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η^4-Pyrone iron(0)carbonyl complexes as effective CO-releasing molecules (CO-RMs)

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Abstract—The CO-releasing properties of iron(0)tricarbonyl complexes bearing a 2-pyrone ligand have been evaluated. In this report, we demonstrate that the intrinsic stability of the (η^4-2-pyrone)$Fe(CO)_3$ complex influences the extent and rate of CO release, which is affected by the presence of a halogen substituent on the 2-pyrone ring. The cell viability index has been highlighted for the active carbon monoxide-releasing molecules (CO-RMs), demonstrating that these complexes and related derivatives are a promising new class of compounds with potential therapeutic applications.

The development of carbon monoxide-releasing molecules (CO-RMs) has become a recent target for therapeutic intervention.[1] It is well known that CO is generated in mammals during the degradation of heme by constitutive (HO-2) and inducible (HO-1) heme oxygenase enzymes. It is responsible for important physiological functions and is a fundamental signaling mediator.[2] CO possesses vasodilatory properties,[3] controls the proliferation of vascular smooth muscle cells,[4] suppresses the rejection of transplanted hearts,[5] mediates potent anti-inflammatory effect,[6] and promotes protection against ischemic tissue injury.[7] When low concentrations of CO gas are administered, beneficial therapeutic effects have been observed, although the associated toxicity and inherent poor selectivity of CO, as a gas, are clearly not ideal. The method of choice, potentially, for taking advantage of the biological role of CO, is to utilize a 'CO-carrier' such as a metal carbonyl complex. Motterlini et al. have previously shown that certain transition metal carbonyl complexes function as carbon monoxide-releasing molecules (CO-RMs) in biological systems.[8]

Dimanganese decacarbonyl (CORM-1) and tricarbonyldichloro ruthenium(II)-dimer (CORM-2) were the first identified complexes to demonstrate an inherent capacity to liberate CO in biological models and promote relaxation of blood vessels in vitro, attenuate coronary vasoconstriction in isolated hearts, and reduce acute hypertension in vivo.[9] The synthesis of tricarbonylchloro(glycinato)ruthenium(II) (CORM-3), the first prototypic water-soluble CO-RM, confirmed the pharmacological effects of metal carbonyls in mediating protection against ischemia and myocardial infarction,[10] vasodilatation, and hypotension[11] as well as prevention of organ graft rejection following heart transplantation.[10b] More recently, transition metal carbonyls and other classes of CO-RMs have been used to assess the role of CO gas as an important signaling factor in a variety of experimental models.[12] Thus, the identification of other metal carbonyls that have the ability to release CO, and are biologically compatible, will provide important information on the chemistry of these compounds in aqueous environments and implement the design of novel pharmaceuticals for therapeutic purposes.

Here we report on the chemical and biological features of a group of 2-pyrone iron(0)carbonyl complexes. They

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represent the first reported CO-RMs containing iron and an η^4-coordinated diene ligand.

2-Pyrone (2*H*-pyran-2-one) **1**, and related compounds, are ubiquitous in nature (Fig. 1).[13] Natural and non-natural 2-pyrone compounds exhibit diverse bioactivity.[14] For example, substituted 2-pyrones **2** inhibit human ovarian carcinoma (A2780) and human chronic myelogenous leukemia (K562) cell lines at the sub-micromolar level (IC_{50}), exhibiting insignificant toxicity in normal cells.[15] In these cellular systems, the 2-pyrone appears to behave as a pro-drug, where carbonyl ring-opening leads to the bioactive form.[16] 6-Chloro-2-pyrones inhibit yeast cholesterol esterase from *Candida cylindracea* in a similar manner.[17]

Complexation of the 2-pyrone in an η^4-diene-like fashion to an irontricarbonyl unit '$Fe(CO)_3$' serves to activate the 2-pyrone ring-system (Fig. 2). The synthesis of such compounds, although challenging, is made possible by the employment of $Fe_2(CO)_9$[18] or Grevels' reagent,[19] $[Fe(coe)_2(CO)_3]$ (coe = *cis*-cyclooctene).[20]

To rationalize the CO-releasing activity of complexes **3a**–**c**, several new (η^4-2-pyrone)$Fe(CO)_3$ complexes: **3d**,**e**, and **g** were synthesized according to Scheme 1.[21]

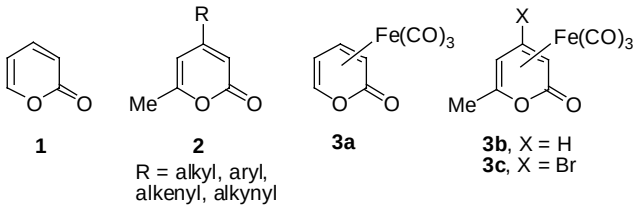


Figure 1. 2-Pyrones and related 2-pyrone metal carbonyl complexes.

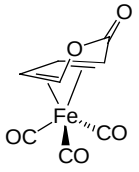
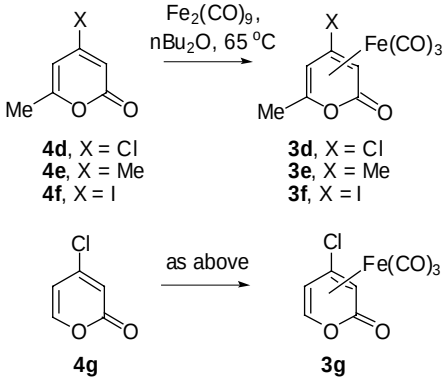


Figure 2. 'Bent-form' of parent 2-pyrone on η^4-coordination to Fe^0.



Scheme 1. Synthesis of novel (η^4-2-pyrone)$Fe(CO)_3$ complexes.

4-Chloro-**4d**, 4-iodo-**4f**, and 4,6-dimethyl-2-pyrone **4e** were reacted with Fe_2CO_9 in *n*-Bu_2O at 65 °C. Complex **3d** was isolated in 13% yield (based on recovered **4d**), and some hydrodechlorination was seen affording **3b** as a minor product (2%).[22] Complex **3e** was produced in 22% yield (based on recovered **4e**). We have previously established that Grevels' reagent may be used to access complexes such as **3c** in higher yield.[20] However, the difficulty in handling this reagent led us to revert to the more traditional preparative method for these preliminary studies.

The coordination mode in the solid state (X-ray),[23] as in the solution state, is very similar to complexes **3a**–**c**.[20] Hydrodeiodonation in **3f** leads to the isolation of **3b** in 7% yield, exclusively. Complexation of 4-chloro-2-pyrone **4g** provides **3g** in 32% yield (based on recovered **4g**) with no observable hydrodechlorination.

The amount of CO released from 2-pyrone carbonyls was assessed by measuring the conversion of deoxymyoglobin (deoxy-Mb) to carbon monoxide myoglobin (MbCO), as previously described.[9–12] The typical spectra of deoxy-Mb and MbCO are represented in Figure 3A. As shown, deoxy-Mb (50 μM) is rapidly converted into MbCO after bubbling CO gas (1%) for 2 min into the solution. A similar profile is observed when deoxy-Mb is reacted with 60 μM **3c** (see Fig. 3B) and the amount of MbCO formed over time is reported in Figure 3C. From the fitted curve it is calculated that the initial rate of CO release from **3c** is approximately 0.19 μM/min. For comparison, under thermal conditions $Fe(CO)_5$ does not result in any appreciable formation of MbCO over a similar period of time—light activation is required to initiate CO release in this case.[9]

The bioactive properties of **3c** were confirmed by examining the effect of this compound on vessel relaxation. Transverse ring sections were prepared from thoracic aortas of male adult Sprague–Dawley rats (350 g) and mounted in an organ bath containing oxygenated (95% O_2 and 5% CO_2) Krebs–Henseleit buffer.[11] The extent of vasorelaxation over time elicited by two consecutive additions of **3c** (100 μM) was assessed in aortic rings pre-contracted with phenylephrine (1 μM). As shown in Figure 3D, **3c** produced a 42% relaxation after the first addition and a further 35% decrease in tension was observed following the second addition. These results are in agreement with the vasodilatory role of CO liberated from primary transition metal carbonyl sources, for example, $Mn_2(CO)_{10}$.[8,9] The potential toxic effects of CO-RMs that were capable of releasing CO were also tested. RAW246.7 murine macrophages (involved in immune response) were incubated for 24 h and the cell viability was determined using an Alamar blue assay as previously described.[24]

As shown in Table 1, the IC_{10} (concentration at which 10% of cells are not viable) for **3c** is 132 μM. This indicates that the concentration of **3c** used for causing vasorelaxation (100 μM) is not toxic and confirms that the pharmacological effect of **3c** is due to the liberated CO. In addition, the small degree of toxicity caused by

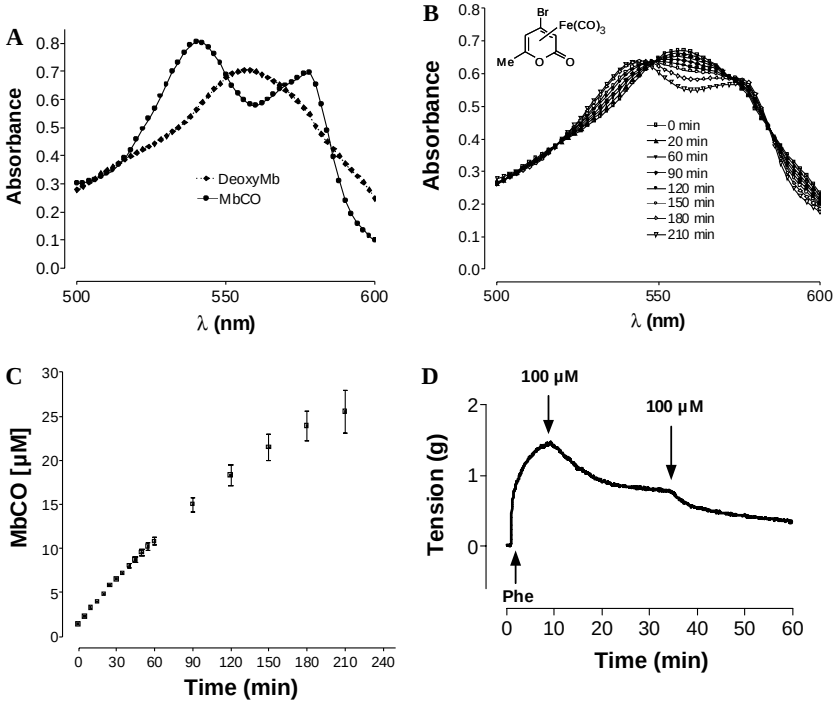


Figure 3. Rate of CO release and vasodilatory properties of **3c**. The release of CO from **3c** was detected by measuring the conversion of deoxy-Mb to MbCO (see A). **3c** (60 μM) was added to a phosphate buffer solution (pH 7.4), the spectra recorded (see B), and the formation of MbCO quantified over time (see C). The vasorelaxation properties of **3c** were evaluated after addition to an isolated rat aortic ring preparation pre-contracted with phenylephrine Phe (see D).

Table 1. Amount of CO released from 2-pyrone complexes and their effect on cell viability[a]

Compound	CO released (μM) after 5 min	CO released (μM) after 60 min	IC_{10} (μM)
3a	0	0	NT
3b	0	0	NT
3c	2.3 (±0.1)	10.8 (±0.4)	132
3d	0.8 (±0.1)	3.1 (±0.4)	89
3e	0	0	NT
3g	0.8 (±0.3)	1.7 (±0.7)	95

[a] The 2-pyrone complexes (60 μM) were added to a solution of phosphate buffer containing deoxy-myoglobin (50 μM) and MbCO was measured after 5 and 60 min. IC_{10}, the concentration at which 10% of cells are not viable, was also tested in cultured RAW264.7 murine macrophages. Data are expressed as means ± SEM of three independent experiments for CO release and six independent experiments for cell viability. NT = not tested.

3c is in the same order of magnitude of a previously reported CO-RM, tricarbonylchloro(glycinato) ruthenium(II) (CORM-3).[10]

Other compounds that released CO were CO-RMs **3d** and **g**; after 60 min, the CO released was 3.1 and 1.7 μM, respectively. The cell viability index (IC_{10}) of these compounds is also included for comparison. Complexes **3a**,**b**, and **e** did not release any detectable CO.

Clear differences exist between the CO-RMs evaluated. A comparison of **3b** and **c** demonstrates the importance of the bromide substituent at the 4-position of the 2-pyrone. The CO release parallels the qualitative observation that in $CDCl_3$ solution, **3b** is more stable than **3c**.[20]

Alteration of this substituent to a chloride **3d** shows a notable loss in CO-releasing activity. Replacement of the halogen for a methyl substituent **3e** results in complete loss of CO-releasing activity, whereas removal of the methyl group at the 6-position **3g** results in some loss of activity (compare with **3d**; after 60 min, 3.1 μM for **3d** and 1.7 μM for **3g**). Unfortunately, we have thus far been unable to access the irontricarbonyl complex of 4-bromo-2-pyrone for comparison with **3c**.[25]

It should be noted that CO release from the uncomplexed 2-pyrones, under the experimental conditions employed, is not observed—the CO released originates from CO ligated to iron (in this series of compounds).

The clear advantages in the employment of (η^4-2-pyrone)$Fe(CO)_3$ complexes are: (1) their decreased toxicity, particularly when compared against the general toxicological data available for $Fe(CO)_5$ and $Fe_2(CO)_9$; (2) stability in the solid state; (3) the potential changes possible through alteration of the steric properties of the 2-pyrone ligand. Furthermore, $Fe(CO)_5$ being highly flammable is difficult to handle and is light and air sensitive. Crucially, the biological compatibility of the 2-pyrone makes it an ideal choice as an organic ligand, and presumably as a leaving group, as its degradation pathways are established.[13,26]

The finding that non-halogenated 2-pyrone ironcarbonyl complexes do not release CO under thermal conditions suggests that in these systems, the halogen substituent is required for spontaneously controlled CO release activity.

In agreement with the nomenclature now in use for classifying bioactive CO-carriers, compound **3c** has been termed CORM-F3, as it is the first iron-containing compound that shows a good CO release profile and promising biological activities.

In summary, we have detailed the first CO-releasing properties of iron(0)tricarbonyl complexes bearing a 2-pyrone ligand. It appears that the intrinsic stability of the (η^4-2-pyrone)$Fe(CO)_3$ complex influences the extent and rate of CO release, a feature which is greatly affected by the presence of a halogen substituent on the 2-pyrone ring. The cell viability index has been highlighted for the active CO-RMs, demonstrating that these complexes and related derivatives are a promising new class of carbon monoxide-releasing molecules with potential therapeutic applications.

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22. The low yields of the desired complex **3d** are due to the limited reactivity of the halogenated 2-pyrone with Fe_2CO_9. There is a subtle balance between further reaction to produce a higher yield of **3d** and the dehydrohalogenation side reaction to give **3b**, that is, extended reaction times produce increasing quantities of **3b**. We also note the formation of Fe_3CO_{12} (dark green) in these reactions which elutes with hexane on a silica column (Note: Fe_2CO_9 sublimes from the crude mixture of **3d**/**3b**/Fe_2CO_9 and Fe_3CO_{12}). No other iron-containing species have been detected in these reactions, but we can safely assume that iron halide complexes (uncharacterized) are formed in the hydrodehalogenation process.
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