Facile Acid Hydrolysis of *p*-Chlorobenzaldoxime and Its Oral Inefficacy

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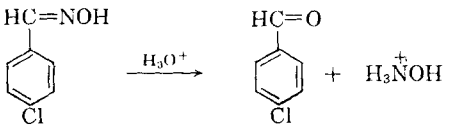
The kinetics of acid hydrolysis of *p*-chlorobenzaldoxime have been studied. A polarographic assay for reactant and product has been devised. The oxime readily hydrolyzes in acid solution to form an equilibrium mixture with the products. It is expected that the half-life of this oxime would be less than 20 minutes in the human stomach and that the oral inefficacy may be ascribed to this mode of degradation.

OXIMES have been shown to have interesting pharmacological properties (1). In recent animal work at our laboratories, a typical oxime, *p*-chlorobenzaldoxime (PCBO), was inactive as a muscle relaxant (2, 3) when administered orally but active when administered parenterally. The facile hydrolysis of the oximes to *p*-chlorobenzaldehyde (PCB) and hydroxylamine at the pH of the stomach may be a possible explanation.



This paper considers the appropriate assay for such kinetics of hydrolysis and the validity of this possible explanation of oral inefficacy.

EXPERIMENTAL

Choice of Assay Method.—The change of the ultraviolet absorption spectra of *p*-chlorobenzaldoxime in hydrochloric acid solution was extremely small and surprisingly fast. The asymptotic value of the spectra with time was too close to the initial spectra. This made it necessary to investigate a more sensitive method of following the possible transformation of the *p*-chlorobenzaldoxime in hydrochloric acid solution to the *p*-chlorobenzaldehyde and the hydroxylamine hydrochloride.

The method chosen was polarography with the Leeds and Northrup dropping mercury electrode Electrochemograph.

Typical polarographic waves for the reactant and the possible products are given for 10^{-3} *M* solutions in 10% ethanol, 0.100 *M* hydrochloric acid at 25° in Fig. 1. Curve A is the diffusion current in microamperes, i_D, *vs.* volts for *p*-chlorobenzaldoxime, $E_{1/2} = -0.64$; i_D/M at -0.75 v. is $(10.98 - 0.90) \div 10^{-3} = 10.08 \times 10^3$ μa./mole/L. where the blank has 0.90 μa. at -0.75 v. Curve B for *p*-chlorobenzaldehyde shows an $E_{1/2} = -0.855$ v., i_D/M at -1.00 v. is $(3.50 - 1.08) \div 10^{-3} = 2.42 \times 10^3$ μa./mole/L. where the blank has 1.08 μa. at -1.00 v. Curve C for the hydroxylamine at 10^{-3} *M* indicates that this product of the hydrolysis will not significantly interfere with the polarographic assay of the *p*-chlorobenzaldoxime and the *p*-chlorobenzaldehyde. Table I gives the $E_{1/2}$ values after accounting for the i_D of the blank solutions for these two compounds in various hydrochloric acid solutions.

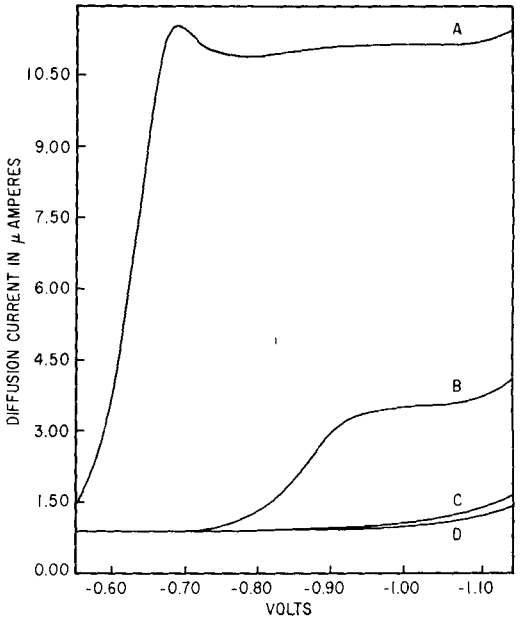


Fig. 1.—Polarographic waves at $10^{-3}M$ in 0.100 *M* hydrochloric acid and 10% ethanol-water at 25°. Curve A, *p*-chlorobenzaldoxime; curve B, *p*-chlorobenzaldehyde; curve C, hydroxylamine hydrochloride; and curve D, blank solution.

Figure 2 is the calibration curve for the i_D at -0.75 v. for *p*-chlorobenzaldoxime in 0.100 *M* hydrochloric acid, 10% ethanol, 25°, as a function of the oxime concentration. The i_D values are corrected for the i_D of the blank under these conditions. The slope or the specific i_D is 0.0633 μa./mcg./ml.

Figure 3 is a typical series of polarograms with time of the reacting solution, 10^{-3} *M* in *p*-chloro-

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TABLE I.—HALF-WAVE POTENTIALS, $E_{1/2}$, OF *p*-CHLOROBENZALDOXIME AS A FUNCTION OF HYDROCHLORIC ACID MOLARITY AT 25° IN 10% ETHANOL

$E_{1/2}$	*M* HCl
−0.658	0.05
−0.648	0.075
−0.638	0.10
−0.627	0.15
−0.621	0.20

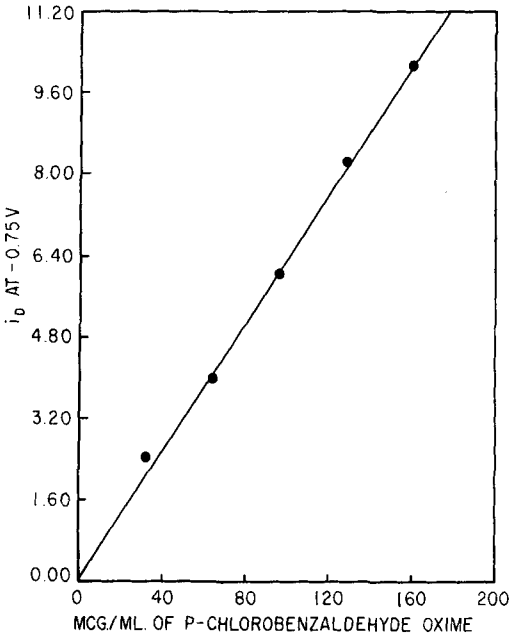

Fig. 2.—Calibration curve for *p*-chlorobenzaldoxime in 0.100 *M* hydrochloric acid, 10% ethanol-water at 25°; diffusion current, i_D in μa., *vs.* concentration in mcg./ml.

benzaldoxime, 0.20 *M* hydrochloric acid, 25°, 10% ethanol. A maximum appeared at −0.68 v. which necessitated the addition of gelatin. The decrease of the wave height characterized by an $E_{1/2}$ of −0.64 is associated with the hydrolysis of the *p*-chlorobenzaldoxime whereas the increase of the wave characterized by an $E_{1/2}$ of −0.84 is associated with the appearance of the hydrolysis product, *p*-chlorobenzaldehyde.

Procedure.—*p*-Chlorobenzaldoxime (PCBO), molecular weight = 155.58, synthesized and recrystallized by G. H. Smith and A. J. Lemin of these laboratories, was titrated and had pKa's of 10.35 in 20% ethanol, 10.70 in 50% ethanol. An 80-mg. quantity of PCBO was weighed into a tared 50-ml. volumetric flask and diluted to volume with nitrogen-purged ethanol. Ten milliliters of the resultant solution was pipetted into a 100-ml. volumetric flask which contained hydrochloric acid plus 2 ml. 0.1% gelatin solution. Subsequently, the solution was diluted up to volume with sufficient hydrochloric acid so that the resultant molarities were 10^{-3} in PCBO, and 0.050, 0.075, 0.150, or 0.200 *M* in hydrochloric acid and 10% in ethanol. The solutions were assayed on the polarograph immediately and at timed intervals thereafter. The solution was maintained in a 25° constant temperature bath between polarographic assays and was degassed with nitrogen for approximately 3 minutes before each run.

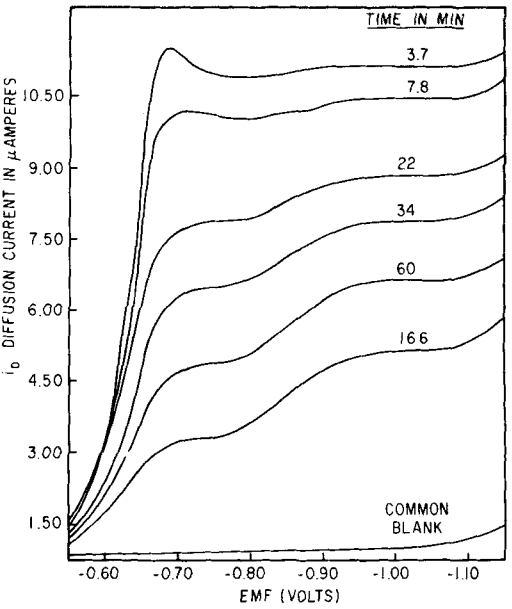

Fig. 3.—Polarographic waves of *p*-chlorobenzaldoxime in 0.200 *M* hydrochloric acid, 10% ethanol-water at 25° at various times.

CALCULATIONS AND RESULTS

The i_D diffusion current at −0.75 and −1.00 v. for the PCBO solutions decreased with time, the latter less than the former since the plateau of the wave representing the *p*-chlorobenzaldehyde concentration appeared prior to −1.00 volts. This wave does not interfere with the −0.75 v. reading. Typical plots of these i_D values as functions of time are given at the top of Fig. 4. The net i_D, i.e., i_D (−1.0 v.) $-i_D$ (−0.75 v.), representing the appearance of the benzaldehyde is given in the bottom of Fig. 4.

The fact that the i_D at −0.75 v. did not approach the i_D of the blank at infinite time indicated that this is an equilibrium reaction.

The plots of Fig. 5 are typical examples on the assumption of an apparent first-order transformation of PCBO to, and in equilibrium with, PCB at constant hydrochloric acid. Curve A represents the first-order disappearance of PCBO by the expression

$$\log [i_D - (i_D)_\infty] = \frac{-(k_1 + k_2)}{2.303} t + \log [(i_D)_0 - (i_D)_\infty] \quad \text{(Eq. 1)}$$

where the i_D values are read at −0.75 v., $(i_D)_0$ is the initial reading, and $(i_D)_\infty$ the reading in μa. at infinite time. Curve B represents the first-order appearance of PCB by the expression

$$\log \{[(i_D)_\infty(-1.0 \text{ v.}) - (i_D)_\infty(-0.75 \text{ v.})] - [i_D(-1.0 \text{ v.}) - i_D(-0.75 \text{ v.})]\} = -\frac{(k_1 + k_2)}{2.303} t + \text{constant} \quad \text{(Eq. 2)}$$

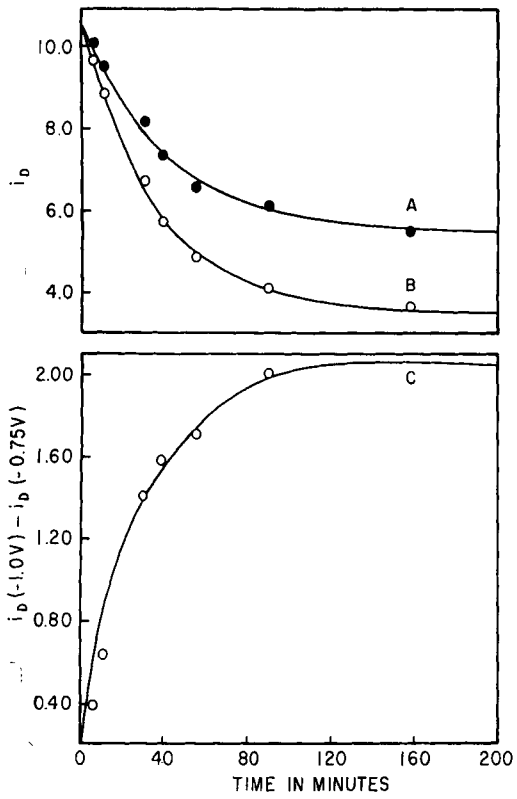

Fig. 4.—Polarographic diffusion current, i_D, of 10^{-3} M p-chlorobenzaldoxime in 0.150 M hydrochloric acid 10% ethanol-water at 25° against time of reading: ○, −0.75 v., ●, −1.00 v.

When it is realized that the data for curve B is obtained from the subtraction of small differences of relatively large values, the empirical use of the assumed model is not unwarranted.

From the slopes of plots similar to curve A, Fig. 5, the sum of the forward and backward rate constants, $k_1 + k_2$, can be calculated as per the postulated equilibrium

$$p\text{-chlorobenzaldoxime} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} p\text{-chlorobenzaldehyde} \qquad \text{(Eq. 3)}$$

where

$$\frac{k_1}{k_2} = \frac{[\text{Benzaldehyde}]}{[\text{Benzaldoxime}]} = K \qquad \text{(Eq. 4)}$$

where K is defined as the equilibrium constant. This K may be estimated from

$$K = \frac{(i_D)_0 - (i_D)_\infty}{(i_D)_\infty} \qquad \text{(Eq. 5)}$$

for all i_D at −0.75 v. From Eqs. 1, 4, and 5; k_1' k_2, and K can be calculated and are given in Table II.

The k_1, k_2, and K values of Table II are plotted in Fig. 6 and dependence on hydrochloric acid concentration may be estimated as

$$k_1\,(\text{min.}^{-1}) = 0.045\,[\text{HCl}] + 0.0134 \qquad \text{(Eq. 6)}$$

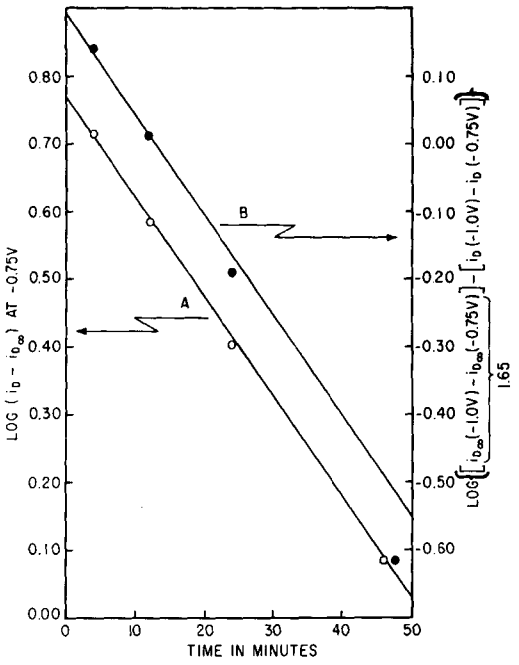

Fig. 5.—Apparent first-order rate plots for the hydrolysis of $10^{-3}M$ p-chlorobenzaldoxime (PCBO) in 0.075 M hydrochloric acid, 10% ethanol-water at 25°. Curve A is for the disappearance of PCBO; curve B is for appearance of p-chlorobenzaldehyde.

TABLE II.—RATES k_1 AND k_2 IN MIN.$^{-1}$ AND EQUILIBRIUM (K) CONSTANTS FOR THE ASSUMED FIRST-ORDER (FORWARD AND REVERSE) TRANSFORMATION OF p-CHLOROBENZALDOXIME $\underset{k_2}{\overset{k_1}{\rightleftharpoons}}$ p-CHLOROBENZALDEHYDE IN 10% ETHANOL, 25°, $10^{-3}M$ IN PCBO. K' IS WHEN THE BACK REACTION IS ASSUMED SECOND ORDER WITH RESPECT TO PCB AND HYDROXYLAMINE

[HCl]	$k_1 + k_2$	k_1	k_2	K	$10^4K'$
0.050	0.0326	0.0152	0.0174	0.870	4.35
0.075	0.0340	0.0185	0.0155	1.194	6.99
0.150	0.0312	0.0211	0.0102	2.071	15.0
0.200	0.0273	0.0216	0.0057	3.778	32.1

A possible alternate dependency is given by the dashed line. The other estimated constants are

$$k_2\,(\text{min.}^{-1}) = -0.00751\,[\text{HCl}] + 0.021 \qquad \text{(Eq. 7)}$$

$$K = 17.5\,[\text{HCl}] \qquad \text{(Eq. 8)}$$

Actually, a more elegant postulation of the equilibrium would be

$$\text{PCBO} \underset{k_2'}{\overset{k_1}{\rightleftharpoons}} \text{PCB} + \text{Hydroxylamine} \qquad \text{(Eq. 9)}$$

and

$$K' = \frac{[\text{PCB}]\,[\text{Hydroxylamine}]}{[\text{PCBO}]} \qquad \text{(Eq. 10)}$$

where

$$[\text{PCB}] = [\text{Hydroxylamine}] \qquad \text{(Eq. 11)}$$

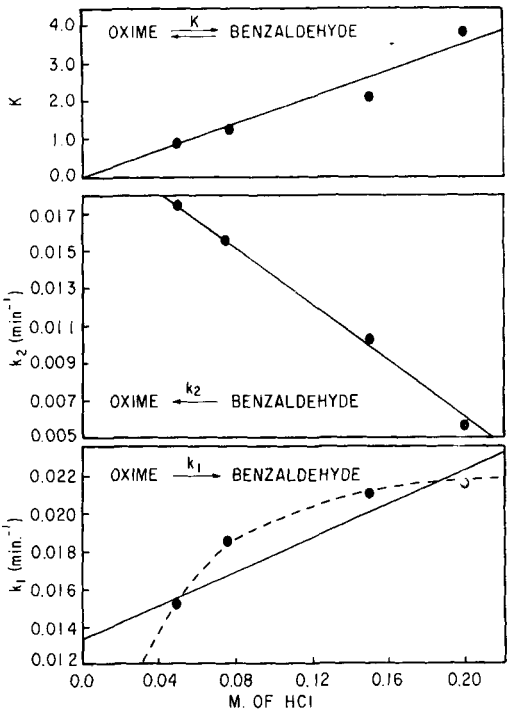

Fig. 6.—Plots of apparent equilibrium constant, K and rate constants, k_1 and k_2, for the simplified equilibrium

$$\text{PCBO} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{PCB}$$

as a function of hydrochloric acid molarity.

so that an equation comparable to Eq. 5 would be

$$K' = \frac{\{(i_D)_0 - (i_D)_\infty \div 10^{-4}\}^2}{(i_D)_\infty \div 10^{-4}} = \frac{\{(i_D)_0 - (i_D)_\infty\}^2 \times 10^4}{(i_D)_\infty} \quad \text{(Eq. 12)}$$

where

$$i_D/C = 10^4\ \mu\text{a.}/M/L. \quad \text{(Eq. 13)}$$

Values for K' are also given in Table II. The plots to determine rate constants could be based on the more elegant and complete expression (4)

$$2.303 \log \frac{[\text{PCBO}]_0 [\text{PCB}]_{eq} + [\text{PCB}]\{[\text{PCBO}]_0 - [\text{PCB}]_{eq}\}}{[\text{PCBO}]_0\{[\text{PCB}]_{eq} - [\text{PCB}]\}} = \frac{kt\{2[\text{PCBO}]_0 - [\text{PCB}]_{eq}\}}{[\text{PCB}]_{eq}} \quad \text{(Eq. 14)}$$

However, since the Eq. 1 satisfactorily fits the data, the simplified assumption of opposing first-order reactions is adequate for the purposes of this study.

DISCUSSION

For our purposes k_1 is given at 25° in 10% ethanol by Eq. 6.

The hydrolysis of PCBO is an equilibrium reaction under the conditions studied. On ingestion into the stomach, however, it is reasonable to postulate the removal of end products PCB and hydroxylamine, so that the hydrolysis tends to be complete.

If the acid concentration of the stomach is assumed to be 0.05 M, the apparent first-order rate constant may be estimated as 0.015 min.$^{-1}$ from Eq. 6 or the half-life for the hydrolysis of the PCB oxime would be

$$t_{1/2} = \frac{2.303 \log 0.5}{k} = \frac{(0.69)}{0.015} = 46 \text{ min.} \quad \text{(Eq. 15)}$$

This is at 25° and in 10% ethanol. It is to be expected that at 37° or body temperature the rate would be at least doubled so that $t_{1/2}$ = 23 min. at 37°. Also, in truly aqueous system the rate should be further increased. Thus, it may be estimated that the half-life of PCBO under the physiological conditions of the human stomach would be less than 20 minutes, and the oral inefficacy of such oximes may be attributed to acid hydrolysis in the stomach.

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