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Two New Flavonoids and Other Constituents in Licorice Root: Their Relative Astringency and Radical Scavenging Effects

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Four compounds, including two new flavonoids, were isolated from Si-pei licorice (licorice from the north-western region of China). The structures of the two new flavonoids, named glycyrrhisoflavanone and glycyrrhisoflavone, were (*S*)-7,8′-dihydroxy-2′,2′-dimethyl-5-methoxy-[3,6′-bi-2*H*-1-benzopyran]-4(3*H*)-one (**6**) and 3-[3,4-dihydroxy-5-(3-methyl-2-butenyl)phenyl]-5,7-dihydroxy-4*H*-1-benzopyran-4-one (**9**). Glycyrrhisoflavone was found to be one of the tannic substances by the measurement of the binding activity to hemoglobin (relative astringency). Licochalcone B (**1**) was isolated from the fraction which showed the highest binding activity to hemoglobin among the fractions obtained by centrifugal partition chromatography of the extract of Sinkiang licorice (licorice from Sinkiang in China). Licochalcone B also showed the highest activity as a radical scavenger in the experiment using 1,1-diphenyl-2-picrylhydrazyl radical, among ten tested compounds obtained from several licorices. The order of the radical scavenging effects was the same as the order of the inhibitory effects on the 5-lipoxygenase-dependent peroxidation in arachidonate metabolism [licochalcone B (**1**) > licochalcone A (**3**) ≫ isoliquiritigenin (**14**) > liquiritigenin (**13**)].

Keywords——glycyrrhisoflavanone; glycyrrhisoflavone; isoflavonoid; licochalcone B; licorice; *Glycyrrhiza*; Leguminosae; tannin; radical scavenger; centrifugal partition chromatography

Tannins and related plant components, which show binding activities to proteins, have also been found to show biological activities in various experimental systems,[1] including inhibitory effects on lipid peroxidation in rat liver mitochondria and microsomes,[2] in which the radical scavenging action of tannins is considered to participate.[3] Recent investigations revealed that besides tannins,[4] some licorice flavonoids[5] also show inhibitory effects on the peroxidation induced by 5-lipoxygenase in arachidonate metabolism.

Our preliminary survey of the binding activity to hemoglobin indicated that the extracts of licorice of several origins[6] showed the *RAG* (relative astringency based on geraniin)[7] values of about 0.1 (Table I), which are equivalent to about 10% geraniin content. The components which show the binding activity have been isolated, and their radical scavenging effects (which may induce inhibition of peroxidation[3]) have been determined. Sinkiang licorice, having the highest *RAG* value of the extract, and Si-pei licorice, which is one of the most frequently used licorices in traditional Chinese medicine (kampo medicine) in Japan, were subjected to fractionation in the present investigation.

Results and Discussion

Components of Sinkiang Licorice

The extract of Sinkiang licorice (licorice from Sinkiang, China)[6] showed the highest *RAG* value (0.14) among five kinds of licorice of different origins (Table I). Upon

TABLE I. The *RAG* Values of the Extracts of Five Kinds of Licorice[a]

	RAG value
Si-pei licorice	0.10
Tong-pei licorice	0.11
Sinkiang licorice	0.14
Licorice from the Soviet Union	0.09
Licorice from Afghanistan	0.13

a) Extracts were prepared by homogenizing each licorice in acetone–water (7:3, v/v).

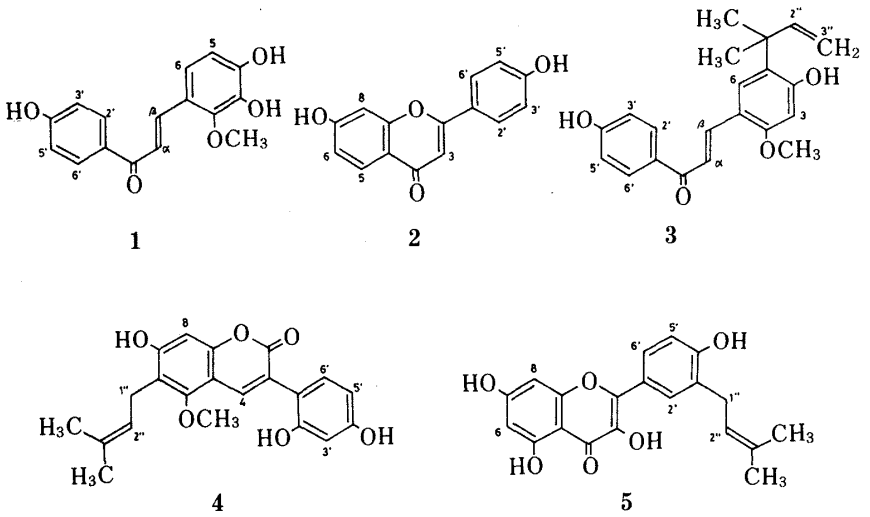


Chart 1

fractionation of the extract of Sinkiang licorice by centrifugal partition chromatography (CPC),[8] licochalcone B (**1**)[9] was isolated from the fraction of the highest *RAG* value, and 4′,7-dihydroxyflavone (**2**)[10] and licochalcone A (**3**)[9] were also isolated. The *RAG* value of licochalcone B (0.40) was relatively high, while those of licochalcone A (0.04) and 4′,7-dihydroxyflavone (0.04) were low.

Components of Si-pei Licorice

Among four compounds isolated from Si-pei licorice (licorice from the north-western region in China)[6] in the present investigation, two were identified as glycycoumarin (**4**)[11] and isolicoflavonol (**5**).[11] A new compound (**6**), named glycyrrhisoflavanone, was obtained as colorless needles. The electron impact mass spectrum (EI-MS) of **6** showed the molecular ion peak at *m/z* 368, which corresponds to the molecular formula $C_{21}H_{20}O_6$. The ^{1}H nuclear magnetic resonance (^{1}H-NMR) spectrum of **6** (300 MHz, in acetone-d_6) showed signals of A-ring protons [δ 6.12 (d, J=2 Hz, H-8), 6.03 (d, J=2 Hz, H-6)] and C-ring protons [δ 4.59 (dd, J=6, 11 Hz, H-2$_A$), 4.57 (dd, J=5, 11 Hz, H-2$_B$), 3.61 (dd, J=5, 6 Hz, H-3)] of an isoflavanone skeleton. The signals observed at δ 6.70 (d, J=2 Hz) and 6.52 (d, J=2 Hz) are attributable to the B-ring of the isoflavanone skeleton, and the signals observed at δ 6.33 (d, J=10 Hz), 5.70 (d, J=10 Hz) and 1.39 (6H, s) are assignable to an isoprenoid side chain which forms a part of the 2,2-dimethylchromene moiety.[12] The presence of a methoxyl group in **6** was also shown by a signal at δ 3.77 (3H, s).

Acetylation of **6** with acetic anhydride and pyridine afforded a diacetate (**7**) which showed the M^+ ion at *m/z* 452 in EI-MS. This diacetate showed a ^{1}H-NMR spectrum which is analogous to that of licoisoflavanone triacetate (**8**),[10b] except for the presence of the signals of

ortho-coupled protons of the B-ring (H-7 and H-8 of the 2,2-dimethylchromene moiety) in the spectrum of **8** in place of the *meta*-coupled protons [δ 6.83 (d, $J=2$ Hz), 6.86 (d, $J=2$ Hz)] in that of **7**, and the fact that the signal of the methoxyl group [δ 3.88 (3H, s)] in **7** is substituted by that of an acetyl group in **8**. The presence of a 6-substituted 2,2-dimethyl-8-hydroxychromene moiety (or corresponding 8-methoxy derivative) in **6** was thus indicated, as an *O*-functional group should biogenetically[13] most probably be at C-4′ of the B-ring of the isoflavonoid. This assignment was substantiated by the ^{13}C-NMR spectrum of **6** as described in the experimental section.

The location of the methoxyl group in **6** was deduced to be C-5 by an NOE (nuclear Overhauser effect) measurement. When the methoxyl signal (δ 3.77) in the ^{1}H-NMR spectrum of **6** was irradiated, a 10% increase of the peak area of H-6 was observed, while the peak area of H-8 was not changed.

The (*S*)-configuration of C-3 in **6** was shown by the negative Cotton effect at 338 nm in the CD (circular dichroism) spectrum.[14] The structure of glycyrrhisoflavanone could therefore be formulated as structure **6**, (*S*)-7,8′-dihydroxy-2′,2′-dimethyl-5-methoxy-[3,6′-bi-2*H*-1-benzopyran]-4(3*H*)-one.

Another new compound (**9**), named glycyrrhisoflavone, was obtained as colorless needles. The EI-MS of **9** showed the molecular ion peak at *m/z* 354, which corresponds to the molecular formula $C_{20}H_{18}O_6$. The ^{1}H-NMR spectrum (500 MHz, in acetone-d_6) of **9** showed the signals of *meta*-coupled aromatic protons [δ 6.39 (d, $J=2$ Hz), 6.26 (d, $J=2$ Hz)] and of a proton attached to an sp_2 carbon [δ 8.10 (1H, s)], which are assignable to H-8, H-6 and H-2 of an isoflavone skeleton, along with the signals of protons of a γ,γ-dimethylallyl (= 3-methyl-2-butenyl) group [δ 5.36 (br t, $J=7$ Hz), 3.35 (2H, d, $J=7$ Hz), 1.72 (3H, s), 1.70 (3H, s)]. The signals of the B-ring protons of the isoflavone skeleton at δ 7.01 (d, $J=2$ Hz) and δ 6.83 (d, $J=2$ Hz), are *meta*-coupled with each other. A signal (δ 13.06) of a chelated hydroxyl proton (OH at C-5) was also observed. Acetylation of **9** with acetic anhydride and pyridine afforded a tetraacetate (**10**) which showed the M^+ ion at *m/z* 522 in EI-MS. Therefore, the compound (**9**) is an isoflavone which has a γ,γ-dimethylallyl group and four hydroxyl groups. In fact, the ^{1}H-NMR data of **9** are analogous to the reported data of licoisoflavone A (**11**)[15] [= 3′-γ,γ-dimethylallyl-2′,4′,5,7-tetrahydroxyisoflavone], except for the presence of the *meta*-coupled protons of the B-ring instead of the *ortho*-coupled protons in **11**.

Treatment of **9** with HCl induced cyclization[15] of the γ,γ-dimethylallyl group with a hydroxyl group, to afford **12** which showed *m/z* 354 (M^+) in EI-MS. This cyclization indicates

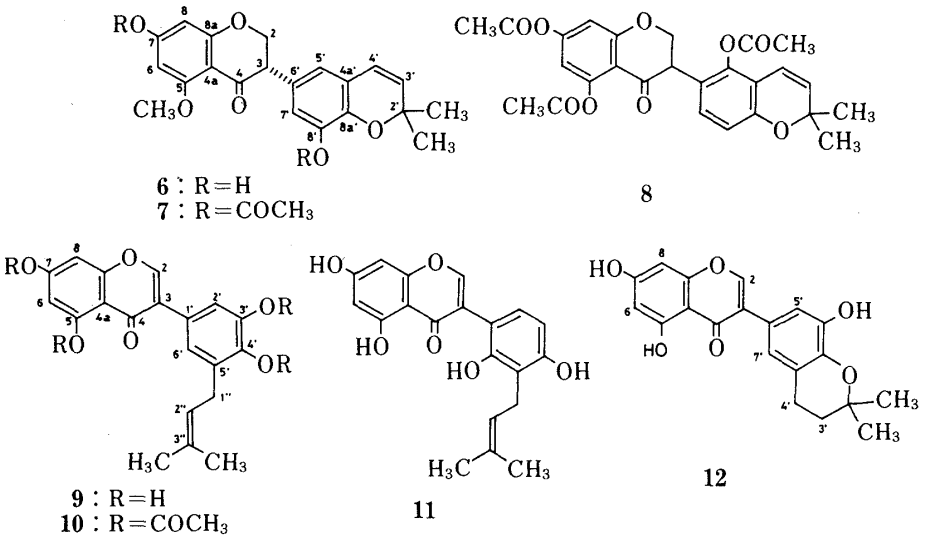


Chart 2

that the γ,γ-dimethylallyl group is located *ortho* to one of the hydroxyl groups on the B-ring. Therefore, the *meta*-coupling of the B-ring protons in the ^{1}H-NMR spectrum of **9**, and the biogenetical likelihood[13] that an *O*-functional group exists at the 4′-position on the B-ring of the isoflavonoid, lead to the structure **9** of glycyrrhisoflavone. The ^{13}C-NMR spectrum of this compound was also consistent with this structure, as shown in the experimental section.

Among the four compounds obtained from Si-pei licorice, glycyrrhisoflavone shows the highest *RAG* value (0.23), which is comparable with the values of several hydrolysable tannins such as pedunculagin (*RAG* 0.24) and corilagin (*RAG* 0.22).[7a]

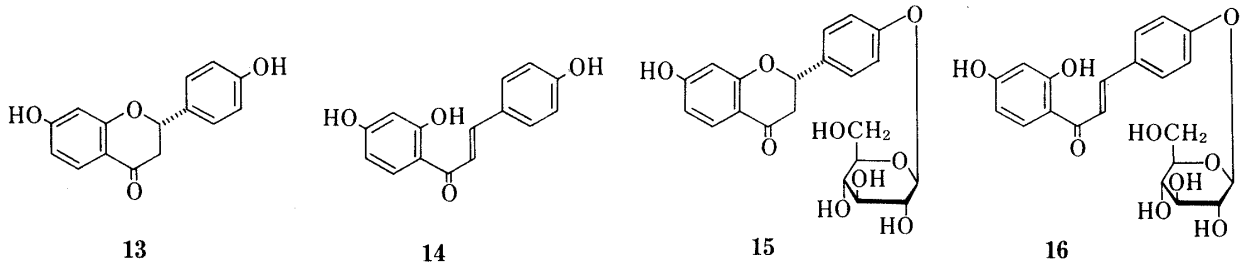
Relationship between the *RAG* Values and Structures of the Components in Licorice

The *RAG* values of the components including liquiritigenin (**13**), isoliquiritigenin (**14**), liquiritin (**15**) and isoliquiritin (**16**), which are four representative licorice flavonoids, are shown in Table II. Although isoliquiritigenin was reported to be an inhibitor of phenylalanine ammonia-lyase,[16] and it was suggested that the inhibition is based on the non-specific binding of the compound to the enzyme,[16] the *RAG* value of this chalcone, which should be related to the non-specific binding to protein, is very low.

Licochalcone B (**1**) and glycyrrhisoflavone (**9**), both of which showed *RAG* values higher than those of the other components of licorice, have three or more hydroxyl groups, two of which form an *ortho*-diphenol structure, to which a double bond is conjugated.

Although it is inappropriate to call these flavonoids "tannins," they should be responsible to some extent for the activity as "tannin" of the licorice extracts. It may also be presumed that some components active as "tannin" in plant extracts could be flavonoids, and that some activities found for tannins may be found for such flavonoids, too.

High-performance liquid chromatography (HPLC) of the licorice extracts revealed that the contents of the flavonoids which show high *RAG* values are not high [licochalcone B (**1**), 1.0% in the 70% acetone extract of Sinkiang licorice; glycyrrhisoflavone (**9**), 0.094% in the 70%



13 **14** **15** **16**

Chart 3

TABLE II. The *RAG* Values of Licorice Components and Their Scavenging Effects on 1,1-Diphenyl-2-picrylhydrazyl Radical

	RAG value	EC_{50} ($\times 10^{-5}$ M)
Licochalcone B (**1**)	0.40	2.2
4′,7-Dihydroxyflavone (**2**)	0.04	>100
Licochalcone A (**3**)	0.04	12
Glycycoumarin (**4**)	0.13	4.1
Isolicoflavonol (**5**)	0	4.0
Glycyrrhisoflavanone (**6**)	0.07	36
Glycyrrhisoflavone (**9**)	0.23	3.8
Liquiritigenin (**13**)	0.06	>100
Isoliquiritigenin (**14**)	0	96
Liquiritin (**15**)	0	>100
Isoliquiritin (**16**)	0	>100

acetone extract of Si-pei licorice; glycycoumarin (**4**), 0.23% in the extract of Si-pei licorice]. Thus, further investigation of the components contributing to the *RAG* values of the licorice extracts is in progress.

The Radical Scavenging Effects of the Components in Licorice

In the course of studies on the mechanism of the inhibitory effects[2] of tannins and related polyphenols on lipid peroxidation, it was found that the radical scavenging action of these compounds participates in the inhibition of the autoxidation of methyl linoleate initiated by irradiated azobisisobutyronitrile,[3b] and also in the inhibition of lipoxygenase-dependent peroxidation of linoleic acid.[3a] The order of the inhibitory effects of these compounds on the lipoxygenase-dependent peroxidation coincided with the order of the radical scavenging activity of the same compounds in experiments using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.[3a]

The scavenging activities of the licorice flavonoids on the DPPH radical are shown in Table II. Licochalcone B (**1**) showed the strongest activity, while the activity of another chalcone, isoliquiritigenin (**14**), was low. The activities of these flavonoids presumably depend on the stability of the corresponding flavonoid radical, as found for tannins.[3] It is probable that the *ortho*-diphenol structure in **1** contributes to the stabilization of its radical, as in caffeic acid and its derivatives.[3a]

The inhibition of the formations of 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) and 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid (5,12-diHETE) in the arachidonate metabolism of human polymorphonuclear leukocyte by licorice flavonoids **1**, **3**, **13** and **14**,[5] and the identity of the orders of the inhibitory effects on the formations of 5-HETE and 5,12-diHETE [licochalcone B (**1**) > licochalcone A (**3**) ≫ isoliquiritigenin (**14**) > liquiritigenin (**13**)] were reported.[5] As the order of the scavenging activity of these compounds on DPPH radical coincides with the order of inhibition of formation of the metabolites described above, which are regarded as the products of 5-lipoxygenase-dependent peroxidation, the radical scavenging actions of the licorice flavonoids probably also participate in the inhibition of arachidonate metabolism.

Experimental

Ultraviolet (UV) spectra were recorded on a Hitachi 200-10 spectrophotometer and infrared (IR) spectra on a JASCO A-102 spectrometer. EI-MS were recorded on a Shimadzu LKB-9000 GC-MS spectrometer. High-resolution mass spectra (HR-MS) were recorded on a JEOL GMS-HX100 machine. Optical rotations were measured on a JASCO DIP-4 polarimeter and CD spectra on a JASCO J-500A spectropolarimeter. NMR spectra were recorded on a Hitachi R22-FTS spectrometer (90 MHz for ^{1}H), with tetramethylsilane as an internal standard; chemical shifts are given in δ values (ppm). A Varian VXR-500 instrument (500 MHz for ^{1}H), the SC-NMR Laboratory of Okayama University, and a Varian VXR-300 instrument (300 MHz for ^{1}H, and 75 MHz for ^{13}C), were also used. CPC was performed on a Sanki L-90 machine equipped with twelve cartridges, developing with $CHCl_3$–MeOH–H_2O (7 : 13 : 8, normal-phase development) at 700 rpm. Kieselgel 60 PF_{254} (Merck) was used for analytical and preparative thin layer chromatography (TLC) (0.25 and 0.5 mm, respectively). Solvent systems used were as follows: (A) $CHCl_3$–acetone–HCOOH (155 : 33 : 12), (B) $CHCl_3$–MeOH (10 : 1), (C) *n*-hexane–$CHCl_3$–acetone (6 : 3 : 1) and (D) $CHCl_3$–acetone–HCOOH (16 : 2 : 1). The plates were visualized by UV irradiation (254 and 365 nm). HPLC was performed on a YMC A312 (ODS) column (6 mm i.d. × 15 cm) with the solvent system (a) MeCN–H_2O–AcOH (35 : 60 : 5) or (b) MeOH–H_2O–AcOH (60 : 35 : 5) at 40 °C in an oven. Detection was effected by UV absorption measurement at 280 nm or at 254 nm, and the flow rate was set at 1.3 ml/min.

Estimation of *RAG* Values——The *RAG* values were determined by the reported method[7a] with a slight modification.[7b] To 0.8 ml of a solution of a test compound in 20% MeOH, 0.4 ml of 0.2 M phosphate buffer (pH 6.0) and 0.8 ml of hemoglobin solution (abjusted to give an absorbance of *ca.* 2.5 at 578 nm) obtained by thawing frozen hemolyzed human blood, were added. The mixture was shaken vigorously and left to stand for 1 h. After centrifugation at 10000 *g* for 10 min, the absorbance at 578 nm of the supernatant was measured. The *RA* values were calculated by applying the following equation:

$$RA = W_{TA}/W_{TC}$$

in which W_{TA} means the amount of tannic acid required to give a 50% decrease of the absorbance from that of the blank solution [consisting of 20% MeOH (0.8 ml), 0.2 M phosphate buffer (0.4 ml) and hemoglobin solution (0.8 ml)], and W_{TC} means the amount of the test compound required to give a 50% decrease of the absorbance. The *RAG* values are the *RA* values of test compounds relative to the *RA* value of geraniin.

Estimation of the Effects on DPPH Radical[3a]——An MeOH solution (4 ml) of a test compound was added to an MeOH solution (1 ml) of DPPH (final concentration of DPPH: 2.0×10^{-4} M). The mixture was shaken vigorously and left to stand for 30 min, and then the absorbance of the resulting solution was measured. The scavenging activity on DPPH radical was expressed as EC_{50}, which is the concentration of the test compound required to give a 50% decrease of the absorbance from that of the blank solution [consisting of MeOH (4 ml) and DPPH solution (1 ml)].

Preparation of the Licorice Extracts——Si-pei licorice and Tong-pei licorice (licorice from the north-eastern region in China)[6] were purchased from Tochimoto-tenkai-do Co., Ltd., Osaka, Japan, and Sinkiang licorice, licorice from the Soviet Union[6] and licorice from Afghanistan[6] were kindly supplied by Maruzen Kasei Co., Ltd. Each of these kinds of licorice (10 g) was homogenized in 70% acetone (50 ml × 3), and after centrifugation, the combined supernatant was evaporated. Yields of the crude extracts were as follows: Si-pei licorice (30%), Tong-pei licorice (31%), Sinkiang licorice (24%), licorice from the Soviet Union (23%) and licorice from Afghanistan (21%).

Isolation of the Components of Sinkiang Licorice——The extract of Sinkiang licorice with aqueous acetone was partitioned between Et_2O and H_2O. The aqueous layer was extracted with EtOAc and *n*-BuOH, successively. Yields of these fractions from the aqueous acetone extract after evaporation and their *RAG* values were as follows: Et_2O extract (24%, 0.18), EtOAc extract (8%, 0.12), *n*-BuOH extract (22%, 0.08) and H_2O layer (46%, 0.01). The Et_2O extract (3 g) was subjected to CPC to afford fr. I-V. Fr. IV (0.41 g), which showed the highest *RAG* value (0.27) among these fractions, was further chromatographed over Kieselgel 60 with $CHCl_3$–MeOH (98 : 2) to give licochalcone B (**1**) (122 mg) and 4′,7-dihydroxyflavone (**2**) (6 mg). Fr. II (0.57 g) gave licochalcone A (**3**) (112 mg) after an analogous treatment. The other fractions were found to be complex mixtures.

Licochalcone B (1)[9]——Yellow needles, mp 195 °C. HPLC [solvent system (a)]: t_R (retention time) 3.8 min. EI-MS *m/z*: 286 (M^+), 255 ($[M-OCH_3]^+$). UV λ_{max}^{EtOH} nm (log ε): 205 (4.34), 262 (3.92), 360 (4.35). IR ν_{max}^{KBr} cm^{-1}: 1630 (C=O), 1600, 1590. ^{1}H-NMR (90 MHz, in acetone-d_6) δ: 3.87 (3H, s, OCH_3), 6.72 (1H, d, *J*=8.5 Hz, H-6), 6.97 (2H, d, *J*=8.5 Hz, H-3′ and H-5′), 7.31 (1H, d, *J*=8.5 Hz, H-5), 7.69 (1H, d, *J*=16 Hz, H-α), 8.00 (1H, d, *J*=16 Hz, H-β), 8.05 (2H, d, *J*=8.5 Hz, H-2′ and H-6′).

4′,7-Dihydroxyflavone (2)[10]——Pale yellow crystals, mp > 300 °C. EI-MS *m/z*: 254 (M^+). UV λ_{max}^{EtOH} nm (log ε): 207 (4.37), 231 (4.14), 255 (sh, 3.88), 312 (sh, 4.21), 329 (4.27). ^{1}H-NMR (90 MHz, in CD_3OD) δ: 6.67 (1H, s, H-3), 6.9—7.0 (4H, H-6, H-8, H-3′ and H-5′), 7.87 (2H, d, *J*=8.5 Hz, H-2′ and H-6′), 7.97 (1H, d, *J*=8.5 Hz, H-5). Diacetate: mp 188 °C. ^{1}H-NMR (90 MHz, in $CDCl_3$) δ: 2.34, 2.37 (3H each, s, 2 × $COCH_3$), 6.78 (1H, s, H-3), 7.17 (1H, dd, *J*=8.5, 2.5 Hz, H-6), 7.27 (2H, d, *J*=8.5 Hz, H-3′ and H-5′), 7.41 (1H, d, *J*=2.5 Hz, H-8), 7.93 (2H, d, *J*=8.5 Hz, H-2′ and H-6′), 8.25 (1H, d, *J*=8.5 Hz, H-5).

Licochalcone A (3)[9]——Yellow needles, mp 101 °C. EI-MS *m/z*: 338 (M^+), 307 ($[M-OCH_3]^+$). UV λ_{max}^{MeOH} (log ε): 204 (4.42), 232 (sh, 4.07), 255 (3.97), 311 (4.02), 377 (4.29). IR ν_{max}^{KBr} cm^{-1}: 1640 (C=O), 1600, 1590. ^{1}H-NMR (90 MHz, in acetone-d_6) δ: 1.45 (6H, s, 2 × CH_3), 3.86 (3H, s, OCH_3), 5.33 (1H, d, *J*=10 Hz, H-3″$_A$), 5.36 (1H, d, *J*=18 Hz, H-3″$_B$), 6.21 (1H, dd, *J*=10, 18 Hz, H-2″), 6.44 (1H, s, H-6), 6.94 (2H, d, *J*=8.5 Hz, H-3′ and H-5′), 7.47 (1H, s, H-3), 7.56 (1H, d, *J*=15 Hz, H-α), 7.95 (2H, d, *J*=8.5 Hz, H-2′ and H-6′), 8.00 (1H, d, *J*=15 Hz, H-β). Comparisons with an authentic sample on TLC [solvent system (A), *Rf* 0.59] and on HPLC [solvent system (b), t_R 13.8 min] confirmed the identity of the compound.

Isolation of the Components of Si-pei Licorice——After removal of oily materials by immersion in hexane, Si-pei licorice was extracted with EtOAc. A portion (15 g) of the EtOAc extract was subjected to chromatography over Kieselgel 60 (2.2 cm i.d. × 57 cm); elution with increasing amounts of methanol in chloroform gave frs. 1—6. Fr.2 (1.66 g, *RAG* 0.20) was further chromatographed over Kieselgel using $CHCl_3$–MeOH (99 : 1) as an eluant, and over MCI gel CHP-20P using MeOH–H_2O (6 : 4), to afford glycyrrhisoflavanone (**6**, 18 mg) and glycyrrhisoflavone (**9**, 33 mg). Glycycoumarin (**4**, 101 mg) was isolated from fr. 3 (1.13 g) by column chromatography over Kieselgel with $CHCl_3$–MeOH (99 : 1) and over MCI gel with MeOH–H_2O (7 : 3). In a separate experiment, a portion (4.7 g) of the EtOAc extract was subjected to droplet countercurrent chromatography [3.2 mm i.d. × 1.2 m × 100 glass tubes, $CHCl_3$–MeOH–H_2O (7 : 13 : 8), descending method], to yield six fractions. The second fraction (164 mg) was subjected to chromatography over MCI gel using MeOH–H_2O (7 : 3), and the fraction eluted after glycyrrhisoflavanone and glycyrrhisoflavone was purified by preparative TLC using solvent system (B) to afford isolicoflavonol (**5**, 6 mg).

Glycyrrhisoflavanone (6)——Colorless needles (from MeOH), mp 224 °C. $[\alpha]_D$ −59° (*c*=1, acetone). TLC [solvent system (A)]: *Rf* 0.68. *Anal.* Calcd for $C_{21}H_{20}O_6 \cdot 1/2CH_3OH$: C, 67.18; H, 5.77. Found: C, 67.39; H, 5.24. EI-MS *m/z*: 368 (M^+). HR-MS *m/z*: 368.1245 (Calcd for $C_{21}H_{20}O_6$, 368.1260). UV λ_{max}^{EtOH} (log ε): 210 (sh, 4.48), 227 (4.62), 282 (4.37), 320 (infl., 3.77). IR ν_{max}^{KBr} cm^{-1}: 1660 (C=O), 1590. CD (MeOH) [θ] (nm): +17900 (225), −14000 (236), −7500 (256), +20900 (285), −5400 (338). ^{1}H-NMR (300 MHz, in acetone-d_6) δ: 1.39 (6H, s, 2 × CH_3), 3.61 (1H, dd, *J*=5, 6 Hz, H-3), 3.77 (3H, s, OCH_3), 4.57 (1H, dd, *J*=5, 11 Hz, H-2$_B$), 4.59 (1H, dd, *J*=6, 11 Hz, H-2$_A$), 5.70 (1H, d, *J*=10 Hz, H-3′), 6.03 (1H, d, *J*=2 Hz, H-6), 6.12 (1H, d, *J*=2 Hz, H-8), 6.33 (1H, d, *J*=10 Hz, H-4′), 6.52 (1H, d, *J*=2 Hz, H-7′), 6.70 (1H, d, *J*=2 Hz, H-5′). ^{13}C-NMR (75 MHz, acetone-d_6) δ: 27.8 (2C, 2 × CH_3), 53.0

(C-3), 56.0 (OCH_3), 71.6 (C-2), 77.1 (C-2′), 94.0 (C-8), 96.3 (C-6), 105.9 (C-4a), 116.7 (C-5′), 118.1 (C-7′), 122.4 (C-6′), 123.0 (C-4′), 130.3 (C-4a′), 131.9 (C-3′), 146.2 (C-8′), 164.1, 164.8 (2C), 165.4 (C-5, C-7, C-8a and C-8a′), 188.3 (C-4).

Diacetate (**7**): mp 77 °C. $[\alpha]_D$ −48° (c=0.23, acetone). TLC [solvent system (C)]: *Rf* 0.25. EI-MS *m/z*: 452 (M^+). HR-MS *m/z*: 452.1473 (Calcd for $C_{25}H_{24}O_8$, 452.1471). ^{1}H-NMR (300 MHz, in $CDCl_3$) δ: 1.40 (6H, s, 2×CH_3), 2.28, 2.31 (3H each, s, 2×$COCH_3$), 3.77 (1H, t, J=7 Hz, H-3), 3.88 (3H, s, OCH_3), 4.63 (2H, d, J=7 Hz, H-2), 5.62 (1H, d, J=10 Hz, H-3′), 6.28 (1H, d, J=10 Hz, H-4′), 6.30 (1H, d, J=2 Hz, H-6), 6.40 (1H, d, J=2 Hz, H-8), 6.83, 6.86 (each 1H, d, J=2 Hz, H-5′ and H-7′).

Glycyrrhisoflavone (9)——Colorless needles (from $CHCl_3$), mp 185 °C. TLC [solvent system (A)]: *Rf* 0.70. HPLC [solvent system (b)]: t_R 11.7 min. *Anal.* Calcd for $C_{20}H_{18}O_6 \cdot 1/2\,H_2O$: C, 66.11; H, 5.27. Found: C, 66.56; H, 4.89. EI-MS *m/z*: 354 (M^+). HR-MS *m/z*: 354.1091 (Calcd for $C_{20}H_{18}O_6$, 354.1103). UV λ_{max}^{MeOH} nm (log ε): 210 (4.58), 262 (4.50), 290 (sh, 4.12). IR ν_{max}^{KBr} cm^{-1}: 1650 (C=O), 1620, 1570. ^{1}H-NMR (500 MHz, in acetone-d_6) δ: 1.70, 1.72 (3H each, s, 2×CH_3), 3.35 (2H, d, J=7 Hz, H-1″), 5.36 (1H, br t, J=7 Hz, H-2″), 6.26 (1H, d, J=2 Hz, H-6), 6.39 (1H, d, J=2 Hz, H-8), 6.83 (1H, d, J=2 Hz, H-2′), 7.01 (1H, d, J=2 Hz, H-6′), 8.10 (1H, s, H-2), 13.06 (1H, s, 5-OH). ^{13}C-NMR (75 MHz, in acetone-d_6) δ: 17.9 (CH_3), 25.9 (CH_3), 94.4 (C-8), 99.8 (C-6), 106.2 (C-4a), 114.8 (C-2′). 122.1 (C-6′), 122.7, 124.3 (C-1′ and C-5′), 123.7 (C-2″), 128.9 (C-3), 132.3 (C-3″), 144.2, 144.9 (C-3′ and C-4′), 154.3 (C-2), 159.0, 163.9, 164.9 (C-5, C-7 and C-8a), 181.7 (C-4).

Tetraacetate (**10**): mp 64 °C. TLC [solvent system (C)]: *Rf* 0.28. EI-MS *m/z*: 522 (M^+). HR-MS *m/z*: 522.1497 (Calcd for $C_{28}H_{26}O_{10}$, 522.1527). ^{1}H-NMR (500 MHz, in $CDCl_3$) δ: 1.67, 1.71 (3H each, s, 2×CH_3), 2.26, 2.28, 2.32, 2.40 (3H each, s, 4×$COCH_3$), 3.25 (2H, d, J=7 Hz, H-1″), 5.19 (1H, br t, J=7 Hz, H-2″), 6.84, 7.16, 7.20, 7.22 (1H each, d, J=2 Hz, H-6, H-8, H-2′ and H-6′), 7.88 (1H, s, H-2).

Treatment of Glycyrrhisoflavone (9) with HCl——Concentrated HCl (0.35 ml) was added to a methanol solution (0.35 ml) of glycyrrhisoflavone (**9**, 5 mg), and the mixture was left to stand for 32 h. After evaporation of the solvent, the residue was subjected to preparative TLC with solvent system (D), to yield **12** (3 mg), as colorless crystals (from MeOH–H_2O). The crystals softened at 143 °C and decomposed at 199 °C. TLC [solvent system (D)]: *Rf* 0.85. *Anal.* Calcd for $C_{20}H_{18}O_6 \cdot 1/2H_2O$: C, 66.12; H, 5.23. Found: C, 66.02; H, 5.29. EI-MS *m/z*: 354 (M^+). HR-MS *m/z*: 354.1108 (Calcd for $C_{20}H_{18}O_6$, 354.1103). UV λ_{max}^{MeOH} nm (log ε): 209 (4.55), 220 (sh, 4.43), 262 (4.50), 292 (sh, 4.09). IR ν_{max}^{KBr} cm^{-1}: 1650 (C=O), 1620, 1580. ^{1}H-NMR (500 MHz, in acetone-d_6) δ: 1.34 (6H, s, 2×CH_3), 1.85 (2H, t, J=7 Hz, H-3′), 2.79 (2H, t, J=7 Hz, H-3″), 6.27 (1H, d, J=2 Hz, H-6), 6.40 (1H, d, J=2 Hz, H-8), 6.82 (1H, d, J=2 Hz, H-7′), 6.92 (1H, d, J=2 Hz, H-6′), 8.14 (1H, s, H-2), 13.06 (1H, s, 5-OH).

Glycycoumarin (4)[11]——Yellow needles, mp 231 °C. HPLC [solvent system (b)]: t_R 10.0 min. EI-MS *m/z*: 368 (M^+). UV λ_{max}^{EtOH} nm (log ε): 211 (4.61), 230 (sh, 4.28), 253 (3.93), 261 (sh, 3.91), 285 (3.80), 353 (4.09). IR ν_{max}^{KBr} cm^{-1}. 1700—1660 (C=O), 1610. ^{1}H-NMR (90 MHz, in DMSO-d_6), δ: 1.63, 1.73 (3H each, s, 2×CH_3), 3.72 (3H, s, OCH_3), 5.16 (1H, t, J=7 Hz, H-2″), 6.29 (1H, dd, J=2, 8 Hz, H-5′), 6.37 (1H, d, J=2 Hz, H-3′), 6.60 (1H, s, H-8), 7.10 (1H, d, J=8 Hz, H-6′), 7.81 (1H, s, H-4). The signal of two protons at C-1″ overlaps the signal of water (δ 3.2—3.3).

Isolicoflavonol (5)[11]——Pale yellow needles, mp 119 °C. EI-MS *m/z*: 354 (M^+). UV λ_{max}^{EtOH} (log ε): 205 (4.54), 230 (sh, 4.20), 252 (4.24), 270 (sh, 4.15), 296 (sh, 3.88), 326 (sh, 3.99), 370 (4.33). IR ν_{max}^{KBr} cm^{-1}: 1655 (C=O), 1620, 1600. ^{1}H-NMR (90 MHz, in DMSO-d_6) δ: 1.69 (6H, s, 2×CH_3), 3.28 (2H, d, J=7 Hz, 2×H-1″), 5.30 (1H, t, J=7 Hz, H-2″), 6.14, 6.36 (1H each, d, J=2 Hz, H-6-and H-8), 6.92 (1H, d, J=8 Hz, H-5′), 7.86 (1H, dd, J=2, 8 Hz, H-6′), 7.90 (1H, d, J=2 Hz, H-2′).

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assignments for the commercial materials are generally accepted: Si-pei licorice, *Glycyrrhiza glabra* L. var. *grandulifera* REG. *et* HERD. (?); Tong-pei licorice, *G. uralensis* FISCH. *et* DC.; Sinkiang licorice, *G. inflata* BATALIN; licorice from the Soviet Union, *G. glabra* L.; licorice from Afghanistan, *G. glabra* L. See, M. Hattori, K. Miyachi, Y.-Z. Shu, N. Kakiuchi and T. Namba, *Shoyakugaku Zasshi*, **40**, 406 (1986).

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