(10) H. C. J. Ottenheijm, S. P. J. M. Van Nipsen, and M. J. Sinnige, *Tetrahedron Lett.*, 1899 (1976).
(11) R. Vince, J. Brownell, and C. K. Lee, *Biochem. Biophys. Res. Commun.*, **75**, 563 (1977).
(12) P. F. Wiley and F. A. MacKellar, *J. Org. Chem.*, 41, 1858 (1976).
(13) M. Dixon, *Biochem. J.*, **55**, 170 (1953).
(14) C. Coutsogeorgopoulos, J. T. Miller, and D. M. Hann, *Nucleic Acid Res.*, **2**, 1053 (1975).
(15) P. H. Duquette, C. L. Ritter, and R. Vince, *Biochemistry*, **13**, 4855 (1974).
(16) While this manuscript was in preparation, the synthesis of **6a** was reported by C. C. L. Lin and R. J. Dubois, *J. Med. Chem.*, **20**, 337 (1977).

[1-Deaminopenicillamine,4-threonine]oxytocin, a Potent Inhibitor of Oxytocin[1]

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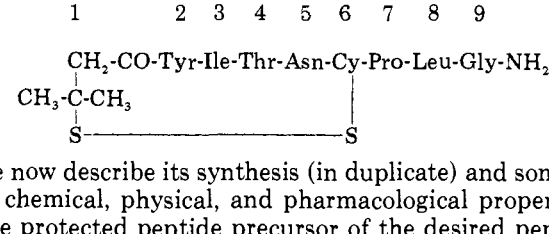
Received September 1, 1977

[1-Deaminopenicillamine,4-threonine]oxytocin was prepared in duplicate from *S*-benzyl-3-mercapto-3,3-dimethylpropanoyl-Tyr(Bzl)-Ile-Thr(Bzl)-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 (I) by removal of the Bzl-protecting groups with Na–NH_3, followed by cyclization of the resulting disulfhydryl compound with $K_3Fe(CN)_6$. The analogue was purified by desalting on Sephadex G-15 in 50% acetic acid and gel filtration on Sephadex G-15. The protected peptide I was synthesized (a) by the solid-phase method and (b) by a combination of solid-phase synthesis and an [8 + 1] coupling in solution. The analogue has no detectable agonist activity in rat vasopressor or isolated rat uterus assays. It has an antivasopressor pA_2 of 6.67 ± 0.09. It is a potent inhibitor of the in vitro oxytocic response to oxytocin and has a pA_2 value of 7.46 ± 0.04. (Material from the repeat synthesis has a pA_2 value of 7.59 ± 0.08.) Thus the substitution of threonine for glutamine in the antagonist [1-deaminopenicillamine]oxytocin (pA_2, 7.14 ± 0.05) has effected a twofold increase in inhibitory potency. [1-Deaminopenicillamine,4-threonine]oxytocin is one of the most potent inhibitors of oxytocin known to date.

The peptide analogues [1-L-penicillamine]-, [1-D-penicillamine]-, and [1-deaminopenicillamine]oxytocin are potent inhibitors of the oxytocic response in vitro to oxytocin and structurally related compounds.[2,3] Other analogues of oxytocin bearing a 3-mercapto-3,3-dialkylpropanoic acid in the 1 position also inhibit the oxytocic and avian vasodepressor responses to oxytocin.[4,5] The antioxytocic potency increases with increasing size and/or lipophilicity of the alkyl substituent. Inhibitory potencies were determined and expressed as pA_2 values. pA_2 values have been defined[6] as the negative logarithm to the base 10 of the average molar concentration of an antagonist which will reduce the biological response to $2x$ units of agonist to equal the response given by x units of agonist in the absence of antagonist. Among the antagonists of oxytocin containing dialkyl substitutions in the 1 position, [1-(3-mercapto-3,3-cyclopentamethylenepropanoic acid)]oxytocin is the most potent, with a pA_2 value of 7.43 when tested against oxytocin on the isolated rat uterus.[5] The 3,3-diethyl and 3,3-dimethyl (deaminopenicillamine) analogues were reported to have pA_2 values of 7.24[4] and 6.94,[2,3] respectively.

The introduction of threonine into position 4 of oxytocin has been shown to modify its spectrum of pharmacological activities in a remarkable manner. Oxytocic activity relative to oxytocin was significantly enhanced while vasopressin characteristics were diminished. [4-Threonine]oxytocin is thus a highly potent and specific oxytocic agent.[7,8] The enhanced oxytocic activity could result from (a) increased affinity for the smooth muscle receptors, (b) increased intrinsic activity subsequent to binding, or (c) relative resistance to enzymatic inactivation in the vicinity of the receptors. If the enhanced activity is due to greater binding resulting from the presence of the threonine residue at position 4, then, it was reasoned, the substitution of threonine in an oxytocin antagonist might also increase its binding and consequently give rise to an enhancement of its antagonist properties.

In the light of this reasoning it appeared worthwhile to explore the effect of a threonine/glutamine interchange in an oxytocin antagonist in the hope of obtaining a peptide with enhanced antagonist potency. Since it had been shown that [1-deaminopenicillamine]oxytocin is a slightly more effective antagonist than [1-L-penicillamine]oxytocin, their reported pA_2 values being 6.94 and 6.86, respectively,[3] it was decided to substitute threonine for glutamine in the former peptide. Thus [1-deaminopenicillamine,4-threonine]oxytocin was designed according to this rationale. It has the following structure.



We now describe its synthesis (in duplicate) and some of its chemical, physical, and pharmacological properties. The protected peptide precursor of the desired peptide analogue was synthesized (a) by development of the entire acyloctapeptide sequence upon the Merrifield resin,[9,10] using previously described procedures,[7,11] and (b) with the final acylation step conducted in solution, using a dicyclohexylcarbodiimide–*N*-hydroxybenzotriazole preactivation method.[12,13] Deblocking[7,14] and purification[7,15] were carried out by previously described methods.

Results and Discussion

The pharmacological properties of [1-deaminopenicillamine,4-threonine]oxytocin were determined by a variety of biological assays. This analogue has weak antidiuretic

0022-2623/78/1821-0179$01.00/0

Table I. Antagonistic Properties of [1-Deaminopenicillamine,4-threonine]oxytocin (1), [1-Deaminopenicillamine]oxytocin (2), and [1-(3-Mercapto-3,3-cyclopentamethylenepropanoic acid)]oxytocin (3)

	Antivasopressor pA_2	Antioxytocic pA_2, no Mg^{2+}
1a	6.67 ± 0.09	7.46 ± 0.04
1b		7.59 ± 0.08
2	6.27[a]	7.14 ± 0.05
3	"Weak"[a]	7.43[a]

[a] Values reported by Nestor et al.[5]

activity, about 0.02 U/mg, when injected iv into rats under ethanol anesthesia.[16] It is also a weak agonist in the rat milk ejection assay[17] and on isolated strips of rat mammary gland,[18] with a potency of about 4 U/mg in both assay systems. The analogue has no detectable agonistic activity in rat vasopressor[19] or isolated rat uterus assays in the absence of 0.5 mM Mg^{2+}.[20]

[1-Deaminopenicillamine,4-threonine]oxytocin was tested for antagonistic activities on the isolated rat uterus in a Mg^{2+}-free medium and pA_2 values were estimated by Schild's method.[6] Antivasopressor pA_2 values were estimated essentially as described by Dyckes et al.[21] Antioxytocic pA_2 values were also estimated for a sample of [1-deaminopenicillamine]oxytocin[2] generously supplied by Dr. W. Y. Chan of Cornell University Medical College. The USP Posterior Pituitary Reference Standard was used as an agonist in these assays.

Estimates of antivasopressor and antioxytocic pA_2 values appear in Table I. [1-Deaminopenicillamine,4-threonine]oxytocin appears to be approximately twice as potent an inhibitor of oxytocin on the rat uterus in the absence of Mg^{2+} as is [1-deaminopenicillamine]oxytocin.

The difference between antioxytocic pA_2 values estimated on the isolated rat uterus in the absence of Mg^{2+} (Table I) appears statistically significant ($p < 0.001$). This is further confirmed by comparing the mean pA_2 values estimated from assays on eight uterine horns with which pA_2 values were determined for both analogues. In every such assay the pA_2 for [1-deaminopenicillamine,4-threonine]oxytocin (preparation Ia) was greater than that for [1-deaminopenicillamine]oxytocin. The mean difference was 0.38 ± 0.06. Respective pA_2 estimates by these assays were 7.50 ± 0.09 and 7.12 ± 0.08. The difference is statistically significant ($p < 0.01$) either by paired analysis or by comparing mean values.

The discrepancy between our present estimate of an antioxytocic pA_2 of 7.14 for [1-deaminopenicillamine]-oxytocin and the pA_2 of 6.94 reported by Vavrek et al.[4] is largely due to differing methods of calculation. We followed Schild's procedure[6] and averaged pA_2 values calculated from the effective molar concentration of the antagonist determined for each assay group. Vavrek et al.[4] averaged the effective molar concentrations and then calculated the pA_2 as the negative logarithm of the mean concentration. If we analyze our data in that manner (Table II) the difference in molar concentrations is clearly not statistically significant. There is a significant difference ($p < 0.005$) between the effective molar concentrations of [1-deaminopenicillamine,4-threonine]oxytocin and [1-deaminopenicillamine]oxytocin. The effective molar concentrations and the pA_2 values calculated from them appear nearly identical for [1-deaminopenicillamine,4-threonine]oxytocin and [1-(3-mercapto-3,3-cyclopentamethylenepropanoic acid)]oxytocin.

Several oxytocin analogues, including [4-threonine]-oxytocin, have been observed to depress the accumulation

Table II. Values of Mean Effective Molar Concentrations (M) and pA_2 Values Calculated from These for the Analogues Listed in Table I Assayed on the Isolated Rat Uterus in the Absence of Mg^{2+}

	Lit. values		Present study	
	M × 10^8	pA_2	M × 10^8	pA_2
1a			4.0 ± 0.3 (38)[a]	7.40
1b			4.3 ± 1.0 (30)	7.36
2[b]	11.6 ± 0.9 (36)	6.94	9.1 ± 1.5 (31)	7.04
3[c]	3.7 ± 0.2 (33)	7.43		

[a] Means ± SEM, numbers of assay groups in parentheses. [b] From Vavrek et al.[4] [c] From Nestor et al.[5]

of radioactivity from labeled oxytocin by segments of rat uterus in proportion to their uterotonic potency.[22] This, and the inability of some prostaglandins to compete for uptake with the labeled hormone, indicates the presence of a receptor specific for oxytocin and supports our earlier speculation that the enhanced oxytocic activity of [4-threonine]oxytocin relative to oxytocin might be a consequence, in part, of an enhanced affinity for oxytocin receptors. The observed enhancement of the antioxytocic potency of [1-deaminopenicillamine,4-threonine]oxytocin relative to [1-deaminopenicillamine]oxytocin brought about by the replacement of glutamine by threonine in position 4 might also reflect increased receptor binding, in this instance nonproductive of oxytocic response. In this regard it is significant that a dissociation constant for the antagonist–receptor complex estimated from binding studies in which [1-deaminopenicillamine,4-threonine]-oxytocin competes with labeled oxytocin[23] is in excellent agreement with the dissociation constant calculated from the pA_2 values of 7.46 and 7.59 reported here.

In [1-(3-mercapto-3,3-dialkylpropanoic acid)]oxytocin derivatives it is known that antioxytocic potency is increased as the 3,3-dialkyl group is varied from dimethyl (deaminopenicillamine) through diethyl to cyclopentamethylene.[4,5,21] Since [1-deaminopenicillamine,4-threonine]oxytocin has proved to be as potent an oxytocic inhibitor as [1-(3-mercapto-3,3-cyclopentamethylenepropanoic acid)]oxytocin, further investigation of the threonine/glutamine interchange in this series of antagonists should be of interest.

Experimental Section

The procedure of "solid phase" synthesis conformed to that published.[7,9,10,13] Amino acid derivatives, including Boc-Gly resin, were supplied by Schwarz Bioresearch, with the exception of Boc-Tyr(Bzl) which was purchased from the Fox Chemical Co. Reagents and solvents were analytical grade. The elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. The analytical results for the elements indicated by their symbols were within ±0.4% of the theoretical values. For amino acid analysis,[24] samples (~0.5 mg) were hydrolyzed with constant boiling HCl (400 μL) containing phenol (20 μL) in evacuated sealed ampules for 18 h at 110 °C. The hydrolysates were analyzed with a Beckman/Spinco amino acid analyzer Model 121C. Ratios are referred to Gly = 1.00. Optical rotations were measured with a Bellingham Stanley Ltd. Model A polarimeter. R_f values refer to chromatography on silica gel (0.25 mm, Brinkman Silplate) in the following solvent systems: A, butan-1-ol–acetic acid–water (4:1:5, v/v, upper phase); B, butan-1-ol–water (3.5% in acetic acid, 1.5% in pyridine) (1:1, v/v, upper phase); C, butan-1-ol–acetic acid–water–pyridine (15:3:3:10, v/v). Loads of 25–50 μg were applied and chromatograms were of minimum length, 10 cm. The chloroplatinate reagent, chlorine–potassium iodide–tolidine, and iodine vapor were used for detection.

***S*-Benzyl-3-mercapto-3,3-dimethylpropanoyl-Tyr(Bzl)-Ile-Thr(Bzl)-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 (I). Preparation a, by Total Synthesis on Resin.** Boc-Gly resin (4.4 g,

1.7 mmol of Gly) was subjected to four cycles of deprotection, neutralization, and coupling to yield Boc-Asn-Cyz(Bzl)-Pro-Leu-Gly-resin (5.79 g, weight gain 790 mg, 90% of theory). A portion of this peptidyl resin (1.95 g, ~0.57 mmol of peptide) was converted to the acyloctapeptide resin (2.30 g, weight gain 350 mg, 95% of theory) in four cycles of deprotection, neutralization, and coupling, with *S*-benzyl-3-mercapto-3,3-dimethylpropanoic acid[2] as the carboxy component in the final coupling step. The resin was ammonolyzed[11] and the product extracted with dimethylformamide (DMF). The solvent was evaporated in vacuo, and the residue was triturated with methanol and washed exhaustively with ether to yield the crude product (495 mg) which was reprecipitated from a filtered solution in a minimum quantitiy of warm DMF by the addition of methanol–ether (1:1 v/v) to give the acyloctapeptide amide Ia (330 mg, 38% based upon initial glycine content of the resin): mp 222–226 °C dec; R_f (A) 0.80, R_f (B) 0.95, R_f (C) 0.74 (chromatograms showing some "tailing"); $[\alpha]^{22}_D$ −28° (*c* 1, DMF). Anal. ($C_{72}H_{94}N_{10}O_{12}S_2$) C, H, N. Amino acid analysis gave the following molar ratios: Tyr, 0.98, Ile, 0.95; Thr, 0.95; Asp, 1.03; Cys(Bzl), 0.89; Pro, 0.98; Leu, 0.99; Gly, 1.00; NH_3, 2.2.

I. Preparation b, by a Combination of Solid-Phase and Solution Methods. Boc-Tyr(Bzl)-Ile-Thr(Bzl)-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2[25] (312 mg, 0.25 mmol) was dissolved in cold TFA (3 mL) and left to stand at room temperature for 1 h with occasional vortex mixing. Cold ether (20 mL) was added and the precipitated material centrifuged and washed with ether (three times, aliquot 10 mL) by successive centrifugation and decantation, the precipitate being well suspended in each wash by vortex mixing. The product was dried in vacuo over sodium hydroxide pellets. This material was dissolved in DMF (~2 mL) and *N*-methylmorpholine (75 μL) was added gradually to give a solution of pH ~7 to moist pH paper, the atmosphere above which also gave an alkaline reaction to moist pH paper.[26] A solution of *S*-benzyl-3-mercapto-3,3-dimethylpropanoic acid[2,27] (141 mg, 0.63 mmol) and *N*-hydroxybenzotriazole monohydrate (145 mg, 0.95 mmol) in DMF (1.5 mL) was cooled in ice and treated with a solution of DCCI (130 mg, 0.63 mmol) in dichloromethane (0.25 mL). A DMF washing (0.25 mL) brought the total volume of the reaction mixture to 2.5 mL. This mixture was left to stand at room temperature for 1 h with occasional mixing. The precipitated dicyclohexylurea was centrifuged and supernatant (1.25 mL, ~0.32 mmol of acylating agent) was added to the neutralized solution of the octapeptide derivative.[12] After a reaction time of 2.5 h a spot of the reaction mixture on filter paper gave only a very faint ninhydrin color. The reaction mixture was set aside at room temperature overnight during which time the atmosphere within the reaction vessel maintained an alkaline reaction to moist pH paper.[26] The gel which formed was broken by the addition of aqueous acetic acid (5%, 20 mL) and vigorous stirring. The precipitated material was centrifuged and washed with water, ethanol (twice), and ether (twice), aliquot 10 mL, by successive centrifugation and decantation, the precipitate being well suspended in each wash. A solution of the crude product in a minimum quantity of hot DMF was diluted tenfold with boiling methanol. The hot solution, upon cooling to room temperature, deposited the acyloctapeptide derivative Ib as a white precipitate which was filtered and washed with methanol: yield 292 mg (84%); mp 240–241 °C; $[\alpha]^{22}_D$ −28° (*c* 1, DMF). Anal. ($C_{72}H_{94}N_{10}O_{10}S_2 \cdot H_2O$) C, H, N. Amino acid analysis gave Tyr, 0.98; Ile, 0.98; Thr, 0.92; Asp, 0.98; Cys(Bzl), 1.00; Pro, 1.22; Leu, 0.92; Gly, 1.00; NH_3, 2.09. Preparations Ia and Ib were indistinguishable by TLC.

[1-Deaminopenicillamine,4-threonine]oxytocin (IIa). A solution of the protected acyloctapeptide amide Ia (150 mg, 0.11 mmol) in sodium-dried, and redistilled, ammonia (~250 mL) was treated, at the boiling point and with stirring, with sodium from a stick of the metal contained in a small bore glass tube,[7,14] until a light blue color persisted in the solution for several seconds as opposed to its spontaneous discharge (~0.02 mL of sodium consumed). The solution was evaporated and the residue taken up in aqueous acetic acid (0.2%, 300 mL) with vigorous stirring. Glacial acetic acid was added to give a solution of pH 6.5; then an excess of potassium ferricyanide solution (0.01 M, 19.5 mL) was added gradually, with vigorous stirring. The yellow solution was stirred for 5 min with anion-exchange resin (BioRad AG 3-X4A, chloride form, ~5 g damp weight) and filtered through a bed of the resin (~40 g damp weight). The bed was washed with aqueous acetic acid (0.2%, 200 mL) and the combined filtrate and washings were lyophilized. The freeze-dried residue was desalted on Sephadex G-15 (column 100 × 2.7 cm) eluting with aqueous acid (50%), with a flow rate ~16 mL h^{-1}.[15] The eluate was monitored for absorbance at 280 nm and fractionated. The fractions comprising the major peak were pooled and lyophilized, and the residue (53.5 mg) was further subjected to gel filtration with Sephadex G-15 (column 100 × 1.2 cm) eluting with aqueous acetic acid (0.2 M, flow rate ~20 mL h^{-1}).[15] The free peptide analogue IIa was isolated from the fractions comprising the single symmetrical peak (detected by absorbance at 280 nm) by lyophilization: yield 30 mg (~32%); R_f (A) 0.49, R_f (B) 0.47, R_f (C) 0.86; $[\alpha]^{22}_D$ −52° (*c* 0.5, 1 M acetic acid). Amino acid analysis gave Tyr, 1.02; Ile, 0.95; Thr, 0.99; Asp, 1.07; $^1/_2$Cys, 0.05; Pro, 0.98; Leu, 1.00; Gly, 1.00; NH_3, 1.70. A peak unresolved from the artifact of the pH 3.25–4.25 buffer change was assumed due to the mixed disulfide of 3-mercapto-3,3-dimethylpropanoic acid and cysteine.[14] Analysis following performic acid oxidation prior to hydrolysis[28] gave a Cys(O_3H)–Gly ratio of 1.00:1.00, the trace of $^1/_2$Cys and the peak presumed due to the mixed disulfide having collapsed.

[1-Deaminopenicillamine,4-threonine]oxytocin (IIb). Treatment of the acyloctapeptide Ib (100 mg) as detailed above for IIa yielded the analogue IIb (48 mg), indistinguishable from preparation IIa by TLC: $[\alpha]^{24}_D$ −46° (*c* 0.5, 1 M acetic acid). Amino acid analysis gave Tyr, 1.00; Ile, 0.97; Thr, 0.94; Asp, 1.02; $^1/_2$Cys, trace; Pro, 1.09; Leu, 0.99; Gly, 1.00; NH_3, 1.65. An additional peak, assumed due to the mixed disulfide of 3-mercapto-3,3-dimethylpropanoic acid, was not resolved fully from the artifact caused by the buffer change pH 3.25–4.25.[14] Analysis following performic acid oxidation prior to hydrolysis[28] gave a Cys(O_3H)–Gly ratio of 0.94:1.00, the trace of $^1/_2$Cys and the peak assumed due to the mixed disulfide having collapsed.

Acknowledgment. This work was supported in part by research grants from the National Institutes of Child Health and Human Development (No. HD 06351), the National Institute of Arthritis, Metabolism and Digestive Diseases (No. AM 01940), the National Heart and Lung Institute (No. HL 12738), and the National Science Foundation (No. BMS 71-01238). The authors wish to thank Mrs. Esther Coy for technical assistance in the early stages of this work and Mrs. Cindy Licata for assistance in the preparation of the manuscript.

References and Notes

(1) (a) Preliminary reports on this work have been presented at the Fourth American Peptide Symposium, New York, N.Y., June 1975; M. Manning, J. Lowbridge, and W. H. Sawyer in "Peptides, Chemistry, Structure and Biology", R. Walter and J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1976, p 737; and at the 60th Meeting of the Federation of American Societies for Experimental Biology, Anaheim, Calif., April 1976; (b) M. Manning, J. Lowbridge, J. Haldar, and W. H. Sawyer, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **36**, 1848 (1977); (c) the symbols for the amino acids follow the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, **247**, 977 (1972).
(2) H. Schulz and V. du Vigneaud, *J. Med. Chem.*, **9**, 647 (1966).
(3) W. Y. Chan, R. Fear, and V. du Vigneaud, *Endocrinology*, **81**, 1267 (1967).
(4) R. J. Vavrek, M. F. Ferger, G. A. Allen, D. H. Rich, A. T. Blomquist, and V. du Vigneaud, *J. Med. Chem.*, **15**, 123 (1972).
(5) J. J. Nestor, Jr., M. F. Ferger, and V. du Vigneaud, *J. Med. Chem.*, **18**, 284 (1975).
(6) H. O. Schild, *Br. J. Pharmacol.*, **2**, 189 (1947).
(7) M. Manning, E. J. Coy, and W. H. Sawyer, *Biochemistry*, **9**, 3925 (1970).
(8) W. H. Sawyer and M. Manning, *Endocrinology*, **49**, 151 (1971).
(9) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963).
(10) R. B. Merrifield, *Biochemistry*, **3**, 1385 (1964).

(11) M. Manning, *J. Am. Chem. Soc.*, **90**, 1348 (1968).
(12) M. F. Ferger and W. Y. Chan, *J. Med. Chem.*, **18**, 1020 (1975).
(13) M. Manning, J. Lowbridge, J. Haldar, and W. H. Sawyer, *J. Med. Chem.*, **19**, 376 (1976).
(14) D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *J. Biol. Chem.*, **237**, 1563 (1962).
(15) M. Manning, T. C. Wuu, and J. W. M. Baxter, *J. Chromatogr.*, **38**, 396 (1968).
(16) W. H. Sawyer, *Endocrinology*, **63**, 694 (1958).
(17) G. W. Bisset, B. J. Clark, J. Haldar, M. C. Harris, G. P. Lewis, and M. Rocha e Silva, Jr., *Br. J. Pharmacol. Chemother.*, **31**, 537 (1967).
(18) I. Polacek, I. Krejci, and J. Rudinger, *J. Endocrinol.*, **38**, 13 (1967).
(19) J. Dekanski, *Br. J. Pharmacol.*, **7**, 567 (1952).
(20) R. A. Munsick, *Endocrinology*, **66**, 451 (1960).
(21) D. F. Dyckes, J. J. Nestor, Jr., M. F. Ferger, and V. du Vigneaud, *J. Med. Chem.*, **17**, 250 (1974).
(22) M. S. Soloff, T. L. Swartz, M. Morrison, and M. Saffran, *Endocrinology*, **92**, 104 (1973).
(23) M. Soloff, *Br. J. Pharmacol.*, **57**, 381 (1976).
(24) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).
(25) Prepared using solid-phase methodology, the octapeptide derivative gave satisfactory elemental and amino acid analysis and was indistinguishable by TLC from a previous preparation.[13]
(26) M. Bodanzsky, M. Kondo, C. Y. Lin, and G. F. Sigler, *J. Org. Chem.*, **39**, 444 (1974).
(27) The propanoic acid derivative used in preparation Ib was a gift from Dr. M. F. Ferger, Department of Chemistry, Cornell Univeristy, New York, N.Y.
(28) S. Moore, *J. Biol. Chem.*, **238**, 235 (1963).

Linked Aryl Aryloxypropanolamines as a New Class of Lipid Catabolic Agents

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Received June 22, 1977

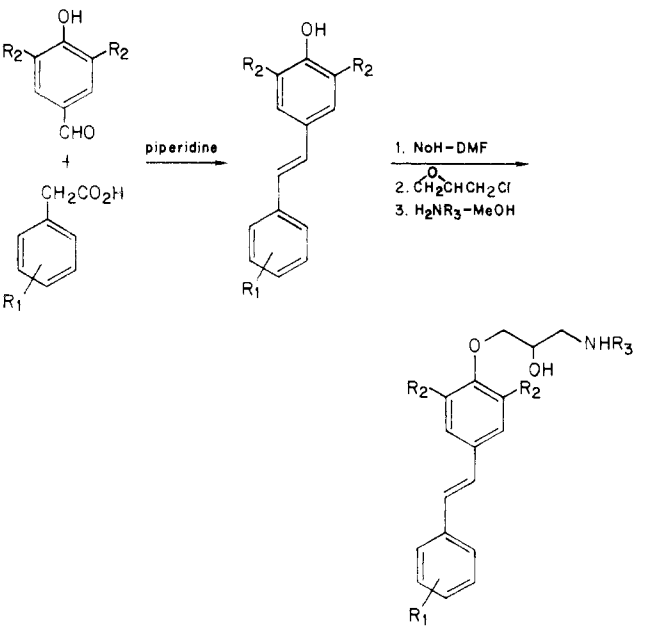
The synthesis of a series of stilbene, biaryl, tolane, diaryl ether, sulfide, sulfoxide, and sulfone oxypropanolamines as potential antiobesity agents is described. These compounds were evaluated in a mouse lipid catabolism screen, and the more active members of the series, **4**, **57**, and **58**, were further investigated in rats and dogs. 1-(2,6-Di-*tert*-butyl-4-*trans*-styrylphenoxy)-3-isopropylamino-2-propanol (**4**) possessed considerable lipid catabolic activity in mice and caused a significant reduction in the body weight of rats after 5 weeks and of dogs after 6 weeks. Only hematological irregularities in a chronic toxicity study precluded further development of this compound as an alternative antiobesity treatment.

The existing methods for the treatment of obesity rely heavily on the use of anorectic drugs, particularly the phenethylamine group[1,2] and more recently mazindol.[3] An alternative approach to this treatment would be by the use of calorigenic or lipid-catabolic drugs which would lower the carcass fat content of individuals.

We have been evaluating compounds for their lipid-catabolizing effects in rodents. The term "lipid catabolizing" is used to describe the ability to cause a net lipid loss, initially demonstrated by a reduction in uterofat and subsequently confirmed by carcass analysis. This effect is achieved other than by a simple reduction in food intake. Weak lipid-catabolizing activity of 2,4,6-tri-*tert*-butylphenol was observed in these laboratories and this encouraged the systematic and progressive modification of this structure leading to the preparation and evaluation of a series of linked diaryl oxypropanolamines, which is the subject of this report. The goal for this research effort was a compound which reduced body fat and consequently body weight without drastically affecting food intake.

Chemistry. The stilbene propanolamines were prepared by the route outlined in Scheme I. Condensation of the appropriately substituted arylacetic acid with a substituted *p*-hydroxybenzaldehyde was carried out in xylene with piperidine as a basic catalyst.[4] The crude phenolic *trans*-stilbene was usually purified by chromatography on a short column of Florisil. Sodium hydride in dimethylformamide was used to generate the sodium salts of the hydroxystilbenes and reaction with epichlorohydrin in refluxing dimethylformamide gave the glycidyl ethers in moderate to good yield. These epoxides reacted with the appropriate amine in methanol giving the desired propanolamines. Compounds **1–4**, **7–32**, and **34–51**

Scheme I



were prepared using this procedure and are listed in Table I. The 4-hydroxystilbene **33** was prepared by pyridine hydrochloride demethylation of **32**.

Compound **4** was initially selected for further biological evaluation and several derivatives of it were prepared. The stilbene double bond in **4** was readily hydrogenated over palladium on carbon giving the dihydro derivative **52**. Compound **4** was resolved using both *d*- and *l*-*p*-toluoyltartaric acids. Solutions of **4** in various solvents were

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