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Group VI transition metal carbonyl hydrosulfides $Na[M(CO)_5(SH)]$ (M = Cr, Mo, W) as water-soluble H_2S-releasing agents

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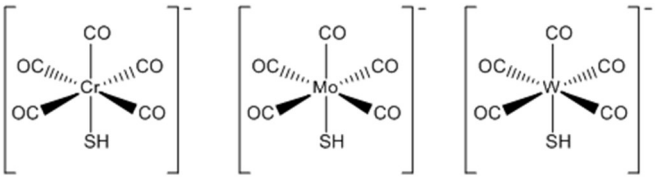
A class of water-soluble Group 6 transition metal carbonyl complexes carrying the SH^- ligand $Na[M(CO)_5(SH)]$ (M = Cr, Mo, W) is prepared and shown to release H_2S under mild conditions with no decarbonylation. Preliminary studies on mammary epithelial cells have shown that both $Na[Mo(CO)_5SH]$ reactant and $Mo(CO)_5$(solvent) product are non-cytotoxic.

The noxious gas, hydrogen sulfide (H_2S), commonly formed by commensal bacteria, has been shown to be a biologically important signalling molecule involving in a myriad of cellular processes such as muscle relaxation[1], neuromodulation[2], blood pressure regulation and insulin release[3]. In mammalian tissues, H_2S is synthesized from L-cysteine via cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE) enzymes. Together with nitric oxide (NO) and carbon monoxide (CO), these three gases form a class of endogenous messenger molecules collectively known as gasotransmitters[4]. The role of hydrogen sulfide as a small messenger molecule mirrors that of NO as both transmit information via the modification of sulfhydryl groups on the cysteine residues of their respective target proteins. In the case of H_2S, this modification is termed protein sulfhydration.

The extensive development of CO- and NO-releasing molecules enables the effects of these gasotransmitters on the cells and tissues to be examined. However, to date, H_2S-releasing molecules have been limited to light-activated organic dithiolate compounds such as *N*-(benzoylthio)benzamide and persulfide-based derivatives[5], simple organic sulfides such as diallyl disulfide and diallyl trisulfide[6] or inorganic salts, such as sodium hydrosulfide (NaSH). For example NaSH releases H_2S in an uncontrolled manner under aqueous conditions while endogenous H_2S is produced at a much slower rate and at a lower concentration. Hence, NaSH does not accurately mimic the effects of naturally-produced endogenous H_2S. Not only that, research progress with H_2S is impeded by the reactivity of free gaseous H_2S with atmospheric oxygen forming different oxidative products and rendering the storage, manipulation and production of the gas particularly challenging. In addition, inhalation as a delivery option is also not viable. If H_2S is not suitably administered, the gas causes not only nausea and irritation of the mucus lining but is also potentially lethal[7]. To circumvent this problem, the search for water-soluble molecules that can release free hydrogen sulfide gas in a controlled fashion continues to be actively pursued by many research groups[8]. Herein we report the synthesis and characterisation of a series of water-soluble Group VI transition metal carbonyl hydrosulfides capable of releasing free H_2S in a controlled manner upon hydrolysis.

The Group 6 transition metal carbonyl hydrosulfide complexes $Na[M(CO)_5SH]$ (M = Cr, Mo, W; Scheme 1) were prepared by a modification of a previously published procedure[9]. Instead of a thermal reaction, a methanol solution containing the transition metal hexacarbonyl, $M(CO)_6$ with sodium hydrosulfide (NaSH) was irradiated under a broadband UV source. The ν_{CO} IR bands obtained for the resulting complex suggests a pattern typical of C_{4v} symmetry, albeit being red-shifted due to the negative charge on the metal complex (Figure 1). The uv-visible spectra of the complexes show broad absorption down to 600-700 nm. ESI mass spectrometry carried out on all three complexes shows the characteristic molecular ion signal, M^- along with signals corresponding to CO loss species from the parent ion. All three $Na[M(CO)_5SH]$ complexes are water-soluble.

It is worth noting that under thermal conditions, the dinuclear-bridged $Na[\mu\text{-}HS[M(CO)_5]_2]$ are generated instead, consistent with previous studies[9]. Under photolytic conditions, the infrared data taken immediately after syntheses showed the presence of the monomeric complexes only. However $Na[Cr(CO)_5SH]$ appears to convert to its dimer after several hours, based on mass spectrometry evidence (see Supporting Information). Hence the three complexes should be used immediately after syntheses for reliable comparison of their reactivity.



Scheme 1 Structures of the three Group 6 transition metal hydrosulfides.

Comparing the three metal hydrosulfides, $Na[Mo(CO)_5SH]$ appears to be most stable as a solid in air, hence it is chosen for more detailed studies. When dissolved in water or wet methanol, the complex slowly hydrolyses at room temperature, although the hydrolysis can be accelerated by the addition of strong acids. Regardless of the acid strength, $Mo(CO)_5(MeOH)$ is found to be the major product in methanol solvent, as characterised by its IR and uv-visible absorption spectra (Figure 1b). Although the IR spectra of the product could not be obtained in water, comparison of the UV-visible spectrum of $Mo(CO)_5(MeOH)$ spectrum in methanol with that of the species formed after acidification suggests that $Mo(CO)_5(H_2O)$ is the most likely product in aqueous solution[10].

We have also used pH changes of an aqueous solution of NaSH to be an indicator of H_2S gas formation since pH is expected to increase due to the concomitant formation of sodium hydroxide;

$$NaSH + H_2O \rightleftharpoons NaOH + H_2S$$

Although HS^- is a weak base (pKa ≈7), it can be protonated in water. Evolution of H_2S, aided by its low water-solubility shifts the equilibrium position to the right in accordance with Le Chatelier's Principle. Indeed, the pH of the solution is determined to be around 11 only a few minutes after NaSH hydrolysis in water. The contribution of the sparingly-soluble H_2S gas to pH changes in water is negligible due to its low solubility[11]. By inference, metal hydrosulfides are expected to undergo a similar reaction;

$$Na[Mo(CO)_5SH] + H_2O \rightleftharpoons NaOH + Mo(CO)_5(H_2O) + H_2S$$

However the pH increase is much slower and reaching similar values only after 60 minutes for the same concentration of $Na[Mo(CO)_5SH]$ as NaSH (see Figure 2).

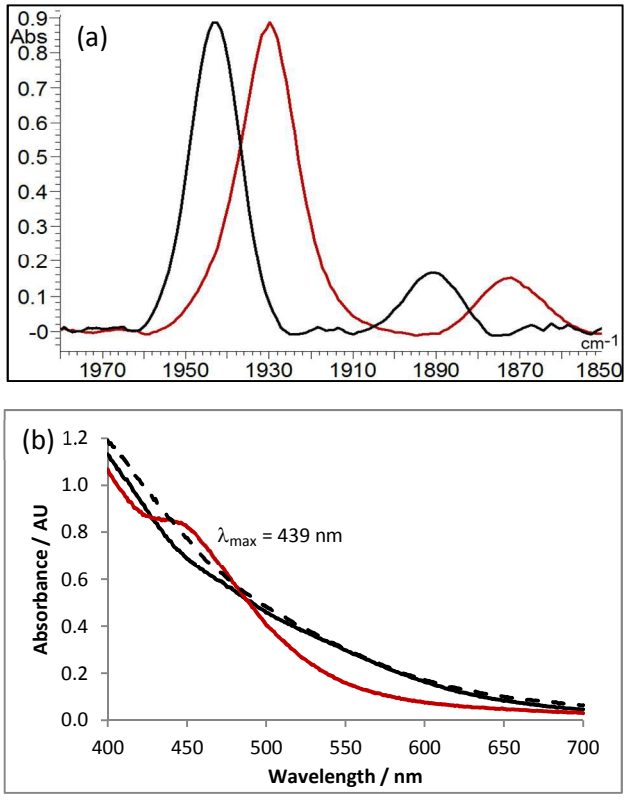


Fig. 1 (a) Infrared spectra of $Na[Mo(CO)_5SH]$ (red) and $[Mo(CO)_5(MeOH)]$ (black) in methanol. (b) UV-visible spectra of $Na[Mo(CO)_5SH]$ (red), $[Mo(CO)_5(MeOH)]$ (black) in methanol and $[Mo(CO)_5(H_2O)]$ (black dashed) in water.

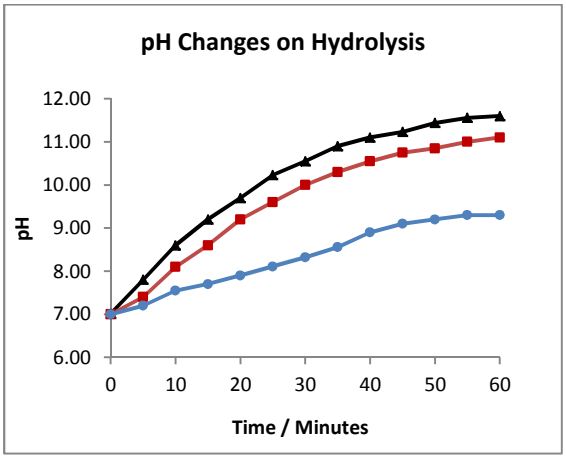


Fig. 2 Changes in pH upon hydrolysis of the Cr(black), Mo(red) and W(blue) hydrosulfides as monitored using pH meter over a period of one hour in water.

The release of hydrogen sulfide gas from $Na[Mo(CO)_5SH]$ has been detected quantitatively via gas-phase infrared spectroscopy by monitoring its intense bending vibrational mode centred at 1180 cm^{-1} in the headspace above the aqueous solution (Figure 2). For the IR measurements, the headspace was directly connected to a 15cm-long gas cell equipped with CaF_2 windows for IR transmission. Since the solubility of H_2S in water is very low[11], most of the gas would be released into the headspace hence minimising the error in determining its concentration. This method also allows continuous monitoring of the gas *in situ* without the need to remove aliquots from the solution. The absorbance of the band is first calibrated by using a standard pressure of H_2S generated stoichiometrically from the reaction of a strong acid on a known quantity of solid NaSH. Care is taken such that the absorbance is proportional to the concentration according to Beer's law[12].

As seen in Figure 3, the release of H_2S in the dark occurs gradually over a period of 40 minutes for $Na[Mo(CO)_5SH]$ in a pH 6.5 phosphate buffer solution. The rate of H_2S production reaches a plateau close to one mole equivalent of the amount of $Na[Mo(CO)_5SH]$ present initially in the aqueous solution. Interestingly, no CO spectrum (ν_0=2143cm^{-1}) was detected which indicates that the acidification process only affects the SH^- ligand. However if the mixture is subjected to photolysis as well, the CO signals will begin to emerge. The result of the work conducted in the dark is also consistent with $Mo(CO)_5(H_2O)$ being the major product since both reactant and product contain the same number of CO ligands. Thus $Na[Mo(CO)_5SH]$ acts purely as a H_2S-releasing molecule and not as a CORM (CO-releasing molecule) under ambient non-photolytic conditions.

From the results obtained via infrared and pH measurements, it can be proposed that the release of H_2S from the three metal hydrosulfides follows the order Cr ≈ Mo > W. According to rate of evolution of H_2S as monitored via IR spectroscopy in Figure 3, the chromium complex releases H_2S fully in 25 minutes; the molybdenum complex requires 40 minutes and tungsten complex takes much more than an hour. The pH measurements in Fig. 2 lend further support to the proposed trend in the rate of hydrolysis of the metal hydrosulfides. The slower rate of H_2S evolution together with non-quantitative release may be due to the stronger W-SH binding compared to the 1st and 2nd row congeners[13]. Alternatively, the

stronger W-L interaction may result in some of the evolved H_2S binding to the metal to generate $W(CO)_5(H_2S)$ thereby resulting in non-quantitative H_2S release in this case.

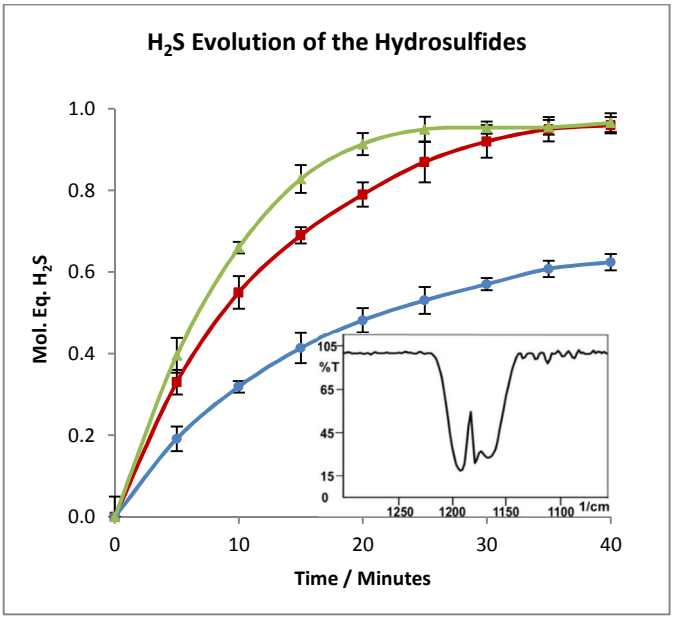


Fig. 3 Changes in IR absorbance of H_2S release of Mo(red), Cr(green) and W(blue) vs time in a pH 6.5 phosphate buffer solution containing 0.005M of $Na[M(CO)_5SH]$. Inset: Gas phase IR spectrum showing the P, Q, R branches of H_2S

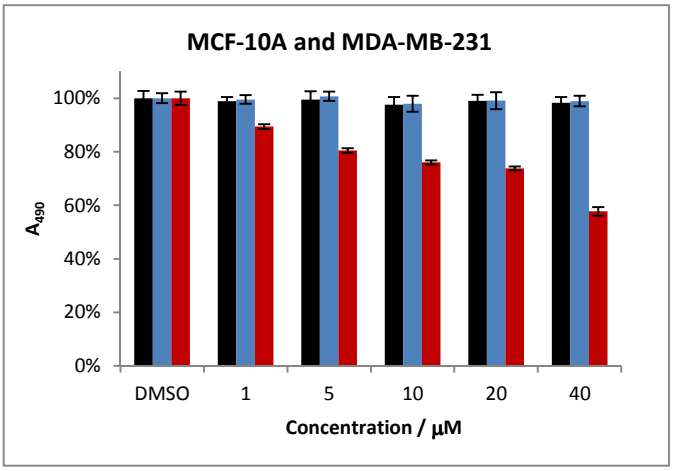


Fig. 4 Viability of mammary epithelial cells MCF-10A (■) and breast cancer cell MDA-MB-231 (■) after 24 h incubation with $Na[Mo(CO)_5SH]$, as determined by the MTS assay. Incubation was also performed with $[Mo(CO)_5(MeOH)]$ on MCF-10A only (■). Cell viabilities have been normalized with DMSO as a control.

We have also evaluated the anti-cancer and cytotoxic properties of $Na[Mo(CO)_5SH]$ towards MCF-10A mammary epithelial cells and well as MDA-MB-231 breast adenocarcinoma cells. Treatment of the cells with concentrations ranging from 1µM to 40µM of the molybdenum hydrosulfide did not lead to any observable cytotoxicity over a period of 24 hours, as concluded from the MTS assay (Fig. 4). Interestingly, $Na[Mo(CO)_5SH]$ exhibited anti-proliferative properties towards the cancer cells, although the established IC50 value is in the mid-micromolar range. We believe that these metal hydrosulfides should have minimal undesirable effect on the cells during the duration of H_2S release in the cell based on our preliminary data. More importantly, the effect of the product as exemplified by $Mo(CO)_5(MeOH)$ on MCF-10A normal cells was also tested and found to be non-toxic over the concentration range and conditions (blue bar in Figure 4).

In conclusion, a class of Group 6 metal hydrosulfides that is capable of releasing hydrogen sulfide gas in a controlled manner has been synthesized and characterized. These complexes are stable in the solid state, are water-soluble, and do not exhibit cytotoxicity towards mammary epithelial cells. In addition, they do not release their CO ligands under the conditions employed for the release of H_2S. These complexes can indeed serve as selective hydrogen-sulfide releasing molecules. Infrared, UV-visible and pH measurements were employed to monitor the hydrogen sulfide release and all three methods placed the rates of H_2S release in the order Cr ≈ Mo > W.

Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental procedure, analytical data for synthesized complexes and cytotoxicity studies. See DOI: 10.1039/c000000x/

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Table of content entry

Group VI metal carbonyl hydrosulfides as water-soluble H_2S-releasing molecules