm ($\varepsilon = 8800$). The proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra of **2** showed signals due to three anomeric protons and three anomeric carbons, which indicated the presence of two β-glycosidic and one ester β-glycosidic linkages in **2**.

Treatment of licorice-saponin A3 (**2**) with 9% hydrogen chloride in methanol liberated glycyrrhetic acid (**5**), methyl glucuronide, and methyl D-glucoside. On the other hand, alkaline hydrolysis of **2** yielded glycyrrhizin (**1**); thus it has become evident that **2** is a β-D-glucoside of **1** connected through an ester linkage. In order to determine the location of the glucoside linkage, **2** was treated with diazomethane to afford the dimethyl ester (**2a**). Further treatment of **2a** with sodium borohydride in methanol yielded two products; **2b** and **1b** (Chart 2). Methanolysis of the former **2b** gave glycyrrhetic acid (**5**) and methyl glucoside. From these findings, it has been proved that the ester glucoside linkage in **2** is located at the 20β-carboxyl moiety in **1**.

In order to provide additional chemical evidence of the structure of licorice-saponin A3 (**2**), **2** was synthesized from glycyrrhizin (**1**) as shown in Chart 3. Thus, glycyrrhizin (**1**) was first treated with 9% hydrogen chloride in methanol at room temperature to give a dimethyl ester, which was then acetylated to afford a dimethyl ester pentaacetate (**1c**) in 97% yield from **1**. Glycosidation of **1c** with 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl bromide (**8**) in the presence of mercuric dicyanide in dry benzene furnished **2c** in 82% yield. Successive treatment of **2c** with 0.25% sodium methoxide in methanol at room temperature (deacetylation only) and then with 0.2% potassium carbonate in 50% aqueous methanol (hydrolysis of the dimethyl ester part) finally provided licorice-saponin A3 (**2**) in 62% yield from **2c**.[10]

Based on the above evidence, the structure of licorice-saponin A3 has been determined as 3-*O*-[β-D-glucuronopyranosyl(1→2)-β-D-glucuronopyranosyl]-30-*O*-β-D-glucopyranosylglycyrrhetic acid (**2**), which is in good accord with the ^{13}C-NMR spectral analysis of **2a** and **2b** (Table I).

TABLE I. ^{13}C-NMR Data for **1a**, **2a**, **2b**, **3a** and **4a** (at 22.5 MHz, in Pyridine-d_5, δ_C)

		1a	2a	2b	3a	4a
Sapogenol moiety	C-3	89.2	89.0	88.6	89.5	89.5
	C-11	199.0	199.3	199.5	47.8	125.5[a]
	C-12	128.7	128.2	128.4	122.9	126.7[a]
	C-13	168.7	169.1	169.1	144.7	135.8
	C-18	48.5	47.9	47.9	48.6	135.8
	C-30	176.5	175.4	175.5	177.3	178.4
3-*O*-β-D-Glucurono or glucopyranosyl moiety	C-1′	104.5	104.3	104.6	105.0	104.8
	C-2′	84.0	83.7	82.8	84.4	84.3
	C-3′	76.2[a]	76.0[a]	77.5[a]	76.3[a]	76.5
	C-4′	72.2	72.2[b]	71.3	72.6[b]	72.4[b]
	C-5′	77.1	77.0	77.5[a]	77.4[c]	77.2[c]
	C-6′	169.5[b]	169.6[c]	62.4[c]	170.1[d]	169.8[d]
2′-*O*-β-D-Glucurono or glucopyranosyl moiety	C-1″	106.3	106.1	105.4	106.8	106.6
	C-2″	75.9[a]	75.8[a]	76.5	76.4[a]	76.5
	C-3″	77.1	77.0	77.7[a]	77.6[c]	77.4[c]
	C-4″	72.4	72.3[b]	71.3	72.9[b]	72.7[b]
	C-5″	77.1	77.0	77.5[a]	76.7[a]	77.2[c]
	C-6″	169.7[b]	169.7[c]	62.4[c]	170.3[d]	170.1[d]
30-*O*-β-D-Glucopyranosyl moiety	C-1‴		95.4	95.5		
	C-2‴		73.6	73.7		
	C-3‴		78.7	78.9		
	C-4‴		70.7	70.9		
	C-5‴		78.1	78.2		
	C-6‴		61.8	62.0[c]		

a—*d*) Assignments may be interchangeable within the same column.

Licorice-Saponin B2 (3) Licorice-saponin B2 (**3**) was also obtained as colorless prisms. The IR spectrum of **3** showed the absorption bands of hydroxyl and carboxyl groups (3400, 1720 cm^{-1}), whereas the ^{1}H-NMR spectrum showed signals due to two β-oriented anomeric protons. Treatment of a trimethyl ester of licorice-saponin B2 (**3a**), prepared by diazomethane methylation of **3**, with 9% hydrogen chloride in methanol yielded 11-deoxoglycyrrhetic acid methyl ester (**6a**)[11] and methyl glucuronide.

Detailed comparison of the ^{13}C-NMR data for a trimethyl ester of licorice-saponin B2 (**3a**) and glycyrrhizin trimethyl ester (**1a**),[12] has shown that signals due to carbohydrate moieties and aglycone moieties are observed with similar chemical shifts, with some exceptions, thus suggesting licorice-saponin B2 (**3**) to be 11-deoxoglycyrrhizin.

In preliminary pharmacological examinations, licorice-

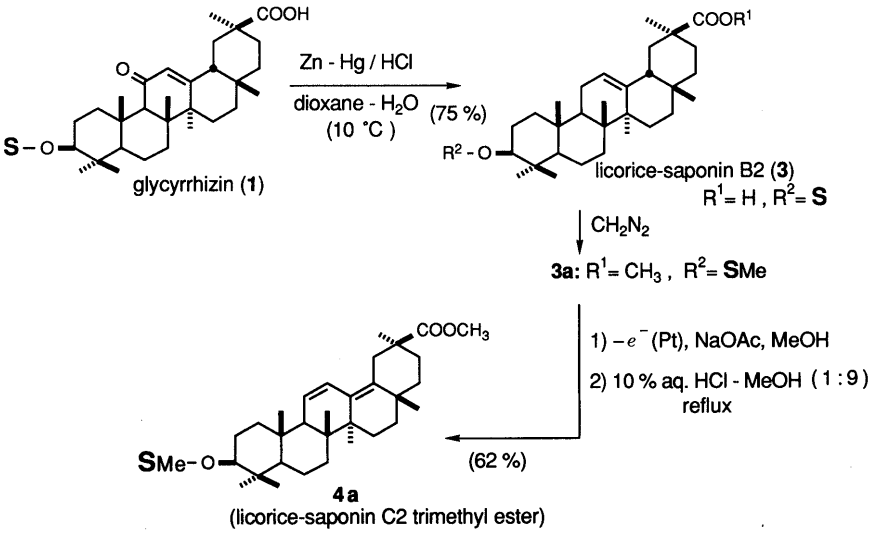

Chart 4

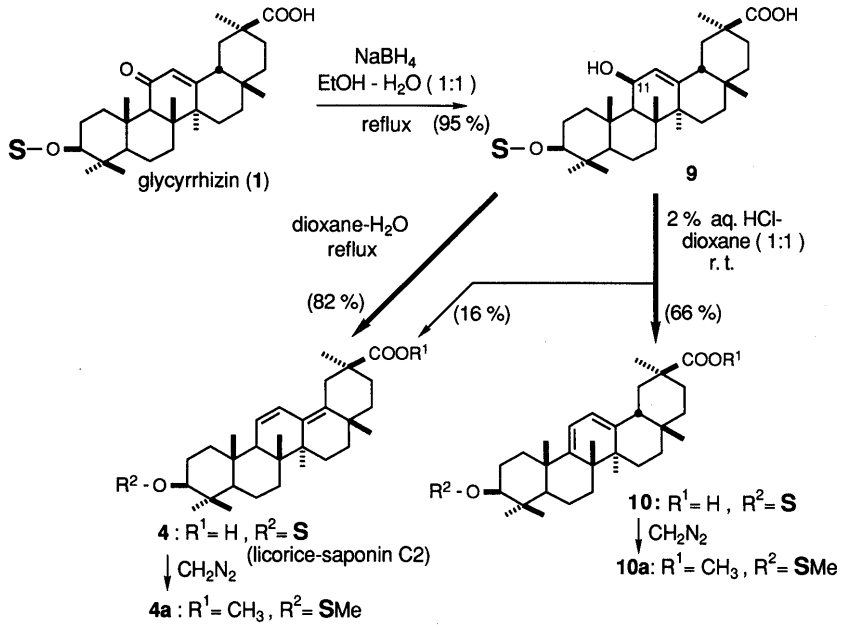

Chart 5

saponin B2 (**3**) was found to promote restoration of hepatocyte function in carbon tetrachloride-intoxicated rats (both *in vitro* and *in vivo*).[13] In order to examine further the restoration-promoting activity of **3**, a larger amount of **3** was required. For this reason, and to confirm the structure **3**, a synthesis of **3** from abundantly available glycyrrhizin (**1**) was carried out by use of Clemmensen reduction.[14] Thus, treatment of **1** with zinc amalgam and hydrochloric acid in dioxane and water at 10 °C furnished **3** in 75% yield. Although **3** has already been synthesized,[14] this is the first report of isolation of this compound in nature. It was named licorice-saponin B2 (**3**).[9a]

Licorice-Saponin C2 (4) Licorice-saponin C2 (**4**) was also isolated as colorless prisms. The UV spectrum of **4** showed the presence of a heteroannular diene chromophore by a characteristic triplet with maxima at 241, 249, and 259 nm, while the IR spectrum showed diene and carboxyl absorption bands (1640, 1710 cm^{-1}). The ^1H-NMR spectrum of a trimethyl ester (**4a**), which was prepared by diazomethane methylation of **4**, showed signals assignable to two β-anomeric protons and two olefinic protons at C-11 and C-12. Furthermore, methanolysis of **4a** liberated methyl glucuronide and 3β-hydroxyoleana-11,13(18)-dien-30-oic acid methyl ester (**7a**).[15]

To shed light on the structure of the carbohydrate portion of licorice-saponin C2 (**4**), the trimethyl ester (**4a**) was first treated with sodium borohydride in methanol and subsequently subjected to complete methylation.[16] Methanolysis of the final product liberated methyl 2,3,4,6-tetra-*O*-methylglucopyranoside and methyl 3,4,6-tri-*O*-methylglucopyranoside.

Based on the above-mentioned evidence and examination of the ^{13}C-NMR data for the trimethyl ester of licorice-saponin C2 (**4a**) (Table I), licorice-saponin C2 (**4**) was presumed to have an 11,13(18)-diene chromophore in its sapogenol moiety and to have the same carbohydrate moiety as glycyrrhizin (**1**) and licorice-saponin B2 (**3**). To verify this presumption, a chemical conversion of **1** to **4**

was undertaken.

Thus, it was found that sodium borohydride treatment of **1** in ethanol and water under reflux gave the 11β-alcohol (**9**) in excellent yield. Treatment of **9** with a 1 : 1 mixture of 2% aqueous hydrochloric acid and dioxane at room temperature yielded a homoannular diene analog (**10**) (in 66% yield) and licorice-saponin C2 (**4**) (in 16% yield). Since the direct separation of **10** and **4** was not easy, the both compounds were separated after diazomethane methylation as the respective trimethyl esters, **10a** and **4a**. The structure of **10** was substantiated by its spectral properties (UV, IR, ^{1}H- and ^{13}C-NMR, see Experimental). On the other hand, treatment of the 11β-alcohol (**9**) with dioxane and water under reflux afforded **4** selectively (in 82% yield).

The conversion of glycyrrhizin (**1**) to licorice-saponin C2 (**4**) was also achieved by way of licorice-saponin B2 (**3**). Thus, Clemmensen reduction of **1** furnished **3** as described above. Licorice-saponin B2 trimethyl ester (**3a**), prepared by diazomethane methylation of **3**, was subjected to anodic oxidation[17] and the resulting product was then treated with 10% aqueous hydrochloric acid in methanol under reflux to afford licorice-saponin C2 trimethyl ester (**4a**) in 62% yield from **3a**.

From the accumulated findings mentioned above, the structure of licorice-saponin C2 has been ascertained as 3-*O*-[β-D-glucuronopyranosyl(1→2)-β-D-glucuronopyranosyl]oleana-11,13(18)-dien-30-oic acid (**4**) as shown. Finally, it should be noted here that, in the conversion from glycyrrhizin (**1**) and licorice-saponin B2 (**3**) to licorice-saponin C2 (**4**), the reactions were carried out without protection of the hydroxyl moieties in **1** and **3**.

Experimental

The instruments used for obtaining physical data and the experimental conditions for chromatography were the same as described in our previous paper[1] except for the following. The specific rotations were obtained with a JASCO DIP-181 or a DIP-370 digital polarimeter. The FAB-MS were obtained with a JEOL JMS SX-102 double beam high resolution mass spectrometer and a JMA DA-6000 data system by a direct inlet method. The primary fast atom bombardment gas was Xe gas, and the accelerating volatages of primary atoms and secondary ions were 3 and 10 kV, respectively. The samples were dissolved in MeOH or *m*-nitrobenzyl alcohol as a matrix, and injected. Polyethylene glycols (No. 1000 and No. 1540) were used as standard samples for high-resolution mass spectrometry.

Isolation of Licorice-Saponins, Glycyrrhizin, and Flavonoid Glycosides The air-dried root of *Glycyrrhiza uralensis* (from Dong Bei, China, cut, 5 kg) was 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 (1.5 kg), which was partitioned into an ethyl acetate–water (1 : 1) mixture (6 l). Removal of water from the water phase under reduced pressure below 40 °C yielded the aqueous extract (1.1 kg). A part of the aqueous extract (200 g) was subjected to reversed-phase silica gel column chromatography (μ-Bondapak C_{18} 1 kg, with gradient elution: H_2O : MeOH = 9 : 1—1 : 9) to furnish three fractions: fr. 1 (eluted with H_2O : MeOH = 9 : 1—7 : 1, mainly sugars, 75 g), fr. 2 (7 : 1—6 : 1) mainly sugars and flavonoid glycosides, 48 g), and fr. 3 (6 : 1—3 : 1, saponins, 50 g), and later eluates (2 : 1—1 : 9, 16 g). Silica gel column chromatography [gradient elution with $CHCl_3$: MeOH : H_2O = 10 : 3 : 1 (lower phase)—5 : 3 : 1] of fr. 2 and subsequent semi-preparative HPLC (Zorbax ODS 4.6 mm × 25 cm, MeOH : H_2O = 5 : 1), provided liquiritin, liquiritin apioside, liquiritigenin 7,4′-diglucoside, and isoliquiritin apioside, in 0.60, 0.60, 0.10, and 0.54% yield from the air-dried root, respectively. Silica gel column chromatography [gradient elution with $CHCl_3$: MeOH : H_2O = 10 : 3 : 1 (lower phase)—6 : 4 : 1] followed by preparative HPLC (Zorbax BP-ODS 9.6 mm × 25 cm, CH_3CN : 1% AcOH = 65 : 35), provided glycyrrhizin (**1**), licorice-saponins A3 (**2**), B2 (**3**), C2 (**4**), D3, E2, F3, G2, H2, J2, and K2 in 3.608, 0.029, 0.004, 0.005, 0.007, 0.012, 0.002, 0.003, 0.007, 0.002, and 0.002% yields from the air-dried root, respectively.

Licorice-Saponin A3 (**2**), mp 198—199 °C (colorless fine prisms from MeOH–H_2O), $[\alpha]_D^{23}$ +69° (c = 1.1, MeOH). *Anal.* Calcd for $C_{48}H_{72}O_{21}\cdot 3H_2O$: C, 55.53; H, 7.60. Found: C, 55.52; H, 7.58. UV λ_{max}^{MeOH} nm (ε): 249 (8800). IR ν_{max}^{KBr} cm^{-1}: 3360, 3000—2500 (br), 1741, 1716, 1650, 1041. ^{1}H-NMR (500 MHz, pyridine-d_5 + D_2O) δ: 0.89, 1.01, 1.19, 1.21, 1.25, 1.34, 1.38 (all 3H, s), 3.00 (1H, br d, J = *ca.* 13 Hz, 18-H), 3.35 (1H, dd, J = 4.7, 11.8 Hz, 3-H), 5.01 (1H, d, J = 7.6 Hz, 1′-H), 5.36 (1H, d, J = 7.8 Hz, 1″-H), 5.92 (1H, s, 12-H), 6.30 (1H, d, J = 8.2 Hz, 1‴-H). ^{13}C-NMR (22.5 MHz, pyridine-d_5) δ_C: 89.0 (C-3), 199.3 (C-11), 128.4 (C-12), 168.9 (C-13), 47.9 (C-18), 37.6 (C-22), 175.3 (C-30), 104.5 (C-1′), 106.2 (C-1″), 95.5 (C-1‴), 171.6, 172.0 (C-6′, C-6″), 62.5 (C-6‴).

Licorice-Saponin B2 (**3**), mp 209—210 °C (colorless fine prisms from MeOH), $[\alpha]_D^{23}$ +54° (c = 0.3, MeOH). *Anal.* Calcd for $C_{42}H_{64}O_{15}\cdot H_2O$: C, 60.02; H, 8.00. Found: C, 60.28; H, 8.07. IR ν_{max}^{KBr} cm^{-1}: 3400, 3000—2800 (br), 1720, 1050. ^{1}H-NMR (500 MHz, pyridine-d_5 + D_2O) δ: 0.83, 0.89, 0.92, 1.20, 1.28, 1.36, 1.39 (all 3H, s), 3.33 (1H, dd, J = 4.6, 11.9 Hz, 3-H), 5.03 (1H, d, J = 7.7 Hz, 1′-H), 5.39 (1H, d, J = 7.6 Hz, 1″-H), 5.49 (1H, br s, 12-H). ^{13}C-NMR (22.5 MHz, pyridine-d_5) δ_C: 88.7 (C-3), 122.0 (C-12), 144.3 (C-13), 48.8 (C-18), 178.3 (C-30), 104.1 (C-1′), 105.7 (C-1″), 171.1, 172.6 (C-6′, C-6″).

Licorice-Saponin C2 (**4**), mp 249—251 °C (colorless fine prisms from MeOH), $[\alpha]_D^{23}$ −120° (c = 0.2, MeOH). *Anal.* Calcd for $C_{42}H_{62}O_{15}\cdot 3H_2O$: C, 58.61; H, 8.03. Found: C, 58.77; H, 8.18. UV λ_{max}^{MeOH} nm (ε): 241 (14100), 249 (15800), 259 (10200). IR ν_{max}^{KBr} cm^{-1}: 3400, 3000—2850 (br), 1710, 1640, 1020. ^{1}H-NMR (500 MHz, pyridine-d_5 + D_2O) δ: 0.80, 0.88, 0.92, 1.05, 1.25, 1.31, 1.38 (all 3H, s), 5.00 (1H, d, J = 7.3 Hz, 1′-H), 5.37 (1H, d, J = 7.4 Hz, 1″-H), 5.92 (1H, br d, J = *ca.* 11 Hz, 11-H), 6.36 (1H, br d, J = *ca.* 11 Hz, 12-H). ^{13}C-NMR (22.5 MHz, pyridine-d_5) δ: 89.2 (C-3), 125.8, 127.2 (C-11, C-12), 135.2, 135.9 (C-13, C-18), 39.6 (C-22), 179.3 (C-30), 103.7 (C-1′), 105.8 (C-1″), 171.1, 171.4 (C-6′, C-6″).

Methanolysis of Licorice-Saponin A3 (2) A solution of **2** (20 mg) in 9% HCl–MeOH (5 ml) 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 [developing with benzene : ethyl acetate = 1 : 1, $CHCl_3$: MeOH = 10 : 1, benzene : MeOH = 5 : 1, $CHCl_3$: MeOH : H_2O = 7 : 3 : 1 (lower phase)] with authentic glycyrrhetic acid (**5**), methyl glucuronide, and methyl glucoside, respectively. The whole products were then purified by column chromatography (silica gel 2 g, $CHCl_3$: MeOH : H_2O = 6 : 4 : 1) to furnish methyl glucoside; this was dissolved in 3% aqueous HCl and the mixture was heated under reflux for 2 h. The reaction mixture was neutralized with Amberlite IRA-2 (OH^-) and evaporation of the solvent from the filtrate provided glucose (2.8 mg), $[\alpha]_D^{24}$ +45° (c = 0.28, 24 h after dissolving in H_2O).

Alkaline Hydrolysis of Licorice-Saponin A3 (2) A solution of **2** (100 mg) in 1% KOH–MeOH (10 ml) was stirred at room temperature for 3 h. The reaction mixture was neutralized with Dowex 50W × 8 (H^+) and filtered to remove the resin. Evaporation of the solvent from the filtrate under reduced pressure gave a solid, which was crystallized from MeOH to afford glycyrrhizin (**1**, 59 mg), mp 212—214 °C, $[\alpha]_D^{23}$ +44° (c = 0.8, EtOH). This was identical with an authentic sample by mixed melting point determination and IR and TLC comparisons [$CHCl_3$: MeOH : H_2O = 6 : 4 : 1, *n*-BuOH : AcOH : H_2O = 4 : 1 : 5 (upper phase)].

Diazomethane Methylation of Licorice-Saponin A3 (2) An ice-cooled solution of **2** (30 mg) in MeOH (5 ml) was treated with ethereal diazomethane (*ca.* 30 ml) until the yellow color persisted. The solution was left stading for 1 h, then the solvent was removed under reduced pressure to furnish the dimethyl ester (**2a**).

2a: mp 206—208 °C (colorless fine prisms from MeOH), $[\alpha]_D^{23}$ +75° (c = 1.1, MeOH). *Anal.* Calcd for $C_{50}H_{76}O_{21}\cdot H_2O$: C, 58.25; H, 7.57. Found: C, 58.21; H, 7.77. UV λ_{max}^{MeOH} nm (ε): 249 (8900). IR ν_{max}^{KBr} cm^{-1}: 3420, 1740, 1650, 1058. ^{1}H-NMR (500 MHz, pyridine-d_5 + D_2O) δ_C: 0.81, 1.06, 1.16, 1.33, 1.37 (all 3H, s), 1.27 (3H × 2, s), 3.06 (1H, br d, J = *ca.* 12 Hz, 18-H), 3.30 (1H, dd, J = 4.3, 11.6 Hz, 3-H), 3.70, 3.84 (both 3H, s, $-OCH_3$ × 2), 4.96 (1H, d, J = 7.6 Hz, 1′-H), 5.38 (1H, d, J = 7.8 Hz, 1″-H), 5.96 (1H, s, 12-H), 6.36 (1H, d, J = 8.2 Hz, 1‴-H). ^{13}C-NMR (22.5 MHz, pyridine-d_5) δ_C: 51.6, 51.7, 52.0 ($-O\underline{C}H_3$), and other signals as given in Table I.

$NaBH_4$ Reduction of the Dimethyl Ester (2a) A solution of **2a** (100 mg) in MeOH (8 ml) was treated with $NaBH_4$ (150 mg) and the whole was stirred at room temperature for 1 h. The reaction mixture was neutralized with Dowex 50W × 8 (H^+) and resin was removed by filtration. The product, obtained by evaporation of the solvent from the filtrate under reduced pressure, was subjected to reversed-phase silica gel column

chromatography (μ-Bondapak C_{18} 10 g, MeOH : H_2O = 1 : 1) to afford **2b** (63 mg) and **1b** (23 mg).

2b: mp 222—224 °C (colorless fine prisms from MeOH), $[\alpha]_D^{23}$ +20° (c = 0.5, MeOH). *Anal.* Calcd for $C_{48}H_{76}O_{19}\cdot 4H_2O$: C, 56.03; H, 8.17. Found: C, 56.18; H, 8.11. UV λ_{max}^{MeOH} nm (ε): 249 (10700). IR ν_{max}^{KBr} cm^{-1}: 3500—3200 (br), 1741, 1652, 1071. ^{1}H-NMR (500 MHz, pyridine-d_5 + D_2O) δ: 0.82, 1.07, 1.14, 1.25, 1.26, 1.32, 1.36 (all 3H, s), 3.02 (1H, br d, J = *ca.* 12 Hz, 18-H), 3.33 (1H, br dd, J = *ca.* 4, 11 Hz, 3-H), 4.88 (1H, d, J = 7.3 Hz, 1′-H), 5.33 (1H, d, J = 7.3 Hz, 1″-H), 5.96 (1H, s, 12-H), 6.36 (1H, d, J = 7.9 Hz, 1‴-H). ^{13}C-NMR: as given in Table I.

1b: mp 223—225 °C (colorless fine prisms from MeOH), $[\alpha]_D^{23}$ +26° (c = 0.4, MeOH). *Anal.* Calcd for $C_{44}H_{60}O_{16}\cdot 3H_2O$: C, 61.23; H, 6.37. Found: C, 61.18; H, 6.31. UV λ_{max}^{MeOH} nm (ε): 249 (10300). IR ν_{max}^{KBr} cm^{-1}: 3530—3200 (br), 1650, 1075. ^{1}H-NMR (90 MHz, pyridine-d_5 + D_2O) δ: 0.82, 1.01, 1.13, 1.30, 1.36 (all 3H, s), 1.25 (3H × 2, s), 3.06 (1H, br d, J = *ca.* 12 Hz, 18-H), 3.33 (1H, br dd, J = *ca.* 4, 11 Hz, 3-H), 4.91 (1H, d, J = 7.3 Hz, 1′-H), 5.28 (1H, d, J = 7.3 Hz, 1″-H), 6.02 (1H, s, 12-H). ^{13}C-NMR (22.5 MHz, pyridine-d_5) δ_C: 88.7 (C-3), 199.4 (C-11), 128.3 (C-12), 169.1 (C-13), 47.9 (C-18), 39.5 (C-22), 65.3 (C-30), 104.7 (C-1′), 83.0 (C-2′), 77.5, 77.7 (C-3′, C-3″), 71.4 (C-4′), 78.0, 78.1 (C-5′, C-5″), 62.6 (C-6′, C-6″), 105.4 (C-1″), 76.6 (C-2″), 71.6 (C-4″).

Methanolysis of 2b A solution of **2b** (2 mg) in 9% HCl–MeOH (0.5 ml) was heated under reflux for 3 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 powder and the reaction products in the filtrate were identified by TLC [developing with benzene : ethyl acetate = 1 : 1, $CHCl_3$: MeOH = 10 : 1, $CHCl_3$: MeOH : H_2O = 7 : 3 : 1 (lower phase)] with authentic glycyrrhetic acid (**5**) and methyl glucoside.

Preparation of 1c from Glycyrrhizin (1) A solution of **1** (100 mg) in 9% HCl–MeOH (2 ml) was stirred at room temperature for 1 h. The reaction mixture was neutralized with Dowex 1 × 2 (OH^-) and the resin was removed by filtration. The residue, obtained by evaporation of the solvent from the filtrate under reduced pressure, was dissolved in Ac_2O–pyridine (1 : 2, 4.5 ml) and the solution was stirred at room temperature for 3 h. The reaction mixture was poured into ice-water and the whole was extracted with ethyl acetate. Work-up of the extract in the usual manner gave a product, which was purified by column chromatography (silica gel 10 g, *n*-hexane : ethyl acetate = 1 : 1) to furnish **1c** (125 mg, 97%).

1c: mp 183—185 °C (colorless fine prisms from EtOH), $[\alpha]_D^{23}$ +45° (c = 0.75, MeOH). *Anal.* Calcd for $C_{54}H_{76}O_{21}\cdot 2H_2O$: C, 59.11; H, 7.35. Found: C, 59.22; H, 7.31. UV λ_{max}^{MeOH} nm (ε): 249 (10300). IR ν_{max}^{KBr} cm^{-1}: 1750, 1710, 1651, 1371, 1029. ^{1}H-NMR (500 MHz, pyridine-d_5 + D_2O) δ: 0.81, 1.24, 1.28, 1.34, 1.40 (all 3H, s), 1.11 (3H × 2, s), 2.00, 2.01, 2.02, 2.08, 2.17 (all 3H, s, acetyl methyl × 5), 3.69, 3.81 (each 3H, s, $-OCH_3$ × 2), 4.96 (1H, d, J = 7.6 Hz, 1′-H), 5.34 (1H, d, J = 7.9 Hz, 1″-H), 5.97 (1H, s, 12-H).

Preparation of 2c from 1c In the presence of $Hg(CN)_2$ (48 mg, 0.19 mmol), a solution of **1c** (100 mg) in dry benzene (2 ml, 0.094 mmol) was treated with 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl bromide (**8**, 100 mg, prepared from 2.5 eq D-glucose) and the whole mixture was heated under reflux for 3 h. The reaction mixture was then passed through a glass filter to remove inorganic material. The filtrate was evaporated under reduced pressure to give a product, which purified by column chromatography (silica gel 10 g, *n*-hexane : ethyl acetate = 1 : 1) to afford **2c** (108 mg, 82%).

2c: mp 176—178 °C (colorless needles from EtOH), $[\alpha]_D^{23}$ +43° (c = 0.70, $CHCl_3$). *Anal.* Calcd for $C_{68}H_{96}O_{30}\cdot 3H_2O$: C, 56.36; H, 7.09. Found: C, 56.18; H, 6.98. UV λ_{max}^{MeOH} nm (ε): 249 (6500). IR ν_{max}^{KBr} cm^{-1}: 1745, 1655, 1368, 1075, 1035. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.83, 1.10, 1.11, 1.20, 1.24, 1.27, 1.34 (all 3H, s), 2.00, 2.01, 2.02, 2.08, 2.17 (all 3H, s, acetyl methyl × 5), 3.69, 3.81 (each 3H, s, $-OCH_3$ × 2), 4.95 (1H, d, J = 7.7 Hz, 1′-H), 5.33 (1H, d, J = 7.9 Hz, 1″-H), 5.94 (1H, s, 12-H), 6.33 (1H, d, J = 8.2 Hz, 1‴-H).

Conversion of 2c to Licorice-Saponin A3 (2) A solution of **2c** (100 mg, 0.072 mmol) in 0.25% NaOMe–MeOH (1 ml) was stirred at room temperature for 30 min. After neutralization with Dowex 50W × 8 (H^+), the mixture was filtered to remove the resin. Removal of the solvent from the filtrate under reduced pressure gave a product (crude **2a**), which was dissolved in 0.2% K_2CO_3–50% aqueous MeOH (1 : 1, 2 ml) and the solution was stirred at room temperature for 2 h. The reaction mixture was neutralized with Dowex 1 × 2 (OH^-) and filtered to remove the resin. Evaporation of the solvent from the filtrate under reduced pressure yielded a residue, which was purified by column chromatography (silica gel 8 g, $CHCl_3$: MeOH : H_2O = 6 : 4 : 1) to afford licorice-saponin A3 (**2**, 57 mg, 62% from **2c**), identical with a natural specimen ($[\alpha]_D$ and spectral properties).

Diazomethane Methylation of Licorice-Saponin B2 (3) Giving 3a An ice-cooled solution of **3** (20 mg) in MeOH (3 ml) was treated with ethereal diazomethane (*ca.* 30 ml) until the yellow color presisted. The solution was left standing for 1 h, then the solvent was evaporated off under reduced pressure to give **3a** (21 mg).

3a: mp 171—172 °C (colorless needles from MeOH), $[\alpha]_D^{23}$ +51° (c = 0.7, MeOH). *Anal.* Calcd for $C_{45}H_{70}O_{15}\cdot H_2O$: 62.19; H, 8.35. Found: C, 62.21; H, 8.27. IR ν_{max}^{KBr} cm^{-1}: 3280, 1740, 1720, 1060. ^{1}H-NMR (500 MHz, pyridine-d_5, δ): 0.87, 0.92, 0.98, 1.12, 1.19, 1.24, 1.31 (all 3H, s), 3.70, 3.72, 3.82 (all 3H, s, $-OCH_3$ × 3), 4.94 (1H, d, J = 7.6 Hz, 1′-H), 5.33 (1H, d, J = 7.9 Hz, 1″-H), 5.38 (1H, br s, 12-H). ^{13}C-NMR (22.5 MHz, pyridine-d_5) δ_C: 51.7, 51.9, 52.0 ($-O\underline{C}H_3$) and other signals as given in Table I.

Methanolysis of 3a A solution of **3a** (10 mg) in 9% HCl–MeOH (0.5 ml) was heated under reflux for 3 h. After cooling, the reaction mixture was neutralized with Dowex 1 × 2 (OH^-) and the product was identified by TLC [benzene : MeOH = 5 : 2, $CHCl_3$: MeOH : H_2O = 7 : 3 : 1 (lower phase)] to contain methyl glucuronide. The reaction mixture was then filtered to remove the resin and the filtrate was evaporated under reduced pressure to yield a residue which was purified by column chromatography (silica gel 1 g, *n*-hexane : ethyl acetate = 1 : 1) to furnish 11-deoxoglycyrrhetic acid methyl ester (**6a**, 3 mg). **6a** thus obtained was identified by comparison of melting point, IR, and ^{1}H-NMR data (in pyridine-d_5) with those reported.[10]

6a: mp 244—246 °C (colorless needles from EtOH), $[\alpha]_D^{23}$ +75.4° (c = 0.6, $CHCl_3$). High-MS: Found: 470.3740; Calcd for $C_{31}H_{50}O_3$ (M^+): 470.3761. IR ν_{max}^{KBr} cm^{-1}: 3350, 1740, 1072. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.78, 1.13 (each 3H × 2, s), 0.93, 0.95, 0.99 (all 3H, s), 3.21 (1H, br dd, J = *ca.* 5, 9 Hz, 3-H), 3.68 (3H, s, $-OCH_3$), 5.27 (1H, dd, J = 3.6, 5.6 Hz, 11-H). EI-MS m/z (%): 470 (M^+, 3.3), 262 (100).

Clemmensen Reduction of Glycyrrhizin (1) Giving Licorice-Saponin B2 (3) A solution of glycyrrhizin (**1**) monoammonium salt (1 g) in 70% dioxane–water (5 ml) was first treated with zinc amalgam (2 g, prepared from zinc powder and mercuric chloride) and then 10% aqueous HCl (12 ml) was added dropwise over a period of 10 min. The reaction mixture was further stirred at 10 °C for 2 h and the zinc amalgam was removed by decantation. After dilution with ice-water (200 ml), the reaction mixture was extracted with *n*-butanol twice (60 ml each). The combined *n*-butanol extract was neutralized with Amberlite IRA 400 (OH^-) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure yielded a residue, which was purified by column chromatography (silica gel 30 g, $CHCl_3$: MeOH : H_2O = 6 : 4 : 1) to furnish licorice-saponin B2 (**3**, 0.75 g, 75%).

Diazomethane Methylation of Licorice-Saponin C2 (4) Giving the Trimethyl Ester (4a) A solution of **4** (20 mg) in MeOH (2 ml) was treated with ethereal diazomethane (*ca.* 15 ml). The reaction mixture was left standing for 1 h, then worked up as described above to give **4a** (21 mg).

4a: mp 174—176 °C (colorless fine prisms from MeOH), $[\alpha]_D^{23}$ −110° (c = 0.30, MeOH). *Anal.* Calcd for $C_{45}H_{68}O_{15}\cdot 2H_2O$: C, 61.07; H, 8.20. Found: C, 60.94; H, 8.18. UV λ_{max}^{MeOH} nm (ε): 242 (13000), 250 (14600), 259 (9300). IR ν_{max}^{KBr} cm^{-1}: 3400, 1720, 1640, 1080. ^{1}H-NMR (500 MHz, pyridine-d_5 + D_2O) δ: 0.78, 0.92, 1.06, 1.10, 1.12, 1.18, 1.32 (all 3H, s), 3.71, 3.74, 3.82 (all 3H, s, $-OCH_3$ × 3), 4.98 (1H, d, J = 7.3 Hz, 1′-H), 5.34 (1H, d, J = 7.3 Hz, 1″-H), 5.62 (1H, br d, J = *ca.* 10 Hz, 11-H), 6.41 (1H, br d, J = *ca.* 10 Hz, 12-H). ^{13}C-NMR (22.5 MHz, pyridine-d_5) δ_C: 51.8, 51.9, 52.0 ($-O\underline{C}H_3$) and other signals as given in Table I.

Methanolysis of 4a A solution of **4a** (10 mg) in 9% HCl–MeOH (0.5 ml) was heated under reflux for 3 h. After cooling, the reaction mixture was neutralized with Dowex 1 × 2(OH^-) and the product was identified by TLC [benzene : MeOH = 5 : 2, $CHCl_3$: MeOH : H_2O = 7 : 3 : 1 (lower phase)] to contain methyl glucuronide. The whole mixture was then filtered to remove the resin and a residue, obtained after usual work-up, was purified by column chromatography (silica gel 1 g, *n*-hexane : ethyl acetate = 1 : 1) to provide 3β-hydroxyoleana-11,13(18)-dien-30-oic acid methyl ester (**7a**, 3 mg) which was identified by comparing the melting point, IR, and ^{1}H-NMR data with reported values.[14]

7a: mp 263—264 °C (colorless needles from MeOH), $[\alpha]_D^{23}$ −40° (c = 0.2, $CHCl_3$). High-MS: Found: 468.3595; Calcd for $C_{31}H_{48}O_3$ (M^+): 468.3603. UV λ_{max}^{MeOH} nm (ε): 241 (12800), 250 (13500), 259 (8100). IR ν_{max}^{KBr} cm^{-1}: 3300—3000 (br), 1725, 1650, 1650, 1080. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.71, 0.78, 0.89, 0.96, 0.99, 1.07, 1.09 (all 3H, s), 3.23 (1H, br dd, J = *ca.* 5, 11 Hz, 3-H), 3.69 (3H, s, $-OCH_3$), 5.55 (1H, dd, J = 3.6, 9.8 Hz, 11-H), 6.35 (1H, br d, J = *ca.* 10 Hz, 12-H). EI-MS m/z (%): 468 (M^+, 100), 469 (M^+ + 1, 34).

$NaBH_4$ Reduction of 4a Followed by Complete Methylation and Methanolysis A solution of the trimethyl ester (**4a**, 5 mg) in MeOH (1 ml)

was treated with $NaBH_4$ (2 mg) and the mixture was stirred at room temperature (23 °C) for 1 h. The reaction mixture was neutralized with Dowex 50W × 8 (H^+) and the resin was removed by filtration. A residue, obtained after work-up as described above, was purified by column chromatography [silica gel 1 g, $CHCl_3$: MeOH : H_2O = 7 : 3 : 1 (lower phase)] to give a product (4.5 mg). The product was then dissolved in dimethyl sulfoxide (DMSO) (0.5 ml) and the solution was treated with a dimsyl carbanion solution (1 ml, prepared with NaH 100 mg and DMSO 2 ml). The mixture was stirred in the dark at room temperature (23 °C) for 1 h, then treated with methyl iodide (1 ml), and the whole was stirred for a further 2 h. The reaction mixture was poured into ice-water and the whole was extracted with ethyl acetate. The ethyl acetate extract was washed with saturated saline, then dried over $MgSO_4$ and filtered. Evaporation of the solvent under reduced pressure afforded a product (6.7 mg). Without further purification, the product was dissolved in 9% HCl–MeOH (1 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, $CHCl_3$: MeOH = 50 : 1) and GLC with methyl 2,3,4,6-tetra-*O*-methylglucopyranoside and methyl 3,4,6-tri-*O*-methylglucopyranoside. GLC conditions: 5% BDS 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: methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, 15 min 46 s, 18 min 10 s and methyl 3,4,6-tri-*O*-methylglucopyranoside, 5 min 30 s, 7 min 40 s.

$NaBH_4$ Reduction of Glycyrrhizin (1) Giving 9 A solution of **1** (1 g) in ethanol–water (1 : 1, 20 ml) was treated with $NaBH_4$ (2.0 g) and the whole mixture was heated at 90 °C under reflux for 1 h. After cooling, the reaction mixture was neutralized with Dowex 50W × 8 (H^+) and the resin was removed by filtration. Evaporation of the solvent under reduced pressure furnished **9** (950 mg, 95%).

9: mp 208—210 °C (colorless fine needles from MeOH), $[\alpha]_D^{23}$ +18.3° (c = 1.80, MeOH). High-resolution FAB-MS: Found 847.4070; Calcd for $C_{42}H_{64}O_{16}Na$ [$(M+Na)^+$]: 847.4092. IR ν_{max}^{KBr} cm^{-1}: 3700—3000 (br), 1717, 1627, 1035. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.87, 1.07, 1.13, 1.32, 1.39 (all 3H, s), 1.15 (3H × 2, s), 4.45 (1H, dd, J = 6.5, 6.5 Hz, 11α-H), 4.91 (1H, d, J = 7.6 Hz, 1′-H), 5.31 (1H, d, J = 7.6 Hz, 1″-H), 5.67 (1H, d, J = 6.5 Hz, 12-H). ^{13}C-NMR (22.5 MHz, pyridine-d_5) δ_C: 89.6 (C-3), 84.2 (C-11), 128.2 (C-12), 145.9 (C-13), 179.2 (C-30), 103.9 (C-1′), 105.4 (C-1″), 171.9, 172.3 (C-6′, C-6″). FAB-MS m/z (positive, %): 847 [$(M+Na)^+$, 2], 825 [$(M+H)^+$, 1.1], 437 [$(M-sugars+H)^+$, 100].

Conversion of 9 Giving 4a and 10a A solution of **9** (500 mg) in 2% aqueous HCl–dioxane (1 : 1, 10 ml) was stirred at room temperature (24 °C) for 2 h. After neutralization with Amberlite IRA-400 (OH^-), the mixture was filtered to remove the resin. Evaporation of the solvent under reduced pressure gave a product (410 mg), which was shown to be a mixture of two isomeric dienes by its ^{1}H-NMR spectrum. Since direct separation was unsuccessful, the product (300 mg) was treated with diazomethane and subjected to column chromatography (silica gel 10 g, $CHCl_3$: MeOH : H_2O = 6 : 4 : 1) to furnish a trimethyl ester of the 9,12-homodiene derivative (**10a**, 210 mg, 66% from **9**) and the trimethyl ester of the licorice-saponin C2 (**4a**, 52 mg, 16% from **9**), which was identical with the authentic compound described above (mixed up, IR, ^{1}H- and ^{13}C-NMR).

10a: mp 180 °C (colorless fine needles from MeOH), $[\alpha]_D^{23}$ +27.6° (c = 1.72, MeOH). High-resolution FAB-MS: Found: 871.4483; Calcd for $C_{45}H_{68}O_{15}Na$ [$(M+Na)^+$]: 871.4461. UV λ_{max}^{MeOH} nm (ε): 245 (13300), 251 (14800), 276 (7200). IR ν_{max}^{KBr} cm^{-1}: 3440, 1727, 1660, 1035. ^{1}H-NMR (500 MHz, pyridine-d_5 + D_2O, δ: 0.88, 1.07, 1.13, 1.14, 1.15, 1.17, 1.31 (all 3H, s), 3.70, 3.74, 3.85 (all 3H, s, $-OCH_3$ × 3), 4.93 (1H, d, J = 7.3 Hz, 1′-H), 5.30 5.31 (1H each, both d, J = 7.3 Hz, 11,12-H), 5.37 (1H, d, J = 7.6 Hz, 1″-H). ^{13}C-NMR (22.5 MHz, pyridine-d_5) δ_C: 89.1 (C-3), 154.9 (C-9), 115.7 (C-11), 125.5 (C-12), 146.0 (C-13), 178.3 (C-30), 104.6 (C-1′) 106.5 (C-1″), 169.8, 170.0 (C-6′, C-6″). FAB-MS m/z (positive, %): 871 [$(M+Na)^+$, 13], 433 [$(M-sugars+H)^+$, 100].

Selective Conversion of 9 Giving Licorice-Saponin C2 (4) A solution of **9** (500 mg) in dioxane–water (1 : 1, 10 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^-) and the resin was filtered off. A product, obtained by evaporation of the filtrate under reduced pressure, was purified by column chromatography (silica gel 20 g, $CHCl_3$: MeOH : H_2O = 6 : 4 : 1) to furnish **4** (420 mg, 82%), which was identical with the authentic compound on the basis of mixed melting point determination and comparison of IR, ^{1}H- and ^{13}C-NMR data. Under these reaction conditions, **10** was not detected as a reaction product.

Anodic Oxidation of 3a Followed by Acidic Treatment Giving 4a A solution of **3a** (100 mg) in MeOH (20 ml) was treated with sodium acetate (250 mg) and subjected to constant-current electrolysis (Pt anode, current 40 mA/cm^2, 2 h, 25 °C). After neutralization with Dowex 50W × 8 (H^+), the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure gave an oxidation product (103 mg), which was concluded to be a mixture of 11α-OAc and 11α-OCH_3 derivatives from an examination of the ^{1}H-NMR spectrum. Without separation, the product was dissolved in MeOH (9 ml), treated with 10% aqueous HCl (1 ml), and then stirred at room temperature (23 °C) for 1 h. The reaction mixture was poured into saturated saline and the whole was extracted with *n*-butanol. The *n*-butanol extract was successively washed with saturated aqueous $NaHCO_3$ and water, then the solvent was evaporated off under reduced pressure. The residue thus obtained was purified by reversed-phase column chromatography (μ-Bondapak C_{18} 5 g, MeOH : H_2O = 4 : 1) to furnish licorice-saponin C2 trimethyl ester (**4a**, 61 mg, 62%), which was identical with the authentic compound on the basis of mixed melting point determination and IR, ^{1}H- and ^{13}C-NMR spectral comparisons.

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