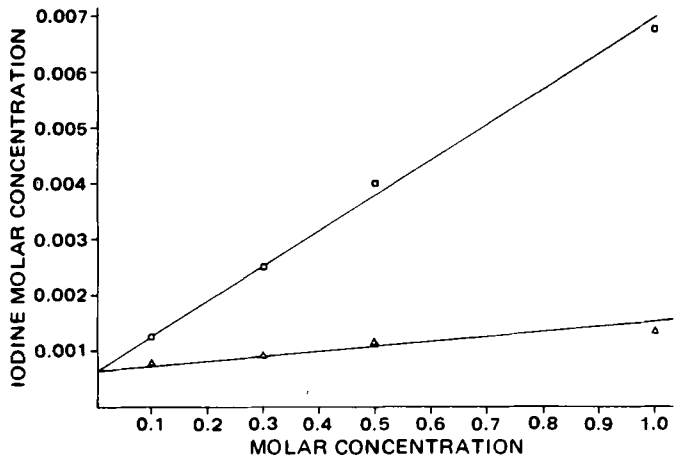

Figure 5—*Plot showing the increase in the solubility of iodine as a function of added sodium chloride (△) and sodium bromide (□).*

from the slope of the lines of Fig. 5 were 10.6 and 2.4 for bromide and chloride, respectively, in good agreement with reported values (7).

Figures 1 and 2 show the UV absorbances of solutions containing 3×10^{-4} *M* I_2 and varying concentrations of sodium bromide and sodium chloride. A linear relationship between the absorbance at the corresponding wavelengths and the concentration of the added halide salts was observed. It is apparent from the data of Figs. 1 and 2 that the change in the magnitude of the absorbance as a function of the added halide was due to changes in the concentration of the complexes formed in solution.

The concentrations of I_2Br^- and I_2Cl^- can be calculated as a function of added halides using Eq. 1 (Tables I and II). The calculated molar absorptivities were 3.27×10^4 and 2.3×10^4 M^{-1} cm^{-1} for I_2Br^- and I_2Cl^-, respectively.

Table III shows the results obtained for the determination of sodium chloride tablets by the USP and spectrophotometric methods. The agreement between the two methods was excellent. In practice, it is advisable to determine the unknown together with a known sample using the same iodine concentration in both samples.

REFERENCES

(1) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 457.

(2) W. M. MacNevin, B. B. Baker, and R. D. McIver, *Anal. Chem.*, **25**, 274 (1953).

(3) I. M. Kolthoff and V. A. Stenger, "Volumetric Analysis," 2nd ed., Interscience, New York, N.Y., 1947.

(4) R. Belcher, A. M. MacDonald, and E. Parry, *Anal. Chim. Acta*, **16**, 524 (1957).

(5) R. Rechnitz, *Chem. Eng. News*, **45**, 146 (1967).

(6) G. A. Rechnitz, M. R. Kresz, and A. Sloan, *Anal. Chem.*, **38**, 1786 (1966).

(7) P. J. Durrant and B. Durrant, "Introduction to Advanced Inorganic Chemistry," Wiley, New York, N.Y., 1962, p. 933.

(8) "Remington's Pharmaceutical Sciences," 14th ed., Mack Publishing Co., Easton, Pa., 1970, p. 1180.

Stability of 10-Acetylphenothiazine

H. ROSEBOOM ˣ **and A. D. FÖRCH**

Received April 7, 1978, from the *Farmaceutisch Laboratorium Rijksuniversiteit Utrecht, Catharijnesingel 60, Utrecht, The Netherlands.* Accepted for publication August 30, 1978.

Abstract □ The degradation kinetics of 10-acetylphenothiazine were studied after isolation and identification of its degradation products, phenothiazine, phenothiazine-5-oxide, 3*H*-phenothiazine-3-one, and 7-(10′-phenothiazinyl)-3*H*-phenothiazine-3-one. The acetyl group was not present in any degradation product. The degradation rate was independent of oxygen but highly pH dependent, indicating a specific hydrogen-ion-catalyzed hydrolysis of 10-acetylphenothiazine to give phenothiazine, which was oxidized. The experimental values for the phenothiazine concentration were in good agreement with values calculated on the basis of this assumption, both in the presence and absence of oxygen.

Keyphrases □ 10-Acetylphenothiazine—stability and degradation kinetics, pH dependency □ Phenothiazines—10-acetylphenothiazine, stability and degradation kinetics, pH dependency □ Degradation—10-acetylphenothiazine, kinetics, effect of pH

The kinetics and mechanism of the oxidative degradation of phenothiazine and its 10-methyl derivative were described previously (1, 2). In this paper, the influence of the introduction of an acetyl group on stability is investigated. The 10-acetyl derivative can be considered as a model compound for the phenothiazines having a carbonyl function next to the nitrogen atom; these compounds possess antiarrhythmic properties (3). The electron-withdrawing properties of the acetyl group could possibly have a strong stabilizing effect on the phenothiazine ring system.

EXPERIMENTAL

Materials—10-Acetylphenothiazine[1] (I) was recrystallized from 96% ethanol, mp 204°, and had a molar absorptivity at 257 nm of 10,194. TLC of the recrystallized product in various systems showed only one spot, so no further purification was performed.

All other materials were as previously described (1).

Isolation and Identification of Degradation Products—Compound I was dissolved in 52.5% ethanol, buffered to an apparent pH of 2.8 with sodium acetate–hydrochloric acid (4), and stored in the dark at 85° for 3 days after saturation with oxygen. The degradation products were extracted with carbon tetrachloride and isolated by TLC and column chromatography. Silica gel and aluminum oxide were used as adsorbents, and the solvents were ether, petroleum ether, chloroform, acetone, and their mixtures. The best separation was obtained with aluminum oxide and ether and silica gel and chloroform, both giving five zones. When the degradation was carried out at pH 1.7, the same products were found by TLC as at pH 2.8.

To isolate the degradation products, the carbon tetrachloride extract was evaporated to dryness under reduced pressure at room temperature and the residue was dissolved in ether. This solution was put onto a silica gel column, and the column was then eluted with ether until the colored products had left the column. Because one degradation product was not eluted with ether, the elution was continued with acetone, giving a solution that contained only one degradation product.

The ether eluate was evaporated to dryness under reduced pressure at room temperature, and the residue was dissolved in ether–petroleum ether (1:3). This solution was put onto an aluminum oxide column, and

[1] 10-Acetylphenothiazine was a gift from Dr. C. D. M. ten Berge, Farmaceutisch Laboratorium, Groningen, The Netherlands.

0022-3549/79/0400-0515$01.00/0

Table I—Apparent First-Order Rate Constants, $k_1 \times 10^3$ hr^{-1}, for the Degradation of 10-Acetylphenothiazine in 52.5% Ethanol at 85° at Various pH Values

pH	Oxygen-Saturated Medium	Oxygen-Free Medium
1.9	17.9	17.7
2.1	11.1	—
2.4	5.5	—
2.8	2.4	2.3

the products were then eluted successively with 150 ml of ether–petroleum ether (1:3), 150 ml of ether–petroleum ether (1:1), and 100 ml of ether–acetone (1:1). The eluate was collected in eight fractions of 50 ml, and the fractions were analyzed by TLC as already described.

Since none of the fractions gave spots with R_f values different from those of the products in the carbon tetrachloride extract, the products appeared to be stable in the isolation procedure. Fractions that contained more than one product were purified further by TLC. The isolated products were then identified by comparing their TLC properties and their UV, IR, and mass spectra with those of known compounds.

Quantitative Analysis of I and Its Degradation Products—Phenothiazine (II), 3*H*-phenothiazine-3-one (III), and 7-(10′-phenothiazinyl)-3*H*-phenothiazine-3-one (IV) were determined spectrophotometrically after they were separated on an aluminum oxide column as described previously (5) with the following modification. Since the expected phenothiazine concentrations were much lower than in the previous studies, the eluate containing phenothiazine was not diluted with ethanol but was evaporated to dryness under reduced pressure. Then the residue was dissolved in 50 ml of ethanol, and the absorbance was measured at 254 nm in a 1-cm cell with ethanol as a blank.

Compound I, which eluted at the same time as III in the system used, was determined by measuring the absorbance at 257 nm of the solution containing I and III after a 10-fold dilution with ethanol. The absorbance of this solution at 504 nm is directly proportional to the concentration of III. Therefore, by multiplying this concentration by the molar absorptivity of III at 257 nm and dividing by the dilution factor, the absorbance at 257 nm caused by III can be calculated and subtracted from the measured absorbance at this wavelength. This difference is directly proportional to the I concentration.

This method is reasonably accurate when the I concentration is much larger than the III concentration, as in these studies. With higher III concentrations, a different procedure would be necessary. Recovery studies with samples containing the various products in various concentrations showed good results: 92.7 ± 1.3% for I, 94 ± 2% for II, 91 ± 2% for III, and 92 ± 2% for IV.

Kinetic Studies—Solutions of I were prepared by dissolving 200 mg in 400 ml of a mixture of ethanol and sodium acetate–hydrochloric acid buffers (4). The pH of these buffers was measured at 85°. The solution was placed in small, screw-capped bottles, saturated with oxygen or oxygen-free nitrogen, and kept in the dark at 85°. The influence of pH on the kinetics was studied in the presence and absence of oxygen.

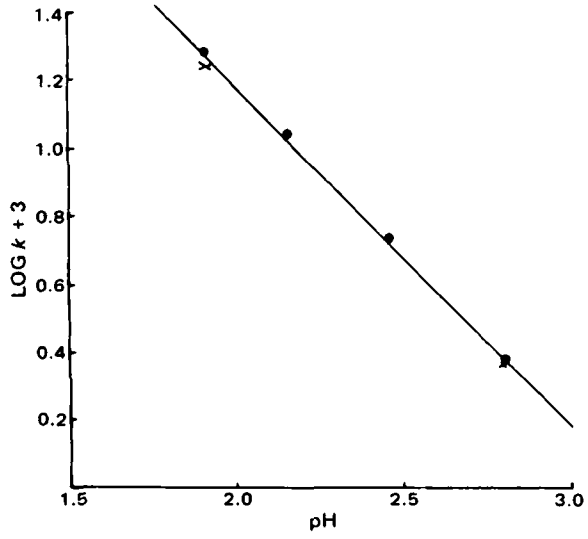

Figure 1—*Plot of log* k versus *pH for the degradation of 10-acetylphenothiazine in 52.5% ethanol at 85° in the presence* (●) *and absence* (×) *of oxygen.*

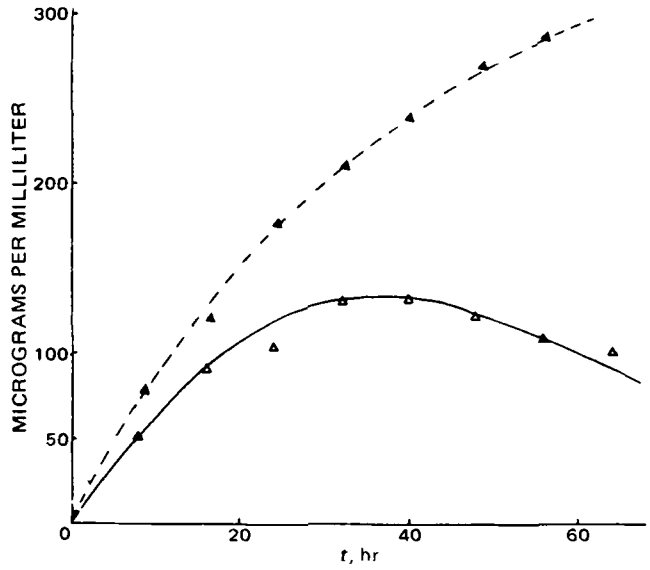

Figure 2—*Plot of the formation of phenothiazine* versus *time in the degradation of 10-acetylphenothiazine in 52.5% ethanol, pH 1.9, at 85°. The solid line was calculated according to Eq. 1 (oxygen saturated), and the dashed line was calculated according to Eq. 2 (oxygen free). The points are experimental values obtained in the presence* (△) *or absence* (▲) *of oxygen.*

RESULTS AND DISCUSSION

Identification of Degradation Products—In the silica gel and chloroform system, the R_f values of the five products were 0.05, 0.12, 0.19, 0.40, and 0.73; in the aluminum oxide and ether system, the R_f values were 0.03, 0.10, 0.24, 0.30, and 0.46, respectively. One product had a very low R_f value in both systems, so its isolation by column chromatography in one of these systems would require a long time and a large volume of solvent. Therefore, this product was separated from the other products by eluting them from a silica gel column with ether and then eluting this compound with acetone.

The product was compared with phenothiazine-5-oxide in various TLC systems, and the same R_f values were obtained for both compounds. Furthermore, their UV, IR, and mass spectra were similar, indicating that the product with the lowest R_f value was phenothiazine-5-oxide. Although phenothiazine-5-oxide and 10-acetylphenothiazine-5-oxide could possibly have the same chromatographic properties and similar spectra, the fact that the parent peak in the mass spectrum was at *m/e* 215, in agreement with the empirical formula $C_{12}H_9NOS$, shows that this product did not have the acetyl group present.

Comparison of the other products with I–IV, as described previously (1), showed that the compound with R_f 0.12 in silica gel and chloroform was identical with IV; the product with R_f 0.19 was identical with III, the product with R_f 0.40 was identical with I, and the one with R_f 0.70 was identical with II.

Kinetic Studies—Under all circumstances, the degradation was first order with respect to 10-acetylphenothiazine. Table I gives the observed rate constants for the degradation at various pH values in 52.5% ethanol saturated with oxygen. The plot of log *k versus* pH (Fig. 1) gives a straight line with a slope equal to −1, indicating that the degradation is a specific hydrogen-ion-catalyzed reaction. Solutions of I with pH 6.9, stored at 85° in an oxygen atmosphere, showed no significant degradation over 11 days.

Table I also lists the observed rate constants for the degradation under the same conditions but in an oxygen-free medium. The rate constants were essentially the same in the presence and absence of oxygen. This result indicates that the degradation of 10-acetylphenothiazine is a specific hydrogen-ion-catalyzed hydrolysis, giving phenothiazine and acetic acid; the phenothiazine is then oxidized. This pattern was also indicated by the fact that no other degradation products were found than those that were obtained in the oxidative degradation of phenothiazine.

If the first step in the degradation is a hydrolytic cleavage, then the

phenothiazine concentration should change with time in the presence of oxygen according to:

$$P = aP_0 \frac{k_1}{k_1 - k_2} (e^{-k_2 t} - e^{-k_1 t}) \quad \text{(Eq. 1)}$$

where P is the phenothiazine concentration, aP_0 is the initial concentration of I, k_1 is the rate constant for I degradation, and k_2 is the rate constant for phenothiazine degradation. At the various pH values, the phenothiazine concentrations were calculated using the values for k_1 given in Table I and the value of 19.7×10^{-3} hr^{-1} for k_2 (1). The calculated curves were in good agreement with the observed ones; a typical example is given in Fig. 2. The small differences were probably due to the experimental conditions being not exactly the same as in the previous studies.

In the absence of oxygen, the phenothiazine concentration can be calculated from the equation:

$$P = aP_0(1 - e^{-k_1 t}) \quad \text{(Eq. 2)}$$

With the evaluated k_1 values, the phenothiazine concentration was calculated as a function of time. Values in good agreement with the experimental ones were obtained (Fig. 2).

From these findings, it can be concluded that the first step in the degradation of I is its hydrolytic cleavage, giving II, which is degraded further by oxidation. This increase in stability of the phenothiazine ring system is probably caused by two factors: the steric effect of the introduction of a side chain at the nitrogen atom (1) and the electron-withdrawing properties of the acetyl group, which decrease the electron density in the ring system.

Interpretation of the data obtained for the formation of III and IV is difficult. Their formation proceeds *via* a number of consecutive reactions (2), their concentrations are rather small so they cannot be measured accurately, and they are unstable at the pH values used. There is, however, no reason to assume that their formation proceeds *via* pathways different from the ones elucidated previously.

REFERENCES

(1) H. Roseboom and J. H. Perrin, *J. Pharm. Sci.*, **66**, 1392 (1977).

(2) *Ibid.*, **66**, 1395 (1977).

(3) P. Danilo, Jr., W. B. Langan, M. R. Rosen, and B. F. Hoffman, *Eur. J. Pharmacol.*, **45**, 127 (1977).

(4) H. T. S. Britton, "Hydrogen Ions," 4th ed., vol. 1, Chapman and Hall, London, England, 1955, p. 357.

(5) H. Roseboom and J. A. Fresen, *Pharm. Acta Helv.*, **50**, 64 (1975).

Drug Absorption from *In Situ* Rat Small Intestine during Metoclopramide Administration

VESA M. K. VENHO

Received May 22, 1978, from the *Department of Pharmacology, University of Helsinki, Siltavuorenpenger 10, SF-00170 Helsinki 17, Finland.* Accepted for publication August 30, 1978.

Abstract □ The effect of metoclopramide on the absorption of drugs in solution in the small intestinal lumen of rats *in situ* was studied. Metoclopramide in doses up to 50 mg/kg sc did not significantly modify the disappearance of isoniazid and quinidine from the small intestinal lumen. At the end of the absorption experiments, quinidine in the whole blood of the experimental animals was increased after metoclopramide. The blood level did not correlate to the drug disappearance from the intestinal lumen. The results probably differ from those obtained when drugs are given orally to subjects treated with metoclopramide.

Keyphrases □ Metoclopramide—effect on absorption of other drugs, *in situ* rat small intestine preparation □ Absorption—drugs from *in situ* rat small intestine, effect of metoclopramide administration □ Isoniazid—absorption from rat small intestinal lumen, effect of metoclopramide administration □ Quinidine—absorption from rat small intestinal lumen, effect of metoclopramide administration

Metoclopramide modifies the absorption of orally administered drugs (1, 2). Its effects are mainly due to changes in gastric emptying, resulting in an accelerated, but not necessarily totally increased, absorption when measured by urinary drug excretion (1). Decreased intestinal drug absorption, probably due to rapid GI transit, also was reported (3).

EXPERIMENTAL

To study whether metoclopramide has effects on the absorption of drugs already in solution in the small intestinal lumen, experiments were carried out in urethan-anesthetized (1–1.5 g/kg im) rats using an *in situ* absorption technique (4, 5) whereby the drug disappearance from a buffer (pH 6.0) in the small intestinal lumen can be measured. Depending on the weight of the rats (200–250 g), 10–15 ml of warm (37°) buffer was introduced into the intestinal lumen through a polyethylene cannula in the proximal duodenum.

Samples were taken from this "intestinal fluid," expelled at 10-min intervals, either through the other cannula in the distal ileum or through the proximal cannula in the duodenum. At the end of the 40-min experiment, whole blood was drawn by heart puncture and the whole small intestine was removed as a sample.

Metoclopramide[1], 50 mg/kg sc, was administered to the anesthetized rats 30 min before the absorption experiment; isoniazid (neutral drug) and quinidine sulfate (basic drug) were used as the test drugs. Smaller doses of metoclopramide (5–10 mg/kg) also were used.

Isoniazid was assayed spectrophotometrically (6), and quinidine was assayed fluorometrically (7).

RESULTS AND DISCUSSION

Figure 1 shows the disappearance of quinidine from the small intestinal lumen in control and metoclopramide-treated (50 mg/kg) rats. A similar plot was made for isoniazid. The absorption half-lives of isoniazid and quinidine, measured from the semilogarithmic plot of drug concentration in the intestinal lumen (intestinal fluid) *versus* time, are presented in Table I.

No statistically significant differences in absorption half-lives could be demonstrated, although the quinidine absorption half-life seemed to be somewhat longer after metoclopramide than in controls. However, in spite of an unchanged absorption half-life, the blood level of quinidine, but not of isoniazid, at the end of the 40-min experiment was statistically significantly increased by metoclopramide, 50 mg/kg (Table I). After 5–10 mg of metoclopramide/kg, the blood quinidine level (0.62 ± 0.03 μg/ml, two experiments) seemed to be also higher than in controls, but blood isoniazid level was the same as in controls.

With solids, a decreased intestinal motility allows more time for the drugs to be dissolved, tending to increase absorption and vice versa (8).

[1] Primperan, H. Lundbeck & Co., Copenhagen, Denmark.

0022-3549/79/0400-0517$01.00/0