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Bispilocarpic acid monoesters as prodrugs of pilocarpine: II. Physicochemical properties and kinetics of hydrolysis in aqueous solution

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Summary

The physicochemical properties and degradation kinetics of bispilocarpic acid monoesters were studied in order to assess the suitability of these compounds as prodrugs of pilocarpine. The esters show variable increases in lipophilicity relative to pilocarpine as evaluated by drug partitioning between 1-octanol and phosphate buffer at pH 7.40 and by the capacity factor (k') of reversed-phase liquid chromatography. The degradation kinetics of the compounds were studied in aqueous solutions as a function of pH and temperature to determine their stability and suitability as prodrugs of pilocarpine. The bispilocarpic acid monoesters were hydrolyzed to yield pilocarpine in quantitative amounts in aqueous solution. Although bispilocarpic acid monoesters showed maximal stability in acidic solutions, the shelf-lives at pH 4.20 and at 4°C were less than 10 days. Consequently, it may be difficult to prepare ready-to-use aqueous solutions at acceptable pH (pH > 4.0).

Introduction

The prodrug concept has been widely used to improve the delivery characteristics of drugs. Application of the prodrug approach is, however, difficult when the aim is to design a prodrug for aqueous formulations such as eyedrops. A successful ophthalmic prodrug should meet several requirements at the same time. It should have sufficient aqueous solubility and stability, be capable of efficient penetration across the cornea and be convertible to the parent drug in the cornea. In addition, the compound must be nontoxic and nonirritant.

The major reasons for using this prodrug approach in ocular delivery can be classed as follows (Lee and Li, 1989): to improve drug efficacy (carbonic anhydrase inhibitors, steroids, antivirals, and beta-blockers), to decrease the systemic side-effects (timolol, phenylephrine and terbutaline) and to change the duration of action (pilocarpine and adrenalone).

Bodor (1977) has described quaternary ammonium salts of pilocarpine and Bundgaard et al. (1985, 1986a,b) investigated diesters of pilocarpic

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acid as possible prodrugs of pilocarpine. Pilocarpic acid diesters have also been found to be efficient in vivo by measuring their miotic activity in rabbits (Mosher et al., 1987).

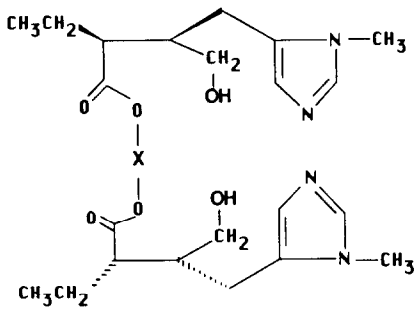
We prepared bispilocarpic acid monoesters, a new class of pilocarpine prodrugs, in order to minimize the formation of the pro-moiety per mol of active pilocarpine and to achieve optimal ocular delivery characteristics. As a part of our evaluation of the potential use of pilocarpine prodrugs in improving the ocular bioavailability of pilocarpine, the present study was carried out in order to study the kinetics of bispilocarpic acid monoesters in aqueous solution as a function of pH and temperature. The ionization constants and lipophilicities of the bispilocarpic acid monoesters were also determined.

Materials and Methods

Apparatus

Liquid chromatography (LC) was performed with a system consisting of a Beckman programmable solvent module 116, a Beckman programmable UV detector 166 (set at 215 nm), System gold data module (Beckman, San Ramon, U.S.A.), Marathon autosampler equipped with column thermostat (Spark Holland, AJ Emmen, the Netherlands) and a Rheodyne 7080-080 loop (20 μl) injector. A deactivated Supelcosil LC8-DB (15 cm $\times$ 4.6 mm i.d., 5 μm) and an LC18-DB (25 cm $\times$ 4.6 mm i.d., 5 μm) reversed-phase column (Supelco, Bellefonte, U.S.A.) were used with various mobile phases for LC analysis.

An Orion SA 520 pH meter (Boston, U.S.A.) equipped with a combination pH electrode was used for pH determination.



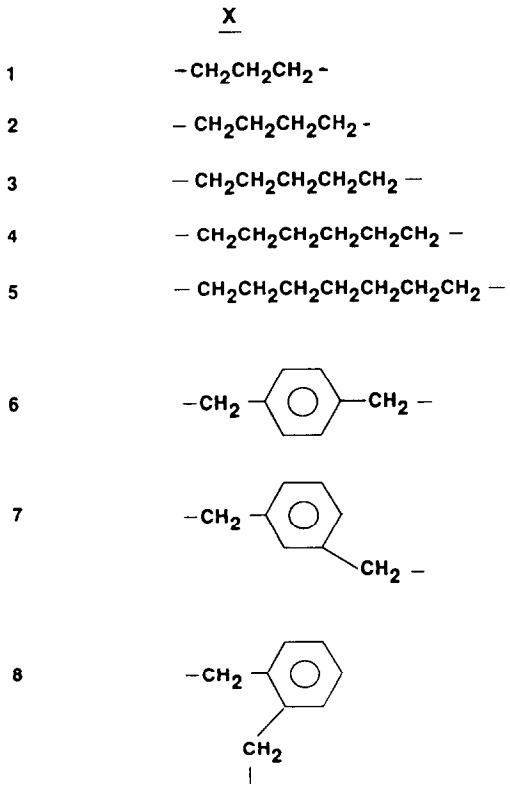


Fig. 1. Structures of bispilocarpic acid monoesters studied.

Chemicals

All the bispilocarpic acid monoesters investigated (Fig. 1) were synthesized and identified as described in the preceding article (Järvinen et al., 1992). Pilocarpine hydrochloride was kindly supplied by Huhtamäki Oy Leiras (Tampere, Finland) and isopilocarpine nitrate was obtained from Aldrich (Steinheim, Germany). Pilocarpic acid and isopilocarpic acid were prepared according to the method of Bundgaard and Hansen (1982). The reagents for various buffer solutions were commercially available and HPLC-grade solvents were used.

Determination of ionization constant

The ionization constants (pK_a values) of pilocarpine and bispilocarpic acid monoesters **1–8** were determined by titration of 2 mM solutions

of the compounds in a mixture of water and ethanol (50%/50%) with 0.05 N hydrochloric acid at room temperature. Ionization constants were obtained from conventional titration curves where volume of titrant was plotted against pH.

Determination of partition coefficients

The apparent partition coefficients (P) for pilocarpine and bispilocarpic acid monoesters **1–8** were evaluated from the distribution of the compounds between 1-octanol and phosphate buffer (0.16 M, pH 7.40, $\mu = 0.5$ adjusted with potassium chloride) using the so-called 'shake-flask' technique (Leo et al., 1971).

Phosphate buffer and 1-octanol phases were saturated before use by stirring vigorously for 24 h at room temperature. The aqueous and octanol phases were separated and the monoesters were dissolved in the aqueous phase at a suitable concentration. The volumes of the octanol and aqueous phases were selected such that the monoester concentration of the aqueous phase could readily be determined before and after distribution. The buffer/1-octanol mixture was shaken strongly for 5 min to achieve complete distribution equilibrium. After separation of the aqueous and 1-octanol phases by centrifugation, 50 μl of 5 M HCl was added to 2.0 ml of buffer phase (also to the solution which was not subjected to the distribution method) in order to adjust the pH to a value below 5 and to minimize the degradation of bispilocarpic acid monoester. The concentrations of the bispilocarpic acid monoesters in the aqueous phase before and after distribution were immediately measured by LC. The column was RP-8 Supelcosil at 40°C and the isocratic solvent system was 0.02 M KH_2PO_4, (pH 4.5)-methanol (50%-50%) at a flow rate of 1.0 ml/min. The column effluent was monitored at 215 nm. The apparent partition coefficients (P) were calculated from Eqn 1:

$$P = \left(\frac{C_i - C_a}{C_a}\right) \cdot \left(\frac{V_a}{V_o}\right) \qquad (1)$$

where C_i and C_a represent the initial solute concentration in the aqueous buffer phase and the corresponding concentration after distribution, respectively, and V_a and V_o denote the respective volumes of the aqueous and 1-octanol phases. Determinations were carried out in triplicate for each bispilocarpic acid monoester.

Capacity factors (k') of RP-LC were also employed in the evaluation of the lipophilicity of the bispilocarpic acid monoesters. A Supelcosil RP-8 column (see above) thermostated to 40°C was used as the stationary phase and the mobile phase consisted of a mixture of 0.02 M KH_2PO_4, pH 4.5 and methanol (29%-71%). Flow rate was 1.0 ml/min. All bispilocarpic acid monoesters and pilocarpine were dissolved in the mobile phase at concentrations of 0.106–0.175 mg/ml. The capacity factor, k', was calculated from Eqn 2:

$$k' = (t_r - t_0)/t_0 \qquad (2)$$

where t_r is the retention time of the compound and t_0 denotes the column dead time. The column dead time was determined by using sodium nitroprusside as t_0 marker.

Kinetic measurements

The hydrolysis of the bispilocarpic acid monoesters was studied in aqueous solutions at various temperatures (37, 50, 60 and 70°C) and pH values (4.20, 6.00, 7.40 and 9.00). Phosphate buffers of 0.16 M at an ionic strength (μ) of 0.5 were used.

For solubility reasons, the compounds were first dissolved in 2.0 ml of ethanol. The reactions were initiated by adding 50.0 ml pre-heated buffer solution, the final concentration of the bispilocarpic monoesters being about 0.1 mM. The solutions were kept in a water bath at constant temperature and, at appropriate intervals, 1.0-ml samples were taken and mixed with 1.0 ml phosphate solution (pH 4.20) in order to terminate the degradation of the monoester. The monoesters were analyzed by measuring the peak areas using the LC method described in the determination of partition coefficients. Quantitation of pilocarpine, isopilocarpine, pilocarpic acid and isopilocarpic acid formed upon hydrolysis was carried out by measuring the peak area by LC using a

deactivated Supelcosil LC18-DB column according to a previous method (Järvinen et al., 1991).

The pseudo-first-order rate constants (k_{obs}) for the overall degradation of the monoesters were calculated from the slopes of the plots of the logarithm of residual monoester vs time using linear regression (r value 0.98–1.00, most often being 1.00). The pseudo-first-order rate constants (k_f) for the formation of pilocarpine were determined from the slopes of linear plots (r value 0.99–1.00, most often being 1.00) of the logarithm of unformed pilocarpine ($\log[\text{pilocarpine}_{max} - \text{pilocarpine}_t]$) vs time.

Results and Discussion

pK_a values of bispilocarpic acid monoesters

Bispilocarpic acid monoesters are insufficiently soluble in water for a reliable titration to be constructed. Consequently, it was necessary to titrate the sample in a water-organic solvent mixture. In order to obtain comparable results all prodrugs were titrated in a water-ethanol mixture (50%-50%). The results all prodrugs were titrated in a water-ethanol mixture (50%-50%). The ionization constants (pK_a value) for bispilocarpic acid monoesters are listed in Table 1.

TABLE 1

pK_a values, apparent partition coefficients (P) and reversed-phase LC capacity factors (k') of pilocarpine and bispilocarpic acid monoesters

Compound	pK_a [a]	Log P [b] (RSD) (%)	k'
Pilocarpine	6.30	0.01 (34.3)	0.41
1	6.40	0.68 (6.65)	0.61
2	6.35	1.16 (1.32)	0.66
3	6.30	1.57 (1.46)	0.75
4	6.30	1.84 (3.38)	0.89
5	6.30	2.67 (2.64)	1.13
6	6.25	2.18 (1.10)	0.81
7	6.30	1.99 (1.73)	0.83
8	6.30	2.03 (2.46)	0.83

[a] pK_a values of compounds were determined in a mixture of ethanol and water (50%-50%).

[b] Apparent partition coefficient between 1-octanol and phosphate buffer of pH 7.40.

The pK_a values of monoesters **1–8** varied from 6.25 to 6.40, most frequently being 6.30. Under the same test conditions, the pK_a value of pilocarpine was 6.30 while it is 7.05 in water (Florence and Attwood, 1988). This difference is due to the effect of ethanol in the titration solution (Parke and Davis, 1954). We observed linear regression between the decrease in alcohol concentration (from 50 to 0%) and the pK_a value of pilocarpine. In our test, the pK_a value of pilocarpine in water was 7.00. The pK_a values of bispilocarpic acid monoesters in water can be estimated to be about 7.0. Opening of the lactone ring and the uniting of two pilocarpic acid molecules with a spacer have no effect upon the ionization of the imidazole moiety of bispilocarpic acid.

Lipophilicity of pilocarpine and bispilocarpic acid monoesters

Apparent partition coefficients (P) are currently the most frequently employed indication of hydrophobicity. Apparent partition coefficients for bispilocarpic acid monoesters and pilocarpine were obtained from the equilibrium distribution of the compounds between 1-octanol and phosphate buffer (pH 7.40) at room temperature (Table 1). The results show that all the bispilocarpic monoesters studied are more lipophilic ($\log P = 0.68–2.67$) than the parent compound, pilocarpine ($\log P = 0.01$). It can also be seen that by varying the spacer chain between the two pilocarpic acid molecules, it is possible to obtain bispilocarpic acid monoesters of various lipophilicities. Consequently, the corneal permeability of bispilocarpic acid monoesters could be optimized. The reliability of the shake-flask method depends on the careful control of several experimental factors. The relative standard deviations (RSD) for monoesters **2–8** were satisfactory (below 4%), but for monoester **1** over 6%.

The shake-flask technique has several drawbacks: it is time-consuming, has a limited range of applicability and may cause degradation of the compounds during the test. An alternative way of measuring lipophilicity is the RP-LC method (El Tayar et al., 1985). In our study the capacity factors (k') determined were consistent with the

results obtained using the shake-flask method (Table 1). Through reversed-phase chromatographic methods, it may be possible to achieve very good estimates of log P rapidly with very little loss of the compound.

Kinetics in aqueous solution

The rate of hydrolysis of the bispilocarpic acid monoesters was investigated in aqueous buffer solutions in the pH range 4.20–9.00 at temperatures of 37, 50, 60 and 70°C. Under the experimental conditions employed, bispilocarpic acid monoesters underwent two-step hydrolysis to yield pilocarpine quantitatively: firstly, the disappearance of bispilocarpic acid monoester and progressive appearance of pilocarpic acid monoester, and secondly, the disappearance of pilocarpic acid monoester and progressive appearance of pilocarpine (Fig. 2). The extent of formation of isopilocarpine during hydrolysis varied between 2 and 20% depending on the monoester, usually being about 5%. Inactive pilocarpic acid and isopilocarpic acid were not found in the test. Fig. 3 shows as an example the time courses for the disappearance of 1,4-xylylene bispilocarpate (compound **6**) and the concurrent appearance of pilocarpine (sum of pilocarpine and isopilo-

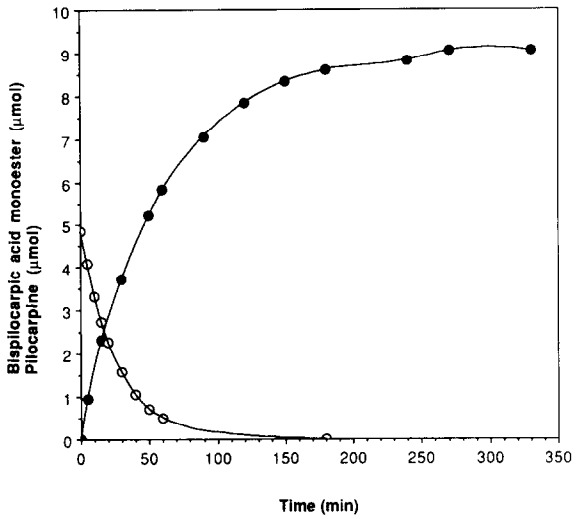


Fig. 3. Plot showing decomposition of bispilocarpic acid monoester **6** (○) and concurrent formation of pilocarpine (●) in the degradation of ester at pH 7.40 and at 37°C.

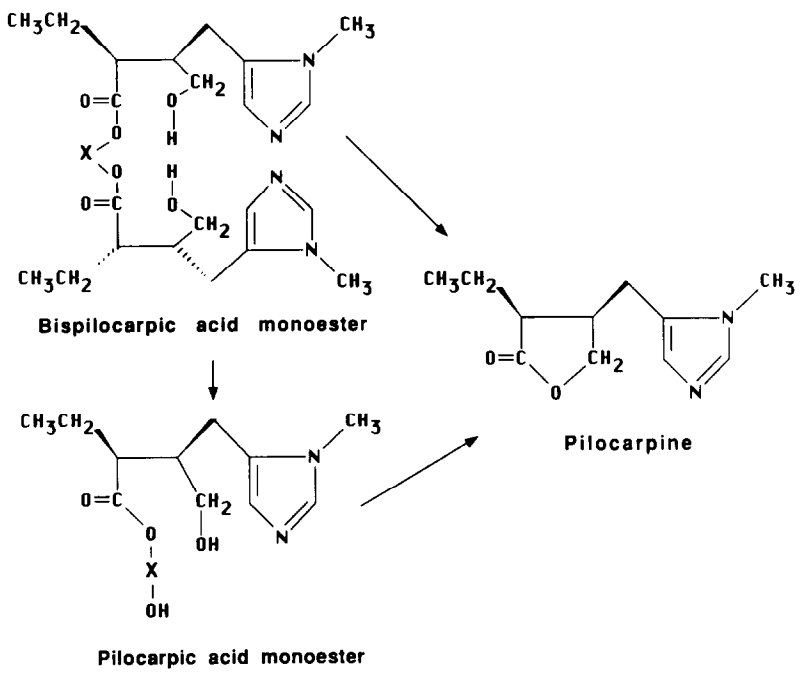


Fig. 2. Degradation of bispilocarpic acid monoester in aqueous solution.

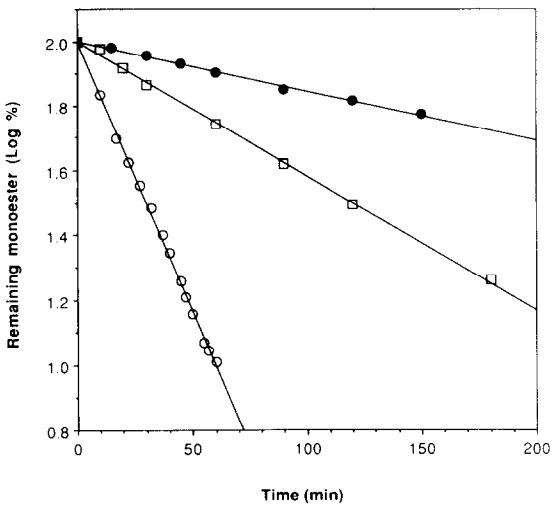


Fig. 4. First-order plots for the hydrolysis of bispilocarpic acid monoesters **1** (□), **5** (●) and **6** (○) in aqueous solution at pH 7.40 and 37°C.

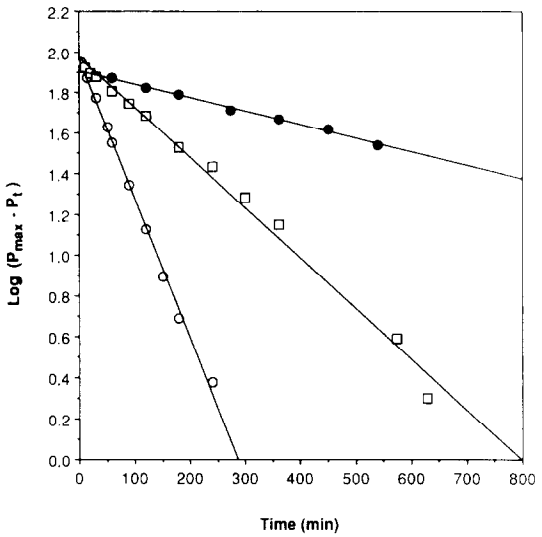


Fig. 5. First-order plots for the formation of pilocarpine from bispilocarpic acid monoesters **1** (□), **5** (●) and **6** (○) in aqueous solution at pH 7.40 and at 37°C. P_{max} and P_t represent the percentage amounts of pilocarpine formed at infinite and at time t, respectively.

carpine). The results show that the formation of pilocarpine was practically complete (≈ 93%) when the initial purity of the bispilocarpic acid monoester (≈ 95%) is taken into account.

At constant pH and temperature the disappearance of all bispilocarpic acid monoesters followed first-order kinetics over several half-lives. Typical first-order plots for the degradation of monoesters in aqueous solution (pH 7.40) at 37°C are shown in Fig. 4 and the corresponding plots for the formation of pilocarpine are depicted in Fig. 5. The rate constants for the degradation of monoester (k_{obs}) and formation of pilocarpine (k_f) are listed in Table 2. The value of $f_{50\%}$ represents the time at which 50% of the total pilocarpine has been formed and was calculated from the slopes of the linear plots of the formation of pilocarpine. It can be seen from the data that by appropriate variation of the spacer chain between the pilocarpic acid molecules, it is possible to change the rate of formation of pilocarpine and consequently to control and modify the release of pilocarpine. Furthermore, these data help in the determination of the optimal structures for use as a starting material in the synthesis of new double-prodrugs of pilocarpine, bispilocarpic acid diesters.

The influence of pH on the rates of hydrolysis at 37°C is illustrated in Fig. 6. The lowest pH studied was pH 4.20, since it is near to the lowest acceptable pH for eye-drop formulation. In the investigated pH range, the pH-rate profile indicates that the hydrolysis reactions are subject to

TABLE 2

Rate data for hydrolysis of various bispilocarpic acid monoesters in aqueous buffer of pH 7.40 at 37°C

Compound	k (min^{-1})	$t_{1/2}$ (min)	k_f (min^{-1})	$f_{50\%}$ (min)
1	0.00967	72	0.00576	120
2	0.00438	158	0.00299	231
3	0.00553	125	0.00299	231
4	0.00461	150	0.00176	393
5	0.00345	201	0.00155	489
6	0.03800	18	0.01589	44
7	0.03639	19	0.01336	52
8	0.02787	25	0.02372	29

k, rate constant for degradation of monoester at pH 7.40 and 37°C; $t_{1/2}$, half-time of degradation of monoester at pH 7.40 and 37°C; k_f, rate constant for formation of pilocarpine at pH 7.40 and 37°C; $f_{50\%}$, time at which 50% of total pilocarpine is formed at pH 7.40 and 37°C.

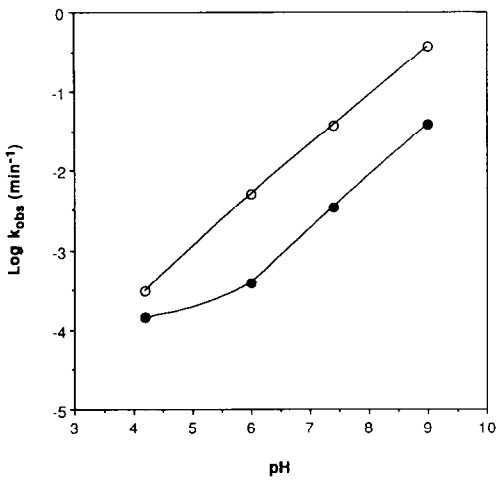


Fig. 6. pH-rate profiles for the hydrolysis of bispilocarpic acid monoesters **5** (●) and **6** (○) in aqueous solution at 37°C.

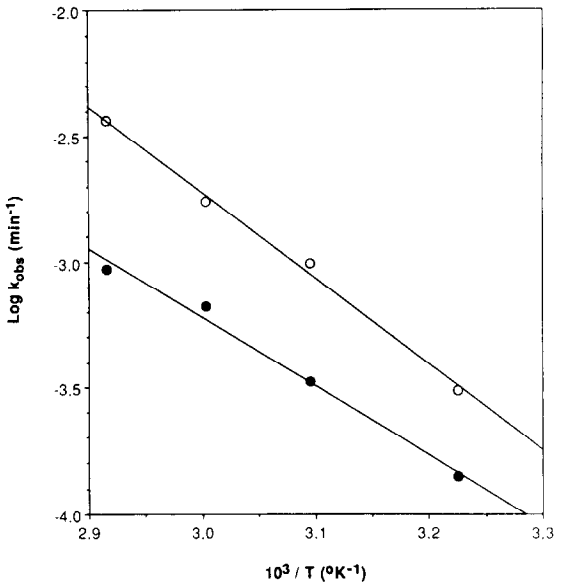


Fig. 7. Arrhenius plots of the rates of hydrolysis of bispilocarpic acid monoesters **5** (●) and **6** (○) in aqueous buffer solution at pH 4.20.

specific base catalysis. The rate data for all the bispilocarpic acid monoesters at different pH values and at 37°C are listed in Table 3.

The effect of temperature on the rate of hydrolysis of the bispilocarpic acid monoesters was studied at pH 4.20 and 6.00 over the temperature range 37–70°C. In Fig. 7 the rate data obtained for monoesters **5** and **6** at pH 4.20 are plotted according to the Arrhenius equation (Eqn 3).

$$\log k = \log A - \frac{E_a}{2.303R} \cdot \frac{1}{T} \qquad (3)$$

where A is the frequency factor, E_a denotes the apparent energy of activation, R is the gas constant and T represents the absolute temperature (in K). On the basis of the equation obtained from the plot in Fig. 7, it was possible to estimate the shelf-life ($t_{10\%}$, time required to degrade 10% of the monoester) of the solution at various temperatures. The shelf-life for bispilocarpic acid monoester **6** was estimated to be 112 h at pH 4.20 and 7 h at pH 6.00 for a solution stored at 4°C. The corresponding values for monoester **5** were 132 h (pH 4.20) and 129 h (pH 6.00).

TABLE 3

Half-times of bispilocarpic acid monoesters in aqueous solution of different pH at 37°C

Compound	$t_{1/2}$ (min)			
	pH 4.20	pH 6.00	pH 7.40	pH 9.00
1	4040	618	72	4
2	7206	1136	158	10
3	4378	1474	125	14
4	3759	1509	150	14
5	4909	1792	201	18
6	2256	137	18	1.9
7	2930	167	19	1.1
8	1210	201	25	1.4

Conclusions

This study has demonstrated that it is possible to design bispilocarpic acid monoesters which have a greater lipophilicity than pilocarpine, which is a prerequisite for improved corneal penetration. All the monoesters hydrolyzed spontaneously to regenerate pilocarpine in aqueous solution. The compounds are most stable at pH 4.20, but even at this pH, it is impossible to design ready-to-use aqueous eye-drop formulations of bispilocarpic acid monoesters with an acceptable shelf-life. Consequently, this kind of

prodrug can be formulated only in non-aqueous vehicles.

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