Methocarbamol Degradation in Aqueous Solution

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Abstract □ The kinetics of the hydrolysis of methocarbamol to the corresponding diol guaifenesin in aqueous solution was studied. Methocarbamol was rather stable in acidic media but easily hydrolyzed in alkaline solution. The formation of an unknown compound, proved to be an isomer of methocarbamol [the 3-(2-methoxyphenoxy)-propanediol 2-carbamate] is involved. The amounts of methocarbamol and the two degradation products resulting from storage of methocarbamol in various buffer solutions over a pH range of 8.0 to 10.0 at 70–80 °C (ionic strength, 0.5 M), were followed as a function of time by a reversed-phase HPLC stability-indicating method to clarify the degradation pathway of methocarbamol in alkaline solutions. Analysis of the concentration–time profiles reveals that base-catalyzed methocarbamol hydrolysis proceeded mainly through the formation of its isomer. The observed degradation rates followed approximately pseudo-first-order kinetics at constant pH and temperature.

Methocarbamol (**M**), a carbamate structural analogue of the aryl glycerol ethers, is a centrally active muscle relaxant used in the symptomatic treatment of muscoloskeletal disorders.[1] It was patented in 1956[2] and it is quoted in *U.S. Pharmacopeia*.[3] Combination products containing, in addition, an analgesic (e.g., aspirin) are also available. **M** may be given orally or by injection. The USP injection has a pH of 3.5 to 6.0.

Aqueous solutions of **M** were shown to be unstable under certain conditions;[4,5] however, no information has been reported regarding its stability or degradation kinetics. The aim of this study was the development of a new reversed-phase HPLC (RP-HPLC) method that is rapid and stability indicating to investigate the hydrolysis of **M** in various pH buffer solutions, and determine the degradation products of **M**. The quantitation of these products allowed the proposal of a degradation pathway of **M** in alkaline media in which an unknown compound was involved. This compound was proved to be the isomer of **M** (**iM**).

Experimental Section

Materials—**M** was kindly supplied by "Santa"-N.Balanos S. A. Pharmaceutical Laboratories (Athens, Greece). Guaifenesin (**D**) was obtained after the alkaline (NaOH) hydrolysis of **M**. The secondary isomer of **M** (**iM**) was synthesized by a known method.[4] All other chemicals, including buffer components, were reagent grade and were used without further purification. Organic solvents were HPLC grade. The water used was deionized and filtered with a MilliQ-Plus water purifying system (Millipore Corporation, France).

Kinetic Method—Citrate, phosphate, and borate buffer solutions with varying pH were used in all experiments. These solutions were 0.2 M with respect to citrate, phosphate, or borate and were adjusted to a total ionic strength (μ) of 0.5 M with potassium chloride. The pH did not change during the study. A stock solution of acetanilide (**Ac**, 4 mg/mL) was prepared and stored in the refrigerator until further use as an internal standard. Appropriate amounts of **M** were dissolved in the buffer solution. The reaction flasks, sealed with a condenser, were immediately placed in a water bath (Tempette Thermoregulator, TE8D, Techne Company) that also protected the solution from light. At appropriate time intervals, three 450-μL aliquots of the reaction mixture were removed from the reaction vessel and immediately added to three test tubes, each containing 50 μL of the internal standard stock solution. The tubes were immersed in ice to stop the reaction. A fixed volume (10 μL) of these samples was assayed chromatographically to determine the concentrations of **M**, **iM**, and **D**.

Analytical Procedures—Analyses were performed with a Waters liquid chromatograph (Waters Associates, Milford, MA; model 590) equipped with a reversed-phase 3 × 3 CR C-18 column (Perkin-Elmer 0258-0164), a Rheodyne 717S injector fitted with a 10-μL loop, and a multiwavelength UV detector (Lambda-Max, model 481) set at 274 nm. The deaerated mobile phase employed in the HPLC separation consisted of a water:acetonitrile (9:1, v/v) solution containing 1% acetic acid. The samples were injected into the HPLC system and detected at an appropriate absorbance units full scale (AUFS) range of 0.01–0.05. The chromatography was performed at room temperature, and the flow rate was 0.9 × 2.5 mL/min (740–760 psi). The absorbances of **M** and its degradation products were recorded using a strip-chart recorder (BBC Goerz Metrawatt, model SE120) at a chart speed of 0.5 cm/min. Quantitation was performed by the peak response ratio method, using acetanilide (**Ac**) as the internal standard.

Standard solutions of **M**, **iM**, and **D** at a concentration range of 0.05 to 1.2 mg/mL were prepared for calibration. The internal standard stock solution was added before each injection, at a concentration of 0.31 mg/mL. The standard curves were obtained from least-squares linear regression by plotting the peak height ratio (**M**/**Ac**, **iM**/**Ac**, **D**/**Ac**, respectively) versus concentration (mg/mL). Linearity results were good, with correlation coefficients (r) > 0.999.

Results and Discussion

Chromatography—The RP-HPLC method permitted the analysis of intact **M** in the presence of its hydrolytic degradation products. **M** was stable in acidic media (pH 3.0) and when it degrades, the only degradation product is the diol **D**. After storage at 60 °C (μ, 0.5M) in pH 3.0 solution for 12 days, 85% of **M** was intact, whereas 15% was converted to **D**. After storing under the same conditions for 30 days, the remaining amount of **M** was 67%, whereas the amount of **D** was 33%. However, when **M** degrades in the presence of different buffers over the pH range 6–10, another peak in addition to those due to **M** and **D** appears; this indicates that the degradation mechanism is not the same as in alkaline media. Figure 1 shows a typical chromatogram of partially degraded **M** in alkaline media. Peak 2, with a retention time of 2.1 min, is due to the internal standard and peak 3, with a retention time of 2.85 min, is attributed to the diol **D**. The small peak 4, with a retention time of 4 min, was proved to be due to the secondary isomer of **M**, 3-(*o*-methoxyphenoxy)propanediol 2-carbamate (**iM**). This product is already known as a side reaction product of **M** synthesis.[6] To confirm this structure, the preparation of **iM** was carried out.[4] The identity of **iM** from either source was verified by comparison of the retention times, which were absolutely identical for the HPLC method described above as well as for the official USP method.[3,7] Finally, peak 5, which is eluted relatively close to peak 4 (retention time, 4.7 min), is due to **M** itself.

Kinetic Studies—To check the degradation pathway of **M** in alkaline media, the kinetics of **M** loss and the formation of **iM** and **D** were simultaneously followed by HPLC. Because it was found that **M** was converted to **iM** and, judging from the change in the chromatograms for the reaction solution, **M** degradation probably may proceed as depicted in Scheme 1.

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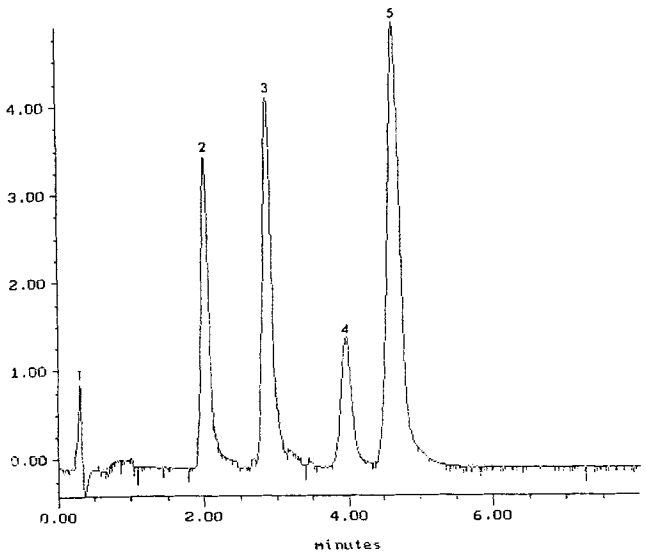
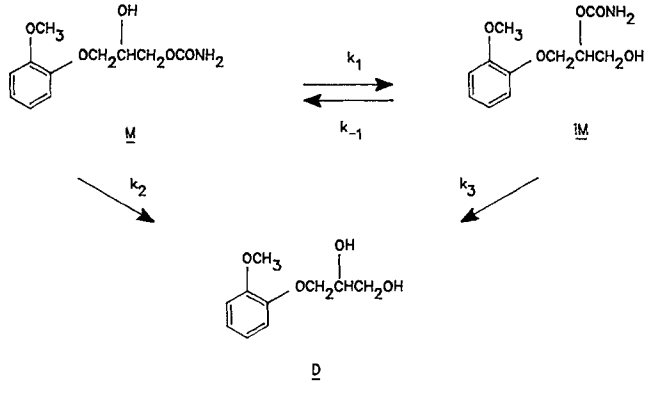


Figure 1—A typical chromatogram of a partially degraded sample of **M** in alkaline solution, showing the formation of the two degradation products. Key: (**1**) solvent front; (**2**) acetanilide (internal standard); (**3**) guaifenesin; (**4**) 3-(2-methoxyphenoxy)propanediol 2-carbamate; (**5**) methocarbamol. Chromatographic conditions are given in the *Experimental Section*.



Scheme 1

The usual semilogarithmic plots of the percentage remaining amount of intact **M** versus time at alkaline pH values (Figure 2) show that the temperature and the pH affected the overall disappearance of **M**, and the reaction rates followed pseudo-first-order kinetics at constant pH, temperature, μ, and buffer species concentrations. The regression lines were reasonably linear under all pH conditions ($r > 0.99$). The observed constants (k_{obs}) for the total degradation of **M**, calculated from the slopes of the semilogarithmic concentration–time plots are listed in Table 1.

Theoretically, the **iM** concentration–time profile should rise exponentially, traverse a maximum, descend, and finally flatten out exponentially. However, the experimental data for **iM**, obtained under all experimental conditions, exhibit a monotinically decreasing pattern (Figure 3). In other words, the concentration of **iM** increases sharply in the commencement of the process, and, therefore, experimental data points in the ascending limb of the curve are not available. Under these circumstances, the concentration–time data found in various experimental conditions for each one of the studied species (**M**, **iM**, and **D**) were separately treated on the basis of a monoexponential model, assuming first-order kinetics. Equations 1–3 were used with a nonlinear least-squares regression analysis method (PC-STATGRAF program[8]).

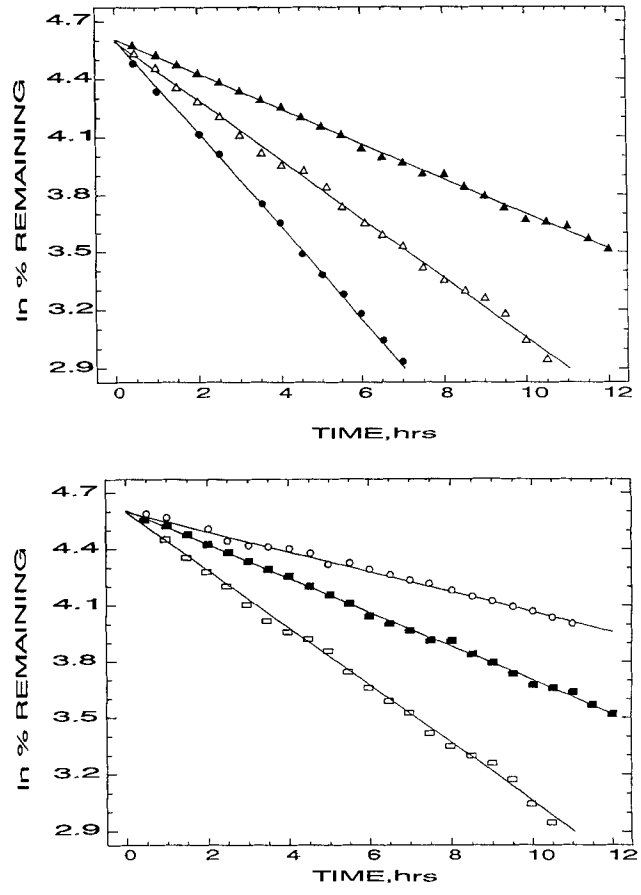


Figure 2—Apparent first-order plots for the degradation of **M** at various pH values and $\mu = 0.5$. Key: (●) pH 10, 80 °C; (□) pH 10, 75 °C; (△) pH 10, 70 °C; (■) pH 9, 80 °C; (▲) pH 8, 80 °C; (○) pH 8, 75 °C. The data are normalized to a value of 100 at zero time. The initial concentration of **M** was 3.1 mM. Points represent the experimental data and the solid lines were drawn using linear least-squares regression analysis.

Table 1—Effect of pH and Temperature on the Observed Degradation Rate Constants for Methocarbamol[a] in Aqueous Buffer Solutions[b]

pH	Temperature, °C	k_{obs}, $h^{-1} \pm$ SD
10	80	0.242 ± 0.0088
10	75	0.156 ± 0.0063
10	70	0.090 ± 0.0039
9	80	0.053 ± 0.0027
8	80	0.039 ± 0.0008
8	70	0.029 ± 0.0024

[a] Initial concentration, 3.1 mM. [b] The rate constants were calculated according to eqs 1–3 by nonlinear least squares regression analysis and from the slopes of the semilogarithmic form of eq 1 by linear regression analysis.

$$[\mathbf{M}] = [\mathbf{M}]_0 e^{-\lambda_1 t} \quad (1)$$

$$[\mathbf{iM}] = [\mathbf{M}]_0 A e^{-\lambda_2 t} \quad (2)$$

$$[\mathbf{D}] = [\mathbf{M}]_0 (1 - B\, e^{-\lambda_3 t}) \quad (3)$$

In eqs 1–3, $[\mathbf{M}]_0$ is the initial **M** concentration; [**M**], [**iM**], and [**D**] are the concentrations of **M**, **iM**, and **D**, respectively, λ_1, λ_2, and λ_3 are first-order rate constants; and A and B are dimentionless positive numbers. The three independent computer fittings gave excellent results ($r > 0.99$) and revealed virtually

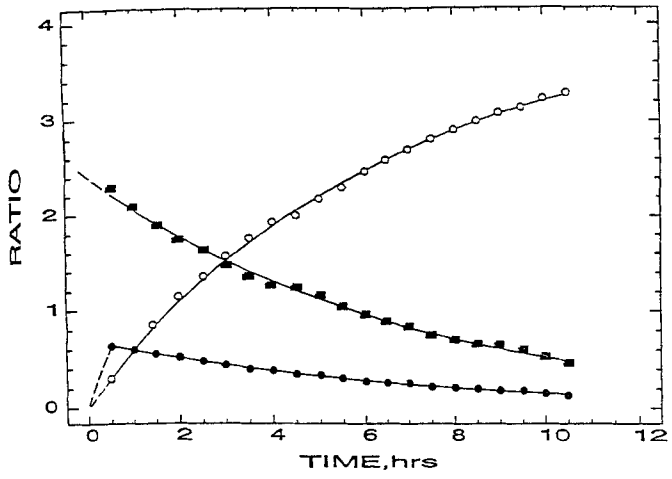


Figure 3—A typical plot showing the time course of peak height ratios of **M** (■), 3-(2-methoxyphenoxy)propanediol 2-carbamate (**iM**, ●), and guaifenesin (**D**, ○), during the degradation of **M** in pH 10.0 borate solution and 75 °C. The symbols represent the experimental data and the solid lines are computer-generated simulation curves based on eqs 1–3.

identical estimates for λ_1, λ_2, and λ_3. For example, for **M** degradation at pH 10 and 75 °C, the following computer-generated constants were obtained: $\lambda_1 = 0.156$ (±0.0025) h^{-1}, $\lambda_2 = 0.159$ (±0.0024) h^{-1}, $\lambda_3 = 0.158$ (±0.0183) h^{-1}, $A = 0.282$ (±0.0018), and $B = 1.272$ (±0.0496).

Similar results were obtained under all experimental conditions investigated. Figure 3 shows a typical fit of the data for the disappearance of **M** and the appearance of **iM** and **D** at pH 10.0 and 75 °C. The close agreement between the theoretical lines and the experimental values for all three species suggests that the model and the rate constants are valid.

Because [**M**] and [**iM**] decline in parallel ($\lambda_1 = \lambda_2 = k_{obs}$), it is rather obvious that this common value is, in reality, the rate constant of the disappearance of **M** and also the rate constant for the formation of **iM**. Consequently, the concentration versus time curve of **iM** is described by the so-called flip-flop model[9] because λ_2 was estimated from the data points of the descending limb of the curve. Furthermore, it is apparent that the same formation rate constant ($\lambda_2 = \lambda_3 = k_{obs}$) was found for both **iM** and **D**. Thus, the buildup of the intermediate **iM** can account completely for the loss of **M**, and the diol formation can account completely for **iM** loss.

According to Scheme 1, the alkaline hydrolysis of **M** may be described with an open, two-compartment model with **iM** degraded to **D** or reconverted to **M**. However, the above-mentioned experimental results strongly suggest that the k_{-1} and k_2 rate constants in Scheme 1 are, in fact, negligible. It seems likely, therefore, that **M** is predominantly converted to **iM** through an irreversible reaction, and that **iM** is hydrolyzed rapidly to **D**. Accordingly, the degradation processes of Scheme 1 essentially become the following two consecutive first-order reactions:

$$\mathbf{M} \xrightarrow{k_1} \mathbf{iM} \xrightarrow{k_3} \mathbf{D}$$

Based on this reaction scheme, a plot of **iM** concentration versus time will be biexponential and it can be described by the following equation:

$$[\mathbf{iM}] = [\mathbf{M}]_0 A(e^{-k_1 t} - e^{-k_3 t}) \quad (4)$$

In eq 4, k_1 and k_3 are the rate constants defined in Scheme 1. The above-mentioned rapid increase of **iM** concentration reflects its almost spontaneous hydrolysis to the diol **D**. It is therefore obvious that for $k_3 \gg k_1$, eq 4 can be approximated validly with eq 2 shortly after the commencement of the experiment. Thus, $k_1 = k_{obs}$ and the buildup of **iM** is the rate-controlling step of the degradation of **M** in the entire pH range studied.

Conclusions

M has a remarkable resistance to hydrolysis in acidic media, in contrast to a poor stability under alkaline conditions. This enhanced reactivity of **M** has been attributed to its irreversible conversion to **iM** during the course of the hydrolysis. This isomerization can be assumed as the rate-limiting step of the whole process because, under every combination of experimental conditions studied, it was found that **iM** arising from **M** is susceptible to a rapid hydrolytic cleavage. Consequently, it was indicated that the major degradation pathway for **M** under these conditions is through the formation of **iM**.

References and Notes

1. Harvey Stewart, C. In *Remington's Pharmaceutical Sciences*, 18th rev.; Gennaro, A. R., Ed.; Mack: Easton, PA, **1990**, p 922.
2. Murphey, R. S. U.S. Patent 2 770 649, 1956; *Chem. Abstr.* **1957**, *22*, 7413h.
3. U.S. Pharmacopeia, 22nd rev.; U.S. Pharmacopeial Convention: Rockville MD, **1990**; pp 852–853.
4. Baizer, Manuel M.; Clarc, John R.; Swidinsky, John *J. Org. Chem.* **1957**, *22*, 1595–1599.
5. Schmid, O.; Voak, D. *Monatsh.* **1963**, *94* (*2*), 339–358.
6. Lunsford, Carl D.; Mays, Richard P.; Richman, John A., Jr.; Murphey, Robert S. *J. Am. Chem. Soc.* **1960**, *82*, 1166–1167.
7. Everett, Richard L. *J. Assoc. Off. Anal. Chem.* **1984**, *67* (*2*), 225–227.
8. STATGRAPHICS Version 4, Statistical Graphics system by Statistical Graphics Corporation.
9. Gibaldi, M.; Perrier, D. In *Pharmacokinetics*, 2nd Ed.; Marcel Dekker: New York, **1982**; p 35.

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