tablets but has the advantage of markedly improved stability. In contrast to conventional tablets, which often develop marked intertablet dose variation within 1 month, the stabilized tablet maintains its content uniformity for long periods, even at 37 and 45°, thus assuring a more uniform and predictable dose to the patient.

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NOTES

Synthesis of Analogs Related to Pilocarpine

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Abstract □ Pilocarpine analogs with modification of the lactone ring were synthesized, and preliminary pharmacological evaluations indicate that several of the synthesized compounds possess interesting cholinergic activity.

Keyphrases □ Pilocarpine analogs—synthesized and screened for cholinergic activity □ Cholinergic activity—pilocarpine analogs with modified lactone rings synthesized and screened □ Structure–activity relationships—pilocarpine analogs synthesized and screened

Several naturally occurring alkaloids, *e.g.*, pilocarpine and muscarine, have been shown to possess potent cholinergic activity. This activity has been associated with the direct action of these substances on cholinergic receptors at synaptic junctions in target organs.

The nature of the cholinergic receptor is still not well understood. Presumably, acetylcholine must geometrically orient itself in such a manner that it binds to the receptor group at two points—the cationic and esteratic sites. Waser (1) postulated that the attachment of muscarinic agents to cholinergic receptors in smooth muscle involved two oxygen atoms and a quaternary nitrogen, having an optimum distance of approximately 4 Å separating the quaternary nitrogen from the ether or carbonyl oxygen. Jones (2) proposed that pilocarpine could assume a conformation similar to that of the active group postulated for the muscarinic receptor in ganglia.

A review of the literature indicates that structural requirements for cholinergic activity in pilocarpine analogs have not been studied adequately. It has been reported that an intact lactone ring is essential for activity. Moreover, the C-2 ethyl substitution on the lactone ring also has been reported as necessary for activity. Demethylation of pilocarpine on the imidazole moiety (pilocarpidine) showed reduced physiological activity (3). Brochmann-Hanssen *et al.* (4) found that various amine analogs, substituted for the imidazole moiety of pilocarpine, produced compounds with reduced activity. Quaternization of the imidazole ring giving the methyl iodides of both pilocarpine and isopilocarpine were synthesized (5) but their biological activity has not been reported. Ben Bassat *et al.* (6) presented data indicating that quaternization of pilocarpine derivatives on the imidazole moiety with a benzyl group possessed anticholinergic activity. This activity was augmented by the addition of *para*-substituents such as bromo, chloro, and methyl groups.

It appears that compounds possessing parasympathomimetic activity must contain a cationic site in the form of either a protonated tertiary or quaternary nitrogen separated from a region of high electron density approximately 4 Å away. Whether this site of high electron density in pilocarpine analogs is located about the carbonyl oxygen or the ether oxygen has not been determined. It appeared that minor alterations in the

lactone ring would provide useful information regarding the nature of the cholinergic receptor. It was proposed that the removal of the carbonyl function would further serve to provide information regarding the importance of that group without significantly altering the geometry of the pilocarpine molecule. In addition, it was felt that the lactam and *N*-methyllactam analogs should be prepared to determine whether the nitrogen in the lactam ring could be involved in an interaction with the receptor group.

RESULTS AND DISCUSSION

The methods used in the conversion of lactones to lactams generally involved the reaction of a lactone with ammonia in various polar solvents (7–9). It is possible that in the aminolysis of pilocarpine, either a hydroxyamide or a lactam derivative could occur. Gresham *et al.* (10) found that the reaction of methylamine with β-lactones in acetonitrile favored the formation of the β-amino acid, which subsequently could be cyclized to the lactam. In this study, however, when pilocarpine was reacted with anhydrous ammonia in acetonitrile at 0°, only the hydroxyamide derivative (I) was obtained. The hydroxyamide derivative was also obtained using anhydrous ammonia in absolute methanol, 95% ethanol, or aqueous ammonia. Reactions of pilocarpine with aqueous solutions of methylamine or isopropylamine gave similar results, yielding the respective *N*-methylhydroxyamide (II) or *N*-isopropylhydroxyamide (III) derivatives (Scheme I).

The lactam analog (IV) was successfully prepared by a reaction of pilocarpine (VII) with liquid ammonia at 200–210° according to the method of Späth and Lintner (11) (Scheme I). Similarly, the *N*-methyllactam analog (V) was prepared by the reaction of liquid methylamine and pilocarpine (VII) at 225° for 2 hr.

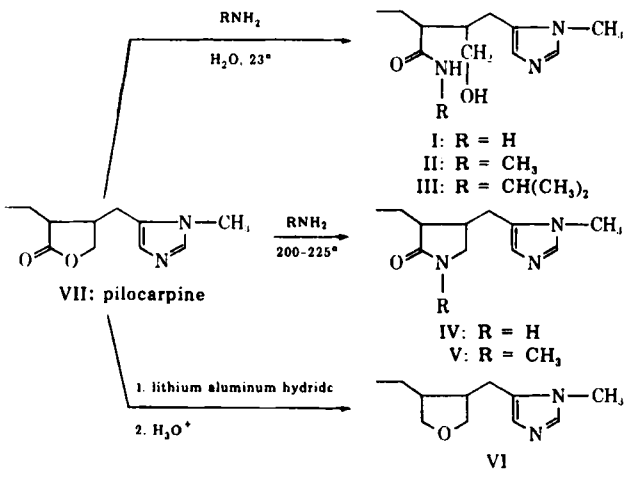
Pilocarpine reduction was accomplished using lithium aluminum hydride in anhydrous tetrahydrofuran (Scheme I). The IR spectra indicated a disappearance of the normal carbonyl peak. Elemental analysis was consistent with the calculated values for the tetrahydrofuran analog (VI).

The NMR spectrum of I indicated a two-proton doublet at δ 3.35 (—CH_2OH), whereas the spectrum of pilocarpine (VII) indicated a two-proton multiplet at δ 4.12 (—CH_2O—). In addition, the NMR spectrum of II indicated an absorption at δ 2.60 (N—CH_3, amide).

The NMR of IV gave a two-proton multiplet at δ 3.10–3.51. These data are consistent with the C-5 (N—CH_2—) protons reported for 2-pyrrolidone. Compound V gave a three-proton singlet at δ 2.90 (N—CH_3 lactam).

The appearance of two doublets at δ 3.48–3.61 and 3.98–4.12 (4H) for VI is consistent for the C-2 and C-4 protons on the tetrahydrofuran moiety.

Specific rotations (Table I) and NMR evidence do not clearly establish whether isomerization occurred during the synthesis of Compounds IV and V. The C-3 and C-4 protons on the 2-pyrrolidone ring of IV and V were assigned δ 2.19 (m, 1H, CH) and 2.52 (m, 1H, CH) and δ 2.28 (m, 1H, CH) and 2.50 (m, 1H, CH), respectively. The C-3 and C-4 protons on the tetrahydro-2-furanone ring of pilocarpine were assigned δ 2.33 (m, 1H, CH) and 2.83 (m, 1H, CH). The NMR peaks for the C-3 and C-4 protons in isopilocarpine were not clearly shown. Thus, these data are not sufficiently definitive to indicate whether isomerization had occurred during the synthetic procedure.



Scheme I

PHARMACOLOGY

Results of the pharmacological studies indicate that there is no significant parasympathomimetic activity for Compounds I, II, and III. However, the administration of IV and V in comparable doses to the pilocarpine control resulted in a sialogogue activity greater than pilocarpine itself. This activity could be antagonized by the administration of 0.4 mg./kg. i.v. of atropine. The administration of IV and V did not induce vomiting and defecation, with only a few drops of urine being discharged. However, unlike pilocarpine, IV and V produce hypertension which is not modified by the administration of atropine during the hypertensive phase.

The intravenous administration of VI resulted in sialogogue activity which could be antagonized by the administration of 0.4 mg./kg. i.v. atropine. When 3.0 mg./kg. VI was administered, a transient hypotension was produced, followed by a hypertensive phase with an attendant bradycardia. The cardiac rate became erratic with a fall in blood pressure. Atropine (0.4 mg./kg. i.v.) did not reverse the cardiovascular effects and the animal died in cardiac arrest. Compound VI exerts a more potent effect on the cardiovascular system than equal doses of pilocarpine.

The preliminary pharmacological evaluation of pilocarpine analogs in which the lactone ring was modified further substantiates the observation that an intact ring structure (lactone or its equivalent) is necessary for biological activity.

EXPERIMENTAL[1]

The pharmacological activity of pilocarpine analogs was determined in male dogs weighing between 13 and 20 kg.; the dogs were previously anesthetized with 30 mg./kg. sodium pentobarbital. Three experiments were run on each compound tested. The anesthetized animal was secured to a dog board; the electroencephalogram (EEG), through fronto-temporal leads, and the electrocardiogram (ECG), through lead I, were recorded using a physiograph. Blood pressure measurements were made through a cannulated left femoral artery, and the right femoral vein was exposed for injection of drug. Estimations of salivary flow were determined by tilting the head of the animal in such a manner that all salivary secretions would drain into a container.

All compounds to be tested were prepared in isotonic saline in concentrations expressed as the free base. The primary objective of the pharmacological study was to compare cholinergic activity of the analogs with the parent compound pilocarpine. A single dose of 3 mg./kg. was used for each compound tested.

Aminolysis of Pilocarpine by Ammonia, Methylamine, and Isopropylamine—Three grams (12.25 mmoles) of pilocarpine hydrochloride (VII) was suspended in 25 ml. of ammonium hydroxide (28%), and the mixture was stirred at room temperature. The crystals of VII slowly dissolved in the mixture. After 30 min., precipitation occurred in the form of a white crystalline suspension. Stirring was continued for an additional 2 hr., at which time a heavy white suspension was obtained. The suspension was filtered and the product was recrystallized from a minimum amount of 28% ammonium hydroxide, yielding 2.05 g. (74%) of 2-ethyl-3-hydroxymethyl-4-

[1] Melting points were determined on a Fisher-Johns melting-point apparatus and are uncorrected. Elemental microanalyses were performed by Elek Microanalytical Laboratory, Torrance, Calif., and C. F. Geiger, Ontario, Calif. All compounds were dried over phosphorus pentoxide under vacuum at 100° for at least 4 hr. IR spectra were run as mineral oil mulls between sodium chloride windows or as potassium bromide pellets on a Perkin-Elmer Infracord, model 137, and were consistent with proposed structures. Thin-layer chromatograms were run on Gelman ITLC (sg) in a solvent system composed of *n*-butanol–*n*-butyl ether–acetic acid (58:40:10) and were detected by spraying with Dragendorf's reagent. NMR spectra were run on a Hitachi Perkin-Elmer spectrometer, model R-24, and were consistent with assigned structures. Optical rotations were determined in a Cenco-Kern Polarmeter using water as a solvent at 23°.

Table I—Specific Rotations of Pilocarpine and Its Derivatives

Compound	$[\alpha]_D^{23}$
Pilocarpine nitrate (VII)	79.0° (c 1.0)
Isopilocarpine nitrate	36.9° (c 1.0)
Amide derivative (I)	47.4° (c 1.0)
Lactam nitrate (IV)	42.2° (c 1.0)
N-Methyllactam (V)	47.4° (c 1.0)
Tetrahydrofuran analog (VI)	26.3° (c 1.0)

(3-methyl-4-imidazolyl)-*n*-butyramide (I), m.p. 195–196°; IR_{max}: 1650 (C=O, amide) and 3200 (NH) cm.$^{-1}$; NMR (dimethyl sulfoxide-d_5): δ 3.35 (d, 2H, CH_2OH).

Anal.—Calc. for $C_{11}H_{19}N_3O_2$: C, 58.64; H, 8.50; N, 18.65. Found: C, 58.85; H, 8.66; N, 18.50.

Similarly, the *N*-methylamide derivative (II) was synthesized using a 30% aqueous solution of methylamine. The product was recrystallized from absolute ethanol, giving 2.25 g. (77%) of 2-ethyl-3-hydroxymethyl-4-(3-methyl-4-imidazolyl)-*N*-methyl-*n*-butyramide (II), m.p. 215–216°; IR_{max}: 1620 (C=O, amide) and 3200 (NH) cm.$^{-1}$; NMR (D_2O): δ 2.60 (s, 3H, N—CH_3, amide).

Anal.—Calc. for $C_{12}H_{21}N_3O_2$: C, 60.22; H, 8.84. Found: C, 60.34; H, 8.88.

The isopropylamide derivative (III) was prepared by stirring 3.0 g. of VII with a mixture of 25 ml. of isopropylamine and 10 ml. of water at room temperature until VII dissolved. The solution was placed in the refrigerator overnight at 4°. The solution was heated on a steam bath to remove the excess isopropylamine. Ten milliliters of water and decolorizing charcoal were added, and the mixture was filtered while hot. Upon cooling, fine white needles of 2-ethyl-3-hydroxymethyl-4-(3-methyl-4-imidazolyl)-*N*-(2-methylethyl)-*n*-butyramide (III) were obtained. The yield was 1.78 g. (55%), m.p. 224°; IR_{max}: 1640 (C=O, amide) and 3330 (NH) cm.$^{-1}$.

Anal.—Calc. for $C_{14}H_{25}N_3O_2$: C, 62.89; H, 9.42. Found: C, 62.74; H, 9.27.

3-Ethyl-4-(3-methyl-4-imidazolyl)methyl-2-pyrrolidone Nitrate (IV)—Crystals of VII were dried in a desiccator over calcium chloride under vacuum. To 2.0 g. (12.25 mmoles) of dried VII in a Parr high pressure reaction bomb was added an excess of liquid ammonia (approximately 10 ml.). The reaction bomb was sealed and heated in an oil bath at 200–210° for 2 hr. The reaction bomb was cooled to −5° and then vented, allowing the excess ammonia to escape. The reaction mixture was evaporated under vacuum, leaving an oily residue. To this residue was added 25 ml. of chloroform. The solution was dried with anhydrous calcium chloride and filtered. The chloroform filtrate was evaporated, leaving an oily residue which was dissolved in absolute ethanol and acidified with nitric acid. Upon acidification, IV precipitated. The product was recrystallized twice from absolute ethanol, giving 1.67 g. (53%) of 3-ethyl-4-(3-methyl-4-imidazolyl)methyl-2-pyrrolidone nitrate (IV), m.p. 160.5–161.5°; IR_{max}: 1675 (C=O, lactam) cm.$^{-1}$; NMR ($CDCl_3$): δ 2.72 (d, 2H, CH_2) and 6.70 (s, 1H, NH, lactam).

Anal.—Calc. for $C_{11}H_{18}N_4O_4$: C, 48.88; H, 6.71; N, 20.73. Found: C, 49.04; H, 6.87; N, 21.25.

The procedure for the preparation of 1-methyl-3-ethyl-4-(3-methyl-4-imidazolyl)methyl-2-pyrrolidone nitrate (V) is similar to that presented for the preparation of IV, substituting anhydrous liquid methylamine for ammonia with the reaction being carried out at 225° for 2 hr. The product was worked up in the usual way and recrystallized twice from absolute ethanol, yielding 1.85 g. (53%) of V, m.p. 174.5–175° dec.; IR_{max}: 1670 (C=O, lactam) cm.$^{-1}$; NMR ($CDCl_3$): δ 2.90 (s, 3H, CH_3 lactam) and 3.12–3.52 (m, 2H, C-5 N—CH_2).

Anal.—Calc. for $C_{12}H_{20}N_4O_4$: C, 50.69; H, 7.09; N, 19.71. Found: C, 50.41; H, 7.32; N, 20.21.

3-Ethyl-4-(3-methyl-4-imidazolyl)methyl Tetrahydrofuran Nitrate (VI)—Anhydrous ammonia was passed into a suspension of 5.0 g. (20.4 mmoles) of VII in 50 ml. of chloroform at 0°. The precipitate of ammonium chloride was filtered, and the chloroform solution evaporated to dryness under reduced pressure, leaving an oily residue of pilocarpine free base.

One gram (26.40 mmoles) of lithium aluminum hydride[2] was added with stirring to 50 ml. of dry tetrahydrofuran in a three-necked flask fitted with a condenser and dropping funnel. The reaction mixture was protected from moisture with drying tubes on all openings. The previously prepared pilocarpine base dissolved in dry tetrahydrofuran was added to the solution of lithium aluminum hydride over 3 hr. at 0°. After the reaction period, water was carefully added to the mixture. The resultant suspension was filtered and the tetrahydrofuran was evaporated under reduced pressure. The oily residue was refluxed with 25 ml. of 10% sulfuric acid for 2 hr. The reaction mixture was cooled to room temperature, adjusted to pH 9 with ammonium hydroxide, and then extracted with four 25-ml. portions of chloroform. The combined chloroform extracts were dried with calcium sulfate, filtered, and evaporated to near dryness, giving an oily residue. The residue was dissolved in 20 ml. of absolute ethanol and acidified with nitric acid. The alcoholic solution was gently heated to boiling, decolorized with charcoal, and filtered. Upon cooling, 2.0 g. (38% based on pilocarpine hydrochloride) of VI was obtained, m.p. 144–146°; IR_{max}: 1550, 1600 (C=C, C=N) cm.$^{-1}$; NMR (D_2O): δ 3.48–3.61 and 3.98–4 12 (2d, 4H, C-2 and C-5 CH_2).

Anal.—Calc. for $C_{11}H_{18}N_3O_4$: C, 51.35; H, 7.44. Found: C, 51.33; H, 7.59.

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[2] Alpha Inorganic.