

Phenolic Constituents of Licorice. VIII.¹⁾ Structures of Glicophenone and Glicoisoflavanone, and Effects of Licorice Phenolics on Methicillin-Resistant *Staphylococcus aureus*

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Two new phenolic compounds, glicophenone (**1**) and glicoisoflavanone (**2**), were isolated from commercial licorice, and their structures were elucidated on the basis of spectroscopic data. Antibacterial assays of licorice phenolics for *Staphylococcus aureus*, including four strains of methicillin-resistant *S. aureus* (MRSA), and also for *Escherichia coli* K12 and *Pseudomonas aeruginosa* PAO1, were then examined. Two compounds among them, 8-(γ,γ -dimethylallyl)-wighteone (**21**) and 3'-(γ,γ -dimethylallyl)-kievitone (**28**), showed remarkable antibacterial effects [minimum inhibitory concentrations (MICs), 8 $\mu\text{g}/\text{ml}$] on the MRSA strains and methicillin-sensitive *S. aureus*. Licochalcone A (**14**), gancaonin G (**20**), isoangustone A (**24**), glyasperins C (**30**) and D (**31**), glabridin, (**32**), licoricidin (**33**), glycoumarin (**34**) and licoumarone (**40**) showed antibacterial effects on the MRSA strains with MIC values of 16 $\mu\text{g}/\text{ml}$. Effects on the β -lactam resistance of the MRSA strains were also examined, and licoricidin (**33**) noticeably decreased the resistance of the MRSA strains against oxacillin, as shown by the reduction in the MICs of oxacillin (lower than 1/128—1/1000 in the presence of 8 $\mu\text{g}/\text{ml}$ of **33**, and 1/8—1/32 in the presence of 4 $\mu\text{g}/\text{ml}$ of **33**). Mechanistic study suggested that **33** does not inhibit the formation of penicillin-binding protein 2' (PBP2'), but affects the enzymatic function of PBP2'.

Key words licorice; glicophenone; glicoisoflavanone; licoricidin; methicillin-resistant *Staphylococcus aureus*; oxacillin

Much of the recent research on licorice constituents has indicated the pharmacological importance of phenolic compounds, together with saponins, in the medicinal use of licorice.²⁻⁶⁾ We also reported inhibitory effects on oxidative enzymes, radical-scavenging effects and the antiviral effect of licorice phenolics.⁷⁾

Since the antibiotic-resistance of bacteria is one of the most serious problems in clinical medicine today, development of new drugs against the drug-resistant bacteria or suppression of the drug-resistance in bacteria is desired. Previously we reported that some low-molecular-weight phenolics (rhubarb anthraquinones and aglycones of naphthalene glycosides of cassia seeds) showed antibacterial effects on methicillin-resistant *Staphylococcus aureus* (MRSA).⁸⁾ On the other hand, the effects of licorice phenolics on various microbes have been reported.⁹⁻¹³⁾

We therefore examined the effects of licorice phenolics on MRSA. In the course of this study, we isolated two new compounds named glicophenone (**1**) and glicoisoflavanone (**2**), along with known compounds, and found that various licorice phenolics have potent antibacterial effects on MRSA and methicillin-sensitive *S. aureus* (MSSA). Among the compounds which showed potent antibacterial effects on the MRSA strains, an isoflavan noticeably reduced the resistance of MRSA against a β -lactam antibiotic oxacillin. This paper deals with the structural elucidation of the new compounds, and the antibacterial effects of licorice phenolics, especially those on MRSA.

Results and Discussion

Structures of Glicophenone and Glicoisoflavanone

The licorice used in this study is a commercial variety, and its source plant was tentatively assigned to be *Glycyrrhiza uralensis* on the basis of similarity of the high-performance

liquid chromatography (HPLC) profile to that reported for *G. uralensis*.¹⁴⁾ An ethyl acetate extract from the licorice was subjected to centrifugal partition chromatography (CPC)¹⁵⁾ and/or column chromatography, and the fractions were further purified by preparative TLC or preparative HPLC to give glicophenone (**1**), glicoisoflavanone (**2**), and 32 known compounds.

Glicophenone (**1**) was obtained as colorless needles. High-resolution electron-impact (EI) MS indicated its molecular formula, C₂₀H₂₂O₆. The UV spectrum is similar to that of licoriphenone (**3**).¹²⁾ The ¹H-NMR spectrum indicated the presence of tri-substituted [δ : 6.31 (1H, d, $J=2.5$ Hz; H-3), 6.43 (1H, dd, $J=2.5, 8.5$ Hz; H-5), 8.01 (1H, d, $J=8.5$ Hz; H-6)] and penta-substituted [δ : 6.29 (1H, s; H-5')] benzene rings. The spectrum also showed signals due to methylene [δ : 4.21 (2H, s; H-8)] and methoxyl protons [δ : 3.62 (3H, s)], along with those attributable to a γ,γ -dimethylallyl group [δ : 3.25 (2H, br d, $J=7$ Hz; H-1''), 5.18 (1H, br t, $J=7$ Hz; H-2''), 1.63, 1.73 (3H each, br s; *gem*-dimethyl at C-3'')]. The pattern of these signals is very similar to that for licoriphenone (**3**), except for the number of methoxyl signals. The EI-MS showed fragment ion peaks at m/z 137 and 221, indicating the presence of a methoxyl group on the B-ring (Fig. 1). The ¹³C chemical shifts of B-ring carbons [δ : 107.9 (C-1'), 159.5 (C-2'), 114.0 (C-3'), 156.2 (C-4'), 99.7 (C-5'), 155.3 (C-6')] of **1** were similar to those of the corresponding carbons of glicoricone (**4**)¹⁶⁾ [δ : 106.2 (C-1'), 159.1 (C-2'), 114.2 (C-3'), 157.7 (C-4'), 100.9 (C-5'), 156.6 (C-6')], suggesting the same substitution pattern of this benzene ring as that of **4**. The nuclear Overhauser effect spectroscopy (NOESY) measurement of **1** showed cross peaks due to the nuclear Overhauser effects (NOEs) of the methoxyl group with H-8 (methylene), H-1'' and H-2'' (γ,γ -dimethylallyl group), as indicated by the arrows in the formula. The loca-

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tion of the methoxyl group in **1** was thus determined to be at C-2'.

Glicoisoflavanone (**2**) was obtained as colorless needles. Its molecular formula $C_{22}H_{24}O_6$ was indicated by its high-resolution EI-MS. The UV spectrum was characteristic of isoflavanone. The 1H -NMR spectrum also showed signals of the isoflavanone skeleton as follows: δ : 4.38 (1H, dd, $J=6$, 13 Hz; H-2), 4.90 (1H, dd, $J=10.5$, 13 Hz; H-2), 4.33 (1H, dd, $J=6$, 10.5 Hz; H-3) (C-ring); 7.76 (1H, d, $J=2.5$ Hz; H-5), 6.56 (1H, dd, $J=2.5$, 8.5 Hz; H-6), 6.40 (1H, d, $J=8.5$ Hz; H-8) (tri-substituted benzene ring); 6.33 (1H, s; H-5') (penta-substituted benzene-ring). Signals due to a γ,γ -dimethylallyl group [δ : 3.25 (2H, m; H-1''), 5.18 (1H, br t, $J=5$ Hz; H-2''), 1.64, 1.73 (3H each, br s; *gem*-dimethyl at C-3'')] and two methoxyl groups [δ : 3.66, 3.74 (3H each, s)] were also shown. The fragment ions m/z 136 and 247 shown in the EI-MS suggested that the tri-substituted and penta-substituted benzene rings are respectively attributed to the A and B rings of the isoflavanone structure (Fig. 1), and the two methoxyl groups are on the B-ring. The ^{13}C chemical shifts of the A-ring [δ : 115.9 (C-4a), 130.0 (C-5), 110.9 (C-6), 164.7 (C-7), 103.5 (C-8), 164.5 (C-8a)] and B-ring [δ : 108.9 (C-1'), 159.7 (C-2'), 114.9 (C-3'), 159.2 (C-4'), 96.5 (C-5'), 155.7 (C-6')] carbons of **2** are closely similar to the corresponding carbons of the A-ring of 4'-methoxy-7-hydroxyisoflavanone (**5**)¹⁷ [δ : 115.3 (C-4a), 130.2 (C-5), 111.4 (C-6), 165.1 (C-7), 103.4 (C-8), 164.4 (C-8a)] and the B-ring of licoriphenone (**3**) [δ : 108.6 (C-1'), 159.2 (C-2'), 114.9 (C-3'), 158.7 (C-4'), 96.1 (C-5'), 155.7 (C-6'), in acetone- d_6], respectively. The NOESY spectrum of **2** showed cross peaks of the methoxyl signal at δ 3.66 with H-2 (C-ring), H-1'' and H-2'' (γ,γ -dimethylallyl group) (indicated by the arrows in the formula). On the other hand, the methoxyl signal at δ 3.74 showed cross peaks with H-5' and protons of the γ,γ -dimethylallyl group in the NOESY spectrum. These NOEs substantiated the substitution pattern of the B-ring. The circular dichroism (CD) spectrum of **2** showed a positive Cotton effect at 328 nm, indicating¹⁸ the *R*-configuration at C-3. Structure **2** was thus assigned to glicoisoflavanone.

Antibacterial Effects of Licorice Phenolics on MRSA and MSSA Antibacterial effects on four strains of MRSA and a strain of MSSA, and also on *Escherichia coli* K12 and *Pseudomonas aeruginosa* PAO1, were evaluated for licorice phenolics of various types using the liquid dilution method.⁸ Minimum inhibitory concentrations (MICs) of the tested compounds are shown in Table 1.

Flavanones and Chalcones Among the tested compounds, flavanones **6**–**9** did not show antibacterial effects on MRSA and MSSA (MIC >128 μ g/ml). Most of the chalcones such as licochalcone B (**15**) and tetrahydroxymethoxychalcone (**12**) showed weak or negligible effects (MIC 64, 128 or >128 μ g/ml). However, licochalcone A (**14**) showed antibacterial effects on both MRSA and MSSA with MICs of 16 μ g/ml.

Isoflavones, Isoflavanones and Isoflavans An isoflavone, 8-(γ,γ -dimethylallyl)-wighteone (**21**), and an isoflavanone, 3'-(γ,γ -dimethylallyl)-kievitone (**28**), showed potent antibacterial effects on MRSA and MSSA (MIC 8 μ g/ml). Isoflavones, gancaonin G (**20**) and isoangustone A (**24**), and isoflavans, glyasperins C (**30**) and D (**31**), glabridin (**32**) and licoricidin (**33**), showed antibacterial effects on MRSA and

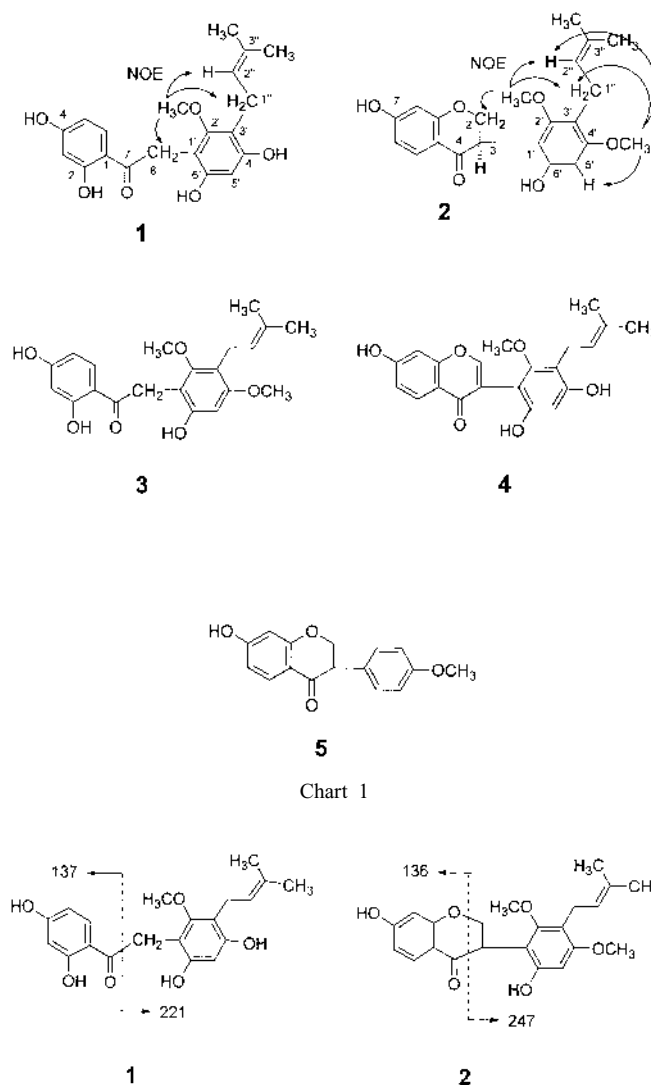


Fig. 1. Mass Fragmentation of **1** and **2** in Their EI-MS

MSSA with MICs of 16 μ g/ml, while isowighteone (**22**) (isoflavone) had MICs of 16 and 32 μ g/ml for MSSA and MRSA, respectively. The MICs of licoisoflavanone (**27**) (isoflavanone) for MRSA and MSSA were 32 μ g/ml. The other isoflavones, isoflavanones and isoflavans, including glicoisoflavanone (**2**), showed MICs of 32–128 μ g/ml for MRSA and MSSA, or negligible effects (MIC >128 μ g/ml).

3-Arylcoumarins and Others Among the 3-arylcoumarins and other phenolic compounds, glycycomarin (**34**), licocoumarone (**40**) (MIC 16 μ g/ml), licoarylcoumarin (**36**), licoriphenone (**3**) (MIC 16–32 μ g/ml), and glicophenone (**1**) (MIC 32 μ g/ml) showed antibacterial effects on MSSA and MRSA.

Structure-Activity Relationships Antibacterial effects of flavanones isolated from leguminous plants on MRSA have been reported,¹⁹ and potent anti-MRSA activity was correlated with the presence of an aliphatic or lavandulyl group, in addition to the substitution pattern of the phenolic hydroxyl groups. Compounds **21** and **28**, which showed MIC values of 8 μ g/ml for MSSA and MRSA, have two γ,γ -dimethylallyl groups, and all of the compounds with the MICs of 16 μ g/ml have at least one γ,γ -dimethylallyl or equivalent (α,α -dimethylallyl or dimethylpyrane) group. On the other

Table 1. MICs of Licorice Phenolics for MRSA, MSSA, *Escherichia coli* and *Pseudomonas aeruginosa* ($\mu\text{g/ml}$)

Compounds	MRSA OM481	MRSA OM505	MRSA OM584	MRSA OM623	MSSA 209P	<i>E. coli</i> K12	<i>P. aeruginosa</i> PAO1
Flavanones							
Liquiritigenin (6)	>128	>128	>128	>128	>128	>128	>128
Liquiritin (7)	>128	>128	>128	>128	>128	>128	>128
6''-O-Acetyllicuritin (8)	>128	>128	>128	>128	>128	>128	>128
Naringenin (9)	>128	>128	>128	>128	>128	>128	>128
Chalcones							
Isoliquiritin apioside (10)	>128	>128	>128	>128	>128	>128	>128
Isoliquiritin (11)	>128	>128	>128	>128	>128	>128	>128
Tetrahydroxymethoxychalcone (12)	>128	>128	>128	>128	>128	>128	>128
Echinatin (13)	128	64	64	64	64	>128	>128
Licochalcone A (14)	16	16	16	16	16	>128	>128
Licochalcone B (15)	128	128	128	128	128	>128	>128
Isoliquiritigenin (16)	128	128	128	128	128	>128	>128
Isoflavones							
Glycyrrhisoflavone (17)	64	64	32	32	32	>128	>128
Semilicoisoflavone B (18)	64	64	64	32	32	>128	>128
Genistein (19)	>128	>128	>128	>128	>128	>128	>128
Glicoricone (4)	64	64	64	64	64	>128	>128
Gancaonin G (20)	16	16	16	16	16	>128	>128
8-(γ,γ -Dimethylallyl)-wighteone (21)	8	8	8	8	8	>128	>128
Isowighteone (22)	32	32	32	32	16	>128	>128
Glisoflavone (23)	64	64	64	64	64	>128	>128
Isoangustone A (24)	16	16	16	16	16	>128	>128
Isoflavanones							
Glycyrrhisoflavanone (25)	64	64	32	32	32	>128	>128
Glyasperin F (26)	64	64	64	64	32	>128	>128
Licoisoflavanone (27)	32	32	32	32	32	>128	>128
Glicoisoflavanone (2)	64	64	32	32	32	>128	>128
3'-(γ,γ -Dimethylallyl)-kievitone (28)	8	8	8	8	8	>128	>128
Isoflavans							
(3 <i>R</i>)-Vestitol (29)	128	128	128	128	128	>128	>128
Glyasperin C (30)	16	16	16	16	16	>128	>128
Glyasperin D (31)	16	16	16	16	16	>128	>128
Glabridin (32)	16	16	16	16	16	>128	>128
Licoricidin (33)	16	16	16	16	16	>128	>128
3-Arylcoumarins							
Glycoumarin (34)	16	16	16	16	16	>128	>128
Licopyranocoumarin (35)	>128	>128	128	128	>128	>128	>128
Licoarylcoumarin (36)	32	32	32	32	16	>128	>128
Glycyrin (37)	128	128	128	128	128	>128	>128
Isolicopyranocoumarin (38)	>128	>128	>128	>128	>128	>128	>128
Glycyrin permethyl ether (39)	>128	>128	>128	>128	>128	>128	>128
Others							
Licocoumarone (40)	16	16	16	16	16	>128	>128
Glicophenone (1)	32	32	32	32	32	>128	>128
Licoriphenone (3)	32	32	32	16	16	>128	>128
Glycyrol (41)	>128	>128	>128	>128	>128	>128	>128
Isoglycyrol (42)	>128	>128	>128	>128	>128	>128	>128

hand, the glycosides tested exhibited negligible effects on MRSA and MSSA. These results implied participation of their lipophilicity in the antibacterial effects of the phenolic compounds on *S. aureus* strains.

The order of strength of the antibacterial activity, glycoumarin (34) > glycyrin (37) > glycyrin permethyl ether (39), suggested requirements of phenolic hydroxyl groups in the molecule for the antibacterial effects. A difference in the antibacterial activity between glycoumarin (34) (MIC 16 $\mu\text{g/ml}$) and glycyrol (41) (MIC >128 $\mu\text{g/ml}$) is also attributable to the difference in the number of phenolic hydroxyl groups. However, in this case, rigidity in the bond between the coumarin structure and B-ring of glycyrol may be related to the decrease of the activity.

Antibacterial properties of various types of licorice pheno-

lics described above may suggest some usefulness of licorice in the treatment of diseases responsible for *S. aureus*, at least in the intestines.

Effects of Licorice Phenolics on *E. coli* and *P. aeruginosa* None of the tested compounds showed antibacterial effects on *E. coli* K12 and *P. aeruginosa* PAO1 (MIC >128 $\mu\text{g/ml}$).

Effects of Licorice Phenolics on the Resistance of MRSA against Oxacillin Recently, the suppression of bacterial resistance against β -lactam antibiotics by several phenolic compounds,^{20–22} including tea catechins and hydrolyzable tannins, have been shown. We therefore examined the effects of licorice phenolics on the MICs of oxacillin for MRSA.

Oxacillin in the absence of the phenolic compounds

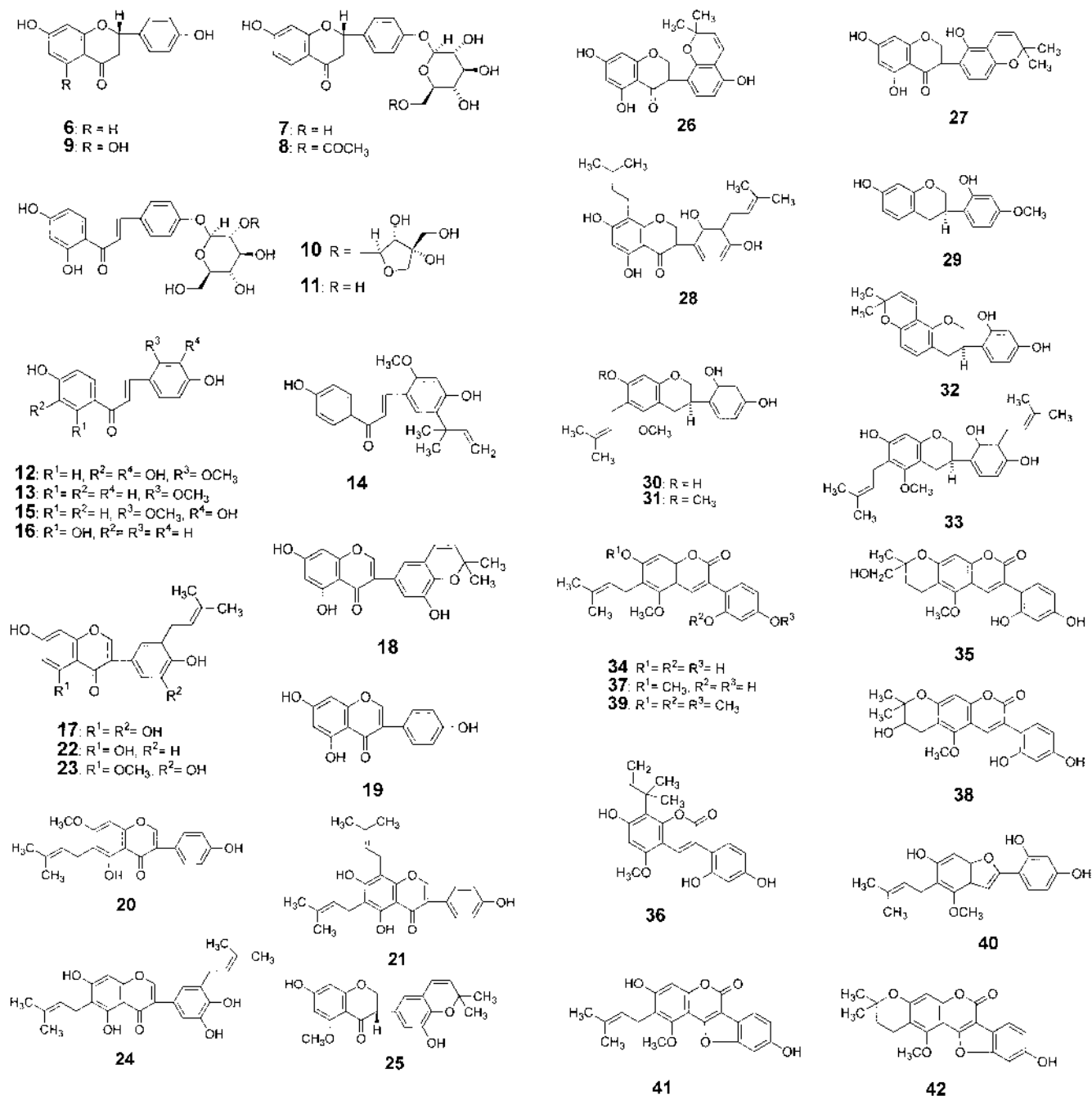


Chart 2

showed MICs of 64–512 $\mu\text{g/ml}$ for the four MRSA strains, while the MIC for MSSA 209P was $<0.5 \mu\text{g/ml}$. However, in the presence of 16 $\mu\text{g/ml}$ of glycofenone (**1**), one of the newly isolated compounds, the MICs of oxacillin for the MRSA strains decreased to 1/2–1/8 of the values in the absence of glycofenone (**1**). Isowightone (**22**) (16 $\mu\text{g/ml}$) reduced the MICs of oxacillin to 1/4–1/8, and isoangustone A (**24**) reduced them to 1/2–1/4. Other tested licorice phenolics, except for glycoumarin (**34**), had an analogous effect on at least two MRSA strains (Table 2).

However, the effects of licoricidin (**33**) were much stronger. In the presence of 8 $\mu\text{g/ml}$ of licoricidin, the MICs of oxacillin decreased to lower than 1/128–1/1000 of the values in the absence of the compound. Even the presence of

4 $\mu\text{g/ml}$ of licoricidin decreased the MICs of oxacillin to 8–16 $\mu\text{g/ml}$.

The effect of licoricidin (**33**) on the growth curve of one of the MRSA strains, OM481, was then examined. As shown in Fig. 2, the amount of the bacterium after 24 h incubation in the presence of both of oxacillin (10 $\mu\text{g/ml}$) and licoricidin (8 $\mu\text{g/ml}$) was about 1/100 of that in the absence of them (control), while oxacillin alone (10 $\mu\text{g/ml}$) or licoricidin alone (8 $\mu\text{g/ml}$) did not cause such an inhibition of the bacterial growth.

In order to clarify the mechanism of the reduction of MICs of oxacillin, the effect of licoricidin (**33**) on the formation of penicillin-binding protein 2' (PBP2') was examined, since the formation of the enzymatic protein PBP2', which cat-

alyzes cell wall construction, causes the resistance of MRSA against the β -lactams.

The MRSA strain OM481 was incubated in the presence of licoricidin at a concentration of 8 $\mu\text{g}/\text{ml}$, where licoricidin showed the reduction of the MICs of oxacillin. After the incubation of MRSA, the bacterium was subjected to the slide latex agglutination assay to examine whether PBP2' was formed.

As a result, the agglutination due to the formation of

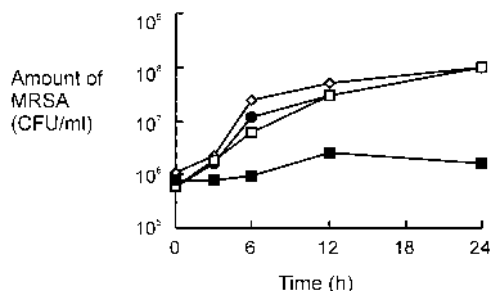


Fig. 2. Growth Curves for MRSA OM481 in the Absence (—◇—) and in the Presence of Oxacillin (1 $\mu\text{g}/\text{ml}$) (—●—), Licoricidin (8 $\mu\text{g}/\text{ml}$) (—□—) or Oxacillin (1 $\mu\text{g}/\text{ml}$) Plus Licoricidin (8 $\mu\text{g}/\text{ml}$) (—■—)

PBP2' was observed analogously to that in the absence of licoricidin or in the presence of oxacillin (1 $\mu\text{g}/\text{ml}$) (Fig. 3). Therefore, this compound restored the antibacterial effect of oxacillin without affecting the PBP2' formation. Although the mechanism for the restoring effect is still unclear, licoricidin may affect the enzymatic function of PBP2'. Assuming that the other PBPs are still available for the bacteria in the presence of licoricidin, oxacillin might work well leading the marked decrease of its MIC. However, other possible mechanisms such as an increase in the affinity of oxacillin to PBP2' by licoricidin may not be excluded.

Experimental

^1H - and ^{13}C -NMR spectra were measured on a Varian VXR-500 instrument (500 MHz for ^1H and 125.7 MHz for ^{13}C) in acetone- d_6 . Chemical shifts are given in δ values (ppm) based on the chemical shifts of solvent signals (δ_{H} 2.04, δ_{C} 29.8).

Isolation of Phenolic Compounds from Licorice Licorice (2 kg) (purchased from Tochimoto-tenkai-do, Osaka) was pulverized and extracted with *n*-hexane (61 \times 3) and ethyl acetate (61 \times 3), successively. The ethyl acetate extract (87 g) was subjected to counter-current distribution (CHCl_3 -MeOH- H_2O , 7 : 13 : 8, $n=3$, $r=3$) to separate six fractions, S1—S6. A portion (20 g) of fraction S6 (the fraction containing compounds of the lowest polarity among the six fractions) (75.9 g) was subjected to CPC (CHCl_3 -MeOH- H_2O , 7 : 13 : 8, reversed-phase development). Fractions

Table 2. Effects of Licorice Phenolics on the MICs of Oxacillin for MRSA Strains ($\mu\text{g}/\text{ml}$)

Compounds	MRSA OM481	MRSA OM505	MRSA OM584	MRSA OM623	MSSA 209P
Oxacillin alone	512	64	256	512	<0.5
Oxacillin plus					
Chalcones					
Licochalcone A (14) (8 $\mu\text{g}/\text{ml}$)	128	128	64	128	<0.5
Licochalcone B (15) (64 $\mu\text{g}/\text{ml}$)	128	64	16	64	<0.5
Isoflavones					
Glicoricone (4) (32 $\mu\text{g}/\text{ml}$)	512	64	64	128	<0.5
Isowightone (22) (16 $\mu\text{g}/\text{ml}$)	64	16	64	64	<0.5
Glisoflavone (23) (32 $\mu\text{g}/\text{ml}$)	128	64	32	128	<0.5
Isoangustone A (24) (8 $\mu\text{g}/\text{ml}$)	256	32	128	128	<0.5
Isoflavanones					
3'-(γ,γ -Dimethylallyl)-kievitone (28) (4 $\mu\text{g}/\text{ml}$)	256	64	64	256	<0.5
Isoflavans					
Glabridin (32) (8 $\mu\text{g}/\text{ml}$)	256	128	128	128	<0.5
Licoricidin (33) (8 $\mu\text{g}/\text{ml}$)	<0.5	<0.5	<0.5	<0.5	<0.5
Licoricidin (33) (4 $\mu\text{g}/\text{ml}$)	16	8	16	16	<0.5
3-Arylcoumarins					
Glycoumarin (34) (8 $\mu\text{g}/\text{ml}$)	1024	64	256	256	<0.5
Others					
Glicophenone (1) (16 $\mu\text{g}/\text{ml}$)	256	32	64	64	<0.5

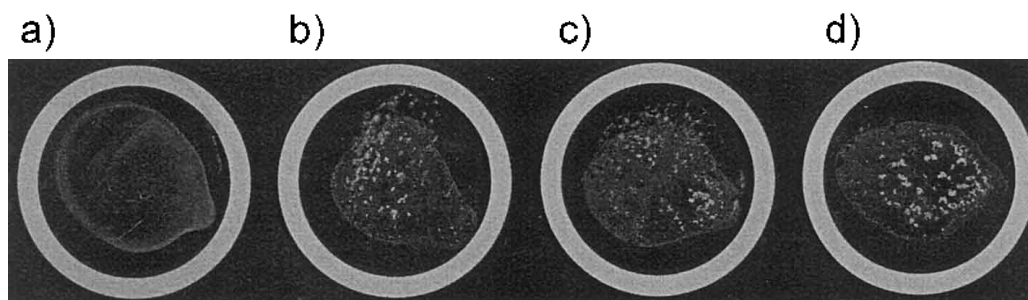


Fig. 3. Formation of PBP2' in MRSA OM481 in the Absence and in the Presence of Oxacillin or Licoricidin

a) MSSA 209P, b) MRSA OM481 in the absence of oxacillin and licoricidin, c) MRSA OM481 in the presence of oxacillin (1 $\mu\text{g}/\text{ml}$), d) MRSA OM481 in the presence of licoricidin (8 $\mu\text{g}/\text{ml}$). Detection of PBP2' was effected with anti-PBP2' monoclonal antibody-sensitized latex (MRSA Screen, Denka Seiken). The MSSA 209P did not show the formation of PBP2', while the other three showed the formation of PBP2'.

from CPC were chromatographed on Fuji gel ODS G3 and MCI gel CHP-20P, and further purified by preparative TLC on silica gel and preparative HPLC [YMC-A324 (5 μ m, 10 mm i.d. \times 300 mm), CH₃CN–H₂O–AcOH (60:35:5)] to give glycycomarin (**34**),²³ glisoflavone (**23**),²⁴ glycyrol (**41**),²⁵ licoisoflavone (**27**),²⁶ isowightone (**22**),²⁷ glycyrin (**37**),²⁸ isoangustone A (**24**),¹² glyasperin D (**31**),²⁹ glicophenone (**1**), licoricidin (**33**)³⁰ and 3'-(γ , γ -dimethylallyl)-kiewitone (**28**).³¹ A part of fraction S6 was chromatographed on YMC-gel SIL-120-S50 and Fuji gel ODS G3, then purified by preparative TLC to give glyasperin F (**26**).³² Yields of the phenolics from the ethyl acetate extract were: **34** (0.26%), **23** (0.059%), **41** (0.19%), **27** (0.023%), **22** (0.009%), **37** (0.012%), **24** (0.18%), **31** (0.029%), **1** (0.004%), **33** (0.15%), **28** (0.072%) and **26** (0.026%).

Analogous treatments of commercial licorice in separate experiments gave isoglycyrol (**42**)²⁵ (0.008% from an ethyl acetate extract), licoriphenone (**3**)¹² (0.016%), glycyrrhisoflavone (**25**)²³ (0.015%), glycyrrhisoflavone (**17**)²³ (0.052%), glicoisoflavone (**2**) (0.007%), liquiritigenin (**6**)³³ (0.006%), glyasperin C (**30**)²⁹ (0.014%), licopyranocoumarin (**35**)²⁴ (0.032%), glicoricone (**4**)¹⁶ (0.001%), semilicoisoflavone B (**18**)¹ (0.29%), liquiritin (**7**)³⁴ (0.21%), isoliquiritin apioside (**10**)³⁵ (0.016%), isoliquiritin (**11**)³⁵ (0.019%), 6''-O-acetyllicoumarin (**8**)³⁶ (0.022%), tetrahydroxymethoxychalcone (**12**)¹ (0.007%), naringenin (**9**)¹ (0.023%), genistein (**19**)¹⁶ (0.016%), echinatin (**13**)¹⁶ (0.021%), licocoumarone (**40**) (0.022%),²⁴ (3R)-vestitol (**29**)¹ (0.024%), gancaonin G (**20**)³⁷ (0.042%), 8-(γ , γ -dimethylallyl)-wightone (**21**)³⁸ (0.008%), in addition to the compounds described above.

Glicophenone (**1**): Colorless needles, mp 145 °C. EI-MS m/z : 358 (M^+ , 38%), 221 (74%), 165 (24%), 137 (100%). High-resolution EI-MS m/z : 358.1460 (M^+ ; Calcd for C₂₀H₂₂O₆, m/z 358.1416). UV λ_{max}^{MeOH} nm (log ϵ): 209 (4.64), 230 (sh, 4.21), 276 (4.13), 313 (3.63). ¹H-NMR: see text. ¹³C-NMR δ : 17.9 (CH₃ at C-3''), 23.5 (C-1''), 25.8 (CH₃ at C-3''), 34.4 (C-8), 61.6 (OCH₃), 99.7 (C-5'), 103.5 (C-3), 107.9 (C-1'), 108.6 (C-5), 112.4 (C-1), 114.0 (C-3'), 125.4 (C-2''), 130.3 (C-3''), 133.6 (C-6), 155.3 (C-6'), 156.2 (C-4'), 159.5 (C-2'), 165.3 (C-2), 166.1 (C-4), 204.3 (C-7).

Glicoisoflavone (**2**): Colorless needles, mp 102 °C. [α]_D^{-3°} (c =2, MeOH). CD (MeOH) [θ] (nm): +9200 (215), +5800 (227), -6500 (300), +3500 (328). EI-MS m/z : 384 (M^+ , 40%), 366 ([M–H₂O]⁺, 100%), 247 (5%), 136 (7%), 115 (21%). High-resolution EI-MS m/z 384.1630 (M^+ ; Calcd for C₂₂H₂₄O₆, 384.1573). ¹H-NMR: see text. ¹³C-NMR δ : 17.9 (CH₃ at C-3''), 23.6 (C-1''), 25.8 (CH₃ at C-3''), 45.3 (C-3), 55.8 (2OCH₃ at C-4'), 62.3 (–OCH₃ at C-6'), 70.6 (C-2), 96.5 (C-5'), 103.5 (C-8), 108.9 (C-1'), 110.9 (C-6), 114.9 (C-3'), 115.9 (C-4a), 125.1 (C-2''), 130.0 (C-5), 130.6 (C-3''), 155.7 (C-6'), 159.2 (C-4'), 159.7 (C-2'), 164.5 (C-8a), 164.7 (C-7), 191.2 (C-4).

Estimation of Antibacterial Effects of Licorice Phenolics on MRSA Strains Four MRSA strains used in this study are clinical isolates from Okayama University hospital.²¹ Phenolic compounds of which the isolation procedure is not described here, licochalcones A (**14**)²³ and B (**15**),²³ isoliquiritigenin (**16**),¹ glabridin (**32**),¹¹ licoarylcoumarin (**36**)²⁴ and isolicopyranocoumarin (**38**),¹ were obtained as described in previous reports. Glycyrin permethyl ether (**39**)²⁸ was prepared from glycycomarin (**34**). The MICs of tested compounds for the bacterial strains were determined using 10⁴ colony forming unit (CFU)/well of bacterial solution on 96-well plates in a way reported previously.⁸

Effects of Licorice Phenolics on the MICs of Oxacillin for MRSA Strains In the presence of each phenolic compound at concentrations lower than its MIC value, the lowest concentration of oxacillin which did not cause turbidity due to bacterial proliferation was estimated in a way analogous to that described above.

Effects of the Addition of Licoricidin on the Inhibitory Activity of Oxacillin against the Growth of MRSA OM481 The MRSA OM481 strain, which was maintained in the laboratory of the Department of Microbiology, was precultured overnight in a Mueller–Hinton (MH) medium containing Ca²⁺ (50 mg/l) and Mg²⁺ (25 mg/l) ions. A bacterial solution (0.2 ml) of absorbance 0.6–0.7 at 650 nm, prepared upon incubation of the precultured bacteria, was diluted with the MH medium (1.8 ml), and 50 μ l portions of the solution were then added to the MH medium (5 ml each) in test tubes. The bacterial solution (ca. 10⁶ CFU/ml) in the tubes was incubated with and without licoricidin (**33**) and/or oxacillin at 32 °C for 24 h. The bacteria in each tube was incubated on Nutrient agar plates at 32 °C for 24 h to estimate the amounts of the bacteria.²¹

Detection of PBP2' in MRSA OM481 in the Presence of Oxacillin or Licoricidin A portion (0.2 ml) of the precultured solution of MRSA OM481 was added to the MH medium (4.8 ml) containing Ca²⁺ (50 mg/l) and Mg²⁺ (25 mg/l) ions, and the solution was incubated until the ab-

sorbance at 650 nm attained 0.6–0.7 in the presence of oxacillin (1 μ g/ml) or licoricidin (8 μ g/ml). The bacterial solution was then centrifuged at 10000 rpm for 5 min, and the precipitated bacteria was washed with 0.05 M phosphate buffer (pH 7.0) twice. A part of the bacterial mass was extracted with 1 M NaOH (0.2 ml) in a boiling-water bath for 3 min, then left to stand at room temperature. A solution of 0.5 M KH₂PO₄ (0.05 ml) was added, and the mixture was centrifuged at 4500 rpm for 5 min. The supernatant was diluted with 9-fold water, and 50 μ l of the resulting solution was treated with anti-PBP2' monoclonal antibody-sensitized latex (MRSA Screen, Denka Seiken Co.) (15 μ l) on a test card for 3 min to show the presence/absence of PBP2' by agglutination on the card.²²

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