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O,O'-(1,4-Xylylene) bispilocarpic acid esters as new potential double prodrugs of pilocarpine for improved ocular delivery. II. Physicochemical properties, stability, solubility and enzymatic hydrolysis

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Summary

Various *O,O'*-(1,4-xylylene) bispilocarpic acid esters, i.e. bispilocarpic acid diesters, were evaluated as water-soluble double prodrugs of pilocarpine. All the prodrug derivatives (log $P = 2.76–7.03$) were more lipophilic than pilocarpine (log $P = 0.01$) as determined from partitioning between 1-octanol and buffer (pH 7.40) or from LC capacity factors. The bispilocarpic acid diester fumarates were shown to be more water-soluble prodrugs than previously described pilocarpic acid diester fumarates. The aqueous stability of the derivatives was investigated as a function of pH and temperature. Maximal stability was achieved in acidic solutions. The shelf-life of *O,O'*-dipropionyl (1,4-xylylene) bispilocarpate fumarate was 469 days at pH 6.0 and 4 °C. Hence, the bispilocarpic acid diester prodrugs possess sufficient aqueous stability to allow formulation of ready-to-use solutions. The diesters were hydrolyzed enzymatically to yield bispilocarpic acid monoester which cyclized to the parent pilocarpine in quantitative amounts. The half-lives of diesters in human plasma varied from 2 to 94 min, being highly dependent on the ester group. It appears that bispilocarpic acid diesters are a promising group of new pilocarpine prodrugs that offer possibilities from the results in stability, solubility, lipophilicity, and enzymatic hydrolysis tests.

Introduction

Pilocarpine is widely used topically in the eye to control the elevated intraocular pressure associated with glaucoma. However, pilocarpine exhibits an extremely low ocular bioavailability; only 1–3% or less of an instilled pilocarpine dose gains access to the internal eye structures (Lee and Robinson, 1979). Since the extent of penetration of pilocarpine across the cornea is very limited, due mainly to its low lipophilicity, delivery of pilocarpine to the eye can be improved using prodrug derivatives (Bundgaard et al., 1986a, b).

A successful pilocarpine prodrug should be more lipophilic than pilocarpine in order to facilitate improved corneal penetration and have sufficient aqueous solubility and stability at neutral or

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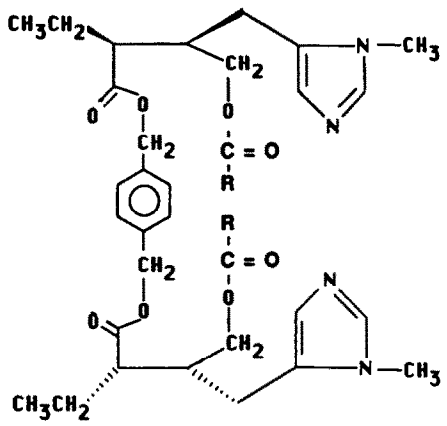
slightly acidic pH to avoid decreased penetration due to ionization and corneal irritation. The prodrug should also release pilocarpine at a reasonable rate to prolong its duration of action and, finally, it should not be toxic itself.

Pilocarpic acid diesters have been reported to be potential prodrugs (Bundgaard et al., 1985, 1986a,b; Järvinen et al., 1991d) and to have miotic activity in rabbits (Mosher et al., 1987), however, low water solubility and irritation of the eye have been observed (Mosher, 1986). We have reported bispilocarpic acid monoesters to be too unstable in aqueous solution to obtain aqueous formulations (Järvinen et al., 1991e) and the bispilocarpic acid diesters were prepared in order to improve the aqueous stability of the corresponding monoesters.

The purpose of the present study was to provide experimental data for evaluation of the potential applicability of *O,O'*-(1,4-xylylene)bispilocarpic acid esters (i.e. bispilocarpic acid diesters), the new water-soluble prodrugs of pilocarpine. The ionization constants, lipophilicity, and aqueous solubility of the prodrug derivatives were determined. Degradation kinetics of the compounds were investigated as a function of pH and temperature in aqueous solution in order to establish the possibilities of formulating ready-to-use eye-drop solutions. Furthermore, enzymatic lability of the double prodrugs was examined in human plasma to demonstrate that bispilocarpic acid diesters undergo enzymatic hydrolysis and form bispilocarpic acid monoesters which spontaneously cyclize to active pilocarpine.



	R
1.	$-CH_3$
2.	$-CH_2CH_3$
3.	$-CH_2CH_2CH_3$
4.	$-CH_2CH_2CH_2CH_3$
5.	$-CH_2COOCH_3$
6.	
7.	

Fig. 1. Structures of the *O,O'*-(1,4-xylylene) bispilocarpic acid esters, i.e. bispilocarpic acid diesters, studied.

Materials and Methods

Chemicals

All *O,O'*-(1,4-xylylene)bispilocarpic acid esters, i.e. bispilocarpic acid diesters (Fig. 1), were synthesized and identified as described previously (Järvinen et al., 1991b). Pilocarpine hydrochloride was kindly supplied by Huhtamäki Oy Leiras (Tampere, Finland). Isopilocarpine nitrate was obtained from Aldrich (Steinheim, Germany). Pilocarpic acid and isopilocarpic acid were prepared according to a method described earlier (Bundgaard and Hansen, 1982). Monobasic potassium phosphate, disodium phosphate dihydrate, sodium bicarbonate, 1-octanol and sodium nitroprusside were purchased from Merck (Darmstadt, Germany) and sodium chloride and methanol (HPLC grade) were from J.T. Baker (Deventer, The Netherlands).

Apparatus

Liquid chromatography (LC) was performed with a system consisting of a Beckman pro-

grammable solvent module 116, a Beckman programmable UV detector 166 (set at 215 nm), System Gold data module (Beckman Instruments Inc., San Ramon, U.S.A.), Marathon autosampler equipped with column thermostat (Spark Holland, Emmen, The Netherlands) and a Rheodyne 7080-080 loop (20 μl) injector. Deactivated Supelcosil LC8-DB (15 cm × 4.6 mm i.d., 5 μm) and LC18-DB (25 cm × 4.6 mm i.d., 5 μm) reversed-phase columns (Supelco, Bellefonte, U.S.A.) were used.

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

Determination of ionization constants

The ionization constants (pK_a values) of pilocarpine and bispilocarpic acid diesters 1–7 (as free base) were determined by titration of 2 mM solutions of the compounds in a mixture of water and ethanol (50:50, %) with 0.05 N HCl at room temperature. The ionization constants were obtained from conventional titration curves, where volume of titrant was plotted against pH.

Measurement of partition coefficients

The lipophilicity of the bispilocarpic acid diester fumarates was evaluated from the distribution of the compounds between 1-octanol and phosphate buffer studied using the 'shake-flask' technique (Leo et al., 1971) or by measuring the capacity factors (k') of the compounds by reversed-phase liquid chromatography (RP-LC).

The apparent partition coefficients (P) for bispilocarpic acid diesters **1**, **2**, **5** and **6** were measured using a 1-octanol/buffer system. The phosphate buffer (0.16 M, pH 7.40 and ionic strength (μ) = 0.5 adjusted with sodium chloride) and 1-octanol phases were saturated before use by stirring vigorously for 24 h at room temperature. The aqueous and 1-octanol phases were separated and the compounds were dissolved in the aqueous phase at a suitable concentration. The volumes of the 1-octanol and aqueous phases were selected so that the diester concentration of the aqueous phase could be readily determined. The buffer/1-octanol mixtures were shaken for 60 min to achieve equilibrium. After separation of the aqueous and 1-octanol phases, the aqueous phase was centrifuged at 4000 rpm for 5 min in order to achieve complete separation. The concentrations of bispilocarpic acid diesters in the aqueous phase before and after distribution were measured by LC. The quantitation of the compounds was achieved by measuring the peak area in relation to the standard solutions chromatographed under the same conditions. The chromatographic conditions were as follows: column, RP-8 Supelcosil (see Apparatus) thermostated to 40 °C; isocratic solvent system, 0.02 M KH_2PO_4 (pH 4.5): methanol (29–71%); flow rate, 1.0 ml/min. The proportion of methanol may be increased for very lipophilic compounds in order to reduce the retention time without losing separation. The column effluent was monitored at 215 nm. The apparent partition coefficients (P) were calculated from Eqn 1:

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

where C_i and C_a represent the initial (i) and equilibrium (a) solute concentrations of the aqueous buffer phase; V_a denotes the volume of the aqueous and V_0 the volume of the 1-octanol phase. Triplicate apparent partition coefficient determinations were made for each compound.

The apparent partition coefficients (log P) and capacity factors of RP-LC have been determined for pilocarpine, two pilocarpic acid monoesters (unpublished data), 11 pilocarpic acid diesters (Järvinen et al., 1991d), eight bispilocarpic acid monoesters (Järvinen et al., 1991e) and bispilocarpic acid diesters **1**, **2**, **5** and **6**. A linear relationship was found between log k' and log P for various types of pilocarpine prodrugs. Log P values for very lipophilic prodrug derivatives (compounds **3**, **4** and **7**) were estimated based on RP-LC capacity factors because the conventional shake-flask method could not be used due to the low aqueous solubility of the compounds. LC analysis was performed as in the measurement of partition coefficients. The capacity factor (k') of each compound was calculated from Eqn 2:

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

where t_r is the retention time of the compound and t_0 denotes the elution time of sodium nitroprusside, a compound not retained on the column.

Solubility measurements

The pH-solubility profile of *O,O'*-dipropionyl (1,4-xylylene) bispilocarpate fumarate (compound **2**) was determined by the phase-solubility technique at room temperature. The solubility of two pilocarpic acid diester fumarates (*O*-propionyl and *O*-benzoyl pilocarpic acid benzyl ester fumarate) (Järvinen et al., 1991a) was also determined for the purpose of comparison.

941 mg of compound **2** (fumarate salt) was dissolved with 13.0 ml of 0.9% NaCl and a saturated solution was achieved with a resulting pH of 2.95. The solution was stirred on a mechanical spindle for 30 min, the pH of the solution was measured, and samples of 750 μl were withdrawn and filtered (Millipore 0.45 μm). The filter was found not to absorb diesters when it had been saturated with 1 ml of solution. After filtration, an aliquot of the filtrate was diluted with an appropriate amount of the mobile phase used in the LC method and the mixture then submitted to LC analysis as described for measurement of partition coefficients. After sampling, the pH of the solution was increased by the dropwise addition of 0.1–5 M NaOH until a pH of 11.96 was reached (taking samples at appropriate intervals). The solution was mixed for 30 min after each addition of NaOH. The solubility-pH profiles for pilocarpic acid diester fumarates were determined identically.

Hydrolysis in aqueous solutions

Hydrolysis of the bispilocarpic acid diesters **1–7** was studied in aqueous buffer solutions at constant temperature (37, 50, 60, 70 °C) and at different pH values (4.20, 6.00, 7.40, 9.00). The buffers contained 0.16 M phosphate, the ionic strength (μ) being adjusted to 0.5 with NaCl.

Solutions of the bispilocarpic acid diester fumarates were prepared by dissolving the appropriate amount of the compound in 2.0–5.0 ml of ethanol followed by 50.0 ml of the pre-heated buffer. Ethanol was used for solubility reasons. The solution was placed in a thermostated water bath maintained at constant temperature and at appropriate intervals 1.0-ml samples were taken and mixed with 2.0 ml of 0.1 M sodium phosphate solution (pH 6.0). The remaining bispilocarpic acid diester was assayed according to the peak areas measured by the LC method described above under measurement of partition coefficients. For very lipophilic compounds, the amount of methanol in the mobile phase may be increased in order to shorten the retention times of the compounds.

The pseudo-first-order rate constants (k_{obs}) and half-lives ($t_{1/2}$) for the overall degradation of the bispilocarpic acid diesters were determined from the slopes of the linear semilogarithmic plots of remaining bisdiester vs time.

Enzyme hydrolysis in human plasma

The bispilocarpic acid diester fumarates (1–10 μmol) were dissolved in 4.0 ml phosphate buffer (0.16 M, pH 7.40, 37 °C), 16.0 ml pre-heated human plasma was added and the solutions were kept in a water bath at 37 °C. At suitable intervals, samples of 1.0 ml of the plasma-buffer mixture were withdrawn and added to 3.0 ml ethanol in order to deproteinize the plasma. After mixing and centrifugation, the clear supernatant was analyzed for remaining diester by the LC method above (see Measurement of partition coefficients). The pseudo first-order rate constants (k_{obs}) and half-lives ($t_{1/2}$) for degradation were calculated from the slopes of linear plots of the logarithm of remaining bispilocarpic acid diesters vs time using linear regression.

Formation of pilocarpine in serum solutions was assayed using a different procedure. A 1.0 ml sample of clear supernatant was evaporated to dryness under a stream of air. The residue was dissolved in 500–750 μl of mobile phase (see below), mixed and analyzed by LC. The LC conditions were as follows: column, RP-18 Supelcosil (see Apparatus) thermostated to 40 °C; isocratic solvent system, 5% KH_2PO_4 (pH 2.50, adjusted with phosphoric acid): methanol (97:3, %); flow-rate, 1.5 ml/min. The column effluent was monitored at 215 nm. This procedure also permits determination of isopilocarpine, pilocarpic acid

and isopilocarpic acid with satisfactory separation (Järvinen et al., 1991d). The pseudo-first-order rate constants for formation (k_f) of pilocarpine were determined from the slopes of linear plots of the logarithm of unformed pilocarpine ($\log[\text{pilocarpine}_{max}-\text{pilocarpine}_t]$) vs time.

Results and Discussion

pK_a values of bispilocarpic acid diesters

The most useful methods for ascertaining pK_a values are potentiometric titration and spectrometric determination (Cookson, 1974). The solubility requirements for aqueous potentiometric titrations are often too demanding and hence nonaqueous or mixed-aqueous solvents are required. Alcohols, dioxane and dimethylformamide have proved useful for this purpose (Parke and Davis, 1954).

The pK_a values of all the bispilocarpic acid diesters were determined for the free base due to interference from fumaric acid and in mixtures of water/ethanol (50:50, %) because of the poor water solubility of the free diesters. The ionization constants (pK_a value) for bispilocarpic acid diesters (free base) are listed in Table 1. The pK_a values of the compounds varied from 5.80 to 6.03, being most often 6.00. Comparable pK_a values have been observed in the case of *O,O'*-dicarboxylate (dibenzyl) bispilocarpates (Järvinen et al., 1991c). The pK_a value of pilocarpine in 50% ethanol has been reported to be 6.30 and in water 7.00 (Järvinen et al., 1991d). In addition, a linear relationship was evident for regression between ethanol concentration and the pK_a value of pilocarpine (Järvinen et al., 1991d). Based on these results, it can be estimated that the pK_a values of the bispilocarpic acid diesters amount to about 6.7 in water. The results show that the linking of spacer chains between the carboxyl groups of two pilocarpic acid molecules has a similar effect on the pK_a values to that between the alcoholic groups of two pilocarpic acids. The pK_a values of pilocarpic acid diesters have been reported to be 6.9 in water (Järvinen et al., 1991d).

TABLE 1

Apparent partition coefficients (P) and LC capacity factors (k') of bispilocarpic acid diesters at 22 °C

Compound	pK_a [a]	Log *P*	*k'*
Pilocarpine [d]	6.30	0.01	0.41
1	6.03	3.04 [b]	1.40
2	5.80	4.08 [b]	2.30
3	6.00	5.47 [c]	4.01
4	6.00	6.87 [c]	7.39
5	6.00	2.76 [b]	1.13
6	6.00	4.20 [b]	2.47
7	5.80	6.37 [c]	5.93

[a] pK_a values of the compounds were determined in a mixture of ethanol and water (50%:50%).
[b] Apparent partition coefficient between 1-octanol and aqueous buffer of pH 7.40.
[c] Log *P* value estimated from the equation: $\log P = 5.27 \cdot \log k' + 2.29$.
[d] From Järvinen et al. (1991d).

Lipophilicity of bispilocarpic acid diesters

Reversed-phase LC (RP-LC) capacity factors and the apparent partition coefficients for bispilocarpic acid diester fumarates as determined using the 1-octanol/pH 7.40 buffer system or estimated from reversed-phase LC capacity factors are listed in Table 1. Due to the high lipophilicity of the prodrug derivatives **3**, **4** and **7**, the log *P* values of these compounds were estimated using a Collander type equation, viz., $\log P_{oct} = a \log k' + b$ (Tayar et al., 1985), which has previously been reported to be $\log P_{oct} = 5.27 \cdot \log k' + 2.29$, $r = 0.98$ for pilocarpine and different types of pilocarpine prodrugs (Järvinen et al., 1991c). The results demonstrate that the prodrug derivatives have greater lipophilicity (log *P* = 2.76–7.03) in comparison with pilocarpine (log *P* = 0.01) and that the lipophilicity of the prodrugs can readily be varied over a wide range by changing the R moiety. Another means of modifying the lipophilicity of the bispilocarpic acid diester fumarates is by changing the spacer chain between pilocarpic acid as described earlier (Järvinen et al., 1991e).

Solubility of bispilocarpic acid diester fumarates

Due to their weakly basic character (the pK_a values 6.7), bispilocarpic acid diesters are more soluble in acidic than in basic solutions. Although

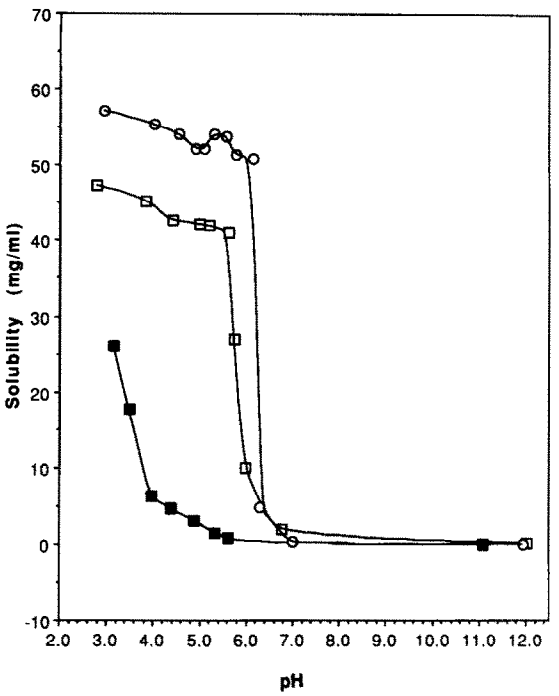


Fig. 2. Aqueous solubility-pH profiles for *O,O'*-dipropionyl(1,4-xylylene)bispilocarpate fumarate (○), *O*-propionyl pilocarpic acid benzyl ester fumarate (□), and *O*-benzoyl pilocarpic acid benzyl ester fumarate (■) at room temperature.

the solubility of the compounds can be increased by salt formation (e.g. with fumaric acid), it is obvious that increased lipophilicity is generally accompanied by impaired aqueous solubility. Consequently, it may be difficult to prepare aqueous eye-drop formulations of adequate drug concentration and non-irritant solution pH from lipophilic prodrugs.

The pH-solubility profiles for *O,O'*-dipropionyl (1,4-xylylene) bispilocarpate fumarate (log $P = 4.08$), *O*-propionyl pilocarpic acid benzyl ester fumarate (log $P = 3.24$), and *O*-benzoyl pilocarpic acid benzyl ester fumarate (log $P = 4.57$) are shown in Fig. 2. For example, the solubilities of these prodrug derivatives at pH 4.2 were 2.3 (equivalent with pilocarpine solution), 1.7 and 0.25%, respectively, and at pH 6.0, the corresponding values were 2.1, 0.4 and 0.04%. Based on these data, the bispilocarpic acid diester structure was clearly more water-soluble than those of the pilocarpic acid diesters with corresponding lipophilicities.

The improved water solubility of these new bispilocarpic acid diester fumarates allows the preparation of aqueous eye-drops containing sufficient drug concentrations using the pH range 5–6. It is very difficult to make eye drops from pilocarpic acid diesters due to their low water solubility. A low pH value for eye-drops may decrease the ocular bioavailability of the drug due to induced lacrimation and rapid precorneal loss of drug (Lee and Robinson, 1986). Lacrimal fluid has poor buffering capacity (Longwell et al., 1976; Carney and Hill, 1979) and if prodrug is instilled in acidic eye-drops, the precorneal pH returns very slowly to neutrality, which hinders the corneal permeability of the prodrug. Based on these reasons, the possibility of formulating eye-drops at pH values closer to neutral pH is an important advantage of bispilocarpic acid diesters.

Stability in aqueous solution

The kinetics of hydrolysis of the bispilocarpic acid diester fumarates **1–7** were studied at 37 °C in aqueous buffer solutions over the pH range 4.2–9.0. At constant temperature, the overall degradation of bispilocarpic acid diesters followed strict pseudo-first-order kinetics.

The effect of pH on the rates of hydrolysis is shown in Fig. 3 in which the logarithm of the observed pseudo-first-order rate constant (k_{obs}) is plotted vs pH. The shapes of the pH-rate profiles demonstrate that bispilocarpic acid diesters are subject to specific base-catalyzed hydrolysis and hence are most stable in acidic solutions. The lowest pH studied was pH 4.20, since this is close to the lowest acceptable pH value for eye-drop formulations. The half-lives of all the bispilocarpic acid diesters at different pH values are listed in Table 2.

The stability of *O,O'*-dipropionyl (1,4-xylylene) bispilocarpate fumarate was also studied at temperatures of 50, 60 and 70 °C in order to establish the stability of the compound under different storage conditions. In Fig. 4 the rate data obtained for *O,O'*-dipropionyl (1,4-xylylene) bispilocarpate fumarate at pH 4.20, 6.00, 7.40 and 9.00

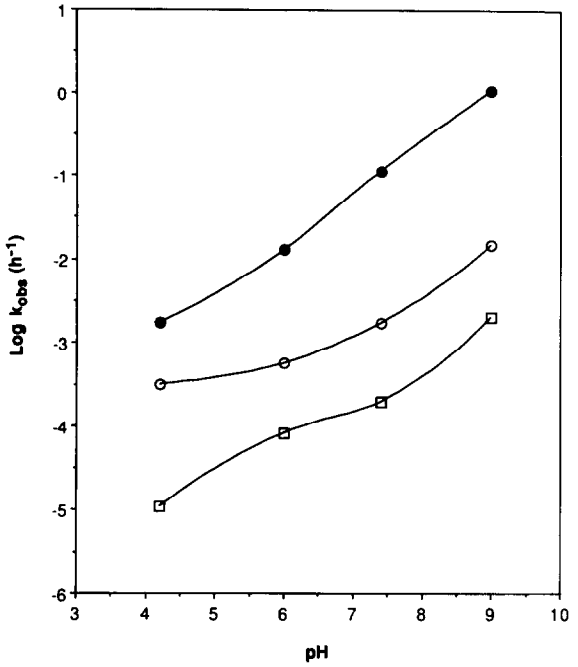


Fig. 3. The pH-rate profiles for the overall degradation of bispilocarpic acid diester **1** (●), **2** (○) and **6** (□) in aqueous solution at 37 °C.

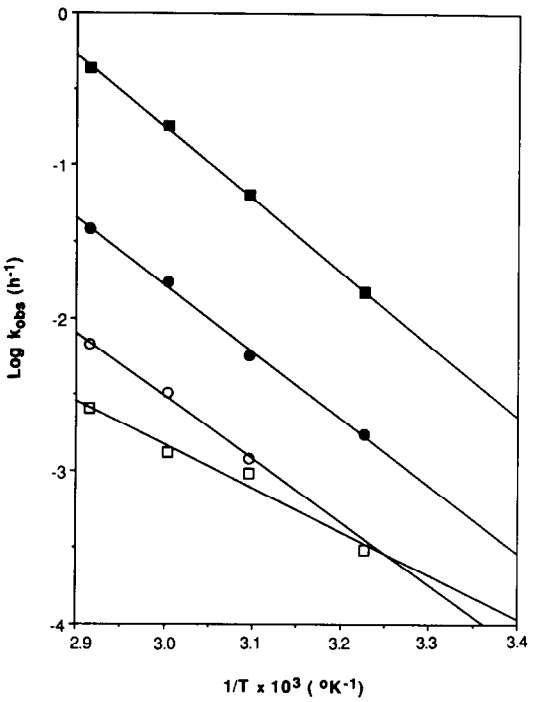


Fig. 4. Arrhenius plots of the rates of hydrolysis of *O,O'*-dipropionyl(1,4-xylylene)bispilocarpate fumarate in aqueous buffer solution of pH 9.00 (■), 7.40 (●), 6.00 (○) and 4.20 (□).

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 represents the gas constant and T is the absolute temperature.

On the basis of the linear relationship between log k_{obs} and $1/T$, it is possible to estimate the rate constant (k_{obs}) at different storage temperatures. The shelf-life ($t_{10\%}$, time required for loss of 10% bispilocarpic acid diester) of the compounds at constant temperature can be calculated from the equation $t_{10\%} = 0.104/k_{obs}$. The calculated shelf-lives for *O,O'*-dipropionyl (1,4-xylylene) bispilocarpate fumarate are listed in Table

TABLE 2

Half-lives ($t_{1/2}$) of bispilocarpic acid diesters at different pH at 37 °C

Ester	$t_{1/2}$ (h)			
	pH 4.20	pH 6.00	pH 7.40	pH 9.00
1	3964	1161	274	34
2	2203	1214	400	47
3	5993	1114	177	137
4	–	–	1010	7
5	405	54	6	0.7 (39 min)
6	63323	8445	3545	339
7	1969	1130	412	8

TABLE 3

Estimated values of $t_{10\%}$ for O,O'-propionyl(1,4-xylylene)bispilocarpate fumarate in aqueous solutions (μ = 0.5) of pH 4.20, 6.00, 7.40 and 9.00

pH	$t_{10\%}$ (days)		
	4 °C	15 °C	25 °C
4.20	157	64	30
6.00	469	62	24
7.40	124	31	10
9.00	19	4	1

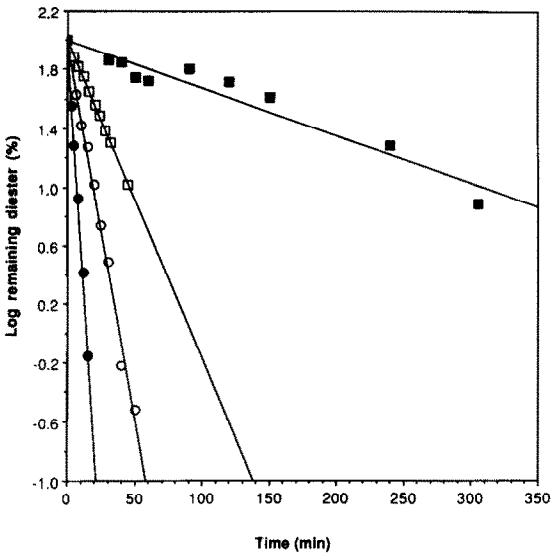


Fig. 5. Pseudo-first-order plots for the hydrolysis of bispilocarpic acid diester **5** (●), **2** (○), **6** (□) and **7** (■) in 80% human plasma solutions (pH 7.40) at 37 °C.

3. The results show that the shelf-life at room temperature is limited even in acidic solutions (30 days at pH 4.20 and 24 days at pH 6.00), however, at 4 °C it is possible to achieve acceptable shelf-life in aqueous solution (over 1 year). Furthermore, it should be noted that a number of other

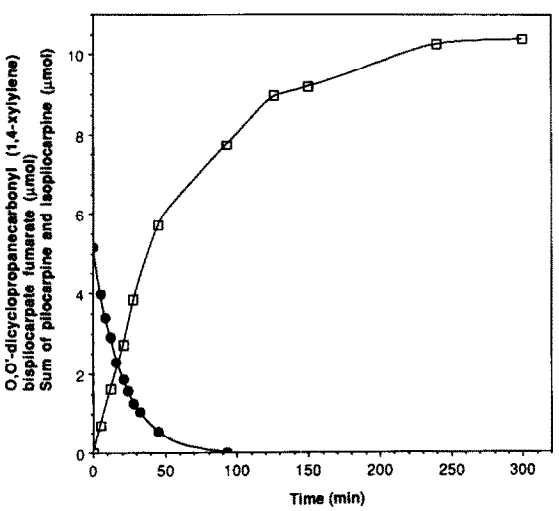


Fig. 6. Time course of disappearance of *O,O'*-dicyclopropanecarbonyl (1,4-xylylene)bispilocarpate (●) and appearance of pilocarpine (□) in 80% human plasma (pH 7.40) at 37 °C.

TABLE 4

Rate data for the hydrolysis of the bispilocarpic acid diesters in 80% human plasma solution (pH 7.40) at 37 °C

Ester	k_{obs} (min^{-1})	$t_{1/2}$ (min)	k_f (min^{-1})	$f_{50\%}$ (min)
1	0.07370	9	0.02142	32
2	0.11837	6	0.02948	24
3	0.06149	11	0.01958	35
4	0.05734	12	0.01658	42
5	0.31942	2	0.02441	28
6	0.04998	14	0.01500	46
7	0.00737	94	0.00750	91

k_{obs}, rate constant for degradation of diester; $t_{1/2}$, half-life for degradation of diester; k_f, rate constant for formation of pilocarpine; $f_{50\%}$, time by which 50% of total pilocarpine has been formed.

bispilocarpic acid diesters (e.g. compounds **1**, **3** and **6**) may be more stable in aqueous solution than *O,O'*-dipropionyl (1,4-xylylene) bispilocarpate fumarate.

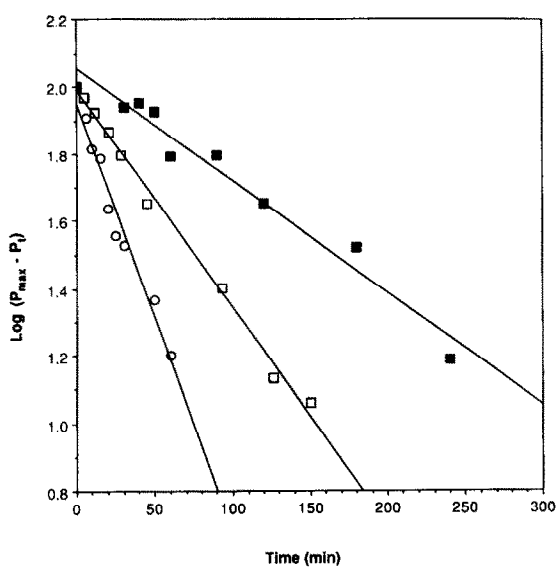


Fig. 7. First-order-plots for the formation of pilocarpine from bispilocarpic acid diester fumarate **2** (○), **6** (□) and **7** (■) in 80% human plasma (pH 7.40) at 37 °C. P_{max} and P_t represent the percentage amounts of the sum of pilocarpine and isopilocarpine formed at infinite time and at time t, respectively.

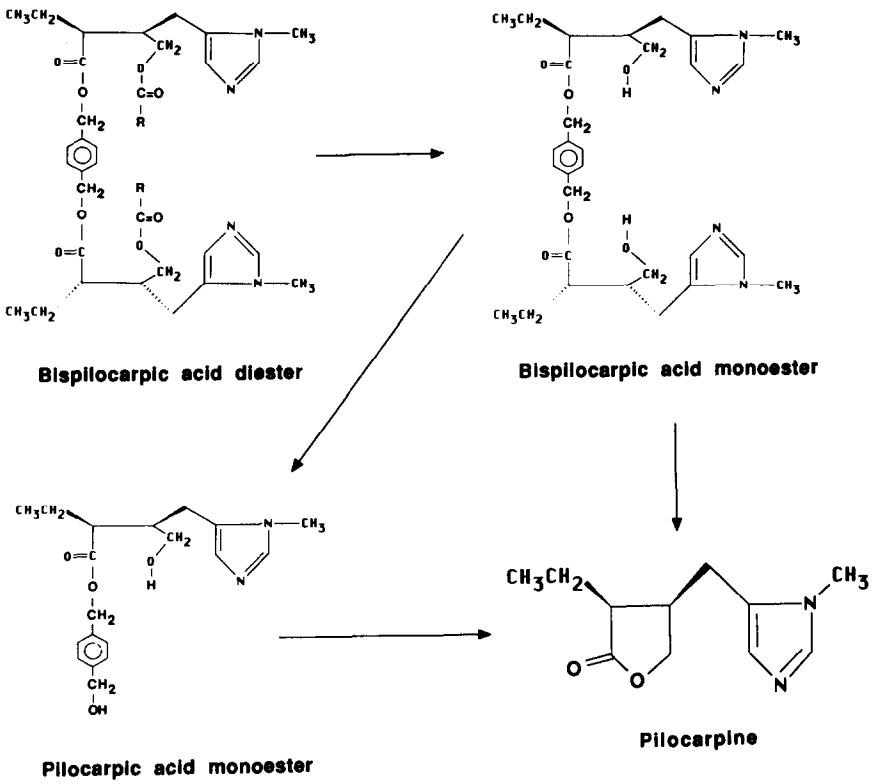


Fig. 8. Bioconversion of bispilocarpic acid diester; diester hydrolyzes enzymatically to monoester, which cyclizes nonenzymatically to pilocarpine.

Enzyme hydrolysis in human plasma

The susceptibility of bispilocarpic acid diesters to undergo enzymatic hydrolysis was investigated in human plasma diluted to 80% with phosphate buffer (pH 7.40 and $\mu = 0.5$).

For all compounds, good pseudo-first-order kinetics were observed over several half-lives. Typical pseudo-first-order plots are shown in Fig. 5 and the pseudo-first-order rate constants (k_{obs}) and half-lives ($t_{1/2}$) for bispilocarpic acid diesters are listed in Table 4. The results show that bispilocarpic acid diesters are very susceptible to enzyme-catalyzed hydrolysis and that enzymatic degradation of diester can be controlled by changing the R moiety ($t_{12} = 2$–94 min).

Pilocarpine formation has been studied by the RP-LC method described earlier (Järvinen et al., 1991d), which also allows one to determine isopilocarpine, pilocarpic acid and isopilocarpic acid. Fig. 6 shows the disappearance of *O,O'*-dicyclopropanecarbonyl (1,4-xylylene) bispilocarpate fumarate and the formation of pilocarpine (sum of pilocarpine and isopilocarpine) in 80% human plasma at 37 °C. The amount of isopilocarpine in this case was about 7%. It is clearly observed that 1 mol bispilocarpic acid diester releases 2 mol pilocarpine and that the conversion of diester to pilocarpine reaches completion during the course of the experiment (5.2 μmol ⇒ 10.4 μmol) (Fig. 6).

Typical pseudo-first-order plots of the formation of pilocarpine in human plasma are depicted in Fig. 7, where the amount of pilocarpine formed is plotted vs time. The rate constant of formation (k_f) of pilocarpine and time at which 50% of the total pilocarpine has been formed ($f_{50\%}$) are listed in Table 4. The $f_{50\%}$ values are dependent on both enzymatic hydrolysis in the R moiety and the subsequent chemical hydrolysis of bispilocarpic acid monoester (Järvinen et al., 1991e).

The concept of $f_{50\%}$ is a useful and informative way to describe to conversion of a prodrug derivative to its parent drug. Especially when several steps are involved in the formation of the parent drug, $f_{50\%}$ should be used instead of half-lives ($t_{1/2}$) of the prodrug. In the current study, however, $t_{1/2}$ and $f_{50\%}$ correlate with each other (Table 4), since bispilocarpic acid monoester (1,4-xylylene bispilocarpate) was present in all cases (Fig. 1).

Conclusions

Esterification of bispilocarpic acid monoester may be a useful approach to obtain water-soluble and stable double prodrugs in order to improve the corneal permeability of pilocarpine. All *O,O'*-(1,4-xylylene)bispilocarpic acid esters, i.e. bispilocarpic acid diesters, are more lipophilic than pilocarpine and the diester structure readily undergoes enzymatic hydrolysis to bispilocarpic acid monoesters, which cyclize spontaneously to pilocarpine. The results indicate that 1 mol bispilocarpic acid diester releases 2 mol pilocarpine, and that the rate of enzymatic hydrolysis can be varied by changing the R moiety. The main drawback of bispilocarpic acid monoesters, instability in aqueous solution, has been overcome through blocking the free hydroxyl group by esterification. Consequently, bispilocarpic acid diester fumarates are rather stable in aqueous solution at pH 4–6, which is an acceptable pH range in eye drops. Moreover, the bispilocarpic acid diester fumarates are clearly more soluble in water than the corresponding pilocarpic acid diester fumarates and hence solutions with therapeutically sufficient concentrations may be achieved within the pH range 5–6.

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