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The kinetics and mechanism of the hydrolysis of cyclopentolate hydrochloride in alkaline solutions

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Abstract

Cyclopentolate hydrochloride (Cy · HCl) is an ester of a substituted benzeneacetic acid, having *N,N*-dimethylaminoethanol as the alcohol moiety. A reversed-phase HPLC assay was employed to investigate the kinetics of degradation of Cy · HCl. The influence of pH, buffers, and temperature was studied in alkaline solutions. The degradation follows (pseudo) first-order kinetics at 50°C. Results indicate that it degrades very rapidly at higher pH values. Phenylacetic acid and α-(1-hydroxycyclopentyl)benzeneacetic acid were isolated and identified as the degradation products. The reaction mechanism appears to follow a parallel scheme where phenylacetic acid and α-(1-hydroxycyclopentyl)benzeneacetic acid are formed simultaneously. It is proposed that α-(1-hydroxycyclopentyl)benzeneacetic acid is formed by normal ester hydrolysis. Phenylacetic acid is formed via a six-membered transition state and its formation requires the assistance of the hydroxyl group from the adjacent cyclopentanol moiety.

Keywords: Kinetics; Hydrolysis; Ester; Reaction mechanism; Parallel mechanism

1. Introduction

Cyclopentolate hydrochloride (Cy · HCl) is a cycloplegic and mydriatic agent widely used for diagnostic purposes. Some other compounds in this group are atropine, homatropine, scopolamine and tropicamide. Cy · HCl is used in eye drops to produce cycloplegia and mydriasis. It acts more quickly than atropine and has a shorter duration of action; the maximum effect is produced in 20–60 min after instillation of one to two drops of 0.5% solution. Accommodation recovers within 24 h but may be hastened if one or two drops of a 2% solution of pilocarpine nitrate is instilled (Ophthalmic Drug Facts, 1989; Remington's Pharmaceutical Sciences, 1990). The structure of Cy · HCl is shown in Fig. 1. It is an ester of a substituted benzeneacetic acid and *N,N*-dimethylaminoethanol. A cyclopentanol group is attached to the benzylic carbon and the nitrogen atom on the ester moiety is responsible for its pK_a. The benzylic carbon is chiral and the dl mixture was used for the kinetic study.

Martin and Pohloud-Fabini (1981a,b, 1982) studied the stability of cyclodrine hydrochloride, which is similar in structure to Cy · HCl, the only

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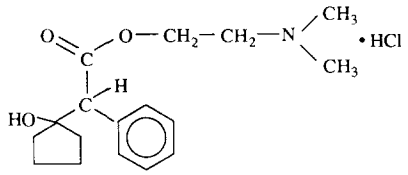
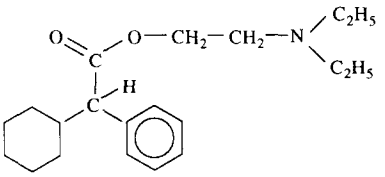


Cyclopentolate Hydrochloride



Drofenine

Fig. 1. The structure of Cy·HCl and a structurally similar compound, drofenine.

difference being that it has an *N,N*-diethyl group rather than an *N,N*-dimethyl group on the nitrogen of the alcohol moiety. They found that the drug was stable under acidic conditions and hydrolyzed rapidly in alkaline solutions. α-(1-Hydroxycyclopentyl)benzeneacetic acid (hereafter referred to as the β-hydroxy acid) and *N,N*-dimethylaminoethanol were found to be the main degradation products by TLC. Attempts by Martin and Pohloud-Fabini to detect the cyclopentyl residue (which is presumably released in the form of cyclopentanol during the cleavage of the β-hydroxy acid) failed. Since there was no reported literature dealing with a systematic study of the chemical stability and mechanism of degradation of the drug, the kinetics and mechanism of hydrolysis of Cy · HCl were studied in alkaline solutions.

2. Materials and methods

Cy · HCl, phenylacetic acid, cyclopentanone and drofenine were obtained from Sigma Chemicals. *N,N*-dimethylaminoethanol was obtained from Aldrich. Chloroform-*d* 99.8 atom% D was obtained from Aldrich. Methanol and acetonitrile were HPLC grade and were obtained from EM Science. Ethyl acetate, reagent grade was obtained from EM Science. All other chemicals and buffers were reagent grade and were used without further purification. All solutions were prepared using double-distilled water.

2.1. Instrumentation

The HPLC separations were conducted isocratically using a liquid chromatograph (Shimadzu, Model LC-6A), variable-wavelength UV spectrophotometer (Shimadzu, Model SPD-6A), auto-injector (Shimadzu, Model SIL-9A), Chromatopac Electronic Integrator (Shimadzu, Model C-R3A), diode-array detector (Shimadzu, Model SPD-M6A) interfaced to a personal computer (80386 workstation, Parallel Port) and software (Shimadzu, SPD-M6A Software). The separations were accomplished using a reversed-phase Waters μ-Bondapak™ phenyl column (3.9 × 300 mm) and quantitation was by peak area measurement.

The separations of the hydrolysis products of Cy · HCl were performed on a semi-preparative HPLC system including a solvent delivery pump (Shimadzu, Model LC-610), variable-wavelength UV spectrophotometer (Shimadzu, Model SPD-6A), Chromatopac Electronic Integrator (Shimadzu, Model CR-501), and reverse-phase semi-preparative column (Shimadzu, Shim-Pak 5 μm ODS 20 × 250 R and D Kit) using a 10 ml loop.

^{1}H-NMR and ^{13}C-NMR spectra were determined in deuterated chloroform ($CDCl_3$) on a Bruker WM360 spectrometer equipped with Fourier transform accessories. Chemical shifts (δ ppm) were reported in parts per million (ppm) downfield from tetramethylsilane (TMS).

Infrared spectra were taken of the solid samples as potassium bromide pellets, and spectra were recorded over a wave number range of 4000–400 cm^{-1} on an FTIR spectrometer equipped with a DTGS detector, plotter and data station (Nicolet, Model 5DXB).

The mass spectrometric analyses were per-

formed on a Hewlett Packard Mass Spectrometer (Model 5970 Mass Selective Detector) with an HP 5890 Series II Gas Chromatograph, equipped with a Hewlett Packard-1 Column (12 m × 0.2 mm i.d.) in the electron impact mode. The temperature was held at 60°C for 1 min, then increased at a rate of 25°C/min up to 200°C, and held. Mass analysis for one of the degradation products was also performed on a ZAB Mass Spectrometer with xenon as the carrier gas. The mass was analyzed in the POS FAB mode in a thioglycerol/glycerol matrix (2:1), using methylene chloride as the solvent (University of Kansas, Mass Spectrometry Laboratory).

EPR spectra were recorded using a Bruker EPR spectrophotometer (ESP 300) equipped with an aqueous sample cell (3 mm i.d. quartz sample tube). The modulation frequency was 100 kHz, modulation amplitude 1 G and the time constant was 655 ms.

Molecular mechanical calculations were performed with the program SYBYL (version 5.3), which uses the Tripos force field (version 5.2) on a Silicon Graphics 4D120GTX Graphics workstation (SYBYL, 1989). Energy minimizations were carried out using the MAXMIN 2 energy minimizer with its default values. The structure was optimized until the energy change from one iteration to the next was less than 0.05 kcal.

The pH of the buffers was measured with a Beckman Model Φ70 pH Meter with a Beckman combination electrode (Model 3981). The pK_a of Cy · HCl was determined at 50°C using a Mettler DL21 Titrator (Mettler Instrument Corp., Highstown, NJ) and temperature was maintained at 50.0 ± 0.1°C with circulating water baths (Fisher, Model 7300 and Haake D1).

2.2. pK_a determination by titration

50 ml of Cy · HCl solution (approx. 1 mM) was titrated at an ionic strength of 0.2 M, under a nitrogen atmosphere with 0.095 N sodium hydroxide using a Mettler DL21 titrator. Once the titration data were collected, analysis was performed using Gran's method (Rossotti and Rossotti, 1965).

2.3. Kinetic studies

Cy · HCl was added to different aqueous buffer solutions (at concentrations of 0.4–0.6 mM), which had been previously equilibrated to 50°C in screw-topped test tubes in a circulating water bath. The buffer solutions of different pH values and buffer concentration were adjusted to a constant ionic strength of 0.20 M with sodium chloride. The effects of buffer concentrations were examined by varying the concentration of the buffers while maintaining a constant pH. Samples periodically withdrawn during a kinetic run were quenched using a pH 4.7 acetate buffer solution and then stored at −20°C. These samples were then later analyzed for the degradation of the drug, and also for the appearance of the degradation products with the aid of a reversed-phase HPLC system. The mobile phase consisted of 80%, 50 mM acetate buffer, pH 4.7 and 20% acetonitrile. The flow rate was 2.0 ml/min and the wavelength for detection was set at 254 nm. In preliminary studies it was shown that the reaction followed first-order kinetics for 3–4 half-lives and no significant change in pH ($\Delta pH < 0.2$) was found at the end of kinetic runs.

2.4. Isolation and characterization of the degradation products

Analyses of degraded solutions of Cy · HCl showed that cyclopentolate degrades to give four major degradation products. Two of these products, phenylacetic acid and cyclopentanone, could be identified by comparison with authentic samples.

Cy · HCl was degraded in 0.1 N NaOH at 50°C. The solution was analyzed periodically until all the drug had been completely degraded. Once degradation was complete, the pH of the solution was brought down to ~ pH 4.0 with glacial acetic acid to quench the reaction and to prevent the degradation products from undergoing further decomposition. Two of the degradation products, phenylacetic acid and the β-hydroxy acid, were then isolated on a semi-preparative HPLC and their structures were identified by ^{1}H-MR and ^{13}C-NMR, IR and mass spectrometric analysis.

Cyclopentanone was identified by spiking a partially degraded solution of Cy · HCl on the HPLC column. The UV spectra of the standard and the degraded samples were matched utilizing the on-line diode-array detector, and the UV spectral match provided proof of the identity of this degradation product.

2.5. EPR spectroscopic studies

Two different studies were performed to detect free radicals by EPR spectroscopy. Cy · HCl (10mM) and spin-trapping agent DMPO (5,5-dimethylpyrroline *N*-oxide, 50 mM) were dissolved in pH 8.8 borate buffer. The resulting solution was divided into two parts. With one part, the EPR spectrum was recorded at room temperature. The other part of the solution was immersed in a 50°C water bath for 15 min and the EPR spectrum was then recorded (Chalfont et al., 1968; Janzen and Blackburn, 1968). In another experiment, Cy · HCl (10 mM) was dissolved in pH 8.8 borate buffer. 2 ml of this solution was transferred to a 3 mm i.d. quartz sample tube and the tube was immersed into liquid nitrogen at −196°C. The solution in the glass tube was immediately frozen to give a 'solid glass' and this was then analyzed by EPR spectroscopy for evidence of free radicals (Broxton and Duddy, 1981).

3. Results and discussion

3.1. Effect of pH on Cy · HCl reactivity

Preliminary degradation studies of Cy · HCl in 0.1 N HCl showed that Cy · HCl degrades very slowly at room temperature. The rate of degradation was measured by following the disappearance of the Cy · HCl peak and the appearance of the degradation product peaks in HPLC chromatograms as shown in Fig. 2. The pseudo-first-order rate constant (k_{obs}) for the hydrolysis of the drug was obtained from the slopes of linear plots of ln(peak area or concentration of the drug) vs time. When a compound C degrades to form products B and D in a parallel mechanism,

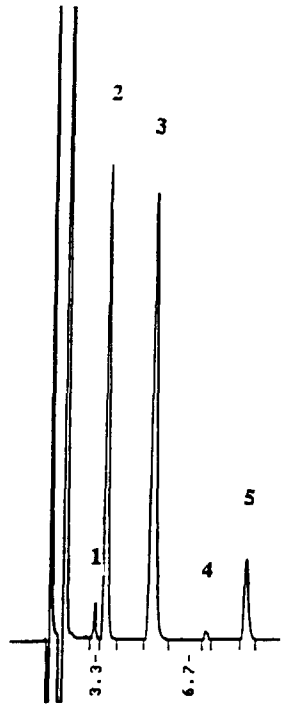


Fig. 2. HPLC chromatogram showing the separation of Cy · HCl from its degradation products. (1) Cyclopentanone, RT 3.4 min; (2) phenylacetic acid, RT 3.7 min; (3) β-hydroxy acid, RT 5.4 min; (4) degradation product 4, RT 8.2 min; (5) Cy · HCl, RT 12.6 min. Wavelength of detection at 254 nm

the rate constants for the parallel appearance of the degradation products can be obtained from the plot of the concentrations of the degradation products vs $1 - e^{-k_{obs}t}$ (Eq. 1).

$$k_{obs} = k_1 + k_2$$

$$\frac{B}{C_0} = \frac{k_1}{k_1 + k_2}\{1 - e^{-(k_1+k_2)t}\} \text{ or} \qquad (1)$$

$$\frac{D}{C_0} = \frac{k_2}{k_1 + k_2}\{1 - e^{-(k_1+k_2)t}\}$$

where C_0 is the initial concentration of C and k_1 and k_2 denote the rate constants for formation of B and D (β-hydroxy acid and phenylacetic acid), respectively.

The slope of such a line is a fraction of k_{obs} obtained above. Hence, knowing k_{obs} the individual rate constants for each of the parallel pathways can be determined. HPLC peak areas can

be substituted for concentrations if the ratio of the molar absorptivities of the two compounds in question (B/C_0) or (D/C_0) is close to unity. The β-hydroxy acid is not available commercially and thus an authentic sample could not be used to construct the standard curve routinely. An HPLC standard plot was, however, made when the β-hydroxy acid was isolated as the degradation product. The parallel rate equation was fitted to a set of data using the peak areas and the actual concentrations. The slopes (fraction of the k_{obs}) obtained were similar, indicating that the molar absorptivities of the three compounds are similar at 254 nm. To verify this point, the molar absorptivities of Cy · HCl and phenylacetic acid in the mobile phase, from Beer's plots of absorbance vs molar concentration were 0.14746 and 0.14748, giving a molar ratio of 0.9998. The UV spectra of Cy · HCl, phenylacetic acid and β-hydroxy acid, obtained from the on-line photo-diode-array detector were almost identical as the molecular group (*N,N*-dimethylaminoethanol) leaving Cy · HCl to produce degradation products (phenylacetic acid and the β-hydroxy acid) does not contain UV absorbing chromophores. At 254 nm, absorption is primarily due to the aromatic ring. The fact that data fitted to the parallel rate equation using peak areas give slopes which sum up to 1 is added evidence that such a substitution is possible, without significant error.

Table 1
Reaction conditions and observed pseudo-first-order rate constants (k_{obs}) for hydrolysis of Cy·HCl at various pH values, 50°C, $I = 0.2$ M

pH	Buffer species	[Buffer] (M)	k_{obs} (h^{-1})	k_o [a] (h^{-1})
7.54	Tris	0.04	0.167	
		0.10	0.192	0.154
		0.16	0.21	
8.00	borate	0.04	0.316	
		0.10	0.330	0.312
		0.16	0.334	
8.38	Tris	0.10	0.413	
		0.16	0.394	0.410
		0.20	0.413	
8.85	Caps	0.04	0.542	
		0.10	0.541	0.537
		0.16	0.551	
9.00	borate	0.01	0.532	
		0.10	0.601	0.53
		0.16	0.634	
9.27	Caps	0.04	0.657	
		0.10	0.672	0.654
		0.16	0.673	
9.57	borate	0.01	0.648	
		0.04	0.786	0.665
		0.10	1.02	
		0.16	1.05	
10.01	Caps	0.04	1.3	
		0.10	1.45	1.19
		0.16	1.61	
10.64	NaOH/NaCl		2.31	2.49
			2.68	
10.79	NaOH/NaCl		3.62	3.57
			3.51	
10.95	NaOH/NaCl		5.18	5.27
			5.56	
			5.08	

[a] Rate constant extrapolated to zero buffer concentration.

Data for k_{obs} under different pH conditions (Table 1), extrapolated to zero buffer concentrations, are plotted to give the pH-rate profile (Fig. 3). Buffer catalysis was observed for the hydrolysis of Cy · HCl, although it was minimal in some instances, and depended on the buffer system and pH. For instances where the rate of hydrolysis exhibited buffer dependence, linear plots were obtained when k_{obs} was plotted against the total buffer concentration at constant pH. At pH values greater than 10.0, the rate increases in a linear fashion with a slope of 1.03 indicating specific base-catalyzed degradation. The kinetic model (Eq. 2) for base-catalyzed ester hydrolysis was fitted to the data and the rate constants obtained are listed in Table 2.

$$k_{obs} = \frac{k_{BH^+}K_w}{([H^+] + K_a)} + \frac{k_B K_w K_a}{[H^+]([H^+] + K_a)} \qquad (2)$$

where k_{BH^+} is the second-order rate constant for specific base catalysis of the protonated form and k_B represents the second-order rate constant for specific base catalysis of the unprotonated form.

The kinetically determined pK_a, 7.9, is similar to that obtained potentiometrically (7.63) at 50°C at an ionic strength of 0.2 M. An inflection in the curve demonstrates that the ionized and unionized forms of the cyclopentolate molecule undergo specific base-catalyzed hydrolysis at different rates.

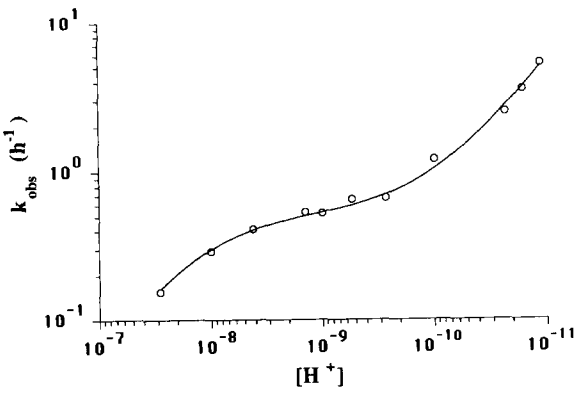


Fig. 3. k_{obs} (h^{-1}) vs [H^+] for hydrolysis of Cy·HCl under alkaline conditions at 50°C, $I = 0.2$ M.

Fig. 4 shows the quantitative appearance of the degradation products formed on hydrolysis of Cy · HCl at pH 8.0, 50°C and I = 0.2 M. At each time point, the sum of the percentages of phenylacetic acid, β-hydroxy acid and of cyclopentolate remains nearly constant, verifying that one is able to account for most of the degradation of Cy · HCl in aqueous solutions. As indicated in Fig. 4, the drug degrades exponentially and the degradation products are formed in an exponential fashion. Phenylacetic acid and cyclopentanone are formed in a 1:1 ratio. In the mechanisms postulated later, we note that both these degradation products are formed via the same reaction pathway.

Since the two main degradation products of hydrolysis of Cy · HCl are formed in a parallel fashion, the activation energies, E_{OH}, of the specific base-catalyzed reaction for each of these reactions were calculated separately (Table 3) (Connors, 1982). The E_{OH} at pH 10.01 was calculated according to experimental approach 1 while that at pH 10.95 according to experimental approach 3 in Connor's paper. Fig. 5 is the Arrhenius plot obtained at pH 10.01 Caps buffer (k_{obs}, k_1 and k_2 are extrapolated to zero buffer concentrations for each of the parallel pathways). Increasing the temperature did not result in the formation of other degradation products.

Table 2
Values of the coefficients generated from the fit of the experimental data to Eq. 2 for hydrolysis of Cy·HCl (reaction at 50°C, $I = 0.2$ M)

Parameter	Value (h^{-1})	Error (h^{-1})
k^+_{BH}	5.89E+04	6.2E+03
k_B	450	12
pK_a	7.9	
R	0.999	

3.2. Mechanism of hydrolysis of Cy · HCl in alkaline conditions

Under alkaline conditions, cyclopentolate undergoes hydrolysis by a $B_{AC}2$ mechanism to give the β-hydroxy acid. The likely mechanism of degradation is illustrated in Fig. 6. Here the rate-determining step is an attack by hydroxide ion on the carbonyl carbon to give a tetrahedral intermediate which yields the β-hydroxy acid and *N,N*-dimethylaminoethanol as the products. The $\Delta S^{\ddagger}$ values obtained suggest that a bimolecular attack is the rate-determining step. The average $\Delta S^{\ddagger}$ obtained for the hydrolysis of ethyl acetate (in a bimolecular step) in aqueous sodium hydroxide was −26.55 e.u. (D'Silva and Notari, 1981). A negative entropy results from an increase in order and may arise from bringing together previously distinct species, reducing conformational degrees of freedom, or increasing solvation due to charge development in the transition state.

Table 3
Activation parameters for Cy · HCl, 50°C, $I = 0.2$ M

pH	Mode of reaction (formation of)	Activation energy (E_{OH}) (kcal/mol)	Enthalpy of activation ($\Delta H^{\ddagger}$) (kcal/mol)	Activation entropy ($\Delta S^{\ddagger}$) (e.u.)	Activation free energy ($\Delta G^{\ddagger}$) (kcal/mol)
10.01	phenyacetic acid	16.5	15.9	−12.1	19.8
10.95		19.8	19.1	−4.1	20.4
10.01	ß-hydroxy acid	11.8	11.3	−27.8	20.2
10.95		10.8	10.1	−34.4	21.3

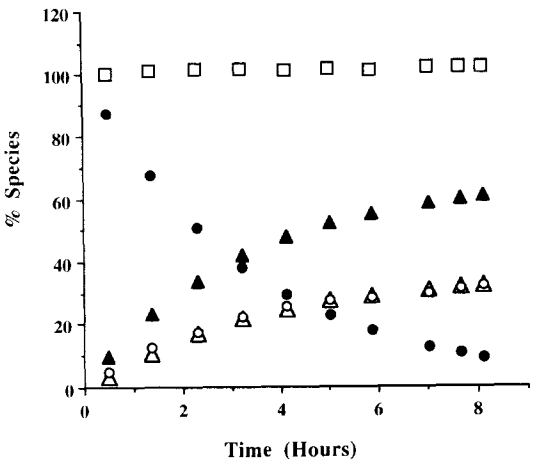


Fig. 4. Quantitative appearance of the degradation products on hydrolysis of Cy·HCl at pH 8.0, $I = 0.2$ M. (●) Cy·HCl; (▲) β-hydroxy acid; (△) phenylacetic acid; (○) cyclopentanone; (□) sum.

Degradation product 4 is formed in very small quantities (as observed by peak area measurements), and is only observed after sufficient degradation of Cy · HCl has already occurred. It was not isolated, but the UV spectrum of the HPLC peak using a diode-array spectrophotometer, indicated a shift in λ_{max} to a longer wavelength. The most plausible explanation is the dehydration of the β-hydroxy acid leading to a spectral shift due to extended conjugation. The degradation product 4 is observed in the acidic to neutral (pH 7–8) conditions and does not occur under the alkaline conditions.

Phenylacetic acid is one of the unexpected degradation products of the hydrolysis of Cy · HCl. There is a possibility that such a degradation product might be formed by a free radical reaction, as it results from the breaking of a strong carbon-carbon bond. To explore the possibility of a free radical reaction, EPR studies were conducted in a pH 8.8 borate buffer, in the presence of excess spin-trapping agent DMPO. If a free radical is being formed in the reaction, the highest prospect is the formation of the carbanion radical at the substituted methyl carbon. This radical can then be resonance stabilized with the benzene group and the double bond of the carbonyl group making the radical sufficiently stable to be detected. No carbanion free radicals could be detected by these studies at room temperature and at 50°C. EPR experiments were also conducted at −196°C. No free radicals could be detected at this temperature, suggesting that a free radical mechanism is not a major route of degradation of Cy · HCl.

EPR spectroscopy, however, does not completely rule out a free radical mechanism. If radicals have a transient half-life, their presence may not be detected even by EPR spectroscopy. Since oxygen can act as a propagating agent in free radical mechanisms, the effect of oxygen on the degradation of Cy · HCl was studied (Table 4). A high concentration of borate buffer (0.2 M) was used to keep the pH constant in the presence of a number of antioxidants. The results in Table 4 indicate that light does not accelerate the reaction. The head spaces above the solutions were purged with nitrogen gas and the bottles were clamped with rubber stoppers. Solutions were periodically removed using a syringe. The rate does not change significantly even in presence of the different antioxidants. This is further evidence that the free radical mechanism is not a major degradation pathway for Cy · HCl. The small differences in rates can be attributed either to experimental error, or to minor changes in pH in the presence of the antioxidants.

Fig. 6 also shows the proposed reaction mechanism for formation of phenylacetic acid in alka-

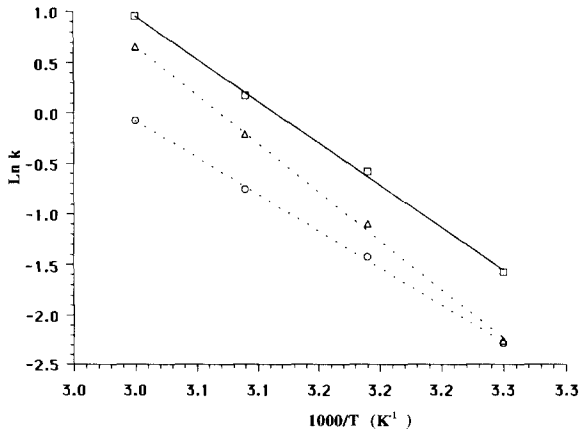


Fig. 5. Arrhenius plot of ln k vs $1/T$ at pH 10.01, $I = 0.2$ M. (□) k_{obs}, (△) k_1, (○) k_2.

Table 4
Effect of oxygen on the degradation of Cy·HCl at pH 8.86, 50°C in a 0.2 M borate buffer

Reaction medium	Rate constant (h^{-1})			
	Clear vial	Final pH	Amber vial	Final pH
Control	0.52	8.92	0.52	9.00
0.1% thioglycollic acid	0.48	8.77	0.50	8.77
0.1% cysteine HCl	0.50	8.76	0.47	8.79
0.1% Na thiosulfate	0.54	8.96	0.54	8.93
0.1% EDTA	0.50	8.92	0.52	8.90
0.1% thiourea	0.49	8.97	0.52	8.91
Oxygen saturated	0.55	8.86	0.56	8.86

line solutions. Attack by the hydroxide ion leads to the carbonyl oxygen developing a negative charge in the transition state. This negatively charged carbonyl oxygen abstracts a proton from the hydrogen of the hydroxyl group of the cyclopentanol, with the transfer of six pairs of electrons in a cyclic fashion to give the products cyclopentanone, phenylacetic acid and *N,N*-dimethylaminoethanol. At a pH of 10.01, the activation entropy is negative, −12.1 e.u., suggesting a bimolecular hydroxide ion attack. The more positive value for entropy for the cyclic pathway results from less solvent orientation since hydrogen bonding is occurring intramolecularly.

Phenylacetic acid and cyclopentanone are formed in a 1:1 molar ratio as they are produced in the same reaction. That such a six-membered structure is possible can be shown from a molecular modeling study of Cy · HCl (Fig. 7). The conformation shown in Fig. 7 is the global minimum energy conformation obtained for the cyclopentolate, i.e., the conformation at which the energy of

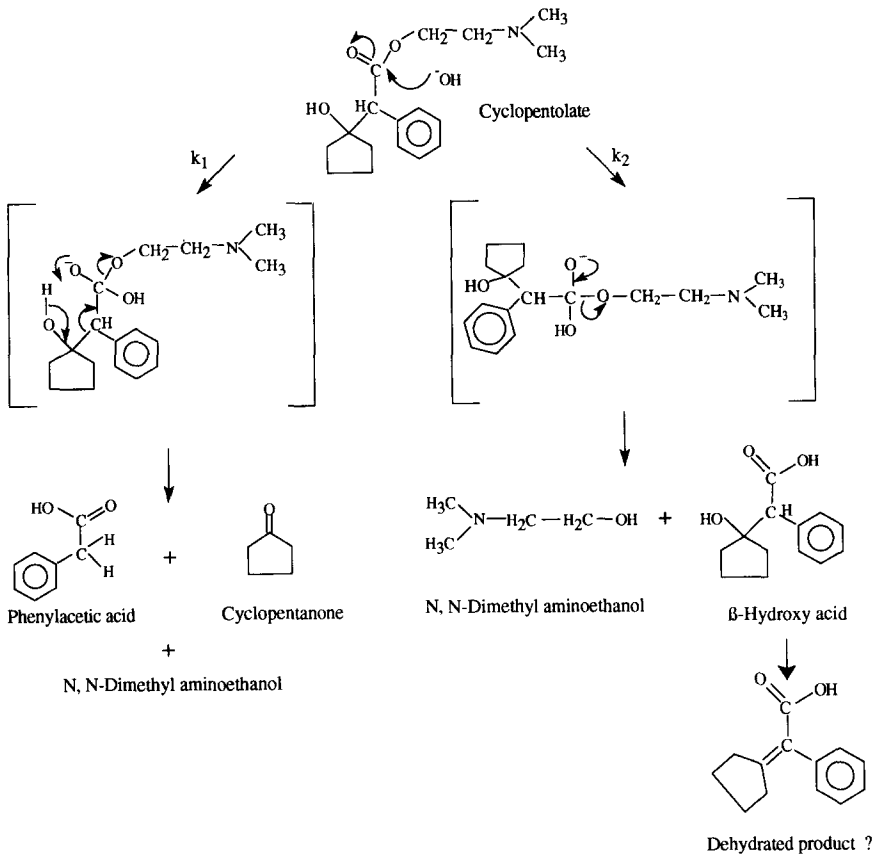


Fig. 6. Proposed parallel reaction mechanism for formation of phenylacetic acid and β-hydroxy acid under alkaline conditions.

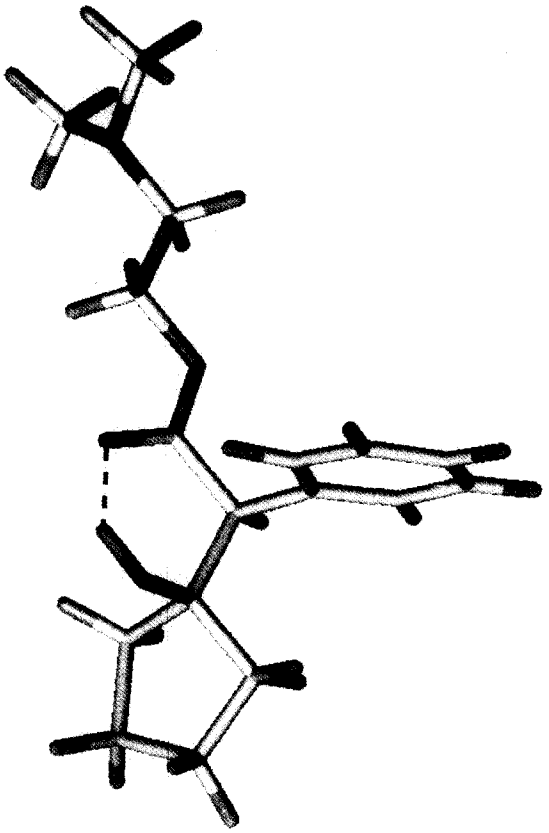


Fig. 7. The global minimum energy conformation obtained for cyclopentolate by a molecular modeling study. The minimum energy conformation indicates that a hydrogen bond can be introduced between the hydroxyl group of the cyclopentanol moiety and the carbonyl oxygen of the ester giving a stable six-membered structure.

the structure is at a minimum. In the minimum energy structure, a hydrogen bond could be introduced between the hydroxyl group of the cyclopentanol moiety and the carbonyl oxygen of the ester giving a stable six-membered structure. The distance between the oxygen and hydrogen atoms is 1.67 Å, within hydrogen bonding distance. One of the drawbacks of the molecular modeling program is that it does the computation of the minimum energy in the gas phase. In solution, the possibility of hydrogen bonding with water molecules remains but it is sufficient to say at this juncture that a six-membered ring can be formed in solution, also.

Additional evidence that a cyclic six-membered ring is necessary for the formation of phenylacetic acid comes from the study of a structurally similar compound, drofenine (Fig. 1). Drofenine has a cyclohexyl group attached to the substituted methyl group of the ester rather than a cyclopentanol group. The notable feature in the structure of drofenine is the absence of the hydroxyl group on the cyclohexane moiety. A cyclohexyl group is similar in conformation to a cyclopentyl group. Drofenine also has the *N,N*-diethyl group rather than the *N,N*-dimethyl group on the nitrogen atom. This group would not be expected to affect the reaction mechanism as it is far away from the reaction center. No phenylacetic acid is formed from drofenine on hydrolysis, and this gives added support to the conclusion that a cyclic six-membered ring is necessary for phenylacetic acid formation.

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