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Synthesis and Cholinergic Activity of Some Structural Analogs of Pilocarpine†

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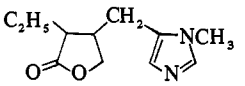
In an attempt to clarify the structural requirements of cholinergic activity of pilocarpine, the preparation of 14 analogs was successfully achieved through the Michael addition of the anions derived from 2-picoline, 4-picoline, 4-methylpyrimidine, and 2-methylpyrazine to $\Delta^{\alpha,\beta}$-butenolide, α-methyl- and α-ethyl-$\Delta^{\alpha,\beta}$-butenolide, cyclopentenone, and 2-ethylcyclopent-2-enone. Attempts to generate anions derived from 1,2-dimethylimidazole and 1,2-dimethylpyrrole were unsuccessful. The synthesis and cholinergic activity of these compounds are discussed. Only 3-ethyldihydro-4-(4-pyrimidylmethyl)-2(3*H*)-furanone (**9**) displayed parasympathomimetic activity.

The action of pilocarpine on the parasympathetic nervous system has been widely studied and this alkaloid has achieved limited use in the treatment of glaucoma. Surprisingly, little data are available in the literature with regard to structure-activity relationship requirements for its cholinergic activity. Pilocarpine is assumed to interact with the muscarinic receptor. Molecular configurations of pilocarpine were presented[1] indicating possible modes of receptor binding. The major binding sites have been assumed to be the lactone ring oxygen and carbonyl oxygen atoms and the imidazole nitrogen atoms in the protonated form.[2] Pilocarpine occurs naturally as the cis



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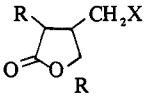
isomer and is more potent than the trans isomer (isopilocarpine).[3] The lactone ring appears to be essential for activity[4,5] while the imidazole ring can be cleaved without completely destroying activity.[6] A number of quaternary salt derivatives of pilocarpine have been recently shown to antagonize the effects of acetylcholine.[7] Although pilocarpine possesses a dual mode of action,[8] we felt it of interest to examine certain structural analogs in an attempt to clarify the binding sites of pilocarpine to the muscarinic receptor. We anticipated that modification of the imidazole ring, the lactone ring oxygen atom, and the alkyl group in the α position of the lactone ring would lead to derivatives which, depending upon their cholinergic activities, could provide information relating to the importance of these functions in cholinergic receptor binding.

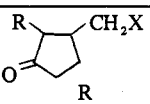
The imidazole ring of pilocarpine can be considered to possess two different types of nitrogen atoms, *viz.*, a pyridine-type nitrogen and a pyrrole-type nitrogen. Delocalization of the positive charge between these nitrogen atoms has been demonstrated upon protonation of 1-methylimidazole[9] and upon quaternization of pilocarpine.[2] To determine whether or not a delocalized positive charge is a requirement for cholinergic activity, we synthesized analogs of pilocarpine in which the imidazole ring is replaced by a pyridine ring while at the same time examining the importance of the ethyl group in the α position of the lactone ring (Table I). The role of the lactone ring oxygen atom could be examined by replacing the oxygen atom with a methylene group to give the corresponding

Table I. γ-Butyrolactone Derivatives



Compd	R	X
2	H	2-Pyridyl
3	CH_3	2-Pyridyl
4	C_2H_5	2-Pyridyl
5	H	4-Pyridyl
6	CH_3	4-Pyridyl
7	C_2H_5	4-Pyridyl
8	H	4-Pyrimidyl
9	C_2H_5	4-Pyrimidyl
10	H	2-Pyrazinyl
11	C_2H_5	2-Pyrazinyl

Table II. Cyclopentanone Derivatives



Compd	R	X
12	H	2-Pyridyl
13	C_2H_5	2-Pyridyl
14	H	4-Pyridyl
15	C_2H_5	4-Pyridyl

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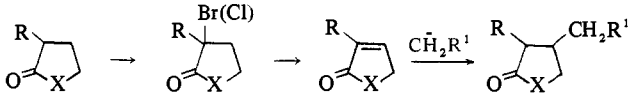
cyclopentanone analogs (Table II). Binding of these derivatives to the receptor at this site of the molecule is obviously no longer possible (*e.g.*, hydrogen bonding) but the integrity of the five-membered ring system is maintained.

Chemistry. The synthesis of these analogs was based on our observation that anions derived from 2- and 4-methylpyridine would add in a Michael fashion to $\Delta^{\alpha,\beta}$-butenolide and 2-cyclopentenone although in low yields.[10] This method was extended to α-substituted $\Delta^{\alpha,\beta}$-butenolides and 2-ethyl-2-cyclopentenone. Additionally, we have found that carbanions derived from 4-methylpyrimidine and 2-methylpyrazine serve as good nucleophiles in Michael addition reactions.

The general approach utilized in the synthesis of these analogs is outlined in Scheme I.

Scheme I



R = H, CH_3, or C_2H_5
X = O or CH_2

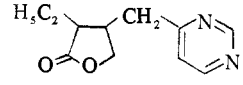
R^1 = 2- or 4-pyridyl, 4-pyrimidyl, or 2-pyrazinyl

The $\Delta^{\alpha,\beta}$-butenolides were prepared by dehydrohalogenation of the corresponding α-bromo derivatives with triethylamine in ether which, in turn, were prepared by bromination of the appropriate butyrolactone. α-Ethyl-γ-butyrolactone was prepared by treating α-acetyl-γ-butyrolactone with ethyl iodide in ethanolic sodium ethoxide modifying somewhat the procedure of Reppe, *et al.*[11] The other butyrolactones were commercially available. 2-Ethylcyclopentanone was prepared by the alkylation of the pyrrolidine enamine of cyclopentanone with ethyl *p*-toluenesulfonate but in only 22% yield. Even lower yields (6%) were obtained using ethyl iodide. Subsequent chlorination with sulfuryl chloride afforded 2-chloro-2-ethylcyclopentanone which partially dehydrohalogenated on vacuum distillation. Complete dehydrohalogenation was achieved with triethylamine in ether. 2-Cyclopentenone is commercially available.

The acidity of the α-hydrogen atoms of 2- and 4-picoline is widely recognized. The corresponding picolyl anions were generated with sodium amide and added to the conjugated carbonyl systems. To our knowledge, anions derived from 4-methylpyrimidine and 2-methylpyrazine have not been previously employed in Michael addition reactions. We found that these anions could be generated with sodium amide and would undergo Michael addition to the conjugated lactones and ketones employed in this series. This approach suffered only from the fact that the yields of products obtained were very low. 1,4 rather than 1,2 addition occurred as was evident from the ir spectra of the products. Carbonyl shifts from normal conjugated carbonyl to saturated carbonyl frequencies were readily apparent. The reported yields (Experimental Section) could have been adjusted upward by taking into consideration recovered 2- and 4-picoline, 4-methylpyrimidine, or 2-methylpyrazine. Attempts to generate carbanions from 1,2-dimethylimidazole (as a model compound for the synthesis of pilocarpine) and 1,2-dimethylpyrrole using both sodium amide and phenylsodium were unsuccessful.

The Michael addition of carbanions to cyclic C=CC=O systems has been shown to be stereoselective giving cis addition in some instances and trans addition in others.[12] The products of the reaction assume the most thermodynamically stable configuration. While we have been unable to prove conclusively the stereochemistry of our addition products because of overlapping multiplets of methylene and methine protons in the nmr spectra, we have assumed our products to exist mainly in the trans configuration. This is a reasonable assumption when one considers the rapid conversion of pilocarpine to isopilocarpine under basic conditions.[13] We are currently exploring methods of converting **2-15** to the cis forms. Although our compounds would not appear to possess the same configuration as pilocarpine, we felt it of interest to investigate the muscarinic activity of these analogs.

Pharmacology. Compounds **2-15** were tested for muscarinic activity as the nitrate salts in the guinea pig ileum. Activity was compared to that of acetylcholine at 5.5×10^{-6} *M*. Compounds **2**, **4-8**, **10-12**, and **15** were inactive at concentrations of 4 and 8×10^{-4} *M* and did not antagonize the effects of acetylcholine. Compounds **3**, **13**, and **14** at these concentrations surprisingly showed weak spasmolytic actions similar to that produced by papverine. Only **9** possessed muscarinic activity. On comparison of 50% maximum effects, **9** possessed muscarinic activity at 7.7×10^{-5} *M* equivalent to that produced by acetylcholine at 1.3×10^{-6} *M* (equipotent molar ratio = 60). However, **9**, like pilocarpine,[14] is a partial agonist (intrinsic activity = 0.7-0.8). Additionally, this activity was blocked by atropine and partially blocked by hexamethonium. It thus appears that **9** possesses a dual mechanism of action as does pilocarpine.[14] It is of interest that only **9** showed a parasympathomimetic effect since it differs from pilocarpine (with the exception of stereochemistry) in that the imidazole ring of pilocarpine has been replaced by a pyrimidine ring. The 1,3 relationship of nitrogen atoms in **9** and pilocarpine is similar. The relationship of the nitrogens in the pyrazine analog **11** is a 1,4 relationship and this compound is inactive. Compounds containing only one basic nitrogen atom (**2-7**) possess no muscarinic activity. The cyclopentanone analogs were also inactive (**12-15**).



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Experimental Section

Melting points were obtained on a Mel-Temp apparatus and are corrected. Ir data were recorded on a Perkin-Elmer 257 spectrophotometer and nmr data with a Jeolco C-60-HL spectrometer. Elemental analyses were performed by Alfred Bernhardt Mikroanalytisches Laboratorium, West Germany. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Refractive indices were obtained on a Bausch & Lomb apparatus. $\Delta^{\alpha,\beta}$-Butenolide was prepared by the method of Price and Judge.[15] The 2- and 4-picoline, 4-methylpyrimidine, and 2-methylpyrazine used were obtained commercially. Compounds **2**, **5**, **12**, and **14** were prepared as previously described.[10]

α-Methyl-$\Delta^{\alpha,\beta}$-butenolide. By following the general procedure used to prepare α-bromo-γ-butyrolactone,[15] 25.0 g (0.25 mol) of α-methyl-γ-butyrolactone, 2.9 g (0.093 g-atom) of red phosphorous, and 83.9 g (0.526 mol) of bromine yielded 25.0 g of a brown liquid. When the liquid was distilled at 56-58° (0.6 mm), 22 g (50%) of a colorless liquid was obtained [ir (liquid film) 1775 (C=O) and 1750 cm^{-1} (C=O $\Delta^{\alpha,\beta}$-butenolide)] indicating a mixture of brominated and dehydrobrominated products. By following the procedure used for the preparation of $\Delta^{\alpha,\beta}$-butenolide, 21.0 g of the mixture dissolved in 80 ml of anhydrous Et_2O and 14.2 g (19.1 ml, 0.14 mol) of triethylamine in 20 ml of anhydrous Et_2O were refluxed for 18 hr. After working

Table III. Physical Data

No.	% yield	Picrate mp,[a] °C	Formula[b]
3	6	150–152	$C_{11}H_{13}NO_2$
4	10	164–166	$C_{12}H_{15}NO_2$
6	7	121–123	$C_{11}H_{13}NO_2$
7	10	198–201	$C_{12}H_{15}NO_2$
8	11	171–173	$C_9H_{10}N_2O_2$
9	11	128–130	$C_{11}H_{14}N_2O_2$
10	10	140–141	$C_9H_{10}N_2O_2$
11	10	154–156	$C_{11}H_{14}N_2O_2$
13	12	190–192	$C_{13}H_{17}NO$
15	11	180–182	$C_{13}H_{17}NO$

[a] Recrystallized from EtOH. [b] All compounds were analyzed for C, H, and N as the free base.

up the reaction mixture, the crude residue (12.5 g) was distilled at 58–60° (2.25 mm) to yield 8.5 g (74%) of a colorless liquid [lit.[16] bp 97–97.6° (20 mm)].

α-Ethyl-γ-butyrolactone. A modified procedure similar to that of Reppe[11] was used. A solution of NaOEt prepared from 46.1 g (2 g-atoms) of Na metal in 1000 ml of absolute EtOH was added to a cooled (5°) mixture of 256.2 g (2.0 mol) of α-acetyl-γ-butyrolactone and 228.8 g (2.1 mol) of ethyl iodide. The mixture was stirred for 2 hr at room temperature, refluxed for 8 hr, and cooled. The inorganic salts were removed by filtration and the filtrate was concentrated under reduced pressure. The residue was dissolved in 500 ml of H_2O saturated with NaCl and extracted with Et_2O (2 × 500 ml). The combined ethereal extracts were dried over $MgSO_4$ and evaporated under reduced pressure. The residue was distilled at 112–115° (29 mm) to yield 151.0 g (66%) of a colorless liquid, n^{30}D 1.4350.

α-Ethyl-Δ$^{\alpha,\beta}$-butenolide. According to the procedure described for the preparation of α-bromo-γ-butyrolactone, 125.5 g (1.10 mol) of α-ethyl-γ-butyrolactone, 13.4 g (0.43 g-atom) of red phosphorous, and 390.0 g (133 ml, 2.44 mol) of bromine gave on work-up a residue which was distilled at 120–125° (4.5 mm) to yield 152.0 g (71.6%) of a colorless liquid: n^{30}D 1.4915; ir (liquid film) 1775 cm^{-1} (C=O saturated cyclic γ-lactone); nmr (CCl_4) δ 1.16 (t, 3, CH_3CH_2), 2.06 (q, 2, CH_3CH_2), 2.55 (m, 2, CH_2 at C_3), and 4.48 (m, 2, CH_2O at C_4). The product suffered partial dehydrohalogenation on distillation at 88–90° (0.4 mm) to yield a mixture of brominated and dehydrobrominated products as indicated by ir (liquid film) 1775 (C=O) and 1750 cm^{-1} (C=O Δ$^{\alpha,\beta}$-butenolide). Complete dehydrobromination was accomplished by refluxing the mixture and 97.0 g (0.96 mol) of triethylamine in 500 ml of anhydrous Et_2O for 18 hr. After working up the reaction mixture, the crude liquid residue, 86.5 g, was distilled at 124–126° (35 mm) to yield 67.6 g (75.4%) of a colorless liquid, n^{30}D 1.4627. *Anal.* ($C_6H_8O_2$) C, H.

2-Ethylcyclopentanone. A mixture of 91.5 g (0.66 mol) of cyclopentanone pyrrolidine enamine (prepared by the method of Stork[17]) and 13.6 g (0.68 mol) of ethyl *p*-toluenesulfonate in 250 ml of dry C_6H_6 was refluxed. After working up the reaction mixture, fractional distillation yielded 15.0 g (22.3%) of the product: bp 158–161° (lit.[18] bp 160–165°); n^{28}D 1.4375 (lit.[18] n^{20}D 1.4399); ir (liquid film) 1740 cm^{-1} (C=O cyclic five-membered ketone).

2-Ethyl-2-cyclopentenone. According to the method of Nedenskov, Taub, and Ginsburg,[19] a solution of 39.3 g (0.35 mol) of 2-ethylcyclopentanone in 200 ml of CCl_4 was slowly added to a solution of 31.5 ml (52.5 g, 0.38 mol) of sulfuryl chloride in 60 ml of CCl_4 over a period of 1 hr with cooling. Stirring was continued for an additional 2 hr and the mixture was stored overnight in the dark. The solvent was removed under reduced pressure and the residue was distilled at 70–74° (17 mm) to yield 31.8 g (62%) of a colorless liquid [lit.[19] bp 78–82° (20 mm)]; n^{22}D 1.4775 (lit.[18] n^{30}D 1.4658); ir (liquid film) 1755 (C=O saturated cyclic five-membered α-halogenated ketone), 1700 (C=O Δ$^{\alpha,\beta}$-unsaturated five-membered ketone), and 1650 cm^{-1} (C=C); nmr (CCl_4) showed a mixture of chlorinated and dehydrochlorinated products in a ratio of 5:6 respectively as determined by the ratio of the olefinic protons. Dehydrochlorination was accomplished by refluxing 29.2 g of the mixture and 24.3 g (0.24 mol) of triethylamine in 100 ml of anhydrous Et_2O overnight. After working up the reaction mixture, the residue was distilled at 68–74° (17 mm) to yield 14.4 g (65.3%) of the product [lit.[20] bp 69–72° (16 mm)]; n^{24}D 1.4755 (lit.[20] n^{20}D 1.4729).

General Procedure for Preparation of 3, 4, 6–11, 13, and 15. The title compounds were prepared by the general procedure described previously.[10] The crude reaction mixtures were purified by chromatography on neutral alumina. The products were obtained as oils from which the solid picrate derivatives were prepared. The ir and nmr spectral data were consistent with the proposed structures. Appropriate physical data and reaction yields are summarized in Table III.

Pharmacology. Terminal ileum from guinea pigs (300–500 g) was suspended in Krebs solution in a 30-ml organic bath aerated with 95% O_2 and 5% CO_2 at 37° Contractions of the ileum were recorded on a E and M Model 4 physiograph. Test compounds were given in doses of 80 and 160 μg/ml (expressed in molar concentration in test). Response of the ileum was compared to that of 5.5 × 10^{-6} *M* acetylcholine (1 μg/ml) allowing 4 min between successive comparisons.

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