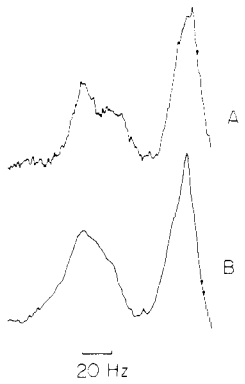

Figure 2. 220-MHz NMR spectra of the α-CH_2 region of (A) phosphatidylserine and (B) phosphatidylethanolamine in mixed micelles with Triton X-100 at pH 6.0 and Triton/phospholipid molar ratios of 4:1. The extra peak furthest upfield arises primarily from the methylene protons adjacent to vinylic groups in the unsaturated fatty acyl chains of the natural phospholipids.

in Figure 1A. Irradiation of the phospholipid β-CH_2 region narrows the α-CH_2 peaks slightly: the narrower decoupled peak is 2.35 ppm downfield from TSP with $\Delta\nu_{1/2}$ = 8-9 Hz, while the broader peak is 20 Hz further upfield (2.26 ppm from TSP) with $\Delta\nu_{1/2}$ = 15-17 Hz (B). The α-CH_2 signals of **1** are compared with those of lysophosphatidylcholine (C) and palmitic acid (D) also in Triton micelles. Each of these products of phospholipase A_2 hydrolysis shows only one resonance in the α-CH_2 region; the line widths ($\Delta\nu_{1/2}$ = 17-18 Hz) correspond to that of the narrow peak in the undecoupled phospholipid spectrum ($\Delta\nu_{1/2}$ = 17-18 Hz).

The nonequivalence of the α-CH_2s may be due to (i) an enhancement of the inherent chemical nonequivalence of the α-CH_2 groups of the two fatty acid chains or (ii) a slow chemical exchange between two distinct micellar environments. The latter could occur if the packing is such that the α-CH_2 group can be in either of two conformations. In this case, the population in each environment (or the exchange rate) should be variable by changing the temperature, pH, surfactant/phospholipid ratio, or phospholipid chain composition. We found that the relative chemical shifts and intensities of the two α-CH_2 resonances in Triton/phosphatidylcholine mixed micelles are not affected by temperature (from 20 to 55 °C),[9] by the surfactant/phospholipid ratio (mole ratios of 2.5:1 to 11:1 Triton:**1**), by changing the phospholipid fatty acid composition (dipalmitoyl- and egg phosphatidylcholine both show the same pattern), or by pH (2 to 9). These experiments suggest that the α-CH_2 pattern exhibited by **1** is an inherent characteristic of the phospholipid conformation in the mixed micelle and not the result of slow exchange between different micelle structures.

The assignment of the two α-CH_2 peaks to the *sn*-1 and *sn*-2 fatty acyl chains was made using a phospholipid in which the *sn*-2 α-CH_2 group was substituted with deuterium as shown in Figure 1E. A single peak was observed at 2.26 ppm. Thus, the broader upfield peak in the spectrum of **1** is assigned to the α-CH_2 group of the *sn*-1 chain, and the narrower downfield peak is assigned to the *sn*-2 chain.

Phosphatidylcholine purified from egg yolk shows the same splitting pattern in the α-CH_2 region when solubilized by Triton X-100 as described for **1**. This suggests that the conformations adapted by the fatty acyl groups are the same for natural phosphatidylcholines containing unsaturated fatty acids as for **1**. Phosphatidylserine in Triton micelles also shows an α-CH_2 region identical with that of **1** as shown in Figure 2A. In both phosphatidylcholine and phosphatidylserine, the peak assigned to the α-CH_2 group of the *sn*-1 chain is shifted upfield from the *sn*-2 chain. An upfield shift could indicate a more hydrophobic environment.[10] The line width of the group is also larger, suggesting more restricted motion or nonequivalence of the two protons of the α-CH_2 group. Surprisingly, phosphatidylethanolamine in Triton does not show the same well-defined α-CH_2 pattern as phosphatidylserine or phosphatidylcholine (Figure 2B). Only one broadened, but somewhat skewed, peak is observed, indicating that the ethanolamine polar group favors a lipid conformation in the mixed micelles in which the environment of the α-CH_2 groups is different from that in phosphatidylcholine or phosphatidylserine.

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(10) (a) The x-ray crystal structure of phosphatidylethanolamine[10b] and ^2H NMR studies on phosphatidylcholine multibilayers[10c] suggest that the conformation of the phospholipid in these structures is such that the α-CH_2 of the *sn*-1 fatty acyl chain is perpendicular to the surface and more buried than the α-CH_2 of the *sn*-2 chain which is parallel to the surface. (b) P. B. Hitchcock, R. Mason, K. M. Thomas, and G. G. Shipley, *Proc. Natl. Acad. Sci. U.S.A.*, 71, 3036 (1974). (c) A. Seelig and J. Seelig, *Biochim. Biophys. Acta*, **406**, 1 (1975).

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Nature of Hg^{2+}-L-Cysteine Complexes Implicated in Mercury Biochemistry

Sir:

L-Cysteine complexes of Hg^{2+} are urinary excretory products of certain mercurial diuretics[1] and have been directly implicated in the transport of Hg^{2+} across membranes,[2] the binding of Hg^{2+} to kidney proteins,[3] and the role of metallothioneine as a possible detoxificating agent for low levels of inorganic mercury.[4] Despite this, little is known of the structures of Hg^{2+}-L-cysteine complexes and as a recent review indicates[5] even the formulae and stoichiometries of the simplest compounds are controversial. We have carried out detailed synthetic, spectroscopic, and x-ray structural studies of Hg^{2+}-L-cysteine complexes to establish structural details of

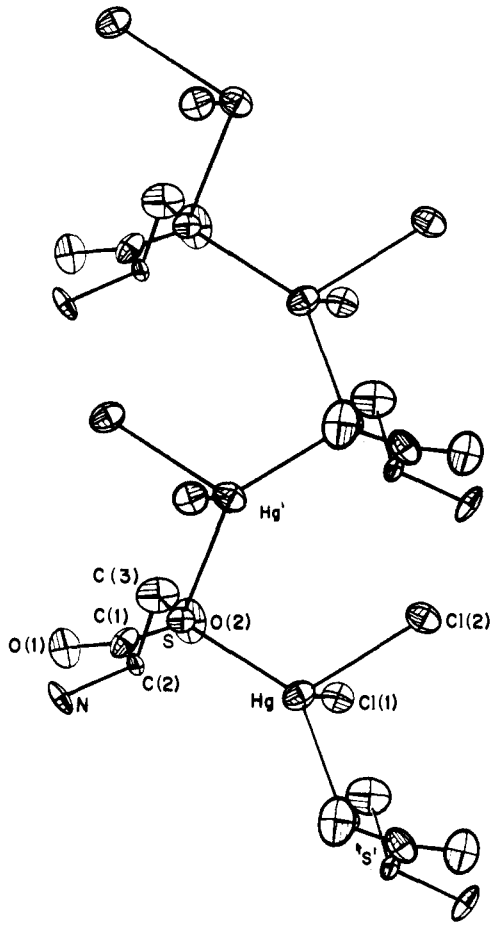


Figure 1. Perspective drawing of **1** showing atomic labeling scheme. Important bond lengths follow: Hg-S, 2.490 (4); Hg-S′, 2.453 (4); Hg-Cl(1), 2.582 (4); Hg-Cl (2), 2.645 (5); S-C(3), 1.83 (2); C(1)-O(1), 1.20 (2); C(1)-O(2), 1.32 (2); Hg-Hg′, 3.878 (1) Å. Important bond angles follow: S-Hg-S′, 136.0 (1); Cl(1)-Hg-Cl(2), 91.4 (1); S-Hg-Cl(1), 103.8 (1); S-Hg-Cl(2), 96.3 (1); S′-Hg-Cl(1), 108.4 (1); S′-Hg-Cl (2), 111.9 (1); Hg-S-Hg′, 103.4 (O)°.

relevance to inorganic mercury binding in biochemistry and more specifically to aid in the design of antidotes for mercury poisoning. The use of cysteine, penicillamine, and their derivatives in chelation therapy for metal poisoning has stimulated recent efforts to characterize the relevant metal complexes.[6]

Reaction of $HgCl_2$ (1.0 g) in ethanol (20 mL) with L-cysteine (0.45 g) in water (20 mL) gave a white precipitate. Dissolution in a minimum of HCl and crystallization afforded clear prisms identified by microanalysis[7] as $HgCl_2[SCH_2CH(NH_3)COOH]$ (**1**). From very dilute solutions **1** can also be obtained, without addition of acid, by crystallization of mother liquor after removal of the initial precipitate. The bromide analogue of **1** can be prepared similarly from $HgBr_2$. Weissenberg and precession photography indicated that **1** crystallizes in the orthorhombic acentric space group, $P2_12_12_1$ with a unit cell of dimensions $a = 14.699$ (3), $b = 8.017$ (2), $c = 7.025$ (1) Å. The experimental density of 3.16 g cm^{-3} agrees with a value of 3.150 g cm^{-1} calculated for $M = 392.66$ with $Z = 4$. The structure solution and refinement[8] were based on 1198 observed reflections (Mo Kα radiation; $2.5° \leq 2\theta \leq 65°$; $I \geq 3\sigma(I)$) from a total of 1742 independent intensities measured by counter methods.[9] Convergence was attained with $R = 0.043$ and $R_w = 0.050$. A perspective view of the molecule is shown in Figure 1. The principal structural feature of biochemical significance is the strong, almost symmetrical sulfur bridge between tetrahedrally coordinated mercury atoms. The Hg-S bond lengths to the bridging cysteine molecules (Hg-S of 2.490 (4) and Hg-S′ of 2.453 (4) Å) are distinctly shorter than the Hg-Cl bonds (average 2.614 (5) Å) or the values usually associated with tetrahedrally coordinated mercury[10] and are even comparable with the short Hg-S bond lengths (2.460 (3) Å) in $HgCl_2$(6-mercaptopurine)$_2$ where the mercapto ligands are terminal.[11] The large S-Hg-S′ angle (136.0 (1)°) and correspondingly small Cl(1)-Hg-Cl(2) angle (91.4 (1)°) together with the bond distances suggest a tendency for mercury to maximize bonding to sulfur. Indeed **1** may be considered as an intermediate en route to an essentially two-coordinate compound with two strong digonal Hg-S (cysteine) bonds. This is borne out by the conversion of **1** to $Hg[SCH_2CH(NH_3)COO][SCH_2CH(NH_3)COOH]Cl\cdot 0.5H_2O$ (**2**) in hot water. **2** can also be synthesized directly from $HgCl_2$ (1.0 g) and L-cysteine (0.90 g) in 50:50 aqueous ethanol. Needles of **2** are monoclinic, space group $C2$, with $a = 24.181$ (8), $b = 5.093$ (3), $c = 12.006$ (6) Å; $\beta = 118.83$ (3)°. With $Z = 4$ the calculated density of 2.494 g cm^{-3} for $M = 486.36$ compares favorably with the measured density of 2.50 g cm^{-3}. From a total of 2094 measured reflections, 1770 with intensities $I \geq 3\sigma(I)$ were employed in the structure solution. Refinement[8] has proceeded to R and R_w values of 0.041 and 0.049, respectively. The mercury atom (Figure 2) is coordinated to the sulfur atoms of two cysteine molecules (average Hg-S, 2.342 (4) Å) in an approximately linear array (S(1)-Hg-S(2), 169.8 (1)°) with the chloride present as essentially chloride ion (Hg-Cl, 3.232 (5) Å).

A charge balance suggests the possibility of different states of ionization for the amino acids. Actually the structural data suggest that O(2) is symmetrically hydrogen bonded to O(3) of a neighboring molecule giving a strongly hydrogen-bonded polymer. Thus the O(2)··O(3) nonbonded distance of 2.44 (2) Å is typical[12] of symmetrical O··H··O bonds, and the C(1)-O(2) (1.28 (2) Å) and C(4)-O(3) (1.25 (2) Å) bond lengths are virtually equivalent. Furthermore, the environments of O(2) and O(3) are very similar with angles C(1)-O(2)··O(3) and C(4)-O(3)··O(2) of 120.0 (6) and 117.9 (8)°, respectively.

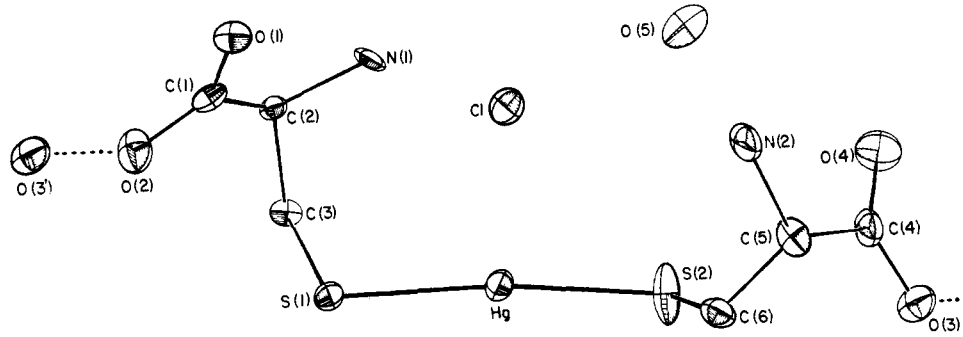
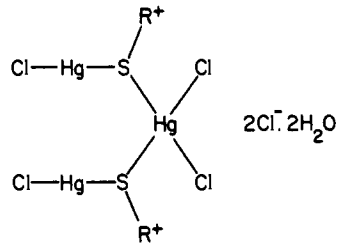


Figure 2. A perspective drawing of **2** showing the atomic labeling scheme. Important bond lengths follow: Hg-S(1), 2.355 (3); Hg-S(2), 2.329 (5); Hg-Cl, 3.232 (5); O(2)-O(3′), 2.44 (2) Å. Important bond angle follows: S(1)-Hg-S(2), 169.8 (1)°. O(5) is an oxygen atom of a molecule of water of crystallization.

Finally the ν(CO) frequency in **2** (1672 cm^{-1}) is intermediate between values in free carboxylic acids and carboxylate anions.

Our structural and synthetic results for **1** and **2** allow a reassessment of previous literature data on Hg^{+2}-L-cysteine complexation. Under neutral or slightly acid conditions the most stable complex is **2.** Since the chloride in **2** is ionic, it is likely that structurally similar compounds with different anions would be obtainable from other Hg^{+2} salts. Complex **2** appears to be identical with a compound obtained via different routes by Neville and Drakenberg.[15] Moreover, facile removal of HCl from **2** without change in mercury coordination would seem possible to generate $Hg[SCH_2CH(NH_3)COO]_2$, which is the usual formula assumed for "mercury cysteinate".[1]

The observation of strong sulfur bridging in **1** and ionic halide in **2** provides a ready explanation for the existence of mercury complexes with the unusual stoichiometries Hg_2L_2·, Hg_3L_2·, Hg_2L_2HCl·, $Hg_3L_2Cl_2$·, and $Hg_3L_2Cl_6{\cdot}2H_2O$[16] which have been suggested either in solution[17] or the solid state.[18] Thus a likely formulation for $Hg_3L_2Cl_6{\cdot}2H_2O$ is **3** with two



3

sulfur bridges, a tetrahedral mercury atom as in **1,** two two-coordinate mercury atoms, chloride ion, and water of crystallization as in **2.** A single bridging sulfur ligand, one terminal sulfur bonded amino acid, and two bicoordinate mercury atoms can likewise be expected for Hg_2L_2HCl. We also note that the much higher affinity of thioneine for Hg^{2+} than $MeHg^{+4}$ may be explicable on the basis that at least two sulfur sites are available for strong binding to the same metal ion in thioneine (cf. **1** and **2**). Methylmercury appears to have only a very weak residual Lewis acidity when coordinated linearly to one sulfhydryl site.[19] The polarity of complexes such as **2** contrasts sharply with the essentially nonpolar $MeHg^{+}$-L-cysteine complex,[19] a fact of obvious relevance to membrane permeabilities and biotransport mechanisms.[4] Furthermore, the association of chloride ion with the polar bis(amino acid)mercury unit in **2** and the coordination of chloride to mercury in **1** may explain the significantly different distributions[20] of inorganic and methylmercury between red blood cells and plasma in whole blood. The high Cl^- concentrations in plasma suggest a role for this ion in the distribution of Hg^{2+} in the body.

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(9) All intensity data were collected on a General Electric XRD-6 Datex automated diffractometer operating at 298K in the θ–2θ scan mode with a scan rate of 2° min^{-1} for **1** and 1° min^{-1} for **2** and with a scan width determined by the equation $\Delta\theta = \pm(0.9 \pm 0.43 \tan\theta)$.
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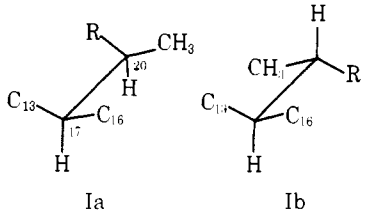
Further Arguments on the Stereochemistry of Sterols at C-20

Sir:

A number of recent communications concern C-20 conformation in sterols.[1-5] Nes[1-3] suggests a solution to this important problem based on (1) comparison of chemical shift differences for cholesterol and isocholesterol with those for analogous stereoisomeric $\Delta^{17(20)}$ olefins, (2) assumption of similar stereochemistry in the saturated and unsaturated analogues, and (3) conclusions regarding the ground-state populations of rapidly equilibrating conformers based on the stereochemistry of products derived from them. We wish to present evidence that (1) the olefin is not a valid model for NMR analysis of the saturated system, (2) the C-17–C-20 rotational barrier may be quite small and there still be the observed NMR differences, and (3) arguments on ground-stage populations of conformers such as those presented are thermodynamically unsound.

Nes[1] has concluded from molecular models that cholesterol, which has the 20*R* configuration, should be most stable in either conformation Ia or Ib, with Ib preferable. The reasons for



Ia Ib

R = isohexyl

seriously considering Ia are somewhat puzzling since it is eclipsed and has maximized hindrance between the largest substituents. Conformation Ib would, indeed, appear to be the best of the staggered rotamers.

In the case of 20-isocholesterol (20*S*), it is less clear-cut