Interaction of 3-Quinuclidinol and Its Derivatives with Acetylcholinesterase

R. PYTTEL and J. B. ROBINSON▲

Abstract ☐ The preparation of R-(−)-3-quinuclidinol and its acetate ester is reported. These compounds, together with their racemates, were tested as inhibitors and substrates of the enzyme acetylcholinesterase. The results suggest that the enzyme active site is stereospecific toward R-(−)-3-quinuclidinol hydrochloride when acting as an inhibitor. Both (±)- and R-(−)-3-acetoxyquinuclidine hydrochloride are substrates of the enzyme, but the S-enantiomer probably has some affinity for the enzyme also.

Keyphrases ☐ 3-Quinuclidinol and derivatives—tested as inhibitors and substrates of acetylcholinesterase ☐ Acetylcholinesterase—3-quinuclidinol and derivatives tested as substrates and inhibitors ☐ R-(−)-3-Acetoxyquinuclidine hydrochloride—tested as substrate of acetylcholinesterase

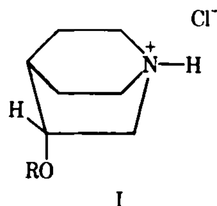
The activity of the enantiomers of 3-acetoxyquinuclidine methiodide, when tested as muscarinic agonists or as substrates for the enzyme acetylcholinesterase (1), has been shown to reside predominantly in the enantiomer of the R-configuration (2). However, the tertiary amine derivative, (±)-3-acetoxyquinuclidine hydrochloride, has been shown to be a more potent muscarinic agonist than its quaternary analog (3, 4) and to act as a substrate for the enzyme acetylcholinesterase (5).

The present work reports the preparation of R-(−)-3-quinuclidinol hydrochloride (I: R = H) and R-(−)-3-acetoxyquinuclidine hydrochloride (I: R = CH_3CO), together with the corresponding racemates, and the testing of these compounds as inhibitors and substrates, respectively, of the enzyme acetylcholinesterase.

EXPERIMENTAL[1]

(±)-3-Quinuclidinol [m.p. 220–222°, lit. (6) m.p. 221–223°] hydrochloride [m.p. >300° dec., lit. (6) m.p. >300°] was resolved according to literature methods (1, 6) using camphor-10-sulfonic acid. Four recrystallizations gave a salt, $[\alpha]_D^{22}$ −0.73° (c, 6.8, water), from which R-(−)-3-quinuclidinol was isolated, m.p. 219–220° (benzene), $[\alpha]_D^{22}$ −36° (c, 3.1, 1 *N* HCl) [lit. (1) $[\alpha]_D^{22}$ −37.1° (c, 3.0, 1 *N* HCl)]. The hydrochloride had m.p. >300° dec. (ethanol), $[\alpha]_D^{22}$ −27.6° (c, 1.1, water).

On acetylation (1), R-(−)-3-quinuclidinol gave R-(+)-3-acetoxyquinuclidine, b.p.$_{11}$ 101–102°; $[\alpha]_D^{22}$ +24.1° (c, 2.5, water) [lit. (1) $[\alpha]_D^{22}$ +28.5 (c, 3.0, ethanol)]. The IR spectrum (liquid film) was



I

Table I—Enzymatic Hydrolysis and Inhibition of Ox Erythrocyte Acetylcholinesterase

Substance	$K_{m\,app} \times 10^4\,M$	$V_{max} \times 10^7$ *M*/min.	$K_i \times 10^3\,M$
Acetylcholine	2.7	9.5	—
(±)-3-Quinuclidinol hydrochloride	—	—	3.4
R-(−)-3-Quinuclidinol hydrochloride	—	—	1.74
(±)-3-Acetoxyquinuclidine hydrochloride	11.0	1.9	—
R-(−)-3-Acetoxyquinuclidine hydrochloride	4.2	1.8	—

identical to that of an authentic sample. The hydrochloride salt had m.p. 200–201°, $[\alpha]_D^{22}$ −4.50° (c, 1.8, CH_3OH).

(±)-3-Acetoxyquinuclidine had a boiling point of 103–104°/10 mm. [lit. (7) b.p. 113–115°/11 mm.] and an IR spectrum (liquid film) identical to that of an authentic sample.

Bovine erythrocyte acetylcholinesterase[2] was used, and the compounds were tested as substrates or inhibitors as previously described (1, 8). All solutions of quinuclidine derivatives were freshly prepared immediately before use, and the solutions were adjusted to pH 7.4 with the appropriate titrant before being made up to the required volume. Values of $K_{m\,app}$, V_{max}, and K_i were calculated from conventional double-reciprocal plots (Table I).

DISCUSSION

Both (±)- and R-(−)-3-quinuclidinol hydrochloride are weak competitive inhibitors of the enzyme-catalyzed hydrolysis of acetylcholine, the interaction being highly stereoselective in favor of the enantiomer having the R-configuration. Theoretical calculations (9) suggest that the S-(+)-enantiomer has some very weak inhibitory activity, but the precision of such calculations under the present conditions is extremely limited.

Tests of the substrate specificity of 3-acetoxyquinuclidine hydrochloride show a similar selectivity, the activity being resident primarily in the R-(−)-enantiomer. The derived Lineweaver–Burk plots for the racemate and R-(−)-enantiomer do suggest, however, some slight enzyme inhibitory effect of the S-(+)-enantiomer and this is reflected in the computed values of V_{max} and $K_{m\,app}$ (Table I). Thus, S-(+)-3-acetoxyquinuclidine hydrochloride would appear to have some affinity for the enzyme active site. This is in contrast to the previously reported results (1) on the enantiomers of 3-acetoxyquinuclidine methiodide, in which the value of $K_{m\,app}$ was, within experimental error, equal for the (±)- and R-(−)-enantiomers (8.12 and 7.95 × 10^{-4} *M*, respectively) and V_{max} for the R-(−)-enantiomer was approximately double that of the racemate (1.50 and 0.835 × 10^{-6} *M*/min., respectively), indicating a lack of affinity of S-(+)-3-acetoxyquinuclidine methiodide for the enzyme active site.

REFERENCES

(1) J. B. Robinson, B. Belleau, and B. Cox, *J. Med. Chem.*, **12**, 848(1969).

(2) B. Belleau and P. Pauling, *ibid.*, **13**, 737(1970).

(3) M. D. Mashovsky and C. A. Zaitseva, *Arzneim.-Forsch.*, **18**, 320(1968).

[1] Melting points were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected. IR spectra were recorded on a Beckman IR-8 spectrophotometer, and optical rotations were recorded on a Bellingham and Stanley model D polarimeter.

[2] Sigma.

(4) A. K. Cho, D. J. Jenden, and S. I. Lamb, *J. Med. Chem.*, **15**, 391(1972).
(5) K. B. Shaw, *Can. J. Chem.*, **43**, 3264(1965).
(6) L. H. Sternback and S. Kaiser, *J. Amer. Chem. Soc.*, **74**, 2215 (1952).
(7) C. A. Grob, A. Kaiser, and E. Renk, *Helv. Chim. Acta*, **40**, 2170(1957).
(8) J. B. Kay and J. B. Robinson, *J. Pharm. Pharmacol.*, **21**, 145 (1969).
(9) H. J. Schaeffer, R. N. Johnson, M. A. Swartz, and C. F. Schwender, *J. Med. Chem.*, **15**, 456(1972).

ACKNOWLEDGMENTS AND ADDRESSES

Received June 28, 1972, from the *Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada.*
Accepted for publication October 27, 1972.
Supported by Grant MA-4033, Medical Research Council of Canada.
▲ To whom inquiries should be directed.

Biochemical Interactions of Dimethyl Sulfoxide I: Respiratory Inhibition

ROBERT L. BEAMER▲, JAMES E. WYNN, and RAFAEL E. LEDESMA*

Abstract ☐ The effect of dimethyl sulfoxide upon rabbit liver homogenates was determined with the Warburg respirometer. Lineweaver–Burk treatment of the data indicated that the respiration was competitively inhibited by dimethyl sulfoxide.

Keyphrases ☐ Dimethyl sulfoxide—effect on rabbit liver homogenates, Warburg respirometer ☐ Respiratory inhibition—dimethyl sulfoxide, effect on rabbit liver homogenates, Warburg respirometer ☐ Warburg respirometer—effect of dimethyl sulfoxide on rabbit liver homogenates

During recent years there has been widespread interest in the clinical effects and applications of dimethyl sulfoxide. Toxicological studies have demonstrated a low acute toxicity, as indicated by higher LD_{50} values from intravenous, oral, and subcutaneous administration (1, 2).

Long-term administration of dimethyl sulfoxide produced changes in tissue morphology, such as reduced relucency of the lens cortex in dogs (3) and changes in hepatic cell morphology in rats (4). Teratogenic effects of dimethyl sulfoxide have been observed in various experimental animals (*e.g.*, chicks and golden hamsters) (5–7). However, few investigations have been reported concerning the effects of dimethyl sulfoxide on biochemical systems (8). This study was designed to provide preliminary data concerning the effects of dimethyl sulfoxide at the enzyme level.

EXPERIMENTAL

Materials—*Dimethyl Sulfoxide*—Experimental drug grade dimethyl sulfoxide[1] was redistilled under reduced pressure (100° at 2 mm.).

Serum Substrate—The substrate was prepared by diluting lyophilized bovine serum[2] to a specific gravity of 1.0210 with glass-redistilled water.

Apparatus—*The Warburg Instrument*—A Warburg respirometer[3], equipped with one-arm, 15-ml. reaction vessels with center wells, was used. The center wells contained 0.2 ml. of 15% KOH and a Whatman No. 1 filter paper wick. The amplitude of flask travel was 4 cm. at a rate of 60 c.p.m.; the water bath temperature was 37°. Warburg manometers were filled with Kreb's manometric fluid (9, 10), density 1.0334.

C-H-N Analyzer—A carbon-hydrogen-nitrogen analyzer[4] was utilized to determine the nitrogen content of animal tissue.

Preparation of Animal Tissues—Four albino rabbits, weighing approximately 1.4 kg. (3 lb.) each and in apparent good health, were quarantined for 2 weeks; they were fed rabbit pellets[5] and given water *ad libitum*. Each day they were examined for symptoms of disease.

At the end of the quarantine period, the animals were sacrificed by placing them in a closed container with dry ice. The livers immediately were removed, rinsed in cold Ringer's solution, weighed, inspected for gross abnormalities, and placed in cold (0–4°) 0.06 *M* sodium phosphate buffer at pH 7.4 (1 ml. buffer/g. wet tissue). The wet liver appeared normal and weighed 54.80–56.51 g. Small segments of each liver were dried at 100°, and their dry-to-wet ratios and nitrogen contents were determined. The mean value of the dry-to-wet weight ratio was 0.7735 with a standard deviation of 0.256; the mean nitrogen content was 10.62% with a standard deviation of 0.21.

The livers were homogenized individually in a cold Virtis homogenizer, pooled, and further homogenized in a test tube homogenizer[6]. The homogenates were placed in test tubes, sealed with sulfide-free rubber stoppers, and rinsed with glass double-distilled water. The homogenates were stored in a freezer.

Inhibition Studies—Inhibition studies were performed using the Warburg technique (11). The homogenates were thawed for approximately 60 min. at 6°. A constant liver homogenate volume containing 3.84×10^{-2} g. of nitrogen was used throughout. The flasks and their contents were incubated for 10 min. at 37° prior to initiating the reaction. The lyophilized serum substrate concentration for apparent maximal liver respiration (apparent V_{max}) (12) was found to be 1.7×10^{-2} g./ml.

Studies were then performed to determine the minimal concentration of dimethyl sulfoxide that would produce significant respiratory inhibition. The results were analyzed statistically and the kinetics of inhibition were evaluated by Lineweaver–Burk plots con-

[1] Crown Zellerbach Corp.
[2] Nutritional Biochemicals Corp.
[3] Model 18U, Precision Scientific.
[4] Hewlett-Packard model 185.
[5] Purina.
[6] Model 8335, Ace Glass Co.