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Laboratory note

Hydroxypyridone iron chelators, L1 and analogues. An ^{17}O NMR study

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Abstract – Some iron chelators of the class of hydroxypyridones, including the experimental drug 1,2-dimethyl-3-hydroxy-4-pyridone (L1), have been studied by ^{17}O NMR spectroscopy. The reciprocal influence of the keto and hydroxy groups has been examined in order to gain a better comprehension of their bonds. A strong intramolecular hydrogen bond between 3-OH and 4-CO groups has been observed for L1 and its analogues. A comparison has also been made on the data of three different solvents. © 1999 Éditions scientifiques et médicales Elsevier SAS

iron chelators / L1 / hydrogen bond / ^{17}O NMR

1. Introduction

Humans have a very limited capacity for iron excretion [1], even when iron stores are markedly increased. Thus, congenital or acquired iron-loading anaemias can cause severe tissue damage and eventual death, unless the iron overload is removed. One such disease, β-thalassaemia, still has a very large diffusion in our region of Sardinia. Desferrioxamine (DFO) is the only iron-chelating agent currently in widespread clinical use. Its administration, however, requires cumbersome and expensive methods of slow subcutaneous or intravenous infusion. This fact has expedited the research for oral iron chelators [2]. One of the most promising among them, 1,2-dimethyl-3-hydroxy-4-pyridone (L1) [2, 3], is currently in clinical tests by our group.

Dioxobidentate ligands are generally iron (III) ligands [4], and the majority of them incorporate the carbonyl and the hydroxy groups. It would be interesting to directly investigate these groups, which actually bond to the metal atom. Moreover, one of the most useful concepts [5] to develop new chelating agents is the theory of hard and soft bases [6]. Its application is helped by a knowledge of the electronic (and charge) distribution, e.g. in the bases [5].

A very valuable method to study oxygenated groups is ^{17}O NMR spectroscopy [7], and both carbonyl and hydroxy groups have been extensively studied by this technique [8–11]. We thus decided to apply such a method to the hydroxypyridone iron chelators, L1 and analogous molecules, as a starting point for a systematic study of actual and possible new iron chelating drugs.

2. Chemistry

Structures of studied compounds are shown in *figure 1* and their ^{17}O NMR spectroscopic data are shown in *table I*. The OH structure has also been used for the groups in positions 2 or 4, without any sign of a preference between the hydroxyl or the carbonyl structure.

^{17}O NMR shifts of the 3-OH group in H_2O/D_2O are quite similar for all the derivatives bearing this group except for **5**. The observed shifts are in the range of the phenols [12–15], albeit **1**–**3** are among the most shielded ones.

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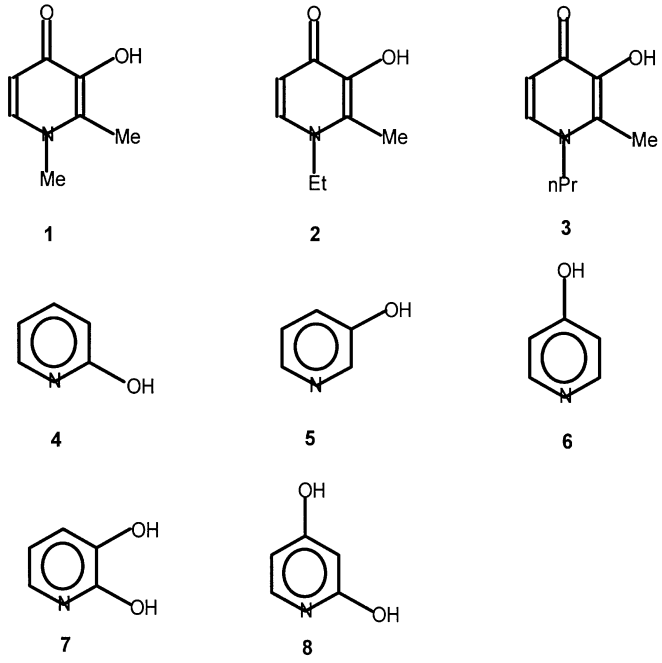


Figure 1. Structures of studied compounds.

The comparatively strong deshielding noted for **5** is only partly due to the lack of an intramolecular hydrogen bond with a contiguous carbonyl group and/or to a different extent of intermolecular hydrogen bonding with the solvent. The intramolecular hydrogen bond between the 3-hydroxy and 4-keto groups of **1**–**3** should scarcely influence the chemical shift of the 3-hydroxy group, within 10–15 ppm, as discussed e.g. in two studies on salicylaldehydes and salicylanilides by Boykin and co-workers [12, 13].

Phenol itself in H_2O has a δ = 77 ppm shift [14]. Its shift ranges, in some solvents, from 73.5 (MeCN) [12] to 83.6 ppm (DMSO-d_6) [15]. All these values are thus more similar among themselves, and to those obtained for **5** in pyridine-d_5 and in $CDCl_3$, than to the shift of **5** in H_2O/D_2O. These data on phenol also suggest that intermolecular hydrogen bonding is not a sufficient cause for the observed deshielding.

Without re-opening a somewhat old and controversial problem [16–20] on the real structure of **5**, we agree on an equilibrium, in H_2O solution, between **5a** and **5b** with an estimated ca. 1:1 ratio [20] *(figure 2)*.

According to the Karplus-Pople equation [21], structure **5b** with an increased electronic density at the oxygen

Table I. ^{17}O NMR chemical shifts (ppm) of studied compounds in H_2O/D_2O, C_5D_5N and $CDCl_3$.[a]

Compound	2 OH (H_2O/D_2O)	2 OH (C_5D_5N)	2 OH ($CDCl_3$)	3 OH (H_2O/D_2O)	3 OH (C_5D_5N)	3 OH ($CDCl_3$)	4 OH (H_2O/D_2O)	4 OH (C_5D_5N)	4 OH ($CDCl_3$)
1				54	53.5	50.6	224	306.2	280.3
2				60	51.0	47.5	229.6	305.0	277.4
3				59	53.6	52.1	227.5	303.0	280.3
4	224.7	268.6	245.7						
5				105.2	77.7	78.0			
6							266.3	251.9	ca. 290
7	ca. 215	252.2	b	ca. 65	69.8	b			
8	218	272	b				104	110	b

a) Linewidths range 200–600 Hz, but for **5** (880 Hz) and **6** (ca. 3 000Hz) in $CDCl_3$ and for **7** and **8** in H_2O/D_2O (not measurable, very faint signals).
b) Insoluble, not measured.

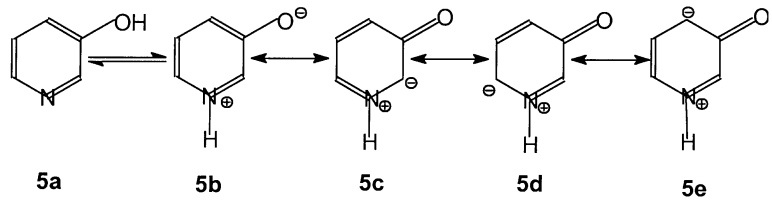
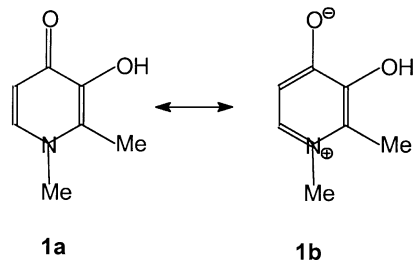


Figure 2. Structures of compound **5**.

atom should cause an upfield shift. The observed deshielding can be due to a considerable weight of resonance for structures **5c**–**e**, which are alike to the commonly accepted resonance structures of the phenol and phenate anion. Actually, sodium phenate in H_2O solution, in a trial experiment, has given a strong deshielding (146 ppm) compared to phenol (ca. 77 ppm). An important C–O double bond character has thus to be taken into account for the observed deshielding of **5**.

Compounds **1**–**3** and **7**, in H_2O/D_2O, for their 3-hydroxy groups, show a narrow shift range, 54–65 ppm, and are thus more shielded than most other phenols [12–15]. Several causes could co-operate to give this result. As discussed, the influence of intramolecular hydrogen bonding can partly account for this shielding, even if a possible inductive effect of the ring nitrogen should operate in an opposite sense. Whilst to the best of our knowledge data for meta-substituted phenols are lacking in the literature, an indication can be obtained from the reported data on anisoles [23]. In their case, if we assume a *meta* nitro group as a comparable substitute of the ring nitrogen of our compounds, a 15 ppm deshielding has been observed. Moreover, *para*-substitued phenols are similarly deshielded by electron-withdrawing groups [12]. The two contributions to the chemical shifts seem opposite and comparable in magnitude. The influence of the adjacent "carbonyl" group is thus apparent, irrespectively of its 2– or 4– position. Its presence causes the resonance structures with a C–O double bond in the 3– position to become negligible, allowing an understanding of the shielding observed compared to other phenols [12–15]. This point is also important for Fe(III) co-ordination, since the hardness as a donor of the oxygen atom is increased.

Derivatives **4** and **6** are known to exist in solution mainly as 2– and 4–pyridones [20]. The ^{17}O NMR spectrum of **4** in MeCN has been reported [24] and its shift (269.5 ppm) is in good agreement with our value in pyridine-d_5 *(table I)*. The observed shifts of the 4-carbonyl group of derivatives **1**–**3** are thus in substantial agreement with the usually accepted pyridone structure of these compounds. We observe that in H_2O/D_2O the C=O groups of **1**–**3** are much more upfield than in the other two solvents, particularly in pyridine-d_5 *(table I)*. On the contrary, 3–OH groups do not show any significant or systematic variation with the solvent. All these observations are coherent with a strong intermolecular hydrogen bond formation, at the carbonyl level, in H_2O/D_2O but without a rupture of the intramolecular hydrogen bond. An alternative and/or complementary



1a **1b**

Figure 3. Resonance formulas of compound **1**.

cause of the observed shielding could be an increased importance of the resonance formulas type **1b** for **1**–**3** *(figure 3)*.

Again, structures type **1b** should increase the hardness of the oxygen atom.

The importance of the intermolecular hydrogen bond is evident by **4**, which shows the same trend of variation of the shifts with all the solvents. Its $\Delta\delta$ values *(table II)* however are smaller than those of the other derivatives. Data for **6** are difficult to interpret. On going from H_2O/D_2O to less polar solvents, a retreat towards the hydroxy tautomer would be predicted [25]. On the other hand, a strong intermolecular hydrogen bonding between the CO group and the solvent is present in H_2O/D_2O and its effect is shielding [26]. The two effects are thus counteracting and it is not possible to know a priori which one will prevail. Moreover, in $CDCl_3$ there is surely an increased importance of self-association, as shown by the quite large value of the line-width of the signal.

Spectra of **8** clearly show that only one of the substituents is in the keto form. In accordance with literature [27], we can assign a 2-pyridone structure at this derivative.

Table II. Chemical shifts differences ($\Delta\delta$)[a] of *CO* groups of selected compounds.

Compound	$\Delta\delta_{P\text{-}W}$ (ppm)	$\Delta\delta_{C\text{-}W}$ (ppm)	$\Delta\delta_{P\text{-}C}$ (ppm)
1	+82.2	+56.3	+25.9
2	+75.4	+47.8	+27.6
3	+75.5	+52.8	+22.7
4	+43.9	+21.0	+22.9
6	–14.4	+23.7	–38.1

a) Subscripts: P–W, chemical shifts difference between C_5D_5N and H_2O/D_2O solutions, C–W, chemical shifts difference between $CDCl_3$ and H_2O/D_2O solutions, P–C, chemical shifts difference between C_5D_5N and $CDCl_3$ solutions.

3. Conclusion

In this report we have shown the potential of ^{17}O NMR spectroscopy, at natural isotopic abundance, for studying the bonds and solvent interactions in small oxygen-containing molecules, like the drug L1. It has been possible to determine that the intramolecular hydrogen bond between the 3–OH and the 4–CO groups in this molecule is strong enough not to be broken even in aqueous solution. The mutual interactions between hydroxyl and carbonyl groups have been examined, obtaining indications of the relative importance of their resonance structures and their influence on their ability to act as Fe(III) complexing agents. These data can thus facilitate both the understanding of the activity of L1 as well as the development of new and more efficient iron chelating drugs. The importance of these points is evident from very recent literature data [28, 29]. A possible hepatotoxicity of L1 has in fact been reported [28] from one of the main groups involved in clinical experimental use of this drug and this point has been challenged in an editorial paper [29], stressing the importance of ascertaining the safety and efficacy of this agent.

4. Experimental

All studied derivatives are known compounds. **1**–**3** have been prepared according to Kontoghiorghes procedure [30]. **4**–**8** were purchased from Aldrich™ and used without further purification.

^{17}O NMR spectra were recorded, in the Fourier transform mode, on a Varian VXR 300 spectrometer equipped with a Sun 3/60 computer and with a 10 mm broad band probe at 65 °C (probe temperature = 338 K) for H_2O/D_2O and pyridine-d_5 solutions and at 45 °C (probe temperature = 318 K) for $CDCl_3$ solutions and at natural isotopic abundance. Saturated solutions were used in all experiments. The instrumental settings were: 40.662 MHz frequency, spectral width 36 KHz, acquisition time 10 ms, pre-acquisition delay 100 μs, pulse angle 90° (pulse width 28 μs). Number of scans varied largely (4×10^4–3×10^6) as a function of solvent and solubility. The spectra were recorded with sample spinning and without lock. The signal to noise ratio was in most cases improved by applying a 30 Hz exponential broadening factor (l.b.) to the FID prior to Fourier transformation. In some experiments (H_2O/D_2O solutions of **1**–**3** and **7** and $CDCl_3$ solution of **6**) an l.b. up to 120 Hz was necessary. The data point resolution was improved by zero filling to 16 K data points. Chemical shifts are expressed in ppm and referred to external tap water by the substitution method. The reproducibility of the chemical shift data is estimated to be ± 1 ppm (± 3 ppm when l.b. = 120 Hz has been used).

Acknowledgements

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