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THE SYNTHESIS OF o-AMINOPHENYL SULFATE METABOLITES OF THE ONCOLYTIC SULFONYLUREAS

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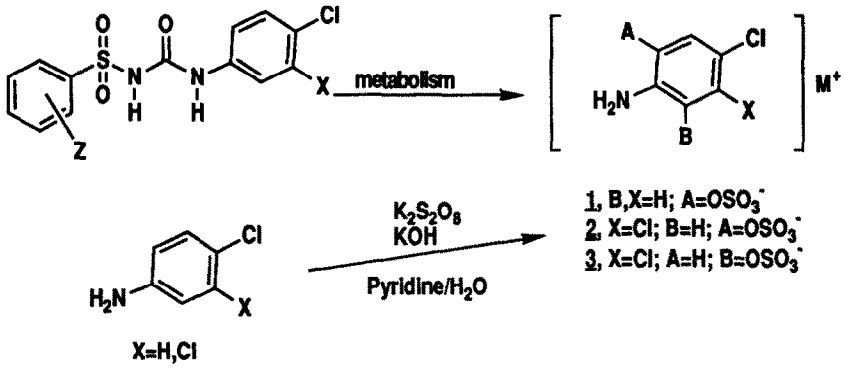
Abstract. Application of the Boyland-Sims oxidation to 4-chloro- and 3,4-dichloroaniline is reported.

Recent reports from these laboratories have detailed the discovery and synthetic development of the oncolytic diarylsulfonylureas, culminating in the clinical trials of N-(5-indanesulfonyl)-N'-(4-chlorophenyl)urea, sulofenur.[1] Sulofenur has been reported to produce the o-aminophenyl sulfate of p-chloroaniline 1 as a urinary metabolite in man, monkey, rat and mouse.[2] In a mouse model, urinary levels of 1 have been viewed as evidence for the intermediacy of p-chloroaniline formation and have been linearly correlated with methemoglobinemia, one of the dose-limiting toxicities of sulofenur.[3] Synthetic 1 was required for verification of its structure assignment and for the measurement of 1 in urine by HPLC analysis. In addition, continued exploration of the SAR and metabolism of related sulfonylureas, containing the component 3,4-dichloroaniline, required the synthesis of the respective o-aminophenyl sulfates 2,3.



Our efforts focused on the reported alkaline persulfate oxidation of anilines[4] rather than the sulfation of aminophenols because of the availability of the requisite anilines and the expected ease of separation of the o-aminophenyl sulfate products from the aniline reactants.

In practice, treatment of p-chloroaniline with a molar equivalent of potassium persulfate in 2N KOH/pyridine solution provided 1 as the potassium salt, which crystallized from ethanol in a straightforward fashion to analytical purity. It is of interest to note that direct sulfation of 4-chloro-2-hydroxyaniline with trimethylamine-SO_3 complex also produced 1 in useful yields.[5]

The nonsymmetrical 3,4-dichloroaniline produced a mixture of the potassium salts of 2 and 3 which were separated by preparative reverse phase HPLC. In an effort to simplify the isolation of 2 and 3, the persulfate oxidation was run using THF as a cosolvent in place of pyridine. Suprisingly, none of the desired sulfates 2 and 3 were observed in this crude reaction extract by reverse phase HPLC.

This report details the one-step synthesis of o-aminophenyl sulfates from anilines in a useful, although unoptimised yield. Clearly, the advantage of this methodology lies in its application to those anilines whose corresponding o-aminophenols are not readily available, and to those situations, such as that described above, where the two isomeric o-aminophenyl sulfates are required for metabolite structure verification.

Experimental.
NMR spectra were acquired on a GE QE-300 spectrometer at 300 MHz (proton) and 75 MHz (carbon). Coupling constants are reported in Hz. Analytical reverse phase HPLC was performed on a Waters 600E system with a Radial-Pak™ 8NVC18 (8mmX100mm, 4μm) with C18 precolumn, employing a gradient solvent program utilizing 25% acetonitrile/0.025 M pH 7.0 $NaPO_4$ buffer at 1.5 mL/min flow rate for 5 min followed by linear change to 60% acetonitrile over 5 min.Preparative reverse phase HPLC was performed on 3 tandem Nova-Pak® HR-C18 (40x100 mm, 6μm, 60 Å) cartridges using water as the mobile phase. All UV detection was at 254 nm.

The persulfate oxidation of 3,4-dichloroaniline.
A mixture of 3,4-dichloroaniline (16.2 g, 100 mmol), pyridine (100 mL) and 2N KOH solution (100 mL) was treated dropwise, over 1 h, with an aqueous solution of $K_2S_2O_8$ (27.0 g, 100 mmol, in approximately 700 mL of water). After stirring 15 h at room temperature, the reaction solution was decanted from some insoluble tar and evaporated to a solid under vacuum (45°C). This solid was triturated with Et_2O (5x200 mL). The remaining solid was triturated with MeOH (5x75 mL) and the combined triturant evaporated to yield 7.0 g of brown solid that consisted mainly of 2,3 by reverse phase HPLC analysis (1:1). This was slurried with ~50 mL of methanol and the undissolved solid collected, dried (2.3 g), and separated by reverse phase HPLC (H_2O) to provide 384 mg of 2 and 541 mg of 3 ;

2: mp 221°C; ^{1}H NMR (d_6-DMSO) δ 5.09 (s, 2H, NH_2), 6.82 (s, 1H, ArH), 7.19 (s, 1H, ArH); ^{13}C NMR (d_6-DMSO) δ 115.1, 115.7, 122.9, 125.8, 139.1, 141.6; IR(KBr) 3439, 3357, 1628, 1488, 1284, 1225 and 1048 cm^{-1}; UV(H_2O) $\lambda_{max}(\varepsilon)$ 208(33719), 242(9233), 299(2617); FAB(-)MS 256, 258, 260 (M-K$^+$); Anal. Calcd for $C_6H_4Cl_2K_1N_1O_4S_1$: C,H,N,S;

3: mp 213-215°C; ^{1}H NMR (d_6-DMSO) δ 5.43 (s, 2H, NH_2), 6.59 (d, 1H, J=8.8, ArH), 6.99 (d, 1H, J=8.8, ArH); ^{13}C NMR (d_6-DMSO) δ 114.4, 117.4, 125.5, 127.0, 137.3, 143.2; IR(KBr) 3428, 3349, 1621. 1588, 1471, 1254 and 1059 cm^{-1}; UV(H_2O) $\lambda_{max}(\varepsilon)$ 207.2(29814), 242.4(8005), 298(1922); FAB(-)MS 256, 258, 260 (M-K$^+$); Anal. Calcd for $C_6H_4Cl_2K_1N_1O_4S_1$:C,H,N,S.

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5. In this experiment, 4-chloro-2-hydroxyaniline was heated (50°C) with an excess of trimethylamine-SO_3 complex in 4 equiv of 2N KOH solution and gave, after recrystallization, 24% of 1.