THE INTERACTION OF THE ACETATES AND CARBAMATES OF SOME HETEROCYCLIC ALCOHOLS WITH ACETYLCHOLINESTERASE[1]

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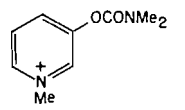
ABSTRACT

The *N,N*-dimethylcarbamates of 3-quinuclidinol, 1-methyl-3-piperidinol, and 1-methyl-4-piperidinol have been prepared and found to be weak inhibitors of acetylcholinesterase. The acetates of the above alcohols were also prepared and found to be moderately good substrates for this enzyme.

The elucidation of the structure (1) of the carbamate physostigmine (eserine), a potent anticholinesterase, led to a search for simpler analogues and a large number of alkyl- and dialkylcarbamates have been prepared and tested for anticholinesterase activity (2). Physostigmine contains a methylcarbamate group attached to an aromatic ring, and perhaps for this reason, the vast majority of the synthetic compounds also contain a carbamate group attached to an aromatic nucleus. An additional structural feature which seems to be necessary for high activity is another nitrogen atom (usually quaternized), which may be incorporated in the aromatic ring as in pyridostigmine (I), or directly attached to it as in neostigmine (II). Since there appears to be very little information in the literature concerning aliphatic carbamates (2), the anticholinesterase activities of the *N,N*-dimethylcarbamates of the saturated alcohols 3-quinuclidinol, 1-methyl-3-piperidinol, and 1-methyl-4-piperidinol have been assessed by measurement of their pI_{50}



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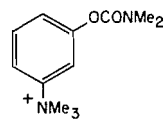

II

values on bovine acetylcholinesterase (the pI_{50} is the negative logarithm of the molar concentration of inhibitor which causes 50% inhibition of the enzyme activity in the system used). In addition, some experiments were performed to investigate the nature of the carbamate inhibition. The acetates of the above alcohols were also prepared and their effectiveness as substrates for acetylcholinesterase determined (by measurement of their Michaelis constants) to provide a comparison with the effectiveness of the carbamates as inhibitors of this enzyme.

1-Methyl-3-piperidyl *N,N*-dimethylcarbamate (III) was prepared from 1-methyl-3-piperidinol and dimethylcarbamyl chloride in pyridine solution (3) and this method proved satisfactory for the preparation of the corresponding 4-isomer (V). Both compounds discolored on storage and were further purified through their picrates, but V still turned slowly brown, even when stored in the freezer. Although some properties of 3-quinuclidinyl *N,N*-dimethylcarbamate (VII) (and its methiodide) have been discussed (4), its preparation does not appear to have been described in the literature. Repetition of the above reaction using 3-quinuclidinol gave a poor yield of impure carbamate and much

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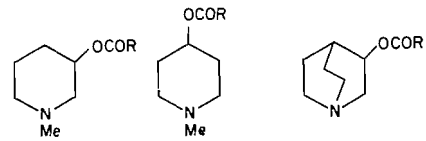

III R = NMe_2 V R = NMe_2 VII R = NMe_2
IV R = Me VI R = Me VIII R = Me

starting material remained. Separation of the mixture proved to be difficult and after a number of experiments it was apparent that pyridine was not a suitable solvent for this reaction. The required carbamate was eventually obtained in good yield using 2 moles of 3-quinuclidinol and 1.15 moles of dimethylcarbamyl chloride in chloroform solution, either at reflux temperature for 3 h or preferably at room temperature for 20 h. The distilled product showed strong absorption in the infrared at about the expected frequency (1 700 cm^{-1}) for a carbamate carbonyl group (5) and also weaker carbonyl absorption at about 1 760 cm^{-1}, and was conveniently purified, leading to material showing only a single carbonyl peak (1 702 cm^{-1}) through the picrate. The compound absorbing at 1 760 cm^{-1} appeared, albeit in very small yield, in all preparations of the carbamate; the compound was not separable from the carbamate by distillation, although it was separated by chromatography on alumina; it showed two carbonyl peaks in the infrared at 1 760 and 1 705 cm^{-1}, the latter more intense, and the spectrum differed in other respects from that of VII. Titration with hydrochloric acid indicated a pK_a (in aqueous ethanol) very similar to that (8.5) of VII and an equivalent weight of 280, and nitrogen analysis indicated a molecular weight of 288 for three nitrogen atoms. A structure for this compound has not been deduced from the available data.

The acetates (IV, VI, VIII) were prepared from the appropriate alcohol and acetic anhydride (6). Like the corresponding carbamate, 1-methyl-4-acetoxypiperidine (VI) discolored at room temperature, but after two months its infrared spectrum had not changed significantly. Although all three acetates were sufficiently stable at the pH (7.4) at which the kinetic measurements were made, they were unstable in aqueous solution at pH 9–10 and gave erratic equivalent weights on titration with hydrochloric acid. For example VIII (equiv. wt. 169) on rapid titration in 0.1 *N* saline solution gave an apparent equivalent weight of 210 and after standing for 5 h in the same solvent, a value of 338. Satisfactory equivalent weights were obtained in acetonitrile solution.

The pI_{50} values (25°, 2 min incubation time) obtained for the three carbamates, together with the Michaelis constants (K_m) and the V_{max} values, relative to acetylcholine = 1.00, for the corresponding acetates are given in Table I. The acetates are moderately good substrates for acetylcholinesterase, (the K_m for the acetylcholine–acetylcholinesterase system is 4.5×10^{-4} *M* (7)) but the carbamates are poor inhibitors and the pI_{50}

TABLE I

Values of pI_{50} for the inhibitors ($ROCONMe_2$) as well as Michaelis constants and V_{max} (relative to acetylcholine = 1.00) for the substrates (ROCOMe)

R	$ROCONMe_2$ pI_{50}	ROCOMe K_m (*M*)	ROCOMe V_{max}
3-Quinuclidinyl	2.75	$2.52 \pm 0.4 \times 10^{-3}$	0.69
1-Methyl-3-piperidyl	2.51	$2.44 \pm 0.27 \times 10^{-3}$	0.59
1-Methyl-4-piperidyl	2.40	$1.59 \pm 0.27 \times 10^{-3}$	0.42

values are considerably lower than that of physostigmine sulfate, which under the same conditions was found to be 6.26. It has been shown (8) that the inhibition of acetylcholinesterase by carbamates leads to a carbamylated enzyme, but the inhibition produced by the carbamates in the present work is weak enough to suggest that the heterocyclic nitrogen atom and not the carbamate group is almost wholly responsible. Support for this argument was obtained when under the same conditions the pI_{50} of 1-methylpiperidine was found to be 2.50.

In contrast to the physostigmine analogues referred to above, the carbamates described here do not contain a quaternary nitrogen atom. However, since at pH 7.4 these tertiary amine carbamates are predominantly cationic (approximate pK_a values determined by titration in saline were found to be 8.7, 9.1, and 9.5 for III, V, and VII respectively), conversion to the methiodide would not be expected to increase the inhibition (9), hence their weakness as inhibitors cannot be due to this. An example of an aliphatic carbamate with anticholinesterase activity is dimethylcarbamyl choline, but it is less potent than I, and especially II (8). Since the stereochemistry of dimethylcarbamyl choline must be favorable for reaction with the enzyme, its comparatively weak inhibitory power is probably caused by the carbonyl carbon atom being less electrophilic than that in I or II. The carbamates described here also contain a similar weakly electrophilic carbon atom, and in addition their stereochemistry is less favorable than that of dimethylcarbamyl choline since the acetates are weaker substrates than acetylcholine; hence the weakness of the carbamates as inhibitors is perhaps not surprising.

A limited number of experiments was performed with the present carbamates to investigate whether the inhibition was competitive or non-competitive. The rate of hydrolysis of acetylcholine was measured in the absence and presence of carbamate and the results fitted to a Lineweaver and Burk plot (10). Although in every case both the slopes and $1/v$ intercepts of the lines for the uninhibited and inhibited reactions were different, implying non-competitive inhibition, extrapolation of the lines to the $1/S$ axis showed that the K_m of the reaction was changed in the presence of inhibitor, indicating that the inhibition was neither completely competitive nor non-competitive (11).

EXPERIMENTAL

3-Quinuclidinyl N,N-Dimethylcarbamate

Dimethylcarbamyl chloride (24.6 g, 0.23 mole) was added to a solution of 3-quinuclidinol (50.8 g, 0.4 mole) in dry chloroform (250 ml), and the mixture was stirred at room temperature for 20 h. The 3-quinuclidinol hydrochloride was filtered off and dried (32.7 g, 0.2 mole) and the chloroform solution was washed with water (2 × 50 ml) and dried ($MgSO_4$). After removal of the chloroform the product was distilled to give a colorless liquid (27 g, 68%), b.p. 64–67° at 0.003–0.005 mm, n_D^{25} 1.4859. The picrate was prepared from the carbamate (8.6 g) in 50% ethanol–acetone (v/v). After two crystallizations from the same solvent mixture, there were obtained 10.2 g (52%) of yellow prisms, m.p. 174–177°.

Anal. Found: C, 45.13; H, 5.05; N, 16.30. $C_{16}H_{21}N_5O_9$ requires C, 44.97; H, 4.95; N, 16.30.

The picrate (10.5 g) was dissolved in cold saturated lithium hydroxide solution (50 ml) and the solution was extracted with chloroform (5 × 25 ml). The combined extracts were washed with water (50 ml) and dried ($MgSO_4$), the solvent was removed, and the residue was distilled to give 3-quinuclidinyl *N,N*-dimethylcarbamate (2.89 g, 62%) b.p. 75–78° at 0.05 mm, n_D^{25} 1.4481.

Anal. Found: C, 60.72; H, 9.25; N, 14.08; equiv. wt. 201. $C_{10}H_{18}N_2O_2$ requires C, 60.58; H, 9.15; N, 14.13; equiv. wt. 198.

1-Methyl-3-piperidyl N,N-Dimethylcarbamate

The method of Biel *et al.* (3) was used and the product was further purified through the picrate. The picrate was prepared from the carbamate (11.8 g) in 50% ethanol–acetone (v/v) and after one crystallization was obtained as yellow prisms (18.15 g, 69%) m.p. 162–164° (unchanged on recrystallization).

Anal. Found: C, 43.61; H, 5.36; N, 16.88. $C_{15}H_{21}N_5O_9$ requires C, 43.37; H, 5.10; N, 16.86.

The picrate (11.8 g) was converted to the free base as above. The crude product was distilled to give

1-methyl-3-piperidyl *N,N*-dimethylcarbamate (6.75 g, 81%), b.p. 83–84° at 1.1 mm, n_D^{25} 1.4640 (lit. b.p. 101–103 at 3 mm (3)).

Anal. Found: C, 57.99; H, 9.94; N, 15.20; equiv. wt. 191. $C_9H_{18}N_2O_2$ requires C, 58.03; H, 9.74; N, 15.04; equiv. wt. 186.

1-Methyl-4-piperidyl N,N-Dimethylcarbamate

This compound was prepared (65%) from 1-methyl-4-piperidinol and dimethylcarbamyl chloride following Biel *et al.* (3) and was further purified through the picrate. The picrate was prepared from the carbamate (5.6 g) in 50% ethanol–acetone (v/v) and after one crystallization was obtained as yellow needles (7.7 g, 58%), m.p. 198–200°, raised to 201–203° after two recrystallizations.

Anal. Found: C, 43.63; H, 5.19; N, 16.66. $C_{15}H_{21}N_9O_9$ requires C, 43.37; H, 5.10; N, 16.86.

The picrate (7.7 g) was converted to the free base in the usual way. The crude product was distilled to give 1-methyl-4-piperidyl *N,N*-dimethylcarbamate (2.53 g, 73%), b.p. 52–53° at 0.05 mm, n_D^{25} 1.4637.

Anal. Found: C, 57.88; H, 9.67; N, 15.13; equiv. wt. 191. $C_9H_{18}N_2O_2$ requires C, 58.03; H, 9.74; N, 15.04; equiv. wt. 186.

3-Acetoxyquinuclidine

The method of Grob *et al.* (6) was used. The product had b.p. 81–83° at 2.2 mm, n_D^{25} 1.4770 (lit. b.p. 113–115 at 11 mm, n_D^{25} 1.4675 (6)).

Anal. Found: C, 63.56; H, 9.05; N, 8.08; equiv. wt. 175. $C_9H_{15}NO_2$ requires C, 63.88; H, 9.05; N, 8.08, equiv. wt. 169.

1-Methyl-3-acetoxypiperidine

This compound (12, 13) was made by the method of Grob *et al.* (6) from 1-methyl-3-piperidinol and acetic anhydride; b.p. 76–78° at 11 mm; n_D^{25} 1.4470; equiv. wt. 160, calcd. 157 (lit. b.p. 32–40° at 0.2 mm; n_D^{25} 1.4471 (13); b.p. 72° at 11 mm, n_D^{20} 1.4512 (12)).

1-Methyl-4-acetoxypiperidine

The reaction of 1-methyl-4-piperidinol and acetic anhydride, following Grob *et al.* (6) gave the required compound (60%), b.p. 77–79° at 11 mm, n_D^{25} 1.4480.

Anal. Found: C, 61.09; H, 9.71; N, 8.65; equiv. wt. 160. $C_8H_{15}NO_2$ requires C, 61.12; H, 9.62; N, 8.91; equiv. wt. 157.

After several crystallizations from ethanol–acetone, the picrate was obtained as pale yellow needles, m.p. 170–171°.

Anal. Found: C, 43.20; H, 4.65; N, 14.66. $C_{14}H_{18}N_4O_9$ requires C, 43.52; H, 4.70; N, 14.50.

Kinetics

Kinetic measurements were made with a Polarad automatic recording titrator, model AT-2A at pH 7.4 and 25 ± 0.1 °C. The enzyme used was bovine erythrocyte acetylcholinesterase obtained from Winthrop Laboratories, New York. A stock solution, prepared by dissolving in water (5 ml) the material contained in one vial (20 000 units), was suitably diluted as required. The solvent used was water, 0.15 *M* in sodium chloride and 0.04 *M* in magnesium chloride, and the titrant was 0.018 *M* sodium hydroxide.

Determination of Michaelis Constants (K_m)

The acetates, made up in dilute hydrochloric acid and then brought to pH 7.4, were used in the following concentration ranges: IV, 2.0–6.0 × 10^{-3} *M*; VI, 3.0–14.0 × 10^{-3} *M*; VIII, 1.0–10.0 × 10^{-3} *M*. Initial rates of hydrolysis (v) were measured at a number of substrate concentrations (S) using enough dilute enzyme solution to ensure hydrolysis rates of about 0.010–0.015 ml sodium hydroxide/min. The volume of the reaction mixture was 10.2–10.6 ml depending upon the volume of substrate solution used. The Michaelis constant was calculated from a Lineweaver and Burk (10) plot ($1/v$ vs. $1/S$) using the method of least squares to obtain the best line.

Determination of pI_{50} Values

The three carbamates and 1-methylpiperidine were made up in dilute hydrochloric acid, and physostigmine sulfate was made up in water; the solutions were then adjusted to pH 7.4. Concentration ranges for the various inhibitors were: III, 0.95–4.55 × 10^{-3} *M*; V, 1.50–5.30 × 10^{-3} *M*; VII, 0.90–4.0 × 10^{-3} *M*; 1-methylpiperidine, 1.70–6.75 × 10^{-3} *M*; physostigmine sulfate, 4.0–8.0 × 10^{-7} *M*. Acetylcholine bromide (initial concentration approximately 8.3 × 10^{-4} *M*) was used as substrate, and the initial rate of hydrolysis was measured using enough dilute enzyme solution to produce a sodium hydroxide uptake of about 0.05 ml/min. The initial rate of hydrolysis was then measured after incubating the enzyme for 2 min with different concentrations of inhibitor before addition of the substrate. Concentrations of inhibitor and substrate were corrected for the total volume of water in the cell (10–11 ml). Inhibitor concentrations were then plotted against sodium hydroxide uptake and the inhibitor concentration at one half the sodium hydroxide uptake for the uninhibited reaction was read off.

Nature of Carbamate Inhibition

The carbamates were made up in dilute hydrochloric acid as before and the following concentrations were used: III, 1.7 × 10^{-3} *M*; V, 4.3 × 10^{-3} *M*; VII, 1.6 × 10^{-3} *M*. The substrate was acetylcholine bromide

which was used in the concentration range 2.0–10.0 $\times$ 10^{-4} *M*. Initial rates of hydrolysis were determined at a number of substrate concentrations, and then determined again after the enzyme had been incubated for 2 min with inhibitor. To determine the nature of the inhibition, $1/v$ was plotted against $1/S$ for both the inhibited and uninhibited reactions.

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