Factors Influencing Decomposition Rate of Amitriptyline Hydrochloride in Aqueous Solution

R. P. ENEVER ˣ, A. LI WAN PO, and E. SHOTTON

Abstract □ The degradation rate of amitriptyline hydrochloride in buffered aqueous solution containing various additives was determined. The oxidation was a free radical-mediated process, and the rate was accelerated by the presence of metal-ion contaminants. Glass ampuls, particularly amber ones, in which the solutions were stored were the major source of these contaminants. Edetate disodium stabilized the solution, but the primary antioxidants propyl gallate and hydroquinone were less effective. Sodium metabisulfite accelerated the decomposition, and it is postulated that there was direct attack by metabisulfite at the olefinic double bond in the drug molecule.

Keyphrases □ Amitriptyline hydrochloride—degradation in aqueous solution, effect of metal-ion contaminants, edetate disodium, and antioxidants □ Degradation—amitriptyline hydrochloride in aqueous solution, effect of metal-ion contaminants, edetate disodium, and antioxidants □ Metal-ion contaminants—effect on degradation of amitriptyline hydrochloride in aqueous solution □ Edetate disodium—effect on degradation of amitriptyline hydrochloride in aqueous solution □ Antioxidants, various—effect on degradation of amitriptyline hydrochloride in aqueous solution

A previous study (1) showed that amitriptyline hydrochloride decomposes in aqueous solution, with the production of dibenzocycloheptanone, 3-(propa-1,3-dienyl)-1,2:4,5-dibenzocyclohepta-1,4-diene, and 3-(2-oxoethylidene)-1,2:4,5-dibenzocyclohepta-1,4-diene. Subsequent work concerned the identification of factors influencing the rate of this oxidative process. The present report outlines this work and describes the use of chelating agents and antioxidants in stabilizing the system.

EXPERIMENTAL

Materials—The following chemicals were used: amitriptyline hydrochloride[1], citric acid[2], dibasic sodium phosphate dihydrate[2], potassium chloride[2], sodium hydroxide[2], hydroquinone[2], edetate disodium[2], ferric chloride[2], ether[2], propyl gallate[2], sodium metabisulfite[2], and chlorpromazine hydrochloride[3].

The purity of amitriptyline hydrochloride was checked by the British Pharmacopoeia (BP) method (2), and the drug was used without further purification. The pH 3.0 and 5.0 McIlvaine citric acid–phosphate buffers[4] (3) were prepared with double-distilled water and adjusted to an ionic strength of 0.5 with potassium chloride.

Assay of Amitriptyline Hydrochloride Solutions—The drug concentration was determined by GLC[5] using a 1.5-m (5-ft) × 0.6-cm (0.25-in.) i.d. glass column packed with 3% (w/w) SE-30[6] on 80–100-mesh Chromosorb W AW/DCMS[6]. The column temperature was 250° (injection port setting 6.5); the flow rates of hydrogen, air, and nitrogen were 80, 300, and 50 ml/min, respectively.

To produce a calibration curve for the assay, 1 ml of a 2-mg/ml solution of amitriptyline hydrochloride in the appropriate pH 3.0 or 5.0 buffer was pipetted into a 10-ml volumetric flask and 1 ml of a 2-mg/ml chlorpromazine hydrochloride solution (internal standard) was added. One milliliter of a 0.5-g/ml sodium hydroxide solution was added to liberate the bases, and the mixture was allowed to equilibrate with 7 ml of ether overnight in a stoppered flask previously flushed with nitrogen. Subsequently, 2 μl of the ethereal extract was injected into the chromatograph.

The procedure was repeated with amitriptyline hydrochloride solutions of 0.2, 0.4, 0.6, 0.8, 1.2, 1.4, 1.6, and 1.8 mg/ml. A linear response (slope 1.635) was obtained when the peak height ratio of amitriptyline to chlorpromazine was plotted against amitriptyline hydrochloride concentration. The test solutions of amitriptyline hydrochloride were assayed in a similar manner.

Assay of Iron in Aqueous Solution—The ferric content of the aqueous solutions was determined by atomic absorption spectroscopy[7] using an iron lamp and a wavelength of 2483 nm. A calibration curve was produced using freshly prepared solutions containing 1, 2, 3, 4, and 5 ppm of ferric ions in double-distilled water. Test solutions were assayed without further dilution.

Degradation of Amitriptyline Hydrochloride Solutions—Aliquots of 2 ml of 0.2% (w/v) solutions of amitriptyline hydrochloride in pH 3.0 or 5.0 buffer containing the appropriate additive were distributed into 10-ml clear and amber glass ampuls[8] and stored at 80° in constant-temperature water baths[9] in the dark. Samples were withdrawn at intervals up to 30 days and assayed for amitriptyline hydrochloride content.

RESULTS AND DISCUSSION

Figure 1 shows the results obtained for an amitriptyline solution sealed

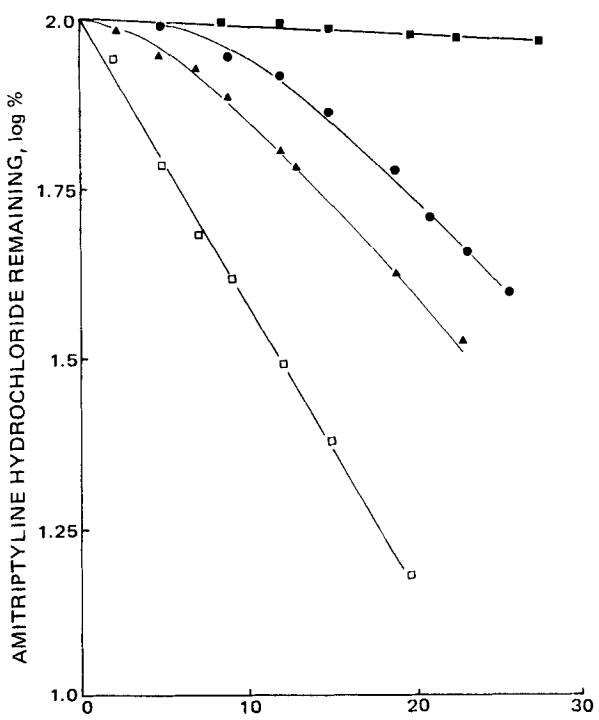

Figure 1—*Influence of metallic ions and edetate disodium on the decomposition rate of 0.2% (w/v) amitriptyline hydrochloride in pH 3.0 buffer at 80°. Key:* □, *containing* 2×10^{-4} *M ferric ions;* ■, *containing 0.1% (w/v) edetate disodium;* ▲, *containing* 2×10^{-4} *M cupric ions; and* ●, *control solution in amber ampuls (Batch BB242).*

[1] Merck Sharp and Dohme, Hoddesdon, Herts, England.
[2] Analar grade reagents, B.D.H. Chemicals Ltd., Poole, Dorset, England.
[3] May and Baker Ltd., Dagenham, Essex, England.
[4] The pH was checked with a model 290 Mk. 2 pH meter, Pye Unicam, Cambridge, England.
[5] Model 105 gas chromatograph equipped with flame-ionization detector, Pye Unicam, Cambridge, England.
[6] Perkin-Elmer Ltd., Beaconsfield, Bucks, England.
[7] Model 103 atomic absorption spectrometer, Perkin-Elmer, Beaconsfield, Bucks, England.
[8] Neutral glass, type Q (B.S.795:1961), Adelphi Tubes Ltd., London, England.
[9] SX 10, Grant Instruments, Cambridge, England.

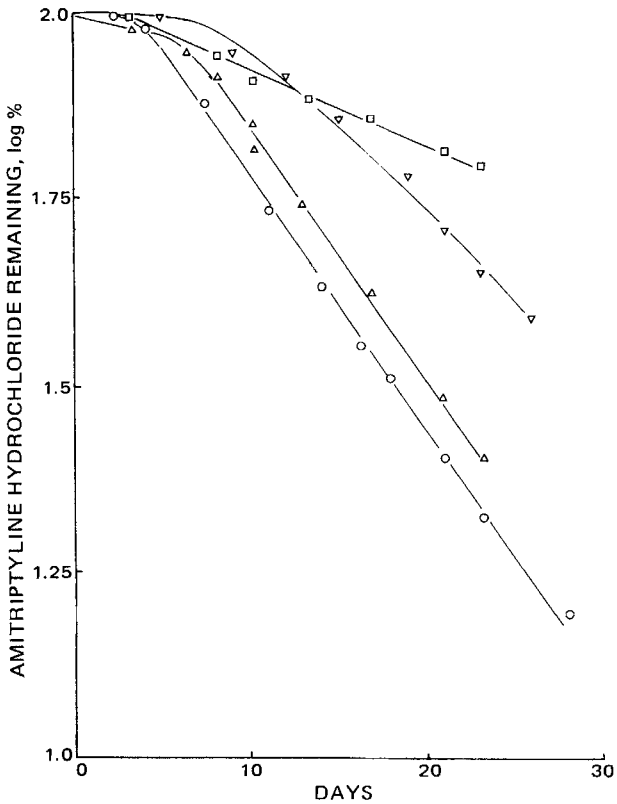

Figure 2—*Influence of glass type and batch of ampuls on decomposition of 0.2% (w/v) amitriptyline hydrochloride in pH 3.0 buffer at 80°. Key: □, clear glass ampuls (Batch 5018/10R); ○, amber glass ampuls (Batch HP65); △, amber glass ampuls (Batch HP67); and ▽, amber glass ampuls (Batch BB242).*

under air in amber ampuls. An initial slow decomposition rate was followed by a relatively faster rate ($k \sim 4.99 \times 10^{-2}$ day^{-1}). Decomposition was not measurable when a similar solution was sealed under nitrogen. Thus, the data are consistent with a free radical oxidation mechanism, since such a reaction normally exhibits an initial lag phase during which free radicals accumulate. To confirm the process, the sensitivity of the reaction to catalysts and inhibitors was investigated.

Addition of cupric acetate or ferric chloride (2×10^{-4} *M*) to amitriptyline hydrochloride solutions accelerated the decomposition rate (Fig. 1). The lag phase was removed with the ferric ions and greatly reduced with the cupric ions. The rate constants for the rapid phase of the reaction were increased to 0.0564 and 0.0854 day^{-1} with cupric and ferric ions, respectively. A reduction in the apparent activation energy of autoxidation processes by metal ions is well known (4), and such a positive catalytic effect on the decomposition of pharmaceutical compounds also was reported (5, 6).

The decomposition rate of amitriptyline hydrochloride solutions was reduced significantly by the addition of 0.1% (w/v) edetate disodium prior to storage (Fig. 1). The results obtained with this chelating agent confirm that metallic-ion impurities are a major cause of degradation. The source of impurities was at first thought to be the buffer salts, since they can contain significant amounts of metallic-ion contaminants (6). However, in the present work, metallic impurities leached from the containers were more important.

The iron content of amber glass ampuls is probably considerably greater than that of clear ampuls (7). Since amitriptyline is photolabile (8), however, such solutions probably would be stored in amber containers. The effect of different batches of such ampuls on the degradation rate is shown in Fig. 2; the rate was much greater in amber ampuls than in clear glass and there was considerable batch-to-batch variation. The amounts of iron leached from one batch[10] of amber ampuls into a 0.2% (w/v) amitriptyline hydrochloride solution in pH 3.0 buffer at 80° after 1, 8, 16, 19, 23, and 30 days were 0.8, 1.10, 1.35, 1.40, 1.58, and 1.80 ppm, respectively. There was a linear increase in iron concentration with time (rate of 0.034 ppm/day).

[10] Batch HP65.

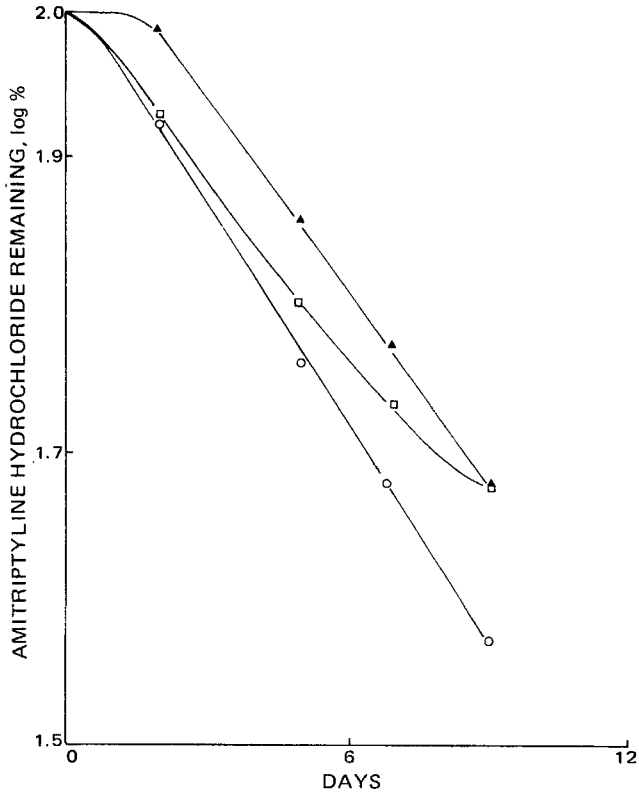

Figure 3—*Influence of addition of a decomposing solution or hydrogen peroxide to 0.2% (w/v) amitriptyline hydrochloride in pH 5.0 buffer at 80° in amber ampuls (Batch HP65). Key: ▲, 2 ml of 0.2% (w/v) amitriptyline hydrochloride solution; □, 2 ml of solution prepared by adding 1 ml of decomposing solution to 99 ml of fresh 0.2% (w/v) amitriptyline hydrochloride solution; and ○, 2 ml of solution prepared by adding 5 μl of 30% (w/w) hydrogen peroxide to 100 ml of fresh 0.2% (w/v) amitriptyline hydrochloride solution.*

Further support for the postulated free radical oxidation was obtained by adding a small volume of a partially decomposed solution to a fresh amitriptyline hydrochloride solution. Figure 3 shows that the lag phase was removed, although the chain propagation rate changed little. A similar result was obtained by adding 5 μl of a 30% (w/w) hydrogen per-

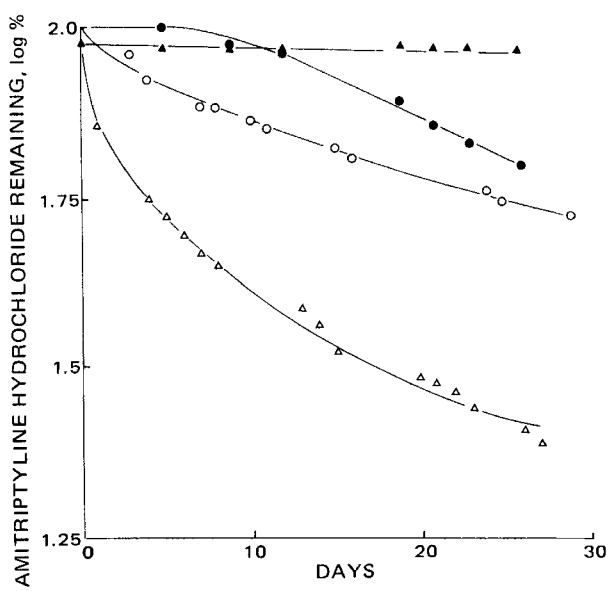

Figure 4—*Effect of antioxidants and sodium metabisulfite on the decomposition rate of 0.2% (w/v) amitriptyline hydrochloride in pH 3.0 buffer at 80°. Key: ●, control solution; ▲, containing 0.05% (w/v) propyl gallate; ○, containing 0.01% (w/v) hydroquinone; and △, containing 0.121% (w/v) sodium metabisulfite.*

oxide solution to 100 ml of fresh 0.2% (w/v) amitriptyline hydrochloride solution. The hydrogen peroxide content was insufficient to cause this marked degree of decomposition by direct oxidation.

Addition of 0.05% (w/v) of the antioxidant propyl gallate to an amitriptyline hydrochloride solution initially caused a 10% fall in drug concentration but subsequently completely stabilized the solution (Fig. 4). Hydroquinone, 0.01% (w/v), was less effective, since it abolished the lag phase and only appeared to slow the reaction after 15 days. The antioxidants appeared to break the chain reaction. With propyl gallate at least, however, there was evidence of a direct reaction with amitriptyline hydrochloride.

The reducing agent sodium metabisulfite was not effective in stabilizing the solution. Figure 4 shows that 0.121% (w/v) sodium metabisulfite produced an immediate fall in the drug concentration and a subsequent acceleration in the decomposition rate. This process did not follow first-order kinetics at any stage. The concentration of sodium metabisulfite was equimolar with amitriptyline hydrochloride (6.37×10^{-3} *M*), and the data fit a simple second-order kinetic plot.

Bisulfite-mediated reactions were reported previously (9–13), and a reaction mechanism similar to the thiamine cleavage shown previously (14, 15) is possible in the drug's side chain. However, using the isolation and identification techniques outlined previously (1), dibenzocycloheptanone was the only decomposition product present, suggesting that direct attack at the olefinic double bond in the molecule is more likely. Such an affinity for these bonds by sodium bisulfite was demonstrated and explained (16) on the basis of a free radical mechanism.

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ACKNOWLEDGMENTS AND ADDRESSES

Received August 6, 1976, from the *Department of Pharmaceutics, School of Pharmacy, University of London, London, WC1N 1AX, England.*

Accepted for publication September 17, 1976.

The authors are grateful to Merck Sharp and Dohme for the gift of amitriptyline hydrochloride.

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Polyamine Metabolism I: Synthesis of Dansyl Derivatives of *N*-(Monoaminoalkyl)- and *N*-(Polyaminoalkyl)acetamides and Elucidation in Urine of a Cancer Patient

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Abstract □ The dansyl derivatives of *N*-(monoaminoalkyl)- and *N*-(polyaminoalkyl)acetamides were synthesized and unequivocally characterized. TLC of the dansyl derivatives obtained from human urine indicated the presence of *N*-[3-[(4-aminobutyl)amino]propyl]acetamide (N^1-acetylspermidine), *N*-[4-[(3-aminopropyl)amino]butyl]acetamide (N^8-acetylspermidine), and *N*-(4-aminobutyl)acetamide (*N*-acetylputrescine) in appreciable amounts. The dansyl derivatives of N^1-acetylspermidine, N^8-acetylspermidine, and *N*-acetylputrescine were isolated and purified using various chromatographic methods. The mass spectra of these compounds were similar to those of authentic samples, which confirmed the identity of these compounds and established the presence of N^8-acetylspermidine as well as N^1-acetylspermidine and *N*-acetylputrescine in human urine.

Keyphrases □ Polyamines—*N*-(monoaminoalkyl)- and *N*-(polyaminoalkyl)acetamides, determined in human urine, dansyl derivatives synthesized □ *N*-(Aminoalkyl)acetamides—determined in human urine, dansyl derivatives synthesized □ Dansyl derivatives—of *N*-(monoaminoalkyl)- and *N*-(polyaminoalkyl)acetamides synthesized □ TLC—determination, dansyl derivatives of *N*-(monoaminoalkyl)- and *N*-(polyaminoalkyl)acetamides, human urine □ High-pressure liquid chromatography—determination, dansyl derivatives of *N*-(monoaminoalkyl)- and *N*-(polyaminoalkyl)acetamides, human urine

The diamine 1,4-butanediamine (putrescine) (I) and the polyamines *N*-(3-aminopropyl)-1,4-butanediamine (spermidine) (II) and *N,N'*-bis(3-aminopropyl)-1,4-butanediamine (spermine) (III) are present in all animal and plant tissues, and at least one of these is present in all microorganisms (1). Studies of both normal and neoplastic rapid growth systems indicate that the synthesis and accumulation of polyamines are elevated shortly after a stimulus inducing proliferation. Furthermore, the levels of these amines were elevated in the urine of cancer patients (2–10). These amines are excreted in human urine mainly as conjugates, which yield the free amines after hydrolysis (2).

Although several studies determined the levels of total