Kinetics and Mechanism of Hydrolysis of Efavirenz

Michael B. Maurin,[1,4] Susan M. Rowe,[1] Karl Blom,[2] and Michael E. Pierce[3]

Received November 23, 2001; accepted January 29, 2002

Purpose. To determine the kinetics and mechanism of hydrolysis of efavirenz [(S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one] in aqueous solutions.

Methods. The solution stability was examined at 60°C and an ionic strength of 0.3 M over the pH range of 0.6 to 12.8. The loss of efavirenz and the appearance of degradants were followed with a reverse-phase high-performance liquid chromatography assay. Characterization of the degradants was accomplished with liquid chromatography-mass spectrometry.

Results. The degradation of efavirenz followed apparent first-order kinetics over the pH range of 0.6 to 12.8 at 60°C. The catalytic effect of phosphate and borate buffers was negligible while acetate and citrate demonstrated buffer catalysis. The overall rate constant indicated a pH minimum (the pH of maximum stability) of approximately 4. Mass spectra data identified a degradant with a molecular weight consistent with hydrolysis of the cyclic carbamate to the corresponding amino alcohol. The degradation route was confirmed with spiking experiments with an authentic sample of the amino alcohol indicating that the carbamate hydrolysis pathway was the predominant reaction throughout the pH range studied. Subsequent degradation of the amino alcohol proceeded at the extremes of the pH range studied via rearrangement to the quinoline.

Conclusions. The pH-rate profile was consistent with a combination of a V-shaped profile in the pH range of 0–9 and a sigmoid-shaped profile in the pH range of 4–13. The plateau that began at pH 10–11 is a result of the ionization of the amine of the carbamate inhibiting the base-catalyzed hydrolysis of efavirenz, given that the ionized form of the carbamate is resonance-stabilized toward hydroxide-catalyzed degradation. Thus, increasing the pH resulted in a parallel decrease in the unionized fraction and increase in hydroxide ion concentration resulting in a constant k_{obs} value.

KEY WORDS: efavirenz; solution stability; carbamate hydrolysis.

INTRODUCTION

Efavirenz [(S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one; Fig. 1] is an HIV-1 reverse transcriptase inhibitor approved in combination with other antiretrovirals for the treatment of HIV-1 infection (1). HIV-1 reverse transcriptase catalyzes the replication of viral RNA to render double-stranded DNA and thus is a crucial element in the viral replication process (2,3). In head-to-head comparative clinical trials, efavirenz, in combination with two nucleoside analog reverse-transcriptase inhibitors, has demonstrated superior effectiveness to protease inhibitors plus nucleosides (4,5).

Following a once-daily dosing of efavirenz in combination with AZT and 3TC (each dosed twice daily), 64% of antiretroviral naïve patients achieved maximal HIV-RNA suppression (defined as less than 50 viral copies per mL of plasma) by intent-to-treat analysis at week 48 (4). This group had an average CD4 cell count elevation from baseline of 201 cells/mm^3. This compared to 43% for the control arm of indinavir (three times daily) plus AZT and 3TC.

The physical chemical properties of efavirenz were described previously (6). It is a crystalline nonhygroscopic lipophilic (log octanol water partition coefficient of 5.4) material with an aqueous solubility of 9.2 μg/mL (pH 8.7) at 25°C. The aqueous solubility increased as the pH increased above 9, consistent with the loss of the proton on the amine of the carbamate. As part of the pharmaceutical development of an oral liquid dosage form for use in pediatrics or in adults with difficulty swallowing, a thorough understanding of the solution stability is tantamount to the development of a dosage form with maximal chemical stability. The objectives of this study were to evaluate the aqueous stability of efavirenz as a function of pH and buffer species and to identify the degradation mechanism.

MATERIALS AND METHODS

Materials

Efavirenz (lot SB706-001) and its degradants were prepared by Chemical Process R&D (7,8) and were used as received. The water was house-deionized water that was passed through a Nanopure II (Barnstead) ion-exchange cartridge system and had a specific resistance of greater than 17 MΩ-cm. All solvents were high-performance liquid chromatography (HPLC) grade. All other reagents were of analytical grade.

Solution Kinetics

A 500-μg/mL stock solution of efavirenz was prepared in methanol. The stock solution was diluted 100-fold with buffer. The methanol dilution in buffer was prepared in triplicate. The buffers had a constant ionic strength of 0.3 M, which was maintained with the addition of potassium chloride and based on calculation at 60°C. The electrode was calibrated at 60°C for pH measurement. At the extremes of pH, hydrochloric acid at pH 0.6, 1.0, and 2.1 and sodium hydroxide at pH 11.9 and 12.8, provided sufficient buffer capacity. However, buffers were necessary to maintain the pH over the range of 3.0–9.2. The buffer systems included citrate at pH 3.0, 3.5, and 4.5, acetate at pH 4.0 and 5.0, phosphate at pH 6.5 and 7.4, and borate at pH 9.2. All buffers were evaluated at three buffer concentrations to determine the catalytic effects of the various buffers and to enable extrapolation to zero buffer concentration at any given pH. The buffer was added at room temperature because the samples reached 60°C after placement into the stability oven (Model LDX1-42, Despatch Industries, Inc.). For those pH conditions where degradation was rapid, preheated buffer was added to the sample. The

[1] Pharmacy R&D, DuPont Pharmaceuticals Company, Wilmington, Delaware 19880-0400.
[2] Chemical and Physical Sciences, DuPont Pharmaceuticals Company, Wilmington, Delaware 19880-0400.
[3] Chemical Process R&D, DuPont Pharmaceuticals Company, Deepwater, New Jersey.
[4] To whom correspondence should be addressed. (e-mail: b.maurin@dupontpharma.com)

0724-8741/02/0400-0517/0

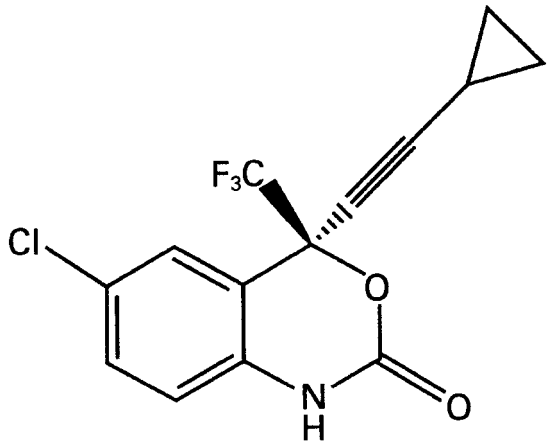

Fig. 1. The chemical structure of efavirenz.

solution was pipetted into 2-mL glass vials (Serum vials 621130-2, Kimble Glass), sealed with teflon-faced stoppers (4416-50, West Company), and placed upright into storage boxes to protect the compound from light. At appropriate intervals, samples were removed from the oven and cooled to room temperature. For pH 9.2 to 12.8, where the rate of degradation was rapid, the sample was immediately quenched with acetonitrile 60% (v/v) in 0.05 M citrate buffer pH 3 and cooled to room temperature.

Chromatographic Methods and Liquid Chromatography–Mass Spectrometry

Concentrations were measured with an isocratic HPLC method. Separation was achieved with a Zorbax Rx-C_8 column 25 cm × 4.6 (MacMod Chromotography), with the temperature maintained at 35°C (Column Heater Module and Temperature Control Module, Waters Chromatography). The mobile phase was composed of acetonitrile 60% (v/v) in trifluoroacetic acid 0.05% (v/v) in water. A flow rate of 1.4 mL/min was used (HPLC Pump, Model 590, Waters Chromotography). Ultraviolet detection was used at 250 nm (HP1050 Series Variable Wavelength Detector, Hewlett-Packard). Data acquisition was completed with a VAX-based program that calculated the sample concentration from a standard curve using peak area (Multichrom®, Fisons Instruments). The standards were prepared before each analysis.

The degradant was characterized with LC-MSI with ESI (Finnigan MAT TSQ7000 LC-MS-MS & HP 1090 LC VG 70-VSE High Resolution Mass Spectrometer). Separation was achieved with a Zorbax SB-C_{18} 15 cm narrow bore column (Mac Mod Chromotography) with the temperature maintained at 45°C. The mobile was composed of acetonitrile 70% (v/v) in acetic acid 0.1% (v/v) in water. A flow rate of 0.25 mL/min was used. Confirmatory studies employed authentic standards to match HPLC retention times.

RESULTS AND DISCUSSION

The degradation of efavirenz followed apparent first-order kinetics. The apparent first-order degradation of efavirenz at a constant ionic strength of 0.3 M and 60°C is provided as a function of pH in Fig. 2a–b. The disappearance of efavirenz (E) from solution can be described with the following equation;

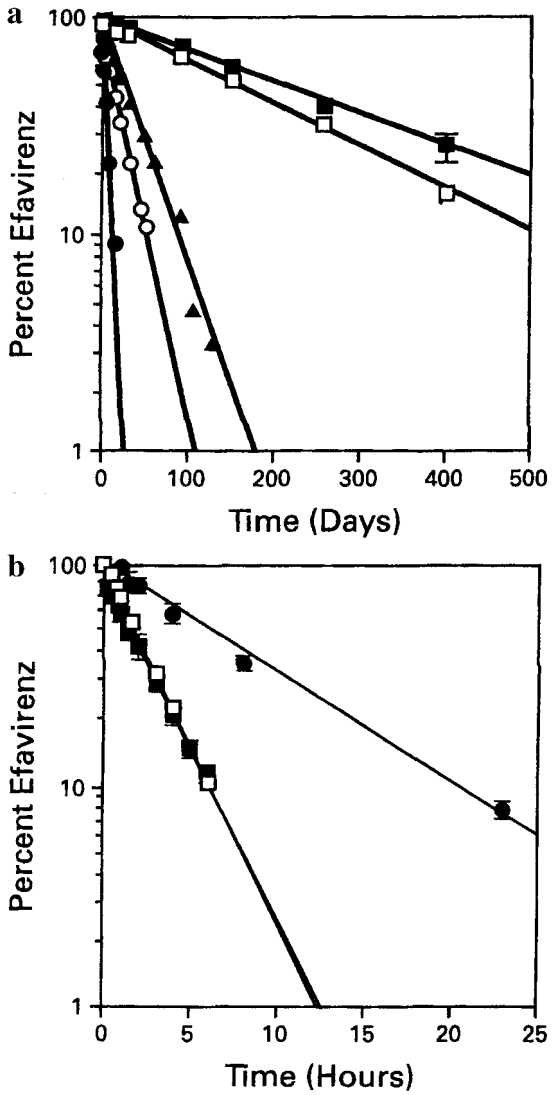

Fig. 2. The apparent first-order degradation of efavirenz at a constant ionic strength of 0.3 M and 60°C is provided as a function of pH. Data points and errors bars are the mean and standard deviation, respectively, of the three solution stability replicates at each time point. The degradation profiles are 0.1 M acetate, pH 4.0 (■); 0.1 M acetate, pH 5.0 (□); 0.1 M phosphatem pH 6.5 (▲); 0.1 N HCl, pH 1.0 (○); and 0.1 M phosphate, pH 6.5 (●) in (a) and 0.1 M borate, pH 9.24 (●); 0.01 N NaOH, pH 11.9 (□); and 0.1 N NaOH, pH 12.8 (■) in (b).

$$\frac{dE}{dt} = k_{obs}[E] \quad (1)$$

where dE/dt is the rate of efavirenz degradation, k_{obs} is the observed first order rate constant, and [E] is the efavirenz concentration. The rate constants were calculated with least square regression analysis and are provided in Table I. The rate constant values are presented as the mean and standard deviation of the three replicates.

Under those conditions where buffer catalysis was observed (pH 3.0–5.0), the observed rate constants were extrapolated to zero buffer concentration to permit the determination of k′. For those conditions where buffers were employed and no buffer catalysis was observed (pH 6.5–9.2), the mean value for the rate constant for the three buffer concentrations

Table I. Solution Stability of Efavirenz at 60°C

Buffer	k_{obs} (day^{-1})[a]	
0.3 N hydrochloric acid pH 0.6	0.106	(0.001)
0.1 N hydrochloric acid pH 1.0	0.0540	(0.0016)
0.01 N hydrochloric acid pH 2.1	0.00745	(0.00015)
0.05 M citrate pH 3.0	0.00338	(0.00014)
0.10 M citrate pH 3.0	0.00364	(0.00002)
0.25 M citrate pH 3.0	0.00405	(0.00023)
0.05 M citrate pH 3.5	0.00346	(0.00025)
0.10 M citrate pH 3.5	0.00348	(0.00009)
0.25 M citrate pH 3.5	0.00335	(0.00015)
0.05 M acetate pH 4.0	0.00341	(0.00024)
0.1 M acetate pH 4.0	0.00338	(0.00023)
0.25 M acetate pH 4.0	0.00355	(0.00012)
0.05 M citrate pH 4.5	0.00369	(0.00009)
0.1 M citrate pH 4.5	0.00435	(0.00019)
0.18 M citrate pH 4.5	0.00428	(0.00023)
0.05 M acetate pH 5.0	0.00372	(0.00020)
0.1 M acetate pH 5.0	0.00406	(0.00006)
0.25 M acetate pH 5.0	0.00461	(0.00021)
0.05 M phosphate pH 6.5	0.0270	(0.0023)
0.1 M phosphate pH 6.5	0.0305	(0)
0.25 M phosphate pH 6.5	0.0273	(0.0004)
0.05 M phosphate pH 7.4	0.163	(0.002)
0.1 M phosphate pH 7.4	0.157	(0.003)
0.135 M phosphate pH 7.4	0.130	(0.005)
0.05 M borate pH 9.2	2.70	(0.02)
0.1 M borate pH 9.2	2.70	(0.05)
0.25 M borate pH 9.2	3.08	(0.08)
0.01 N sodium hydroxide pH 11.9	8.92	(0.07)
0.1 N sodium hydroxide pH 12.8	8.58	(0.14)

[a] Mean (SD) n = 3.

was used at each pH to generate the k′ value for the rate profile. The pH-rate profile is presented in Fig. 3.

The pH-rate profile was consistent with a combination of a V-shaped profile in the pH range of 0–9 and a sigmoid-shaped profile in the pH range of 4–13. The plateau that began at pH 10–11 is a result of the ionization of the amine of the carbamate inhibiting the base-catalyzed hydrolysis of efavirenz, given that the ionized form of the carbamate is resonance-stabilized toward hydroxide-catalyzed degradation (9, Fig. 4). Thus, increasing the pH resulted in a parallel decrease in the unionized fraction and increase in hydroxide ion concentration resulting in a constant rate of degradation.

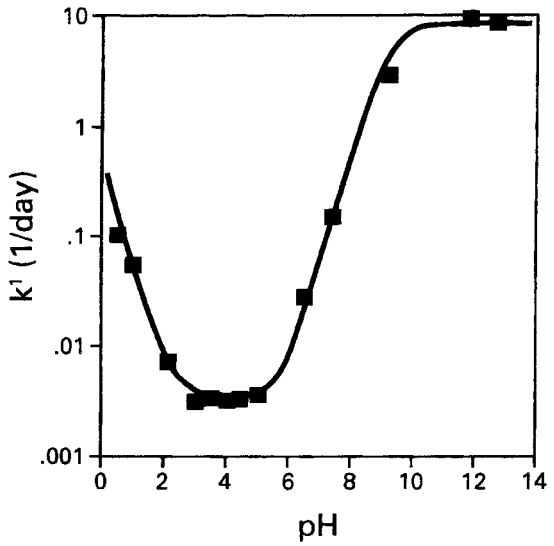

Fig. 3. pH-rate profile for the degradation of efavirenz at 60°C. All rate constants (k′) have been extrapolated to zero buffer concentration. The solid line is generated from a non-linear model fit of the rate constants (Eq.3).

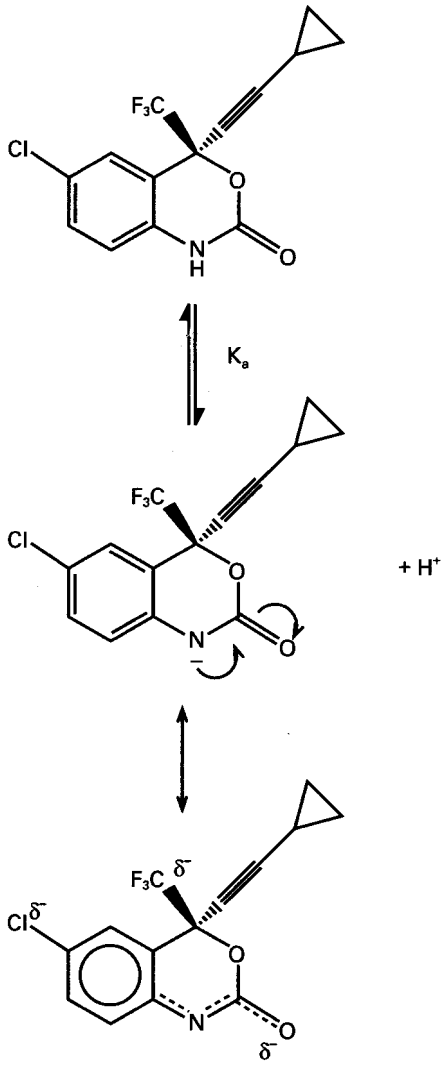

Fig. 4. Ionization of the amine proton of the carbamate functionality and stabilization of the negative charge present on the ionized species through resonance with the extended π-electron system of the benzene ring and through inductive electron-with-drawing effects of electronegative substituents.

The rate equation for the loss of efavirenz can be described as follows

$$\text{rate} = k_H[H+][E] + k_0[E] + k_{OH}[OH-][E] \quad (2)$$

$$E \overset{K_a}{\rightleftharpoons} E^- + H^+$$

$$k_{obs} = (k_H[H+] + k_0 + k_{OH}K_W/[H+])([H+])/(K_a + [H+]) \quad (3)$$

where $[H^+]$, K_w, and K_a are the hydrogen ion concentration, the ion product of water at 60°C, and the ionization constant of efavirenz at 60°C, respectively, and k_H, k_0, and k_{OH} are the rate constants for specific acid, water, and specific base ca-

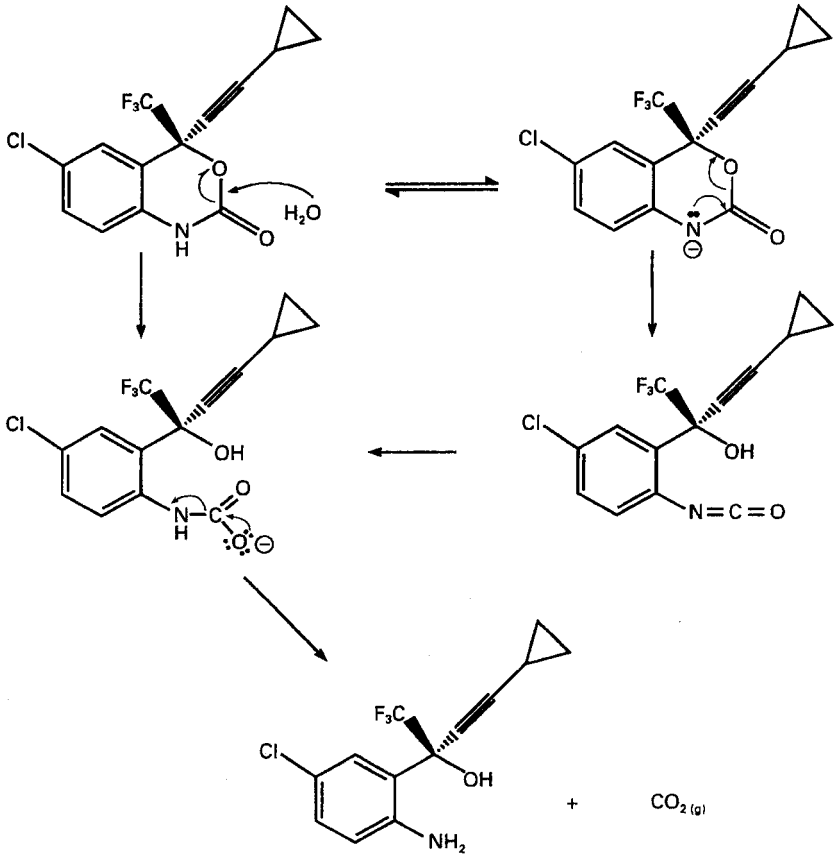

Fig. 5. Proposed degradation pathway for the carbamate hydrolysis of efavirenz.

talysis, respectively. Values for K_a, k_H, k_0, and k_{OH} were determined to be 7.76×10^{-10}, 4.61×10^{-1} M^{-1} day^{-1}, 3.30×10^{-3} day^{-1}, and 6.28×10^4 M^{-1} day^{-1}, respectively, using non-linear (WinNonLin) rate equation fit of the data (Fig. 3). The pK_a value for efavirenz at 60°C determined from non-linear regression analysis was 9.1. The pK_a value decreased by one unit from the value that was reported for 25°C consistent with the effect on increased temperature on the ionization of a proton donor (8).

Samples from the sodium hydroxide studies were utilized to identify the molecular weight of the degradation products with LC-MS. Efavirenz had a molecular weight of 315.7 that was consistent with the elemental composition of $C_{14}H_9NO_2F_3C1$. The principal degradant had a molecular weight of 290.1 that was consistent with the M+1 molecular ion of the amino alcohol (calculated molecular weight of 289.7) and an assigned elemental composition of the ion of $C_{13}H_{12}NOF_3C1$ consistent with the gain of H_2O and loss of CO_2 that would result from hydrolysis of the cyclic carbamate. The retention time of an authentic sample of the amino alcohol was consistent with the retention time of the degradation product indicating that the carbamate hydrolysis pathway is the predominant reaction throughout the pH range studied. The hydrolysis of the carbamate may have proceeded through two kinetically indistinguishable mechanisms. The proposed degradation mechanisms are presented in Fig. 5. Hydrolysis may have proceeded via direct attack on the carbonyl forming the carbamic acid intermediate or via an elimination-addition forming the isocyanate intermediate, consistent with the classical methods for carbamate hydrolysis (10,11). It would be expected that hydrolysis via attack on the carbonyl would predominate under acidic conditions while the elimination-addition forming the isocyanate intermediate would predominate under basic conditions especially based on the leaving group (stabilized by the inductive effect of the CF_3). Previous studies of the synthesis of efavirenz postulated

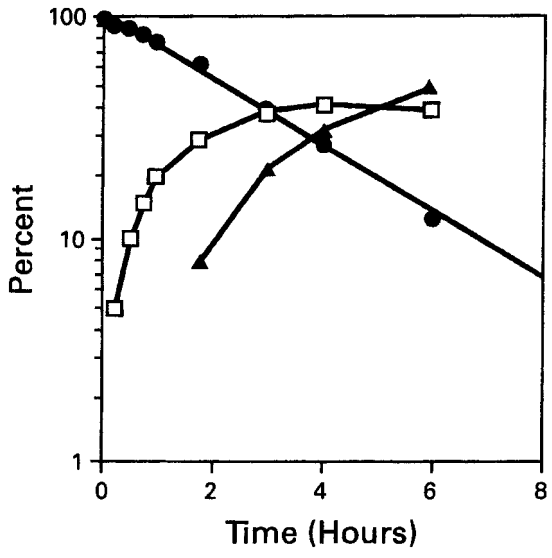

Fig. 6. Percent time profile of efavirenz (●), degradant 1 (□), and degradant 2 (▲) at pH 11.9 at a constant ionic strength of 0.3 M and an initial efavirenz concentration of 16 μM at 60°C.

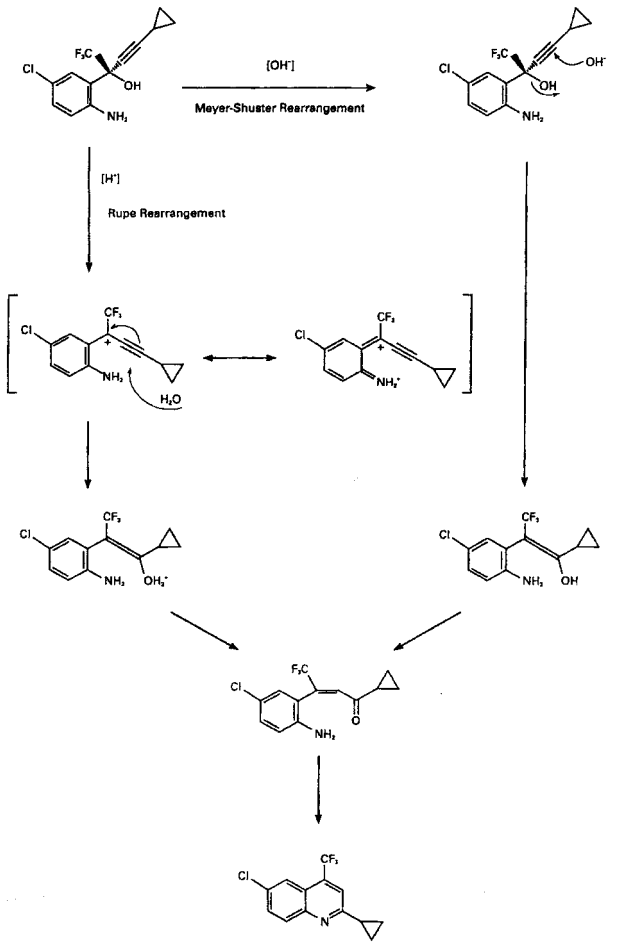

Fig. 7. Proposed degradation pathway for the Rupe/Meyer–Shuster rearrangement of the amino alcohol degradant of efavirenz.

that the reaction proceeded through an unseen isocyanate (7). Both mechanisms are expected to contribute to the hydrolysis with each expected to generate the same unstable carbamic acid intermediate. It is not unexpected to not observe either the carbamic acid or the isocyanate intermediate. The isocyanate expect to be trapped immediately by an internal nucleophile whereas the unstable carbamic acid degraded to the corresponding amino alcohol and CO_2.

Subsequent loss of the amino alcohol (degradant 1) resulted in the formation of degradant 2. The kinetics are depicted in Fig. 6. Degradant 2 had a molecular weight of 272.1 and an assigned elemental composition of $C_{13}H_9NF_3C1$ by LC-MS consistent with the degradation to the quinoline via the Rupe rearrangement and commensurate loss of water (Fig. 7). The inductive effect of the CF3 group destabilized the tertiary carbonium ion. The rearrangement predominated under acidic (Rupe Rearrangement) or basic conditions (Meyer–Shuster Rearrangement) (12). A rationalization for the rearrangement under either acidic or basic conditions is attributed to the stabilization of the carbonium ion by the anilino group as shown in Fig. 7.

ACKNOWLEDGMENTS

The authors acknowledge the expert assistance of Amita Joshi, PhD, with the WinNonLin application used for the non-linear regression analysis and Ms. Lisa Frasher in the preparation of this manuscript.

REFERENCES

1. Physicians Desk Reference®, 1154–1159, (2001).
2. R. A. Spence, W. M. Kati, K. S. Anderson, and K. A. Johnson. Mechanism of Inhibition of HIV-1 Reverse Transcriptase by Nonnucleoside Inhibitors. *Science* **267**:988–993 (1995).
3. E. DeClercq. HIV inhibitors targeted at the reverse transcriptase. *AIDS Res. Hum. Retroviruses* **8**:119–134 (1992).
4. S. Staszewski, J. Morales-Ramirez, K. T. Tashima, A. Rachlis, D. Skiest, J. Stanford, R. Stryker, P. Johnson, D. F. Labriola, D. Farina, D. J. Manion, and N. M. Ruiz. Efavirenz Plus Zidovudine and Lamivudine, Efavirenz Plus Indinavir, and Indinavir Plus Zidovudine and Lamivudine in the Treatment of HIV-1 Infection in Adults. *N. Eng. J. Med.* **341**:1865–1873 (1999).
5. M. Albrecht, D. Katzenstein, R. Bosch, S. Liou, and S. Hammer. ACTG 364: Virologic Efficacy of Nelfinavir and/or Efavirenz in Combination with New Nucleoside Analogs in Nucleoside Experienced Subjects. 6th Conference on Retrovirurses and Opportunistic Infections, Abstract 489 (1999).
6. S. M. Rowe, L. G. Fontalbert, S. R. Rabel, and M. B. Maurin. Physical Chemical Properties of Efavirenz. AAPS Annual Meeting and Exposition, *AAPS PharmSci Supplement* (1999).
7. L. A. Radesca, Y. S. Lo, J. R. Moore, and M. E. Pierce. Synthesis of HIV-1 reverse transcriptase inhibitor DMP 266. *Synth. Commun.* **27**:4373–4384 (1997).
8. M. E. Pierce, R. L. Parsons, Jr., L. A. Radesca, Y. S. Lo, S. Silverman, Q. Islam, J. R. Moore, A. Choudhury, J. M. D. Fortunak, D. Nguyen, S. Morgan, W. Davis, P. N. Confalone, C. Y. Chen, R. D. Tillyer, L. Frey, L. Tan, F. Xu, D. Zhao, A. S. Thompson, E. G. Corley, E. J. J. Grabowski, and P. J. Reider. practical asymmetric synthesis of efavirenz (DMP 266), an HIV-1 reverse transcriptase inhibitor. *J. Org. Chem.* **63**:8536–8543 (1998).
9. S. R. Rabel, M. B. Maurin, S. M. Rowe, and M. Hussain. Determination of the pK_a and pH-solubility behavior of an ionizable cyclic carbamate, (S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3, 1-benzoxazin-2-one (DMP 266). *Pharm. Dev. Tech.* **1**:91–95 (1996).
10. A. Williams and K. T. Douglas. Elimination-addition mechanisms of acyl transfer reactions. *Chem. Rev* **75**:627–649 (1975).
11. A. Vigroux, M. Bergon, C. Bergonzi, and P. Tisnes. A general acid-catalyzed anion breakdown associated with an E1cB reaction in the hydrolysis of aryl *N*-(substituted phenylsulfonyl)carbamates. *J. Am. Chem. Soc.* **116**:11787–11796 (1994).
12. M. Picquet, A. Fernandez, C. Bruneau, and P. H. Dixneuf. Efficient ruthenium-catlysed synthesis of 3-hydroxy-1-propen-1-yl benzoates: en route to an improved isomerization of 2-propyn-1-ols into α,β-unsaturated aldehydes. *Eur. J. Org. Chem.* **1**:2361–2366 (2000).