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COMMUNICATION

Anaerobic Stability of Aqueous Physostigmine Solution

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ABSTRACT

The anaerobic stability of physostigmine was studied in this report. Physostigmine and two important degradants, eseroline and rubreserine, were identified by a high-performance liquid chromatography (HPLC) system using a photodiode array detector and a UV detector at the retention times of 12.4, 9.2, and 7.9 min. Under anaerobic treatment, the minimum degradation rate constant was found at pH 3.4, evaluating from pH-rate profiles at 45°C, 55°C, and 70°C with the pH range 2.4–6.8. The degradation of physostigmine was suggested as specific acid-base catalysis. Anaerobic activation energies of K_H, K_{OH}, *and* K_{obs} *were 9.4, 8.3, and 17.9 kcal/mol, respectively. The ratios of observed aerobic and anaerobic rate constants were 2–33 at 88°C, which had a trend to increase ratios as pH increased in the range 2.4–5.2. The shelf life of physostigmine solution was estimated to be 4 years at room temperature under anaerobic conditions at pH 3.4.*

Key Words: *Degradation; Physostigmine; Stability.*

INTRODUCTION

Physostigmine is an alkaloid that easily penetrates the brain-blood barrier (BBB) to produce central nervous effects. Recent reports have highlighted the potential of physostigmine in the treatment of Alzheimer's disease (AD) for patients classified as physostigmine responders (1). Physostigmine has some effects to promote memory and cognition and reduce the deterioration in AD patients (2,3). Due to physostigmine, with a biological half-life of only 30 min (4,5), a large dose of physostigmine is required to obtain suitable treatment duration, which

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also caused side effects. Therefore, it is impractical to use conventional dosage forms of physostigmine in the treatment of patients with AD. In addition, physostigmine is labile in light, oxygen, and alkaline environments (6–8). Two important degradants, eseroline and rubreserine, have been reported. Yang and Wilken (9) reported that the stability of physostigmine could be significantly improved during autoclaving under an anaerobic condition. However, until now, the stability of physostigmine at anaerobic conditions has not been completely studied. Therefore, there have been restrictions in the application of the manufacturing preparation of physostigmine. Thus, investigation of the degradation of physostigmine and its relevant degradants in anaerobic conditions might be beneficial for designing a stable drug delivery system for physostigmine for the therapy of AD.

This study was to investigate the pH and temperature effects on the stability of physostigmine under anaerobic conditions. Eseroline, a degradant of physostigmine, was synthesized and also measured in this study. Thermodynamic parameters related to physostigmine degradation were also assessed for further evaluation of the effect of oxygen on the stability of physostigmine.

EXPERIMENTAL

Materials

Physostigmine was purchased from Sigma (St. Louis, MO). Ethyl morphine (Narcotic Bureau, Taipai, ROC) was used as the internal standard in high-performance liquid chromatography (HPLC) analysis. Methanol, triethylamine, glacial acetic acid (E. Merck, Darmstadt, Germany), and sodium camphor sulfonic acid (Chinese Camphor Co., Taiwan, ROC) were used in the preparation of the HPLC mobile phase. Other chemicals and reagents were either HPLC or analytical grade.

Preparation of Eseroline

The preparation of eseroline was modified from the Ellis method (5). Briefly, the process is as follows. Physostigmine was hydrolyzed in 10% sodium hydroxide solution and extracted by N_2 saturated ether. After evaporation and recrystallization, purified eseroline was filled with N_2 gas and sealed. The structure of eseroline was confirmed by nuclear magnetic resonance (NMR) and infrared (IR) spectra, with a melting point at 127°C.

Factors of Study

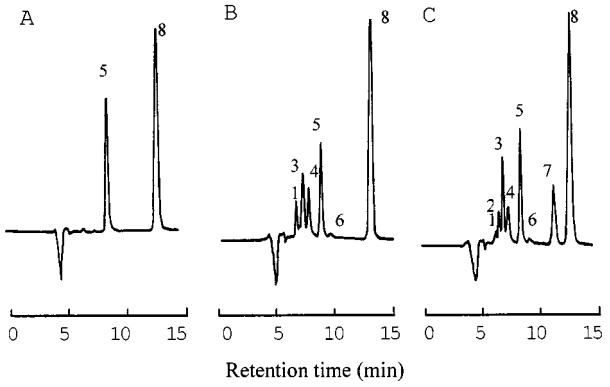
Oxygen, temperature, and pH effects were investigated in this study. Citrate buffers with pH 2.7 to 5.2 and phosphate buffers with pH 6.4 to 7.5 were used in the study. The total concentration of buffers was 0.02 M. The ionic strength was adjusted to 0.5 M using potassium chloride. The initial concentration of physostigmine was 60 μg/ml. Varying pH of physostigmine solutions (2 ml) were pipetted into 2-ml ampoules and sealed after purging with or without N_2 gas. Ampoules were placed in ovens at constant temperatures of 45°C, 55°C, 70°C, and 88°C ± 0.2°C. Samples were collected at suitable time intervals and frozen at −20°C before analysis.

High-Performance Liquid Chromatography Analysis

Samples of the study were analyzed with an HPLC system. The HPLC system consisted of a pump (Jasco, 880-Pu, Tokyo, Japan), an autosampler (Jasco, 855-As) with a 50-μl injection loop, a UV detector (Jasco, 875-UV) monitored at 245 nm or a photodiode array detector (SPD M-10A, Shimadzu, Kyoto, Japan), and an integrator (SIC 12). The mobile phase of the HPLC system, run at ambient temperature, was composed of 350 ml of methanol and 650 ml of 0.5 M acetate buffer containing 0.005 M sodium camphor sulfonic acid (pH 3.2). A reverse-phase C_{18} column (Vercopak, 5ODS, 4.6 mm × 250 mm) was used to separate physostigmine from its degradants. Ethyl morphine was used as the internal standard.

RESULTS AND DISCUSSION

Figure 1 shows the HPLC chromatograms of physostigmine and its related degradants in aerobic and anaerobic experimental conditions. Including eseroline, six degradants were found in the HPLC system. Most degradants had shorter retention times than physostigmine. Figure 1D shows the UV spectra of physostigmine and its two important degradants eseroline and rubreserine, which were determined simultaneously with a photodiode array detector during the HPLC analysis. All three peak purities are more than 99%. Rubreserine (peak 3) shows an absorption peak between wavelength of 350 and 600 nm, while the other degradants do not show any significant absorptions in this region. The identical UV spectrum was reported by Ellis (6). In the HPLC system



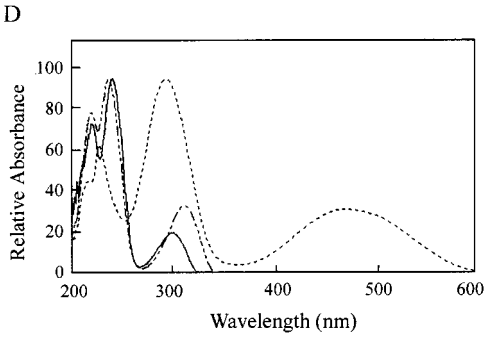


Figure 1. Chromatograms and UV spectra of physostigmine and its degradants eseroline and rubreserine. (A) Aqueous physostigmine solution was degraded at pH 11 for 3 min under N_2 gas purging at 25°C; (B) aqueous physostigmine solution was degraded at pH 11 for 3 min without N_2 gas purging at 25°; (C) aqueous physostigmine solution (2 ml, pH 5.2) was sealed in a 10-ml ampoule without N_2 gas purging at 88°C for 5 days; (D) UV spectra of physostigmine, eseroline, and rubreserine determined by a photodiode array in HPLC analysis. —, physostigmine; — –, eseroline; ---, rubreserine. Peaks 1, 2, 4, 6 are unidentified degradants; peak 3, rubreserine; peak 5, eseroline; peak 7, physostigmine; peak 8, ethyl morphine (internal standard).

of the study, rubreserine, eseroline, physostigmine, and internal standard (ethyl morphine) had retention times of 7.9, 9.2, 12.4, and 13.8 min, respectively. Physostigmine and eseroline have λ_{max} at 244 and 246 nm, respectively. Both compounds have very similar UV absorption patterns within UV 220–280 nm, but they are different within UV 280–350 nm.

The pH-rate profiles for physostigmine under anaerobic conditions determined at three temperatures of 45°C, 55°C, and 70°C are shown in Fig. 2. The observed degra-

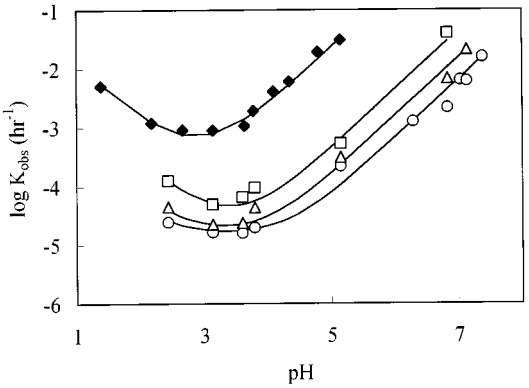


Figure 2. The pH-rate profiles of physostigmine in aqueous buffer solutions (0.02 M) with constant ionic strength ($\mu = 0.5$) and purging with or without N_2 gas. The observed degradation rate constants were determined by fitting the equation $K_{obs} = K_H[H^+] + K + K_{OH}[OH^-]$. Anaerobic treatment: ○, 45°C; △, 55°C; □, 70°C; aerobic treatment: ◆, 88°C.

dation rate constants K_{obs} of physostigmine (Table 1) were described as the following equation:

$$K_{obs} = K_H[H^+] + K + K_{OH}[OH^-] \quad (1)$$

where K_H, K_{OH}, and K represent specific acid-, base-, and solvent-catalyzed rate constants, respectively. K_{obs} of the three temperatures were fitted to Eq. 1 to determine related rate constants, which are listed in Table 1. The minimum of K_{obs} was found at pH 3.4. Fletcher and Davies (7) reported that the pH of maximum stability exists at pH 2.2–3.0 under aerobic condition at 90°C.

In the study, we also determined the degradation of physostigmine under aerobic conditions (Fig. 1) at 88°C from pH 1.4 to 5.2. The minimum of K_{obs} was found at pH 2.8. It seems that the minimum K_{obs} for degradation of physostigmine in anaerobic conditions was found at higher pH in comparison with the results studied in aerobic conditions.

The temperature effect on the degradation of physostigmine could be determined by the following Arrhenius equation:

$$K_{obs} = A \exp(-E_a/RT) \quad (2)$$

where A is a constant, R is the gas constant, E_a is the activation energy, and T is the absolute temperature. The results are listed in Table 1. The activation energies of K_{obs} of physostigmine under anaerobic and aerobic conditions were 17.9 and 17.5 kcal/mole, respectively (Ta-

Table 1

Rate Constants and Thermodynamic Parameters of Physostigmine Determined in Varying pH and Temperatures Under Aerobic or Anaerobic Treatments

	With N_2 Purging			Without N_2 Purging	*Ea*
	45°C	55°C	70°C	88°C	(Kcal/mol)
K_{obs} (hr^{-1})					
pH					
1.4	—	—	—	5.1×10^{-3}	
2.2	—	—	—	1.2×10^{-3}	
2.4	2.5×10^{-5}	4.5×10^{-5}	1.2×10^{-4}	8.2×10^{-4}	
3.1	1.7×10^{-5}	2.2×10^{-5}	5.0×10^{-5}	8.9×10^{-4}	
3.6	1.6×10^{-5}	2.4×10^{-5}	6.5×10^{-5}	1.1×10^{-3}	
3.8	2.0×10^{-5}	4.4×10^{-5}	1.6×10^{-4}	1.9×10^{-3}	17.9
	(2.3×10^{-5})[b]	(6.3×10^{-5})[b]	(2.9×10^{-4})[b]		17.5
4.1	—	—	—	4.1×10^{-3}	
4.3	—	—	—	6.1×10^{-3}	
4.8	—	—	—	1.9×10^{-2}	
5.2	2.2×10^{-4}	3.1×10^{-4}	5.3×10^{-4}	3.1×10^{-2}	
6.3	1.3×10^{-3}	—	—	—	
6.8	2.2×10^{-3}	6.9×10^{-3}	4.0×10^{-2}	—	
7.0	6.5×10^{-3}	—	—	—	
7.1	6.2×10^{-3}	2.1×10^{-2}	—	—	
7.4	1.6×10^{-2}	—	—	—	
K_H^c ($hr^{-1}M^{-1}$)	3.3×10^{-3}	6.4×10^{-3}	2.8×10^{-2}		9.4
K (hr^{-1})	1.4×10^{-5}	1.5×10^{-5}	2.5×10^{-5}		13.0
K_{OH} ($hr^{-1}M^{-1}$)	6.5×10^{4}	1.6×10^{5}	4.4×10^{5}		8.3

—: no determination.

[a] Observed degradation rate constants of physostigmine at varying pH

[b] Stability test without N_2 gas treatment.

[c] Fit the equation $K_{obs} = K_H[H^+] + K + K_{OH}[OH^-]$; K_H = specific acid-catalyzed constant; K = solvent-catalyzed constant; and K_{OH} = specific base-catalyzed constant.

ble 1). The activation energy for hydrolyzing the carbamate group of neostigmine has been reported to be 15.7 kcal/mole (10). Activation energies of K_H and K_{OH} are about equal (i.e., 9.4 and 8.3 kcal/mol, respectively), suggesting that the influence of temperature on these two rate constants is similar. Chiang et al. (11) reported that the activation energy of K_{OH} for the hydrolysis of diacetyl nadolol was 9.78 kcal/mole. The slopes of the pH-rate profiles for K_{OH} were close to 1 for pH 4–6 (Fig. 2), which coincided with the proposed specific-base catalysis.

In the oxygen effect study for pH 2.4–5.2, we found that degradation rate constants of physostigmine without nitrogen purging were much higher than those determined under anaerobic conditions. Physostigmine is hydrolyzed to eseroline, then to rubreserine by oxidization. The formation of rubreserine needs to spend 1/2 molecular oxygen. The ratios of degradation rate constants for aerobic and anaerobic treatments ranged from 2 to 33 (Table 2). As pH increased, the ratios also increased. Yang and Wilkin (12) reported that physostigmine, under anaerobic conditions, was more stable than under aerobic conditions with antioxidants during the process of autoclaving at 123°C from pH 3.0 to 5.3. Asker and Harris (8) reported that, for photo-catalyzing degradation of physostigmine, the ratio of rate constants of physostigmine solution treated without and with antioxidant (sodium thiosulfite) is 2.1 at pH 4.5. Thus, oxygen is one of most important factors that affect the decomposition of physostigmine.

Table 2

Effect of Oxygen on the Decomposition of Physostigmine at 88°C

pH	Rate Constant (hr^{-1})		Ratio (Aerobic/ Anaerobic)
	Aerobic	Anaerobic[a]	
2.4	8.2×10^{-4}	3.4×10^{-4}	2.4
3.1	8.9×10^{-4}	9.7×10^{-5}	9.2
3.6	1.1×10^{-3}	1.5×10^{-4}	7.0
3.8	1.9×10^{-3}	2.6×10^{-4}	7.2
5.2	3.1×10^{-2}	9.3×10^{-4}	33.4

[a] Estimated by Arrhenius equation from Table 1 data.

CONCLUSION

The degradation of physostigmine was catalyzed by specific acid and specific base under anaerobic conditions with a minimum degradation rate constant at pH 3.4. Activation energies for both K_H and K_{OH} are about equal, with values of 9.4 and 8.3 kcal/mol, respectively, from 45°C to 70°C in the pH range 2.4–4.8. The effect of temperature on these two rate constants is similar.

The degradation rate constant of physostigmine under aerobic condition was 2 to 33 times higher than that obtained under anaerobic condition at pH 2.4–5.2. Thus, the physostigmine delivery system could be prepared under anaerobic conditions. The shelf life of physostigmine solution was estimated to be 4 years at room temperature under anaerobic conditions with pH 3.4.

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