

[Chem. Pharm. Bull.]
[29(5)1398—1402(1981)]

Decomposition and Stabilization of Drugs. XIX.¹⁾ Kinetic Studies on the Hydrolysis of Bencyclane Fumarate

KAZUO KIGASAWA, HIROAKI SHIMIZU, SHIGERU HAYASHIDA,
and MITSUYO FUJINO

*Research Laboratories, Grelan Pharmaceutical Co., Ltd., 2-12-3
Sakurashinmachi, Setagaya-ku, Tokyo*

(Received July 25, 1980)

Bencyclane fumarate (I) was decomposed in aqueous buffer solution from pH 1.2 to 6.3 in order to examine its stability and mechanism of hydrolysis.

It was found that I was hydrolyzed with pseudo first-order kinetics, and the pH-rate profile was a straight line of slope -1. The activation energy (E_a) was calculated to be 33.0 kcal/mol from Arrhenius plots.

Further examination of the decomposition mechanism through kinetic analysis suggested a new pathway where I first formed a carbonium ion intermediate in an acidic solution then decomposed immediately into an alcohol and two olefins by hydration and dehydration, respectively.

Keywords—bencyclane fumarate; hydrolysis of bencyclane fumarate; decomposition routes and mechanism of bencyclane fumarate; 1-benzylcycloheptanol; 1-benzylcycloheptene and 1-benzylidenecycloheptane

Bencyclane fumarate, 3-[(benzyl cycloheptyl)oxy]-N,N-dimethylpropanolamine fumarate (I), was developed by Pallos²⁾ in 1965 as an antispasmodic and vasodilator agent.

In a study of the stability and decomposition mechanism of I in an acidic solution, Simonyi and Kekesy³⁾ reported that when heated under sterile conditions in an acidic solution, I was decomposed into four compounds, 1-benzylcycloheptanol (II), 3-(dimethylamino)propanol (III), 1-benzylcycloheptene (IV) and 1-benzylidenecycloheptane (V). However, there was no experimental support for their suggestion that the olefin compounds (IV) and (V) were formed from the alcoholic compound (II) by an elimination reaction.

There are few comprehensive studies on the stability and hydrolysis mechanism of I in an acidic solution or on the decomposition of drugs bearing a cyclo alkyl ether bond.

This paper describes the identification of new decomposition processes of I in an acidic solution, in addition to those known up to now, by kinetic analysis of the hydrolysis.

Experimental

Bencyclane Fumarate (I)—The material was purchased from Wako Pure Chemical Industries and recrystallized from water. mp 128—130°. ²⁾

1-Benzylcycloheptanol (II)—II was synthesized by the reaction of cycloheptanone and Grignard reagent prepared from benzylchloride and magnesium. ^{3,4)} mp 44—46°, NMR (CDCl₃) δ : 2.73 (2H, s, PhCH₂), 1.35 (1H, s, OH), IR ν_{max} cm⁻¹: 3400 (OH). Anal. Calcd for C₁₄H₂₀O: C, 82.30; H, 9.87. Found: C, 82.34; H, 9.80.

The Mixture of 1-Benzylcycloheptene (IV) and 1-Benzylidenecycloheptane (V)—II (5 g) was dissolved in 100 ml of 1 N HCl and heated on a boiling water bath for 3 hr. The resulting solution was extracted with benzene and extract was concentrated after being dried over anhydrous MgSO₄. The oily residue was chromatographed on a silica gel column with cyclohexane.

Concentration of the collected cyclohexane fractions yielded a mixture of IV and V. Due to difficulty in further separation, IV and V were treated in this study as a mixture with a constant ratio (determined by GLC and NMR: IV/V=5.7/1).

Buffer Solution—The buffer solution was prepared from HCl, H₃PO₄, CH₃COOH, CH₃COONa, NaOH, NaH₂PO₄, Na₂HPO₄ of guaranteed JIS grade. The ionic strength (μ) was adjusted to 0.5 with KCl.

Kinetic Procedure—Solutions of I at the concentration of $7.5 \times 10^{-4} \text{ M}$ in various buffers were sealed in ampoules.

The samples were heated at suitable intervals in a water bath kept at $40 \pm 1^\circ$ and $60 \pm 1^\circ$ and in an oily bath at $95 \pm 1^\circ$.

At suitable intervals, a sample was withdrawn, rapidly cooled in ice-water to stop the reaction, allowed to stand at room temperature and analyzed.

Analytical Procedure—To 2 ml of sample, 0.5 ml of 10% NaOH and 0.5 ml of diphenhydramine·HCl solution (containing $1.57 \times 10^{-3} \text{ M}$) and 2 ml of benzene solution (containing $4.61 \times 10^{-4} \text{ M}$, *n*-eicosane) were added (diphenhydramine was added as an internal standard for I and *n*-eicosane for II, IV and V).

The mixture was shaken for 20 min and centrifuged at 3000 rpm for 10 min, then 3 μl of the supernatant was chromatographed on a Shimadzu GC-4CM gas chromatograph equipped with FID. A glass column, $2 \text{ m} \times 3 \text{ mm}$ i.d., was packed with 3% OV-17 coated on Chromosorb-W (80—100 mesh).

The column and detector temperatures were 200° and 235° , respectively. The flow rate of N_2 was 45 ml/min. The peak areas were calculated with a Shimadzu model ITG-4A integrator.

The peak area ratios of I, II, IV and V were linearly related to the concentration from 0.1 to $2.0 \mu\text{g}$, and the relative retention times of I, II, IV and V were *ca.* 10, 4.5, 2.0 and 2.5 min, respectively.

Thin-layer Chromatography—Separation was carried out with the developing solvent, cyclohexane: isopropanol: conc. NH_3 water = 80: 20: 1 (v/v), on plates coated with Kiesel gel 60 F₂₅₄ purchased from Merck Inc. Spots were located by exposure to UV light (2536 Å) or by treatment with Dragendorff reagent.⁵⁾

Results and Discussion

The Reaction Order

Decomposition products formed during the hydrolysis of I at the most acidic condition of pH 1.2 and $95 \pm 1^\circ$ were examined by TLC. Four spots corresponding to II, III, IV and V

were seen. The amounts of these decomposition products increased with the passage of time. When I was hydrolyzed at 60° and pH 1.2, 2.0 or 3.3, a plot of the logarithm of residual amounts of I against time was linear within the working pH range (Fig. 1).

In addition to this linearity, the independence of the hydrolysis rates on the initial concentrations indicated that the hydrolysis of I was a pseudo first-order reaction (Fig. 2).

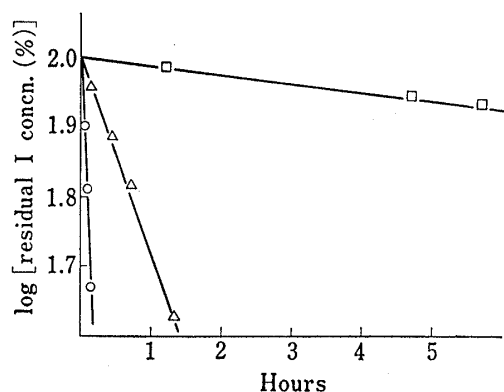


Fig. 1. Pseudo First-order Plots of the Hydrolysis of I at 60° ($\mu=0.5$)

pH 1.2; —○—, pH 2.0; —△—, pH 3.3; —□—.

to study the effect of ionic strength on the hydrolysis of I. The primary salt effect found in Fig. 3 required that the present experiment be carried out at constant ionic strength, which was adjusted to an arbitrary value of 0.5 with KCl.

pH-Rate Profile

The determination of the rate constants of I at 60° with various buffer concentrations ($\text{CH}_3\text{COOH}-\text{CH}_3\text{COONa}$ Michaelis buffer, $\mu=0.5$) showed that the rate constants were independent of the concentrations.

The pH-rate profile was examined in buffer solution ($\mu=0.5$) from pH 1.2 to 6.5 at 40° , 60° and 95° .

The linear plot of slope -1 between the logarithm of the rate constants and pH led to equation (1), which indicates that hydrogen ion-catalyzed decomposition is dominant under the conditions of the present study.

$$\log k_{\text{H}^+} = \log k_{\text{obs.}} + \text{pH} \quad (1)$$

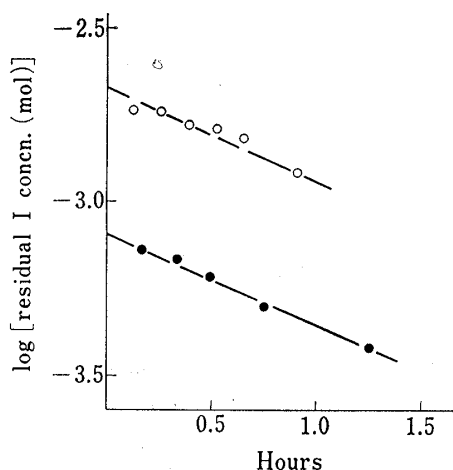


Fig. 2. Effect of Initial Concentration on the Hydrolysis of I at 60° and pH 2.0 ($\mu = 0.5$)

—○—; 2.10×10^{-3} M/L, —●—; 8.02×10^{-4} M/L.

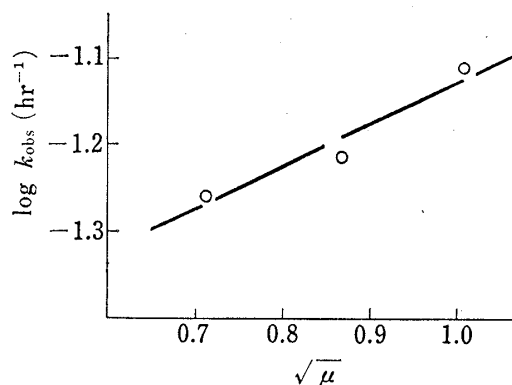


Fig. 3. Effect of Ionic Strength on the Hydrolysis of I at 60° and pH 2.9

Equation (1) gives apparent hydrogen ion catalysis constants (k_{H^+}) of 3.18×10^3 , 5.19×10 and $2.99 \text{ M}^{-1} \text{ hr}^{-1}$ at 95°, 60°, 40°, respectively.

I was found to follow the Arrhenius equation, since the plots of the logarithm of the hydrogen ion catalysis constants k_{H^+} against $1/T$ were linear. The activation energy, calculated from the slope of this plot, was equal to 33.0 kcal/mol.

The Pathway and Mechanism of the Reaction

In the hydrolysis of I in hydrochloric acid solution, Simonyi *et al.*³⁾ indicated that the alcoholic derivative (II) was first produced then decomposed further into the unsaturated derivatives IV and V (Chart 1).

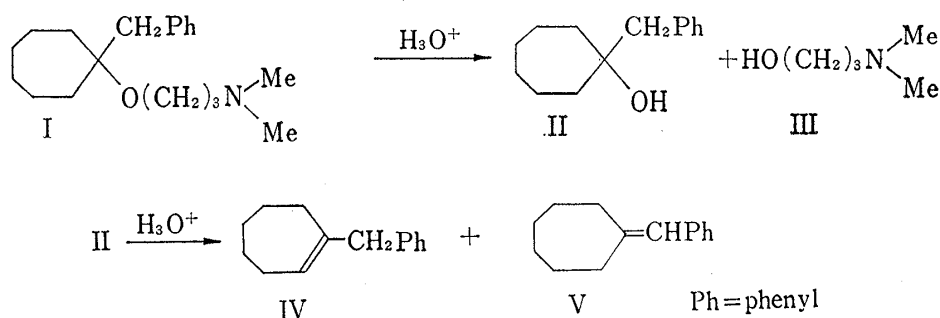


Chart 1

On the other hand, the cleavage of the ether bond through the carbonium cation is well known,⁶⁾ while Wilt and Roberts⁷⁾ reported that the reaction of 1-phenyl cycloalkyl carbinyl arenesulfonates in acetic acid yielded the 1-benzyl cycloalkyl cation as an intermediate which immediately underwent elimination reaction to form unsaturated 1-benzyl cycloalkenes and 1-benzylidene cycloalkanes.

Taking these reaction into consideration, we consider that in an acidic solution the hydrolysis of I may be initiated by the cleavage of its ether bond, *i.e.* the formation of the benzyl cycloheptyl cation, followed by the formation of II as well as IV and V through elimination and addition reactions.

Further, II may undergo dehydration to produce IV and V (Chart 2).

If the above assumptions are correct and each reactant is produced by a pseudo first-order reaction, the rate of each reaction may be expressed as follows.

$$\frac{d[I]}{dt} = -(k_1 + k_2 + k_3)[I] = -K_1[I] \quad (2)$$

where K_1 is the sum of k_1 , k_2 and k_3

$$\frac{d[II]}{dt} = k_1[I] - (k_4 + k_5)[II] = k_1[I] - K_2[II] \quad (3)$$

where K_2 is the sum of k_4 and k_5

$$\frac{d[IV]}{dt} = k_2[I] + k_4[II] \quad (4)$$

$$\frac{d[V]}{dt} = k_3[I] + k_5[II] \quad (5)$$

When $[I]_0$, $[II]_0$, $[IV]_0$, and $[V]_0$ represent the initial concentrations of I, II, IV and V at $t=0$, respectively, equation (2) can be written as equation (6).

$$[I] = [I]_0 e^{-K_1 t} \quad (6)$$

Similarly, equations (7), (8) and (9) can be obtained from equations (3), (4), and (5), respectively.

$$[II] = k_1[I]_0 (e^{-K_1 t} - e^{-K_2 t}) / (K_1 - K_2) \quad (7)$$

$$[IV] = [I]_0 \{ (k_2(K_1 - K_2) - k_4 k_1) (1 - e^{-K_1 t}) / K_1 (K_1 - K_2) + k_4 k_1 (1 - e^{-K_2 t}) / K_2 (K_1 - K_2) \} \quad (8)$$

$$[V] = [I]_0 \{ (k_3(K_1 - K_2) - k_5 k_1) (1 - e^{-K_1 t}) / K_1 (K_1 - K_2) + k_5 k_1 (1 - e^{-K_2 t}) / K_2 (K_1 - K_2) \} \quad (9)$$

When I was hydrolyzed at pH 1.2 and $60 \pm 1^\circ$, the residual amount of I and the amount of II formed were plotted against the reaction time as shown in Fig. 4. The amounts of IV and V were similarly plotted.

$K_1 (=k_1 + k_2 + k_3)$ was calculated to be 2.99 hr^{-1} from the residual amount of I, since I was hydrolyzed obeying a pseudo first-order reaction.

When I is exhausted, namely $k_1[I] \rightleftharpoons 0$, equation (3) leads to equation (10) which indicates that $[II]$ decreases according to pseudo first-order kinetics.

$$\frac{d[II]}{dt} = -K_2[II] \quad (10)$$

The plot of the logarithm of the concentration of II against time was linear and $K_2 (=k_4 + k_5)$ was calculated to be $3.84 \times 10^{-1} \text{ hr}^{-1}$ from the residual amount of II.

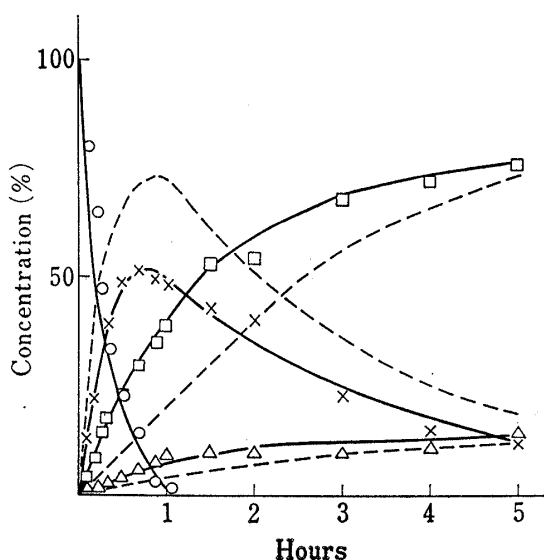


Fig. 4. The Hydrolysis of I at pH 1.2 and 60° ($\mu=0.5$) [Experimental data: $\bigcirc=I$, $\times=II$, $\square=IV$, $\triangle=V$]

The Solid lines were calculated on the basis of the decomposition routes of I shown in Chart 2 ($k=1.96$, $k_2=1.07$, $k_3=1.29 \times 10^{-1}$, $k_4=3.27 \times 10^{-1}$, $k_5=5.73 \times 10^{-2} \text{ hr}^{-1}$), while dotted lines were calculated from the routes in Chart 1: $I \rightarrow II \rightarrow IV$ ($K_1=K_1=2.99$, $k_2=k_3=0$, $k_4=3.27 \times 10^{-1}$, $k_5=5.73 \times 10^{-2} \text{ hr}^{-1}$).

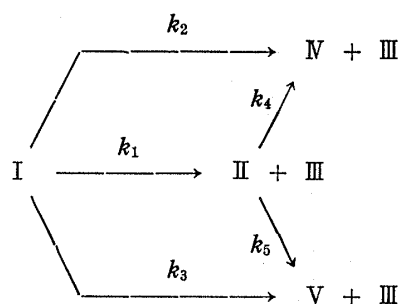


Chart 2

When II synthesized independently as an authentic sample was heated at pH 1.2 and $60\pm 1^\circ$ with an initial concentration of 2.12×10^{-3} M, it was found that II was decomposed with a pseudo first-order rate constant K_2 of 3.70×10^{-1} hr $^{-1}$.

The agreement of the above two decompositions of II supported the validity of equation (10) and the rate constant K_2 was concluded to be 3.84×10^{-1} hr $^{-1}$.

Next, the conversion between IV and V in acidic solution will be discussed.

Our finding that more *endo*-compound IV was obtained than *exo*-compound V in the decomposition of II is consistent with reports^{4,8)} that more *endo*- than *exo*-compounds were obtained in the decomposition of simple cyclic hydrocarbons.

Further, Collines and Schaeffer⁹⁾ showed that *exo* 1-benzylidene cyclopentane was converted into *endo* 1-benzyl cyclopentene in a phosphoric acid aqueous solution.

A purified mixture of IV and V was heated at pH 1.2 and $60\pm 1^\circ$ for 12 hr. GLC and NMR determinations of the ratios of IV and V before and after heating of the mixture showed a constant ratio, indicating that no conversion between IV and V occurred under the conditions of the present study.

This led us to conclude that the ratio of the rate constants of formation of IV and V from II corresponded to that of their final concentrations (5.7/1) in an acidic solution, and consequently the contribution ratio to K_2 of 5.7/1 yielded k_4 and k_5 values of 3.27×10^{-1} hr $^{-1}$ and 5.73×10^{-2} hr $^{-1}$, respectively.

Substitution of the data based on the assumption that I decomposed according to the pathway, I $\rightarrow$ II $\rightarrow$ IV and V as drawn in chart 1, *i.e.*, $K_1=k_1=2.99$ hr $^{-1}$, $k_4=3.27\times 10^{-1}$ hr $^{-1}$, $k_5=5.73\times 10^{-2}$ hr $^{-1}$, $k_2=k_3=0$, into equations (7), (8) and (9) gave the theoretical changes in the concentrations of I, II, IV and V shown by dotted lines in Fig. 4, which are not in agreement with the experimental data.

It was apparent that the calculated values were considerably higher than the experimental ones for II and lower for IV and V (Fig. 4).

These discrepancies indicated that other pathways had to be considered. The validity of our assumed pathway shown in Chart 2 was therefore examined as follows. Under conditions where $K_1\approx k_1$ was essential to the above pathway, substitution of the experimental concentrations of II, IV and V at 15 min after the beginning of the decomposition of I and values of K_2 , k_4 and k_5 ($K_2=3.84\times 10^{-1}$, $k_4=3.27\times 10^{-1}$ and $k_5=5.73\times 10^{-2}$) into equations (7), (8) and (9) yielded k_1 , k_2 and k_3 values of 1.96 hr $^{-1}$, 1.07 hr $^{-1}$ and 1.29×10^{-1} hr $^{-1}$, respectively.

The changes in the concentrations of I, II, IV and V at intervals were then calculated by substituting the calculated values of k_1 , k_2 , k_3 , k_4 and k_5 into equations (6), (7), (8) and (9). The good agreement of these changes in concentrations with the experimental ones shown by solid lines in Fig. 4 supported the validity of the new pathway.

It may be deduced from our findings that I was predominantly decomposed in a pseudo first-order reaction catalyzed by the hydrogen ion over the pH range of 1.2 to 6.3.

References and Notes

- 1) Part XVIII: K. Kigasawa, H. Shimizu, H. Ohtani, S. Hayashida, and T. Ishiodori, *Yakugaku Zasshi*, **99**, 402 (1979). This report was presented at the 100 th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, Apr. 1980.
- 2) L. Pallos, Hung. Patent 151865 (1965) [*C.A.*, **62**, 16125b (1965)].
- 3) I. Simonyi and Kekesy, *Pharmazie.*, **22**, 23 (1967).
- 4) G.R. Newkom and Y.W. Allen, *J. Chem. Education.*, **50**, (5), 373 (1973).
- 5) E. Eosgay-Kovacs and A. Fischer, *Pharmazie.*, **30**, 609 (1975).
- 6) a) E. Knobloch and M. Profiva, *Chem. Comm.*, **19**, 976 (1954); b) M. Profiva and J.O. Jilek, *Chem. Comm.*, **19**, 732 (1954); c) H. Nogami and M. Horioka, *Yakugaku Zasshi*, **81**, 79 (1960).
- 7) J.W. Wilt and D.D. Roberts, *J. Org. Chem.*, **27**, 3434 (1962).
- 8) a) H.C. Brown and J.H. Brewsyer, *J. Am. Chem. Soc.*, **76**, 467 (1954); b) B. Raymond, *J. Org. Chem.*, **22**, 439 (1957); c) V.J. Trayeli and W.L. Hergnother, *J. Org. Chem.*, **29**, 123 (1964).
- 9) C.J. Collines and H.J. Schaeffer, *J. Am. Chem. Soc.*, **78**, 124 (1956).