FOUR NEW ISOPRENOID-SUBSTITUTED DIBENZOYLMETHANE DERIVATIVES, GLYINFLANINS A, B, C, AND D FROM THE ROOTS OF GLYCYRRHIZA INFLATA[1]

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

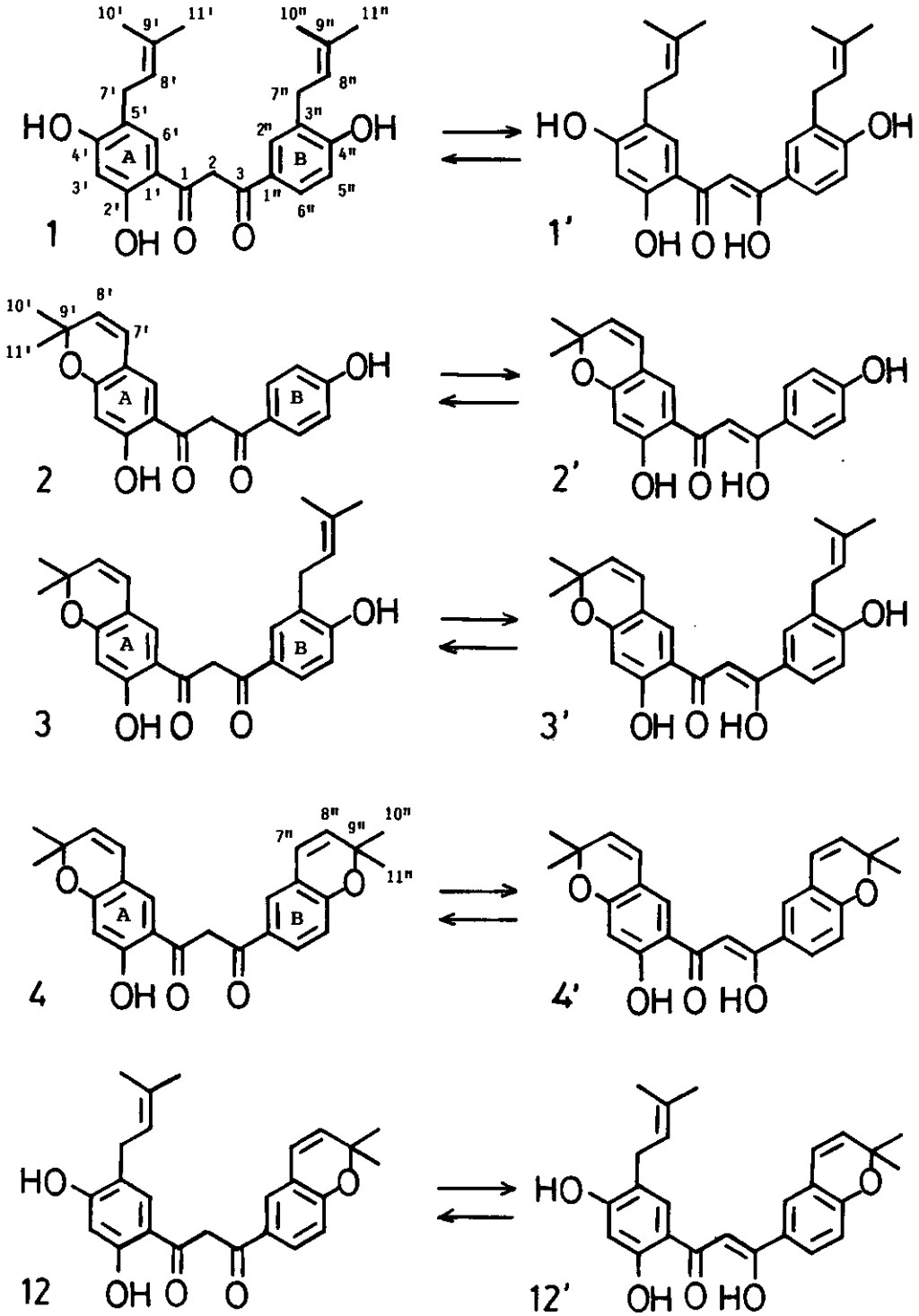
a) Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274, Japan
b) School of Pharmaceutical Sciences, Beijing Medical University, Beijing 100083, P. R. China

Abstract —— Four new isoprenoid-substituted dibenzoylmethane derivatives, glyinflanins A (**1**), B (**2**), C (**3**), and D (**4**) were isolated from the roots of Glycyrrhiza inflata. The structures of glyinflanins A - D were elucidated by spectroscopic and chemical methods.

We reported earlier the structures of isoprenoid-substituted phenols from Xibei licorice (Glycyrrhiza species, Seihoku Kanzo in Japanese), the aerial parts of G. uralensis Fisch. et DC., and the aerial parts and roots of G. pallidiflora Maxim.[2,3] In the continuation of the investigation, we studied the phenolic constituents of the roots of G. inflata Bat. (Leguminosae), which is one of the main species of Xibei licorice.[4] On the phenolic constituents of the roots, Yang and Liu reported the isolation of eight known compounds.[5] In the present study four new isoprenoid-substituted dibenzoylmethane derivatives, glyinflanins A (**1**), B (**2**), C (**3**), and D (**4**) were isolated along with two known compounds, glabrone (**5**)[6] and lico-

chalcone A (**6**).[7]

Glyinflanin A (**1**),[8] yellow needles, mp 100-102 °C, $C_{25}H_{28}O_5$, gave a dark green color with methanolic ferric chloride test. The uv spectrum of **1** resembles the spectra of dibenzoylmethane derivatives, licodione (**7**),[9] and 5'-prenyl-licodione (**8**).[10] Under uv illumination, the orange fluorescence of this compound (**1**) on tlc plate changed to blue after spraying with sulfuric acid and subsequent heating slightly. This color reaction indicates that **1** is a 2'-hydroxydibenzoylmethane derivative such as **7** and **8**.[9,10] The mass spectrum of **1** shows the characteristic fragment ions at m/z 205.0870 ($C_{12}H_{13}O_3$, **1a**) and 189.0864 ($C_{12}H_{13}O_2$, **1b**).[9,10] The ^1H and ^{13}C nmr spectra (acetone-d_6) of glyinflanin A appeared as the equilibrium mixture of a dibenzoylmethane and a ß-hydroxychalcone (a tautomer of dibenzoylmethane) moiety in the solution (Tables 1 and 2).[10,11] The ^1H nmr spectrum of the compound shows singlet signals at δ 4.54 (0.8H) and 6.85 (0.6H), disappeared with the addition of CD_3OD, which are attributed to the α-proton (C-2-H) of dibenzoylmethane existing in the equilibrium mixture of keto and enol forms (Table 1).[10,11] The signals of hydrogen-bonded hydroxy groups appeared at δ 12.27 (0.6H), 12.31 (0.4H), and 15.78 (0.6H) along with the signal of two hydroxyl groups [δ 9.14 (2H, br s)]. All other signals appeared as pairs arising from the two tautomeric structures. In the ^1H nmr spectrum, the ratio of two tautomers (keto and enol forms) was changed with the concentration, when 10 mg of sample in 0.6 ml of acetone-d_6, the ratio is about 2:3, and when 60 mg of sample in 0.6 ml of the same solvent, the ratio is about 1:1. The ^{13}C nmr spectrum of the compound was analyzed by using gated decoupling with NOE, heteronuclear shift correlation spectroscopy (C,H-COSY), and correlation spectroscopy via long-range coupling (COLOC), and by comparing the spectrum with those of licodione (**7**),[11] broussochalcone A (**9**),[12] and gancaonin Q (**10**)[2] as shown in Table 2. The analysis of the ^1H and ^{13}C nmr spectra revealed the presence of two 3-methyl-2-butenyl (prenyl) groups in the compound (Tables 1 and 2). In the ^1H nmr spectrum, the aromatic protons appeared as two pairs of


1
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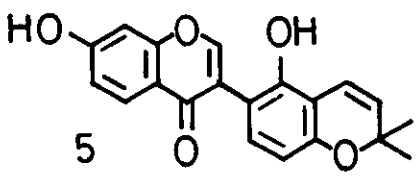

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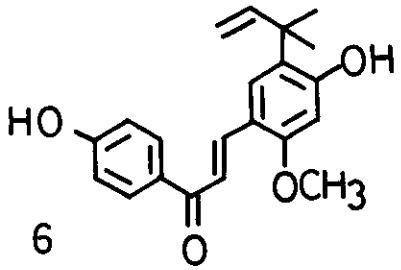

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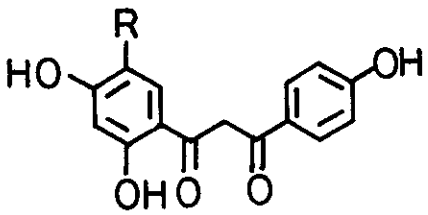

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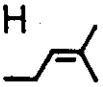
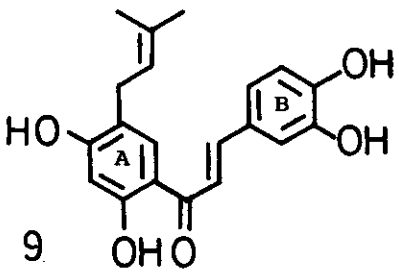
8: R = 



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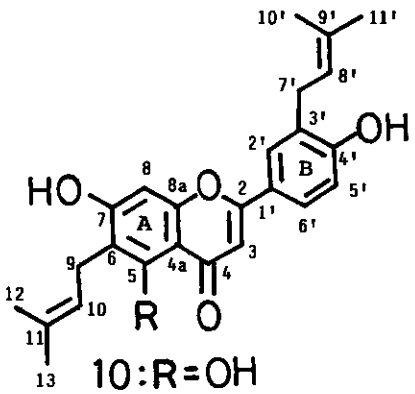

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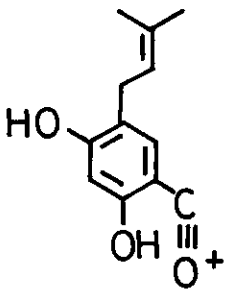
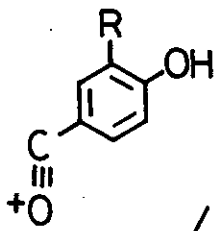
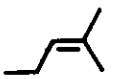


1a



1b: R = 

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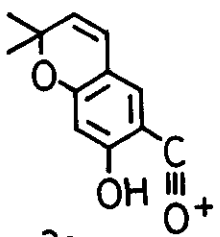
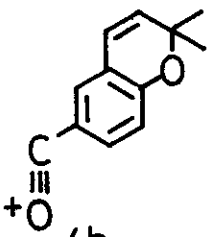
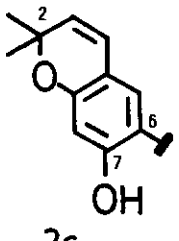


2a



4b



2c

Table 1. ^{1}H Nmr data of **1**-**4** in acetone-d_6 (400 MHz)

proton No.	**1**[a] (enol form)	(keto form)	**2**[b] (enol form)	(keto form)
2	6.85 (0.6H, s)	4.54 (0.8H, s)	7.02 (0.8H, s)	4.68 (0.4H, s)
3'	6.40 (0.6H, s)	6.37 (0.4H, s)	6.26 (0.8H, s)	6.25 (0.2H, s)
6'	7.71 (0.6H, s)	7.59 (0.4H, s)	7.87 (0.8H, s)	7.69 (0.2H, s)
2"	7.80 (0.6H, d)	7.84 (0.4H, d) (2",6")	7.98 (1.6H, d)	7.96 (0.4H, d)
5"	6.97 (0.6H, d)	6.95 (0.4H, d) (3",5")	6.98 (1.6H, d)	6.96 (0.4H, d)
6"	7.74 (0.6H, dd)	7.79 (0.4H, dd)		
7'	3.29 (1.2H, br d)	3.21 (0.8H, br d)	6.42 (0.8H, d)	6.37 (0.2H, d)
7"	3.40 (1.2H, br d)	3.37 (0.8H, br d)		
8'	5.32-5.42 (1.6H, m)	5.26 (0.4H, br t)	5.71 (0.8H, d)	5.70 (0.2H, d)
8"				
9'-Me_2	1.62, 1.63 (each 1.8H, br s), 1.71 (1.8H, br s)		1.44 (4.8H, s)	1.43 (1.2H, s)
9"-Me_2	1.74 (4.2H, br s), 1.75 (3.6H, br s)			
3-OH	15.78 (0.6H,br s)		15.70 (0.8H, br s)	
2'-OH	12.27 (0.6H, br s)	12.31 (0.4H, s)	12.49 (0.8H, br s)	12.55 (0.2H, s)
OH	9.14 (2H, br s)		9.30 (1H, br s)	

proton No.	**3**[c] (enol form)	(keto form)	**4**[d] (enol form)	(keto form)
2	6.99 (0.7H, s)	4.63 (0.6H, s)	7.00 (0.7H, s)	4.66 (0.6H, s)
3'	6.27 (0.7H, s)	6.23 (0.3H, s)	6.26 (0.7H, s)	6.25 (0.3H, s)
6'	7.80 (0.7H, s)	7.69 (0.3H, s)	7.81 (0.7H, s)	7.66 (0.3H, s)
2"	7.84 (0.7H, d)	7.81 (0.3H, d)	7.78 (0.7H, d)	7.77 (0.3H, d)
5"	6.99 (0.7H, d)	6.96 (0.3H, d)	6.85 (0.7H, d)	6.83 (0.3H, d)
6"	7.80 (0.7H, dd)	7.78 (0.3H, dd)	7.86 (0.7H, dd)	7.85 (0.3H, dd)
7'	6.40 (0.7H, d)	6.37 (0.3H, d)	6.41 (0.7H, d)	6.37 (0.3H, d)
8'	5.70 (0.7H, d)	5.69 (0.3H, d)	5.71 (0.7H, d)	5.70 (0.3H, d)
9'-Me_2	1.43 (4.2H, s)	1.42 (1.8H, s)	1.45 (2.1H, s)	1.44 (0.9H, s)
7"	3.39 (1.4H, br d)	3.36 (0.6H, br d)	6.49 (0.7H, d)	6.48 (0.3H, d)
8"	5.36 (0.7H, br t)	5.34 (0.3H, br t)	5.84 (0.7H, d)	5.82 (0.3H, d)
9"-Me_2	1.73 (2.1H, br s)	1.69 (0.9H, br s) 1.74 (3H, br s)	1.47 (2.1H, s)	1.46 (0.9H, s)
3-OH	15.70 (0.7H, br s)		15.60 (0.7H, br s)	
2'-OH	12.48 (0.7H, br s)	12.55 (0.3H, s)	12.37 (0.7H, br s)	12.46 (0.3H, s)
OH	9.28 (0.7H, br s)	9.38 (0.3H, br s)		

a: Measured at 50 °C, 10 mg of the sample/0.6 ml of acetone-d_6. b: Measured at 24 °C, 20 mg/0.6 ml. c: Measured at 24 °C, 10 mg/0.6 ml. d: Measured at 50 °C, 2 mg/0.6 ml. Coupling constants: **1**, $J_{2",6"}$=2 Hz, $J_{5",6"}$=8.5 Hz, $J_{7',8'}$=$J_{7",8"}$=7 Hz; **2**, $J_{2",3"}$=9 Hz, $J_{7',8'}$=10 Hz; **3**, $J_{2",6"}$=2 Hz, $J_{5",6"}$=8 Hz, $J_{7',8'}$=10 Hz, $J_{7",8"}$=7 Hz; **4**, $J_{2",6"}$=2 Hz, $J_{5",6"}$=8 Hz, $J_{7',8'}$=$J_{7",8"}$= 10 Hz.

Table 2. ^{13}C Nmr data of **1-4** and related compounds (acetone-d_6, 100 MHz)

C No.	1 (enol)	1 (keto)	9[a]	10[b]	2 (enol)	2 (keto)	3 (enol)	3 (keto)	4 (enol)	4 (keto)
1	194.80	200.25			194.85	200.92	194.50	200.98	194.01	200.91
2	91.24	50.18			91.35	49.67	91.26	49.78	91.82	49.71
3	177.84	193.15			177.81	193.07	178.37	193.11	177.46	193.24
1'	112.75	114.11	114.3		113.49	114.69	113.55	114.69	113.56	114.78
2'	164.18	164.76	165.8		165.77	166.20	165.71	166.21	165.90	166.24
3'	103.98	103.33	103.5		105.04	104.56	105.04	104.56	105.11	104.62
4'	163.04	163.76	163.5		160.90	161.56	160.78	161.56	161.07	161.64
5'	121.71	121.48	121.3		114.89	114.95	114.94	114.82	114.98	114.78
6'	130.98	133.27	132.2		128.21	130.64	128.06	130.61	128.29	130.69
7'	28.67	28.17	----[e]		121.84	121.64	121.87	121.65	121.85	121.69
8'	123.87	123.01	124.0		129.66	129.79	129.66	129.80	129.82	129.90
9'	133.14[c]	132.57[d]	132.2		78.63	78.90	78.60	78.89	78.75	78.97
10'	17.92	17.75	17.9		28.69	28.75	28.68	28.75	28.75	28.80
11'	25.85	25.70	25.8		28.69	28.75	28.68	28.75	28.75	28.80
1"	126.02	130.01	(1')	123.43	125.57	130.12	125.61	129.93	125.01	130.79
2"	129.50	131.55	(2')	128.85	130.30	132.09	129.66	131.46	132.57	132.49
3"	129.30	129.91	(3')	129.91	116.53	116.22	129.20	129.79	132.03	131.41
4"	160.05	160.94	(4')	159.53	162.56	163.29	160.78	161.10	157.99	158.71
5"	116.03	115.73	(5')	116.34	116.53	116.22	116.05	115.62	117.33	117.05
6"	127.23	129.54	(6')	126.57	130.30	132.09	127.52	129.55	126.25	128.29
7"	29.12	28.87	(7')	29.05			29.16	28.84	122.29	122.18
8"	123.18	122.89	(8')	123.27			123.18	122.85	129.33	129.68
9"	133.37[c]	133.37[d]	(9')	133.28			133.02	133.32	78.45	78.58
10"	17.92	17.84	(10')	17.91			17.92	17.82	28.56	28.62
11"	25.85	25.85	(11')	25.88			25.89	25.89	28.56	28.62

a: Data from reference 12 (22.5 MHz). b: Data from reference 2. c,d: Signals may be interchanged in each column. e: The signal was overlapped with the solvent.

singlet signals and one pair of ABX type signals. The above ^1H nmr spectrum suggests that the A ring has the partial structure of the 2,4,5-trisubstituted phenyl moiety and the B ring is of the 3,4-disubstituted phenyl moiety. In the ^{13}C nmr spectrum, the oxygenated carbon atoms in the A ring were observed in the range of δ 163.04-164.76 suggesting that the oxygenated aromatic carbon atoms are located at the meta position each other.[13] The chemical shifts of the B ring carbon atoms in the enol form were similar to those of relevant atoms of the B ring of gancaonin Q (**10**) except C-1" (Table 2). Furthermore, glyinflanin A was converted to prenyllicoflavone A (**11**)[14] by heating in dry benzene.

Thus, the structure of the keto form of glyinflanin A is characterized as

formura (**1**).[15]

In the ^{13}C nmr spectrum of glyinflanin A, the signal of C-1" of the enol form appeared noticeably upfield (δ 126.02) compared to the signal of C-1" of the keto form (δ 130.01).[17] The enol carbon signal of C-3 (δ 177.84)[17] shows long-range correlation to C-2" proton (δ 7.80, d) in COLOC spectrum. Therefore, the structure of the enol form of glyinflanin A is characterized as formula (**1'**).

Glyinflanin B (**2**),[18] yellow needles, mp 151-152 °C, $C_{20}H_{18}O_5$, was suggested to be a dibenzoylmethane derivative by its uv spectrum[9,10,19] and the color reaction.[9,10] The ^1H and ^{13}C nmr spectra (acetone-d_6) of glyinflanin B also appeared as the equilibrium mixture of a dibenzoylmethane (**2**) and a β-hydroxychalcone moiety (**2'**) in the solution (Tables 1 and 2). The analysis of the ^1H nmr spectrum of the compound shows the presence of a 2,2-dimethylpyran ring [enol form (**2'**); δ 1.44 (4.8H, s), 5.71, 6.42 (each 0.8H, d, J = 10 Hz), keto form (**2**); δ 1.43 (1.2H, s), 5.70, 6.37 (each 0.2H, d, J = 10 Hz)]. The aromatic protons appeared as two pairs of singlet signals and one pair of A_2B_2 type signals. The mass spectrum of **2** shows characteristic fragment ions at m/z 203.0728 ($C_{12}H_{11}O_3$, **2a**) and 121.0304 ($C_7H_5O_2$, **2b**). The ^{13}C nmr spectrum of **2** (Table 2) was analyzed by using gated decoupling with NOE and by comparison with the spectra of **1** and **7**. In the ^{13}C nmr spectrum, the oxygenated aromatic carbons in the A ring were observed in the range of δ 160.90-166.20 indicating that the oxygenated carbon atoms are located at the meta position each other. This result suggests that the structure of the A ring is the 6-substituted 2,2-dimethyl-7-hydroxychromene moiety (**2c**). The chemical shifts of the carbon atoms of the B ring were similar to those of relevant atoms of **7**.[11]

Thus, the structure of the keto form of glyinflanin B is characterized as formula (**2**).

In the ^{13}C nmr spectrum of glyinflanin B, the signal of C-1" of enol form appeared noticeably upfield (δ 125.57) compared to the signal of C-1" of keto form (δ 130.12).[17] Therefore, the structure of the enol form of

glyinflanin B is deduced to formula (**2'**).

Glyinflanin C (**3**),[18] yellow needles, mp 90-91°C, $C_{25}H_{26}O_6$, was regarded as a dibenzoylmethane derivative by its uv spectrum[9,10,19] and the color reaction.[9,10] The ^1H and ^{13}C nmr spectra (acetone-d_6) of glyinflanin C also appeared as the equilibrium mixture of a dibenzoylmethane (**3**) and a ß-hydroxychalcone (**3'**) moiety in the solution (Tables 1 and 2). The analysis of the ^1H nmr spectrum shows the presence of a 2,2-dimethylpyran ring and a prenyl group. The aromatic protons appeared as two pairs of singlet signals and one pair of ABX type signals. The mass spectrum of **3** shows the characteristic fragment ions at m/z 203.0728 (**2a**) and 189.0916 (**1b**). The ^{13}C nmr spectrum of the compound (Table 2) was analyzed by using C,H-COSY and by comparison with the spectra of **1** and **2**. In the spectrum, the chemical shifts of the carbon atoms of the A ring were in agreement with those of the relevant carbon atoms of **2**, while the chemical shifts of the carbon atoms of the B ring were consistent with those of the relevant carbon atoms of **1**. Furthermore, the cyclization of **1** with 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) was performed to afford glyinflanin C as the main product.

Thus, the structure of the keto form of glyinflanin C is characterized as formula (**3**), and the enol form is deduced to formula (**3'**).[20]

Glyinflanin D (**4**), yellow needles, mp 89-90°C, $C_{25}H_{24}O_5$, was suggested to be a dibenzoylmethane derivative by its uv spectrum[9,10,19] and the color reaction.[9,10] The ^1H and ^{13}C nmr spectra (acetone-d_6) of the compound also appeared as the equilibrium mixture of a dibenzoylmethane (**4**) and a ß-hydroxychalcone (**4'**) moiety in the solution (Tables 1 and 2). The analysis of the ^1H nmr spectrum shows the presence of two 2,2-dimethylpyran rings [enol form (**4'**); δ 1.45, 1.47 (each 2.1H, s), 5.71, 5.84, 6.41, 6.49 (each 0.7H, d, J = 10 Hz), keto form (**4**); δ 1.44, 1.46 (each 0.9H, s), 5.70, 5.82, 6.37, 6.48 (each 0.3H, d, J = 10 Hz)]. The aromatic protons appeared as two pairs of singlet signals and one pair of ABX type signals. The mass spectrum of **4** shows the characteristic fragment ions at m/z 203

(**2a**) and 187.0704 ($C_{12}H_{11}O_2$, **4b**). In the ^{13}C nmr spectrum of the compound (Table 2), the chemical shifts of the carbon atoms of the A ring were in agreement with those of the relevant atoms of **2** and **3**. Further, the cyclization of **3** with DDQ was performed to give glyinflanin D.

Thus, the structure of the keto form of glyinflanin D is characterized as formula (**4**), and the enol form is deduced to formula (**4'**).

EXPERIMENTAL

The general procedures followed as described in our previous papers.[2,3] The following instruments and chemicals were used: melting points; Yazawa's micromelting point apparatus (hot-stage type), ^1H and ^{13}C nmr spectra; JEOL JNM-GX-400 and JEOL JNM-EX-400 NMR Spectrometers, mass spectra, JEOL JMS-D-300 and JEOL JMS-DX-303 Mass Spectrometers, and uv spectra: Shimadzu UV-265 Spectrophotometer. For tlc (silica gel), Wakogel B-5F and B-5FM were used. For preparative tlc (silica gel), Wagogel B-5F was used.

Plant Material

The roots of Glycyrrhiza inflata Bat. (Leguminosae) were collected in Jinta County, Gansu Province, China in July, 1986. The morphological and histlogical studies of the plant material is reported in previous paper.[21] The sample has been deposited in the drug museum of the Department of Pharmacognosy, School of Pharmaceutical Sciences, Beijing Medical University.

Isolation of Phenolic Compounds from G. inflata

The dried roots of G. inflata (2 kg) were powdered and exhaustively extracted with EtOH (20 l) at room temperature (for 3 days) by using continuous extractor. Evaporation of the extract to dryness yielded 200 g of a residue. The residue (200 g) was absorbed on Amberlite XAD-2 (500 g). The resin was washed with water (5 l) and then dried at room temperature. The dried resin was put into column and eluted successively with n-hexane (2 l), benzene (4 l), benzene-acetone=8:1 (4 l), benzene-acetone=1:1 (4 l), acetone (2 l), and MeOH (2 l). The benzene eluate was evaporated to give a residue (17 g). This residue (17 g) was chromatographed on silica gel (150 g), successively with n-hexane-acetone= 100:1 ⟶100:16 (fr. 1-10), n-hexane-acetone=10:2 ⟶ 10:3 (fr. 11-19), and n-hexane-acetone=100:35 ⟶1:1 (fr. 20-37) as an eluent, each fraction (eluted volume 200 ml) being monitored by tlc. The fraction 1 (eluent; n-hexane-acetone=100:1, yield: 0.18 g) was purified by preparative tlc [solvent system, n-hexane-$CHCl_3$=8:1 (multiple developments;

x7), n-hexane-ethyl ether=8:1 (x4)], then by hplc (solvent; n-hexane-AcOEt=18:1, column; Senshu Pak SSC-silica 4251-N, 5μ, 1 x 25 cm, detector: 254 nm) to give glyinflanin D (**4**, 2 mg). The fraction 5 (n-hexane-acetone=100:6, 0.3 g) and fraction 6 (n-hexane-acetone=100:8, 0.4 g) were combined and purified by preparative tlc [$CHCl_3$ (x3), n-hexane-AcOEt=4:1 (x3), n-hexane-ethyl ether=6:1 (x5)] to give glyinflanin C (**3**, 10 mg). The fraction 7 (n-hexane-acetone=10:1, 0.6 g) was purified by preparative tlc [n-hexane-acetone=8:1 (x3), $CHCl_3$-ethyl ether=30:1 (x3), n-hexane-AcOEt=5:1 (x4), $CHCl_3$ (x6)] to give glyinflanin B (**2**, 20 mg) and glabrone (**5**, 900 mg). The fraction 8 (n-hexane-acetone=100:12, 1.8 g) was purified by preparative tlc (n-hexane-acetone=3:1, n-hexane-AcOEt=5:2, $CHCl_3$-ethyl ether=25:1) to give glyinflanin A (**1**, 200 mg) and glabrone (**5**, 400 mg). The fractions 12-15 (n-hexane-acetone=4:1, 1.5 g), fractions 16-19 (n-hexane-acetone=10:3, 0.4 g), and fractions 20-23 (n-hexane:acetone=100:35, 2.1 g) were combined and purified by preparative tlc [$CHCl_3$-AcOEt=8:1 (x3)] to give licochalcone A (**6**, 2.3 g). Identification of the known compounds (**5** and **6**) was carried out by comparison of the physical and spectral data of those compounds with the relevant published data.

Glyinflanin A (1)

Compound (**1**) was recrystallized from n-hexane-ethyl ether to give yellow needles, mp 100-102°C. $FeCl_3$ test: dark green. Uv λ_{max}^{MeOH} nm (log ε): 203 (4.20), 230 (sh 3.85), 286 (3.78), 342 (3.58), 386 (3.90), 395 (sh 3.89), EI-Ms (probe) 70 eV, m/z (rel. int.): 409 $[M+1]^+$ (16%), 408 $[M]^+$ (56), 391 (4), 321 (19), 307 (9), 298 (4), 205 (15), 189 (100), 146 (6), 133 (13). HR-Ms m/z: 408.1942 $[M]^+$ ($C_{25}H_{28}O_5$ requires: 408.1937).

Glyinflanin B (2)

Compound (**2**) was recrystallized from n-hexane-ethyl ether to give yellow needles, mp 151-152°C. $FeCl_3$ test: dark green. Uv λ_{max}^{MeOH} nm (log ε): 223 (4.63), 232 (4.62), 249 (4.62), 265 (4.65), 347 (4.49), 398 (4.68). EI-Ms, m/z: 399 $[M+1]^+$ (7%), 338 $[M]^+$ (32), 324 (19), 323 (84), 321 (4), 204 (16), 203 (100), 187 (31), 185 (7), 161 (5), 160 (5), 131 (5), 122 (3), 121 (39), 93 (10). HR-Ms, m/z: 338.1163 $[M]^+$ ($C_{20}H_{18}O_5$ requires: 338.1154).

Glyinflanin C (3)

Compound (**3**) was recrystallized from benzene-$CHCl_3$-n-hexane to give yellow prisms, mp 90-91°C, $FeCl_3$ test: dark green. Uv λ_{max}^{MeOH} nm (log ε): 224 (4.51), 232 (4.51), 248 (4.48), 258 (4.48), 351 (4.23), 399 (4.44), EI-Ms, m/z: 407 $[M+1]^+$ (16%), 406 $[M]^+$ (52), 392 (10), 391 (85), 229 (4), 204 (20), 203 (100), 190 (7), 189 (53), 188 (6), 187 (27), 185 (5), 160 (5), 133 (28). HR-Ms, m/z: 406.1805 $[M]^+$ ($C_{25}H_{26}O_5$ requires: 406.1780).

Glyinflanin D (**4**)

Compound (**4**) was recrystallized from benzene-$CHCl_3$ to give yellow prisms, mp 89-90 °C. $FeCl_3$ test: dark green. Uv ν_{max}^{MeOH} nm (log ε): 226 (4.73), 233 (sh 4.73), 259 (4.69), 357 (4.39), 399 (4.65). EI-Ms, m/z: 405 $[M+1]^+$ (9%), 404 $[M]^+$ (24), 390 (12), 389 (43), 213 (4), 203 (11), 188 (13), 187 (100), 144 (10). HR-Ms, m/z: 404.1612 $[M]^+$ ($C_{25}H_{24}O_5$ requires: 404.1624).

Formation of Prenyllicoflavone A (**11**) from Glyinflanin A (**1**)

Glyinflanin A (**1**, 15 mg) was dissolved in dry benzene (9 ml) and refluxed for 28 h. The reaction product was purified by preparative tlc (n-hexane-AcOEt=3:1, 2:1) to give prenyllicoflavone A (**11**, 1 mg, 7%) and the starting material (**1**, 11 mg, 73%). Compound (**11**) was recrystallized from n-hexane-acetone to give pale yellow prisms, mp 128-130 °C. $FeCl_3$ test: olive. Uv ν_{max}^{MeOH} nm: 240 (sh), 258, 315 (sh), 335. EI-Ms, m/z: 390 $[M]^+$ (96%). HR-Ms, m/z: 390.1840 $[M]^+$ ($C_{25}H_{26}O_4$ requires: 390.1831). ^1H Nmr (acetone-d_6): δ 1.73, 1.75 (each 3H, br s, Me), 1.76 (6H, br s, Me), 3.41 (4H, br d, J = 7 Hz, C-9-Hx2 and C-7'-Hx2), 5.36-5.44 (2H, m, C-10-H and C-8'-H), 6.57 (1H, s, C-3-H), 7.20 (1H, d, J = 8 Hz, C-5'-H), 7.05 (1H, s, C-8-H), 7.72 (1H, dd, J = 2 and 8 Hz, C-6'-H), 7.77 (1H, s, C-5-H), 7.78 (1H, d, J = 2 Hz, C-2'-H), 9.45 (2H, br s, OHx2). ^{13}C Nmr (acetone-d_6): δ 17.87, 17.92 (C-12 and C-10'), 25.92, 25.95 (C-13 and C-11'), 28.65, 29.09 (C-9 and C-7'), 102.89 (C-8), 105.70 (C-3), 116.25 (C-5'), 117.72 (C-4a), 122.82, 123.14 (C-10 and C-8'), 123.93 (C-1'), 126.16, 126.23 (C-5 and C-6'), 123.38 (C-6), 128.57 (C-2'), 129.77 (C-3'), 133.20, 133.47 (C-11 and C-9'), 157.10 (C-8a), 159.10 (C-4'), 161.10 (C-7), 163.83 (C-2), 177.41 (C-4).

Cyclization of Glyinflanin A (**1**) with DDQ (Formation of **3**, **4**, and **12**)

A mixture of **1** (52 mg, 0.13 mmol), DDQ (15 mg, 0.07 mmol), and dry dioxane (2 ml) in dry benzene (13 ml) was refluxed for 8 h. The reaction product was purified by preparative tlc [n-hexane-AcOEt=4:1 (x3)] to give **3** (8 mg, 16%), **4** (2 mg, 4%), **12** (less than 0.5 mg), and the starting material (**1**, 8 mg, 16%). Compound (**3**) was identified with glyinflanin C by tlc analysis and ^1H nmr spectrum. Compound (**12**) was obtained as yellow powder, and its ^1H nmr spectrum showed that this compound existed as keto form (**12**) and enol form (**12'**) in acetone-d_6. The mass spectrum was not available owing to inorganic impurity from silica gel. ^1H Nmr (acetone-d_6, at 24 °C), enol form: δ 1.47 (4.2H, s, C-9"-Me), 1.73, 1.74 (each 2.1H, br s, C-9'-Me), 3.28 (1.4H, br d, J = 7 Hz, C-7'-H), 5.33 (0.7H , br t, J = 7 Hz, C-8'-H), 5.86 (0.7H, d, J = 10 Hz, C-8"-H), 6.41 (0.7 H, s, C-3'-H), 6.50 (0.7 H, d, J = 10 Hz, C-7"-H), 6.863 (0.7H, d, J = 8.5 Hz, C-5"-H), 6.99 (0.7H, s, C-2-H), 7.75 (0.7H, s, C-6'-H), 7.80 (0.7H, d, J = 2 Hz, C-2"-H), 7.85 (0.7H, dd, J = 2 and 8.5 Hz, C-6"-H), 12.26 (0.7H, br s, C-2'-OH), 15.75 (0.7H, br s, C-3-OH), keto form: 1.44 (1.8H, s,

C-9"-Me), 1.60, 1.61 (each 0.9H, br s, C-9'-Me), 3.21 (0.6H, br d, J = 7 Hz, C-7'-H), 4.63 (0.6H, s, C-2-H), 5.25 (0.3H, br t, J = 7 Hz, C-8'-H), 5.85 (0.3 H, d, J = 10 Hz, C-8"-H), 6.38 (0.3H, s, C-3'-H), 6.49 (0.3H, d, J = 10 Hz, C-7"-H), 6.86 (0.3H, d, J = 8.5 Hz, C-5"-H), 7.83 (0.3H, d, J = 2 Hz, C-2"-H), 7.58 (0.3H, s, C-6'-H), 7.87 (0.3H, dd, J = 2 and 8.5 Hz, C-6"-H), 12.35 (0.3 H, s, C-2'-OH).

Cyclization of Glyinflanin C (**3**) with DDQ (Formation of **4**)

A mixture of **3** (11 mg, 0.03 mmol), DDQ (6 mg, 0.03 mmol), and dry dioxane (2 ml) in dry benzene (10 ml) was refluxed for 10 h. The reaction product was purified by preparative tlc [n-hexane-AcOEt=3:1 (x3)] to give **4** (3 mg, 27%) and the starting material (**3**, 2 mg, 18%).

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