steam bath in a stream of N_2. The residue was extracted with EtOAc. The EtOAc extract was washed with saturated NaCl, dried (Na_2SO_4), and evaporated to dryness to afford a viscous yellow oil. The oil was triturated with ether containing a small amount of EtOAc to yield 160 mg of crude 3b, mp 196–203°.

The ether–EtOAc trituration solution was evaporated to dryness, and the residual oil was chromatographed on 50 g of SiO_2. Elution with 30% EtOAc–70% C_6H_6 afforded 384 mg of solid which was crystallized from EtOAc–hexane to yield 227 mg of 3b, mp 219.5–222°. Further crystallization raised the melting point to 224.5–228.5°; λ^{KBr} 2.82, 2.98, 5.61, 5.98, 6.19 μ; λ_{max}^{MeOH} 240–241 nm (ε 14,350): nmr (Hz) 345, 240, 164 (OH), 73, 66. In D_2O the original broad signal at 240 Hz (16-H) appeared as a pair of doublets at 245, 240, 238, and 232 Hz; $[\alpha]D$ +67.2°. *Anal.* ($C_{22}H_{30}O_4$) C, H.

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Synthesis and Some of the Pharmacological Properties of [4-Leucine]-8-lysine-vasopressin and [1-Deamino,4-leucine]-8-lysine-vasopressin†

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[4-Leucine]-8-lysine-vasopressin ([4-Leu]-LVP) and its 1-deamino analog have been synthesized by classical methods and tested for a number of biological activities. The compounds exhibited, respectively, 1–2 units/mg and 5–6 units/mg of antidiuretic activity, 1.33 units/mg and 0.55 unit/mg of pressor activity, and negligible oxytocic activity. [4-Leu]-LVP had no avian vasodepressor activity, while the 1-deamino analog possessed 4.6 units/mg. Both compounds inhibited the oxytocic effects of oxytocin. [4-Leu]-LVP inhibited the avian vasodepressor effects of oxytocin as well, but not (as does [4-Leu]oxytocin) the antidiuretic effects of vasopressin. The low antidiuretic potencies of the two compounds are in marked contrast to the extremely high potencies (707 and 729 units/mg, respectively) of [4-aminobutyric acid]-LVP and its 1-deamino analog, compounds which differ from the 4-leucine compounds only in the absence of two methyl groups in the 4 position.

In the course of studies on the relationship of structure to the biological activity of the posterior pituitary hormones, a series of analogs of oxytocin and deaminooxytocin (Figure 1) has been prepared in which hydrophobic, aliphatic amino acid residues have been substituted for the glutamine residue in position 4.§ These studies have demonstrated that the glutamine carboxamide group contributes to but is not essential for biological activity. Indeed, the formal replacement of this group by a hydrogen atom to give [4-decarboxamido]oxytocin ([4-α-aminobutyric acid]-oxytocin) yielded an analog which possesses high oxytocic and avian vasodepressor (AVD) activities.[3] All of the compounds in this series of analogs have extremely low or negligible antidiuretic (ADH) and rat pressor activities. In fact, [4-leucine]oxytocin was actually found to possess the opposite effects (*i.e.*, diuretic and depressor effects) in the rat. Moreover, it exhibits potent anti-ADH activity (*i.e.*, it

†This work was supported in part by Grants HL-11680 (V. du V.) and HL-09795 (W. Y. C.) from the U. S. Public Health Service. All optically active amino acid residues are of the L variety. The symbols for the amino acid residues follow the recommendations (1971) of the IUPAC-IUB Commission on Biochemical Nomenclature.[1] Abbreviations: *i*-Pr_2NEt, *N,N*-diisopropylethylamine; TFA, trifluoroacetic acid; DMF, dimethylformamide; Mpa, the β-mercaptopropionyl residue; ADH, antidiuretic hormone. All melting points were determined in capillary tubes and are corrected. The Boc-protected intermediates invariable decomposed upon melting but without discoloration. Plates of silica gel G were used for thin-layer chromatograms which were developed in the following solvent systems: (A) $CHCl_3$–MeOH, 9:1; (B) BuOH–HOAc–H_2O, 3:1:1; (C) BuOH–pyridine–H_2O, 20:10:11; (D) MeOH–pyridine–H_2O, 12:1:7. Where analyses are indicated only by symbols of the elements, analytical results obtained for the elements were within ±0.4% of the theoretical values.

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§A list of these analogs with individual references has been presented in a table by Flouret and du Vigneaud.[2]

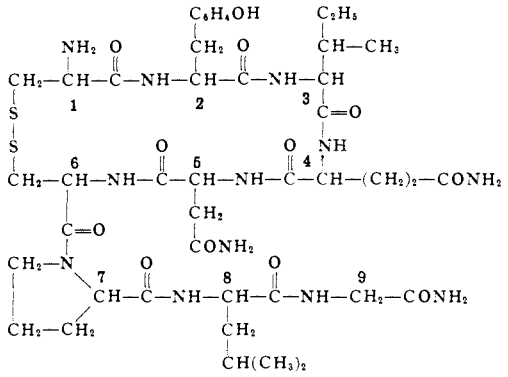

Figure 1. Structure of oxytocin with numbers indicating the position of the individual amino acid residues. Lysine-vasopressin has a phenylalanine residue instead of an isoleucine residue at position 3 and a lysine residue instead of a leucine residue at position 8. Arginine-vasopressin has a phenylalanine residue at position 3 and an arginine residue at position 8. In the deamino analogs, the NH_2 at position 1 is replaced with H.

inhibits the antidiuretic activity of arginine-vasopressin).[4] On the other hand, [1-deamino,4-leucine]oxytocin does not possess diuretic activity, although its ADH activity is almost negligible.[5]

In the course of parallel studies with respect to position 4 of 8-lysine-vasopressin (LVP) and its deamino analog (Figure 1), [4-α-aminobutyric acid]-LVP and [1-deamino,4-α-aminobutyric acid]-LVP were synthesized.[6] Both have lower levels of oxytocic and AVD activities compared to LVP; both show antidiuretic potencies considerably above that of LVP and at the same time pressor potencies well below that of LVP. In the case of [1-deamino,4-α-aminobutyric acid]-LVP, the ratio of antidiuretic (729 units/mg) to pressor (3.5 units/mg) activity is about 200:1, compared to a 1:1 ratio for LVP (240 units/mg *vs.* 266 units/mg, respectively).[6] Because of these surprising findings on the 4-aminobutyric acid analogs of LVP and deamino-LVP and those on the anti-ADH activity of [4-leucine]oxytocin, it was of great interest to us to investigate the pharmacological properties of [4-leucine]-LVP and [1-deamino,4-leucine]-LVP.

The protected nonapeptide precursors of [4-leucine]-LVP and [1-deamino,4-leucine]-LVP have been synthesized by classical stepwise methods,[7] utilizing active esters at each stage of chain elongation. The *tert*-butyloxycarbonyl group[8] was used throughout for the protection of α-amino functions, except in the protected nonapeptide of [4-leucine]-LVP, for which the benzyloxycarbonyl group[9] was employed. The sulfur atoms of the cysteine residues and the phenolic oxygen atom of the tyrosine residue were protected with benzyl groups, and the ε-amino function of the lysine residue was protected by a tosyl group. All of the protecting groups were removed from the nonapeptide intermediates by the use of sodium in liquid ammonia.[10] After oxidation of the disulfhydryl solutions by aeration[11a] in the case of [4-leucine]-LVP and by Weygand reagent[11b] in the case of [1-deamino,4-leucine]-LVP, the analogs were purified by ion-exchange chromatography.[11a,12] partition chromatography on Sephadex G-25,[13] and gel chromatography on Sephadex G-25.[14]

[4-Leucine]-LVP and [1-deamino,4-leucine]-LVP were tested for biological activities using a four-point assay design[15] and employing the U.S.P. posterior pituitary reference standard. Rat pressor assays were performed on anesthetized male rats as described in the U. S. Pharmacopeia.[16] Assays for antidiuretic activity were carried out on male rats by the method of Jeffers Livezey, and Austin[17] as modified by Sawyer.[18] Oxytocic activity was assayed on isolated uteri of rats in natural estrus by the method of Holton[19] as modified by Munsick[20] employing Mg-free van Dyke-Hastings solution as the bathing fluid. Avian vasodepressor assays were performed on conscious chickens by the method of Coon[21] as modified by Munsick, Sawyer, and van Dyke.[22] Both compounds were also tested for inhibitory properties in each of these systems.

[4-Leucine]-LVP exhibited 1.33 units/mg of rat pressor activity and 1-2 units/mg of antidiuretic activity. Similarly low potencies were found for [1-deamino,4-leucine]-LVP: 0.55 unit/mg of pressor activity and 5-6 units/mg of antidiuretic activity. When tested for oxytocic activity, both [4-leucine]-LVP and the 1-deamino analog had negligible potencies but both inhibited the action of oxytocin at virtually the same dose levels, being about one-tenth as strong inhibitors as [1-deaminopenicillamine]oxytocin.[23] The average molar concentration ($\bar{M}$) needed to reduce the response to $2x$ units of oxytocin (agonist) to the response to x units was 1.03×10^{-6} and $1.14 \times 10^{-6} M$, respectively, for the two analogs. The $\bar{M}$ for [1-deaminopenicillamine]-oxytocin was 1.16×10^{-7}.[24] [4-Leucine]-LVP showed an AVD response of approximately 1 unit/mg and also inhibited the AVD response to synthetic oxytocin ($\bar{M} = 4.9 \times 10^{-7}$), being roughly one-fortieth as strong an inhibitor as [1-deaminopenicillamine]oxytocin.[24] [1-Deamino,4-leucine]-LVP exhibited an AVD potency of 4.60 units/mg and did not inhibit the AVD activity of oxytocin.

The two compounds were also tested for natriuretic activity in rats by the techniques used by Chan, *et al.*[4a,c] Because both possess antidiuretic activity, albeit very weak compared to LVP, the doses which could be injected into the animal without producing a marked antidiuresis were very small: 0.015 μg/100 g of body weight for [4-leucine]-LVP and 0.002 μg/100 g of body weight for [1-deamino,4-leucine]-LVP. At these low dose levels, the natriuretic activity of the two compounds was either mild or not discernible. In a few preliminary experiments, osmotic diuresis was induced in the rats by a continuous iv infusion of 5% mannitol to counteract the antidiuretic effect of [4-leucine]-LVP. Under this experimental condition, higher doses of [4-leucine]-LVP (0.5 μg/100 g of body weight, comparable to the effective natriuretic dose of [4-leucine]oxytocin) could be injected, and a marked increase in urinary excretion of sodium was observed. Because the experimental conditions were different, it is not possible to assess the comparative natriuretic potencies of [4-leucine]oxytocin and [4-leucine]-LVP from these data.

As has been pointed out, [4-leucine]oxytocin has no antidiuretic activity and on the contrary inhibits the antidiuretic action of vasopressin.[4] It was therefore of great interest to determine whether [4-leucine]-LVP also possesses an anti-ADH activity. The effect of [4-leucine]-LVP on the antidiuretic action of lysine- or arginine-vasopressin was determined as previously described for [4-leucine]oxytocin.[4] Since [4-leucine]-LVP has an antidiuretic activity of 1-2 units/mg, only subthreshold antidiuretic doses were employed in these experiments. Doses of [4-leucine]-LVP up to 5.0×10^{-4} μg/100 g priming with an infusion rate of 0.5×10^{-4} μg/100 g/min had no measurable anti-ADH activity. At the upper limit of this infusion rate, an antidiuretic effect was observed as indicated by a decrease in free-water clearance. However, urine output was either unchanged or slightly increased because of the natriuresis induced by the high infusion rate of [4-leucine]-LVP.

[4-Leucine]oxytocin has greatly reduced potencies in those activities (oxytocic and AVD) in which oxytocin itself is high and opposite or inhibitory properties in those activities (ADH and pressor) in which oxytocin is low.[4] Similarly, [4-leucine]-LVP has greatly reduced potencies in those activities (ADH and pressor) most characteristic of its parent hormone and exhibits inhibitory properties in those activities (oxytocic and AVD) in which LVP itself is low. Thus it seems that the 4-leucine analogs retain their affinity for the hormone receptor sites and can become inhibitors in cases where the initial activity of the hormone is not too high.

Perhaps the most interesting outcome of the present work on [4-leucine]- and [1-deamino,4-leucine]-LVP is the dramatic change in antidiuretic activity in comparison with that of the corresponding 4-α-aminobutyric acid analogs: from 707 units/mg for [4-α-aminobutyric acid]-LVP to 1.2 units/mg for [4-leucine]-LVP and from 729 units/mg to 5-6 units/mg for the corresponding deamino analogs. This is particularly striking when one considers that leucine differs from α-aminobutyric acid only in that two methyl groups have replaced the two hydrogens on the γ carbon. A preliminary nmr study on the two α-aminobutyric acid analogs in comparison with LVP has already been reported.[25] This work is being extended to [4-leucine]-LVP and [deamino,4-leucine]-LVP in the hope of being able to correlate conformational changes with the large changes in biological activity.

Experimental Section

Boc-Lys(Tos)-Gly-OEt (1). HCl · Gly-OEt (4.61 g, 33 mmol) in $CHCl_3$ (45 ml) was neutralized with *i*-Pr_2NEt (5.74 ml). The solution was stirred, Boc-Lys(Tos)-ONp[26] (15.6 g, 30 mmol) was added in an additional 45 ml of $CHCl_3$, and the temperature of the reaction mixture was elevated to 35° for 3 days. The solvent was then removed *in vacuo*. The residual yellow oil was dissolved in EtOAc (200 ml), washed with 150-ml portions of 0.5 *N* NH_4OH (6 times), H_2O, 1 *N* HCl and brine, and then dried ($MgSO_4$). Upon removal of the solvent *in vacuo* the product crystallized: 13.2 g; mp 97–100°; homogeneous by tlc (A). An analytical sample was obtained by two crystallizations from 95% EtOH: mp 97–99°; $[\alpha]^{24}D$ −10.9° (*c* 2.2, HOAc). *Anal.* ($C_{22}H_{35}N_3O_7S$) C, H, N.

Boc-Pro-Lys(Tos)-Gly-OEt (2). Crystallized 1 (2.43 g, 5.0 mmol) was added to 5 ml of ice-cold, anhydrous trifluoroacetic acid (TFA) for removal of the Boc-protecting group. The suspension was allowed to come to room temperature. When the solid had entirely dissolved, the solvent was removed *in vacuo* and the resultant oil triturated to a white powder under Et_2O. This powder was collected, washed thoroughly with Et_2O, and dried *in vacuo* over KOH, 2.43 g. The product was suspended in EtOAc (10 ml) at 0° with Boc-Pro-ONSu[27] (1.72 g, 5.5 mmol) and stirred for 15 min. Next, *i*-Pr_2NEt (0.87 ml) was added, and the reaction was allowed to stir at room temperature for 65 hr. At this point a quantitative Kaiser test[28] showed no more than 0.2% unreacted primary amine, and the reaction mixture was dense with crystals (needles) which were collected, washed with cold EtOAc, and dried *in vacuo*: 1.85 g; mp 119–125°; $[\alpha]^{24}D$ −60.9° (*c* 1.1, HOAc); homogeneous by tlc (A). Two more crops (0.62 g) of equal purity were obtained by washing the filtrate of the reaction mixture (diluted to 30 ml with EtOAc) with 0.5 *N* NH_4OH and 1 *N* HCl as described for 1, evaporation of the dried ($MgSO_4$) solvent, and crystallization of the residue from EtOAc. An analytical sample was prepared by recrystallization from 95% EtOH: mp 125–127°; $[\alpha]^{25}D$ −61.5° (*c* 3.1, HOAc). *Anal.* ($C_{27}H_{42}N_4O_8S$) C, H, N.

Boc-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 (3). A solution of **2** (7.98 g, 13.7 mmol) in absolute EtOH (80 ml) was saturated at 0° with dry NH_3. The flask was sealed and allowed to stand at room temperature for 90 hr. The solvent was removed *in vacuo* and the resultant oil reevaporated from EtOH (20 ml) to a white foam. This product was dissolved in hot $CHCl_3$ (20 ml) and refrigerated overnight. A small amount of insoluble material was removed by filtration and the filtrate evaporated *in vacuo* to an oil which was reevaporated from EtOH (20 ml) to give a brittle white foam (6.81 g). Attempts to crystallize this protected tripeptide amide were unsuccessful and a sample (5.5 g, 10 mmol) was dissolved in TFA (16 ml) for removal of the Boc group as described previously. The resulting material was added to a solution of *i*-Pr_2NEt (1.72 ml) in EtOAc (50 ml). After 10 min an additional 0.35 ml of *i*-Pr_2NEt was added and the suspension stirred for 90 min, until most of the suspended matter had dissolved. Next, Boc-Cys(Bzl)-ONp[29] (5.20 g, 12 mmol) was added. All starting materials had dissolved within another 90 min. The reaction mixture was left at room temperature overnight and was a solid mass by morning. The product was collected and washed with cold EtOAc and then recrystallized from EtOAc: 5.09 g, mp 115–124°; $[\alpha]^{20}D$ −24.5° (*c* 2.1, $CHCl_3$); homogeneous by tlc (A). An analytical sample was prepared by crystallization from EtOH and from EtOAc: mp 125–128°, softens at 118°; $[\alpha]^{24}D$ −25.4° (*c* 1.1, $CHCl_3$). *Anal.* ($C_{35}H_{50}N_6O_8S_2$) C, H, N.

Boc-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 (4). The Boc group was removed from a sample of **3** (5.03 g, 6.73 mmol) with TFA (11 ml). The resulting product was dissolved in DMF (50 ml) with Boc-Asn-ONp[30] (2.75 g, 7.8 mmol) and the solution cooled to 0° After the addition of *i*-Pr_2NEt (1.12 ml), the reaction mixture was allowed to stir at room temperature, and additional 0.22-ml portions of *i*-Pr_2NEt were added at 1.5 and 20 hr. After 40 hr, tlc (B) of the reaction mixture showed no detectable free amine tetrapeptide. The solvent was removed *in vacuo* to a yellow sludge which was suspended in 95% EtOH. The product was collected by filtration and washed thoroughly with 95% EtOH and EtOAc and then dried *in vacuo*: 5.01 g; $[\alpha]^{23}D$ −47.7° (*c* 2.1, DMF); homogeneous by tlc (B). When heated slowly from 148°, the compound shriveled at 153° and melted at 200°. This product was used directly in the next step of chain elongation. An analytical sample was prepared by crystallization from 80% EtOH: mp 200.5–201.5°; $[\alpha]^{23}D$ −47.2° (*c* 1.2, DMF). *Anal.* ($C_{39}H_{56}N_8O_{10}S_2$) C, H, N.

Boc-Leu-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 (5). The Boc group was removed from 4 (2.15 g, 2.5 mmol) by the usual procedure The product was dissolved in DMF (12.5 ml) with Boc-Leu-ONp[31] (1.06 g, 3.0 mmol) and the solution was cooled to 0°. After the addition of *i*-Pr_2NEt (0.43 ml), the reaction mixture was allowed to come to room temperature. After 90 min an additional 0.11 ml of *i*-Pr_2NEt was added. After 24 hr the solution was diluted with an equal volume of 95% EtOH, and within 30 min it became thick with crystalline precipitate. More 95% EtOH was added to a total of 5 vol, and after stirring 1 hr the product was collected and thoroughly washed with 95% EtOH and EtOAc and then dried *in vacuo*: 1.90 g; mp 207–208.5°; $[\alpha]^{23}D$ −43.2° (*c* 1.6, DMF); homogeneous by tlc (B). A second crop (0.32 g) of comparable purity was obtained by concentrating the filtrate *in vacuo* and diluting with EtOAc and then collecting the precipitate and washing thoroughly with 95% EtOH and EtOAc. Both crops were utilized directly for the next step of chain elongation. An analytical sample was recrystallized from 80% EtOH: mp 210–210.5°; $[\alpha]^{23}D$ −43.5° (*c* 1.0, DMF). *Anal.* ($C_{45}H_{67}N_9O_{11}S_2$) C, H, N.

Boc-Phe-Leu-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 (6). The Boc group was removed from **5** (1.92 g, 1.96 mmol) by the usual procedure. The product was dissolved in DMF (10 ml) with Boc-Phe-ONp[32] (0.90 g, 2.3 mmol), and the solution was cooled to 0°. After the addition of *i*-Pr_2NEt (0.34 ml), the mixture was allowed to stir at room temperature and the reaction followed at intervals by the quantitative Kaiser test method. After 2 hr another 0.084 ml of *i*-Pr_2NEt was added. After 18 hr a Kaiser test indicated that less than 0.3% of the original primary amine content was still present. The product was isolated in the manner described for **5**: 2.10 g; mp 216.5–218.5°; $[\alpha]^{22}D$ −36.2° (*c* 0.86, DMF); homogeneous by tlc (B, C). This product was used directly in the next step of chain elongation. An analytical sample was crystallized from DMF by the addition of 1.5 vol of 95% EtOH: mp 222–223.5°; $[\alpha]^{25}D$ −37.8° (*c* 1.1, DMF). *Anal.* ($C_{54}H_{76}N_{10}O_{12}S_2$) C, H, N.

Boc-Tyr(Bzl)-Phe-Leu-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 (7). The Boc group was removed from 6 (2.89 g, 2.57 mmol) in the usual manner. The product was dissolved in DMF (9 ml) containing *i*-Pr_2NEt (0.44 ml) at 0°. An excess of Boc-Tyr(Bzl)-ONp[33] (1.64 g, 3.3 mmol) was added to this solution, and the mixture was allowed to stir to room temperature. After 45 min another 0.11 ml of *i*-Pr_2NEt was added. By 17 hr the entire mixture had solidified. The product was isolated in the manner described for **5**: 3.40 g; mp 229–231°; $[\alpha]^{25}D$ −34.3° (*c* 1.0, DMF); homogeneous by tlc (B, C). This material was used directly for the preparation of the two remaining intermediates. An analytical sample was crystallized from DMF by the addition of 5 vol of 95% EtOH: mp 232.5–233.5°; $[\alpha]^{24}D$ −35.5° (*c* 1.3, DMF). *Anal.* ($C_{70}H_{91}N_{11}O_{14}S_2$) C, H, N.

Z-Cys(Bzl)-Tyr(Bzl)-Phe-Leu-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 (8). The Boc group was removed from 7 (0.69 g, 0.5 mmol)

in the usual manner. The product was dissolved in DMF (30 ml) at 0° with Z-Cys(Bzl)-ONp[34] (0.28 g, 0.6 mmol). Next, *i*-Pr_2NEt (0.088 ml) was added and after 5 min the solution was allowed to stir to room temperature. The reaction mixture soon became thick with precipitate and had virtually solidified within 1 hr. After 3.5 hr the product was isolated from the solidified reaction mixture in the manner described for **5**: 0.73 g; mp 236.5–238°; $[\alpha]^{21}D$ −37.5° (*c* 1.2, DMF). Tlc (B, C) of this material showed in each case a slight spot at the origin accompanying the product (R_f 0.70, 0.67, respectively). No component was detected corresponding to the deblocked octapeptide. Despite this apparent impurity, the above product was successfully utilized in the synthesis of the desired analog. An analytical sample was reprecipitated from DMF containing 1% HCOOH. *Anal.* ($C_{83}H_{100}N_{12}O_{15}S_3$) C, H, N.

[4-Leucine]-8-lysine-vasopressin (9). A sample of **8** (200 mg, 125 μmol), which had been lyophilized from glacial HOAc and dried thoroughly *in vacuo*, was dissolved in boiling liquid NH_3 (350 ml, freshly distilled from Na). The solution was treated with Na in small amounts until a blue color pervaded the reaction mixture, lasting 20–30 sec. The excess Na was then discharged with a few crystals of NH_4Cl, the NH_3 was removed *in vacuo*, and the resulting residue was dissolved in 0.1% aqueous TFA (250 ml). This solution was adjusted to pH 7 with 2 *N* NH_4OH and aerated for 1 hr at room temperature. The course of the oxidation was followed by the Ellman test for -SH.[35] The solution was concentrated *in vacuo* and lyophilized. The product was dissolved in 4 ml of the upper phase of the solvent system 1-BuOH–95% EtOH–3.5% aqueous HOAc containing 1.5% pyridine (7:2:9) and purified by partition chromatography on a column (2.8 × 55 cm) of Sephadex G-25 (100–200 mesh). Fractions of 5 ml were collected at an average flow rate of 23 ml/hr. The eluted peptide materials were detected by the Folin–Lowry method.[36] The major product emerged at R_f 0.37 and was collected in such a way as to separate it from both faster and slower moving material. The desired material was recovered by dilution with H_2O, concentration *in vacuo*, and lyophilization. It was further purified by ion-exchange chromatography on a column (1.2 × 53 cm) of IRC-50 in 0.5 *M* NH_4OAc (pH 6.38).[11a] Fractions of 2.6 ml were collected at a flow rate of 8.8 ml/hr, and eluted materials were detected by their uv absorption at 280 nm. The product emerged as a broad peak in fractions 17–30, separated from a smaller peak in fractions 9–13. The contents of tubes 17–30 were pooled, adjusted to pH 4, and desalted on a column (1.2 × 15 cm) of IRC-50 (H^+).[37] The product was isolated from the column eluate in the usual manner and then chromatographed in 0.2 *N* HOAc on Sephadex G-25 in a 2.8 × 70 cm column at a flow rate of 30 ml/hr. The product emerged as a single symmetrical peak at 79% of the total column volume and was isolated by lyophilization: 64 mg; $[\alpha]^{24}D$ −12.6° (*c* 0.5, 1 *N* HOAc); homogeneous on tlc (B, C). *Anal.* ($C_{47}H_{68}N_{12}O_{11}S_2 \cdot 2C_2H_4O_2 \cdot 2H_2O$) C, H, N. Amino acid analysis[38] gave the following molar ratios: Lys, 0.95; NH_3, 2.07; Asp, 1.00; Pro, 1.05; Gly, 1.01; Leu, 0.97; Tyr, 0.90; Phe, 0.96. Cystine was determined as cysteic acid on a separate sample that was oxidized with performic acid for 4 hr at 0°[39] prior to the usual HCl hydrolysis: molar ratio cysteic acid, 2.14.

Mpa(Bzl)-Tyr(Bzl)-Phe-Leu-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 (10). The Boc group was removed from **7** (0.55 g, 0.4 mmol) in the usual manner. The product was dissolved in DMF (3 ml) with Mpa(Bzl)-ONp[40] (0.15 g, 0.48 mmol). This solution was cooled in an ice bath and *i*-Pr_2NEt (0.07 ml) was added with stirring. After 15 min the reaction was allowed to come to room temperature and was left stirring overnight. By morning it was a solid mass, and the product was isolated in the manner described for **5**: 0.51 g; mp 242–245.5°; $[\alpha]^{24}D$ −35.0° (*c* 1.2, DMF); homogeneous by tlc (B, D). An analytical sample was crystallized from hot DMF by the addition of an equal volume of 95% EtOH: mp 245.5–246°; $[\alpha]^{26}D$ −37.1° (*c* 1.0, DMF). *Anal.* ($C_{75}H_{93}N_{11}O_{13}S_2 \cdot H_2O$) C, H, N.

[1-Deamino,4-leucine]-8-lysine-vasopressin (11). A sample of crystalline **10** (121 mg, 0.083 μmol) was dissolved in liquid NH_3 (125 ml, freshly distilled from Na) and treated with Na until a persistent (20 sec) blue color pervaded the solution. This color was discharged with a little NH_4Cl, and the solution was concentrated *in vacuo* to a few milliliters and then lyophilized. The residue was dissolved in H_2O (115 ml) and acetone (115 ml) and treated with ICH_2CH_2I by the Weygand[11b] procedure. Oxidation was complete within 15 min. After removal of the acetone *in vacuo*, the solution was desalted and the product isolated as described for **9**, 75 mg. This material was subjected to ion-exchange chromatography on a column (1.06 × 116 cm) of Bio-Rex 70 in 0.5 *M* NH_4OAc (pH 5.85).[41] Fractions of 2.9 ml were collected at a flow rate of 8.7 ml/hr. The major peak was detected by uv absorption at 280 nm and emerged in fractions 35–50, separated from only traces of other uv-absorbing impurities. The material isolated from the peak following desalting in the usual manner was subjected to gel filtration in 0.2 *N* HOAc on Sephadex G-25. It emerged at 86% of the total column volume: 35 mg; $[\alpha]^{25}D$ −95.4° (*c* 0.5, 1 *N* HOAc); homogeneous by tlc (B, C). *Anal.* ($C_{47}H_{67}N_{11}O_{11}S_2 \cdot C_2H_4O_2 \cdot 3H_2O$) C, H, N. Amino acid analysis gave the following molar ratios: Cys (as cysteic acid), 0.99; Asp, 1.02; Pro, 0.96; Gly, 1.05; Leu, 1.00; Tyr, 0.96; Phe, 1.00; Lys, 0.98; NH_3, 2.03.

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Note Added in Proof. A very important series of papers has recently appeared on the synthesis and pharmacological properties of [4-leucine]arginine-vasotocin, a natriuretic analog of arginine-vasotocin.[42]

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Trimethylammonium Phenyl Ketones. Actions on the Cholinergic Receptor and Acetylcholinesterase†

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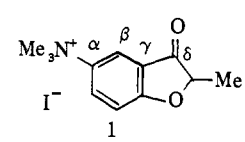
The syntheses of models for a study of acetylcholinesterase (AChE) binding are described. 6-(*N,N,N*-Trimethylammonium)indan-1-one iodide (**4**) as a muscarinic agent possessed $^1/_{100}$ the activity of acetylcholine. As an inhibitor of AChE (eel) the calculated K_i for 4 was $1.6 \times 10^{-7} M$. 4-(*N,N,N*-Trimethylammonium)indan-1-one iodide (**5**), 3-(*N,N,N*-trimethylammonium)propiophenone iodide (**6**), and 3-(*N,N,N*-trimethylammonium)butyrophenone iodide (**7**) were weakly muscarinic ($<^1/_{1000}$ ACh) and had K_i's ranging from 2 to $5 \times 10^{-6} M$ as inhibitors of AChE.

Pfeiffer,[1] in one of the earliest studies relating the acetylcholine (ACh) structure to biological actions, described the interatomic distances in ACh between the proposed binding sites. Further refinements in understanding the diverse biological properties of ACh have recently been directed at a description of the relative positions of the carbon, oxygen, and quaternary nitrogen of ACh that elicit a particular response. Excluding the "nicotinic action" two receptor responses related to ACh structure that are of current interest are the "muscarinic structure" and the "esterase structure."[2]

Archer and coworkers[3] advanced the trans relationship of the O-C-C-N portion of ACh as the muscarinic structure. Related in terms of the torsional angle, the O and N viewed along the C-C bond approaches 180° or is antiperiplanar. Additional support for the 140-180° muscarinic structure comes from the potent cyclopropyl analog study of Chiou and coworkers[4] (137° torsional angle[5]), dimethylacetylcholines,[6] decalins,[7,8] decahydroquinolines,[9] and bicyclo[2.2.2]octane[10] analogs. These interpretations contrast with those investigators favoring a 60-90° torsional angle for muscarinic action.[11-24]

Conclusions regarding the esterase structure, the optimal torsional angle for substrate activity using acetylcholinesterase (AChE), are not as divergent. Values ranging from 150 to 180° as derived from physical measurements and analog activities are generally quoted.[6-9,19-25]

Biological testing of a series of benzo analogs of muscarine revealed potent inhibition of AChE by **1**. A K_i of $2.5 \times 10^{-8} M$ calculated for both the butyryl (BChE) and acetylcholinesterase revealed that **1** was bound 10,000 times more effectively than the substrate ACh ($K_m = 4 \times 10^{-4} M$ for BChE and $1 \times 10^{-4} M$ for AChE). In contrast, the 7-isomer **2** was much less potent: $K_i = 2.5\text{-}3.8 \times 10^{-4}$ for BChE and



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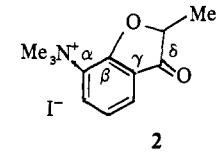

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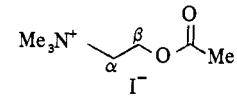
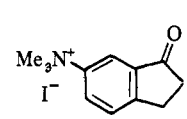


3

AChE. Additional analogs were desired to evaluate the requirement for a rigid 180° torsional angle imposed on the N-C_α-C_β-C_γ and C_α-C_β-C_γ-C_δ chains in **1** and **2**, those portions of the inhibitor simulating the analogous N-C_α-C_β-O and C_α-C_β-O-C fragments in ACh (**3**). Furthermore, the ether oxygen and the 2-methyl group were questionable requirements for binding of **1** and **2** to AChE. The carbonyl and the quaternary ammonium groups considered to be the two most important binding sites for AChE were retained in the analogs **4-7** synthesized.



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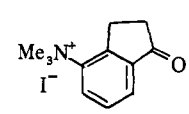

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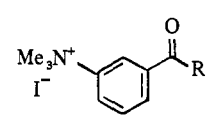

6, R = Et
7, R = *n*-Pr

Nitration of 1-indanone gave a 7:1 mixture of **6** and 4-nitroindan-1-one (**8**, **9**) separated by silica gel chromatography.[26] Nmr evidence in support of these assignments is derived from the downfield shift of the benzylic protons ($\Delta \sim \delta$ 0.24) of **9** compared to **8** attributed to the diamagnetic anisotropic effect[27] of the ortho nitro in **9**. Further, double irradiation of the H-5 or H-7 proton signals of **9** collapses the H-6 triplet (δ 7.62, $J = 8$ cps) to a doublet ($J_{6,7} = J_{5,6} = 8$ cps). Reductive methylation[28] of **8** and **9**

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