was dried ($MgSO_4$) and concentrated in vacuo to give a crystalline residue. This was recrystallized (Et_2O) to give (1*R*,4*S*,5*R*)-**17b** as colorless crystals: mp 99.0–100.0 °C; $[\alpha]^{23}_D$ -39.9° (*c* 0.91, $CHCl_3$); IR (neat) 3436, 2984, 1770, 1718 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz), δ 0.77 (ddd, 1 H, *J* = 3.5, 4.0, 5.0 Hz), 1.19 (ddd, 1 H, *J* = 5.0, 8.0, 9.0 Hz), 1.50 (s, 9 H), 1.88 (ddd, 1 H, *J* = 4.0, 6.0, 8.0 Hz), 2.00 (dddd, 1 H, *J* = 1.5, 3.5, 6.0, 9.0 Hz), 2.31 (t, 1 H, *J* = 6.0 Hz), 3.82 (ddd, 1 H, *J* = 4.0, 6.0, 11.0 Hz), 3.86 (ddd, 1 H, *J* = 4.0, 6.0, 11.0 Hz), 4.11 (dt, 1 H, *J* = 1.5, 4.5, 4.5 Hz); MS (SIMS) *m/z* 228 $(M + H)^+$, 172, 128. Anal. Calcd for $C_{11}H_{17}O_4N$: C, 58.14; H, 7.54; N, 6.16. Found: C, 58.04; H, 7.67; N, 6.13. To a solution of **17b** in MeOH (5 mL) was added LiOH (14 mg, 0.57 mmol). The solution was stirred at room temperature for 16 h. Then it was extracted with EtOAc. The extract was dried ($MgSO_4$) and concentrated under reduced pressure to give an oily residue. This was purified by column chromatography on silica gel (Et_2O) to give **8d** (111 mg, 83% from **17a**) as a colorless amorphous solid: $[\alpha]^{23}_D$ -52.7° (*c* 1.09, $CHCl_3$); IR (neat) 3384, 2984, 1718 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.14 (ddd, 1 H, *J* = 5.0, 8.0, 9.5 Hz), 1.17 (ddd, 1 H, *J* = 5.0, 5.5, 7.0 Hz), 1.42 (s, 9 H), 1.55 (dddd, 1 H, *J* = 7.0, 7.5, 9.0, 9.5 Hz), 1.80 (ddd, 1 H, *J* = 5.5, 8.0, 9.0 Hz), 2.80 (br s, 1 H), 3.62 (dddd, 1 H, *J* = 3.5, 6.0, 7.5, 7.5 Hz), 3.70 (dd, 1 H, *J* = 6.0, 11.0 Hz), 3.70 (s, 3 H), 3.83 (dd, 1 H, *J* = 3.5, 11.0 hz), 4.93 (br s, 1 H); MS (SIMS) *m/z* 260 $(M + H)^+$, 204, 160. Anal. Calcd for $C_{12}H_{21}O_5N$: C, 55.58; H, 8.16; N, 5.40. Found: C, 55.44; H, 8.29; N, 5.40.

(4*S*)-4-(*N*-(*tert*-Butoxycarbonyl)amino)-5-pentanolide (18a). A solution of **14a** (1.00 g, 4.04 mmol) and CSA (10 mg) in benzene (500 mL) was refluxed for 3 h. The mixture was washed with aqueous $NaHCO_3$ and water and dried ($MgSO_4$). Concentration *in vacuo* gave a crystalline residue. This was crystallized (Et_2O) to give **18a** (799 mg, 92%) as colorless crystals: mp 104.0–104.5 °C; $[\alpha]^{23}_D$ -37.4° (*c* 1.09, $CHCl_3$); IR (neat) 3356, 2984, 1756, 1690 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.44 (s, 9 H), 1.87 (m, 1 H), 2.22 (m, 1 H), 2.58 (dt, 1 H, *J* = 7.5, 7.5, 17.0 Hz), 2.66 (dt, 1 H, *J* = 7.0, 7.0, 17.0 Hz), 4.03 (m, 1 H), 4.19 (dd, 1 H, *J* = 5.0, 11.5 Hz), 4.40 (ddd, 1 H, *J* = 0.5, 4.0, 11.5 Hz), 4.72 (br s, 1 H); MS (SIMS) *m/z* 216 $(M + H)^+$, 160, 116. Anal. Calcd for $C_{10}H_{17}O_4N$: C, 55.80; H, 7.96; N, 6.51. Found: C, 55.81; H, 8.03; N, 6.36.

(5*S*)-5-(*N*-(*tert*-Butoxycarbonyl)amino)-5,6-dihydro-2-pyrone (19). To a solution of **18a** (3.00 g, 13.9 mmol) in THF (70 mL) was added, successively, Me_3SiCl (3.85 mL, 30.7 mmol) and lithium hexamethyldisilazide (30.7 mL of a 1 *M* solution in THF, 30.7 mmol) at -78 °C. The solution was stirred for 15 min, then was added to a solution of $Pd(OAc)_2$ (3.74 g, 16.7 mmol) in CH_3CN (80 mL). The mixture was stirred at room temperature for 45 min and then quenched with aqueous NH_4Cl. The resulting suspension was filtered. The filtrate was concentrated *in vacuo* to give an oily residue. This was dissolved in Et_2O (100 mL), and the solution was washed with brine. The Et_2O solution was dried, and the solvent was evaporated *in vacuo* to give an oily residue. This, upon purification by column chromatography on silica gel (Et_2O/hexane, 3:1), gave **19** (2.08 g, 70%) as colorless crystals: mp 128.0–129.0 °C; $[\alpha]^{23}_D$ +113° (*c* 1.07, $CHCl_3$); IR (neat) 3340, 2988, 1724, 1686 cm^{-1}; ^1H NMR ($CDCl_3$, 100 MHz), δ 1.47 (s, 9 H), 4.2–4.6 (m, 3 H), 4.80 (br s, 1 H), 6.70 (d, 1 H, *J* = 10 Hz), 6.86 (dd, 1 H, *J* = 5, 10 Hz); MS (SIMS) *m/z* 214 $(M + H)^+$, 158, 114. Anal. Calcd for $C_{10}H_{15}O_4N$: C, 56.33; H, 7.09; N, 6.57. Found: C, 56.15; H, 7.07; N, 6.43.

(1*S*,5*S*,6*R*)-5-(*N*-(*tert*-Butoxycarbonyl)amino)-3-oxabicyclo[4.1.0]heptan-2-one (9c). To a suspension of $Pd(OAc)_2$ (21 mg, 0.094 mmol) and **19** (200 mg, 0.938 mmol) in Et_2O (30 mL) was added, drop by drop, a solution of CH_2N_2 in Et_2O at room temperature over 2 h. The mixture was then filtered. The filtrate was concentrated *in vacuo* to give an oily residue. This, upon purification by flash column chromatography on silica gel (Et_2O/hexane, 3:1), gave **9c** as colorless crystals (98 mg, 46%).

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Nuclear Magnetic Resonance Study of the Kinetics of the Penicillamine/Bis(penicillamine) Selenide Symmetrical Exchange Reaction

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The kinetics of reaction of the thiolate form of D-penicillamine (β,β-dimethylcysteine, PSH) with bis(D-penicillamine)selenide (PSSeSP) have been characterized over a wide pH range in aqueous solution by NMR line broadening techniques. Because of the steric bulk of the substituents on the β-carbon of penicillamine, the rate of nucleophilic reaction at the sulfur of PSSeSP is much less than at selenium, making it possible for the first time to characterize quantitatively the kinetics for reaction of a thiolate at the selenium of the bis(alkylthio) selenide of a biological molecule. Rate constants are reported for reaction of the amino and ammonium forms of PS^- with the various amino protonated forms of PSSeSP, e.g. the rate constant for reaction of the (NH_3^+, CO_2^-, S^-) form of PSH with the (NH_3^+, NH_3^+, CO_2^-, CO_2^-) form of PSSeSP is 9.7×10^5 L/mol·s. The PSH/PSSeSP interchange reactions are approximately 10^6 faster than the analogous reaction of 2-methyl-2-propanethiol with bis(*tert*-butylthio) selenide. The large magnitude of the rate constants suggests that nucleophilic reaction at the selenium of bis(alkylthio) selenides, which are thought to be formed initially in the incorporation of inorganic selenium into living systems, could be important in their conversion to other selenium-containing biomolecules.

Introduction

The reaction of selenious acid with thiol groups to form bis(alkylthio) selenides (or selenotrisulfides) (eq 1) is thought to be one of the principal pathways by which inorganic selenium is initially incorporated into biological systems.[1-6] Once formed, the bis(alkylthio) selenides are consumed by various reactions, although the nature of these reactions is not known. In a study of the reactivity

$$3RSH + H_2SeO_3 \rightarrow RSSeSR + RSSR + 3H_2O \quad (1)$$

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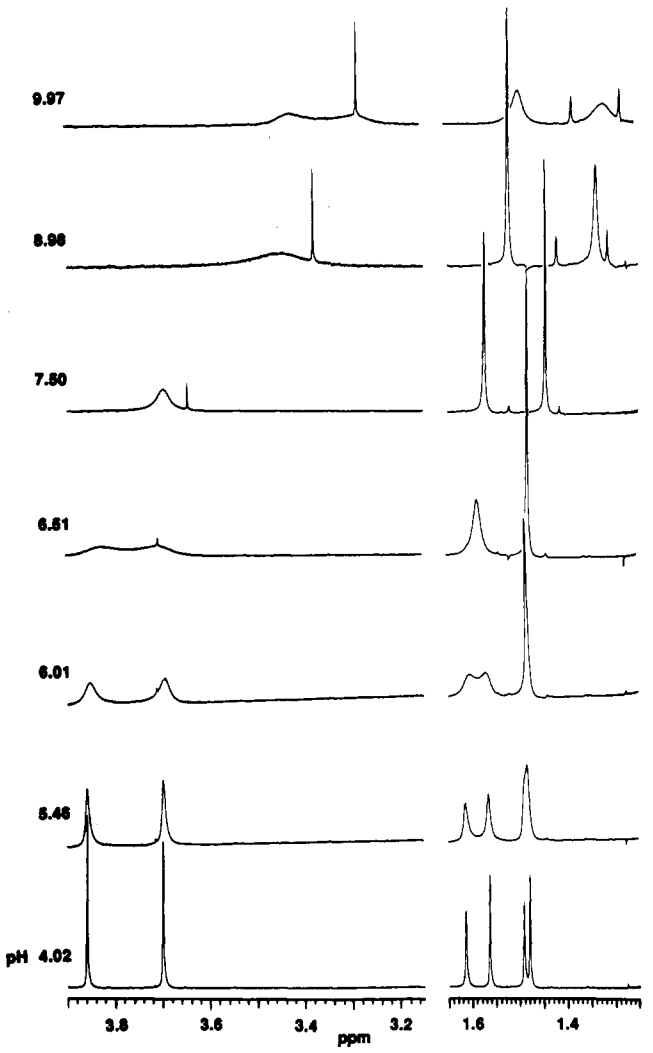

Figure 1. The methine (3.1–3.9 ppm) and methyl (1.25–1.65 ppm) regions of the ^{1}H NMR spectra of aqueous solutions containing 0.010 M PSH, 0.005 M PSSeSP, and 0.3 M KCl at 25 °C. The two regions are plotted with different vertical scales. The assignment of resonances in the pH 4.02 spectrum is as follows: 3.860 ppm, methine proton of PSSeSP; 3.699 ppm, methine proton of PSH; 1.614 and 1.491 ppm, methyl protons of PSSeSP; 1.564 and 1.479 ppm, methyl protons of PSH. The additional, sharp resonances which are present in spectra at pH 6.01 and higher are due to PSSP, as discussed in the text.

of the bis(alkylthio) selenides of 1-butanethiol, 2-propanethiol, and 2-methyl-2-propanethiol with nucleophiles, Kice and Slebocka-Tilk[7] found that organic thiols can react with bis(alkylthio) selenides at either sulfur (eq 2a) or selenium (eq 2b).

$$R^*SH + RSSeSR \rightarrow R^*SSR + RSSeH \quad (2a)$$

$$R^*SH + RSSeSR \rightarrow RSH + R^*SSeSR \quad (2b)$$

In both reactions, the thiolate (RS^-) rather than the undissociated thiol (RSH) reacts with the bis(alkylthio) selenide. When R and R* are *n*-butyl, reaction of RS^- at the sulfur is considerably faster than at selenium, whereas, when both are *tert*-butyl, the rate constant for reaction at selenium is 0.3 M^{-1} s^{-1} as compared to a rate constant of 0.011 M^{-1} s^{-1} for reaction at sulfur at 25 °C.

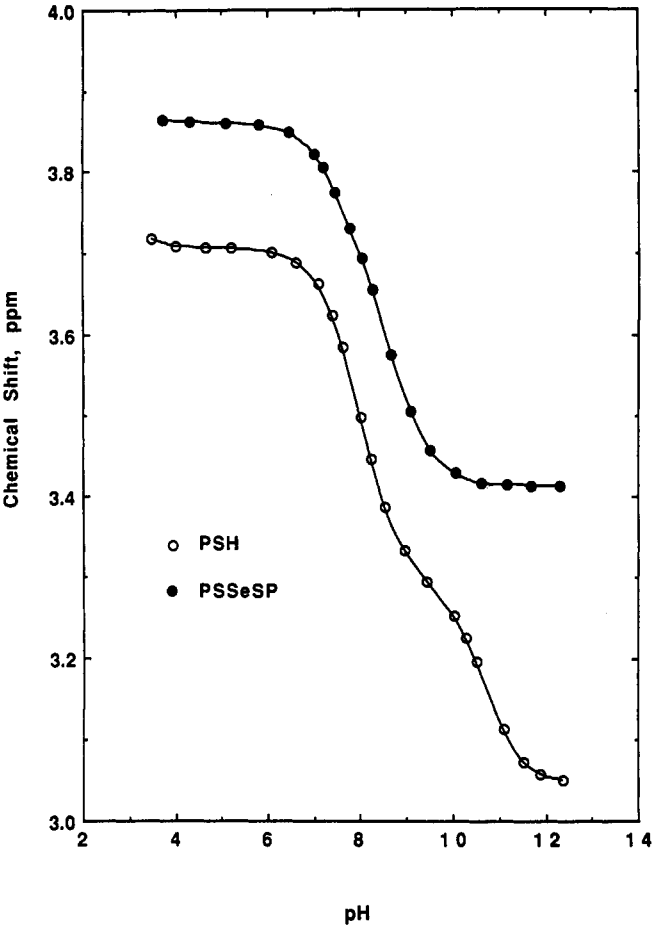

Figure 2. pH dependence of the chemical shifts of the resonances for the methine protons of PSH and PSSeSP: 25 °C, 0.3 M KCl.

Because of the importance of bis(alkylthio) selenides as intermediates in the incorporation of inorganic selenium into biological systems, we have initiated studies of their chemical properties in aqueous solution.[8,9] During the course of ^{1}H NMR studies of aqueous solutions containing D-penicillamine (β,β-dimethylcysteine, PSH)[10] and its bis(alkylthio) selenide (PSSeSP),[11] we observed exchange broadening and coalescence of the PSH and PSSeSP resonances, which indicates that exchange of the penicillamine moiety between PSH and PSSeSP is much faster than the rate of exchange (eq 2b) reported by Kice and Slebocka-Tilk for the *t*-BuSH/*t*-BuSSeSBu-*t* system.[7] Because of the limited amount that is known about the chemistry of bis(alkylthio) selenides, we have characterized the kinetics of the nucleophilic displacement reaction of PSH at the selenium of PSSeSP by NMR line broadening techniques. The results suggest that nucleophilic substitution by thiolates at the selenium in bis(alkylthio) selenides of biological thiols is much faster than found by Kice and Slebocka-Tilk for bis(butylthio) selenides.[7]

Results and Discussion

Representative ^{1}H NMR spectra are shown in Figure 1 for a solution containing 10 mM PSH and 5 mM PSSeSP as a function of pH. At pH 4.02, the spectrum is a composite of the spectra of PSH and PSSeSP, each of which

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(9) Killa, H. M. A.; Rabenstein, D. L. *J. Chromatogr.* **1989**, *465*, 359–368.
(10) Structure A in Scheme I.
(11) Structure B in Scheme II.

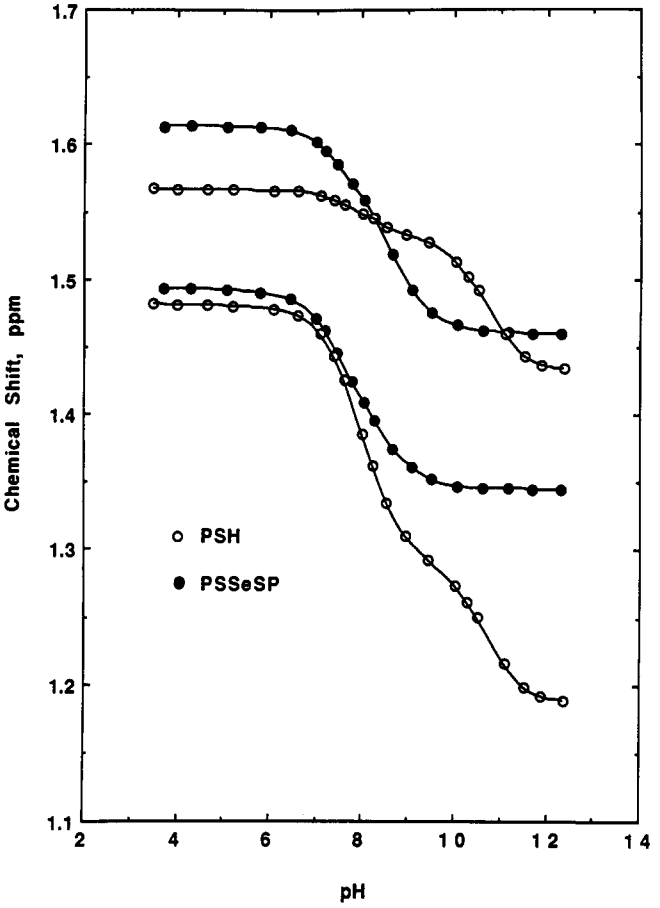

Figure 3. pH dependence of the chemical shifts of the methyl protons of PSH and PSSeSP: 25 °C, 0.3 M KCl.

consists of a singlet resonance for the methine proton and two singlets for the nonequivalent methyl groups. As the pH is increased, the resonances broaden and coalesce. The methine resonances coalesce to a single resonance, whereas the four methyl resonances coalesce to two exchange-averaged resonances. Because the chemical shift separation between the three pairs of resonances is different (Figures 2 and 3), the pH at which the resonances coalesce and the widths of the exchange-averaged resonances at a particular pH are different for the three different pairs of exchanging resonances. As the pH is increased above ~8, the exchange-averaged resonances broaden and, in the case of the methine resonance, separate into two distinct resonances at pH 9.97. The results indicate exchange of the penicillamine moiety between the PSH and PSSeSP forms:

$$\text{P*SH} + \text{PSSeSP} \rightleftharpoons \text{P*SSeSP} + \text{PSH} \qquad (3)$$

where P and P* are equivalent.

Examination of the spectra in Figure 1 also indicates that, at pH greater than approximately 6, additional resonances appear in the methine and methyl regions, and that the intensity of these resonances increases as the pH is increased. These additional resonances are due to penicillamine disulfide (PSSP). They are not observed in spectra of PSSeSP or PSH alone, which indicates that the PSSP is formed by reaction of PSH with PSSeSP by an additional pathway, presumably by reaction of PSH at a sulfur of PSSeSP.[7,12] No resonances which could be assigned to $PSSe^-$ were observed. However, in some experiments the solutions developed a faint reddish color, which might indicate the formation of elemental selenium by further reaction of any $PSSe^-$ formed by reaction of PSH at the sulfur of PSSeSP.

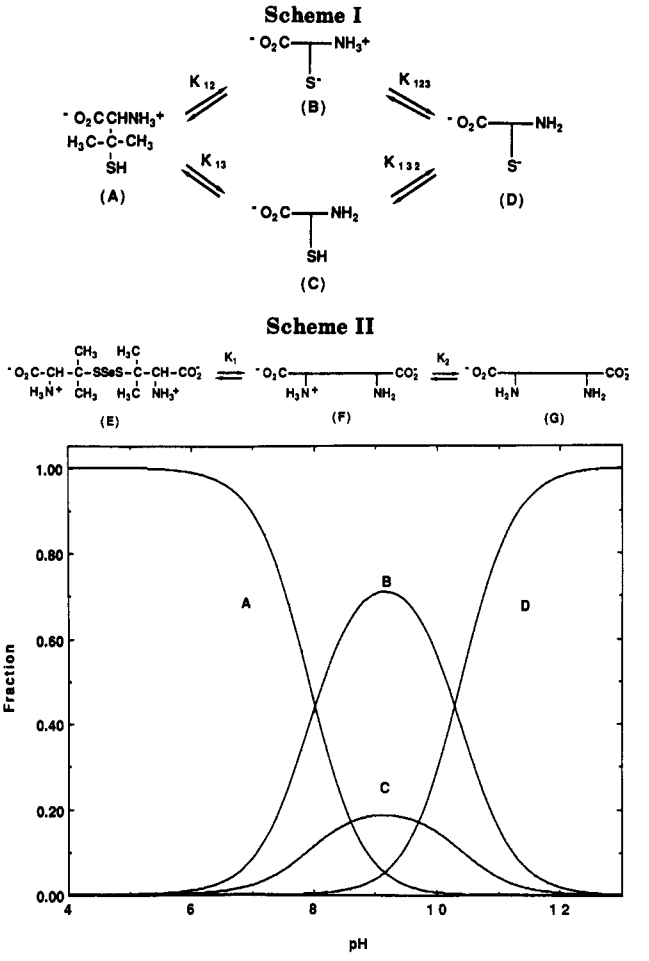

Figure 4. Fractional concentrations of the penicillamine species identified in Scheme I as a function of pH.[11]

The kinetics of the symmetrical PSH/PSSeSP exchange reaction (eq 3) were characterized by determining the mean lifetimes of the PSH and the penicillamine moieties in PSSeSP from exchange-broadened spectra of the type shown in Figure 1. Mean lifetimes determined for PSH, τ_{PSH}, over a wide range of pH values are listed in Table I.[13] The mean lifetime of PSH is related to the rate of exchange by the equation:

$$1/\tau_{PSH} = -\frac{d[PSH]}{dt}\frac{1}{[PSH]} \qquad (4)$$

The exchange reaction was determined to be first-order in both PSH and PSSeSP by measuring the mean lifetimes of PSH and the penicillamine moieties of PSSeSP at different concentrations of PSH and PSSeSP at pH 5.00. Thus,

$$-\frac{d[PSH]}{dt} = k[PSH][PSSeSP] \qquad (5)$$

As the pH is increased from 4.26, $1/\tau_{PSH}$ increases, indicating an increase in the rate of the reaction as acidic groups on PSH and/or PSSeSP are titrated. Kice and Slebocka-Tilk found that the thiol-deprotonated form of

(12) The concentrations of both PSH and PSSeSP were found to decrease upon formation of PSSP and by amounts which indicate a net reaction of PSH with PSSeSP at a 2:1 stoichiometry. Thus, it is proposed that PS^- reacts with PSSeSP to form PSSP and $PSSe^-$, which in turn reacts with PS^- to form PSSP and Se^{2-} (or HSe^-).

(13) The presence of PSSP was found to have no effect on the kinetics of the reaction of PSH with PSSeSP.

Table I[a,b]

pH	$\tau_{PSH,experimental}$ (s)	$\tau_{PSH,predicted}$ (s)
4.26	0.816	0.867
4.53	0.388	0.382
4.74	0.238	0.216
4.91	0.138	0.141
5.01	0.116	0.111
5.14	0.0835	0.0815
5.24	0.0639	0.0635
5.35	0.0479	0.0500
5.45	0.0402	0.0402
5.52	0.0323	0.0377
6.01	0.0110	0.0109
6.26	0.00596	0.00658
6.41	0.00409	0.00469
6.57	0.00314	0.00334
6.95	0.00298	0.00297
7.51	0.00092	0.00137
9.01	0.00260	0.00256
9.50	0.00465	0.00532
9.96	0.0101	0.00964
10.48	0.0121	0.0109
12.51	0.00849	0.00845

[a] 25 °C, 0.3 M KCl. [b] Data over the pH range 4.26–6.57 are for solutions containing 0.010 M PSH and 0.010 M PSSeSP. Data over the pH range 6.95–12.51 for 0.010 M PSH and 0.005 M PSSeSP. Concentrations were corrected to account for formation of PSSP.

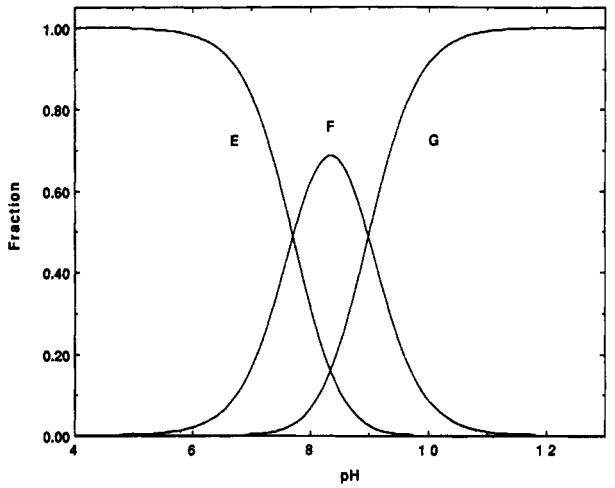

Figure 5. Fractional concentrations of the PSSeSP species identified in Scheme II as a function of pH.[11]

2-methyl-2-propanethiol is the species responsible for exchange in the *t*-BuSH/*t*-BuSSeSBu-*t* system.[7] It also is well established that thiol-deprotonated species are the reactive species in thiol/disulfide exchange reactions.[14] Over the pH range studied here, there are two thiol-deprotonated forms of PSH, B and D, in Scheme I. The fractional concentrations of the four PSH species in Scheme I are plotted as a function of pH in Figure 4.[13] The protonated forms of PSSeSP present over this pH range are shown in Scheme II, and their fractional concentrations are plotted as a function of pH in Figure 5.[15]

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(15) Fractional concentrations for PSH were calculated using the following values for the acid dissociation constants: pK_{12} = 8.03, pK_{13} = 8.61, pK_{123} = 10.29, and pK_{132} = 9.70. Backs, S. J.; Rabenstein, D. L. *Inorg. Chem.* **1981**, *20*, 410–415. Those for PSSeSP were calculated using the following acid dissociation constants; pK_1 = 7.71 and pK_2 = 8.99.[8]

Table II[a]

rate constant	value (L/mol·s)	rate constant	value (L/mol·s)
k_1	9.7 (±0.07) × 10^5	k_4	≤7.1 × 10^7
k_2	0.9 × 10^5 to 1.9 × 10^5	k_5	1.0 × 10^5 to 2.0 × 10^5
		k_6	2.7 (±0.3) × 10^4
k_3	≤1.0 × 10^4		

[a] 25 °C, 0.3 M KCl.

Assuming that the two thiolate forms of PSH can react with the three protonated forms of PSSeSP, the complete rate expression is

$$-\frac{d[PSH]}{dt} = k_1[B][E] + k_2[B][F] + k_3[B][G] + k_4[D][E] + k_5[D][F] + k_6[D][G] \quad (6)$$

where the letters represent species identified in Schemes I and II. Using the fractions defined in Figures 4 and 5, the concentrations of the different forms can be expressed in terms of the total concentrations of PSH and PSSeSP

$$-\frac{d[PSH]}{dt} = (k_1\alpha_B\alpha_E + k_2\alpha_B\alpha_F + k_3\alpha_B\alpha_G + k_4\alpha_D\alpha_E + k_5\alpha_D\alpha_F + k_6\alpha_D\alpha_G)[PSH][PSSeSP] \quad (7)$$

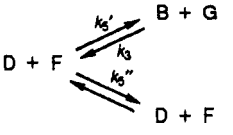
where α represents fractional concentration. Dividing both sides by the concentration of PSH, we obtain eq 8.

$$\frac{1}{\tau_{PSH}} = (k_1\alpha_B\alpha_E + k_2\alpha_B\alpha_F + k_3\alpha_B\alpha_G + k_4\alpha_D\alpha_E + k_5\alpha_D\alpha_F + k_6\alpha_D\alpha_G)[PSSeSP] \quad (8)$$

Examination of the plots in Figures 4 and 5 indicates that the product $\alpha_B\alpha_E$ is much larger than the other α products at a pH of 6 or less. A value of 9.7 (±0.07) × 10^5 L/mol·s is obtained for k_1 from the slope of a plot of $1/\tau_{PSH}$ vs $\alpha_B\alpha_E$[PSSeSP] using the data for the pH range 4.26–6.01 in Table I. At pH >6.01, exchange reactions involving other species become important.

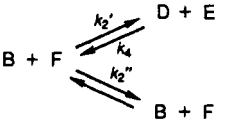
Examination of the α products indicates that at pH 12.51, the $\alpha_D\alpha_G$ product is much larger that the other α products. Assuming the k_6 term in eq 8 dominates at this pH, a value of 2.7 (±0.3) × 10^4 L/mol·s is calculated for k_6.

Evaluation of rate constants k_2–k_5 is more complicated due to the relationship between k_2 and k_4 and between k_3 and k_5. Specifically, species D and F can react to form B + G or products equivalent to the reactants:



(9)

i.e. k_5 is the sum of rate constants k_5' and k_5'' in eq 9. Applying the principles of microscopic reversibility, it can be shown that $k_3 = k_5K_{123}/K_2 = 0.05k_5'$ where K_2 and K_{123} are acid dissociation constants defined in Schemes I and II. Thus $k_3 \leq 0.05k_5$, depending on the relative magnitudes of k_5' and k_5''. Similarly, k_2 is a sum of two rate constants (k_2' and k_2'' in eq 10),



(10)

and it can be shown that $k_4 = K_1k_2'/K_{123} = 380k_2'$. Thus,

$k_4 \leq 380k_2$. Substitution of these relationships into eq 8, and using the above values for k_1 and k_6, the values listed in Table II were obtained for k_2–k_5.

The fit of the experimental lifetime data to the above model is indicated by the agreement between the experimental and predicted τ_{PSH} values in Table I. The predicted values were calculated by assuming that $k_2 = k_2''$ and $k_5 = k_5''$; however, the same agreement is obtained if k_2'' is some fraction of k_2 and reaction k_4 is important, etc.

The rate constants in Table II indicate that the rate of displacement of PS^- from PSSeSP by nucleophilic reaction of PS^- at selenium is strongly dependent on the protonation state of the amino groups of the reactants, with $k_1 > k_2 > k_3$ and $k_4 > k_5 > k_6$. In the first series, the aminoprotonated form of penicillamine (species B in Scheme I) reacts with PSSeSP having both, one, and no amino groups protonated, respectively, while in the second series the amino form of penicillamine (species D) reacts with the same PSSeSP species. The decrease in the magnitude of the rate constant in each series suggests that the amino form of PS^- is a poorer leaving group than the ammonium form. This is consistent with the significant decrease in acidity of the SH group of penicillamine upon deprotonation of the ammonium group ($pK_{12} = 8.03$, $pK_{132} = 9.70$ in Scheme I) and the well-established dependence of leaving group properties on acidity in similar nucleophilic reactions involving sulfur and seleno groups.[7,16] Increased electrostatic repulsion between reactants as the ammonium groups of PSSeSP are deprotonated might also be responsible for some of the decrease observed in each series.

Nucleophilic reaction of PS^- at the selenium of PSSeSP is much faster than reaction at one of the sulfurs to form PSSP. For example, approximately 50% of the penicillamine in a solution which initially contained 10 mM PSH and PSSeSP at pH 11.1 is converted to PSSP after 12.5 h. By contrast, the half-life of a penicillamine moiety of PSSeSP in a solution containing 10 mM PSH and 5 mM PSSeSP at pH 10.48 before exchange by nucleophilic reaction of PSH at the selenium is 8.4×10^{-3} s. In their study of the reaction with 1-butanethiol, 2-propanethiol, and 2-methyl-2-propanethiol with their bis(alkylthio) selenides, Kice and Slebocka-Tilk found that *n*-BuS^- reacts considerably faster at the sulfur of *n*-BuSSeSBu-*n* than at the selenium, whereas the reverse is true for the reaction of *t*-BuS^- with *t*-BuSSeSBu-*t*, and they attributed the differences to steric effects.[7] Similar decreases in rate constants due to steric effects have been observed for thiol/disulfide exchange reactions involving penicillamine.[14g] Thus, the much smaller rate for reaction of PS^- at sulfur as compared to selenium of PSSeSP can be attributed to the steric bulk of the substituents on the quaternary β-carbon of penicillamine.

The most striking result from this study is that the rate of nucleophilic reaction of PS^- at the selenium of PSSeSP is so much faster than the rate of nucleophilic reaction of *t*-BuS^- at the selenium of *t*-BuSSeSBu-*t*. For example, rate constant k_1 is 3×10^6 larger than the rate constant for the reaction of *t*-BuS^- with *t*-BuSSeSBu-*t* at 25 °C. Kice and Slebocka-Tilk[7] accounted for an unexpectedly large rate for the reaction of *i*-PrS^- with *i*-PrSSeSPr-*i* in terms of a two-step reaction sequence in which (A) R^*SSe^- reacts at a sulfur of RSSeSR to form R*SSeSR and $RSSe^-$ and (B) R^*SSe^- (or R*SSeH) reacts with RS^- (or RSH) to form $RSSe^-$ (or RSSeH) and R^*S^- (or R*SH), with the overall rate of exchange controlled by A. For such a reaction sequence to account for the observed rate of transfer of penicillamine between PSH and PSSeSP would require that some of the PSSeSP has been converted to $PSSe^-$ and that the rate constant for reaction A is extremely large. For example, although there is no evidence in the NMR spectra for the presence of $PSSe^-$, if we assume that 1% of the PSSeSP has been converted to $PSSe^-$ by some process and that the overall rate is controlled by reaction A, the rate constant for reaction A would have to be $>10^6$ L/mol·s and that for reaction B would have to be $>10^8$ L/mol·s to account for the observed kinetics at pH 4.26 in Table I. The rate for reaction A for the *t*-BuSH/*t*-BuSSeSBu-*t* system is predicted to be extremely slow because of steric effects.[7] Considering that the steric effects will be similar for the PSH/PSSeSP system and that the rate of the reaction of PS^- at the sulfur of PSSeSP is observed to be very slow, if seems unlikely that reaction A can proceed with a rate constant this large and thus that the reaction sequence A and B can account for the observed kinetics for the exchange of penicillamine between PSH and PSSeSP. Furthermore, the rate of exchange via $PSSe^-$ would depend on the concentration of $PSSe^-$, which presumably would vary with the age of the solution. Experimentally, it is observed that the value obtained for rate constant k_1 is highly reproducible and does not depend on the age of the solution.

Although we do not have an explanation for the much larger rate constants observed here for the reaction of PS^- with PSSeSP as compared to that reported by Kice and Slebocka-Tilk for the reaction of *t*-BuS^- with *t*-BuSSeSBu-*t*, it should be pointed out that rate constants of similar magnitude have been observed previously for other nucleophilic displacement reactions involving organoselenium. For example, the rate constant for the symmetrical selenol/diselenide interchange reaction involving $D_3N^+CH_2CH_2Se^-/D_3N^+CH_2CH_2SeSeCH_2CH_2ND_3^+$ is 1.8×10^7 L/mol·s at 25 °C, as compared to 68 L/mol·s for the symmetrical interchange reaction for the analogous sulfur compounds.[17] Even though the origin of the large difference between the rates of nucleophilic reaction of PS^- at the selenium of PSSeSP and *t*-BuS^- at the selenium of *t*-BuSSeSBu-*t* has not been established, the results of this study suggest that reaction at the selenium of bis(alkylthio) selenides of biological thiols could be important in their conversion to other selenium-containing biomolecules.

Experimental Section

Chemicals. D-Penicillamine was used as received from Sigma Chemical Co. after its purity was checked by ^1H NMR. Selenious acid (H_2SeO_3) was used as received from Aldrich Chemical Co. Bis(D-penicillamine) selenide was synthesized by the procedure described by Nakagawa et al.[18] No impurities were observed in the ^1H NMR spectrum of the PSSeSP.

Solutions were prepared in 95% H_2O/5% D_2O; 0.3 M KCl was added to maintain a constant ionic strength. The solvent was degassed by bubbling with either nitrogen or argon before adding penicillamine and PSSeSP, and then the solution was bubbled with nitrogen or argon while the pH was adjusted and samples removed for NMR measurement.

NMR Measurements. ^1H NMR spectra were measured at 500 MHz. The water resonance was suppressed by a selective decoupler saturation pulse prior to the observation pulse. Preexchange lifetimes for PSH and the penicillamine moieties of PSSeSP were obtained from exchange-broadened and coalesced resonances by matching of experimental and computer-simulated

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spectra. Spectra were simulated using Bloch equations modified for chemical exchange.[19]

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Effect of Bay Region Methyl Group on Reactions of *anti*-Benz[*a*]anthracene 3,4-Dihydrodiol 1,2-Epoxides with DNA†

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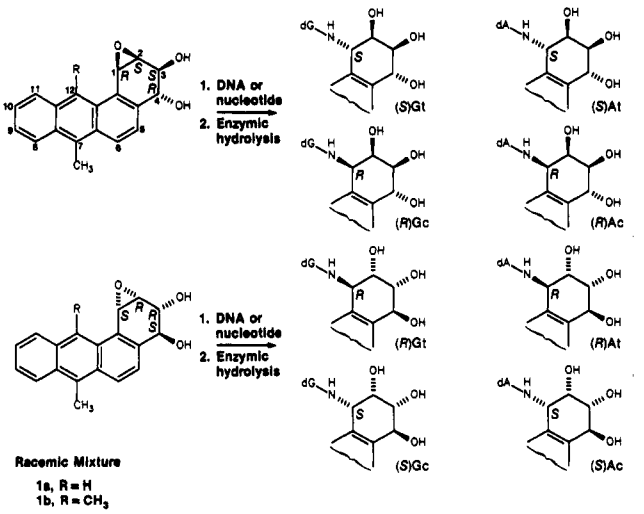
The NMR spectroscopic characterization of seven 7-methylbenz[*a*]anthracene–deoxyribonucleoside adducts and eight 7,12-dimethylbenz[*a*]anthracene–deoxyribonucleoside adducts, derived from the reaction of the corresponding *anti*-dihydrodiol epoxides and deoxyguanylic and deoxyadenylic acids, is described. The epoxide ring is opened by the purine amino groups to yield both cis and trans products from each enantiomer in the racemic dihydrodiol epoxides. Circular dichroism and NMR spectra allow the conformations of the products to be established. Interesting differences between the products from the two hydrocarbons are as follows: the dimethyl derivative is distributed fairly evenly over adenine and guanine residues in DNA, whereas guanine is the principal site for reaction of the monomethyl derivative; the conformation of the tetrahydro ring system is similar in trans products for both hydrocarbons with the hydrogens on C_3 and C_4 being pseudodiaxial; in cis adducts, these hydrogens are pseudodiaxial for 7,12-dimethylbenz[*a*]anthracene adducts but pseudodiequatorial for the 7-methylbenz[*a*]anthracene adducts; in reactions with nucleotides, trans adducts predominate for 7-methylbenz[*a*]anthracene derivatives but trans and cis adducts form to similar extents for 7,12-dimethylbenz[*a*]anthracene derivatives. This latter differs substantially from previous findings with other bay region substituted hydrocarbons where cis adducts have been obtained only in low yields.

Introduction

Polycyclic aromatic hydrocarbons express carcinogenic and mutagenic properties through covalent reactions with cellular DNA.[1] These reactions are mediated by metabolically generated bay region dihydrodiol epoxides[2,3] that aralkylate DNA following the chemistry first described for 7-(bromomethyl)benz[*a*]anthracene, i.e., the amino groups of the DNA bases are the principal sites of reaction.[4] However, the dihydrodiol epoxide chemistry is complex because of the different stereoisomers of the dihydrodiol epoxides and the fact that the epoxide ring can open to give cis or trans products.[5] Although the identity of the major adducts of the *anti*-dihydrodiol epoxides of benzo[*a*]pyrene was established soon after their discovery,[6] quantities of adducts from this[7] and other epoxides,[8] sufficient for detailed structural characterizations, have been prepared only recently through reactions of synthetic dihydrodiol epoxides with high concentrations of deoxy-

Scheme I. Structures of Adducts Formed in DNA and Nucleotide Reactions



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