Chemical Studies of Chinese Licorice-Roots. II.[1] Five New Flavonoid Constituents from the Roots of *Glycyrrhiza aspera* PALL. Collected in Xinjiang

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From the air-dried roots of *Glycyrrhiza aspera* PALL. (Leguminosae) collected in Xinjiang province, China (known as a kind of "Shinkyo-Kanzo"), five new flavonoid compounds named glucoisoliquiritin apioside (1) (a chalcone bisdesmosidic triglycoside), 7-*O*-apioglucosyl-7,4′-dihydroxyflavone (5) (a flavone diglycoside), dehydroglyasperin C (7) (a prenylated isoflav-3-ene derivative), asperopterocarpin (9) and 1-methoxyphaseollidin (12) (an *O*-prenylpterocarpan and a *C*-prenylpterocarpan, respectively), were isolated together with four known saponins, one known flavonoid *O*-glycoside, two known flavonoid *C*-glycosides, and seven flavonoids. The structures of the new compounds have been elucidated on the basis of their chemical and physicochemical properties. Some pterocarpan compounds showed moderate cytotoxic activity against KB and L1210 cells.

Key words *Glycyrrhiza aspera*; Leguminosae; licorice root; chalcone bisdesmoside; isoflavene prenylated; pterocarpan prenylated

In the course of our chemical studies on the constituents of botanically identified various Chinese licorice-roots, we have so far characterized saponins of *Glycyrrhiza uralensis* from northeastern China[4] and *G. inflata* from Xinjiang province,[5] and saponins and flavonoids of *G. uralensis* from Xinjiang.[6] Afterwards, we investigated the chemical constituents of the air-dried roots of *G. glabra* L., which were collected in Xinjiang province of China, and elucidated five new flavonoid derivatives which included glucoliquiritin apioside (**4**), prenyllicoflavone A, shinflavanone, shinpterocarpin, and 1-methoxyphaseollin (**11**).[1]

As a continuation of these investigations, we have examined the constituents of the air-dried roots of *Glycyrrhiza aspera* PALL. called "粗毛甘草" in China, which were collected in Xinjiang province of China,[7] and characterized five new flavonoids. They are a chalcone bisdesmosidic triglycoside named glucoisoliquiritin apioside (**1**), a flavone diglycoside 7-*O*-apioglucosyl-7,4′-dihydroxyflavone (**5**), a prenylated isoflav-3-ene derivative dehydroglyasperin C (**7**), an *O*-prenylpterocarpan asperopterocarpin (**9**), and a *C*-prenylpterocarpan 1-methoxyphaseollidin (**12**). This paper presents a full account of the structure elucidation of these new flavonoid constituents.

The isolation of the chemical constituents from the Xinjiang air-dried roots of *Glycyrrhiza aspera* was carried out through the procedure as shown in Fig. 1.

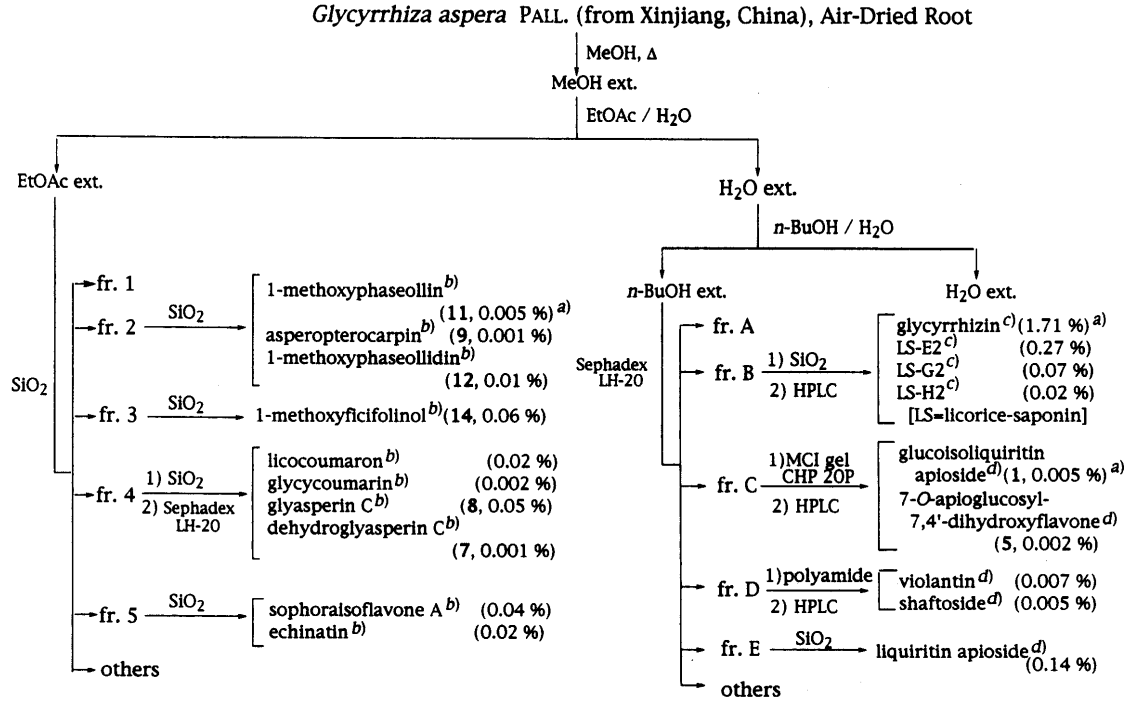

Fig. 1

a) Isolated yield from the air-dried roots, *b*) flavonoid, *c*) saponin, *d*) flavonoid glycoside

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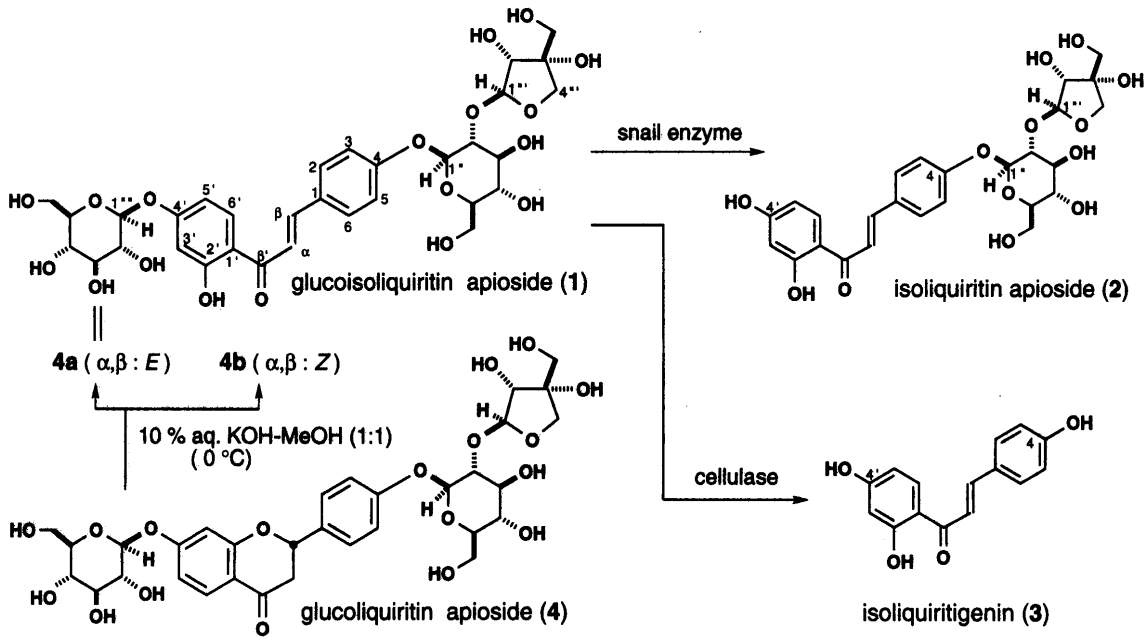

Fig. 2

At the beginning, the methanolic extract of the roots was partitioned into an ethyl acetate and water mixture. The water soluble portion was then partitioned into butanol, and the butanol soluble portion was subjected to Sephadex LH-20 chromatography to provide six fractions (fractions A—E and later eluate). Repeated separation of fr. B, by means of ordinary-phase silica gel column chromatography and subsequent high-performance liquid chromatography (HPLC), furnished four known saponins (glycyrrhizin, licorice-saponins E2[4b], G2[4b], and H2[4b]). Chromatographic separation of fr. C by means of high porous polymer gel column (MCI gel CHP 20P) and reversed-phase HPLC afforded two new compounds: glucoisoliquiritin apioside (**1**) and 7-*O*-apioglucosyl-7,4′-dihydroxyflavone (**5**). Fraction D was subjected to polyamide column chromatography and subsequent HPLC to furnish two known flavonoid *C*-glycosides (violantin[8] and shaftoside[9]), while liquiritin apioside was obtained from fr. E by chromatography on ordinary-phase silica gel column.

On the other hand, the ethyl acetate-soluble portion was subjected to ordinary-phase silica gel column chromatography repeatedly and subsequent Sephadex LH-20 column chromatography to provide a new isoflav-3-ene derivative dehydroglyasperin C (**7**), together with the related known compound glyasperin C (**8**),[10] two new *O*- and *C*-prenylated pterocarpans: asperopterocarpin (**9**) and 1-methoxyphaseollidin (**12**), two known pterocarpans: 1-methoxyphaseollin (**11**)[1] and 1-methoxyficifolinol (**14**),[11] two known coumarins: licocoumaron[12] and glycycoumarin,[12] a known isoflavone sophoraisoflavone A,[13] and a known chalcone echinatin.[14]

Glucoisoliquiritin Apioside (1) The molecular composition $C_{32}H_{40}O_{18}$ of glucoisoliquiritin apioside (**1**), obtained as a white fine powder, was determined from the *quasi*-molecular ion peak observed at *m/z* 713.2295 [$(M+H)^+$] by high-resolution (HR) fast atom bombardment mass spectrum (FAB-MS) analysis. The proton magnetic resonance (^{1}H-NMR) spectrum of **1** showed three anomeric proton signals [δ 5.58 (1H, d, J=7.6 Hz), δ 5.80 (1H, d, J=7.0 Hz), and δ 6.58 (1H, br s)] which suggested **1** to be a triglycoside.

Table 1. ^{13}C-NMR Data for Glucoisoliquiritin Apioside (**1**) and Isoliquiritin Apioside (**2**) (in DMSO-d_6, δ_C)

		1[a]	**2**[b]
Chalcone moiety	C-1′	115.7	113.0
	C-2′	164.7	165.9
	C-3′	103.6	102.8
	C-4′	164.7	166.0
	C-5′	110.5	108.6
	C-6′	132.4	133.1
	C-β	143.3	143.3
	C-α	119.2	119.5
	C-β′	192.4	191.5
	C-1	129.0	128.7
	C-2,6	130.9	130.8
	C-3,5	117.2	116.6
	C-4	160.4	159.4
4-*O*-β-D-Glucopyranosyl moiety	C-1″	99.1	98.7
	C-2″	77.2	77.1
	C-3″	76.2[c]	76.3
	C-4″	70.0[d]	70.2
	C-5″	76.3[c]	76.3
	C-6″	59.7[e]	59.7
2″-*O*-β-D-Apiofuranosyl moiety	C-1‴	108.7	108.6
	C-2‴	77.2	77.1
	C-3‴	80.0	79.4
	C-4‴	74.8	74.1
	C-5‴	64.5	64.5
4′-*O*-β-D-Glucopyranosyl moiety	C-1⁗	100.0	
	C-2⁗	73.6	
	C-3⁗	77.0[c]	
	C-4⁗	70.2[d]	
	C-5⁗	76.8[c]	
	C-6⁗	59.6[e]	

a) Measured at 67.8 MHz, *b*) measured at 22.5 MHz, *c*—*e*) assignments may be interchangeable within the same column.

The carbon-13 (^{13}C-) NMR spectrum of **1** showed the carbon signals ascribable to an isoliquiritigenin (**3**) moiety, a β-glucopyranosyl moiety, and a β-apiofuranosyl(1→2)-β-glucopyranosyl moiety. The presence of the β-apiofuranosyl structure in **1** was assumed from the chemical shift of the

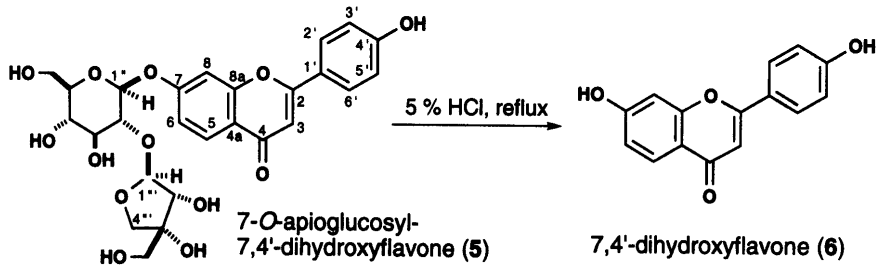

Fig. 3

apiosyl anomeric carbon signal observed at δC 108.7[5] (Table 1).

In order to clarify the location of the two sugar moieties (one glucopyranose and one 2-apiofuranosylglucopyranose), glucoisoliquiritin apioside (**1**) was subjected to enzymatic partial hydrolysis using snail enzyme[15] to afford isoliquiritin apioside (**2**).[6] This finding has led us to assume that compound **1** is a 4′-*O*-glucopyranoside of isoliquiritin apioside (**2**). Finally, our hypothesis was verified by direct chemical correlation between **1** and glucoliquiritin apioside (**4**),[1] namely the correlation between chalcone and flavanone. Thus, treatment of **4** with 10% KOH aq.-EtOH (1 : 1), under ice-water cooled conditions, provided two geometric isomers: **4a** (α,β: *E*) and **4b** (α,β: *Z*) in a 2 : 1 ratio. One of the reaction products **4a** was found identical with **1** by direct comparison of ^{1}H-NMR, IR, and UV spectra and optical rotation values. Consequently, the structure of glucoisoliquiritin apioside has been determined as 4-*O*-[β-D-apiofuranosyl(1‴→2″)-β-D-glucopyranosyl]-4′-*O*-β-D-glucopyranosylisoliquiritigenin (**1**).

7-*O*-Apioglucosyl-7,4′-dihydroxyflavone (5) 7-*O*-Apioglucosyl-7,4′-dihydroxyflavone (**5**) was isolated as a yellowish oily compound. From the HR FAB-MS, the molecular composition of **5** was determined as $C_{26}H_{28}O_{13}$. Comparison in detail of the ^{13}C-NMR data for the sugar moieties of **5** and isoliquiritin apioside (**2**) has suggested that **5** possesses the same 2-apiofuranosylglucopyranosyl moiety as **2** (Table 2). Hydrolysis of **5** with 5% aqueous HCl liberated D-glucose and D-apiose, together with 7,4′-dihydroxyflavone (**6**).[16] Furthermore, investigation of the ^{13}C-NMR data for **5** in comparison with those for **6**, has shown the presence of a glycosylation shift[17] around the C-7 signal, thus the sugar moiety was determined to be attached at the C-7 hydroxyl. Consequently, the structure of **5** has been clarified as 7-*O*-[β-D-apiofuranosyl-(1‴→2″)-β-D-glucopyranosyl]-7,4′-dihydroxyflavone.

Dehydroglyasperin C (7) Dehydroglyasperin C (**7**) was isolated as a yellowish oily substance. From the HR electron-impact mass spectrum (EI-MS), the molecular composition of **7** was defined as $C_{21}H_{22}O_5$. In 1992, Nomura and co-workers[10] investigated phenolic constituents of the roots of *Glycyrrhiza aspera* from Xinjiang, China and elucidated a then-new isoflavane named glyasperin C (**8**). Since the molecular composition of **8**, $C_{21}H_{24}O_5$, corresponded to a dihydro type of **7**, the ^{1}H-NMR (Table 3) and ^{13}C-NMR (Table 4) data for **7** were investigated in detail in comparison with those for **8**. It has been suggested that **7** is a 3,4-dehydro analog of **8**. In order to determine the location of the *cis*-double bond, a heteronuclear multiple-bond connectivity (HMBC) experiment of **7** was undertaken to reveal the correlations between the C-3 carbon signal (δ_C 129.4) and two proton signals due to 4-H (δ 6.47) and 6′-H (δ 7.13), together with the correlation between the C-5 carbon signal (δ_C 159.6) and the methoxyl proton signal (δ 3.73) (Fig. 4). Based on these findings, the structure of dehydroglyasperin C has been determined to be 3-(2′,4′-dihydroxyphenyl)-6-(3″,3″-dimethylallyl)-7-hydroxy-5-methoxy-2*H*-chromene (**7**).

Table 2. ^{13}C-NMR Data for 7-*O*-Apioglucosyl-7,4′-dihydroxyflavone (**5**), 7,4-Dihydroxyflavone (**6**) and Isoliquiritin Apioside (**2**, Sugar Moiety)

		5	**6**	($\Delta\delta_5$—δ_6)	**2**
Flavone moiety	C-2	164.2	164.2		
	C-3	105.8	105.8		
	C-4	177.2	177.4		
	C-4a	119.3	117.8	(+1.5)	
	C-5	127.0	127.5	(−0.5)	
	C-6	115.6	115.5	(+0.1)	
	C-7	162.3	163.4	(−1.1)	
	C-8	104.4	103.5	(+0.9)	
	C-8a	157.4	158.1	(−0.7)	
	C-1′	122.7	122.7		
	C-2′,6′	128.6	128.6		
	C-3′,5′	116.8	116.8		
	C-4′	162.3	162.2		
β-D-Glucopyranosyl moiety	C-1‴	98.0			99.7
	C-2‴	79.1			80.6
	C-3‴	78.3			78.3[a]
	C-4‴	71.2			71.0
	C-5‴	78.3			78.4[a]
	C-6‴	62.2			61.8
β-D-Apiofuranosyl moiety	C-1⁗	110.6			110.2
	C-2⁗	77.6			77.6
	C-3⁗	80.9			80.6
	C-4⁗	76.4			75.4
	C-5⁗	66.4			65.9

67.8 MHz, pyridine-d_5, δ_C. *a*) Assignments may be interchangeable.

Asperopterocarpin (9) and 1-Methoxyphaseollidin (12) The molecular composition of asperopterocarpin (**9**), obtained as a yellowish oily substance, was defined as $C_{21}H_{22}O_5$ by the HR EI-MS analysis. In the ^{1}H-NMR spectrum of **9**, four characteristic one-proton signals were observed at δ 3.34 (m) due to 6a-H, at δ 3.60 (dd, J=10.9, 11.2 Hz) and δ 4.17 (dd, J=5.2, 10.9 Hz) assignable to 6-H_2, and at δ 5.61 (d, J=6.2 Hz) assignable to 11a-H, thus **9** being suggested to have a pterocarpan skeleton.

Investigation of the ^{1}H-NMR and ^{13}C-NMR data (Table 5) for asperopterocarpin (**9**), in comparison with those for medicarpin (**10**)[1,18] and 1-methoxyphaseollin (**11**),[1] has led us to determine that **9** has a 1,2,4-trisubstituted [at δ 6.30 (1H, dd, J=2.0, 7.9 Hz), δ 6.40 (1H, d, J=2.0 Hz), δ 7.05 (1H, d, J=7.9 Hz)] and a 1,2,3,5-tetrasubstituted [at δ 6.10

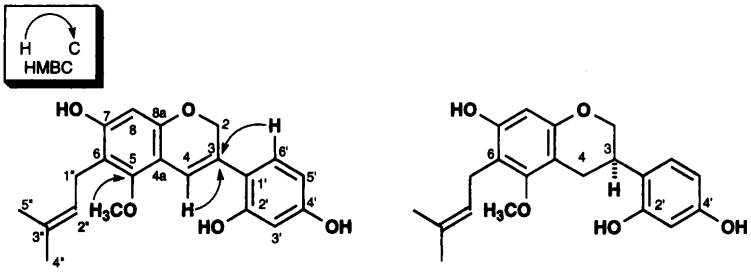

Fig. 4

Table 3. ^{1}H-NMR Data for Dehydroglyasperin C (**7**) and Glyasperin C (**8**)

	7	8
2-H	4.92 (s)	3.96 (dd, J=10.2, 10.2) 4.19 (ddd, J=2.4,3.3,10.2)
3-H		3.39 (m)
4-H (or 4-H_2)	6.47 (s)	2.83 (dd, J=10.8, 15.9) 2.92 (ddd, J=2.4, 5.4, 15.9)
8-H	6.22 (s)	6.17 (s)
3′-H	6.45 (d, J=2.3)	6.46 (d, J=2.4)
5′-H	6.28 (dd, J=2.3, 8.2)	6.35 (dd, J=2.4, 8.2)
6′-H	7.13 (d, J=8.2)	6.89 (d, J=8.2)
1″-H_2	3.28 (m)	3.28 (m)
2″-H	5.25 (dd, J=5.6, 6.9)	5.25 (br t, J=*ca.* 7)
4″-H_3	1.76 (s)	1.75 (s)
5″-H_3	1.65 (s)	1.65 (s)
5-OCH_3	3.73 (s)	3.70 (s)

270 MHz, acetone-d_6, δ, J in Hz.

Table 4. ^{13}C-NMR Data for Dehydroglyasperin C (**7**) and Glyasperin C (**8**)

	7	8
C-2	69.0	70.4
C-3	129.4	32.3
C-4	116.6	26.6
C-4a	110.9	108.4
C-5	159.6	158.3
C-6	115.6	114.4
C-7	156.8	155.4
C-8	99.9	99.8
C-8a	154.4	154.5
C-1′	100.0	119.8
C-2′	157.2	156.7
C-3′	104.2	103.6
C-4′	157.4	158.3
C-5′	108.5	107.7
C-6′	130.3	128.7
C-1″	23.6	23.3
C-2″	130.9	130.3
C-3″	125.4	125.3
C-4″	26.3	25.9
C-5″	18.3	17.9
—OCH_3	62.7	60.6

67.8 MHz, acetone-d_6, δ_C.

(1H, d, J=2.3 Hz), δ 6.16 (1H, d, J=2.3 Hz)] aromatic rings, and one methoxyl group (δ 3.88, 3H, s). Furthermore, from the presence of significantly deshielded methylene signals observed at δ 4.47 (1H, d, J=6.6 Hz) and δ_C 64.8 (triplet), **9** has been suggested to have a prenylated hydroxy (namely a 3,3-dimethylallyloxy) moiety.

The HMBC experiment of **9** showed the correlations between the deshielded methylene proton signal at δ 4.47 (1′-H) and the carbon signal at δ_C 157.3 (C-3), thus substantiating the 3,3-dimethylallyloxy group being attached at C-3. Further correlations, observed between several other protons and carbons as shown in Fig. 5, have indicated that a hydroxyl group attaches at C-9 whereas a methoxyl group attaches at C-1 of asperopterocarpin (**9**). As for the absolute configuration of **9**, it has been generally accepted that the absolute configuration of a pterocarpan compound may be assumed from the sign of the optical rotation.[19] In the case of asperopterocarpin (**9**), the specific rotation was found as -189 (in $CHCl_3$), thus the absolute configuration of **9** was assumed to be 6a*R* and 11a*R*.

Consequently, the chemical structure of asperopterocarpin has been determined to be 3-(3′,3′-dimethylallyloxy)-9-hydroxy-1-methoxy-[6a*R*,11a*R*]pterocarpan (**9**).

Another pterocarpan derivative, 1-methoxyphaseollidin (**12**), was obtained as a yellowish oily substance and the molecular composition was defined as $C_{21}H_{22}O_5$ from the HR EI-MS analysis. Detailed comparison of the ^{1}H-NMR and ^{13}C-NMR data (Table 5) for **12**, with those for medicarpin (**10**), 1-methoxyphaseollin (**11**) and phaseollidin (**13**),[20] has led us to assume that **12** has a pterocarpan skeleton with one methoxyl group and one *C*-substituted prenyl (*i.e.* a 3,3-dimethylallyl) moiety, such as a monomethoxylated analog of **13**. In order to define the locations of the methoxyl and the prenyl groups, correlation spectroscopy *via* long-range coupling (COLOC) experiments of **12** were carried out, which disclosed the presence of correlations between proton and carbon signals as shown in Fig. 5, so that the locations of the methoxyl group at C-1 and the prenyl moiety at C-10 have been clarified.

This plane structure of 1-methoxyphaseollidin (**12**) was already reported in 1977 by Preston,[21] however the absolute configurations at C-6a and C-11a were not discussed there. Here again, the specific rotation of **12**, $[\alpha]_D$ -165, has led to the assumption that the absolute configurations in question are 6a*R* and 11a*R*, the same as in asperopterocarpin (**9**).

From the above-mentioned evidence, the structure of 1-methoxyphaseollidin has been determined to be 3,9-dihydroxy-10-(3′,3′-dimethylallyl)-1-methoxy-[6a*R*,11a*R*]pterocarpan (**12**).

In 1993, Dange *et al.*[20] reported that phaseollidin (**13**), isolated from the bark of an Ethiopian endemic plant *Erythrina burana* (Leguminosae), exhibited moderate cytotoxic activitiy against Chinese hamster ovary cells. As reported in the previous[1] and present papers, we have so far isolated sev-

Table 5. ^{13}C-NMR Data for Asperopterocarpin (**9**), Medicarpin (**10**), 1-Methoxyphaseollin (**11**), 1-Methoxyphaseollidin (**12**) and Phaseollidin (**13**) (in $CDCl_3$, δ_C)

	Carbons	**9**[a]	**10**[a]	**11**[a]	**12**[a]	**13**[b]
Pterocarpan moiety	C-1	161.0	132.3	158.1	158.4	132.4
	C-2	93.0	109.9	92.7	92.6	110.0
	C-3	157.3	157.3	161.3	161.2	158.5
	C-4	94.0	103.7	96.1	95.9	103.7
	C-4a	157.3	156.7	157.4	157.2	157.4
	C-6	66.4	66.6	66.5	66.3	66.6
	C-6a	38.7	39.6	39.0	39.2	40.1
	C-6b	118.8	119.2	119.1	118.6	118.7
	C-7	119.2	124.8	123.7	122.2	121.6[c]
	C-8	98.7	97.0	108.5	108.0	108.2
	C-9	160.8	161.2	155.4	157.8	156.5
	C-10	107.4	106.6	106.4	110.3	110.9
	C-10a	161.0	160.7	153.7	155.7	155.5
	C-11a	75.6	78.7	76.8	75.2	78.2
	C-11b	101.5	112.5	101.5	101.8	112.6
3-(3′,3′-dimethyl-allyloxy) moiety	C-1′	64.8				
	C-2′	124.6				
	C-3′	138.5				
	C-4′	25.8				
	C-5′	18.2				
10-(3′,3′-dimethyl-allyl) moiety	C-1′				23.2	23.1
	C-2′				121.5	122.3[c]
	C-3′				135.1	134.4
	C-4′				25.8	25.6
	C-5′				17.7	17.9
6′,6′-dimethylpyran moiety	C-4′			117.0		
	C-5′			129.3		
	C-6′			75.9		
	6′,6′-dimethyl			27.9, 29.8		
	9-OCH_3		55.6			
	1-OCH_3	55.9		60.2	55.7	

a) Measured at 67.8 MHz. *b*) Cited in reference 20 (100 MHz). *c*) Assignment may be interchangeable, so that this assignment was made only by measurement of distortionless enhancement by polarization transfer (DEPT) experiment in reference 20.

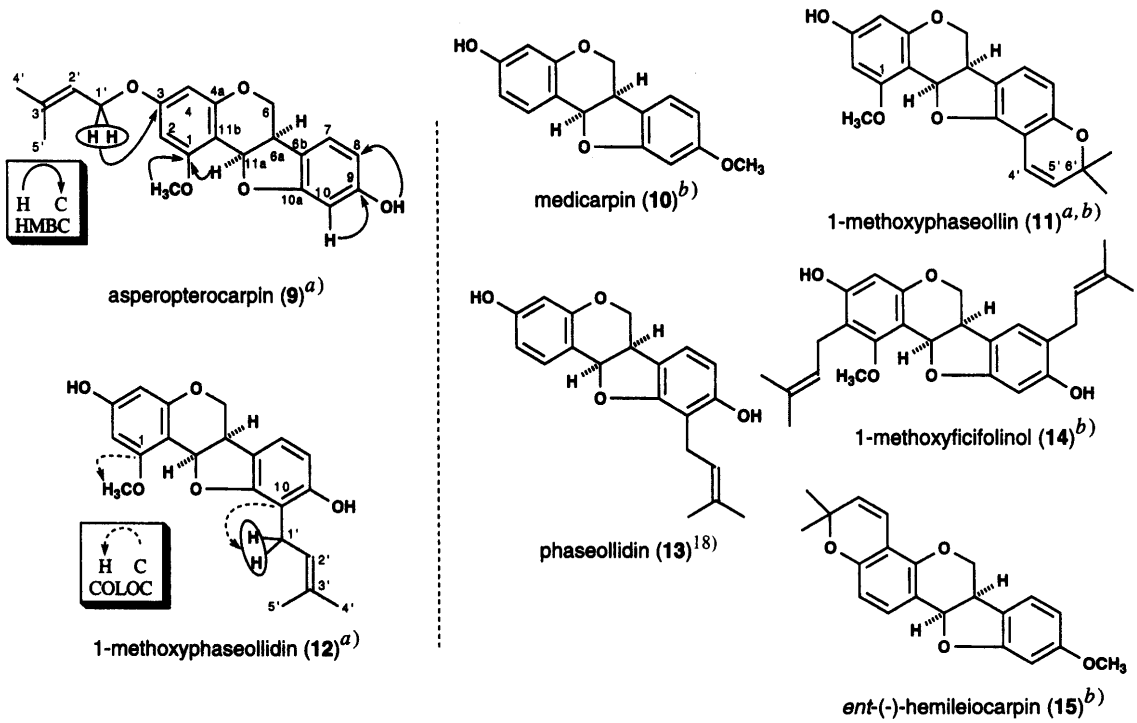

Fig. 5

a) Isolated from Xinjiang *Glycyrrhiza aspera* (in the present work). *b*) Isolated from Xinjiang *G. glabra*.[1]

eral pterocarpans: asperopterocarpin (**9**), medicarpin (**10**),[1] 1-methoxyphaseollin (**11**),[1] 1-methoxyphaseollidin (**12**), 1-methoxyficifolinol (**14**) and *ent*-(−)-hemileiocarpin (**15**),[1] from two Xinjiang licorice-roots: *Glycyrrhiza aspera* and *G. glabra*.[1] Among these six pterocarpan compounds (**9**, **10**, **11**, **12**, **14**, **15**), **12** and **14** (a major flavonoid in *G. aspera*), were found to exhibit moderate cytotoxic activities: IC_{50} for **12**: 5.7 μg/ml (KB cells) and 4.7 μg/ml (L1210 cells) and for **14**: 8.0 μg/ml (KB), 4.6 μg/ml (L1210). We are currently engaged in comparative analyses of flavonoid, flavonoid glycoside and saponin constituents in various Chinese licorice roots, and the composition of these constituents in the roots of *G. aspera* have been observed to be fairly characteristic, which will be reported in due course.

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 Ten Flavonoids, Four Saponins, and Five Flavonoid Glycosides The air-dried roots of *Glycyrrhiza aspera* (from Xinjiang province, China, cut, 0.3 kg)[7] were extracted three times with MeOH (1 l each) under reflux. Evaporation of the solvent under reduced pressure from the combined extract gave the MeOH extract (60 g). The extract (60 g) was then partitioned into an ethyl acetate–water (1 : 1) mixture (1 l). Removal of the solvent from the water phase and the ethyl acetate phase, respectively under reduced pressure below 40 °C, yielded the aqueous extract (39.4 g) and the ethyl acetate extract (20.6 g). The ethyl acetate extract (18 g) was subjected to silica gel column chromatography [SiO_2 200 g, gradient elution with hexane–ethyl acetate (10 : 1→0 : 1), ethyl acetate and methanol] to provide six fractions: fraction 1 (fr. 1) [eluted with hexane–ethyl acetate (10 : 1→7 : 1), 2.8 g], fraction 2 (fr. 2) [eluted with hexane–ethyl acetate (7 : 1→5 : 1), 1.2 g], fraction 3 (fr. 3) [eluted with hexane–ethyl acetate (5 : 1), 0.4 g], fraction 4 (fr. 4) [eluted with hexane–ethyl acetate (5 : 1→3 : 1), 1.4 g], fraction 5 (fr. 5) [eluted with hexane–ethyl acetate (3 : 1→2 : 1), 1.7 g], and later eluates combined [eluted with ethyl acetate and methanol, 9.5 g]. Silica gel column chromatography (SiO_2 50 g, hexane : $CHCl_3$=20 : 1) of fr. 2 (1.2 g) afforded 1-methoxyphaseollin (**11**), asperopterocarpin (**9**), and 1-methoxyphaseollidin (**12**) in 0.005, 0.001, and 0.01% yields from the air-dried roots, respectively. Silica gel column chromatography (SiO_2 50 g, benzene : acetone=40 : 1) of fr. 3 (0.4 g) afforded 1-methoxyficifolinol (**14**) in 0.05% yield. Silica gel column chromatography [SiO_2 80 g, benzene : acetone= 6 : 1] of fr. 4 (1.4 g) followed by Sephadex LH-20 column chromatography (Sephadex LH-20 80 g, $CHCl_3$: MeOH=1 : 2) afforded licocoumaron, glycycoumarin, glyasperin C (**8**), and dehydroglyasperin C (**7**) in 0.02, 0.004, 0.02, and 0.01% yields, respectively. Finally, silica gel column chromatography [SiO_2 50 g, hexane : acetone=2 : 1] of fr. 5 (1.7 g) provided sophoraisoflavone A and echinatin in 0.04 and 0.02% yields, respectively.

Next, the above-described aqueous extract (39.4 g) was partitioned into butanol–water (1 : 1) mixture (1.2 l). Removal of the solvent from the each phase, under reduced pressure below 40 °C, provided butanol extract (8.7 g) and aqueous extract (30.7 g), respectively.

The butanol extract (8.7 g) was subjected to Sephadex LH-20 column chromatography (Sephadex LH-20 250 g, MeOH) to furnish six fractions: fr. A (0.5 g), fr. B (1.0 g, mainly saponins), fr. C (3.5 g), fr. D (1.7 g), fr. E (0.4 g), and later eluates (1.0 g). Silica gel column chromatography [SiO_2 40 g, gradient elution with $CHCl_3$: MeOH : H_2O=10 : 3 : 1 (lower phase)→6 : 4 : 1] of fr. B (1.0 g) and subsequent *semi*-preparative HPLC separation (Cosmosil $5C_{18}$, 10 mm (i.d.)×25 cm (L.), CH_3CN : 1% aqueous AcOH=15 : 85) provided glycyrrhizin, licorice-saponins E2, G2, and H2 in 1.71, 0.27, 0.07 and 0.02% yields from the roots, respectively. High porous polymer gel chromatography (MCI gel CHP 20P, 200 g, MeOH : H_2O=1 : 1) followed by *semi*-preparative HPLC [Cosmosil $5C_{18}$, 10 mm (i.d.)×25 cm (L.), CH_3CN : 1% aqueous AcOH=15 : 85) provided glucoisoliquiritin apioside (**1**) and 7-*O*-apioglucosyl-7,4′-dihydroxyflavone (**5**) in 0.005 and 0.002% yields, respectively. Polyamide column chromatography [polyamide C-200 (Wako Pure Chemical) 100 g, MeOH] of fr. D (1.7 g) and subsequent *semi*-preparative HPLC separation [Zorbax SIL, 10 mm (i.d.)×25 cm (L.), $CHCl_3$: MeOH : H_2O=65 : 40 : 10) provided violantin and shaftoside in 0.007 and 0.005% yields from the roots, respectively. Finally, silica gel column chromatography (SiO_2, 30 g, $CHCl_3$–MeOH–H_2O=6 : 4 : 1) of fr. E (0.4 g) afforded liquiritin apioside in 0.14% yield from the roots.

Glucoisoliquiritin apioside (**1**), a white powder, $[\alpha]_D$ −86.2 (c=0.20, MeOH, 23 °C). HR FAB-MS (positive) *m/z*: Found: 713.2295; Calcd for $C_{32}H_{41}O_{18}$ [$(M+H)^+$]: 713.2293. UV (MeOH) λ_{max} nm (ε): 360 (1800); (+NaOAc): 360. IR (KBr) ν_{max} cm^{-1}: 3339, 1599, 1385, 1126, 1028. ^{1}H-NMR (270 MHz, C_5D_5N) δ: 3.10—3.77 (18H, m, sugar protons), 5.58 (1H, d, J=7.6 Hz, 1″-H), 5.80 (1H, d, J=7.0 Hz, 1‴-H), 6.58 (1H, br s, $W_{1/2}$=*ca.* 8 Hz, 1‴-H), 6.93 (1H, d, J=8.9 Hz, 5′-H), 7.11 (1H, br s, 3′-H), 7.46 (2H, d, J=8.9 Hz, 3-H, 5-H), 7.63 (2H, d, J=8.9 Hz, 2-H, 6-H), 7.78 (1H, d, J=15.2 Hz, α-H), 8.07 (1H, d, J=15.2 Hz, β-H), 8.14 (1H, d, J=8.9 Hz, 6′-H). ^{13}C-NMR: as given in Table 1. FAB-MS (positive) *m/z*: 713 $(M+H)^+$, 735 $(M+Na)^+$, 751 $(M+K)^+$.

7-*O*-Apioglucosyl-7,4′-dihydroxyflavone (**5**), a yellowish oil. HR FAB-MS *m/z*: Found: 549.1630, Calcd for $C_{26}H_{29}O_{13}$ [$(M+H)^+$]: 549.1608. UV (MeOH) λ_{max} nm (e): 254sh, 303sh 328 (7400); (+NaOMe): 283, 303, 388; (+NaOAc): 280, 304, 384. IR (KBr) ν_{max} cm^{-1}: 3329, 1604, 1383, 1178, 1028. ^{1}H-NMR (270 MHz, C_5D_5N) δ: 3.07—3.65 (10H, m. sugar protons), 5.75 (1H, d, J=7.6 Hz, 1″-H), 6.61 (1H, br s, $W_{1/2}$=*ca.* 8 Hz, 1‴-H), 7.01 (1H, s, 3-H), 7.18 (2H, d, J=8.3 Hz, 3′-H, 5′-H), 7.42 (1H, dd, J=2.0, 8.6 Hz, 6-H), 7.63 (1H, d, J=2.0 Hz, 8-H), 7.90 (2H, d, J=8.3 Hz, 2′-H, 6′-H), 8.34 (1H, d, J=8.6 Hz, 5-H). ^{13}C-NMR: as given in Table 2. FAB-MS (positive) m/z (%): 549 $(M+H)^+$, 555 $(M+Li)^+$, 571 $(M+Na)^+$.

Dehydroglyasperin C (**7**), a yellowish oil. HR EI-MS *m/z*: Found: 354.0048; Calcd for $C_{21}H_{22}O_5$ (M^+): 354.0038. UV (MeOH) λ_{max} nm (ε): 289 (12000), 329 (14000). IR (KBr) ν_{max} cm^{-1}: 3351, 2967, 1699, 1616, 1514, 1167, 839. ^{1}H-NMR: as given in Table 3. ^{13}C-NMR: as given in Table 4. EI-MS *m/z* (%): 354 (M^+, 100), 339 [$(M-CH_3)^+$, 14], 322 [$(M-CH_3OH)^+$, 20].

Asperopterocarpin (**9**), a yellowish oil, $[\alpha]_D$ −189 (c=0.28, $CHCl_3$, 22 °C). HR FAB-MS *m/z*: Found: 355.1520; Calcd for $C_{21}H_{23}O_5$ [$(M+H)^+$]: 355.1560. UV (MeOH) λ_{max} nm (ε): 236 (20000), 286 (5200). CD (MeOH, c=6.0×10^{-3}, 20 °C): $[\theta]_{284}$+1.3×10^4 (pos. max.), $[\theta]_{272}$ 0, $[\theta]_{238}$ −1.0×10^5 (neg. max.). IR (KBr) ν_{max} cm^{-1}: 3404, 2918, 1616, 1591, 1496. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.74, 1.79 (each 3H, both s, 4′-H_3, 5′-H_3), 3.34 (1H, m, 6a-H), 3.60 (1H, dd, J=10.9, 11.2 Hz, 6β-H), 3.88 (1H, s, 1-OCH_3), 4.17 (1H, dd, J=5.2, 10.9 Hz, 6α-H), 4.47 (2H, d, J=6.6 Hz, 1′-H), 5.47 (1H, t, J=6.6 Hz, 2′-H), 5.61 (1H, d, J=6.2 Hz, 11a-H), 6.10 (1H, d, J=2.3 Hz, 4-H), 6.16 (1H, d, J=2.3 Hz, 2-H), 6.30 (1H, dd, J=2.0, 7.9 Hz, 8-H), 6.40 (1H, d, J=2.0 Hz, 10-H), 7.05 (1H, d, J=7.9 Hz, 7-H). ^{13}C-NMR: as given in Table 5. FAB-MS (positive) *m/z*: 355 [$(M+H)^+$].

1-Methoxyphaseollidin (**12**), a yellowish oil, $[\alpha]_D$ −165 (c=2.63, $CHCl_3$, 20 °C). HR EI-MS *m/z*: Found: 354.1444; Calcd for $C_{21}H_{22}O_5$ (M^+): 354.1454. UV (MeOH) λ_{max} nm (ε): 287 (15000), 330sh. CD (MeOH, c=1.1×10^{-2}, 20 °C): $[\theta]_{326}$+8.0×10^3 (pos. max.), $[\theta]_{306}$ 0, $[\theta]_{290}$−2.5×10^4 (neg. max). IR (KBr) ν_{max} cm^{-1}: 3400, 2913, 1620, 1587. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.73, 1.78 (each 3H, both s, 4′-H_3, 5′-H_3), 3.35 (1H, m, 6a-H), 3.44 (2H, d, J=6.9 Hz, 1′-H_2), 3.60 (1H, dd, J=10.9, 11.2 Hz, 6β-H), 3.83 (3H, s, 1-OCH_3), 4.15 (1H, dd, J=4.9, 10.9 Hz, 6α-H), 5.27 (1H, t, J=6.9 Hz, 2′-H), 5.56 (1H, d, J=6.3 Hz, 11a-H), 6.01 (1H, d, J=2.0 Hz, 2-H), 6.07 (1H, d, J=2.0 Hz, 4-H), 6.35 (1H, d, J=7.9 Hz, 8-H), 6.93 (1H, d, J=7.9 Hz, 7-H). ^{13}C-NMR: as given in Table 5. EI-MS *m/z* (%): 354 (M^+, 100), 339 [$(M-CH_3)^+$, 68], 311 [$(M-CH_3-H_2O)^+$, 10].

Partial Hydrolysis of Glucoisoliquiritin Apioside (1) with Snail Enzyme A solution of **1** (5 mg) in distilled water (5 ml) was treated with snail enzyme[15] and the whole was incubated with gentle stirring at 37 °C for 2 d. After addition of butanol (5 ml), the whole mixture was heated in a boiling water bath for 2 min. The whole was taken in a separatory funnel and the butanol phase was separated. The aqueous phase was extracted with butanol twice (3 ml each time) and the combined butanol phases were evaporated to dryness under reduced pressure. Preparative TLC (TLC plate: Merck #5744, Pre-Coated Silica gel $60GF_{254}$, 0.5 mm thickness, developed with $CHCl_3$: MeOH : H_2O=6 : 4 : 1), of the butanol extract furnished isoliquiritin apioside (**2**, 3 mg) which was identical with an authentic sample by TLC co-chromatography [1) SiO_2 plate developed with $CHCl_3$: MeOH : H_2O=6 : 4 : 1, 2) reversed-phase SiO_2 plate Merck RP-18 developed with MeOH : H_2O=2 : 3)], optical rotation, IR (KBr), and ^{1}H-NMR (DMSO-d_6) comparisons.

Hydrolysis of Glucoisoliquiritin Apioside (1) with Cellulase A solution of **1** (6 mg) in distilled water (5 ml) was treated with cellulase (Sigma #C9422 from *Trichoderma virire*, *ca.* 20 mg) and the whole was incubated with gentle stirring at 37 °C for 2 d. After addition of ethyl acetate (10 ml) and water (5 ml), the whole mixture was transfered to a separatory funnel and the ethyl acetate phase was separated. The aqueous phase was extracted with ethyl acetate twice (5 ml each time) and the combined ethyl acetate phases were evaporated to dryness under reduced pressure to furnish

isoliquiritigenin (**3**, 2 mg) which was identical with an authentic sample[6] by TLC co-chromatography [1) SiO_2 plate developed with $CHCl_3$: MeOH = 5 : 1, 2) SiO_2 plate developed with hexane : ethyl acetate = 1 : 1)] and IR (KBr) and ^{1}H-NMR ($CDCl_3$) comparisons.

Alkaline Treatment of Glucoliquiritin Apioside (4) Giving Glucoisoliquiritin Apioside (1) A 10% aqueous potassium hydroxide solution (5 ml) was slowly added dropwise to an ice-cooled solution of **4** (15 mg) in ethanol, and the reaction mixture was stirred for 3 h at room temperature (24 °C). After cooling in an ice-bath, the reaction mixture was diluted with water (10 ml) and neutralized with 1 N HCl. The whole was then extracted with butanol (8 ml each time) twice. The butanol extracts were combined and the solvent was evaporated under reduced pressure. The residue was dissolved in water (1 ml) and the solution was adsorbed into Sep-Pak C18 (Waters), then eluted with MeOH-H_2O (2 : 1, 20 ml). Evaporation of the solvent from the eluate under reduced pressure gave the residue, a mixture of *E*- and *Z*-chalcone glycosides (12.5 mg), which was subjected to ^{1}H-NMR analysis without further purification. The ratio of signal intensities due to α-H of the *E* analog (δ 7.79, J=15.2 Hz) and α-H of the *Z* analog (δ 7.60, J=12.8 Hz) was *ca.* 2 : 1. Then, the mixture (12 mg) was subjected to polyamide column chromatography (polyamide C-200, 30 g, CH_3CN : H_2O = 5 : 1) to afford **4a** (*E* analog, 7.2 mg) and **4b** (*Z* analog, 2.8 mg). **4a** thus obtained was identical with **1** by comparison of optical rotation: $[\alpha]_D$ −80 (c=0.72, MeOH, at 22 °C), IR (KBr), and ^{1}H-NMR (DMSO-d_6) spectra with those of **1** isolated by us above.

Acidic Hydrolysis of 7-*O*-Apioglucosyl-7,4′-dihydroxy-flavone (5) A solution of **5** (15 mg) in 5% aqueous HCl (1.5 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was treated with water (10 ml) and neutralized with Ag_2CO_3 powder, then the whole was filtered to remove the inorganic material. The reaction solution was extracted with ethyl acetate twice (5 ml each time) to separate the aglycone (in the ethyl acetate phase) and sugars (in the aqueous phase). The combined ethyl acetate phases were washed with saturated aqueous NaCl, then dried over $MgSO_4$. After removal of the inorganic solid, the ethyl acetate solution was evaporated to dryness under reduced pressure to furnish 7,4′-dihydroxyflavone (**6**, 5.0 mg). **6** thus obtained was identical by comparison of UV, IR (KBr) and ^{1}H-NMR (pyridine-d_5) data with those reported.[16] The aqueous phase (containing sugars) was evaporated under reduced pressure to give the sugar mixture (7.8 mg). The residue (7.8 mg) was subjected to silica gel column chromatography (SiO_2 2 g, $CHCl_3$: MeOH : H_2O = 6 : 4 : 1) to afford D-apiose (2.3 mg) and D-glucose (2.6 mg). Optical rotations were measured at 24 h after dissolving in H_2O in a quartz cell: D-apiose, $[\alpha]_D$ +8 (c=0.23, 22 °C); D-glucose, $[\alpha]_D$ +46 (c=0.26, 22 °C).

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References and Notes

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