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THE STABILITY OF 1-CYCLOPROPYLMETHYL-5-(o-FLUOROPHENYL)-7-CHLORO-1,3-DIHYDRO-2H-1,4-BENZODIAZEPIN-2-ONE (KB-509) IN AQUEOUS SOLUTION

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SUMMARY

The main degradation of 1-cyclopropylmethyl-5-(o-fluorophenyl)-7-chloro-1,3-dihydro-2H-1,4-benzodiazepin-2-one (KB-509) in aqueous solution involves a two-step consecutive mechanism. The first step is the hydrolysis of KB-509, yielding 5-chloro-cyclopropylmethylamino-2′-fluorobenzophenone (1) and glycine. The second step is the ring closure of the benzophenone derivative to give 2-chloro-10-cyclopropylmethyl-9-acridanone (2) at all pH regions.

INTRODUCTION

1-Cyclopropylmethyl-5-(o-fluorophenyl)-7-chloro-1,3-dihydro-2H-1,4-benzodiazepin-2-one (KB-509) belongs to the 1,4-benzodiazepine class of tranquillizing agents (Yamamoto et al., 1970) similar to oxazepam and diazepam. Recently the aqueous solution stability of oxazepam, diazepam (Wesley et al., 1977) and nitrazepam (Wesley et al., 1977) were reported. In this report we describe the stability and degradation mechanism of KB-509 which has an ortho-fluorophenyl substituent at the C-5 position different from diazepam or nitrazepam.

MATERIALS AND METHODS

Compound purity was verified by GLC and TLC. KB-509 was obtained from Sumitomo Chemical Company Ltd. Diazepam, 5-chloro-2-cyclopropylmethylamino-2′-fluorobenzophenone (1), 2-chloro-10-cyclopropylmethyl-9-acridanone (2) were used upon recrystallization and other chemicals were of reagent grade quality. Deionized distilled water was used in all experiments. The ionic strengths of all buffered solutions were adjusted to 1.0 with sodium chloride. The kinetic measurements of KB-509 were carried out by the following procedure. Ten milliliters of stock solution of KB-509 in ethanol (3.03×10^{-3} M) was brought up to 1000 ml with the appropriate buffer solution. Ten milliliters of the final solution was then placed in a sealed glass ampoule. Temperature was controlled at $100 \pm 2.5^{\circ}$C by immersing these ampoules into a constant temperature oil bath. The ampoules were withdrawn at suitable time intervals and cooled to room temperature. The solution was transferred into a separatory funnel and extracted with two portions of

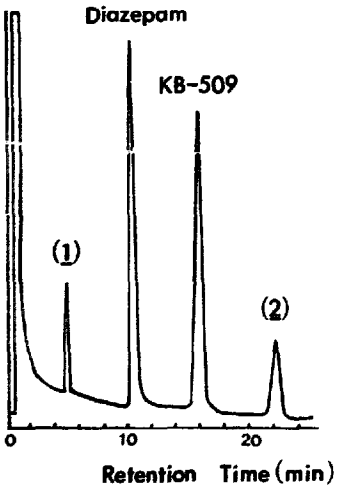


Fig. 1. Gas chlormatogram of KB-509 and its degradation products, (1) and (2).

20 ml chloroform. Five milliliters of internal standard solution (ethanolic solution of diazepam, 3.68×10^{-5} M) was added to the chloroform layer, and the solvent was removed under reduced pressure. The residue was diluted with ethanol and injected into a gas chromatograph (Shimadzu FID gas chromatograph, Model GC-4C PF). The operating conditions of GLC were as follows: 2% OV-17 2 m glass column, column temperature 260°C, injection and detector temperature 280°C, injection amount 2 μl, carrier gas pressure (N_2) 1.1 kg/cm^2. The representative gas chromatogram of the intact KB-509 and its degradation products (1) and (2) is shown in Fig. 1. The pH measurements were made using a direct reading, digital pH meter (Hitachi digital pH meter, Model F-7 DE).

RESULTS AND DISCUSSION

Rate of degradation

The concentration change of KB-509 was measured by GLC at various time intervals. The initial concentration was substituted for the ratio of the peak height of H_0(KB-509) to H_0(diazepam), where H_0(KB-509) was the peak height of KB-509 (retention time, 17 min) at zero time and H_0(diazepam) was peak height of diazepam (retention time, 11 min). The concentration of KB-509 at t hours was calculated by the following equation:

$$[\text{KB-509}]_t = \frac{H_t(\text{KB-509})/H_t(\text{diazepam})}{H_0(\text{KB-509})/H_0(\text{diazepam})} \times 3.03 \times 10^{-5}\ \text{M} \qquad (1)$$

where $[\text{KB-509}]_t$ was the concentration of KB-509 at t h, H_t(KB-509) was the peak height of KB-509 at t h, and H_t(diazepam) was the peak height of diazepam added as internal standard at t h.

The logarithm of the concentration of KB-509 at any time was plotted against time. The kinetic measurements were carried out in duplicate at each run, and the coefficients of variation of the concentration of intact KB-509 were all less than 0.6%. Only the average values are reported. Typical first-order plots for the hydrolysis of KB-509 are shown as Fig. 2. The apparent first-order rate constants were calculated from the slopes of the plots of Eqn. 2:

$$\log \frac{[\text{KB-509}]_t}{3.03 \times 10^{-5}} = -\frac{kt}{2.30} \tag{2}$$

where k was the apparent first-order rate constant for the hydrolysis of KB-509.

The rate constants derived from these plots are presented in Table 1. These log k values can be used to plot a log k–pH profile for the reaction (Fig. 3). The pH of maximum stability was about 5. The profile suggested that the overall degradative rate represented a summation of a relatively large number of separate reactions. The study for theoretical pH–rate profile is now in progress and will be reported in the next paper.

Isolation and identification

The same procedure as for the kinetic measurement was used and the chloroform extract without internal standard was concentrated and then subjected to TLC analysis using Kieselgel 60 F_{254} (Merck). The samples were spotted and developed approximately 20 cm with acetone–chloroform (1 : 5) in a closed tank. Over the entire pH range, a KB-509 spot (R_f 0.49) and three spots of degradation products (R_f 0.65, R_f 0.60 and origin) were detected using a UV (253 nm) lamp or ninhydrin aerosol spray.

For isolation and identification of the top spot (R_f 0.65), a degradation reaction of

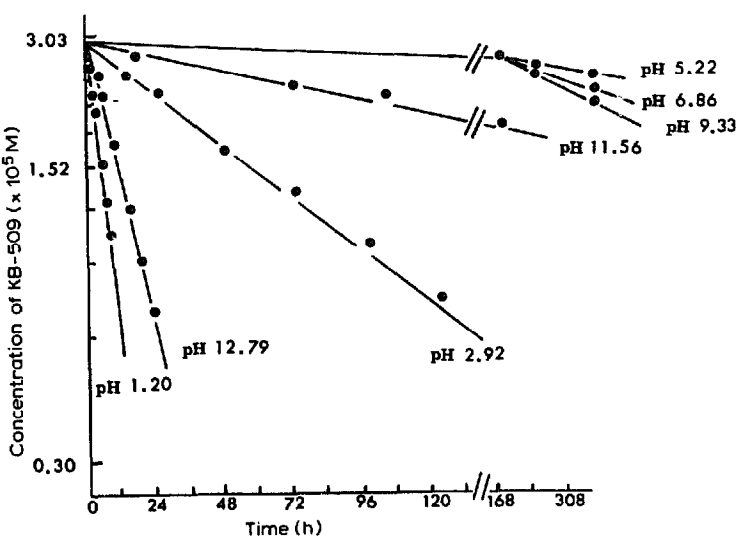


Fig. 2. Stability of KB-509 in aqueous solution at 100°C.

TABLE 1

APPARENT FIRST ORDER RATE CONSTANT (k) FOR KB-509 AT 100°C

pH	Buffer composition	k, h^{-1}	r^b
1.20 (1.23) [a]	0.1 M HCl	1.22×10^{-1}	0.99
2.92 (3.00) [a]	53 ml of CH_3COOH^- 25 ml of 1.3 M CH_3COONH_4	1.14×10^{-2}	0.99
5.22 (5.22) [a]	4.2 ml of 0.2 M CH_3COOH^- 15.8 ml of 0.2 M CH_3COONa	4.30×10^{-4}	0.99
6.86 (6.88) [a]	52.5 ml of 0.067 M $Na_2HPO_4^-$ 47.5 ml of 0.067 M KH_2PO_4	7.96×10^{-4}	0.99
9.42 (9.33) [a]	32 ml of 0.2 M NaOH 25 ml of 0.2 M H_3BO_3 25 ml of 0.2 M KCl 118 ml of H_2O	9.92×10^{-4}	0.99
11.56 (11.33) [a]	50 ml of 0.1 M Na_2HPO_4 24.5 ml of 0.1 M NaOH	2.98×10^{-3}	0.99
12.79 (12.79) [a]	0.1 M NaOH	6.27×10^{-2}	0.99

[a] pH value of final solution

[b] Correlation coefficient calculated from a linear regression analysis of the data.

KB-509 was carried out on a large scale. Two grams of KB-509 was dissolved in 1 N HCl aqueous solution (about 50 ml) and the solution was refluxed for 3 h. After cooling, the reaction mixture was extracted with chloroform. The solvent was removed under reduced pressure and the residue was dissolved in 5 ml of n-hexane–ethylacetate (5 : 1) mixture. Column chromatography was applied for separation of the degradation product. Wakogel

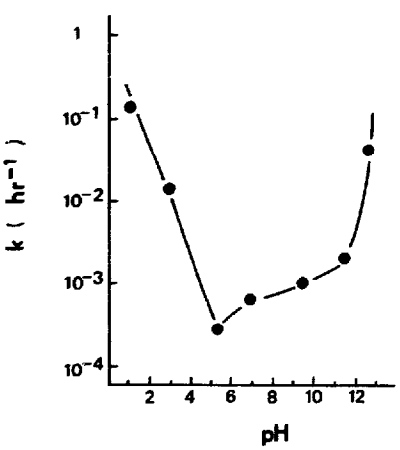


Fig. 3. Log k–pH profile for the hydrolysis of KB-509 at 100°C.

C-200 was used as support and n-hexane–ethylacetate (5 : 1) mixture as eluate. From the eluent containing the top spot (R_f 0.65), the solvent was removed under reduced pressure, the residue was recrystallized from methanol, and the yellow crystals were obtained at m.p. 75.2–75.8°C. From the spectral data and elemental analysis (Table 2), this degradation product was identified as 5-chloro-2-cyclopropylmethylamino-2′-fluorobenzophenone (1).

Further, we attempted to isolate and identify the other degradation reaction of KB-509 for the middle spot (R_f 0.60). Five grams of KB-509 was dissolved in acetic acid (about 50 ml) and the solution was heated at 120°C for 60 h. This reaction was followed by TLC. After 60 h this degradation product was remarkably accumulated compared with the others; the solution was then cooled, neutralized with sodium bicarbonate and extracted with chloroform. The solvent was removed under reduced pressure and ethylether was added to the residue for crystallization. This isolated component was recrystallized from methanol and pale yellow needles were obtained at m.p. 116.8–169.2°C. From the spectral data and elemental analysis (Table 3), this degradation product was identified as 2-chloro-10-cyclopropylmethyl-9-acridanone (2).

For identification of the origin spot, experiments with a different developing solvent were made. This component was detected as a red spot (R_f 0.25) with an ethanol–water (7 : 3) mixture using ninhydrin aerosol spray. Its R_f value agreed with that of an authentic glycine standard.

Further reaction of hydrolysis product

The concentration changes of the intact KB-509, and the degradation products, (1) and (2) at pH 1.20 and 12.75 were measured and are shown in Figs. 4 and 5. It was assumed from these results that KB-509 might decompose according to a first-order–first-order consecutive reaction: KB-509 → (1) → (2) (Fig. 6). This further reaction is very interesting, because there has been no report about this ring closure reaction in 1,4-benzodiazepine derivatives which have no ortho-fluorophenyl substituent at the C-5 position. The ring closure reaction of benzophenone derivatives to 9-acridanone has been presented by Sternbach (Fryer et al., 1963), and it has been shown that no ring closure took place in the case of ortho-chlorobenzophenone derivatives. Consequently, the specific further

TABLE 2

SPECTRAL DATA AND ELEMENTAL ANALYSIS OF THE DEGRADATION PRODUCT (1)

NMR (in $CDCl_3$)δ:	0.2–1.4 (5 H, multiplet, c-C_3H_5), 3.1 (2 H, doublet of doublet, J_{-CH} = 6.8 Hz, J_{-NH} = 6.0 Hz, $-CH_2$-c-C_3H_5), 6.6–7.6 (7 H, multiplet, aromatic protons), 8.9 (1 H, broad singlet, NH proton)
IR ν_{max}^{KBr} cm^{-1}:	3320 (N–H), 1610 (C=O)
MS m/e:	303 (M^+), 288, 166, 123, 95, 75, 55(base)
Anal. Calcd. for $C_{17}H_{15}ClFNO$:	C, 67.22; H, 4.98, N, 4.61%
Found:	C, 67.15; H, 4.97; N, 4.63%

TABLE 3

SPECTRAL DATA AND ELEMENTAL ANALYSIS OF THE DEGRADATION PRODUCT (2)

NMR (in $CDCl_3$)δ:	0.4–1.6 (5 H, multiplet, c-C_3H_5), 4.3 (2 H, doublet, J = 5.4 Hz, $-CH_2$-c-C_3H_5), 7.1–7.7 (5 H, multiplet, aromatic protons), 8.3–8.5 (2 H, multiplet, aromatic protons)
IR ν_{max}^{KBr} cm^{-1}:	1638 (C=O), 1598 (C=C)
MS m/e:	283 (M^+), 242, 229, 149, 55 (base)
Anal. Calcd. for $C_{17}H_{14}ClFNO$:	C, 71.96; H, 4.97, N, 4.94%.
Found:	C, 71.77; H, 5.24; N, 4.90%.

reaction is considered to be attributable to the strong nucleophilicity in the aromatic amine. In practice, (1) was easily converted to (2) in acetic acid at 120°C and this fact also suggests the consecutive reaction. By applying the data of Figs. 4 and 5 to the first-order–first-order consecutive reaction, the rate constants k_1 and k_2 were graphically calculated. That is to say, concentration of (1) follows Eqn. 3:

$$[(1)]_t = \frac{[\text{KB-509}]_0 k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \quad (3)$$

where $[(1)]_t$ is the concentration of (1) at t h and $[\text{KB-509}]_0$ is the initial concentration of KB-509.

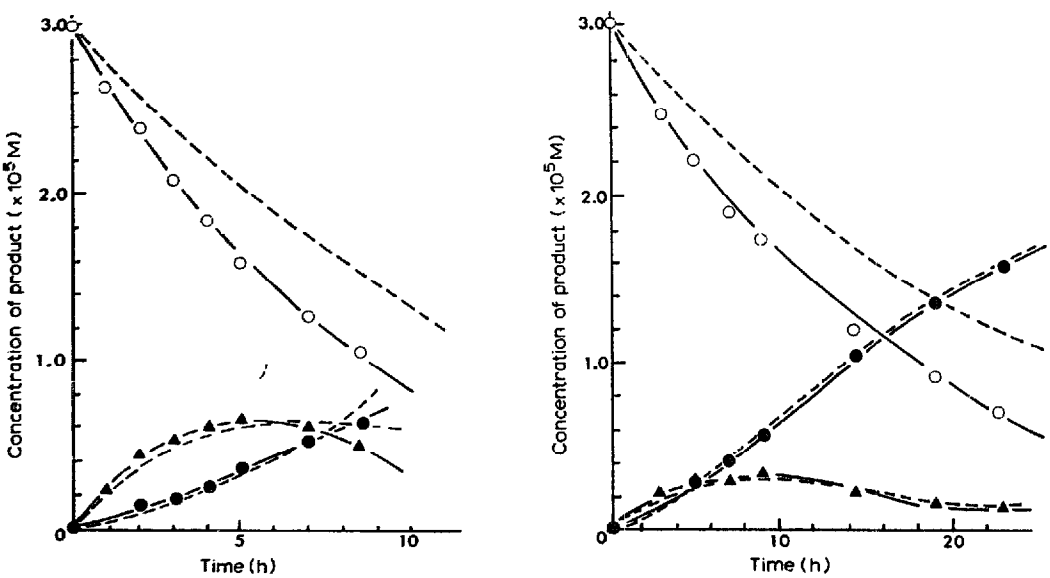
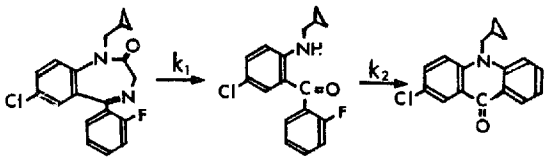


Fig. 4. (Left) Concentration changes of KB-509 (o———o), degradation products (1) (▲———▲) and (2) (●———●) at pH 1.20 and 100°C. Theoretical curve of consecutive reaction, k_1 8.0×10^{-2} h^{-1}, k_2 2.0×10^{-1} h^{-1} is shown by (- - - - -).

Fig. 5. (Right) Concentration changes of KB-509 (o———o), degradation products (1) (▲———▲) and (2) (●———●) at pH 12.79 and 100°C. Theoretical curve of consecutive reaction, k_1 4.0×10^{-2} h^{-1}, k_2 2.8×10^{-1} h^{-1} is shown by (- - - - -).



KB-509 (1) (2)

Fig. 6. Reaction scheme of degradation of KB-509.

If k_2 is much larger than k_1, and t is sufficiently large, Eqn. 3 approximates Eqn. 4:

$$[(1)]_t = \frac{[\text{KB-509}]_0 k_1}{k_2 - k_1} (-e^{-k_1 t}) \tag{4}$$

Consequently, k_1 was obtained from the slope of a plot of log $[(1)]_t$ vs. t for the loss of (1) and k_2 was calculated from the intercept. The values obtained are: $k_1 = 8.0 \times 10^{-2}\ h^{-1}$, $k_2 = 2.0 \times 10^{-1}\ h^{-1}$ at pH 1.20 and $k_1 = 4.0 \times 10^{-2}\ h^{-1}$, $k_2 = 2.8 \times 10^{-1}\ h^{-1}$ at pH 12.79. The theoretical curves (dotted curves) in Figs. 4 and 5 were obtained by using Eqns. 3, 5 and 6.

$$[\text{KB-509}]_t = [\text{KB-509}]_0 e^{-k_1 t} \tag{5}$$

$$[(2)]_t = [\text{KB-509}]_0 - [\text{KB-509}]_t - [(1)]_t \tag{6}$$

where $[\text{KB-509}]_t$ and $[(2)]_t$ are theoretical concentrations at t h. Compared with the apparent rate constant of KB-509 in Table 1, k is greater than k_1 at both pH values. This fact suggests that other degradation reactions occur together with the reaction shown in Fig. 6. In practice, three minor products were detected by TLC analysis of the solution of final run at pH 1.20 (R_f values 0.05, 0.23 and 0.33 with acetone–chloroform (1 : 5) mixture), but a further analysis was not carried in this report.

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