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Microbial Growth-Promotion Activity of 3–Hydroxymonoazine- and *N*-Hydroxydiazine-Type Heterocycles

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Three 3–hydroxymonoazine- and three *N*–hydroxydiazine-type heterocycles were tested whether they act as artificial siderophores toward *Aureobacterium flavescens* JG–9 (ATCC No. 25091). Among them, 1–hydroxy–3,5,6–trimethyl–2(1*H*)–pyrazinone (**3**) showed the highest growth-promotion activity comparable to desferrioxamine B (DFB), a natural trihydroxamate siderophore, at 48.5 μM or above, followed by 1–hydroxy–5,6–dimethyl–2(1*H*)–pyrazinone (**2**), 1–hydroxy–4,6–dimethyl–2(1*H*)–pyrimidinone (**1**), and 3–hydroxy–2–methyl–1–phenyl–4(1*H*)–pyridinone (**6**), while 3–hydroxy–1,2–dimethyl–4(1*H*)–pyridinone (**5**) did not show the bioactivity. These results are the first examples of *N*–hydroxydiazine-type heterocycles acting as artificial siderophores for *A. flavescens* JG–9.

Key words——3–hydroxymonoazine- and *N*–hydroxydiazine-type heterocycles; microbial growth-promotion activity; artificial siderophore

INTRODUCTION

Siderophores are low-molecular-weight iron-scavenging agents secreted by microorganisms, which have commonly three sets of hydroxamate or catecholate bidentate ligands as iron chelators.[1–3] Since siderophores are potential therapeutic agents for iron overloaded disease, many siderophore analogues have been synthesized to elucidate the detailed mechanism of the siderophore-mediated iron uptake and to map membranal siderophore-iron complex receptors by testing their growth-promotion activity toward siderophore auxotrophs such as *A. flavescens* JG–9.[4—8]

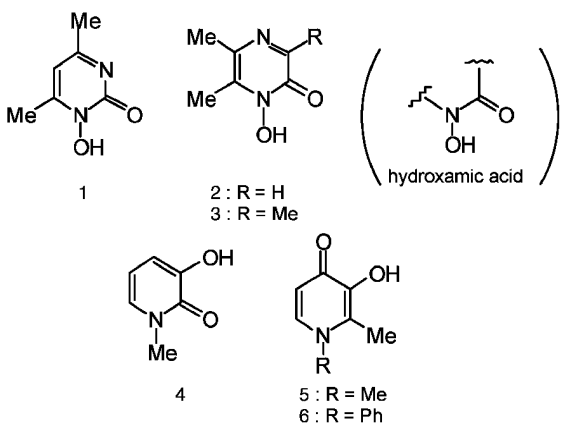

Fig. 1. Structures 1—6

In this context, we have intensively investigated synthesis and characterization of iron(III) complexes, including iron(III)–chelating property and kinetics on iron(III) removal from human transferrin, of *N*–hydroxydiazines such as 1–hydroxy–2(1*H*)–pyrimidinone and -pyrazinone, and 3–hydroxy-monoazine such as 3–hydroxy–4(1*H*)–pyridinone.[9—12]

We have been interested in whether these *N*–hydroxydiazine-type heterocycles act as artificial siderophores, because they can be regarded as cyclic hydroxamic acids. Therefore, we report here the first example of microbial growth-promotion activity of *N*–hydroxydiazine-type heterocycles (**1**—**3**) for Gram-positive *A. flavescens* JG–9. The growth-promotion activity of 3–hydroxymonoazine-type heterocycles such as 3–hydroxy–2(1*H*)–pyridinone (**4**) and –4(1*H*)–pyridinones (**5** and **6**) is also tested, because this strain is known to be quite omnivorous as regards the hydroxamate siderophores and even uses, in the proper level, of synthetic chelating agents.[13,14]

MATERIALS AND METHODS

Materials

1–Hydroxy–4,6–dimethyl–2(1*H*)–pyrimidinone (**1**),[15] 1–hydroxy–5,6–dimethyl–2(1*H*)–pyrazinone (**2**),[15] 1–hydroxy–3,5,6–trimethyl–2(1*H*)–pyrazinone (**3**),[16] 3–hydroxy–1–methyl–2(1*H*)–pyridinone (**4**),[17] 3–hydroxy–1,2–dimethyl–4(1*H*)–pyridi-

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none (**5**),[18] and 3-hydroxy-2-methyl-1-phenyl-4(1*H*)-pyridinone (**6**)[18] were synthesized according to literature methods. Titration experiments in conjunction with electronic spectroscopies showed 3 : 1 stoichiometry to iron(III) for all compounds. DFB was purchased from Ciba Geigy Ltd. as its mesylate, whose trade name is Desferal®.

Growth Response Studies The test compounds were prepared as 5-mM stock solutions in water, whose pH were adjusted about 7, and were added to 3.0 mL of sterile ATCC medium 424[14,19] to give desired final concentrations (0.03, 0.54, 11.3, 24.4, 48.5, or 234 μM) of the compounds. The culture including bidentate ligands was inoculated with *A. flavescens* JG-9, and then shaken at 30°C at 165 rpm for 12 hours. Aliquots were taken out and diluted with water, whose turbidity was measured at 630 nm to determine the bacteria density. The optical densities (OD_{630}) measured were corrected for those of control experiments without a siderophore.

Growth Curve Studies The culture including *A. flavescens* JG-9 and appropriate concentrations of the test compounds (**1**; 234 μM, **2**; 48.5 μM, **3**; 24.4 μM, **4**; 234 μM, **5**; 234 μM, **6**; 24.4 μM, and DFB; 24.4 μM) were prepared and shaken in a similar way described above. Aliquots were taken out at appropriate intervals and the corrected OD_{630} values were determined.

RESULTS AND DISCUSSION

The growth-promotion activities of the compounds **1**—**6** were examined in liquid medium with *A. flavescens* JG-9, and DFB was used as a positive control. Figure 2 showed the dose response of *A. flavescens* JG-9 strain to heterocycles **1**—**6** and DFB after 12-hour incubation at 30°C. In the concentration range employed, DFB exhibited maximal growth as reported previously.[19] Among six heterocycles, 2(1*H*)-pyrazinone (**3**) exhibited almost the same growth-promotion activity as natural siderophore DFB at final concentration of 48.5 μM. 2(1*H*)-Pyrazinone (**2**) also showed a good bioactivity, although such high concentration as 234 μM was required. 2(1*H*)-Pyrimidinone (**1**) and 2(1*H*)-pyridinone (**4**) did not promote the growth of the JG-9 strain at 48.5 μM, but under incubation at 234 μM these compounds did show the activity. Interestingly, 1-phenyl-4(1*H*)-pyridinone (**6**) showed appreciable activity (increasing OD_{630}) at 24.4 μM, but it showed negative OD_{630} at 234 μM. This may be attributable that 4(1*H*)-pyridinone (**6**) has a certain antimicrobial activity at such higher concentration. 1-Methyl-4(1*H*)-pyridinone (**5**) did not promote growth of the strain at all.

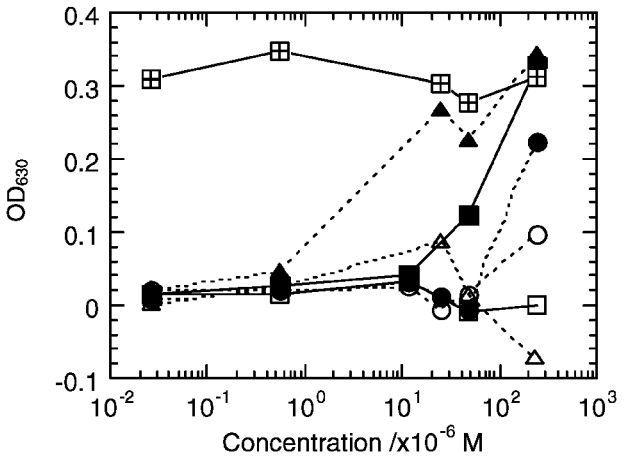

Fig. 2. Growth Response of *A. flavescens* JG-9 to 1(●), 2(■), 3(▲), 4(○), 5(□), 6(△), and DFB(⊞) after 12 h Incubation in ATCC Medium 424 at 30°C.

Thus, with appropriate concentrations, all hydroxyazine-type heterocycles except compound **5** can act as artificial siderophores for *A. flavescens* JG-9, as shown in Figure 3. The same results were obtained when bidentate ligands were used as their iron(III) complexes, indicating that these bidentate ligands scavenge adventitious ferric iron in the medium and form their iron(III) complexes during incubation. As expected, compound **3** exhibited remarkable growth promotion activity comparable to DFB. Compounds **1**, **2**, and **4** also promoted the microbial growth with the given concentrations for each, although the growth levels in the stationary phase decreased. Nagasaki *et al* have found the analogous decrement of the growth in the stationary state in the growth experiment with a photoresponsive siderophore.[19] They considered this phenomenon as that the availability of ferric ion in the initial phase rather than in the logarithmic phase is significant for determining the growth of this strain. Therefore, our results may also be attributed to scant availability of ferric iron with these ligands in the initial phase. The positive bioactivity of compounds **4** and **6** suggests that we cannot simply predict whether a hydroxyazine-type heterocycle acts as an artificial siderophore judging from its structural feature.

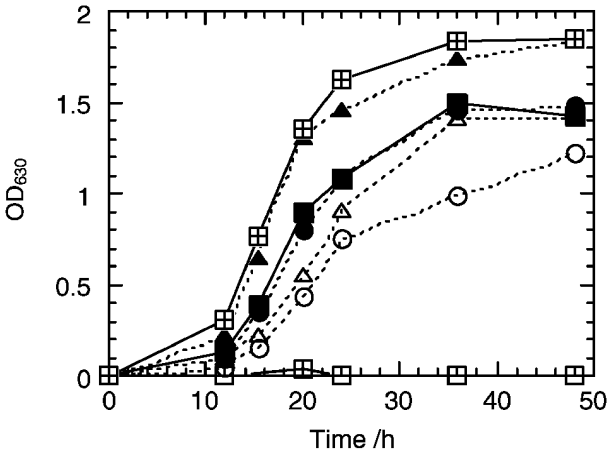

Fig. 3. Growth Curves of *A. flavescens* JG–9 with Appropriate Concentrations of 1 (●; 234 μM), 2 (■; 48.5 μM), 3 (▲; 24.4 μM), 4 (○; 234 μM), 5 (□; 234 μM), 6 (△; 24.4 μM), and DFB (⊞; 24.4 μM) in ATCC Medium 424 at 30°C

CONCLUSION

In conclusion, we have demonstrated, for the first time, the growth-promotion activity of 3–hydroxymonoazine- and *N*–hydroxydiazine-type heterocycles toward *A. flavescens* JG–9 and revealed that 1–hydroxy–3,4,6–trimethyl–2(1*H*)–pyrazinone (**3**) exhibited the highest growth-promotion activity. The present results also indicate that 3–hydroxy–2(1*H*)–pyridinones, 1–hydroxy–2(1*H*)–pyrimidinones, and 1– hydroxy–2(1*H*)–pyrazinones act as artificial siderophores, although the minimum concentration of the growth-promotion of these heterocycles is far greater than that of natural DFB.

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