Phenolic Constituents of Licorice. III.[1] Structures of Glicoricone and Licofuranone, and Inhibitory Effects of Licorice Constituents on Monoamine Oxidase

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Two new phenolic compounds, glicoricone (3) and licofuranone (4), were isolated from a species of licorice brought from the northwestern region of China, and their structures were assigned.

Among the twelve licorice constituents examined for the inhibition of monoamine oxidase (MAO), six compounds, 3, 4, genistein (6), licopyranocoumarin (7), licocoumarone (14) and glycyrrhisoflavone (15), inhibited the enzyme with the IC_{50} (concentration required for 50% inhibition of the enzyme activity) values of 6.0×10^{-5}—1.4×10^{-4} M. Glycyrrhizin (1) also inhibited MAO with the IC_{50} value of 1.6×10^{-4} M.

Keywords glicoricone; licofuranone; genistein; licocoumarone; licopyranocoumarin; glycyrrhizin; *Glycyrrhiza*; licorice; monoamine oxidase; enzyme inhibitor

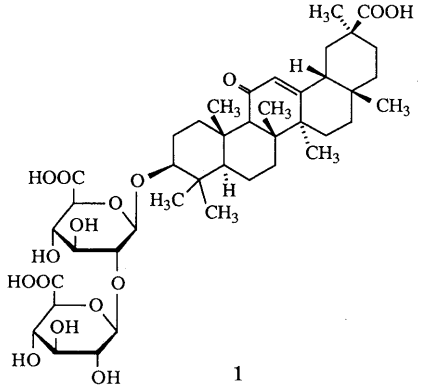
Although glycyrrhizin (**1**) is known to be the main constituent of licorice (root and rhizome of *Glycyrrhiza* spp.), a large number of phenolic constituents of root, rhizome and overground part of *Glycyrrhiza* spp., have been found recently.[1] In addition to the structures of these phenolic constituents, their pharmacological activities have also been investigated.[1-4] The inhibition of the lipoxygenase-dependent peroxidation in the arachidonate metabolism is one such activity of licorice phenolics.[4a] In a previous paper, we reported that several licorice phenolics showed inhibitory effects on xanthine oxidase (XOD) which catalyzes the oxidation of hypoxanthine and xanthine into uric acid.[1] This enzyme has been considered to participate in the oxidative damage of the living body through the generation of superoxide.[5] The participation of monoamine oxidase (MAO) in the oxidative stress in the nervous system has also been pointed out.[6] Synthetic MAO inhibitors, which have been used for depression, are accompanied by some adverse effects, and therefore attempts have been made to find natural MAO inhibitors[7-10]; among those tested has been isoliquiritigenin (**2**),[8] a licorice constituent. We report here the isolation and structure elucidation of two additional new phenolic constituents of licorice, and also the inhibitory effects of several licorice constituents on MAO.

Results and Discussion

Isolation of Two New Phenolics from Licorice The licorice imported from the northwestern region of China (Seihokukanzo in the Japanese markets[11]) and frequently used for Japanese traditional Kampo medicine, was defatted with *n*-hexane and then extracted with EtOAc. The EtOAc extract was subjected to centrifugal partition chromatography (CPC)[12] and column chromatography to yield two new compounds, which we have named glicoricone (**3**) and licofuranone (**4**), together with echinatin (**5**),[13] genistein (**6**)[14] and licopyranocoumarin (**7**).[1]

Structures of the Two New Compounds Glicoricone (**3**) was obtained as colorless needles. The high-resolution electron-impact mass (EI-MS) spectrum of **3** indicated the molecular formula $C_{21}H_{20}O_6$. The ultraviolet (UV) spectrum of **3** [λ_{max} nm (log ε): 209 (4.55), 240 (sh, 4.27), 247 (4.24), 285 (4.02) and 305 (sh, 3.94)] is similar to the reported spectra of isoflavones, especially that of licoricone (**8**)[15] possessing a B-ring with the phloroglucinol-type substitution pattern. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **3** showed a 1H singlet in the low field (δ 8.10), which is characteristic of H-2 of the isoflavone structure. The spectrum also showed the signals of a tri-substituted benzene ring [δ 6.95 (1H, d, J=2 Hz), 7.03 (1H, dd, J=2, 9 Hz), 8.07 (1H, d, J=9 Hz)], a penta-substituted benzene ring [δ 6.32 (1H, s)], a γ,γ-dimethylallyl group [δ 1.64, 1.74 (3H each, br s; *gem*-dimethyl at C-3″), 3.29 (2H, br d, J=7 Hz; H-1″), 5.25 (1H, br t, J=7 Hz; H-2″)] and a methoxyl group [δ 3.41 (3H, s)].

Methylation of **3** afforded licoricone dimethyl ether (**9**),[15]



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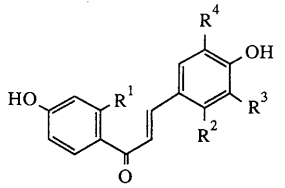
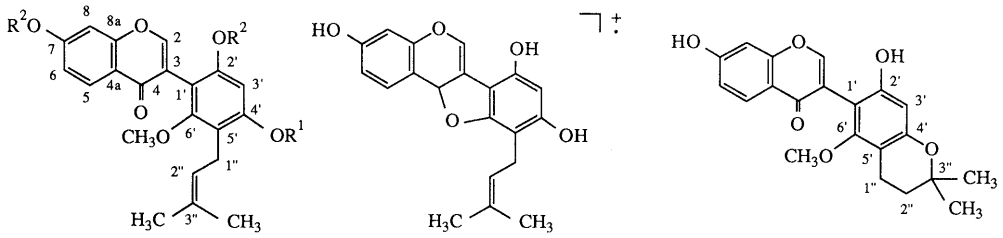


2 : R^1= OH, R^2= R^3= R^4= H
5 : R^1= H, R^2= OCH_3, R^3= R^4= H
16 : R^1= R^3= H, R^2= OCH_3,
R^4= -$C(CH_3)_2$-CH=CH_2
17 : R^1= R^4= H, R^2= OCH_3, R^3= OH

Chart 1

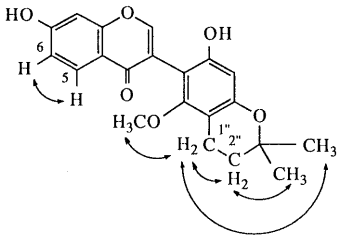


3 : $R^1 = R^2 = H$
8 : $R^1 = CH_3$, $R^2 = H$
9 : $R^1 = R^2 = CH_3$

3a

10

Chart 2



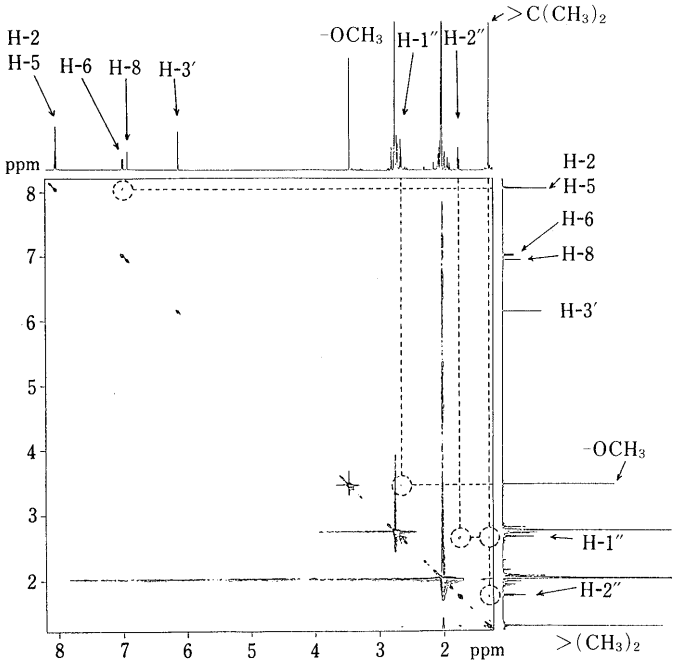


Fig. 1. The ROESY Spectrum of **10** (30 °C, in Acetone-d_6)

The dashed line circles indicate the cross peaks attributable to NOEs shown by the arrows in the structural formula. The signals at around δ 2.8 and δ 2.0 in the accompanying 1D spectrum are those of water and the solvent, respectively.

indicating that the structure of glicoricone has three hydroxyl groups in place of three of the four methoxyl groups of **9**.

The EI-MS spectrum of **3** showed an $[M-31]^+$ peak (relative intensity, 40%) ascribable to the fragment ion **3a**, and indicated that the location of the methoxyl group in **3** is *ortho* to C-1′ on the B-ring.[15,16]

Treatment of **3** with HCl caused cyclization between the γ,γ-dimethylallyl group and the adjacent hydroxyl group, to give **10**. The rotating flame Overhauser enhancement

TABLE I. One-Bond and Long-Range ^1H–^{13}C Correlation Data for Glicoricone (**3**)

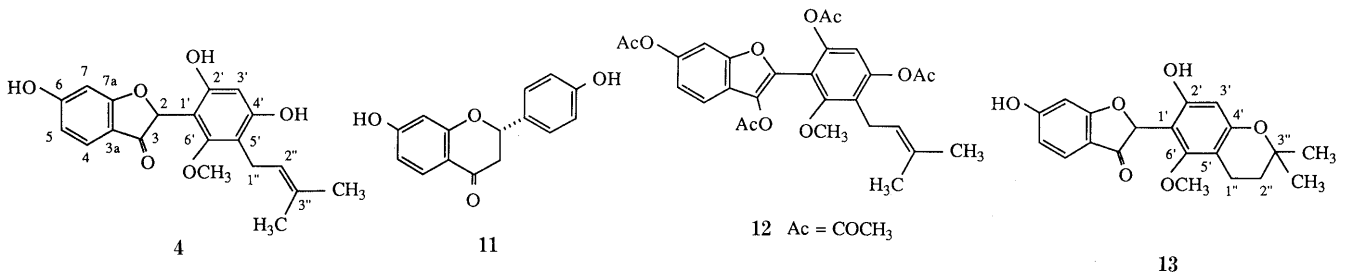
Carbon	δ_C	Correlated proton	
		Proton coupled *via* one bond (δ_H)	Proton coupled *via* two or three bonds[a]
C-2	157.0	8.10	
C-3	119.6		H-2
C-4	177.6		H-2/H-5[b]
C-4*a*	118.2		H-6/H-8[c]
C-5	128.4	8.07	
C-6	115.9	7.03	H-8[d]
C-7	163.4		H-5
C-8	103.2	6.95	H-6[e]
C-8*a*	158.8		H-5
C-1′	106.2		H-2, H-3′
C-2′	156.6		H-3′
C-3′	100.9	6.32	
C-4′	157.7		H-3′, H-1″
C-5′	114.2		H-3′, H-1″
C-6′	159.1		$OC\underline{H}_3$, H-1″
C-1″	23.5	3.29	
C-2″	125.1	5.25	
C-3″	130.4		$=C(C\underline{H}_3)_2$
$=C(C\underline{H}_3)_2$	17.9	1.74	
	25.8	1.64	
OCH_3	61.0	3.41	

500 MHz for ^1H-NMR and 125.7 MHz for ^{13}C-NMR, in acetone-d_6. *a*) The average J_{CH} value for the long-range couplings was set at 7 Hz. *b, c*) Unresolved cross peak ascribable to the couplings with the two protons. *d, e*) The cross peak due to the long-range coupling which is expected to be observed was overlapped by the nearby cross peak due to the one-bond coupling.

spectroscopy (ROESY) spectrum of **10** (Fig. 1) showed a cross peak, due to the nuclear Overhauser effect (NOE) between the methoxyl group and the signal of the methylene protons at C-1″ of the newly formed pyrane ring. On the other hand, no cross peak was observed between the methoxyl group and the aromatic protons. The location of the methoxyl group *ortho* to the aromatic protons is thus excluded, and structure **10**, in which the methoxyl group is at C-6′, is the only one which satisfies these findings.

Structure **3**, in which the methoxyl group is at C-6′, was thus assigned for glicoricone. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **3** is also consistent with this structure. The assignments of the ^{13}C signals were confirmed by the one-bond and long-range ^1H–^{13}C correlation spectral data shown in Table I.

Licofuranone (**4**) was obtained as colorless needles. The $[M+H]^+$ ion peak in the high-resolution fast-atom bom-



4 11 12 Ac = $COCH_3$ 13

Chart 3

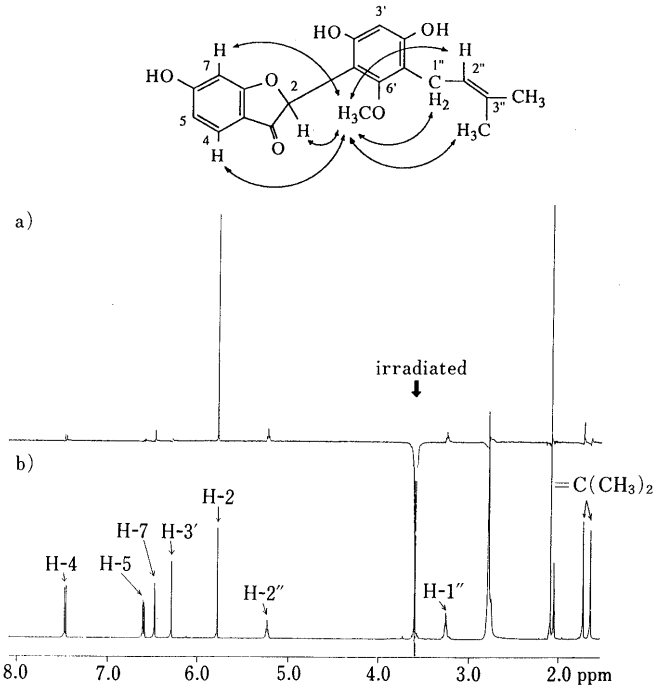


Fig. 2. The NOE Difference Spectrum of **4** (40 °C, in Acetone-d_6)

a) Difference spectrum with the irradiation of the methoxyl signal. b) Reference spectrum.

TABLE II. One-Bond and Long-Range ^1H–^{13}C Correlation Data for Licofuranone (**4**)

Carbon	δ_C	Correlated proton	
		Proton coupled *via* one bond (δ_H)	Proton coupled *via* two or three bonds[a]
C-2	81.4	5.77	
C-3	198.8		
C-3*a*	115.7		H-5
C-4	125.6	7.45	
C-5	111.2	6.60	H-7
C-6	166.5		H-4
C-7	99.0	6.47	
C-7*a*	174.9		H-4
C-1′	108.3		H-3′
C-2′	156.7		H-2
C-3′	99.6	6.28	
C-4′	158.6		
C-5′	113.8		H-3′
C-6′	161.0		
C-1″	23.3	3.23	
C-2″	124.9	5.21	
C-3″	130.7		
$=C(CH_3)_2$	17.9	1.71	
	25.8	1.63	
OCH_3	62.9	3.58	

500 MHz for ^1H-NMR and 125.7 MHz for ^{13}C-NMR, in acetone-d_6. *a*) The average J_{CH} value for the long-range couplings was set at 7 Hz. Several cross peaks expected to be observed were lacking in the long-range ^1H–^{13}C correlation spectrum because of a limitation in quantity of the available sample.

bardment mass (FAB-MS) spectrum of **4** indicated its molecular formula $C_{20}H_{20}O_6$. The UV spectrum of **4** [λ_{max} nm (log ε): 212 (4.69), 232 (sh, 4.32), 272 (4.12), 317 (3.92) and 331 (sh, 3.84)] resembles those of flavanones such as liquiritigenin (**11**).[17] The ^1H-NMR spectrum of **4** also showed the presence of two aromatic rings [δ 6.47 (1H, d, J=2 Hz), 6.60 (1H, dd, J=2, 8.5 Hz), 7.45 (1H, d, J=8.5 Hz) (tri-substituted benzene ring), and 6.28 (1H, s; penta-substituted benzene ring)] and an aliphatic methine [δ 5.77 (1H, br s; H-2)], and also a γ,γ-dimethylallyl group [δ 1.63, 1.71 (3H each, br s; *gem*-dimethyl at C-3″), 3.23 (2H, m, H-1″), 5.21 (1H, br t, J=7 Hz; H-2″)] and a methoxyl group [δ 3.58 (3H, br s)]. However, this spectrum lacks the signals of the methylene protons at C-3 of flavanones.

The ^{13}C-NMR spectrum of **4** showed a ketone carbon signal at δ 198.8, and also the signals of a methine carbon [δ 81.4 (C-2)], a γ,γ-dimethylallyl group [δ 17.9, 25.8 (*gem*-dimethyl at C-3″), 23.3 (C-1″), 124.9 (C-2″) and 130.7 (C-3″)], a methoxyl group (δ 62.9) and twelve carbons of the two aromatic rings.

Acetylation of **4** afforded a tetra-acetate **12**, as indicated by its molecular-ion peak (*m/z* 524) in the EI-MS spectrum. The ^1H-NMR spectrum of **12** showed the signals of four acetyl groups [δ 2.12, 2.28, 2.29 and 2.31 (3H each, s)], along with those of two aromatic rings [δ 7.09 (1H, dd, J=2, 8.5 Hz), 7.41 (1H, d, J=2 Hz), 7.53 (1H, d, J=8.5 Hz); δ 6.89 (1H, s)], a methoxyl group [δ 3.50 (3H, s)] and a γ,γ-dimethylallyl group [δ 1.67, 1.77 (3H each, s; *gem*-dimethyl at C-3″), 3.33 (2H, d, J=6.5 Hz; H-1″), 5.12 (1H, br t, J=6.5 Hz; H-2″)]. The signal of the methine proton at C-2 was absent in this spectrum. Therefore, it is considered that the number of hydroxyl groups in **4** is three, and one of the four acetyl groups in **12** was introduced after tautomerization of the keto-form of **4** to the enol-form, as occurs in the acetylation of a 2-arylbenzofuranone.[18]

These findings indicate that **4** has a 2-arylbenzofuranone structure with three hydroxyl groups, a methoxyl group and a γ,γ-dimethylallyl group.

The treatment of **4** with HCl afforded **13**. The formation of a new pyrane ring in **13** indicates that the location of the γ,γ-dimethylallyl group in **4** is *ortho* to one of the hydroxyl groups on the same aromatic ring.

The NOE difference spectrum of **4** (Fig. 2) showed a distinctive NOE of H-2 upon the irradiation of the methoxyl

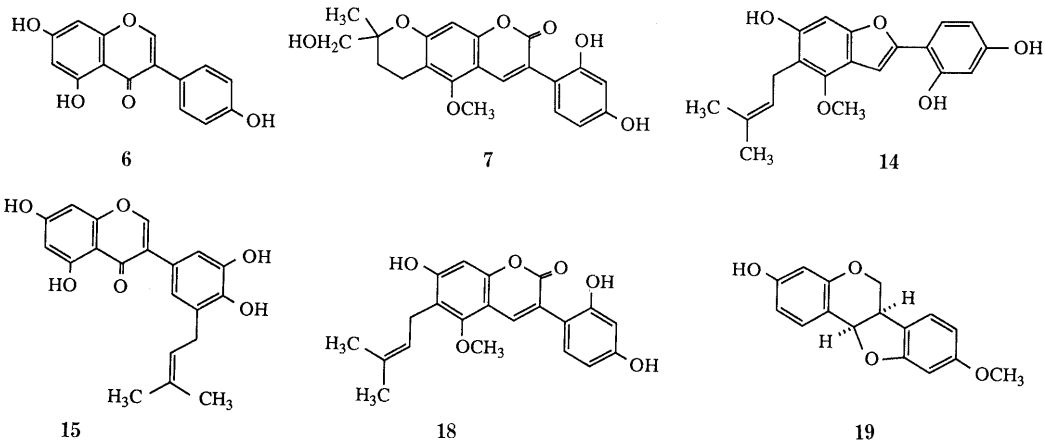


Chart 4

signal, indicating that the methoxyl group is *ortho* to C-1′ on the B-ring. This irradiation also enhanced NOEs of H-1″, H-2″ and a methyl signal of the γ,γ-dimethylallyl group, to indicate that the γ,γ-dimethylallyl group is *ortho* to the methoxyl group.

The ^{13}C chemical shifts of the B-ring carbons of **4** [δ 108.3 (C-1′), 156.7 (C-2′), 99.6 (C-3′), 158.6 (C-4′), 113.8 (C-5′), 161.0 (C-6′) (B-ring)] are closely similar to those of the B-ring carbons of **3** [δ 106.2 (C-1′), 156.6 (C-2′), 100.9 (C-3′), 157.7 (C-4′), 114.2 (C-5′), 159.1 (C-6′)].

These data indicate the identity of the substitution pattern on the B-ring in **4** with that in **3**.

The coupling pattern of the ABX protons (thus assigned to be the A-ring protons) in the ^1H-NMR spectrum of **4** indicates that the one remaining hydroxyl group on the A-ring is at C-6 or at C-5 of the benzofuranone structure. Considering that an *O*-functional group on the A-ring in the reported licorice flavonoids is, without exception, at C-7 or the corresponding position, we assigned the hydroxyl group at C-6.

Structure **4** thus proposed for licofuranone was substantiated by the ^1H–^{13}C correlation spectral data shown in Table II. Each chemical shift of the C-ring carbons (C-2, C-3, C-3a, C-7a) in the ^{13}C-NMR spectrum of **4**, especially the noticeable downfield shift of C-7a (δ 174.9), from the corresponding carbons of flavanones[19,20] or isoflavanones,[3d,21] is consistent with the constrained furanone structure.

In spite of the presence of an asymmetric center in the molecule, isolated **4** was optically inactive. This is attributable to the racemization caused by the tautomerization between the keto-form and the enol-form described above, although the ^1H- and ^{13}C-NMR spectra of **4** did not show duplication of the signals due to this tautomerization.

Inhibitory Effects of Licorice Constituents on MAO The inhibitory effects of twelve licorice constituents, **1**—**4**, **6**, **7**, licocoumarone (**14**),[22] glycyrrhisoflavone (**15**),[4b] licochalcones A (**16**)[23] and B (**17**),[23] glycycoumarin (**18**)[24] and (−)-medicarpin (**19**),[3e] on MAO were examined, and the results are summarized in Table III.

Among the tested compounds, six, **3**, **4**, **6**, **7**, **14** and **15** showed inhibition with the IC_{50} values of 6.0×10^{-5}—1.4×10^{-4} M. Although the inhibitory activity of **1** against MAO was not strong (IC_{50}: 1.6×10^{-4} M), it cannot be ignored because of its abundant presence in most species of licorice.[25]

It is noticeable that **14**, which was the strongest inhibitor of XOD among the tested compounds,[1] is again the most

Table III. Inhibitory Effects of Licorice Constituents on MAO

Compound	IC_{50}[a] (M)
Isoflavone	
Glycyrrhisoflavone (**15**)	9.5×10^{-5}
Glicoricone (**3**)	1.4×10^{-4}
Genistein (**6**)	9.5×10^{-5}
Chalcone	
Licochalcone A (**16**)	$>2.0\times10^{-4}$
Licochalcone B (**17**)	$>2.0\times10^{-4}$
Isoliquiritigenin (**2**)	$>2.0\times10^{-4}$
3-Arylcoumarin	
Glycycoumarin (**18**)	$>2.0\times10^{-4}$
Licopyranocoumarin (**7**)	1.4×10^{-4}
2-Arylbenzofuran	
Licocoumarone (**14**)	6.0×10^{-5}
Licofuranone (**4**)	8.7×10^{-5}
Pterocarpan	
(−)-Medicarpin (**19**)	$>2.0\times10^{-4}$
Saponin	
Glycyrrhizin (**1**)	1.6×10^{-4}
Others	
Harmane hydrochloride	5.7×10^{-5}
Quinine sulfate	6.4×10^{-5}

a) Concentration required for 50% inhibition.

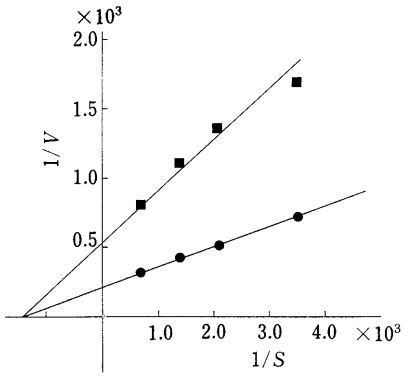


Fig. 3. Inhibitory Effects of **14** on MAO

Lineweaver–Burk plots in the absence (●—●) and in the presence (■—■, 6.0×10^{-5} M) of **14**. *V*, μmol substrate metabolized/mg enzyme/min; *S*, molar concentration of substrate.

potent inhibitor of MAO among the compounds tested in the present study. The kinetic analysis using Lineweaver–Burk plots (Fig. 3) indicated that **14** inhibited MAO non-competitively at the concentration of 6.0×10^{-5} M.

Chalcones **16** and **17**, which remarkably inhibit XOD,[1] showed only weak or practically no inhibition against MAO. Another chalcone, **2**, which was reported to be a stronger inhibitor of MAO than harmane hydrochloride,[8] showed only very weak inhibition (IC_{50}: 9.0×10^{-4} M) in our experiment. This difference in the inhibitory activity of **2** may be due to the difference of the origin of the enzyme. The poor inhibitory activity of the chalcones suggests strongly that the mechanism of the inhibition by the phenolic constituents against MAO is different from the mechanism proposed for the inhibition of phenylalanine ammonia-lyase[26] by chalcones.

Experimental

UV spectra were recorded on a Hitachi 200-10 spectrophotometer. EI-MS and FAB-MS spectra were recorded on a VG 70-SE instrument. ^{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); chemical shifts were based on those of the solvent signals (acetone-d_6: δ_H 2.04 and δ_C 29.8; chloroform-d: δ_H 7.24), and given in δ values (ppm) from tetramethylsilane. A JEOL GSX-500 spectrometer was also used for recording the ^{1}H-NMR spectra. CPC was performed on a Sanki L-90 machine (Nagaoka-kyo, Kyoto) equipped with twelve 1000E cartridges. Analytical thin layer chromatography (TLC) was conducted on Kieselgel 60 F254 plates (0.2 mm thick) and preparative TLC (PTLC) on Kieselgel 60 PF254 plates (0.5 mm thick). Solvents for TLC were A ($CHCl_3$–MeOH, 10:1), B (toluene–acetone–HCOOH, 36:6:1), C (*n*-hexane–EtOAc, 2:1), D ($CHCl_3$–acetone–HCOOH, 16:2:1) and E ($CHCl_3$). High-performance liquid chromatography (HPLC) was performed on a Merck LiChrospher cartridge column with CH_3CN–H_2O–AcOH (70:25:5) at 40 °C, and the flow rate was set at 1.2 ml/min. An MCPD-350 instrument (Otsuka Electronics) was used for the HPLC detection.

Isolation of Phenolic Constituents from Licorice Licorice (2 kg) (Seihoku-kanzo; Tochimoto-tenkai-do, Osaka) was pulverized and then successively extracted with *n*-hexane (6 l × 3) and EtOAc (6 l × 3), and solvents were evaporated off. A portion (20 g) of the EtOAc extract (87 g) was subjected to CPC [$CHCl_3$–MeOH–H_2O (7:13:8); reversed-phase development; flow rate, 3 ml/min], to separate fractions I—V. Fraction II (473 mg) was further separated by column chromatography on MCI gel CHP-20P (1.1 × 38 cm) with 60% MeOH and then by PTLC with solvent A, to give licopyranocoumarin (**7**) (19 mg) and licofuranone (**4**) (9 mg), and also two crude compounds. The latter two were respectively purified by PTLC with solvent B, and gave echinatin (**5**) (2 mg) and genistein (**6**) (2 mg). Fraction IV (717 mg) of CPC was purified by column chromatography on MCI gel CHP-20P (with 60% MeOH), and by PTLC with solvent B, to give glicoricone (**3**) (20 mg).

Glicoricone (3) Colorless needles, mp 192 °C. *Anal.* Calcd for $C_{21}H_{20}O_6 \cdot 1/2H_2O$: C, 66.83; H, 5.60. Found: C, 66.16; H, 5.35. EI-MS: *m/z* 368 {$[M]^+$, 100% (relative intensity)}, 353 ($[M-CH_3]^+$, 37%), 337 ($[M-OCH_3]^+$, 40%), 313 ($[M-(CH_3)_2C=CH]^+$, 63%). High-resolution EI-MS: *m/z* 368.1257 ($[M]^+$; Calcd for $C_{21}H_{20}O_6$, 368.1260). UV λ_{max}^{MeOH} nm (log ε): 209 (4.55), 240 (sh, 4.27), 247 (4.24), 285 (4.02) and 305 (sh, 3.94). ^{1}H-NMR (acetone-d_6) δ: 1.64, 1.74 (3H each, br s; *gem*-dimethyl at C-3″), 3.29 (2H, br d, J=7 Hz; H-1″), 3.41 (3H, s, OCH_3), 5.25 (1H, br t, J=7 Hz; H-2″), 6.32 (1H, s; H-3′), 6.95 (1H, d, J=2 Hz; H-8), 7.03 (1H, dd, J=2, 9 Hz; H-6), 8.07 (1H, d, J=9 Hz, H-5), 8.10 (1H, s; H-2). ^{13}C-NMR: See, Table I.

Methylation of 3 Ethereal diazomethane (0.4 ml) was added to an EtOH solution (0.4 ml) of **3** (2.2 mg), and the mixture was left to stand for 12 h. The solvent was distilled off and the residue was purified by PTLC with solvent C, to give **9** (1.1 mg). EI-MS: *m/z* 410 ($[M]^+$). ^{1}H-NMR (30 °C, acetone-d_6) δ: 1.64, 1.73 (3H each, br s, *gem*-dimethyl at C-3″), 3.26, 3.29 (1H each, dd, J=7, 14 Hz), 3.46, 3.71, 3.89, 3.97 (3H each, s; 4 × OCH_3), 5.19 (1H, br t, J=7 Hz, H-2″), 6.54 (1H, s; H-3′), 7.05 (2H, m; H-6 and H-8), 7.88 (1H, s; H-2), 8.05 (1H, d, J=9 Hz; H-5). ^{1}H-NMR (chloroform-d)[15] δ: 1.65, 1.73 (3H each, br s; *gem*-dimethyl at C-3″), 3.25, 3.33 (1H each, dd, J=7, 14 Hz), 3.45, 3.74, 3.85, 3.90 (3H each, s; 4 × OCH_3), 5.18 (1H, br t, J=7 Hz; H-2″), 6.34 (1H, s; H-3′), 6.86 (1H, d, J=2.5 Hz; H-8), 6.96 (1H, dd, J=2.5, 8.5 Hz; H-6), 7.78 (1H, s, H-2), 8.19 (1H, d, J=8.5 Hz; H-5). This compound was identical with licoricone dimethyl ether (**9**)[15] prepared from licoricone (**8**)[15] [mixed mp, EI-MS, ^{1}H-NMR, TLC (solvent C, *Rf* 0.20) and HPLC (retention time, 5.8 min). The UV spectra recorded using the MCPD detector were also identical].

Treatment of 3 with HCl Conc. HCl (0.1 ml) was added to an MeOH solution (0.5 ml) of **3** (0.7 mg), and the mixture was left to stand for 36 h. The solvent was evaporated, and the residue was purified by PTLC using solvent D, to give **10** (0.7 mg), mp 138 °C. EI-MS: *m/z*: 368 ($[M]^+$). ^{1}H-NMR (acetone-d_6) δ: 1.30 (6H, s; *gem*-dimethyl at C-3″), 1.77 (2H, t, J=7 Hz; H-2″ × 2), 2.68 (2H, t, J=7 Hz; H-1″ × 2), 3.48 (3H, s; OCH_3 at C-6′), 6.14 (1H, s; H-3′), 6.94 (1H, d, J=2 Hz; H-8), 7.02 (1H, dd, J=2, 9 Hz; H-6), 8.06 (1H, d, J=9 Hz; H-5).

Licofuranone (4) Colorless needles, mp 162 °C, $[\alpha]_D$ 0° (c=3, acetone). *Anal.* Calcd for $C_{20}H_{20}O_6 \cdot 1/2H_2O$: C, 65.74; H, 5.79. Found: C, 65.22; H, 5.62. High-resolution FAB-MS: *m/z* 357.1385 ($[M+H]^+$; Calcd for $C_{20}H_{20}O_6+H$, 357.1338). UV λ_{max}^{MeOH} nm (log ε): 212 (4.69), 232 (sh, 4.32), 272 (4.12), 317 (3.92), 331 (sh, 3.84). ^{1}H-NMR (acetone-d_6) δ: 1.63, 1.71 (3H each, br s; *gem*-dimethyl at C-3″), 3.23 (2H, m, H-1″), 3.58 (3H, br s; OCH_3 at C-6′), 5.21 (1H, br t, J=7 Hz; H-2″), 5.77 (1H, br s; H-2), 6.28 (1H, s; H-3′), 6.47 (1H, d, J=2 Hz; H-7), 6.60 (1H, dd, J=2, 8.5 Hz; H-5), 7.45 (1H, d, J=8.5 Hz; H-4). ^{13}C-NMR: See Table II.

Acetylation of 4 A mixture of **4** (1 mg), acetic anhydride (0.2 ml) and pyridine (0.2 ml) was left to stand overnight at room temperature, and then the solvent was removed by evaporation. The residue was purified by PTLC with solvent E, to give a tetra-acetate, **12** (0.7 mg), mp 108 °C. EI-MS: *m/z* 524 ($[M]^+$). ^{1}H-NMR (acetone-d_6) δ: 1.67, 1.77 (3H each, s; *gem*-dimethyl at C-3″), 2.12, 2.28, 2.29, 2.31 (3H each, s; $OCOCH_3$ × 4), 3.33 (2H, d, J=6.5 Hz; H-1″), 3.50 (3H, s; OCH_3 at C-6′), 5.12 (1H, br t, J=6.5 Hz; H-2″), 6.89 (1H, s; H-3′), 7.09 (1H, dd, J=2, 8.5 Hz; H-5), 7.41 (1H, d, J=2 Hz; H-7), 7.53 (1H, d, J=8.5 Hz; H-4).

Treatment of 4 with HCl A mixture of **4** (0.7 mg) in MeOH (0.5 ml) and conc. HCl (0.1 ml) was left to stand for 36 h. The solvent was then evaporated, and the residue was purified by PTLC using solvent D, to give **13** (0.7 mg), mp 148 °C. High-resolution EI-MS: *m/z* 356.1282 ($[M]^+$; Calcd for $C_{20}H_{20}O_6$, 356.1260). ^{1}H-NMR (acetone-d_6) δ: 1.28, 1.29 (6H in total; *gem*-dimethyl at C-3″), 1.74 (2H, t, J=7 Hz; H-2″ × 2), 2.63 (2H, t, J=7 Hz; H-1″ × 2), 3.55 (3H, br s; OCH_3 at C-6′), 5.79 (1H, s; H-2), 6.10 (1H, s; H-3′), 6.47 (1H, d, J=2 Hz, H-7), 6.61 (1H, dd, J=2, 8.5 Hz; H-5), 7.47 (1H, d, J=8.5 Hz; H-4).

Estimation of Inhibitory Effects of Licorice Phenolics on MAO The reagents other than licorice constituents were purchased from the following companies: MAO (from bovine plasma), Sigma; benzylamine hydrochloride, Tokyo Kasei; harmane hydrochloride and quinine sulfate, Nacalai Tesque. The inhibitory activity of each compound was determined by the reported method[10] with a slight modification. A mixture consisting of an MAO solution [0.012 U/ml in 0.1 M phosphate buffer (pH 7.4); 25 μl], a tested compound solution [175 μl; in water–dimethylsulfoxide (8:2, by volume)], 0.1 M phosphate buffer (pH 7.4) (350 μl) and water (300 μl), was preincubated for 15 min at 37 °C. An aqueous solution (0.06 M; 25 μl) of benzylamine hydrochloride was then 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 (25 μl), and the absorbance at 250 nm of the reaction mixture was measured. The blank was prepared in an analogous way, except that the MAO solution was added after the addition of HCl. Inhibitory activity (%) was calculated using the following equation, and was given as the mean value of triplicate experiments.

$$\text{inhibitory activity } (\%) = (1 - B/A) \times 100$$

where A is the enzyme activity in the absence of the tested compound, and B is that in the presence of the compound.

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