Phenolic Constituents of Licorice. II.[1] Structures of Licopyranocoumarin, Licoarylcoumarin and Glisoflavone, and Inhibitory Effects of Licorice Phenolics on Xanthine Oxidase[2]

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An anti-HIV (human immunodeficiency virus) phenolic constituent, licopyranocoumarin (4), and two other new phenolics named licoarylcoumarin (5) and glisoflavone (6) were isolated from Si-pei licorice (a commercial licorice; root and stolon of *Glycyrrhiza* sp. from the north-western region of China) using droplet countercurrent chromatography and centrifugal partition chromatography, and their structures were assigned based on chemical and spectroscopic data. Kaempferol 3-*O*-methyl ether (7) and licocoumarone (8) were also isolated from the licorice. The inhibitory effects of ten licorice phenolics on xanthine oxidase were examined. Licochalcone B (1), glycyrrhisoflavone (2), 8 and licochalcone A (19) showed 50% inhibition at the concentration of $1.3—5.6 \times 10^{-5}$ M.

Keywords licopyranocoumarin; licoarylcoumarin; glisoflavone; kaempferol 3-*O*-methyl ether; licocoumarone; *Glycyrrhiza*; licorice; xanthine oxidase; droplet countercurrent chromatography; centrifugal partition chromatography

In our previous investigation on the licorice components with tannin-like activity of binding with protein, several phenolics of low molecular weight such as licochalcone B (**1**) were found to bind with hemoglobin.[1] Another characteristic activity of tannins, the scavenging of free radicals,[3,4] was exhibited by some licorice phenolics.[1] Structures of glycyrrhisoflavone (**2**),[1] glycyrrhisoflavanone (**3**)[1] and licopyranocoumarin (**4**),[2] among which **2** and **4** showed anti-HIV (human immunodeficiency virus) activity,[2] were also reported. Further study on the licorice components led us to the isolation of four additional phenolics including two new compounds named licoarylcoumarin (**5**) and glisoflavone (**6**), from Si-pei licorice (Seihoku-kanzou in Japanese; a commercial licorice from the north-western region of China).[5]

Xanthine oxidase, which induces gout through the formation of uric acid, also causes oxidative damage of tissues in the living body through generation of the superoxide anion radical,[4] and various phenolics have been reported to inhibit this enzyme.[6] Since much attention has been concentrated recently on various biological activities of licorice phenolics,[7—9] we have examined the inhibitory effects of the licorice phenolics on this enzyme.

We describe here the isolation of five phenolics and the elucidation of their structures, including the experimental details in the case of **4**, and also the inhibitory effects of licorice phenolics on xanthine oxidase.

Results and Discussion

Isolation of Five Phenolics from Si-pei Licorice The licorice phenolics in the present experiment were isolated as follows. A part of the ethyl acetate extract of Si-pei licorice was subjected to droplet countercurrent chromatography (DCCC) with normal-phase development (descending mode). Further separation of a DCCC fraction by column chromatography on MCI gel CHP-20P, followed by preparative thin layer chromatography (PTLC) on silica gel, gave licopyranocoumarin (**4**) along with kaempferol 3-*O*-methyl ether (**7**).[10] Another portion of the ethyl acetate extract was subjected to centrifugal partition chromatography (CPC)[11] with reversed-phase development, and the CPC fractions were purified by column chromatography on Toyopearl HW-40 and/or MCI gel CHP-20P, and subsequent PTLC, to give two new compounds, licoaryl-

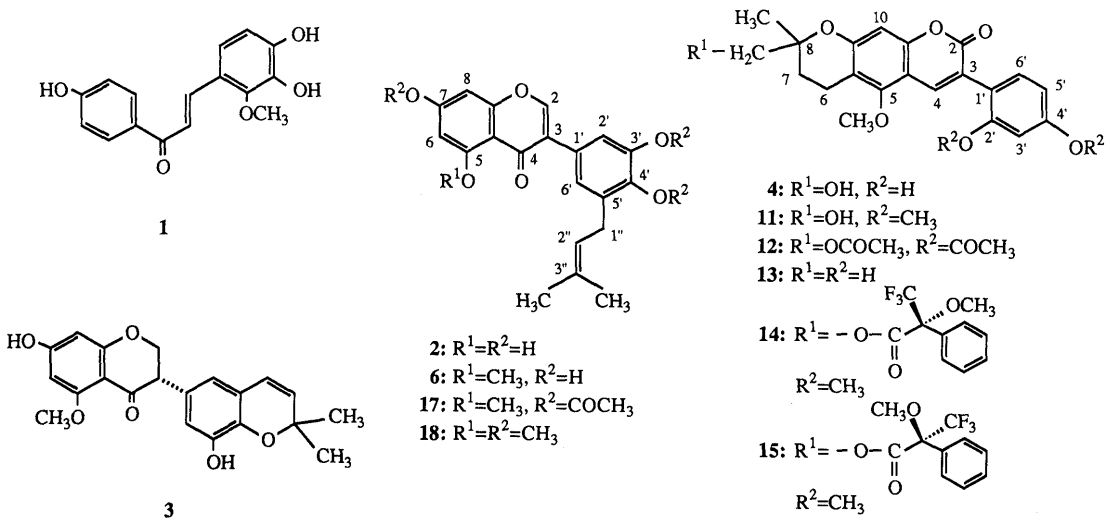

Chart 1

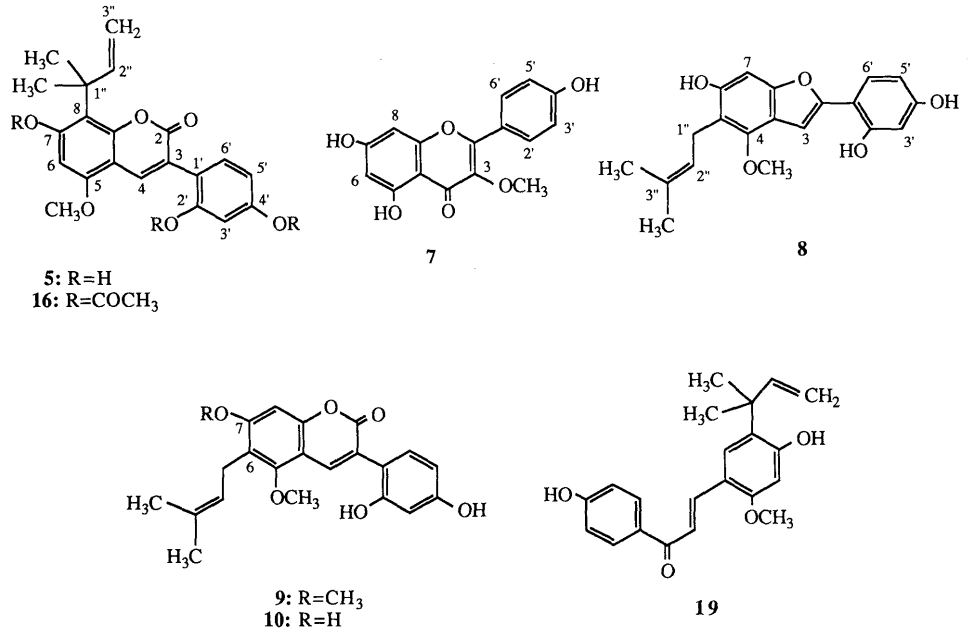

Chart 2

coumarin (**5**) and glisoflavone (**6**), together with licocoumarone (**8**).[7]

Structures of Licopyranocoumarin, Licoarylcoumarin and Glisoflavone Licopyranocoumarin (**4**), mp 137 °C, $[\alpha]_D$ + 14° ($c=1$, acetone), was isolated as yellow needles. The high-resolution electron-impact mass spectrum (HR-MS) of **4** indicated the molecular formula $C_{21}H_{20}O_7$ for this compound. The ultraviolet (UV) spectrum of **4** [λ_{max}^{MeOH}: 211 (log ε 4.62), 262 (sh, 3.94) and 352 nm (4.21)] was analogous to those of 3-arylcoumarins, glycyrin (**9**)[12] and glycycoumarin (**10**).[1,13] The proton nuclear magnetic resonance (^{1}H-NMR) spectrum (in acetone-d_6) of **4** also showed the presence of the 3-arylcoumarin skeleton [two 1H singlets (δ 7.97, H-4; 6.50, H-10) and ABX protons (δ 6.42, dd, $J=2$, 8 Hz, H-5′; 6.47, d, $J=2$ Hz, H-3′; 7.21, d, $J=8$ Hz, H-6′)], along with a methoxyl signal (δ 3.91, 3H, s). The presence of an isoprenoid moiety in **4** was shown by signals of a CH_2–CH_2 system [δ 2.89 (dt, $J=17$, 6 Hz, H-6), 2.82 (ddd, $J=6$, 9, 17 Hz, H-6), 2.0 (H-7, in part overlapped with the solvent signals) and 1.82 (dt, $J=14$, 6 Hz, H-7)], a methyl group [δ 1.31 (3H, s)], and a hydroxymethyl group [δ3.63 and 3.56 (each d, $J=11$ Hz)]. Treatment of **4** with diazomethane afforded a methylate (**11**), $C_{23}H_{24}O_7$, which shows three methoxyl signals at δ 3.84, 3.83 and 3.79 (3H each, s) in its ^{1}H-NMR spectrum, indicating that the methylate has two additional methoxyl groups. Acetylation of **4** afforded a triacetate (**12**), $C_{27}H_{26}O_{10}$. Therefore, **4** has two phenolic hydroxyl groups, and an alcoholic hydroxyl group and a methoxyl group in the molecule.

Treatment of glycycoumarin (**10**) with HCl induced cyclization, forming an ether bond between the isoprenoid side chain (γ,γ-dimethylallyl group) at C-6 and the hydroxyl group at C-7, to give a pyranocoumarin (**13**), $C_{21}H_{20}O_6$. This compound closely resembles **4** in the ^{1}H-NMR spectra, except for the presence of a 6H singlet at δ 1.36 (signal of a *gem*-dimethyl group) in **13**, in place of a 3H singlet (at δ 1.31) and methylene protons of a hydroxymethyl group (at δ 3.56 and 3.63) in **4**. These data indicate a structure in which a hydroxymethyl group replaces a methyl group in the *gem*-dimethyl group of the pyranocoumarin (**13**) for licopyranocoumarin. A nuclear Overhauser effect (NOE) was observed for the H-4 signal at δ7.97 (11%) upon irradiation of the methoxyl signal at δ 3.91 in the ^{1}H-NMR spectrum of **4**. Location of the methoxyl group at C-5 in **4** was thus confirmed.

Based on these results, structure **4** was assigned for licopyranocoumarin.[2,14] Although the absolute configuration at C-8 of **4** has not yet been determined, signals of the methylenoxy group at C-8 in the ^{1}H-NMR spectra of the esters (**14** and **15**) of **11**, which were produced with (*R*)- and (*S*)-forms of α-methoxy-α-trifluoromethylphenylacetic acid (MTPA),[15] indicate that **11** is partially (*ca.* 5%) racemized. Therefore, **4** exists as a partial racemate in the extract, if it was not racemized during the purification and the methylation.

Licoarylcoumarin (**5**) was isolated as yellow needles, mp 160 °C. The HR-MS of **5** indicated the molecular formula $C_{21}H_{20}O_6$ for this compound. The UV spectrum of **5** was also characteristic of the 3-arylcoumarin skeleton. The ^{1}H-NMR spectrum (in acetone-d_6) of **5** showed the presence of an α,α-dimethylallyl group [δ 6.38 (dd, $J=11$, 17.5 Hz, H-2″), 5.01 (dd, $J=1.5$, 17.5 Hz, H-3″), 4.92 (dd, $J=1.5$, 11 Hz, H-3″) and 1.68 (6H, s, $CH_3 \times 2$)], a methoxyl group [δ 3.89 (3H, s)], and also a 3-(2,4-dihydroxyphenyl)coumarin skeleton [δ 8.07 (s, H-4), 7.20 (d, $J=8$ Hz, H-6′), 6.46 (s, H-6), 6.45—6.43 (2H, m, H-3′ and H-5′)].

Acetylation of **5** afforded a triacetate **16**. Upon irradiation of the methoxyl signal at δ 3.94 in the ^{1}H-NMR spectrum of **16**, NOE was observed at the H-4 signal at δ 8.08 (5%), and at the H-6 signal at δ 6.66 (28%). Therefore, structure **5** in which the methoxyl group is at C-5 and the α,α-dimethylallyl group is at O-8 of the 3-arylcoumarin skeleton, is formulated for licoarylcoumarin, based on the biogenetical consideration[12] that the 3-arylcoumarins, coumestans and related isoflavonoids[16] in licorice have an O-functional group at C-7 or a corresponding po-

TABLE I. Inhibitory Effects of Licorice Phenolics on Xanthine Oxidase

Compound	IC_{50}[a] (M)
Chalcone	
Licochalcone B (**1**)	3.0×10^{-5}
Licochalcone A (**19**)	5.6×10^{-5}
Isoflavone	
Glycyrrhisoflavone (**2**)	5.3×10^{-5}
Glisoflavone (**6**)	$>1.0\times10^{-4}$
Isoflavanone	
Glycyrrhisoflavanone (**3**)	$>1.0\times10^{-4}$
3-Arylcoumarin	
Licopyranocoumarin (**4**)	$>1.0\times10^{-4}$
Licoarylcoumarin (**5**)	$>1.0\times10^{-4}$
Glycycoumarin (**10**)	$>1.0\times10^{-4}$
2-Arylbenzofuran	
Licocoumarone (**8**)	1.3×10^{-5}
Flavone	
Kaempferol 3-*O*-methyl ether (**7**)	$>1.0\times10^{-4}$
Allopurinol	1.6×10^{-7}

a) Concentration for 50% inhibition.

sition. The ^{13}C-NMR spectrum of licoarylcoumarin (see Experimental) is also consistent with the assigned structure **5**.

Glisoflavone (**6**), mp 199 °C, was isolated as colorless needles. The HR-MS of **6** indicated the molecular formula $C_{21}H_{20}O_6$ for this compound. The UV spectrum is similar to that of glycyrrhisoflavone (**2**)[1] with a slight blue-shift of a peak [262 nm (**2**)→256 nm (**6**)]. The ^{1}H-NMR spectrum of **6** indicates the presence of a γ,γ-dimethylallyl group [δ 5.36 (br t, J=7 Hz), 3.34 (2H, br d, J=7 Hz), 1.71, 1.69 (3H each, br s)], a methoxyl group [δ 3.84 (3H, s)] and an isoflavone skeleton [δ 7.85 (s, H-2), 6.43 (d, J=2 Hz, H-6), 6.42 (d, J=2 Hz, H-8), 6.77 (d, J=2 Hz, H-2′), 6.96 (d, J=2 Hz, H-6′)]. Acetylation of **6** gave a triacetate (**17**), indicating that **6** has three hydroxyl groups. Methylation of **6** gave **18**, which was identical with the permethyl derivative of **2**. Therefore, glisoflavone has the structure, in which a methoxyl group replaces one of the hydroxyl groups of **2**.

Upon irradiation of the methoxyl signal (δ 3.90) in the ^{1}H-NMR spectrum of the acetate **17**, an NOE (23%) was observed for the H-6 signal at δ 6.79, while no effect was observed on the H-8 signal. Based on these results, the structure **6** was assigned for glisoflavone.

Inhibitory Effects of Licorice Phenolics on Xanthine Oxidase Inhibitory effects of ten phenolics isolated from Sinkiang licorice [**1** and licochalocone A (**19**)[1,17]] and Si-pei licorice [**2**, **3**, **4**, **5**, **6**, **7**, **8** and **10**] on xanthine oxidase were tested, and the results are summarized in Table I. Four compounds among them, **1**, **2**, **8** and **19** showed 50% inhibition at the concentration of 1.3—5.6×10^{-5} M, although the inhibitory effects of these compounds were weaker than that of allopurinol, a remedy for gout.

The inhibitory effects of these phenolics may be due to the non-specific binding of these compounds to the enzyme protein.[18] The inhibitory effects of the two chalcones, **1** and **19**, are explicable in terms of covalent binding with amino acids in the enzyme protein, in a way analogous to the proposed mechanism for the inhibition of phenylalanine ammonia-lyase by chalcones.[18] However, the effects of the other phenolics are not attributable to covalent-bond formation. It is also noticeable that licocoumarone (**8**), which was reported to inhibit lipid peroxidation,[7] was most effective in inhibiting this oxidative enzyme.

Experimental

UV and infrared (IR) spectra were recorded on a Hitachi 200-10 spectrophotometer and on a JASCO A-102 spectrometer, respectively. Mass spectra (MS) were recorded on a JEOL GMS-HX100 spectrometer, a VG 70-SE instrument or a Shimadzu LKB-9000 machine. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian VXR-500 instrument (500 MHz for ^{1}H-NMR and 125.7 MHz for ^{13}C-NMR) unless otherwise mentioned, and chemical shifts are given in δ values (ppm) from tetramethylsilane. CPC was performed on a Sanki L-90 machine equipped with twelve cartridges, of which the total inner volume is 850 ml. PTLC was conducted on Kieselgel PF_{254} (0.5 mm thick) with solvent system A (chloroform–acetone–formic acid, 80 : 17 : 3), B (chloroform–MeOH, 10 : 1), C (hexane–ethyl acetate, 3 : 2), D (chloroform–acetone–formic acid, 16 : 2 : 1) or E (chloroform–MeOH, 49 : 1) as the developer. High-performance liquid chromatography (HPLC) was performed on a LiChrospher RP-18 (5 μm) (4 × 250 mm) cartridge in an oven set at 40 °C, with acetonitrile–H_2O–acetic acid (8 : 11 : 1) as an eluant. Detection was effected by a Shimadzu SPD-6A spectrophotometric detector at 280 nm, and the flow rate was 1.3 ml/min.

Isolation of Phenolics from Si-pei Licorice Powdered Si-pei licorice (500 g) was defatted with hexane, and then extracted with ethyl acetate. A portion (8.4 g) of the ethyl acetate extract (23.9 g) was subjected to droplet countercurrent chromatography (3.2 mm i.d. × 1.2 m × 95 glass tubes; descending mode) using chloroform–MeOH–H_2O (7 : 13 : 8), to separate fractions I—VII. Fraction V (190 mg) was subjected to column chromatography on MCI gel CHP-20P (1.1 cm × 38 cm) using 70% MeOH as eluant; 400-drop fractions were collected. Combined fractions 18—23 (28 mg) were purified by PTLC with solvent system A, and further purified by PTLC with solvent system B (developed twice), to give licopyranocoumarin (**4**) (18 mg). Combined fractions 42—75 (20 mg) were purified by PTLC with solvent system A, to afford kaempferol 3-*O*-methyl ether (**7**) (5 mg). Another portion (10 g) of the ethyl acetate extract was subjected to CPC (reversed-phase development) using chloroform–MeOH–H_2O (7 : 13 : 8), to separate fractions I—V. Fraction II (306 mg) from CPC was purified by column chromatography on MCI gel CHP-20P (1.1 × 38 cm) with 70% MeOH and then by PTLC with solvent system B (developed twice), to give licocoumarone (**8**) (15 mg). Fraction IV (283 mg) of CPC was purified by column chromatography on Toyopearl HW-40 (fine grade) (with 70% EtOH), and on MCI gel CHP-20P (with 70% MeOH→80% MeOH), to give crude licoarylcoumarin (**5**) and crude glisoflavone (**6**). They were further purified by PTLC with solvent system A, to afford 8 mg of **5** and 4 mg of **6**.

Licopyranocoumarin (4) Yellow needles, mp 137 °C. $[\alpha]_D$ +14° (c=1, acetone). *Anal.* Calcd for $C_{21}H_{20}O_7\cdot1/2H_2O$: C, 64.11; H, 5.38. Found: C, 64.01; H, 5.72. HR-MS m/z: 384.1175 ([M]$^+$; Calcd for $C_{21}H_{20}O_7$, 384.1209). UV λ_{max}^{MeOH} nm (log ε): 211 (4.62), 262 (sh, 3.94), 352 (4.21). IR ν_{max}^{KBr} cm^{-1} 1695, 1610, 1570. ^{1}H-NMR (acetone-d_6) δ: 1.31 (3H, s, CH_3 at C-8), 1.82 (1H, dt, J=14, 6 Hz, H-7), 2.0 (H-7, in part overlapping with the solvent signals), 2.82 (1H, ddd, J=6, 9, 17 Hz, H-6), 2.89 (1H, dt, J=17, 6 Hz, H-6), 3.56, 3.63 (1H each, d, J=11 Hz, CH_2OH at C-8), 3.91 (3H, s, OCH_3 at C-5), 6.42 (1H, dd, J=2, 8 Hz, H-5′), 6.47 (1H, d, J=2 Hz, H-3′), 6.50 (1H, br s, H-10), 7.21 (1H, d, J=8 Hz, H-6′), 7.97 (1H, s, H-4). ^{13}C-NMR (acetone-d_6) δ: 17.0 (C-6), 21.9 (CH_3 at C-8), 27.6 (C-7), 62.5 (OCH_3 at C-5), 68.2 (CH_2OH at C-8), 79.1 (C-8), 100.5 (C-10), 104.4 (C-3′), 108.0 (C-5′), 108.2 (C-5a), 113.2, 115.5 (C-1′, C-3), 122.6 (C-4a), 132.7 (C-6′), 137.6 (C-4), 154.3, 156.3, 157.1, 158.9, 159.8 (C-2′, C-4′, C-5, C-9a, C-10a), 161.8 (C-2).

Triacetate (**12**): mp 87 °C. HR-MS m/z: 510.1491 ([M]$^+$; Calcd for $C_{27}H_{26}O_{10}$, 510.1526). ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, s, CH_3 at C-8), 1.82 (1H, dt, J=14, 6 Hz, H-7), 1.96 (1H, ddd, J=6, 9, 14 Hz, H-7), 2.10, 2.16, 2.29 (3H each, s, $OCOCH_3$ × 3), 2.77 (1H, ddd, J=6, 9, 17 Hz, H-6), 2.83 (1H, dt, J=17, 6 Hz, H-6), 3.84 (3H, s, OCH_3 at C-5), 4.12, 4.17 (1H each, d, J=12 Hz, CH_2OAc at C-8), 6.62 (1H, s, H-10), 7.04 (1H, d, J=2 Hz, H-3′), 7.06 (1H, dd, J=2, 8 Hz, H-5′), 7.43 (1H, d, J=8 Hz, H-6′), 7.84 (1H, s, H-4).

Methylation of Licopyranocoumarin (4) Ethereal diazomethane (1 ml) was added to an EtOH solution (0.5 ml) of **4** (13 mg), and the mixture was left to stand for 16 h. The solvent was distilled off and the residue was purified by PTLC with solvent system B, to afford the methylate **11** (6 mg), mp 73 °C, $[\alpha]_D$ +18° (c=1, acetone). HR-MS m/z: 412.1493 ([M]$^+$; Calcd

for $C_{23}H_{24}O_7$, 412.1522). ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, s, CH_3 at C-8), 1.76 (1H, ddd, J=4.5, 6, 13.5 Hz, H-7), 1.86 (1H, br t, J=6 Hz, CH_2OH), 2.02 (1H, ddd, J=6, 11, 13.5 Hz, H-7), 2.75 (1H, ddd, J=6, 11, 17 Hz, H-6), 2.89 (1H, ddd, J=4.5, 6, 17 Hz, H-6), 3.61, 3.69 (1H each, dd, J=6, 11.5 Hz, CH_2OH), 3.79, 3.83, 3.84 (3H each, s, $OCH_3\times3$), 6.53—6.55 (2H, H-3′, H-5′), 6.60 (1H, s, H-10), 7.31 (1H, d, J=8 Hz, H-6′), 7.85 (1H, s, H-4).

Esterification of 11 with (*R*)-MTPA *N,N′*-Dicyclohexylcarbodiimide (Wako) (2.2 mg) dissolved in dichloromethane (80 μl) was added to a solution (170 μl) of **11** (2.0 mg), 4-dimethylaminopyridine (Wako) (0.6 mg) and (*R*)-MTPA (Fluka) (1.2 mg) in dichloromethane at 0 °C, and the reaction mixture was shaken for 9 d at room temperature. After removal of the solvent with an N_2 stream, the residue was purified by PTLC with solvent system C, to give the ester **14** (1.6 mg). HR-MS m/z: 628.1913 ($[M]^+$; Calcd for $C_{33}H_{31}O_9F_3$, 628.1921). ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, s, CH_3 at C-8), 1.80 (1H, dt, J=14, 6 Hz, H-7), 1.94 (1H, ddd, J=6, 9, 14 Hz, H-7), 2.74 (1H, ddd, J=6, 9, 17 Hz, H-6), 2.80 (1H, dt, J=17, 6 Hz, H-6), 3.52 (3H, br s, OCH_3 of MTPA moiety), 3.77, 3.83, 3.83 (3H each, s, $OCH_3\times3$), 4.32, 4.42 (1H each, d, J=11 Hz, CH_2O at C-8), 6.53—6.56 (3H, m, H-3′, H-5′, H-10), 7.31 (1H, d, J=8 Hz, H-6′), 7.35—7.41 (3H, m, phenyl protons of MPTA moiety), 7.51 (2H, br d, J=7 Hz, phenyl protons of MTPA moiety), 7.84 (1H, s, H-4). A 2H singlet (δ 4.36) due to the methylenoxy group at C-8 of the ester of the minor enantiomer of **11** was also observed. The peak areas of the methylenoxy group signals indicated that the ratio, major enantiomer : minor enantiomer, is 19 : 1.

Esterification of 11 with (*S*)-MTPA Compound **15** was synthesized from **11** and (*S*)-MTPA in a way analogous to that described above. HR-MS m/z: 628.1916 ($[M]^+$; Calcd for $C_{33}H_{31}O_9F_3$, 628.1921). ^{1}H-NMR ($CDCl_3$) δ: 1.35 (3H, s, CH_3 at C-8), 1.80 (1H, dt, J=14, 7 Hz, H-7), 1.89 (1H, dt, J=14, 7 Hz, H-7), 2.77 (2H, t, J=7 Hz, H-6), 3.53 (3H, br s, OCH_3 of MTPA moiety), 3.79, 3.83, 3.83 (3H each, s, $OCH_3\times3$), 4.36 (2H, s, CH_2O at C-8), 6.53—6.56 (3H, m, H-3′, H-5′, H-10), 7.31 (1H d, J=8 Hz, H-6′), 7.35—7.41 (3H, m, phenyl protons of MPTA moiety), 7.52 (2H, br d, J=7 Hz, phenyl protons of MTPA moiety), 7.84 (1H, s, H-4). Two doublets (δ 4.32 and 4.42, J=11 Hz) due to the methylenoxy group at C-8 of the minor enantiomer ester were also observed.

Treatment of Glycycoumarin (10) with HCl Concentrated HCl (0.3 ml) was added to an MeOH solution (0.7 ml) of **10** (10 mg), and the mixture was left to stand for 35 h. The solvent was evaporated off, and the residue was purified by PTLC using solvent system D, to give a pyranocoumarin **13** (3 mg), mp 235 °C (dec.). *Anal.* Calcd for $C_{21}H_{20}O_6$: C, 66.84; H, 5.61. Found: C, 67.26; H, 5.60. HR-MS m/z: 368.1256 ($[M]^+$; Calcd for $C_{21}H_{20}O_6$, 368.1260). ^{1}H-NMR (acetone-d_6) δ: 1.36 (6H, s, *gem*-dimethyl at C-8), 1.87 (2H, t, J=7 Hz, H-7), 2.84 (2H, t, J=7 Hz, H-6), 3.91 (3H, s, OCH_3 at C-5), 6.43 (1H, dd, J=2.5, 8.5 Hz, H-5′), 6.47 (1H, d, J=2.5 Hz, H-3′), 6.50 (1H, s, H-10), 7.21 (1H, d, J=8.5 Hz, H-6′), 7.97 (1H, s, H-4). ^{13}C-NMR (acetone-d_6) δ: 17.6 (C-6), 26.9 ($CH_3\times2$ at C-8), 32.3 (C-7), 62.5 (OCH_3 at C-5), 76.4 (C-8), 100.5 (C-10), 104.3 (C-3′), 108.0 (C-5′), 108.1 (C-6), 112.8, 115.6 (C-1′, C-3), 122.5 (C-4a), 132.8 (C-6′), 137.6 (C-4), 154.4, 156.4, 157.1, 158.9, 159.8 (C-2′, C-4′, C-5, C-9a, C-10a), 161.8 (C-2).

Licoarylcoumarin (5) Yellow needles, mp 160 °C. *Anal.* Calcd for $C_{21}H_{20}O_6\cdot3/2H_2O$: C, 63.79; H, 5.86. Found: C, 64.26; H, 5.57. HR-MS m/z: 368.1289 ($[M]^+$: Calcd for $C_{21}H_{20}O_6$, 368.1259). UV λ_{max}^{MeOH} nm (log ε): 210 (4.63), 258 (sh, 4.07), 267 (4.11), 356 (4.21). IR ν_{max}^{KBr} cm^{-1}: 1650, 1595, 1570. ^{1}H-NMR (acetone-d_6) δ: 1.68 (6H, s, *gem*-dimethyl at C-1″), 3.89 (3H, s, OCH_3 at C-5), 4.92 (1H, dd, J=1.5, 11 Hz, H-3″), 5.01 (1H, dd, J=1.5, 17.5 Hz, H-3″), 6.38 (1H, dd, J=11, 17.5 Hz, H-2″), 6.43—6.45 (2H, m, H-3′, H-5′), 6.46 (1H, s, H-6), 7.20 (1H, d, J=8 Hz, H-6′), 8.07 (1H, s, H-4). ^{13}C-NMR (acetone-d_6) δ: 41.6 (C-1″), 56.3 (OCH_3 at C-5), 97.1 (C-6), 104.7 (C-3′), 105.2 (C-8), 108.3 (C-5′), 109.6 (C-3″), 112.9, 115.5 (C-1′, C-3), 120.2 (C-4a), 132.5 (C-6′), 138.0 (C-4), 150.3 (C-2″), 155.3, 156.0, 157.2, 159.6 160.8 (C-2′, C-4′, C-5, C-7, C-8a), 162.3 (C-2). The solvent signals overlap the signal of the *gem*-dimethyl group at C-1″.

Triacetate (**16**): mp 176 °C. HR-MS m/z: 494.1532 ($[M]^+$; Calcd for $C_{27}H_{26}O_9$, 494.1577). ^{1}H-NMR (acetone-d_6) δ: 1.62 (6H, s, *gem*-dimethyl at C-1″), 2.13, 2.22, 2.28 (3H each, s, $OCOCH_3\times3$), 3.94 (3H, s, OCH_3 at C-5), 4.91 (1H, dd, J=1, 10.5 Hz, H-3″), 4.94 (1H, dd, J=1, 17.5 Hz, H-3″), 6.28 (1H, dd, J=10.5, 17.5 Hz, H-2″), 6.66 (1H, s, H-6), 7.09 (1H, d, J=2 Hz, H-3′), 7.14 (1H, dd, J=2, 8.5 Hz, H-5′), 7.61 (1H, d, J=8.5 Hz, H-6′), 8.08 (1H, s, H-4).

Glisoflavone (6) Colorless needles, mp 199 °C. *Anal.* Calcd for $C_{21}H_{20}O_6\cdot3/2H_2O$: C, 63.79; H, 5.86. Found: C, 63.91; H, 5.44. HR-MS m/z: 368.1241 ($[M]^+$; Calcd for $C_{21}H_{20}O_6$, 368.1259). UV λ_{max}^{MeOH} nm (log ε): 208 (4.52), 256 (4.36), 288 (sh, 4.05). IR ν_{max}^{KBr} cm^{-1}: 1630, 1575. ^{1}H-NMR (acetone-d_6) δ: 1.69, 1.72 (3H each, br s, *gem*-dimethyl at C-3″), 3.34 (2H, br d, J=7 Hz, H-1″×2), 3.84 (3H, s, OCH_3 at C-5), 5.35 (1H, br t, J=7 Hz, H-2″), 6.42 (1H, d, J=2 Hz, H-8), 6.43 (1H, d, J=2 Hz, H-6), 6.77 (1H, d, J=2 Hz, H-2′), 6.96 (1H, d, J=2 Hz, H-6′), 7.85 (1H, s, H-2). ^{13}C-NMR (acetone-d_6) δ: 17.9, 25.9 (*gem*-dimethyl at C-3″), 29.1 (C-1″), 56.3 (OCH_3 at C-5), 95.8 (C-8), 97.2 (C-6), 109.8 (C-4a), 115.1 (C-2′), 122.1 (C-6′), 123.9 (C-2″), 124.4, 126.7 (C-1′, C-5′), 128.5 (C-3), 132.1 (C-3″), 143.8, 144.7 (C-3′, C-4′), 150.8 (C-2), 160.5, 162.8, 163.0 (C-5, C-7, C-8a), 174.8 (C-4).

Triacetate (**17**): mp 135 °C. HR-MS m/z: 494.1515 ($[M]^+$; Calcd for $C_{27}H_{26}O_9$, 494.1577). ^{1}H-NMR (acetone-d_6) δ: 1.70, 1.71 (3H each, br s, *gem*-dimethyl at C-3″), 2.26, 2.30, 2.31 (3H each, s, $OCOCH_3\times3$), 3.29 (2H, br d, J=7 Hz, H-1″×2), 3.90 (3H, s, OCH_3 at C-5), 5.24 (1H, br t, J=7 Hz, H-2″), 6.79 (1H, d, J=2 Hz, H-6), 6.92 (1H, d, J=2 Hz, H-8), 7.33, 7.35 (1H each, d, J=2 Hz, H-2′, H-6′), 8.18 (1H, s, H-2).

Methylation of Glycyrrhisoflavone (2) Ethereal diazomethane (0.6 ml) was added to an EtOH solution (0.3 ml) of **2** (3 mg), and the mixture was left to stand overnight. Then, the solvent was distilled off, and the residue was purified by PTLC with solvent system E, to afford the methylate **18** (2 mg), mp 92 °C. HR-MS m/z: 410.1751 ($[M]^+$; Calcd for $C_{24}H_{26}O_6$, 410.1730). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.71, 1.72 (3H each, br s, *gem*-dimethyl at C-3″), 3.36 (2H, d, J=7 Hz, H-1″×2), 3.82, 3.88, 3.90, 3.94 (3H each, s, $OCH_3\times4$), 5.27 (1H, br t, J=7 Hz, H-2″), 6.38, 6.45 (1H each, d, J=2 Hz, H-6, H-8), 6.81, 7.10 (1H each, d, J=2 Hz, H-2′, H-6′), 7.77 (1H, s, H-2).

Methylation of Glisoflavone (6) Treatment of an EtOH solution of **6** (2 mg) with ethereal diazomethane, followed by purification by PTLC gave a product (1 mg) which was identified as **18** by mixed melting point determination, co-TLC, and comparison of the ^{1}H-NMR data.

Kaempferol 3-*O*-Methyl Ether (7)[10] Pale yellow needles, mp 271 °C. EI-MS (LR) [electron-impact mass spectrum; low resolution mode] m/z: 300 {$[M]^+$, 100% (relative intensity)}, 299 ($[M-H]^+$, 77%), 282 ($[M-H_2O]^+$, 16%), 271 ($[M-HCO]^+$, 19%), 269 ($[M-CH_3O]^+$, 10%), 257 ($[M-CH_3CO]^+$, 42%), 153 ($[C_7H_4O_4+H]^+$, 15%), 121 ($[C_7H_5O_2]^+$, 29%). UV λ_{max}^{MeOH} (log ε): 208 (4.42), 218 (infl., 4.30), 267 (4.25), 295 (sh, 4.05), 351 (4.23). IR ν_{max}^{KBr} cm^{-1}: 1645, 1610, 1560. ^{1}H-NMR (acetone-d_6) δ: 3.86 (3H, s, OCH_3), 6.25 (1H, d, J=2 Hz, H-6), 6.49 (1H, d, J=2 Hz, H-8), 7.01 (2H, d, J=9 Hz, H-3′, H-5′), 8.02 (2H, d, J=9 Hz, H-2′, H-6′), 12.80 (1H, s, chelated OH at C-5). An NOE (4%) of the signal at δ 8.02 (H-2′, H-6′), upon irradiation of the methoxyl signal (δ 3.86), indicated the location of the methoxyl group at C-3.

Licocoumarone (8)[7] Colorless needles, mp 182 °C. EI-MS (LR) m/z: 340 {$[M]^+$, 100% (relative intensity)}, 285 ($[M-(CH_3)_2C=CH]^+$, 64%), 269 (74%). UV λ_{max}^{MeOH} nm (log ε): 213 (4.52), 322 (4.46), 336 (4.40). IR ν_{max}^{KBr} cm^{-1}: 1620, 1600. ^{1}H-NMR (acetone-d_6) δ: 1.63, 1.77 (3H each, br s, *gem*-dimethyl at C-3″), 3.39 (2H, br d, J=7 Hz, H-1″×2), 4.01 (3H, s, OCH_3 at C-4), 5.24 (1H, br t, J=7 Hz, H-2″), 6.47 (1H, dd, J=2, 8 Hz, H-5′), 6.54 (1H, d, J=2 Hz, H-3′), 6.75 (1H, br s, H-7), 7.28 (1H, d, J=1 Hz, H-3), 7.66 (1H, d, J=8 Hz, H-6′). The location of the methoxyl group (C-4) was indicated by an NOE (7%) of the H-3 signal upon irradiation of the methoxyl signal (δ 4.01). The product was identical with authentic licocoumarone on HPLC (retention time, 13.6 min), and on TLC (solvent system B, *Rf* 0.39).

Estimation of Inhibitory Effects of Licorice Phenolics on Xanthine Oxidase The inhibitory activity of each compound was determined by the reported method[6a] with a slight modification. A solution of xanthine oxidase (from cow's milk; Boehringer Mannheim) [0.04 U/ml in 0.1 M phosphate buffer (pH 7.4); 40 μl] was added to a solution consisting of 0.1 M phosphate buffer (pH 7.4) (360 μl) and a solution of a test compound in H_2O–dimethylsulfoxide (8 : 2, by volume) (200 μl), and the mixture was preincubated for 10 min at 37 °C. Then, an aqueous solution (1×10^{-4} M; 600 μl) of xanthine (Wako) was added to the mixture and the resulting solution was incubated for 30 min at 37 °C. The enzyme reaction was stopped by adding 1 N HCl (200 μl), and the absorbance at 295 nm of the reaction mixture was measured. The blank solution was prepared in an analogous way, except that the solution of xanthine oxidase was added after the addition of HCl. Inhibitory activity (%) was calculated in the same way as reported,[6a] and is given as the mean value of triplicate experiments.

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