Photoamidation of *N*-Acetyl-2-chlorotyrosine Methyl Ester and 3-Chlorophenol†

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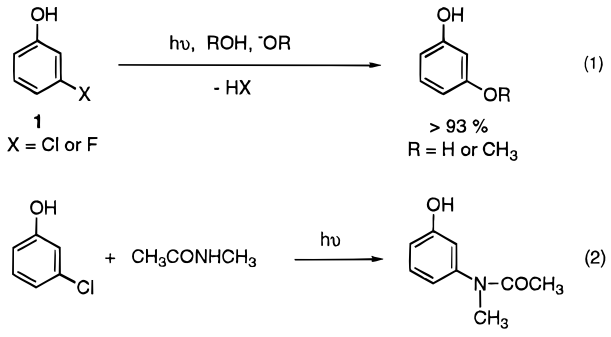
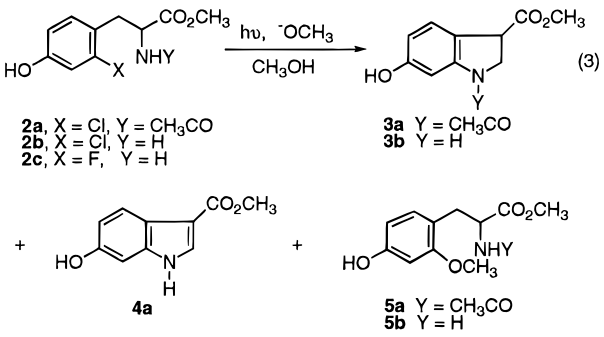
Aryl halides are stable in nucleophilic displacement reactions. 4-Chlorophenols undergo a variety of photochemical reactions to yield a plethora of products, while 3-chlorophenol undergoes photohydrolysis in water to form resorcinol.[1] Since the excited state of phenols is more energetic than that of the corresponding phenoxides, excited phenols are known to ionize to excited phenoxide.[2] On the basis of our experience in the photochemistry of 4-haloindoles[3] and the charge distributions of the L_b excited state of benzene,[4] the powerful electron-releasing excited phenoxide ion will delocalize a substantial portion of its charge to the meta-position.[5] Localization of charge at the position bearing the halogen substituent may lead to its activation and subsequent displacement (see also Scheme 1).

We irradiated 3-fluoro- and 3-chlorophenol, **1**, and found that they undergo photomethanolysis to yield resorcinol monomethyl ether in excellent yield (94%, reaction 1). In examining the scope of photosubstitution of 3-halophenols, we discovered that these 3-halophenols undergo photoamidation by *N*-methylacetamide (reaction 2), a very weak nucleophile, to yield 3-(*N*-methyl-*N*-acetylamino)phenol (77%) together with a small amount of phenol (6%). A competitive study between methanol and *N*-methylacetamide in intermolecular photosubstitution indicates that methanol is more reactive than *N*-methylacetamide by at least 1 order of magnitude.



Peptide linkages are inert toward chemical modifications under ordinary laboratory conditions, tyrosine often plays an important role in the biochemistry of proteins,[6] halotyrosines may be readily incorporated in lieu of tyrosine into proteins by the method of molecular biology,[7] and Ar−X bonds are stable in the ground state. Therefore, our next goal is to evaluate photoamidation of 2-halotyrosine derivatives as a possible entry to photoaffinity labels for tyrosine in protein chemistry.[8] This communication deals with the photochemistry of 3-halophenols and derivatives of 2-halotyrosines.

When DL-*N*-acetyl-2-chlorotyrosine methyl ester[9] (2×10^{-3} M, **2a**) in HPLC grade methanol (100 mL) containing 1 equiv of sodium methanolate was irradiated with a Hanovia 450 W Hg-arc through a Pyrex filter under argon, about 40% of the substrate was consumed after 1 h. The products were isolated by semipreparative HPLC using a silica gel column and chloroform:methanol (94/6, v/v) as the eluent. Three new products were isolated: **3a**, **4a**, and **5a**, in 35%, 11%, and 45% yield, respectively (reaction 3). They were characterized unambiguously by



elemental and spectroscopic analyses.[10] The formation of **4a** has an induction period and was detected only after an appreciable amount of **3a** was present; **4a** is thus likely to be a secondary product formed from the irradiation of **3a**. The formation of **3a**, methyl 1-acetyl-6-hydroxy-2-indolinecarboxylate, is an intramolecular photoamidation of an aryl halide, a novel chemical reaction. This reaction is competitive vs the formation of **5a**, the intermolecular methanolysis with the solvent, methanol. Since methanol is much more reactive than *N*-methylacetamide, a secondary amide in intermolecular photosubstitution, the formation of **3a** vs **5a** must be controlled by a prevailing steric factor. We also studied the photochemistry of DL-2-chlorotyrosine methyl ester, **2b**, under the same experimental conditions. The relative yields of intramolecular amination products, **3b** (19%) and **4a** (17%), to the intermolecular solvolysis product **5b** (41%) are also approximately 1:1. Since amines are much stronger nucleophiles than amides, the results again indicate that the intramolecular process is controlled by the stereochemistry of the substrate and not by the nucleophilicity of the substituting reagent. A parallel study of the photochemistry of L-2-fluorotyrosine

† Dedicated to Professor Morris S. Kharasch on the centennial of his birth.

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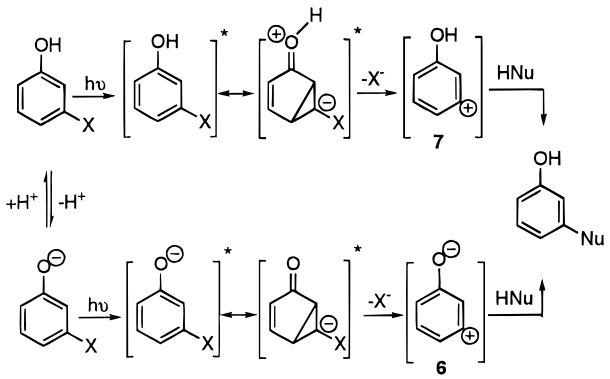
methyl ester **2c** indicates that the fluoro compound is less reactive than the 2-chloro compound in the intramolecular amination; i.e., the yields of **3b**, **4a**, and **5b** were 13%, 15%, and 58%, respectively.

Our experimental results suggest that the reactions proceed via a highly reactive electron-deficient intermediate. When the photolyses of 3-chlorophenol was carried out in acetonitrile containing 1−2% of water or methanol, arylation of acetonitrile occurred and (*N*-acetylamino)-phenol was isolated as the major product (45−75% yield). *N*-Alkylamides may be prepared from the alkylation of nitriles under acidic conditions via the carbocation intermediate, the Ritter reaction.[11] Photoarylations of amides and nitriles suggest that these reactions proceed via a cationic intermediate **7** (or a solvent separated ion-pair). This intermediate may be formed from either the excited phenol or the excited phenoxide (Scheme 1). The photoamidation is novel because the reaction takes place under neutral conditions, and it is an arylation.

Photoaffinity labeling is a powerful technique in exploring protein:substrate interactions.[8] However, common photoaffinity labels such as azides and diazo compounds are chemically labile to acidic reagents used in peptide and polynucleotide syntheses,[8] and many are toxic to common bacteria and cell lines used in molecular biology. These photoaffinity labels cannot be incorporated into aromatic amino acids in peptides and proteins, and are thus incompatible in the study of protein−biopolymer interactions. Aryl−halogen bonds are stable in the ground state, yet halogens *meta* to the phenol in 2-halotyrosines readily undergo photosubstitutions. Depending on their steric environment, they may be substituted competitively by a polar OH or NH group and by a non-nucleophilic amide, *the* linkage of amino acids in proteins. We had synthesized two insulins in which the B-25 phenylalanine, a position crucial in the receptor binding, was substituted by 3-fluorotyrosine and by 4-fluorophenylalanine.[12] These modified insulins were found to be as active as insulin in both the hormone assay and the receptor-binding assay.[12] Photosubstitution of 2-halotyrosines, therefore, offers a novel approach to photoaffinity labeling by the interactions of these haloaromatic amino acids with all neighboring amino acids, polar and apolar, in proteins. The photochemical interactions of 2-halotyrosyl derivatives and 3-halophenols with other weak nucleophiles will be explored.

Scheme 1



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(10) Analytical data: Compound **3a**: mp 207−8 °C. Anal. Calcd for $C_{12}H_{13}NO_4$: C, 61.28; H, 5.53; N, 5.96. Found: C, 61.13; H, 5.50; N, 5.88. MS (EI): 235(19), 193(17), 134(100). ^{1}H NMR (methanol-d_4, 500 MHz, 22 °C): two conformers (A:B = 1:3): conformer A: δ 6.97 (d, J = 8 Hz, 1H), 6.72 (s, 1H), 6.42(d, J = 8 Hz, 1H), 5.05 (d, J = 11 Hz, 1H), 3.67 (s, 3H), 3.38 (dd, J = 11 and 15.5 Hz, 1H), 2.91 (d, J = 15.5 Hz, 1H), 2.42 (s, 3H); conformer B: δ 6.92 (d, J = 8 Hz, 1H), 6.70 (s, 1H), 6.42 (d, J = 8 Hz, 1H), 5.13 (d, J = 11 Hz, 1H), 3.72 (s, 3H), 3.50 (dd, J = 11 and 15.5 Hz, 1H), 3.12 (d, J = 15.5 Hz, 1H), 2.41 ppm (s, 3H). At 55 °C, all peaks broaden, and the COOCH peaks at δ 3.7 and the CH_3CON peaks at δ 2.4 coalesce into single peaks. Compound **3b**: It resisted all efforts for recrystallization so far and was readily converted into **3a** by acetylation. MS (EI): 193(31), 134(100), 116-(11). ^{1}H NMR (methanol-d_4, 500 MHz): δ 6.75 (d, J = 8 Hz, 1H), 6.12 (d, J = 2.5 Hz, 1H), 6.07 (dd, J = 8 and 2.5 Hz, 1H), 4.29 (dd, J = 10.5 and 6 Hz, 1H), 3.64 (s, 3H), 3.18 (dd, J = 10.5 and 15.5 Hz, 1H), 3.02 (dd, J = 6 and 15.5 Hz). Compound **4a**: mp 165−8 °C. Anal. Calcd for $C_{10}H_9NO_3$: C, 62.83; H, 4.71; N, 7.33. Found: C, 62.83; H, 4.81; N, 7.22. MS (EI): 191(79), 159(100), 131(53), 105(29), 51(15). λ_{max}-(methanol), 319 nm. Fluorescence, E_{max}(methanol), 381 nm. ^{1}H NMR (methanol-d_4, 500 MHz), δ 7.33 (d, J = 9 Hz, 1H), 6.98 (s, 1H), 6.70 (d, J = 2 Hz, 1H), 6.57 (dd, J = 9 and 2 Hz), 3.81 (s, 3H). Compound **5a**: mp 155−7 °C. Anal. Calcd for $C_{13}H_{17}NO_5$: C, 58.42; H, 6.37; N, 5.24. Found: 57.98; H, 6.18; N, 5.21. MS (EI): 267(8), 208(74), 177(16), 137-(100), 107(38). ^{1}H NMR (methanol-d_4, 500 MHz): δ 6.77 (d, J = 8 Hz, 1H), 6.31 (d, J = 2 Hz, 1H), 6.20 (dd, J = 8 and 2 Hz), 4.54 (dd, J = 8.5 and 6.5 Hz, 1H), 3.77 (s, 3H), 3.62 (s, 3H), 3.02 (dd, J = 13 and 8.5 Hz, 1H), 2.77 (dd, J = 13 and 8.5 Hz), 1.89 (s, 3H). Compound **5b**: Due to the difficulty involved in the isolation of **5b** by HPLC, it was first acetylated with acetic anhydride to **5a** which was isolated and characterized as such.

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