The pyran ring isopentene group: an overlooked antimicrobial active group in prenylated flavonoids

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**ABSTRACT**

Prenylated flavonoids show antibacterial activity towards 
*Staphylococcus aureus* (*S. aureus*). Previous studies have suggested that the prenyl side-chain is an important active group for antimicrobial activity. However, prenylated flavonoids also often contain a pyran ring isopentene group. Few studies have explored the contribution of the pyran ring isopentene group to antibacterial activity. In this study, the antibacterial activities of structurally related flavonoid compounds from mulberry root bark were studied by detecting the minimum inhibitory concentration (MIC) and colony counting. These flavonoid compounds all exhibited antibacterial activities against *S. aureus* ATCC6538, *S. aureus* ATCC25923 and methicillin-resistant *S. aureus* (MRSA) ATCC43300 with MIC values of 7.3–248.2 \(\mu\)mol/L, 7.3–330.9 \(\mu\)mol/L, and 7.3–330.9 \(\mu\)mol/L, respectively. Structure-activity relationship analyses demonstrated that the pyran ring isopentene group plays an important role in antibacterial activity. Thus, the pyran ring isopentene group is an overlooked antimicrobial active group in prenylated flavonoids.

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1. Introduction

*Staphylococcus aureus* (*S. aureus*) is a pathogen that causes vomiting, diarrhea, endocarditis, and skin and soft tissue infections (Turner et al. 2019). Worryingly, the emergence and spread of drug-resistant bacteria, such as methicillin-resistant *S. aureus*...
(MRSA), poses increasing risks to human health (Yelin and Kishony 2018). Therefore, it is important to search for new antibacterial agents to address this problem.

Prenylated flavonoid natural products combine a flavonoid skeleton with prenyl group(s) (Yang et al. 2015) and show significant antibacterial activity (Araya-Cloutier et al. 2018; Pang et al. 2019). However, few studies have analyzed the structure–activity relationships of prenylated flavonoids against pathogens. A previous study showed that the prenyl side-chain and the phenolic hydroxyl group are both bacteriostatic active groups (Zuo et al. 2018). However, some prenylated flavonoids contain a pyran ring isopentene group. We wanted to investigate whether the pyran ring isopentene group contributes to the antimicrobial activity of prenylated flavonoids to the same degree as the prenyl side-chain. This unanswered question limited the use of pyran ring prenylated flavonoids as antimicrobial agents.

In the present study, we assessed the antibacterial activities of different types of prenylated flavonoids extracted from mulberry (Morus alba L., family Moraceae) trees. The contribution of the pyran ring isopentene group to the inhibitory effect against S. aureus was assessed. This research sheds light on the importance of the pyran ring isopentene group for the antibacterial activity of these flavonoid compounds.

2. Results and discussion

2.1. MIC Values of flavonoids against S. aureus

As shown in Table S1, six types of flavonoids all exhibited antibacterial activities against S. aureus ATCC6538, S. aureus ATCC25923 and MRSA ATCC43300 with MIC values of 7.3–248.2 μmol/L, 7.3–330.9 μmol/L, and 7.3–330.9 μmol/L, respectively. For ATCC6538, mulberrin showed the strongest antibacterial activities among these flavonoid compounds. The MIC value of mulberrin toward S. aureus ATCC 6538 was 7.3 μmol/L, 3.1 μg/mL, which is even better than that of ampicillin (9.0 μmol/L, 3.1 μg/mL), followed by morusin (14.9 μmol/L, 6.3 μg/mL), cyclomulberrin (47.6 μmol/L, 20.0 μg/mL), cyclomorusin (59.8 μmol/L, 25.0 μg/mL), and morusinol (228.0 μmol/L, 100.0 μg/mL). The MIC value of morin was the highest (248.2 μmol/L, 75.0 μg/mL).

The antibacterial activities of different flavonoid compounds against S. aureus ATCC 25923 generally followed the same trend as that of the inhibition effects on S. aureus ATCC 6538. Mulberrin also showed the strongest antibacterial activities among these flavonoid compounds. These flavonoid compounds exhibited the antibacterial activities in the following decreasing order: mulberrin (7.3 μmol/L) > morusin (14.9 μmol/L) > cyclomulberrin (29.7 μmol/L) > cyclomorusin (59.8 μmol/L) > morusinol (114.0 μmol/L) > morin (330.9 μmol/L). The MIC value of ampicillin toward S. aureus ATCC 25923 was 2.2 μmol/L. Interestingly, a previous study also suggested that mulberrin showed better antibacterial activity than morusin toward S. aureus (Sohn et al. 2004).

For MRSA, the MIC value of morin was the highest (330.9 μmol/L, 100.0 μg/mL) among these compounds. The antibacterial activities of these prenylated flavonoids are all better than morin. Mulberrin has the best antibacterial activity toward MRSA, with the lowest MIC value (7.3 μmol/L, 3.1 μg/mL), followed by morusin (29.8 μmol/L, 12.5 μg/mL), cyclomulberrin (59.5 μmol/L, 25.0 μg/mL), cyclomorusin (179.3 μmol/L, 75.0 μg/mL), and morusinol (228 μmol/L, 100.0 μg/mL). These results also suggest that
prenylated flavonoids show strong antibacterial activity against MRSA. However, the MIC value of ampicillin toward MRSA (71.6 μmol/L, 25.0 μg/mL) showed a weaker antibacterial activity than mulberrin and morusin. Notably, a previous study also determined the antibacterial activities of mulberrin, morusin, and morin against a different MRSA strain. The MIC values of mulberrin, morusin, and morin against MRSA T144 are 2, 8, and 32 μg/mL, respectively (Wu et al. 2019). Although the results suggest that the antibacterial activities of mulberrin, morusin, and morin on MRSA are slightly better than this study, our results showed the same order of antibacterial activities.

Overall, these prenylated flavonoid compounds exhibited antibacterial activities toward three kinds of *S. aureus* in the following decreasing order: mulberrin > morusin > cyclomulberrin > cyclomorusin > morusinol.

### 2.2. Bacterial growth after exposure to flavonoid compounds

To determine the antibacterial effects of structurally similar flavonoid compounds, the growth profiles of MRSA treated with these compounds were determined by counting the bacterial colonies at different time points (Figure S1). After exposure to mulberrin (6.3 μg/mL) for 8 h, the number of colonies decreased significantly, which demonstrates that mulberrin had a stronger inhibitory effect against MRSA than morusin (6.3 μg/mL) and cyclomulberrin (6.3 μg/mL). The number of bacterial colonies at different time points was lower after exposure to morusin than after exposure to cyclomulberrin, which demonstrates that morusin had stronger inhibitory effects than cyclomulberrin against MRSA. After treatment with cyclomorusin (75.0 μg/mL) for 4 h, the number of bacterial colonies decreased from 5.5 log_{10} CFU/mL to 3.7 log_{10} CFU/mL. However, after exposure to morusinol (75.0 μg/mL) for 4 h, the number of colonies decreased from 5.5 log_{10} CFU/mL to 4.0 log_{10} CFU/mL. Generally, cyclomorusin showed stronger inhibitory effects than morusinol against MRSA at all time points. In addition, morusinol had a stronger inhibitory effect than morin against MRSA, although the MIC values of morusinol and morin were both 100 μg/mL. Overall, the trends in MRSA growth inhibition by flavonoids were the same as those detected in the MIC analyses.

### 2.3. The structural relationships of the flavonoid compounds

The structural relationships of the flavonoid compounds analyzed in this study are shown in Figure 1. After cyclization, morusin is transformed into cyclomorusin. This change results in the loss of a phenolic hydroxyl group, which causes the antibacterial activity to decrease slightly (Figure S2(B)). After the same cyclization reaction, mulberrin is transformed to cyclomulberrin, and this also results in a slight decrease in antibacterial activity (Figure S2(C)). These results confirmed that a phenolic hydroxyl group is an active group for inhibiting the growth of bacteria. Morin showed antibacterial activities toward different kinds of *S. aureus*. The phenolic hydroxyl group may contribute significantly to the activity. However, once isopentene groups were added to morin at the C3 and C8 positions to convert it into mulberrin, the antibacterial activities toward different kinds of *S. aureus* became significantly stronger (Figure S2(A)). Furthermore, after an additional reaction at the C3 position, morusin was transformed to morusinol, where the isopentene group had been converted into an
alcoholic hydroxyl group, and the antibacterial activity decreased significantly (Figure S2(B)). These results suggest that isopentene groups may promote the antibacterial activity of flavone, which is in accordance with a previous study (Wu et al. 2019). Therefore, the prenyl side-chain and phenolic hydroxyl have both been shown to be bacteriostatic active groups, and the prenyl side-chain groups contribute important effects to the antibacterial activities.

Although prenylated flavonoids contain prenyl side-chains, they also commonly contain pyran ring isopentene groups. However, previous studies on the structure-activity relationship of prenylated flavonoids against pathogens have mainly focused on the prenyl side-chain. Few studies have explored the contribution of the pyran ring isopentene group to antibacterial activity. To study this, the structural characteristics of pyran ring isopentene groups and prenyl side-chains were analyzed. A pyran ring isopentane group can be converted to a prenyl side-chain group and a phenolic hydroxyl group by a ring-opening reaction. As Figure S2(D) shows, morusin can be converted into mulberrin using this ring-opening reaction. The rest of these compounds look the same; the only difference is that the pyran ring isopentane group has been converted into a prenyl side-chain group and a phenolic hydroxyl group. The antibacterial activities of mulberrin are slightly better than morusin. Interestingly, as shown in Figure S2(E), the structural differences between cyclomulberrin and cyclomorusin are the same as for mulberrin and morusin, and, consequently, the difference in antibacterial activities followed the same trend as those of mulberrin and morusin. The results suggest that the contribution of a prenyl side-chain group and a phenolic hydroxyl group to antibacterial activities are only slightly better than that of a pyran ring isopentane group (Figure S2(F)). This is because the prenyl side-chain group and phenolic hydroxyl group are both antibacterial active groups. Therefore, pyran ring isopentane groups also showed strong antibacterial activity, which may be equal to the prenyl side-chain groups.
3. Conclusion

This study showed that five types of prenylated flavonoids exhibited strong antibacterial activities toward different kinds of S. aureus. The prenyl side-chain and phenolic hydroxyl group are both bacteriostatic active groups, and the prenyl side-chain groups contribute important effects to the antibacterial activities. Pyran ring isopentene groups may also contribute significant inhibitory effects toward S. aureus, similar to the prenyl side-chain; however, these contributions have been previously overlooked. These results will provide some guidance for selecting prenylated flavonoids for antibacterial applications.

Disclosure statement

No potential competing interest was reported by the authors.

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