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Biological Sulfate Mono-Esters Can Be Mimicked By Synthesizing Useful Models

* **Mohamed Suliman Sasi**

Abstract: The research project aim is to make efficient models of arylsulfate monoesters to study the corresponding enzymatic catalysis of biological sulfate monoesters. However, there are some conditions should be considered in order to design a suitable active site model to monitor the progress of the reaction, such as the pK_a of leaving group. An easy approach to the synthesis of arylsulfate monoesters was developed. The wanted phenolic leaving group and chlorosulfuric acid were used in the synthesis of aryl sulfate monoesters. The direct and high-yielding synthesis of arylsulfate monoesters can be obtained from this procedure. Designed models of sulfate monoesters were successfully synthesized to quantify the effects of pK_a of leaving group during sulfuryl transfer catalysis.

***Keywords*:** Biological sulfate monoesters, Sulfuryl Transfer catalysis.

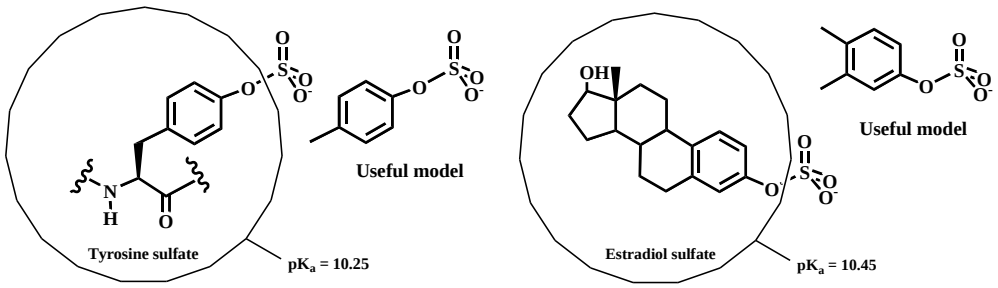

Fig. 1: Some of sulfate monoesters in biological systems. [1-3]

* Mohamed S. Sasi is with the department of Chemistry, Education faculty, Misurata University, Misurata, Libya. (fax: +218512632517; e-mail: msasi40@ yahoo.com).

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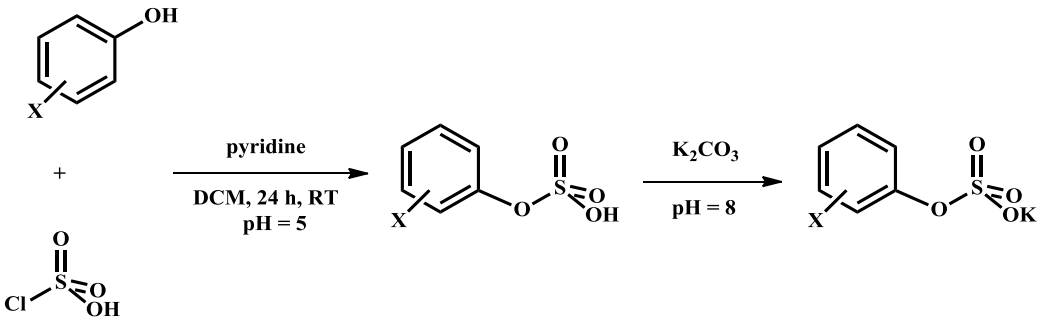
Introduction

Sulfate monoester mono-anions are important in a number of biological functions, controlling the activity level of enzymes and hormones, molecular recognition and cell signaling. For example, sulfating of protein tyrosine residues can function as a modulator of protein-protein interactions. [4,5] Fig.1 shows some of the common sulfate monoesters in biological processes. Most of the enzymes usually react in a specific range of pH and temperature. In fact, prepared models are usually more flexible to study and more appropriate to observe the adaptations and modifications on the substrate during the reactions. [6- 8] Different properties and conditions can be added or changed on models in a stepwise fashion to facilitate quantifying and improving the contribution of each function much easier than in real enzymes. Suitable models of enzymes can give us a good approximation of the enzymatic behaviour in the active site (where the function group actually reacts with the nucleophiles) in a number of biological functions. [9-11]

MATERIALS AND METHODS

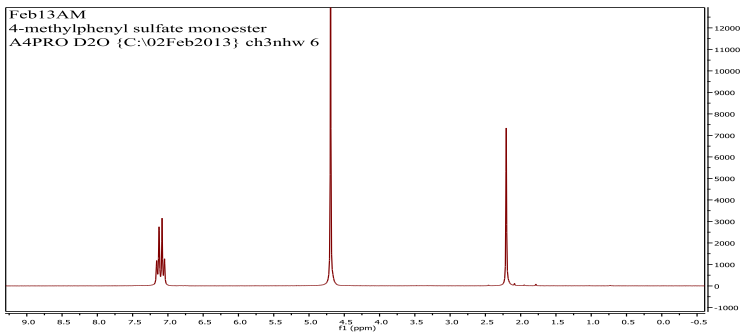


Scheme 1: General procedure for preparing a range of sulfate monoesters. [12]

Useful models for sulfate monoesters have been successfully prepared by using a typical procedure as shown in scheme 1 to yield sulfate monoester potassium salts. Selected phenol or alcohol (15 mmol) was dissolved in pyridine (20 ml) leave them stirring on ice at 0 °C. Chlorosulfonic acid (1ml, 15mmol) was dissolved in dichloromethane (10ml), and then the mixture was added dropwise into phenol solution under argon with stirring. The reaction was stirred several hours at 0°C, then stirring overnight at room temperature. The reaction was quenched by adding potassium carbonate solution (0.04 M in H_2O) the pH adjust to 8. The obtained white solid was extracted and washed by diethyl ether then dissolved in water and adjust the pH to 5 by acetic acid. Diethyl ether was added to remove the remaining phenol. The aqueous layer was collected and removing the solvent to obtain the substrate. The pure sulfate salt was obtained by recrystallizing from ethanol. [13,14]

NMR and Mass Spectra for Prepared Compounds:

Fig. 2 shows 1H NMR for 4-methyl phenyl sulfate potassium salt: the pKa of this leaving group is 10.25. 1H NMR (250 MHz, D2O) δ/ppm 2.35 (3 H, s, CH_3), 7.15 - 7.33 (4 H, m, Ar - H). Mass Spectra (ES^-) m/z = 187 $[M\text{-}K]^-$. HRMS required for $C_7 H_7 O_4 S$: 187.9519; found 187.9512.



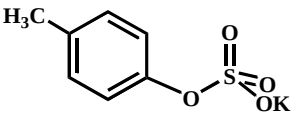


Fig. 2: 1H NMR Spectrum for 4-Methyl phenyl sulfate potassium salt.

Fig. 3 shows mass spectra analysis for 4-methyl phenyl sulfate potassium salt (ES^-) m/z = 263 $[M-K]^-$. HRMS required for $C_7H_7O_4S$: 187.0061; found 187.065.

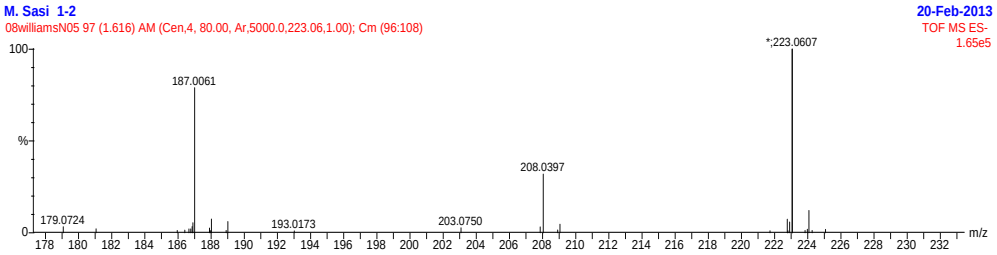


Fig. 3: Mass Spectrum for 4-Methyl phenyl sulfate potassium salt.

Fig. 4, 5 show 1H NMR and F19 spectra for trifluoroethyl sulfate potassium salt: the pKa of this leaving group is 12.4. ^1H NMR δ_H (250 MHz, D_2O) 4.45 - 4.25 (2 H, m, CH_2 - O), δ_F (250 MHz, D_2O) -75.43 (3 F, t, $^3J_{FF}$ 15 C-F_3). Mass Spectra (ES^-) m/z = 180 ($[M-K]^-$). HRMS (ES^-): 180.0548, $C_2H_2O_4F_3S$ requires 180.0541.

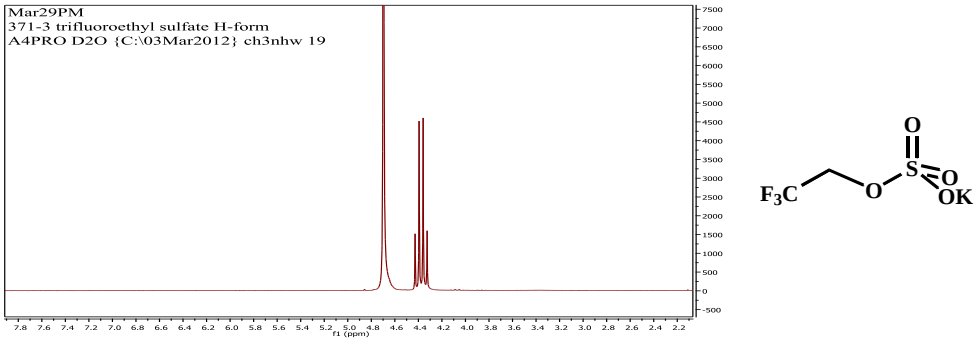


Fig. 4: 1H NMR Spectrum for trifluoroethyl sulfate potassium salt.

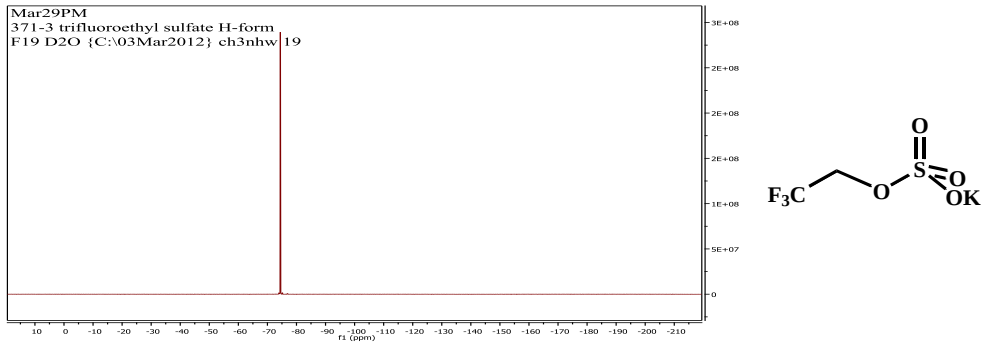


Fig. 5: F19 Spectrum for trifluoroethyl sulfate potassium salt.

RESULTS AND DISCUSSION

Our interest in the synthesis of aryl sulfates stemmed from our desire to prepare useful models to mimic the hydrolysis of biological sulfate monoesters as shown in Fig. 1. These compounds are designed to act as the behavior of real biological leaving groups in biological substrates by replacing the real one by phenolic group with similar acidity of pKa. [15,16] Wanted aryl sulfate monoesters were synthesized from the reaction of substituted phenols with chlorosulfonic acid in the presence of pyridine under argon gas with stirring. The white solid was extracted with diethyl ether then dissolved in water. The target aryl sulfate was obtained by removing the solvent, recrystallized from ethanol and dried under vacuum to give pure aryl sulfate monoesters.

By preparing well designed models of biological sulfate monoesters, we can obtain a better estimate for the stability of biological sulfate monoester monoanions towards hydrolytic S–O bond cleavage; tyrosine sulfate and estradiol sulfate. The kinetic parameters of *arylsulfatase* (PAS) enzyme catalyzed hydrolysis of 3,4-Dimethylphenyl sulfate (pK_a 10.34) could be used

to estimate the catalytic proficiency of *arylsulfatase* enzyme to catalyze the hydrolysis of estradiol sulfate (pK_a 10.45). Also, 4-methylphenyl sulfate (pK_a 10.25) seems to be very close pK_a to the tyrosine as a leaving group with a pK_a 10.3. From these models can be estimated the high proficiency of *pseudomonas aeruginosa arylsulfatase* to accelerate S-O bond cleavage in the hydrolysis of biological sulfate monoester mono-anions. [17,18]

CONCLUSION

In this work, an easy approach to synthesize arylsulfate monoesters was developed by using chlorosulfuric acid. Wanted phenolic leaving group and chlorosulfuric acid were successfully used in the synthesis, to yield designed arylsulfate monoesters. The direct and high-yielding synthesis of substituted phenyl sulfate esters can be obtained from this approach. Series of sulfate esters can be also synthesized to quantify the effects of pK_a of leaving group during sulfuryl transfer reactions. Efficient models of arylsulfate monoesters could provide a better estimate for the real enzymatic catalysis of biological sulfate monoesters.

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