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## Abstract

Potentiometric study of phenacyl alcohol reveals it to form a 2:1 complex with calcium in aqueous solution, with a formation constant ($\log_{10} \beta$) of 3.93 at 37 °C. In contrast, magnesium appears not to form any complexes under analogous conditions, while strontium and barium form complexes of both 1:1 and 2:1 ligand:metal stoichiometry. The efficiency of complexation of calcium by this simple α-hydroxy ketone and its high ability to discriminate between calcium and magnesium are both rather remarkable given the simplicity of its binding site.

## Introduction

Although the anthracycline-type antibiotics (*e.g.*, tetracycline), the anti-inflammatory corticosteroids (*e.g.*, hydrocortisone), and the promising anti-tumor agent, doxorubicin (adriamycin), appear to present greater structural diversity than similarity (Figure 1), each offers the possibility for interaction with metal ions through a variety of potential chelate sites. Common to each of these drugs and their analogs is the α-hydroxy ketone functionality, and it is for this reason that we have initiated an exploration of the metal chelation ability of this functional group. As magnesium and calcium play significant roles in numerous biological processes[1] and are present in comparatively high concentration in both serum and intercellular fluids,[2] we have directed our attention to the study of the interactions of both simple model compounds and pharmacologically relevant α-hydroxy ketones with the group 2 metal ions.

Calcium and other group 2 metal ion complexation by tetracycline and its derivatives has received a fair bit of attention,[3-11] and selective calcium chelation by the α-hydroxy ketone functionality has been demonstrated for some anthracyclines.[12] Interest in this area has been driven in large part by the demonstrated physiological interplay between tetracycline and calcium, leading to such effects as altered drug uptake and distribution,[13] decreased bacteriostatic action,[14] tooth staining in newborns of women administered the drug during the second or third trimesters of pregnancy and in children,[15] and inhibition of osteogenesis and mineralization in developing bone tissue.[16]

Much less studied are the corticosteroid metal complexes, although a number of suggestive physiological links between these therapeutic agents and calcium are apparent, including increased bone resorption in patients receiving long-term corticosteroid treatment, possible leading to the disruption of calcium homeostasis and osteoporosis,[17,18] impairment of intestinal calcium uptake,[19,20] and alteration of receptor binding[21] and cell

membrane transport[22] of hydrocortisone upon calcium chelation. In addition, physiological levels of calcium increase the water solubility of hydrocortisone,[23] and given the critical dependence of serum concentration (and thus dosage) on drug solubility, this issue merits further investigation. In a non-physiological study, calcium chelation was also inferred to influence the sterocontrol of the chemical reduction of corticosteroids.[24]

Adriamycin has been used in the treatment of certain types of cancers since the early 1960's. Little is known about specific interactions between adriamycin and calcium, but it has been demonstrated that both adriamycin and the related anthracycline-type antitumor antibiotic, daunomycin, inhibit calcium transport in cells.[25]

Our earlier studies[26] have demonstrated the ability of phenacyl alcohol (2-hydroxy-1-phenyl-1-ethanone, α-hydroxyacetophenone), chosen as a convenient and structurally simple α-hydroxy ketone, to complex calcium ion in non-aqueous solution, and have elucidated the solid-state structural details of the resulting complex. We now report quantitative studies of the stoichiometry, efficiency, and selectivity of complexation of the group 2 metal ions by phenacyl alcohol in aqueous solution.

## Experimental Section

**Materials**. Phenacyl alcohol was prepared as described[26] and recrystallized three times from water, affording material of mp 85-86 °C, then stored under an inert atmosphere. All other reagents, of >99.9% purity, were obtained commercially (Aldrich Chemical CO.), dried *in vacuo*, and stored under an inert atmosphere. Potassium hydroxide solutions were prepared with deionized (Barnstead) water and titrated with a standard HCl solution, using an Orion model 720A pH/EMF meter. These solutions were stored under soda lime and used within 48 h, as Gran plots[27] showed > 0.5% $CO_2$ contamination in solutions stored for longer periods of time.

**$pK_a$ and Metal Affinity Determinations**. At least five duplicate titrations were carried out for each determination, using the Orion model 720A pH/EMF meter, and Orion 91-01 Ag/AgCl internal reference half cell pH electrode and an Orion 90-01 single junction Ag/AgCl reference electrode. Potassium chloride (0.5 M) was used as the background electrolyte,[28] and the electrode system was calibrated at this concentration and 37 °C. The titration vessel, consisting of a sealed 100 mL jacketed beaker with entry ports for the electrodes, a digital thermometer probe, and the titration burette, and inlet and outlet ports for nitrogen, was maintained at 37.0 ± 0.2 °C by a thermostatted bath. The temperature of the titration mixture was monitored with a VWR 4000 NIST Traceable digital thermometer.

The $pK_a$ of phenacyl alcohol was determined by titration of a solution of *ca.* 0.100 g of phenacyl alcohol (0.734 mmol), 0.35 - 0.45 g of KCl (4.7 - 6.0 mmol), and 2.00 mL of 0.097 M HCl in deionized water (total solution volume 30.00 mL) with 0.12 - 0.14 M KOH. Establishment of both temperature and pH equilibrium was monitored during all titrations; near the inflection point, equilibration required up to *ca.* 30 min. The ion product of water ($pK_w$) was calculated to be 13.625 at 37 °C.[29,30,31] Metal affinity determinations were carried out analogously, but in the presence of the metal chloride under study at *ca.* 20 mM concentration. Average concentrations and pH ranges used for the potentiometric titrations are summarized in Table 1, and a summary of $pK_a$ and affinity values, as calculated from the titration data using the PKAS and BEST computer programs,[32] is presented in Table 2.

## Results and Discussion

Our earlier structural studies of the phenacyl alcohol complex of calcium chloride demonstrated a 2:1 ligand:metal stoichiometry, and this stoichiometry appeared on the basis of NMR titration experiments to be maintained in chloroform and methanol solution as

well.[26] The potentiometric analyses reported in the present report were carried out in an aqueous medium in order to mimic physiological conditions, and this solvent change, as well as the use of group 2 metals other than calcium, necessitated analysis of the solution speciation profiles for phenacyl alcohol with each of the group 2 metals studied. Most importantly, the quantitative determination of speciation profiles is critical for successful potentiometric determination of binding affinities, as calculated affinities are highly dependent upon speciation.

Potentiometric titration of phenacyl alcohol afforded a $pK_a$ (required data for the computational analysis of metal binding affinities) of 12.96 at 37 °C. Although perhaps surprisingly low, this value compares favorably with the value of 13.33 at 25 °C calculated using Taft constants and vibrational frequency analysis.[33] Confirmation that alcohol deprotonation is responsible for the observed $pK_a$ was obtained through potentiometric analysis of the corresponding methyl ether (*i.e.*, 2-methoxyacetophenone), which displayed a much higher $pK_a$ (18.55) consistent with methylene group deprotonation.

Starting with a speciation set comprising all combinations of protonated and deprotonated 1:1 and 2:1 ligand:metal complexes, free metal ions and their mono- and dihydroxides, free hydroxide, and free protons, species were systematically added and deleted while calculationally assaying the goodness of fit between the observed titration data and those calculated for each particular speciation set. Calculations were performed using the computer programs PKAS and BEST,[32] which minimize the standard deviation of fit between the observed and calculated pH values by solving a set of mass balance equations for each titration point. Initial speciation sets for each of the metal ions in this study included the metal hydroxides, as the group 2 metals readily form hydrolysis complexes in water.[34] Final speciation sets, summarized in Table 3 and graphically represented in Figures 2-4,[35] in which phenacyl alcohol is represended by LH, were found

in the case of magnesium to include only the monohydroxide, consistent with literature reports that only $Mg(OH)^+$ is formed at low concentrations.[34]

Complexation of magnesium by phenacyl alcohol appears to be negligible under all conditions examined. However, phenacyl alcohol displays a readily measurable affinity for calcium (Table 2). The dominant calcium complex formed in aqueous solution is of 2:1 ligand:metal stoichiometry, in accord with our earlier solid-state and nonaqueous solution studies. Strontium and barium form significant amounts of both 1:1 and 2:1 ligand:metal complexes with phenacyl alcohol, with the 2:1 barium complex representing that of highest formation constant (Table 2).

Comparison of these results with those obtained for the far more extensively studied and structurally related α-hydroxy carboxylates is illustrative. While simple carboxylates[36] generally display formation constants with the group 2 metals which decrease monotonically with increasing ionic radius,[37] α-hydroxy carboxylates display highest formation constants for calcium. This reversal of selectivity for calcium *vs.* magnesium is reportedly due to a change in chelation mode, with the smaller magnesium ion bound only to the carboxylate group, while the larger calcium (and strontium and barium) ion binds through an "α-chelation" mode[38] to the carboxylate and α-hydroxy groups.[39] In the case of phenacyl alcohol, magnesium is apparently too small to be chelated efficiently by the α-hydroxy ketone functionality and interacts too weakly for detection, if at all, with the (monodentate) alcohol or ketone functionalities. The calcium ion apparently provides the most optimal "fit" to the phenacyl alcohol chelate ring, as demonstrated by the near-ideal bond angles and distances found in the solid-state structure of the 2:1 complex, but may suffer from some ligand-ligand steric interactions.[40] Relief of the latter through chelation to the progressively larger strontium and barium ions, with this steric relief played off against loss of the optimum chelate ring "fit" observed for calcium, may account for the

increased affinity for barium *vs.* calcium. Comparison of the formation constants for the 1:1 strontium and barium complexes, in which such ligand-ligand steric repulsion is not present, supports this contention - the formation constants are nearly identical (Table 2).

Although the formation constant for the 2:1 calcium complex (log $\beta = 3.93$), is rather modest in comparison with well-known calcium binders such as EDTA[41] and the polyether antibiotic A23187 (calcimycin),[42] it is quite remarkable in comparison to other bidentate chelators (Table 4).[43] Indeed, the formation constant for the calcium complex of phenacyl alcohol substantially exceeds that of the tridentate chelator, iminodiacetate.[41] The fact that phenacyl alcohol displays such efficient binding even in aqueous solution, which usually results in diminished binding constants when compared to nonaqueous solvents,[44] is also noteworthy. Equally noteworthy is the high level of discrimination between calcium and magnesium displayed by this remarkably simple chelating agent, with comparable to superior Ca/Mg selectivity (as well as Ca/Sr and Ca/Ba) to far more complex designed calcium binding agents,[45] suggesting potential use of the α-hydroxyketone unit as a specific recognition site in a new generation of calcium-selective synthetic ionophores.

## Summary

The 2:1 ligand:calcium stoichiometry revealed in our earlier solid-state and organic solvent studies of phenacyl alcohol is maintained in aqueous solution, while strontium and barium form detectable amounts of complexes of both 1:1 and 2:1 ligand:metal stoichiometry. Phenacyl alcohol displays both a remarkable calcium affinity and a remarkable level of discrimination between calcium and magnesium in aqueous solution, with magnesium forming no detectable complexes. Fairly high pH is required to form significant quantities of the metal complexes in aqueous solution, although our earlier studies demonstrated formation of the 2:1 calcium complex in neutral chloroform and methanol, suggesting that calcium complexes of α-hydroxy ketones may be accessible

under near-physiological conditions. Results of our studies of the corticosteroids and of synthetic ionophores based on the α-hydroxy ketone functionality will be presented in due course.

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Table 1. Average Concentrations and pH Ranges for $pK_a$ and Formation Constant Determinations for Phenacyl Alcohol

| Titration Type | Average Initial Concentration ($mol\text{-}L^{-1} \times 10^{-2}$) | | Average pH Range | Number of Data Points |
|---|---|---|---|---|
| | Metal | Phenacyl Alcohol | | |
| pKa | - | 2.32 | 2.27 - 11.19 | 28 |
| $Mg^{2+}$ | 2.07 | 2.63 | 2.30 - 11.46 | 30 |
| $Ca^{2+}$ | 2.22 | 2.28 | 2.18 - 11.04 | 30 |
| $Sr^{2+}$ | 2.46 | 2.32 | 2.21 - 11.47 | 29 |
| $Ba^{2+}$ | 2.28 | 2.38 | 2.28 - 11.50 | 32 |

Table 2. Formation Constants for 1:1 and 2:1 Complexes of Phenacyl Alcohol with Group 2 Metals

| Metal | Formation Constant ($\log_{10} \beta$) | | Standard Deviation |
|---|---|---|---|
| | ML | $ML_2$ | |
| $Mg^{2+}$ | not detected | not detected | - |
| $Ca^{2+}$ | not detected | 3.93 | ± 0.03 |
| $Sr^{2+}$ | 2.48 | 2.87 | ± 0.02 |
| $Ba^{2+}$ | 2.24 | 4.82 | ± 0.03 |

Table 3. Speciation Summary for Phenacyl Alcohol (LH) with Group 2 Metals

| Metal | Speciation |
|---|---|
| $Mg^{2+}$ | $H^+$, $OH^-$, $MgOH^+$, $L^-$, LH |
| $Ca^{2+}$ | $H^+$, $OH^-$, $Ca(OH)_2$, $L^-$, LH, $CaL_2$ |
| $Sr^{2+}$ | $H^+$, $OH^-$, $SrOH^+$, $Sr(OH)_2$, $L^-$, LH, $SrL^+$, $SrL_2$ |
| $Ba^{2+}$ | $H^+$, $OH^-$, $BaOH^+$, $Ba(OH)_2$, $L^-$, LH, $BaL^+$, $BaL_2$ |

Table 4. Representative Calcium and Magnesium Complex Formation Constants (β)

| Chelator | log β ($Ca^{2+}$) | log β ($Mg^{2+}$) | Reference |
|---|---|---|---|
| $CH_3CO_2H$ | 1.18 | 1.27 | 43 |
| $H_2NCH(CH_3)CO_2H$ | 1.24 | 1.96 | 43 |
| $H_2NCH_2CO_2H$ | 1.39 | ~2 | 43 |
| $HOCH_2CO_2H$ | 1.62 | 1.33 | 43 |
| $CH_3CONHOH$ | 2.4 | NR[a] | 43 |
| $NH(CH_2CO_2H)_2$ | 2.6 | 3.0 | 41 |
| $O(CH_2CO_2H)_2$ | 3.4 | 1.8 | 41 |
| phenacyl alcohol | 3.93 | - | this work |
| A23187 | 6.5 | 6.9 | 42 |
| EDTA | 10.6 | 8.8 | 41 |

[a]NR = Not reported.

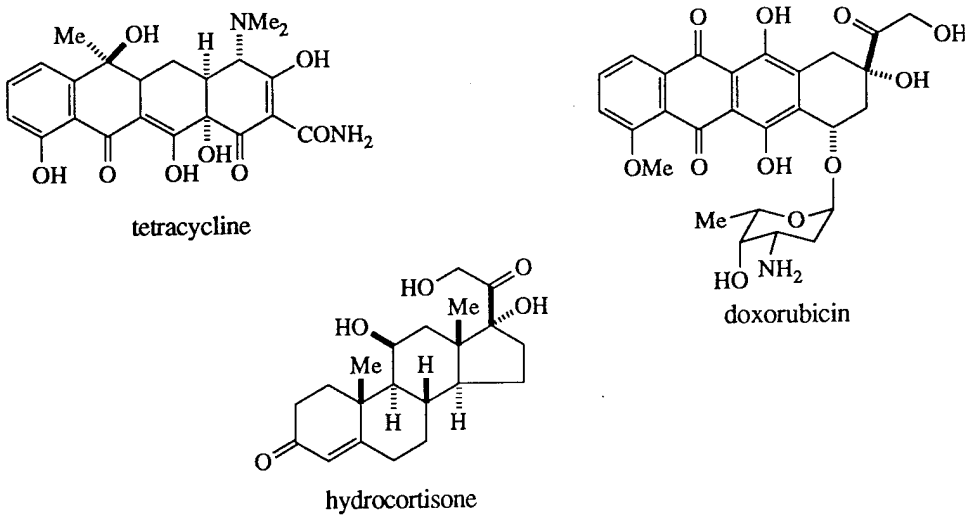
## Figure Captions

Figure 1. Representative Pharmaceuticals Bearing the α-Hydroxy Ketone Functionality

Figure 2. Speciation Plot for the Phenacyl Alcohol - Calcium System

Figure 3. Speciation Plot for the Phenacyl Alcohol - Strontium System

Figure 4. Speciation Plot for the Phenacyl Alcohol - Barium System

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