FIVE NEW ISOPRENOID-SUBSTITUTED FLAVONOIDS, GLYASPERINS F, G, H, I, AND J FROM THE ROOTS OF GLYCYRRHIZA ASPERA[1]

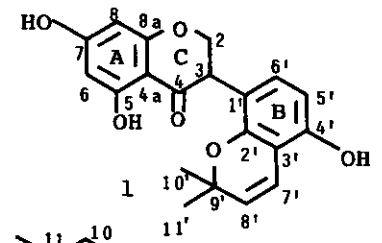
Lu Zeng,[a] Toshio Fukai,[a] Taro Nomura,*,[a] Ru-Yi Zhang,[b] and Zhi-Cen Lou[b]

a) Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274, Japan

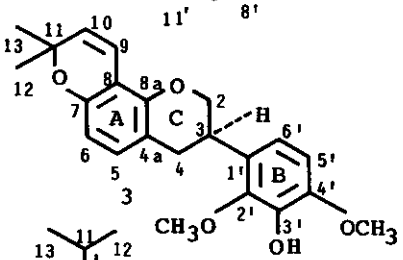
b) School of Pharmaceutical Sciences, Beijing Medical University, Beijing 100083, P. R. China

Abstract——Five new isoprenoid-substituted flavonoids, glyasperins F (**1**), G (**2**), H (**3**), I (**4**), and J (**5**) were isolated from the roots of Glycyrrhiza aspera. The structures of glyasperins F-J were elucidated by spectroscopic methods.

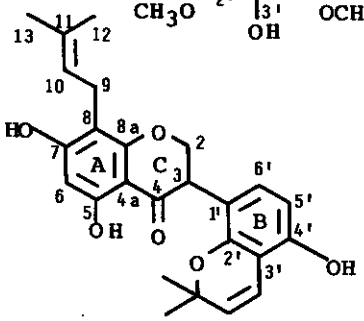
In the previous papers, we reported the isolations and structure determinations of isoprenoid-substituted flavonoids, gancaonins A-E[2] and L-Q,[3,4] from the aerial parts of Glycyrrhiza uralensis Fisch. (Leguminosae), gancaonins F-I[5] from the Xibei licorice (Glycyrrhiza spp., Seihoku Kanzo in Japanese), gancaonin J and flavonoid, gancaonin K[6] from the roots of G. pallidiflora Maxim.; prenylated dihydrostilbenes, gancaonins R-T[4] and prenylated dihydrophenanthrenes, gancaonins U and V[4] from the aerial parts of G. uralensis; isoprenoid-substituted dibenzoylmethanes, glyinflanins A-D[7] from the roots of G. inflata Bat.; and prenylated flavonoids, glyasperins A-D[8] from the roots of G. aspera Pall. To continue the research, we report here another five new isoprenoid-substituted flavonoids, glyasperins F (**1**), G (**2**), H (**3**), I (**4**), and J (**5**) from the roots of G. aspera.



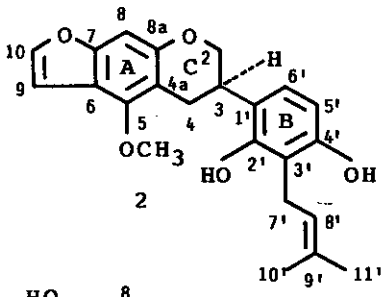
1



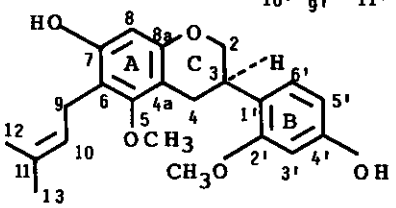
3



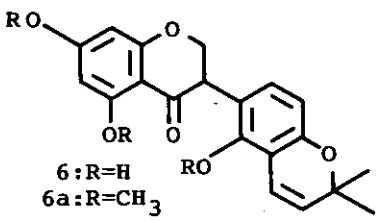
5



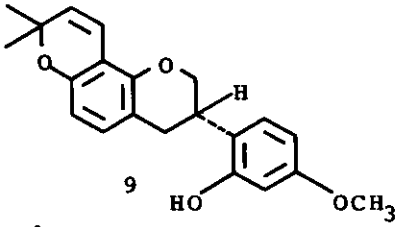
2



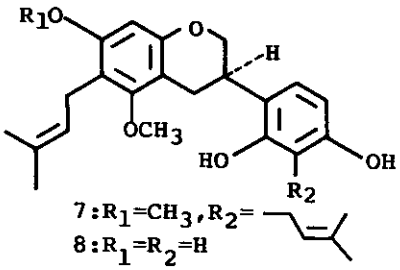
4



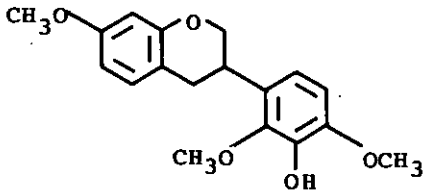
6:R=H
6a:R=CH_3



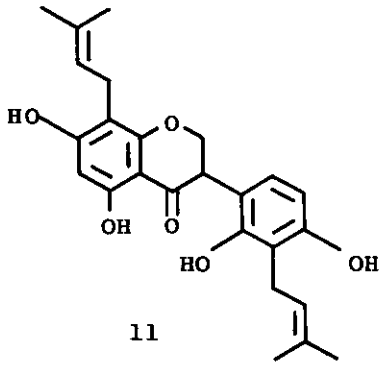
9



7:R_1=CH_3, R_2=
8:R_1=R_2=H



10



11

Figure 1

Table 1. ^{13}C Nmr data of **1**, **5**, and related compounds (**6**, **6a**, and **11**) in acetone-d_6, 100 MHz

C	1	1a		6	6a	5		11	
2	71.25	71.28	(DDd)§,a	70.86	70.77	71.24	(DDd)c	70.94	(Td)
3	47.66	49.49	(Dm)	46.98	48.40	47.62	(Dm)	46.46	(Dm)
4	198.02	188.85	(Sm)	198.25	189.18	198.71	(Sm)	198.87	(Sm)
4a	103.54	107.83	(St)	103.08	107.29	103.82	(St)	102.77	(St)
5	164.57	163.50	(Sm)	164.46	163.64	163.21	(Sdd)	163.53	(Sdd)
6	97.23	94.31	(Dd)	97.02b	94.31	96.50	(Dd)	96.54	(Dd)
7	168.12	166.24	(Sm)	167.58	166.56	164.58	(Sm)	165.36	(Sm)
8	95.96	93.72	(Dm)	95.75b	93.63	108.12	(Sm)	108.30	(Sm)
8a	165.73	165.80	(Sm)	165.80	166.01	161.31	(Sm)	161.05	(Sm)
9						22.10	(Td)	22.05	(Td)
10						123.90	(Dm)	123.81	(Dm)
11						131.20	(Sm)	131.31	(Sm)
12						17.82	(Qm)	17.83	(Qm)
13						25.88	(Qm)	25.88	(Qm)
1'	115.09	111.30	(Sm)	116.35	112.95	115.08	(Sm)	115.45	(Sm)
2'	152.10	152.18	(Sm)	151.72	154.30	152.46	(Sm)	154.89	(Sm)
3'	110.46	118.00	(St)	111.52	122.80	110.35	(Sm)	117.03	(Sm)
4'	153.73	155.12	(Sm)	154.37	155.95	153.60	(Sdt)	156.43	(Sm)
5'	108.50	103.70	(D)	109.63	112.95	108.39	(D)	108.44	(D)
6'	131.14	131.05	(Dd)	130.30	131.31	131.23	(Dd)	126.91	(Dd)
7'	117.83	117.55	(D)	117.47	118.03	117.75	(D)	23.27	(Td)
8'	129.22	129.81	(Dm)	129.98	130.44	129.24	(Dm)	123.69	(Dm)
9'	77.08	76.96	(Sm)	76.19	76.36	77.02	(Sm)	131.61	(Sm)
10'	27.81	27.43	(Qm)	27.87	27.88	27.75	(Qm)	17.95	(Qm)
11'	28.25	28.02	(Qm)	27.96	27.92	28.20	(Qm)	25.88	(Qm)
MeO		55.96	(Q)		56.08				
		56.00	(Q)		56.19				
		56.18	(Q)		62.95				

§: Capital letters refer to the coupling pattern resulting from directly bonded proton(s) and lowercase letters to long-range ^{13}C-^{1}H coupling. a: J=150, 147 and 6 Hz. b: The assignments were made by long-range ^{13}C-^{1}H correlation spectroscopy. c: J=150, 148 and 5 Hz.

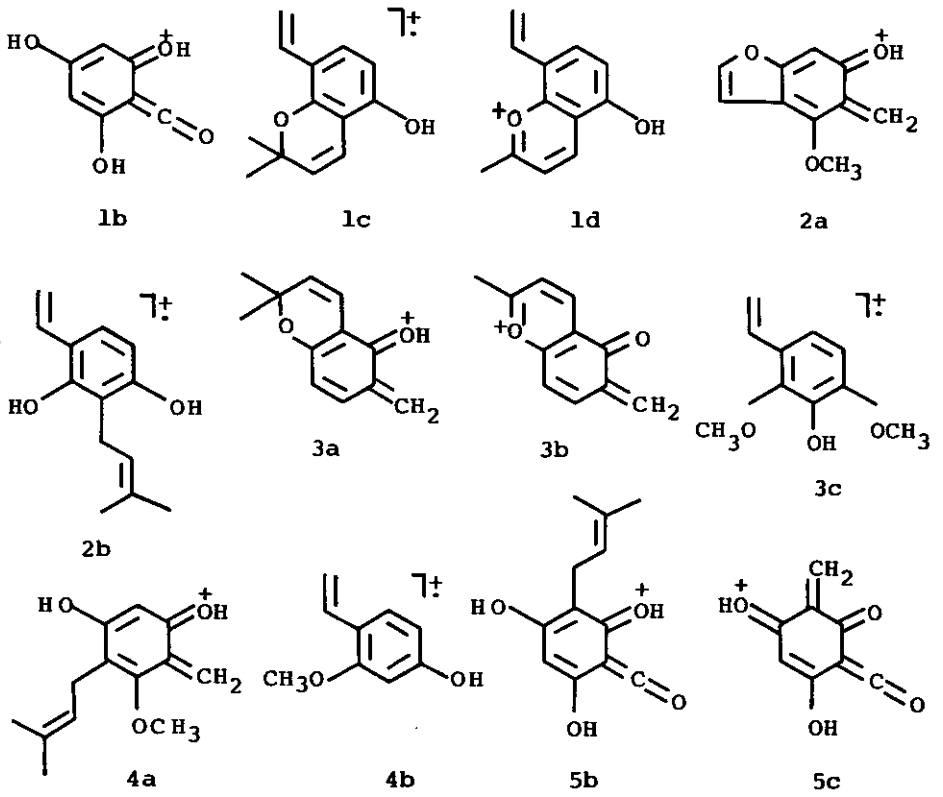


Figure 2

Glyasperin F (**1**) was recrystallized from acetone to form colorless needles, mp 164-166 °C, $C_{20}H_{18}O_6$, $[\alpha]_D^{20}$ -4.6°. The uv spectrum showed that the compound (**1**) was either a flavanone or an isoflavanone derivative.[8,9] The ^{1}H nmr spectrum of **1** exhibited the signals for an isoflavanone skeleton, in which the methylene protons at C-2 appeared as a double boublet (δ 4.39) and a triplet (δ 4.52), and the methine proton at C-3 as a double doublet (δ 4.17), respectively. The ^{1}H nmr spectrum also showed the signals for the following protons: protons in a 2,2-dimethylpyran moiety, a pair of meta coupling aromatic protons, a pair of ortho coupling aromatic protons, and a hydrogen-bonded hydroxyl proton. In the mass spectrum of **1**, the fragment ions at m/z 153 (**1b**), 202 (**1c**), and 187.0574 ($C_{12}H_{11}O_2$, **1d**) were observed as shown in Figure 2. Compairing the ^{13}C nmr spectrum of **1** with that of

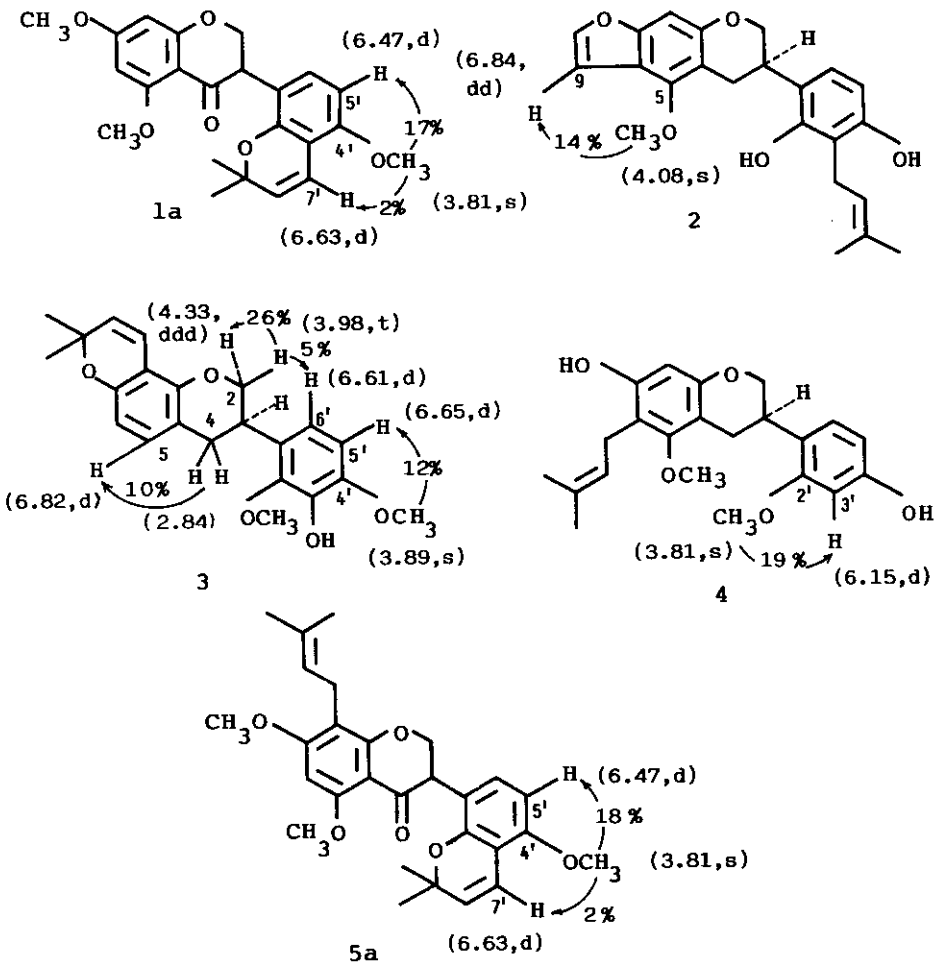


Figure 3 NOE values for **1a**, **2**, **3**, **4**, and **5a**, measured in acetone-d_6 (**1a**, **4**, and **5a**) and $CDCl_3$ (**2** and **3**)

licoisoflavanone (**6**), the chemical shifts for all the carbon atoms of two compounds were found to be nearly the same, except that the C-1', C-3', and C-5' showed 1.26, 1.06, 1.13 ppm differences respectively, indicating that two compounds were structural isomers with different substituted pattern in the B ring. The compound (**1**) was then methylated to form the trimethyl ether (**1a**). In the ^{13}C nmr spectrum of **1a**, the signals for methoxyl groups were observed at δ 55.96, 56.00, and 56.18 respectively (Table 1), which

revealed these methoxyl groups were mono- or none-ortho substituted,[10] and the NOE effects were found between one of the methoxyl groups and C-5' and C-7' protons (Figure 3). All the above evidence indicated that the compound (**1**) bore a hydroxyl group at C-4' position in the B ring.

Thus, the structure of glyasperin F is proposed as the formula (**1**) except for the absolute configuration at C-3 position.[11]

Glyasperin G (**2**) was obtained as an amorphous powder, $C_{23}H_{24}O_5$, $[\alpha]_D^{20}$ +8.3°. The uv spectrum of **2** showed the maxima at 259 and 283 nm. The ^1H nmr spectrum of **2** (in $CDCl_3$) exhibited the signals for an isoflavan skeleton, in which the methylene protons at C-2 appeared as a triplet (δ 4.04) and a doublet of double doublet (δ 4.33), the methine proton at C-3 as a multiplet (δ 3.48), and the methylene protons at C-4 as a double doublet (δ 2.87) and a doublet of double doublet (δ 3.12), respectively. The ^1H nmr spectrum of **2** also showed the signals for the following protons: protons in a 3,3-dimethylallyl (prenyl) group, protons in a methoxyl group, two hydroxyl groups (observed as singlet signals in acetone-d_6 only), aromatic protons (AB type, J=8 Hz), an aromatic proton (doublet due to long-range coupling), and a pair of olefinic protons, of which the chemical shifts (δ 7.43 and 6.84, J=2 Hz) were found to be similar to those for the corresponding protons at C-2 and C-3 in benzofuran ring which appear at δ 7.52 and 6.66 (J=2.5 Hz), respectively.[12] The mass spectrum of **2** gave the fragment ions at m/z 177.0574 ($C_{10}H_9O_3$, **2a**) and 204 (**2b**) indicating that the methoxyl group and furan ring connected to the A ring, and two hydroxyl and the prenyl groups located in the B ring. In the ^{13}C nmr spectrum of **2**, the oxygenated aromatic carbon atoms were observed in the range of δ 155.61-156.67 (Table 2), showing that all the oxygenated aromatic carbon atoms were located each other at meta position,[13] and the signal of methoxyl group appeared at δ 59.80 (di-ortho substituted methoxyl group),[10] which revealed that the substituted groups in the A ring were 5-methoxyl, 7-oxygenated and 6-alkylated moieties; and also the chemical shifts for the

Table 2. ^{13}C Nmr data of **2**, **3**, **4**, and related compounds (**7**, **8**, **9**, and **10**) in acetone-d_6

C	2	7	3	3[a]	9[b]	10[c]	4	8
2	70.73	70.51	71.31	70.56	70.7		70.43	70.37
3	31.97	31.98	32.64	31.72	31.7		32.05	32.26
4	27.37	27.07	32.24	31.64	30.6		26.76	26.61
4a	109.52	109.01	115.45	114.35	114.4		108.33	108.38
5	151.61	157.91	130.15	129.15	129.1		158.34	158.32
6	111.37	115.46	108.00	106.52	102.7[d]		114.46	114.39
7	156.67	157.83	150.58	149.72	150.3		155.49	155.43
8	93.68	96.26	109.35	109.93	109.9		99.83	99.82
8a	154.27	154.64	152.87	151.95	154.2[e]		154.46	154.48
9	105.51	23.15	117.38	117.00	116.9		23.35	23.33
10	143.63	125.04	129.71	129.00	128.0		125.33	125.31
11		130.32	76.09	75.60	75.7		130.31	130.27
12		17.83	27.79	27.53	27.6		17.61	17.90
13		25.86	28.01	27.83	27.8		25.85	25.85
1'	120.67	120.58	117.34	116.92		116.2	111.46	119.80
2'	154.20	154.01	146.80	145.37		146.0	158.34	156.73
3'	116.37	116.19	140.23	138.70	105.6[d]	139.2	99.59	103.58
4'	155.33	155.22	148.51	146.67	151.6[e]	147.7	159.26	158.30
5'	108.35	108.27	109.35	108.71		107.5	107.36	107.72
6'	125.25	125.04	128.00	127.54		126.8	128.42	128.70
7'	23.32	23.23						
8'	123.94	123.78						
9'	131.87	131.78						
10'	17.97	17.94						
11'	25.85	25.83						
MeO	59.80	60.60	56.53	56.25			60.58	60.57
		55.75	60.88	61.05			55.72	

a: Measured in $CDCl_3$. b: See reference 16, measured in $CDCl_3$. c: See reference 17, measured in DMSO-d_6. d, e: The assignments may be interchangeable.

B ring and the prenyl group of **2** were found to be in agreement with those for the relevent carbon atoms of licorisoflavan A (**7**),[14] which indicated that the B ring of **2** was substituted by 2',4'-dihydroxyl and 3'-prenyl groups. The substitution of methoxyl group and the connection of benzofuran ring were further supported by NOE experiments (see Figure 3) and decoupling experiment. When the C-9 proton signal (δ 6.84, dd) was irradiated, the doublet signal at δ 6.75 (for C-8 proton) changed to a singlet. The absolute configuration of **2** was assigned to be 3-(R) with CD spectrum in which the positive Cotton effect exhibited at 290 nm.[15]

The structure of glyasperin G is therefore established as the formula (**2**).

Glyasperin H (**3**) was recrystallized from acetone to give colorless prisms, mp 58-60 °C, $C_{22}H_{24}O_5$, $[\alpha]_D^{20}$ +8.0 °. The ^1H nmr spectrum ($CDCl_3$) showed that the compound (**3**) was also an isoflavan derivative in which the methylene protons at C-2 appeared as a triplet (δ 3.98) and a doublet of double doublet (δ 4.33), the methine proton at C-3 as a multiplet (δ 3.54), and the methylene protons at C-4 as a doublet of double doublet (δ 2.84) and a double doublet (δ 2.93), respectively. The ^1H nmr spectrum of **3** also showed the signals for the following protons: protons in a 2,2-dimethylpyran ring, protons of two methoxyl groups, a pair of AB type aromatic protons (J=8 Hz), and a hydroxyl proton (observed in acetone-d_6 only). The mass spectrum of **3** gave the fragment ions at m/z 189 (**3a**), 173.0642 ($C_{11}H_9O_2$, **3b**), and 180 (**3c**), indicating that the 2,2-dimethylpyran ring was located in the A ring, and one hydroxyl group and two methoxyl groups were located in the B ring. In the ^{13}C nmr spectrum of **3** (in $CDCl_3$), the chemical shifts for the A ring and the 2,2-dimethylpyran ring were found to be in agreement with those for the relevent carbon atoms of (-)-4'-O-methylglabridin (**9**),[16] and the chemical shifts for the B ring to be consistent with that for the relevent carbon atoms of mucromulatol (**10**).[17] The positions substituted by methoxyl groups were first deduced from the chemical shifts of the ^{13}C nmr spectrum of **3** in which one of them appeared at δ 56.25 indicating that this

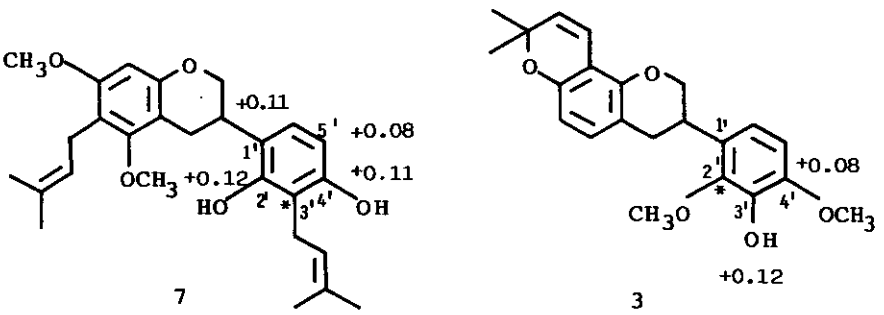


Figure 4 Deuterium induced isotope shift ^{13}C nmr experiments of **7** and **3**. Each 5 mg of samples were measured in 0.6 ml of acetone-d_6, added one drop of the mixture of D_2O and H_2O in 1:1. *These carbon signals were observed as broad singlet.

methoxyl group attached to C-4' position, another at δ 61.50 indicating that this methoxyl group attached either to C-2' or C-3' position.[10] The substituted pattern and the existence of 4'-methoxyl group in the B ring, and the connection of 2,2-dimethylpyran moiety in the A ring were further supported by NOE experiments as shown in Figure 3. The position of 2'- or 3'-hydroxyl group was determined with designed deuterium induced isotope shift in ^{13}C nmr experiments.[18] In the case of model compound, licorisoflavan A (**7**), when the sample was measured in the acetone-d_6, added one drop of the mixture of H_2O and D_2O in 1:1, the signals of carbon atoms substituted by hydroxyl groups and two adjacent carbon atoms shifted upfield. And in the case of the compound (**3**), the signals for oxygenated carbon atoms shifted upfield, whereas the signal for C-1' atom remained the same, which meant that the hydroxyl group was attached to C-3' position. The absolute configuration of **3** was assigned to be 3-(R) with CD spectrum in which the positive Cotton effect exhibited at 277 nm.[15] Consequently, the structure of glyasperin H is represented by the formula (**3**).

Glyasperin I (**4**) was obtained as an amorphous powder, $C_{22}H_{26}O_5$, $[\alpha]_D^{20}$ -5.3°, negative to Gibbs test. The uv, ^1H, and ^{13}C nmr spectra showed that the compound (**4**) was a monomethyl ether of glyasperin C (**8**).[8] The mass spectrum

of **4** gave the fragment ions at m/z 221 (**4a**) and 150 (**4b**). Comparing the ^{13}C nmr spectrum of **4** with that of **8**, the chemical shifts for the carbon atoms in the A ring and prenyl group in two compounds were found to be almost the same, which revealed that the A ring of **4** was also substituted by 7-hydroxyl-5-methoxyl-6-prenyl groups. Furthermore, the NOE experiment was performed (see Figure 3), the result indicated that the methoxyl group in the B ring was substituted in C-2' position. The absolute configuration of **4** was assigned to be 3-(R) with CD spectrum in which the positive Cotton effect exhibited at 288 nm.[15]

Thus, the stucture of glyasperin I is concluded to be the formula (**4**).

Glyasperin J (**5**) was obtained as an amorphous powder, $C_{25}H_{26}O_6$, $[\alpha]_D^{20}$ -39°, positive to ferric chloride ($FeCl_3$) test and negative to Gibbs reagent. The ^1H nmr specturm of **5** exhibited the signals for an isoflavanone skeleton in which the methylene protons at C-2 appeared as a double doublet (δ 4.48) and a triplet (δ 4.56), the methine proton at C-3 as a double doublet (δ 4.19), respectively. The ^1H nmr spectrum also showed the signals for the following protons: protons of a prenyl group, protons of a 2,2-dimethylpyran ring, an isolated aromatic proton, vicinal aromatic protons (AB type, J=8 Hz), two hydroxyl protons, and a hydrogen-bonded hydroxyl proton. The mass spectrum of **5** gave the fragment ions at m/z 221 (**5b**), 165 (**5c**), 202 (**1c**), and 187 (**1d**), indicating that the prenyl group and two hydroxyl gorups were located in the A ring, and the 2,2-dimethylpyran ring and a hydroxyl group were substituted in the B ring. In the ^{13}C nmr spectrum of **5**, the chemical shifts for the A ring carbon atoms and prenyl group were found to be in agreement with those for the relevent carbon atoms of 3'-(γ,γ-dimethylallyl)-kievitone (**11**),[8,19] and the chemical shifts for the B ring carbon atoms and 2,2-dimethylpyran ring to be consistent with those for the relevent carbon atoms of glyasperin F (**1**) as shown in the Table 1. The position of prenyl group in the A ring was determined by measuring ^{13}C nmr spectrum using gated decoupling with NOE technique, in which the C-6

signal at δ 96.50 was observed as a double doublet, ^{1}J=161 Hz and $^{3}J_{C(6)\text{-}OH(5)}$=7 Hz. And the substituted pattern in the B ring was further supported by NOE experiment on the trimethyl ether of **5** (**5a**) as shown in the Figure 3.

Thus, the structure of glyasperin J is proposed as the formula (**5**) except for the absolute configuration at C-3 position.[11]

EXPERIMENTAL

The general procedures followed, and the instruments and chemicals were used as described in the previous paper.[7] *For preparative tlc (silica gel), Wakogel B-5F was used. Digital* resolutions on ^{1}H and ^{13}C nmr measurements were 0.18 and 1.5 Hz respectively.

Plant materials

The roots of Glycyrrhiza aspera were used as described in the previous paper.[7] The voucher specimen has been deposited in the drug museum of the Department of Pharmacognosy, School of Pharmaceutical Sciences, Beijing Medical University, P. R. China.

Isolation of phenolic compounds from Glycyrrhiza aspera roots

The extract were used similarly as described in the previous paper.[7] The benzene eluate (33.6 g) from Amberlite XAD-2 was subjected to a silica gel (260 g) column chromatography (column A) and eluted with n-hexane (fraction 1-2), n-hexane-benzene=5:1→1:7 (fr. 3-9), benzene (fr. 10-12), benzene-ethyl ehter=20:1→1:5 (fr. 13-27), benzene-acetone=8:1→1:2 (fr. 28-33). The fractions (500 ml each) were monitored by tlc. The fraction 9 (eluent, n-hexane-benzene=1:7, yield 0.39 g) was purified by preparative tlc (solvent system, n-hexane-ethyl ehter=4:1, multiple developments, x3, n-hexane-acetone=5:1, x3) to give licorisoflavan A (**7**, 0.25 g), glyasperin G (**2**, 2 mg), and glyasperin H (**3**, 1.9 mg). The fraction 14 was subjected to a silica gel (100 g) column chromatography (column B) and eluted with n-hexane-acetone=19:1→1:1 (fr. 1-17). The fraction 6 was purified by preparative tlc (solvent system, $CHCl_3$-AcOEt=7:1, $CHCl_3$-AcOEt=10:1, x5, benzene-acetone =3:1, x4, benzene-acetone=3:1, x4, benzene-ethyl ether=6:1, x4) to give glyasperin I (**4**, 1.9 mg) and glyasperin J (**5**, 17 mg). The fraction 15 of column A (eluent, benzene-ethyl ether=10:1, 1.96 g) was subjected to a silica gel (100 g) column chromatography and eluted with n-hexane-acetone=100:1→2:1 (fr. 1-19). The fraction 8 was purified by preparative tlc (solvent system, n-hexane-AcOEt=6:1, x6, benzene-ethyl ether=6:1, x4, $CHCl_3$-acetone =20:1, x3) to give glyasperin F (**1**, 11 mg).

Glyasperin F (1)

The compound (1) was recrystallized from acetone to give colorless needles, mp 164-166°C, positive to $FeCl_3$ test (brown), $[\alpha]_D^{20}$ -4.6°(c=0.5, $CHCl_3$-acetone=1:1). Uv ν_{max}^{MeOH} nm (log ε): 287 (4.37), 322 (sh 3.80). Uv $\nu_{max}^{MeOH+AlCl_3}$ nm (log ε): 310 (4.81), 370 (3.98). Uv $\nu_{max}^{MeOH+AcONa}$ nm (log ε): 290 (4.48), 325 (4.80). Uv $\nu_{max}^{MeOH+MeONa}$ nm (log ε): 324 (4.86). EI-Ms (probe) 70 eV, m/z (rel. int.): 355 $[M+1]^+$ (6), 354 $[M]^+$ (17), 341 (7), 340 (36), 339 (100), 272 (6), 202 (4), 188 (12), 187 (55), 186 (5), 173 (8), 153 (14), 150 (11), 69 (5). HR-Ms, m/z: 354.1073 $[M]^+$ ($C_{20}H_{18}O_6$, requires: 354.1098). ^1H Nmr (acetone-d_6): δ 1.34, 1.35 (each 3H, CH_3), 4.17 (1H, dd, J=6 and 11 Hz, C-3-H), 4.39 (1H, dd, J=6 and 11 Hz, C-2-H), 4.52 (1H, t, J=11 Hz, C-2-H), 5.62 (1H, d, J=10 Hz, C-8'-H), 5.95, 5.97 (each 1H, d, J=2 Hz, C-6, 8-H), 6.40 (1H, d, J=8 Hz, C-5'-H), 6.68 (1H, d, J=10 Hz, C-7'-H), 6.85 (1H, d, J=8 Hz, C-6'-H), 12.40 (1H, br, OH).

Trimethyl ether of glyasperin F (1a)

A mixture of the compound (1) (5 mg), dimethyl sulfate (0.5 ml), and anhydrous potassium carbonate, and acetone (20 ml) was refluxed for 1 h, and treated as usual. The compound (1a) was obtained as an amorphous powder. EI-Ms, m/z: 396 $[M]^+$. ^1H Nmr (acetone-d_6): δ 1.33, 1.34 (each 3H, s, CH_3), 3.81, 3.83, 3.87 (each 3H, s, OCH_3), 3.99 (1H, dd, J=5 and 11 Hz, C-3-H), 4.40 (1H, dd, J=5 and 11 Hz, C-2-H), 4.52 (1H, t, J=11 Hz, C-2-H), 5.64 (1H, d, J=10 Hz, C-8'-H), 6.13, 6.22 (each 1H, d, J=2 Hz, C-6, 8-H), 6.47 (1H, d, J=8 Hz, C-5'-H), 6.63 (1H, d, J=10 Hz, C-7'-H), 6.93 (1H, d, J=8 Hz, C-6'-H).

Glyasperin G (2)

The compound (2) was obtained as an amorphous powder, negative to $FeCl_3$ test, $[\alpha]_D^{20}$ +8.3° (c=0.19, $CHCl_3$). Uv ν_{max}^{MeOH} nm (log ε): 259 (4.01), 283 (3.84). EI-Ms m/z: 381 $[M+1]^+$ (27), 380 $[M]^+$ (100), 204 (12), 203 (21), 202 (10), 201 (6), 192 (13), 191 (44), 190 (21), 189 (47), 188 (39), 187 (18), 178 (13), 177 (84), 176 (28), 175 (10), 173 (9), 162 (11), 161 (28), 147 (36), 135 (32), 133 (18), 131 (11), 115 (6), 91 (14), 77 (13), 69 (7). HR-Ms, m/z: 380.1594 $[M]^+$ ($C_{23}H_{24}O_5$, requires: 380.1617). ^1H Nmr (acetone-d_6): δ 1.66 (3H, br d, J=1 Hz, CH_3), 1.78 (3H, br s, CH_3), 2.89 (1H, dd, J=10 and 16 Hz, C-4-H), 3.05 (1H, ddd, J=2, 5 and 16 Hz, C-4-H), 3.45 (2H, d, J=7 Hz, C-7'-H_2), 3.47 (1H, m, C-3-H), 4.00 (1H, t, J=10 Hz, C-2-H), 4.10 (3H, s, OCH_3), 4.25 (1H, ddd, J=2, 3 and 10 Hz, C-2-H), 5.26 (1H, br t, J=7 Hz, C-8'-H), 6.45 (1H, d, J=8 Hz, C-5'-H), 6.66 (1H, d, J=1 Hz, C-8-H), 6.85 (1H, d, J=8 Hz, C-6'-H), 7.03 (1H, dd, J=1 and 2 Hz, C-9-H), 7.18 (1H, s, OH), 7.61 (1H, d, J=2 Hz, C-10-H), 8.12 (1H, s, OH). ^1H Nmr ($CDCl_3$): δ 1.79 (3H, br d, J=1 Hz, CH_3), 1.85 (3H, br s, CH_3), 2.87 (1H, dd, J=11 and 16 Hz, C-4-H), 3.12 (1H, ddd, J=2, 5 and 16 Hz, C-4-H), 3.44 (2H, d, J=7 Hz, C-7'-H_2), 3.48 (1H, m, C-3-H), 4.04 (1H, t, J=10 Hz, C-2-H), 4.08

(3H, s, OCH_3), 4.33 (1H, ddd, J=2, 4 and 10 Hz, C-2-H), 5.28 (1H, br t, J=7 Hz, C-8'-H), 6.37 (1H, d, J=8 Hz, C-5'-H), 6.75 (1H, d, J=1 Hz, C-8-H), 6.84 (1H, dd, J=1 and 2 Hz, C-9-H), 6.86 (1H, d, J=8 Hz, C-6'-H), 7.43 (1H, d, J=2 Hz, C-10-H). CD (c=2.97 $x10^{-4}$ mol/l, MeOH): $[\theta]_{214}$ 0, $[\theta]_{228}$ +1.1 $x10^6$, $[\theta]_{240}$ +3.4 $x10^5$, $[\theta]_{254}$ +6.2 $x10^5$, $[\theta]_{276}$ +5.1 $x10^4$, $[\theta]_{290}$ +1.3 $x10^5$.

Glyasperin H (3)

The compound (**3**) was recrystallized from acetone to give colorless prisms, mp 58-60 °C, negative to $FeCl_3$ test, $[\alpha]_D^{20}$ +8.0°(c=0.10, $CHCl_3$). Uv ν_{max}^{MeOH} nm (log ε): 279 (4.21), 290 (sh 4.10), 310 (sh 3.72). EI-Ms, m/z: 369 $[M+1]^+$ (6), 368 $[M]^+$ (24), 354 (24), 353 (100), 189 (5), 180 (4), 174 (7), 173 (41), 167 (12), HR-Ms, m/z: 368.1633 $[M]^+$ ($C_{22}H_{24}O_5$, requires: 368.1617). ^1H Nmr (acetone-d_6): δ 1.38, 1.39 (each 3H, s, CH_3), 2.80-2.86 (1H, overlap with water, C-4-H), 2.93 (1H, ddd, J=2, 11 and 16 Hz, C-4-H), 3.47 (1H, m, C-3-H), 3.83, 3.88 (each 3H, s, OCH_3), 4.00 (1H, t, J=10 Hz, C-2-H), 4.29 (1H, ddd, J=2, 3 and 10 Hz, C-2-H), 5.64 (1H, d, J=10 Hz, C-10-H), 6.30 (1H, dd, J=1 and 8 Hz, C-6-H), 6.62 (1H, dd, J=1 and 10 Hz, C-9-H), 6.67 (1H, dd, J=8 Hz, C-6'-H), 6.74 (1H, d, J=8 Hz, C-5'-H), 6.84 (1H, d, J=8 Hz, C-5-H), 7.53 (1H, s, OH). ^1H Nmr ($CDCl_3$): δ 1.41, 1.43 (each 3H, s, CH_3), 2.84 (1H, ddd, J=2, 5 and 15 Hz, C-4-H), 2.93 (1H, ddd, J=1, 10 and 15 Hz, C-4-H), 3.54 (1H, m, C-3-H), 3.89, 3.90 (each 3H, s, OCH_3), 3.98 (1H, t, J=10 Hz, C-2-H), 4.33 (1H, ddd, J=2, 4 and 10 Hz, C-2-H), 5.56 (1H, d, J=10 Hz, C-10-H), 6.37 (1H, dd, J=1 and 8 Hz, C-6-H), 6.61 (1H, d, J=9 Hz, C-6'-H), 6.64 (1H, dd, J=1 and 10 Hz, C-9-H), 6.65 (1H, d, J=9 Hz, C-5'-H), 6.82 (1H, d, J=8 Hz, C-5-H). CD (c=1.69 $x10^{-4}$ mol/l): $[\theta]_{214}$ 0, $[\theta]_{226}$ -2.5 $x10^6$, $[\theta]_{234}$ 0, $[\theta]_{236}$ +5.6 $x10^5$, $[\theta]_{242}$ 0, $[\theta]_{244}$ -1.2 $x10^5$, $[\theta]_{277}$ +4.4 $x10^5$, $[\theta]_{300}$ +1.5 $x10^5$.

Glyasperin H (4)

The compound (**4**) was obtained as an amorphous powder, negative to $FeCl_3$ and Gibbs tests, $[\alpha]_D^{20}$-5.3 °(c=0.095, $CHCl_3$). Uv ν_{max}^{MeOH} nm (log ε): 281 (3.77). EI-Ms, m/z: 371 $[M+1]^+$ (24), 370 $[M]^+$ (100), 355 (20), 351 (18), 302 (5), 233 (10), 222 (11), 221 (79), 205 (8), 191 (5), 189 (5), 178 (5), 177 (24), 165 (30), 164 (6), 163 (9), 151 (6), 150 (38), 149 (8), 138 (5), 137 (33), 135 (21), 107 (7), 69 (7). HR-Ms, m/z: 370.1781 $[M]^+$ ($C_{22}H_{26}O_5$, requires: 370.1773). ^1H Nmr (acetone-d_6): δ 1.65 (3H, br d, J=1 Hz, CH_3), 1.74 (3H, br s, CH_3), 2.78 (1H, dd, J=11 and 16 Hz, C-4-H), 2.87 (1H, ddd, J=2, 6 and 16 Hz, C-4-H), 3.28 (1H, m, C-9-H_2), 3.38 (1H, m, C-3-H), 3.69, 3.81 (each 3H, s, OCH_3), 3.92 (1H, t, J=10 Hz, C-2-H), 4.15 (1H, ddd, J=2, 3 and 10 Hz, C-2-H), 5.25 (1H, br t, J=7 Hz, C-10-H), 6.17 (1H, s, C-8-H), 6.41 (1H, dd, J=2 and 8 Hz, C-5'-H), 6.51 (1H, d, J=2 Hz, C-3'-H), 7.01 (1H, d, J=8 Hz, C-6'-H), 8.11, 8.19 (each 1H, br s, OH). CD (c=3.76 $x10^{-4}$ mol/l): $[\theta]_{213}$ 0, $[\theta]_{233}$ +3.5 $x10^5$, $[\theta]_{228}$ 0, $[\theta]_{232}$ -1.5 $x10^5$, $[\theta]_{254}$ -2.7 $x10^4$, $[\theta]_{276}$ -1.9 $x10^5$,

$[\theta]_{284}$ 0, $[\theta]_{288}$ +1.2 x10^5, $[\theta]_{296}$ 0.

Glyasperin J (5)

The compound (5) was obtained as an amorphous powder, positive to $FeCl_3$ test (brown) and negative to Gibbs test, $[\alpha]_D^{20}$ -39°(c=0.69, MeOH). Uv ν_{max}^{MeOH} nm (log ε): 290 (4.82), 340 (sh 3.46). Uv $\nu_{max}^{MeOH+AlCl_3}$ nm (log ε): 275 (sh 3.98), 314 (4.34), 387 (3.54). Uv $\nu_{max}^{MeOH+AcONa}$ nm (log ε): 289 (4.07), 330 (4.21). Uv $\nu_{max}^{MeOH+MeONa}$ nm (log ε): 330 (4.37). EI-Ms, m/z: 423 $[M+1]^+$ (8), 422 $[M]^+$ (26), 408 (26), 407 (100), 354 (6), 340 (27), 339 (29), 285 (5), 221 (30), 203 (6), 202 (7), 189 (9), 188 (11), 187 (71), 173 (12), 165 (29), 153 (7), 150 (19), 137 (10), 69 (5). HR-Ms, m/z: 422.1694 $[M]^+$ ($C_{25}H_{26}O_6$, requires: 422.1722). ^1H Nmr (aectone-d_6): δ 1.337, 1.340 (each 3H, s, CH_3), 1.64 (3H, br d, J=1 Hz, CH_3), 1.73 (3H, br s, CH_3), 3.24 (2H, br d, J=7 Hz, C-9-H_2), 4.19 (1H, dd, J=6 and 11 Hz, C-3-H), 4.48 (1H, dd, J=6 and 11 Hz, C-2-H), 4.56 (1H, t, J=11 Hz, C-2-H), 5.21 (1H, br t, J=7 Hz, C-10-H), 5.63 (1H, d, J=10 Hz, C-8'-H), 6.06 (1H, s, C-6-H), 6.40 (1H, d, J=8 Hz, C-5'-H), 6.68 (1H, d, J=10 Hz, C-7'-H), 6.89 (1H, d, J=8 Hz, C-6'-H), 8.60, 9.50 (each 1H, br, OH), 12.36 (1H, s, C-5-OH).

Trimethyl ether of glyasperin J (5a)

A mixture of the compound (5) (5 mg), dimethyl sulfate (0.5 ml), and anhydrous patassium carbonate, and acetone (20 ml) was refluxed for 1 h, and treated as usual. The compound (5a) was obtained as an amorphous powder. EI-Ms, m/z: 464 $[M]^+$. ^1H Nmr (acetone-d_6): δ 1.32, 1.35 (each 3H, s, CH_3), 1.63 (3H, br d, J=1 Hz, CH_3), 1.73 (3H, br s, CH_3), 3.25 (2H, br d, J=7 Hz, C-9-H_2), 3.81, 3.86, 3.95 (each 3H, s, OCH_3), 3.99 (1H, dd, J=6 and 11 Hz, C-3-H), 4.43 (1H, dd, J=6 and 11 Hz, C-2-H), 4.50 (1H, t, J=11 Hz, C-2-H), 5.15 (1H, br t, J=7 Hz, C-10-H), 5.64 (1H, d, J=10 Hz, C-8'-H), 6.39 (1H, s, C-6-H), 6.47 (1H, d, J=8 Hz, C-5'-H), 6.63 (1H, d, J=10 Hz, C-7'-H), 6.94 (1H, d, J=8 Hz, C-6'-H).

ACKNOWLEDGEMENT

We are grateful to Mr. T. Takakuwa, JASCO Co. Ltd., for CD spectral measurement.

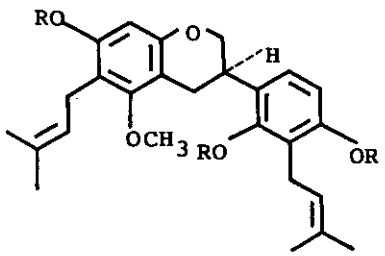
REFERENCES AND NOTES

1. Part 9 in the series 'Phenolic Constituents of Glycyrrhiza Species.'

For part 8: see reference 8.

2. T. Fukai, Q.-H. Wang, and T. Nomura, Heterocycles, 1989, **29**, 1369.
3. T. Fukai, Q.-H. Wang, M. Takayama, and T. Nomura, Heterocycles, 1990, **31**, 373.
4. T. Fukai, Q.-H. Wang, and T. Nomura, Phytochemistry, 1991, **30**, 1245.
5. T. Fukai, Q.-H. Wang, T. Kitagawa, K. Kusano, T. Nomura, and Y. Iitaka, Heterocycles, 1989, **29**, 1761.
6. T. Fukai, Q.-H. Wang, R. Inami, and T. Nomura, Heterocycles, 1990, **31**, 643.
7. L. Zeng, T. Fukai, T. Kaneki, T. Nomura, R.-Y. Zhang, and Z.-C. Lou, Heterocycles, 1992, **34**, 85.
8. L. Zeng, T. Fukai, T. Nomura, R.-Y. Zhang, and Z.-C. Lou, Heterocycles, 1992, **34**, 575.
9. T. J. Mabry, K. R. Markham, and M. B. Thomas, 'The Systematic Identification of Flavonoids,' Springer, Berlin, 1970, p. 33.
10. K. S. Dhami and J. B. Stothers, Can. J. Chem., 1966, **44**, 2855.
11. The compounds (**1**) and (**5**) were obtained as optical active forms, but the CD spectra of both compounds showed no valuable Cotton effects in the range of 330-350 nm, since the racemization may occur under relatively mild conditions (see reference 20).
12. E. Pretsch, T. Clerc, J. Seibl, and W. Simon, 'Tabellen zur Strukturaufklärung organischer Verbindungen mit spektroskopischen Methoden,' Zweite Auflage, Springer, Berlin, 1981, p. H325.
13. K. R. Markham, V. M. Chari, and T. J. Mabry, 'The Flavonoids: Advances in Research,' eds by J. B. Harborne and T. J. Mabry, Chapman & Hall, New York, 1982, p. 21.
14. The absolute configuration of licorisoflavan A (**7**) at C-3 position was determined as 3-(R) with the following experiment. The compound (**7**) and licoricidin(**12**)[21] were methylated by dimethyl sulfate and potassium carbonate in acetone to form permethylated products (**7a**, m/z 466 $[M]^+$)

and (**12a**, m/z 466 $[M]^+$), respectively. The compounds (**7a**) and (**12a**) showed the same optical rotatory, $[\alpha]_D^{20}$ +19.4°(c=0.23, $CHCl_3$), indicating that two compounds have same configuration at C-3 position.



7a=**12a**, R=CH_3

12, R=H

15. a) K. Kurosawa, W. D. Ollis, B. T. Redman, I. O. Sutherland, H. M. Alves, and O. R. Gottlieb, Phytochemistry, 1978, **17**, 1423. b) F. R. van Heerden, E. V. Brandt, and D. G. Roux, J. Chem. Soc., Perkin Trans. I, 1978, 137.

16. O. Castro, J. Lopez, A. Vergara, F. R. Stermitz, and D. R. Gardner, J. Nat. Prod., 1986, **49**, 680.

17. M. O. Hamburger, G. A. Cordell, P. Tantivatana, and N. Ruangrungsi, J. Nat. Prod., 1987, **50**, 696.

18. Y. Shirataki, I. Yokoe, M. Noguchi, T. Tomimori, and M. Komatsu, Chem. Pharm. Bull., 1988, **36**, 2220 and references cited therein.

19. M. J. O'Neill, S. A. Adesanya, M. F. Roberts, and I. R. Pantry, Phytochemistry, 1986, **25**, 1315.

20. P. M. Dewick, 'The Flavonoids: Advances in Research,' eds by J. B. Harborne and T. J. Mabry, Chapman & Hall, New York, 1982, p. 535.

21. T. Fukai, M. Toyono, and T. Nomura, Heterocycles, 1988, **27**, 2309.

Received, 14th May, 1992