Saponin and Sapogenol. XLVIII.[1] On the Constituents of the Roots of *Glycyrrhiza uralensis* FISCHER from Northeastern China. (2). Licorice-saponins D3, E2, F3, G2, H2, J2, and K2

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Following the characterization of licorice-saponins A3 (2), B2 (3), and C2 (4), the chemical structures of licorice-saponins D3 (5), E2 (6), F3 (7), G2 (8), H2 (9), J2 (10), and K2 (11), seven of the ten oleanane-type triterpene oligoglycosides isolated from the air-dried roots of *Glycyrrhiza uralensis* FISCHER collected in the northeastern part of China, were investigated. On the basis of chemical and physicochemical evidence, the structures of licorice-saponins D3, E2, F3, G2, H2, J2, and K2 have been determined to be expressed as 3β-[α-L-rhamnopyranosyl(1→2)-β-D-glucuronopyranosyl(1→2)-β-D-glucuronopyranosyloxy]-22β-acetoxyolean-12-en-30-oic acid (5), 3-*O*-[β-D-glucuronopyranosyl(1→2)-β-D-glucuronopyranosyl]glabrolide (6), 3-*O*-[α-L-rhamnopyranosyl(1→2)-β-D-glucuronopyranosyl-(1→2)-β-D-glucuronopyranosyl]-11-deoxoglabrolide (7), 24-hydroxyglycyrrhizin (8), 3-*O*-[β-D-glucuronopyranosyl-(1→2)-β-D-glucuronopyranosyl]liquiritic acid (9), 24-hydroxy-11-deoxoglycyrrhizin (10), and 3β-[β-D-glucuronopyranosyl(1→2)-β-D-glucuronopyranosyloxy]-24-hydroxyoleana-11,13(18)-dien-30-oic acid (11), respectively.

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

In the previous paper,[1] we reported the isolation of ten licorice-saponins together with glycyrrhizin (**1**) and several known flavonoids, from the air-dried root of *Glycyrrhiza uralensis* FISCHER collected in the northeastern part of China, and we also described the elucidation of the chemical structures of three of them, namely licorice-saponins A3 (**2**), B2 (**3**), and C2 (**4**). As a continuation of that study, we now present a full account of the structure elucidation of the remaining seven licorice-saponins, *i.e.* licorice-saponins D3 (**5**), E2 (**6**), F3 (**7**), G2 (**8**), H2 (**9**), J2 (**10**), and K2 (**11**).[2]

Licorice-saponin D3 (5) Licorice-saponin D3 (**5**) was obtained as a white amorphous powder. It showed no absorption maximum in its ultraviolet (UV) spectrum. The infrared (IR) spectrum of **5** showed absorption bands ascribable to acetoxyl and carboxyl functions (1730, 1712 cm^{-1}) and strong broad absorption bands (3700—3200, 1065 cm^{-1}) suggestive of glycosidic structure. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **5** showed a signal due to an acetoxyl methyl group at δ 2.16 (3H, s) and three anomeric proton signals at δ 5.02 (d, J=7.4 Hz), 5.34 (d, J=7.6 Hz), and 6.30 (br s).

Methylation of licorice-saponin D3 (**5**) with ethereal diazomethane gave a trimethyl ester (**5a**). Methanolysis of **5a** with 9% hydrogen chloride–methanol liberated the triterpenoid aglycone methyl ester (**12a**) together with methyl D-glucuronide and methyl L-rhamnoside. The ^{1}H-NMR spectrum of **12a** showed two signals, one assignable to a hydroxy-bearing axial methine proton at δ 3.24 (dd, J=5.2, 11.5 Hz, 3α-H), and the other to an acetoxy-bearing equatorial methine proton at δ 4.61 (dd, J=2.8, 3.4 Hz, 22α-H), together with signals due to seven tertiary methyls, one acetoxyl group and one methoxycarbonyl group. The mass spectrum (MS) of **12a** gave the molecular ion peak at m/z 528 (M^+) together with the fragment ion peaks at m/z 320 (**i**) and m/z 260 (**ii**) deriving from the D/E ring and an ion peak at m/z 208 (**iii**) from the A/B ring, all of which were formed through the characteristic retro-Diels Alder fragmentation at the C ring in the olean-12-ene skeleton of **12a**. The ^{1}H-NMR spectrum of **12b**, prepared by ordinary acetylation of **12a** with acetic anhydride–pyridine, showed signals due to the two methine protons on acetoxy-bearing carbons at δ 4.51 (dd, J=4.3, 11.5 Hz, 3α-H) and 4.60 (dd, J=2.8, 3.0 Hz, 22α-H). In the MS of **12b**, in addition to the molecular ion peak at m/z 570, the prominent fragment ion peaks (**i**, **ii**) from the D/E ring and the ion peak m/z 250 (**iv**) from the A/B ring, were observed.

To figure out the structure chemically, the aglycone methyl ester (**12a**) was treated with 10% H_2SO_4–50% aqueous EtOH (1:1) under reflux to provide 11-deoxoglabrolide (**13**),[3] which possesses a 30,22β-lactone moiety. Consequently, it has become clear that the methoxycarbonyl group and the axial acetoxyl group in **12a** are located at the 20β and 22β positions, respectively. Based on the above-mentioned evidence, the structure of the aglycone methyl ester has been clarified as 22β-acetoxyolean-11-en-30-oic acid methyl ester (**12a**).

To shed light on the structure of the oligosaccharide moiety of licorice-saponin D3 (**5**), the trimethyl ester (**5a**) was first subjected to complete methylation[4] and subsequently to treatment with sodium borohydride. Methanolysis of the final product liberated methyl 3,4-di-*O*-methylglucopyranoside (**a**) and methyl 2,3,4-tri-*O*-methylrhamnopyranoside (**b**) in a 2:1 ratio. Detailed comparison of the ^{13}C-NMR data for **5a** with those for the methyl esters of licorice-saponin B2 (**3**)[1] and soyasaponin I,[5] has led us to assign the carbon signals of **5a** as given in Table I. Furthermore, the ^{13}C–^{1}H coupling constants, 171 Hz for the α-L-rhamnopyranosyl moiety and 160 Hz for the two β-D-glucuronopyranosyl moieties, which were observed at the anomeric carbon signals in the ^{13}C-NMR spectrum of **5a**, have made clear the anomeric configurations in the oligosaccharide part of licorice-saponin D3. Thus, the structure of licorice-saponin D3 has been determined to be 3β-[α-L-rhamnopyranosyl(1→2)-β-D-glucuronopyranosyl(1→2)-β-D-glucuronopyranosyloxy]-22β-acetoxy-

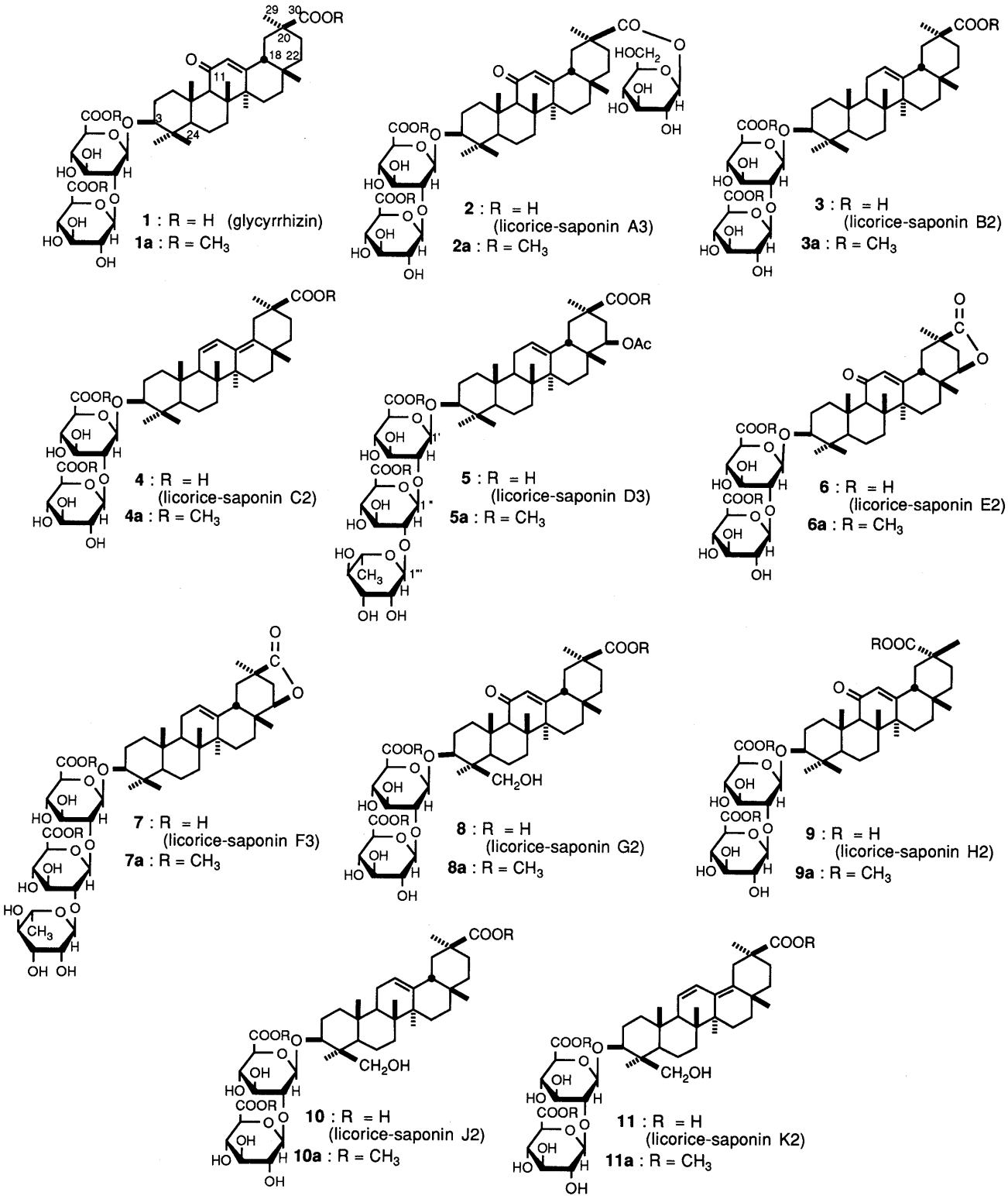

Fig. 1

olean-11-en-30-oic acid (**5**).

Licorice-saponin E2 (6) Licorice-saponin E2 (**6**), obtained as colorless needles of mp 218—219 °C, showed a UV absorption maximum at 250 nm ($\varepsilon = 12700$) which was ascribable to the conjugated enone chromophore. The IR spectrum of **6** showed absorption bands due to hydroxyl (3400—3000 cm^{-1}), δ-lactone (1780 cm^{-1}) and conjugated enone (1724, 1645 cm^{-1}) moieties. Methanolysis of **6** under reflux provided glabrolide (**14**)[6] and methyl glucuronide. The ^{1}H-NMR spectrum of **6** showed signals due to two anomeric protons at δ 5.02 (d, $J = 7.6$ Hz) and 5.38 (d, $J = 7.6$ Hz) which indicated β-anomeric configuration of the two glucuronide moieties.

Diazomethane methylation of **6** furnished the dimethyl ester (**6a**). Reduction of **6a** with sodium borohydride and subsequent complete methylation followed by methanolysis, liberated methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**c**) and methyl 3,4,6-tri-*O*-methylglucopyranoside (**d**) in a 1 : 1

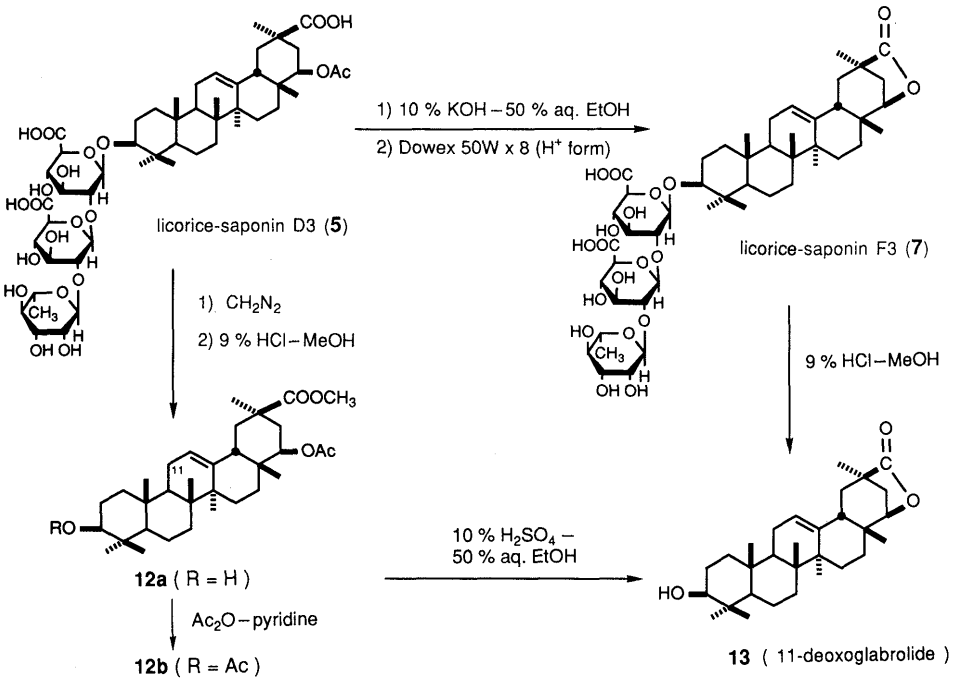

Fig. 2

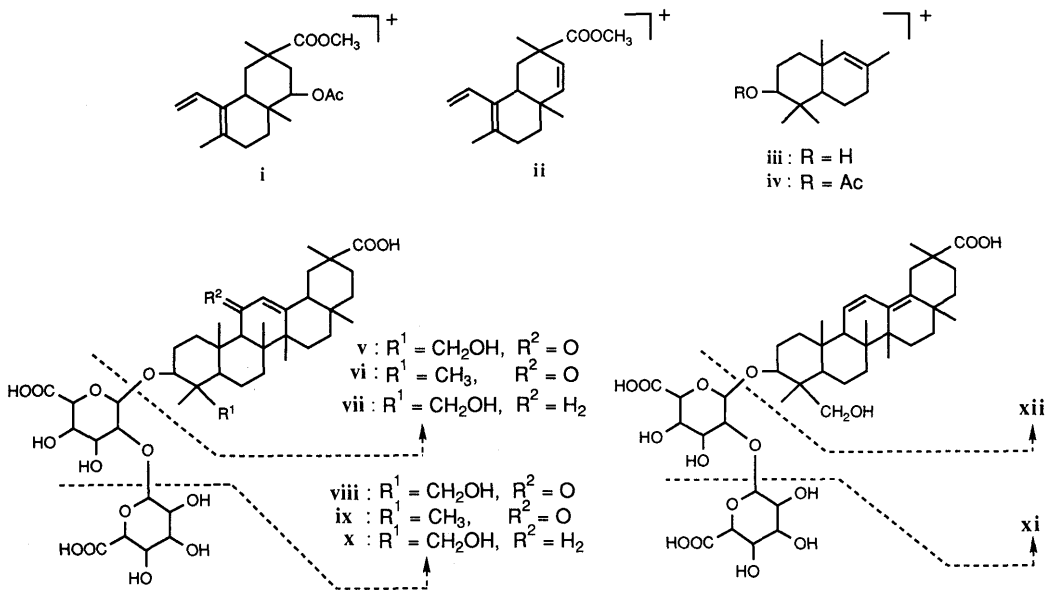

Fig. 3

ratio. Based on the above-mentioned evidence and a detailed comparison of the ^{13}C-NMR data for **6a** with those for licorice-saponin B2 methyl ester (**3a**),[1] the structure of licorice-saponin E2 has been determined as 3-*O*-[*β*-D-glucuronopyranosyl(1→2)-*β*-D-glucuronopyranosyl] glabrolide (**6**).

Licorice-saponin F3 (7) Licorice-saponin F3 (**7**) was obtained as colorless needles of mp 214—217 °C. The IR spectrum of **7** showed the presence of hydroxyl groups and a *γ*-lactone moiety. Methanolysis of **7** yielded 11-deoxoglabrolide (**13**)[3] together with methyl glucuronide and methyl rhamnoside.

The ^{1}H-NMR spectrum of **7** showed signals assignable to three anomeric protons at δ 4.96 (d, J=7.6 Hz), 5.68 (d, J=6.9 Hz), and 6.10 (br s). The carbon signals due to the sugar moieties in the ^{13}C-NMR spectrum of **7a**, which was prepared by diazomethane methylation of **7**, were superimposable on those in the spectrum of licorice-saponin D3 trimethyl ester (**5a**). Complete methylation of **7a** with dimsyl carbanion and subsequent sodium borohydride reduction followed by methanolysis, liberated two methyl glycosides (**a** and **b** in a 2 : 1 ratio), the same as those obtained from **5a** (*vide supra*). Consequently, **7a** was presumed to be a 30,22*β*-lactone analog in the sapogenol moiety of **5a**. Finally, the structure of licorice-saponin F3 (**7**) has been established by chemical derivation from above-described licorice-saponin D3 (**5**). Thus, alkaline hydrolysis of **5** with 10% potassium hydroxide and subsequent treatment of the product with acidic resin provided **7**, exclusively. Based on the accumulated evidence, the structure of licorice-saponin

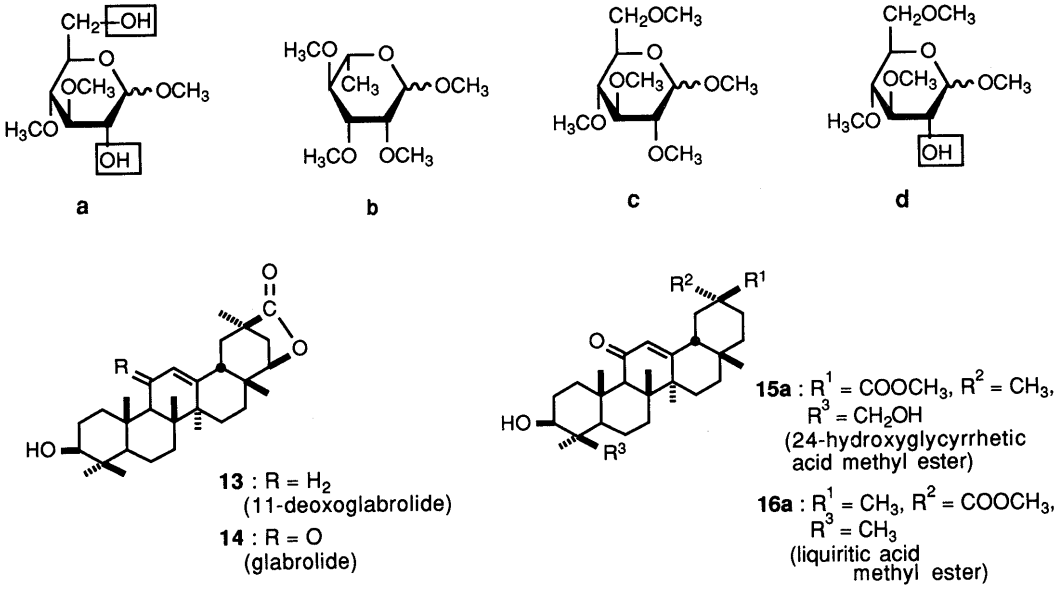

Fig. 4

TABLE I. ^{13}C-NMR Data for **1a**, **5a**, **6a**, **7a**, **8a**, **9a**, **10a**, and **11a** (δ_C at 22.5 MHz, Pyridine-d_5)

		1a	5a	6a	7a	8a	9a	10a	11a
Sapogenol moiety	C-3	89.2	89.9	89.0	89.9	89.9	89.2	89.8	89.8
	C-11	199.0	23.6	198.8	23.6	198.9	199.0	23.7	125.3
	C-12	128.7	122.2	129.6	124.8	128.6	128.6	122.2	126.4
	C-13	168.7	143.6	164.3	140.6	168.7	168.4	144.3	135.2
	C-18	44.5	44.0	44.8	44.1	44.2	44.0	44.4	135.2
	C-22	38.1	77.5[a]	84.0[b]	84.3	38.2	37.1	36.2	36.2
	C-24	16.5	16.4	16.4	16.3	63.1	16.2	62.8	62.4
	C-29	28.4	29.1	28.0	28.0	28.0	178.1	29.1	28.1
	C-30	176.5	177.2	179.3	180.1	176.5	19.3	177.0	178.2
3-*O*-*β*-D-Glucuronopyranosyl moiety	C-1′	104.5	104.7	104.6	104.7	104.1	104.9	104.0	104.0
	C-2′	84.0	79.1	83.8[b]	79.1	81.9	84.3	81.1	80.8
	C-3′	75.9[b]	76.2[b]	76.2[c]	76.3[b]	75.2	76.4[b]	76.3[b]	76.6
	C-4′	72.2	72.2[c]	72.2[a]	72.2[c]	72.2	72.5[c]	72.1	72.1
	C-5′	77.1	77.9[a]	77.2	77.9[a]	77.2	77.3[a]	77.4[c]	77.7[b]
	C-6′	169.5[c]	169.6[d]	169.7[d]	170.1[d]	169.6	170.1[d]	169.6	169.8
2′-*O*-*β*-D-Glucuronopyranosyl moiety	C-1″	106.3	102.4	106.2	102.4	105.5	106.8	104.7	104.5
	C-2″	76.2[b]	78.2	76.1	78.2	76.5[b]	76.6[b]	76.6[b]	76.6
	C-3″	77.1	76.7[b]	77.2	76.6[b]	76.8[b]	77.4[a]	77.0[c]	77.0[b]
	C-4″	72.4	72.9[c]	72.5[a]	72.8[c]	72.2	72.9[c]	72.1	72.1
	C-5″	77.1	77.5[a]	77.2	77.6[a]	77.2	77.6[a]	77.4[c]	77.4[b]
	C-6″	169.7[c]	169.8[d]	169.8[d]	169.8[d]	169.6	170.2[d]	169.6	169.8
2″-*O*-*α*-L-Rhamnopyranosyl moiety	C-1‴		101.6		101.6				
	C-2‴		71.9		71.8				
	C-3‴		72.9		72.8				
	C-4‴		73.9		73.9				
	C-5‴		69.2		69.1				
	C-6‴		18.5		18.5				

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

F3 has been elucidated as 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucuronopyranosyl(1→2)-*β*-D-glucuronopyranosyl]-11-deoxoglabrolide (**7**).

Licorice-saponin G2 (8) Licorice-saponin G2 (**8**), obtained as colorless prisms of mp 229—230 °C, was shown to possess a conjugated enone moiety and a carboxyl group by consideration of its UV and IR spectra. The positive fast atom bombardment MS (positive FAB-MS) of **8** showed the quasimolecular ion peaks at *m/z* 861 $(M+Na)^+$ and *m/z* 839 $(M+H)^+$ in addition to the fragment ion peaks at *m/z* 663 $(\mathbf{viii}+H)^+$, *m/z* 487 $(\mathbf{v}+H)^+$, and *m/z* 469 $(\mathbf{v}-H_2O+H)^+$, while the negative FAB-MS of **8** showed the quasimolecular ion peak at *m/z* 837 $(M-H)^-$ and the fragment ion peaks at *m/z* 661 $(\mathbf{viii}-H)^-$ and *m/z* 485 $(\mathbf{v}-H)^-$. In the ^{13}C-NMR spectrum of the trimethyl ester (**8a**), prepared by diazomethane methylation of **8**, the carbon signals were observed with very similar chemical shifts to those observed in the spectrum of glycyrrhizin trimethyl ester (**1a**) except for several signals ascribable to the 24-hydroxyl moiety.[7]

Methanolysis of **8a** yielded 24-hydroxyglycyrrhetic acid methyl ester (**15a**)[8] and methyl glucuronide, whereas

methanolysis of **8a**, after sodium borohydride reduction and subsequent complete methylation, liberated two methyl glycosides (**c** and **d**) in a 1 : 1 ratio. Consequently, the structure of licorice-saponin G2 has been clarified as 24-hydroxyglycyrrhizin (**8**).

Licorice-saponin H2 (9) Licorice-saponin H2 (**9**) was also isolated as colorless prisms of mp 209—210 °C. It showed a UV absorption maximum and IR absorption bands attributable to the conjugated enone moiety and the carboxyl function which were characteristically similar to those observed in the spectra of glycyrrhizin (**1**). The molecular formula ($C_{42}H_{62}O_{16}$) of **9**, clarified from the quasimolecular ion peak observed in the positive FAB-MS and by high-resolution MS (high-MS) measurement, was found to be identical with the molecular formula of **1**. The ^{1}H-NMR spectrum of **9** showed signals assignable to two β-anomeric protons at δ 4.97 (d, J = 7.6 Hz) and δ 5.35 (d, J = 7.6 Hz). Methanolysis of **9a**, which was obtained by diazomethane methylation of **9**, provided liquiritic acid methyl ester (**16a**)[9] and methyl glucuronide. On the other hand, methanolysis of **9a**, after sodium borohydride reduction and subsequent methylation, liberated two methyl glycosides (**c** and **d**) in a 1 : 1 ratio.

Based on the above-mentioned evidence and detailed comparison of the ^{13}C-NMR data for **9a** with those for **1a**, the structure of licorice-saponin H2 has been determined as 3-*O*-[β-D-glucuronopyranosyl(1→2)-β-D-glucuronopyranosyl]liquiritic acid (**9**).

Licorice-saponin J2 (10) The IR spectrum of licorice-saponin J2 (**10**), which was obtained as colorless prisms of mp 263—265 °C, showed absorption bands of hydroxyl and carboxyl groups. Here again, the molecular formula ($C_{42}H_{64}O_{16}$) of **10** was clarified from the quasimolecular ion peaks observed in the positive and negative FAB-MS and by high-MS measurement. Thus, the quasimolecular ion and fragment ion peaks [positive FAB-MS m/z: 847 $(M+Na)^+$, 825 $(M+H)^+$, 455 (**vii** $-H_2O+H)^+$; negative FAB-MS m/z: 823 $(M-H)^-$, 647 (**x** $-H)^-$, 471 (**vii** $-H)^-$] were observed in the FAB-MS of **10**.

Diazomethane methylation of **10** afforded the trimethyl ester (**10a**). In the ^{13}C-NMR spectrum of **10a**, the carbon signals assignable to the aglycone part were very similar to those observed in the spectra of licorice-saponin G2 trimethyl ester (**8a**) except for several signals attributable to the 12-en-11-one moiety. As for the carbon signals assignable to the oligosaccharide moiety, they were shown to be superimposable on those of **8a**. Based on this evidence, **10a** has been presumed to be an 11-deoxo-analog of **8a**. In order to verify this presumption, licorice-saponin G2 (**8**) was subjected to Clemmensen reduction under the same conditions as employed for the chemical derivation of licorice-saponin B2 (**3**) from glycyrrhizin (**1**). Licorice-saponin J2 (**10**) was obtained in good yield. Thus, the structure of licorice-saponin J2 has been determined as 24-hydroxy-11-deoxoglycyrrhizin (**10**).

Licorice-saponin K2 (11) Licorice-saponin K2 (**11**) was also obtained as colorless prisms of mp 207—209 °C. The UV spectrum of **11** showed the presence of a heteroannular diene chromophore by a characteristic triplet with maxima at 241 nm (ε = 13000), 249 (ε = 15000), and 259 (ε = 9200), while the IR spectrum showed hydroxyl, diene, and carboxyl absorption bands. These physicochemical properties of **11** led us to presume a resemblance of the structure of **11** to that of licorice-saponin C2 (**4**).[1] However, **11** was more polar than **4** as judged from the behavior of both compounds on thin-layer chromatography (TLC).

The molecular formula ($C_{42}H_{62}O_{16}$) of licorice-saponin K2 (**11**) was determined from its positive and negative FAB-MS data and by high-MS measurement. Thus, in the FAB-MS of **11**, the quasimolecular ion and fragment ion peaks [positive FAB-MS m/z: 845 $(M+Na)^+$, 823 $(M+H)^+$, and 453 (**xii** $-H_2O+H)^+$; negative FAB-MS m/z: 821 $(M-H)^-$, 645 (**xi** $-H)^-$, and 469 (**xii** $-H)^-$] were observed. Detailed comparison of the ^{13}C-NMR data for the trimethyl ester **11a**, which was prepared by diazomethane methylation of **11**, with those for licorice-saponin C2 trimethyl ester (**4a**)[1] has led us to presume the presence of a heteroannular 11,13-diene moiety in licorice-

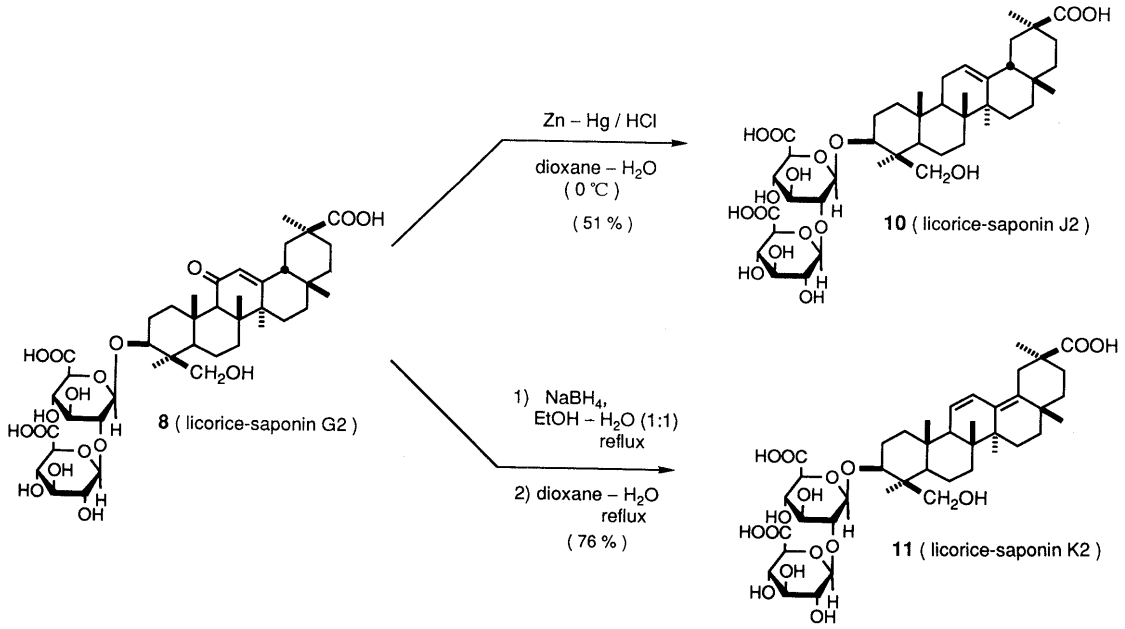

Fig. 5

saponin K2 (**11**).

In our previous paper,[1] we reported a facile chemical conversion method starting from olean-12-ene and olean-12-en-11-one type triterpene oligoglycosides to oleana-11,13(18)-diene type triterpene oligoglycosides. In order to corroborate chemically the structure of licorice-saponin K2 (**11**), this chemical conversion method was applied to licorice-saponin G2 (**8**) to provide **11**. Thus, **8** was treated with the sodium borohydride under ice-cooling and the resulting product was further treated with dioxane–H_2O under reflux to furnish **11** in 76% yield.

Based on the accumulated evidence, the structure of licorice-saponin K2 has been determined as 3β-[β-D-glucuronopyranosyl(1→2)-β-D-glucuronopyranosyloxy]-24-hydroxyoleana-11,13(18)-dien-30-oic acid (**11**).

Experimental

The instruments used for obtaining physical data and experimental conditions for chromatography were the same as described in our previous paper.[1,5b]

Isolation of Licorice-saponins D3 (5), E2 (6), F3 (7), G2 (8), H2 (9), J2 (10), and K2 (11) Isolation procedures for licorice-saponins D3 (**5**), E2 (**6**), F3 (**7**), G2 (**8**), H2 (**9**), J2 (**10**), and K2 (**11**) were as described in our previous paper.[1]

Licorice-saponin D3 (**5**): White amorphous powder, $[\alpha]_D^{20}$ −5.0° (c=0.15, MeOH). *Anal.* Calcd for $C_{50}H_{76}O_{21}\cdot 3H_2O$: C, 56.27; H, 7.74. Found: C, 56.16; H, 7.68. IR ν_{max}^{KBr} cm^{-1}: 3700—3200 (br), 2940, 1730, 1712, 1410, 1065. ^{1}H-NMR (500 MHz, pyridine-d_5+D_2O) δ: 0.89, 0.91, 0.99, 1.13, 1.21, 1.28, 1.38 (all 3H, s), 1.79 (3H, d, J=5.7 Hz, rhamnosyl methyl), 2.16 (3H, s, acetyl methyl), 3.28 (1H, dd, J=4.2, 11.0 Hz, 3α-H), 4.59 (1H, dd, J=2.6, 3.4 Hz, 22α-H), 5.02 (1H, d, J=7.4 Hz, 1′-H), 5.34 (1H, d, J=7.6 Hz, 1″-H), 5.44 (1H, br s, 12-H), 6.30 (1H, br s, 1‴-H).

Licorice-saponin E2 (**6**): mp 218—219 °C (colorless needles from MeOH), $[\alpha]_D^{20}$ +68.0° (c=0.2, MeOH). *Anal.* Calcd for $C_{42}H_{60}O_{16}\cdot 2H_2O$: C, 58.87; H, 7.52. Found: C, 58.62; H, 7.50. UV λ_{max}^{MeOH} nm (ε): 250 (12700). IR ν_{max}^{KBr} cm^{-1}: 3400—3000 (br), 2929, 1780, 1724, 1645, 1385, 1010. ^{1}H-NMR (500 MHz, pyridine-d_5+D_2O, 40 °C) δ: 0.79, 1.06, 1.21, 1.22, 1.34, 1.40, 1.42 (all 3H, s), 3.02 (1H, br d, J=*ca.* 12 Hz, 18-H), 3.35 (1H, dd, J=4.4, 11.2 Hz, 3α-H), 5.02 (1H, d, J=7.6 Hz, 1′-H), 5.38 (1H, d, J=7.6 Hz, 1″-H), 5.94 (1H, s, 12-H).

Licorice-saponin F3 (**7**): mp 214—217 °C (colorless needles from MeOH), $[\alpha]_D^{20}$ −20.0° (c=0.1, MeOH). *Anal.* Calcd for $C_{48}H_{72}O_{19}\cdot 5H_2O$: C, 55.27; H, 7.86. Found: C, 54.97; H, 7.81. IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (br), 2940, 1760, 1720, 1456, 1340. ^{1}H-NMR (500 MHz, pyridine-d_5+D_2O, 40 °C) δ: 0.71, 0.75, 1.11, 1.14 (all 3H, s), 1.26 (3H×2, s), 1.55 (3H, d, J=6.4 Hz, rhamnosyl methyl), 3.27 (1H, dd, J=4.4, 10.8 Hz, 3α-H), 4.96 (1H, d, J=7.6 Hz, 1′-H), 5.40 (1H, br s, 12-H), 5.68 (1H, d, J=6.9 Hz, 1″-H), 6.10 (1H, br s, 1‴-H).

Licorice-saponin G2 (**8**): mp 229—230 °C (colorless fine prisms from MeOH), $[\alpha]_D^{20}$ +34.0° (c=0.12, MeOH). High-resolution FAB-MS (positive): Calcd for $C_{42}H_{62}NaO_{17}$ $(M+Na)^+$: 861.3886. Found: 861.3848. UV λ_{max}^{MeOH} nm (ε): 249 (10560). IR ν_{max}^{KBr} cm^{-1}: 3500—3000 (br), 2910, 1720, 1648, 1385, 1040. ^{1}H-NMR (500 MHz, pyridine-d_5+D_2O, 40 °C) δ: 0.78, 1.05, 1.20, 1.34, 1.43, 1.48 (all 3H, s), 2.99 (1H, br d, J=*ca.* 14 Hz, 18-H), 3.52 (1H, dd, J=4.8, 11.0 Hz, 3α-H), 4.58, 4.68 (each 1H, both d, J=10.6 Hz, 24-H_2), 5.64 (1H, d, J=7.0 Hz, 1″-H), 5.94 (1H, s, 12-H). The anomeric proton (1′-H) signal overlapped with the water proton signal. FAB-MS m/z: 861 $[(M+Na)^+]$, 839 $[(M+H)^+]$, 663 $[(\mathbf{viii}+H)^+]$, 487 $[(\mathbf{v}+H)^+]$, 469 $[(\mathbf{v}-H_2O+H)^+]$ (positive); 837 $[(M-H)^-]$, 661 $[(\mathbf{viii}-H)^-]$, 485 $[(\mathbf{v}-H)^-]$ (negative).

Licorice-saponin H2 (**9**): mp 209—210 °C (colorless fine prisms from MeOH), $[\alpha]_D^{20}$ +31.3° (c=0.21, MeOH). High-resolution FAB-MS (positive): Calcd for $C_{42}H_{62}NaO_{16}$ $(M+Na)^+$: 845.3934. Found: 845.3957. UV λ_{max}^{MeOH} nm (ε): 248 (10650). IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (br), 2920, 1725, 1645, 1386, 1200, 1040. ^{1}H-NMR (500 MHz, pyridine-d_5+D_2O, 40 °C) δ: 0.87, 1.06, 1.19, 1.20, 1.35, 1.36, 1.38 (all 3H, s), 3.00 (1H, br d, J=*ca.* 13 Hz, 18-H), 3.35 (1H, dd, J=4.0, 10.2 Hz, 3α-H), 4.97 (1H, d, J=7.6 Hz, 1′-H), 5.35 (1H, d, J=7.6 Hz, 1″-H), 5.79 (1H, br s, 12-H). FAB-MS m/z: 845 $[(M+Na)^+]$, 823 $[(M+H)^+]$, 647 $[(\mathbf{ix}+H)^+]$, 471 $[(\mathbf{vi}+H)^+]$, 453 $[(\mathbf{vi}-H_2O+H)^+]$ (positive); 821 $[(M-H)^-]$, 645 $[(\mathbf{ix}-H)^-]$, 469 $[(\mathbf{vi}-H)^-]$ (negative).

Licorice-saponin J2 (**10**): mp 263—265 °C (colorless fine prisms from MeOH), $[\alpha]_D^{23}$ +21.0° (c=0.18, MeOH). High-resolution FAB-MS (positive): Calcd for $C_{42}H_{64}NaO_{16}$ $(M+Na)^+$: 847.4092. Found: 847.4087. IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (br), 2930, 1720, 1648, 1405, 1385, 1040. ^{1}H-NMR (500 MHz, pyridine-d_5, 40 °C) δ: 0.82, 0.84, 0.91, 1.18, 1.24, 1.45 (all 3H, s), 3.45 (1H, dd, J=4.3, 9.8 Hz, 3α-H), 4.50, 4.56 (each 1H, both d, J=9.0 Hz, 24-H_2), 5.00 (1H, d, J=7.6 Hz, 1′-H), 5.35 (1H, br s, 12-H), 5.65 (1H, d, J=7.0 Hz, 1″-H). FAB-MS m/z: 847 $[(M+Na)^+]$, 825 $[(M+H)^+]$, 455 $[(\mathbf{vii}-H_2O+H)^+]$ (positive); 823 $[(M-H)^-]$, 647 $[(\mathbf{x}-H)^-]$, 471 $[(\mathbf{vii}-H)^-]$ (negative).

Licorice-saponin K2 (**11**): mp 207—209 °C (colorless fine prisms from MeOH), $[\alpha]_D^{25}$ +28.0° (c=0.20, MeOH). High-resolution FAB-MS (positive): Calcd for $C_{42}H_{62}NaO_{16}$ $(M+Na)^+$: 845.3934. Found: 845.3945. UV λ_{max}^{MeOH} nm (ε): 241 (13000), 249 (15000), 259 (9200). IR ν_{max}^{KBr} cm^{-1}: 3500—3100 (br), 2928, 1690, 1629, 1395, 1050. ^{1}H-NMR (500 MHz, pyridine-d_5, 40 °C) δ: 0.72, 0.88, 1.07, 1.33, 1.37, 1.45, 1.47 (all 3H, s), 3.50 (1H, dd, J=4.2, 9.6 Hz, 3α-H), 4.48, 4.58 (each 1H, both d, J=9.8 Hz, 24-H_2), 5.04 (1H, d, J=7.6 Hz, 1′-H), 5.54 (1H, br d, J=*ca.* 12 Hz, 11-H), 5.64 (1H, d, J=6.9 Hz, 1″-H), 6.52 (1H, br d, J=*ca.* 12 Hz, 12-H). FAB-MS m/z: 845 $[(M+Na)^+]$, 823 $[(M+H)^+]$, 453 $[(\mathbf{xii}-H_2O+H)^+]$ (positive); 821 $[(M-H)^-]$, 645 $[(\mathbf{xi}-H)^-]$, 469 $[(\mathbf{xii}-H)^-]$ (negative).

Diazomethane Methylation of Licorice-saponin D3 (5) An ice-cooled solution of **5** (20 mg) in MeOH (2 ml) was treated with ethereal diazomethane (*ca.* 15 ml) until the yellow color persisted. The solution was left standing for 1 h, then the solvent was removed under reduced pressure to furnish the trimethyl ester (**5a**, 21 mg).

5a: mp 221—223 °C (colorless fine needles from MeOH), $[\alpha]_D^{25}$ −5.0° (c=0.10, MeOH). *Anal.* Calcd for $C_{53}H_{82}O_{21}\cdot 3H_2O$: C, 57.38; H, 7.99. Found: C, 57.27; H, 7.98. IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (br), 2950, 1733, 1440, 1372, 1243. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.90, 0.93, 0.98, 1.16, 1.20, 1.29, 1.39 (all 3H, s), 1.81 (3H, d, J=5.8 Hz, rhamnosyl methyl), 2.10 (3H, s, acetyl methyl), 3.28 (1H, dd, J=4.2, 11.0 Hz, 3α-H), 3.77, 3.78, 3.81 (all 3H, s, OCH_3×3), 4.57 (1H, dd, J=2.8, 3.4 Hz, 22α-H), 5.01 (1H, d, J=7.3 Hz, 1′-H), 5.49 (1H, br s, 12-H), 5.76 (1H, d, J=7.6 Hz, 1″-H), 6.39 (1H, br s, 1‴-H). ^{13}C-NMR (22.5 MHz, pyridine-d_5) δ_C: 170.1 (acetyl carbonyl), 21.8 (acetyl methyl), 51.5×2, 51.6 (OCH_3×3), and other signals as given in Table I; J_{C-H} value (δ): 171 Hz (101.6, C-1‴), 160 Hz (102.4, C-1″), 160 Hz (104.7, C-1′).

Methanolysis of 5a A solution of **5a** (25 mg) in 9% HCl–dry MeOH (2 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-2 (OH^- form) and the resin was removed by filtration. After removal of the solvent from the filtrate, the product was purified by column chromatography [SiO_2 2 g, *n*-hexane–AcOEt (2:1), $CHCl_3$–MeOH–H_2O (7:3:1, lower phase)] to furnish **12a** (11 mg), methyl glucuronide (8 mg), and methyl rhamnoside (6 mg).

Methyl glucuronide and methyl rhamnoside, thus obtained from **5a**, were identified respectively by co-TLC [developing with $CHCl_3$–MeOH–H_2O (7:3:1, lower phase), aqueous saturated 1-BuOH, and benzene–MeOH (5:2)] with authentic samples prepared by methanolysis of D-glucuronic acid and L-rhamnose.

Furthermore, methyl glucuronide (8 mg) obtained from **5a** was dissolved in MeOH (1 ml) and the solution was treated with $NaBH_4$ (3 mg). The whole mixture was stirred at room temperature (23 °C) for 1 h and then neutralized with Dowex 50W×8 (H^+ form). After removal of the solvent from the filtrate, 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 yielded D-glucose (4.7 mg), $[\alpha]_D^{24}$ +42° (c=0.28, 24 h after dissolving in H_2O). Methyl rhamnoside (6 mg) obtained from **5a** 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-rhamnose (5 mg), $[\alpha]_D^{24}$ +8.0° (c=0.50, 24 h after dissolving in H_2O).

12a: mp 232—234 °C (colorless fine needles from MeOH), $[\alpha]_D^{23}$ −68° (c=0.20, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{33}H_{52}O_5$ (M^+): 528.3825. Found: 528.3831. IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (br), 2940, 1760, 1720, 1460, 1384, 1372, 1025. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.79, 0.96, 0.99, 1.01, 1.15, 1.18, 1.27 (all 3H, s), 1.98 (3H, s, acetyl methyl), 3.24 (1H, dd, J=5.2, 11.5 Hz, 3α-H), 3.68 (3H, s, OCH_3), 4.61 (1H, dd, J=2.8, 3.4 Hz, 22α-H), 5.40 (1H, t-like, J=*ca.* 3.7 Hz, 12-H). EI-MS m/z (%): 528 (M^+, 5), 468 (M^+ − AcOH, 100), 320 (**i**, 8.2), 260 (**ii**, 45.3), 208 (**iii**, 3.4).

Acetylation of 12a An ice-cooled solution of **12a** (3 mg) in pyridine (1 ml) was treated with Ac_2O (0.5 ml) and the whole mixture was left standing at room temperature (22 °C) for 3 h. The reaction mixture was

poured into ice-water and the whole was extracted with AcOEt. Work-up of the extract in the usual manner gave **12b** (3 mg).

12b: mp 201—203 °C (colorless fine needles from MeOH), $[\alpha]_D^{23}$ +68° (c=0.20, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{35}H_{54}O_5$ (M^+): 570.3918. Found: 570.3893. IR ν_{max}^{KBr} cm^{-1}: 2940, 1764, 1758, 1720, 1460, 1384, 1370, 1025. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.79, 0.88, 0.89, 0.98, 0.99, 1.18, 1.26 (all 3H, s), 1.98, 2.06 (each 3H, both s, acetyl methyls), 3.68 (3H, s, OCH_3), 4.51 (1H, dd, J=4.3, 11.5 Hz, 3α-H), 4.60 (1H, dd, J=2.8, 3.0 Hz, 22α-H), 5.40 (1H, t-like, J=*ca.* 3.7 Hz, 12-H). EI-MS m/z (%): 570 (M^+, 1.5), 320 (**i**, 18.8), 260 (**ii**, 100), 250 (**iv**, 4.2).

Acidic Treatment of 12a A solution of **12a** (5 mg) in 10% H_2SO_4–50% aqueous EtOH (1 : 1, 4 ml) was heated under reflux for 4 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with aqueous saturated $NaHCO_3$ and saturated saline, then dried over $MgSO_4$. After removal of the desiccant by filtration, the filtrate was evaporated under reduced pressure to give **13**, which was shown to be identical with 11-deoxoglabrolide by comparison of melting point, IR and ^{1}H-NMR data (in pyridine-d_5) with reported values.[3]

13: mp 271—274 °C (colorless fine needles from isopropanol–EtOH), $[\alpha]_D^{24}$ +57° (c=0.20, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{30}H_{46}O_3$ (M^+): 454.3443. Found: 454.3435. IR ν_{max}^{KBr} cm^{-1}: 3400—3100 (br), 2900, 1760, 1720, 1460, 1384, 1370, 1025. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.79, 0.97, 1.00, 1.16 (all 3H, s), 0.94, 1.16 (each 3H × 2, both s), 3.19 (1H, dd, J=5.4, 11.0 Hz, 3α-H), 4.15 (1H, d, J=5.2 Hz, 22α-H), 5.27 (1H, t-like, J=*ca.* 3.6 Hz, 12-H). EI-MS m/z (%): 454 (M^+, 0.9), 436 [$(M-H_2O)^+$, 1.2], 246 (100), 208 (11.8).

Complete Methylation of 5a Followed by $NaBH_4$ Reduction and Methanolysis A solution of **5a** (5 mg) in dimethyl sulfoxide (DMSO) 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 CH_3I (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 AcOEt. The AcOEt extract was washed with saturated saline, then dried over $MgSO_4$ and filtered. Evaporation of the solvent under reduced pressure afforded a product (10 mg), which was dissolved in MeOH (2 ml). The solution was treated with $NaBH_4$ (3 mg) and the mixture was stirred at room temperature (24 °C) for 2 h. The reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and filtered. Removal of the solvent from the filtrate under reduced pressure gave a product, which was dissolved in 9% HCl–dry MeOH (2 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 whole was filtered to remove the inorganic material. After removal of the solvent from the filtrate, the products were identified by TLC [benzene–acetone (2 : 1), $CHCl_3$–MeOH (50 : 1)] and GLC with methyl 3,4-di-*O*-methylglucopyranoside (**a**) and methyl 2,3,4-tri-*O*-methylrhamnopyranoside (**b**). The composition of these two methyl glycosides was determined from the GLC peak areas. GLC analysis: conditions i) 5% butane disuccinate (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: **a** 38 min 5 s, 46 min 4 s; **b** 1 min 25 s.

Methanolysis of Licorice-saponin E2 (6) A solution of **6** (4 mg) in 9% HCl–dry MeOH was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Dowex 1 × 2 (OH^- form) and the product was identified by co-TLC [1-BuOH–AcOEt–H_2O (4 : 1 : 5, upper phase), $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower phase)] with authentic methyl glucuronide. After removal of the solvent from the filtrate, the residue was subjected to column chromatography [SiO_2 500 g, *n*-hexane–AcOEt (1 : 1)] to furnish **14** (3 mg), which was identified by comparison of its melting point, $[\alpha]_D$, and IR data with reported values.[6]

14: mp 263—264 °C (colorless needles from MeOH), $[\alpha]_D^{24}$ −40° (c=0.20, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{31}H_{48}O_3$ (M^+): 468.3603. Found: 468.3595. UV λ_{max}^{MeOH} nm (ε): 250 (12700). IR ν_{max}^{KBr} cm^{-1}: 3400—3000 (br), 2910, 1725, 1650, 1380, 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), 2.92 (1H, br d, J=*ca.* 12 Hz, 18-H), 3.23 (1H, br d, J=5.4, 11.2 Hz, 3α-H), 4.21 (1H, d, J=4.2 Hz, 22α-H), 5.55 (1H, br s, 12-H). EI-MS m/z (%): 468 (M^+, 100), 469 (M^+ + 1, 34).

Diazomethane Methylation of Licorice-saponin E2 (6) An ice-cooled solution of **6** (25 mg) in MeOH (2 ml) was treated with ethereal diazomethane (*ca.* 15 ml) until the yellow color persisted. The reaction mixture was left standing for 1 h and then evaporated under reduced pressure to give the dimethyl ester (**6a**, 25 mg).

6a: mp 232—234 °C (colorless fine needles from MeOH), $[\alpha]_D^{24}$ +65° (c=0.30, MeOH). *Anal.* Calcd for $C_{46}H_{68}O_{16}\cdot 3H_2O$: C, 59.33; H, 8.01. Found: C, 59.13; H, 8.00. UV λ_{max}^{MeOH} nm (ε): 250 (11000). IR ν_{max}^{KBr} cm^{-1}: 3500—3100 (br), 2920, 1733, 1440, 1372, 1243. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.80, 1.11, 1.12, 1.27, 1.31, 1.34, 1.44 (all 3H, s), 3.04 (1H, br d, J=*ca.* 12 Hz, 18-H), 3.32 (1H, dd, J=4.6, 11.3 Hz, 3α-H), 3.75, 3.85 (each 3H, both s, OCH_3 × 2), 4.92 (1H, d, J=7.6 Hz, 1′-H), 5.20 (1H, d, J=8.2 Hz, 1″-H), 5.97 (1H, br s, 12-H). ^{13}C-NMR (22.5 MHz, pyridine-d_5) δ_C: 51.5, 51.6 (OCH_3 × 2), and other signals as given in Table I.

$NaBH_4$ Reduction of 6a Followed by Complete Methylation and Methanolysis A solution of **6a** (5 mg) in MeOH (2 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^+ form) and the resin was removed by filtration. A residue, obtained after work-up as described above, was purified by column chromatography [SiO_2 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 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 CH_3I (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 AcOEt. The AcOEt extract was worked up as described above to afford a product (6.8 mg). Without further purification, this product was dissolved in 9% HCl–dry 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 co-TLC [benzene–acetone (2 : 1), $CHCl_3$–MeOH (50 : 1)] and GLC with methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**c**) and methyl 3,4,6-tri-*O*-methylglucopyranoside (**d**). The ratio of these two methyl glycosides was determined from the GLC peak areas. GLC analysis: condition i), t_R: **c**, 15 min 16 s, 18 min 10 s; **d**, 5 min 30 s, 7 min 40 s.

Methanolysis of Licorice-saponin F3 (7) A solution of **7** (10 mg) in 9% HCl–dry MeOH (1.0 ml) was heated under reflux for 3 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 powder and filtered. The product in the filtrate was identified by TLC [1-BuOH–AcOEt–H_2O (4 : 1 : 5, upper phase), $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower phase)] with authentic methyl glucuronide. After removal of the solvent from the filtrate, the product was purified by column chromatography [SiO_2 1 g, *n*-hexane–AcOEt (1 : 1)] to furnish 11-deoxoglabrolide (**13**, 3 mg). Compound **13** thus obtained was confirmed to be identical with an authentic sample, which was prepared by acidic treatment of **12a**, by mixed melting point determination and IR (KBr) and ^{1}H-NMR ($CDCl_3$) comparisons.

Diazomethane Methylation of Licorice-saponin F3 (7) An ice-cooled solution of **7** (20 mg) in MeOH (2 ml) was treated with ethereal diazomethane (*ca.* 15 ml) until the yellow color persisted. The solution was left standing for 1 h, then the solvent was removed under reduced pressure to furnish the dimethyl ester (**7a**, 22 mg).

7a: mp 207—209 °C (colorless fine needles from MeOH), $[\alpha]_D^{23}$ −14° (c=0.30, MeOH). High resolution FAB-MS (positive): Calcd for $C_{50}H_{76}NaO_{19}$: 1003.4882. Found: 1003.4901. IR ν_{max}^{KBr} cm^{-1}: 3500—3100 (br), 2949, 1745, 1440, 1384, 1221. ^{1}H-NMR (500 MHz, pyridine-d_5 + D_2O)δ: 0.78, 0.79, 0.92, 1.06, 1.14, 1.15, 1.28 (all 3H, s), 1.70 (3H, d, J=5.8 Hz, rhamnosyl methyl), 3.21 (1H, dd, J=4.0, 11.0 Hz, 3α-H), 3.68, 3.72 (each 3H, both s, OCH_3 × 2), 4.92 (1H, d, J=7.2 Hz, 1′-H), 5.44 (1H, br s, 12-H), 5.67 (1H, d, J=7.3 Hz, 1″-H), 6.20 (1H, br s, 1‴-H). ^{13}C-NMR (22.5 MHz, pyridine-d_5) δ_C: 51.6, 51.7 (OCH_3 × 2), and other signals as given in Table I. FAB-MS m/z: (positive) 1003 [$(M+Na)^+$]; (+Li) 987 [$(M+Li)^+$]; (negative) 979 [$(M-H)^-$], 965 [$(M-CH_3)^-$], 833 [(M − rhamnosyl moiety)$^-$].

Complete Methylation of 7a Followed by $NaBH_4$ Reduction and Methanolysis A solution of **7a** (5 mg) in DMSO (1 ml) 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 and worked up as described above for the methylation analysis of **5a**. The product (10 mg) was dissolved in MeOH (2 ml) and the solution was treated with $NaBH_4$ (3 mg). The whole mixture was stirred at room temperature (24 °C) for 2 h and the reaction mixture was neutralized with Dowex 50W × 8 (H^+ form), then filtered. Evaporation of the solvent from the filtrate gave a product, which was dissolved in 9% HCl–dry MeOH (2 ml). This 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 co-TLC and GLC (under the same conditions as described above for the methylation analysis of **5a**) with methyl glycosides **a** and **b**.

Alkaline Hydrolysis of Licorice-saponin D3 (5) Followed by Acidic

Treatment Giving Licorice-saponin F3 (7) A solution of **5** (20 mg) in MeOH (5 ml) was treated with 10% KOH (5 ml) and the whole was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure furnished a product which was purified by column chromatography [SiO_2 5 g, $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower phase)] to give **7** (12 mg). Compound **7** thus obtained was concluded to be identical with authentic licorice-saponin F3 on the basis of mixed melting point determination and comparison of $[\alpha]_D$, IR (KBr), and ^{1}H-NMR (pyridine-d_5) data.

Diazomethane Methylation of Licorice-saponin G2 (8) An ice-cooled solution of **8** (30 mg) in MeOH (2 ml) was treated with ethereal diazomethane solution (*ca.* 15 ml) until the yellow color persisted. The reaction mixture was left standing for 1 h and then the solvent was evaporated under reduced pressure to furnish the trimethyl ester (**8a**, 30 mg).

8a: mp 176—178 °C (colorless fine needles from MeOH), $[\alpha]_D^{24}$ +36° (c=0.15, MeOH). High-resolution FAB-MS (positive): Calcd for $C_{45}H_{68}NaO_{17}$: 903.4354. Found: 903.4296. UV λ_{max}^{MeOH} nm (ε): 248 (9460). IR ν_{max}^{KBr} cm^{-1}: 3450—3100 (br), 2897, 1720, 1648, 1380, 1007. ^{1}H-NMR (500 MHz, pyridine-d_5 + D_2O) δ: 0.77, 1.06, 1.20, 1.22, 1.41, 1.46 (all 3H, s), 2.99 (1H, br d, J=*ca.* 14 Hz, 18-H), 3.50 (1H, dd, J=5.0, 11.6 Hz, 3α-H), 3.75, 3.79, 3.87 (all 3H, s, OCH_3 × 3), 4.47, 4.53 (each 1H, both d, J=10.2 Hz, 24-H_2), 4.94 (1H, d, J=7.9 Hz, 1′-H), 5.55 (1H, d, J=7.6 Hz, 1″-H), 5.86 (1H, br s, 12-H). ^{13}C-NMR (22.5 MHz, pyridine-d_5) δ_C: 51.4, 51.9, 51.9 (OCH_3 × 3), and other signals as shown in Table I. FAB-MS m/z: (positive) 903 [(M + Na)$^+$]; (+Li) 887 [(M + Li)$^+$]; (negative) 879 [(M − H)$^-$], 865 [(M − CH_3)$^-$].

Methanolysis of 8a A solution of **8a** (5 mg) in 9% HCl–dry MeOH (0.5 ml) was heated under reflux for 1 h. The reaction mixture was neutralized with Dowex 1 × 2 (OH^- form) and the product was shown by TLC (as described above for the methanolysis of **5a**) to contain methyl glucuronide. After removal of the resin by filtration, the filtrate was evaporated under reduced pressure to furnish a product. The product was purified by column chromatography [SiO_2 500 mg, *n*-hexane–AcOEt (2 : 3)] to furnish 24-hydroxyglycyrrhetic acid methyl ester (**15a**, 3 mg), which was identified by comparison of its melting point, $[\alpha]_D$ and IR (KBr) data with reported values.[8]

15a: mp 246—248 °C (colorless fine needles from MeOH), $[\alpha]_D^{24}$ +125.5° (c=0.30, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{31}H_{48}O_5$ (M^+): 500.3667. Found: 500.3633. UV λ_{max}^{MeOH} nm (ε): 248 (10500). IR ν_{max}^{KBr} cm^{-1}: 3600—3400 (br), 2960, 1729, 1653, 1618, 1455, 1155, 1038. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.80, 1.08, 1.10, 1.15, 1.25, 1.36 (all 3H, s), 2.94 (1H, br d, J=*ca.* 13 Hz, 18-H), 3.23 (1H, dd, J=4.5, 10.0 Hz, 3α-H), 3.69 (3H, s, OCH_3), 4.20, 4.35 (each 1H, both d, J=10.0 Hz, 24-H_2), 5.60 (1H, br s, 12-H). EI-MS m/z (%): 500 (M^+, 16.5), 453 (52.1), 317 (100).

$NaBH_4$ Reduction of 8a Followed by Complete Methylation and Methanolysis A solution of **8a** (5 mg) in MeOH (2 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^+ form) and the resin was removed by filtration. A residue, obtained after work-up as described above, was purified by column chromatography [SiO_2 1 g, $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower phase)] to furnish a product (4.2 mg). This product was dissolved in DMSO (1 ml) and the solution was treated with a dimsyl carbanion solution (1 ml, prepared as described above). The mixture was stirred in the dark at room temprature (23 °C) for 1 h, then treated with CH_3I (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 AcOEt. After work-up of the AcOEt extract in the usual manner, the product (5.2 mg) was dissolved in 9% HCl–dry 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 co-TLC and GLC with methyl glycosides **c** and **d** as described above in the methylation analysis of **6a**.

Diazomethane Methylation of Licorice-saponin H2 (9) An ice-cooled solution of **9** (20 mg) in MeOH (2 ml) was treated with ethereal diazomethane (*ca.* 15 ml) and the solution was left standing for 1 h. Evaporation of the solvent from the solution gave the trimethyl ester (**9a**, 20 mg).

9a: mp 169—170 °C (colorless fine needles from MeOH–H_2O), $[\alpha]_D^{23}$ +29° (c=0.20, MeOH). High-resolution FAB-MS (positive): Calcd for $C_{45}H_{68}NaO_{16}$ [(M + Na)$^+$]: 887.4405. Found: 887.4333. UV λ_{max}^{MeOH} nm (ε): 249 (10900). IR ν_{max}^{KBr} cm^{-1}: 3550—3100 (br), 2900, 1722, 1645, 1435. ^{1}H-NMR (500 MHz, pyridine-d_5 + D_2O) δ: 0.87, 1.09, 1.13, 1.31, 1.36 (all 3H, s), 1.26 (3H × 2, s), 3.04 (1H, br d, J=*ca.* 13 Hz, 18-H), 3.37 (1H, dd, J=4.6, 10.0 Hz, 3α-H), 3.79, 3.80, 3.91 (all 3H, s, OCH_3 × 3), 4.95 (1H, d, J=7.3 Hz, 1′-H), 5.33 (1H, d, J=7.7 Hz, 1″-H), 5.71 (1H, br s, 12-H). ^{13}C-NMR (22.5 MHz, pyridine-d_5) δ_C: 51.8, 51.9, 52.0 (OCH_3 × 3), and other signals as given in Table I. FAB-MS m/z: (positive) 887 [(M + Na)$^+$]; 865 [(M + H)$^+$]; (negative) 863 [(M − H)$^-$].

Methanolysis of 9a A solution of **9a** (7 mg) in 9% HCl–dry MeOH (0.5 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Dowex 1 × 2 (OH^- form) and the product was identified by TLC with methyl glucuronide as described above. After removal of the resin by filtration, the filtrate was evaporated under reduced pressure to give a product, which was purified by column chromatography [SiO_2 500 mg, *n*-hexane–AcOEt (1 : 1)] to furnish liquiritic acid methyl ester (**16a**, 4 mg). Compound **16a** was identified by comparison of its melting point, $[\alpha]_D$, and IR (KBr) data with reported values.[9]

16a: mp 223—225 °C (colorless fine needles from EtOH), $[\alpha]_D^{23}$ +65° (c=0.40, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{31}H_{48}O_4$ (M^+): 484.3634. Found: 484.3603. UV λ_{max}^{MeOH} nm (ε): 248 (10500). IR ν_{max}^{KBr} cm^{-1}: 3600—3300 (br), 2965, 1728, 1656, 1455, 1380, 1038. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.00, 1.34, 1.36 (all 3H, s), 0.80, 1.14 (each 6H, both s), 2.98 (1H, br d, J=*ca.* 13 Hz, 18-H), 3.30 (1H, dd, J=5.0, 10.0 Hz, 3α-H), 3.69 (3H, s, OCH_3), 5.64 (1H, br s, 12-H). EI-MS m/z (%): 484 (M^+, 85), 485 (M^+ + 1, 26), 262 (100).

$NaBH_4$ Reduction of 9a Followed by Complete Methylation and Methanolysis A solution of **9a** (5 mg) in MeOH (2 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^+ form) and worked up as described above to give a residue, which was purified by column chromatography [SiO_2 1 g, $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower phase)] to furnish the product (4.2 mg). This product was dissolved in DMSO (0.5 ml) and the solution was treated with a dimsyl carbanion solution (1 ml). The mixture was stirred in the dark at room temperature (23 °C) for 1 h, then treated with CH_3I (1 ml), and the whole was stirred for a further 2 h. Work-up of the reaction mixture as described above gave a product which was dissolved in 9% HCl–dry 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 co-TLC and GLC (as described above) with methyl glycosides **c** and **d**.

Diazomethane Methylation of Licorice-saponin J2 (10) An ice-cooled solution of **10** (15 mg) in MeOH (2 ml) was treated with ethereal diazomethane (*ca.* 10 ml) until the yellow color persisted. The reaction mixture was left standing for 1 h and then evaporation of the solvent from the solution under reduced pressure gave the trimethyl ester (**10a**, 15 mg).

10a: mp 198—199 °C (colorless fine needles from MeOH), $[\alpha]_D^{23}$ +23° (c=0.20, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3650—3200 (br), 2930, 1730, 1626, 1387, 1116. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.83, 0.84, 1.00, 1.18, 1.31, 1.36 (all 3H, s), 3.46 (1H, dd, J=5.0, 9.2 Hz, 3α-H), 3.79, 3.80, 3.88 (all 3H, s, OCH_3 × 3), 4.51, 4.62 (each 1H, both d, J=10.2 Hz, 24-H_2), 5.02 (1H, d, J=7.3 Hz, 1′-H), 5.31 (1H, br s, 12-H), 5.70 (1H, d, J=7.0 Hz, 1″-H). ^{13}C-NMR (22.5 MHz, pyridine-d_5) δ_C: 51.5, 51.6, 51.8 (OCH_3 × 3), and other signals as given in Table I.

Clemmensen Reduction of Licorice-saponin G2 (8) Giving Licorice-saponin J2 (10) A solution of licorice-saponin G2 (**8**) (100 mg) in 70% dioxane–water (5 ml) was first treated with zinc amalgam (100 mg, prepared from zinc powder and mercuric chloride) and then 10% aqueous HCl (2 ml) was added dropwise over a period of 10 min. The reaction mixture was further stirred at 0 °C for 30 min and the zinc amalgam was removed by filtration with a glass filter. After dilution with ice-water (40 ml), the reaction mixture was extracted with 1-butanol twice (20 ml each). The combined 1-butanol extract was neutralized with Amberlite IRA 400 (OH^- form) 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 (SiO_2 8 g, $CHCl_3$: MeOH : H_2O = 6 : 4 : 1) to furnish licorice-saponin J2 (**10**, 51 mg, 51%). Compound **10** thus obtained was shown to be identical with authentic licorice-saponin J2 on the basis of mixed melting point determination and comparison of IR (KBr) and ^{1}H-NMR (pyridine-d_5) data.

Diazomethane Methylation of Licorice-saponin K2 (11) A solution of **11** (15 mg) in MeOH (2 ml) was treated with ethereal diazomethane (*ca.* 10 ml) until the yellow color persisted. The reaction mixture was left standing for 1 h and then the solvent was removed under reduced pressure to furnish the trimethyl ester (**11a**).

11a: mp 179—181 °C (colorless fine needles from MeOH), $[\alpha]_D^{23}$ +27° (c=0.16, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3500—3120 (br), 2940, 1731, 1621, 1386, 1059. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.87, 1.09, 1.13, 1.31, 1.36 (all 3H, s), 1.26 (3H × 2, s), 3.46 (1H, dd, J=4.4, 10.0 Hz, 3α-H), 3.79, 3.80, 3.91 (all 3H, s, OCH_3 × 3), 4.46, 4.56 (each 1H, both d, J=10.2 Hz, 24-H_2),

4.95 (1H, d, $J=7.3$ Hz, 1′-H), 5.33 (1H, d, $J=7.7$ Hz, 1″-H), 5.52 (1H, br d, $J=ca.$ 12 Hz, 11-H), 6.49 (1H, br d, $J=ca.$ 12 Hz, 12-H). ^{13}C-NMR (22.5 MHz, pyridine-d_5) δ_C: 51.5, 51.6, 52.0 ($OCH_3 \times 3$), and other signals as given in Table I.

Conversion of Licorice-saponin G2 (8) to Licorice-saponin K2 (11) A solution of licorice-saponin G2 (**8**, 50 mg) in EtOH–H_2O (1 : 1, 3 ml) was treated with $NaBH_4$ (300 mg) and the mixture was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and the resin was removed by filtration. Removal of the solvent from the filtrate gave a product which was dissolved in dioxane–H_2O (1 : 1, 3 ml) and the solution was heated under reflux for 1 h. Evaporation of the solvent from the reaction solution gave a product, which was purified by column chromatography (SiO_2 5 g, $CHCl_3$: MeOH : H_2O = 6 : 4 : 1) to furnish **11** (38 mg, 76%). Compound **11** thus obtained was shown to be identical with authentic licorice-saponin K2 on the basis of mixed melting point determination and comparison of IR (KBr), ^{1}H- and ^{13}C-NMR (both in pyridine-d_5) data.

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