STRUCTURES OF SIX ISOPRENOID-SUBSTITUTED FLAVONOIDS, GANCAONINS F, G, H, I, GLYCYROL, AND ISOGLYCYROL FROM XIBEI LICORICE (GLYCYRRHIZA SP.)[1]

Toshio Fukai,[a] Qing-Hua Wang,[a] Taro Kitagawa,[a] Kazunao Kusano,[a] Taro Nomura,*[,a] and Yoichi Iitaka[b,2]

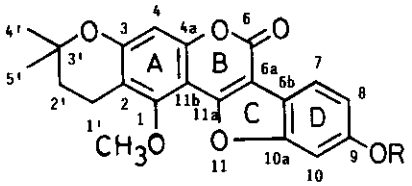
a) Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi-shi, Chiba 274, Japan

b) School of Medicine, Teikyo University, 359 Ohtsuka, Hachioji-shi, Tokyo 192-03, Japan

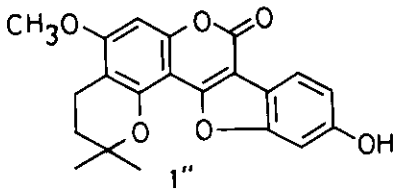
Abstract —— Four new isoprenoid-substituted flavonoids, gancaonins F, G, H, and I, along with two known compounds, glycyrol and isoglycyrol, were isolated from the Xibei licorice (Glycyrrhiza sp.). Structures of gancaonins F, G, H, and I were shown to be **3-6**, respectively, on the basis of spectral evidence. From the X-ray crystallographic analysis, spectroscopic data, and chemical evidence, the structures **1'** and **2'** for isoglycyrol and glycyrol should be revised to the structures **1** and **2**, respectively.

Licorice, the root of various species of Glycyrrhiza (Leguminosae), has been used for a long time as one of the most important crude drugs. On the constituents of phenolic compounds of Xibei licorice (Glycyrrhiza sp., Seihoku Kanzo in Japanese), many investigators reported a series of isoprenoid-substituted flavonoids.[3-6] We have also reported prenylated flavonoids from Xibei licorice[7] and the aerial parts of Glycyrrhiza uralensis FISCH.[1] In continuation of these studies, we examined the phenolic constituents of Xibei licorice, and describe here the characterization of four new isoprenoid-substituted flavonoids, gancaonins F (**3**), G (**4**), H (**5**), and I (**6**) together with the revised structures of two known compounds, isoglycyrol (**1**) and glycyrol (**2**).[8]

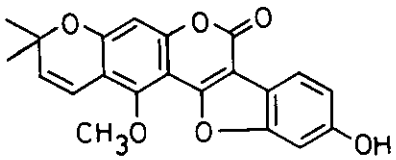
From the benzene extract of the crude drug, four kinds of new isoprenoid-substi-



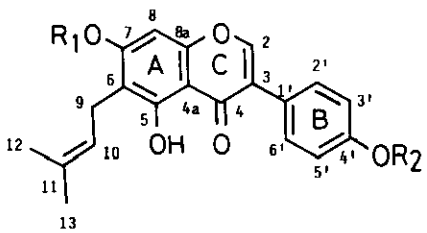
1 : R = H
1a: R = $COCH_3$



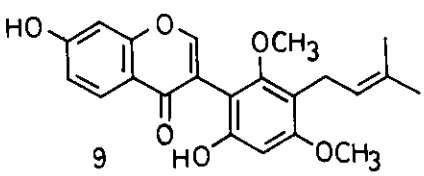
1''



3

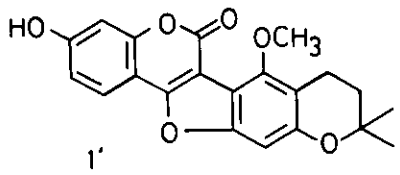


4 : R_1 = CH_3, R_2 = H
10 : R_1 = R_2 = H
11 : R_1 = H, R_2 = CH_3

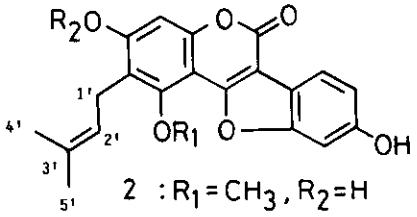


9

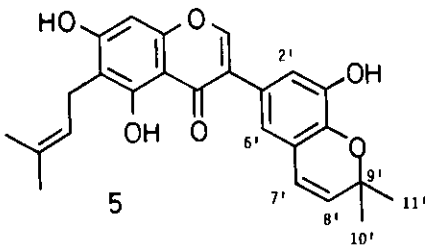
Fig. 1



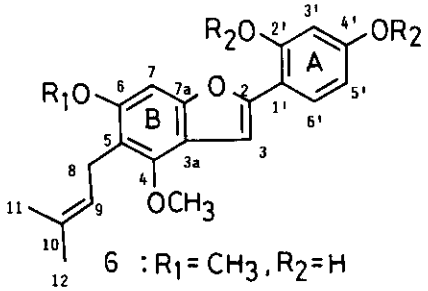
1'



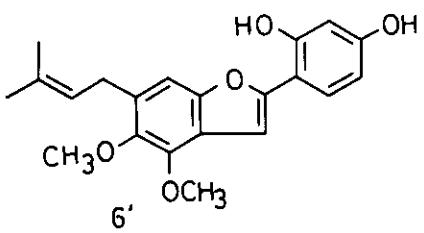
2 : R_1 = CH_3, R_2 = H
2' : R_1 = H, R_2 = CH_3
2a: R_1 = R_2 = CH_3



5



6 : R_1 = CH_3, R_2 = H
6a : R_1 = CH_3, R_2 = $COCH_3$
12 : R_1 = R_2 = H



6'

tuted flavonoids, gancaonins F (**3**), G (**4**), H (**5**), and I (**6**), along with known compounds, isoglycyrol (**1**),[8] glycyrol (**2**),[8] 5-O-methylglycyrol (**2a**),[8] licoricidin (**7**),[7,9] kumatakenin (**8**),[10] and licoricone (**9**)[11] were isolated by column chromatography and preparative tlc.

Compound **1** (isoglycyrol) was obtained as colorless needles, mp 303-306 °C (colorless prisms, mp 313-315 °C), $C_{21}H_{18}O_6$, negative to ferric chloride test. The uv spectrum of **1** resembled those of coumestan derivatives,[8] and their ir spectrum showed an absorption band at 1705 cm^{-1}. The ^1H nmr spectrum showed the signals of the following protons: 1) protons in a 2,2-dimethyldihydropyran ring, δ1.34 (6H, s), 1.81, 2.78 (each 2H, t, J = 6.5 Hz), 2) an aromatic proton, δ6.63 (1H, s), 3) ABC type aromatic protons, δ6.95 (1H, dd, J = 2 and 8.5 Hz), 7.16 (1H, d, J = 2 Hz), 7.69 (1H, d, J = 8.5 Hz), and 4) protons of a methoxyl group, δ3.95 (3H, s). Treatment of **1** with acetic anhydride in pyridine gave a monoacetate (**1a**). In the ^1H nmr spectrum of **1a**, the ABC type protons showed remarkable downfield shifts as compared with the spectrum of **1**. These results suggest that **1** has a 1,2,4-trisubstituted benzene ring and a pentasubstituted benzene ring in the structure, and that a hydroxyl group is located in the trisubstituted ring. In the ^{13}C nmr spectrum of **1**, the signals of all the oxygenated carbon atoms were observed at δ152-158 suggesting that the oxygenated aromatic carbon atoms are located at the meta position to each other (Table 1).[12] The methoxyl carbon atom observed at δ61.42 suggested the methoxyl group to be diortho-substituted.[13] From these results, two possible structures, **1** and **1'**, were proposed for the compound (**1**). In order to determine the definite structure, the X-ray crystallographic analysis of **1** was carried out, and the formula **1** was confirmed as the structure of the compound (**1**) (Fig. 2). On the other hand, the compound (**1**) was proved to be identical with authentic isoglycyrol (**1"**)[8] by comparing the ^1H and ^{13}C nmr spectra with each other. All these results indicated that the structure (**1"**) for isoglycyrol should be revised to **1**.[14]

Compound **2** (glycyrol) was obtained as colorless needles, mp 252-255 °C, $C_{21}H_{18}O_6$, negative to ferric chloride test. The uv spectrum of **2** resembled that of **1**. In the presence of sodium acetate, the uv spectrum of **2** showed a bathochromic shift while not in the spectrum of **1**. The ^1H nmr spectrum of **2** showed the signals of the following protons: 1) protons of a γ,γ-dimethylallyl (prenyl) group, δ1.65 (3H, br s), 1.77 (3H, br s), 3.34 (2H, br d, J=7 Hz), 5.19 (1H, br t, J = 7 Hz), 2) an aromatic proton, δ6.78 (1H, s), 3) ABC type aromatic protons, δ6.96 (1H, dd, J = 2

Table 1 ^{13}C Nmr data of **1-6** and **10-12** in DMSO-d_6 *

C§	1	2	3	C	4	10[a]	11[b]	5	C	6	12[c]
1	153.62[d]	153.84[e]	150.92[d]	2	154.01	153.8	153.70[y]	153.74[y]	2	150.87	150.4
2	112.99[e]	119.66	112.11[v]	3	122.72	122.3	122.03	122.09	3	100.26[z]	100.3
3	157.43[f]	159.48[f]	156.20[w]	4	180.50	180.4	180.19	180.11	3a	113.68	112.6
4	100.67[g]	99.20[q]	100.74	4a	105.45	104.4	104.45	104.30	4	155.42	153.1
4a	152.81[h]	152.89[h]	154.09[x]	5	157.54	159.0	158.99	158.85	5	115.08	114.1
6	157.27[i]	157.44[i]	157.05[i]	6	111.94	111.2	111.37	111.19	6	150.05	150.1
6a	102.83[j]	102.20[r]	103.15[r]	7	163.06	162.1	162.11	162.04	7	89.53	92.2
6b	114.38	114.30	114.16	8	90.24	93.0	93.08	92.96	7a	153.26	153.0
7	120.50	120.43	120.57	8a	156.01	155.5	155.46	155.32	1'	108.81	108.9
8	114.14[k]	113.97[s]	114.27[s]	1'	121.36	121.5	123.31	122.94	2'	155.37	155.2
9	157.11[l]	156.90[l]	157.21[l]	2'	130.13	130.3	130.13	121.95	3'	102.97	102.9
10	98.53[m]	98.46[t]	98.57[t]	3'	115.20	115.2	113.81	145.05	4'	158.30	158.1
10a	156.28[n]	156.10[n]	156.32[n]	4'	157.97	157.5	159.29	140.10	5'	107.11	107.0
11a	157.80[o]	158.14[u]	157.69[u]	5'	115.20	115.2	113.81	121.34	6'	126.61	126.5
11b	100.42[p]	99.72[p]	101.36[p]	6'	130.13	130.3	130.13	117.55	8	22.27	22.3
1'	16.30	22.03	114.92	9	21.06	21.2	21.12	21.02	9	123.61	123.9
2'	31.24	122.49	131.50	10	121.99	122.3	122.35	122.21	10	129.72	129.3
3'	75.93	130.82	77.73	11	130.92	130.7	130.54	130.51	11	17.51	17.6
4'	26.46	17.67	27.77	12	17.59	17.8	17.62	17.57	12	25.42	25.7
5'	26.46	25.40	27.77	13	25.39	25.6	25.37	25.31	4-OCH_3	59.83	59.7
1-OCH_3	61.42	62.29	63.10	7"				117.12	6-OCH_3	55.99	
				8"				131.15			
				9"				75.98			
				10"				27.51			
				11"				27.51			
				OCH_3	56.40		55.21				

*: Compounds **1-6** and **11** were measured at 60 °C, digital resolution, **1**: 0.52 Hz, **2-6, 11**: 0.73 Hz, §: the number of carbons; according to D.M. Dewick (ref. 25) and J.L. Ingham (ref. 26), a): data from K.R. Markham et al. (ref. 12), b): data from T. Fukai et al. (ref. 1), c): Some signals were not assigned in original report; data from S. Demizu et al. (ref. 6), d):qd-like (J=ca. 3 and 7 Hz), e): multiplet, f): td, ^2J=4, ^3J=3 Hz, g): d: d, ^1J=165 Hz, h): d, ^2J=4 Hz, i): singlet, j): d, ^3J=1.2 Hz, k): dd, ^1J=160 Hz, ^3J=4 Hz, l): ddd, ^2J=2 and 4 Hz, ^3J=9 Hz, m): ddd, ^1J=164 Hz, ^3J=4 Hz, ^4J=0.7 Hz, n): dd, ^2J=3, ^3J=10 Hz, o): d, ^4J=1.8 Hz, p): d, ^3J=5 Hz, q): d, ^1J=164 Hz, r): d, J=2 Hz, s): dd, J=5 and 161 Hz, t): dd, J=5 and 163 Hz, u) d, J=1.5 Hz, v): ddd, J=2, 5 and 8 Hz, w): t, J=4 Hz, x): d, J=5 Hz, y): d, ^1J=198 Hz, z): d, ^1J=180 Hz.

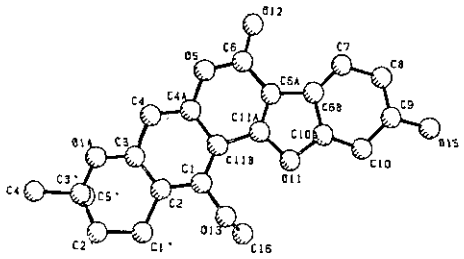


Fig. 2

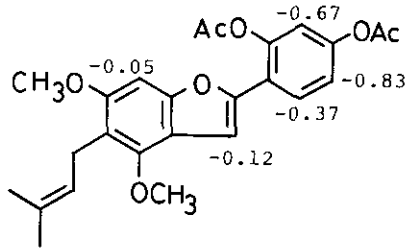


Fig. 3 Acetylation shifts of **6a**

and 8 Hz), 7.17 (1H, d, J = 2 Hz), 7.71 (1H, d, J = 8 Hz), and 4) protons of a methoxyl group, δ3.90 (3H, s). The chemical shifts of the ABC type protons were similar to those of the relevant protons of **1**. In the ^{13}C nmr spectrum of **2**, the chemical shifts of all the carbon atoms, except those of the carbon atoms at the C-2 and C-3 positions and the isoprenoid moiety, were similar to those of the relevant carbon atoms of **1** (Table 1). From the chemical shift of the methoxyl carbon atom, the methoxyl group seems to be di<u>ortho</u>-substituted.[13] The compound (**2**) was correlated with **1** by treating **2** with concentrated hydrochloric acid in methanol.[8] The compound (**2**) was identical with authentic glycyrol (**2'**)[8] by comparing the ^{1}H and ^{13}C nmr spectra with each other. These results indicate that the structure **2'** for glycyrol should be revised to the structure **2**.[14]

Gancaonin F (**3**) was obtained as colorless prisms, mp 290-291 °C, $C_{21}H_{16}O_6$, negative to ferric chloride test. The uv spectrum of **3** resembled those of **1** and **2**. The ^{1}H nmr spectrum showed the signals of the following protons: 1) protons of a 2,2-dimethylpyran ring, δ1.45 (6H, s), 5.96 (1H, d, J = 10 Hz), 6.69 (1H, d, J = 10 Hz), 2) an aromatic proton, δ6.81 (1H, s), 3) ABC type aromatic protons, δ6.97 (1H, dd, J = 2 and 8 Hz), 7.19 (1H, d, J = 2 Hz), 7.73 (1H, d, J = 8 Hz), and 4) protons of a methoxyl group, δ3.96 (3H, s). The chemical shifts of all the carbon atoms, except those of the carbon atoms at the C-1, C-2, and C-3 positions and the isoprenoid moiety, were similar to those of the relevant carbon atoms of **2**. Gancaonin F (**3**) was derived from **2** by treating **2** with palladium chloride in methanol-H_2O (9:1) solution.[15] From these results, the structure of gancaonin F is represented by the formula **3**.

Gancaonin G (**4**) was obtained as colorless needles, mp 95-98 °C, $C_{21}H_{20}O_5$, positive to ferric chloride test, and to Gibbs test. The uv spectrum of **4** resembled those of isoflavone derivatives,[16] and showed no bathochromic shift in the presence of sodium acetate. The uv spectrum showed no bathochromic shift immediately after addition of aluminum chloride, while bathochromic shift was observed one hour

after.[1,17] The ^{1}H nmr spectrum of **4** showed a characteristic singlet signal at δ8.20 (C-2-H), and showed the signals of the following protons: 1) protons of a prenyl group, δ1.64 (3H, br s), 1.77 (3H, br s), 3.33 (2H, br d, J = 7 Hz), 5.21 (1H, br t, J = 7 Hz), 2) an aromatic proton, δ6.61 (1H, s), 3) A_2B_2 type aromatic protons, δ6.91 (2H, d, J = 9 Hz), 7.46 (2H, d, J = 9 Hz), 4) protons of a methoxyl group, δ3.97 (3H, s), and 5) proton in a hydrogen-bonding hydroxyl group, δ13.20 (1H, s). In the ^{13}C nmr spectrum, the chemical shifts of the carbon atoms in the B-ring were similar to those of the relevant carbon atoms of wighteone (**10**)[12] (Table 1), and the signal at δ90.24, assigned to the signal of a carbon atom in the A-ring, was observed as doublet. These results suggest the prenyl group to be located at the C-6 position.[12] The comparison of the chemical shifts of the carbon atoms of **4** with those of **10** indicates the O-methylation effect on the carbon atoms in the A-ring. The similar result was reported by Fujita et al. in the case of silvaticol.[18] From these results, the structure of gancaonin G is represented by the formula **4**.

Gancaonin H (**5**) was obtained as colorless prisms, mp 205-206 °C, $C_{25}H_{24}O_6$, positive to both ferric chloride and Gibbs tests. The uv spectrum of **5** resembled those of isoflavone derivatives,[16] and showed a bathochromic shift in the presence of sodium acetate. The uv spectrum showed a bathochromic shift two hours after adding aluminum chloride to the solution, while not immediately after the addition.[1,17] The ^{1}H nmr spectrum of **5** showed the signals of the following protons: 1) protons of a prenyl group, δ1.63 (3H, br s), 1.73 (3H, br s), 3.24 (2H, br d, J = 6 Hz), 5.18 (1H, br t, J = 6 Hz), 2) protons of a 2,2-dimethylpyran ring, δ1.40 (6H, s), 5.76 (1H, d, J = 10 Hz), 6.38 (1H, d, J = 10 Hz), 3) three aromatic protons, δ6.46 (1H, s), 6.91 (1H, d, J = 2 Hz), 6.72 (1H, d, J = 2 Hz), 4) an olefinic proton, δ8.31 (1H, s), and 5) proton in a hydrogen-bonding hydroxyl group, δ13.22 (1H, s). The ^{13}C nmr spectrum of **5** showed that the chemical shifts of the carbon atoms in the A-ring, and those of the prenyl carbon atoms were similar to those of the relevant carbon atoms of wighteone (**10**) and gancaonin A (**11**)[1] (Table 1). The 3',4'-di-oxygenated phenyl structure for the B-ring of **5** was supported by the chemical shifts of the two oxygenated carbon atoms (δ140.10 and 145.05).[12] A 3',4'-di-hydroxy-5'-prenylphenyl structure for the B-ring of **5** was excluded on the basis of discrepancy in the chemical shifts of the carbon atoms in the B-ring of **5** and of glycyrrhisoflavone (5,7,3',4'-tetrahydroxy-5'-prenylisoflavone).[4,19] From these results, the structure of gancaonin H is represented by the formula **5**.

Gancaonin I (**6**) was obtained as colorless prisms, mp 67-70 °C/125-127 °C, $C_{21}H_{22}O_5$,

negative to ferric chloride test, while positive to Gibbs test. The uv spectrum of **6** resembled those of 2-arylbenzofuran derivatives.[20,21] Treatment of **6** with acetic anhydride in pyridine gave a diacetate (**6a**). The ^{1}H nmr spectrum of **6** showed the signals of the following protons: 1) protons of a prenyl group, δ1.62 (3H, br s), 1.73 (3H, br s), 3.30 (2H, br d, J = 7 Hz), 5.13 (1H, br t, J = 7 Hz), 2) protons of two methoxyl groups, δ3.81 (3H, s), 3.96 (3H, s), 3) ABC type aromatic protons, δ6.37 (1H, dd, J = 2 and 8.5 Hz), 6.48 (1H, d, J = 2 Hz), 7.56 (1H, d, J = 8.5 Hz), and 4) long-range coupled two protons,[22] δ6.92 (1H, br d, J = ca. 0.7 Hz), 7.17 (1H, d, J = 0.7 Hz). Comparison between the ^{1}H nmr spectra of **6** and its diacetate (**6a**) indicated that the acetylation of the hydroxyl groups caused remarkable downfield shifts of the ABC type protons (Fig. 3).[23] In the ^{13}C nmr spectrum of **6**, the chemical shifts of the carbon atoms in the A-ring were similar to those of the relevant carbon atoms of licocoumarone (**12**).[6] By using gated decoupling with nOe technique, the signals at δ150.05 (m) and 155.42 (m, overlapping with the signal at δ155.37) were assigned as the signals of the carbon atoms substituted with a methoxyl group. A location of one of the methoxyl groups was confirmed to be the C-4 position by the following long-range selective ^{1}H decoupling (LSPD) technique. When the proton at δ7.17 (C-3-H)[21,24] was weakly irradiated, the signal at δ155.42 changed its shape. From these results, two possible structures (**6** and **6'**) were suggested for gancaonin I. In the case of **6'**, the chemical shifts of the carbon atoms of the methoxyl groups could be observed at ca. 60 ppm.[13] From the chemical shifts of the methoxyl groups of **6** (δ55.99 and 59.83), the formula **6** is more favorable than the formula **6'** (Table 1). From the above results, the structure of gancaonin H is represented by the formula **6**.

EXPERIMENTAL

Abbreviations: s=singlet, d=doublet, dd=double doublet, t=triplet, m=multiplet, br=broad, sh=shoulder, infl.=inflectiom. The general procedures followed as described in our previous paper.[1] The following instruments were used: melting points; Yazawa or Mitamura micro-melting point apparatuses, uv spectra; Shimadzu UV-265 spectrophotometer, ir spectra; Hitachi 260-30 IR spectrophotometer, ms: JEOL JMS-D 300 or JMS-DX 303 Spectrometer, ^{1}H and ^{13}C nmr spectra; JEOL JNM GX-400 FT NMR spectrometer.

Isolation of Six Isoprenoid-Substituted Flavonoids, Isoglycyrol (**1**), Glycyrol (**2**), Gancaonins F (**3**), G (**4**), H (**5**), and I (**6**) from Xibei Licorice (Glycyrrhiza sp.).

Xibei (northwest) licorice (4.8 kg) imported from the People's Republic of China was extracted with n-hexane (20 l), benzene (20 l), and acetone (20 l), successively. Evaporation of the benzene and

acetone solutions to dryness yielded 110 g and 150 g of the residue, respectively. The benzene extract (110 g) was extracted with methanol (300 mlx2) at room temperature . The methanol solution was concentrated to afford the residue (100 g). This residue (100 g) was chromatographed on silica gel (600 g) successively with n-hexane (Fr. 1-4), n-hexane-benzene=1:1 (Fr. 5-10), benzene (Fr. 11-60), benzene-acetone=99:1 (Fr. 61-72), and benzene-acetone=49:1 (Fr.73-110) as the eluent, each fraction (eluent volume 500 ml) being monitored by tlc. The fractions 19-21 were evaporated to give 3.4 g of residue, from which licoricidin (**7**, mp 161 °C, 500 mg) and gancaonin G (**4**, 12 mg) were obtained by preparative tlc (silica gel, solvent system, acetone-benzene=1:10, ethyl acetate-benzene=1:6, ether-benzene=1:10). The fractions 44-60 were evaporated to give 5.6 g of residue, from which kumatakenin [**8**, mp 261 °C (from acetone-benzene), 20 mg], 5-O-methylglycyrol (3-O-methylglycyrol)[14,27] [**2a**, mp 265 °C (from acetone), 26 mg], gancaonins F (**3**, 3 mg), I (**6**, 30 mg), compounds **1** (**1**, 23 mg), and 2 (**2**, 3 mg) were obtained by preparative tlc (silica gel, n-hexane-acetone=3:2, n-hexane-ethyl acetate=2:1, benzene-ethyl acetate=10:1). The fractions 98-109 were evaporated to give 8.5 g of residue. This residue (8.5 g) was purified by column chromatography [silica gel (60 g), benzene-ether mixture] and preparative tlc (silica gel, ether-n-hexane= 3:1), successively, to give licoricone [**9**, mp 244-245 °C (from acetone-benzene), 5 mg].
The acetone extract (150 g) of the crude drug was purified by column chromatography (silica gel, benzene-acetone mixture) and preparative tlc (silica gel), successively, to give licoricidin (**7**, 250 mg), kumatakenin (**8**, 18 mg), licoricone (**9**, 145 mg), and compound **2** (**2**, 160 mg). Physical and spectral data of these known compounds were identified with the relevant published data.

Compound 1 (1, Isoglycyrol).

Compound **1** was recrystallized from benzene-acetone to give colorless prisms, mp 313-315 °C (from acetone to give colorless needles, mp 303-306 °C). $FeCl_3$ test: negative. Uv λ_{max}^{MeOH} nm (log ε): 210 (4.48), 225 (sh 4.32), 247 (4.26), 255 (sh 4.15), 348 (4.36), 360 (sh 4.30). Uv $\lambda_{max}^{MeOH+AcONa}$: no shift. Ir ν_{max}^{KBr} cm^{-1}: 3250, 1705, 1630, 1585, 1500. EI-Ms (probe), 70 eV, m/z (relative intensity): 367 $[M+1]^+$ (24%), 366 $[M]^+$ (100), 311 (76), 296 (18), 267 (8). High-resolution ms (HR-Ms), m/z: 366.1109 $[M]^+$ ($C_{21}H_{18}O_6$ requires: 366.1103). ^1H Nmr (50 mg/0.6 ml of DMSO-d_6): δ1.34 (6H, s, C-3'-CH_3x2), 1.81 (2H, t, J = 6.5 Hz, C-2'-Hx2), 2.78 (2H, t, J = 6.5 Hz, C-1'-Hx2), 3.95 (3H, s, OCH_3), 6.63 (1H, s, C-4-H), 6.95 (1H, dd, J = 2 and 8.5 Hz, C-8-H), 7.16 (1H, d, J = 2 Hz, C-10-H), 7.69 (1H, d, J = 8.5 Hz, C-7-H), 10.04 (1H, br s, OH); (2 mg/0.6 ml of DMSO-d_6); δ6.75 (1H, s, C-4-H). The crystal data for the X-ray crystallographic analysis of **1** were obtained as follows: $C_{21}H_{18}O_6$; molecular weight = 366; monoclinic; space group $P2_1/n$; lattice constants, a = 9.016 (5) Å, b = 22.367 (12) Å, C = 8.805 (5) Å, α=β=γ=93.29 (5)°; V=1772.7 $Å^3$; Z = 4; the final R value, 0.098.

Compound 1 Monoacetate (1a, Isoglycyrol Monoacetate).

A mixture of **1** (5 mg), acetic anhydride (0.2 ml), and pyridine (0.2 ml) was kept at room temperature for 10 min and treated as usual. Compound **1** monoacetate was crystallized from benzene to give **1a** (2 mg), colorless needles, mp 209 °C [from methanol, mp 178-179 °C/201-202 °C (double melting point)]. EI-Ms (probe), 70 eV, m/z (rel. int.): 409 $[M+1]^+$ (13%), 408 $[M]^+$ (51), 366 (100), 311 (51), 296 (11), 267 (5). ^1H Nmr (DMSO-d_6): δ1.36 (6H, s, C-3'-CH_3x2), 1.86 (2H, t, J = 6 Hz, C-2'-Hx2), 2.83 (2H, t, J = 6 Hz, C-1'-Hx2), 2.33 (3H, s, OAc), 3.98 (3H, s, OCH_3), 6.78 (1H, s, C-4-H), 7.28 (1H, dd, J = 2 and 8 Hz, C-8-H), 7.78 (1H, d, J = 2 Hz, C-10-H), 7.94 (1H, d, J = 8 Hz, C-7-H).

Compound 2 (2, Glycyrol).

Compound 2 was recrystallized from benzene-acetone to give colorless needles, mp 252-255°C. $FeCl_3$ test: negative. Uv λ_{max}^{MeOH} nm (log ε): 212 (4.54), 227 (4.51), 244 (4.37), 255 (sh 4.26), 347 (4.45), 358 (sh 4.40). Uv $\lambda_{max}^{MeOH+AcONa}$: 245 (sh 4.30), 265 (sh 4.20), 315 (sh 3.86), 345 (sh 4.32), 365 (4.40), 385 (sh 4.26). Ir ν_{max}^{KBr} cm^{-1}: 3450, 3380, 1715, 1635, 1615, 1590. EI-Ms (probe), 70 eV, m/z (rel. int.): 367 $[M+1]^+$ (25%), 366 $[M]^+$ (100), 351 (3), 335 (6), 311 (86), 296 (21), 280 (8). HR-Ms, m/z: 366.1111 $[M]^+$ ($C_{21}H_{18}O_6$ requires: 366.1103). ^1H Nmr (DMSO-d_6): δ1.65, 1.77 (each 3H, br s, C-3'-CH_3), 3.34 (2H, br d, J = 7 Hz, C-1'-Hx2), 3.90 (3H, s, OCH_3), 5.19 (1H, br t, J = 7 Hz, C-2'-H), 6.78 (1H, s, C-4-H), 6.96 (1H, dd, J = 2 and 8 Hz, C-8-H), 7.17 (1H, d, J = 2 Hz, C-10-H), 7.71 (1H, d, J = 8 Hz, C-7-H), 10.07 (br, OH).

Formation of Compound 1 (1, Isoglycyrol) from Compound 2 (2, Glycyrol).

A mixture of 2 (10 mg), 35% HCl (5.5 ml), and methanol (20 ml) was refluxed for 2 h and treated as usual. The reaction product was purified by preparative tlc (silica gel, ethyl acetate-benzene=1:6) to give compound 1 [mp 312-315°C (from acetone-benzene), 4 mg]. Compound 1 thus obtained was identical with the authentic sample on the basis of ir spectral comparison and mixed melting point determination.

Gancaonin F (3).

Gancaonin F (3) was recrystallized from acetone-benzene to give colorless prisms, mp 290-291°C. $FeCl_3$ test: negative. Uv λ_{max}^{MeOH} nm (log ε): 203 (4.24), 240 (infl. 4.34), 248 (sh 4.40), 255 (4.43), 280 (sh 3.93), 293 (3.88), 330 (sh 4.08), 355 (4.26), 367 (4.23). Uv $\lambda_{max}^{MeOH+AcONa}$: no shift. Ir ν_{max}^{KBr} cm^{-1}: 3400 (sh), 3280, 1715, 1630, 1505. EI-Ms (probe), 70 eV, m/z (rel. int.): 365 $[M+1]^+$ (14%), 364 $[M]^+$ (54), 349 (100), 334 (47), 175 (6), 167 (9), 153 (3), 125 (2). HR-Ms, m/z: 364.0953 $[M]^+$ ($C_{21}H_{16}O_6$ requires: 364.0947). ^1H Nmr (DMSO-d_6): δ1.45 (6H, s, C-3'-CH_3x2), 3.96 (3H, s, OCH_3), 5.96 (1H, d, J = 10 Hz, C-2'-H), 6.69 (1H, d, J = 10 Hz, C-1'-H), 6.81 (1H, s, C-4-H), 6.97 (1H, dd, J = 2 and 8 Hz, C-8-H), 7.19 (1H, d, J = 2 Hz, C-10-H), 7.73 (1H, d, J = 8 Hz, C-7-H), 10.10 (1H, br s, OH).

Formation of Gancaonin F (3) from Compound 2 (2, Glycyrol).

A mixture of 2 (12 mg), $PdCl_2$ (7 mg), and 90% methanol (4 ml) was kept at room temerature for 4 days and treated as usual. The reaction product was purified by preparative tlc (silica gel, ethyl acetate-benzene=1:6) to give 3 [mp 286-290°C (from acetone-benzene), 7 mg]. Gancaonin F thus obtained was identical with authentic sample on the basis of ir, ^1H and ^{13}C nmr spectral comparisons and mixed melting point determination.

Gancaonin G (4).

Gancaonin G (4) was recrystallized from methanol to give colorless needles, mp 95-98°C. $FeCl_3$ test: dark green. Gidds test: positive. Uv λ_{max}^{MeOH} nm (log ε): 211 (4.59), 268 (4.61), 335 (sh 3.64). Uv $\lambda_{max}^{MeOH+AcONa}$: no shift. Uv $\lambda_{max}^{MeOH+AlCl_3}$ (after 1 h): 210 (4.66), 275 (4.61), 320 (sh 3.98), 380 (3.42). Ir ν_{max}^{KBr} cm^{-1}: 3400, 1655, 1620, 1515. EI-Ms (probe), 70 eV, m/z (rel. int.): 353 $[M+1]^+$ (15%), 352 $[M]^+$ (62), 337 (22), 323 (9), 309 (100), 297 (94), 118 (5). HR-Ms, m/z: 352.1291 $[M]^+$ ($C_{21}H_{20}O_5$ requires: 352.1311). ^1H Nmr (acetone-d_6): δ1.64, 1.77 (each 3H, br s, C-11-CH_3), 3.33

(2H, br d, J = 7 Hz, C-9-Hx2), 5.21 (1H, br t, J = 7 Hz, C-10-H), 6.61 (1H, s, C-8-H), 6.91 (2H, d, J = 9 Hz, C-3'-H and C-5'-H), 7.46 (2H, d, J = 9 Hz, C-2'-H and C-6'-H), 8.20 (1H, s, C-2-H), 8.60 (1H, br s, OH), 13.20 (1H, s, 5-OH).

Gancaonin H (**5**).

Gancaonin H (**5**) was recrystallized from acetone-benzene to give colorless prisms, mp 205-206 °C. $FeCl_3$ test: dark green. Gibbs test: positive. Uv λ_{max}^{MeOH} nm (log ε): 205 (sh 4.40), 216 (4.44), 230 (sh 4.40), 267 (4.57), 300 (infl. 3.73). Uv $\lambda_{max}^{MeOH+AcONa}$: 268.5 (4.54), 332 (3.91). Uv $\lambda_{max}^{MeOH+AlCl_3}$ (after 2 h): 205 (4.52), 220 (4.53), 230 (infl. 4.51), 275.5 (4.58), 315 (sh 4.00), 383 (3.48). EI-Ms (probe), 70 eV, m/z (rel. int.): 421 $[M+1]^+$ (28%), 420 $[M]^+$ (94), 405 (100), 377 (33), 365 (38), 364 (33), 337 (5), 221 (3), 175 (21), 165 (8), 153 (2). HR-Ms, m/z: 420.1585 $[M]^+$ ($C_{25}H_{24}O_6$ requires: 420.1573). ^1H Nmr (DMSO-d_6, 60 °C): δ 1.40 (6H, s, C-9'-CH_3x2), 1.63, 1.73 (each 3H, br s, C-11-CH_3), 3.24 (2H, br d, J = 6 Hz, C-9-Hx2), 5.18 (1H, br t, J = 6 Hz, C-10-H), 5.76 (1H, d, J = 10 Hz, C-8'-H), 6.38 (1H, d, J = 10 Hz, C-7'-H), 6.46 (1H, s, C-8-H), 6.72, 6.91 (each 1H, d, J = 2 Hz, C-2'-H or C-6'-H), 8.31 (1H, s, C-2-H), 8.99 (br, OH), 13.22 (1H, s, 5-OH).

Gancaonin I (**6**).

Gancaonin I (**6**) was recrystallized from chloroform-petroleum ether to give colorless prisms, mp 67-70 °C/125-127 °C (double melting point). Anal. Calcd for $C_{21}H_{22}O_5 \cdot H_2O$: C, 67.73; H, 6.50. Found: C, 67.52; H, 6.54. $FeCl_3$ test: negative. Gibbs test: positive. Uv λ_{max}^{MeOH} nm (log ε): 213 (4.54), 230 (infl. 4.24), 240 (infl. 4.04), 250 (infl. 3.87), 285 (sh 4.16), 295 (sh 4.19), 305 (sh 4.30), 320 (4.51), 335 (4.46). Uv $\lambda_{max}^{MeOH+MeONa}$: 295 (sh 4.14), 330 (4.49), 343 (4.49). Ir ν_{max}^{KBr} cm^{-1}: 3440, 3260, 1620, 1610 (sh), 1600 (sh), 1505. EI-Ms (probe), 70 eV, m/z (rel. int.): 355 $[M+1]^+$ (24%), 354 $[M]^+$ (100), 339 (16), 299 (5), 286 (9). ^1H Nmr (DMSO-d_6, 60 °C): δ 1.62, 1.73 (each 3H, br s, C-10-CH_3), 3.30 (2H, br d, J = 7 Hz, C-8-Hx2), 3.81, 3.96 (each 3H, s, OCH_3), 5.13 (1H, br t, J = 7 Hz, C-9-H), 6.37 (1H, dd, J = 2 and 8.5 Hz, C-5'-H), 6.48 (1H, d, J = 2 Hz, C-3'-H), 6.92 (1H, br d, J = ca. 0.7 Hz, C-7-H), 7.17 (1H, d, J = 0.7 Hz, C-3-H), 7.56 (1H, d, J = 8.5 Hz, C-6'-H); (DMSO-d_6, 24 °C): δ 9.64, 10.22 (each 1H, br s, OH).

Gancaonin I Diacetate (**6a**).

A mixture of **6** (5 mg), acetic anhydride (0.1 ml), and pyridine (0.1 ml) was kept at room temperature for 30 min and treated as usual. Gancaonin I diacetate was crystallized from chloroform-methanol to give colorless needles, mp 104-105 °C. EI-Ms (probe), 70 eV, m/z: 439 $[M+1]^+$ (27%), 438 $[M]^+$ (100), 396 (67), 354 (86), 339 (11), 295 (5), 286 (7). HR-Ms, m/z: 438.1676 $[M]^+$ ($C_{25}H_{26}O_7$ requires: 438.1678). ^1H Nmr (DMSO-d_6, 60 °C): δ 1.62, 1.73 (each 3H, br s, C-10-CH_3), 2.29, 2.39 (each 3H, s, OAc), 3.31 (2H, br d, J = 7 Hz, C-8-Hx2), 3.84, 4.03 (each 3H, s, OCH_3), 5.12 (1H, br t, J = 7 Hz, C-9-H), 6.97 (1H, br s, C-7-H), 7.15 (1H, d, J = 2 Hz, C-3'-H), 7.20 (1H, dd, J = 2 and 8.5 Hz, C-5'-H), 7.29 (1H, br s, C-3-H), 7.93 (1H, d, J = 8.5 Hz, C-6'-H). ^1H Nmr ($CDCl_3$): δ 1.68 (3H, br d, J = 0.9 Hz, C-10-CH_3), 1.80 (3H, br d, J = 0.4 Hz, C-10-CH_3), 2.31, 2.40 (each 3H, s, OAc), 3.41 (2H, br d, J = 7 Hz, C-8-Hx2), 3.88, 4.04 (each 3H, s, OCH_3), 5.20 (1H, br t, J = 7 Hz, C-9-H), 6.79 (1H, br s, C-7-H), 7.04 (1H, d, J = 2 Hz, C-3'-H), 7.08 (1H, d, J = 0.7 Hz, C-3-H), 7.11 (1H, dd, J = 2 and 8.5 Hz, C-5'-H), 7.92 (1H, d, J = 8.5 Hz, C-6'-H).

ACKNOWLEDGEMENT

We are grateful to Prof. T. Saitoh and to Dr. T. Kinoshita, Faculty of Pharmaceutical Sciences, Teikyo University for their kind supply of authentic sample of isoglycyrol and spectral data of isoglycyrol and glycyrol, and of informations of their unpublished data.

REFERENCES AND NOTES

1. Part 3 in the series 'Phenolic Constituents of Glycyrrhiza Sp.' Part 2: T. Fukai, Q.-H. Wang, and T. Nomura, Heterocycles, 1989, **29**, 1369.
2. Present address: Faculty of Pharmaceutical Sciences, Teikyo University, 1091 Suarashi, Sagamiko-machi, Tsukui-gun, Kanagawa 199-01, Japan.
3. S. Shibata and T. Saitoh, J. Indian Chem. Soc., 1978, **55**, 1184 and references cited therein.
4. T. Hatano, H. Kagawa, T. Yasuhara, and T. Okuda, Chem. Pharm. Bull., 1988, **36**, 2090.
5. T. Hatano, T. Yasuhara, K. Miyamoto, and T. Okuda, Chem. Pharm. Bull., 1988, **36**, 2286.
6. S. Demizu, K. Kajiyama, K. Takahashi, Y. Hiraga, S. Yamamoto, Y. Tamura, K. Okada, and T. Kinoshita, Chem. Pharm. Bull., 1988, **36**, 3474.
7. T. Fukai, M. Toyono, and T. Nomura, Heterocycles, 1988, **27**, 2309.
8. T. Saitoh and S. Shibata, Chem. Pharm. Bull., 1969, **17**, 729.
9. S. Shibata and T. Saitoh, Chem. Pharm. Bull., 1968, **16**, 1932.
10. T. Saitoh, T. Kinoshita, and S. Shibata, Chem. Pharm. Bull., 1976, **24**, 1242.
11. M. Kaneda, T. Saitoh, Y. Iitaka, and S. Shibata, Chem. Pharm. Bull., 1973, **21**, 1338.
12. 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 and Hall, London, 1982, Chapter 2.
13. K.S. Dhami and J.B. Stothers, Can. J. Chem., 1966, **44**, 2855.
14. We contributed the abstract paper on the structure determination of compound 1 to the Organizing Committee of 109th Annual Meeting of the Pharmaceutical Society of Japan, (November 19th, 1988). In the paper, we proposed the formula 1 for the structure of compound 1 (Abstract Papers of 109th Annual Meeting of the Pharmaceutical Society of Japan, 1989, Nagoya, Vol. III,

p. 204). After completion of our work of compounds **1** and **2**, Dr. T. Kinoshita, Teikyo Univ. informed us that Teikyo Univ. group had revised the formulae of isoglycyrol and glycyrol from **1'** and **2'** to **1** and **2**, respectively, (a private communication, March 16th, 1989; T. Shiozawa, S. Urata, T. Kinoshita, and T. Saitoh, Chem. Pharm. Bull. in press; T. Shiozawa, T. Kinoshita, S. Urata, and T. Saitoh, 'Abstract Papers of 109th Annual Meeting of Pharmaceutical Society of Japan, 1989, Nagoya, Vol. III, p.159).

15. T. Hosokawa, S. Yamashita, S.-I. Murahashi, and A. Sonoda, Bull. Chem. Soc. Jpn., 1976, **49**, 3662.
16. T.J. Mabry, K.R. Markham, and M.B. Thomas 'The Systematic Identification of Flavonoids,' Springer-Verlag, New York, 1970.
17. E.A. Sherif, R.K. Gupta, and M. Krishnamurti, Tetrahedron Lett., 1980, **21**, 641.
18. M. Fujita, M. Yamada, S. Nakajima, K.-I. Kawai, and M. Nagai, Chem. Pharm. Bull., 1984, **32**, 2622.
19. ^{13}C Nmr data (acetone-d_6) of glycyrrhisoflavone (data from ref. 4): δ 114.8 (C-2'), 122.1 (C-6'), 122.7, 124.3 (C-1', C-5'), 144.2 (C-3'), 144.9 (C-4').
20. T. Kinoshita, T. Saitoh, and S. Shibata, Chem. Pharm. Bull., 1978, **26**, 135.
21. M. Takasugi, S. Nagao, T. Masamune, A. Shirata, and K. Takahashi, Tetrahedron Lett., 1978, 797.
22. J.A. Elvidge and R.G. Foster, J. Chem. Soc., 1963, 590.
23. T. Fukai, Y. Hano, K. Hirakura, T. Nomura, J. Uzawa, and K. Fukushima, Chem. Pharm. Bull., 1985, **33**, 3195.
24. Assignment of the signal was confirmed by consideration of long-range coupling between the C-7-H (δ 6.92),[22] and by the result of selective decoupling between the C-3 carbon atom (δ 100.26, d, J = 180 Hz).
25. D.M. Dewick, 'The Flavonoids: Advances in Research,' eds. by J.B. Harborne and T.J. Mabry, Chapman and Hall, London, 1982, p. 599.
26. J.L. Ingham, 'Progress in the Chemistry of Organic Natural Products,' Vol. 43, eds. by W. Herz, H. Grisebach, and G.W. Kirby, Springer-Verlag, New York, 1983, pp. 181-189.
27. As the structure of glycyrol (**1**) was revised, 5-O-methylglycyrol should be renamed as 3-O-methylglycyrol.

Received, 8th May, 1989