New Water-Soluble Pilocarpine Derivatives with Enhanced and Sustained Muscarinic Activity

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Received April 4, 1991; accepted October 10, 1991

The synthesis of an homologous series of new water-soluble derivatives of pilocarpine is described. The new compounds, referred to as soft quaternary salts, are water soluble by virtue of a cationic ammonium head and their lipophilicity can be modulated by manipulating the size and the nature of the substituent in the inactive portion of the molecule. The miotic activity of the compounds was evaluated after administration to normotensive New Zealand White rabbits. Changes in pupil size indicated a substantial cholinergic effect on the iridal sphincter musculature. The best candidate, compound **20**, which has a 16-carbon side chain, was evaluated for reduction of the intraocular pressure in genetically glaucomatous Beagles. Compound *20* is superior to pilocarpine in both tests, with a potency 10 to 20 times that of the parent compound and a longer duration of action. It is suggested that the new compounds are prodrug forms of pilocarpine which greatly enhance the corneal bioavailability of the parent compound.

KEY WORDS: pilocarpine; prodrugs; soft quaternary salts; intraocular pressure; miotic activity; rabbits; glaucomatous beagle.

INTRODUCTION

Open-angle glaucoma is still a relatively poorly understood condition and consequently there is currently no drug capable of addressing the primary causes of the disease. Several FDA-approved drugs are, however, useful for the treatment of the elevated intraocular pressure (IOP) associated with glaucoma. Of these drugs, pilocarpine (1), a partial agonist of the muscarinic receptor, is the only one that reduces IOP, mainly by increasing the outflow of aqueous humor from the anterior chamber toward the episcleral veinous system of the eye (1,2). The other antiglaucoma agents reduce IOP by decreasing the formation of aqueous humor. There is a strong need for additional antiglaucoma therapy: it is estimated that the IOP in nearly 50% of glaucomatous patients is not adequately controlled by a single therapeutic agent. Pilocarpine is typically effective, even in patients who do not respond well to other antiglaucoma agents. Although it is very effective in reducing IOP, pilocarpine is associated with several side effects and patient dosing-compliance problems. The compliance issue is a consequence of the relatively short duration of action of pilocarpine. This in turn is a consequence of a combination of several factors including its poor permeation properties through the cornea and its sensitivity to corneal esterases and/or epimerases. The toxicity issue is a consequence of the frequent massive dosages necessary to achieve therapeutic efficacy. The successful development of a chemical delivery system for pilocarpine, capable of achieving enhanced and sustained ocular levels, would offer many advantages, including less frequent dosing intervals; decreased dosage, thereby decreasing systemic levels and systemic toxicity; and enhanced and sustained drug delivery to the site of action, thereby optimizing the therapeutic margin of safety of the drug. Due to the dual nature of the cornea, with a lipophilic epithelium and a hydrophilic stroma, the epithelium appears to be rate limiting to the movement of hydrophilic compounds, whereas for lipophilic compounds, the stroma is rate limiting (3). Because of this duality, drugs that have to negotiate the corneal barrier successfully must be both lipid soluble and water soluble. The aim of this research was to synthesize a series of soft quaternary salts, water-soluble metabolic precursors of pilocarpine, and to demonstrate a sustained muscarinic activity over an extended period of time.

MATERIALS AND METHODS

Chemistry

Pilocarpine hydrochloride and chloromethyl pivalate (6) were obtained from Aldrich Chemical Co., chloromethyl benzoate (7) was obtained from Columbia Organics, and chloromethyl chlorosulfate was obtained from Fairfield Chemicals, Blythewood, SC. NMR spectra were obtained on a Varian XL-300 spectrometer. IR spectra were obtained on a Perkin Elmer 1600 FTIR. Elemental analyses were done by Atlantic Microlab (Atlanta, GA). Only the general synthetic procedures are described.

Preparation of Pilocarpine Free Base (1)

Pilocarpine hydrochloride (10.1 g, 41 mmol) was dissolved in water (250 ml) and stirred in an ice bath. Sodium hydroxide (1.65 g, 1 eq) was dissolved in water (200 ml) and added to the pilocarpine hydrochloride solution. The product was extracted with CH_2Cl_2 (5 × 50 ml), then dried over Na_2SO_4, and the solvent was removed. After drying under vacuum, 8.0 g (93%) of thick oil was obtained. IR (film): 3375 s, 3115 s, 2964 s, 1775 s, 1557 m, 1505 s, 1460 s, 1224 s, 1171 s, 1110 s, 1053 s, 1023 s, 981 s, 945 s, 927 s, 817 s, 664 s; ^1H NMR (200 MHz, FT mode, $CDCl_3$) σ: 7.41 (1H, s, H-2), 6.76 (1H, s, H-4), 4.30–4.02 (2H, CH_2O), 3.59 (3H, s, CH_3), 2.80–2.35 (4H, m), 1.88–1.54 (2H, m), 1.14–1.06 (3H, m).

Typical Procedure for Compounds 2–5

Chloromethyl 1-Adamantanecarboxylate (2). Adamantanecarboxylic acid (3.60 g, 20 mmol) was dissolved in water (50 ml) containing $NaHCO_3$ (10 g). Tetrabutylammonium hydrogen sulfate (3.72 g, 0.05 eq) was added, followed by the slow addition of chloromethyl chlorosulfate (3.72 g, 23 mmol) dissolved in CH_2Cl_2 (5 ml). After 4 hr the organic

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0724-8741/92/0300-0372$06.50/0

layer was separated, dried with $MgSO_4$, and concentrated under vacuum. The compound was purified by column chromatography on silica gel using 4% MeOH in CH_2Cl_2 as eluent. IR (film): 2909 s, 2853 s, 1755 s, 1451 m, 1340 m, 1211 s, 1061 s; ^1H NMR (300 MHz, $CDCl_3$) σ: 5.71 (2H, s, CH_2), 2.03 (3H, s), 1.90 (6H, d, J = 2.6 Hz), 1.72 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) σ: 175.34 (CO), 68.78 (CH_2-O), 40.73 (C), 38.37 (CH), 36.37 (CH), 27.85 (CH_2).

Chloromethyl Cyclohexylcarboxylate (3). This was purified by vacuum distillation.

Chloromethyl α-Phenylcyclopentylacetate (4). This was purified by vacuum distillation.

Chloromethyl Adamantaneacetate (5). This was purified by vacuum distillation.

Typical Procedure for Compounds 8–11

Chloromethyl n-*Dodecanoate (8).* A mixture of dodecanoyl chloride (4.38 g, 20 mmol) and paraformaldehyde (0.6 g, 20 mmol) was mixed with a catalytic amount of anhydrous $ZnCl_2$ (2–5 mg) and was stirred and heated under N_2 until a homogeneous solution was obtained. The crude product was purified on Florisil using pet. ether as eluent. IR (film) 2925 s, 2854 s, 1767 s, 1464 m, 1442 m, 1376 w, 1338 w, 1260 m, 1109 s, 1037 s, 722 s; ^1H NMR (300 MHz, FT mode, $CDCl_3$) σ: 5.70 (2H, s, CH_2), 2.32 (2H, t, J = 6 Hz, CH_2–CO), 1.65 (2H, m, CH_2), 1.26 (16H, s, $8CH_2$), 0.89 (3H, m, CH_3); ^{13}C NMR (75 MHz, FT mode, $CDCl_3$) σ: 171.62 (CO), 68.46 (CH_2O), 33.91, 31.87, 29.54, 29.36, 29.29, 29.15, 28.90, 24.49, 22.64, 14.03 (CH_3).

Chloromethyl n-*Tetradecanoate (9).* This was purified on silica gel (60–270 mesh) using pet. ether/CH_2Cl_2 as eluent.

Chloromethyl n-*Hexadecanoate (10).* This was purified on silica gel, using pet. ether:CH_2Cl_2 (8:2).

Chloromethyl n-*Octadecanoate (11).* This was purified by column chromatography, on silica gel, using pet. ether/CH_2Cl_2 (8:2) as eluent.

Typical Procedure for Compounds 12–21

N-Adamantanecarboxymethylpilocarpinium Chloride (12). Pilocarpine (2.08 g, 10 mmol) and chloromethyl adamantanecarboxylate (**2**; 2.28 g, 10 mmol) were stirred and heated gently under N_2. A homogeneous mixture was obtained, which usually solidified into a glassy solid. The product was purified by crystallization from THF. IR (KBr): 3421 w (H_2O), 2980 s, 1768 s, 1568 w, 1454 m, 1219 s, 1175 s, 1050 s; ^1H NMR (300 MHz, FT mode, $CDCl_3$) σ: 9.88 (1H, s), 7.64 (1H, s), 6.24 (2H, s), 4.40–4.34 (1H, m) 4.13–3.93 (1H, d, J = 9 Hz), 4.03 (3H, s), 3.95 (1H, d, J = 9 Hz), 3.12–3.11 (1H, broad signal), 2.81–2.62 (4H, m), 2.01 (3H, s), 1.85 (6H, s), 1.71 (6H, s), 1.12–1.09 (3H, m); ^{13}C NMR (75 MHz, FT mode, $CDCl_3$) σ: 178.12 (CO), 176.71 (CO), 139.09 (C-2), 133.11 (C-4), 119.91 (C-5), 69.91 (CH_2O), 69.77 (CH_2O), 44.35, 40.69, 38.91, 38.34, 36.50, 36.25, 34.51, 27,93, 27,61, 21,47, 18.43, 12.21.

N-*Cyclohexylcarboxymethylpilocarpinium Chloride (13).* This was purified on a column of silica gel using 15% MeOH in CH_2Cl_2 as an eluent.

N-(*α-Phenylcyclopentyl*)-*acetoxymethylpilocarpinium Chloride (14).* The crude product was triturated with ether for 16 h.

N-(*-1-Adamantane*)-*acetoxymethylpilocarpinium Chloride (15).* The crude glassy product was purified by column chromatography on silica gel using a gradient of 5 to 10% MeOH in CH_2Cl_2. This purification was followed by crystallization from an acetone/hexane mixture.

N-*Pivaloyloxymethylpilocarpinium Chloride (16).* Trituration of the crude product with ether yielded a white solid, which was isolated by filtration and dried over P_2O_5.

N-*Benzoyloxymethylpilocarpinium Chloride (17).* The crude product was purified by column chromatography on silica gel using a gradient of 5 to 20% MeOH in CH_2Cl_2, followed by crystallization from THF.

N-*Dodecanoyloxymethylpilocarpinium Chloride (18).* The crude product was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (9:1, then 8.5:1.5). The product was then crystallized twice from THF.

N-*Tetradecanoyloxymethyl Pilocarpinium Chloride (19).* The glassy solid was crystallized twice from THF.

N-*Hexadecanoyloxymethyl Pilocarpinium Chloride (20).* The crude product was triturated with ether followed by two crystallizations from THF.

N-*Octadecanoyloxymethylpilocarpinium Chloride (21).* The crude product was crystallized twice from THF.

Effect on Pupil Size and IOP in New Zealand White Rabbits

Nine normotensive New Zealand White rabbits, weighing between 3 and 4 kg, were evaluated after the instillation of a single drop (50 μl) of the test compounds **12** to **21** and pilocarpine as a standard. The drugs were used at concentrations of from 0.1 to 10%. One eye (the left eye) received a placebo, whereas the fellow eye received the test compound dissolved in the placebo solution. The placebo consisted of a 0.05 *M* phosphate buffer at a slightly acidic pH (pH 6.75). The concentrations used were calculated as equivalent to pilocarpine hydrochloride and are expressed as grams per 100 ml (%). The pupil diameters and the IOP of both eyes were measured in each rabbit just before the application of the eye drops. Intraocular pressure was measured by pneumatonography using a Digital Model 30R pneumatonometer and the pupil size was measured with calipers. The recording times for pupil size were predrug and every 15 min post drug administration. Intraocular pressure was recorded predrug and at 30 min and 2 hr post drug administration.

Effect on IOP in Glaucomatous Beagles

Compound **20** was evaluated against a 2% pilocarpine solution in four glaucomatous beagles ranging in age from 1 to 2 years and in the early stages of inherited primary angle glaucoma. Based on the rabbit study, compound **20** was evaluated at concentrations of 0.1, 0.2, and 0.4%. Pilocarpine and compound **20** were dissolved in a vehicle which consisted of an actual clinical formulation made from Xenon Vision, Inc., by Chesapeake Biological Laboratories, Inc. (Baltimore, MD) and which contained benzalkonium chloride (0.5 ml), sodium chloride (17 g), sodium edetate (2.5 g), sodium phosphate (4.313 g), povidone K-15 (40 g), sodium hydroxide (q.s.) and purified water (to 2.5 L). Intraocular pressures were measured using the Mackay-Marg tonome-

ter. Intraocular pressure was recorded predrug and 1, 2, 4, and 6 hr post drug administration.

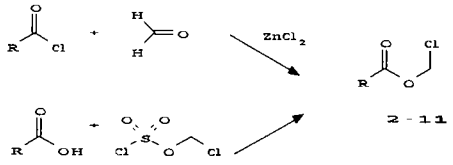
RESULTS

Chemistry

The synthetic route, structural details, and analytical data concerning the various intermediates and target compounds are shown in Figs. 1 and 2. The quaternization reaction occurred on mixing within a range of temperatures between 60 and 90°C. The chloromethyl esters intermediates **2** to **11**, unless stated otherwise, were obtained by reaction of the corresponding acid chloride with paraformaldehyde in the presence of a catalytic amount of anhydrous zinc chloride (4) or from the corresponding carboxylic acid and chloromethyl chlorosulfate under phase transfer conditions (5). The final compounds were obtained as a white hygroscopic material which was difficult to handle, even after crystallization from the appropriate solvent (usually tetrahydrofuran, isopropanol, acetone, or a mixture thereof) or after chromatography on silica gel.

Effect on Pupil Size and IOP in Rabbits

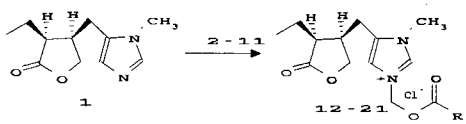
The tolerance (lack of topical irritation) and the changes in intraocular pressure (IOP) and pupil size were determined for 10 compounds in a population of nine albino New Zealand White rabbits. The compounds were tested in a random crossover design. The treatment of the data used Gaussian statistics and Student's *t* test. The data were gathered over a 2-month period and were used to calculate a mean pupil size at time 0 for the left and the right eye of each rabbit. Data were obtained at different time points in the



No.[a]	R	el. analysis	mp/bp (°C)	Yield
2		$C_{12}H_{17}ClO_2$	oil, decomposes during distillation	85
3		$C_8H_{13}ClO_2$	bp: 76-77/12mm	90
4		$C_{14}H_{17}ClO_2$	bp: 137/2-4mm	73
5		$C_{13}H_{19}ClO_2$	bp: 114-119/1mm	66
8	$CH_3(CH_2)_{10}$	$C_{13}H_{25}ClO_2$	low melting solid[b]	63
9	$CH_3(CH_2)_{12}$	$C_{15}H_{29}ClO_2$	low melting solid[b]	60
10	$CH_3(CH_2)_{14}$	$C_{17}H_{33}ClO_2$	mp: 40-42	45
11	$CH_3(CH_2)_{16}$	$C_{19}H_{37}ClO_2$	mp: 47.5-48.7	59

[a] Compounds 6 (R = ter-butyl) and 7 (R = phenyl) were commercially available
[b] Mp less than 25°C

Fig. 1. Synthetic scheme and analytical data for the chloromethyl esters.



No.	R	el. analysis	mp (°C)	Yield[a]	Rf[b]
12		$C_{23}H_{33}ClN_2O_4$	93-98	88	0.13
13		$C_{18}H_{29}ClN_2O_4.H_2O$	64-66	55	0.11
14		$C_{25}H_{33}ClN_2O_4$	60.9-64.3	65	0.23
15		$C_{24}H_{35}ClN_2O_4.H_2O$	75.6-79	64	0.13
16		$C_{17}H_{27}ClN_2O_4$	179.9-185.4	83	0.11
17		$C_{19}H_{23}ClN_2O_4$	66-70	82	0.11
18	$CH_3(CH_2)_{10}$	$C_{24}H_{41}ClN_2O_4.H_2O$	62-64.2	52	0.08
19	$CH_3(CH_2)_{12}$	$C_{26}H_{45}ClN_2O_4.H_2O$	65.2-67	59	0.10
20	$CH_3(CH_2)_{14}$	$C_{28}H_{49}ClN_2O_4.H_2O$	78.8-80.9	42	0.11
21	$CH_3(CH_2)_{16}$	$C_{30}H_{53}ClN_2O_4.H_2O$	88-94	64	0.14

[a] After purification
[b] TLC on silica plates, eluting with MeOH/CH_2Cl_2 (10:90)

Fig. 2. Synthetic scheme and analytical data for the soft quaternary salts.

control eye, and by direct comparison with the value at time 0, it could be assumed that the drugs did not have any effect on the control eye (results not shown). In order to reduce the interindividual variation of miotic responses which is observed within the sample population when the absolute pupil diameter is measured, the results were reported in terms of miotic activity, which is the percentage reduction in pupil size observed at time *t* by comparison with time 0. The miotic activity vs time response curve for selected test compounds is shown in Fig. 3. The miotic activity vs dose is shown in Fig. 4. In Fig. 4, the miotic activity is the peak activity of the test compounds, usually observed 30 min after instillation. In order to make meaningful comparisons, the concentrations are expressed as grams per 100 ml (%) equivalents of pilocarpine hydrochloride. IOP activity was monitored at 30 min and 2 hr following instillation of the eye drops. There was no measurable change in IOP after 2 hr, even with pilocarpine 2%, and the results were not analyzed further. However, as shown in Figs. 3 and 4, changes in pupil size indicated cholinergic effect for all the test compounds. The compounds which compare favorably with pilocarpine are the ones with a lipophilic side chain, i.e., 10 carbon atoms or more. With one of the most effective drugs, compound **20**, the effect of a 0.5% concentration was equivalent to that observed with 2% pilocarpine, representing a fourfold improvement on pilocarpine itself. Some of the compounds were definitely irritating to the conjunctiva, as determined by slit lamp biomicroscopy. Conjunctival hyperemia and lacrimation were observed and were dose related, the highest concentrations producing the most irritation. Corneal changes and aqueous flare were not detected.

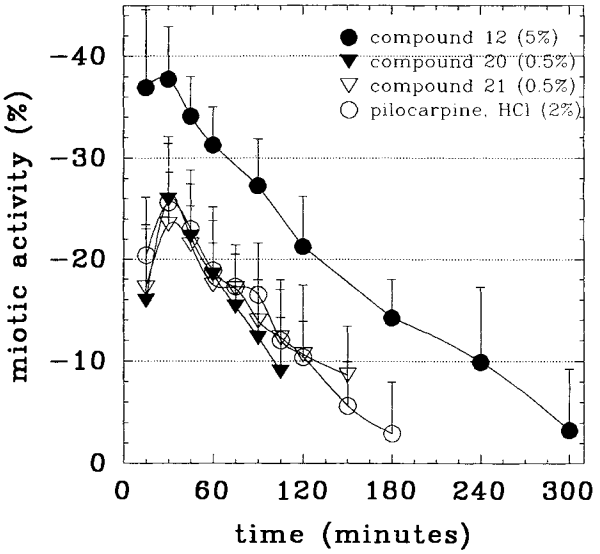


Fig. 3. Miotic activity vs time response curve for selected compounds.

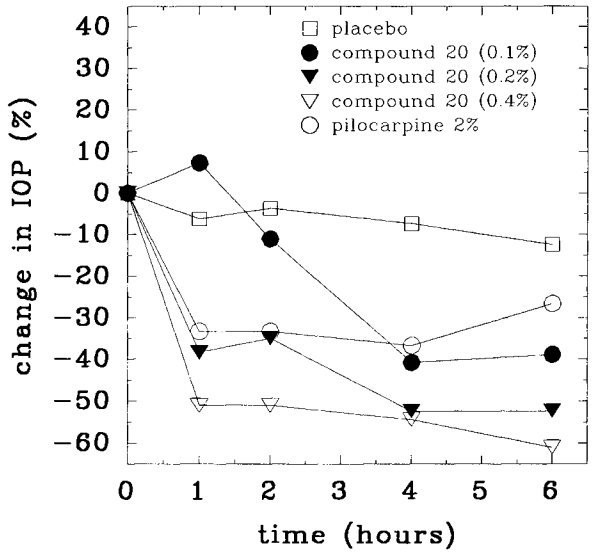


Fig. 5. Effect of compound 20 on IOP in genetically glaucomatous beagles.

IOP Activity in Glaucomatous Beagles

Based on the miotic activity study in rabbits, compound 20 was evaluated in the glaucomatous beagle for IOP reduction and was compared to a 2% solution of pilocarpine dissolved in the same vehicle as the test compound. The IOP was recorded at time 0 and 1, 2, 4, and 6 hr post drug administration. The results are plotted in Fig. 5. A profound dose-related decrease in IOP was observed in the glaucomatous beagles starting within the first hour and continuing throughout the 6 hr of monitoring. The 0.1% concentration of compound 20 had a longer onset of action but was still close to peak activity after 6 hr, while the IOP in the 2% pilocarpine subjects was almost back to original values. The 0.2 and 0.4% concentrations produced even more dramatic effects.

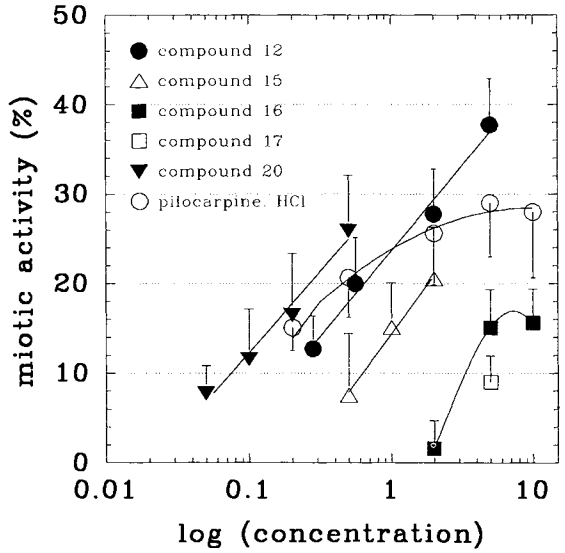


Fig. 4. Peak miotic activity vs dose response curve for selected compounds.

DISCUSSION

In order to assess the activity of the newly developed compounds, the miotic response and IOP activity in rabbits was evaluated. The albino rabbit is well established as an ophthalmic animal model. It is used in many screening tests for ocular drugs and a large drug information base is available for the rabbit that permits convenient comparisons with established ophthalmic drugs. The rabbit screening test permits the measurement of three critical aspects of the new compounds, i.e., irritation, effect on the pupil and effect on intraocular pressure. The changes in IOP in the normotensive rabbit following the administration of the test compounds were not remarkable, even with 2% pilocarpine (results not shown). The lack of IOP activity with pilocarpine in the normotensive rabbit is known and has been documented (6). Miichi and Nagataki (7) even found that pilocarpine increases aqueous humor formation in the rabbit eye, an effect which may be detrimental to the facilitation of aqueous humor outflow. The observed lack of IOP activity in our experiment was therefore anticipated. IOP measurements were nonetheless performed since there was a possibility that the soft quaternary salts would exert an activity of their own before releasing pilocarpine *in situ*. The observed change in pupil size, however, was evidence for the cholinergic effect on the iridal sphincter musculature. Compound 20 was chosen as the best drug candidate of the series for further study in glaucomatous beagles. The choice of compound 20 was based on criteria of miotic activity and ocular irritation in rabbits (Table I). Primary open-angle glaucoma is inherited in the beagle as an autosomal recessive trait (8). This model was chosen because, in general, the glaucomatous beagle has been demonstrated to be a good indicator of many classes of drugs that lower intraocular pressure in humans. Pilocarpine solutions and gels have been successfully used before in single-dose studies in this model and therefore the model is valid (9,10).

Based on the known biological lability of certain quaternary salts (11), and based on preliminary observations

Table I. Miotic Activity of Test Compounds

No.	Concentration (%)[a]	Miotic activity[b]
Pilocarpine	2	25.63
12	0.28	12.77
13	1	9.76
14	2	22.87
15	0.5	7.54
16	10	15.67
17	5	9.01
18	0.5	25.92
19	0.5	24.66
20	0.5	25.95
21	0.5	23.48

[a] Maximum concentration that produced little or no ocular irritation.

[b] Peak activity, expressed as percentage change from control eye.

about quaternary salts of pilocarpine (12), we suggest that the newly developed compounds are transient inactive derivatives of pilocarpine, also known as prodrugs (13). The proposed mechanism for the release of pilocarpine from its prodrug form is illustrated in Fig. 6, where esterases cleave the acyl portion of the side chain in order to form an unstable α-hydroxylammonium salt which spontaneously liberates the tertiary amine. According to this mechanism, the observed muscarinic activity is interpreted as evidence for the intraocular delivery of the active drug. Therefore the enhanced and sustained activity that was observed in rabbits as well as in dogs is also evidence for a dramatic improvement in the ocular bioavailability of pilocarpine. In the past, the approach to improving the corneal permeation of drugs has stressed the importance of the partition coefficient *P*, suggesting that more lipophilic compounds, i.e., higher *P*, have better corneal permeability properties. According to this approach, the new quaternary salts, which have a significant aqueous solubility, should not have a better corneal bioavailability than pilocarpine. However, the results show that they do. The data, when seen in the light of the study by Grass and Robinson (3), suggest that the absolute values of aqueous and lipid solubilities of a solute are more important than the relative values thereof. At the physiological level, this reflects the dual nature of the cornea, with its lipophilic epithelium and its hydrophilic stroma.

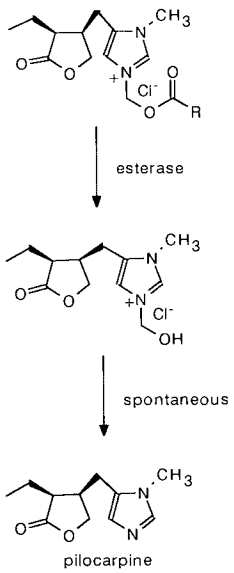


Fig. 6. Suggested mechanism of activation of the new prodrug form of pilocarpine.

CONCLUSION

Our approach was based on the known biological lability of certain quaternary ammonium salts. This type of quaternary ammonium salts also has been referred to as "soft quaternary salts" in the design of biologically labile antimicrobial agents (11). The quaternary salts described in this work are soluble in water by virtue of the cationic ammonium head, while lipophilicity can be modulated by manipulating the size of the side-chain R. This chemical derivatization of drugs produces compounds that are water soluble. The resulting salts are subject to facile enzymatic hydrolysis to generate the starting parent drug. By carefully selecting the nature of the substituent R, the correct hydrophilic/lipophilic balance can be found and the physicochemical parameters of the new compounds can be manipulated in order to optimize corneal transport. This approach departs from the conventional approach by suggesting that individual values of aqueous and lipid solubilities may be more important than the ratio thereof.

ACKNOWLEDGMENTS

Part of this work was accomplished under a Phase I SBIR grant (Grant 1 R43 EY08636-01). The authors wish to acknowledge the financial support of the National Eye Institute, Bethesda, MD, which provided that grant.

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