AAPS PharmSciTech **,** 2000; 1 (3) article TN1
(http://www.pharmscitech.com/)

Competitive Inhibitory Effect of External Nucleophile Concentration on Intramolecular O- to N-Acylation in O-Acetylsalicylamide

Submitted: May 31, 2000; Accepted: August 16, 200; Published: August 25, 2000.

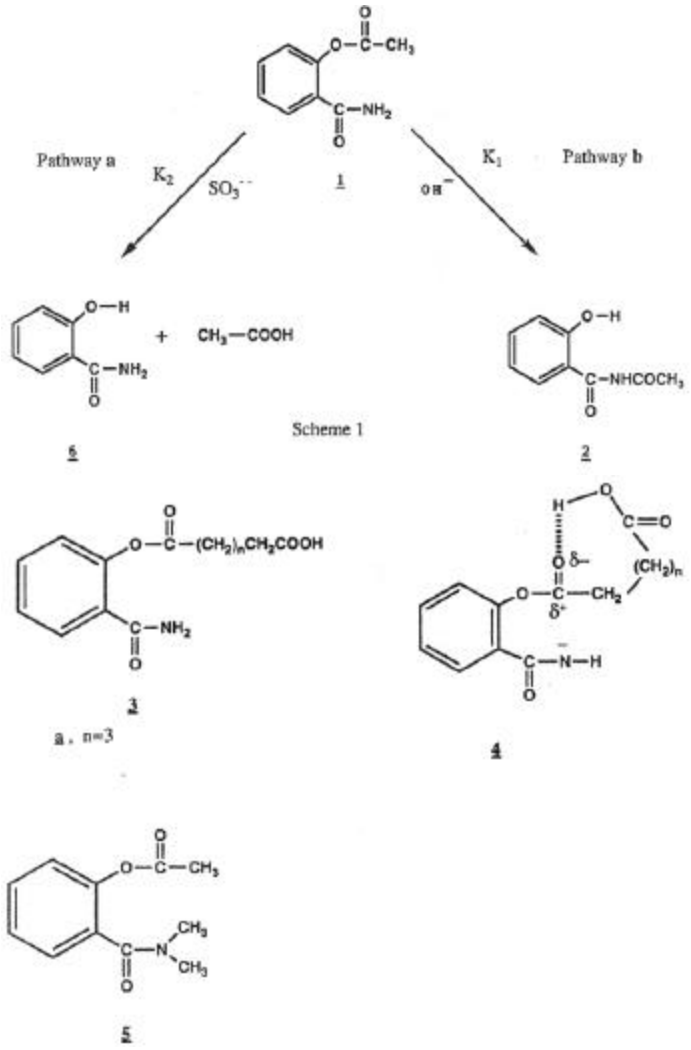
Anwar A. Hussain,* Janan Jona, and Peter A. Crooks

University of Kentucky College of Pharmacy, Lexington, KY, 40536-0082

INTRODUCTION A model esterolytic mechanism involving the cooperative effect of an amide functionality and the cleavage of a neighboring acetyloxy group was previously reported [1,2]. The O- to N- transacylation reaction of O-acetylsalicylamide (compound 1) to give compound 2 is rapid, pH-dependent, and independent of buffer concentration (see Scheme 1). In addition, the rate of such reactions can be greatly enhanced through an intramolecular general acid catalysis mechanism. For example, in compounds containing a terminal carboxylic acid functionality as part of the O-acyl group (i.e., compounds of general structure 3), the involvement of the free carboxylate group in the rate enhancement of O- to N-acylation was postulated to proceed via the transition state 4 [3]. Thus, the O-glutaryl hemiester derivative 3a was found to exhibit a transacylation rate that was 400 times faster than that observed for compound 1 (3a, K_{OH^-} = 440.2 x $10^4 mol^{-1}s^{-1}$; 1, K_{OH^-} = 1.4 x $10^4 mole^{-1}s^{-1}$). Comparison of the reactivity of the hydroxide ion toward the ester functionality with that of the ionized amide function in the intramolecular catalysis was carried out by utilizing the model compound N,N-dimethyl-O-acetyl-salicylamide (5). The half-life for hydrolysis of the ester group of compound 5 was found to be 8 hours, whereas the half-life of compound 1 at the same pH was 13 seconds.

KEYWORDS: O-Acetylsalicylamide, Hydrolysis, Esterolysis, Intramolecular Acylation, Cooperative Effects.

***Corresponding Author:** Anwar A. Hussain, PhD, University of Kentucky College of Pharmacy, Lexington, KY, 40536-0082 . telephone: 859-257-5939; facsimile: 859-257-1954; e-mail: aahuss1@pop.uky.edu



The purpose of this technical note is to report on results from studies that further compare the relative contributions of the intramolecular esterolysis in the presence of external nucleophiles such as sodium bisulfite [1] or acetamide, utilizing quantitative product analysis. The former compound represents a strong

nucleophile, which is known to catalyze ester hydrolysis [4], whereas acetamide was utilized to mimic the *ortho* amido functionality in compound **1**. The results of this study afford important data on the magnitude of intramolecular esterolytic processes involving cooperativity of adjacent functional groups within the same molecule. These data provide detailed information on the stability and mechanism of degradation of O-acyl derivatives of salicylamide in solution. Such information is of value in the design of prodrugs and structural analogs of salicylic acid and salicylamide, and other related drug molecules.

MATERIALS AND METHODS

Salicylamide and acetamide were obtained from Sigma-Aldrich (Milwaukee, WI). O-acetylsalicylamide and N-acetylsalicylamide were prepared as previously described [1]. Sodium hydroxide, sodium bisulfite, spectral grade methanol, spectral grade dioxane, citric acid, potassium dihydrogen phosphate, and phosphoric acid were obtained from Fisher Scientific (Pittsburgh, PA).

Degradation kinetics were carried out at room temperature in 0.2 mol/L phosphate buffer at pH 6.5 in the presence and absence of various concentrations of sodium bisulfite or acetamide. O-acetylsalicylamide concentrations ranged from 0.1 mol/L to 0.5 mol/L. The dependency of the reaction between bisulfite ion and O-acetylsalicylamide on the concentration of bisulfite ion, and the quantitative formation and identification of the reaction products, was determined by adding a solution of O-acetylsalicylamide in spectral grade dioxane to solutions containing sodium bisulfite (0.01 mol/L – 0.50 mol/L) and 0.2 mol/L phosphate buffer at pH 6.5. Similar experiments were carried out to determine the effect of varying concentrations of acetamide on the O- to N- transacylation reaction. At appropriate time points, samples were quenched by adding solutions of 0.2 mol/L citric acid, pH 2.5. The resulting solutions were analyzed by high-performance liquid chromatography (HPLC).

HPLC analyses were carried out using a model 400 Applied Biosystems pump (Ramsey, NJ), a Spectraflow 773 UV detector (Rainin Instruments, Emeryville, CA) operating at 240 nm, an Applied Biosystems integrator (Rainin Instruments, Emeryville, CA), and a Rheodyne 7125 injector (Rheodyne, Cotati, CA). The column packing used for separations was an Adsorbosphere C_8 with 5 µ particle size (Rainin Instruments, Emeryville, CA). The mobile phase consisted of methanol:0.01mol/L KH_2PO_4, pH adjusted to 2.3 with 85% H_3PO_4, 20:80, at a flow rate of 1.0 mL per minute. Retention times for solutes were the following: O-acetylsalicylamide, 4.5 minutes; salicylamide, 7.6 minutes; N-acetylsalicylamide, 19.0 minutes.

RESULTS AND DISCUSSION

The rate of reaction, the pH-dependency, and the effect of concentration of bisulfite ion and acetamide on the reaction pathways illustrated in **Scheme 1** were determined by following the rate of formation of the N-acetylated product **2** and the rate of formation of salicylamide (**6**).

A specific analytical HPLC methodology was utilized, which was capable of separating and quantitating compounds **1**, **2,** and **6** (see Scheme 1). At pH 6.5 and in the presence of 0.5 mol/L bisulfite ion, the predominant degradation route for compound **1** was via pathway **a** (**Figure 1**). The ratio of products **2** and **6** formed from compound **1** was dependent on the concentration of bisulfite ion and on the pH of the medium. In the presence of a much lower concentration of bisulfite ion (i.e., 0.05 mol/L) at pH 6.5, the two products, **2** and **6**, from reaction pathways **b** and **a**, respectively, could be detected (see **Figure 2**). The value of the second-order rate constants K_a and K_b for reactions **a** and **b** at pH 6.5 were $1.1 \times 10^4 mol^{-1}s^{1}$ and $2.3 \times 10^2 mol^{-1}s^{-1}$, respectively. These data would suggest that under the above conditions, to compete with an intramolecular process involving an amide functionality, in concentration terms, about 10^6-fold excess of bisulfite ion over hydroxide ion concentration is required for pathway **a** to effectively compete with pathway **b**. Thus, the predominance of the intramolecular transacylation mechanism involving

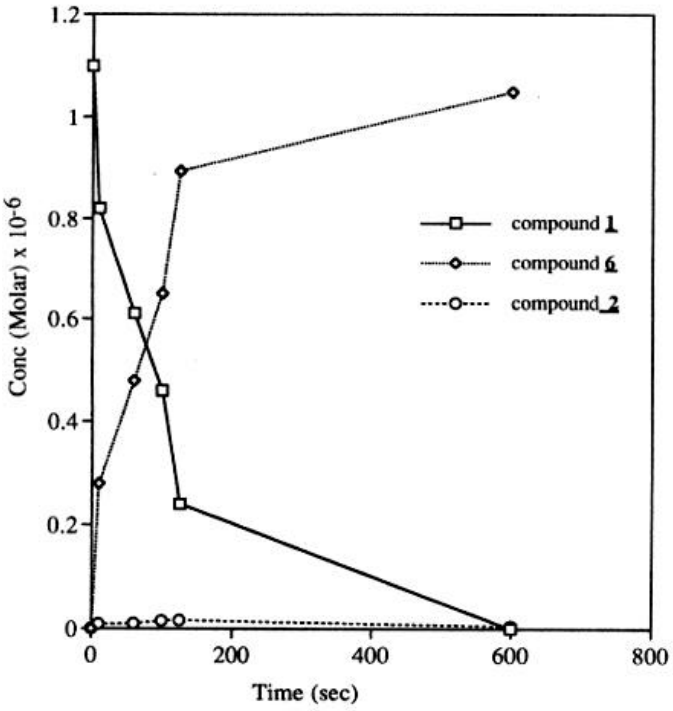


Figure 1: Hydrolysis of compound 1 in the presence of 0.50 mol/L sodium bisulfite at pH 6.5.

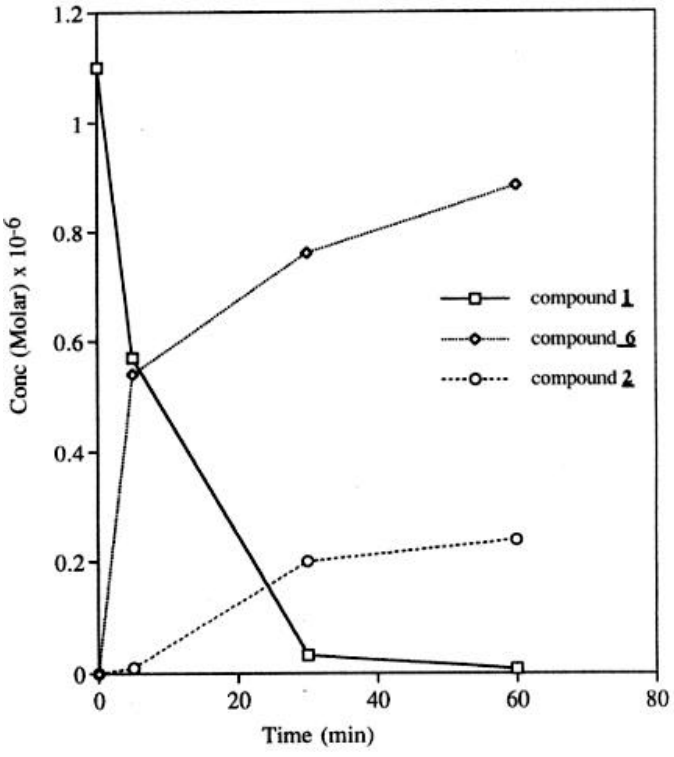


Figure 2: Hydrolysis of compound 1 in the presence of 0.05 mol/L sodium bisulfite at pH 6.5.

attack of the carboxamide anion on the adjacent ester carbonyl group, over normal esterolysis by bisulfite ion, reflects the relative basicity and nucleophilicity of the hydroxide ion compared to the bisulfite ion, and the relative abilities of these anions to generate an ionized carboxamide species.To further compare the reactivity of an amide functional group in an intramolecular process to that of a similar functionality in an intermolecular process, up to 1.0 mol/L of acetamide was used at a pH of 6.5 (pathway **c**). Under these conditions, only the transacylated product from pathway **b** was detected, suggesting that the amide functionality, as an external nucleophile, is completely ineffective in the esterolytic mechanism. These results indicate that ionization of the carboxamide group of O-salicylamide affords an internal nucleophile that participates in an extremely fast O- to N-transacylation reaction.

In biological systems it has become evident that the participation of more than one functional group is usually involved in the catalytic mechanisms associated with enzyme-substrate interactions [5]. Trifunctional amino acids that may be involved in such reactions are those containing amino, carboxylate, thiol, phenolic, hydroxyl, guanidino, or imidazole functionalities. The unique tertiary structure of biologically active proteins affords a molecular scaffolding that allows these functionalized amino acid residues to adopt the unique three-dimensional environment required for the specific catalytic mechanism at the active site of the enzyme. It is also possible that the peptide bond itself could participate in such a catalytic mechanism [6]. The data from this study, together with the transacylation studies on O-glutarylsalicylamide previously carried out in our laboratories, would suggest that the magnitude of the second-order reaction rate in this model system probably approaches that encountered in enzymatic reactions. As far as we are aware, it is one of the fastest esterolytic rates at pH 6.5 ever encountered in such a low molecular weight model system.

REFERENCES

1. Babhair SA, Hussain AA. O-Acetylsalicylamides as possible prodrugs for salicylamide. I. Kinetics and mechanisms of their degradation and reaction with enzymes and sodium bisulfite. *Int J Pharm.* 1983;13:273-286.

2. Tawfiq FA, Dittert LW, Hussain AA, Crooks PA. Kinetics of decomposition of O-acyl derivatives of salicylamide and related compounds. *Int J Pharm.* 1990; 64:43-55.

3. Tawfiq FA, Dittert LW, Hussain AA, Crooks PA. Rearrangement of O-acetylsalicylamides via O to N transacylation: rate enhancement by an adjacent carboxylic acid group. *J Pharm Sci.* 1990;79:651.

4. Munson JW, Hussain, AA, Bilous R. Precautionary note for use of bisulfite in pharmaceutical formulations. *J Pharm Sci.* 1977; 66:1775-1776.

5. Menger FM, Ladika M. Fast hydrolysis of an aliphatic amide at neutral pH and ambient temperature. A peptidase model. *J Amer Chem Soc*. 1988;110:6794-6796.

6. Bernhard SA, Berger A, Carter JH, Katchalski E, Sela M, Shalitin Y. Co-operative effects of functional groups in peptides. I. Aspartyl-serine derivatives. *J Amer Chem Soc*. 1962;84:2421-2434.