

unit, was only slightly less potent ( $IC_{50} = 4 \mu M$ ).<sup>15</sup> *N*-Methylcyclopropylamine **2** was a poor inhibitor of SE and showed only a moderate decrease in [<sup>14</sup>C]lanosterol formation at 100  $\mu M$ . Although not as potent as several related compounds for OSC and SE mixtures,<sup>10</sup> *N*-oxide **2b** was a modest inhibitor of OSC ( $IC_{50} = 40 \mu M$ ), as indicated by an accumulation of [<sup>14</sup>C]squalene epoxide. These results suggest that oxidation of the nitrogen atom to the corresponding *N*-oxide does not occur in vitro. The difference in activity between secondary and tertiary amines **1** and **2** can be attributed to steric perturbation.

The time dependency and irreversibility of inhibition of SE by cyclopropylamine **1** was investigated. Figure 1 shows a time dependency of inactivation with  $K_i = 2.4 \mu M$  and  $k_{inact} = 0.055 \text{ min}^{-1}$ .<sup>16</sup> Addition of excess squalene (ca. 400  $\mu M$ ) to a mixture of **1** (2  $\mu M$ ) and pig liver microsomes caused complete recovery of SE activity, illustrating substrate protection.

Amine **1** was not readily removed from the crude inactivated enzyme mixtures. However, incubation of <sup>3</sup>H-labeled cyclopropylamine **1** (prepared from reductive amination of [<sup>3</sup>H]trisorosqualene aldehyde)<sup>4a</sup> with crude pig liver homogenate, followed by anion-exchange chromatography, resulted in complete recovery of the radiolabeled amine **1** (detected by radio-TLC) and full restoration of SE activity.<sup>17</sup> Thus, cyclopropylamine **1** cannot be a mechanism-based inactivator of SE. Instead, it is probable that there exists a strong electrostatic interaction between enzyme and inhibitor. The protonated amine may mimic the transient positive charge which develops at the C-3 and/or C-2 positions during the epoxidation of squalene.

Secondary amines **4** and **5** analogous to cyclopropylamine **1** are less potent inhibitors of SE by more than 2 orders of magnitude ( $IC_{50} = 200 \mu M$  and  $>400 \mu M$ , respectively). Replacement of alkyl substituents by the cyclopropyl moiety can result in increased inhibition.<sup>18</sup> The reasons for the increased activity of many cyclopropane-containing analogues are poorly understood and involve a combination of steric and electronic effects.<sup>19</sup>

In conclusion, trisorosqualene cyclopropylamine (**1**) is a potent inhibitor of pig liver SE. Because **1** does not appear to be a mechanism-based inactivator of SE, interaction between enzyme and inhibitor is most probably electrostatic in nature. Structural studies of cyclopropylamine variants suggest that there is considerable flexibility for the position of the nitrogen atom. However, the strong requirement of the cyclopropyl moiety for SE inhibition suggests that there also exists a specific, but as yet undefined, interaction between the cyclopropyl ring and SE.

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**Supplementary Material Available:** Experimental details for syntheses of **1**, **2**, **2b**, **3**, and eight other compounds and for enzyme experiments (8 pages). Ordering information is given on our current masthead page.

(14) 2-Aza-2,3-dihydrosqualene (**6**), the most potent inhibitor of vertebrate squalene epoxidase thus far described in the literature (Ryder, N. S.; Dupont, M.-C.; Frank, I. *FEBS Lett.* **1986**, *204*, 239-242), was tested for pig liver SE inhibition. Under our assay conditions, this analogue was less potent than **1**, with  $IC_{50} = 20 \mu M$ .

(15) In contrast, the trisorosqualene alcohol is 10-fold more potent than its truncated tetranor analogue. See: Sen, S. E.; Prestwich, G. D. *J. Med. Chem.*, submitted.

(16) Cyclopropylamine **1** was incubated for 0.5, 5, 7, and 10 min at inhibitor concentrations of 0, 0.4, 1, and 2  $\mu M$ .

(17) Sedimentation of nonsolubilized, inactivated microsomes, dialysis, and ultrafiltration of an inactivated enzyme mixture all failed to restore SE activity. However, an independent observation (M. Bai, unpublished results) showed that a variety of amine-containing inhibitors of SE could be removed from reversibly inhibited SE preparations by using ion-exchange chromatography on DEAE-Sephadex.

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## A Trigonal Planar $[Zn(SR)_3]^{1-}$ Complex. A Possible New Coordination Mode for Zinc-Cysteine Centers

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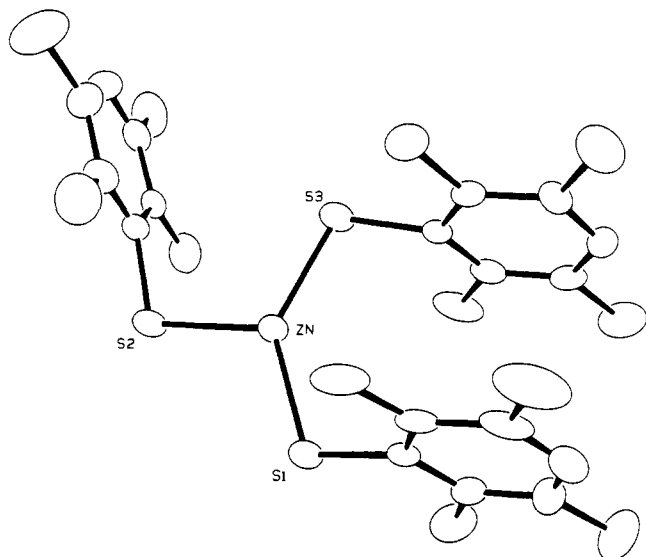
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The recent discovery of the "zinc finger" structural motif and its wide occurrence in nucleic acid binding proteins has greatly increased interest in zinc-cysteine coordination.<sup>1-3</sup> We have been studying the coordination chemistry of zinc and spectroscopically observable metals with similar structural requirements to serve as models for zinc-cysteine metalloproteins.<sup>4-7</sup> Herein, we report the synthesis and structure of the first example of a trigonal planar  $[M(SR)_3]^{1-}$  complex of zinc.

The reactions of  $ZnSO_4 \cdot 7H_2O$  with 5 equiv of lithium 2,3,5,6-tetramethylbenzenethiolate ( $LiS-2,3,5,6-Me_4C_6H$ ) in  $CH_3CN$  and 1 equiv of (*n*-Pr<sub>4</sub>N)Br gave a white crystalline compound, which was recrystallized from  $CH_3CN$ . The <sup>1</sup>H NMR spectrum of the product indicated the empirical ratio of one (*n*-Pr)<sub>4</sub>N cation per three thiolate ligands and no resonance for  $CH_3CN$ . An X-ray crystal structure established the monomeric, three-coordinate nature of the anion in  $[(n-Pr)_4N][Zn(S-2,3,5,6-Me_4C_6H)_3]$  (Figure 1).<sup>8</sup> The sum of the three S-Zn-S angles equals 360.0°, indicative of the planar  $ZnS_3$  coordination. The distortions from 3-fold symmetry (S1-Zn-S2, 110.11 (7)°; S1-Zn-S3, 134.10 (8)°; S2-Zn-S3, 115.78 (7)°) result from the stacking interactions between two of the aromatic rings.

This result is surprising with respect to the known coordination chemistry of zinc<sup>9</sup> and particularly its coordination with thiolate ligands.<sup>10</sup> Three coordination is extremely rare for Zn(II) and Cd(II),<sup>11-13</sup> although it is more commonly observed for other d<sup>10</sup> transition metals, Hg(II), Cu(I), Ag(I), and Au(I).<sup>14</sup> The structure of **1** must be considered in relationship to other structures that would have been suggested by precedent. It is not a  $[(RS)_2Zn(\mu_2-SR)_2Zn(SR)_2]^{2-}$  dimer as has been characterized for SR = SEt and SPh or a  $Zn_4(\mu_2-SR)_6L_4$  adamantane cluster.<sup>10,15,16</sup> It has previously been demonstrated with a number of different metals that ortho-disubstituted aromatic thiolates have a reduced tendency to bridge metal centers.<sup>4,17</sup> This effect is not

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- (8)  $[(n-Pr)_4N][Zn(S-2,3,5,6-Me_4C_6H)_3]$ ,  $ZnS_3N_4C_{24}H_{67}$ , crystallizes in the monoclinic space group  $P2_1/n$  with  $a = 11.029$  (2) Å,  $b = 18.499$  (3) Å,  $c = 21.588$  (5) Å,  $\beta = 96.05$  (2)°,  $V = 4380$  (3) Å<sup>3</sup>,  $Z = 4$ . Final least-squares refinement using 2947 unique reflections with  $I > 3\sigma(I)$  gave  $R(R_w) = 0.045(0.056)$ .
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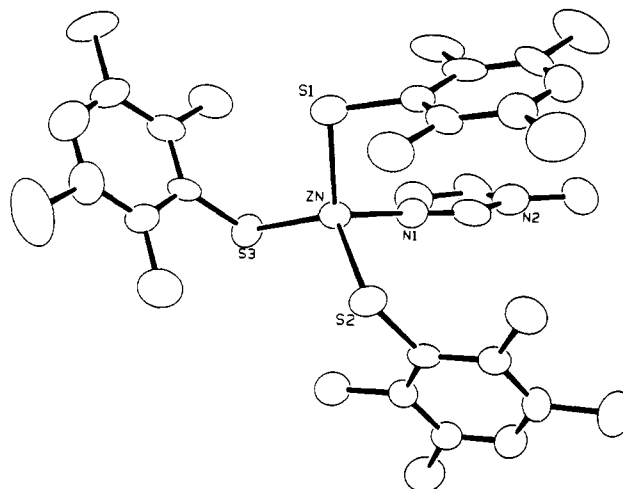


**Figure 1.** ORTEP diagram of  $[\text{Zn}(\text{S}-2,3,5,6\text{-Me}_4\text{C}_6\text{H})_3]^{1-}$  (**1**). Selected bond distances (Å) and angles (deg): Zn-S1, 2.230 (2); Zn-S2, 2.243 (2); Zn-S3, 2.217 (2); S1-Zn-S2, 110.11 (7); S1-Zn-S3, 134.10 (8); S2-Zn-S3, 115.78 (7); Zn-S1-C11, 107.7 (2); Zn-S2-C21, 103.7 (2); Zn-S3-C31, 108.0 (2).

absolute as the corresponding Cd complex has the dimeric structure,  $[(n\text{-Pr}_4\text{N})_2[\text{Cd}_2(\text{S}-2,3,5,6\text{-Me}_4\text{C}_6\text{H})_6]]$ .

Compound **1**, although it was prepared in and recrystallized from  $\text{CH}_3\text{CN}$ , does not add a molecule of solvent to give a tetrahedral  $[\text{Zn}(\text{SR})_3(\text{CH}_3\text{CN})]^{1-}$  complex. The analogous cobalt complex, which was crystallized in a manner identical with the crystallization of **1**, was previously shown to be four-coordinate,  $[\text{Co}(\text{S}-2,3,5,6\text{-Me}_4\text{C}_6\text{H})_3(\text{CH}_3\text{CN})]^{1-}$ .<sup>17a</sup> This is another example of a difference in the coordination chemistry of Co(II) and Zn(II) thiolate complexes;  $[\text{Zn}(\text{SR})_2(\text{bpy})]$  is four-coordinate while  $[\text{Co}(\text{SR})_2(\text{bpy})]$  adds  $\text{CH}_3\text{CN}$  to give the five-coordinate complex,  $[\text{Co}(\text{SR})_2(\text{bpy})(\text{CH}_3\text{CN})]$ .<sup>4,6</sup>

The steric capacity of the thiolate ligands in **1** does not prevent the addition of a fourth ligand to the zinc. The addition of 1-methylimidazole to the reaction mixture yields  $[(n\text{-Pr}_4\text{N})[\text{Zn}(\text{S}-2,3,5,6\text{-Me}_4\text{C}_6\text{H})_3(1\text{-Me-imid})]]$  (**2**).<sup>7</sup> The crystal structure of **2** (Figure 2) shows a distorted tetrahedral  $\text{ZnS}_3\text{N}$  unit with Zn-S = 2.33 (2) Å and Zn-N = 2.06 (1) Å.<sup>18</sup> This  $[\text{Zn}(\text{SR})_3(\text{N})]$  unit reproduces the proposed  $[\text{Zn}(\text{S-Cys})_3(\text{His})]$  coordination in the gene32 protein and retroviral gag proteins.<sup>19-21</sup> The reaction of 8 equiv of  $\text{KS}-2,3,5,6\text{-Me}_4\text{C}_6\text{H}$  with  $\text{ZnCl}_2$  in  $\text{CH}_3\text{CN}$  gives  $\text{K}_2[\text{Zn}(\text{S}-2,3,5,6\text{-Me}_4\text{C}_6\text{H})_4]$  (**3**), which has been structurally characterized to contain the  $[\text{Zn}(\text{SR})_4]^{2-}$  unit. The large difference



**Figure 2.** ORTEP diagram of  $[\text{Zn}(\text{S}-2,3,5,6\text{-Me}_4\text{C}_6\text{H})_3(1\text{-Me-imid})]^{1-}$  (**2**). Selected bond distances (Å) and angles (deg): Zn-S1, 2.328 (4); Zn-S2, 2.351 (3); Zn-S3, 2.298 (4); Zn-N1, 2.06 (1); S1-Zn-S2, 110.2 (1); S1-Zn-S3, 110.9 (1); S2-Zn-S3, 119.8 (1); S1-Zn-N1, 107.3 (3); S2-Zn-N1, 102.0 (4); S3-Zn-N1, 105.4 (4); Zn-S1-C11, 100.7 (3); Zn-S2-C21, 111.1 (4); Zn-S3-C31, 101.5 (4).

in the Zn-S distances in **1** (2.23 (1) Å) and in **2** (2.33 (2) Å) and **3** (2.36 Å) reflects the change in coordination number. The coordination of a fourth ligand to  $[\text{Zn}(\text{SR})_3]^{1-}$  necessitates a considerable change in the Zn-S distance. The difference in the Zn-S distances in **2** and **3** fits the observed trend for bond distances in  $[\text{M}(\text{SR})_x(\text{imidazole})_{(4-x)}]$  complexes.<sup>6</sup>

The formation of  $[\text{Zn}(\text{SR})_3]^{1-}$  results from the electron-donating nature of the thiolate ligands, together with their moderate steric hindrance. These rather unremarkable conditions suggest that low-coordinate complexes should become more common in the coordination chemistry of zinc. The factors that control the equilibria between the  $[\text{M}_2(\text{SR})_6]^{2-}$ ,  $[\text{M}(\text{SR})_3]^{1-}$ , and  $[\text{M}(\text{SR})_3\text{L}]^{n-}$  complexes for various M(II) ions are the subject of continuing investigation.

There are several biochemical implications to these observations: (1) trigonal  $[\text{Zn}(\text{S-Cys})_3]$  should be considered as a possible coordination mode in zinc-cysteine proteins;<sup>22</sup> (2) three-coordinate zinc intermediates deserve more consideration in proposed mechanisms for zinc-containing enzymes; (3) cobalt(II) substitution in zinc-cysteine proteins may not always give isostructural centers; and (4) the EXAFS signature of a three-coordinate  $[\text{Zn}(\text{S-Cys})_3]$  center would be short Zn-S distances in a total  $\text{ZnS}_x$  coordination sphere.

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**Supplementary Material Available:** Tables of crystallographic parameters, atomic coordinates, thermal parameters, and bond distances and angles for **1** and **2** (22 pages); observed and calculated structure factors for **1** and **2** (20 pages). Ordering information is given on any current masthead page.

(18)  $[(n\text{-Pr}_4\text{N})[\text{Zn}(\text{S}-2,3,5,6\text{-Me}_4\text{C}_6\text{H})_3(1\text{-Me-imid})]\cdot\text{CH}_3\text{CN}]$  (**2**),  $\text{ZnS}_3\text{N}_4\text{C}_{48}\text{H}_{76}$ , crystallizes in the triclinic space group  $P\bar{1}$  with  $a = 11.398$  (9) Å,  $b = 22.694$  (8) Å,  $c = 10.396$  (5) Å,  $\alpha = 93.04$  (4)°,  $\beta = 107.49$  (6)°,  $\gamma = 93.86$  (6)°,  $V = 2551$  (5) Å<sup>3</sup>,  $Z = 2$ . Final least-squares refinement using 2493 unique reflections with  $I > 3\sigma(I)$  gave  $R(R_w) = 0.056(0.067)$ .

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