Vinyl Ethers of Choline and Congeners[1]

Joseph G. Cannon,* Aleem Gangjee,

Division of Medicinal Chemistry and Natural Products, College of Pharmacy

John Paul Long, and Albert John Allen

Department of Pharmacology, College of Medicine, The University of Iowa, Iowa City, Iowa 52242.
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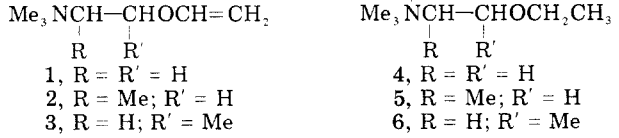
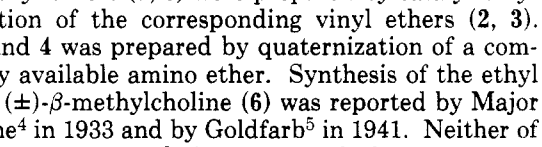
The vinyl ethers of choline and of its α- and β-methyl homologues were prepared to determine their cholinergic effects and to determine whether a separation of the dual physiologic activity (nicotinic and muscarinic) reported for the vinyl ether of choline could be achieved by this modification. A literature method of vinyl transetherification of amino alcohols has been studied and modified. The ethyl ethers of choline and of α- and β-methylcholine were prepared for comparison with the vinyl ethers. Two independent, unequivocal syntheses of the ethyl ether of β-methylcholine have been accomplished. This study showed that the two literature methods for synthesis of this compound are equivocal, and, hence, the biological data reported for this compound in the older literature may not be valid. Certain of the ethers showed marked nicotinic or muscarinic activities.

Acetylcholine and muscarone have long been considered to be unique with respect to their high order of dual nicotinic–muscarinic potency. Separation of these two physiological actions has been achieved in acetylcholine by introducing a methyl group into an appropriate position in the choline moiety; acetyl-α-methylcholine is a predominantly nicotinic agent, whereas acetyl-β-methylcholine is predominantly muscarinic. In 1970, Chiou[2] reported that the vinyl ether of choline (1) demonstrates prominent nicotinic and muscarinic activities and he suggested that, because this compound is not a substrate for cholinesterases, it might be a useful tool for the study of cholinergic physiology. Chiou[2] noted that the ethyl ether of choline, the most potent of the choline saturated ethers, has been reported to be weaker than acetylcholine and the vinyl ether of choline, and he speculated that the vinyl group may play some important biological role.

In the present work, the vinyl ethers (1–3) of choline and of (±)-α- and -β-methylcholines were prepared in order further to investigate the effect of α- and β-methyl substitution of choline and to evaluate the role of unsaturated ether groups in cholinergic activities. For comparison purposes, the ethyl ethers (4–6) of choline and its *dl*-*C*-methyl homologues were synthesized.



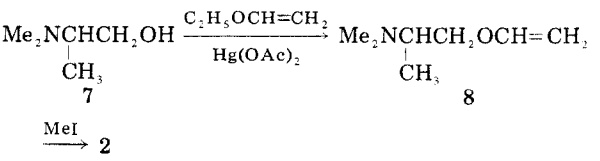
1, R = R′ = H
2, R = Me; R′ = H
3, R = H; R′ = Me



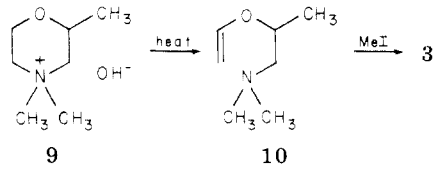
4, R = R′ = H
5, R = Me; R′ = H
6, R = H; R′ = Me

The vinyl ether of (±)-α-methylcholine (2) was prepared by vinyl transetherification of 2-dimethylamino-1-propanol, according to a modified procedure of Watanabe and Conlon[3] (Scheme I). However, when this procedure was attempted on 1-dimethylamino-2-propanol, only a 4% yield of the vinyl ether was obtained. Watanabe and Conlon[3] have proposed side reactions in vinyl transetherifications of amino alcohols, involving the lone electron pair on the nitrogen. In the present work, it was speculated that if these electrons were rendered less available (e.g., by incorporation of the amino function into a carbamate moiety), the transetherification reaction might proceed more readily. Vinyl transetherification of 1-(*N*-ethoxycarbonylamino)-2-propanol (19) gave an 18% yield of the vinyl ether of this system. However, all attempts to remove the carbamate functionality by reductive procedures or hydrolysis failed; either unchanged starting material was recovered or intractable tars resulted. A satisfactory yield of 3 was realized by Hofmann degradation of the *N*,*N*-dimethyl quaternary salt of 2-methylmorpholine (9) (Scheme II).

Scheme I. Preparation of the Vinyl Ether of α-Methylcholine



Scheme II. Preparation of the Vinyl Ether of β-Methylcholine



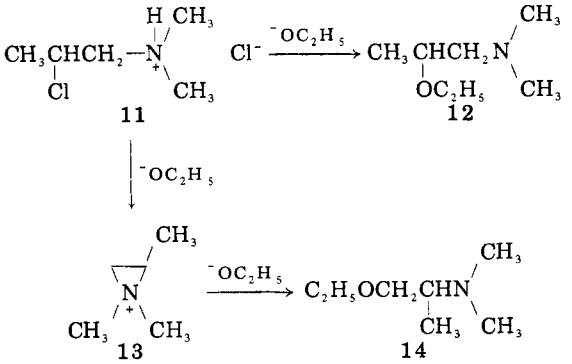
A noteworthy feature of the NMR spectra of all of the vinyl ethers prepared was the presence of a doublet of doublets centered at δ 6.30–6.63 and assigned to $-OCH{=}CH_2$.

The ethyl ethers (5, 6) were prepared by catalytic hydrogenation of the corresponding vinyl ethers (2, 3). Compound 4 was prepared by quaternization of a commercially available amino ether. Synthesis of the ethyl ether of (±)-β-methylcholine (6) was reported by Major and Kline[4] in 1933 and by Goldfarb[5] in 1941. Neither of these groups presented rigorous proof of structure nor evidence for homogeneity of their products, and the synthetic sequences employed by both groups seemed to include highly equivocal steps, which might lead to a product other than a β-methylcholine derivative or to a mixture of products. Goldfarb[5] described a synthesis for 1-(*N*,*N*-dimethylamino)-2-ethoxypropane (12), involving treatment of the corresponding haloamine hydrochloride (11) with sodium ethoxide. When the Goldfarb sequence was repeated in the present study, a liquid tertiary amine was isolated, whose boiling point was identical with that reported by Goldfarb;[5] quaternization of this material gave a solid whose ir and NMR spectra were identical with similar spectra of the ethyl ether of *dl*-α-methylcholine (5). It is concluded that Goldfarb's purported β-methylcholine derivative was predominantly if not entirely the α-methyl isomer, and it is suggested that treatment of 11 with excess ethoxide leads, not to a simple displacement to give 12, but rather to formation of an aziridinium ion (13), which

Table I. Muscarinic and Nicotinic Activities Relative to Acetylcholine

Compd	Rel muscarinic act. (95% C.I.)		Rel nicotinic act. (95% C.I.), dog blood pressure[b]
	Guinea pig ilea	Dog blood pressure	
Acetylcholine	1.0[a]	1.0[c]	1.0[d]
1	0.09 (0.06-0.18)	1.13 (0.51-6.2)	1.93 (1.11-4.14)
2	0.001 (0.0007-0.002)	0.06 (0.01-0.14)	3.74 (1.82-5.79)
3	0.02 (0.01-0.04)	0.74 (0.31-2.74)	Inactive at 2 mg/kg
4	0.15 (0.10-0.19)	1.03 (0.41-2.07)	0.90 (0.08-1.91)
5	0.003 (0.002-0.004)	0.08 (0.03-0.17)	1.13 (0.64-1.66)
6	0.03 (0.02-0.04)	0.64 (0.27-1.89)	Inactive at 2 mg/kg

[a] Doses used in assay were 2.5, 5, and 10 ng/ml. [b] Dogs were pretreated with atropine sulfate, 0.5 mg/kg. [c] Doses used in assay were 1 and 4 μg/kg. [d] Doses used in assay were 0.3 and 0.6 mg/kg.

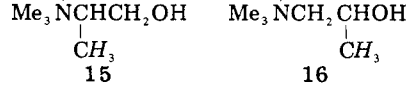


is attacked by ethoxide at the less hindered position, to give **14**.

In the present work, (±)-1-dimethylamino-2-hydroxypropane was treated with ethyl bromide in a Williamson ether synthesis to give an ethyl ether which, upon quaternization, gave a product identical to **6**, prepared by reduction of the corresponding vinyl ether (**3**), and different from the product of the Goldfarb sequence. Thus, two unequivocal syntheses verify the identity of the isomeric *C*-methylcholine ethyl ethers reported herein.

Hunt and Renshaw[6] evaluated the biological activities of the purported β-methylcholine ether prepared by Goldfarb. There are discrepancies between the data in this report and those in one by Simonart[7] who evaluated a product prepared by Major and Kline.[4] It is concluded that this older literature contains no account of biological effects of authentic, pure samples of ethyl ethers of α- and β-methylcholines.

The NMR spectra of α- and β-methylcholine ethers were used in confirming the positions of the *C*-methyl groups. The protons of the β-methyl groups in **3** and **6** appeared as sharp, well-defined signals, slightly upfield from the α-methyl signals of **2** and **5**, which were quite broad. The position and character of the α-methyl signals were consistent with the proximity of the α-methyl group to the quaternary nitrogen. An authentic sample of α-methylcholine (**15**) showed a broad CH_3 doublet at δ 1.40, whereas authentic β-methylcholine (**16**) showed a sharp *C*-methyl doublet at δ 1.16.



Pharmacology. Results. Table I shows the potencies relative to acetylcholine. When the guinea pig ileum assay was used, all of the compounds were significantly less active than acetylcholine. However, with the depressor response serving as the assay procedure, four of the compounds were not significantly different in activity from acetylcholine; the vinyl and ethyl ethers of α-methylcholine were decidedly less potent in this assay than the other compounds tested. A rank order listing of the compounds' potencies in the two assays does not appear to be different. The difference in assay potencies between the two procedures may be related to the cholinesterase substrate properties of acetylcholine. In none of the experiments was potentiation of acetylcholine noted. Atropine sulfate (0.2 mg/l.) significantly antagonized the compounds' actions using the guinea pig preparation. In the dog, no pressor responses were seen with doses used in the above assays, and atropine (0.2 mg/kg) abolished all depressor responses. Contrary to Chiou's[2] statement, the vinyl ether of choline was not found to be more potent than the ethyl ether (**4**) in the two muscarinic assays studied; **1** was somewhat more potent in the nicotinic assay. Methyl substitution of the α position of choline in its vinyl and its ethyl ethers greatly diminished muscarinic potencies; β-methyl substitution also resulted in diminution of activity in both ethers, but to a much lesser extent, and both β-methyl systems (**3**, **6**) retained marked muscarinic agonist effect in the dog blood pressure assay.

In anesthetized dogs, if the animals were pretreated with atropine sulfate (0.5 mg/kg), and the doses of agents increased, pressor responses were seen with compounds **1**, **2**, **4**, and **5**. All of these compounds were equal to or more active than acetylcholine as pressor agents. The pressor responses were blocked by pretreatment with hexamethonium bromide (10 mg/kg), administered intravenously. As with acetyl-β-methylcholine, substitution on the β-carbon greatly diminishes nicotinic activity. However, nicotinic activity is retained or somewhat enhanced by substitution of the α-carbon with methyl.

Experimental Section

Boiling points are uncorrected. Melting points were determined in open glass capillaries using a Thomas-Hoover Uni-Melt apparatus or a Mettler FP5 automated melting point apparatus programmed for a 3°/min rise and are corrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn., and by the Microanalytical Service, College of Pharmacy, University of Iowa. Where analyses are indicated by symbols of the elements, the analytical results were within ±0.4% of the theoretical values. NMR spectra were recorded on a Varian Associates T-60 instrument (Me_4Si) and are reported in parts per million (δ). Ir spectra were obtained with Beckman IR 10 or Perkin-Elmer IR 267 spectrometers. Column chromatographic separations were performed with Fisher A-540 alumina.

Pharmacology. Methods. The muscarinic activity relative to acetylcholine was calculated using the following two bioassay procedures. (1) Guinea pigs weighing 300–600 g were sacrificed by a blow to the head, and a portion of the ileum just prior to the ileocecal valve was removed. One end of the tissue was connected by a thread to a hook, and the other end was connected by a thread to a force-displacement transducer (Grass Instrument Co.). The ileum was placed in a 15-ml organ bath filled with Tyrode's solution which was oxygenated with 95% O_2–5% CO_2 and maintained at 37°. A tension of 1 g was placed on the ileum. Contractions were induced by adding no more than 0.2 ml of drug solution. (2) The second procedure involved bioassay of the depressor responses induced by the compounds in dogs an-

esthetized with barbital sodium (250 mg/kg). Barbital sodium was selected because of the stability of the blood pressure. The left femoral arterial blood pressure was measured using a Statham arterial transducer and changes in blood pressure were recorded using a Beckman RS recorder. Solutions of acetylcholine and test compounds were administered rapidly (volume ≤ 1.0 ml) into the right femoral vein. The order of administration of the compounds was randomized. The potency calculations were based on the percent depressor response relative to the blood pressure just prior to administration of the compound.

The ability of atropine sulfate (0.2 mg/kg) to antagonize the depressor responses in five dogs was evaluated. Relative potencies and their 95% confidence intervals were calculated by the method of Finney[8] and were based on molar amounts of the cation.

2-(*N,N*-Dimethylamino)ethyl Vinyl Ether (17). *N,N*-Dimethylmorpholinium iodide[9] (100 g, 0.412 mol) in 580 ml of H_2O and 72 ml of MeOH was stirred for 1 h with 134 g (0.575 mol) of freshly prepared[10] Ag_2O; then the mixture was filtered as rapidly as possible. The clear filtrate was concentrated to one-fourth its volume at 60–80° under reduced pressure. The resulting syrup was heated under reflux in an oil bath at 175° for 1.5 h, cooled, diluted with 50 ml of H_2O, and distilled. One fraction, bp 100° (750 mm), was basic to pH paper and was saturated with NaOH pellets, and the resulting mixture was extracted repeatedly with Et_2O and the combined extracts were dried ($MgSO_4$). Filtration and evaporation of the Et_2O left a pale oil which was distilled at 124° (750 mm) to give 12.5 g (20%) of a colorless liquid: lit.[9] bp 124°. Anal. ($C_6H_{13}NO$) C, H, N.

2-(*N,N*-Dimethylamino)ethyl Vinyl Ether Methiodide (1). Compound **17** (5.0 g, 0.048 mol) was stirred in an ice bath with 12 g (0.084 mol) of MeI in 25 ml of anhydrous Et_2O. After standing overnight at room temperature, the solid which separated was recrystallized from *n*-BuOH–hexane to afford 9.3 g (83%) of white crystals: mp 184–185°; NMR (Me_2SO-d_6) δ 6.63 (d of d, 1 H, C*H*=CH_2). Anal. ($C_7H_{16}INO$) C, H, N.[11]

(±)-2-(*N,N*-Dimethylamino)-1-propyl Vinyl Ether (8). 2-(*N,N*-Dimethylamino)-1-propanol (10.3 g, 0.1 mol) was added with stirring to 150 ml of ethyl vinyl ether (purified by distillation from Na) containing 2.5 g (0.007 mol) of recrystallized (anhydrous EtOH) mercuric acetate. The resulting solution was heated under reflux under N_2 for 48 h; then it was cooled and 4 drops of glacial AcOH were added. Stirring at 30° was continued for 3 h; then an equal volume of Et_2O was added and this solution was washed with 15 ml of 5% KOH and dried (K_2CO_3). Removal of the solvent left a liquid residue. TLC analysis (alumina; Me_2CO–$CHCl_3$, 1:1) of this indicated two components, one of which was identified by its R_f value to be starting material. This mixture was chromatographed on 230 g of alumina and was eluted with Me_2CO–$CHCl_3$ (1:1). Thirty-eight 20-ml fractions were collected; fractions 14–26 were pooled, on the basis of TLC analysis, the majority of the solvent was removed, and the liquid residue was used directly in the next step.

(±)-2-(*N,N*-Dimethylamino)-1-propyl Vinyl Ether Methiodide (2). Crude **8** (5 g) was treated with 10 g (0.07 mol) of MeI in 20 ml of anhydrous Et_2O as described for **1**. The crude product was recrystallized from *n*-BuOH–hexane to afford 6.5 g (24%) of white crystals: mp 152°; NMR (Me_2SO-d_6) δ 1.40 (broad d, 3 H, CHC*H*$_3$), 6.6 (d of d, 1 H, C*H*=CH_2). Anal. ($C_8H_{18}INO$) C, H, N.

(±)-2,4-Dimethylmorpholine Methiodide (18). A procedure of Sommer et al.[12] was used. To a cooled mixture of 2 g (0.02 mol) of 2-methylmorpholine,[13] 3.7 g (0.02 mol) of tri-*n*-butylamine, and 7 ml of EtOAc was added dropwise 8.52 g (0.06 mol) of MeI. The mixture was stirred at 30° in a stoppered flask for 12 h. The white solid which separated was collected on a filter and was washed several times with EtOAc and then with anhydrous Et_2O. It was recrystallized from anhydrous EtOH to give 4.2 g (80%) of white needles: mp 208–209°; NMR (D_2O) δ 3.26 [s, 6 H, N(C*H*$_3$)$_2$]. Anal. ($C_7H_{16}INO$) C, H, N.

(±)-1-(*N,N*-Dimethylamino)-2-propyl Vinyl Ether (10). Compound **18** (195 g, 0.76 mol), 1800 ml of H_2O, 255 ml of MeOH, and 185 g (0.8 mol) of freshly prepared Ag_2O were stirred for 1 h; then the mixture was filtered. The filtrate was concentrated to 1 l. at 70–80° under reduced pressure, and then it was heated under reflux for 2 h in an oil bath. The mixture was then cooled and distilled. The fraction, bp 100° (750 mm), was basic to pH paper. This distillate was repeatedly extracted with Et_2O and the combined extracts were dried ($MgSO_4$). Filtration and removal of Et_2O from the filtrate afforded a yellow liquid which was distilled at 127–128° (750 mm) to yield 28 g (31%) of a colorless liquid: NMR ($CDCl_3$) δ 1.16 (d, 3 H, CHC*H*$_3$), 2.30 [s, 6 H, N(C*H*$_3$)$_2$], 2.56 (m, 2 H, NC*H*$_2$), 4.13 (m, 3 H, C*H*CH$_3$, CH=C*H*$_2$), 6.31 (d of d, 1 H, C*H*=CH_2). A mass spectrum exhibited a peak at *m/e* 129, corresponding to $C_7H_{15}NO$.

(±)-1-(*N,N*-Dimethylamino)-2-propyl Vinyl Ether Methiodide (3). Method A. Compound **10** (2.0 g, 0.015 mol) in 15 ml of anhydrous Et_2O was treated with 2.8 g (0.019 mol) of MeI as described for **1**. The crude product was recrystallized from *n*-BuOH–hexane to give 3.5 g (84%) of white needles: mp 97.7–99.7°; NMR (Me_2SO-d_6) δ 1.16 (d, 3 H, CHC*H*)3), 3.10 [s, 9 H, N(CH$_3$)$_3$], 6.50 (d of d, 1 H, C*H*=CH_2). Anal. ($C_8H_{18}INO$) C, H, N.

Method B. Vinyl transetherification was carried out by the method of Watanabe and Conlon,[3] using the modification of Church et al.[14] *N,N*-Dimethylamino-2-propanol (10.3 g, 0.10 mol), 150 ml of ethyl vinyl ether (redistilled from Na), and 2.5 g (0.007 mol) of mercuric acetate were heated under reflux under N_2 for 72 h at a bath temperature of 60°. The mixture was then cooled and 0.2 ml of AcOH was added and the resulting mixture was stirred for 3 h. An equal volume of anhydrous Et_2O was added and the solution was washed with 15 ml of 5% KOH. The organic phase was dried ($MgSO_4$) and filtered, and the filtrate was evaporated to yield a light yellow liquid. TLC analysis (alumina, Me_2CO–$CHCl_3$, 1:1) showed the presence of two components, one of which was identified by its R_f value as starting material. The mixture was chromatographed on 230 g of alumina and was eluted with Me_2CO–$CHCl_3$ (1:1). Thirty-nine 15-ml fractions were collected and were monitored by TLC. Fractions 12–27 were pooled and most of the solvent was removed. A cooled solution of the residue in 15 ml of anhydrous Et_2O was treated with 2.8 g (0.019 mol) of MeI. The resulting white solid was recrystallized from *n*-BuOH–hexane to yield 1.08 g (4%) of product: mp 97.8°. Ir and NMR spectra of this material were superimposable upon similar spectra of the product of method A. A mixture melting point determination of the products of methods A and B showed no depression.

(±)-1-(*N*-Ethoxycarbonylamino)-2-propanol (19). To a vigorously stirred solution of 140 g (2.0 mol) of 1-amino-2-propanol and 358 g (3.38 mol) of anhydrous Na_2CO_3 in 1 l. of H_2O was added dropwise 182 g (1.66 mol) of ethyl chloroformate. After all of the ethyl chloroformate was added, concentrated HCl was added carefully until the solution was acidic to pH paper. The solution was extracted repeatedly with Et_2O and the combined extracts were dried ($MgSO_4$). Filtration and evaporation of the filtrate gave a yellow liquid which was distilled, bp 92–94° (0.25 mm), to yield 204 g (73%) of a colorless liquid: ir (neat) 1680 cm^{-1} (C=O). Anal. ($C_6H_{13}NO_3$) C, H, N.

(±)-1-(*N*-Ethoxycarbonylamino)-2-propyl Vinyl Ether (20). Compound **19** (50 g, 0.35 mol), 5 g (0.013 mol) of mercuric acetate, and 300 g (4.17 mol) of ethyl vinyl ether were heated under reflux for 16 h. To the cooled solution, 200 ml of 10% Na_2CO_3 was added with stirring, which was continued for 1 h. The organic phase was separated and dried ($MgSO_4$); then it was filtered and evaporated to afford a yellow liquid. TLC analysis (alumina, Me_2CO–$CHCl_3$, 1:1) showed the presence of three components. This mixture was chromatographed on 230 g of alumina and was eluted with Me_2CO–$CHCl_3$ (1:1). Thirty-six 20-ml fractions were collected, and these were monitored by TLC. Fractions 12–29 were pooled and the solvent was evaporated to leave a liquid which was shown by TLC to contain two components. This material was distilled through a 16-cm Vigreux column to give 12 g (18%) of a homogeneous liquid: bp 52–53° (0.05 mm); NMR ($CDCl_3$) δ 6.35 (d of d, 1 H, C*H*=CH_2). Anal. ($C_8H_{15}NO_3$) C, H, N.

2-(*N,N*-Dimethylamino)ethyl Ethyl Ether Methiodide (4). 2-Ethoxyethylamine (4.45 g, 0.05 mol), 18.5 g (0.1 mol) of tri-*n*-butylamine, and 100 ml of EtOAc were cooled in an ice bath and 30 g (0.22 mol) of MeI was added dropwise with stirring. The reaction was stirred at room temperature for 24 h, and then the solid which had separated was collected on a filter and recrystallized from *n*-BuOH–hexane to give 7.8 g (61%) of a white solid: mp 166.7° (lit. mp 159°,[15] 160–165°[16]). Anal. ($C_7H_{18}INO$) C, H, N.

(±)-2-(*N,N*-Dimethylamino)-1-propyl Ethyl Ether Methiodide (5). Compound **2** (0.25 g, 0.0092 mol) in 50 ml of anhydrous EtOH was hydrogenated at room temperature in the presence of 0.25 g of 10% Pd/C at an initial pressure of 50 psig. The reduction was complete in 1 h. Removal of the catalyst and evaporation of the solvent under reduced pressure afforded a white solid which was recrystallized from *n*-BuOH–hexane to give 0.212 g (85%) of white crystals: mp 148.3°; NMR (Me_2SO-d_6) δ 1.26 (center) (m, 6 H, $CHCH_3$, CH_2CH_3), 3.13 [s, 9 H, $N(CH_3)_3$]. Anal. ($C_8H_{20}INO$) C, H, N.

Literature[5] Reaction of Dimethylaminoisopropyl Chloride (11) with Sodium Ethoxide. To a solution of 79 g (0.5 mol) of dimethylaminoisopropyl chloride hydrochloride in 500 ml of anhydrous EtOH was added a solution of 25.3 g (1.1 g-atom) of Na in 500 ml of anhydrous EtOH. The reaction mixture was heated on a H_2O bath for 24 h; then it was cooled and filtered. The filtrate was distilled through a 32-cm Vigreux column to give 28 g (43%) of a clear liquid: bp 133–135° (750 mm) (lit.[5] bp 133–135°). This product (2.5 g, 0.019 mol) in 20 ml of anhydrous Et_2O was treated with 3.5 g (0.025 mol) of MeI and the white solid which formed was recrystallized from amyl alcohol–amyl acetate to give 4.6 g (86%) of a white powder: mp 145–146° (lit.[5] mp 144.5°). An ir spectrum (Nujol) of this material was superimposable upon a similar spectrum of **5**. An NMR spectrum (Me_2SO-d_6) was superimposable upon a similar spectrum of **5**, showing the characteristic broadening of the CCH_3 multiplet at δ 1.26.

(±)-1-(*N,N*-Dimethylamino)-2-propyl Ethyl Ether (21). Method A. Compound **10** (2.0 g, 0.015 mol) in 50 ml of anhydrous EtOH was hydrogenated at room temperature in the presence of 0.25 g of 10% Pd/C at an initial pressure of 50 psig. The calculated amount of H_2 was taken up in 1.5 min, and the reaction was allowed to continue for an additional 2 min. Filtration and concentration of the filtrate under reduced pressure gave 15 ml of crude product which was used in the next step without purification.

Method B. A mixture of 54 ml of Me_2SO (distilled from CaH) and 4.5 g of a 57% mineral oil dispersion of NaH (which had been washed three times with hexane) was heated at 70–75° until evolution of H_2 ceased (ca. 45 min). This deep gray mixture and 0.05 g of triphenylmethane were added to 9 g (0.087 mol) of 1-(*N,N*-dimethylamino)-2-propanol in 10 ml of Me_2SO. The resulting deep red-brown solution was treated with 13.6 g (0.088 mol) of diethyl sulfate, and the resulting mixture was stirred with cooling for 10 min. Then, 15 ml of CH_2Cl_2 and 15 ml of H_2O were added; the aqueous layer was separated and extracted repeatedly with CH_2Cl_2. The combined organic phases were washed several times with H_2O and dried ($MgSO_4$). Filtration and evaporation of the filtrate afforded a gummy residue and a supernatant liquid which were extracted with anhydrous Et_2O to give, after removal of the Et_2O, a yellow liquid for which TLC analysis (alumina, Me_2CO–$CHCl_3$, 1:1) showed two components. This material was chromatographed on 230 g of alumina and eluted with Me_2CO–$CHCl_3$ (1:1). Forty-two 15-ml fractions were collected and monitored by TLC. Fractions 12–33 were pooled and the solvent was evaporated to give a light yellow liquid which was used in the next step without purification.

(±)-1-(*N,N*-Dimethylamino)-2-propyl Ethyl Ether Methiodide (6). The products **21** of methods A and B were quaternized separately as described for **1**. The material from method B afforded a dark brown liquid which solidified upon standing in the cold. This was washed several times with anhydrous Et_2O and was recrystallized from *n*-BuOH–hexane to afford 0.24 g (1%) of white crystals: mp 97–98°; NMR (Me_2SO-d_6) δ 1.13 (m, 6 H, $CHCH_3$, CH_2CH_3), 3.20 [s, 9 H, $N(CH_3)_3$]. Anal. ($C_8H_{20}INO$) C, H, N. The material from method A gave a brown solid which upon recrystallization from *n*-BuOH–hexane gave 3.4 g (81%) of white crystals: mp 99.8°. NMR (Me_2SO-d_6) of this material was identical with that of the quaternization of the method B product.

References and Notes

(1) (a) A preliminary account of this work was presented at the 170th National Meeting of the American Chemical Society, Chicago, Ill., August 25, 1975. (b) Abstracted in part from a thesis submitted by A.G. in partial fulfillment of the requirements for the Ph.D. degree, University of Iowa, 1975.
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Analogues of Luteinizing Hormone Releasing Factor Modified at Positions 2, 6, and 10

Nicholas C. Ling,* Jean E. Rivier, Michael W. Monahan, and Wylie W. Vale

The Salk Institute, Neuroendocrinology Laboratory, La Jolla, California 92037.

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Eighteen analogues of luteinizing hormone releasing factor (LRF, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) were synthesized. The ten agonistic analogues were [D-Lys^6], [D-Orn^6], [D-Lys^6,des-Gly^{10},Pro^9-NHEt], [D-Orn^6,-des-Gly^{10},Pro^9-NHEt]-LRF plus their respective lauric acid conjugates as well as [(N^{ϵ}-Ac)-D-Lys^6] and [(N^{δ}-Ac)-D-Orn^6]-LRF. The eight antagonistic analogues were [des-His^2,D-Lys^6], [des-His^2,D-Orn^6], [des-His^2,D-Lys^6,des-Gly^{10},Pro^9-NHEt], [des-His^2,D-Orn^6,des-Gly^{10},Pro^9-NHEt]-LRF as well as their respective lauric acid conjugates. Biological activities of these analogues were determined in vitro, using LRF as the standard for the agonists and [des-His^2,D-Ala^6]-LRF for the antagonists. The potency of the agonists ranged from 1 to 17 times the activity of LRF while the antagonists had between 1 and 3 times the potency of [des-His^2,D-Ala^6]-LRF.

Following the discovery in our laboratory that substitution of luteinizing hormone releasing factor (LRF, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2[1]) with D-alanine at the 6 position rendered the resulting compound more potent,[2a] many analogues incorporating this modification as well as other D-amino acids have been synthesized. Monahan et al.[2a] have replaced the glycine at the 6 position with D-valine and D-proline while Vil-