Identification of Chlorothiazide and Hydrochlorothiazide UV-A Photolytic Decomposition Products

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Abstract □ Methanol solutions of hydrochlorothiazide and chlorothiazide were irradiated with fluorescent UV-A lamps in order to simulate degradation under normal conditions. The degradation products were identified by comparison to synthetic standards featuring electrospray ionization mass spectroscopy, ultraviolet spectroscopy, and high performance liquid chromatography. The standards were characterized by high resolution fast atom bombardment MS and ^{1}H NMR. The photolysis of chlorothiazide resulted in photodehalogenation products exclusively, while the irradiation of hydrochlorothiazide primarily yielded photodehalogenation products with significant yields of photodehydrogenation products and minor amounts of thermal hydrolysis products.

Introduction

Hydrochlorothiazide (**1**) and chlorothiazide (**1A**) have proven to be clinically effective diuretics for more than 35 years since their original syntheses.[1,2] Degradation of these thiazides by hydrolysis has been well-studied.[3] Thiazides **1** and **1A** have been reported to be potent photosensitizers which increase the sensitivity of skin to solar radiation.[4] The irradiation of **1** at wavelengths greater than 310 nm (medium-pressure mercury lamp with glass filter)[5] and the irradiation of **1** and **1A** at 313 nm (high pressure mercury lamp with filters)[6] have been reported.

The International Conference on Harmonization (ICH) has developed guidelines for photostability studies. The ICH Photostability Expert Working Group has proposed guidelines for optional photolysis sources.[7] One of the optional ICH lamp sources was defined as a UV-A fluorescent lamp with wavelengths ranging from 300 to 400 nm. This lamp source simulates the UV portion of sunlight filtered through window glass.[8] To study the photodegradation of **1** and **1A** due to UV radiation that might be encountered during storage, manufacture, and skin exposure, we have photolyzed methanol solutions of these thiazides under the ICH-defined UV-A lamp and identified the degradation products. In the photolysis of hydrochlorothiazide we did not find after exhaustive effort the principal hydrolysis product reported by Tamat and Moore.[5] In addition they[5] failed to find the photodehydrogenation products we identified. In the photolysis of chlorothiazide Ulvi and Tammilehto[6] failed to find the photodehalogenation products we identified. We have synthesized independently a standard for each degradation product reported (Figure 1). Toxicity studies will be reported separately.

Experimental Section

Part 1—*Photolysis Method*—Methanol solutions of **1** or **1A** were presaturated with nitrogen by bubbling for 60 min to remove molecular oxygen. The deoxygenated thiazide solution was immediately pipetted into a quartz photolysis cuvette (1 cm × 1 cm × 3 cm), Starna Cells, Atascadero, CA. The cuvette was immediately capped with a rubber septum. The cuvette was centrally and horizontally located, 3 cm underneath two UV-A 24 inch, 20 watt Sylvania fluorescent lamps, Model F20T12/BLB, Starbeam Supply, St. Louis, MO. The lamps were mounted horizontally, parallel, and 6 cm apart. The cuvette solution was irradiated. Aliquots were analyzed immediately by HPLC−MS and HPLC−UV. Several hours later after photolysis termination they were analyzed again by HPLC−UV.

HPLC−MS—Chromatography was performed on a YMC, Inc. (Wilmington, NC) J sphere ODS-M80 HPLC column (2 × 250 mm). Chromatography conditions for electrospray analysis started with 0% acetonitrile then changed by a linear gradient to 30% acetonitrile in 20 min. A buffer concentration of 0.1% formic acid was maintained throughout the run. A Hewlett-Packard (Palo Alto, CA) Model 1050 HPLC pump was employed to provide a linear gradient and constant flow rate of 200 mL/min. The entire column effluent passed into the standard ion source of a Finnigan Model TSQ-7000 triple-quadrupole mass spectrometer (San Jose, CA). Nitrogen was used as a nebulizing gas and the capillary temperature was 260 °C. The instrument was scanned over the range of 180−500 amu at 1 s/scan.

HPLC−UV—A Waters 600 HPLC pump was employed to provide a linear gradient and constant flow rate of 1.0 mL/min. The mobile phase was pumped through a Hewlett-Packard 1090M (Palo Alto, CA) ChemStation equipped with a diode array detector, a Rheodyne manual injector (Model 7125), and a Beckman Ultrasphere, C-18, 5 μm HPLC column (150 × 4.6 mm). The wavelength was monitored at 270 nm. The chromatographic conditions for analysis started with 0% acetonitrile then changed by a linear gradient to 30% acetonitrile over a period of 12 min. A phosphoric acid solution (0.1%) was maintained throughout the run.

High Resolution MS—Spectra were measured on a MS-50 three-sector (KRATOS, Ramsey, NJ) mass spectrometer. Samples were dissolved in methanol and mixed with 4-nitrobenzyl alcohol (Aldrich, St. Louis, MO) prior to introduction to the mass spectrometer. The ionization mode was FAB with Argon as a primary beam. The spectra were recorded twice for each sample by the peak matching method; a mixture of glycerol and CsI was used as a cocalibrant.

High Resolution NMR—Proton NMR spectra were recorded on a Unity-Plus 500 (Varian, Palo Alto, CA) spectrometer. All NMR samples were dissolved in methanol-d_4 (Cambridge Isotope Laboratories, Woburn, MA) and referenced to residual protonated methanol at 3.30 ppm.

*Photolysis of Hydrochlorothiazide (**1**)*—A 9.6 mg/mL methanol solution of **1** (Mylan Pharmaceuticals, Morgantown, WV) was added to fill a photolysis cuvette. The solution was irradiated according to the photolysis method previously described. At 3 h intervals over a 21 h period, a 25 mL aliquot was removed via syringe to be diluted with 1.00 mL of an internal standard solution for HPLC−UV analysis. (The internal standard solution was prepared by dissolving 380 mg of ethylparaben in 1.00 L of 0.1% phosphoric acid aqueous). The above photolysis experiment was repeated using the same concentration of **1** in methanol without deoxygenation.

*Photolysis of Chlorothiazide (**1A**)*—A 1.2 mg/mL methanol solution of **1A** (Merck, Sharp & Dohme, West Point, PA) was added to fill a photolysis cuvette. The solution was photolyzed according to the photolysis method previously described. At 24 h intervals over a 5 day photolysis period a 75 mL aliquot was removed via syringe and diluted with 75 mL of internal standard solution for HPLC−UV analysis. (The internal standard solution was prepared as described

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above.) The above photolysis experiment was repeated using the same concentration of **1A** in methanol without deoxygenation.

Part 2—*Synthesis of Thiazide Derivatives*—Synthetic thiazide derivatives described below (Scheme 1) were dissolved in methanol and applied to tapered, preparative TLC plates (Analtech, Newark, DE). The plates were developed in ethyl acetate:hexane 80:20 eluant. The proper band was scraped and eluted with ethyl acetate for analysis. Synthetic standards showed greater than 98% purity by HPLC–UV analysis.

*4-Amino-6-iodo-1,3-benzenedisulfonamide (**6**)*—Chlorosulfonic acid (40 mL) was reacted with *m*-iodoaniline (8 g) by analogy to reported procedures.[1,2] The final product was recrystallized from boiling water to yield intermediate **6** (4 g, 29% yield) as a colorless solid: ESI-MS (M − H) 376 (100).

*4-Amino-1,3-benzenedisulfonamide (**5**)*—Into 100 mL of anhydrous ethanol was dissolved **6** (100 mg). The solution was photolyzed according to the photolysis method previously described except that a 100 mL, 35 cm × 2.5 cm quartz tube (Ace Glass, Louisville, KY) was employed with a microspin bar for stirring during photolysis. The dark solution was purified by preparative TLC to afford **5** (12 mg, 18% yield): ESI-MS (M − H) 250 (100); high-resolution FAB (M + H) $C_6H_{10}N_3O_4S_2$ calcd 252.0112, found 252.0122; ^{1}H NMR (CD_3-OD) δ 6.88 (1H, d, J = 8.79 Hz, 5), 7.70 (1H, dd, J = 2.44, 8.79 Hz, 6), 8.20 (1H, d, J = 2.44 Hz, 2).

*2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-Dioxide (**3A**)*—Standard **5** (17 mg) was refluxed for 2 h with 5 mL of formic acid, by analogy to reported procedures.[2] The final purification by preparative TLC afforded **3A** (3.5 mg, 20% yield) as a colorless solid: ESI-MS (M − H) 260 (100); high-resolution FAB (M + H) $C_7H_8N_3O_4S_2$ calcd 261.9956, found 261.9964; ^{1}H NMR (CD_3OD) δ 7.39 (1H, d, J = 8.79 Hz, 5), 7.83 (1H, s, 3) 8.07 (1H, dd, J = 8.79, 1.96 Hz, 6) 8.33 (1H, d, J = 1.96, 8).

*3,4-Dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-Dioxide (**3**)*—Standard **5** (34 mg) was reacted with 6 mg of paraformaldehyde by analogy to reported procedures.[1] Final purification by preparative TLC resulted in **3** (5.9 mg, 17%) as a colorless solid: ESI-MS (M − H) 262 (100); high-resolution FAB (M + H) $C_7H_{10}N_3O_4S_2$ calcd 264.0113, found 264.0116; ^{1}H NMR (CD_3OD) δ 4.76 (2H, s, 3), 6.84 (1H, d, J = 8.79 Hz), 7.71 (1H, dd, J = 2.44, 8.79 Hz, 6), 8.05 (1H, d, J = 2.44 Hz, 8).

*4-Amino-6-methoxy-1,3-benzenedisulfonamide (**4**)*—Chlorosulfonic acid (70 g) and *m*-anisidine (8.0 g) were reacted as described by reported procedures[5] to give **4** (4.95 g, 27% yield). The final purification by a series of hot methanol extractions produced a slightly brown solid: ESI-MS (M − H) 280 (100); high-resolution FAB (M + H) $C_7H_{12}N_3O_5S_2$ calcd 282.0218, found 282.0210; ^{1}H NMR (CD_3OD) δ 3.93 (3H, s, OCH_3), 6.48 (1H, s, 5), 8.15 (1H, s, 2).

*6-Methoxy-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-Dioxide (**2**)*—Standard **4** (100 mg) was reacted with paraformaldehyde (27.5 mg) as described by reported procedures.[5] Final purification by preparative TLC afforded **2** (31.5 mg, 30% yield): ESI-MS (M − H) 292 (100); high-resolution FAB (M + H) $C_8H_{12}N_3O_5S_2$ calcd 294.0218, found 294.0213; ^{1}H NMR (CD_3OD) δ 3.92 (3H, s, OCH_3), 4.74 (2H, s, 3), 6.40 (1H, s, 5), 7.97 (1H, s, 8).

*6-Methoxy-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-Dioxide (**2A**)*—Standard **4** (25 mg) was heated to reflux for 1.5 h with 5 mL of formic acid by analogy to reported procedures.[2] The final product **2A** (6.8 mg, 26.3% yield) was purified by preparative TLC as a colorless solid: ESI-MS (M − H) 290 (100); high-resolution FAB (M + H) $C_8H_{10}N_3O_5S_2$ calcd 292.0062, found 292.0060; ^{1}H NMR (CD_3-OD) δ 4.05 (3H, s, OCH_3), 6.89 (1H, s, 5), 7.85 (1H, s, 3), 8.25 (1H, s, 8).

Results

Hydrochlorothiazide (1)—After **1** was photolyzed for 24 h, the resulting solution was analyzed directly by HPLC–MS. The chromatogram generated (Figure 1) shows the relative intensities of each of the seven degradants and the starting material. In Scheme 1 the decomposition reactions can be divided into three types: photodehalogenation, photodehydrogenation, and thermal hydrolysis.

Photodehalogenation—Photodehalogenation is the primary degradation process in which the Cl of **1** is replaced by H to afford **3** or by OCH_3, from the methanol solvent, to produce **2**. After a 24 h photolysis, the concentrations of **3** and **2** were nearly equal, as determined by integration of the HPLC–MS chromatogram. From Figure 2, the photolysis of **1** as followed by HPLC–UV shows that **3** and **2** are major photolysis products. A discussion of the photoreaction mechanisms of **1** has been reported.[5] Parallel photolysis reactions in which the methanol solution of **1** was not deoxygenated resulted in lower yields of **2** and **3**. A discussion of the mechanisms of how oxygen scavenges free radicals in photoreactions has been reported.[9] The photodehalogenation reactions of **1** we observed were consistent with those reported by other groups.[5,6]

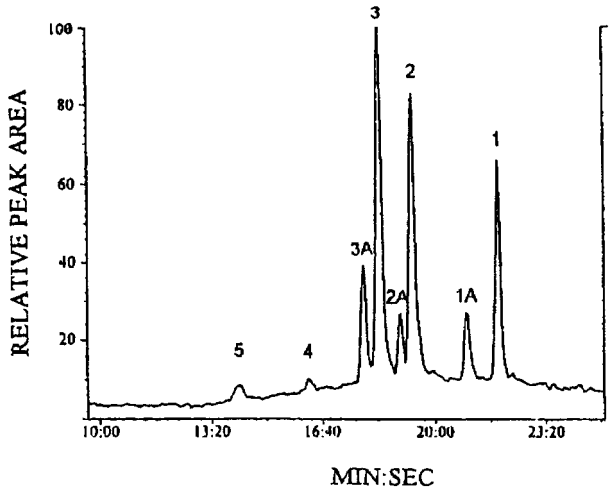
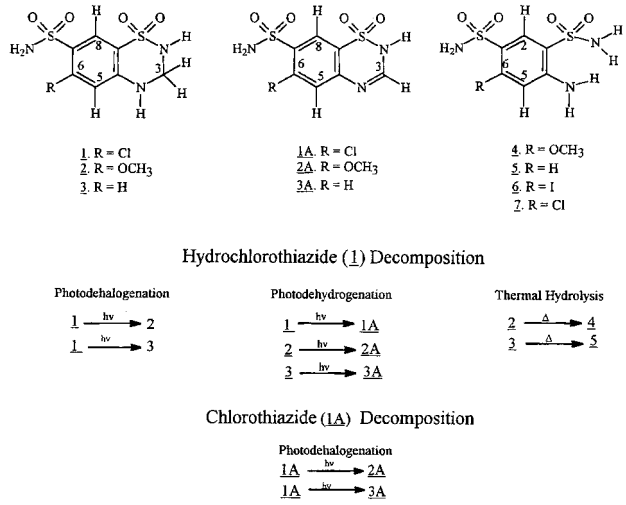


Figure 1—HPLC–MS chromatogram of hydrochlorothiazide photolysis products after 24 h. See Scheme 1 for peak component identification.



Scheme 1—Hydrochlorothiazide (**1**), chlorothiazide (**1A**), their derivatives and reactions.

Photodehydrogenation—The photodehydrogenation reactions, in which hydrothiazides **1**, **2**, and **3** were dehydrogenated to thiazides **1A**, **2A**, and **3A**, are shown in Scheme 1. After 21 h of hydrochlorothiazide photolysis, the yields of photodehalogenation products **2** and **3** were many times greater than the yields of photodehydrogenation products **1A**, **2A**, and **3A**, as viewed by HPLC–UV integration (Figure 2). When the above photolysis of **1** was repeated without deoxygenating the methanol solution, the yields of the photodehydrogenation products were lowered. Previous photolysis studies[5,6] of **1** do not report photodehydrogenation reactions.

Thermal Hydrolysis—The hydrolysis of thiazides **2** and **3** afforded the ring-opened thiazides **4** and **5** (Scheme 1). Photolysis aliquots of **1** were analyzed immediately by HPLC–

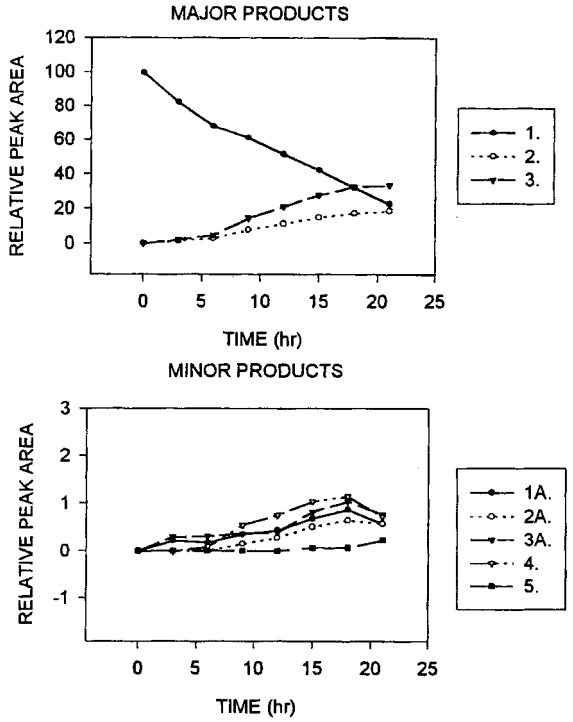


Figure 2—Photolysis of hydrochlorothiazide (**1**) monitored by HPLC–UV.

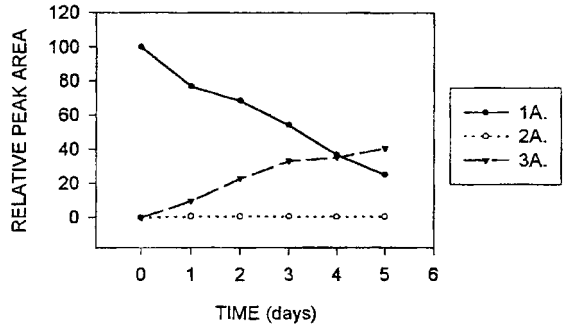


Figure 3—Photolysis of chlorothiazide (**1A**) monitored by HPLC–UV.

UV (Figure 2) and showed yields of **4** and **5** to be many times lower than the yields of photodehalogenation products **2** and **3**. After the photolysis aliquots were allowed to stand in the dark for a few hours, the aliquots were analyzed again by HPLC−UV. The results showed that the rate of hydrolysis to **4** and **5** in the dark was approximately equal to the rate of hydrolysis to **4** and **5** under UV light exposure. It appears that the hydrolyses of **3** and **2** to **5** and **4** go well without UV light. Previous studies[5] report that the rate of photohydrolysis of **1** was approximately equal to the rate of photodehalogenation reactions. Although considerable effort was made to identify the hydrolysis product **7** in the photolysate of **1**, the reported major photolysis product[5] was not observed by HPLC−UV, HPLC−MS, or HPLC coinjection experiments.

Chlorothiazide (1A)—*Photodehalogenation*—After **1A** was irradiated for 2 days, the resulting solution was analyzed by HPLC−MS. From the (M − H) molecular ions of each degradant, *m/z* 260 and 290, structures **3A** and **2A**, respectively, were proposed on the basis of photodehalogenation reactions (Scheme 1). Standards **2A** and **3A** were synthesized and shown to be identical to the corresponding degradants by HPLC coinjection experiments and by HPLC−MS and HPLC−UV. From Figure 3, after 5 days, the yield of **3A** (Cl replacement by H) is many times the yield of **2A** (Cl replacement by OCH_3) as viewed by HPLC−UV integration. As a way of confirming the structure, by use of the diode array detector, the UV spectrum of the standard **3A** was superimposed on the the UV spectrum of the photoproduct **3A** (Figure 4). Previous photolysis studies report no photodehalogenation products of **1A**.[6]

Discussion

A discussion of a previously reported photolysis of hydrochlorothiazide (**1**)[5] is presented. The source of UV light employed was a medium-pressure mercury vapor lamp with glass filter. The primary photoprocesses were photodehalogenation and photohydrolysis reactions. The rates of the two reactions were about equal. No photodehydrogenation reactions were reported. Some of the degradates were characterized by GC−MS. Standards were synthesized to help identify some of the photoproducts reported. The photomechanisms involving radical cation intermediates were discussed.

Our hydrochlorothiazide (**1**) photolysis results differ significantly from those of previously reported studies.[5,6] The most significant difference is that Tamat and Moore[5] report hydrolyzed **1** (compound **7**, Scheme 1) as a major photolysis product. Ulvi and Tammilehto[6] reported detecting hydrolysis product **7** in small amounts. Compound **7** was not detected in any of our hydrochlorothiazide photolysis studies.

Differences in the photolysis sources may explain this apparent discrepancy. Tamat and Moore[5] and Ulvi and Tammilehto[6] utilized medium- and high-pressure mercury vapor photolysis sources, respectively. In our hydrochlorothiazide photolysis studies, a fluorescent UV-A photolysis source was employed. The fluorescent source intensity wavelength distribution differs remarkably from that of mercury vapor sources.[8,10]

Specifically, the UV-A radiation emitted by a medium-pressure mercury vapor source is dominated by radiation at 313 and 365 nm.[10] Although a high-pressure mercury vapor source emits continuous radiation throughout the UV-A spectral region, Ulvi and Tammilehto[6] using filters only utilized radiation near 313 nm. In contrast, a fluorescent UV-A source emits a broad continuous radiation band centered at about 350 nm with less than 1% of the radiation at or below 315 nm.[8] Thus, it is possible that photohydrolysis of hydrochlorothiazide (**1**) to compound **7** is produced predominately by 313 nm radiation from the mercury vapor photolysis sources. This high-energy radiation is only a minor component of the radiation from a fluorescent UV-A photolysis source.

Our studies indicate a hydrochlorothiazide photodehydrogenation process, yielding small amounts of compounds **1A**, **2A**, and **3A** (Scheme 1). Tamat and Moore[5] did not observe this photolysis process. It is likely that small amounts of these compounds were simply not detected by Tamat and Moore's[5] isocratic HPLC method monitored at 254 nm. Our HPLC monitoring system required gradient elution and small particle (5 μm) C-18 stationary phase conditions to resolve the dehydrogenation products from the corresponding saturated species (Scheme 1, compounds **1**−**3**). Further, in our studies the HPLC monitoring wavelength of 270 nm, rather than 254 nm used by Tamat and Moore, offers greater sensitivity for detecting photodehydrogenation products (see Figure 4, UV spectra of compounds **3** and **3A**).

The unique photodegradation product distributions for **1** and **1A** we have reported can be attributed to the UV-A lamps employed and the method of product detection and identification. The photolysates generated in these experiments were analyzed directly by HPLC−MS and HPLC−UV with no pretreatment or derivatization. The sensitivity observed was sufficient to identify and quantify decomposition products at

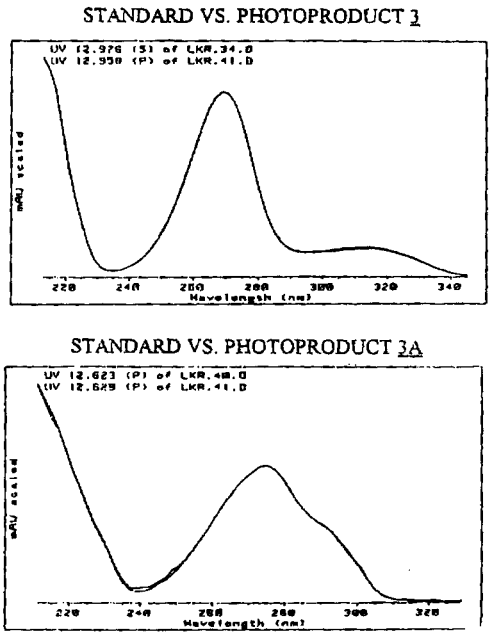


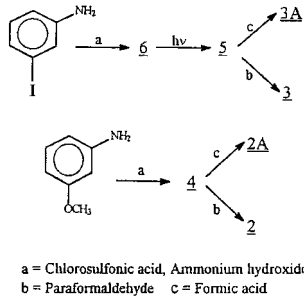
Figure 4—Representative UV spectral match of standards and photoproducts.

very low concentration, for example **4** and **5** in Figure 1. The (M − H) molecular ion suggested the structure of the photodegradant. If the tentatively identified structure was logical from the standpoint of known photochemical processes, a standard was synthesized and shown to be identical to the corresponding photodegradant by HPLC coinjection experiments and by HPLC−MS and HPLC−UV. By use of a diode array detector, the UV spectrum of the standard was superimposed on the UV spectrum of the corresponding photoproduct to confirm its identity (Figure 4). The structure of the standard was further defined by high-resolution FAB-MS and by ^{1}H NMR.

Previous investigators have expressed difficulty in synthesizing standard **3**.[5] We attempted unsuccessfully to make standard **3** by the synthetic route previously reported.[5] Eventually we solved the problem by following our own route (Scheme 2). The key step in our synthesis of derivative **3** was the preparative photolysis (photodehalogenation) of the iodo intermediate **6** to give standard **5**.

Conclusions

The primary photochemical process in the photolysis of thiazides **1** and **1A** employing a UV-A lamp defined by ICH is photodehalogenation. Irradiation of **1** also produces significant amounts of photodehydrogenation products and thermal hydrolysis products. The results of our photolysis of hydrochlorothiazide (**1**) differ from those reported by Tamat



Scheme 2—Synthesis of thiazide derivatives.

and Moore[5] in that we did not find hydrolysis product **7** after diligent effort by several methods. In addition they[5] did not find the dehydrogenation products which we identified. The results of our photolysis of chlorothiazide (**1A**) differ from those reported by Ulvi and Tammilehto[6] in that we identified photodehalogenation products. The differences in product distribution are attributed mainly to differences in the emissions of the lamps employed.

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