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Quaternary Pilocarpine Derivatives as Potential Acetylcholine Antagonists. 2. Alterations in the Lactone and Imidazole Moieties[1]

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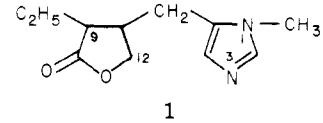
In order to investigate the chemical behavior of pilocarpine, as well as the factors which determine its pharmacological activity, systematic and specific structural changes involving the lactone and imidazole moieties have been performed. Series of model compounds with cyclic or open-chain structures and a variety of N-3 bonded chains obtained from previously prepared anticholinergic derivatives of pilocarpine have been synthesized. The changes included N-3 chains of different lengths with an acetylcholine-like structure, the introduction of nucleophilic groups such as ketoxime, hydroxamic, or both at the side chain, or following hydroxylaminolysis of the lactone, respectively. Specific structural alterations could be obtained by reacting with free hydroxylamine under carefully controlled conditions, and the existence of syn and anti isomers was disclosed in certain cases. The new groups in the pilocarpine derivatives influenced their degree of antagonism to acetylcholine. Several compounds displayed some antidotal activity.

The action of pilocarpine (1) on the parasympathetic nervous system has been extensively investigated; however, its use is presently still limited to the treatment of glaucoma. This alkaloid, isolated from the leaves of the South American shrubs *Pilocarpus jaborandi* and *Pilocarpus microphyllus* Stapf., has been the subject of structure–activity studies.[2-4] Pilocarpine occurs naturally as the cis isomer which is much more potent than the *trans*-isopilocarpine.[5] It is assumed to interact with the muscarinic receptor, and molecular configurations indicating possible modes of receptor binding were presented.[6] Several binding sites in the lactone and imidazole rings have been assumed,[7] and the lactone appears to be essential for the cholinergic activity[8,9] whereas cleavage of the imidazole ring does not completely suppress this activity.[9] In certain systems *d*-pilocarpine, known as a typical parasympathomimetic drug, may act as an anticholinergic.[2] We have previously reported the synthesis of a series of quaternary derivatives of pilocarpine, most



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of which antagonized to a different degree the effect of acetylcholine (AcCh).[1] As a continuation, we wish to report in the present paper a systematic study involving the synthesis of new derivatives, in which characteristic groups have been added (oxime, hydroxamic, or both) in a search for a synergistic effect to that of atropine against organophosphate intoxication, as well as cholinolytics.

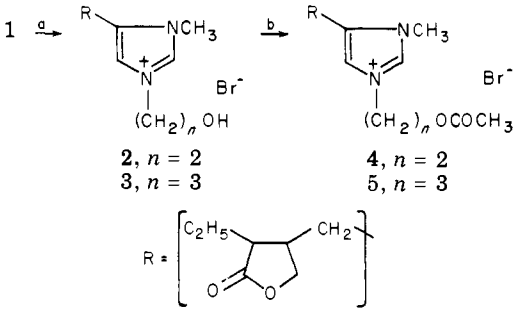
Chemistry. Derivatives **4** and **5** were designed to have the N-3 side chain structurally similar to acetylcholine, the

Table I. Physical Properties of Quaternary *d*-Pilocarpine Derivatives with a Hydroxyl, Acetoxy, and Ketoxime Group in the Side Chain and of ω-Halogenoacetophenones 17-19

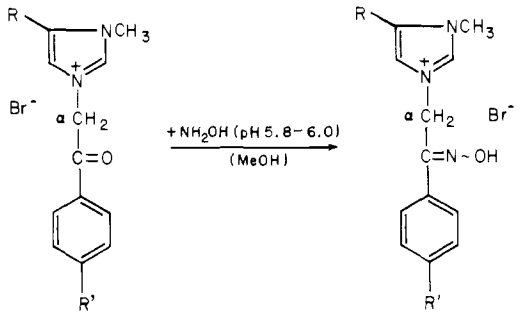
Compd[a]	Crystalline appearance	Mp, °C	$[\alpha]^{24}D$, deg	Formula[c]
2	Transparent prisms	120	+88	$C_{13}H_{21}BrN_2O_3$
3	Soft solid		+74.8	$C_{14}H_{23}BrN_2O_3$
4	Oil		+64.8	$C_{15}H_{23}BrN_2O_4$
5	Oil		+53.6	$C_{16}H_{25}BrN_2O_4$
9	White stars	149-151[b]	+42.8	$C_{19}H_{23}Br_2N_3O_3$
10	Brown prisms	103-105[b]	+38	$C_{19}H_{23}BrN_4O_5$
11	Yellowish stars	167-169	+39.2	$C_{25}H_{28}BrN_3O_3$
17	White needles	113-115[b]		$C_8H_7BrClNO$
18	Yellow needles	120-122		$C_8H_7BrN_2O_3$
19	White needles	153-154		$C_{14}H_{12}ClNO$
20	White prisms	95-98[b]	+54	$C_{19}H_{24}ClN_3O_3$
21	White prisms	176-178[b]	+49.2	$C_{19}H_{23}BrClN_3O_3$
22	White needles	182-184	+52	$C_{25}H_{28}ClN_3O_3$

[a] Compound 16 was prepared according to ref 13. [b] Evolution of gas. [c] Satisfactory analyses for C, H, N, Br, and, if present, Cl were obtained for all compounds; the results were within ±0.6% of the theoretical values.

Scheme I



Scheme II. Method a



ring nitrogen corresponding to the quaternary N of the choline moiety. This preparation was performed in order to observe the influence of possible active groups on the cholinergic activity of pilocarpine.

Since direct quaternization of the alkaloid with the corresponding haloorganic reagent[1] was not successful, the reaction was carried out, as shown in Scheme I, by reacting first with the reagents having a terminal hydroxyl group, preparing initially compounds **2** and **3**. The reaction took place best in 2-methoxyethanol as solvent. Acetylation was then performed, better results being obtained when the reaction with $(Ac)_2O$ was carried out at 80°, producing the required products **4** and **5**.

The ir spectra of **2** and **3** (Nujol) showed the appropriate band at 3320 cm^{-1} (OH associated),[10] and in those of **4** and **5** the presence of the lactone ring and one acetate group was indicated by ν_{max} 1770 and 1740 cm^{-1}, respectively. The latter group was also confirmed by a three-proton NMR signal at δ 2.04 and by the mass spectrum, a characteristic peak for M - CH_3Br being observed.[11]

The conversion of the ketone group to a ketoxime in the N-3 phenacyl chain of the previously prepared[1] anticholinergic compounds **6-8** was carried out (Scheme II) in order to examine the role of the ketone group and the ketoxime anion on the degree of antagonism to AcCh or on the reactivation of phosphorylated AcChE. Two approaches were used: (a) by treating the quaternary active ketones **6-8** with free NH_2OH in methanol at pH 5.8-6.0 (Scheme II), conditions which left the lactone unaltered as shown by the double quartet for the 12-CH_2 in the NMR, J = 9.0 Hz. The use of free NH_2OH ensured the formation of the ketoximes **9-11** without inorganic byproducts, simplifying their purification. Compound **6** was very slow to react, whereas **7** was faster and gave good yields. (b) In the second method the corresponding ω-halo ketoximes **16-19** were prepared first; then by nucleophilic substitution with **1** the desired quaternary ketoximes **10** and **20-22** were obtained (Scheme III). The ω-halo ketoximes were prepared by reacting the corresponding ketones **12-15** with NH_2OH·HCl in aqueous methanol. Interestingly, in certain cases a transhalogenation at the ω position was observed to take place, the bromine in **13** and **15** being displaced by chlorine.[12] This transhalogenation was found to be independent of the ketoxime formation reaction; indeed, when ketone **13** was treated in methanol with NaCl under the same conditions, *p*-bromophenacyl chloride was obtained. Its reaction with NH_2OH produced the ketoxime **17**. The para substituent seems to be a determinant factor in the process, since **14** with the electron-withdrawing NO_2 group gave **18** which retained the bromine atom. The experimental data indicated that method b afforded products of higher purity. The physical data for the ω-haloacetophenone oximes and the corresponding pilocarpine ketoximes are collected in Table I.

The NMR spectra of compounds **16-19** in $(CD_3)_2SO$* showed the signal of the α-methylenic hydrogens at δ 4.77-4.86 and the signal of the =NOH downfield at δ 11.95-12.16. No paired signals for these protons related to the two possible geometrical isomers could be observed; however, this is not a sufficient indication yet for the existence of only one isomer.[14]

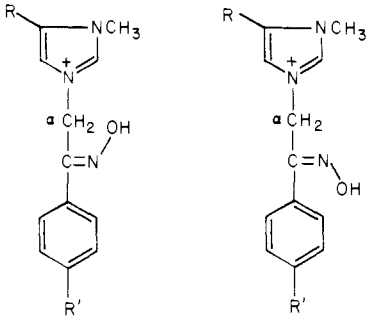
The generation of a formal positive charge during the formation of the quaternary ketoximes **10** and **20-22** induced a characteristic shift of the C=N stretching vibration from 1610 cm^{-1} in **16-19** to 1640-1650 cm^{-1} with an increase of intensity. In parallel, the NMR spectra showed a downfield shift of the α-CH_2 singlet from δ 4.77-4.86 to δ 5.47-5.56. In these spectra one could well disclose a mixture of geometrical isomers: two signals at low field for the hydroxyl hydrogens and two neighboring singlets at higher field for the α-CH_2. The syn or anti

Table II. Chemical Shifts of the Characteristic Hydrogens in Syn and Anti Isomers of Compounds 9-11 and 20-22 in $(CD_3)_2SO$ (δ Units)

Compd	α-CH_2 (s)		=NOH (s)		Major isomer[c]
	Syn	Anti	Syn	Anti	
9	5.47	5.58	11.80	12.54	Anti
10[a]	5.53	5.67	12.10	13.03	Anti
10[b]	5.56	5.65	11.95	12.70	Syn
11	5.55	5.63	12.20	13.10	Anti
20	5.48	5.57	11.80	12.67	Syn
21	5.50	5.58	11.97	12.75	Syn
22	5.54	5.62	11.90	12.70	Syn

[a] Method a. [b] Method b. [c] According to the integration of the signal area. (s) = singlet.

configuration of the two isomers could be determined by taking advantage of the presence of the phenyl group. Due to the induced magnetic field of the aromatic ring, a deshielding effect on the proton located in the vicinity of the ring could be expected.[15,16]



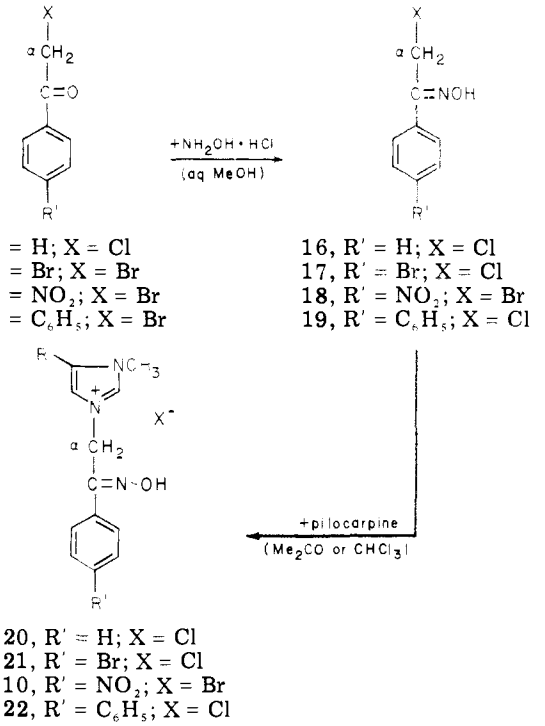
syn anti

Of the two isomers, the hydroxyl proton signal of the anti should appear, therefore, at lower field. Furthermore, by comparing the signal areas of the =NOH and α-CH_2 in both isomers, it was found that the latter group signal at higher field corresponds to the syn isomer.[17] The NMR data for compounds **9–11** and **20–22** are presented in Table II. The direct oximation method a led to the preferential formation of the anti isomer.

The role of the γ-lactone on the pharmacological activity of pilocarpine and its derivatives has not yet been systematically investigated.[18] Irreversible opening of the lactone had been obtained by derivation of the 12-OH and of the carboxyl group.[3,19] More recently the 12-hydroxypilocarpinhydroxamic acid was prepared.[11]

In the present work a number of derivatives of pilocarpine synthesized earlier in our laboratory, and exhibiting high antagonism to AcCh,[1] were converted to the corresponding open-chain 12-hydroxyhydroxamic acids. This was carried out in order to investigate the role of the lactone function and of the nucleophilic hydroxamic group[20,21] on the degree of antagonism to AcCh or for

Scheme III. Method b



12, R′ = H; X = Cl
13, R′ = Br; X = Br
14, R′ = NO_2; X = Br
15, R′ = C_6H_5; X = Br

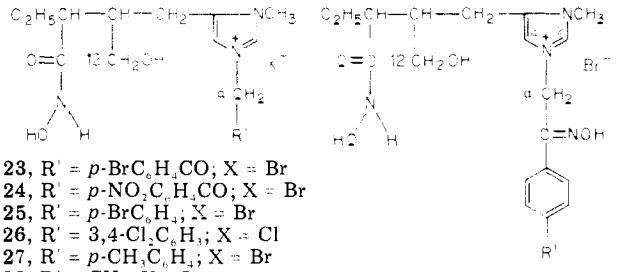
16, R′ = H; X = Cl
17, R′ = Br; X = Cl
18, R′ = NO_2; X = Br
19, R′ = C_6H_5; X = Cl

20, R′ = H; X = Cl
21, R′ = Br; X = Cl
10, R′ = NO_2; X = Br
22, R′ = C_6H_5; X = Cl

antidotal action against organophosphate intoxication, respectively.

Optimal conditions for hydroxylaminolysis of the lactone without interaction with the ketone group in the N-3 phenacyl chain were obtained; thus, the open-chain keto acids **23** and **24** were prepared by treating **6** and **7** with NH_2OH at pH 8.8–9.0.

The other quaternary acids **25–28** were prepared at pH



23, R′ = *p*-BrC_6H_4CO; X = Br
24, R′ = *p*-$NO_2C_6H_4CO$; X = Br
25, R′ = *p*-BrC_6H_4; X = Br
26, R′ = 3,4-$Cl_2C_6H_3$; X = Cl
27, R′ = *p*-$CH_3C_6H_4$; X = Br
28, R′ = CH_3; X = I

29, R′ = Br
30, R′ = NO_2
31, R′ = C_6H_5

7.8–8.0 when the opening of the lactone was rapid. Most acids were hygroscopic, melting with evolution of gas followed by solidification and decomposition of the melt (Lossen rearrangement of the hydroxamic group).[22] Their physical properties are collected in Table III. Ir spectra in neat showed the characteristic bands: ν 3450 (free NH),

Table III. Physical Data of Some 12-Hydroxypilocarpiniumhydroxamic Acids[a]

Compd	Crystalline appearance	Mp, °C[b]	$[\alpha]^{24}D$, deg	Formula[d]
23	White prisms	107-108	+27.2	$C_{19}H_{25}Br_2N_3O_4$
24	Orange prisms	98-100	+30.4	$C_{19}H_{25}BrN_4O_6$
25	White crystals	47-48	+15.2	$C_{18}H_{25}Br_2N_3O_3$
26	White stars	95-113	+19.6	$C_{18}H_{24}Cl_3N_3O_3$
27	White prisms	70-71	+17.6	$C_{19}H_{28}BrN_3O_3$
28	Yellow oil	*c*	+30.6	$C_{13}H_{24}IN_3O_3$
29	Brown prisms	62-64	+12	$C_{19}H_{26}Br_2N_4O_4$
30	Orange prisms	50-52	+13.6	$C_{19}H_{26}BrN_5O_6$
31	Yellowish crystals	125-127	+8.4	$C_{25}H_{31}BrN_4O_4$

[a] All acids gave red-violet complexes with $FeCl_3$. [b] Melt with evolution of gases. [c] Crystallizes at −5°. [d] All compounds were analyzed for C, H, N, Br, and, if present, Cl; the results were within ±0.6% of the theoretical values.

Table IV. Relative Activity of the Spasmogenic Compounds on the Isolated Guinea Pig Ileum

Compd	Spasmogenic act.[a]	Compd	Spasmogenic act.[a]
Pilocarpine	1[b]	5	0.05
Isopilocarpine	0.02	7	0.01
2	0.1	24	0.1
3	0.05	30	0.01
4	0.1		

[a] The spasmogenic activity was suppressed in the presence of 10 ng/ml of atropine sulfate. [b] Contraction elicited by 0.6 μg/ml of pilocarpine.

3200 (OH bonded[23]), 1650 (C=O amidic), and 920 cm^{-1} (NO). For **23** and **24** the ketone C=O absorption at 1690 cm^{-1} appeared as a shoulder due to overlapping with the amidic C=O. The NMR spectra of **23–28** in $(CD_3)_2SO$ clearly showed the broad signals of NOH (δ 10.74–10.82) and amidic NH (δ 6.38–6.77) for the hydroxamic group and the doublet (J = 6 Hz) of the 12-CH_2 (δ 3.36–3.42). The singlet of α-CH_2 was located in the same location as in the closed ring analogues confirming that **23** and **24** are indeed keto acids; a ketoxime would result in a marked upfield shift of this singlet.

The preparation of 12-hydroxypilocarpiniumhydroxamic acids having a ketoxime in the N-3 chain was next undertaken. The new acids **29–31** were prepared by hydroxylaminolysis of the lactone in the corresponding ketoximes **9–11** at about pH 8. Interestingly, the optical rotations of all open-chain acids (Table III) have lower values than the corresponding analogues with a lactone ring (Table I). Ir spectra (neat or Nujol) showed superposition of the C=N ketoxime band over the hydroxamic C=O. The NMR spectra showed all the signals of the characteristic hydrogens and the integration indicated that the anti isomer was predominant.

Pharmacology. The compounds tested which showed spasmogenic activity are presented in Table IV. In all cases this activity was fully antagonized by 10 ng/ml of atropine sulfate introduced into the bath 1 min before the test substance. In addition, most of the compounds were tested for antagonism of AcCh-induced spasm of isolated guinea pig ileum as well as antidotes against tetraethylpyrophosphate (TEPP) intoxication in mice (Table V). None of the compounds (**2–5**) possessing bonding of N-3 with di- or trimethylenic chains having terminal hydroxyl or acetoxy group antagonized the spasmogenic activity of AcCh. On the other hand, compounds **3** and **5** with three $-CH_2-$ groups in contrast with those possessing only two $-CH_2-$ groups (**2–4**) showed antidotal activity when given together with atropine. This antidotal action was surprising and difficult to explain. It could be speculated that it was derived from some additional antagonism, besides that of atropine, against accumulated acetylcholine due to organophosphate intoxication.

Interesting data for the quaternary ketoximes **9–11** and **20–22** were obtained. The antagonism to AcCh of **9–11** was higher than that of the parent ketones (**6–8**),[1] as for **10** a total conversion of the cholinergic activity was attained, conversion of **7** to **10**. The ketoxime bromides displayed higher antagonism to AcCh than the corresponding chlorides. Compound **24** retained the high spasmogenic activity of its closed-ring analogue.[1] It was found that the open-chain 12-hydroxy-*d*-pilocarpinhydroxamic acid[11] was less spasmogenic than pilocarpine itself since at a concentration of 10 μg/ml no contraction of the ileum was obtained whereas pilocarpine caused contraction at 0.6 μg/ml. Except for **25**, all hydroxamic acids exhibited a net reduction in their antagonism to AcCh compared to the analogues having a lactone structure (ref 1 and **9–11**). As an extreme example, compound **30** lacks anticholinergic activity whereas the analogue **10** displayed high antagonism to AcCh. Finally, isopilocarpine exhibited 50 times less contracting activity than pilocarpine. Except for compounds **20** and **21**, the ketoxime group did not contribute to antidotal activity. The antidotal action of **25–27** might be ascribed, therefore, to the presence of the hydroxamic group.

Table V. Anticholinergic Action of Some Pilocarpine Derivatives

Compd	Antagonism of AcCh induced contraction of guinea pig ileum		Antidotal action[c]	
	μg/ml[a]	%[b]	Dose,[d] mg/kg	Surviving mice[e]
Atropine SO_4	0.03	100	25	0
2	0.1	0		
3	0.4	0	100	3
4	0.1	0		
5	0.4	0	100	3
6	10	90		
7	1	0		
8	10	100		
9	10	100		
10	10	90		
11	10	100		
20	10	30	50	3
21	10	50	25	1
22	10	80		
23	10	80 (90)		
24	0.1	0 (0)		
25	10	95 (85)	100	1
26	10	25 (90)	100	3
27	10	55 (78)	100	3
28	10	55 (65)		
29	10	55 (100)		
30	2	0 (90)		
31	10	53 (100)		

[a] Final concentration in the bathing fluid. [b] Percent reduction of the control response to AcCH, average of two separate experiments; values in parentheses refer to results with analogues having a lactone structure.[1] [c] Compounds **2**, **4**, **6–11**, **22–24**, and **28–31** did not display antidotal action against TEPP intoxication. [d] Control animals injected with these amounts did not show abnormalities within 24 h. [e] Average of two experiments with five animals each.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer Infracord 137 spectrophotometer equipped with a NaCl prism, generally in KBr pellets, and whenever the compounds were oils, soft solids, or hygroscopic, the direct sandwich or Nujol method was used. NMR spectra were measured on a Varian A-60 for a 10–15% solution in $(CD_3)_2SO$. Mass spectra were taken on an Atlas CH-4 spectrometer by the direct inlet method and under the conditions: electron energy, 70 eV; emission current, 10–20 μA. For the quaternary compounds the interpretations of the spectra were reported elsewhere.[11] Optical rotations were measured with a Perkin-Elmer 141 polarimeter; the values refer to EtOH solutions (0.25%). For TLC, chromatoplates coated with cellulose (Riedel De Haën, 0.25 mm) were irrigated in a mixture of *n*-BuOH–HOAc–H_2O (4:0.5:5) and developed with an iodoplateate solution. The halogen content of the products was determined by potentiometric titration. Elemental analyses were performed by the microanalytical laboratory of our Institute (Mr. R. Heller). Where analyses are indicated only by symbols of the elements, the analytical results for those elements were within ±0.6% of the theoretical values. *d*-Pilocarpine free base was obtained from its hydrochloride[1] (Plantex Ltd. Nathanya, Israel). The preparation of **6–8** and the closed-ring analogues of compounds **25–28** was described elsewhere.[1] The ω-halogenoacetophenones **12–15** were obtained from commercial sources

(Fluka). Quaternization was carried out in dried air-tight vessels, and high temperatures were avoided since isomerization of the alkaloid may then take place. For each run the pilocarpine was previously dried (P_2O_5, 48 h) and the haloorganic reagents were of high purity.

Anhydrous Me_2CO, 2-methoxyethanol, EtOH, $CHCl_3$, and Et_2O were used as reaction medium and for purification or crystallization of the products.

3-(2′-Hydroxyethyl)-*d*-pilocarpinium Bromide (2) and 3-(3′-Hydroxypropyl)-*d*-pilocarpinium Bromide (3). To 4.16 g (0.02 mol) of 1 dissolved in 35 ml of 2-methoxyethanol, 2.84 ml (0.04 mol) of 2-bromoethanol or 3.62 ml (0.04 mol) of 3-bromopropanol was added to prepare **2** and **3**, respectively. The reaction mixture was heated to 75° (oil bath) for 12 h (compound **2**) or to 60° for 36 h (compound **3**) and allowed at room temperature up to 8 days. The solvent was removed under reduced pressure (70°) resulting in a colorless oil for **2** or yellowish oil for **3**. The products were purified by repeated triturations with Et_2O and Et_2O–$CHCl_3$ (3:1) and stored in a refrigerator (7 days). Further washing with Et_2O afforded 6.34 g (92%) of prisms for **2** and 5.15 g (74%) of soft solid for **3**. Crystallization of **2**: dissolution in MeOH, filtration, addition of Et_2O up to turbidity, and cooling. Compound **2**: ir (Nujol) 3320 (broad, OH), 1770 (C=O, lactone), and 1050 cm^{-1} (OH); NMR δ 5.34 (s, OH), 9.4 (s, H-2), 7.9 (s, H-4), and 3.9 (s, NCH_3). Compound **3**: ir (Nujol) same bands as observed for **2**; NMR δ 9.4 (s, H-2), 7.85 (s, H-4), and 3.87 (s, NCH_3), the signal for OH was detected by exchange with D_2O.

Preparation of 4 and 5 by Acetylation of 2 and 3. To 0.66 g (0.002 mol) of **2** or 0.69 g (0.002 mol) of **3**, 6 ml (0.06 mol) of $(Ac)_2O$ was added with stirring. The solution was heated to 80° for 48 h and excess $(Ac)_2O$ removed by repeated triturations with Et_2O. For purification, the compound was dissolved in Me_2CO and filtered and cold Et_2O added to turbidity: yield, 0.73 g (97%) of **4** and 0.75 g (96%) of **5**, both oily; stored under Et_2O.

3-(2′-Acetoxyethyl)-*d*-pilocarpinium bromide (4): ir (Nujol) 1770 (C=O), 1740 (COO), and 1240 cm^{-1} (CO); NMR δ 2.06 (s, 3, CH_3COO), 9.42 (s, H-2), and 7.9 (s, H-4); mass spectrum for M – CH_3Br, *m/e* 280 (calcd 280.32).

3-(3′-Acetoxypropyl)-*d*-pilocarpinium bromide (5): ir (Nujol) bands as reported for 4; NMR δ 2.02 (s, 3, CH_3COO), 9.38 (s, H-2), and 7.8 (s, H-4); mass spectrum for M – CH_3Br, *m/e* 294 (calcd 294.34).

3-(*p*-Bromophenacylketoxime)-*d*-pilocarpinium Bromide (9) and 3-(*p*-Nitrophenacylketoxime)-*d*-pilocarpinium Bromide (10). Ketone **6** (1.94 g, 0.004 mol) (to prepare **9**) or 1.8 g of ketone **7** (to prepare **10**) was dissolved with gentle heating in a mixture of MeOH (40 ml) and water (4 ml). Separately for each run, to a suspension of 0.35 g (0.005 mol) of dried and finely powdered NH_2OH·HCl in absolute MeOH (15 ml), a cooled solution of NaOMe (3%) was added dropwise with stirring to pH 6. The suspension (at –5°) was *directly* filtered into the precooled above solution, and the pH adjusted to 5.8. The mixture was left at room temperature (48 h), followed by heating to 40° (4 h) (for the preparation of **10** no heat was used, total reaction time 30 h). The solvent was removed; the residue was dried on P_2O_5, dissolved in a cold mixture of absolute EtOH (25 ml) and Me_2CO (5 ml), and filtered and again the solvents were removed. This purification was repeated several times. Compound **9**: the residue (1.6 g, 79%) was crystallized by repeated triturations with Et_2O and stored in a refrigerator; recrystallized from absolute EtOH–Me_2CO (5:2). Compound **10**: the viscous orange oil was triturated and crystallized in cold Et_2O (48 hr) (1.72 g, 78%); recrystallization by dissolving in cold EtOH (absolute)–Me_2CO (3:1), filtration, and adding Et_2O to turbidity.

3-(*p*-Phenylphenacylketoxime)-*d*-pilocarpinium Bromide (11). To a solution of 1.94 g (0.004 mol) of ketone **8** in MeOH (80 ml) and water (5 ml), free NH_2OH prepared as described for **9** and **10** was added. The reaction was carried out as for **10**. The product (1.52 g, 76%) crystallized by trituration with Et_2O. Recrystallization: dissolution with gentle heating in $CHCl_3$–absolute EtOH (1:3), filtration, and addition of cold Et_2O up to turbidity.

4-Bromo-ω-chloroacetophenone Oxime (17). A solution of 4.17 g (0.06 mol) of NH_2OH·HCl in 10 ml of water was added to 5.56 g (0.02 mol) of ketone **13** dissolved (50°) in 150 ml of MeOH. The reaction mixture was left at 50° for 4 days, reduced to one-half of its volume, and poured onto 500 ml of cold water. The precipitate was filtered, washed with water, and dried over P_2O_5 in vacuo: yield 4.75 g (95%). Crystallization: dissolution in MeOH, filtration, and addition of cold water up to turbidity.

4-Nitro-ω-bromoacetophenone Oxime (18) and 4-Phenyl-ω-chloroacetophenone Oxime (19). The reaction mixture was prepared as described for **17**. The ketones were 4.48 g (0.02 mol) of **14** in 80 ml of MeOH (to prepare **18**) and 5.5 g of **15** in 20 ml of $CHCl_3$ and 350 ml of MeOH (to prepare **19**). The precipitation and purification procedures were as for compound **17**. For **18** the reaction mixture was kept at 50° for 2 h and at room temperature for 36 h. The product (3.9 g, 75%) was recrystallized from hot water–MeOH (1:2). For **19** the reaction mixture was kept at 60° for 12 h and at room temperature for 72 h. The product (4.42 g, 90%) was recrystallized from hot MeOH.

Transhalogenation of 13 with Sodium Chloride. **13** (0.56 g, 0.002 mol) in 25 ml of MeOH and 0.35 g (0.006 mol) of NaCl in 5 ml of water were mixed and heated to 50° for 48 h. The product was *p*-bromophenacyl chloride: mp 116–117° (lit. 116–118°). Anal. (C_8H_6BrClO) C, H, Br, Cl.

Preparation of the Quaternary Ketoximes 20–22. General Procedure. A solution of 0.01 mol of the corresponding ω-halogenoacetophenone oxime **16–19** in 25 ml of Me_2CO was added dropwise to a heated (45°) solution of 1.04 g (0.005 mol) of dried pilocarpine in 15 ml of Me_2CO.

3-(Phenacylketoxime)-*d*-pilocarpinium Chloride (20) and 3-(*p*-Bromophenacylketoxime)-*d*-pilocarpinium Chloride (21). **16** (1.69 g) (to prepare **20**) or 2.49 g of **17** (to prepare **21**) was used. The clear reaction mixture was left at 45° for 20 min (turbidity) and at room temperature for 18 h. Finally the solvent was removed. In the case of compound **20**, the oily residue crystallized from cold Et_2O (4 days). Trituration with Et_2O–$CHCl_3$ (2:1) and washing with Et_2O afforded 1.62 g (86%) of a white solid. In the case of compound **21** the precipitate was triturated several times with Me_2CO and Et_2O: yield 2 g (87%).

3-(*p*-Phenylphenacylketoxime)-*d*-pilocarpinium Chloride (22). **19** (2.46 g) was used and the reaction mixture was left at 45° for 4 h (turbidity) and at room temperature up to 72 h. The Me_2CO was removed and the oily residue crystallized by trituration with Me_2CO and Et_2O: yield 1.9 g (82%).

3-(*p*-Nitrophenacylketoxime)-*d*-pilocarpinium Bromide (10) (Method b). **18** (2.59 g) in 40 ml of $CHCl_3$ (dried over KOH) was added portionwise to 1.04 g of pilocarpine in 30 ml of $CHCl_3$. After 24 h at room temperature the solvent was removed. The orange oily residue crystallized (several days) in Et_2O–$CHCl_3$ (5:2) and was washed with the same solvents: yield 2.34 g (92%).

3-(*p*-Bromophenacyl)-12-hydroxy-*d*-pilocarpinium-hydroxamic Acid Bromide (23). To a suspension of 0.56 g (0.008 mol) of NH_2OH·HCl in 15 ml of absolute EtOH, a solution of NaOEt (3%) was added dropwise to pH 8.5 and, after cooling (–5°), the mixture was directly filtered into a flask containing a precooled solution of 2.92 g (0.006 mol) of compound **6** in 75 ml of EtOH. The pH was adjusted to 9 and the reaction mixture was left at 0° for 4 h and at room temperature for 12 h. The solvent was removed (40°); the foamy residue was dried. Crystallization: dissolution in cold Me_2CO–absolute EtOH (1:5), filtration, addition of Et_2O up to turbidity, and cooling (48 hr). performed twice, yield 2.86 g (91.5%).

3-(*p*-Nitrophenacyl)-12-hydroxy-*d*-pilocarpinium-hydroxamic Acid Bromide (24). The experimental procedure was as for compound **23**. **7** (2.72 g, 0.006 mol) and 0.56 g (0.008 mol) of NH_2OH·HCl were used; duration, 8 h at 0° and 4 h at room temperature. Purification and crystallization: dissolution in cold absolute EtOH–*i*-PrOH (1:3), filtration, distillation of the solvents (50°), and storage of the residue in a refrigerator under Et_2O, yield 2.82 g (95%).

3-(*p*-Bromobenzyl)-12-hydroxy-*d*-pilocarpiniumhydroxamic Acid Bromide (25). To a suspension of 0.84 g (0.012 mol) of NH_2OH·HCl in 20 ml of absolute EtOH, a solution of NaOEt (3%) was added dropwise up to pH 7.8–8.0 and, after cooling (–5°), the mixture was directly filtered into the precooled solution of 1.84 g (0.004 ml) of 3-(*p*-bromobenzyl)-*d*-pilocarpinium bromide[1] in 40 ml of EtOH. The reaction mixture was left for 24 h at room temperature and the pH was kept at 8. The solvent was removed

(40°) under reduced pressure, the foamy residue was dissolved in 25 ml of cold EtOH–*i*-PrOH (1:3) and filtered, and the volume was reduced. Addition of 30 ml of Et_2O and cooling (48 h) precipitated 1.96 g (92.6%) of **25**. Recrystallization: dissolution in Me_2CO–absolute EtOH (1:3), addition of Et_2O to turbidity, and cooling.

3-(3′,4′-Dichlorobenzyl)-12-hydroxy-*d*-pilocarpiniumhydroxamic Acid Chloride (26), 3-(*p*-Methylbenzyl)-12-hydroxy-*d*-pilocarpiniumhydroxamic Acid Bromide (27), and 3-Ethyl-12-hydroxy-*d*-pilocarpiniumhydroxamic Acid Iodide (28). The molar ratio of the reagents, the procedure of preparation of the reaction mixture, and the time of reaction were as described for compound **25**. Compound **26**: 1.62 g of 3-(3′,4′-dichlorobenzyl)-*d*-pilocarpinium chloride[1] and 0.84 g of NH_2OH·HCl were used, yield 1.64 g (94%). Crystallization: dissolution with heating in Me_2CO–absolute EtOH (1:3), cooling, and addition of Et_2O up to turbidity. Compound **27**: 1.58 g of 3-(*p*-methylbenzyl)-*d*-pilocarpinium bromide[1] and 0.84 g of NH_2OH·HCl were used. The product (1.7 g, 97%) was crystallized as described for **26**. Compound **28**: 1.46 g of 3-ethyl-*d*-pilocarpinium iodide[1] and 0.84 g of NH_2OH·HCl were used, yield 1.58 g (93%) of soft solid which crystallized in cold Et_2O (2 weeks).

3-(*p*-Bromophenacylketoxime)-12-hydroxy-*d*-pilocarpiniumhydroxamic Acid Bromide (29). To a suspension of 0.56 g (0.008 mol) of NH_2OH·HCl in 15 ml of absolute EtOH, a solution of NaOEt (3%) was added dropwise to pH 8. After cooling (−5°) the mixture was directly filtered onto a precooled solution of 2 g (0.004 mol) of compound **9** in 45 ml of EtOH (pH 7.5–7.8). The mixture was left at 0° for 2 h and at room temperature for 24 h and filtered and the solvent evaporated (35°) under reduced pressure leaving a soft solid. Crystallization: dissolution in cold absolute EtOH–*i*-PrOH (1:3), filtration, concentration to a few milliliters, addition of Et_2O, and cooling, yield 1.8 g (84%). These manipulations were repeated 2–3 times.

3-(*p*-Nitrophenacylketoxime)-12-hydroxy-*d*-pilocarpiniumhydroxamic Acid Bromide (30) and 3-(*p*-Phenylphenacylketoxime)-12-hydroxy-*d*-pilocarpiniumhydroxamic Acid Bromide (31). The molar ratio of the reagents, the procedure of preparation of the reaction mixture, and the time of reaction were as described for compound **29**. Compound **30**: 1.98 g of compound **10** and 0.56 g of NH_2OH·HCl were used. Dissolution of the residue in some cold EtOH, filtration, addition of Et_2O to turbidity, and cooling (1 week) afforded 1.76 g (87.9%). Compound **31**: 2 g of compound **11** dissolved at 40° and 0.56 g of NH_2OH·HCl were used. The foamy residue was dissolved in 30 ml of absolute MeOH, Darco treated (50°, 30 min), and filtered and the solvent was removed. Dissolution of the new residue in absolute EtOH–*i*-PrOH (1:1), filtration, evaporation of the solvents, and cooling in Et_2O (several days) afforded 1.76 g (83%) of crystals. The product was then recrystallized.

Pharmacology. The spasmogenic activity was determined on a segment of guinea pig terminal ileum suspended in a 5-ml organ bath filled with Tyrode solution at 35°. Contractions were recorded on a kymograph with an isometric lever which gave 16× magnification. Contact time was 30 sec and time cycle 2 min. Contractions were induced with the reference compound pilocarpine (0.6 μg). The test compounds were applied in different concentrations, and the relative potency to pilocarpine was calculated by comparing the height of contractions. Antagonism to AcCh was determined by first eliciting control contractions with 0.1 μg of AcCh. A maximal dose of 10 μg or, in case that the compound tested exhibited spasmogenic activity, the maximal amount (which by itself did not cause contraction) was introduced, and 1 min afterward AcCh was added without washing. Antagonism was expressed as percent reduction of the control response to AcCh and represents the average of two separate assays. All values given are final concentrations per milliliter of bath fluid.

The antidotal action against tetraethylpyrophosphate (TEPP) poisoning was tested in male mice of 20-g body weight. The compounds were dissolved in saline or propylene glycol according to their solubility and injected at a volume of 0.2 or 0.05 ml, respectively. In preliminary experiments the maximal dose (up to 100 mg/kg), which together with atropine sulfate (25 mg/kg) did not cause any observable symptoms, was determined in groups of four mice. This dose was then injected ip simultaneously with the same amount of atropine sulfate 5 min prior to sc administration of $3 \times LD_{50}$ of the poison (groups of five mice). In order to compare the protective action of the newly synthesized substances with that of a known antidote, separate groups of mice were injected with 40 mg/kg of pralidoxime methanesulfonate (P2S) and 25 mg/kg of atropine sulfate. This drug combination conferred full protection whereas atropine sulfate alone did not protect the animals at all against this challenge.

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