Improved Delivery through Biological Membranes IX: Kinetics and Mechanism of Hydrolysis of Methylsulfinylmethyl 2-Acetoxybenzoate and Related Aspirin Prodrugs

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Received August 25, 1980, from the **Department of Pharmaceutical Chemistry, School of Pharmacy, University of Kansas, Lawrence, KS 66045*, and the ‡*Department of Medicinal Chemistry, College of Pharmacy, J. Hillis Miller Health Center, University of Florida, Gainesville, FL 32610.* Accepted for publication November 24, 1980.

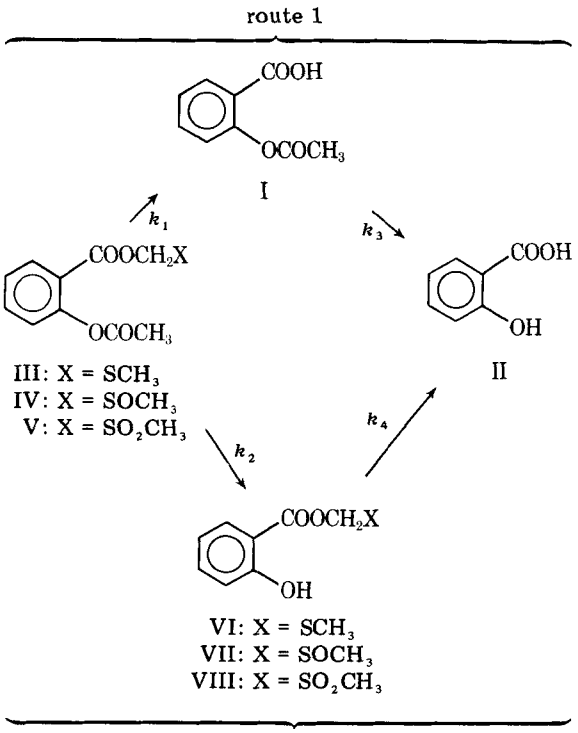
Abstract □ The complex kinetics and mechanism of the hydrolysis of the methylthiomethyl, methylsulfinylmethyl, and methylsulfonylmethyl 2-acetoxybenzoates, novel aspirin prodrugs, were studied. The pH profiles for the related salicylates and benzoates also were determined. Based on the activation parameters, isotope effects, and other data, it was established that the methylthiomethyl esters hydrolyze *via* a unimolecular alkyl–oxygen cleavage. The methylsulfinylmethyl and methylsulfonylmethyl 2-acetoxybenzoates undergo neutral hydrolysis of esters at pH > 4 to form aspirin, while water acts as a general base; but at lower pH, a different mechanism takes place and the *o*-acetyl group cleaves first, releasing the corresponding salicylates.

Keyphrases □ Methylsulfinylmethyl 2-acetoxybenzoate—kinetics and mechanism of hydrolysis □ Prodrugs, aspirin—methylsulfinylmethyl 2-acetoxybenzoate, kinetics and mechanism of hydrolysis □ Hydrolysis—kinetics and mechanism established for methylsulfinylmethyl 2-acetoxybenzoate and other related aspirin prodrugs

The results of the *in vitro* enzymic cleavage studies and the *in vivo* blood level data (1) indicate that the novel aspirin derivatives of the methylsulfinylmethyl and related ester type are promising, true aspirin prodrugs. These observations warranted a detailed study of the complex kinetics and mechanism of the chemical hydrolysis of these esters. Theoretically, the new prodrugs can hydrolyze in two steps *via* two routes (Scheme I).

Thus, the pH profiles for the hydrolysis of III–V and VI–VIII were determined and compared to that of aspirin and simple related benzoates [methylthiomethyl benzoate (IX), methylsulfinylmethyl benzoate (X), and methylsulfonylmethyl benzoate (XI)]. The various activated esters (methylthiomethyl, methylsulfinylmethyl, and methylsulfonylmethyl) and the *o*-phenolacetates may have different mechanisms of hydrolysis at the various pH values. The influence of ionic strength, dielectric constant, activation parameters, and solvent isotope effects also was studied to elucidate the mechanism of the hydrolysis and to explain the shapes of the pH profiles.

Comparison of the chemical and the enzymic hydrolysis revealed interesting differences.



Scheme I

EXPERIMENTAL

Instrumentation—The high-pressure liquid chromatographic (HPLC) method (1) was used for the determination of aspirin (I), salicylic acid (II), the aspirin derivatives (III–V), and the salicylates (VI–VIII), except at pH > 9.55. At these pH values, the hydrolytic rate constants were determined directly spectrophotometrically at 349 nm using a spectrophotometer[1] equipped with a thermostated cell compartment maintained at 50.8° by a circulating water bath.

The phenolic pKa values of VI–VIII were determined[1] spectrophotometrically at room temperature. Four ovens were used in the determination of the activation parameters. A research pH meter[2] was equipped with a combination glass electrode standardized at the temperature at which the kinetic data were obtained.

Materials—All buffer solutions for kinetic studies were prepared from deionized water that was boiled for 5 min to liberate the dissolved gases and cooled under nitrogen. Sufficient water was added to weighed amounts of various buffer salts to yield a solution of the desired molarity and ionic strength. The buffer solutions were thermostated at 50.8° where the pH and volumes were adjusted. All buffer solutions were stored under nitrogen in a refrigerator and used within 1 week. Where the buffer capacity was questionable, the pH was checked following each kinetic run. All chemicals used were analytical grade.

Determination of Hydrolytic Rate Constants—For the determination of the hydrolysis rate constants of aspirin (I), its derivatives (III–V), and related compounds (VI–XI), 50 μl of a concentrated acetonitrile solution of each ester was added separately to 10 ml of buffer, previously equilibrated to the desired temperature in a water bath, and mixed thoroughly to give an initial concentration of 5×10^{-4} mole/liter. All reactions were run under pseudo-first-order conditions. Aliquots (100 μl) were injected into the column at various intervals, and the pseudo-first-order rate constants were determined from the disappearance of the compound by linear regression of natural logarithm of the peak height *versus* time plots. The half-life, correlation coefficient, and standard error

[1] Cary 219.

[2] Orion model 701.

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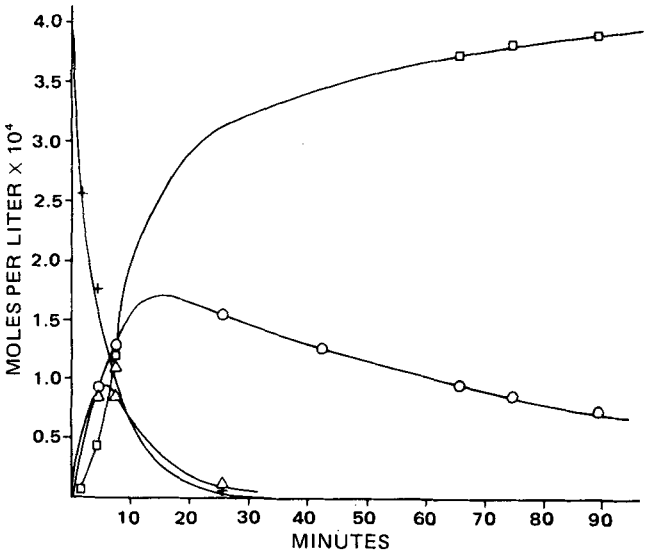


Figure 1—*Kinetics of hydrolysis of methylthiomethyl 2-acetoxybenzoate (III) (+) in 0.01* M *carbonate buffer (pH 9.55) at 50.8° and 0.1* M *ionic strength to form methylthiomethyl 2-hydroxybenzoate (VI) (△), aspirin (I) (○), and salicylic acid (II) (□).*

of the rate constant were calculated for each run (2).

At lower pH values, where hydrolysis was slow, the reaction mixture was introduced into ampuls of 1 or 2 ml, sealed under nitrogen, and immersed in a thermostated bath. Individual ampuls were withdrawn at various times, and the concentrations were determined using HPLC. At higher pH values, where the disappearance of absorbance at 349 nm was followed, 20 μl of a concentrated solution of the reactant in acetonitrile was added to 3 ml of buffer in a 1-cm quartz cell previously equilibrated to 50.8° in the cell compartment of the spectrophotometer. The cell was closed and shaken to ensure mixing. The pseudo-first-order rate constant was determined by linear regression of $\ln(A_t - A_\infty)$ *versus* time, where A_∞ is the absorbance at 349 nm after 10 half-lives and A_t is the absorbance at time t.

Determination of Phenolic pKa Values of VI–VIII—The pKa values for the 2-hydroxybenzoates (VI–VIII) were determined spectrophotometrically at room temperature (23°). Five tris(hydroxymethyl)-aminomethane and three carbonate buffers of ionic strength 0.1 *M* (using sodium chloride) were prepared at 23° in the pH 8.38–10.14 range. The UV absorption of the undissociated and dissociated forms was determined in 0.1 *N* HCl and 0.01 *M* Na_2CO_3 solutions. A solution (100 μl) of the compound in acetonitrile was added to 3.00 ml of buffer at time zero. Due to the rapid hydrolysis of the ester(s), the decrease in absorbance at 23° was followed at a given wavelength for a short time, and the absorbance at time zero was found by extrapolation. The pKa values were calculated using $pKa = pH - \log(a_{obs} - a_{HA})/(a_{A^-} - a_{obs})$, where a_{obs} is the absorbance of the solution tested, a_{HA} is absorbance of the unionized species, and a_{A^-} is the absorbance of the ionized species. The pKa of methylthiomethyl 2-hydroxybenzoate (VI) was 9.60 ± 0.01; it was 9.00 ± 0.2 and 8.91 ± 0.02 for methylsulfinylmethyl 2-hydroxybenzoate (VII) and methylsulfonylmethyl 2-hydroxybenzoate (VIII), respectively.

Determination of Activation Parameters for III and VI–VIII Hydrolysis—The change in the reaction rates with temperature for the hydrolysis of VI–VIII in 0.1 *N* HCl (pH 1.1) and of III in unbuffered aqueous solution (pH 5) was studied. The ionic strength was maintained at 0.1 *M* with sodium chloride. Because of the slow reaction, the activation parameters for the hydrolysis of VII and VIII were determined using solutions of the compounds sealed in ampuls and stored at constant temperature for various intervals in four ovens maintained at 35.8, 44.0, 61.0, and 102.8°, respectively. The activation parameters for III and VI hydrolysis were determined by measuring the pseudo-first-order rate constants at 50–30°.

Influence of Ionic Strength and Dielectric Constant—The effect of the solvent ionic strength (0.05–0.5 *M* NaCl) on III hydrolysis in unbuffered aqueous solutions was studied at 50.8°. The effect of the solvent dielectric constant on the reaction rate also was investigated at 50.8° in an aqueous solution containing up to 30% dioxane. The dielectric constant of each water–dioxane mixture was calculated at 50.8° (3). The pseudo-first-order rate constants were determined using HPLC to follow the disappearance of III as a function of time.

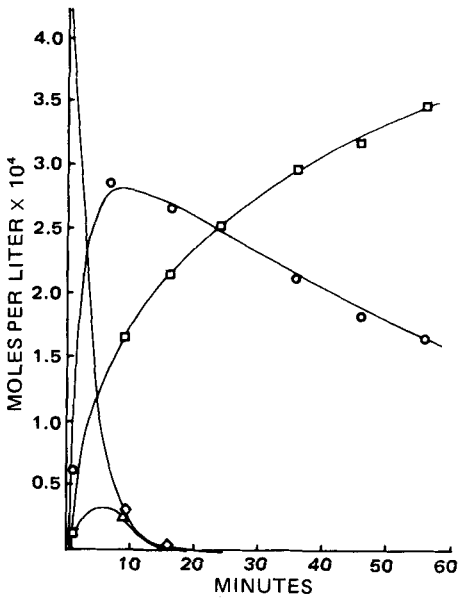


Figure 2—*Kinetics of hydrolysis of methylsulfinylmethyl 2-acetoxybenzoate (IV) in 0.01* M *carbonate buffer (pH 9.55) at 50.8° and 0.1* M *ionic strength to form methylsulfinylmethyl-2-hydroxybenzoate (VII) (△), aspirin (○), and salicylic acid (□).*

Solvent Isotope Effect—The deuterium solvent isotope effects on the hydrolysis rate of III, VII, and VIII were measured at 50.8° and ionic strength 0.1 *M*. The pseudo-first-order rate constants were determined as described. The hydrolysis rate constants for VII and VIII were determined in 0.1 *N* DCl solution in deuterium oxide (pD 0.9).

RESULTS AND DISCUSSION

The hydrolysis rate constants were all first order for the 2-acetoxybenzoates (III–V). The pseudo-first-order rate constants (k_{obs}) for the three 2-acetoxybenzoates were determined by HPLC following the disappearance of the compound as a function of time at pH 1.13, 2.64, 4.00, 7.96, and 9.55 at four buffer concentrations at 50.8 ± 0.2° and ionic strength 0.1 *M*. The kinetics of the disappearance of III and the formation of the various hydrolysis products (I, II, and VI) are exemplified in Fig. 1 (pH 9.55 at 50.8°). Under the same conditions, IV gave only traces of the salicylate (VII) (Fig. 2) while the most reactive V hydrolyzed only to aspirin (Fig. 3).

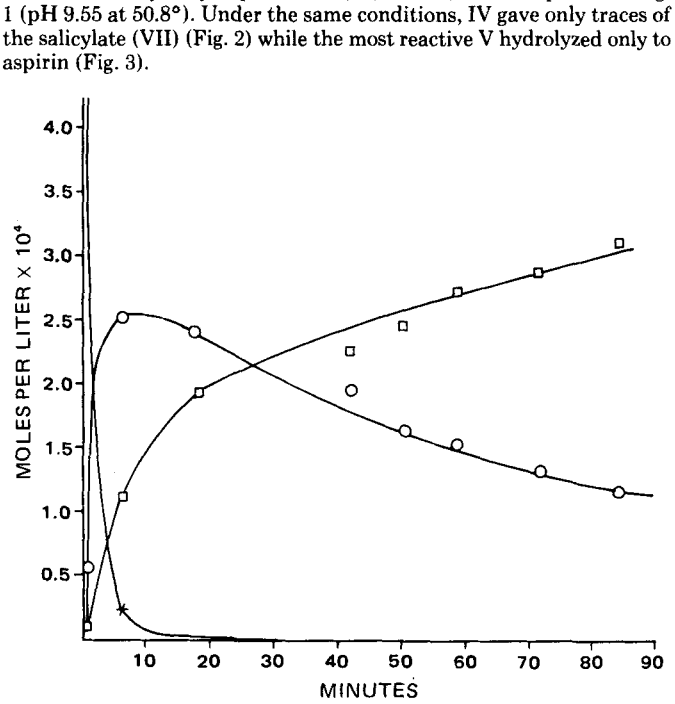


Figure 3—*Kinetics of hydrolysis of methylsulfonylmethyl 2-acetoxybenzoate (V) (+) in 0.01* M *carbonate buffer (pH 9.55) at 50.8° and 0.1* M *ionic strength to form aspirin (○) and salicylic acid (□).*

Table I—Pseudo-First-Order Hydrolytic Rate Constants at Zero Buffer Concentration (k_0) for the Disappearance of Methylthiomethyl (III), Methylsulfinylmethyl (IV), and Methylsulfonylmethyl (V) 2-Acetoxybenzoates at 50.8 ± 0.2° and 0.1 *M* Ionic Strength

Buffer	pH	III		IV		V	
		k_0, min^{-1}	$t_{1/2}$, min	k_0, min^{-1}	$t_{1/2}$, hr	k_0, min^{-1}	$t_{1/2}$, hr
0.100 *N* HCl	1.13	8.26×10^{-2}	8.39	1.15×10^{-3}	10.0	0.90×10^{-4}	11.7
Monochloroacetate	2.64	5.68×10^{-2}	12.2	5.56×10^{-5}	208	5.15×10^{-5}	224
Succinate	4.00	6.10×10^{-2}	11.4	2.60×10^{-5}	444	1.95×10^{-5}	592
Tris(hydroxymethyl)aminomethane	7.96	6.39×10^{-2}	10.8	9.30×10^{-3}	1.24	1.27×10^{-2}	0.91
Carbonate	9.55	0.17	4.1	0.32	0.04	0.48	0.02

Table II—Acid-Catalyzed, Base-Catalyzed, and Spontaneous Hydrolytic Rate Constants for Methylthiomethyl (III), Methylsulfinylmethyl (IV), and Methylsulfonylmethyl (V) 2-Acetoxybenzoates [a]

Rate Constant [b]	III	IV	V
k_H	2.97×10^{-1}	1.48×10^{-2}	1.42×10^{-2}
k_{H_2O}	6.06×10^{-2}	2.35×10^{-5}	1.86×10^{-5}
k_{OH}	5.61×10^{2}	1.75×10^{3}	2.50×10^{3}

[a] Conditions of 50.8 ± 0.2° and ionic strength 0.1 *M* (sodium chloride). [b] k_H is the specific acid hydrolytic rate constant in liters per mole minute, k_{H_2O} is the spontaneous hydrolytic rate constant in $minutes^{-1}$, and k_{OH} is the specific base hydrolytic rate constant in liters per mole minute.

The k_{obs} values were plotted against the buffer concentration, and the rate constants at zero buffer concentration (k_0) were obtained by linear regression (Table I). Only the carbonate buffer had any effect on the hydroxide-ion-catalyzed hydrolysis of III (slope = 2.50 ± 0.30) and IV (slope = 2.00 ± 0.01), but it had little effect on V hydrolysis. Tris(hydroxymethyl)aminomethane catalyzed the hydrolysis of IV (slope = 0.201 ± 0.007) and V (slope = 0.264 ± 0.009) but showed a very small effect on III (slope = 7.8 ± 0.4 × 10^{-2}). Succinate buffer had a significant effect on the hydrolysis of V (slope = 3.15 ± 0.49 × 10^{-4}) but little or no effect on the hydrolysis of the other two 2-acetoxybenzoate esters. The monochloroacetate buffer had a much smaller effect. The main degradation products at each pH were identified by HPLC to be the compounds according to Scheme I. At pH 4.00, 7.96, and 9.55, the main hydrolysis product was aspirin, which hydrolyzed further to salicylic acid (route 1, Scheme I); at pH 1.13, IV and V hydrolyzed to form only the corresponding 2-hydroxybenzoate esters (VII and VIII) (route 2), which are stable at low pH; no aspirin was formed. Compound III hydrolyzed mainly through route 1 at all pH values studied. The pH profiles for III–V, together with aspirin (I), are shown in Fig. 4. The curves for III–V can be described as a simple ester hydrolysis by:

$$k_0 = k_H a_{H^+} + k_{H_2O} + k_{OH} a_{OH^-} \qquad \text{(Eq. 1)}$$

where k_{H_2O} is the rate constant for spontaneous water hydrolysis and k_H and k_{OH} are the rate constants for catalysis of the hydrolysis by hydronium and hydroxide ions, respectively.

The k_H, k_{H_2O}, and k_{OH} values in Table II were calculated from the pseudo-first-order rate constants given in Table I using pKw = 13.26 at 50° (4). At pH 7.96, k_1 (Scheme I) was calculated by measuring the appearance of aspirin (Table III). In the hydrolysis of IV and V, tris(hydroxymethyl)aminomethane mainly affected k_2 but had little effect on k_1. At 0.04 *M* tris(hydroxymethyl)aminomethane concentration, ~50% of the hydrolysis went through route 1; but at zero buffer concentration, this route increased to ~90%. About 90% of the hydrolysis of III occurred *via* route 1 at all buffer concentrations; thus, its hydrolysis was catalyzed only very slightly by this buffer.

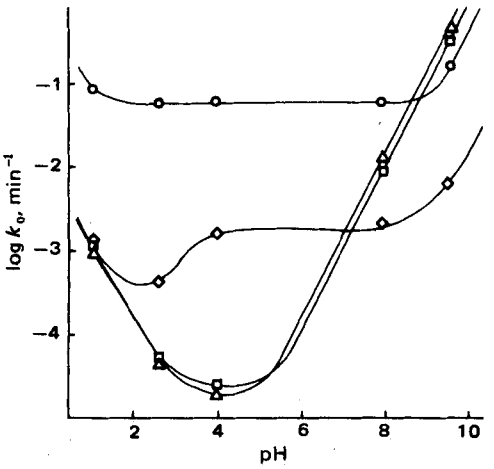


Figure 4—*Experimentally determined pH–rate profiles for hydrolysis of the methylthiomethyl (III) (○), methylsulfinylmethyl (IV) (□), and methylsulfonylmethyl (V) (△) 2-acetoxybenzoates at 50.8 ± 0.2° and 0.1 M ionic strength. The smooth curves from pH 0.5 to 10 were calculated using Eq. 1 and the constants given in Table II. The experimentally determined pH profile of aspirin (I) (◇) was included for comparison. The curve was calculated using Eq. 5.*

Hydrolysis of the possible intermediate salicylates (VI–VIII) also was studied. All the pseudo-first-order rate constants (k_{obs}) for the hydrolysis of the 2-hydroxybenzoates (VI–VIII) were determined at pH 1.13, 2.02, 2.64, 4.00, 5.97, 6.86, 7.96, 8.82, 9.55, 10.90, and 11.86 at four buffer concentrations at 50.8 ± 0.2° and ionic strength 0.1 *M*. The rate constants at zero buffer concentration (k_0) were obtained by extrapolation (Table IV).

Of the seven buffers, only the two trimethamine buffers had any significant effect on the hydrolysis rate. In the case of VI, this catalyzing effect was rather small at pH 7.96, and no effect was observed at pH 8.82. At pH 7.96, the slopes were 1.50 ± 0.06, 1.01 ± 0.08, and 2.23 ± 0.39 for VI, VII, and VIII, respectively. The pH rate profiles (Fig. 5) were fitted by computer to:

$$k_0 = f_{acid} k_{H_2O} + f_{base} k'_{H_2O} \qquad \text{(Eq. 2)}$$

where k_{H_2O} and k'_{H_2O} are the rate constants for the spontaneous water hydrolysis of the undissociated and dissociated reactant, respectively,

Table III—Pseudo-First-Order Hydrolytic Rate Constants of Methylthiomethyl (III), Methylsulfinylmethyl (IV), and Methylsulfonylmethyl (V) 2-Acetoxybenzoates in Tris(hydroxymethyl)aminomethane Buffers at pH 7.96, 50.8 ± 0.1°, and 0.1 *M* Ionic Strength

Buffer, *M*	III			IV			V		
	k' [a], min^{-1}	k_1, min^{-1}	Route 1 [b], %	k' [a], min^{-1}	k_1, min^{-1}	Route 1 [b], %	k' [a], min^{-1}	k_1, min^{-1}	Route 1 [b], %
0.01	6.57×10^{-2}	6.2×10^{-2}	94	1.12×10^{-2}	8.1×10^{-3}	72	1.54×10^{-2}	1.2×10^{-2}	78
0.02	6.65×10^{-2}	6.1×10^{-2}	92	1.35×10^{-2}	8.0×10^{-3}	59	1.77×10^{-2}	1.2×10^{-2}	68
0.03	6.74×10^{-2}	5.4×10^{-2}	80	1.53×10^{-2}	8.1×10^{-3}	53	2.07×10^{-2}	1.4×10^{-2}	68
0.04	6.80×10^{-2}	6.3×10^{-2}	93	1.73×10^{-2}	8.2×10^{-3}	47	2.32×10^{-2}	1.3×10^{-2}	56
Zero	6.50×10^{-2}	6.1×10^{-2}	94	9.30×10^{-3}	8.0×10^{-3}	86	1.27×10^{-2}	1.2×10^{-2}	94
Slope	7.8×10^{-2}	4×10^{-2}		0.20	4×10^{-3}		0.26	5×10^{-2}	
r	0.997			0.999			0.999		

[a] $k' = k_1 + k_2$ (Scheme I). [b] Percent *via* route 1 = $(k_1/k') \times 100$.

Table IV—Pseudo-First-Order Hydrolytic Rate Constants at Zero Buffer Concentration (k_0) for Methylthiomethyl (VI), Methylsulfinylmethyl (VII), and Methylsulfonylmethyl (VIII) 2-Hydroxybenzoates at 50.8 ± 0.2° and 0.1 *M* Ionic Strength (Sodium Chloride), Obtained by Following the Disappearance of the Compound

Buffer	pH	VI		VII		VIII	
		k_0, min^{-1}	$t_{1/2}$, min	k_0, min^{-1}	$t_{1/2}$, min	k_0, min^{-1}	$t_{1/2}$, min
0.10 *N* NaOH	11.86	0.108	6.42	0.510	1.36	0.632	1.10
0.01 *N* NaOH	10.90	0.158	8.77	0.523	1.30	0.512	1.35
Carbonate	9.55	—	—	0.4	1.7	0.6	1.2
Tris(hydroxymethyl)aminomethane	8.82	0.307	2.27	0.148	4.68	0.202	3.67
Tris(hydroxymethyl)aminomethane	7.96	0.334	2.08	4.54×10^{-2}	15.3	6.26×10^{-2}	11.1
Phosphate	6.86	—	—	5.87×10^{-3}	1.18×10^{2}	1.09×10^{-2}	63.6
Succinate	5.97	—	—	6.94×10^{-4}	9.99×10^{2}	1.47×10^{-3}	4.72×10^{2}
Succinate	4.00	0.26	2.7	2.95×10^{-5}	2.35×10^{4}	4.12×10^{-5}	1.68×10^{4}
Monochloroacetate	2.64	0.329	2.11	—	—	—	—
0.01 *N* HCl	2.02	0.324	2.14	2.20×10^{-5}	3.15×10^{4}	3.40×10^{-5}	2.04×10^{4}
0.100 *N* HCl	1.13	0.349	1.99	2.26×10^{-5}	3.07×10^{4}	3.42×10^{-5}	2.03×10^{4}

and f_{acid} and f_{base} are the fractions of the total reactant in acidic and basic form:

$$f_{acid} = \frac{a_{H^+}}{a_{H^+} + k_a} \quad \text{(Eq. 3)}$$

$$f_{base} = \frac{k_a}{a_{H^+} + k_a} \quad \text{(Eq. 4)}$$

where k_a is the dissociation constant of the phenol group estimated from the pH hydrolytic rate profiles. By using the computer fitting, the pKa, k_{H_2O}, and k'_{H_2O} values for VI–VIII were obtained (Table V).

The pKa values obtained from the pH–rate profiles at 50.8° were in reasonable agreement with the values obtained spectrophotometrically at 23° and pH 9.60, 9.00, and 8.91, respectively. The pKa values for phenols and the pKa_2 values for salicylic acid decrease somewhat with increasing temperature in this temperature range (5, 6).

To complete the hydrolytic sequence, the hydrolytic rate constants for aspirin were determined by HPLC at pH 1.13, 2.64, 4.00, 7.96, and 9.55 at 50.8 ± 0.2° and ionic strength 0.1 *M* (Table VI). Only the carbonate buffer significantly influenced the hydrolysis rate (slope = 0.34 ± 0.04). According to Edwards (7, 8), k_0 for aspirin hydrolysis may be expressed by:

$$k_0 = \frac{a_{H^+}}{a_{H^+} + k_a}(k_H a_{H^+} + k_{H_2O}) + \frac{k_a}{a_{H^+} + k_a}(k'_{H_2O} + k'_{OH} a_{OH^-}) \quad \text{(Eq. 5)}$$

where k_a is the dissociation constant of aspirin and k_H, k_{H_2O}, k'_{H_2O}, and k'_{OH} are the rate constants for the acid-catalyzed, spontaneous, and base-catalyzed hydrolysis of aspirin in acidic and basic forms, respectively. The k_H, k_{H_2O}, and k'_{H_2O} values were calculated from the pseudo-first-order rate constants at pH 1.13, 2.64, and 4.00 using pKa = 3.5 and applying the method of determinants (9). The value of k_{OH} was obtained at pH 9.55. The value obtained for k_H was 1.32×10^{-2} liter/mole/min; for k_{H_2O}, it was 1.92×10^{-4} min^{-1}; for k'_{H_2O}, it was 2.11×10^{-3} min^{-1}; and for k'_{OH}, it was 32.25 liters/mole/min. All of these values agree with the literature values (10).

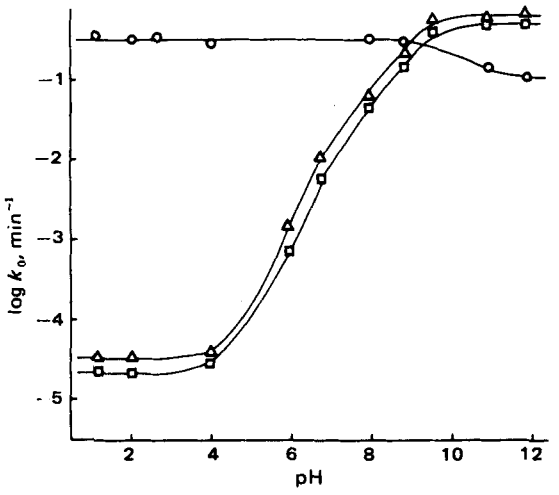


Figure 5—*The pH–rate profile for hydrolysis of methylthiomethyl 2-hydroxybenzoate (VI) (○), methylsulfinylmethyl 2-hydroxybenzoate (VII) (□), and methylsulfonylmethyl 2-hydroxybenzoate (VIII) (△) at 50.8 ± 0.2° and 0.1* M *ionic strength. The curves were calculated using Eqs. 1–4 based on the experimental data.*

To determine the hydrolysis mechanism of the various species (since the pH profile implies differences in the mechanism), additional investigations were conducted. Thus, the pseudo-first-order rate constants (k_{obs}) for the hydrolysis of IX–XI were determined by HPLC in 0.05 *M* buffers at pH 2.6, 4.0, 6.8, 7.9, and 9.2 at 25.5° (Table VII). The methylthiomethyl benzoate (IX) has a wide pH-independent region from pH 2.5 to 7, where k_{obs} was ~7.7×10^{-4} min^{-1}. The methylsulfinylmethyl (X) and methylsulfonylmethyl (XI) benzoates hydrolyzed at much slower rates, the pseudo-first-order rate constants at pH 4.0 being only 1.6×10^{-5} and 1.1×10^{-5} min^{-1}, respectively. Next, the change in the reaction rates with temperature for the hydrolysis of the three 2-hydroxybenzoate derivatives (VI–VIII) in dilute hydrochloric acid and of the methylthiomethyl 2-acetoxybenzoate (III) in unbuffered aqueous solution was studied by following the disappearance of the compound (Tables VIII and IX).

The relatively high enthalpies ($\Delta H^{\ddagger}$) and small negative entropies ($\Delta S^{\ddagger}$) of activation of III and VI are typical for unimolecular ester hydrolysis following cleavage of the alkyl–oxygen bond (11). The values for VII and VIII fall into the range of neutral ester hydrolysis by general base catalysis (11) (Table X).

The effect of ionic strength on the spontaneous water hydrolysis of the

Table V—Rate Constants and pKa Values of the Salicylate Esters

Compound	pKa[a]	pKa[b]	k_{H_2O}, min^{-1}	k'_{H_2O}, min^{-1}
VI	10.2	9.60 × 0.01	0.330	0.105
VII	8.85	9.00 × 0.02	2.23×10^{-5}	0.156
VIII	8.70	8.91 × 0.02	3.41×10^{-5}	0.570

[a] Obtained from the hydrolysis rate profiles at 50.8 ± 0.2°. [b] Experimental values obtained at 23°.

Table VI—Pseudo-First-Order Hydrolytic Rate Constants at Zero Buffer Concentration (k_0) for Aspirin (I) at 50.8 ± 0.2° and 0.1 *M* Ionic Strength

Buffer	pH	k_0, min^{-1}	$t_{1/2}$, hr
0.100 *N* HCl	1.13	1.17×10^{-3}	9.87
Monochloroacetate	2.64	4.51×10^{-4}	25.6
Succinate	4.00	1.64×10^{-3}	7.00
Tris(hydroxymethyl)aminomethane	7.96	2.14×10^{-3}	5.40
Carbonate	9.55	6.47×10^{-3}	1.97

Table VII—Pseudo-First-Order Rate Constants for Hydrolysis of Methylthiomethyl (IX), Methylsulfinylmethyl (X), and Methylsulfonylmethyl (XI) Benzoates in 0.5 *M* Buffers at 25.5 ± 0.1°

Buffer	pH	k_{obs}, min^{-1}		
		IX	X	XI
Borate	9.20	1.3×10^{-3}	4.1×10^{-3}	5.9×10^{-3}
Phosphate	7.93	1.3×10^{-3}	1.9×10^{-4}	2.9×10^{-4}
Phosphate	6.81	8.1×10^{-4}	3.2×10^{-5}	4.5×10^{-5}
Acetate	4.00	7.6×10^{-4}	1.6×10^{-5}	1.1×10^{-5}
Monochloroacetate	2.60	7.7×10^{-4}	1.6×10^{-5}	1.1×10^{-5}

Table VIII—Observed Pseudo-First-Order Rate Constants for Hydrolysis of Methylthiomethyl (VI), Methylsulfinylmethyl (VII), and Methylsulfonylmethyl (VIII) 2-Hydroxybenzoates in 0.1 *N* HCl

Temperature	VI, k_{obs}, min^{-1}	Temperature	k_{obs}, min^{-1}	
			VII	VIII
29.88°	$3.15 \pm 0.001 \times 10^{-2}$	35.8°	$5.51 \pm 0.24 \times 10^{-6}$	$1.25 \pm 0.07 \times 10^{-5}$
35.68°	$6.93 \pm 0.05 \times 10^{-2}$	44.0°	$1.36 \pm 0.04 \times 10^{-5}$	$2.03 \pm 0.30 \times 10^{-5}$
42.88°	0.156 ± 0.002	61.0°	$5.64 \pm 0.14 \times 10^{-5}$	$7.16 \pm 0.27 \times 10^{-5}$
50.78°	0.325 ± 0.005	102.8°	$8.04 \pm 0.83 \times 10^{-4}$	$6.13 \pm 0.11 \times 10^{-4}$

aspirin derivative III was investigated at 50.8 ± 0.1°. The small positive slope (0.202 ± 0.009) of the log k_{obs} *versus* $\sqrt{I}$ plot indicates increased stability of the transition state with increasing ionic strength, or decreased stability of the reactant state, or both.

The effect of the solvent dielectric constant (ϵ) on the rate constant of the spontaneous water hydrolysis of III to form aspirin also was studied using water–dioxane mixtures at 50.8°. The dielectric constant of each mixture at this temperature was calculated (3), and the measured k_{obs} values were plotted *versus* $1/\epsilon$. The decrease in the solvent dielectric constant resulted in a decrease in the rate constant, which implies that the transition state is more polar than the reactant(s) (Table XI).

The deuterium solvent isotope effects on the hydrolysis rates of methylthiomethyl 2-acetoxybenzoate (III), methylsulfinylmethyl 2-hydroxybenzoate (VII), and methylsulfonylmethyl 2-hydroxybenzoate (VIII) were measured at 50.8° and 0.1 *M* ionic strength. The hydrolysis rate constant (k_{D_2O}) for III to form aspirin in unbuffered deuterium oxide solution was 5.06×10^{-2} min^{-1} compared to 6.06×10^{-2} min^{-1} in water, which results in an isotope effect (k_{H_2O}/k_{D_2O}) of 1.20. The hydrolysis rate constants for VII and VIII were measured in 0.1 *N* DCl solution in deuterium oxide (pD 0.9) and found to be 1.10×10^{-5} and 1.68×10^{-5} min^{-1} compared to 2.23×10^{-5} and 3.41×10^{-5} min^{-1} in water, respectively. The two 2-hydroxybenzoates showed the same solvent isotope effects, $k_{H_2O}/k_{D_2O} = 2.03$.

Table IX—Observed Pseudo-First-Order Rate Constants (k_{obs}) for Hydrolysis of Methylthiomethyl 2-Acetoxybenzoate (III), Obtained by Following the Disappearance of III as a Function of Time in Aqueous Solution at 0.1 *M* Ionic Strength (Sodium Chloride) and Various Temperatures

Temperature	k_{obs}, min^{-1}
50.78°	$5.68 \pm 0.01 \times 10^{-2}$
44.48°	$3.11 \pm 0.03 \times 10^{-2}$
37.07°	$1.38 \pm 0.01 \times 10^{-2}$
29.20°	$5.06 \pm 0.04 \times 10^{-3}$

Table X—Enthalpies and Entropies of Activation for the Hydrolysis of Methylthiomethyl and Related Esters

Compound	E_a, kcal/mole	$\Delta H^{\ddagger}$, kcal/mole	$\Delta S^{\ddagger}$, e.u.	r
III	21.8 ± 0.7	21.2 ± 0.7	−7.1	0.999
VI	21.7 ± 0.9	21.1 ± 0.9	−4.0	0.998
VII	16.2 ± 0.8	15.6 ± 0.8	−40.1	0.998
VIII	13.5 ± 0.4	12.9 ± 0.4	−48.1	0.999

Table XI—Observed Pseudo-First-Order Rate Constants (k_{obs}) for Hydrolysis of Methylthiomethyl 2-Acetoxybenzoate (III) in Various Water–Dioxane Mixtures at 50.8 ± 0.1°

Dioxane, %	k_{obs}, min^{-1}	ln k_{obs}	ϵ[a]	$1/\epsilon$
0	$5.11 \pm 0.02 \times 10^{-2}$	−2.98	69.60	1.44×10^{-2}
10	$2.35 \pm 0.04 \times 10^{-2}$	−3.75	61.43	1.63×10^{-2}
20	$9.05 \pm 0.10 \times 10^{-3}$	−4.71	53.30	1.88×10^{-2}
30	$3.64 \pm 0.10 \times 10^{-3}$	−5.62	45.27	2.21×10^{-2}

[a] The dielectric constant (ϵ) of each mixture was calculated at 50.8° according to Ref. 3.

Table XII—Relative Rates of Hydrolysis of Methylthiomethyl Benzoate (IX), Methylthiomethyl 2-Acetoxybenzoate (III), and Methylthiomethyl 2-Hydroxybenzoate (VI) at pH 4.00 at the Plateau and 25.5° [a]

Compound	k_{obs}, min^{-1}	Relative Rate	pKa[b]
IX	7.6×10^{-4}	1.0	4.2
III	3.45×10^{-3}	4.5	3.5
VI	1.90×10^{-2}	25	3.0

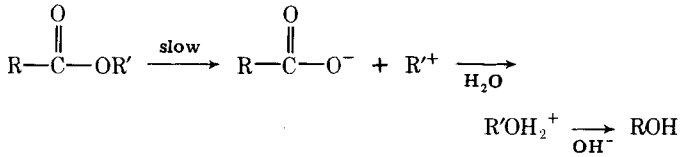
[a] The rate constant for IX was not extrapolated to zero buffer concentration. [b] The pKa of the carboxylic acid.

The pH–rate profiles of III and VI demonstrate large plateaus where the hydrolysis rates were pH independent. For III (Fig. 4), this plateau extended from about pH 2 to 8, with aspirin as the main hydrolytic product. Methylthiomethyl 2-hydroxybenzoate (VI) (Fig. 5) had an even wider plateau, showing no acid- or base-catalyzed regions on the pH profile from pH 1 to 12. The small decrease in the rate at ~pH 10 can be explained by the ionization of the 2-hydroxy group. Similar pH-independent hydrolysis behavior was shown by methylthiomethyl benzoate (IX) (Table VII).

The pH–rate profiles of VII and VIII show two plateaus with a large increase in the rate around the pKa of the 2-hydroxy group (Fig. 5). On the other hand, the pH–rate profiles for methylsulfinylmethyl (IV) and methylsulfonylmethyl (V) 2-acetoxybenzoates do not have these large plateaus but are V-shaped with a minimum at pH 4 (Fig. 4). The hydrolysis pathways, however, are changing with pH.

According to Fife (12), a plateau of the pH-independent rate, as for III, VI, and IX, could be due to: (*a*) attack of both hydronium ions and hydroxide ions, (*b*) water attack on the substrate, or (*c*) spontaneous, uncatalyzed decomposition of the substrate. The first possibility seems to be highly unlikely; if it occurred, the reaction would have a very large negative entropy of activation. Thus, the other two possibilities are left.

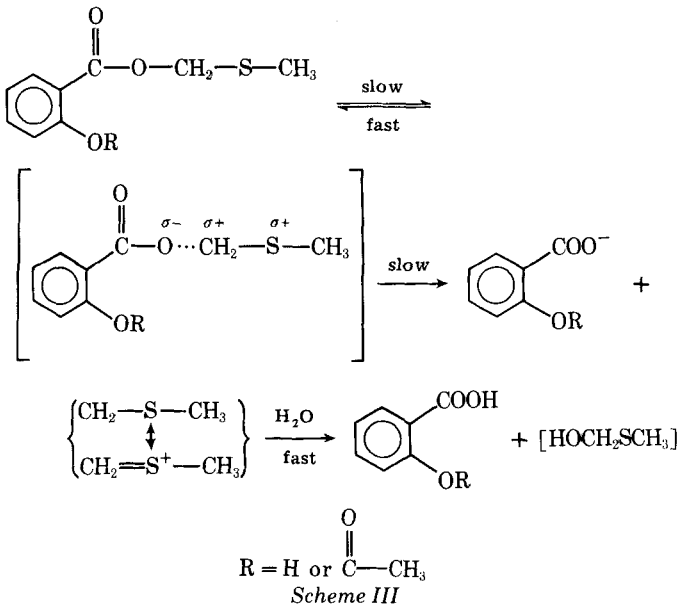
The pH–rate profiles for hydrolysis of both III and VI were obtained by following the disappearance of the compounds with time at various pH values. The main hydrolysis product of III in the plateau region is aspirin, which is hydrolyzed further to salicylic acid. Compounds III and VI have enthalpies of activation ($\Delta H^{\ddagger}$) of 21.2 and 21.1 kcal/mole and entropies of activation ($\Delta S^{\ddagger}$) of −7.12 and −4.04 e.u., respectively, in the pH-independent plateau region. These high enthalpies and low entropies of activation are characteristic for unimolecular reactions, which can be regarded as S_N1 reactions, where the leaving group is the carboxylate anion (11, 13) (Scheme II).



Scheme II

The enhanced solvation due to the charge separation in the transition state compared to the ground state results in a small negative entropy of activation but still is favorable compared to a bimolecular reaction involving water as a reactant (S_N2 reaction). Salomaa (14) studied the kinetics of uncatalyzed hydrolysis of some aliphatic acylals ($RCOOCHROCH_3$) in aqueous solutions and got similar values for the entropy of activation to those obtained in the present study. The hydrolysis rate of methylthiomethyl 2-acetoxybenzoate is highly dependent on the nature of the solvent, being ~14 times greater in water than in a 30% dioxane–water mixture, suggesting considerable charge development in the transition state. Change in ionic strength has a similar effect. Compound III is hydrolyzed with about the same rate in deuterium oxide as in water ($k_{D_2O}/k_{H_2O} = 1.2$). This small solvent isotope effect also is found in the hydrolysis of neutral ester molecules cleaved by alkyl–oxygen fission ($B_{AL}1$) (11, 13). The mechanism can be written as shown in Scheme III.

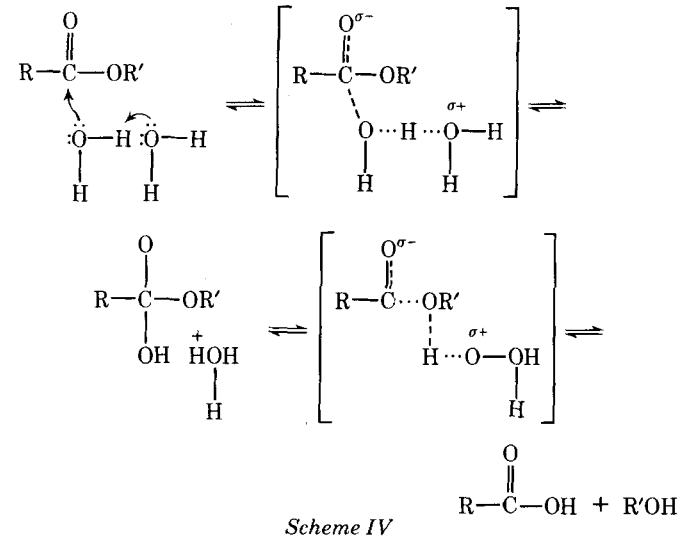
This mechanism would be facilitated by the fact that the carbonium ion formed is resonance stabilized by the neighboring sulfur group. In Table XII, the relative rates on the plateau for IX, III, and VI were listed at 25.5°. The increase in rate can be explained by the decreasing pKa or increased leaving group ability of the corresponding acid. The decrease



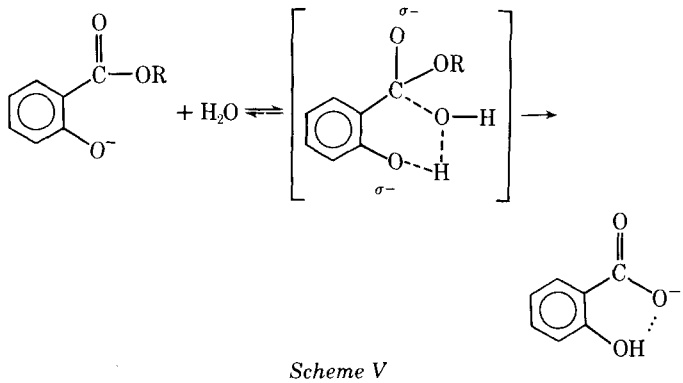
Scheme III

in the hydrolysis rate of VI at pH ~10 can be attributed to ionization of the 2-hydroxy group (pKa ~9.6 at 23°).

In the case of methylsulfinylmethyl (VII) and methylsulfonylmethyl (VIII) 2-hydroxybenzoates, the large negative entropy values and low enthalpies of activation are typical for neutral hydrolysis of esters, where water acts as a general base to assist the addition of a second water molecule to the ester carbonyl group (Scheme IV). The hydrolysis of both compounds show deuterium solvent isotope effect (k_{H_2O}/k_{D_2O}) of 2.03, which also is consistent with this mechanism. The significant rate increase at higher pH values can be explained by an intermolecular catalyzed attack by the ionized phenol group (Scheme V), as suggested by Bender *et al.* (15) in the case of *p*-nitrophenyl salicylate.



Scheme IV



Scheme V

The pH profiles for the aspirin derivatives IV and V (Fig. 4) show the common V-shape at a minimum of pH 4. The hydrolysis pathways, however, change with the pH. Thus, aspirin (I) is the main product at pH > 4, but the relatively stable 2-hydroxybenzoates (VII and VIII) form at lower pH values.

While the methylthiomethyl ester (III) of aspirin at pH 7.96 hydrolyzes almost exclusively (>90%) to aspirin, the same compound in human plasma clearly hydrolyzes *via* both routes; on the other hand, IV apparently goes to aspirin in plasma but follows this route to a significantly smaller proportion in chemical hydrolysis.

As expected, the enzymic hydrolysis is significantly faster in all cases. However, the overall picture underlines the fact that, due to binding, mechanistic, and other differences, the chemical cleavage studies of esters will not necessarily predict the relative rates, possible selectivity, product ratio, *etc.*, obtained in enzymatic conditions.

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