consisting of two straight lines. Carrier-mediated K^+ permeation must thus be directly affected by the molecular motion of the liquid-crystal phase. In contrast, K^+ permeation through the PC/EBBA/**1b** composite membrane was "completely" suppressed below T_{KN} and increased with increasing temperature above T_{KN} (Figure 1): E_a = 15.3 kcal mol^{-1}. We conclude, therefore, that ion permeation below T_{KN} is largely governed by the dispersion state of the carriers.

In Figure 2, we demonstrate the reversible thermocontrol of K^+ permeation through the PC/EBBA/**1b** composite membrane. In response to a temperature change in the water bath (283 → 313 → 283 → 313 K), the rate of K^+ permeation showed an all-or-nothing change. The relatively slow response observed for a change from 313 to 283 K is attributed either to a leakage of K^+ dissolved in the membrane during the 313 K period or to an induction period for reorganization of the gel phase.

Detailed characterization of these and related composite membranes is now under intensive investigation. Of particular interest are (i) the rate of K^+ permeation may be sensitively controlled by a thermoswitch and (ii), since K^+ ion cannot permeate through the PC/EBBA/**1b** composite membrane below T_{KN}, it may be transported against its concentration gradient from the high-temperature cell ($T > T_{KN}$) to the low-temperature cell ($T < T_{KN}$). Statement (ii) would be regarded as a new class of thermodriven active transport. Further elaboration of the present concept might lead to the eventual development of a variety of thermocontrollable membranes.

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Organomercurial Reagents for the Simultaneous Introduction of Mercury and a pH-Sensitive Reporter Functional Group into a Protein Containing No Thiol Group[1]

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We wish to report the synthesis, characterization, and demonstration of the utility of the organomercurial reagents 3-(acetoxymercurio)- and 3-(chloromercurio)-5-nitrosalicylaldehyde, which may be used to silmultaneously introduce a heavy-metal and a pH-sensitive reporter group[2] into a protein bearing no sulhydryl residue. Proteins thus modified may become amenable to X-ray structure analysis[3] and to examination of their quaternary interactions with other proteins bearing free sulhydral groups with which the mercurial-modified protein may react.

The synthesis of 3-(acetoxymercurio) and 3-(chloromercurio) derivatives of 5-nitrosalicylaldehyde was carried out by the scheme

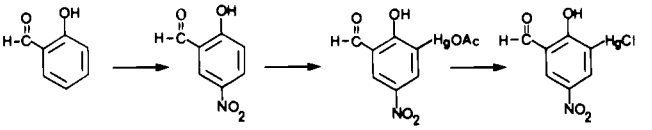

Figure 1. Synthesis of 3-(choloromercurio)- and 3-(acetoxymercurio)-5-nitrosalicaldehyde.

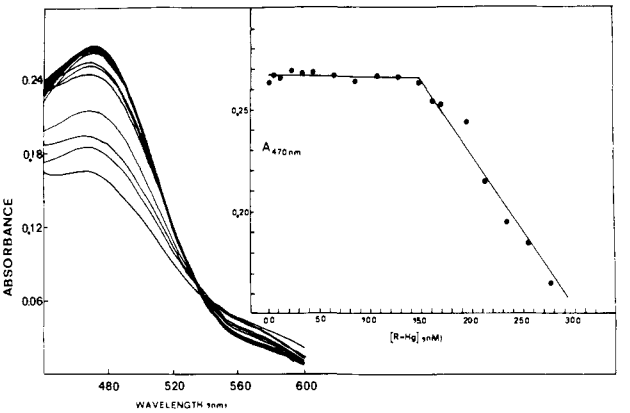

Figure 2. Titration of 2-mercaptoethanol with 3-(acetoxymercurio)-5-nitrosalicaldehyde. Spectra correspond to mercurial concentrations (R–Hg) plotted in the insert as a function of observed absorbance at 470 nm (A_{470}).

Table I. pKs, Spectrophotometric Properties, and Melting Points of Mercurial Reagents, Model Reaction Products, and Neurotoxin Mercurial Derivatives

compounds	mp, °C	pK_a	max(base)	max(acid)	IP
5-nitrosalicyladehyde	126	5.5	360, 386	308	329
3-HgOAc derivative	310 dec	5.4	363, 395	315	332
3-HgOAc–EDTA Complex		7.8			
3-HgCl derivative	252 dec	5.4	365, 395	309	332
Benzyl alcohol derivative		6.9	414	316	366
(α-acetyllysyl)amino-methane derivative		5.7	400	310	345
neurotoxin A		4.8	400	325	352
neurotoxin B		5.1	400	325	348

in Figure 1. Salicylaldehyde was nitrated in nitric and acetic acid and pure 5-nitrosalicylaldehyde (mp 126–127 °C, uncorrected, cf. lit.[4]) obtained after two recrystallizations. The purified 5-nitrosalicylaldehyde (1.50 g, 9 mM) was heated in aqueous potassium hydroxide (26 mL, 0.03 M) at 70 °C while mercuric acetate (2.9 g, 9 mM) in aqueous acetic acid (25 mL, 0.08 M) was added over a 30-min period. During the addition a yellow-brown precipitate formed and was filtered subsequently from the hot solution, washed successively with acetic acid (0.08 M), water, methanol, and diethyl ether, and recrystallized from aqueous acetic acid (450 mL, 0.7 M). White needles were obtained (1.85 g, mp 310 °C dec, uncorrected). Anal. Calcd for $C_9H_7HgNO_6$: C, 25.37; H, 1.66; Hg, 47.12; N, 3.29. Found: C, 25.50; H, 1.73; Hg, 46.83; N, 3.36.

3-(Acetoxymercurio)-5-nitrosalicylaldehyde (1.85 g) could be converted to the 3-(chloromercurio) derivative by dissolution of the former in aqueous potassium hydroxide (900 mL, 0.03 M) and precipitation of the latter subsequent to dropwise neutrali-

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(1) (a) In partial fullfillment of the Ph.D. requirements of Eric Wohlfeil, Wayne State University. (b) Supported by National Institutes of Health, National Institute of Neurological Childhood Disorders and Stroke, NS-14491, and the National Cancer Institute, CA-27674.

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zation with concentrated hydrochloric acid. The precipitate washed with water and dried at 75 °C for 24 h gave 3-(chloromercurio)-5-nitrosalicylaldehyde (1.55 g) in 42% yield based on 5-nitrosalicylaldehyde, mp 252 °C dec, uncorrected. Anal. Calcd for $C_7H_4ClHgNO_4$: C, 20.89; H, 1.00; Cl, 8.82, Hg, 49.89; N, 3.48. Found: C, 20.98; H, 1.00; Cl, 8.49, 8.58; Hg, 50.06, N, 3.44. Although the chloro- and acetoxymercurio forms are probably equivalent for most applications, we have found that the latter is easily purified whereas the former tends to lose chlorine and form higher melting products on attempted recrystallization. However, if the latter is purified first as illustrated here then the initially isolated chloromercury product is quite suitable for use. The open ring position activated by the ortho hydroxyl is suggested to be the site of mercuration as this position is also meta to both the nitro and aldehyde meta-directing substituents. Mercuration in this position is further indirectly supported by the magnitude of the phenolic pK shift of the mercurial-EDTA complex (cf. Table I and ref 11).

Table I summarizes spectroscopic data, pK_a, and melting points for 5-nitrosalicylaldehyde and the two mercurials as well as two model compounds formed and examined in situ after reduction of the mercurials with sodium borohydride alone or in the presence of (α-acetyllysyl)aminomethane. The latter was formed after reduction in the presence of a large excess of amine and separated from traces of the benzyl alcohol analogue product by extraction of the latter from acidified solutions of the products. Also included in Table I are two products formed from the borohydride reduction of Schiff's bases derived from the interaction of the 3-(chloromercurio)-5-nitrosalicylaldehyde and the principal curarimimetic neurotoxin[6] from the venom of the Thailand cobra, *Naja naja siamensis*. Figure 2 shows the results of the titration of a freshly prepared solution of mercaptoethanol (13 nM) with 3-(acetoxymercurio)-5-nitrosalicylaldehyde using the mercurial indicator dye pyridine-2-azo-4'-(*N'*,*N'*-dimethylaniline) to detect excess mercurial.[5]

The pK_a of 3-(acetoxymercurio)-5-nitrosalicylaldehyde is, as expected, similar to that of the 3-(chloromercurio) derivative and to 5-nitrosalicylaldehyde. The relatively large pK_a differences between the ortho hydroxymethyl- and the (alkylamino)-methyl-substituted phenols may be associated with the ionic and hydrogen-bonded stabilization of the phenolic anion by the protonated amine as well as the inductive withdrawing potential of aminomethylammonium (cf. hydroxymethyl). Different microenvironmental effects on the aminomethyl function modified in the neurotoxin evidently lead to the pK_a differences observed in derivatives A and B.[7,8]

We used 3-(chloromercurio)-5-nitrosalicylaldehyde to reductively alkylate amino functions of the principal curarimimetic neurotoxin from the venom of the Thailand cobra, *Naja naja siamensis*. Purification of this protein, which bears no free sulfhydrals, from the lyophillized venom (obtained from the Miami Serpentarium) was carried out by the method of Karlsson et al.[9] The protein (0.72 μM) was incubated with 3-(chloromercurio)-5-nitrosalicylaldehyde (2.0 μM) in sodium phosphate (pH 9) and the Schiff's base adducts reduced with sodium borohydride (40 μM). The solution was acidified (pH 5), exhaustively dialyzed against distilled water, and lyophilized. The product was taken up in phosphate buffer (20 mM, pH 4.80) and chromatographed on phosphocellulose using a concave gradient from 0.02 to 1.0 M phosphate (pH 4.80). Three protein peaks were observed, two of which (A and B) possessed the spectrum of the reduced mercurial reagent. These two fractions were radiolabeled upon reduction with sodium [^{3}H]borohydride. Their specific activities were approximately equal. The third peak eluted at an ionic strength similar to that observed for the native toxin when chromatographed alone.

While fractions A and B had virtually indistinguishable spectra characteristic of the introduction of 1 mol of reagent per mol of protein, the phenolic pK_a's were depressed to differing extents, cf. the model lysylaminomethane adduct. As the crystal structure of this neurotoxin has been determined,[10] it will be of certain interest to survey the microenvironment of each of the residues modified in light of these pK_a depressions. However, variation in the pK_a of nitrophenols introduced into protein is not unexpected. McMurray and Trentham[11] synthesized several mercurial nitrophenols which they used for the modification of protein sulfhydral groups. They showed that these reagents were sensitive probes of the microenvironment of the thiol group.

Furthermore, though not demonstrated here, there may be additional instances in which the reagents reported may find use in protein crystallographic studies. Isomorphous, heavy-metal derivatives are required to solve the phase problem.[12] The ease of formation of derivatives for the curarimimetric toxins suggests that crystals of other proteins formed or stable at neutral to basic pH may interact directly with the reagents to form heavy-metal derivatives without the need to form a stable linkage via borohydride reduction. The Schiff's base reaction, while kinetically favored at higher pH, may be carried out at or near neutral pH. This reaction will be especially favorable for proteins that contain uniquely reactive amino groups. Normally, proteins that do not possess a sulhydral are modified so that one may be introduced. One of several heterobifunctional reagents may be employed.[13-16] The introduced sulfhydryl is then reacted with organomercurials. The class of reagents reported here offers the opportunity to bypass the intermediate step.

Finally, in preliminary experiments we have shown that the two prepared mercurial toxin derivatives retain reactive mercurials capable of mediating the cross-linking of the toxin into sulfhydryl functions in its biological target, the nicotinic acetylcholine receptor.[17-19] Thus, the reagents that are presented here will, we believe, be useful in probing protein-protein interactions.

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The Planar C···C Ring-Opened Form of the Ethylene Oxide Radical Cation. ESR Evidence from Anisotropic ^{13}C Studies

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The nature of the ethylene oxide radical cation has recently attracted considerable interest,[1-7] but the experimental studies using solid-state ESR spectroscopy have led to conflicting interpretations[1,2,7] of the hyperfine data. Essentially, the ^{1}H hy-

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