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RESEARCH PAPERS

KINETIC AND STABILITY STUDY OF AN INVESTIGATIVE ANTIRHINOVIRUS COMPOUND

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ABSTRACT

A hydrolytic acid and base catalyzed ring opening reaction has been demonstrated for a 1,2,4-oxadiazole antiviral compound (WIN 63843) resulting mainly in an amidoxime product. Decomposition products and related impurities were detected using a gradient HPLC method. The hydrolysis reaction was first-order for 35% ethanol/buffer solutions in a 50°C chamber or a light cabinet (1000 ft-candles), the greatest stability being between pH 4 and 6. Furthermore, increasing ethanol concentrations resulted in a great decrease in reaction rates. Therefore, for oral or aerosol solution formulations, light protection, pH control between 4 and 6 and the highest permissible ethanol concentrations would be advantageous. This study has shown that the highly electronegative trifluoromethyl group at the 5 position increases the lability of a 1,2,3-oxdiazole compound.

INTRODUCTION

WIN 63843 in aqueous buffer suspensions (pH 2 to 7.4) is not subject to significant chemical degradation because of its very low solubility in water (<20 ng/mL). It was found to be soluble and very stable in safflower seed oil, corn oil or corn oil-ethanol solutions. Further experiments have shown that the 1 2,4-oxadiazole ring of WIN 63843 in aqueous ethanol solution is susceptible to hydrolysis which is the subject of this report. The 3,5-alkyl and aryl disubstituted 1 ,2,4-oxadiazoles are very stable to heat or strong acids (1 ,2). If this ring is unsubstituted at the 3 or 5

position amidoxime compounds are formed with aqueous acid (2) whereas the 3 unsubstituted 1 2,4-oxadiazoles are easily cleaved by aqueous alkali at room temperature (3). WIN 63843 is a 3-aryl 5-trifluoromethyl disubstituted 1,2,4-oxadiazole. Nucleophilic displacement of electron withdrawing groups has been shown, for example, with 3-phenyl-5-trichloromethyl which in the presence of alkali produces chloroform and the 3-phenyl-5-hydroxyoxadiazole (4). However the main product in our studies with WIN 63843 was the amidoxime compound (WIN 65489) produced by a ring opening hydrolytic reaction. The presence of the trifluoromethyl group at the 5 position in WIN 63843 increases the lability of the 1 2,4-oxadiazole ring.

MATERIALS AND METHODS

Sample Solutions

Aliquots of a stock alcohol solution of WIN 63843 were added to 0.05M buffers of pHs 1, 2, 3, 4, 5, 6, 7, and 8. The drug concentration of each test solution was approximately 50 μg/mL, and the final alcohol concentration was 35% to keep the drug in solution. WIN 63843 solutions having alcohol concentrations of 40%, 60%, 80%, and 100% were similarly prepared with water. Portions of all solutions were placed in 4 mL, teflon lined screw capped vials at three stations: room temperature in the dark (control), light cabinet (1000 ft-candles), and 50°C oven. At regular intervals, the preparations were cooled to room temperature, and sampled for HPLC analysis by either method I or method II. The pH of each solution was measured at sampling intervals with a Beckman ϕ71 pH meter (Beckman Instruments, Fullerton, Ca.). Portions of the 40%, 60%, 80%, and 100% alcohol solutions were diluted with water to 35% ethanol for pH determinations.

HPLC Method I

The HPLC method development and assays of kinetic samples were conducted using the following Varian equipment (Varian Associates, Sunnyvale, CA): 5500 pump, 8085 autoinjector, Polychrom diode array and regular UV-200 detector, and DS 654 data acquisition station. The analytical column was a 10 micron Waters Bondapak 300 x 4.6 mm (Waters Chromatography, Marlborough, MA). The optimized mobile phase contained two reservoirs, A and B, running at 1 mL/min. Reservoir A contained an isopropanol-acetonitrile solution (2:8) and reservoir B contained 0.005M sodium octanesulfonate sodium and 0.5% acetic acid in water. A gradient with reservoirs A and B was set up as follows:

Time	% A	% B
0	45	55
8	45	55
12	80	20
20	80	20
21	45	55
22	45	55

The run time for each sample was 24 minutes and peak areas were integrated at 254 nm. A linearity study was conducted over a range of 15 - 100 μg/mL using a series of standard solutions of WINs 63843, 64239, 61836, 61834, 65489 (initially presumed to be potential degradation products) were prepared in alcohol or acetonitrile and diluted with mobile phase. The structures are shown in Figure 1. Typically, external standards were used at 5 to 10 μg/mL during most assays.

HPLC Method II

In a second HPLC procedure (isocratic), WIN 63843 eluted in 4.8 min. Degradation products were not detectable by this method. This procedure was used because of the limited availability of the instrumentation in method I, the lengthy analysis time of method I, and the rapid analysis obtained by method II. The following Waters equipment (Waters Chromatography, Marlborough, MA) was used: Model 510 solvent pump, Model 712 WISP autoinjector, and 745 Data Module for peak area integration. A C-18 Oft Rx (DU PONT Co., Wilmington, DE, 10 μ, 250 x 4.6 mm) column was used and WIN 63843 was detected at 230 nm with a Spectroflow 757 detector (Applied Biosystems, San Jose, CA). A mobile phase consisting of 70% acetonitrile and 30% 0.05 M ammonium acetate was delivered at 2 mL/min. All samples were diluted with mobile phase, prior to injection.

RESULTS AND DISCUSSION

WIN 63843 was separated from 64239, 61836, 61834, and 65489 as shown in the chromatogram (Figure 2). The retention time for WIN 63843 was 18.9 minutes in Method I. The plots of various standards versus peak areas were linear with correlations of 0.999 or better as shown in Figure 3. Ethanolic solutions of WIN 63843 degrade at 50°C and in the light cabinet according to a first order process as shown in Figures 4,5, and 6. The major product was the putative ring opening hydrolysis compound, WIN 65489. Other product peaks were also observed and separated but were not identified at the time of the study due to lack of proper degradation standards for superimposition. It is apparent from the kinetic data shown in Figure 7 and Table 1 derived from a first order model that the slowest reaction rates occur in the pH range of 4 to 7. Also the agreement between the rate constants obtained using HPLC Method I and Method II is good. The hydrolytic ring-opening reaction, producing mainly the amidoxime compound (WIN 65489), is both acid and base catalyzed. The kinetic profiles are similar for the thermal and light stressed solutions (Figure 7). Half-lives for the light cabinet solutions are 3.7 times longer (range of 3.6 to 3.9) than the 50°C solutions, from pH 1.3 to 8.2. Additional kinetic information (below pH 1.3 and above pH 8.2) is necessary for determination of the acid and basic hydrolytic constants as expressed below.

$$K = K_H [H^+] + K_W + K_{OH} [OH^-]$$

where K_H and K_{OH} are the acidic and basic catalytic constants and K_W is the spontaneous reaction (water constant). However, K_W is about 0.09 inverse

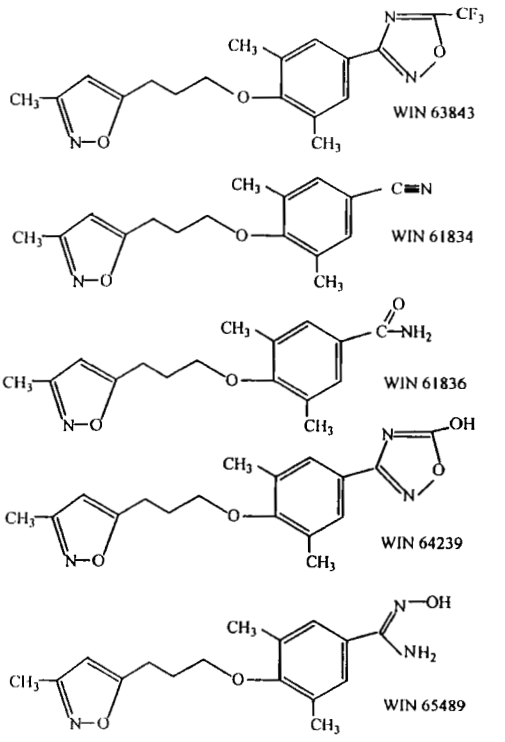

FIGURE 1
Structures of WIN 63843 and Related Compounds.

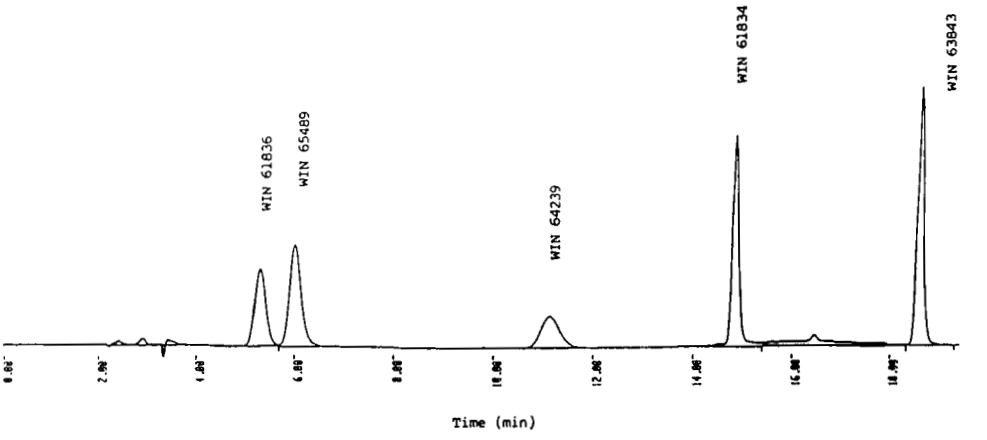

FIGURE 2
Chromatogram of WIN 63843 and Related Compounds.

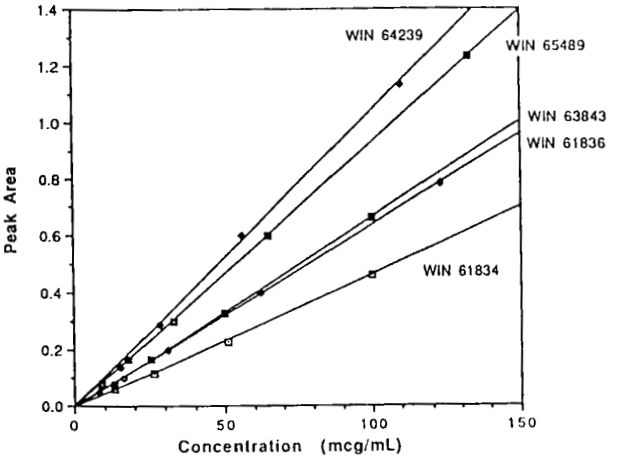

FIGURE 3

Standard Curves of WIN 63843 and the Related Products; WINs 63843, 64239, 65489, and 61836.

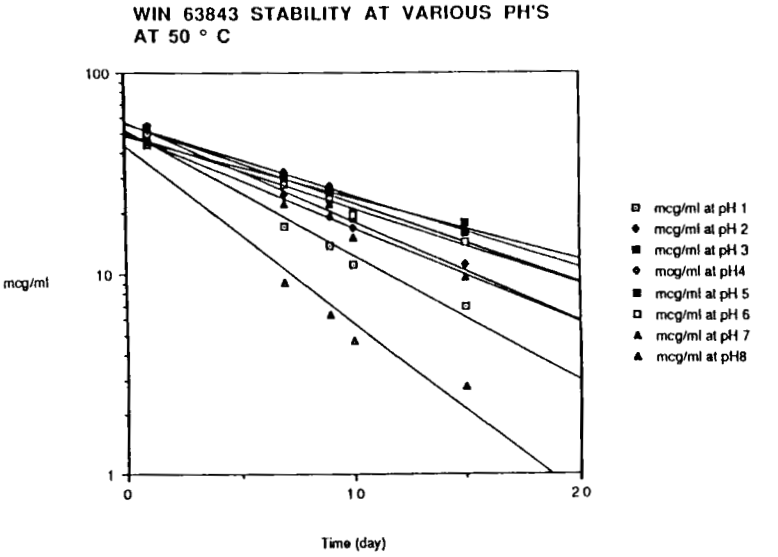

FIGURE 4

Concentration of WIN 63843 in 35% Ethanol/buffer Solutions Exposed to Heat (50° C). HPLC by Method I.

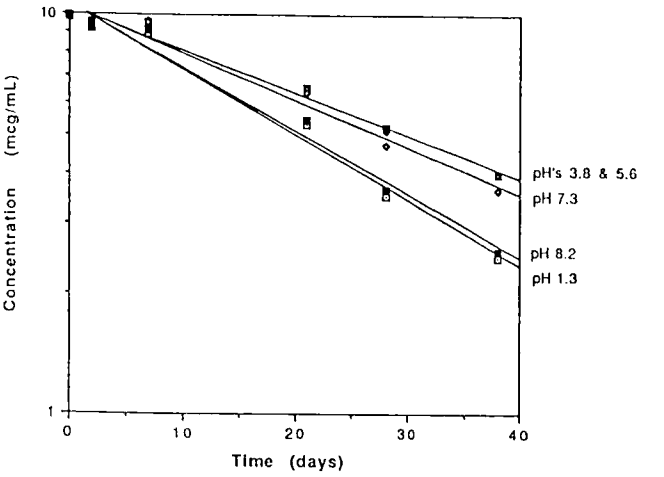

FIGURE 5
Concentration of WIN 63843 in 35% Ethanol/buffer Solutions Exposed to Light (1000 ft-candles). The Solutions of pH 3.8 and 5.6 Had Identical Rate Constants. HPLC by Method II.

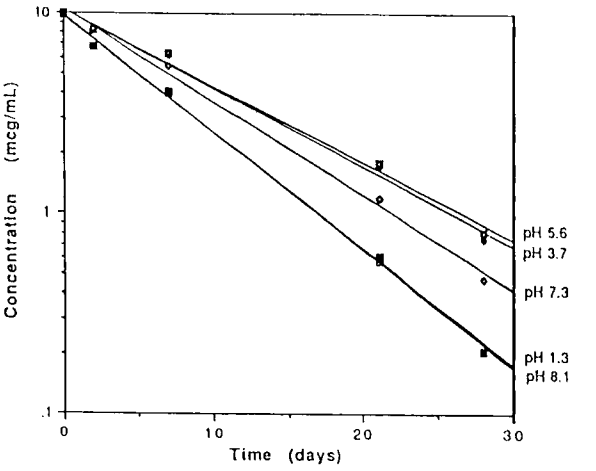

FIGURE 6
Concentration of WIN 63843 in 35% Ethanol/buffer Solutions Exposed to Heat (50° C). The Solutions of pH 1.3 and 8.1 Have Similar Rate Constants. HPLC by Method I I.

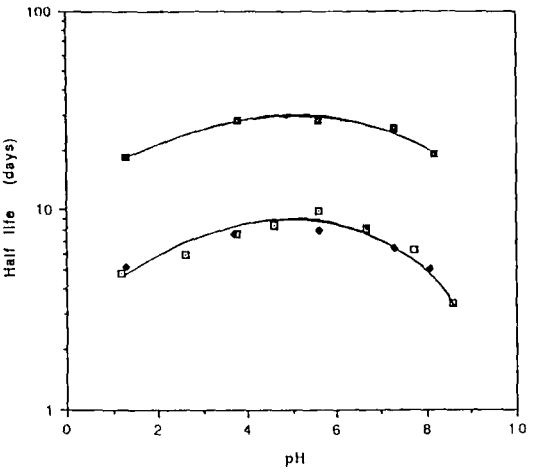

FIGURE 7

WIN 63843 Half Life (days) in 35% Ethanol/buffer Solutions of Various pHs, Subjected to Light (1000ft-candles, room temperature) and to 50° C temperatures. ■ Light; ◘ 50°C, HPLC method I; ♦ 5 0°C, HPLC II.

dcorresponding to a half-life of 7.7 days at 50°C. Previously it was shown that aqueous suspensions of WIN 63843 (4 mg/mL) at pH 2 to pH 7.4 subjected to 50°C for 6 weeks, were completely stable physically and chemically. This is in accordance with the present information on solution kinetics and WIN 63843 solubility. At a solubility of less than 20 ng/mL and a rate constant of 0.10 inverse days the predicted decrease in WIN 63843 concentration after 6 weeks at 50°C would be less than 84 ng/mL or less than 0.002% of the initial concentration for a suspension. The percent decomposition depends on the concentration of suspension.

Identification Of The Degradation Products

The putative ring opening hydrolysis product formed during acid hydrolysis in solutions was found to superimpose with the standard WIN 65489 peak and their UV/Visible spectra were similar (Figure 8). However, the WIN 65489 spectrum is not unique. Therefore since other impurities or unidentified degradation products may have similar retention times, the presence of WIN 65489 should be confirmed by additional methods. WIN 68025, a metabolic product with a retention time of about 6 minutes, elutes slightly ahead of WIN 65489. With samples stressed at pH 5 in the light cabinet, both WINs 68025 and 65489 were present with retentions of about 5 to 6 minutes (Figure 9). In contrast, solutions of WIN 63843 stressed at 50°C without light resulted in one decomposition product, WIN 65489 (Figure 10).

When WIN 63843 solutions in pH 9 to 11.5 buffers were stressed at 50°C, a major peak was observed by HPLC at about 13.5 minutes. This product decomposed over

TABLE I

Rate constants (k, $days^{-1}$), half-life (t 1/2, days), pH, and linearity (R^2) of the lines for WIN 63843 decomposition in various 0.05 M buffers, at 50°C and under 1000 ft-candles of light: (A) results obtained with 50°C samples by HPLC method I, (B) results obtained with 50°C samples by HPLC method II, (C) results obtained with Light Cabinet samples (1000 ft-candles) by HPLC method II.

Measured pH	k	t 1/2	R^2
A.			
1.2	0.142	4.86	0.98
2.6	0.114	6.08	0.99
3.8	0.090	7.70	0.94
4.6	0.083	8.39	0.94
5.6	0.070	9.86	0.93
6.7	0.085	8.17	0.99
7.8	0.108	6.43	0.96
8.6	0.201	3.44	0.96
B.			
1.3	0.133	5.21	0.99
3.7	0.090	7.69	0.99
5.6	0.088	7.89	0.99
7.3	0.108	6.44	0.99
8.1	0.135	5.14	0.99
C.			
1.3	0.037	18.6	0.98
3.8	0.024	28.4	0.98
5.6	0.024	28.4	0.98
7.3	0.027	25.5	0.98
8.2	0.036	19.1	0.98

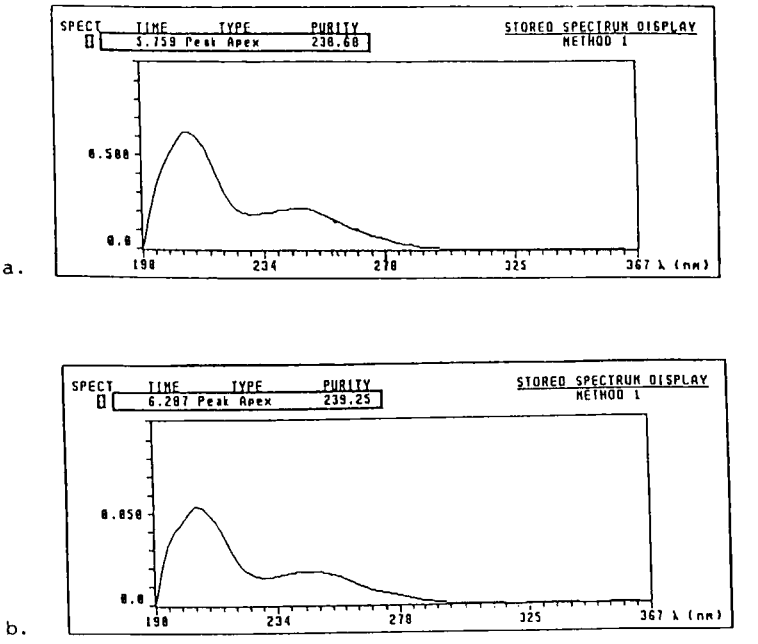

FIGURE 8
UV Scan of WIN 65489 Standard (a), and the Acid Catalyzed Product (b).

FIGURE 9
Chromatogram of WIN 63843 Solution (35% ethanol/buffer) at pH 5.6 after 56 Days Under Light (1000 ft-candles).

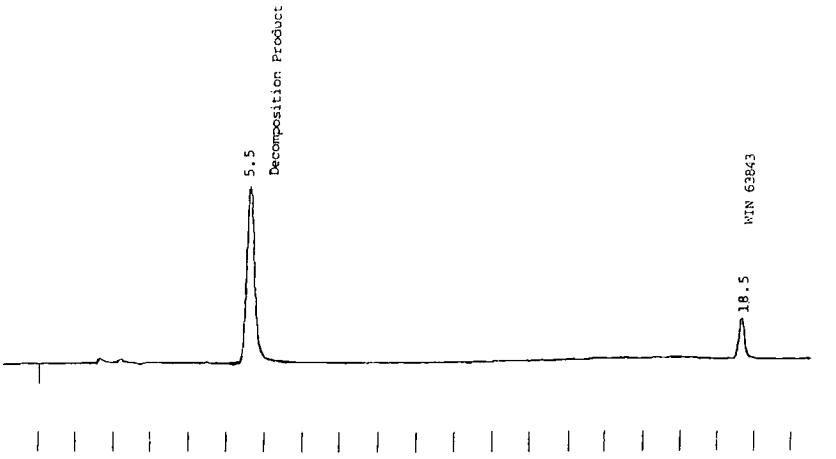

FIGURE 10
Chromatogram of WIN 63843 Solution (35% ethanol/buffer) at pH 5.6 after 9 Days at 50°C

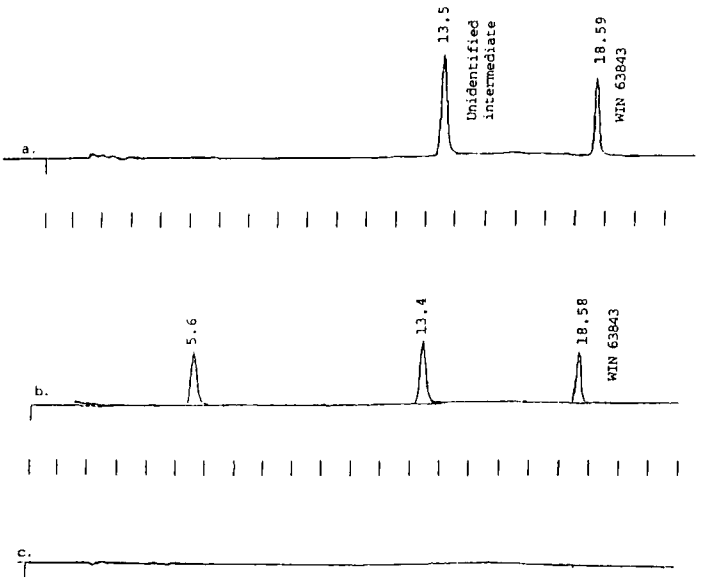

FIGURE 11
a). Chromatogram of WIN 63843 at pH 9.16 at Time Zero; b). Chromatogram of the Same Solution After 6 Hours at 50 °C, with a Decrease of Both the Intermediate, and WIN 63843, and Formation of the Amidine Product; c). Blank Control Chromatogram.

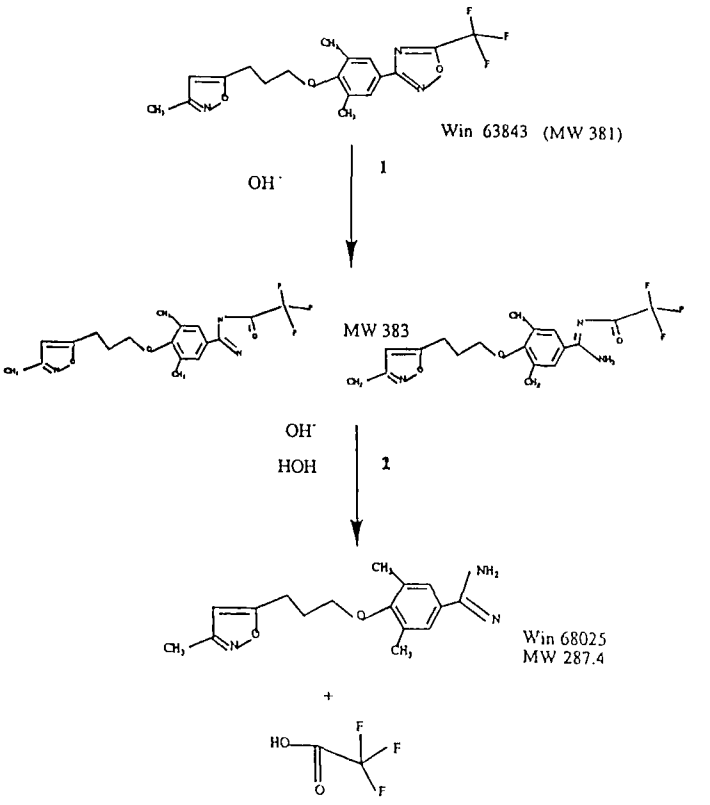

FIGURE 12
Products of WIN 63843 After Exposure to Ammonium Hydroxide (pH = 11.5).

several hours to the amidine, WIN 65489 or amidoxime product, WIN 68025 (Figure 11). In order to identify this degradation product under basic pH conditions, a concentrated solution of WIN 63843 (0.5 mg/mL) at pH 11.5 was prepared with ammonium hydroxide and analyzed by mass spectrometry. The result indicated a molecular weight fragment that corresponded to WIN 68025 (MW 287.4). In addition, ions of molecular weights 382 and 384 were also found. The first corresponds to WIN 63843 (MW of 381) while the second may be a ring opening intermediate that eventually breaks down to form WIN 68025. WIN 65489 was not found by Mass spectrometry in this sample although it cannot be ruled out at other pH conditions. The decomposition mechanism is unclear. Two of the species that may be formed from WIN 63843 have a molecular weight of 383 and are ring opening products shown in Figure 12.

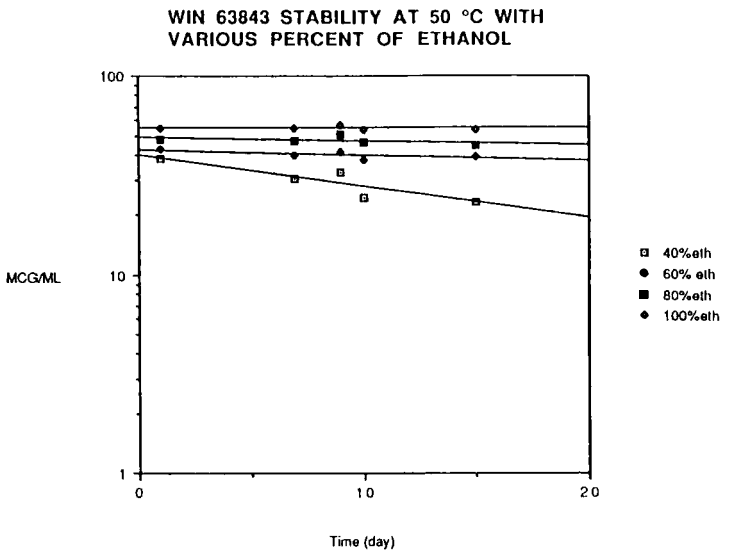

FIGURE 13
Concentration of WIN 63843 in Various Percent Ethanol Solutions at 50 °C.

TABLE 2
WIN 63843 Concentration in Ethanol-water Solutions stored at 50°C

	WIN 63843 Concentration (μg/mL) Percent Ethanol Concentration in Water			
Days	40	60	80	100
1	38.9	42.8	47.8	54.6
7	30.5	40.3	47.7	54.9
9	32.6	41.5	50.8	56.3
10	24.5	37.7	46.6	54.0
15	23.4	39.7	44.6	54.9

Effect Of Ethanol On The Stability Of WIN 63843

The effect of alcohol in the stability of WIN 63843 was studied at 50°C and in the light cabinet (1000 ft- candles), using different concentrations of alcohol in water. Degradation is decreased with increasing concentrations of alcohol (Figure 13, Table 2). In pure ethanol the degradation products from photolysis (peak retention at 7.6 and 9.1 minutes) were quite different from those obtained in aqueous solutions stressed in the light cabinet (compare Figures 11 and 14). The presence of a degradation product at 18.1 minutes superimposable with authentic WIN 64529 standard shows

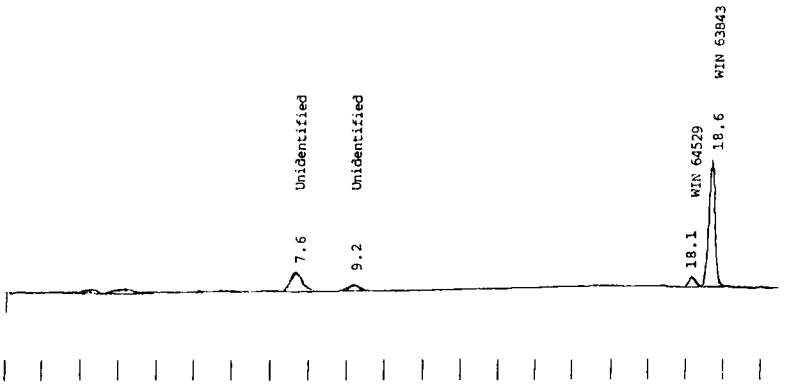

FIGURE 14
Chromatogram of WIN 63843 in 100% Ethanol After 56 Days Exposure To 1000 foot-candles of Light.

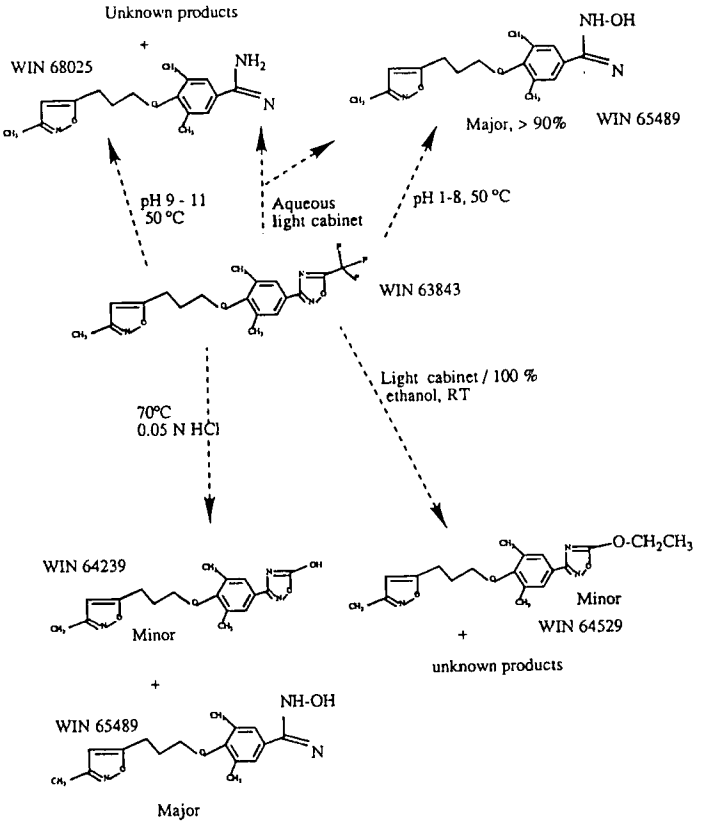

FIGURE 15
Some Degradation Products Of WIN 63843 and Conditions for Their Formation.

TABLE 3
Measured pHs of the 40%, 60%, 80%. and 100% ethanol solutions diluted with water to 35% ethanol

Percent ethanol	pH
40	60
60	6.2
80	6.6
100	7.0

that some ethyl ether product (WIN 64529) is formed possibly from the hydroxy product after the trifluoromethyl group of WIN 63843 is removed under photolytic conditions. In the absence of light at 50°C for up to 2 weeks, the drug is stable in pure ethanol at concentration of 50 μg/mL (Table 2). No degradation product was detected.

The pH's of the 40%, 60%, 80%, and 100% alcohol solutions were constant throughout the 15 day period at all stations. The sample pH range of 5.5 to 7.0 is the range showing greater stability of WIN 63843 in the alcohol buffer solutions (Table 3, Figure 12). These solutions were diluted to 35% alcohol to limit the alcohol interference during pH measurement, while keeping the drug in solution. Only the 40% alcohol solution went through more than one half-life (k of 0.0517 inverse days, half-life of 13.4 days). This half-life is about 1.5 times longer than that observed for the buffered solution containing 35% ethanol at the same pH. WIN 63843 in absolute ethanol solution is very stable, no significant concentration change was detected after 15 days at 50°C.

ACKNOWLEDGEMENTS

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REFERENCES

(1) Behr, LyeII C. The Chemistry of Heterocyclic Compounds. (Richard H. Wiley, Ed.), p. 250, John Wiley & Sons, New York, (1962).
(2) Clapp, Leallyn B, Advances in Heterocyclic Chemistry, p. 95, vol. 20 (A. R. Katritzky and A. J. Boulton, Eds.), New York, Academic Press, (1976).
(3) Gilchrist, Thomas L. Advances in Heterocyclic Chemistry, p. 54, vol. 41 (Alan R. Katritzky, Ed.), New York, Academic Press, (1987).
(4) Clapp, Leallyn B. Op. Cit. p. 102.