were filtrated with suction, washed with *i*-PrOH and $(Et)_2O$, dried, and purified by recrystallization.

In the case of highly insoluble products, 0.04 mol of hydrazide V was dissolved at 60° in 200–400 ml of ethylene glycol, 0.04 mol of 3-(5-nitro-2-furyl)acrolein in 68 ml of tetrahydrofuran was added, and the mixture was heated for 30 min at 60°. Crystallization began at this temperature (3). In some cases, the crude products were obtained after addition of $(Et)_2O$ (8) or H_2O (12) to the mother liquors.

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References

(1) M. C. Dodd and W. B. Stillman, *J. Pharm. Exp. Ther.*, **82**, 11 (1944).
(2) A. Burger, "Medicinal Chemistry," 2nd ed, Interscience, New York, N. Y., 1960, p 96.
(3) *Beilstein*, 4th ed, **4**, 780 (1919).
(4) See ref 3, p 819.
(5) D. F. Elliott, *J. Chem. Soc.*, 589 (1949) [*Chem. Abstr.*, **43**, 9033*b* (1949)]; F. W. Holly and C. A. Stammers, U. S. Patent 2,772,281 (Nov 27, 1956) [*Chem. Abstr.*, **51**, 8145*b* (1957)].
(6) R. W. Chambers and F. H. Carpenter, *J. Amer. Chem. Soc.*, **77**, 1522 (1955) [*Chem. Abstr.*, **50**, 2426*c* (1956)]; G. Losse and H. Jeschkeit, *Chem. Ber.*, **90**, 1275 (1957) [*Chem. Abstr.*, **54**, 13008*d* (1960)].
(7) M. Szekerke, M. Kajtar, and V. Bruckner, *Chem. Abstr.*, **64**, 17706*d* (1966).
(8) *Beilstein*, 4th ed, **14**, 499 (1931).
(9) Organon, Dutch Patent Application 6,509,727 (Jan 30, 1967); Thibaut Gibbs Cie, French Patent 1,121,631 (Aug 22, 1956) [*Chem. Abstr.*, **54**, 1345*c* (1960)].
(10) Institut Organischesko Sinteza an Latvisloi, British Patent 1,164, 588 (Feb 22, 1968).

Quaternary Aminooxy Congeners of Acetyl γ-Homocholine

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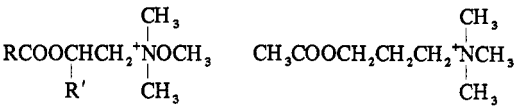
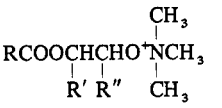
A prior communication[1] described preparation and biological effects of a series of N-methoxylated analogs **1–4** of certain cholinergic agents related to acetylcholine. These methoxyammonium compounds, with one exception, exhibited a low order of cholinergic activity. The unexpected and unexplained dramatically high muscarinic activity of **4**, the *N*-methoxy congener of Bethanechol, suggested further investigation of simple cholinergic agonist molecules possessing an N-O linkage.

Nicolaus, *et al.*,[2] described preparation of **6**, the "reverse" analog of **1**, and a bioisostere of acetyl γ-homocholine (**5**). Compound **6** was described[2] as having "similar activity" to acetylcholine, but quantitative data were not reported. The Nicolaus group also prepared 2-dimethylaminooxyethyl carbamate, the tertiary amine analog of **9**, but this compound was apparently never quaternized for biological testing. Schiatti and Maffii[3] stated that the introduction of an oxygen into the chain of acetylcholine so as to produce **6** has a similar effect as the addition of one more CH_2 group into this position (forming acetyl γ-homocholine) insofar as the affinity of the molecule for acetylcholinesterase is concerned. Both acetyl γ-homocholine and **6** were reported to



1, R = Me; R′ = H
2, R = R′ = Me
3, R = NH_2; R′ = H
4, R = NH_2; R′ = Me

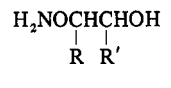
5



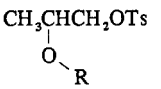
6, R = Me; R′ = R″ = H
7, R = R′ = Me; R″ = H
8, R = R″ = Me; R′ = H
9, R = NH_2; R′ = R″ = H
10, R = NH_2; R′ = Me; R″ = H
11, R = NH_2; R′ = H; R″ = Me

be poorer substrates for the enzyme than was acetylcholine. In the present work, the goal was to prepare the aminooxy systems **6–11** and to evaluate their muscarinic effects.

2-Aminooxyethanol **12** was prepared by a literature method.[2] Alternate methods for **12** were investigated as models for synthesis of the α- and β-methylcholine congeners **13** and **14**. Attempted O-alkylation of *N*-hydroxyurethane with 2-bromoethyl acetate was unsuccessful; Nicolaus, *et al.*,[2] obtained a 10% yield of product in a similar reaction of *N*-hydroxyurethane with 2-chloroethanol.



12, R = R′ = H
13, R = H; R′ = Me
14, R = Me; R′ = H



15a, R = benzyl
b, R = 2-tetrahydropyranyl

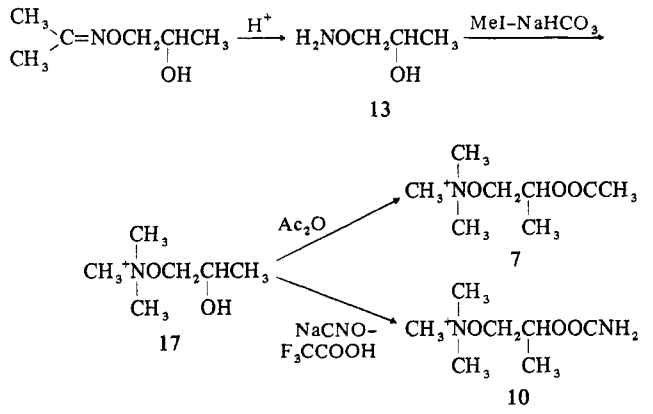
The monotosylate ester of ethylene glycol was treated with the sodium salt of *N*-hydroxyurethane, according to a procedure of Winternitz and Lachazette;[4] none of the desired O-alkylated product was obtained. When the free OH group of ethylene glycol monotosylate was masked as its benzyl ether, the anion of *N*-hydroxyurethane displaced the tosyl group to form the O-alkylated urethane **16** in adequate yield. However, application of the procedure to two monoetherified tosylate esters (**15a,b**) of propane-1,2-diol either gave very low yields or failed completely. Attempted O-alkylation of *N*-hydroxyurethane with a chloroacetone ketal failed. Compound **13** was obtained in good yield by reaction of acetone oxime with propylene oxide, according to a procedure of Bachman and Hokama,[5] followed by hydrolysis of the O-substituted acetone oxime (Scheme I).

The α-methylcholine analog **14** was prepared by alkylation of *N*-hydroxyurethane with ethyl 2-bromopropionate, followed by $LiAlH_4$ reduction (Scheme II).

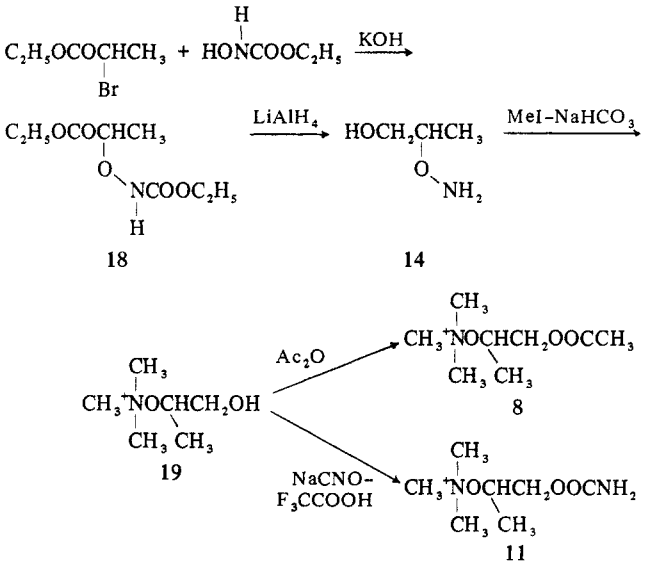
The primary alcohols **12–14** were converted directly to their quaternary ammomium salts with methyl iodide–sodium bicarbonate, and these were esterified at room temperature with acetic anhydride. At higher temperatures, acetic anhydride induced cleavage reactions in the quaternary ammonium systems. The carbamate esters were conveniently prepared by reaction with sodium cyanate and trifluoroacetic acid in methylene chloride. All of the carbamates were extremely hygroscopic and were difficult to purify. Spectral (ir, nmr) data on all compounds were consistent with the proposed structures. No attempt was made to resolve those compounds possessing an asymmetric center.

Pharmacology. Compounds **6–11** and the reference com-

Scheme I. Preparation of Aminooxy Congeners of β-Methylcholine Esters



Scheme II. Preparation of Aminooxy Congeners of α-Methylcholine Esters



pounds were evaluated for muscarinic effects in a superfused guinea pig ileum preparation.[6] Relative potencies and their 95% confidence intervals were estimated by the method of Litchfield and Wilcoxon.[7] At least three animals were used for each comparison and, usually, four doses per compound were administered. Effects were blocked by atropine (0.2 μg/kg) but not by hexamethonium (20 μg/kg); all compounds were much less active than acetylcholine. Compound **6** was approximately $^1/_{10}$ as active as acetyl γ-homocholine, and the carbamate congener of **6** was even less active. Placement of a CH_3 on the choline portion of the esters (either in the α or the β position) invariably severely diminished muscarinic effects. The muscarinic effects of this series do not correlate well with those of acetyl γ-homocholine, as might have been predicted (see Table I).

Experimental Section

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. Where analyses are indicated by symbols of the elements, the analytical results were within ±0.4% of the theoretical values.

***N*-Carbethoxy-O-(2-benzyloxyethyl)hydroxylamine (16).** 2-Benzyloxyethyl *p*-toluenesulfonate[8] (30.6 g, 0.1 mol), *N*-hydroxyurethane[9] (10.5 g, 0.1 mol), and 5.6 g (0.1 mol) of KOH were refluxed for 3 hr in 100 ml of ethanol. At the end of this period, the $FeCl_3$ test on the reaction mixture was only faintly positive. The solution was evaporated, diluted with ether, washed with water, dried ($MgSO_4$), and evaporated. Distillation of the residual oil at 147–149° (0.2 mm) gave 8 g (33%) of product. *Anal.* ($C_{12}H_{17}NO_4$) C, H, N.

***dl*-1-Aminooxy-2-propanol (13).** A mixture of 20 g (0.15 mol) of the 2-hydroxy-1-propyl ether of acetone oxime[5] and 100 ml of concentrated HCl was steam distilled until no more acetone was detectable in the distillate. The pot residue was evaporated under reduced pressure, dissolved in methanol, treated with excess methanolic KOH, and filtered, and the filtrate was evaporated under reduced pressure. The residual oil was distilled at 50° (2 mm) to afford 8.0 g (58%) of product. *Anal.* ($C_3H_8NO_2$) C, H.

***dl*-1-Dimethylaminooxy-2-propanol Methiodide (17).** Compound 13 (7.7 g, 0.083 mol), 71 g (0.5 mol) of methyl iodide, and 16.9 g (0.2 mol) of $NaHCO_3$ were refluxed in 70 ml of ethanol for 3 hr. The reaction mixture was filtered hot and, upon cooling, crystals were deposited which were recrystallized from acetone to yield 16.5 g (80%) of product, mp 135–136°. *Anal.* ($C_6H_{16}INO_2$) C, H, I, N.

***dl*-1-Dimethylaminooxy-2-propyl Acetate Methiodide (7).** Compound 17 (2.5 g, 0.009 mol) was stirred in 10 ml of acetic anhydride until complete solution occurred (7 days). The reaction mixture was poured into excess ether and the solid which separated was recrystallized from ethanol–ether to yield 1.8 g (65%) of crystals, mp 115–117°. Recrystallization from ethanol gave mp 133–134.5°. *Anal.* ($C_8H_{18}INO_2$) C, H, I, N.

***dl*-1-Dimethylaminooxy-2-propyl Carbamate Methiodide (10).** Compound 17 (2.6 g, 0.01 mol) and 1.44 g (90% purity, 0.02 mol) of sodium cyanate were stirred in 60 ml of methylene chloride and 2.28 g (0.02 mol) of trifluoroacetic acid was added dropwise. The reaction mixture was stirred overnight; the resulting precipitate was collected under N_2 in a drybox and dissolved in acetone, the solution was filtered, and the filtrate was treated with ether, which induced separation of a hygroscopic solid which was repeatedly recrystallized from 1-butanol–hexane to yield 0.33 g (11%) of yellow needles, mp 134–136°. *Anal.* ($C_7H_{17}IN_2O_3$) C, H, N.

2-Dimethylaminooxyethyl Carbamate Methiodide (9). This was prepared from 2.47 g (0.01 mol) of 2-dimethylaminooxyethanol methiodide,[2] 1.44 g (0.02 mol) of sodium cyanate, and 2.28 g (0.02 mol) of trifluoroacetic acid in 60 ml of methylene chloride as was described for **10**. Repeated recrystallization from ethanol–ether afforded 0.5 g (18%) of product, mp 120–121°. *Anal.* ($C_6H_{15}INO_3$) C, H, I, N.

***dl*-Ethyl 2-(*N*-Carbethoxyaminooxy)propionate (18).** Ethyl 2-bromopropionate (70 g, 0.386 mol; Aldrich Chemical Co.) was added slowly to 50 g (0.477 mol) of *N*-hydroxyurethane[9] and 27 g (0.48 mol) of KOH in 300 ml of ethanol. The resulting mixture was refluxed overnight, cooled, and filtered, and the filtrate was evaporated under reduced pressure. The residual oil was taken up in ether and this solution was washed with water and dried ($MgSO_4$). Removal of the ether left an oil: bp 125–126° (8 mm); yield 50 g (60%). *Anal.* ($C_8H_{15}NO_5$) C, H, N.

***dl*-2-Aminooxy-1-propanol (14).** Compound 18 (18.1 g, 0.088 mol) in 30 ml of anhydrous ether was added to 5.6 g (0.14 mol) of $LiAlH_4$ in 200 ml of anhydrous ether over 5 hr. Water (25 ml) was added, and the resulting mixture was stirred overnight. It was then filtered and the filtrate was dried ($MgSO_4$) and evaporated to leave an oil. The solid on the filter was extracted in a Sohxlet apparatus with ether for 24 hr; the extract was dried ($MgSO_4$) and filtered, and the filtrate was evaporated to leave an oil. The two oils were combined and were distilled through a "short path" apparatus: bp 47° (0.075 mm); yield, 8.0 g (88%). *Anal.* ($C_3H_9NO_2$) C, H.

Table I. Muscarinic Properties of Quaternary Aminooxy Compounds

Compd no.	Rel muscarinic potency (95% C.I.)
Acetylcholine	1.0
6	0.005 (0.001–0.02)
7	0.00025 (0.00006–0.0007)
8	0.00005 (0.00001–0.0002)
9	0.002 (0.00008–0.009)
10	0.00014 (0.00005–0.008)
11	0.008 (0.00004–0.006)
Acetyl γ-homocholine	0.05[a] (0.008–0.01)

[a] B. C. Barrass, R. W. Brimblecomb, P. Rich, and J. V. Taylor, *Brit. J. Pharmacol.*, 39, 40 (1970), reported that acetyl γ-homocholine was 0.02 as active as acetylcholine.

dl-2-Dimethylaminooxy-1-propanol Methiodide (19). Compound 14 (23.1 g, 0.253 mol) was treated with 213 g (1.5 mol) of methyl iodide and 50.7 g (0.6 mol) of $NaHCO_3$ in 210 ml of ethanol as was described for 17. The crude product was recrystallized from acetone to yield 10 g (16%) of crystals, mp 114–115°. *Anal.* ($C_6H_{16}INO_2$) C, H, I, N.

dl-2-Dimethylaminooxy-1-propyl Acetate Methiodide (8). This was prepared from 2.5 g (0.0095 mol) of 19 and 25 ml of acetic anhydride as was described for 7. The product was recrystallized from acetone: mp 124–125°; yield, 2.6 g (90%). *Anal.* ($C_8H_{18}INO_3$) C, H, I, N.

dl-2-Dimethylaminooxy-1-propyl Carbamate Methiodide (11). This was prepared from 2.6 g (0.01 mol) of 19, 1.44 g (0.02 mol) of sodium cyanate, 2.28 g (0.02 mol) of trifluoroacetic acid, and 60 ml of methylene chloride as was described for 10. The product was recrystallized from ethanol–ether to yield 1.7 g (56%) of crystals, mp 145–146°. *Anal.* ($C_7H_{17}IN_2O_3$) C, H, I, N.

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References

(1) L. L. Darko, J. G. Cannon, J. P. Long, and T. F. Burks, *J. Med. Chem.*, 8, 841 (1965).
(2) B. J. R. Nicolaus, G. Pagani, and E. Testa, *Helv. Chim. Acta*, 45, 358 (1962).
(3) P. Schiatti and G. Maffii, *Boll. Soc. Ital. Biol. Sper.*, 38, 1823 (1962).
(4) F. Winternitz and R. Lachazette, *Bull. Soc. Chim. Fr.*, 644 (1958).
(5) G. B. Bachman and T. Hokama, *J. Amer. Chem. Soc.*, 81, 4223 (1959).
(6) J. G. Cannon, T. L. Gruen, A. B. Rege, and J. P. Long. *J. Med. Chem.*, 15, 71 (1972).
(7) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, 96, 99 (1949).
(8) C. L. Butler, A. G. Renfrew, and M. Clapp, *J. Amer. Chem. Soc.*, 60, 1472 (1938).
(9) A. Hantsch, *Ber.*, 27, 1254 (1894); A. T. Fuller and H. King, *J. Chem. Soc.*, 963 (1947).

Improved Synthesis of DL-Alanosine†

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The antibiotic and antitumor agent alanosine was originally isolated as a levorotatory substance from the fermentation broth of *Streptomyces alanosinicus*.[1,2] The compound was subsequently found to have immunosuppressive activity.[3] The structure of alanosine was established as L-(−)-2-amino-3-(hydroxynitrosamino)propionic acid (L-I) by Lancini, *et al.*,[4] who described a synthesis used to prepare the D, L, and DL forms of the compound. Their approach was based upon the exothermic conversion of the β-chloroalanine derivative DL-II to DL-2-amino-3-(hydroxyamino)-propionic acid (DL-III) by means of hydroxylamine in the absence of solvent. Resolution of *N,N'*-dibenzoyl-DL-III by way of its cinchonine salt gave D-III and L-III upon removal of the blocking groups. This was followed by nitrosation using $NaNO_2$ and cold aqueous HCl to give D-I, L-I, and DL-I, L-I being identical in every respect with alanosine as obtained by fermentation (see Table I).

We now wish to report an improved chemical synthesis of DL-alanosine in which the key intermediate DL-III was prepared in a convenient alternative way through the nitrone DL-IV and in which the final nitrosation step was revised so as to facilitate the preparation of pure DL-alanosine in a stable condition.

Table I

Compd	$XCH_2CHCOOY$ (NHZ) X	Y	Z
I	HO, ON, N–	H	H
II	Cl	CH_3	$COCH_3$
III	HONH–	H	H
IV	$C_6H_5CH=N^{+}(O^{-})$	C_2H_5	$COCH_3$
V	Cl	CH_3	H·HCl
VI	Cl	CH_3	$COOC_2H_5$

Compound DL-IV, obtained from the reaction of DL-II with sodium *anti*-benzaldoximate in absolute ethanol, was hydrolyzed with concentrated HCl to the desired amino acid DL-III. The intermediate DL-IV need not be isolated in this synthesis, it being more efficient to proceed directly from DL-II to DL-III (yield, 66%). In a separate experiment the nitrone DL-IV was isolated (yield, 7%) and characterized as an ethyl ester, indicating that ester interchange had taken place with the ethanol solvent.

This revised approach avoids the potentially hazardous reaction of DL-II with the free base hydroxylamine, which is itself difficult to prepare. Furthermore, we have found our method to be readily adaptable to the preparation of large quantities (*ca.* 100 g) of DL-III. Several attempts to convert DL-II into DL-III using solutions of hydroxylamine in which the base was generated from its hydrochloride gave gummy products which were not identified.

Precedent for the present approach to the introduction of the hydroxylamino group may be found in the publications of Buehler and Brown,[5] Bellasio, *et al.*,[6] and Schoenewaldt, *et al.*[7]

In studies of the synthesis of *N*-hydroxylamino acids Liberek and Palacz[8] noted that only minor racemization was encountered when an optically active α-bromocarboxylic acid was converted to a nitrone using sodium *anti*-benzaldoximate, the predominant stereochemical result being inversion. In this connection it was noted in our experiments that complete racemization occurred when the present method was applied to L-II or L-VI. Our observation that the nitrone reaction mixtures were devoid of optical activity is consistent with the report of Lancini, *et al.*,[4] that L-II gave an optically inactive product after reaction with hydroxylamine.

The final nitrosation step was revised in order to provide a product in which the levels of contamination by residual acetic acid and metallic salts were substantially lowered. This was accomplished by using an absolute minimum of NaOH solution to dissolve the product and by effecting precipitation by adding acetic acid to pH 5.4, instead of 4.0 as previously specified.[4] Product prepared according to the revised procedure was found by nmr to be free of residual acetic acid, to have an undetectable ash content, and to be stable indefinitely at 5°, in contrast to samples purified using the original procedure.

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