Scheme I

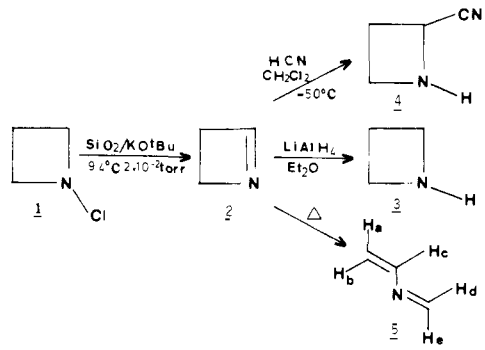

Table I. ^{13}C Chemical Shifts and Coupling Constants of 2, 6, and 7

compound	chemical shift, δ_C[a]			$J_{^{13}C-H}$, Hz		
	C-2	C-3	C-4	^{13}C-2-H	^{13}C-3-H	^{13}C-4-H
1-azetine (2)[b]	187.0	36.1	58.8	182.0	142.0	150.5
cyclobutene (6)	162.3[c]	56.5[c]	56.5[c]	170[d]	140[d]	140[d]
3-phenyl-1-azirine (7)[e]	160.6	28.6		242.5		

[a] Relative to internal Me_4Si. [b] Sample concentration in $CDCl_3$, 15% at -55 °C, recorded on a WP 60 Bruker NMR spectrometer. [c] Reference 10a. [d] Reference 10b. [e] Reference 10c.

led to 1-azetine (**2**), partially at 15 °C (5%), completely at 94 °C (yield >98%, purity >95%).[5] The stability of the pure compound is somewhat greater than initially expected; it can be trapped at liquid nitrogen temperature and revaporized. It is a colorless liquid at -70 °C and polymerizes in a few seconds even in sealed degassed tubes at 20 °C. The half-life of **2** in solution in sealed degassed tubes ($CFCl_3$, 36 °C) is about 90 min or 3 days in the presence of hydroquinone. However, polymerization occurs rapidly with traces of oxygen or acid. Conclusive structural proof for **2** was afforded by (1) its reduction with $LiAlH_4$ in ether at 0 °C to azetidine (**3**) and (2) the addition of HCN at -50 °C in CH_2Cl_2 solution to give the nitrile derivative **4** (Scheme I).[6] Further evidence is provided by the ^1H NMR spectrum (CD_2Cl_2, -60 °C) which exhibits two sharp triplets at δ 3.09 (J = 2.8 Hz) and 4.00 and a singlet at δ 8.22.[7] The ^{13}C NMR parameters are compared with those observed for cyclobutene (**6**) and 3-phenyl-1-azirine (**7**)[8] (Table I). The ^{13}C-2-H and ^{13}C-4-H coupling constant values are larger than those observed in cyclobutene (**6**). This phenomenon could be due to the presence of the adjacent electronegative atom which is known to increase the ^{13}C-H coupling constant.[9]

The strong IR band of **2** at 1570 cm^{-1} (CCl_3F, 25 °C or -196 °C with an optical cryostat) is consistent with the presence of a C=N group in a four-membered ring;[1c] the UV spectrum (*n*-pentane, -60 °C) exhibits a single band at 237 nm (ϵ 80[11]). In spite of numerous attempts, no mass spectrum of **2** could be obtained, presumably due to rapid polymerization. Flash vacuum pyrolysis (FVP) of **2** (450 °C, 10^{-4} torr) in a furnace equipped with an optical cryostat[12] formed the unknown 2-azabutadiene (**5**), as witnessed by its IR spectrum ($\nu_{C=N}$ 1610, $\nu_{C=C}$ 1628 cm^{-1}; the product was trapped on a liquid nitrogen cooled KBr target window). Polymerization of the solid film was observed on warming. Larger samples of **5** were obtained by fitting the FVP apparatus on the vacuum line after the dry ice trap.[4] The ^1H NMR spectrum of **5** [$(CD_3)_2CO$, -60 °C][13] confirms its structure [δ_{H_a} 5.24 (dd, $J_{H_aH_c}$ = 7.1, $J_{H_aH_b}$ = 0.8 Hz); δ_{H_b} 5.56 (dd, $J_{H_bH_c}$ = 14.8 Hz), δ_{H_c} 6.97 (dd); δ_{H_d} 7.31 (d, $J_{H_dH_e}$ = 16.7 Hz; δ_{H_e} 7.59 (d)]. Its mass spectrum exhibits the molecular ion at m/e 55. Polymerization of a dilute solution of **5** takes only a few minutes at 25 °C, but no dimer or trimer products were detected. The thermal transformation of 1-azetine to 2-azabutadiene was predicted by molecular orbital theory to proceed along a pathway similar to that for hydrocarbon analogues.[14] Preceding experimental data[1b,15] support these observations. For the parent compound **2** no retro [2 + 2] process with formation of HCN could be observed even by FVP at higher temperatures (500-700 °C).[16]

The use of SiO_2/*KO-t-Bu* as a solid base on a vacuum line proves to be a powerful technique for the direct synthesis of high-purity samples of very reactive intermediates. Further work is in progress to elaborate the chemical reactivities of 1-azetine and 2-azabutadiene and synthesize other reactive intermediates by similar methods.

Acknowledgment. We thank Dr. B. M. Carden for linguistic cristicism of the manuscript; Professors R. Carrie and P. Guenot for high-resolution mass spectra measurements, and Professor G. Pouzart for recording ^1H NMR spectra. Acknowledgments to these colleagues are also expressed for their valuable comments.

(4) *tert*-Butyl alcohol formed in the reaction was efficiently eliminated by a dry-ice trap fitted on to the vacuum line after the elimination apparatus.

(5) Several grams could be obtained with the same solid base if the addition of chloro amine **1** was regular and slow (1 g/h).

(6) ^1H NMR ($CDCl_3$) δ 4.2 (m, 1 H), 3.6 (m, 2 H), 2.6 (m, 2 H); IR (CD_2Cl_2) ν_{N-H} 3680, 3595, $\nu_{C\equiv N}$ 2208 cm^{-1}. Due to the instability of this product, combustion analysis could not be carried out; by high-resolution MS the molecular weight corresponds to $C_4H_6N_2$. (Anal. Calcd: m/e 82.0531. Found: 82.0530.)

(7) The ^1H NMR spectrum of **2** was deceptively simple. The coupling constant between the vinylic proton and the proton of the adjacent methylene group was too small (<0.2 Hz) to be detected by using a Nicolet NTC 200 NMR spectrometer.

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(11) The extinction coefficient is given at ±10% due to polymerization at the time of introduction of **2** into the cell.

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(16) HCN was independently trapped under the same experimental conditions with the optical cryostat at 77 K or in the trap of the vacuum line.

Use of Singly Modified Cytochrome *c* Derivatives To Determine the Site for Electron Transfer in Reactions with Inorganic Complexes

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Cytochrome *c* (MW = 12400) is a component of the mitochondrial respiratory chain and transfers electrons from cytochrome c_1 to cytochrome *c* oxidase. The heme prosthetic group of horse cyctochrome *c* is almost completely enfolded by a polypeptide chain of 104 amino acids, leaving the edge containg pyrrole rings II and III partly exposed at the "front" surface of the protein. It has been suggested that electron transfer in and out of the protein is via this exposed heme edge.[1] However, the evidence for such a mechanism is only indirect.[2] At pH 7 many amino

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acid residues are ionized and the ferro and ferri forms of the protein are believed to carry net charges of +8 e and +9 e, respectively, in which e is the elementary charge:[3] The distribution of charges on the protein surface is asymmetric, leading to a dipole moment of 325 D for horse ferricytochrome *c* and 308 D for the ferro form. The direction of the dipole moment is not significantly altered upon reduction.[4] Such an electrostatic configuration favors a reaction at the heme edge if the reactant is negatively charged.[3] A positively charged reactant would be guided toward the "back" of the molecule, from where the shortest distance to the prosthetic group is 14 Å through the protein. If the exposed heme edge is the only site for electron transfer on the protein surface, such a positively charged reactant would be required to overcome a Coulombic barrier.

We report here the kinetics studies of oxidation of native horse ferrocytochrome *c* and of four of its singly modified (4-carboxy-2,6-dinitrophenyl)lysine (CDNP-Lys) derivatives, CDNP-Lys-13, -72, -87, and -60, with tris(1,10-phenanthroline)cobalt(III) ($Co(phen)_3^{3+}$) and hexacyanoferrate(III) ($Fe(CN)_6^{3-}$). The 4-aminopyridine complex $Fe(CN)_5(4\text{-}NH_2py)^{2-}$ was also used as oxidant, and $Fe(CN)_5(4\text{-}NH_2py)^{3-}$ and $Fe(CN)_5(imid)^{3-}$ were similarly used as reductants for ferricytochrome *c*. The preparation and characterization of 12 different singly modified CDNP-Lys horse cytochromes *c* have been previously described.[5-7] The modification neutralizes the positive charge at the ε-nitrogen atom of lysine and introduces a negative charge, namely, the carboxyl group at the 4-position of the phenyl ring. Each of these derivatives was shown to be better than 99% pure. Physicochemical characterization demonstrated that the native structure was not perturbed,[6-8] and reduction potentials remain identical.[6] Studies of the reactions of the modified proteins with cyctochrome *c* reductase, cytochrome *c* oxidase, and yeast cytochrome *c* peroxidase have shown that the domains on cytochrome *c* which interact with the enzymes are essentially identical.[4,7,9-12] Lysines-13, -72, -86, and -27 and, to a lesser extent, lysine-87 are involved in these interactions. The domains are centered around the β carbon of phenylalanine-82, the point at which the positive part of the dipole axis crosses the protein surface,[4] close to the exposed heme edge.

Commerically available $K_3[Fe(CN)_6]$ (BDH, AnalaR) was used. The preparation of $[Co(phen)_3]Cl_3 \cdot 7H_2O$ was as described.[13] Complexes $Fe(CN)_5(4\text{-}NH_2py)^{2-}$,[14] $Fe(CN)_5(4\text{-}NH_2py)^{3-}$,[14] and $Fe(CN)_5(imid)^{3-}$[15] were generated in solution by reacting an excess (>10-fold) of free ligand, 4-aminopyridine or imidazole, with the corresponding ammine complex $Fe(CN)_5(NH_3)^{2-}$ or $Fe(CN)_5(NH_3)^{3-}$. The latter were prepared by literature methods.[16,17] Reduced cytochrome *c* was obtained by reaction with sodium ascorbate, followed by gel filtration (Sephadex G-50, 0.7 cm × 30 cm) under O_2-free conditions (N_2 gas). Elution was with pH 7.2 Tris buffer containing sodium chloride.

Table I. Second-Order Rate Constants for the Reactions of Singly Modified CDNP-Lys Horse Cytochromes *c* with Inorganic Complexes[a]

	$10^{-5}k$, M^{-1} s^{-1}				
reaction	native	CDNP-Lys-60	CDNP-Lys-87	CDNP-Lys-72	CDNP-Lys-13
cyt *c*(II) + $Fe(CN)_6^{3-}$	93	81	78	39	33
cyt *c*(II) + $Fe(CN)_5(4\text{-}NH_2py)^{2-}$ [b]	6.8	6.5	6.0	2.6	2.3
cyt *c*(II) + $Co(phen)_3^{3+}$	0.015	0.017	0.019	0.036	0.044
$Fe(CN)_5(4\text{-}NH_2py)^{3-}$ + cyt *c*(III)	38	37	34	25	20
$Fe(CN)_5(imid)^{3-}$ + cyt *c*(III)	3.9	3.4	3.8	1.5	1.4

[a] At 25 °C, pH 7.2 (Tris), except as stated; $I = 0.10$ M (Tris buffer and NaCl). Rate constants have standard deviations of ±3%. [b] pH 9.4 ($4\text{-}NH_2py$ as buffer) in this study.

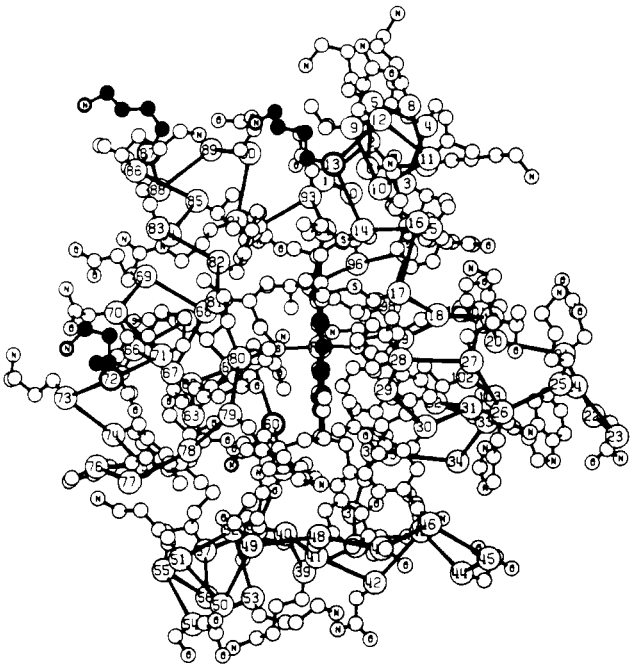

Figure 1. Front view of cytochrome *c* prepared from an electron density map of tuna ferricytochrome *c*, which is known to closely resemble the horse heart protein at a resolution of 2.0 Å according to Swanson et al.[21] The larger circles are the α carbons with the sequence position written in them, while the smaller circles represent the side-chain atoms. The heme, marked in black, is seen edge on, at the front of the molecule. The heavier circles and filled circles mark the lysines which have been modified (lysine-13, -60, -72, and -87). The lysines influencing reactivity are those at positions 13 and 72, above and to the left of the heme edge, respectively.

Kinetic studies at 25 °C were carried out with a Durrum-Gibson stopped-flow spectrophotometer at a total ionic strength of 0.10 M (NaCl, Tris buffer, pH 7.2), except in the case of the oxidant $Fe(CN)_5(4\text{-}NH_2py)^{2-}$, when the pH was 9.4 ($4\text{-}NH_2py$ as buffer). The inorganic redox partner was in ≥10-fold excess of the cytochrome *c*, the concentration range of which was (4–7) × 10^{-7} M for cytochrome *c*(II) and (1–3) × 10^{-6} M for cytochrome *c*(III). These conditions ensure that the reactions proceeded to ≥90% completion.[18] Cytochrome *c* absorbance was monitored at 416 nm, $\epsilon = 1.29 \times 10^5$ M^{-1} cm^{-1} for cyctochrome *c*(II) (Soret-band

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maximum) and 8.9×10^4 M^{-1} cm^{-1} for cytochrome *c*(III), or at 417 nm where the $\Delta\epsilon$ is slightly bigger.[19] First-order rate constants, k_{obsd}, were obtained from the slopes of plots of absorbance changes, $\ln |A_\infty - A_t|$, against time. A linear dependence of k_{obsd} on the concentration of inorganic complex was observed in each case. Second-order constants defined by the rate law

$$\text{rate} = k[\text{cytochrome } c][\text{complex}]$$

are listed in Table I. The values reported for native cytochrome *c* are in reasonable agreement with those reported in the literature.[18,20]

It is concluded that modifications of lysine-13 and lysine-72 but not of lysine-87 and lysine-60 (Figure 1[21]) have an influence on all the reactions investigated. With the positively charged oxidant $Co(phen)_3^{3+}$ there is an approximately twofold increase in the rate constant when the modification is at positions 13 and 72, consistent with the replacement of a positive by a negative charge. That there is an increase shows that the modified residue probably does not sterically hinder the reaction. All the negatively charged redox partners, whether oxidants or reductants, show an approximately twofold decrease in rate constant with the same modified cytochrome *c*. The rate constants reported for CDNP-Lys-60 and -87 cyctochrome *c* are only slightly different from those for native cytochrome *c*, the small effect most likely being attributable to the change in net charge. These observations indicate that a neutral complex could be expected to show little or no response to modification at positions 13 and 72. The present results unequivocally show that the small inorganic complexes investigated transfer electrons to or at the exposed heme edge.

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Liquid Crystal Characterization of Compounds Chiral by Deuterium Substitution

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Acyclic compounds of the type RCHDR′ which are chiral only due to deuterium substitution are very important in studies on chemical and biochemical mechanisms.[1] A major problem faced with the use of these substances is the matter of recognition and characterization of the chirality. Apart from the few cases where optical rotatory dispersion can be observed, the classical optical methods are inapplicable for the characterization of deuterium-labeled chiral compounds because of their low specific optical rotation.

We now present evidence indicating that the determination of the "handedness" of the cholesteric mesophase induced in a nematic liquid by the presence of an asymmetrically deuterated compound of the type RCHDR′ as solute and of the "twisting power", β, can be used for the characterization of acyclic, RCHDR′, chiral compounds.

Table I. Twisting Power (β) of Chiral Deuterium Derivatives Dissolved in a 1:1 (m/m) MBBA-EBBA Mixture

compound	β	compound	β
1a[a,c]	+0.093 ± 0.006	**1b**[a,c]	−0.096 ± 0.007
2a[d]	+0.079 ± 0.006	**2b**[d]	−0.075 ± 0.005
3a[b,e]	−0.113 ± 0.005	**3b**[b,e]	+0.109 ± 0.006

[a] Assumed as enantiomerically pure.[8] [b] Assumed to contain ca. 95% of each enantiomer on the basis of the optical purity of the resolved starting hydroxy amines **5** and **6**. [c] 93% d_1. [d] 96% d_1. [e] 92% d_1.

When a chiral substance is dissolved in a nematic liquid crystal, a cholesteric mesophase is obtained.[2] The cholesteric structure is characterized by its handedness (*P* or *M* helix) and pitch. Equal amounts of enantiomeric solutes of equal optical purity induce helical structures with identical pitch and opposite handedness.[3] Different substances show a different ability to twist the nematic phases. The twisting power of a chiral dopant can be defined as[4]

$$\beta = (pcr)^{-1}$$

where *p* is the pitch (μm), *c* is the concentration (mole of solute/mole of solution), and *r* is the enantiomeric purity of the dopant. The parameter β together with the sign + or − for the *P* helix or *M* helix characterizes the chiral solute in a way similar to the specific optical rotation [α]. However, the physical origin of the two quantities is entirely different.[5]

The origin of the optical rotation depends in fact on the interactions of light with molecules, while the twisting power originates from the interactions between molecules of solute and solvent.

A very small chiral "perturbation" is required in order to induce into a nematic mesophase a cholesteric molecular arrangement which is characterized by its very high macrostructural chirality.

Hence a chirality, not detectable by polarimetric methods owing either to the small quantity of the investigated optically active compound available or to its very low rotatory power, can be, in principle, "amplified" by the phenomenon of the induction of cholesteric mesophases.

Indeed, when the enantiomeric pairs of asymmetrically deuterated compounds **1a–3a** and **1b–3b** were dissolved in nematic solvents, the formation of well-characterized cholesteric mesophases (Table I) was observed.[6] Compound **1a** was prepared by

1a, R = C_6H_5; R′ = $OCOC_6H_4NO_2$-*p*; H_R = H; H_S = ^2H
b, R = C_6H_5; R′ = $OCOC_6H_4NO_2$-*p*, H_R = ^2H; H_S = H
2a, R = $(CH_2)_2C_6H_5$; R′ = OH; H_R = H; H_S = ^2H
b, R = $(CH_2)_2C_6H_5$; R′ = OH; H_R = ^2H; H_S = H
3a, R = C_6H_5; R′ = $CH_2N(Me)COC_6H_5$; H_R = H; H_S = ^2H
b, R = C_6H_5; R′ = $CH_2N(Me)COC_6H_5$; H_R = ^2H; H_S = H

esterification (4-$NO_2C_6H_4COCl$/pyridine/CH_2Cl_2) of (1*S*)-[1-^2H]benzyl alcohol,[8] whereas the 1*R* isomer (**1b**) was prepared as reported for the benzoate[8] by using 4-$NO_2C_6H_4CO_2H$ instead of $C_6H_5CO_2H$. (1*S*)-[1-^2H]3-Phenylpropan-1-ol (**2a**) and its 1*R* isomer (**2b**) which were used in the synthesis of (4*S*)- and (4*R*)-[4-^2H]D,L-homoserine, were enantiomerically pure, within

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