Degradation Kinetics of Tolrestat

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Abstract □ Under stressed conditions, hydrolysis of the trifluoromethyl moiety of tolrestat (**1**) to the dicarboxylic acid analogue (**2**) is the major degradation pathway in solution; >C=S bond hydrolysis of the thioamide moiety with formation of the oxo analogue (**3**) is the major solid-state degradation pathway. Rotamerization and degradation reactions in solution occur simultaneously and follow psuedo first-order kinetics. No appreciable buffer effect on the degradation of tolrestat is observed. The pH–rate profile exhibits specific acid catalysis (k_H) and neutral water catalysis (k_o). When tolrestat in solution and solid state is exposed to fluorescent and UV light, degradation reactions generate similar products to those found in the thermal reaction. No oxygen effect on the degradation reaction is observed.

Tolrestat (AY-27,773; *N*-[[6-methoxy-5-(trifluoromethyl)-1-naphthalenyl]thioxomethyl]-*N*-methylglycine; **1**) is an aldose reductase inhibitor.[1] Tolrestat rapidly forms an equilibrium mixture consisting of rotamers A and B in solution (Scheme I).[2] However, tolrestat is very stable in the solid state.

As part of a tolrestat preformulation study, this investigation was initiated to elucidate a general degradation pattern under highly stressed conditions, such as high temperature (70–120 °C), exposure to UV and fluorescent light, and oxygen environment. This work covers isolation and identification of major degradation products and degradation kinetics.

Experimental Section

Materials and Reagents—Tolrestat (**1**), the oxo analogue (**3**), and 6-methoxy-5-trifluoromethyl-1-naphthoic acid (**5**) were obtained from Wyeth-Ayerst Research. All solvents and reagents used were spectrophotometric and reagent grade.

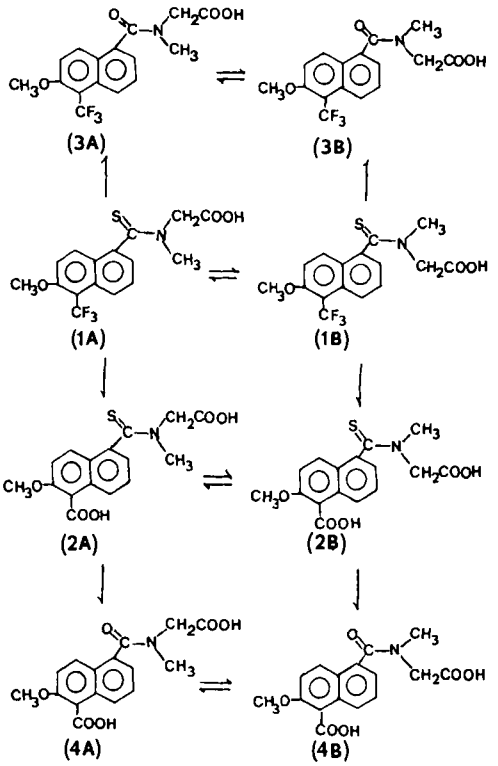
High-Performance Liquid Chromatography Analysis—The HPLC system consisted of a Hewlett-Packard model 1082B LC with a 1040A detector (diode array detector), a 7950B LC terminal, an HP-85 PC, a 82901 flexible disc drive, and a 74470A plotter. This system possesses the capability of instant UV-VIS scanning and storing and retrieving of chromatographic data obtained by monitoring several different wavelengths simultaneously.

The column used had an internal diameter of 150 × 4.6 mm, and was packed with 5-μm Spherisorb ODS reversed-phase material. A flow rate of 1.5 mL/min for the mobile phase (650 mL of 0.05 M KH_2PO_4 solution, 350 mL of CH_3CN, and 5 mL of 1 M tetrabutylammonium hydroxide solution) and a 50-μL injection volume were employed. The variable wavelength detector (1040A Detector) was set at 220 nm.

A once-a-day calibration standard was prepared from 10 mg/mL of stock solution of tolrestat. One milliliter of the stock solution was diluted in 100 mL of methanol, and subsequently 0.5 mL of this solution was diluted with mobile phase to make 10 mL of solution. An appropriate injection volume of the standard solution was chosen to match the amount of sample injected.

After appropriate dilution of the sample with mobile phase solvent, samples were injected into the HPLC system by an autosampler injector. Each peak area was computed automatically by the LC terminal. The column was flushed at the end of each day with water, followed by methanol.

Kinetic Procedures—*Thermal Reaction in Solution*—A stock solution of tolrestat (10 mg/mL) was prepared in methanol and stored in a low actinic volumetric flask. Aliquots taken from the stock solution were diluted with the reaction media to produce final concentrations of 0.01 mg/mL in 0.1 M HCl solution and of 0.2 mg/mL in pH 7.0 phosphate buffer (0.1 M) and 0.1 M NaOH solutions. All sample solutions were purged with nitrogen in 2-mL amber glass ampules and then sealed. Kinetic studies were carried out in constant temperature oil baths at 75, 80, and 95 °C. At designated time intervals, samples were withdrawn. For the reactions in acidic solutions, 1 mL of sample was diluted with 1 mL of mobile phase; for neutral and basic pH systems 1-mL samples were diluted with 4 mL of mobile phase. The diluted samples were subjected to HPLC analysis.



Scheme I

Thermal Reaction in Solid State—Approximately 10-mg portions of tolrestat powder were placed in loosely tightened screw-capped amber glass vials and stored in a 120 °C constant temperature oven. Samples were withdrawn at designated time intervals and dissolved in 3 mL of methanol; after 1 to 200 dilutions were made with the mobile phase, samples were subjected to HPLC analysis.

Photoreaction in Solution—Samples for photodegradation studies were prepared by the same procedures used for solution thermal reaction studies. For the fluorescent light study, samples were sealed in clear glass ampules and exposed to 500 ft-candle of light, where the temperature in the light box was 26 ± 1 °C. For the UV light study, sample solutions were placed in clear glass screw-capped test tubes, immersed in a constant temperature water bath at 25 °C, and exposed to a General Electric sun lamp light (275 W).

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Samples were taken at designated intervals. After appropriate dilutions were made with the mobile phase in a manner similar to that used for the solution thermal reaction studies, samples were subjected to HPLC analysis.

Photoreaction in Solid State—For visible light exposure, tolrestat powder was thinly spread on a petri dish and exposed to 500 ft-candle of light. For UV irradiation, the solid power sample was spread thinly along the wall of a clear screw-capped tube and placed in a constant temperature bath at 25 °C. Then the samples were exposed to a General Electric sun lamp light (275 W). Samples were taken at designated time intervals, diluted appropriately with the mobile phase, and subjected to HPLC analysis.

Oxidative Reaction in Solution—For reactions in alkaline solution, 1 mL of tolrestat stock solution in methanol (10 mg/mL) was diluted to 50 mL with 0.1 M NaOH solution; for reactions in acidic solutions, 100 μL of the stock solution was initially diluted to 1 mL with methanol and then diluted further to 100 mL with 0.1 M HCl solution.

Each of the acidic and alkaline solutions was placed in two amber glass volumetric flasks. One of each pair was purged with oxygen, while the other was purged with nitrogen, stoppered, and sealed with paraffin. The sample flasks were placed in a constant temperature oil bath at 75 °C. Aliquots were taken and appropriate dilutions were made in a manner similar to the thermal reaction in solution. Then, the samples were subjected to HPLC analysis.

Oxidative Reaction in Solid State—Approximately 400 mg of tolrestat powder was placed in a 125-mL Erlenmeyer flask, purged with oxygen for 2 min, and stoppered tightly. The reaction vessel was protected from light and stored at room temperature. Samples were withdrawn at designated time intervals. After each sampling, the reaction vessel was purged with oxygen. Appropriate dilutions were made with mobile phase, after which samples were subjected to HPLC analysis.

Identification of Degradation Products—*Preparation of Solutions*—For the degradation reaction at acidic pH, 40 mg of tolrestat were dissolved in 800 mL of 0.1 M HCl and refluxed for 40 h. For the reaction at neutral and alkaline pH, 3 g of tolrestat were dissolved in 800 mL of pH 7.0 phosphate buffer (0.1 M) and 0.1 M NaOH solution and refluxed for 4 days. The reaction mixture was analyzed by HPLC with scanning of full UV spectra for each peak and spiking with known reference compounds.

Dicarboxylic Acid Analogue (N-[(5-Carboxyl)-6-methoxy-1-naphthalenyl]-thiomethyl-N-methylglycine; 2) of Tolrestat—The solution in 0.1 M NaOH previously refluxed for 4 days at 90 °C was adjusted to pH 4.0 with concentrated HCl and extracted with ether. Rotamers A and B of tolrestat were extracted into the ether layer. The aqueous phase was further acidified to pH 1.0 and extracted with ether. The ether layer was evaporated to dryness, and the resulting residue was triturated in methanol and water and filtered.

Recrystallization of the filtered solid from chloroform:hexane gave off-white crystals, mp 187–188 °C; IR (Nujol): 1720 cm^{-1} (—COOH); UV (H_2O): nm 234 (ε, 47,050), 278 (ε, 13,270), 336 (ε, 3,390); ^1H NMR (acetone-d_6): δ 3.08 (S, 3H, >N—CH_3), 4.00 (S, 3H, —OCH_3), 4.6–5.6 (d, 2H, >N—CH_2), and 7.10–8.35 ppm (m, 5H, naphthyl protons); MS: *m/e* 333 (M^+), 288 [M^+—COOH), 245 [M^+—$N(CH_3)CH_2COOH$].

Anal.—Calc. for $C_{16}H_{15}NO_5S$ (molecular formula for dicarboxylic acid analogue): C, 57.65; H, 4.54; N, 4:20; S, 9.62. Found: C, 57.51; H, 4.52; N, 4.04; S, 9.84.

Oxo Analogue (N-[[6-Methoxy-5-(trifluoromethyl)-1-naphthalenyl]oxomethyl]-N-methylglycine; 3) of Tolrestat—Three grams of tolrestat were heated in the solid state at 120 °C for 10 days. The sample was dissolved in methanol, the methanol was evaporated to dryness, and the residue was dissolved in ether. The ether solution was extracted with 0.1 M NaOH solution. The aqueous phase was subjected to a series of ether extractions by stepwise pH adjustments of the aqueous phase to pH 6, 5, 4.5, and 4.0. Ether extracts were collected from the aqueous layer, which was adjusted to pH 4.0. The combined ether extracts were washed with small portions of pH 4.0 water and then evaporated to dryness. The residue was recrystallized from chloroform:hexane. The UV, IR, and NMR spectra of the sample and authentic oxo analogue reference compound were shown to be identical to each other.

6-Methoxy-5-trifluoromethyl-1-naphthoic Acid (5)—From the previous isolation procedure for the oxo analogue of tolrestat (3), the ether extract fractions were combined from aqueous layers adjusted to pH 6.5 and 4.5. Then, the ether was evaporated to dryness. The residue was dissolved in chromatographic mobile phase and then subjected to HPLC analyses with scanning of full UV spectra for each peak and spiking with the known reference compound. The sample and authentic reference compound were shown to be identical to each other.

Results and Discussion

Degradation Reaction in Aqueous Solution—*Identification of Degradation Products*—A typical HPLC chromatogram for the degradation reaction in 0.1 M NaOH at 90 °C for 3 days is shown in Figure 1. Identification of peaks **1A** and **1B** is based on comparisons of retention times and on-line UV spectra with authentic tolrestat compound. Peaks **1A** and **1B** are identified as rotamers **A** and **B** of tolrestat. Rotational isomers of tolrestat in solution were characterized by NMR in a previous report.[2]

Product 2, corresponding to peaks **2A** and **2B**, was isolated and subjected to structural analyses. The structural information obtained from IR, NMR, UV, MS, and elemental analyses data is sufficient to conclude that products **2A** and **2B** are hydrolyzed products of the trifluoromethyl group of rotamers **1A** and **1B**: [*N*-(5-carboxyl)-6-methoxy-1-naphthalenyl]-thiomethyl-*N*-methylglycine (**2A,B**).

A minor product, peaks labeled as **3A** and **3B** (Figure 1), is present in quantities too small to take on-line UV spectra. However, when the sample was spiked with the authentic oxo analogue, peaks **3A** and **3B** of the sample were precisely overlaid with those for the spiked authentic oxo analogue, confirming **3A** and **3B** as rotamers A and B of the oxo analogue.

A typical HPLC chromatogram for the reaction in 0.1 M HCl at 90 °C for 18 h is shown in Figure 2. The major degradation product peak at a retention time of 1.5 min is confirmed as product 2 (dicarboxylic acid analogue), and a few unknown minor peaks are shown at retention times around 1–2 min. The first eluting peak (4) has on-line absorption maximum at 235 nm, which is similar to product 2. Thus, product 4 is tentatively assigned as the oxo-dicarboxylic acid analogue based on its HPLC retention time being very close to that of the dicarboxylic acid analogue and its UV characteristics. No further attempt was made to characterize the products **4A,B** since they were present in quantities of <1% each. Structures of the rotamers of 1 to 4 are shown in Scheme I.

Reaction Order and Rate Constants—As shown in Figure 3, semilogarithmic plots of the percent total tolrestat (**1A** and **1B**) remaining versus time have a linear relationship (r = 0.99) for 80% of degradation. This indicates that degradation of total tolrestat follows pseudo first-order kinetics at constant pH and temperature conditions. After the rapid rotational equilibrium between **1A** and **1B** is attained, rotamers **1A** and **1B** decompose to other products with about the same degra-

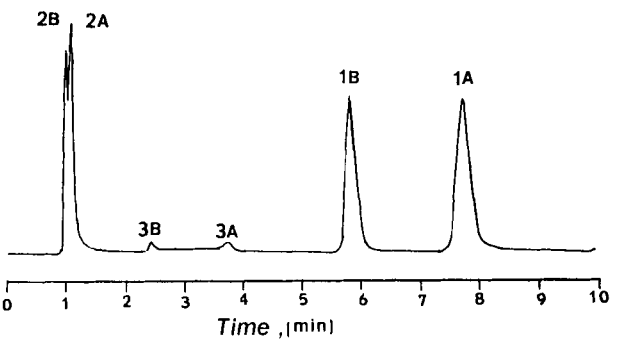


Figure 1—A typical HPLC chromatogram for the degradation reaction of tolrestat in 0.1 M NaOH at 90 °C for 3 days. Key: **1A** and **1B**, rotamers **A** and **B** of tolrestat; **2A** and **2B**, rotamers **A** and **B** of the dicarboxylic acid analogue; **3A** and **3B**, rotamers **A** and **B** of the oxo analogue.

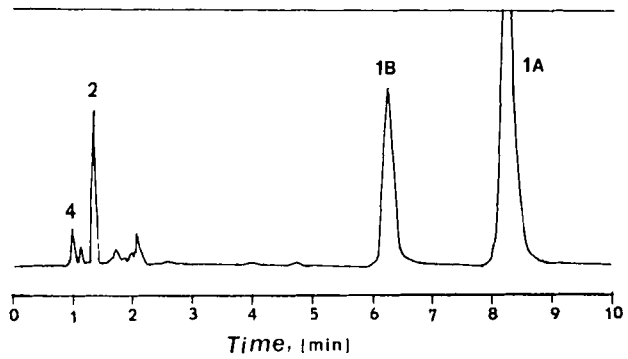


Figure 2—A typical HPLC chromatogram for the degradation reactions of tolrestat in 0.1 M HCl at 90 °C for 18 h. Key: **1A** and **1B**, rotamers **A** and **B** of tolrestat; **2**, dicarboxylic acid analogue; **4A** and **4B**, oxodicarboxylic acid analogue.

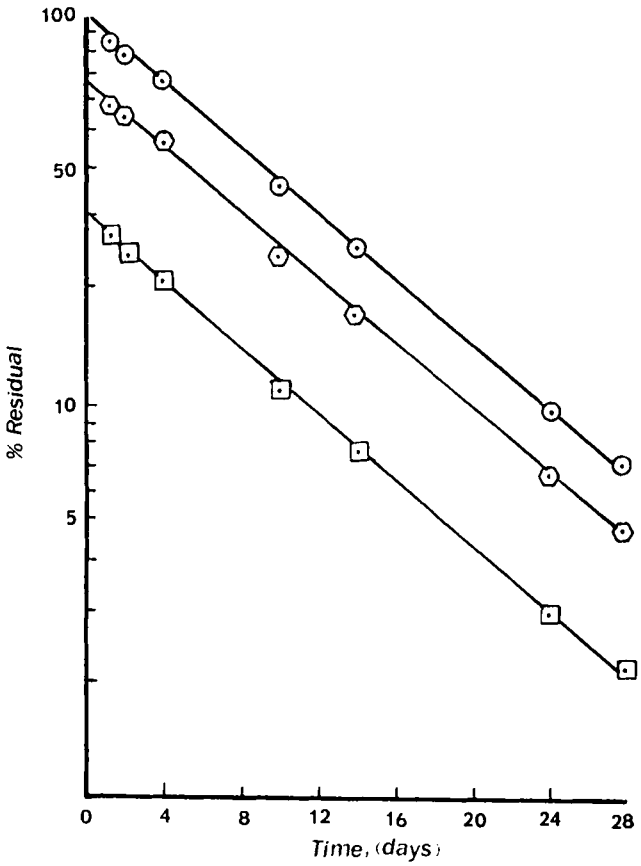


Figure 3—First-order plots for the degradation reaction of tolrestat (1) in 0.1 M HCl at 80 °C. Key: (⊙) total tolrestat (**1A** and **1B**); (⊙) rotamer **A** of tolrestat (**1A**); (⊡) rotamer **B** of tolrestat (**1B**).

dation rate: apparent first-order rate constants for the disappearance of total tolrestat concentration (k_{app}) at pH 13 and at 80 °C are 8.881×10^{-7} s^{-1} for **1A**, 8.451×10^{-7} s^{-1} for **1B**, and 8.713×10^{-7} s^{-1} for total tolrestat.

In comparison, the k_{app} for rotamerizational equilibrium between **1A** and **1B** at equivalent conditions is 1.353×10^{-4} s^{-1}; thus, the rotamerizational equilibrium reaction[2] is ~200 times faster than the degradation reaction (1 → 2 + 3).

Catalytic Effect of Buffer Systems—The catalytic effect of buffer systems used in the kinetic studies (acetate, phosphate, and carbonate buffers) was determined at constant pH, temperature, ionic strength, and concentration of tolrestat; only the buffer concentration varied. No appreciable effect on the degradation of tolrestat was observed for any buffer species used in this study.

Logarithmic Rate–pH Profile—The log k_{app}–pH profiles for degradation of total tolrestat at various temperatures are shown in Figure 4. The pa_H values of HCl solutions were calculated from the mean activity coefficient obtained or extrapolated from the literature data.[3,4] In the rate–pH profile, the k_{app} at pH values <2.0 decreased linearly with increasing pH, with a slope of about unity, suggesting that a specific acid-catalytic reaction is important. The plateau region at pH values >3.0 suggests that a neutral water catalysis is significant. Thus, the profiles were fitted to the following relationship:

$$k_{app} = k_o + k_H a_H \quad (1)$$

where k_o is the first-order rate constant for neutral water catalysis, k_H is the second-order rate constant for specific acid catalysis, and a_H is the activity of hydrogen ion.

The k_o and k_H values were estimated from the best fit of rate–pH profiles; results are shown in Table I. In Figure 4, the line represents the theoretical curve calculated by substituting microscopic constants into eq 1, while the points show experimental results. The reasonable agreement indicates that eq 1 adequately describes the kinetics of solvolytic tolrestat degradation.

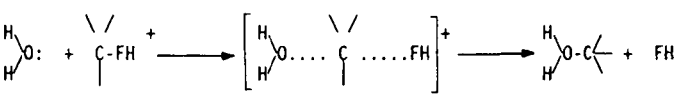
Acid catalysis has been observed in the solvolysis of benzyl,[5] *t*-butyl, *t*-amyl, and cyclohexyl fluorides.[6] Alkyl chlorides, bromides, and iodides rarely show acid catalysis, although a few examples have been observed.[7] The rate-determining steps (r.d.s.) proposed were direct reaction with hydronium ion to give a carbonium ion (A-1 mechanism; eq 2) or reaction of a hydrogen-bonded halide:hydronium ion complex with solvent (A-2 mechanism; eq 3):[8]

$$RF + H_3O^+ \xrightarrow{\text{r.d.s.}} R^+ + HF + H_2O \quad (2)$$

$$H_2O + RF \ldots . H_3O^+ \xrightarrow{\text{r.d.s}} ROH_2^+ + HF + H_2O) \quad (3)$$

Figure 4 shows that the rate of hydrolysis is proportional to the activity of hydronium ion. The slope of a plot of the logarithm of the rate against pa_H is shown to be 1.1 (r = 0.99). Arrhenius plots for the hydrolysis rate of the trifluoromethyl group are shown in Figure 5. The data give linear plots of log k_{app} versus $1/T$: for the uncatalyzed reaction (k_o), E_a = 27 kcal/mol and $\Delta S^{\ddagger}$ = −13 eu at 80 °C; for the acid-catalyzed reaction (k_H), E_a = 28 kcal/mol and $\Delta S^{\ddagger}$ = −2 eu at 80 °C.

For the acid-catalysis reaction, the rate-determining step may be the water attack-to-protonated substrate step, as in the A-2 mechanism. While the $\Delta S^{\ddagger}$ value for the acid-catalyzed reaction is closer to the absolute values for the A-1 than the A-2 reaction, the fact that the −2 eu value is intermediate between the A-1 and A-2 mechanisms requires some comment. Borderline behavior can be explained as due to concurrent operation of S_N1 and S_N2 mechanisms (Scheme II). According to the merged mechanism, borderline behavior is the result of a degree of bond formation to the nucleophile which is not as advanced as is bond breaking to the leaving group, but which is significant enough to prevent formation of an intermediate carbonium ion. This may be due to a uniquely



Scheme II

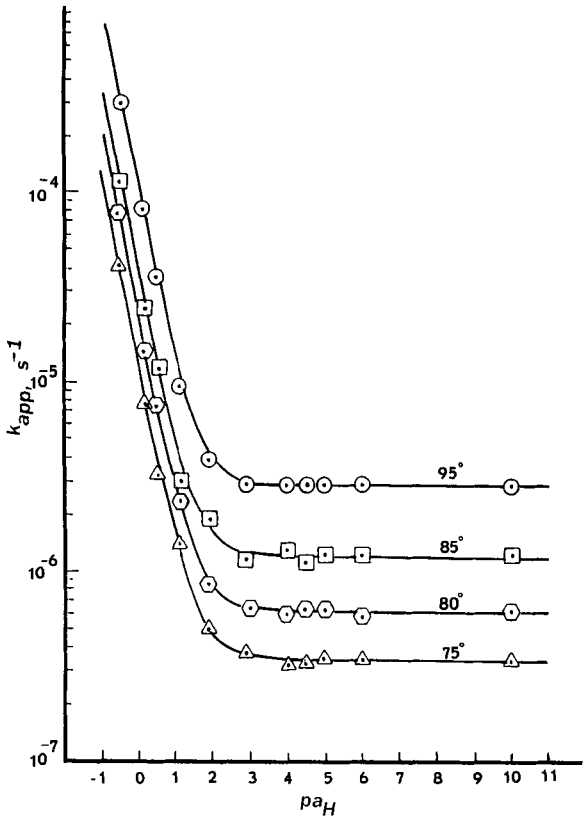


Figure 4—Log k_{app}–pH profiles for the tolrestat degradation reaction at various temperatures, where points are experimental and solid lines represent the theoretical curve generated by eq 1.

Table I—Microscopic Rate Constants and Arrhenius Parameters for Solvolytic Degradation of Tolrestat

Temperature, °C	Microscopic Rate Constant	
	k_o, s^{-1}	k_H, M^{-1} s^{-1}
95	2.90×10^{-6}	1.09×10^{-4}
85	1.23×10^{-6}	3.86×10^{-4}
80	6.32×10^{-7}	2.17×10^{-5}
75	3.49×10^{-7}	1.18×10^{-5}
25	5.20×10^{-10}[a]	1.28×10^{-8}[a]
Arrhenius Parameter		
ln A	24.28	29.47
E_a, kcal/mol	27.03	28.21
$\Delta S^{\ddagger}$, eu at 80 °C	−12.61	−2.30

[a] The values at 25 °C were obtained by extrapolation.

higher degree of solvation in the conjugated acid, or it may be that in the A-1 mechanism there is restriction of rotation about the breaking bond in the transition state. The uncatalyzed reaction (k_o) apparently follows the S_N2 displacement mechanism. The remaining two fluorines may be consecutively hydrolyzed and finally the dehydrated product (2) can be obtained.

Degradation Profile in Solution—A typical concentration–time profile for the degradation of tolrestat and the formation of degradation products 2 and 3 in pH 4.5 acetate buffer solution at 80 °C is presented in Figure 6. After the rapid rotamerizational equilibrium is attained (**1A** ⇄ **1B**), rotamers **1A** and **1B** degrade at the same rate. The decrease of **1A** and **1B** is accompanied by an increase in the dicarboxylic acid analogue (**2A,B**), and the oxo analogue (**3A,B**) rises gradually. Results of NONLIN curve fitting to the experimental data show that the first-order rate constants for product 2 (k_{12})

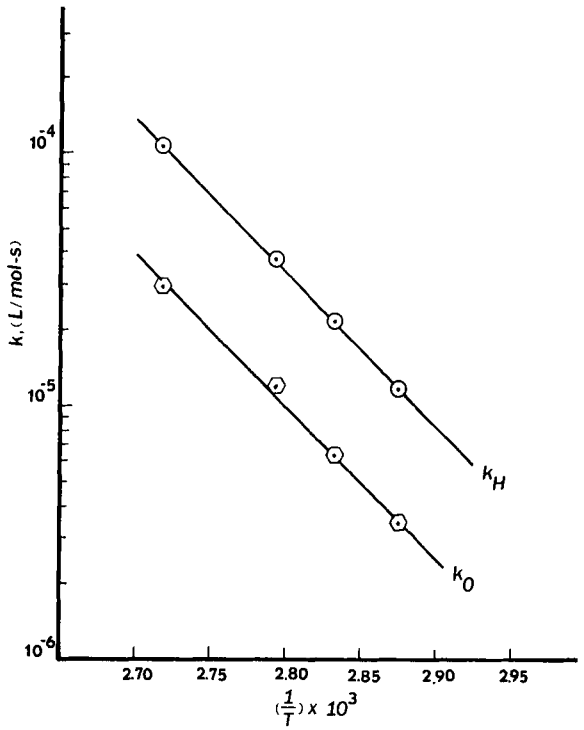


Figure 5—Arrhenius plots for the degradation reaction of tolrestat.

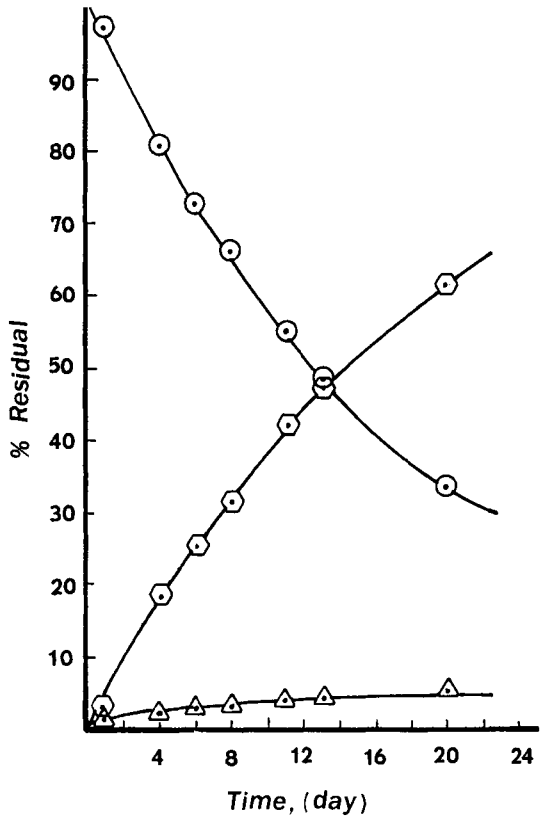
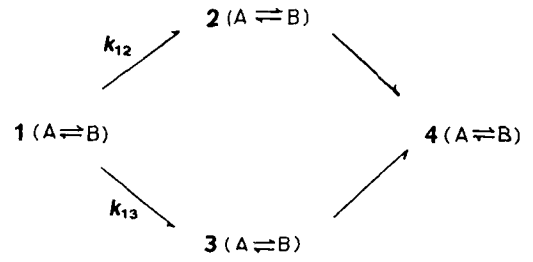


Figure 6—Degradation profile of tolrestat in pH 4.5 acetate buffer solution at 80 °C. Key: (⊙) **1A,B**; (⊙) **2A,B**; (△) **3A,B**.

and product 3 (k_{13}) are $(5.784 \pm 0.049) \times 10^{-7}$ and $(5.238 \pm 0.239) \times 10^{-8}$ s^{-1}, respectively. This profile indicates two parallel first-order reactions, as shown in Scheme III.

A rotamer interconversion kinetics study of **1A** and **1B** was carried out previously.[2] However, no attempt was made to



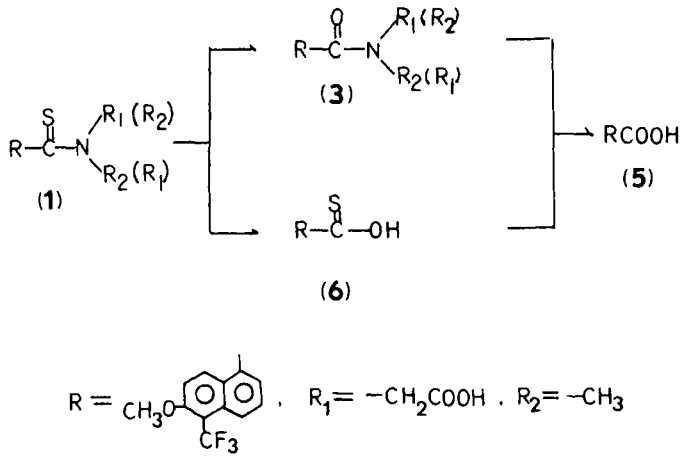
Scheme III

follow the rotamerizational kinetics of **2A** and **2B**, **3A** and **3B**, and **4A** and **4B**. There was no major difference in degradation reaction pattern found under alkaline and neutral pH conditions: major product **2** (rotamer **A** and **B**) and minor product **3** (rotamer **A** and **B**) form as degradation products. However, under acidic reaction conditions, some unknown minor products (peaks at retention times ~1–2 min in Figure 2) were formed as the reaction progressed further. No further attempt was made to characterize them since each was present in amounts of <1%. Evidently, the major degradation pathway is hydrolysis of the trifluoromethyl group at all three different pH conditions.

In addition to hydrolysis of the trifluoromethyl group, hydrolysis of the thioamide group may occur by two parallel reaction pathways, as represented in Scheme IV.[9] In the course of thioamide group hydrolysis, two bonds, the C—N and C—S bonds, must be broken. Depending on which bond is broken first, two intermediates in the reaction, **3** and **6**, are possible.

Hydrolysis of the >C=S bond to form the oxo analogue (**3**) is a very slow process compared with hydrolysis of the trifluoromethyl group: the [3]:[2] ratio in the reaction mixture of 0.1 M NaOH at 80 °C for 21 h is 0.05. The products corresponding to the oxo analogue (**3**) and naphthoic acid (**5**) were not detected at given reaction conditions of pH 7 and 13. However, under acidic reaction conditions, the possibility of forming the corresponding thionaphthoic acid, product **6**, may exist. No definite conclusion can be made as to whether any of the unknown peaks represents these hypothesized products.

The apparent first-order rate constant for the degradation reaction of the oxo analogue (**3**) to the oxo-diacid analogue (**4**) in 0.1 M NaOH at 95 °C was independently determined to be 1.59×10^{-6} s^{-1}, whereas the k_{app} value for degradation of total tolrestat (**1** → **2** + **3**) at equivalent conditions was 3.25 $\times 10^{-6}$ s^{-1}. The rate of trifluoromethyl group hydrolysis is not affected by thioamide or oxoamide groups.



Scheme IV

Fluorescent Light Effect—Exposure to 500 ft-c of light at room temperature (26 ± 1 °C) for 7 weeks yielded products **2** and **3** as the major degradation products formed under acidic and basic conditions. Product **2** was the only major degradation product formed under neutral conditions; product **3** was not observed at neutral conditions.

After rapid rotational equilibrium is attained, tolrestat rotamers **A** and **B** degrade at the same rate. Zero-order kinetics at neutral and alkaline conditions are followed after a 5-day induction period, with half-lives of 4.7 and 4.2 weeks, respectively. However, the zero-order plot for the acidic pH condition (0.1 M HCl) does not maintain linearity after a half-life of 6 weeks. This suggests that an autocatalytic reaction occurs with one of the degradation products. Overall, fluorescent light degradation patterns are similar to those of thermal degradation reactions, except the formation of the oxo analogue (**3**) is more prominent.

Ultraviolet Light Effect—Under the condition of a UV lamp (275 W) at 25 °C for 18 h, products **2** and **3** are found as major products at all three pH (1, 7, and 13) conditions. The reactions at pH 7 and 13 follow zero-order kinetics with half-lives of 19 and 30 h at pH 7 and 13, respectively. However, the reaction at pH 1.0 does not follow zero-order kinetics; rather, it follows first-order kinetics with a half-life of 19 h.

Tolrestat has strong UV absorption at 226, 277, and 337 nm, but no visible absorption. Apparently, the efficiency of UV light in producing a photodegradation reaction is higher than that of the fluorescent light source; the percent degradation decreased with increasing wavelength. Although product **3** (oxo analogue) forms only as a minor product in the thermal reaction (protected from light), it is one of the major degradation products in photodegradation reactions. Higher efficiency of oxo analogue (**3**) formation may be due to the fact that a conjugated UV absorbing chromophore of the thioketo group becomes a part of the reactive excited state under photoexcitation.

Oxygen Effect—Both 0.1 M HCl and 0.1 M NaOH solutions of tolrestat purged with nitrogen and oxygen were studied at 75 °C. After rapid rotational equilibrium is attained, disappearance of tolrestat rotamers **A** and **B** is shown to occur at the same rate, regardless of whether the samples were purged with nitrogen or oxygen. These results indicate that the oxidative degradation reaction of tolrestat should be negligible.

Degradation Reaction in Solid State—*Identification of Degradation Products*—A typical HPLC chromatogram for tolrestat degradation in solid state at 120 °C for 10 days is shown in Figure 7. Peaks **1A** and **1B** are identified as rotamers **1A** and **1B**, based on the comparison of the retention times and on-line UV spectra of the sample with those of authentic compound. Rotamerization of tolrestat in the solid state may not occur under normal conditions.[2] Even at 120 °C, no rotamer **B** (**1B**) formation was observed until 3–4 days of heating; subsequently, a small amount of **1B** appeared in the reaction mixture when the solid began to partially melt. Slow surface degradation of tolrestat powders apparently caused the phase to change to a lower melting solid solution. Solid tolrestat **1A** might be dissolved in a lower melting liquid phase of a degradation product and then rotamerized.

Minor product peaks at **2A** and **2B** are confirmed as rotamers **A** and **B** of the dicarboxylic acid analogue, based on retention time and on-line UV spectra of the sample and authentic compound.

A series of ether extractions from aqueous solutions by gradual change of aqueous pH afforded isolation of products **3A** and **3B**. The UV, IR, and NMR spectra of the sample and

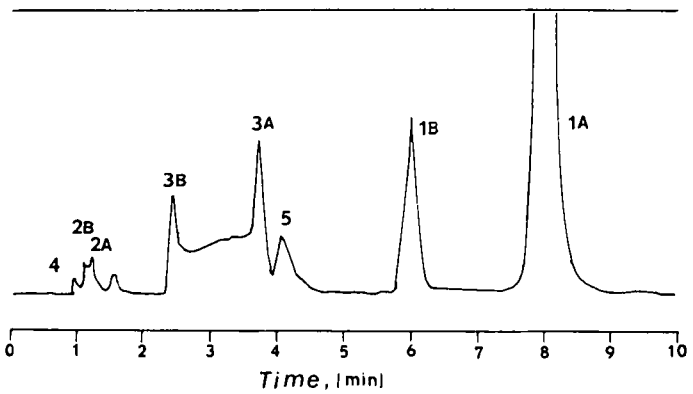


Figure 7—Typical HPLC chromatogram for the degradation reaction in solid state at 120 °C for 10 days. Key: **1A** and **1B**, rotamers **A** and **B** of tolrestat; **2A** and **2B**, rotamers **A** and **B** of dicarboxylic acid analogue; **3A** and **3B**, rotamers **A** and **B** of oxo analogue; **4A** and **4B**, rotamers A and B of oxo-dicarboxylic acid analogue; **5**, naphthoic acid analogue.

authentic oxo analogue are matched to each other to confirm the structure of tolrestat oxo analogues (3A and 3B).

Minor peak 4 has an on-line UV spectrum similar to that of product 2. Based on this result, product 4 is tentatively assigned as the oxo-dicarboxylic acid analogue (4A,B).

Although peak 5 is absent in the solution degradation reaction, it is shown to be present in the solid degradation reaction sample at 120 °C. Peak 5 is confirmed as the corresponding naphthoic acid (5) from the data of HPLC retention time and UV spectrum of the sample and authentic compound.

Degradation Profile in Solid State—The degradation profile at 120 °C is shown in Figure 8. The major degradation products are found to be the oxo analogue (3A,B) and corresponding naphthoic acid (5) of tolrestat. Formation of 1B may be due to the phase change to liquid during the reaction. During the course of the degradation reaction, a mercaptan odor was produced. In an attempt to identify these odor-causing compounds, gas chromatographic (GC) and GC/MS analyses were performed on the head space over a decomposed sample in a sealed Teflon-capped vial stored at 135 °C for 24 h.[10] The volatile products were identified as carbonoxy sulfide

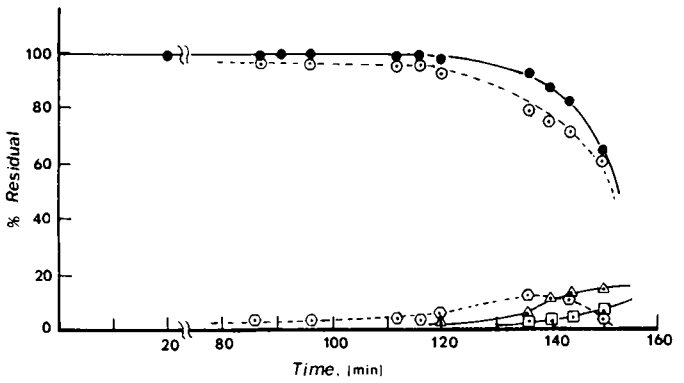
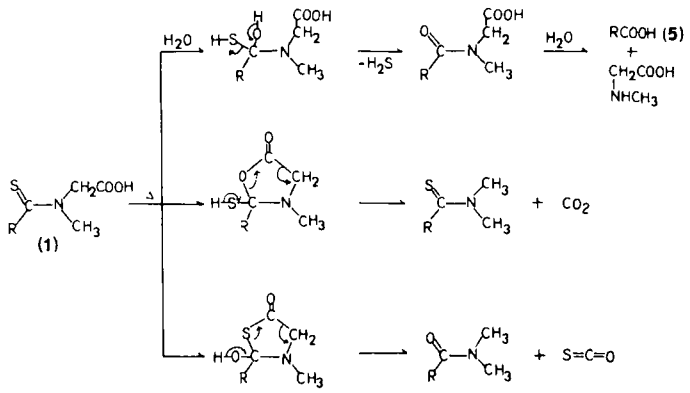


Figure 8—Tolrestat degradation time profile in solid state at 120 °C. Key: (●) **1A,B**; (○) **1A**; (△) **1B**; (◇) **3A,B**; (□), naphthoic acid analogue (**5**).



Scheme V

(COS), H_2S, methylsulfinic acid (CH_3SOH), and CO_2. Based on these product formations, the solid-state thermal degradation pattern is postulated as shown in Scheme V.

Photodegradation Reaction in Solid State—After about a 4-week induction period, photodegradation reactions of tolrestat samples exposed to both UV sun-lamp (275 W) and 500 ft-c of light at 25 °C are followed by zero-order kinetics. Photoreactivity of tolrestat in solid state is substantial; samples of tolrestat exposed to fluorescent and UV light degrade with half-lives of 14 and 17 weeks, respectively, while those stored and protected from light at room temperature for 2 years show practically no degradation.[11]

During the photodegradation reaction, rotamer **1A** in the solid state does not convert to rotamer **1B**. However, rotamers A and B of the oxo analogue (3) and of the dicarboxylic acid analogue (2) are shown as major products. Reasons for formation of rotamers B of 2 and 3 are unknown.

Oxygen Effect in Solid State—The tolrestat powder samples under oxygen environment at room temperature for 4 weeks show no degradation by HPLC analysis.

References and Notes

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