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Flavonoid constituents from *Glycyrrhiza glabra* hairy root cultures

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Abstract

An unusual biflavonoid named licoagrodin was isolated from the hairy root cultures of *Glycyrrhiza glabra* (Leguminosae) along with three prenylated retrochalcones, licoagrochalcones B, C, D, a prenylated aurone, licoagroaurone and four known prenylated flavonoids, licochalcone C, kanzonol Y, glyinflanin B and glycyrdione A. From the glycosidic fraction, a isoflavone glycoside, licoagroside A, and a maltol glycoside, licoagroside B were isolated together with four known isoflavone glycosides, two flavone *C*-glycosides, and three other glycosides. Their structures were elucidated on the basis of spectroscopic evidence.

Keywords: *Glycyrrhiza glabra*; Leguminosae; Hairy root; Flavonoid; Biflavonoid; Aurone; Flavonoid glycoside; Maltol glycoside

1. Introduction

Licorice, the roots and stolons of some *Glycyrrhiza* species is one of the oldest plant medicines that have been used by human beings. Many biological activities such as antimutagenic activity (Ngo et al., 1992; Zani et al., 1993), anti-ulcer effects (Takagi and Ishii, 1967; Takagi et al. 1969; van Marle et al., 1981), protective action against hepatotoxicity (Nose et al., 1994), antitumor promoting activity (Nishino et al., 1986), antimicrobial effects (Mitscher et al., 1980; Demizu et al. 1988; Okada et al., 1989; Haraguchi et al., 1998), etc., were reported. These activities are reported due to two kinds of main constituents, the saponins and flavonoids. One of the flavonoid-rich fractions from the extract of licorice has been developed as an anti-ulcer drug (Aspalon®) in Japan (Japan Pharmceutical Information Center, 1997). Until now, many studies on the flavonoid constituents of *Glycyrrhiza* species have been carried out, with more than 300 flavonoids being reported. Among them, about 70 were isolated from the underground organs of *G. glabra*, one of the most important species of licorice (Nomura and Fukai, 1998).

In our studies on hairy root cultures to produce useful compounds, we investigated the constituents of *G. glabra* hairy root cultures. Although glycyrrhizin, the main saponin of some *Glycyrrhiza* species was not detected in its cultures, a high flavonoid production was nevertheless established, and 10 flavonoids were reported in previous papers (Asada et al., 1998, 1999; Li et al., 1998). Herein, we report the isolation and structural determination of compounds **1**–**20**, which include an unusual biflavonoid together with four retrochalcones, a dihydrochalcone, an aurone, two dibenzoylmethanes, seven flavonoid glycosides, a maltol glycoside and three other glycosides (Fig. 1).

2. Results and discussion

The methanol extract of *G. glabra* hairy root cultures was partitioned between water and ethyl acetate. The EtOAc extract was subjected to silica gel column chromatography and further purified by normal-phase HPLC to give five new flavonoids, licoagrodin (**1**), licoagrochalcone B (**3**), licoagrochalcone C (**4**), licoagrochalcone D (**5**) and licoagroaurone (**7**) and four known flavonoids, licochalcone C (**2**) (Kajiyama et al., 1992), kanzonol Y (**6**) (Fukai et al., 1996, 1997), glyinflanin B (**8**) (Zheng et al., 1992) and glycyrdione A (**9**) (Demizu et al., 1992; Zheng et al., 1992). The water layer was subjected to a Daion HP-20 column chromatography, which was eluted with methanol. The methanol eluate was evaporated in vacuo to give a glycosidic fraction. The fraction was applied to ODS column chromatography and then

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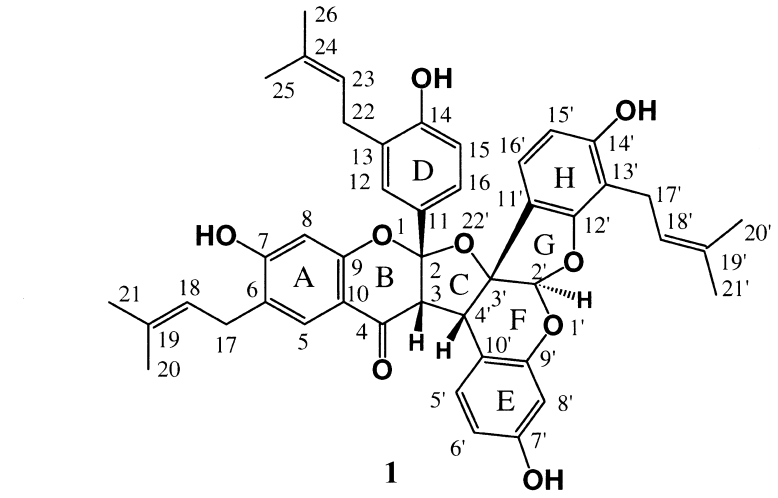

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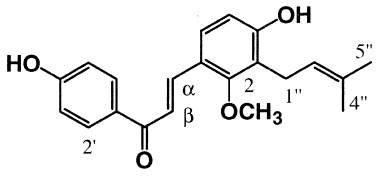

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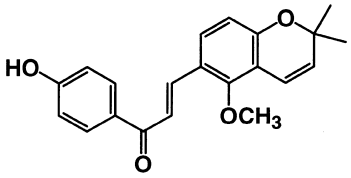

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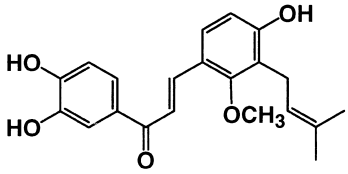

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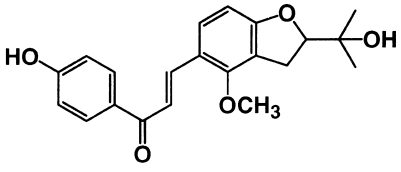

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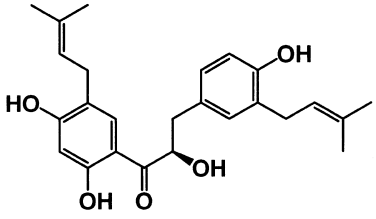

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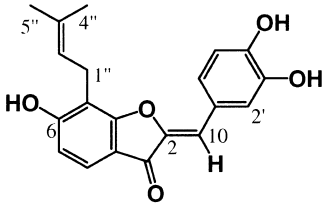

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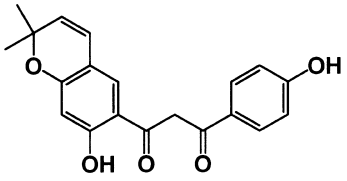

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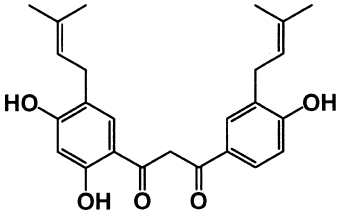

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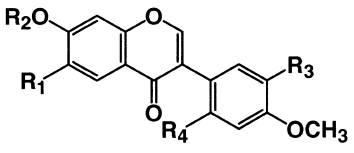

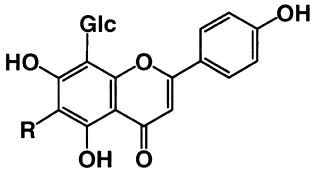

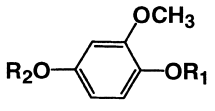

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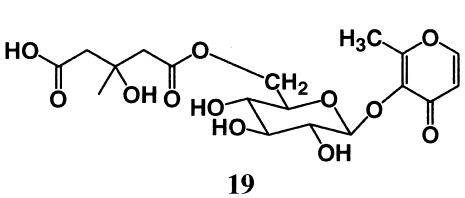
13: R_1=OCH_3, R_2=Glc, R_3=R_4=H
14: R_1=OCH_3, R_2=Glc-6-$OOCCH_2COOH$, R_3=R_4=H

15: R=Glc
16: R=Ara

17: R_1= H, R_2=Glc
18: R_1= Glc, R_2=H



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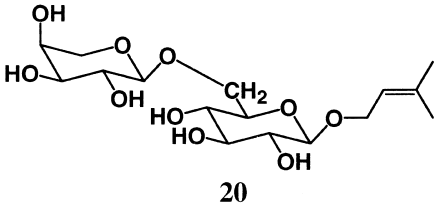

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Fig. 1. Compounds isolated from *G. glabra* hairy root cultures.

purified by reverse phase HPLC to give the new isoflavone glycoside, named licoagroside A (**10**), as well as six known flavonoid glycosides, ononin (**11**) (Kitakawa et al., 1994), calycosin 7-*O*-glucoside (**12**) (Katsura and Yamagishi, 1987), wistin (**13**) (Kajiyama et al., 1994), afrormosin 7-*O*-(6″-malonylglucoside) (**14**) (Kessmann et al., 1990), vicenin-2 (**15**) (Österdahl, 1978), isoschaftoside (**16**) (Österdahl, 1978), the new maltol glycoside, licoagroside B (**19**), and three other known glycosides, tachioside (**17**) (Inoshiri et al., 1987), isotachioside (**18**) (Inoshiri et al., 1987), dimethylallyl 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside (**20**) (Chassagne et al., 1996).

Licoagrodin (**1**) was obtained as a colorless powder, which did not give crystals suitable for X-ray analysis. Its molecular formula, $C_{45}H_{44}O_9$, was established by HR-FAB mass spectral analysis. The IR spectrum showed absorption bands at 3440 and 1660 cm^{-1} due to hydroxyl and carbonyl groups, respectively. The ^1H NMR spectrum of **1** revealed signals indicating three γ, γ-dimethylallyl groups, *ortho*-coupled aromatic protons at δ 6.50 (*d*, *J* = 8.5 Hz), 7.23 (*d*, *J* = 8.5 Hz), two ABX-type aromatic protons at δ 6.80 (*d*, *J* = 8.5 Hz), 7.21 (*dd*, *J* = 8.5, 2.5 Hz), 7.27 (*d*, *J* = 2.5 Hz) and δ 6.45 (*dd*, *J* = 8.5, 2.5 Hz), 6.62 (*d*, *J* = 2.5 Hz), 6.68 (*d*, *J* = 8.5 Hz), two isolated aromatic protons at δ 6.70, 7.56, two coupled methine protons at δ 3.36 (*d*, *J* = 12.0 Hz), 3.93 (*d*, *J* = 12.0 Hz) and an isolated methine proton at δ 6.32. All protonated carbons were assigned by the HMQC spectrum. The ^{13}C NMR spectrum showed 45 carbon signals, including a carbonyl carbon (δ 189.0), 24 carbons due to four aromatic rings, seven of which have an oxygen function, 15 carbons due to three prenyl groups and residual five carbons (Table 1). By analysis of the HMBC spectrum, the carbons on four aromatic rings A, D, E and H were assigned as shown in Fig. 2 and Table 1. Further correlations between the methylene proton signals on the prenyl groups and the quaternary aromatic carbons suggested that the prenyl groups located at C-6 on aromatic ring A, C-13 on aromatic ring D, C-13′ on aromatic ring H, respectively. The HMBC

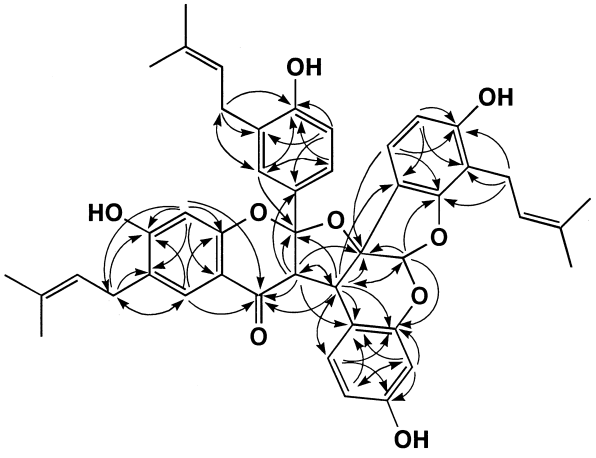

Fig. 2. ^1H-^{13}C long-range correlation by the HMBC spectrum of **1**.

Table 1
^1H NMR and ^{13}C NMR spectral data for licoagrodin (**1**)

Position	^{13}C NMR	^1H NMR	Position	^{13}C NMR	^1H NMR
2	113.2		2′	111.2	6.32 (1H, *s*)
3	63.4	3.36 (1H, *d*, *J* = 12.0 Hz)	3′	93.5	
4	189.0		4′	50.4	3.93 (1H, *d*, *J* = 12.0 Hz)
5	128.3	7.56 (1H, *s*)	5′	131.88	6.68 (1H, *d*, *J* = 8.5 Hz)
6	124.6		6′	111.6	6.45 (1H, *dd*, *J* = 8.5, 2.5 Hz)
7	164.0		7′	159.4	
8	103.4	6.70 (1H, *s*)	8′	106.8	6.62 (1H, *d*, *J* = 2.5 Hz)
9	159.9		9′	152.3	
10	112.2		10′	113.1	
11	129.3		11′	118.3	
12	128.0	7.27 (1H, *d*, *J* = 2.5 Hz)	12′	159.3	
13	128.5		13′	111.3	
14	156.6		14′	158.4	
15	115.5	6.80 (1H, *d*, *J* = 8.5 Hz)	15′	110.2	6.50 (1H, *d*, *J* = 8.5 Hz)
16	125.5	7.21 (1H, *dd*, *J* = 8.5, 2.5 Hz)	16′	123.3	7.23 (1H, *d*, *J* = 8.5 Hz)
17	28.2	3.30 (2H, *dd*, *J* = 14.0, 7.0 Hz)	17′	22.8	3.14 (1H, *dd*, *J* = 14.0, 7.0 Hz)
18	122.9	5.34 (1H, *m*)			3.20 (1H, *dd*, *J* = 14.0, 7.0 Hz)
19	133.1		18′	122.69	5.19 (1H, *m*)
20	17.78	1.71 (3H, *d*, *J* = 1.0 Hz)	19′	131.85	
21	25.9	1.74 (3H, *d*, *J* = 1.0 Hz)	20′	17.74	1.67 (3H, *d*, *J* = 1.0 Hz)
22	28.6	3.25 (2H, *d*, *J* = 7.0 Hz)	21′	25.77	1.57 (3H, *d*, *J* = 1.0 Hz)
23	122.72	5.23 (1H, *m*)			
24	133.4				
25	17.68	1.59 (3H, *d*, *J* = 1.0 Hz)			
26	25.79	1.69 (3H, *d*, *J* = 1.0 Hz)			

spectrum of **1** also showed correlations between the ring A protons at δ 7.56 (5-H), 6.70 (8-H) and the carbonyl carbon at δ 189.0 (C-4), indicating that the carbonyl group is combined with ring A. The carbonyl carbon was further correlated to two coupled methine protons at δ 3.36 (3-H) and 3.93 (4′-H). The 3-H was correlated to C-11 on D-ring at δ 129.3 and to C-10′ on E-ring at δ 113.1 through $^3J_{CH}$, and the 4′-H was correlated to C-10′ and C-11′ on H-ring at δ 118.3 through $^2J_{CH}$ and $^3J_{CH}$. The methine proton at δ 6.32 (2′-H) was correlated to aromatic carbons at δ 152.3 (C-9′) and δ 159.3 (C-12′) on E and H-rings, respectively. Taking account the ^{13}C NMR spectral data and the 24 degrees of unsaturation calculated from the empirical formular of **1**, it was suggested that **1** has four alicyclic rings in addition to four aromatic rings. The D ring, which is bound to the B ring at C-2, was indicated by the correlations of 5-H, 8-H/C-4 and 3-H/C-2, C-4, C-11. The six-membered F ring binding to E-ring was indicated by the correlation of 4′-H/C-2′, C-3′, C-9′, C-10′ and 2′-H/C-3′, C-4′, C-9′, respectively. The five-membered G ring was indicated by the correlations of 2′-H/C-3′, C-9′, C-12′, 16′-H/C-3′, C-12′ and 17′-H/C-12′. The other five-membered ring (C-ring) was suggested by correlation of 3-H/C-2, C-4, C-3′ and 4′-H/C-2, C-3, C-3′, C-10′, C-11′. Thus, the molecular structure of licoagrone (**1**) was as depicted.

The relative stereochemistry of **1** was determined by the NOESY spectrum (Fig. 3). The 3-H showed NOEs with 12-H and 16-H on ring D which suggested a *cis*-junction of the B/C rings. The cross-peak observed between 3-H and 4′-H showed that they are in a *cis*-configuration, and then the 4′-H displayed a NOE with 16′-H but no NOE with 2′-H, suggested *cis*-junctions for the C/F and F/G rings. Base on the above spectral data, the structure of licoagrodin (**1**) was elucidated as shown in Fig. 1.

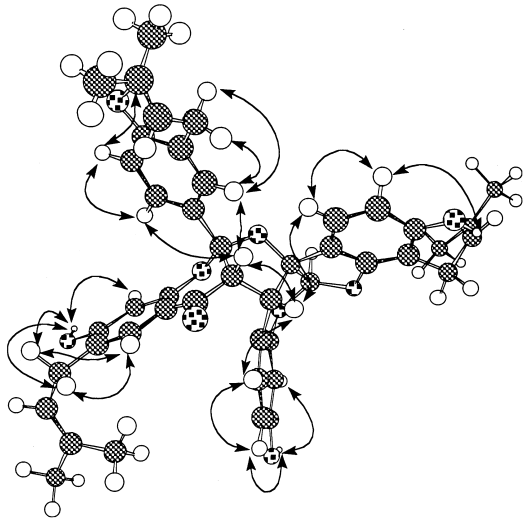

Fig. 3. NOEs detected for **1** by the NOESY spectrum.

Licoagrochalcone B (**3**) was isolated as a yellow powder. Its molecular formula, $C_{21}H_{20}O_4$, was established by HR-EI mass spectral analysis. Its spectroscopic properties are very similar to the known retrochalcone, licochalcone C (**2**) except for the presence of a prenyl group. In the ^{1}H NMR spectrum, **3** showed two one-proton doublets characteristic of trans-olefinic protons of a chalcone at δ 7.77 (*d*, J=15.5 Hz) and 7.98 (*d*, J=15.5 Hz), a methoxy signal at δ 3.83 (3H, s), A_2X_2-type aromatic protons at δ 6.98 (*d*, J=8.5 Hz) and 8.08 (*d*, J=8.5 Hz) on A ring, and an AX-type aromatic system at δ 6.65 (*dd*, J=8.5, 0.5 Hz) and 7.77 (*d*, J=8.5 Hz) on B ring. In the EI–MS spectrum, **3** showed a molecular ion peak at m/z 336 $[M]^+$ which is smaller by 2 mass units than that of **2**. The base peak at m/z 305 $[M-OMe]^+$ suggested that **3** is a 2-methoxychalcone (Saitoh et al., 1975) and the fragment ion peak at m/z 121 indicated the presence of *p*-hydroxybenzoyl group in **3**. The ^{1}H NMR spectrum also exhibited signals due to a dimethylchromene group at δ 1.45 (6H, *s*), 5.84 (1H, *d*, J=10.0 Hz) and 6.67 (1H, *dd*, J=10.0, 0.5 Hz). Long-range coupling (J=0.5 Hz) was also observed between 5-H at δ 6.65 and 1″-H at δ 6.67 indicating that the chromene group was located at C-3 of the B-ring. The above data suggested that the structure of **3** is as shown in Fig. 1. In order to confirm its substitution pattern containing the B-ring, and also for a more accurate assignment of ^{13}C NMR spectral data, HMBC analysis give the long-range correlations shown in Fig. 4. These data supported structure **3**.

Licoagrochalcone C (**4**) was isolated as a yellow powder. Its HR-El mass spectrum displayed a $[M]^+$ at 354.1453, which corresponded to the empirical formula, $C_{21}H_{22}O_5$ (354.1468). The ^{1}H NMR spectrum of **4** was similar to that of **2**, except for ABX-type aromatic proton signals at δ 6.96 (*d*, J=8.5 Hz), 7.63 (*dd*, J=8.5, 2.5 Hz) and 7.64 (*d*, J=2.5 Hz) instead of the A_2X_2-type aromatic proton signals due to the A-ring of **2**. Thus, the structure of licoagrochalcone C was suggested to be **4**. The substitution pattern and assignment of ^{13}C NMR spectral data was confirmed by analysis of the HMBC spectrum as shown in Fig. 4. These data also supported structure **4**.

Licoagrochalcone D (**5**) had the same molecular formula as licoagrochalcone C (**4**) in their HR–EI mass spectra. For the ^{1}H- and ^{13}C-NMR spectra, **5** and **3** showed similar patterns in aromatic proton and carbon signal regions. In the ^{1}H NMR spectrum of **5**, the coupling constants between 1″-H_a and 2″-H (J=10.0 Hz) and 1″-H_b and 2″-H (J=8.0 Hz) suggested that C-3 was substituted by a 2, 3-dihydro-2-(1-hydroxy-1-methylethyl) furan isoprenoid unit (Tahara et al., 1987). The structure of **5** was also confirmed by analysis of the HMBC spectrum as shown in Fig. 4. Thus, the structure of licoagrochalcone D was characterized as formula **5**.

Licoagroaurone (**7**) was obtained as an orange powder. The EI mass spectrum of **7** showed a molecular ion

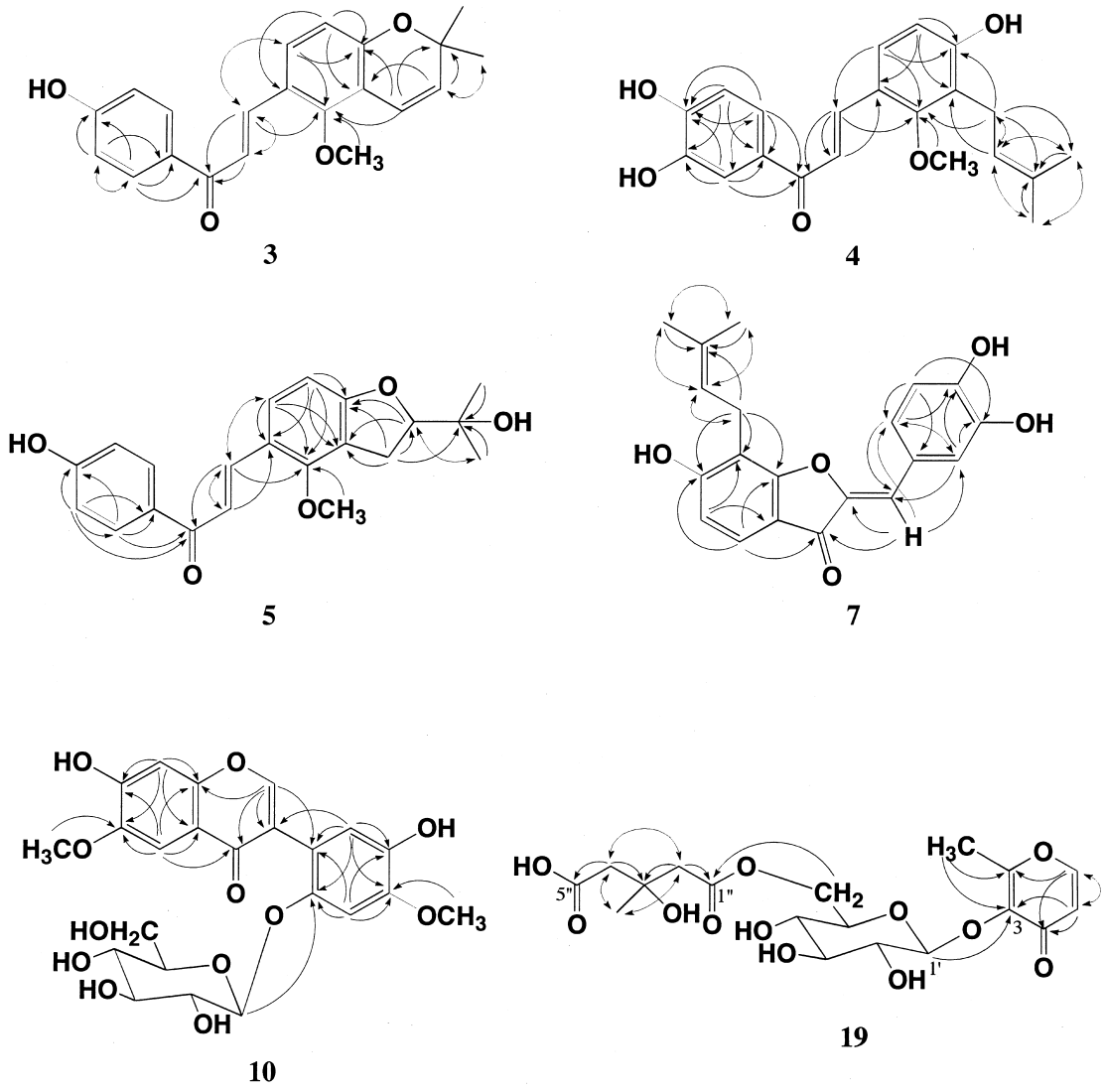

Fig. 4. ^{1}H–^{13}C long-range correlation by the HMBC spectra of compounds **3**–**5**, **7**, **10** and **19**.

peak at *m/z* 338 and the molecular formula was determined as $C_{20}H_{18}O_5$ from the HR-EI mass spectrum. The UV spectrum of **7** exhibited absorption maxima at 256, 267 and 396 nm, resembling that of aurone derivatives (Markham and Mabry, 1975). The ^{1}H NMR spectrum of **7** showed signals indicating an olefinic proton at δ 6.64, a dimethylallyl group at δ 1.69 (3H, *s*), 1.88 (3H, *s*), 3.56 (2H, *d*, $J=7.0$ Hz), 5.41 (1H, *m*), AX-type aromatic protons at δ 6.83 (*d*, $J=8.5$ Hz), 7.46 (*d*, $J=8.5$ Hz) and ABX-type aromatic protons at δ 6.96 (*d*, $J=2.5$ Hz), 7.42 (*dd*, $J=8.5$, 2.5 Hz), 7.61 (*d*, $J=8.5$ Hz). In the ^{13}C NMR spectrum, 20 carbon signals were observed. The above data indicated that **7** is most likely a prenylated aurone. The ^{1}H and ^{13}C NMR spectra of flavones and aurones are similar (Markham and Chari, 1982; Markham and Geiger, 1994), but they can be clearly distinguished from the HMBC spectrum. The olefinic proton was correlated to the carbonyl carbon (δ 182.9), C-2′ carbon (δ 118.8) and 6′ carbon (δ 125.5) through $^{3}J_{CH}$ coupling. The methylene carbon signal of the prenyl group was observed at δ 22.6, which indicated that both *ortho*-positions to the dimethylallyl group were occupied by oxygenated substituents (Fukai and Nomura, 1989), so the location of the prenyl moiety was determined to be at C-7. These substituent patterns were also confirmed by the HMBC spectrum (Fig. 4). The Z-stereochemistry of the double bond at C-10 in **7** was determined by the chemical shift of C-10 (δ 112.0) (Pelter et al., 1979). Thus, the structure of licoagroaurone was established as **7**.

Compound **10** was obtained as a powder. The HR-FAB mass spectrum displayed $[M+H]^{+}$ at *m/z* 493.1360 in agreement with the empirical formula $C_{23}H_{25}O_{12}$. The ^{13}C NMR spectrum indicated the presence of 23 carbon signals, including a carbonyl group at δ 178.3. The ^{1}H NMR spectrum of **10** showed a singlet at δ 8.16 characteristic of 10 2-H resonance of isoflavone derivatives. The spectrum also exhibited four isolated singlets for

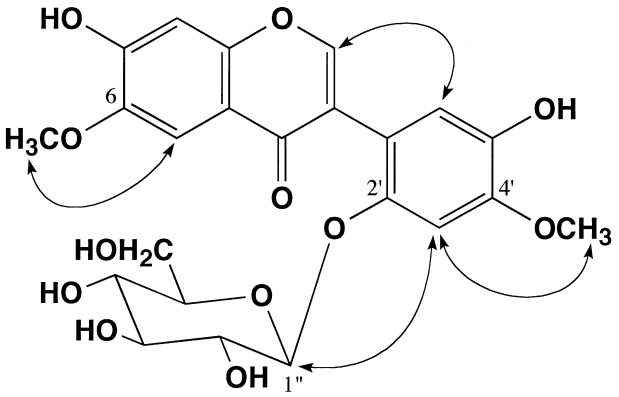

Fig. 5. NOEs observed in the NOE difference spectrum of **10**.

aromatic protons at δ 6.76, 6.94, 7.03, 7.55, indicating that the protones on the A and C rings were both *para*-oriented, as well as two methoxy proton signals at δ 3.90, 3.96 and an anomeric proton signal at δ 4.81 (*d*, $J=8.0$ Hz). The coupling constants of the protons of the sugar moiety in **10** suggested the presence of a β-D-glucopyranose. Among the four aromatic protons, the most deshielded proton (δ 7.55) was readily assigned to 5-H. On irradiation of 2-H at δ 8.16, an NOE was observed for the signal at δ 6.76 from the NOE difference spectrum as shown in Fig. 5, suggesting that the proton at δ 6.76 could be assigned to 6-H′, while the residual aromatic protons at δ 6.94 and 7.03 were assigned to 8-H and 3-H′ based on the analysis of the HMBC spectrum of **10**. Irradiation of the methoxy protons at δ 3.90 and the anomeric proton at δ 4.81 caused NOEs on the 3-H′ signal at δ 7.03 on the B ring, suggesting that the methoxy and glucopyranosyl groups are located at C-2′ or C-4′ of the B-ring. The other methoxy protons at δ 3.96 showed an NOE with 5-H at δ 7.55 indicating that the methoxy group at δ 3.96 was located at C-6. The substitution pattern of **10** was further supported from the HMBC spectrum except for the 4′-methoxy and 2′-glucopyranoyloxy groups as shown in Fig. 4. Considering the methylation pattern of known isoflavonoids from hairy root cultures of *G. glabra*, it is most likely that the methoxy group is located at C-4′. In order to confirm this pattern, the long-range selective proton decoupling spectrum of **10** was measured. On irradiation of 2-H at δ 8.16, $^4J_{CH}$ long range coupling was observed for the carbon signal at δ 150.4, which was assigned to the glucosyloxy bonded carbon by the HMBC spectrum. Therefore, the location of the glucopyranosyl moiety was determined to be at 2′-OH. Thus, **10** was determined as 7, 5′-dihydroxy-6, 4′-dimethoxy-isoflavone-2′-*O*-β-D-glucopyranoside.

Compound **19** was obtained as a powder. The molecular formula of **19** was determined as $C_{18}H_{24}O_{12}$, on the basis of the $[M+H]^+$ at m/z 433.1344 in the HR-FAB mass spectrum. The ^1H and ^{13}C NMR spectra of **19** showed signals due to a 3-hydroxy-3-methylglutaric acid (HMG) group, a 3-hydroxy-2-methyl-γ-pyrone (maltol) group and a β-D-glucopyranose group. In the HMBC spectrum (Fig. 4), a cross-peak was observed between the anomeric proton at δ 4.85 (*d*, $J=8.0$ Hz) and the C-3 carbon at δ 143.3 of the maltol motiety, suggesting that compound **19** contains maltol 3-*O*-β-D-glucopyranoside as a partial structure (Looker and Fisher, 1985). In comparing the ^{13}C NMR spectra of **19** and maltol 3-*O*-β-D-glucopyranoside, acylation shifts were observed for the signals due to C-5′ (ca.−2.9 ppm) and C-6′ (ca.+1.4 ppm) of the glucopyranosyl moiety in **19**, suggesting that the HMG moiety is attached to the C-6′ position of glucopyranosyl moiety. The HMBC spectrum of **19** further supported the linkage site of the HMG moiety to glucopyranose. Namely, the HMBC spectrum showed the cross-peak between the 6′-H protons of glucopyranose (δ 4.21 and 4.45) and the C-1″ carbon of HMG (δ 172.3). Thus, the structure of **19** was determined as maltol 3-*O*-[6-*O*-(3-hydroxy-3-methylglutaroyl)]-β-D-glucopyranoside.

In conclusion, we have isolated five new prenylated flavonoids, a new isoflavone glycoside, a new maltol glycoside and known compounds from the hairy root cultures of *G. glabra*. Among the known flavonoids, kanzonol Y (**6**) (Fukai et al., 1996; Fukai et al., 1997) and ononin (**11**) (Kitakawa et al., 1994) were previously obtained from the root of *G. glabra*, while licochalcone C (**2**) (Kajiyama et al., 1992), glyinflanin B (**8**) (Zheng et al., 1992) and glycyrdione A (**9**) (Zheng et al., 1992; Demizu et al., 1992) were isolated from the root of another *Glycyrrhiza* species, *G. inflata* and vicenin-2 (**15**) (Österdahl, 1978) from that of *G. uralensis*. Compounds **2**–**5** are regarded as retrochalcones, because their oxygenation patterns are the reverse as compared with those of the normal chalcones. The first retrochalcone, echinatin was isolated from the *G. echinata* cell cultures (Furuya et al., 1971) and also reported in the root of *G. glabra* (Kitakawa et al., 1994). It seems that the *G. glabra* hairy root cultures have more prenylation and hydroxylation abilities than *G. glabra* roots in the course of the retrochalcone biosynthesis and thus lead to produce the new compounds **3**–**5**. Aurones have never been isolated from the *Glycyrrhiza* species, so **7** is the first aurone from this species. The biflavonoid (**1**) has a unique structure as a natural product. As for its possible biosynthesis, a hypothetical pathway to **1** is given as shown in Fig. 6 by a radical reaction mechanism. It is very interesting that the isolated flavonoids could be obviously divided into two groups in the course of their biosynthesis. The flavonoids lacking 5-hydroxyl substituent are biosynthesized to isoflavones, then further glycosylated to afford *O*-glucosides (**10**–**14**), while the flavonoids possessing 5-hydroxyl substituent are biosynthsized to flavone *C*-glycosides (**15** and **16**). The reason for this are not clear and need further study.

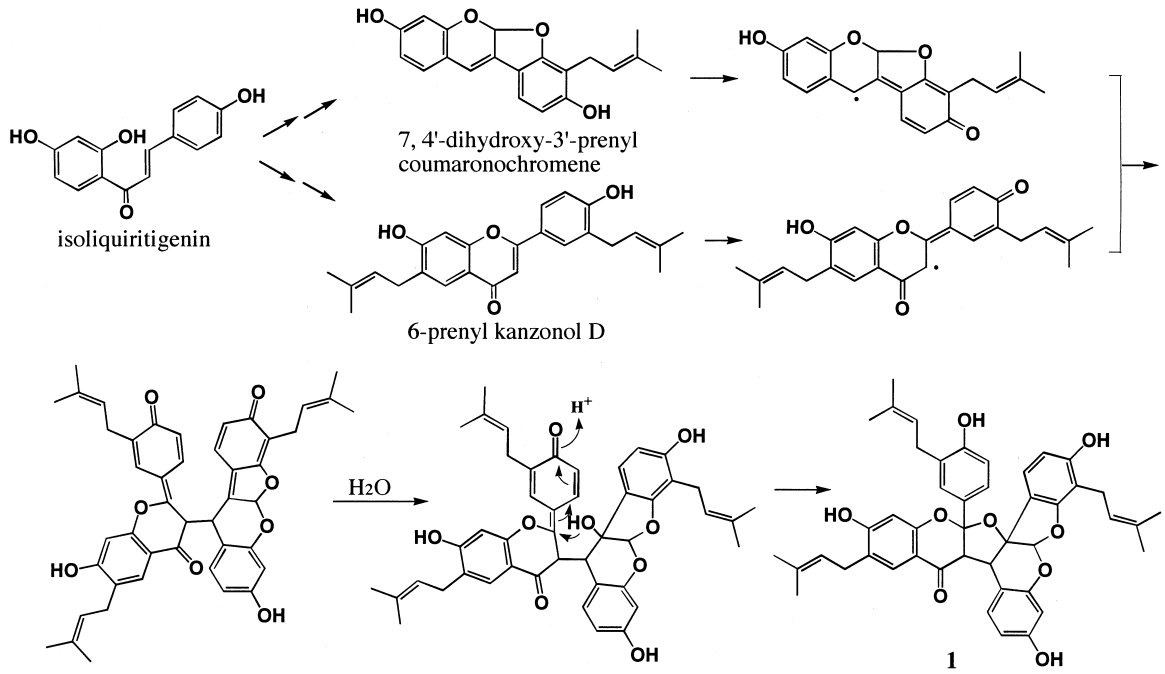

Fig. 6. Hypothetical biosynthesis pathway for the formation of **1** from isoliquiritigenin.

3. Experimental

3.1. General

UV Spectra were obtained with a Hitachi 340 spectrophotometer, whereas the IR spectra were measured with a Jasco FT/IR-200 (by a KBr disk method) spectrometer. Optical rotations were measured with a Jasco DIP-370 digital polarimeter in a 0.5 dm length cell. EI–MS and HR-FAB–MS were taken on a Jeol JMS DX-300 spectrometer. ^{1}H and ^{13}C NMR spectra were measured with a Varian XL-400 spectrometer. For HPLC, a Waters model 510 HPLC system was used. Column chromatography was carried out using Wako-gel C-200 and YMC-gel ODS-AQ. TLC was conducted on Kieselgel $60F_{254}$ plates (Merck).

3.2. Mass culture of G. glabra hairy root cultures

The mass culture conditions of *G. glabra* hairy root were described previously (Asada et al., 1998).

3.3. Separation procedures of flavonoids

The methanol extract obtained as mentioned in the previous paper was partitioned between EtOAc and water. Removal of the solvent from the EtOAc phase under reduced pressure below 40°C yielded the EtOAc extract (50.66 g). The water layer was applied to a Diaion HP-20 column which was eluted with water, followed by methanol. Evaporation of the methanol eluate gave the glycosidic fraction (9.73 g).

The EtOAc fraction was subjected to column chromatography on Wako-gel C-200 (1500 g) and eluted with *n*-hexane:acetone (3:2, v/v) to give six fractions, fr. 1 (1250 ml, 2.2754 g), fr. 2 (250 ml, 1.1072 g), fr. 3 (1000 ml, 6.5808 g), fr. 4 (2000 ml, 6.5556 g), fr. 5 (1500 ml, 3.2771 g), fr. 6 (2000 ml, 3.6993 g) and then the column was washed with MeOH to give fr. 7 (1500 ml, 11.190 g).

Fr. 3 was subject to silica gel column chromatography (*n*-hexane:acetone = 75:25) to give 10 fractions I–X. Fr. V was further purified by repeated HPLC (*n*-hexane–$CHCl_3$–EtOH = 9:10:0.5; *n*-hexane–EtOAc–EtOH = 90:8:1) to give **8** (5.8 mg). Fr. VI was purified by HPLC (*n*-hexane–$CHCl_3$–EtOH = 60:40:1; $CHCl_3$–MeOH = 99:1; *n*-hexane–$CHCl_3$–EtOH = 15:85:0.3) to give **3** (14.8 mg) and **6** (5.4 mg). Fr. VII was purified by HPLC (*n*-hexane–$CHCl_3$–EtOH = 60:40:1; *n*-hexane–isopropanol = 96:4) to give **9** (138.0 mg). Fr. IX was purified by HPLC (*n*-hexane–$CHCl_3$–EtOH = 40:60:1; *n*-hexane–acetone–isopropanol = 90:5:4) to give **2** (39.6 mg).

Fr.4 was subject to silica gel column chromatography (*n*-hexane:acetone = 65:35) to give six fractions I–VI. Fr. VI was further separated by HPLC (*n*-hexane–EtOH = 89:11) to give more six fractions VIa–VIf. Fr. VIc was purified by HPLC (*n*-hexane–EtOAc–EtOH = 80:20:3) to give **4** (9.4 mg) and **7** (9.4 mg). Fr. VId was purified by HPLC (*n*-hexane–$CHCl_3$–EtOH = 10:90:2; hexane–$CHCl_3$–EtOH = 70:30:0.5) to give **1** (9.0 mg) and **5** (6.2 mg).

The glycosidic fraction was subjected to ODS silica gel column chromatography (50 g), eluted with aqueous methanol (0–100%) to give eight fractions, fr. 1 (0.9865 g),fr. 2 (1.3821 g), fr. 3 (1.38289 g), fr. 4 (2.7798 g), fr. 5

(1.1291 g), fr. 6 (0.7685 g), fr. 7 (0.8364 g) and fr. 8 (0.1743 g). Further purification of fractions 1–5 was achieved by repeated HPLC (μ-Bondasphere, 5 μm C18-100 Å, 19×150 mm) to give **10** (28.3 mg), **11** (18.3 mg), **12** (8.8 mg), **13** (33.8 mg), **14** (87.6 mg), **15** (96.8 mg), **16** (31.0 mg), 17 (5.8 mg), **18** (5.8 mg), **19** (55.5 mg), and **20** (20.6 mg).

3.4. Licoagrodin (*1*)

Yellow powder. $[\alpha]_D^{24}$ ±0° (*c* = i.12, MeOH). UV λ_{max}^{MeOH} nm (log ε): 230 sh (4.49), 280 (4.23), 326 (3.93). IR υ_{max}^{KBr} cm^{-1}: 3440, 1660, 1600. EI–MS *m*/*z* (%): 408 (29), 322 (39), 266 (42), 189 (100). HR-FAB–MS: calcd for $C_{45}H_{43}O_9$ ($[M–H]^-$), 727.2907; found: 727.2923. ^{1}H NMR (400 MHz, acetone-d_6) and ^{13}C NMR (100 MHz, acetone-d_6) δ: see Table 1.

3.5. Licoagrochalcone B (*3*)

Yellow powder. UV λ_{max}^{MeOH} nm (log ε): 290 (3.70), 350 (3.73). IR υ_{max}^{KBr} cm^{-1}: 3430, 1630, 1590, 1560. EI–MS *m*/*z* (%): 336 (M^+, 15), 321 (93), 305 (100),121 (27). HR-EI–MS: calcd for $C_{21}H_{20}O_4$ (M^+), 336.1362; found: 336.1374. ^{1}H NMR (400 MHz, acetone-d_6) δ: 1.45 (6H, *s*, 4″ and 5″-CH_3), 3.83(3H, *s*, OCH_3), 5.84 (1H, *d*, *J* = 10.0 Hz, 2″-H), 6.65 (1H, *dd*, *J* = 8.5, 0.5 Hz, 5-H), 6.67 (1H, *dd*, *J* = 10.0, 0.5 Hz, 1″-H), 6.98 (2H, *d*, *J* = 8.5 Hz, 3′ and 5′-H), 7.77 (1H, *d*, *J* = 8.5 Hz, 6-H), 7.77 (1H, *d*, *J* = 15.5 Hz, α-H), 7.98 (1H, *d*, *J* = 15.5 Hz, β-H), 8.08 (2H, *d*, *J* = 8.5 Hz, 2′ and 6′-H). ^{13}C NMR (100 MHz, acetone-d_6) δ: see Table 2.

3.6. Licoagrochalcone C (*4*)

Yellow powder. UV λ_{max}^{MeOH} nm (log ε): 247 (4.10), 360 (4.38). IR υ_{max}^{KBr} cm^{-1}: 3430, 1630, 1590, 1560. EI–MS *m*/*z* (%): 354 (M^+, 1), 323 (64), 137 (100). HR-EI–MS: calcd for $C_{21}H_{22}O_5$ (M^+), 354.1468; found: 354.1453. ^{1}H NMR (400 MHz, acetone-d_6) δ: 1.67 (3H, *d*, *J* = 0.8 Hz, 5″-CH_3), 1.79 (3H, *d*, *J* = 1.0 Hz, 4″-CH_3), 3.40 (2H, *d*, *J* = 7.0 Hz, 1″-H), 3.78 (3H, *s*, OCH_3), 5.27 (1H, *m*, 2″-H), 6.77 (1H, *d*, *J* = 8.5 Hz, 5-H), 6.96 (1H, *d*, *J* = 8.5 Hz, 5′-H), 7.63 (1H, *dd*, *J* = 8.5, 2.5 Hz, 6′-H), 7.64 (1H, *d*, *J* = 2.5 Hz, 2′-H), 7.67 (1H, *d*, *J* = 8.5 Hz, 6-H), 7.67 (1H, *d*, *J* = 15.5 Hz, α-H), 7.98 (1H, *d*, *J* = 15.5 Hz, β-H).^{13}C NMR (100 MHz, acetone-d_6) δ: see Table 2.

3.7. Licoagrochalcone D (*5*)

Yellow powder. $[\alpha]_D^{24}$ −8.7° (*c* 0.23, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 260 (3.83), 300 sh (3.87), 356 (4.16). IR υ_{max}^{KBr} cm^{-1}: 3440, 1630, 1600, 1590. EI–MS *m*/*z* (%): 354 (M^+, 17), 323 (100), 121 (29). HR-EI–MS: calcd for $C_{21}H_{22}O_5$ (M^+), 354.1468; found: 354.1461. ^{1}H NMR (400 MHz, acetone-d_6) δ: 1.25 (3H, *s*, 4″ or 5″-CH_3), 1.28 (3H, *s*, 4″ or 5″-CH_3), 3.43 (1H, *dd*, *J* = 16.0, 10.0 Hz, 1″-H_a), 3.44 (1H, *dd*, *J* = 16.0, 8.0 Hz, 1″-H_b), 4.00 (3H, *s*, OCH_3), 4.71 (1H, *dd*, *J* = 10.0, 8.0 Hz, 2″-H), 6.53 (1H, *d*, *J* = 8.5 Hz, 5-H), 6.96 (2H, *d*, *J* = 8.5 Hz, 3′ and 5′-H), 7.70 (1H, *d*, *J* = 16.5 Hz, α-H), 7.71 (1H, *d*, *J* = 8.5 Hz, 6-H), 8.04 (1H, *d*, *J* = 16.5 Hz, β-H), 8.06 (2H, *d*, *J* = 8.5 Hz, 2′ and 6′-H). ^{13}C NMR (100 MHz, acetone-d_6) δ: see Table 2.

Table 2
^{13}C NMR spectral data for retrochalcones **3**–**5**

	3	**4**	**5**
C=O	188.1	188.2	188.2
C-α	121.2	120.4	120.0
C-β	138.1	139.1	139.2
C-1	121.9	121.1	120.4
C-2	157.1	160.5	157.5
C-3	115.8	123.0	118.0
C-4	157.1	159.6	165.3
C-5	113.9	112.7	105.4
C-6	129.2	127.4	129.8
C-1′	131.7	132.1	131.5
C-2′	131.7	116.1	131.5
C-3′	116.1	145.9	116.0
C-4′	162.5	150.8	162.4
C-5′	116.1	115.6	116.0
C-6′	131.7	122.7	131.5
C-1″	117.2	23.6	29.6
C-2″	131.7	123.9	91.2
C-3″	77.4	131.5	71.4
C-4″	28.1	17.9	25.4[a]
C-5″	28.1	25.8	26.0[a]
OCH_3	63.2	62.7	60.0

[a] Assignments may be interchangeable.

3.8. Licoagroaurone (*7*)

Yellow powder. UV λ_{max}^{MeOH} nm (log ε): 256 (3.99), 267 (3.98), 396 (4.41). IR υ_{max}^{KBr} 1 cm^{-1}: 3440, 1630, 1600. EI–MS *m*/*z* (%): 338 (M^+, 100), 283 (54), 149 (46). HR-EI–MS: calcd for $C_{20}H_{18}O_5$ (M^+), 338.1154; found: 338.1164. ^{1}H NMR (400 MHz, acetone-d_6) δ: 1.69 (3H, *d*, *J* = 0.5 Hz, 5″-CH_3), 1.88 (3H, *d*, *J* = 0.5 Hz, 4″-CH_3), 3.56 (2H, *d*, *J* = 7.0 Hz, 1″-H), 5.41 (1H, *m*, 2″-H), 6.64 (1H, *s*, 10-H), 6.83 (1H, *d*, *J* = 8.5 Hz, 5-H), 6.96 (1H, *d*, *J* = 8.5 Hz, 5′-H), 7.42 (1H, *dd*, *J* = 8.5, 2.5 Hz, 6′-H), 7.46 (1H, *d*, *J* = 8.5 Hz, 4-H), 7.61 (1H, *d*, *J* = 2.5 Hz, 2′-H). ^{13}C NMR (100 MHz, acetone-d_6) δ: 18.1 (C-4″), 22.6 (C-1″), 25.8 (C-5″), 112.0 (C-10), 112.9 (C-5), 113.1 (C-7), 114.9 (C-9), 116.5 (C-5′), 118.8 (C-2′), 122.1(C-2″), 123.4 (C-4), 125.5 (C-6′), 125.7 (C-1′), 132.8 (C-3″), 146.2 (C-3′), 147.4 (C-2), 148.1 (C-4′), 163.6 (C-8), 167.7 (C-6), 182.9 (C-3).

3.9. Licoagroside A (*10*)

Powder, $[\alpha]_D^{24}$ + 75.1° (*c* 0.37, MeOH). UV λ_{max}^{MeOH} nm (log ε): 218 (4.30), 250 (4.07), 294 (3.85), 324 (3.84). IR

υ_{max}^{Kbr} cm^{-1}: 3430, 1630. FAB–MS (positive) *m/z*: 515 $[M+Na]^+$, 493 $[M+H]^+$. HR-FAB–MS (positive) *m/z*: found: 493.1360; calcd for $C_{23}H_{25}O_{12}$ $[M+H]^+$: 493.1346. ^1H NMR (400 MHz, methanol-d_4) δ: 3.24 (1H, *dd*, $J=9.0$, 9.0 Hz, 4″-H), 3.28 (1H, *dd*, $J=9.0$, 8.0 Hz, 2″-H), 3.40 (1H, *ddd*, $J=9.0$, 6.0, 2.0 Hz, 5″-H), 3.42 (1H, *dd*, $J=9.0$, 9.0 Hz, 3″-H), 3.60 (1H, *dd*, $J=12.0$, 6.0 Hz, 6″-H_a), 3.87 (1H, *dd*, $J=12.0$, 2.0 Hz, 6″-H_b), 3.90 (3H, *s*, 4′-OCH_3), 3.96 (3H, *s*, 6-OCH_3), 4.84 (1H, *d*, $J=8.0$ Hz, 1″-H), 6.76 (1H, *s*, 6′-H), 6.94 (1H, *s*, 8-H), 7.03 (1H, *s*, 3′-H), 7.55 (1H, *s*, 5-H), 8.16 (1H, *s*, 2-H). ^{13}C NMR (100 MHz, methanol-d_4) δ: 178.3 (C-4), 156.8 (C-2), 155.1 (C-7), 154.5 (C-9), 150.4 (C-2′), 149.7 (C-4′), 148.7 (C-6), 142.8 (C-5′), 122.6 (C-3), 118.7 (C-6′), 117.9 (C-10), 115.5 (C-1′), 105.4 (C-5), 104.3 (C-1″), 104.0 (C-8), 103.4 (C-3′), 78.4 (C-5″), 78.1 (C-3″), 74.9 (C-2″), 71.6 (C-4″), 62.8 (C-6″), 56.7 (6-OCH_3), 56.6 (4′-OCH_3).

3.10. Licoagroside B (*19*)

Powder, $[\alpha]_D^{24}$ −61.4° (*c* 0.60, MeOH). UV λ_{max}^{MeOH} nm (log ε): 210 (4.04), 254 (4.07). IR υ_{max}^{KBr} cm^{-1}: 3420, 1650, 1620. FAB–MS (positive) *m/z*: 455 $[M+Na]^+$, 433 $[M+H]^+$ HR-FAB–MS (positive) *m/z*: found: 433.1344; calcd for $C_{18}H_{25}O_{12}$ $[M+H]^+$: 433.1346. ^1H NMR (400 MHz, methanol-d_4) δ: 1.35 (3H, *s*, 6″-H), 2.42 (3H, *s*, 7-H), 2.60 (1H, *d*, $J=15.0$ Hz, 4″-H_a), 2.64 (1H, *d*, $J=15.0$ Hz, 4″-H_b), 2.65 (1H, *d*, $J=15.0$ Hz, 2″-H_a), 2.72 (1H, *d*, $J=15.0$ Hz, 2″-H_b), 3.34 (1H, *dd*, $J=9.0$, 8.0 Hz, 4′-H), 3.38 (1H, *dd*, $J=8.0$, 8.0 Hz, 2′-H), 3.40 (1H, *dd*, $J=9.0$, 8.0 Hz, 3′-H), 3.45 (1H, *ddd*, $J=8.0$, 6.0, 2.0 Hz, 5′-H), 4.21 (1H, *dd*, $J=12.0$, 6.0 Hz, 6′-H_a), 4.45 (1H, *dd*, $J=12.0$, 2.0 Hz, 6′-H_b), 4.85 (1H, *d*, $J=8.0$ Hz, 1′-H), 6.45 (1H, *d*, $J=6.0$ Hz, 5-H), 8.01 (1H, *d*, $J=6.0$ Hz, 6-H). ^{13}C NMR (100 MHz, methanol-d_4): δ 177.0 (C-4), 174.8 (C-5″), 172.3 (C-1″), 164.6 (C-2), 157.2 (C-6), 143.3 (C-3), 117.3 (C-5), 104.9 (C-1′), 77.8 (C-3′), 75.9 (C-5′), 75.3 (C-2′), 71.2 (C-4′), 70.6 (C-3″), 64.3 (C-6′), 46.3 (C-2″), 45.9 (C-4″), 27.8 (C-6″), 15.8 (C-7).

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References

Asada, Y., Li, W., Yoshikawa, T., 1998. Isoprenylated flavonoids from hairy root cultures of *Glycyrrhiza glabra*. Phytochemistry 47, 389–392.

Asada, Y., Li, W., Yoshikawa, T., 1999. The first prenylated biaurone, licoagrone from hairy root cultures of *Glycyrrhiza glabra*. Phytochemistry 50, 1015–1019.

Chassagne, D., Crouzet, J., Bayonove, C.L., Brillouet, J., Baumes, R.L., 1996. 6-*O*-α-L-Arabinopyranosyl-β-D-glucopyranosides as aroma precursors from passion fruit. Phytochemistry 41, 1497–1500.

Demizu, S., Kajiyama, K., Takahashi, K., Hiraga, Y., Yamamoto, S., Tamura, Y., Okada, K., Kinoshita, T., 1988. Antioxidant and antimicrobial constituents of licorice: isolation and structure elucidation of a new benzofuran derivative. Chem. Pharm. Bull. 36, 3474–3479.

Demizu, S., Kajiyama, K., Hiraga, Y., Kinoshita, K., Koyama, K., Takahashi, K., Tamura, Y., Okada, K., Kinoshita, T., 1992. Prenylated dibenzoylmethane derivatives from the root of *Glycyrrhiza inflata* (Xinjiang licorice). Chem. Pharm. Bull. 40, 392–395.

Fukai, T., Nomura, T., 1989. Constituents of the Moraceae plants. Part 5. Revised structures of broussoflavonols C and D, and the structure of broussoflavonol E. Heterocycles 29, 2379–2390.

Fukai, T., Sheng, C.B., Horikoshi, T., Nomura, T., 1996. Isoprenylated flavonoids from underground parts of *Glycyrrhiza glabra*. Phytochemistry 43, 1119–1124.

Fukai, T., Cai, C.B., Maruno, K., Miyakawa, Y., Konishi, M., Nomura, T., 1998. "Phenolic constituents of *Glycyrrhiza* species. An isoprenylated flavanone from *Glycyrrhiza glabra* and rec-assay of licorice phenols. Phytochemistry 49, 2005–2013.

Furuya, T., Matsumoto, K., Hikichi, M., 1971. Echinatin, a new chalcone from tissue culture of *Glycyrrhiza echinata*. Tetrahedron Letters , 2567–2569.

Haraguchi, H., Tanimoto, K., Tamura, Y., Mizutani, K., Kinoshita, T., 1998. Mode of antibacterial action of retrochalcones from *Glycyrrhiza inflata* Phytochemistry 48, 125–129.

Inoshiri, S., Sasaki, M., Konda, H., Otsuka, H., Yamasaki, K., 1987. Aromatic glycosides from *Berchemia racemosa*. Phytochemistry 26, 2811–2816.

Japan Pharmaceutical Information Center (Ed.), 1997. Drugs In Japan, Ethical Drugs. Yakugys Jiho Co., Ltd, Tokyo, p. 24.

Kajiyama, K., Demizu, S., Hiraga, Y., Kinoshita, K., Koyama, K., Takahashi, K., Tamura, Y., Okada, K., Kinoshita, T., 1992. Two prenylated retrochalcones from *Glycyrrhiza inflata*. Phytochemistry 31, 3229–3232.

Kajiyama, K., Hiraga, Y., Takahashi, K., Hirata, S., Kobayashi, S., Sankawa, U., Kinoshita, T., 1994. Flavonoids and isoflavonoids of chemotaxonomic significance from *Glycyrrhiza pallidiflora* (Leguminosae). Biochemical Systematics and Ecology 21, 785–793.

Katsura, E., Yamagishi, T., 1987. Quantitative determination of isoflavonoids in *Astragalus* root by high performance liquid chromatography. Hokkaidoritsu Eisei Kenkyushoho 37, 48–52.

Kessmann, H., Edwards, R., Geno, P.W., Dixon, R.A., 1990. Stress responses in alfalfa (*Medicago sativa* L.). V.. Constitutive and elicitor-induced accumulation of isoflavonoid conjugates in cell suspension cultures. Plant Physiol. 94, 227–232.

Kitakawa, I., Chen, W.Z., Hori, K., Harada, E., Yasuda, N., Yoshikawa, M., Ren, J., 1994. Chemical Studies of Chinese Licorice-Roots. I. Elucidation of five new flavonoid constituents from the roots of *Glycyrrhiza glabra* L. collected in Xinjiang. Chem. Pharm. Bull. 42, 1056–1062.

Li, W., Asada, Y., Yoshikawa, T., 1998. Antimicrobial flavonoids from *Glycyrrhiza glabra* hairy root cultures. Planta Medica 64, 746–747.

Looker, J.H., Fisher, M.S., 1985. Synthesis and characterization of the naturally occurring monocyclic γ-pyrone glucosides. Journal of Carbohydrate Chemistry 4, 381–392.

Markham, K.R., Mabry, T.J., 1975. Ultraviolet-visible and proton magnetic resonance spectroscopy of flavonoids. In: Harborne, J.B., Mabry, T.J., Mabry, H. (Eds.), The Flavonoids. Chapman and Hall, London, pp. 45–77.

Markham, K.R., Chari, V.M., 1982. Carbon-13 NMR spectroscopy of flavonoids. In: Harborne, J.B., Mabry, T.J. (Eds.), The Flavonoids: Advances in Research. Chapman and Hall, London, pp. 19–134.

Markham, K.R., Geiger, H., 1994. ^{1}H nuclear magnetic resonance spectroscopy of flavonoids and their glycosides in hexadeuterodimethylsulfoxide. In: Harborne, J.B. (Ed.), The Flavonoids Advances in Research since 1986. Chapman and Hall, London, pp. 441–497.

Mitscher, L.A., Park, Y.H., Clark, D., Beal, J.L., 1980. Antimicrobial agents from higher plants. Antimicrobial isoflavanoids and related substances from *Glycyrrhiza glabra* L. var. typica. J. Nat. Prod. 43, 259–269.

Ngo, H.N., Teel, R.W., Lau, B.H.S., 1992. Modulation of mutagenesis, DNA binding, and metabolism of aflatoxin B_1 by licorice compounds. Nutrition Res. 12, 247–257.

Nishino, H., Yoshioka, K., Iwashima, A., Takizawa, H., Konishi, S., Okamoto, H., Okabe, H., Shibata, S., Fujiki, H., Sugimura, T., 1986. Glycyrrhetic-Acid inhibits tumor-promoting activity of teleocidin and 12-*O*-tetradecanoylphorbol-13-acetate in two-stage mouse skin carcinogensis. Japan J. Cancer Res. 77, 33–38.

Nomura, T., Fukai, T., 1998. Phenolic constituents of licorice (*Glycyrrhiza* Species). In: Herz, W., Kirby, G.W., Moore, R.E., Steglich, W., Tamm, C. (Eds.), Fortschritte der Chemie Organischer Naturstoffe, Vol. 73. Springer-Verlag, New York, pp. 1–140.

Nose, M., Ito, M., Kamimura, K., Shimizu, M., Ogihara, Y., 1994. A comparison of the antihepatotoxic activity between glycyrrhizin and glycyrrhetinic acid. Planta Medica 60, 136–139.

Okada, K., Tamura, Y., Yamamoto, M., Inoue, Y., Takagaki, R., Takahashi, K., Demizu, S., Kajiyama, K., Hiraga, Y., Kinoshita, T., 1989. Identification of antimicrobial and antioxidant constituents from licorice of Russian and Xinjiang origin. Chem. Pharm. Bull. 37, 2528–2530.

Österdahl, B., 1978. Chemical studies on bryophytes. 19. Application of ^{13}C NMR in structural elucidation of flavonoid C-glucosides from *Hedwigia ciliata*. Acta Chemica Scandinavica B 32, 93–97.

Pelter, A., Ward, R.S. and Heller, H.G., 1979. Carbon-13 nuclear magnetic resonance spectra of (*Z*)- and (*E*)-aurones. J. Chem. Soc. Perkin Trans. I, 328–329.

Saitoh, T., Shibata, S., Sankawa, U., Furuya, T., Ayabe, S., 1975. Biosynthesis of echinatin. A new biosynthetical scheme of retrochalcone. Tetrahedron Letters, 4463–4466.

Tahara, S., Misumi, E., Ingham, J.L., Mizutani, J., 1987. Fungal metabolism of the prenylated isoflavone 2,3-dehydrokievitone. Naturforsch. 42c, 1055–1062.

Takagi, K., Ishii, Y., 1967. Peptic ulcer inhibiting properties of a new fraction from licorice root (Fm 100). I. Experiental peptic ulcer and general pharmacology. Arzneim. Forsch. 17, 1544–1547.

Takagi, K., Okabe, S., Saziki, R., 1969. A new method for the production of chronic gastric ulcer in rats and the effect of several drugs on its healing. Japan. J. Pharmacol. 19, 418–426.

van Marle, J., Aarsen, P.N., Lind, A., van Weeren-Kramer, J., 1981. Deglycyrrhizinized licorice (DGL) and the renewal of rat stomach epithelium. Eur. J. Pharmacol. 72, 219–225.

Zani, F., Cuzzoni, M.T., Daglia, M., Benvenuti, S., Vampa, G., Mazza, P., 1993. Inhibition of mutagenicity in *Salmonella typhimurium* by *Glycyrrhiza glabra* extract, glycyrrhizinic acid, 18α- and 18β-glycyrrhetinic acids. Planta Medica 59, 502–507.

Zheng, L., Fukai, T., Kaneki, T., Nomura, T., Zhang, R.Y., Lou, Z.C., 1992. Four new isoprenoid-substituted dibenzoylmethane derivatives, glyinflanins A, B, C, and D from the roots of *Glycyrrhiza inflata*. Heterocycles 34, 85–97.