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MOLECULAR STRUCTURE AND BIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF 3-HYDROXY-2-METHYL-1-(β-D-RIBOFURANOSYL OR PYRANOSYL)-4-PYRIDINONE: POTENTIAL IRON OVERLOAD DRUGS FOR ORAL ADMINISTRATION

Gang Liu,[a,*] Fred W. Bruenger,[a] Scott C. Miller,[a] and Aarif M. Arif[b]

[a]*Radiobiology Division, Building 585 and* [b]*Chemistry Department, University of Utah, Salt Lake City, UT 84112, U. S. A.*

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Abstract: Replacing alkyl groups by sugar moieties at N-1 position of 3-hydroxy-2-methyl-4-pyridinone did not affect the geometry of the iron chelating sites but increased the hydrophilic nature. The formation of a polymer cluster through the intermolecular hydrogen bonds was also revealed by X-ray crystal structure analysis for the first time in all known 3-hydroxy-4-pyridinone crystal structures. Iron removal from ferritin by the title compounds was more efficient than with DFO.

There is a great need for the development of new orally active, nontoxic, iron chelators that can be used for the clinical treatment of transfusional iron overload, the most common heavy metal toxicity with the highest mortality rate worldwide.[1] Moreover, control of iron in the body by chelating drugs may have a significant beneficial effect on tissue damage caused by free radicals.[2] Also, temporary iron depletion may affect tumor growth and if administred as a complementary treatment, may reduce the severity of bacterial and parasite infections because iron is an esssential nutrient for cell growth.[3]

Presently, desferrioxamine (Desferal, DFO) is the only approved drug to treat iron overload conditions clinically, but its use is limited by its high cost, oral inactivity,[4] and toxic side effects.[5] Orally active derivatives of 3-hydroxy-4-pyridinones having an alkyl group in either the N-1/C-2 positions have been developed[6] and investigated in clinical trials.[7] However, most of these compounds proved to be toxic due to their lipophilic character[8] and the cleavage of the N-alkyl substituent in the hydroxypyridinone ring, leading to the formation of catechol-like metabolites.[9] In addition, they were not as efficatious as hoped for, because of their rapid conversion to nonchelating glucuronide metabolites.[10] In order to improve the pharmacological properties, we have recently designed and synthesized 2-methyl (ethyl)-3-hydroxy-4-pyridinone derivatives with a sugar moiety as a carrier attached to the N atom instead of alkyl groups. It is anticipated that the sugar moiety imparts less toxicity to the pyridinone than the alkyl group because the lipophilic nature of the compounds decreases and the cleavage as mentioned above is less likely with a larger substituent at the N-1 position.[9] At the same time, the sugar moiety supports penetration of the intestine and permeation of cell membranes and prevents the hydroxypyridinone from converting to nonchelating metabolites because the glucuronide reaction is now favored on the sugar ring, leaving the hydroxypyridinone active. We believe that the sophisticated design and synthesis of 2-alkyl-3-hydroxy-1-sugar substituent-4-pyridinones may lead to a new type of orally active, nontoxic, iron chelators. To evaluate these novel iron chelating agents, it is necessary to examine whether the sugar substituents did not cause any unfavorable structural changes in the iron chelating sites and to determine their iron binding properties and their

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iron removal abilities. Therefore, we report here the X-ray crystal structure assignment of 3-hydroxy-2-methyl-1-(β-D-ribopyranosyl)-4-pyridinone (**II**), one of the title compounds and their abilities to mobilize iron from ferritin, an important iron storage protein.

The title compounds of 3-hydroxy-2-methyl-1-(β-D-ribofuranosyl)-4-pyridinone (**I**) and 3-hydroxy-2-methyl-1-(β-D-ribopyranosyl)-4-pyridinone (**II**) were synthesized in good yield according to our earlier work[11] and their structrures were shown in Figure 1.

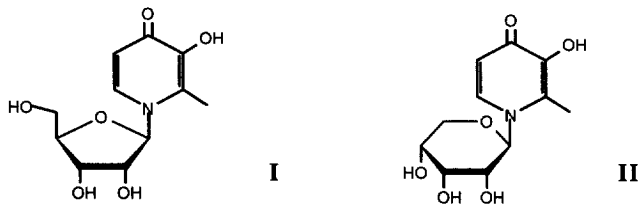

Figure 1. Chemical structures of compounds I and II

The colorless single crystals of **II** were obtained from a solution of $CHCl_3$/MeOH and the crystal and molecular structures were studied by X-ray crystallography.[12] In the molecular structure (Fig. 2), the C3-O2, C2-O1, and C2-C3 bond lengths are 1.269 (6) Å, 1.361 (6) Å, and 1.428 (7) Å, respectively, which compare well with the corresponding bond lengths of 1.271 Å, 1.364 Å, and 1.438 Å in 3-hydroxy-1,2-dimethyl-4-pyridinone.[13] The angles of O2-C3-C2 (121.0 (5)) ° and O1-C2-C3 (119.4 (5)) ° are also in agreement with corresponding angles (120.5 (3) and 119.2 (2) °).[14] The unchanged α-ketohydroxyl geometry in **II** indicated that the substituted sugar group at N-1 position did not affect the mainly quinoid form of the pyridinone ring structure. Therefore, the iron binding affinity of **II** should retain about the same strength as in other 3-hydroxy-4-pyridinones. The molecular structure of **II** shows a β-anomeric configuration of **II** with the hydroxypyridinone in a preferred equatorial position. It is interesting to see that the crystal structure of **II** does not exhibt centrosymmetric hydrogen bonded dimeric units, which are found in all other known 3-hydroxy-4-pyridinone crystal structures.[15] The hydroxyl proton (H1) on the pyridinone ring of one molecule forms a hydrogen bond with the hydroxyl oxygen (O4) on the sugar ring of a second molecule while the hydrogen (H9) on oxygen (O4) forms a hydrogen bond with the sugar hydroxyl oxygen (O6) of a third molecule. The hydrogen (H13) on oxygen (O6) as well as hydrogen (H11) on oxygen (O5) on the sugar ring of the third molecule forms two hydrogen bonds back to the same carbonyl oxygen (O2) on the pyridinone ring of the first molecule. These intermolecular hydrogen bonds[16] crosslink the molecules of **II** forming a polymer cluster within the crystal structure. This is the first report of this type of crystal formation of 3-hydroxy-4-pyridinone compounds. Based on the observation of crystal structure, in which so many hydrogen bonds have been involved, it can be predicated that the title compounds are more hydrophilic than other N-alkyl substituted 3-hydroxy-4-pyridinone derivatives. It is consistent with the evidence of the solubilities of the title compounds in water.

In the titration of the title compounds with iron, purple complexes were formed and these complexes have a chelator iron stoichiometry of approximately 3:1. This was determined spectrophotometrically in Tris (20 mM, pH 7.5, 22 °C) as described in reference 17. This result also indicates that the hydroxy groups on the sugar rings do not involve the iron complexation at physiological condition. The results of the reaction of the title compounds and DFO with ferritin are shown in Figure 3.[18] Both of the title compounds were more effective than DFO in

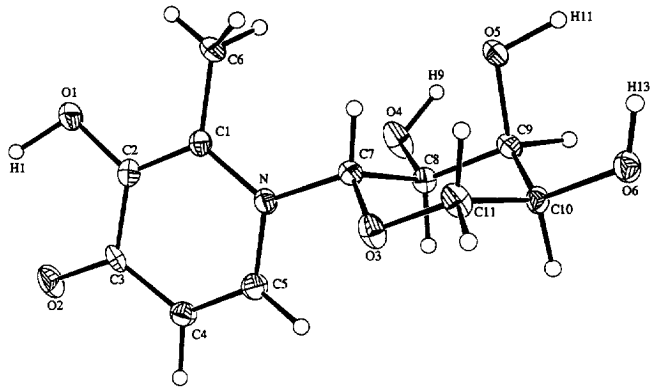

Figure 2. The molecular structure of compound II showing the atom-numbering scheme

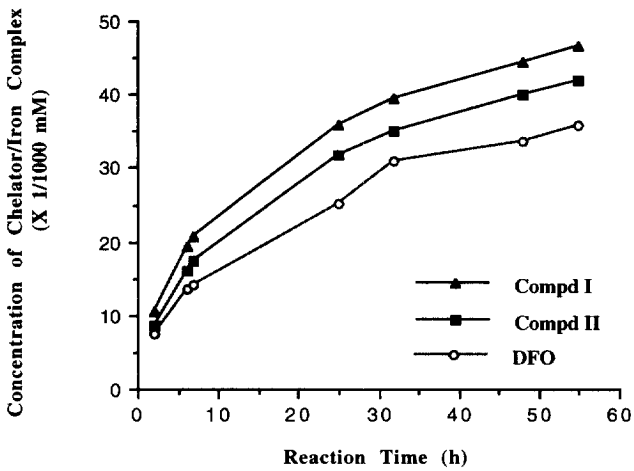

Figure 3. Iron mobilization from ferritin by chelators

removing the iron core from the ferritin protein at physiological pH. The initial rate of removal was faster with the title compounds than with DFO, while the rate after 24 h was about the same. This indicates that the title compounds can penetrate the channels in the ferritin protein shell more efficiently than DFO and that the affinity toward iron is high enough to interact with the internal polynuclear iron core. Additional experiments to study the toxicity and the ability of removal of the metal from iron overloaded rats are underway.

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12. Crystal data: $C_{11}H_{15}NO_6$, FW = 257, Orthorhombic, Space group $P2_12_12_1$ (No. 19), Cell constants: a = 6.994(2) Å; b = 7.697(2) Å; c = 20.833(5) Å, Cell volume: 1121.48 A^3, Z = 4; Calcd Density = 1.523 g/cm^3, Mo-Kα Radiation (0.71073 Å), No. of reflections measured: 1287, 2θ range: 4.00 to 50.00 Degree, Final refinement: R Factor = 0.0533, Weighted R Factor = 0.0633.
    Selected bond lengths (Å): C1-C2, 1.362 (7); C2-C3, 1.428 (7); C3-C4, 1.423 (7); C4-C5, 1.337 (7); N-C1, 1.383 (6); N-C5, 1.365 (6); N-C7, 1.463 (6). Selected angles(°): C1-N-C5, 120.1 (4); C1-N-C7, 121.4 (4); C5-N-C7, 118.5 (4); N-C1-C2, 118.8 (5); N-C1-C6, 120.5 (4); C2-C1-C6, 120.7 (5); O1-C2-C1, 117.7 (5); C1-C2-C3, 122.8 (5); O2-C3-C4, 124.2 (5); C2-C3-C4, 114.8 (5); C3-C4-C5, 121.4 (5); N-C5-C4, 122.0 (5); O3-C7-N, 106.9 (4); N-C7-C8, 113.0 (5).
    Atomic coordinates, bond lengths and bond angles have been deposited at the Cambridge Crystallographic Data Centre.
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16. O1-H1··O4 (2.762 Å (5), 144.0 ° (2)), O5-H11··O2 (2.676 Å (5), 150.6 ° (2)); O6-H13··O2 (2.707 Å (5), 142.2 ° (2)); O4-H9··O6 (2.708 Å (5), 148.7 ° (2)).
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