Hydrolysis Kinetics of Thifensulfuron Methyl in Aqueous Buffer Solutions

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The hydrolysis of thifensulfuron methyl and thifensulfuron were investigated in buffered aqueous solutions with pH values of 4, 5, 9, and 10. Hydrolysis of thifensulfuron methyl was pH dependent and relatively fast both in acidic and alkaline buffer solutions. In the case of thifensulfuron, hydrolysis rates were of the same order of magnitude as thifensulfuron methyl at acidic pH, but very low at alkaline pH. In acidic solutions, cleavage of the sulfonylurea bridge and *O*-demethylation of the methoxy group of the triazine ring occurred concurrently. The resulting intermediates gave two parallel reactions: cleavage of the sulfonylurea bridge and opening of the triazine ring. The relative rates of the different hydrolysis pathways were influenced by the pK_a of compounds. At alkaline pH, thifensulfuron methyl hydrolyzed to thifensulfuron, which was slowly transformed by cleavage of the sulfonylurea bridge and *O*-demethylation.

Keywords: *Sulfonylurea herbicide; hydrolysis; thifensulfuron methyl*

INTRODUCTION

The sulfonylurea herbicides are subject to both chemical hydrolysis and microbial degradation in the soil (Harvey et al., 1985; Beyer et al., 1988). Their degradation rates are related to sulfonylurea structures. All sulfonylurea herbicides degrade relatively rapidly in soil, but half-lives ($t_{1/2}$) of ~1−8 weeks, depending on the specific compounds, have been reported (Brown, 1990). The slowly dissipating sulfonylurea herbicides chlorsulfuron, metsulfuron methyl, and chlorimuron were chemically hydrolyzed in acidic conditions by cleavage of the sulfonylurea bridge and *O*-demethylation of triazine ring (Sabadie, 1990, 1991, 1992; Reiser et al., 1991). The more rapidly dissipating sulfonylurea herbicides have shown different pathways of chemical hydrolysis. The sulfonylurea bridge of tribenuron methyl was easily broken in aqueous solutions (Brown, 1990), whereas rimsulfuron rapidly hydrolyzed in both acidic and alkaline conditions through contraction of the sulfonylurea bridge (Schneiders et al., 1993).

Thifensulfuron methyl rapidly degraded in soil by a microbial mechanism ($t_{1/2}$ = 2−3 days; Smith et al., 1990; Cambon and Bastide, 1992) to give thifensulfuron. In this paper, we report an investigation of the chemical hydrolysis mechanism of thifensulfuron methyl and its transformation products in aqueous buffer solutions (pH 4, 5, 9, and 10). The different reaction rate constants and pathways of hydrolysis were determined.

MATERIALS AND METHODS

Chemicals. Thifensulfuron methyl [methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophene methyl carboxylate, **1**] was a gift of Procida (Marseille, France). Thifensulfuron 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2 thiophene carboxylic acid; **2**] was prepared according to the method of Smith et al. (1990). Methyl 3-sulfamoylthiophene-2-carboxylate (**3**) and 3-sulfamoylthiophene-2-carboxylic acid (**6**) were prepared according to the method of Bastide et al. (1994).

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Methyl 3-[[[[(4-hydroxy-6-methyl-1,3,5-triazin-2yl)amino]-carbonyl]amino]sulfonyl]-2-thiophene methyl carboxylate (**4**) was prepared by mixing concentrated HCl (1 mL; d = 1.19; 37%) and anhydrous tetrahydrofuran (50 mL) at room temperature. After 2 min of stirring, thifensulfuron methyl (**1**, 1 g) was added and the mixture was allowed to stir for 2 h. The white precipitate formed was collected by filtration and recrystallized from ethanol to afford 350 mg of a white solid of **4** (35% yield): mp 227−228 °C; IR (Nujol) υ (cm^{-1}) 1784, 1734, 1716; ^{1}H NMR (DMSO-d_6) δ 2.23 (s, 3H, CH_3), 3.85 (s, 3H, CO_2CH_3), 7.6 (d, 1H, CH), 8.02 (d, 1H, CH), 11 (s, 1H, OH), 12.85 (s, 1H, NH), 13 (s, 1 H, NH).

1-(Methylthiophene-2-carboxylate-3 sulfonyl)-7-acetyltriuret (**5**) was prepared by adding concentrated HCl (0.5 mL) to a solution of thifensulfuron methyl (0.5 g) in 80 mL of methanol. The reaction mixture was stirred at room temperature for 3 days. The precipitate formed was filtered, and **5** was obtained as a white solid (0.1 g): mp 205−206 °C; IR (Nujol) υ (cm^{-1}) 3272, 3175, 1733, 1723, 1698; ^{1}H NMR (DMSO-d_6) δ 2.20 (s, 3H, CH_3), 3.9 (s, 3H, CO_2CH_3), 7.7 (d, 1H, CH), 8.12 (d,1H, CH), 10.3 (s, 1H, NH), 11.08 (s, 1H, NH), 11.09 (s, 1H, NH).

3-(4-Hydroxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)-thiophene-2-carboxylic acid) (**7**) was prepared by dissolving a mixture of thifensulfuron (0.5 g) in anhydrous tetrahydrofuran (50 mL) and concentrated HCl (1.5 mL) and stirring the mixture at room temperature for 19 h. The precipitate was filtered, and **7** was obtained as a white solid (72% yield): mp 285−294 °C; IR (Nujol) υ (cm^{-1}) 3553, 1782, 1716; ^{1}H NMR (DMSO-d_6) δ 2.19 (s, 3H, CH_3), 7.76 (d, 1H, CH), 7.41 (d, 1 H, CH), 10.4 (b, 1H, CO_2H).

1-(Thiophene-2-carboxylic acid-3 sulfonyl)-7-acetyltriuret (**8**) was prepared by dissolving a mixture of thifensulfuron (0.5 g) in anhydrous tetrahydrofuran (50 mL) and concentrated HCl (2 mL) and stirring the mixture at room temperature for 6 days. The precipitate was filtered, and **8** was obtained as a white solid (50% yield): mp >295 °C; IR (Nujol) υ (cm^{-1}) 3334, 1714, 1694, 1682; ^{1}H NMR (DMSO-d_6) δ 2.1 (s, 3H, CH_3), 7.58 (d, 1H, CH), 7.96 (d, 1H, CH).

Buffer Solutions. Five buffer solutions were used. Buffer A (pH 4) consisted of 33 mL of citric acid solution (0.1 M) and 17 mL of Na_2HPO_4 solution (0.2 M) diluted to a total of 100 mL. Buffer B (pH 5) consisted of 24.3 mL of citric acid solution (0.1 M) and 25.7 mL of Na_2HPO_4 solution (0.2 M) diluted to a total of 100 mL. Buffer C (pH 7) consisted of 41.3 mL of KH_2PO_4 (1/15 M) and 58.7 mL of $Na_2HPO_4 \cdot 2H_2O$ (1/15 M). Buffer D (pH 9) consisted of 50 mL of a mixture of both KCl (0.1 M)

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Table 1. Typical HPLC Retention Times of Thifensulfuron Methyl and Metabolite Reference Standards

	HPLC retention time (R_f), min	
compd	method 1[a]	method 2[b]
4	4.61	17.40
3	6.41	14.32
5	7.26	64.43
1	10.93	–
6	3.94	7.53
7	3.20	9.64
8	–	20.51
2	5.45	21.94

[a] Method 1: the mobile phase consisted of water:acetonitrile: acetic acid, 60:40:0.6 (v/v/v); flow rate, 1 mL/min; UV detector, 254 nm. [b] Method 2: **2**, **6**, **7**, and **8** were analyzed with a mobile phase of water:acetonitrile:trifluoroacetic acid (80:20:0.3, v/v/v); flow rate, 1 mL/min; UV detector, 254 nm.

and boric acid (0.1 M) and 20.8 mL of a NaOH (0.1 M) solution diluted to a total of 100 mL. Buffer E (pH 10) consisted of 50 mL of both KCl (0.1 M) and boric acid (0.1 M) and 43.7 mL of solution of NaOH (0.1 M) diluted to 100 mL.

Hydrolysis Rate Determination. The hydrolysis rates were determined by monitoring the rate of disappearance of thifensulfuron methyl (**1**), thifensulfuron (**2**), and **4** and **7** in aqueous buffers A, B, D, and E. All glass apparatus were heat-sterilized by autoclaving, and buffer solutions were sterilized by filtration (Sartorius Minisart NML, 0.22 μm). Aseptic techniques were used throughout the study to maintain sterility.

Two stock solutions containing 4 g·L^{-1} of **1** or 2.1 g·L^{-1} of **2** in acetonitrile were prepared. Compounds **4** and **7** are unstable in acetonitrile, so the stock solutions were prepared in 0.52 and 0.58 g·L^{-1} of buffer C, respectively.

An aliquot of each stock solution was added aseptically to 50 mL of each sterilized buffer solution 0.375 mL of **1** to buffers A, B, D, and E; 0.5 mL of **2** to buffers A and B; 0.4 mL of **2** to buffers D and E; 2.9 mL **4** to buffers A and B; and 2.6 mL of **7** to buffers A, B, D, and E. The solutions were maintained at 28 °C in the dark. Aliquots (0.5 mL) were aseptically removed from each flask at appropriate times, depending on the rate of hydrolysis. Each sample was analyzed by HPLC, and each experiment was run in triplicate.

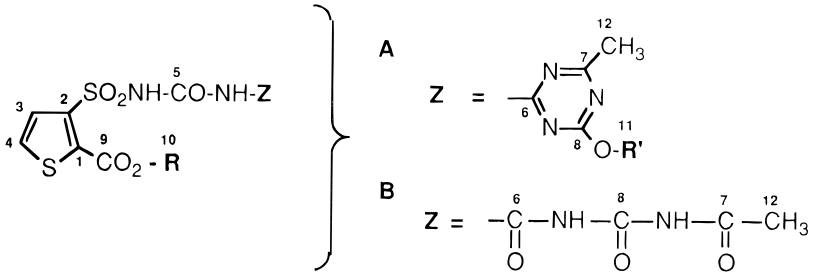
Analytical Methods. Herbicide and metabolite concentrations of aqueous samples were analyzed by a Laboratory Data Control division of Milton Roy (Constametric I) high-performance liquid chromatograph (HPLC) equipped with a Valco injection valve, a UV detector (Monitor III), and a 250 × 4.6 mm Ultrabase 235, C_8, 5 μm analytical column. The typical HPLC retention times for thifensulfuron methyl and potential transformation products are listed in Table 1. The detection limit of each compound was between 0.05 and 0.1 mg·L^{-1}.

RESULTS

Hydrolysis Products. Products **2**, **4**, **5**, **7**, and **8** were synthesized according to standard procedure and identified by IR, ^{1}H NMR (see Materials and Methods), and ^{13}C NMR (Table 2). The structure of **5** was confirmed by X-ray analysis (data not shown).

Hydrolysis Rate of Thifensulfuron Methyl at pH 4 and 5. The hydrolysis rate of thifensulfuron methyl is pH dependent and follows pseudo-first-order kinetics (Table 3). This reaction shows two different pathways (Figure 1): cleavage of the sulfonylurea bridge (path a) to yield **3** and *O*-demethylation of the methoxy group of the triazine ring (path b) giving **4**. This latter compound was transformed either into **3** by cleavage of the sulfonylurea bridge (path c) or into **5** by opening of the triazine ring (path d). The different hydrolysis products were identified on the basis of their HPLC retention times compared with authentic standards

Table 2. ^{13}C NMR Chemical Shifts in DMSO-d_6 (^{13}C Spectra Recorded on a JEOL 400 MHz)



		carbon											
structure	group	1	2	3	4	5	6	7	8	9	10	11	12
A	R = CH_3 R′ = CH_3	133.1	141.4	132.1	133.1	148.5	163.8	170.1	178.4	159.3	53.12	55.22	25.07
A	R = H R′ = CH_3	135.9	140.8	131.6	131.1	149.2	163.9	170.1	178.5	160.6		55.17	25.18
A	R = CH_3 R′ = H	134	141.1	131.9	131.7	149.2	163.3	170.9	153.2	159.9	53.33		21.07
A	R = H R′ = H	135.5	141.1	131.8	131.1	150	163	170.1	153.4	160.6			21.36
B	R = CH_3	133.3	141.3	132.1	131.6	148.1	149.5	173	150.3	159.4	53.27		23.95
B	R = H	113.5	140.7	131.4	131.3	148.2	149.7	173	150.2	160.4			24.01

Table 3. First-Order Rate Constants of 1 and 4 at pH 4, 5, 9, and 10

	$k \times 10^2$ h^{-1} [a]					
	compd **1**			compd **4**		
pH	k_{obs}	k_1	k_2	k_{obs}	k_3	k_4
4	2.41 ± 0.02	1.34 ± 0.06	1.07 ± 0.08	2.37 ± 0.19	0.84 ± 0.06	1.52 ± 0.13
5	0.25 ± 0.02	0.13 ± 0.01	0.12 ± 0.01	0.40 ± 0.06	0.22 ± 0.08	0.18 ± 0.02
9	0.71 ± 0.15					
10	11.55 ± 0.25					

[a] Data are means ± SD of three replicates.

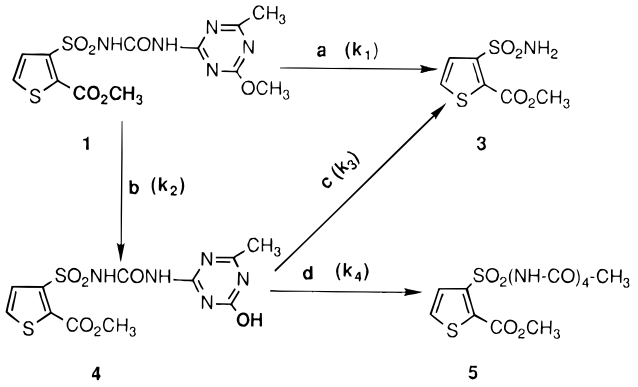


Figure 1. Proposed acidic hydrolysis pathway of thifensulfuron methyl (**1**) in aqueous buffer solutions (pH 4 and 5) at 28 °C (*k* represents reaction rate constants).

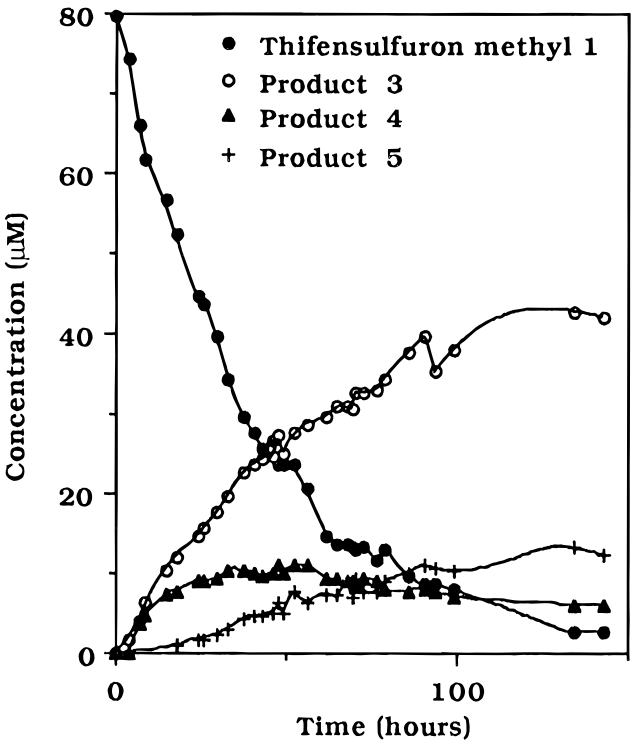


Figure 2. Hydrolysis of thifensulfuron methyl (**1**) in pH 4 buffer solution at 28 °C.

(Table 1). The relative rates of these different reactions can be estimated by three different methods.

*Method 1. Measurement of the Initial Formation Rate of **3** and **4**.* At the beginning of the reaction (pH 4), the product **5** was present at very low concentration (Figure 2), which allows us to neglect this transformation. In this condition, the ratio of the concentrations of **3** and **4** and the ratio of their rate constants are the same.

$$\frac{[\mathbf{3}]}{[\mathbf{4}]} = \frac{k_1}{k_2} \quad \text{and} \quad k_{obs} = k_1 + k_2$$

These ratios give the values $k_1/k_2 = 1.24$, $k_1 = 1.34 \times 10^{-2}$ h^{-1}, and $k_2 = 1.07 \times 10^{-2}$ h^{-1}.

Method 2. Analysis of Final Concentrations of Compounds. When the hydrolysis reaction is completed, the products **3** and **5** are relatively stable and their concentrations do not change. Compound **5** was obtained by hydrolysis pathway d and **3** was formed by paths a and c. The amount of **3** formed by path c was related to the amount of **5** in solution by the ratio k_3/k_4. This ratio was determined by studying the hydrolysis of **4** under the same conditions. In this case

$$[\mathbf{3}] = [\mathbf{3}]_{(\text{path a})} + [\mathbf{3}]_{(\text{path c})}$$

$$\frac{[\mathbf{3}]_{(\text{path c})}}{[\mathbf{5}]} = \frac{k_3}{k_4} \quad \text{and} \quad \frac{[\mathbf{3}]_{(\text{path a})}}{[\mathbf{3}]_{(\text{path c})} + [\mathbf{5}]} = \frac{k_1}{k_2}$$

$$\frac{k_1}{k_2} = \frac{[\mathbf{3}] - \dfrac{k_3[\mathbf{5}]}{k_4}}{\dfrac{k_3[\mathbf{5}]}{k_4} + [\mathbf{5}]}$$

A ratio $k_1/k_2 = 1.33$ was calculated from the latter equation. The k_2 value was 1.03×10^{-2} h^{-1}.

*Method 3. Measurement of the Concentration of the Different Products When **4** is Maximum.* At the reaction time when **4** is maximum

$$-\frac{\mathrm{d}[\mathbf{4}]}{\mathrm{d}t} = 0$$
$$= k_2 (a - x) - (k_3 + k_4)[\mathbf{4}]$$

Here $(a - x)$ is the concentration of the product **1** at $t =$ 48 h, and $k_3 + k_4 = k_{obs}$ (transformation rate of **4**). The k_2 value calculated according to this method is 1.0×10^{-2} h^{-1}.

The three different methods lead to similar k_2 values ($k_2 \approx 1.0 \times 10^{-2}$ h^{-1}). The same hydrolysis pathways were observed at pH 5, although the relative transformation rates were not affected to the same magnitude. The different *k* values were calculated from the same methods as for pH 4 and are reported in Table 3.

Hydrolysis Rate of Thifensulfuron at pH 4 and 5. Three transformation products were detected by HPLC in these reactions and were identified as **6**, **7**, and **8**. In acidic aqueous solution, thifensulfuron (**2**) and thifensulfuron methyl (**1**) hydrolysis pathways were similar; that is cleavage of the sulfonylurea bridge (path e) to yield **6** and conversion of the methoxy group on the triazine ring to hydroxyl function (path f) to give **7**. Further hydrolysis of this latter compound leading to **6** (path g) and **8** (path h) was also observed (Figure 3). The rate constants of the different hydrolysis reactions were calculated by the method previously described for the hydrolysis of **1** (Table 4).

The hydrolysis rate of thifensulfuron is lower at pH 4 (Figure 4) and higher at pH 5 than the respective thifensulfuron methyl hydrolysis rates. The relation between pH and hydrolysis rates of chlorsulfuron and metsulfuron methyl have been reported by Sabadie (1990, 1991) and Dinelli et al. (1994). At pH > pK_a, a linear relation between pH and log *k*, with a slope of ~1 was obtained. At pH < pK_a, the slope was <0.4. The pK_a of thifensulfuron methyl is 4, and the variations in rate constants versus pH are in agreement with the results reported for other sulfonylureas. The pK_a of thifensulfuron was measured as 4.6−4.7. This value explains the low variation of rate constants observed between pH 4 and 5.

The hydrolysis rates of the *O*-demethylated compound **7** were measured at pH 4 and 5, and the rate constants calculated followed first-order kinetics (Table 4). The hydrolysis of product **7** at pH 4 was faster than thifensulfuron **2** hydrolysis, although at pH 5, the hydrolysis

Table 4. First-Order Rate Constants of 2 and 7 at pH 4, 5, 9, 10

	$k \times 10^2$ h^{-1} [a]					
	compd **2**			compd **7**		
pH	k'_{obs}	k'_1	k'_2	k'_{obs}	k'_3	k'_4
4	10.7 ± 0.17	0.70 ± 0.20	0.36 ± 0.06	2.97 ± 0.12	1.55 ± 0.06	1.42 ± 0.06
5	0.45 ± 0.06	0.19 ± 0.02	0.25 ± 0.07	0.55 ± 0.04	0.35 ± 0.03	0.20 ± 0.01
9	0.032 ± 0.009	0.021 ± 0.004	0.012 ± 0.005	0.032 ± 0.004		
10	0.089 ± 0.008		0.071 ± 0.005	0.049 ± 0.01		

[a] Data are means ± SD of three replicates.

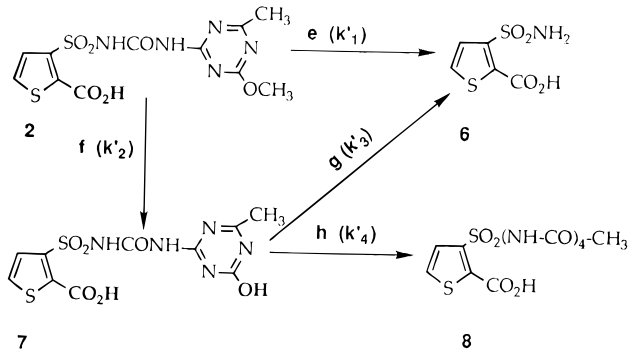


Figure 3. Proposed acidic hydrolysis pathway of thifensulfuron (**2**) in aqueous buffer solutions (pH 4 and 5) at 28 °C (*k'* represents reaction rate constants).

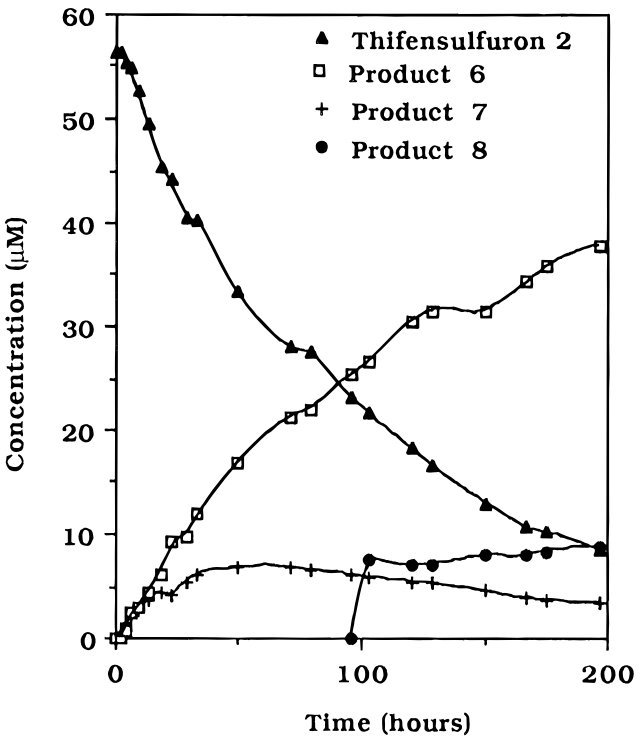


Figure 4. Hydrolysis of thifensulfuron (**2**) in pH 4 buffer solution at 28 °C.

rates of these two compounds, **2** and **7**, were similar. The relatively low hydrolysis rate of thifensulfuron **2** at pH 4 may be related to the pK_a of this compound, as previously explained for **1** and **2**.

Hydrolysis Rate of Thifensulfuron Methyl at pH 9 and 10. The stability of thifensulfuron methyl in alkaline aqueous solutions was pH dependent and followed first-order kinetics (Table 3). However, for this product, we did not observe a contraction of the sulfonylurea bridge as in the case of rimsulfuron (Schneiders et al., 1993), but rather hydrolysis of the ester function (Smith et al., 1990; Cambon et al., 1992). The sole first transformation product found was thifensulfuron **2** (Bastide et al., 1994). This hydrolysis pathway was different from the methyl metsulfuron pathway and could be explained by the difference between thiophene ring and benzene ring activation (Bastide et al., 1994).

Hydrolysis Rate of Thifensulfuron (2) at pH 9 and 10. The two compounds **6** and **7** were obtained by hydrolysis of **2**. In the case of chlorsulfuron and metsulfuron methyl, only the *O*-demethylation reaction was reported (Sabadie, 1991; Reiser et al., 1991). At pH 9 (Figure 5A), the most important pathway was the hydrolysis of the sulfonylurea bridge. At pH 10 (Figure 5B), during a reaction time of <200 h, only **7** appeared. Compound **6** could be formed by subsequent hydrolysis of **7**. To verify this hypothesis, the hydrolysis rates of **7** at pH 9 and 10 were measured, and the *k* values were calculated as 3.2×10^{-4} h^{-1} and 4.86×10^{-4} h^{-1}, respectively. These results partially explain the formation of **6** by hydrolysis of **7** in the hydrolytic reaction of **2**.

DISCUSSION

The stability of thifensulfuron methyl (**1**) in aqueous solution is markedly influenced by pH, and hydrolysis is accelerated by acidic and, to a lesser extent, alkaline conditions. The pH values studied are not necessarily the best choice for correlation with agricultural soils, but some experiments were conducted at pH 6 and 8 with thifensulfuron methyl (**1**), and the hydrolysis rates were low ($t_{1/2} \approx 770$ h at pH 8; $t_{1/2} \approx 2000$ h at pH 6). With these conditions, the hydrolysis pathways were similar to those at others pHs, but it was not possible to accurately determine the rate constant data of these different pathways. Two hydrolysis pathways of thifensulfuron methyl (**1**), observed with acidic conditions, were in agreement with those reported for other sulfonylureas with triazine substituted by a methoxy group (Sabadie, 1990, 1991; Reiser et al., 1991). Two similar hydrolysis pathways were obtained with thifensulfuron **2**. The relative ratio of the two pathways is of the same order of magnitude for the two compounds at pH 4 and 5; the breakdown of the sulfonylurea bridge is only slightly more rapid than the *O*-demethylation reaction.

The hydrolysis of hydroxylated intermediates **4** and **7** also gives two pathways of transformation; they are, hydrolysis of the sulfonylurea bridge and opening of the triazine heterocycle. The latter reaction is pH dependent [k_4(pH 4)/k_4 (pH 5) = 8.4; k'_4 (pH 4)/k'_4 (pH 5) = 7.1 for **4** and **7**, respectively], and the former is less affected by pH [k_3 (pH 4)/k_3 (pH 5) = 3.8; k'_3(pH 4)/k'_3 (pH 5) = 4.4) for **4** and **7**, respectively]. Moreover, the major hydrolysis product of **4** was **5** at pH 4 and **3** at pH 5, whereas **6** was the major product of **7** at pH 5. At pH 4, **7** gave an equal amount of **6** and **8**. In the case of chlorsulfuron and metsulfuron methyl, the selectivity of these different reactions was not pH dependent (Sabadie, 1990, 1991).

The hydrolysis of thifensulfuron methyl (**1**) in alkaline condition is specific to this compound, leading to the

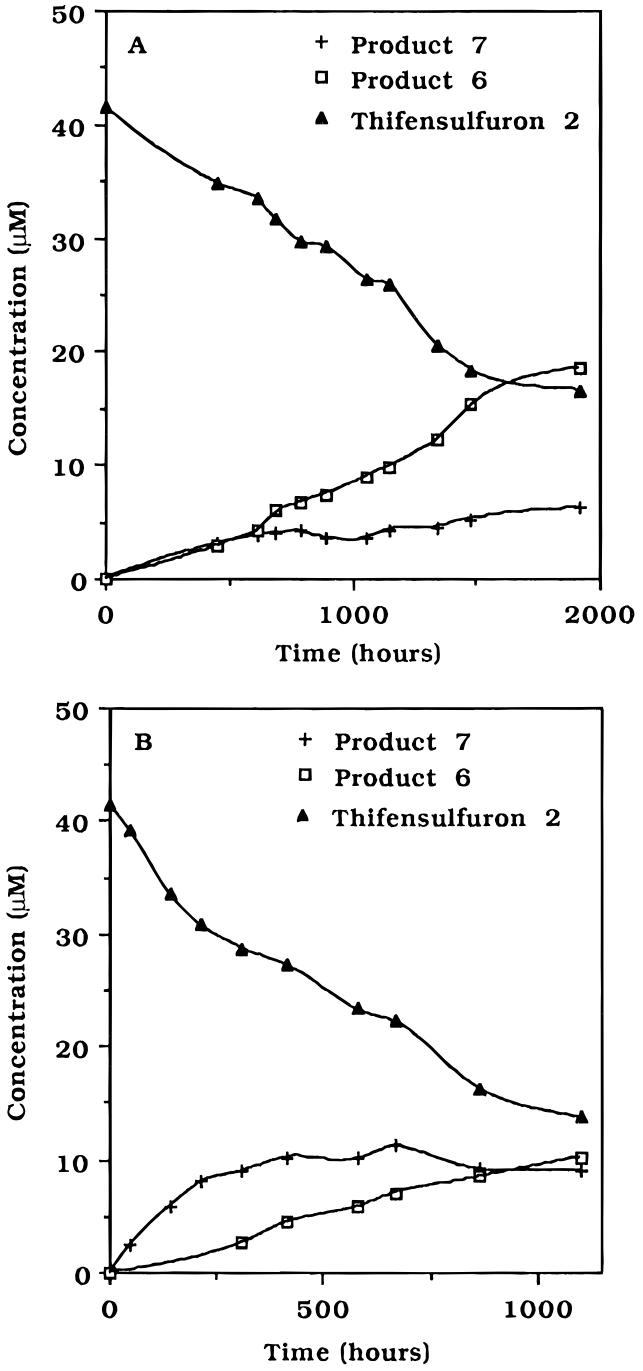


Figure 5. Hydrolysis of thifensulfuron (**2**) in pH 9 (A) and 10 (B) buffer solutions at 28 °C.

corresponding acid. The ester function of other sulfonylurea herbicides was not easily hydrolyzable. The resulting thifensulfuron (**2**) was subsequently slowly hydrolyzed in alkaline conditions. In soil, the first metabolite isolated was thifensulfuron (**2**), produced by biological degradation (Smith et al., 1990; Cambon et al., 1992). Thifensulfuron (**2**) was transformed by chemical processes, and the pathway of transformation could be the same as in aqueous solutions. However, the sole metabolites identified in soil were **6** and **7**. The metabolite **8** was not observed. Either it was not formed or it was easily degraded by microorganisms.

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