Synthesis of *N*-(3,5-Dichlorophenyl)-2-hydroxysuccinimide-*O*-sulfate: A Potential Metabolite of the Nephrotoxicant *N*-(3,5-Dichlorophenyl)succinimide

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The sulfate conjugate **2** of *N*-(3,5-dichlorophenyl)-2-hydroxysuccinimide, a potential metabolite of the nephrotoxicant *N*-(3,5-dichlorophenyl)succinimide, is prepared from the 2-hydroxysuccinimide (**1**) by the reaction with chlorosulfonic acid in chloroform and ether mixture at -78°.

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Succinimide (1*H*-pyrrolidine-2,5-dione) derivatives are widely used as pharmaceutical, industrial and agricultural agents. One succinimide derivative, *N*-(3,5-dichlorophenyl)succinimide (NDPS), was developed in Japan as an agricultural fungicide during the early 1970's [1]. However, NDPS has not been widely used in agriculture because of potential health hazards associated with NDPS exposure. The major toxicity induced by NDPS is nephrotoxicity [2,3], but the chemical species responsible for ultimately inducing renal damage is still unclear.

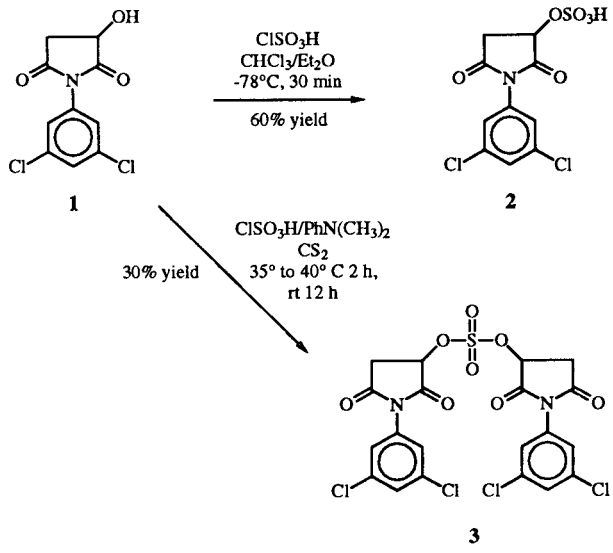
Previous work in our laboratory demonstrated that one or more oxidative metabolite of NDPS was responsible for inducing the nephrotoxic response [4,5]. Two metabolites of NDPS, *N*-(3,5-dichlorophenyl)-2-hydroxysuccinimide (**1**) and *N*-(3,5-dichlorophenyl)-2-hydroxysuccinamic acid (NDHSA) are more potent as nephrotoxicants than NDPS *in vivo* [5]. However, neither **1** nor NDHSA are directly toxic to renal cortical slices, isolated renal tubules or isolated renal mitochondria [5,6]. One explanation for these observations is that the ultimate nephrotoxicant species is derived from further biotransformation of **1** or NDHSA.

In our studies to elucidate the ultimate nephrotoxic chemical species following NDPS exposure, we became interested in the sulfate conjugate of **1**, a potential metabolite of NDPS, as the possible penultimate nephrotoxicant species. The sulfate conjugate [*N*-(3,5-dichlorophenyl)-2-hydroxysuccinimide-*O*-sulfate, **2**] would be expected to accumulate in the kidney *via* the sulfate transporters in the proximal tubular cells (targets of NDPS-induced nephrotoxicity). In kidney cells, **2** could eliminate the sulfate group to liberate *N*-(3,5-dichlorophenyl)maleimide, a potent *in vitro* nephrotoxicant species [6], or react directly with tissue nucleophiles *via* a S_N2 mechanism with loss of a sulfate anion [7] or *via* acylation reactions to induce toxicity. In general, the chemical synthesis of a sulfate conjugate, an important xenobiotic conjugate in living systems, is necessary in order to prove metabolite structure and physicochemical characteristics and to elucidate its biological activity.

Although the synthesis of phenolic sulfate conjugates is relatively straightforward, alkyl sulfate conjugate synthesis can be more difficult to achieve due to the lower reactivity of alcohols *versus* many phenols. The preparation of alkyl sulfates from alcohols is generally performed by the reaction with sulfur trioxide and its derivatives [8]. In general, sulfation of secondary alcohols requires more rigorous reaction conditions than primary alcohols because of slower reaction rates. However, sulfates derived from aliphatic secondary alcohols have been prepared using sulfur trioxide complexes [9], chlorosulfonic acid [8,10] or sulfamic acid-pyridine [11]. Attempted preparation of **2** from **1** under various conditions using trimethylamine-sulfur trioxide complex [9], sulfamic acid with pyridine [11], sulfuric acid and dicyclohexylcarbodiimide [12], or chlorosulfonic acid at 0° to room temperature [8] was unsuccessful. From these reactions, only the starting material **1** and/or unidentified decomposition products were observed. The reaction of **1** with *N,N*-dimethylaniline-sulfur trioxide complex, generated from chlorosulfonic acid and *N,N*-dimethylaniline in carbon disulfide *in situ* [13], resulted in the previously undescribed dimer **3**, which was identified by spectral and elemental analyses.

The sulfation of **1** with chlorosulfonic acid at 0° gave the sulfate conjugate **2**, however the reaction was not complete and the isolation of pure **2** was unsuccessful. In order to obtain **2** as a morpholine salt, the reaction of **1** with chlorosulfonic acid and morpholine at 0° was attempted and a white solid obtained, which proved to be a mixture of **2** as its morpholine salt and morpholine hydrochloride. However, efforts to purify the morpholine salt of **2** were also unsuccessful.

Although numerous standard procedures for preparing alkyl sulfates had failed to yield **2**, **2** was finally prepared in a stable sodium salt form. Because of the extreme readiness of **2** to hydrolyze to the starting compound **1** and/or other degradation products, the reaction of **1** with chlorosulfonic acid at a low temperature (-78°) in an



anhydrous chloroform/ether mixture was attempted. Mild neutralization conditions were also used to minimize hydrolysis of the sulfate group and the succinimide ring. Under these conditions, the product **2** was obtained as the sodium salt. The combination of very low temperature, anhydrous reaction conditions, and neutralization of the reaction mixture to *p*H 7-8 under cold conditions were all key to the successful preparation of the novel conjugate **2**. These results suggest that the synthesis of alkyl sulfate conjugates of nephrotoxicant succinimides and possibly other related heterocyclic compounds require tightly controlled conditions to achieve preparation and isolation of the conjugate.

EXPERIMENTAL

Melting points were determined with a Mel-Temp apparatus and are uncorrected. The ir spectra were obtained using a Perkin-Elmer model 297 spectrophotometer. The ^{1}H-nmr spectra were recorded with a Varian XL-200 spectrometer; the chemical shifts are expressed in δ values downfield from tetramethylsilane as an internal standard. Elemental analyses were performed by Atlantic Microlab, Inc., Georgia, USA.

N-(3,5-Dichlorophenyl)-2-hydroxysuccinimide (**1**) [mp 146-147° (lit [14] 144-145°)] was prepared from 5-carboxymethyl-2-trichloromethyl-4-oxo-1,3-dioxolane and 3,5-dichloroaniline according to the literature [14]. 5-Carboxymethyl-2-trichloromethyl-4-oxo-1,3-dioxolane [mp 176-178° (lit [15] 175°)] was obtained from chloral hydrate and D,L-malic acid.

N-(3,5-Dichlorophenyl)-2-hydroxysuccinimide-*O*-sulfate (**2**).

A solution of 1.04 g (4 mmoles) of **1** in 60 ml of dry chloroform and 40 ml of anhydrous ether was cooled in a dry ice-acetone bath, 0.53 ml (8 mmoles) of chlorosulfonic acid was added dropwise *via* a syringe with stirring under anhydrous conditions, and the mixture was then purged with nitrogen gas to remove the dissolved hydrogen chloride gas. After stirring the mixture for 30 minutes at -78°, 5% sodium bicarbonate solution was added to the mixture to adjust the *p*H to 7-8. The reaction vessel was then removed from the dry ice-acetone bath, placed in an ice bath, and the precipitated white solid filtered immediately. The solid was washed with 50 ml of cold chloroform, and then 100 ml of ice-cold water, and dried. The white solid was resuspended in ice-cold water and washed with ice-cold water to give 0.95 g (60%) of the sodium salt of **2** as the dihydrate, mp 163-172° dec; tlc [silica gel 60 F_{254}, chloroform:ethyl acetate:methanol (4:3:3)], 1 spot, R_f = 0.49; ir (potassium bromide): 3490 (OH), 3080 (aromatic CH), 1720 (C=O), 1580, 1450, 1240 (SO_2), 1050 and 1010 (S-O-C), 780, 730, 670 (aromatic CH) cm^{-1}; ^{1}H-nmr (deuteriodimethyl sulfoxide): δ 3.01 (m, 2H, C_3-H), 3.33 (s, 4H, H-O-H), 5.09 (dd, 1H, C_2-H, J = 5.3 Hz), 7.40 (d, 2H, 2,6-aromatic H, J = 1.8 Hz), 7.71 (t, 1H, 4-aromatic H, J = 1.8 Hz).

Anal. Calcd. for $C_{10}H_6Cl_2NO_6SNa\cdot 2H_2O$: C, 30.16; H, 2.54; Cl, 17.81; N, 3.52; S, 8.05; Na, 5.77. Found: C, 30.07; H, 2.39; Cl, 17.89; N, 3.45; S, 8.09; Na, 5.86.

Bis-[*N*-(3,5-dichlorophenyl)-2-hydroxysuccinimide]-*O*-sulfate (**3**).

To a well-stirred solution of 0.94 ml (1 mmole) of *N,N*-dimethylaniline in 1 ml of carbon disulfide at -16 to -22° under a nitrogen atmosphere and anhydrous conditions, 0.2 ml (2.8 mmoles) of chlorosulfonic acid was added *via* a syringe. After the addition, the mixture was warmed to 35-40° and 0.52 g (1.92 mmoles) of **1** was added in portions over 20 minutes. The mixture was stirred for an additional 2 hours at 35-40° and for 12 hours at room temperature. The mixture was cooled to 0° and poured rapidly into 5 ml of a cold 4 *M* solution of potassium hydroxide with stirring. The precipitate was filtered immediately, washed twice with 10 ml of cold absolute ethanol and dried *in vacuo*. The product **3** was obtained as a white powder in 30% yield (0.33 g), mp >300°; ir (nujol): 3490, 3080 (aromatic CH), 1695 (C=O), 1575, 1405, 1190 (SO_2), cm^{-1}; ^{1}H-nmr (deuteriodimethyl sulfoxide): δ 2.82 (dd, 2H, C_3-H, J = 5.4 Hz), 3.32 (dd, 2H, C_3-H, J = 8.8 Hz), 4.43 (d, 2H C_2-H, J = 7.0 Hz), 7.52 (s, 4H, 2,6-aromatic H), 7.64 (s, 2H, 4-aromatic H).

Anal. Calcd. for $C_{20}H_{12}Cl_4N_2O_8S$: C, 41.26; H, 2.08; Cl, 24.36; N, 4.81; S, 5.51. Found: C, 41.40; H, 1.85; Cl, 24.89; N, 4.77; S, 5.88.

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