Acid and Enzymic Hydrolysis of N-Butylformamide and Its Oral Inefficacy

By EDWARD R. GARRETT† and DENNIS J. WEBER

The kinetics of acid and enzymic hydrolysis of the parenterally pharmacologically active N-butylformamide have been studied by titrimetric and color assay procedures. It is expected that the half-life of the amide under the conditions of the human stomach would exceed 30 hours. The stability of the drug greatly exceeds any stomach retention time.

INITIAL recognition of the pharmacological efficacy of a drug often results from a screening program where the potential agent is parenterally administered. However, this mode of administration severely limits a drug's utility and marketability so that the product potential has to be evaluated with regard to oral efficacy. If a drug is parenterally effective but orally ineffective, a physical-chemical evaluation may possibly demonstrate the reason so that the drug may be properly chemically or pharmaceutically modified (1).

The compound, N-butylformamide, $HCNCH_2-CH_2CH_2CH_3$ (O double-bonded to C; H on N), was shown to have a selective effect on the avoidance behavior of rats similar to that of chlorpromazine when administered parenterally but not when administered orally (2). The purpose of this study was to predict the possible gastric stability of the material for a possible explanation of its oral inefficacy.

EXPERIMENTAL

Kinetics of Acid Hydrolysis.—Weighed amounts of the liquid N-butylformamide, synthesized by R. B. Moffett of these laboratories, were placed in thermally equilibrated 50-ml. volumetric flasks and brought up to volume with thermally equilibrated standard hydrochloric acid, immediately mixed, and maintained in the constant temperature bath. The detailed concentrations and temperatures are given in Table I. Five-milliliter aliquots of the hydrolyzing amide solutions were taken at planned intervals and quenched with sufficient 1 *M* sodium hydroxide so that the excess hydrochloric acid was almost neutralized. To the 6 ml. of the resultant solution was added 25 ml. dimethylsulfoxide, and the resultant mixed solution was cooled to room temperature. This solution was subsequently titrated with standard 0.1 *N* sodium hydroxide on the Precision-Dow recording titrator equipped with glass-saturated calomel electrodes. This solvent mixture gave two sharp inflections, the first one indicative of complete neutralization of the excess hydrochloric acid and the second one indicative of the complete neutralization of the formic acid produced by the acid hydrolysis.

Kinetics of Enzymic Hydrolysis.—Accurately weighed samples of N-butylformamide were diluted to 50 ml. with thermally equilibrated degrading solutions. There were three different degrading solutions used. One contained 0.4 *N* hydrochloric acid and 0.1% w/v pepsin at 40°; another contained 0.01 *N* hydrochloric acid and 0.1% w/v pepsin at 40°; and the third contained a Clark and Lubbs buffer (3) of pH 7.9 at 40° and contained 0.1% w/v trypsin. The pepsin and trypsin were obtained from the Worthington Biochemical Corp.

One-milliliter aliquots were taken at recorded time intervals and their N-butylamine content determined by the method evaluated by Forist and Judy (4) of these laboratories. The spectrophotometric procedure for the determination of amines based on reaction with bromcresol purple in chloroform (5) was applicable to the determination of N-butylamine in chloroform (4). Under the conditions of extraction (0.1 *N* sodium hydroxide) production of butylamine from intact butylformamide was less than 2% over a period of 15 minutes and indicated adequate stability of the amide. Beer's law was followed for butylamine over the range 10^{-4} to 10^{-3} *M*, the concentration range of the samples. Determination of standard butylamine samples gave a mean recovery ± standard deviation of 99.1 ± 3.5% (4). The presence of enzyme was shown not to interfere with the assay.

CALCULATIONS AND RESULTS

The basic expression for the hydrolysis kinetics is

$$d(A - X)/dt = -k[A - X] = -k_{H^+}[H^+][A - X] \quad \text{(Eq. 1)}$$

where A is the initial concentration of the N-butylformamide, X is the concentration of formed formic acid at time t, and k is the pseudo first-order rate constant obtained from the slope of the first-order plot of the integrated expression

$$\log [A - X] = -kt/2.303 + \log [A] \quad \text{(Eq. 2)}$$

Typical plots are given in Fig. 1. The conditions, pseudo first-order and actual second-order rate constants, are given in Table I.

The Arrhenius plot of the logarithm of the rate constant, k_{H^+} in L/M/sec., against the reciprocal of the absolute temperature as per

$$\log k = -(\Delta H_a/2.303R)(1/T) + \log P \quad \text{(Eq. 3)}$$

is given in Fig. 2. The heat of activation is 19.8 Kcal./mole and the log P value is 9.16.

Apparent rate constants for the hydrolysis of N-

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TABLE I.—KINETICS OF ACID HYDROLYSIS OF N-BUTYLFORMAMIDE

Temp., °C.	[HCl]	[N-Butylformamide]	10^6k (sec.$^{-1}$)	$t_{1/2}$, hr.	$10^5k/[HCl] = 10^5 k_{H^+}(L/M/sec.)$
30.0	0.0993	0.0400	0.747	258	0.750
30.0	0.1988	0.0421	1.40	138	0.703
30.0	0.3989	0.0388	2.94	65.6	0.738
39.0	0.4045	0.0331	8.47	22.8	2.08
50.6	0.0994	0.0449	6.69	28.7	6.74
50.5	0.1988	0.0182	14.7	13.1	7.39
50.2	0.1988	0.0182	12.6	15.3	6.34
51.0	0.4045	0.0288	23.1	8.34	5.70
60.0	0.4045	0.0355	63.6	3.02	15.7
70.0	0.0993	0.0210	27.9	6.80	28.1
70.8	0.1988	0.0192	81.9	2.35	41.1
69.6	0.3989	0.0196	120	1.59	30.2

TABLE II.—DATA FROM SUPPOSED ENZYMATIC HYDROLYSIS OF N-BUTYLFORMAMIDE AT 40°

[H$^+$]	w/v % Pepsin	w/v % Trypsin	[*N*-Butylformamide]	10^6k (sec.$^{-1}$)	$t_{1/2}$, hr.	$10^5k/[H^+] = 10^5 k_{H^+}(L/M/sec.)$
0.4117	0.1	..	1.10×10^{-3}	8.60	22.3	2.09
0.0100	0.1	..	1.68×10^{-3}	0.0524	3670	0.524
$10^{-7.9}$	..	0.1	1.07×10^{-3}	*ca.* 0.00	>3670	*ca.* 0.0

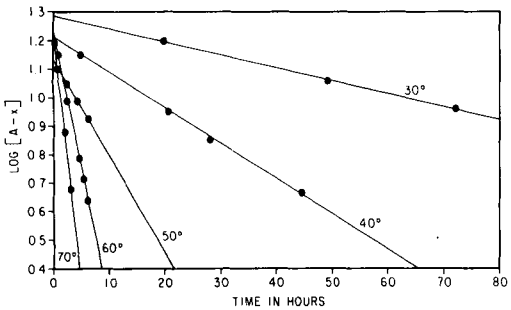

Fig. 1.—Pseudo first-order plots of the hydrolysis of N-butylformamide in 0.4 *N* hydrochloric acid. The temperatures and the initial molarities of *N*-butylformamide were: 30°, 0.0388; 40°, 0.0331; 50°, 0.0288; 60°, 0.0355; and 70°, 0.0196.

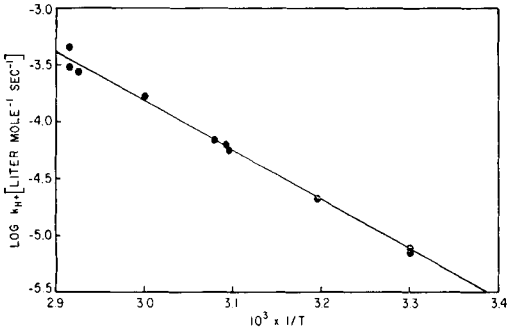

Fig. 2.—Arrhenius plot of the bimolecular rate constant for the acid hydrolysis of N-butylformamide.

butylformamide in the presence of the enzymes trypsin and pepsin are given in Table II. A specific example of a pseudo first-order rate plot is given in Fig. 3. The line is drawn on the basis of the acid hydrolysis equations evaluated in Eq. 2 and shows no enhancement of rate by the presence of the enzyme pepsin. The pH 2 solution of pepsin, a better approximation of stomach conditions, also demonstrated a hydrolysis rate not inconsistent with simple acid hydrolysis. The experiment using trypsin at its optimum pH value also showed no significant enzymic effect.

DISCUSSION

Kinetic analysis of the enzymatic degradation of N-butylformamide showed that the oral inefficacy of the drug cannot be attributed to gastrointestinal degradation by the enzymes, pepsin or trypsin, at their optimum pH values, 2 and 7.8, respectively. Since the half-life of N-butylformamide at 39° in 0.4 *N* hydrochloric acid is 22.8 hours, the inefficacy of oral administration where parenteral administration is effective cannot be attributed to acid degradation of the compound in the gastrointestinal tract. This large half-life proves that the rates of acid degradation are grossly less than any stomach retention time.

This work indicates that the lack of oral activity is not due to enzymatic or solution degradation in the gastrointestinal tract. An alternative explanation is that the drug is orally inactive due to its inability to pass through the lipid-like gastrointestinal mucosa. This is consistent with its high insolubility in fat solvents (6).

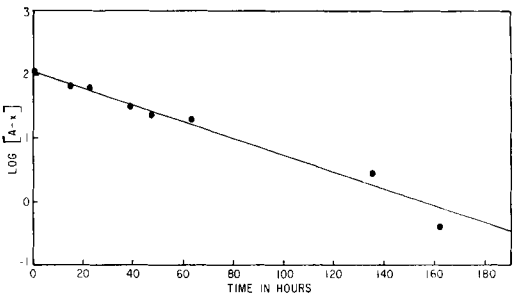

Fig. 3.—Pseudo first-order plot of the hydrolysis of N-butylformamide in the presence of 0.1% w/v pepsin at 40° and 0.411 *M* hydrochloric acid. The drawn line is expected from acid hydrolysis alone.

The heats of activation for the acid hydrolysis of various unsubstituted acylamides listed in the literature (7) are all in the range 19–21 Kcal./mole. These amides include formamide, acetamide, benzamide, propionamide, and butyramide. However, all of these compounds hydrolyzed at much faster rates; for example, formamide has a half-life at 42° in 0.1 *N* hydrochloric acid of 2.2 hours, at 25° of 7.0 hours (7). Apparently substitution on the nitrogen of the amide markedly slows the rate of hydrolysis.

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Preparation of Microcrystalline Progesterone Using Ultrasound

By JOSEPH R. PRINCIPE† and DONALD M. SKAUEN

Microscopic crystals of progesterone were prepared by insonating saturated solutions of the hormone. Suspensions were made of the crystalline precipitates in 1% tyloxapol solution. Individual crystals were measured by projecting photographs on a calibrated screen.

THE DESIRABILITY of obtaining a chemical compound of uniform microscopic size is considered to be of importance to the pharmaceutical industry, particularly in the preparation of suspensions.

The usual methods of preparing suspensions for injection involve size reduction by milling or crystallization from various solvents to produce crystals of microscopic size and uniformity.

This investigation was undertaken to ascertain the suitability of employing ultrasonic energy in the production of microcrystalline progesterone.

EXPERIMENTAL

Saturated solutions of progesterone in solvent mixtures of varying proportions of ethyl alcohol and ethylene glycol at different temperatures were prepared. This solvent was selected because of good solvent action and because viscosity could be varied without the use of a secondary agent.

One-milliliter samples of the supernatant liquid from the saturated solutions were transferred to small hard-glass test tubes, stoppered, and immediately insonated.[1] The contents of the sound chamber was kept at approximately 20° by means of a cold water coil. The effect of length of time of insonation and the effect of power were explored.

Immediately after insonation, the test tubes containing the resulting precipitates were centrifuged, the supernatant discarded, and the crystals allowed to dry before resuspension in a 1% aqueous tyloxapol solution.

Photomicrographs of the uniformly distributed suspended material were made and examined for particle size by a projection technique.

Table I shows typical data obtained by insonating saturated solutions prepared at 55°.

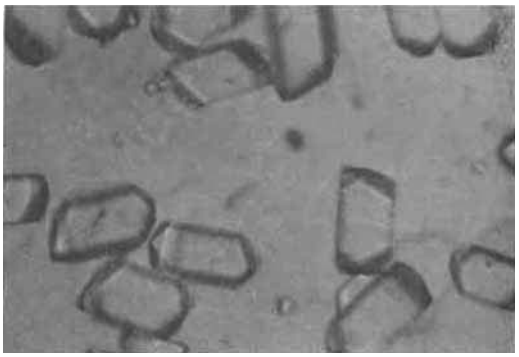

Fig. 1.—Prepared progesterone crystals. Solvent, ethyl alcohol U.S.P., 25 parts, ethylene glycol, 75 parts; insonation time, 5 sec.; arithmetic mean length (μ), 47.3; saturated solution prepared at 55°; plate power, 50 ma.; S.D., 9.4.

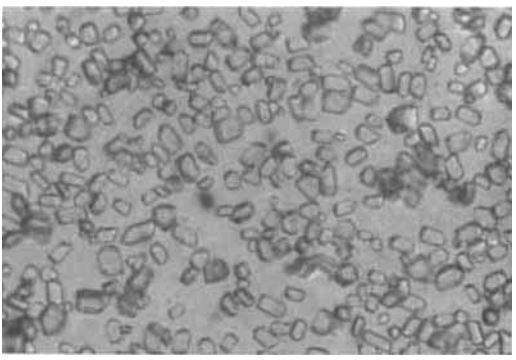

Fig. 2.—Prepared progesterone crystals. Solvent, ethyl alcohol U.S.P., 25 parts, ethylene glycol, 75 parts; insonation time, 5 seconds; arithmetic mean length (μ), 11.0; saturated solution prepared at 55°; plate power, 100 ma.; S.D., 3.3.

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[1] Model G-3, General Electric Ultrasonic Generator.