Open-Chain Analogs of Muscarine Derivatives

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Abstract □ A short series of choline ethers containing a free or esterified OH group was prepared as open-chain analogs of desmethylmuscarine stereoisomers. The acetate ester of one alcohol showed some degree of muscarinic activity. Contrary to the implication of an old literature report, 2-(dimethylaminoethoxy)-ethanol methiodide exhibited a low order of muscarinic activity.

Keyphrases □ Choline ethers—prepared as open-chain analogs of desmethylmuscarine stereoisomers, evaluated for muscarinic activity □ Muscarine derivatives—synthesis and evaluation of open-chain desmethylmuscarine stereoisomer analogs □ Desmethylmuscarine stereoisomer analogs—synthesis of choline ether series, muscarinic activity evaluated

In the course of other work, dimethylaminoethoxyethanol (I) was required. The quaternary salt (II) of this compound closely resembles an open-chain analog of desmethylmuscarine or desmethylepimuscarine (III), which was reported (1) to possess some weak muscarinic properties.

Callsen (2) described the therapeutic effect of II as being similar to that of arecoline, and he suggested possible use as a laxative by subcutaneous injection. The age and questionable validity of this report suggested the evaluation of II for muscarinic effect. The acetate (IV) and carbamate (V) esters were prepared, on the basis that the acetate ester of muscarine is markedly muscarinic (1).

Compound I has been prepared from dimethylamine in 75% yield by treatment with 2-chloroethoxyethanol (3) and in poor yield by treatment with ethylene oxide (4). In the present work, the most convenient method utilized a reaction between dimethylaminoethanol and ethylene oxide in the presence of base. A reaction of dimethylamino-2-propanol with propylene oxide gave a poor yield of VI. The quaternary derivative (VII) was prepared as an open-chain analog of a muscarine-like system.

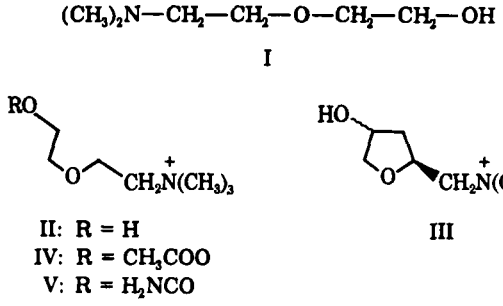
IR and NMR data on all of the compounds prepared were consistent with the structures proposed.

PHARMACOLOGY

The compounds were evaluated for muscarinic effect in a superfused guinea pig ileum preparation (5). Muscarinic activity relative to acetylcholine (Table I) was estimated using the method of Litchfield and Wilcoxon (6). At least three different animals were used for assaying each compound. Muscarinic effects were blocked by atropine (0.2 mcg./ml.) but not by hexamethonium (20 mcg./ml.). The entire series revealed no properties of pharmacological interest; the claim of the older report (2) that II mimics acetylcholine or arecoline with respect to action on smooth muscle tissue was verified, but activity relative to acetylcholine was very low.

Table I—Relative Muscarinic Activity

Compound	Relative Potency (95% *CI*)
Acetylcholine	1.0
II	0.003 (0.0007–0.02)
IV	0.004 (0.0006–0.013)
V	0.0003 (0.00007–0.018)
VII	<0.00001



EXPERIMENTAL[1]

2-(Dimethylaminoethoxy)ethanol (I)—Ethylene oxide (132 g., 3 moles) and sodium methoxide (5 g.) in 1.5 l. of methanol were added slowly to 222.5 g. (2.5 moles) of dimethylaminoethanol in 2 l. of methanol, and the resulting mixture was refluxed for 10 hr. The methanol was removed under reduced pressure, and the liquid residue was distilled at 95° (8 mm.) to afford 120 g. (36%) of product [lit. (4) b.p. 78–79° (3.5 mm. Hg)].

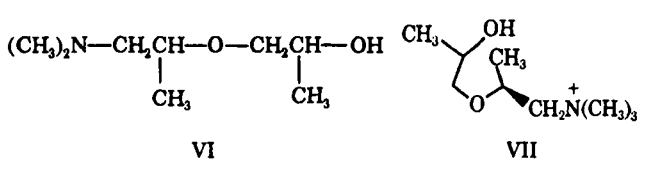
2-(Dimethylaminoethoxy)ethanol Methiodide (II)—Compound I (13.3 g., 0.1 mole) was stirred with a 2 *M* excess of methyl iodide in 100 ml. of ether at room temperature for 24 hr. The solid which separated was recrystallized from *n*-butanol and then from acetone to yield 24 g. (87%) of crystals, m.p. 116–117° [lit. (2) m.p. 116–117°].

2-(Dimethylaminoethoxy)ethyl Acetate Methiodide (IV)—Compound II (2.5 g., 0.0088 mole) was stirred in 25 ml. of acetic anhydride for 10 days at room temperature. The resulting solution was poured into an excess of ether, and the solid which separated was collected and recrystallized from acetone to yield 2.0 g. (72%), m.p. 123–125°.

Anal.—Calc. for $C_9H_{20}INO_2$: C, 34.07; H, 6.30; I, 40.00; N, 4.40. Found: C, 34.16; H, 6.30; I, 39.80; N, 4.50.

2-(Dimethylaminoethoxy)ethyl Carbamate Methiodide (V)—Trifluoroacetic acid (4.56 g., 0.04 mole) was added dropwise with stirring to 5.5 g. (0.02 mole) of II and 3.08 g. (0.04 mole) of sodium cyanate in 100 ml. of methylene chloride. The resulting mixture was stirred at room temperature until solution occurred and then was stirred overnight. The crystals which separated were recrystallized from *n*-butanol–ether to yield 2.5 g. (39%) of needles, m.p. 158–160°.

Anal.—Calc. for $C_8H_{19}IN_2O_3$: C, 30.17; H, 5.90; I, 39.90; N, 8.80. Found: C, 29.96; H, 5.99; I, 40.10; N, 8.70.



[1] Boiling points are uncorrected. Melting points were determined in open glass capillaries using a Thomas-Hoover Uni-Melt apparatus and are corrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn.

1-Dimethylamino-2-(2-hydroxy-1-propyloxy)propane (VI)—1-Dimethylamino-2-propanol (103 g., 1 mole) and 65 g. (1.1 mole) of propylene oxide were refluxed in 800 ml. of methanol for 18 hr. Methanol was evaporated under reduced pressure and the residue was fractionated. The fraction with a boiling point of 65° (0.2 mm. Hg) was the desired product; the yield was 24 g. (15%).

Anal.—Calc. for $C_8H_{19}NO_2$: C, 59.63; H, 11.79. Found: C, 59.86; H, 11.89.

1-Dimethylamino-2-(2-hydroxy-1-propyloxy)propane Methiodide (VII)—Compound VI (1 g., 0.006 mole) and 3 g. (0.021 mole) of methyl iodide in 25 ml. of ether containing a few drops of methanol were kept at 0° for 2 days. The resulting crystals were recrystallized from acetone–ether and then from acetone to afford 0.93 g. (55%) of material, m.p. 147–148°.

Anal.—Calc. for $C_9H_{22}INO_2$: C, 35.64; H, 7.26; I, 41.91; N, 4.60. Found: C, 35.79; H, 7.21; I, 41.72; N, 4.52.

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Colorimetric Method for Determination of Guanazole in Blood and Urine

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Abstract □ A colorimetric method, based on diazotization and coupling, for the determination of guanazole in aqueous solution, blood, and urine is described. The average relative standard deviation of the method is about 0.9%. Determination in blood involves precipitation of proteins with trichloroacetic acid and diazotization and coupling of guanazole in the filtrate. Determination in urine involves separation of guanazole from the diazotizable, naturally occurring urinary aromatic amines using ion-exchange chromatography.

Keyphrases □ Guanazole (3,5-diamino-1,2,4-triazole)—colorimetric analysis in aqueous solution, blood, and urine □ 3,5-Diamino-1,2,4-triazole (guanazole)—colorimetric analysis in aqueous solution, blood, and urine □ Colorimetry—analysis, guanazole in aqueous solution, blood, and urine

Guanazole (3,5-diamino-1,2,4-triazole)[1] is an experimental drug beneficial in the treatment of adult acute leukemia (1–6). No methods for its determination are published in the literature. This paper describes a method for the determination of guanazole in aqueous solution, blood, and urine. The method is based on diazotization and coupling (7, 8).

EXPERIMENTAL

Apparatus—Spectra and absorbance measurements were made with a recording spectrophotometer[2]. Matched cells with a 1-cm. optical path were used.

Materials—Dowex-50W (H^+) (12% cross-linkage, 200–400 mesh) was prepared as previously described (9). Dowex-1 (Cl^-) (10% cross-linkage, 200–400 mesh) was sedimented to remove the very fine and very coarse particles. Dowex-1 (Cl^-) was converted to Dowex-1 formate by passage of sodium formate and formic acid over the resin followed by appropriate water washes.

Guanazole was obtained in bulk[3].

The following reagent grade chemicals were used: concentrated hydrochloric acid, sodium nitrite, ammonium sulfamate, *N*-(1-naphthyl)ethylenediamine dihydrochloride, dimethyl sulfoxide, and trichloroacetic acid.

Procedure for Aqueous Solution—Two milliliters of 5.0 *N* HCl and 2 ml. of the guanazole aqueous solution were added to 2 ml. of dimethyl sulfoxide. Then 0.2 ml. of 0.5% $NaNO_2$ was added to this mixture. Immediately 0.2 ml. of 5.0% ammonium sulfamate was added and mixed thoroughly, followed by 0.2 ml. of 0.5% *N*-(1-naphthyl)ethylenediamine dihydrochloride[4]. After 10 min. the absorbance of the sample was compared with a blank at 504 nm. in the spectrophotometer. Quantitative comparisons were made with a guanazole standard curve prepared in a similar fashion.

Procedure for Blood—Eight milliliters of a 15% solution of trichloroacetic acid was added to 2 ml. of blood. This was then filtered through a prewashed filter paper, which was then washed with a few milliliters of distilled water. The filtrate was made up to 25 ml. in a volumetric flask. Two milliliters of the filtrate was added to 2 ml. of 5.0 *N* HCl, diazotized and coupled as described for the aqueous solution, and compared with a blank made up with water and the other reagents.

Procedure for Urine—Fifty milliliters of the urine sample was passed through a Dowex-1 formate column (1.0 cm. o.d. × 10 cm.) under a pressure of 1.0–2 p.s.i. The column was washed with 50 ml. of deionized water, the effluent and wash were combined, and the pH was adjusted to 1–2. The acidified sample was passed through a Dowex-50W (H^+) column (1.0 × 10 cm.) by gravity. The eluate was discarded, and the column was then washed with 50 ml. of 0.1 *N* HCl. Guanazole was eluted from the column with 200 ml. of 2.5 *N* HCl. This chromatographic procedure separates guanazole from the diazotizable, naturally occurring urinary aromatic amines. An

[1] NSC 1895.
[2] Cary 14.
[3] From the National Cancer Institute, U. S. Public Health Service.
[4] This solution is to be kept in a dark bottle and should be prepared fresh every week.