(15) R. Schwyzer and H. Kappeler, *Helv. Chim. Acta*, **46**, 1150 (1963).
(16) Ap-M (Rohm & Haas) and Prolidase (Miles Laboratories) digestion after digestion with trypsin and chymotrypsin (Worthington Biochemical Co.) in Tris buffer, pH 8.5 at 37°.[13]
(17) Enzymatic digestion; Ser, 1.78; Tyr 1.98; Met, 1.02; Glu, 1.15; His, 0.98; Phe, 1.10; Arg, 3.41; Trp, 1; Gly, 1.98; Lys, 4.51; Pro, 2.94; Val, 3.26 and acid hydrolysis; Ser, 1.91; Tyr, 2.07; Met, 0.96; Glu, 1; His, 0.87; Phe, 1.07; Arg, 3.32; Gly, 2.18; Lys, 4.39; Pro, 3.28; Val, 3.14. This result shows that no detectable racemization has occurred during fragment condensation by the oxidation–reduction process.
(18) Thin-layer chromatography gave a single spot detected by Ehlrich, Pauly, Cl–tolidine, and ninhydrin reactions and uv absorption in 0.1 *N* NaOH was in good agreement with the literature.[15] High voltage electrophoresis gave a single spot in 0.5 *N* HCOOH–2*N* AcOH (1:1) (R_m, 0.72) and pyridine–AcOH–H_2O (10:0.4:90) (0.77) with lysine as reference.
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Mechanism of Anion Exchange of Alkyl Mercurials

Sir:

Anion exchange reactions of methylmercury halides and pseudo halides exhibit second-order kinetic behavior. NMR experiments have established that exchange in the magnetic environment of the methyl group in CH_3HgX–CH_3HgY mixtures involves transfer of the ligand (X and Y) on mercury. Cleavage of the covalent carbon–mercury bond has been excluded by the observation of ^2J ^{199}Hg–^1H coupling under anion exchange conditions.[1,2] Five mechanisms for this rapid anion or potential anion exchange may be considered (eq 1–5).[3-5]

$$RHgX + Y^- \rightleftharpoons RHg^{\cdot}\begin{matrix}X\\ \diagup\\ \diagdown\\ Y\end{matrix} \rightleftharpoons RHgY + X^- \quad (1)$$

$$CH_3\overset{*}{H}g^+ + CH_3HgY \rightleftharpoons CH_3\overset{*}{H}gY + CH_3Hg^+ \quad (2)$$

$$CH_3\overset{*}{H}g^+\|X^- + CH_3HgY \rightleftharpoons CH_3\overset{*}{H}gY + CH_3Hg^+\|X^- \quad (3)$$

$$CH_3\overset{*}{H}g^+\|X^- + CH_3Hg^+\|Y^- \rightleftharpoons CH_3\overset{*}{H}g^+\|Y^- + CH_3Hg^+\|X^- \quad (4)$$

$$CH_3\overset{*}{H}gX + CH_3HgY \rightleftharpoons \left[CH_3\overset{*}{H}g\begin{matrix}X\\ \diagup\\ \diagdown\\ Y\end{matrix}HgCH_3\right] CH_3\overset{*}{H}gY + CH_3HgX \quad (5)$$

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Kinetic evidence[1] based upon an NMR study in aqueous medium of the exchange reaction of CH_3HgCN and OH^- supports a bimolecular anionic mechanism at pH greater than 10.3 (eq 1). A second mechanism was operating at a pH of 9 or below. By the process of eliminating other mechanisms, the exchange was attributed to a "direct" exchange as suggested by eq 5. An exchange process involving methylmercuric ion (eq 2) was ruled unlikely. A mechanism involving the bridged intermediate (**1**) was also preferred for CH_3HgCN–CH_3HgX (X = Cl, Br, I) anion exchange in DMF solvent.[2] In the latter study, pathways involving solvent-separated ions pairs (eq 4) were also considered but not rigorously excluded. One of the basic difficulties in excluding an ionic mechanism in these exchange reactions is the possibility of ionization of RHgX and a rapid diffusion controlled anion exchange involving ions (eq 1 and 2) or reactive solvent-separated ion pairs (eq 3 and 4).[3]

Table I. Activation Parameters for the Exchange of CH_3HgCN with CH_3HgX in DMF

Compound	X	$\Delta H^{\ddagger}$,[a,b]	$\Delta S^{\ddagger}$, eu	$\Delta G^{\ddagger}_{298°K}$[a,b]	$k_{298°K}$[c]
2a	Cl	11.6 ± .2	−18 ± 1	17.1	1.5
2b	OAc	12.1 ± .3	−16 ± 1	16.9	2.2
2c	Br	11.3 ± .2	−17 ± 1	16.3	5.8
2d	SCN	16.1 ± .3	−1 ± 1	16.3	6.1
2e	SCH_3	11.2 ± .2	−13 ± 1	15.2	38.
2f	SC_6H_5	13.0 ± .2	−7 ± 1	15.2	40.
2g	S-*p*-C_6H_5Cl	11.7 ± .2	−11 ± 1	15.1	45.
2h	$SC(CH_3)_3$	11.8 ± .3	−9 ± 1	14.6	98

[a] $\Delta H^{\ddagger}$ and $\Delta G^{\ddagger}$ are given in kilocalories per mole. [b] The error limits given are those derived from least-squares analysis. [c] Second-order rate constants $k(M^{-1}\ sec^{-1})$ were not measured at 298°K but were obtained by extrapolation of a least-squares plot of data obtained at least eight other temperatures. Correlation coefficients of >0.9977 were obtained for each plot. The rate constants were calculated assuming a bimolecular mechanism where rate = $(k_2/2)[CH_3HgX][CH_3HgCN]$ and the observed rate of exchange $1/\tau_{CH_3HgX} = (k_2/2)[CH_3HgCN]$.

We now report a series of NMR experiments involving CH_3HgCN–CH_3HgX anion exchange in DMF solvent. The principal objective of this study was to establish the effect of the ionic character of CH_3HgX on its anion exchange rate with CH_3HgCN. By keeping the CH_3HgCN common to each exchange system, the variation in $\Delta G^{\ddagger}$ should be attributable largely to an enthalpy change if an ionic mechanism obtains or if extensive Hg–X bond breaking is involved in the rate limiting step. However, our data establish that the trend observed for the rates of anion exchange is the opposite to that which would be anticipated on the basis of an ionic pathway involving ionization of CH_3HgX. We also report the first unequivocal evidence excluding an ionic process in the mercaptide anion exchange of RHgSR compounds. These data are consistent with a bridged intermediate or transition state such as **1** (eq 5).

The thermodynamic parameters for exchange, which are summarized in Table I, were obtained by the complete line shape analysis NMR method.[6] Our results are consistent with earlier studies that established that the C–Hg bond was not labile under exchange conditions.[1,2,7] NMR experiments with the CH_3HgCN–$CH_3HgSC_6H_5$ system at three different temperatures each at four different concentrations in DMF established that exchange proceeded by a second-order pathway.

The measured[8] association constants for the formation of CH_3HgX from CH_3Hg^+ and the anions chosen for this study vary over 13 orders of magnitude.[9] The complete ionization of the Hg–X bond has been shown to increase in the order $RS^- < CN^- < Br^- < SCN^- < Cl^- < OAc^-$.[9] The relatively fast rate of exchange for the highly covalent methylmercury mercaptides (**1e–h**) strongly argues against any of the above ionic mechanisms (eq 1–4) being involved in mercaptide anion exchange. The magnitude of the exchange rates we have measured completely exclude all exchange processes involving two solvent separated ion pairs (eq 4). The rate expression, rate = $k_2K_1K_3[CH_3HgX][CH_3HgCN]$, consistent with this mechanism contains equilibrium constants (K_1 and K_3) for the dissociation of both CH_3HgX and CH_3HgCN. If the equilibrium constants (K_1) for the formation of solvent separated

ion pairs (eq 6) do not differ significantly from those reported for complete ionization ($K = 10^{-3}$ to 10^{-16}),[9] then the observed rate of exchange is too rapid for this type of exchange mechanism. Similar arguments tend to preclude

$$CH_3HgX \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} CH_3Hg^+ \,||\, X^- \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} CH_3Hg^+ + X^- \qquad (6)$$

exchange involving one solvent separated ion pair (eq 3) even if a fast second-order diffusion controlled exchange ($k_2 = 10^9$ to 10^{10}) were assumed

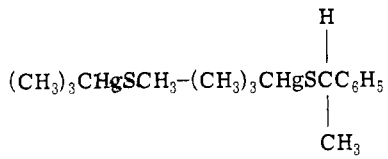
$$\text{rate} = \frac{k_2}{2} K_1[CH_3HgX][CH_3HgCN]$$

However, the ionic mechanism in eq 2

$$\text{rate} = \frac{k_2}{2}(K_1[CH_3HgX])^{1/2}[CH_3HgCN]$$

is not as readily excluded with more ionic substrates. Likewise it is plausible from Simpson's work[1] that at higher acetate ion concentrations the CH_3HgCN-CH_3HgOAc system may undergo exchange by the anionic mechanism in eq 1.

The trend observed with the rate constants also establishes the importance of bridging in the transition state. Thus, the slowest rate observed is for **2a** (Table I) where a chlorine bridge is presumably involved. The faster rate of exchange for the *tert*-butyl mercaptide (**2h**) relative to the methyl mercaptide (**2e**) suggests that electron density at sulfur is more important than steric effects. The apparent importance of bridging in the above reactions suggested that a RHgSR-RHgSR′ exchange would exhibit a rate of exchange sufficiently high that an ionic mechanism could be unequivocally excluded. Our anticipations were realized with the exchange system



These compounds exhibited a line separation for the *tert*-butyl resonance of 13.5–14.5 Hz and were sufficiently soluble in the mixed solvents HCF_2Cl:$HCFCl_2$ (4:1) at a coalescence temperature of −142° to allow a total line shape analysis exchange study. The free energy of activation for this remarkably facile anion exchange was found to be only 5.2 kcal/mol at −138°. Extrapolation of the data obtained at low temperature with several different ratios of substrate to 25° gave $\Delta G^{\ddagger}_{25} = 7.3$ kcal/mol and $\Delta S^{\ddagger} = -12.7$ eu. This exchange reaction also exhibited second-order kinetics with extrapolated $k_2 = 3 \times 10^7$ at 25° calculated on the basis of exchange as in eq 5. Since the equilibrium constant for ionization of a typical RHgSR is $\sim 10^{-16}$ in a polar solvent, any calculated rate constants based upon the ionic processes eq 1–4 for the relatively nonpolar solvent system investigated would be at least 10^4 greater than that of a diffusion controlled process. These data thus provide the first unequivocal example of a bimolecular anion exchange of RHgX with total exclusion of an ionic process. In conclusion, our results provide convincing evidence that all anion exchange reactions of RHgX compounds involving a covalent ligand bonded to mercury proceed via a bimolecular process involving a bridged intermediate such as **1**.[10]

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References and Notes

(1) R. B. Simpson, *J. Chem. Phys.*, **46**, 4775 (1967).
(2) L. L. Murrell and T. L. Brown, *J. Organometal. Chem.*, **13**, 301 (1968).
(3) E. D. Hughes, C. Ingold, F. G. Thorpe, and H. C. Volger, *J. Chem. Soc.*, 1133 (1961), and previous papers.
(4) (a) F. R. Jensen and B. Rickborn "Electrophilic Substitution of Organomercurials", McGraw-Hill, New York, N.Y., 1968; (b) D. S. Matteson, *Organomet. Chem. Rev.*, **4**, 263 (1969); D. S. Matteson, "Organometallic Reaction Mechanisms", Academic Press, New York, N.Y., 1974.
(5) M. H. Abraham, D. Dodd, M. D. Johnson, E. S. Lewis, and R. A. More O'Ferrall, *J. Chem. Soc. B*, 762 (1971).
(6) Theoretical spectra were generated by an IBM 360/65 computer and plotted on a calcomp plotter using a program of Raban and Carlson based on the solution to the (see M. Raban and E. Carlson, *J. Am. Chem. Soc.*, **93**, 685 (1971) and references therein), exchange-modified Bloch equations (program CLAS). The determination of rates of exchange by total line shape analysis involved obtaining complete correspondence between experimental and theoretical spectra. The variance in chemical shifts for the individual compounds was determined as a function of temperature. The entropy and enthalpy of activation were calculated using a double precision linear least-squares program of the Eyring equation with rate constants for each exchange system evaluated at a minimum of eight temperatures. For a pair of species, A and B, of equal concentrations the rate of exchange = $(1/[A])(d[A]/dt) = 1/\tau$ and for this exchange rate = $k_2[A][B]$, $1/\tau_A = k_2[B]$ and $1/\tau_B = k_2[A]$.
$$\ln \frac{k}{T} = \ln \frac{k_B}{h} + \frac{\Delta S^{\ddagger}}{R} - \left(\frac{\Delta H^{\ddagger}}{R}\right)\left(\frac{1}{T}\right)$$
(7) J. V. Hatton, W. G. Schneider, and W. Siebrand, *J. Chem. Phys.*, **39**, 1330 (1963); P. R. Wells, W. Kitching, and R. S. Henzel, *Tetrahedron Lett.*, 1029 (1964); M. D. Rausch and J. R. Van Wazer, *Inorg. Chem.*, **5**, 761 (1964).
(8) (a) G. Schwarzenbach and M. Schellenberg, *Helv. Chim. Acta*, **48**, 28 (1965); (b) R. Scheffold, *ibid.*, 1419 (1967); (c) R. D. Simpson, *J. Am. Chem. Soc.*, **83**, 4711 (1961).
(9) The K_{eq} for the reaction of $CH_3Hg^+ + X^- \rightleftharpoons CH_3HgX$ for $X^- = OAc^-$, Cl^-, SCN^-, Br^-, CN^-, $C_6H_5S^-$, and $HOCH_2CH_2S^-$ are $10^{3.6}$, $10^{5.25}$, $10^{6.05}$, $10^{6.62}$, $10^{14.1}$, $10^{14.67}$, and $10^{16.1}$, respectively, in aqueous solution. Equilibrium constants (K_1) for formation of solvent separated ion pairs are not available. However, it is intuitively reasonable that the same relative order of ionic character should be maintained.
(10) Extended Huckel calculations suggest that the bridged transition state **1** is considerably more stable than an acyclic species. The calculations also provide convincing evidence for significant Hg–Hg bonding in the transition state (unpublished results).

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Irreversible Inhibition of Δ^5-3-Ketosteroid Isomerase by 5,10-Secosteroids

Sir:

Recent studies[1] have shown that remarkably specific irreversible enzyme inhibitors can result from compounds bearing potential reactive groupings which are unmasked at the active site by the target enzyme. This specificity resides in the generation of the alkylating agent by the target enzyme at the active site as a result of the enzyme's normal catalytic process. The process is exemplified by the enzymatic conversion of an acetylenic compound to an allene which can alkylate an active site amino acid residue. The first such example was provided by Bloch[2] who showed that the acetylenic analog of a normal substrate for β-hydroxydecanoyl thioester dehydrase is converted by the enzyme to the corresponding conjugated allenic thioester with rapid alkylation of an active site histidine residue. This approach has been applied to the inhibition of monoamine oxidase[3] and γ-cystathionase.[4]

The enzyme Δ^5-3-ketosteroid isomerase[5] (EC 5.3.3.1) from *Pseudomonas testosteroni* converts C_{19} and C_{21} Δ^5-3-ketosteroids to the corresponding Δ^4-3-ketosteroids. The proposed mechanism[5,6] involves removal of the axial 4β-hydrogen with concomitant enolization to give a $\Delta^{3,5}$-dienol, followed by ketonization with axial reprotonation at C-6. The hydrogen transfer from C-4 to C-6 is intramolecular (Scheme I). This reaction when carried out by mamma-