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Calcium Complexation by Corticosteroids

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Cortisone and hydrocortisone form calcium complexes of 2:1 steroid/Ca^{2+} stoichiometry in solution, with formation constants of 201 ± 1.7 and 184 ± 1.7 M^{-2}, respectively—roughly half the value seen for a simple aromatic α-hydroxy ketone, phenacyl alcohol (341 ± 10.0 M^{-2}). X-ray crystallographic analysis demonstrates maintenance of this 2:1 stoichiometry in the solid state for the phenacyl alcohol and cortisone complexes, while the hydrocortisone complex crystallizes with a 1:1 stoichiometry in the form of linear chains, with the α-hydroxy ketone ligand and C(3) (A-ring) carbonyl groups binding two different calcium ions. In each complex, both in solution and in the solid state, the α-hydroxy ketone moiety serves as a bidentate chelator for calcium. Extensive hydrogen-bonded networks are present in each structure, linking various hydroxyl groups, coordinated and noncoordinated hydrate molecules, and the chloride counterions, in essence representing supramolecular complexation of the hydrated chloride anion.

Introduction

Given the critical role played by calcium in myriad biological processes,[1,2] the study of calcium complexation has become of increasing importance in the understanding of the mechanisms by which these processes occur.[3−6] Many naturally occurring[7] and synthetic[8] chelating agents for calcium have been reported, and a variety of structural motifs leading to efficient complexation of calcium have been revealed.

Robinson et al., as a tangential side note accompanying the synthesis of an anthocyanin flower pigment, provided one of the earliest reports of calcium binding by a specific functional group, reporting the purification of a simple phenacyl alcohol derivative by formation of a crystalline calcium complex.[9] (As a historically interesting aside, recent studies have demonstrated the role of complexation of a variety of metal ions, including calcium, in the establishment of the range of colors produced by the anthocyanins.[10−12]) Calcium complexation by α-hydroxy carboxylates has been well established,[13] but beyond the early Robinson et al. report,[9] only a scattering of reports have addressed the potential for complexation of calcium by α-hydroxy ketones. Han and Monder ascribed the alteration of reaction stereoselectivity in corticosteroid reductions to the formation of a calcium complex,[14,15] and a single report from our group elucidated the structural details of Robinson's original calcium complex.[16] Formation of intensely colored precipitates upon the addition of barium hydroxide to cyclic α-hydroxy ketones[17] may be indicative of the formation of related heavy metal complexes, although the requirement for enolizable ketones and strong bases suggests that these are more complicated systems. A number of recently reported metal-catalyzed transformations may also proceed via intermediacy of metal complexes of α-hydroxy ketones.[18,19]

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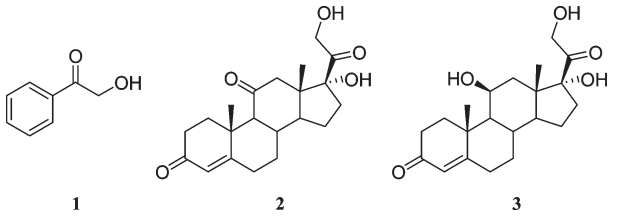

Figure 1. Phenacyl alcohol (**1**), cortisone (**2**), and hydrocortisone (**3**).

Several frequently prescribed pharmaceutical compounds, including corticosteroids and tetracycline antibiotics, contain the α-hydroxy ketone moiety as a part of their structure. Calcium and other group 2 metal ion complexation by tetracycline and its derivatives has received a fair bit of attention,[20−22] and selective calcium chelation by the α-hydroxy ketone functionality has been demonstrated for some anthracyclines.[23] 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,[24] decreased bacteriostatic action,[25] tooth staining in children and in newborns of women administered the drug during the second or third trimesters of pregnancy,[26] and inhibition of osteogenesis and mineralization in developing bone tissue.[27]

Recent reports have linked osteoresorption with long-term steroid use,[28] as well as increased osteoporosis rates and reduced bone mineral density in both adults and children who received long-term corticosteroid therapy for a variety of chronic diseases such as cystic fibrosis, chronic renal insufficiency, leukemia, and Duchenne muscular dystrophy.[29−32] Other suggestive physiological links between the corticosteroids and calcium include impairment of intestinal calcium uptake[33,34] and alteration of receptor binding[35] and cell membrane transport[36] of hydrocortisone upon calcium chelation. In addition, physiological levels of calcium increase the water solubility of hydrocortisone,[37] highlighting a need for further study given the critical dependence of serum concentration (and thus dosage) on drug solubility. In light of these reports, suggesting potential relationships between calcium-related disease conditions and the calcium chelation capabilities of the α-hydroxy ketone moiety, we have carried out a study of the interactions of calcium with phenacyl alcohol (**1**) and two corticosteroids, cortisone (**2**) and hydrocortisone (**3**) (Figure 1). Here, we present the results of solution and solid-state studies of these interactions, reporting the formation constants for calcium complexes and the results of single-crystal X-ray diffraction analyses.

Experimental Section

Cortisone and hydrocortisone were obtained from Aldrich and used without further purification. Phenacyl alcohol was prepared[38] and structurally characterized[16] as previously reported. NMR spectra were recorded on a Varian Inova 300 MHz spectrometer.

Preparation of $[(C_6H_5COCH_2OH)_2Ca(H_2O)_3]^{2+}\cdot 2Cl^-\cdot H_2O$. Evaporation of a methanolic solution of phenacyl alcohol containing 0.5 equiv of $CaCl_2$ afforded the complex as an air-stable solid, mp 106−108 °C. IR (KBr): $\nu_{C=O}$ = 1660, ν_{C-O} = 1093 cm^{-1}. Alternatively, the same complex can be isolated by the addition of excess solid $CaCl_2$ to a refluxing chloroform solution of phenacyl alcohol, followed by filtration and evaporation. Recrystallization from methanol/pentane by slow evaporation or from a chloroform solution by vapor diffusion with hexanes affords colorless, air-stable crystals suitable for single-crystal X-ray analysis, reported previously.[16]

Preparation and Structural Analysis of $[(C_{21}H_{28}O_5)_2Ca(H_2O)_2(C_2H_5OH)_2]^{2+}\cdot 2Cl^-\cdot 2H_2O$. A solution of cortisone in ethanol was stirred with excess solid $CaCl_2$ for several hours. The supernatant was decanted and diluted with toluene, then loosely capped and placed in a 4 °C refrigerator. Slow evaporation in this damp atmosphere resulted in the deposition of large colorless laths. They deteriorated rapidly on exposure to the air, even when coated with adhesive, but were adequately stable when transferred from the mother liquor to Apiezon hydrocarbon grease and then sealed in a capillary. All crystals examined diffracted weakly. The cell parameters and orientation matrix of the data crystal (dimensions 0.09 × 0.20 × 0.50 mm) were obtained from the setting angles of a Rigaku AFC6R diffractometer for 20 centered reflections in the 2θ range 11−14°. The fraction of reflections with $I \geq 3\sigma(I)$ fell from 92% at low angles to 23% in the shell $45° < 2\theta < 50°$. The fall partly resulted from a loss in diffraction intensity over the 24 h of data collection (ca. 15% for standard reflections), for which a correction was applied. Absorption corrections based on azimuthal scans were also made. The distribution of intensities and the systematic absences together defined the space group as $C222_1$. A MITHRIL E-map[39] gave the position of the Ca atom, on a diad axis, and the one independent C1 atom. Most of the C and O atoms were revealed by a cycle of DIRDIF,[40] and the remainder by a difference synthesis after least-squares refinement. Hydrogen atoms bonded to carbon were included at calculated "riding" positions [d(C−H) = 0.95 Å; B(H) = 1.2B_{eq}(C)]. Five of the seven hydrogens bonded to oxygen were located in difference maps and were included at those positions without refinement. The value of a secondary extinction parameter refined to ca. 10^{-8} and was fixed at zero. The TEXSAN program suite, incorporating complex atomic scattering factors, was used in all calculations.[41] Crystallographic data are summarized in Table 1, and full crystallographic information is provided in CIF format as Supporting Information.

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Table 1. Crystal and Structure Determination Data for Calcium Complexes of **1**, **2**, and **3**

	1·$CaCl_2$·$4H_2O$	**2**·$CaCl_2$·$2(CH_3CH_2OH)$ $2H_2O$	**3**·$CaCl_2$·$4H_2O$
formula	$C_{16}H_{24}CaCl_2O_8$	$C_{46}H_{76}CaCl_2O_{16}$	$C_{21}H_{38}CaCl_2O_9$
fw	455.33	996.07	545.49
xtl system	orthorhombic	orthorhombic	orthorhombic
space grp	*Pbcn*	$C222_1$	$P2_12_12_1$
color, habit	colorless block	colorless plate	colorless block
a (Å)	7.374(3)	9.715(5)	6.310(2)
b (Å)	23.314(5)	32.469(7)	14.144(3)
c (Å)	12.458(3)	16.000(5)	29.431(5)
volume	2141.9(11)	5047(3)	2627.0(10)
Z ($Å^3$)	4	4	4
T (K)	120	292	298
R, *wR* (%)	4.19, 7.41	6.4, 4.9	4.6, 5.2
GOF	0.64	1.68	2.21

Preparation and Structural Analysis of $[(C_{21}H_{30}O_5)Ca(H_2O)_4]^{2+}\cdot 2Cl^-$. To a solution of 0.52 g of hydrocortisone (1.4 mmol) in 10 mL of MeOH in a 50 mL round-bottom flask was added 0.10 g of dry $CaCl_2$ (0.90 mmol). The mixture was stirred, affording a clear solution, to which was added 10 mL of xylene. The flask was covered with perforated Parafilm and placed in a cold room at 8 °C. After a seven-week interval, small rectangular clear-white crystals were seen at the solvent interface. By 12 weeks, these crystals were deemed large enough for X-ray diffraction analysis. They were stored under xylene in a sealed vial.[42] A crystal of dimensions 0.36 × 0.40 × 0.47 mm was sealed in a special glass capillary in the presence of the mother liquor. The orientation parameters and cell dimensions were obtained from the setting angles of an Enraf-Nonius CAD-4 diffractometer for 25 centered reflections in the range $13° < \theta < 15°$. The systematic absences together with the acentric distribution of intensities indicated the space group $P2_12_12_1$. A SIR92 E-map[43] showed all of the non-hydrogen atoms. Hydrogen atoms bonded to carbon were approximately located in a difference map and were included at calculated positions, updated after each cycle of refinement, with B(H) set at $1.2B_{eq}$ (C). Hydrogen atoms bonded to oxygen were, if locatable, included at observed positions without refinement. The final difference synthesis was featureless. The TEXSAN program suite,[41] incorporating complex atomic scattering factors, was used in all calculations. Crystallographic data are summarized in Table 1, and full crystallographic information is provided in CIF format as Supporting Information.

Formation Constants. A 10.0 mM solution of the α-hydroxy ketone (600 μL) was added via gastight syringe to a NMR tube fitted with a septum cap. To this solution was added 6 μL of TMS as an internal standard. A 6 mM solution of $Ca(ClO_4)_2\cdot 4H_2O$ in CD_3CN was then titrated in via gastight syringe, such that the ratio of Ca^{2+} to the ligand was increased in increments of 0.01 equiv up to 0.1 equiv; then, a 60 mM solution of $Ca(ClO_4)_2\cdot 4H_2O$ was titrated in via gastight syringe, such that the ratio of Ca^{2+} to the ligand was increased in increments of 0.1 equiv up to 1.3 equiv. At each data point, the chemical shifts of the methylene protons were recorded. By fitting the data using a Microsoft Excel add-in (Equilibrium Expert), formation constants were determined for the Ca^{2+} complexes of each ligand.[44] Each experiment was repeated in triplicate.

Results and Discussion

Solution Studies. Sequential addition of aliquots of a CD_3CN solution of $Ca(ClO_4)_2\cdot 4H_2O$ to a CD_3CN solution of phenacyl alcohol leads to progressive downfield shifts for the methylene and OH resonances, while the other resonances display negligible to imperceptible shifts. The rate of change in chemical shift drops significantly after the addition of the first 0.5 equiv of Ca^{2+}, suggestive of the formation of a complex with a 2:1 ligand-to-Ca^{2+} stoichiometry. This stoichiometry was confirmed through the method of continuous variation,[45,46] in which the chemical shift of an appropriate ^1H resonance is measured as a function of the ligand-to-metal ratio, which is varied while holding constant the total concentration of ligand + metal ([L]+[M]). A plot of $[L]\cdot\Delta/\Delta_{tot}$ versus [L]/([L]+[M]), where Δ is the difference between the observed chemical shift and that of the free ligand and Δ_{tot} is the difference between the extrapolated chemical shift for the fully formed complex and that of the free ligand (i.e., a Job plot), yields a curve approximating an inverted parabola, with the maximum identifying the stoichiometry of the complex. A representative Job plot for phenacyl alcohol interacting with $Ca(ClO_4)_2\cdot 4H_2O$ in CD_3CN is presented in Figure 2. The maximum occurs at a value for [L]/([L]+[M]) of approximately 0.66, corresponding to a 2:1 ratio of ligand to metal.

The 2:1 stoichiometry was additionally confirmed, and the formation constant was quantified, through nonlinear least-squares fitting of the experimental titration data with Equilibrium Expert, an add-in to Microsoft Excel developed by Barbet et al. for the specific purpose of simulation of multiple binding equilibria.[44] Attempted fitting with a 1:1 stoichiometry does not lead to a stable solution, while modeling with a 2:1 stoichiometry quickly converges on a satisfactory solution, affording a formation constant of 341 ± 10 M^{-2}. Modeling with higher ligand/Ca^{2+} ratios gives notably inferior fits, additionally confirming the 2:1 stoichiometry.

Cortisone and hydrocortisone behave very similarly to phenacyl alcohol in these complexation assays, again displaying simple titration curves with a significant change in slope at a 2:1 ligand-to-Ca^{2+} ratio, Job plots with maxima at [L]/([L]+[M]) = 0.66, and Equilibrium Expert simulations requiring a 2:1 stoichiometry. The results of these simulations are summarized in Table 2, where each formation constant, standard deviation (from the least-squares fit), and *r*-squared value is reported as the average of three independent experiments. Since the methylene resonance for phenacyl alcohol appears as a

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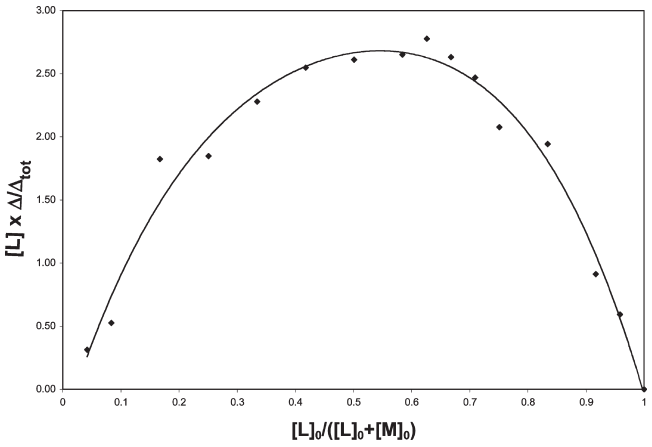

Figure 2. Job plot for phenacyl alcohol + $Ca(ClO_4)_2 \cdot 4H_2O$ in CD_3CN.

Table 2. Formation Constants and Coefficients of Determination for Ca^{2+} Complexes of α-Hydroxy Ketones

ligand	K (M^{-2})	r^2
phenacyl alcohol	341 ± 10.0	0.989
cortisone	201 ± 1.7	0.997
hydrocortisone	184 ± 1.7	0.996

doublet, each independent experiment effectively allowed two determinations of the formation constant. Similarly, the methylene resonance appears as a doublet of quadruplets for cortisone and hydrocortisone, and thus each binding constant was effectively determined eight separate times in each independent experiment.

Differing only in the oxidation state of the C ring, which appears both geometrically and electronically remote from the presumed binding site (vide infra), the two corticosteroids display very similar formation constants. The phenacyl alcohol complex, however, has a notably higher formation constant, nearly twice as large as that seen for the steroids. To the extent that calcium complexation would appear to enhance the carbocationic character of the carbonyl carbon, the benzylic stabilization afforded in the phenacyl alcohol complex (Figure 3) would plausibly appear to be reflected in a higher formation constant.

Cortisone[47] and hydrocortisone[48] are only sparingly water-soluble (0.025 μg mL^{-1} and 0.28 mg mL^{-1}, respectively) and are well-known to localize in lipophilic environments, as highlighted by the reported linear correlation between the logarithm of the apparent first-order rate constant for steroid metabolism and the logarithm of the oil/water partition coefficient for several corticosteroids.[49] Thus, while our formation constant measurements were carried out in acetonitrile rather than in water, this nonaqueous environment may be more relevant to the in vivo environment of the corticosteroids than an aqueous medium, although we do not wish to overstate the potential direct biological or biochemical relevance of these formation constants.

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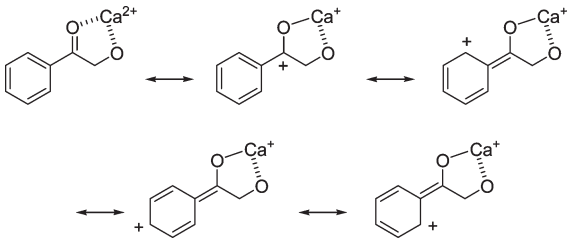

Figure 3. Resonance structures for phenacyl alcohol complex.

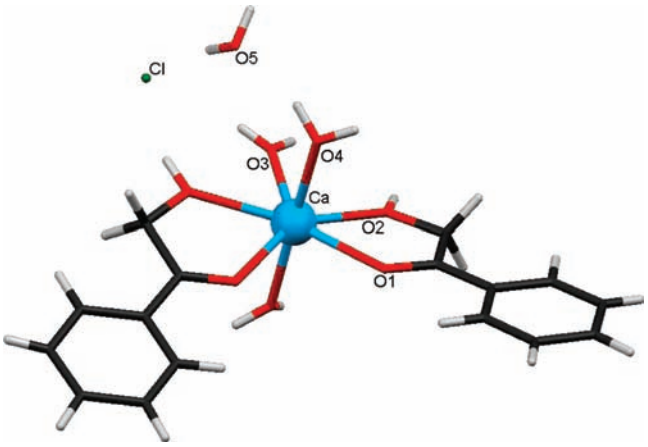

Figure 4. Molecular structure of the Ca^{2+} complex of phenacyl alcohol, $[(C_6H_5COCH_2OH)_2Ca(H_2O)_3]^{2+} \cdot 2Cl^- \cdot H_2O$, omitting one symmetry-equivalent chloride ion.

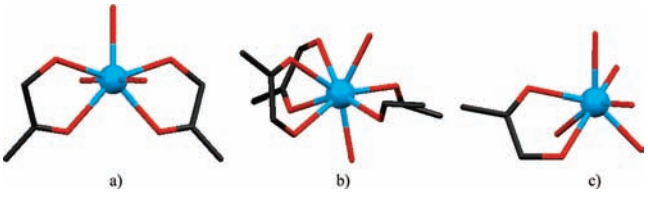

Figure 5. Calcium coordination sphere in Ca^{2+} complexes of (a) phenacyl alcohol (pentagonal bipyramidal), (b) cortisone (square antiprismatic), and (c) hydrocortisone (capped trigonal pyramidal).

Solid-State Studies. Evaporation of either a methanolic solution of phenacyl alcohol containing 0.5 equiv of $CaCl_2$ or a chloroform solution of phenacyl alcohol that has been refluxed with solid anhydrous calcium chloride affords a crystalline solid. This solid displays a comparatively high melting point (106−108 °C) relative to that of phenacyl alcohol itself (85−86 °C). Infrared spectroscopy (KBr pellet) is suggestive of the formation of a complex, with a considerable lowering of the C=O stretching frequency from 1693 cm^{-1} in the free ketol to 1660 cm^{-1} in the complex, as well as a significant broadening of the O−H stretching band and a reduction of the C−O stretching frequency from 1112 to 1093 cm^{-1}. These spectral changes are consistent with a withdrawal of electron density from the α-hydroxy ketone by the Ca^{2+} cation, as represented in Figure 3.

Recrystallization from methanol/pentane by slow evaporation or from chloroform solution by vapor diffusion with hexanes affords colorless, air-stable crystals suitable for single-crystal X-ray analysis. The 2:1 solution stoichiometry of the complex is maintained in the solid state, as

Table 3. Hydrogen Bonding Interactions of Chloride Counterions in Ca^{2+} Complexes of Phenacyl Alcohol, Cortisone, and Hydrocortisone

phenacyl alcohol complex[a] (four-coordinate chloride)			cortisone complex[c] (four-coordinate chloride)			hydrocortisone complex[e] (four-coordinate chloride)			hydrocortisone complex (three-coordinate chloride)		
atoms A−B	distance (Å)	A−H−B angle (deg)	atoms A−B	distance (Å)	A−H−B angle (deg)	atoms A−B	distance (Å)	A−H−B angle (deg)	atoms A−B	distance (Å)	A−H−B angle (deg)
Cl−O(2)	3.039	157.74	Cl−O(17)	3.018	165.76	Cl(2)−O(2)	3.140	174.53	Cl(1)−O(4)	3.220	164.70
Cl−O(3)	3.158	156.81	Cl−O(21)	3.100	165.08	Cl(2)−O(4)	3.220	125.47	Cl(1)−O(5)	3.071	151.50
Cl−O(4)[b]	3.138	172.33	Cl−O(25)	3.151	144.50	Cl(2)−O(5)	2.990	124.99	Cl(1)−O(17)	3.177	134.08
Cl−O(5)	3.100	169.77	Cl−O(25)	3.226	*d*	Cl(2)−O(11)	3.047	174.13			
average	3.109	164.16	average	3.124	158.45	average	3.099	149.78	average	3.156	150.09

[a] Additional H-bonding contact: O(4)−O(5), 2.747 Å. [b] O(4) of symmetry-related molecule at $1-x, 1-y, 1-z$. [c] Additional H-bonding contacts: O(22)−O(25), 2.800 Å; O(3)−O(24), 2.804 Å; O(3)−O(24), 2.902 Å. [d] Relevant H atom not located. [e] Additional H-bonding contacts: O(2)−O(11), 2.840 Å; O(5)−O(21), 2.783 Å.

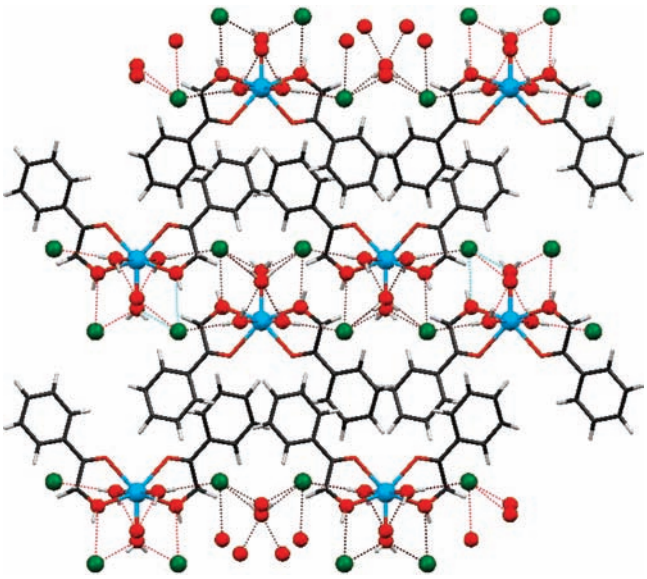

Figure 6. Molecular packing for the Ca^{2+} complex of phenacyl alcohol, including hydrogen-bonding interactions, viewed along the *a* axis (red = oxygen, green = chlorine, light blue = calcium).

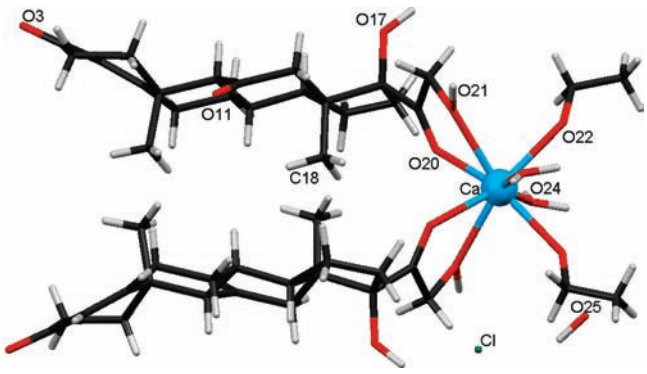

Figure 7. Molecular structure of the Ca^{2+} complex of cortisone, omitting one symmetry-equivalent chloride ion and one symmetry-equivalent noncoordinated water molecule.

demonstrated by single-crystal X-ray diffraction analysis (Figure 4). An overall seven-coordinate complex is formed, with the calcium ion surrounded by two bidentate phenacyl alcohol molecules and three coordinated water molecules. A fourth water molecule and the two chloride counterions are outside the primary coordination shell at distances of 4.16 Å for the water and 4.63 Å

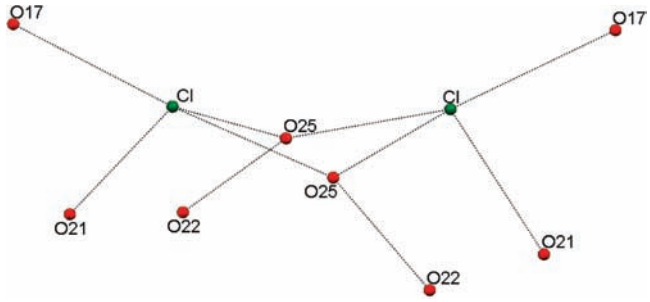

Figure 8. Hydrogen-bonding interactions of counteranions in the Ca^{2+} complex of cortisone.

Figure 9. Hydrogen bonding interactions between A-ring carbonyls and calcium-coordinated water molecules in the Ca^{2+} complex of cortisone.

for the symmetry-equivalent chloride ions. The overall coordination geometry is roughly pentagonal bipyramidal (Figure 5a), with two waters in the axial position (O−Ca−O angle 177.9°) and the two phenacyl alcohols moieties and a third water in the equatorial plane. This geometric formulation is supported by the average $O_{equatorial}-Ca-O_{equatorial}$ bond angle of 72.5° and the average $O_{equatorial}-Ca-O_{axial}$ bond angle of 90.0°.

With a crystallographic 2-fold rotation axis passing through the calcium ion and the oxygen of the water located in the pentagonal plane, the two chloride ions are crystallographically equivalent. Each chloride accepts a total of four hydrogen bonds—one each from the equatorial water, a phenacyl alcohol hydroxyl group, the noncoordinated hydrate, and an axial water of an adjacent complex—in a highly skewed trigonal-pyramidal coordination geometry. The equatorial water donates a total of two hydrogen bonds, one to each of the symmetry-equivalent chlorides. It does not appear to accept hydrogen bonds, although methylene hydrogens of adjacent phenacyl alcohol moieties are rather close, at 2.621 Å (C−H−O angle 165.20°). The axial waters also donate two hydrogen bonds each, to a chloride and to the noncoordinated hydrate, but do not appear to serve as

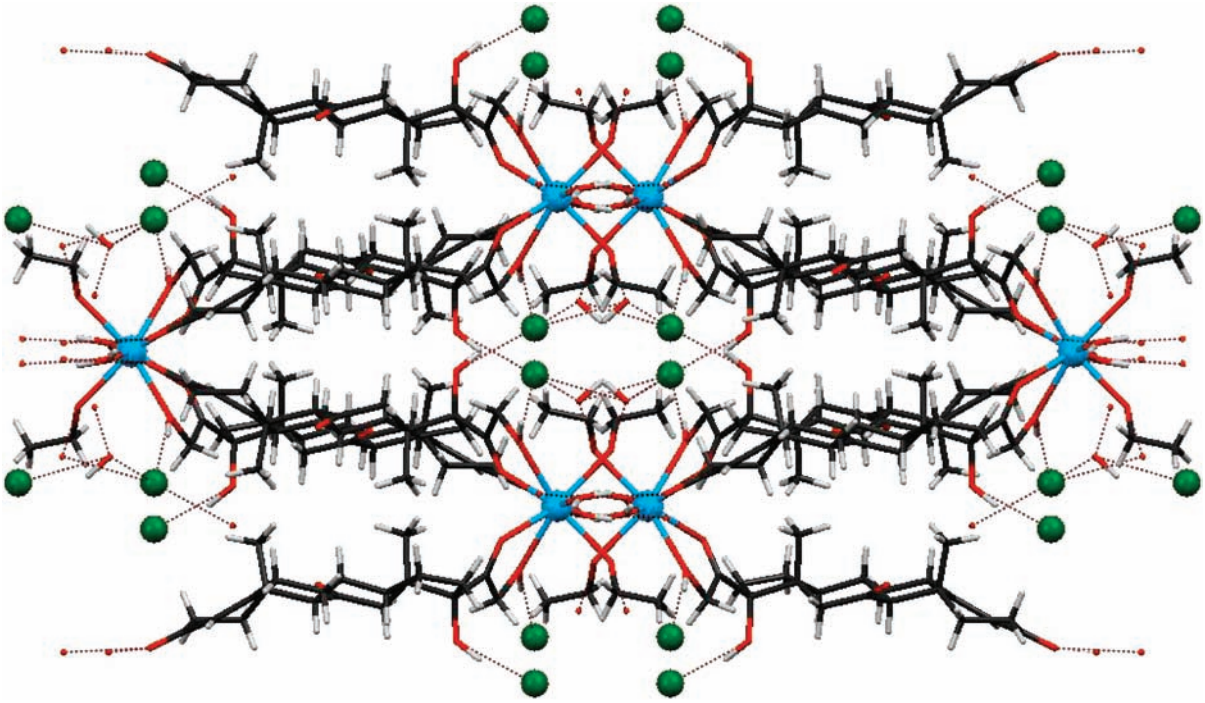

Figure 10. Molecular packing for the Ca^{2+} complex of cortisone, including hydrogen-bonding interactions, viewed along the *c* axis (green = chlorine, light blue = calcium).

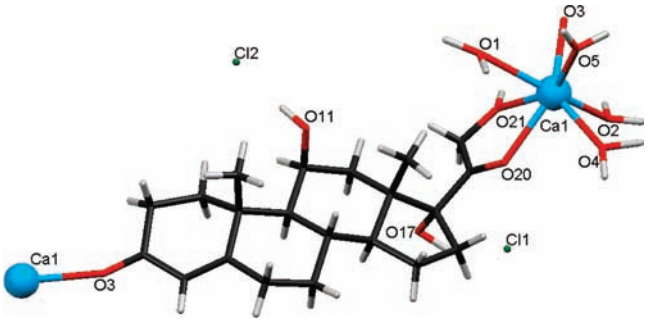

Figure 11. Molecular structure of the Ca^{2+} complex of hydrocortisone.

hydrogen-bond acceptors. The noncoordinated hydrate, in contrast, efficiently participates in four hydrogen bonds, serving as a donor to two chlorides and accepting hydrogen bonds from two axial waters. In the aggregate, these interactions (summarized in Table 3) lead to the formation of hydrogen-bonded sheets oriented in the crystallographic *bc* plane, as depicted in Figure 6.

Crystallization of cortisone in the presence of calcium chloride may be effected from a variety of solvent mixtures, invariably affording very thin crystals that deteriorate rapidly upon removal from the mother liquor. Ultimately, slow evaporation in a humid environment of a toluene/ethanol solution of cortisone and calcium chloride was found to afford crystalline material of sufficient stability to allow coating with Apiezon hydrocarbon grease in a sealed capillary without a significant loss of crystal quality or diffraction intensity. As for the phenacyl alcohol complex, single-crystal X-ray diffraction analysis revealed a 2:1 stoichiometry, with each of the cortisone ligands chelating the calcium ion through the ketone and the side-chain [C(21)] hydroxyl group rather than the steroidal D-ring [C(17)] hydroxyl group (Figure 7).

With a crystallographic 2-fold rotation axis present, the eight-coordinate central calcium ion adopts a square-antiprismatic coordination geometry (distorted somewhat due primarily to the small "bite size" of the α-hydroxy ketone moiety), with each symmetry-equivalent cap comprised of a bidentate cortisone and a molecule each of water and ethanol (Figure 5b).

Two additional water molecules and the two chloride counterions are outside the primary coordination shell, at distances of 4.481 Å for the symmetry-equivalent waters and 5.206 Å for the symmetry-equivalent chloride ions. In a rather complex but beautifully symmetrical set of hydrogen-bonding interactions (Figure 8 and Table 3), these chloride ions accept hydrogen bonds from the cortisone C(17) and C(21) hydroxyl groups, as well as from both of the noncoordinated waters [O(25) in our numbering scheme], which in turn accept hydrogen bonds from the calcium-coordinated ethanol moieties [O(22)]. The chloride ions each accept four hydrogen bonds, with the coordination geometry about the chloride ions far from tetrahedral, and indeed most closely approximated as (very) skewed trigonal-pyramidal, quite similar to that seen in the phenacyl alcohol complex.

Each calcium-bound water molecule [O(24)] donates two hydrogen bonds to the A-ring carbonyl group [O(3)] of two adjacent cortisone molecules, generating yet another 2-fold symmetric substructure, depicted in Figure 9. The full symmetry of the solid-state structure is perhaps most graphically illustrated by the packing diagram, viewed along the *c* axis, in which both the high symmetry and the origin of the rather long crystallographic *b* dimension are readily evident (Figure 10). Relatively close contacts between the angular methyl groups at the CD ring junction are displayed [C(18)···C(18), 3.725 Å; H(20)···H(20), 2.247 Å], suggesting a potential albeit

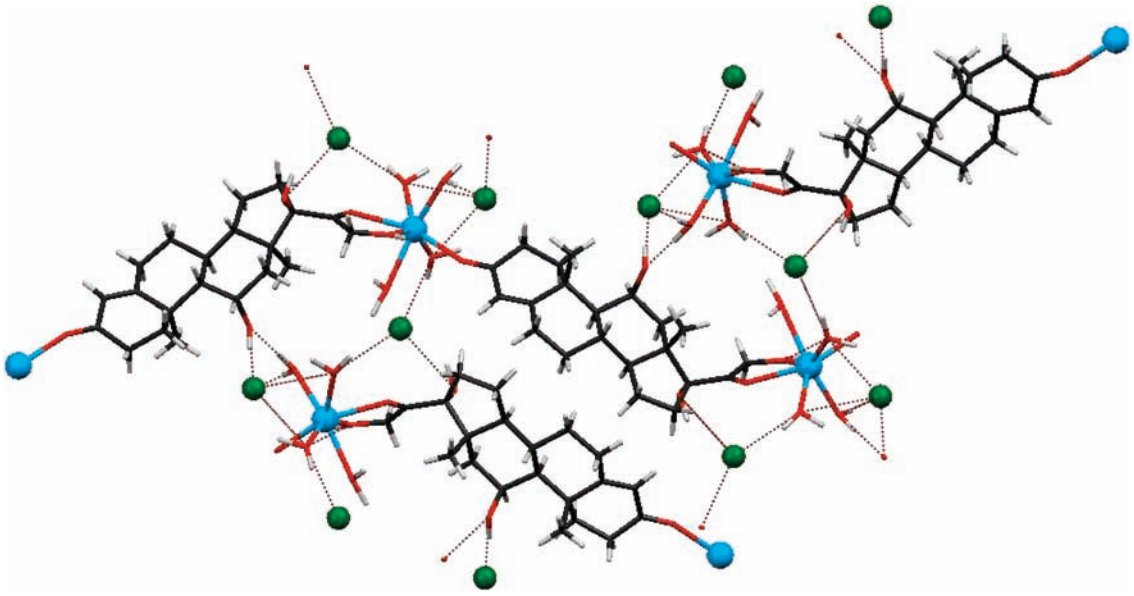

Figure 12. Molecular packing for the Ca^{2+} complex of hydrocortisone, including hydrogen-bonding interactions, viewed along the *a* axis (green = chlorine. light blue = calcium).

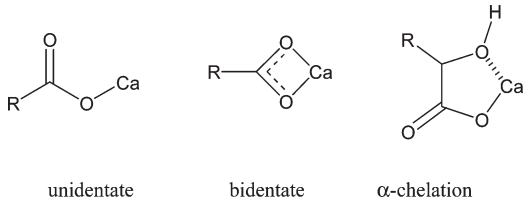

Figure 13. Ligand geometries for calcium complexes of carboxylates and α-hydroxy carboxylates.

presumably minor hydrophobic contribution to the molecular packing forces.

Slow evaporation in a humid environment of a methanolic solution of hydrocortisone and calcium chloride to which had been added xylenes led to the deposition of small prismatic crystals, revealed by single-crystal X-ray diffraction analysis to be a calcium complex (Figure 11). As in the cortisone complex, the calcium ion is coordinated to the ketone and side-chain [O(21)] hydroxyl group rather than the D-ring [O(17)] hydroxyl group, with the metrical parameters associated with this chelation very similar to those in the cortisone complex. Most striking is the crystallization of the hydrocortisone complex in a 1:1 stoichiometry, whereas both cortisone and phenacyl alcohol yielded crystalline complexes with a 2:1 ligand/calcium stoichiometry. In this complex, the calcium ion does interact with a second steroid molecule, but this second interaction is through the C(3) carbonyl group, resulting in infinite chains extending in the *c* direction (Figure 12). In addition to the three hydrocortisone-derived ligands, the calcium ion bears four water ligands, providing overall seven coordination with a distorted capped trigonal-pyramidal geometry (Figure 5c). Two of the water ligands and the ketone carbonyl group do not appear to participate in hydrogen bonding, while each of the other coordinated groups participates in the formation of a complex set of intra- and intermolecular hydrogen-bonding contacts.

The two chloride ions are located outside the primary coordination shell, 3.931 Å and 5.236 Å from the calcium ion. Close contacts between the chloride ions and a number of hydrogen-bond donor groups lead to a rather complex array of hydrogen-bonding interactions. One chloride shows close contacts with two adjacent water ligands on one calcium ion, as well as to a water molecule coordinated to a second calcium ion and the C-11 hydroxyl group of a hydrocortisone, giving an overall coordination geometry around chlorine similar to the skewed trigonal-pyramidal coordination seen in the phenacyl alcohol and cortisone complexes. The other chloride forms only three apparent hydrogen bonds, to a water ligand and the C-17 hydroxyl group associated with one calcium ion and to an additional water ligand on a second calcium ion. The heavy atom distances and angles pertinent to these contacts are summarized in Table 3.

Structures of several calcium complexes of α-hydroxy acids and α-amino acids have been reported in the literature.[50−52] While the reported mode of binding often involves η^1 (unidentate) coordination to a single, deprotonated carboxylate oxygen, it is not unusual to encounter systems where calcium is bound in a bidentate mode, or in an "α-chelation mode" where the metal ion is bound to one carboxylate oxygen and an α-substituent (Figure 13). Other than a previous paper published by our group regarding the phenacyl alcohol−Ca^{2+} system and a very recent report of $CaBr_2$ and $CdCl_2$ complexes of dihydroxyacetone,[53] we are unaware of any complexes of α-hydroxy ketones that have been structurally characterized.

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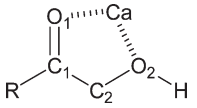
Table 4. Comparison of Selected Structural Metrics for α-Hydroxy Ketones and Their Complexes with Calcium

	atom labels[a]	**1**·0.5H_2O	**1**	**1**·Ca^{2+}	**2**	**2**·Ca^{2+}	**3**	**3**·Ca^{2+}
reference		57	62	this work	54	this work	55	this work
bond length (Å)	C_1═O_1	1.212(2)	1.212	1.227(4)	1.274	1.200(7)	1.212	1.221(6)
	C_2−O_2	1.403(2)	1.404	1.417(4)	1.381	1.424(8)	1.415	1.403(5)
	O_1−Ca			2.415(3)		2.516(5)		2.437(3)
	O_2−Ca			2.389(3)		2.509(5)		2.365(5)
	Ca−O_{H_2O}			2.429(4)		2.367(4)		2.405(4)[b]
	Ca−O_{EtOH}					2.429(5)		
	Ca−Cl			4.630		5.206		5.589
bond angle (deg)	C_1═O_1−Ca			124.4(2)		124.6(5)		121.9
	C_2−O_2−Ca			124.1(2)		122.5(4)		123.6
	O_1−Ca−O_2			64.4(1)		61.1(1)		64.4
	C_1−C_2−O_2	113.6(1)	120.7	107.9(3)	111.89	106.2(5)	112.70	108.4
dihedral (deg)	O_1═C_1−C_2−O_2	5.0(2)	0.8	3.55(4)	6.41	−12.3(9)	−0.62	4.1

[a] For cross-comparison purposes, in this table, the α-hydroxy ketone atoms are labeled as shown here:



[b] Average of four equivalent bonds.

The α-hydroxy ketone moiety consistently functions as a bidentate chelator in each of the three calcium complexes reported here. In each complex, calcium binding appears to be well accommodated by the α-hydroxy ketone functionality, with bond angles very similar to those for the uncomplexed ligand in all cases. As an illustrative example, the CH_2—C═O and HO−CH_2−C bond angles in the calcium complex of cortisone are 120.69° and 106.18°, respectively, as compared to 119.93°and 112.70° in the free steroid.[54] Similarly, these angles in the hydrocortisone complex are 120.23° and 106.41°, respectively (free steroid 118.14° and 112.71°),[55] and in the phenacyl alcohol complex are 118.8° and 107.9°, respectively. The formation of seven- (**1** and **3**) and eight-coordinate (**2**) complexes is not surprising given calcium's proclivity for these coordination numbers,[56] and a strong preference for incorporation of two α-hydroxy ketone ligands is clearly indicated by the solution complexation studies. This stoichiometry is maintained in the solid state for **1** and **2**, while **3** finds an alternative solid-state structure in which each calcium is coordinated by only a single α-hydroxy ketone moiety.

The binding mode of α-hydroxy ketones to the calcium ion may be further described by the distance of the calcium from the plane of the carbonyl group and the length and angle of the calcium−carbonyl bond. In simple carbonyl and α-hydroxy carboxylate complexes, the coordinated calcium ion is frequently found close to the plane of the carboxylate or carbonyl group.[50,51] The calcium-to-carbonyl plane distances in the phenacyl alcohol complex, hydrocortisone complex, and cortisone complex are ca. 0.3, 0.3, and 0.6 Å, respectively. The Ca—O═C angle for these complexes also falls within the expected range for the α-chelation mode (range, 110−130°; phenacyl alcohol complex, 124.4°; hydrocortisone complex, 121.88°; cortisone complex, 124.53°). These complexes also mimic other calcium carbonyl and carboxylate complexes in their Ca−$O_{carbonyl}$ bond length, which is 2.415 Å in the phenacyl alcohol complex, 2.437 Å in the hydrocortisone complex, and 2.517 Å in the cortisone complex.

Other relevant distances and angles in the calcium complexes and, for comparison, the corresponding calcium-free compounds (and, in the case of **1**, a hemihydrate[57]), are summarized in Table 4. Consistent with the infrared spectrum of the calcium complex of phenacyl alcohol (**1**·Ca^{2+}), which displayed a carbonyl stretching frequency of reduced energy relative to the free ligand (cf. Figure 3), the C═O bond length increases upon calcium chelation, although the lengthening is rather subtle (from 1.212 to 1.227 Å). The significant decrease in stretching frequency (by 33 cm^{-1}) parallels the changes seen in the infrared spectra of a variety of aromatic ketones upon complexation by $HgCl_2$, $HgBr_2$, and $CdCl_2$, for which the extent of decrease (20−50 cm^{-1}) was reported to parallel the strength of the O−M interaction.[58] The decrease is significantly larger than the changes seen for N, N-dimethylformamide (DMF) upon coordination by Li^+, Na^+, Mg^{2+}, and Ca^{2+} (10−17 cm^{-1}),[59] while the increase in C═O bond length upon Ca^{2+} coordination is comparable to that seen in the DMF complex.[59−61] The complexity of a critical analysis of these data, however, is highlighted by the structural analyses of the two steroid complexes, for which the C═O bond length is essentially unchanged (**3**) or is decreased (**2**) by Ca^{2+} coordination. (The latter appears rather peculiar, and significant differences in the reported C═O and C−O bond lengths for **2** suggest that the structure of **2** may bear reinvestigation.) Further studies of the spectral and structural implications

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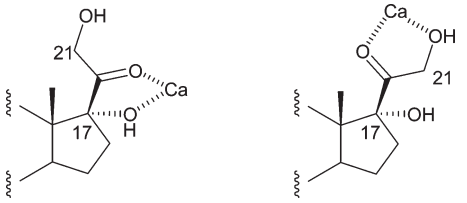

Figure 14. Inferred[14,15] (left) and observed (right) calcium chelation mode for steroidal α-hydroxy ketones.

of calcium coordination to the α-hydroxy ketone functionality are currently underway.

Interestingly, Han and Monder suggested that chelation occurs with the carbonyl and the C(17) hydroxyl group of cortisone, rather than with the C(21) hydroxyl group crystallographically observed for both cortisone and hydrocortisone (Figure 14).[14,15] This proposal, however, was based on the observation of significant effects on the stereoselectivity of C-ring reaction chemistry—effects for which placement of the bulky calcium ion in as close proximity to the C-ring as possible was completely logical—rather than on spectroscopic or structural analysis.

The structural reorganization upon crystallization for the calcium complex of **3**, which clearly displays evidence for formation of a complex of 2:1 stoichiometry in solution but crystallizes to afford a complex containing a single bidentate α-hydroxy ketone ligand per calcium, suggests caution is in order when translating the results of solid-state studies to interpretation of solution behavior. This said, our solution spectroscopic evidence, in particular the significant alteration of the chemical shift of the C(21) methylene resonance upon titration with calcium, supports the conclusion that calcium binds to the less-hindered α-hydroxy ketone in both solution and the solid state.

Summary

Phenacyl alcohol, a prototypical α-hydroxy ketone, and two corticosteroids, cortisone and hydrocortisone, each display solution titration behavior consistent with the formation of calcium complexes of 2:1 ligand/metal stoichiometry. The formation constants for these complexes are modest, ranging from 184 ± 1.7 M^{-2} (hydrocortisone) to 341 ± 10.0 M^{-2} (phenacyl alcohol), but significant given the simplicity of the α-hydroxy ketone functionality in contrast to the more complex architectures often employed for calcium chelation. The relative formation constants for the complexes appear to parallel simple electron density and charge stabilization rationalizations; substituent effect studies are currently underway to explore this issue in greater detail. While these formation constant measurements were carried out in acetonitrile rather than in water, they clearly suggest that the formation of discrete corticosteroid−calcium complexes in the biological milieu should be considered, particularly because of the lipophilic biological environment for the corticosteroids.

Crystallization affords solid calcium complexes in which the α-hydroxy ketone unit consistently functions as a bidentate chelator. The complexes of phenacyl alcohol and cortisone crystallize with 2:1 ligand/metal stoichiometries consistent with solution equilibrium measurements, while hydrocortisone adopts an alternative 1:1 stoichiometry in the solid state, comprised of infinite chains linked through calcium coordination to the α-hydroxy ketone side chain and to the A-ring carbonyl. These results highlight the importance of careful collection and analysis of both solution and solid-state data without making overly simplistic assumptions about the relevance of solution behavior to solid-state structure (or vice versa) without convincing supporting evidence. The formation of solid calcium complexes of these corticosteroids may offer a structural explanation for the empirically observed ability of calcium bromide to precipitate 3-oxosteroids from complex mixtures.[63]

Complex hydrogen-bonding networks link the ligands, water and alcohol molecules, and chloride counterions into intricate superstructures. While the focus of this manuscript is on Ca^{2+} complexation, these hydrogen-bonded superstructures in essence represent supramolecular complexes of the chloride anion, paralleling analogous supramolecular complexes of hydrated cations (e.g., an 18-crown-6 complex of $Zn(H_2O)_6^{2+}$).[64] With this inspiration, we are considering the rational design of molecular vehicles designed to foster complexation of *hydrated* anions, representing a potentially valuable tool in the burgeoning field of anion complexation.[65,66]

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Supporting Information Available: Full details and results of the crystallographic analyses of the calcium complexes of phenacyl alcohol (**1**), cortisone (**2**), and hydrocortisone (**3**) have been deposited in the form of a CIF file. This information is available free of charge via the Internet at http://pubs.acs.org.

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