Pilocarpine Prodrugs I. Synthesis, Physicochemical Properties and Kinetics of Lactonization of Pilocarpic Acid Esters

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Abstract □ Various alkyl and aralkyl esters of pilocarpic acid were synthesized and evaluated as prodrug forms for pilocarpine with the purpose of improving the ocular bioavailability of pilocarpine through increased corneal membrane permeability. The esters were found to undergo a quantitative cyclization to pilocarpine in aqueous solution of pH 3.5–10, the rate of cyclization being a function of the polar and steric effects within the alcohol portion of the esters. The rates of lactonization increased proportionally with the hydroxide ion activity over the pH range studied which is in accord with a reaction mechanism involving intramolecular nucleophilic attack of alkoxide ion on the ester carbonyl moiety. At pH 7.4 and 37°C, half-times of lactonization ranging from 30 min (*p*-chlorobenzyl ester) to 1105 min (*n*-hexyl ester) were observed for the various esters. The esters are markedly more lipophilic than pilocarpine. The results suggested that the pilocarpic acid esters may be potentially useful prodrugs, especially when further derivatized to give in vitro stable pilocarpic acid diesters.

Although pilocarpine (**1**) is widely used as a topical miotic for controlling the elevated intraocular pressure associated with glaucoma the compound presents severe delivery problems. Its ocular bioavailability is very low, only 1–3% or less of an instilled pilocarpine dose gain access to the internal eye structures[1–4] (and references cited therein). This poor bioavailability has been predominantly attributed to rapid loss of the drug from the precorneal area via drainage, systemic conjunctival absorption and vasodilation due to the drug in conjunction with poor permeability of the drug across the corneal membrane.[3,5–8] The poor ability of pilocarpine to permeate the cornea may most likely be ascribed to the low lipophilicity of the drug. Because of the low bioavailability, a large ophthalmic dose is required in order to enable an effective amount of pilocarpine to reach the inner eye receptors and affect an intraocular pressure reduction. This in turn gives rise to concern about systemic toxicity since most of the applied drug is then available for systemic absorption from the conjunctival and nasolacrimal duct.[7–10] The systemic absorption of pilocarpine, amounting to about 50% of the instilled dose,[7,8] may lead to undesired side-effects, e.g. in those patients who display sensitivity to cholinergic agents.

Another delivery problem associated with pilocarpine is its short duration of action. Upon instillation into the eye, the duration of lowering of the intraocular pressure caused by pilocarpine lasts only for about 3 h. As a consequence thereof, the frequency of administration is at an inconvenient 3 to 6 times per day. Patient compliance with such treatment regimens is poor, and failure to comply is likely to contribute to inadequate pressure control and deterioration of vision.[11–13] Furthermore, the frequent administration of massive amounts of pilocarpine is associated with transient peaks of high drug concentration in the eye which in turn results in dose-related ocular side-effects such as myopia and miosis.[14,15]

These shortcomings of pilocarpine are dependent upon the physicochemical properties of the drug and may probably be overcome by the prodrug approach. Such an approach[16] involving transient modification of a drug molecule to give more lipophilic derivatives with increased corneal membrane permeability characteristics has in the past been applied to improve the ocular bioavailability of epinephrine,[17–21] nadolol[22] and various prostaglandins.[23] As shown for an epinephrine prodrug (dipivefrin) the more rapid penetration rate has led to use of a reduced dosage and the occurrence of fewer side-effects.[18]

To be successful a pilocarpine prodrug should exhibit a high lipophilicity in order to enable an efficient penetration through the corneal membrane, should possess sufficient aqueous solubility and stability for formulation as eyedrops, should be converted to the active parent drug within the cornea or once the corneal barrier has been passed and finally should lead to a controlled release and hence prolonged duration of action of pilocarpine.

The purpose of this study was to develop pilocarpine prodrugs with these desirable attributes. Aside from a quaternary prodrug type described in a preliminary report[24] no bioreversible derivatives of pilocarpine have hitherto been reported. We have recently shown[25] that esters of 4-hydroxybutyric acid may be potentially useful prodrug forms for the γ-lactone moiety which occurs in several drugs such as pilocarpine. Simple alkyl esters like methyl or ethyl were found to be more lipophilic than the parent γ-butyrolactone and to undergo a quantitative and apparent specific base-catalyzed lactonization in neutral and alkaline aqueous solution. Therefore, it was thought that this prodrug principle may be applied to pilocarpine. In this paper the syntheses of various esters of pilocarpic acid **2**–**11**[26] and the kinetics and mechanism of their cyclization to pilocarpine in aqueous solution are reported. Furthermore, the lipophilicity of pilocarpine and its precursors has been determined. In subsequent papers the ocular bioavailability characteristics of the pilocarpic acid esters will be reported as well as an evaluation of a further prodrug type, *O*-acylated pilocarpic acid esters. Part of this work has been published in a preliminary communication.[27]

Results and Discussion

Synthesis of Pilocarpic Acid Esters—The esters **2**–**11** were prepared by treating sodium pilocarpate (**13**) with the appropriate alkyl or aralkyl halide in dimethylformamide. The starting material, sodium pilocarpate, was obtained by alkaline hydrolysis of pilocarpine. In addition to the hydrolytic opening of the γ-lactone ring, pilocarpine undergoes epimerization at the α-carbon atom of the lactone ring in aqueous alkaline solution to yield isopilocarpine (**12**) which rapidly hydrolyzes to isopilocarpic acid (**14**)[28] (and references

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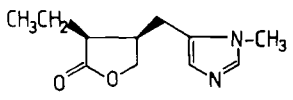

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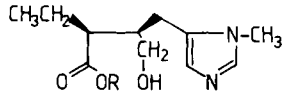
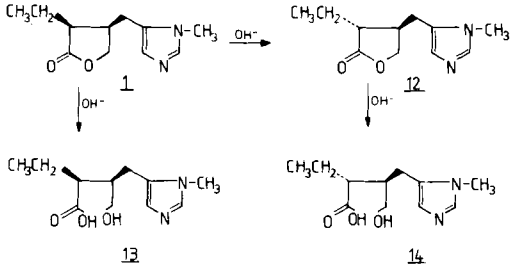
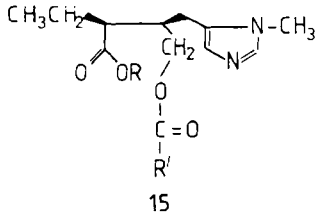


2 R = $-CH_2CH_3$
3 R = $-CH_2CH_2CH_2CH_3$
4 R = $-CH_2CH_2CH_2CH_2CH_2CH_3$
5 R = $-CH_2-C_6H_5$
6 R = $-CH_2-C_6H_4-Cl$
7 R = $-CH_2-C_6H_4-CH_3$
8 R = $-CH_2-C_6H_4-C(CH_3)_3$
9 R = $-CH_2CH_2-C_6H_5$
10 R = $-CH_2-C_6H_4-CH_3$
11 R = $-CH(CH_3)-C_6H_5$





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cited therein). Both the hydrolysis and epimerization of pilocarpine is subject to specific base catalysis[28] and therefore, the relative amounts of pilocarpic acid and isopilocarpic acid formed upon degradation of pilocarpine in alkaline solution does not depend on pH. However, the two simultaneously proceeding processes show a different dependency on the temperature, the relative importance of hydrolysis to epimerization being increased with decreased temperature ranging from 80% at 66°C to 88% at 18°C.[28] Therefore, in order to depress the formation of isopilocarpine and hence isopilocarpic acid the ring opening of pilocarpine was performed at 0–4°C. The product thus obtained contained about 10% of sodium isopilocarpate and at such purity, it proved highly satisfactory as a starting material for the synthesis of optically pure pilocarpic acid esters. It has also been found that potassium pilocarpate is equally suitable for the synthesis of the esters, it being less hygroscopic than the sodium salt. Interestingly, it was found that if pilocarpine was treated with methanolic sodium hydroxide, essentially pure isopilocarpic acid sodium salt was obtained. Under such conditions the epimerization of pilocarpine may apparently be the predominant reaction.

The modest yield (Table I) of pilocarpic acid ester obtained was found to be due to the fact that the imidazole moiety of the esters or the pilocarpic acid salt was also alkylated to some extent by the alkyl or aralkyl halide, yielding quaternary derivatives. During the isolation procedure these materials remain in the aqueous dimethylformamide phase.

Conversion of the Pilocarpic Acid Esters to Pilocarpine—Solutions of the various esters of pilocarpic acid (0.06–10 mg/mL) in buffer solutions (pH 3.5–10) were kept at 37°C and at various times aliquots were removed and analyzed by the HPLC assays for intact esters as well as for pilocarpine, isopilocarpine and the corresponding carpic acids as described (see *Experimental Section* and Fig. 1). The progressive appearance of pilocarpine and disappearance of ester were observed and quantitative measurements showed a virtual complete (97–100%) conversion of the esters to pilocarpine in the pH range investigated (Fig. 2). No peaks in the chromatograms corresponding to isopilocarpine or isopilocarpic acid appeared which shows that a possible epimerization of the esters with subsequent ring closure to give the biologically inactive isopilocarpine does not take place. It was shown separately that the specific HPLC assay[28] would allow a formation of isopilocarpine of 1% to be detected. Hydrolysis

Table I—Physical and Analytical Data of Various Pilocarpic Acid Esters

Compound	Yield, %	mp, °C	Solvent[a]	Formula
2	27	105–106	A	$C_{13}H_{22}N_2O_3$
3	30	89–90	A	$C_{15}H_{26}N_2O_3$
4	36	91–93	A	$C_{17}H_{30}N_2O_3$
5	35	84–85	A	$C_{18}H_{24}N_2O_3 \cdot 0.2H_2O$
6	43	106–107	B	$C_{18}H_{23}ClN_2O_3$
7	40	107–109	A	$C_{19}H_{26}N_2O_3$
8	18	68–69	C	$C_{22}H_{32}N_2O_3$
9	22	74–77	D	$C_{19}H_{26}N_2O_3$
10	35	57–60	E	$C_{19}H_{26}N_2O_3$
11	35	109–110	B	$C_{19}H_{26}N_2O_3$

[a] Solvent of recrystallization; A, chloroform-petroleum ether; B, ethyl acetate; C, ether-petroleum ether; D, ethyl acetate-ether-petroleum ether; E, ether. [b] Elemental analyses are ±0.4% of the theoretical value for the elements listed.

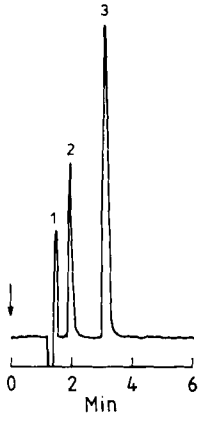


Figure 1—*Chromatogram of a partially degraded aqueous solution (0.05 M phosphate, pH 7.40) of the pilocarpic acid ester 7. Key:* (1) *solvent front;* (2) *pilocarpine;* (3) *7. The flow rate used was 1.6 mL/min.*

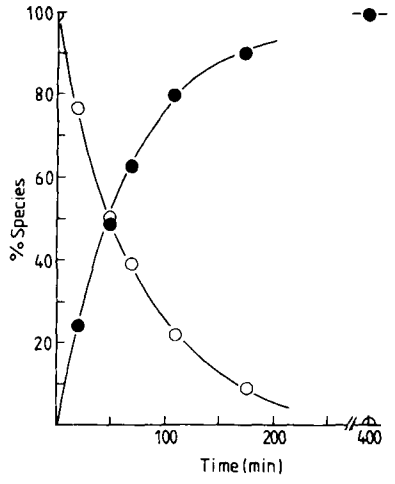


Figure 2—*Time-courses for the disappearance of pilocarpic acid benzyl ester **5** (○) and the appearance of pilocarpine (●) in a 0.05 M phosphate buffer solution (pH 7.40) of **5** at 37 °C.*

of the esters to yield the inactive pilocarpic acid is also a negligible reaction, the amounts formed of this product never exceeding 3%. Thus, it can be concluded that the pilocarpic acid esters undergo a quantitative ring closure in aqueous solution with the formation of pilocarpine.

When the reactions were followed for longer periods the pilocarpine formed was found to slowly disappear with the formation of pilocarpic and isopilocarpic acid. In a separate experiment similar to those reported earlier,[28] the specific base catalytic rate constant (k_{OH}) for the overall degradation (hydrolysis and epimerization) of pilocarpine at 37°C was determined to be 24.6 M^{-1} min^{-1}. By comparing this value with the k_{OH} values for the various pilocarpic acid esters (Table II) it can be estimated that pilocarpine is 42–1,560 times more stable than the pilocarpic acid esters in neutral and alkaline solutions.

Kinetics and Mechanism of the Lactonization—At constant pH and temperature strict first-order kinetics was observed for the lactonization of the pilocarpic acid esters. Some typical first-order plots are shown in Fig. 3. As described above, different experimental or analytical methods were used to follow the reactions. In several cases the rate of ring closure of the esters at a given pH was determined using more than one of the methods and, as seen from the examples given in Table III, the values of the observed pseudo-first-order rate constants (k_{obs}) derived were in favorable agreement.

Table II—Second-Order Rate Constants (k_{OH}) for the Apparent Specific Base-Catalyzed Lactonization of the Pilocarpic Acid Esters 2–11 in Aqueous Solution and Half-Times of Lactonization ($t_{1/2}$) at pH 7.40[a]

Pilocarpic Acid Ester	σ^{*}[b]	k_{OH}, M^{-1} min^{-1}	$t_{1/2}$, min
2	0.00	2.25×10^3	510
3	−0.12	1.40×10^3	820
4	−0.23	1.04×10^3	1105
5	0.75	2.30×10^4	50
6	0.87	3.83×10^4	30
7	0.59	1.49×10^4	77
8	0.52	1.32×10^4	87
9	0.27	5.07×10^3	227
10	0.62	8.28×10^3	139
11	—	2.42×10^3	475

[a] μ = 0.5; 37°C. [b] The Taft polar substituent constant σ^{*} refers to R minus $-CH_2$ in the formula **2–10**.

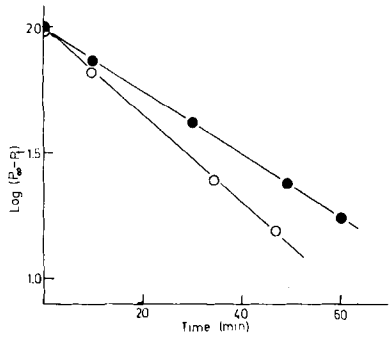


Figure 3—*First-order plots for the cyclization of the benzyl ester (**5**) (●) and p-chlorobenzyl ester (**6**) (○) of pilocarpic acid to pilocarpine in 0.05 M phosphate buffer solution of pH 7.75 (μ = 0.5) at 37 °C. P_∞ and P_t represent the percentage amounts of pilocarpine formed as determined by HPLC at infinity and at time t, respectively.*

Table III—Pseudo-First-Order Rate Constants (k_{obs}, min^{-1}) for the Lactonization of Various Pilocarpic Acid Esters[a] as Determined by Different Methods

Compound	Buffer	Method[b]		
		A	B	C
3	0.05 M Borate (pH 9.62)	0.14	0.15	0.14
	0.05 M Borate (pH 9.20)	0.055	0.052	0.057
5	0.05 M Phosphate (pH 7.40)	—	0.0140	0.0137
6	0.05 M Phosphate (pH 7.40)	0.0228	0.0230	0.0235

[a] μ = 0.5; 37°C; [b] (A) Direct UV spectrophotometry; (B) HPLC of residual pilocarpic acid ester; (C) HPLC of pilocarpine formed.

The rates of lactonization were for all esters found to be independent of acetate, phosphate and borate buffer concentration from 0.02 to 0.10 M at constant ionic strength and thus, no significant general acid-base catalysis appears to be involved. The effect of the ionic strength on the reaction rate was examined with the *p*-chlorobenzyl ester **6**. In neutral and alkaline buffer solutions no effect of ionic strength (0.05–0.6) was observed. At pH 4–6.5 k_{obs} decreased slightly with increasing ionic strength (adjusted with potassium chloride), e.g. k_{obs} was 30% smaller at an ionic strength of 0.56 at pH 6.1 than at an ionic strength of 0.06.

The influence of pH on the lactonization rate is shown in Fig. 4, where the logarithm of the k_{obs} values are plotted against pH. The pH-rate profiles for all the esters are

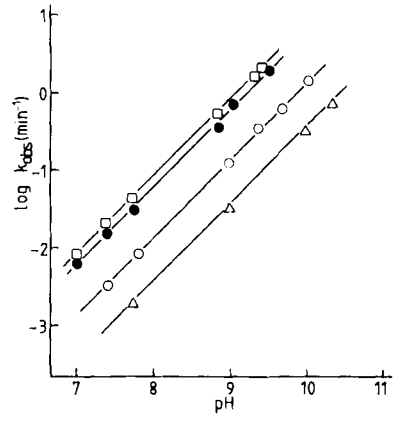


Figure 4—*The pH–rate profiles for the cyclization of the pilocarpic acid esters **3** (△), **5** (●), **6** (□), and **9** (○) to pilocarpine in aqueous solutions at 37 °C (μ = 0.5).*

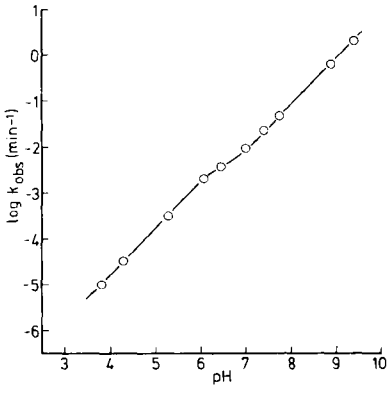


Figure 5—*The pH–rate profile for the cyclization of pilocarpic acid p-chlorobenzyl ester (**6**) to pilocarpine in aqueous solution at 37°C (μ = 0.5).*

straight lines of unity slopes at pH>7, suggesting that the reactions are first-order dependent on hydroxide ions.

The rate expression accounting for the lactonization kinetics of the esters at about pH>7 may thus be formulated as:

$$k_{obs} = k_{OH}a_{OH} \quad (2)$$

where k_{OH} is an apparent hydroxide ion catalytic rate constant for the lactonization and a_{OH} refers to the hydroxide ion activity. This was calculated from the measured pH (at 37°C) according to the following equation:[29]

$$\log a_{OH} = pH - 13.62 \quad (3)$$

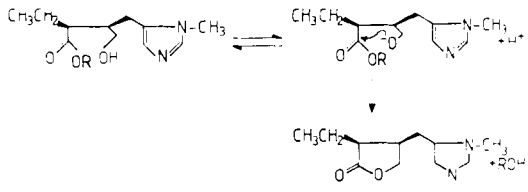
The values of k_{OH} for the various esters studied are listed in Table II together with half-times for the reactions at pH 7.4 and 37°C.

The study of the lactonization kinetics for the *p*-chlorobenzyl ester derivative **6** was extended to include pH values down to pH 3.5. As seen from the pH-rate profile in Fig. 5 the protonated form of the molecule undergoes apparent specific base-catalyzed lactonization at a rate that is slightly greater (~1.85-fold) than that of the free base form. At 37°C and μ = 0.5 the pK_a value of the compound was determined to be 6.78 ± 0.03. The rate equation accounting for the pH dependencies of k_{obs} within the pH-range 3.5–10 may be formulated as:

$$k_{obs} = k_{OH}a_{OH}f_E + k'_{OH}a_{OH}f_{EH^+} \quad (4)$$

where f_E and f_{EH^+} refer to the fractions of the ester occurring on the free base form and as protonated species, respectively (e.g.; $f_E = K_a/(a_H + K_a)$). The values determined for the apparent hydroxide ion catalytic rate constants for the reaction of these different forms are: $k_{OH} = 3.8 \times 10^4$ M^{-1} min^{-1} and $k'_{OH} = 7.0 \times 10^4$ M^{-1} min^{-1} (at 37°C and μ = 0.5). It is also to be expected that the lactonization is specific acid-catalyzed but the reaction was not studied below pH 3.5.

Concerning the mechanism of lactonization of the pilocarpic acid esters the observed occurrence of the apparent hydroxide ion catalysis and insignificant general base catalysis by buffers suggest that the reaction involves pre-equilibrium ionization of the hydroxyl group and intramolecular nucleophilic attack of alkoxide ion on the ester carbonyl moiety as depicted below. This mechanism, which is similar to that previously suggested for the cyclization of alkyl 4-hydroxybutyrates to γ-butyrolactone,[25] is consistent with the observed linearity between k_{obs} and a_{OH} up to a pH of 10.5. The pK_a value of the hydroxyl group in the pilocarpic acid esters might be expected to be about the same as that of ethanol, 15.9,[30] and therefore, in the investigated pH range the concentration of alkoxide ion is directly proportional to the hydroxide ion concentration.



Interestingly, it was found that esters of isopilocarpic acid cyclize much more readily than the corresponding esters of pilocarpic acid. Thus, whereas the half-life of ester **9** at pH 7.4 and 37°C is 227 min the corresponding phenethyl ester of isopilocarpic acid showed a half-life of 13 min at the same conditions. This greater propensity of isopilocarpic acid esters to lactonize parallels the behavior of the isomeric carpic acids in that isopilocarpic acid has been reported[31] to undergo acid-catalyzed cyclization about 30 times as rapid as pilocarpic acid.

In contrast to the ester derivatives simple amides of pilocarpic acid do not undergo a ready cyclization to pilocarpine in aqueous solution. Thus, the benzylamide of pilocarpic acid, prepared by aminolysis of pilocarpine as described by Koda et al.,[32] was found to remain intact after standing in a borate buffer of pH 9.2 for 48 h at 37°C. This difference in reactivity between esters and amides parallels the behavior in other nucleophilic reactions, such as alkaline hydrolysis.

Structural Effects on Lactonization Rate—As appears from the rate data obtained (Table II), the various esters differ greatly in their rates of cyclization to pilocarpine. The reactivity of the pilocarpic acid esters studied is primarily determined by the polar effects exhibited by the alcohol portions of the esters. Except for the 2-methylbenzyl **10** and α-methylbenzyl **11** esters the steric requirements in the alcohol portions of the esters can be considered to be constant and the variation of the rates of lactonization of these ester derivatives can be totally accounted for in terms of the different stability of the leaving alcohol group as expressed by the pK_a values of the alcohols. As seen in Fig. 6 an excellent linear correlation exists between log k_{OH} and the Taft polar substituent parameter σ^*, the latter referring to R in RCH_2OH for the alcohols. Taft[33] and others[34] have previously reported that the pK_a of alcohols is linearly related to

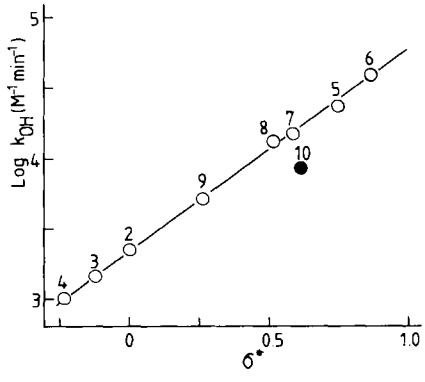


Figure 6—*Plot of log k_{OH} against the Taft polar substituent parameter σ^* for various pilocarpic acid esters (**2–10**). The σ^* values used refer to the substituent connected to the methylene group in the alcohol portion of the esters, e.g., phenyl in the benzyl ester. The values were taken from ref. 34.*

σ^* used in this manner. The regression equation between log k_{OH} and σ^* for these pilocarpic acid esters is given by:

$$\log k_{OH} = 1.44\ \sigma^* + 3.33\ (n=8;\ r=0.998) \quad (5)$$

The corresponding equation between the half-time of pilocarpine formation from these esters at pH 7.40 and 37°C and σ^*, as calculated on the basis of Eq. 5, is given by:

$$\log t_{1/2} = -1.44\ \sigma^* + 2.73\ (t_{1/2} \text{ in min}) \quad (6)$$

This linear free energy relationship established for the lactonization of the pilocarpic acid esters may be highly useful for the prediction of the reactivity of an ester derivative solely on basis of the σ^* value of the appropriate alcohol substituent. A large number of σ^* values are available and have recently been compiled by Perrin et al.[34] As a matter of fact, the preparation and subsequent testing of several of the ester derivatives included in this study were based on such prediction of reactivity made on the basis of the few initially prepared compounds. Robinson and Matheson[35] have previously described a similar linear free energy relationship between alcohol pK_a and hydrolysis rate of various esters.

The 2-methylbenzyl ester **10** shows a lower reactivity than expected on the basis of pure polar effects (cf. Fig. 6). This can most likely be ascribed to steric reasons. Similarly, the sterically hindered α-methylbenzyl ester **11** is ~10-fold less reactive than the benzyl ester **5**.

For the aralkyl esters of pilocarpic acid the rate data could, as expected, also be correlated with the Hammett substituent parameter σ. Figure 7 shows a Hammett plot for these derivatives, the slope being 1.06.

Although the relative reactivity of the pilocarpic acid esters qualitatively parallels that of esters in general it is worthy to note the great dependency of the lactonization rate on the polar effect by the alcohol portion. Thus, whereas the pilocarpic acid benzyl ester is about 16-fold more reactive than the corresponding butyl ester with respect to undergo ring closure, the difference in reactivity of benzyl and butyl esters in intermolecular nucleophilic reactions such as alkaline hydrolysis usually only corresponds to a factor of about 3.[36]

On basis of the data given it is evident that by appropriate variation of the alcohol portion of the esters it is possible to predict and vary the rate of ring closure and hence to control and modify the rate of pilocarpine generation.

Influence of Enzymes on Lactonization—For the evaluation of the pilocarpic acid esters as potential prodrugs of pilocarpine it is important to ascertain whether enzymes such as those present in ocular tissues would be able to hydrolyze the ester linkage at such a rate that the ring closure reaction to yield the parent drug would be seriously depressed. This possibility was investigated for the benzyl **5**, 4-methylbenzyl **7** and 2-phenethyl **9** ester derivatives. Human plasma was used as a model of ocular tissue enzymes based on previous studies.[17,37,38] When incubated in 75% human plasma (pH 7.4) at 37°C the three ester derivatives were found to degrade according to first-order kinetics, the half-lives being 49 min **5**, 83 min **7** and 220 min **9**. These values are very similar to those observed in buffer solutions of pH 7.4 without plasma (Table II). HPLC analysis of the reaction solutions revealed the formation of pilocarpine in stoichiometric amounts. These results show, accordingly, that under physiological conditions of pH and temperature the spontaneous cyclization of these pilocarpic acid esters predominates entirely over a possible enzymatic hydrolysis of the ester groups to give pilocarpic acid which does not cyclize to pilocarpine in neutral and basic solution.

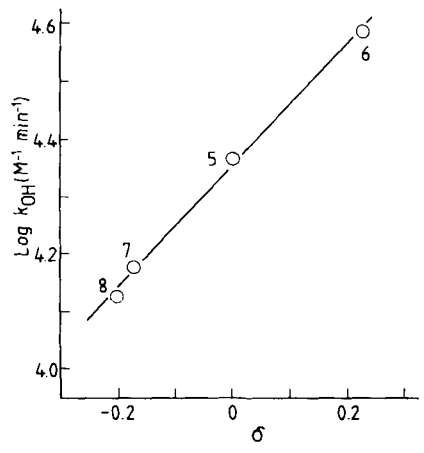


Figure 7—*Hammett plot of the rate data for the pilocarpic acid esters* ***5–8****.*

The Lipophilicity of Pilocarpine and Pilocarpic Acid Esters—The partition or distribution coefficients (P) for pilocarpic acid esters and pilocarpine were measured using the widely used octanol-water system. Since the primary interest was to know the lipophilicity of the esters and pilocarpine at physiological pH a phosphate buffer of pH 7.4 was used as the aqueous phase. At this pH the esters are mostly, but not exclusively, on the free base form. The pK_a values determined for the esters **2–11** at 22°C were in the range 6.94–6.98. The values found for log P are listed in Table IV. Taking the degree of ionization of the esters at pH 7.4 into account the log P values for the free base forms can be calculated from these values by adding 0.15. For pilocarpine a log P value of −0.15 was found (octanol-buffer pH 7.4). The pK_a of pilocarpine was determined to be 7.07 at 22°C and log P for the compound as free base can be calculated to be 0.02. This value agrees well with the log P value (0.03) determined for pilocarpine using a borate buffer of pH 9.2 as the aqueous phase. The results obtained show that the pilocarpic acid esters are much more lipophilic than the parent lactone which is in accord with the predictions made previously with the γ-butyrolactone—4-hydroxybutyric acid ester model system.[25] It can readily be seen that by varying the alcohol portion of the pilocarpic acid esters it is feasible to obtain prodrug derivatives with varying lipophilicity. The log P values found are mutually in good agreement with values calculated on basis of π substituent values.[39] For example, on going from benzyl ester **5** to the 4-methylbenzyl ester **7** or the 4-*tert*-butylbenzyl ester **8**, log P increases with 0.49 and 1.70, respectively, which are equal to π for a methylene group (0.50) and a *tert*-butyl group (1.70). Thus, it is possible to predict the partition coefficients of other pilocarpic acid esters on the basis of the additive substituent

Table IV—Partition Coefficients (P) and Chromatographic Capacity Factors (*k'*) of Pilocarpine and Pilocarpic Acid Esters

Compound	log P[a]	*k'*
Pilocarpine	−0.15	0.22
2	0.58	0.33
3	1.58	0.63
4	2.56	0.96
5	1.82	0.65
6	2.54	0.96
7	2.31	0.91
8	3.52	1.96
9	2.16	0.76
10	2.27	0.87
11	2.08	0.78

[a] Partition coefficient between octanol and 0.05 M phosphate buffer solution of pH 7.40 at 22°C.

principle. The lipophilicity of the derivatives was also evaluated by means of reversed-phase HPLC.[40,41] In this method the capacity factor (k') of a solute is taken as a measure for the relative lipophilicity:

$$k' = (t_r - t_0)/t_0 \quad (7)$$

where t_r is the retention time of the solute and t_0 is the elution time of the solvent. With methanol–0.02 M potassium dihydrogen phosphate (pH ~4.5) (1:3 v/v) as mobile phase the ester derivatives 2–11 showed the k' values given in Table IV. These data also demonstrate the higher lipophilicity of the derivatives in comparison with pilocarpine. As has been observed for other compounds[41] (and references cited therein) a linear relationship existed between log k' and log P for the compounds including pilocarpine.

Predictions of Shelf Lives—Due to their weak basic character the pilocarpic acid esters are readily soluble in aqueous solutions of pH 4–6 and may, like pilocarpine, form salts with various acids. For example, the fumarate salt of the benzyl ester 5 was readily prepared. At such pH values the stability is much increased as compared with that at neutral and basic pH values (Fig. 5). In order to predict the stability of the compounds under conditions similar to those encountered for storage of e.g. aqueous eye-drop formulations the decomposition kinetics of the *p*-chlorobenzyl ester derivative 6 was examined at pH 6.1 and 4.0 over the temperature range 25–70°C and 37–70°C, respectively. At these pH values the predominant reaction is apparent hydroxide ion-catalyzed lactonization of the protonated form of the pilocarpic acid ester. The rate data obtained are plotted according to the Arrhenius equation in Fig. 8. From these plots an activation energy of 13.65 kcal/mol was obtained at both pH values. On the basis of this value and the influence of pH upon k_{obs} as described above it is possible to estimate the shelf life of aqueous solutions of the *p*-chlorobenzyl pilocarpic acid ester at various pH values and temperatures. The calculated shelf lives in terms of $t_{10\%}$, i.e. times for an extent of degradation (i.e. lactonization) of 10%, are listed in Table V. The results show that the shelf life at room temperature is limited and that it may be difficult to prepare ready-to-use solutions with a not too low pH (pH > 4) and possessing an acceptable shelf life. The stability is, however, sufficient to make it possible to make preparations intended to be reconstituted before use. It should be noted that the shelf lives for the other pilocarpic acid esters studied would be somewhat higher than those calculated for the *p*-chlorobenzyl ester since this compound is the most reactive one, cf. Table II.

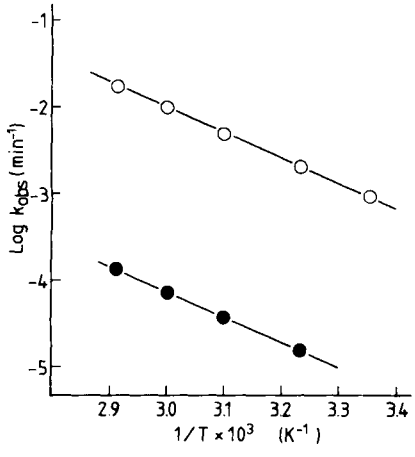


Figure 8—*Arrhenius plot of the rate of cyclization of pilocarpic acid* p*-chlorobenzyl ester (6) to pilocarpine in aqueous buffer solutions of pH 4.0* (●) *and 6.1* (○).

Table V—Predicted Values of $t_{10\%}$ for Aqueous Solutions of Compound 6

pH	$t_{10\%}$, days		
	25°C	15°C	4°C
3.5	318	1.9 years	4.8 years
4.0	103	244	1.5 years
4.5	31.8	69	174
5.0	10.3	22.4	56
5.5	3.2	6.9	17.4

The ocular bioavailability characteristics of the pilocarpic acid esters 2–11 will be reported in the subsequent paper. Another paper will describe how the problem of obtaining a reasonable product stability can be totally overcome by blocking the free hydroxyl group in the pilocarpic acid esters. By esterification of this group compounds (diesters or pro-prodrugs; 15)[26,27] are obtained from which pilocarpine is released in vivo through a sequential process involving enzymatic hydrolysis of the *O*-acyl bond followed by the spontaneous lactonization of the pilocarpic acid monoester as described herein.

Experimental Section

Melting points were determined in capillary tubes and are uncorrected. ^{1}H NMR spectra were run on a Varian 360L instrument using tetramethylsilane as an internal standard. HPLC was carried out with a Spectra-Physics Model 3500 B equipped with a variable-wavelength detector and a 10-μL loop injection valve. A column, 250 × 4 mm, packed with LiChrosorb RP-8, particle size 7 μm, (E. Merck, Darmstadt) was used. Measurements of pH were done at the temperature of study using a Radiometer Type PHM 26 instrument. A Zeiss PMQ II spectrophotometer equipped with a thermostated cell compartment was used for some kinetic measurements. Optical rotations were measured with a Perkin-Elmer 141 polarimeter.

Sodium Pilocarpate (13)—To a solution of pilocarpine hydrochloride (19.6 g, 80 mmol) in 20 mL of water, kept in an ice-water bath, was added 90 mL of ice-cold 2 M NaOH in four portions. The solution was allowed to stand at 0–4°C for 1 h. After neutralizing the excess of NaOH by adding ~20 mL of 1 M HCl (to pH~9), the solution was evaporated under reduced pressure at 40°C. After drying over P_2O_5 under reduced pressure, the resulting residue was slurried in 300 mL of ethanol and stirred for about 10 min at 60°C. After cooling to 4°C, the insoluble sodium chloride was removed by filtration. The filtrate was evaporated under reduced pressure and the residue dried over P_2O_5 at room temperature, to give 20.1 g (95% yield) of sodium pilocarpate as a white semicrystalline, hygroscopic material. HPLC analysis of the compound performed as previously described[28] revealed the presence of 90% of the title compound and 10% of sodium isopilocarpate (14). An analytical sample containing only 2% of the latter was obtained by recrystallization from 2-propanol:acetonitrile:ether, mp. 67–69°C.

Anal.—Calc. for $C_{11}H_{17}N_2O_3Na \cdot 3/4\ H_2O$: C, 50.47; H, 7.12; N, 10.70. Found: C, 50.46; H, 7.11; N, 10.73.

Pilocarpic Acid Esters (2–11)—To a solution of 4 mmol of sodium pilocarpate (containing 10% of sodium isopilocarpate) in 60 mL of dimethylformamide was added dropwise the appropriate alkyl or aralkyl halide (chloride or bromide) (4 mmol) over a period of about 1 h. The solution was stirred at room temperature overnight, poured into water (75 mL) and then extracted with EtOAc (2 × 75 mL). The combined extracts were washed with H_2O (50 mL), 2% $NaHCO_3$ (50 mL) and H_2O (50 mL). After drying over $MgSO_4$, the EtOAc was removed under reduced pressure to give 2–11. Recrystallization was performed as indicated in Table 1. Physical and analytical data for the compounds are also given in Table 1. The ^{1}H NMR spectra of the esters were consistent with the structures.

Fumarate Salt of Pilocarpic Acid Benzyl ester (5)—To a solution of 5 (158 mg, 0.5 mmol) in EtOAc (3 mL) was added a solution of fumaric acid (60 mg, 0.5 mmol) in a mixture (1:1) of ethanol and ether. The solution was evaporated under reduced pressure and the

residue was recrystallized twice from 2-propanol-petroleum ether to give 5 as a colorless monohydrate, mp. 77–78°C.

Anal.—Calc. for $C_{22}H_{28}N_2O_7 \cdot 1\ H_2O$: C, 58.66; H, 6.71; N, 6.23. Found: C, 59.07; H, 6.73; N, 6.54.

Kinetic Studies—Most kinetic measurements were carried out in aqueous buffer solutions at 37.0 ± 0.2°C. The buffers used were acetate (pH 3.5–5.5), phosphate (pH 6–7.8) and borate (pH 8.75–10.35) and, except in those experiments in which buffer effects were specifically investigated, the total concentration of the buffers was 0.05 M. A constant ionic strength (μ) of 0.5 was maintained for each buffer solution by adding a calculated amount of potassium chloride. The rates of lactonization of the pilocarpic acid esters were followed by either HPLC or UV spectrophotometry.

Direct UV Spectrophotometry—In this method reactions were performed in 2.5-mL aliquot portions of borate buffer solutions (pH 9.75–10.35) in a thermostated quartz cuvette and were initiated by adding 25 μL ethanolic stock solutions of the esters to give a final concentration of about 10^{-4} M. The progress of the reaction was followed spectrophotometrically by recording the decrease in absorbance at 225 nm as a function of time. Pseudo-first-order rate constants were determined from the slopes of linear plots of log ($A_t - A_\infty$) against time, where A_t and A_∞ are the absorbance readings at time t and at infinity (i.e., when no further changes in absorbance occurred), respectively.

High-Performance Liquid Chromatography (HPLC)—The reactions were followed using two different HPLC methods. One of these was the method recently developed by Bundgaard and Hansen[28] which allows the simultaneous separation and determination of pilocarpine and its degradation products [isopilocarpine (**12**), pilocarpic acid (**13**) and isopilocarpic acid (**14**)]. Under the chromatographic conditions used, given in the reference cited, pilocarpine and its degradation products were eluted within 15 min whereas the pilocarpic acid esters were retained on the column. Therefore, when using this method the progress of the reaction was followed by determining the appearance of pilocarpine as a function of time. Quantitation of the latter as well as of its degradation products was done from measurement of the peak areas in relation to those of standards chromatographed under the same conditions as previously described.[28] In the kinetic runs, an accurately weighed sample of pilocarpic acid ester (about 10 mg) was dissolved in 400 μL of ethanol, and 10.00 mL of an aqueous buffer solution pre-equilibrated at 37°C was added. The solution was kept at 37°C in a water-bath and at appropriate intervals aliquots were removed and mixed with a pre-determined amount of 2 M HCl to give a pH of ~4. The mixtures (10 μL) were chromatographed within 0.5 h. Pseudo-first-order rate constants for the lactonization were determined from the slopes of linear plots of log ($C_\infty - C_t$) against time, where C_∞ and C_t are the pilocarpine concentrations at infinity (i.e. after 8–10 half-lives) and at time t, respectively.

In the other HPLC method used the progress of the reaction was followed by determining the disappearance of pilocarpic acid ester as a function of time. The reversed-phase column (LiChrosorb RP-8) was eluted at ambient temperature with a mobile phase consisting of CH_3OH and 0.02 M KH_2PO_4 (3:1 v/v) at a rate of 1.2 or 1.6 mL/min. The column effluent was monitored at 215 nm. Under these conditions the pilocarpic acid esters were well separated from pilocarpine and could readily be determined (Fig. 1). Pilocarpic acid and isopilocarpic acid eluted with the solvent front. In this system pilocarpine could not be distinguished from isopilocarpine but such differentiation was possible with the other HPLC method described above. Quantitation of the pilocarpic acid esters was done from measurement of the peak heights in relation to those of standards chromatographed under the same conditions. In the kinetic runs, buffer solutions containing the pilocarpic acid esters at initial concentrations of ~0.06 mg/mL were kept at constant temperature, and at various times 10 μL aliquot portions were chromatographed. Pseudo-first-order rate constants for the lactonization were determined from the slopes of linear plots of the logarithm of residual pilocarpic acid ester against time.

The conversion kinetics was for some compounds also studied in a 0.01 M phosphate buffer pH 7.4 containing 75% human plasma. The initial pilocarpic acid ester concentration was 0.06 mg/mL and at various times 200 μL aliquots of the plasma reaction solution were withdrawn and added to 1000 μL of EtOH. After mixing and centrifugation 10 μL of the clear supernatant were chromatographed as described above.

Measurement of Partition Coefficients—The apparent partition coefficients of pilocarpine and the various pilocarpic acid esters were determined in an octanol-water system at 20–23°C. The aqueous phase was a 0.05 M phosphate buffer solution of pH 7.40; for pilocarpine, a 0.05 M borate buffer solution of pH 9.20 was also used. The buffer solutions and octanol were mutually saturated at 20–23°C before use. The compounds were dissolved in the aqueous buffer phase and the octanol-water mixtures were shaken for 5 min to reach a distribution equilibrium. The volumes of each phase were chosen so that the solute concentration in the aqueous phase, before and after distribution could readily be measured using the aforementioned HPLC methods. Centrifugation for 2 min was used to separate the two phases. Immediately following centrifugation an appropriate amount of 5 M HCl was added to the aqueous phase to give a pH of about 5, thereby quenching the lactonization. The HPLC analysis was then performed within 10 min. During the entire procedure less than 3% pilocarpine was formed from the esters as determined by HPLC. The partition coefficients (P) were calculated from eq. 1:

$$P = \frac{C_i - C_w}{C_w} \times \frac{V_w}{V_o} \qquad (1)$$

where C_i and C_w represent the solute concentrations in the aqueous buffer phase before and after distribution, respectively; V_w represents the volume of the aqueous phase and V_o the volume of the octanol phase. For each compound, determinations were carried out in triplicate, and the P values thereby obtained were reproducible to within ±4%.

Measurement of Ionization Constants—The ionization constants of the pilocarpic acid esters were determined by potentiometric titration of 2×10^{-3} M aqueous solutions containing 4% ethanol of the esters with 0.1 M hydrochloric acid at 22°C. Similarly, the pK_a of pilocarpine was determined by titrating its hydrochloride salt with 0.1 M NaOH.

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