113 °C (1.2 mmHg). This material was stable for several weeks when stored in the dark at 5 °C, but it slowly decomposed on standing at room temperature: ^{1}H NMR ($CDCl_3$) δ 1.65 (s, 9 H), 7.05 (s, 1 H), 7.60 (s, 1 H), 8.45 (s, 1 H); IR (neat) 1735 (ester), 1765 (amide) cm^{-1}; mass spectrum, *m/e* 196 (M^+).

Anal. Calcd for $C_9H_{12}N_2O_3$: C, 55.06; 6.16; N, 14.33. Found: C, 54.77; H, 6.14; N, 14.06.

Preparation of Grignard Reagents. The Grignard reagents were prepared from freshly distilled halide (26.4 mmol) in THF (30 mL) with magnesium (642 mg) below 40 °C. An aliquot was titrated with 0.1 N HCl to determine the molarity.

Grignard Reactions with Imidazolides 1 and 2. The procedures were identical with reagents 1 and 2. The imidazolide (24.0 mmol) was dissolved in THF (75 mL) under nitrogen and cooled to −50 °C in a dry ice/acetone bath. The Grignard solution, (1.0 equiv in 50 mL of THF) was added by dropping funnel over 1 h with stirring. The solution was allowed to come to room temperature over 3 h and poured into ice–water (200 mL). The solution was extracted with ether (a few drops of acetic acid was added if necessary to break up emulsions) and the ether extracts were washed with brine and dried ($MgSO_4$ or K_2CO_3). Removal of solvents under reduced pressure and distillation afforded the α-keto esters.[15] The data on compounds **3–13** are listed in Table I.

Cleavage of *tert*-Butyl Esters to Acids.[14] The procedure for all examples was as follows. The *tert*-butyl ester (500 mg) was added to CF_3CO_2H (5 mL) and stirred in an ice bath. After approximately 60 min, TLC monitoring showed the reaction to be complete and the CF_3CO_2H was removed under reduced pressure. The residue was either recrystallized from benzene/petroleum ether (bp 60–110 °C) (for compounds **14–17**) or purified by bulb-to-bulb distillation (for compounds **18** and **19**).

Acknowledgment. We gratefully acknowledge financial support by the National Institutes of Health, NS 14345, and the Hertz Foundation for a fellowship.

Registry No. 1, 75716-82-4; 2, 75716-83-5; 3, 1603-79-8; 4, 40140-16-7; 5, 34966-48-8; 6, 5524-56-1; 7, 15933-07-0; 8, 7332-98-1; 9, 75716-84-6; 10, 75716-85-7; 11, 75716-86-8; 12, 75716-87-9; 13, 75716-88-0; 14, 611-73-4; 15, 7099-91-4; 16, 7099-88-9; 17, 7163-50-0; 18, 816-66-0; 19, 815-17-8; PhBr, 108-86-1; *p*-$CH_3O(C_6H_4)$Br, 104-92-7; *p*-Cl(C_6H_4)Br, 106-39-8; *p*-$CH_3(C_6H_4)$Br, 106-38-7; EtBr, 74-96-4; *i*-BuCl, 513-36-0; *t*-BuCl, 507-20-0; ethyl oxalyl chloride, 4755-77-5; imidazole, 288-32-4; *tert*-butyl alcohol, 75-65-0; oxalyl chloride, 79-37-8.

(15) Yields were not improved by use of ether as the reaction solvent, by quenching with aqueous ammonium chloride, with pH 7.0 buffer or with aqueous acetic acid at low temperature.

Mechanistic Studies on the Addition of Cysteine to 3,4-Dihydroxyphenylalanine

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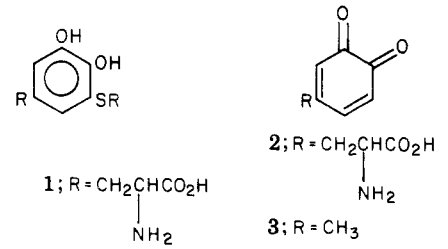
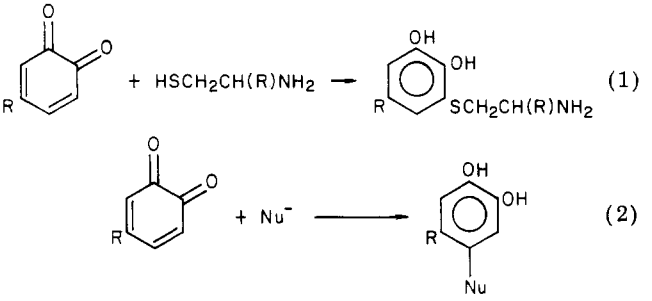
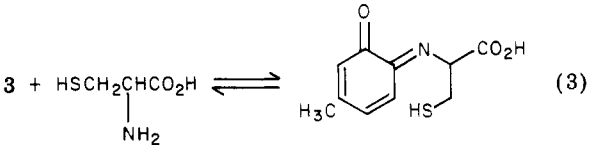
Urine levels of 5-(*S*-cysteinyl)dopa, **1**, are indicative of melanocyte activity,[2] and, as such, have been used for early detection of metastasizing melanoma.[3,4] Cysteinyldopas (the 2-*S*-, 5-*S*-, and 2,5-*S*,*S*-di isomers) are important biosynthetic intermediates of the red-brown polymeric pheomelanin pigments found throughout nature.[5] However, cysteinyldopas are by no means confined to phenomelanocytes; indeed, they are now known to be found in all tyrosinase-containing melanocytes[6] and have been proposed to be detoxification products of dopaquinone,[7] **2**. Herein we communicate preliminary work on the in vitro mechanism of cysteinyldopa formation.



Previous workers have speculated that addition of β-aminoethanethiols to *o*-benzoquinones occurred in a 1,6-fashion as depicted in eq 1.[8] However, no mention was made of the strong literature precendence favoring a general 1,4-addition of nucleophiles as depicted in eq 2.[9] Since the structure of **1** has been confirmed by independent synthesis,[10] a viable explanation for this abnormal addition is needed.



Initially we felt that a rapid preequilibrium, such as depicted in eq 3, might serve to position the incoming



nucleophile so as to favor attack at the 6 position. If this reasoning was correct, substitution of *N*-acetylcysteine for cysteine would dramatically alter the course and products of the reaction. In order to simplify both the isolation and analysis of the products, we decided to use 4-methyl-*o*-quinone, **3**, in place of **2**.

Various molar ratios of *N*-acetylcysteine, **4**, and preformed **3** were mixed together in the absence of oxygen. Inverse and simultaneous addition of the two was also tried. However, after workup of the reaction mixture, no trace of addition of **4** to the quinone could be detected. The same result was obtained if the quinone was prepared

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(2) Agrup, G.; Falck, B.; Kennedy, B-M.; Rorsman, H.; Rosengren, A-M.; Rosengren, E. *Acta Derm.-Vernerol.* **1973**, *53*, 453–454.

(3) Agrup, G.; Hansson, C.; Kennedy, B-M.; Persson, K.; Rorsman, H.; Rosengren, A-M.; Rosengren, E. *Acta Derm.-Vernerol.* **1976**, *56*, 491-492.

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(5) Fattorusso, E.; Minale, L.; Stefano, S. de; Cimino, G.; Nicolaus, R. A. *Gazz. Chim. Ital.* **1969**, *99*, 969–978.

(6) Dryja, T. P.; Albert, D. M.; Rorsman, H.; Rosengren, E.; Reid, T. W. *Exp. Eye Res.* **1977**, *25*, 459–464.

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(8) Prota, G.; Scherillo, G.; Napolano, E.; Nicolaus, R. A. *Gazz. Chim. Ital.* **1967**, *97*, 1451–1478.

(9) Wanzlick, H. W. *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 401–408.

(10) Prota, G.; Scherillo, G.; Nicolaus, R. A. *Gazz. Chim. Ital.* **1968**, *98*, 495–510.

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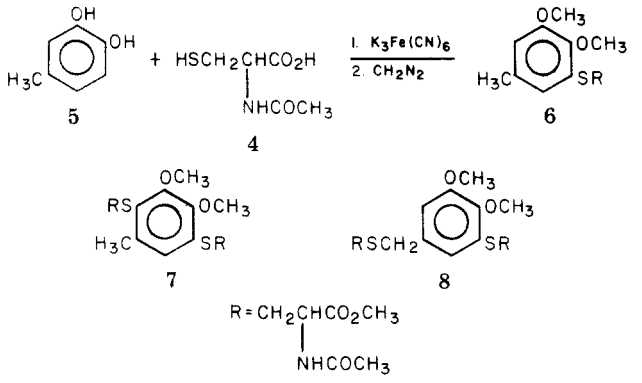
in situ by addition of potassium ferricyanide solution to a mixture of 4 and 4-methylcatechol, 5. However, when equimolar methanolic solutions of 4 and 5 were treated with potassium ferricyanide in the presence of oxygen, three addition products were formed. Workup of the reaction afforded an oil which was treated with excess diazomethane and separated into its components by use of medium-pressure liquid chromatography over silica gel.

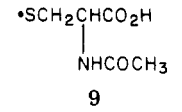
Structural assignments of the addition products are based on analysis of the infrared, mass spectral, and nuclear magnetic resonance data (both proton and carbon) and are herein detailed. The exact mass of the molecular ion of the most abundant coupled product (17.5% overall yield)[11] clearly indicated that it resulted from an addition of one molecule of 4 to 5. The infrared spectrum exhibited bands typical of an aliphatic ester, monosubstituted amide, and aromatic ring. Comparison of the carbon-13 chemical shift and off-resonance decoupling data of this product with those of 4 and 5 leads to the conclusion that the sulfur atom of 5 has become attached to the catechol ring. Thus the only question remaining is at which position of the catechol ring has the sulfur become attached. The aromatic region of the proton NMR spectrum of this compound exhibited a pair of doublets with a coupling constant typical of meta hydrogens (2 Hz). Furthermore, in the carbon-13 gated-decoupled NMR spectrum of this compound the 4-methyl group is observed to be a quartet of triplets (J for the quartet is 125.6 Hz and J for the triplet is 4.4 Hz), a pattern typical of an aromatic methyl group flanked by two ortho hydrogens. Thus, from the above data this product was assigned the structure 6.

Spectral data on the other two products indicated that they resulted from addition of two molecules of 4 to 5. On the basis of comparison of the proton NMR data of these products with those of 4, 5, and 6 they have been assigned the structures 7 and 8 (isolated in 3.6% and 1.0% overall yields, respectively).



Thus, we have established that addition of N-acetylcysteine to 4-methylcatechol in the presence of basic ferricyanide cannot be accounted for mechanistically by a nucleophilic addition of sulfur to the quinone moiety. In addition, we have unambiguously demonstrated that oxygen is necessary for any addition to take place.

In light of our results, we feel that a radical-type mechanism is operating under these conditions. Photolytic generation of the thiyl radical 9 in the presence of 5 did



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not lead to an addition product. Thus, we suggest that the initial addition takes place via a nucleophilic attack of sulfur on the semiquinone of 5. Subsequent oxidation of this addition product to a semiquinone with further attack of 4 would then account for the minor products 7 and 8.

Although a good deal more work is needed to understand the nuances of this mechanism, these preliminary experiments indicate that the addition of cysteinyl residues to catechols takes place neither via an abnormal nucleophilic attack on an o-quinone nor via the preequilibrium depicted in eq 3. Indeed, formation of the biologically important cysteinyldopas probably proceeds through a complex mechanism which involves free radicals. Given the biological role of dopa in particular, and catecholamines in general, it is interesting to speculate as to how significant this type of reactivity is to the in vivo chemistry of this important class of compounds.

Experimental Section

Medium-pressure liquid chromatography (75–100 psi) was performed on a size B Lobar prepacked LiChromprep Si60 column (EM Reagents). The pressure was provided by an FMI Lab Pump, Model RFP. Proton NMR spectra were obtained on a Varian EM 360 spectrometer with $(CH_3)_4Si$ as an internal standard, carbon-13 NMR spectra were recorded on a Brüker WP-80 spectrometer with $(CH_3)_4Si$ as an internal standard, infrared spectra were run on a Perkin-Elmer 457 grating spectrometer, and mass spectra were recorded on an AEI MS-9 spectrometer at 70 eV (in all cases there were no peaks higher than those of the molecular ion).

Over a period of 65 min, a solution of potassium ferricyanide in 2% aqueous sodium bicarbonate (6.58 g of $K_3Fe(CN)_6$ in 200 mL of $NaHCO_3$) was added dropwise to a mechanically stirred solution of freshly sublimed 4-methylcatechol (1.24 g) and N-acetyl-l-cysteine (1.63 g) in 50 mL of methanol. After addition of the ferricyanide solution, the reaction mixture was allowed to stir at room temperature for an additional 3 h. A yellow precipitate formed immediately upon addition of the ferricyanide, which by the end of the reaction had become a green suspension. Methanol was added to the green suspension and the resultant inorganic precipitate removed by filtration. The pH of the filtrate was adjusted to 5 with dilute hydrochloric acid whereupon the resulting red solution was extracted two times with twice its volumn of chloroform and two times with ethyl acetate. The organic layers were combined, the solvent was removed in vacuo, and the resultant oil (2.1 g) was dissolved in 25 mL of methanol and treated with an excess of diazomethane (in ethyl ether). The esterification mixture was allowed to stir overnight whence the solvent and excess diazomethane were removed in vacuo to afford an oil (2.9 g). This oil was separated into seven products by use of medium-pressure liquid chromatography using ethyl acetate/methanol (98/2) as eluant. Only the last three compounds to elute were derived by coupling of 4 to 5; the rest were either catechol–catechol coupling products or oxidation products of cysteine.[12]

N-Acetyl-S-(2,3-dimethoxy-5-methylphenyl)-L-cysteine Methyl Ester. (6). This low-melting solid (mp <40 °C) was the first addition product to be eluted from the column. It was obtained in 17.5% overall yield (0.57 g) and had the following spectral data: IR (neat oil) 3300 (m, br), 2960 (m), 1750 (s), 1665 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.02 (s, 3 H), 2.40 (s, 3 H), 3.48 (d, 2 H), 3.75 (s, 3 H), 3.95 (s, 6 H), 4.95 (m, 1 H), 6.70 (d, 1 H), 6.85 (d, 1 H) 6.95 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 21.2 (q^{13}), 22.8 (q), 35.8 (t), 52.3 (s and t, 2 C), 55.9 (q), 60.7 (q), 113.1 (d), 124.4 (d), 128.6 (s), 134.3 (s), 146.4 (s), 152.9 (s), 169.8 (s), 170.9 (s); mass spectrum for $C_{15}H_{21}NO_5S$ (calcd m/e 327.1140, found m/e 327.1145).

S,S'-(2,3-Dimethoxy-5-methyl-1,4-phenylene)bis[N-acetyl]-L-cysteine Dimethyl Ester. This second addition product to be eluted from the column was isolated as a white

(11) Three products containing both a catechol and cysteine residue were isolated. A number of other products containg only catechol or cysteine residues were isolated. The total material balance based on all products isolated was over 70%.

(12) Nkpa, N. N. Masters Thesis, The Ohio State University, 1979.

(13) This is the designation of the carbon multiplicity in the off-resonance decoupled carbon-13 NMR spectrum: q = quartet, t = triplet, d = doublet, and s = singlet.

crystalline solid (mp 135-137 °C) in 3.6% overall yield (0.18 g) and had the following spectral data: ^{1}H NMR ($CDCl_3$) δ 1.95 (s, 6 H), 2.40 (s, 3 H), 3.36 (d, 4 H), 3.48 (s, 3 H), 3.64 (s, 3 H), 3.85 (s, 3 H), 3.90 (s, 3 H), 4.80 (m, 2 H), 6.70 (br s, 2 H), 6.95 (s, 1 H); mass spectrum for $C_{21}H_{30}N_2O_8S_2$ (calcd *m/e* 502.1443, found *m/e* 502.1453).

***N*-Acetyl-*S*-[[3-[[2-(acetylamino)-3-methoxyoxopropyl]thio]-4,5-dimethoxyphenyl]methyl]-L-cysteine Methyl Ester.** This third addition product to be eluted from the column was isolated as a viscous oil in 1.0% overall yield (0.05 g) and had the following spectral data: ^{1}H NMR ($CDCl_3$) δ 2.07 (s, 6 H), 3.24 (d, 4 H), 3.60 (s, 2 H), 3.80 (s, 6 H), 3.90 (s, 6 H), 4.90 (m, 2 H), 6.71 (d, 1 H), 6.82 (d, 1 H); mass spectrum for $C_{21}H_{30}N_2O_8S_2$ (calcd *m/e* 502.1443, found *m/e* 502.1458).

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Registry No. 4, 616-91-1; 5, 452-86-8; 6, 75625-96-6; 7, 75625-97-7; 8, 75659-24-4.

Salt Effects on the Intramolecular Carboxyl-Catalyzed Hydrolysis of Sulfonamides[1]

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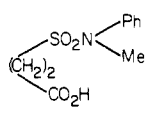
Relatively few studies have been made of solvent and electrolyte effects on intramolecular catalyzed hydrolytic processes.[3] This is surprising since medium effects are believed to play a significant, although probably not dominant,[3b] role in the important class of intramolecular reactions which go on within the enzyme–substrate complex.[4] The present work involves a study of electrolyte effects on the intramolecular carboxyl-catalyzed hydrolysis of the sulfonamides 1 and 2. In previous studies,[1,5] the



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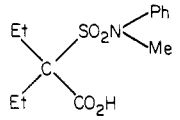

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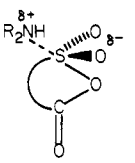

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mechanism of this reaction has been investigated in some detail. All kinetic data are consistent with nucleophilic catalysis, the rate-determining step being breakdown of a pentacovalent sulfur intermediate[1] via a transition state schematically depicted as 3.

Results and Discussion

First-order rate constants for the intramolecular carboxyl-catalyzed hydrolysis of 1 and 2 in a variety of aqueous electrolyte solutions are listed in Table I. Thermodynamic activation parameters are given for hydrolysis in the presence of $MgCl_2$, $CaCl_2$, and *n*-Bu_4NBr. The effect of HCl on the hydrolysis rate is treated as a salt effect[6] since external acid catalysis cannot compete with intramolecular catalysis in 2.0 M HCl at 55 °C. The magnitudes of the salt effects are expressed as k_{obsd}^s/k_{obsd}^o (Table I), which reveal that the hydrolysis of 1 is generally somewhat more affected[7] by the presence of the electrolytes than the hydrolysis of 2. The greater sensitivity of the hydrolysis of 1 to solvation effects was also noted for hydrolysis in some mixed aqueous solvents.[1,8] For example, on going from H_2O to 1:1 (v/v) EtOH–H_2O the rate constant decreases about tenfold for 1 while the rate constant for 2 becomes smaller by a factor of only 4. We assume that this difference is a consequence of the closer proximity of the reacting groups in 2, which implies that the solvation shells of the sulfonamide and carboxyl groups are not independent of each other.[9] Therefore, the solvation change upon transforming the initial state into the transition state 3 will most likely be smaller for 2 than for 1, and this factor may well attenuate the medium effects on the hydrolysis of 2.

As shown in Table I, all electrolytes except *n*-Bu_4NBr accelerate the hydrolysis of 1 and 2. Since studies[1,5,8] of mixed aqueous solvent effects have shown that the rates of hydrolysis of 1 and 2 *decrease* with decreasing dielectric constant (ε) of the medium, it seems evident that an explanation of the salt effects cannot be given in terms of the reduced dielectric constant in the salt solutions.[10] Furthermore, it is also highly unlikely that "structure-making" and "structure-breaking" effects[11] of the ions represent the dominant factor in determining the kinetic salt effects. For both substrates the electrolyte effects of the single ions follow the sequences $Mg^{2+} > Ca^{2+} > H^+ > Na^+ \approx K^+ > NH_4^+ > Cs^+ > Me_4N^+ > n\text{-}Bu_4N^+$ and $Cl^- \approx Br^- > ClO_4^- > HSO_4^-$. These are not the sequences expected for water-structure perturbation.[12]

We suggest that the kinetic salt effects presented in Table I mainly reflect the hydrogen bonding properties of water molecules as modulated by the presence of the electrolytes.[13] This explanation hinges on the notion that water molecules in the electrostatic field of cations will be better hydrogen bond donors than unpolarized water molecules whereas water molecules in the hydration shells of anions will be better hydrogen bond acceptors. The

(1) Part V in the series "Intramolecular Carboxyl-Catalyzed Hydrolysis of Sulfonamides". Part IV: Graafland, T.; Wagenaar, A.; Kirby, A. J.; Engberts, J. B. F. N. *J. Am. Chem. Soc.* **1979**, *101*, 6981.

(2) (a) University of Groningen. (b) University of Cambridge.

(3) See, for example: (a) Bruice, T. C.; Turner, A. *J. Am. Chem. Soc.* **1970**, *92*, 3422; (b) Dafforn, G. A.; Koshland, D. E., Jr. *Ibid.* **1977**, *99*, 7246.

(4) (a) Lumry, R.; Rajender, S. *Biopolymers* **1970**, *9*, 1125. (b) Jencks, W. P. *Adv. Enzymol. Relat. Subj. Biochem.* **1975**, *43*, 219.

(5) Graafland, T.; Engberts, J. B. F. N.; Kirby, A. J. *J. Org. Chem.* **1977**, *42*, 2462.

(6) k_{obsd}^s/k_{obsd}^o = 1.42 for 2 in 2 M HCl: this value is probably too low as a result of the salt effect on k_{obsd}^o in 0.5 M HCl (see Experimental Section).

(7) A rigorous comparison is hindered by the different acidities of the media used for hydrolysis of the two substrates (Experimental Section). However, salt effects measured for the hydrolysis of 1 in the presence of 0.5 N HCl showed that this factor is of minor importance and does not affect the sequences of the salt effects of the single ions.

(8) Graafland, T., unpublished results.

(9) A referee has pointed out that the relative magnitude of the salt effects observed for 1 and 2 might also be affected by the intrinsic difference in volume change upon transferring the initial state into the transition state. See: Long, F. A.; McDevit, W. F. *Chem. Rev.* **1952**, *51*, 119. In terms of this theory the largest effects are expected for 1, but in the absence of ΔV^* values no definite conclusions can be drawn.

(10) It has been calculated that around a single charged ion the dielectric constant at a distance of 6 Å is reduced to 40: Azzam, A. M. *Z. Elektrochem.* **1954**, *58*, 889.

(11) See: Bunton, C. A.; Robinson, L. *J. Am. Chem. Soc.* **1968**, *90*, 5965.

(12) Blandamer, M. J. *Q. Rev., Chem. Soc.* **1970**, *24*, 169.

(13) Compare: (a) Menninga, L.; Engberts, J. B. F. N. *J. Am. Chem. Soc.* **1976**, *98*, 7652 and references cited therein; (b) Breemhaar, W.; Engberts, J. B. F. N. *J. Org. Chem.* **1978**, *43*, 3618.

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