enthalpy change. This is unusual because most noncovalent interactions favored in water are facilitated because of a favorable entropy change, resulting from disruption of solvent structure. However, this change in stability may not directly involve the forces stabilizing the helix in water but may result instead from the phosphate–phosphate repulsions which should be greater in the helix than in the coil where they are more separated.[19] Thus, in water, the negative charges are shielded by the solvent, whereas in ethanol they may become a major destabilizing factor. The exact nature of the stacking interaction in polyriboadenylic acid awaits further investigation.

Acknowledgment. We are grateful for the assistance of Mr. Gary Davenport in the design and construction of the cell used to measure heat capacities and to Mrs. Evelyn R. Stimson for technical assistance with the measurements.

Syntheses and Pharmacological Properties of Selenium Isologs of Oxytocin and Deamino-oxytocin[1-3]

Roderich Walter and W. Y. Chan

Contribution from the Department of Biochemistry, Cornell University Medical College, New York, New York 10021, The Medical Research Center, Brookhaven National Laboratory, Upton, New York 11973, and The Physiology Department, Mount Sinai Medical and Graduate Schools, New York, New York 10029.
Received January 7, 1967

Abstract: Isologs, differing from oxytocin and deamino-oxytocin only in the replacement of the sulfur in position 1 or in position 6, have been synthesized, purified by countercurrent distribution, and tested for pharmacological activities. For the synthesis of the isolog 1-hemi-L-selenocystine-oxytocin, N-carbobenzoxy-Se-benzyl-L-selenocysteine was employed. This intermediate was obtained by the resolution of N-acetyl-Se-benzyl-DL-selenocysteine with hog acylase to yield Se-benzyl-L-selenocysteine which was subsequently carbobenzoxylated. The deamino-1-hemiseleno-oxytocin was synthesized with the use of Se-benzyl-β-selenopropionic acid in place of N-carbobenzoxy-Se-benzyl-L-selenocysteine. In addition, 6-hemi-L-selenocystine-oxytocin and its deamino analog were prepared. For the synthesis of the intermediate tetrapeptide N-carbobenzoxy-Se-benzyl-L-selenocysteinyl-L-prolyl-L-leucylglycinamide, the tripeptide L-prolyl-L-leucylglycinamide was acylated with *p*-nitrophenyl N-carbobenzoxy-Se-benzyl-DL-selenocysteinate, and the resulting diastereoisomeric tetrapeptides were resolved by fractional crystallization. The replacement of one sulfur atom by a selenium atom in position 1 or 6 yielded highly potent isologs of oxytocin and deamino-oxytocin. Upon bioassay, 1-hemi-L-selenocystine-oxytocin was found to possess 362 ± 9 units/mg of rat oxytocic activity, 351 ± 15 units/mg of rabbit milk-ejecting activity, 361 ± 18 units/mg of avian vasodepressor activity, 3.1 ± 0.2 units/mg of rat pressor activity, and 5.7 ± 0.6 units/mg of rat antidiuretic activity. The deamino-1-hemiseleno-oxytocin exhibited 560 ± 34 units/mg of oxytocic activity, 248 ± 8 units/mg of milk-ejecting activity, 613 ± 38 units/mg of vasodepressor activity, 1.7 ± 0.2 units/mg of pressor activity, and 24.0 ± 1.5 units/mg of antidiuretic activity. The comparable values for 6-hemi-L-selenocystine-oxytocin and deamino-6-hemiseleno-oxytocin were 405 ± 5, 398 ± 6, 385 ± 15, 3.8 ± 0.2, and 3.4 ± 0.1 units/mg and 492 ± 5, 397 ± 8, 622 ± 18, 1.1 ± 0.1, and 15.5 ± 1.0 units/mg, respectively.

The synthesis of oxytocin[4] (Figure 1) provided the foundation for investigating contributions of structural features of the molecule to its biological activity. One point of focus in our studies of structure–activity relationships involves questions about the role of the disulfide bond of neurohypophyseal hormones. In view of the chemical and biological analogies and diversities which exist between organic sulfur and selenium compounds,[5] it appeared desirable to investigate from a synthetic and biological standpoint the effect of the replacement of one of the sulfur atoms by a selenium atom in oxytocin and deamino-oxytocin. Thus the synthesis and pharmacological evaluation of 1-hemi-L-selenocystine-oxytocin (1-seleno-oxytocin), 6-hemi-L-selenocystine-oxytocin (6-seleno-oxytocin), and their corresponding deamino analogs were undertaken.[6]

For the syntheses of the key intermediates of the analogs presented herein, the *p*-nitrophenyl ester method[7] was used as introduced in the stepwise syn-

(1) This work was supported by Grants HE-01675 and HE-09795 of the National Heart Institute, by Grant AM-10080 of the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service, and by the U. S. Atomic Energy Commission.

(2) R. W. was responsible for the chemical, and W. Y. C. for the pharmacological aspects of the work presented here.

(3) The following abbreviations have been incorporated into the text: $CHCl_3$, chloroform; C_6H_{12}, cyclohexane; PrOH, 1-propanol; 2-PrOH, 2-propanol; BuOH, 1-butanol; AcOH, acetic acid; DMF, dimethylformamide; THF, tetrahydrofuran; DCC, N,N′-dicyclohexylcarbodiimide.

(4) V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, P. G. Katsoyannis, and S. Gordon, *J. Am. Chem. Soc.*, **75**, 4879 (1953); V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, and P. G. Katsoyannis, *ibid.*, **76**, 3115 (1954).

(5) T. W. Campbell, H. G. Walker, and G. M. Coppinger, *Chem. Rev.*, 50, 279 (1952); A. Shrift, *Federation Proc.*, 20, 695 (1961); D. Dingwall, *J. Pharm. Pharmacol.*, 14, 765 (1962); I. Rosenfeld and O. A. Beath, "Selenium," Academic Press Inc., New York, N. Y., 1964, Chapters 9 and 10; J. Jáuregui-Adell, *Advan. Protein Chem.*, 21, 387 (1966).

(6) A preliminary account of the synthesis and pharmacological evaluation of 6-seleno-oxytocin and its deamino analog has been reported: R. Walter and V. du Vigneaud, *J. Am. Chem. Soc.*, **87**, 4192 (1965).

(7) M. Bodanszky, *Nature*, **175**, 685 (1955).

thesis of oxytocin;[8] the last step was the coupling of *p*-nitrophenyl N-carbobenzoxy-Se-benzyl-L-selenocysteinate and *p*-nitrophenyl Se-benzyl-β-selenopropionate, respectively, with the octapeptide amide, L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide. The Se-benzyl-L-selenocysteine, which, following carbobenzoxylation, was converted to the *p*-nitrophenyl ester, had been obtained by the resolution of N-acetyl-Se-benzyl-DL-selenocysteine[9] with hog acylase I. The enzymatic resolution product exhibited an identical optical rotation with Se-benzyl-L-selenocysteine prepared by another method.[10] The Se-benzyl-β-selenopropionic acid, which in turn was treated with *p*-nitrophenol in the presence of DCC to form the *p*-nitrophenyl Se-benzyl-β-selenopropionate, had been synthesized by the base-catalyzed β-addition of benzylselenol to acrylic acid.

For the synthesis of 6-seleno-oxytocin and its deamino analog the deprotected tripeptide amide, L-prolyl-L-leucylglycinamide,[11] was lengthened with *p*-nitrophenyl N-carbobenzoxy-Se-benzyl-DL-selenocysteinate to the tetrapeptides N-carbobenzoxy-Se-benzyl-L-selenocysteinyl- and N-carbobenzoxy-Se-benzyl-D-selenocysteinyl-L-prolyl-L-leucylglycinamide. At this stage, on the basis of the difference in solubility of the two diastereoisomeric sulfur-containing tetrapeptides,[12] we anticipated that the separation of the corresponding selenium-containing tetrapeptides might succeed. And, in fact, the desired N-carbobenzoxy-Se-benzyl-L-selenocysteinyl-L-prolyl-L-leucylglycinamide resulted from the diastereoisomeric peptides by fractional crystallization in 78% of the theoretical yield; thus the resolution of the DL-selenocysteine and the preparation of the tetrapeptide amide were achieved in one step.[12a] Upon chromatography on a thin-layer silica gel (Kieselgel G, Merck) plate in $CHCl_3$–methanol (9:1) or on paper in $CHCl_3$–C_6H_{12} (7:3) and 2-PrOH–water (2:1), the R_f values of the protected L-tetrapeptide were identical with those of the corresponding sulfur analog. As in the sulfur series,[12] the corresponding D-selenocysteine-containing diastereoisomer traveled faster under identical chromatographic conditions.[13] The protected L-tetrapeptide amide exhibited a somewhat more negative optical rotation and higher melting point than was recorded for the same compound.[13] Decarbobenzoxylation of the tetrapeptide with hydrobromic acid in glacial AcOH was followed by passing the hydrobromide through an anion-exchange resin, yielding Se-benzyl-L-selenocysteinyl-L-prolyl-L-leucylglycinamide. This partially deprotected tetrapeptide amide was lengthened stepwise with the appropriate protected amino acid *p*-nitrophenyl esters to the key intermediates, N-carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-Se-benzyl-L-selenocysteinyl-L-prolyl-L-leucylglycinamide and S-benzyl-β-mercaptopropionyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-Se-benzyl-L-selenocysteinyl-L-prolyl-L-leucylglycinamide.

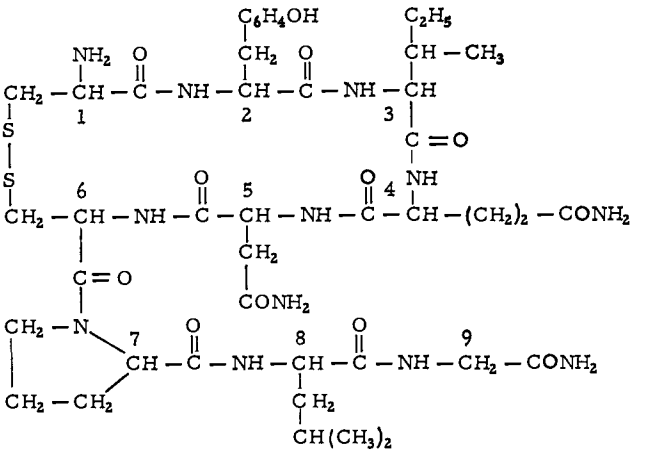

Figure 1. Structure of oxytocin with numbers indicating the position of the individual amino acid residues.

The peptide intermediates for the synthesis of 1- and 6-seleno-oxytocin and their deamino analogs were deprotected with sodium in liquid ammonia according to the method of Sifferd and du Vigneaud[14] as employed in the original synthesis of oxytocin.[4] Following the removal of ammonia the reduced compounds were oxidized at a slightly acidic pH by treatment with potassium ferricyanide as employed for the synthesis of deamino-oxytocin.[15] The ferricyanide and ferrocyanide ions were removed with a cation-exchange resin. Further purification of the 1- and 6-seleno-oxytocins was effected by countercurrent distribution[16] in the solvent system BuOH–PrOH–0.5% AcOH containing 0.1% pyridine (6:1:8). In this solvent system the isologs exhibited identical partition coefficients (*K*). The 1-seleno-oxytocin was purified by countercurrent distribution in a second solvent system, BuOH–benzene–pyridine–0.1% AcOH (6:2:1:9),[17] selected in order to separate the L analog from the 1-D-seleno-oxytocin diastereoisomer, which may have been formed during the acylation of L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide with N-carbobenzoxy-Se-benzyl-L-selenocysteine in the presence of DCC. These solvent systems were chosen since the seleno isologs were expected to exhibit approximative or identical *K* values as compared with the corresponding sulfur compounds, an assumption that was confirmed in this study. Furthermore, the selection was based on the effectiveness of these solvent systems in the purification of oxytocin. Similarly, deamino-1- and deamino-6-seleno-oxytocin were oxidized, deionized, and isolated by countercurrent dis-

(8) M. Bodanszky and V. du Vigneaud, *Nature*, **183**, 1324 (1959).
(9) G. Zdansky, *Arkiv Kemi*, **17**, 273 (1961).
(10) W. Frank, *Z. Physiol. Chem.*, **339**, 202 (1964).
(11) R. A. Boissonnas, S. Guttmann, P.-A. Jaquenoud, and J. P. Waller, *Helv. Chim. Acta*, **38**, 1491 (1955); M. Zaoral and J. Rudinger, *Collection Czech. Chem. Commun.*, **20**, 1183 (1955); W. B. Lutz, C. Ressler, D. E. Nettleton, Jr., and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 167 (1959).
(12) M. Manning and V. du Vigneaud, *Nature*, **87**, 3978 (1965).
(12a) NOTE ADDED IN PROOF. In the meantime a general method for the synthesis of L-selenoncysteine-containing peptides has been developed in our laboratory (D. Theodoropoulos, I. L. Schwartz, and R. Walter, *Tetrahedron Letters*, in press) permitting a more convenient preparation of this tetrapeptide.
(13) No difference was found in the solubility of the protected L-selenocysteinyl tetrapeptide amide as compared with the corresponding sulfur analog, in contrast with the observation reported by W. Frank, *Z. Physiol. Chem.*, **339**, 222 (1964).
(14) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).
(15) V. du Vigneaud, G. Winestock, V. V. S. Murti, D. B. Hope, and R. D. Kimbrough, Jr., *ibid.*, **235**, PC64 (1960); D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *ibid.*, **237**, 1563 (1962); D. Jarvis and V. du Vigneaud, *Science*, **143**, 545 (1964); B. M. Ferrier, D. Jarvis, and V. du Vigneaud, *J. Biol. Chem.*, **240**, 4264 (1965).
(16) L. C. Craig, W. Hausmann, E. H. Ahrens, Jr., and E. J. Harfenist, *Anal. Chem.*, **23**, 1236 (1951).
(17) D. Yamashiro, D. Gillessen, and V. du Vigneaud, *J. Am. Chem. Soc.*, **88**, 1310 (1966).

Table I. Biological Potencies[a,b] of Oxytocin, 1-Seleno-oxytocin, 6-Seleno-oxytocin, and Their Deamino Analogs

Compound	Depressor (fowl)	Oxytocic (rat)	Milk-ejecting (rabbit)	Pressor (rat)	Antidiuretic (rat)
Oxytocin	507 ± 15[c]	546 ± 18[d]	410 ± 16[c]	3.1 ± 0.1[c]	2.7 ± 0.2[c]
Deamino-oxytocin[e]	733 ± 23[c]	684 ± 32[c]	400 ± 8[c]	1.1 ± 0.1[c]	15.0 ± 2.0[c]
1-Seleno-oxytocin	361 ± 18	362 ± 9	351 ± 15	3.1 ± 0.2	5.7 ± 0.6
Deamino-1-seleno-oxytocin	613 ± 38	560 ± 34	248 ± 8	1.7 ± 0.2	24.0 ± 1.5
6-Seleno-oxytocin	385 ± 15	405 ± 5	398 ± 6	3.8 ± 0.2	3.4 ± 0.1
Deamino-6-seleno-oxytocin	622 ± 18	492 ± 5	397 ± 8	1.1 ± 0.1	15.5 ± 1.0

[a] The bioassay methods are described in the Experimental Section. [b] Expressed in USP units/mg ± SE. [c] Value reported by Chan and du Vigneaud; see ref 24. [d] Value reported by W. Y. Chan, M. O'Connell, and S. R. Pomeroy, *Endocrinology*, **72**, 279 (1963), from assays on uteri from rats in natural estrus. The value from assays on uteri from a large number of rats taken at random with regard for stage of the estrous cycle was found to be 486 ± 5 units/mg: Chan and du Vigneaud, ref 24. [e] These values are those earlier reported for amorphous deamino-oxytocin, extensively purified by countercurrent distribution and lyophilized. These values are given in Table I for comparison with the selenium-containing analogs which are isolated in the same manner and are also amorphous. For crystalline deamino-oxytocin the following values were reported: depressor 975 ± 24; oxytocic 803 ± 36; milk-ejecting 541 ± 13; pressor 1.44 ± 0.06; antidiuretic 19.0 ± 1: Ferrier, Jarvis, and du Vigneaud.[15]

tribution. The solvent system BuOH–benzene–0.05% AcOH (3:2:5), in which both isologs exhibited comparable K values, was found satisfactory.

Following purification by countercurrent distribution the contents of the tubes comprising the biologically active peptides (as indicated by the determination of Folin–Lowry color values[18] and avian vasodepressor activity) were combined, concentrated, and lyophilized. The compounds so obtained were evaluated for purity by paper chromatography, gel filtration on Sephadex G-25 in 0.2 N AcOH, amino acid analyses, and elementary analyses.

The selenium-containing isologs were indistinguishable from the corresponding sulfur hormones on paper chromatography in BuOH–AcOH–water (4:1:5, upper phase) and BuOH–AcOH–water (5:1:5, upper phase), although in the case of the selenium isologs a trace of chromogenic material remained at the origin of the chromatograms. This polymeric material was formed at least in part during lyophilization after the countercurrent distribution[19] and during application of the sample onto the paper. This behavior is due in all probability to the greater instability of selenium-containing peptides as compared with isologous sulfur-containing peptides.

Amino acid analyses[20] of acid hydrolysates of the isologs gave the expected molar proportions of amino acids and ammonia, except in the case of the selenium-containing acids which were low due to partial destruction[21] during the hydrolysis procedure. Furthermore, an acid-catalyzed interchange[22] occurred with the selenenyl thiolates during acid hydrolysis yielding in the cases of 1- and 6-seleno-oxytocin a mixture of cystine, selenocystine, 1,6-diamino-3-thio-4-selenohexane-1,6-dicarboxylic acid (the selenenyl thiolate of cysteine and selenocysteine), and in the cases of deamino-1- and deamino-6-seleno-oxytocin a mixture of cystine, 1-amino-3-thio-4-selenohexane-1,6-dicarboxylic acid (the selenenyl thiolate of cysteine and β-selenopropionic acid), 3,4-diselenohexane-1,6-dicarboxylic acid (diselenide of β-selenopropionic acid; ninhydrin negative), and selenocystine, 1-amino-3-seleno-4-thiohexane-1,6-dicarboxylic acid (the selenenyl thiolate of selenocysteine and β-mercaptopropionic acid), and the ninhydrin-negative diselenide of β-selenopropionic acid, respectively.

The 1- and 6-seleno-oxytocins and their corresponding deamino analogs were assayed for some of the biological activities characteristic of the posterior pituitary hormones. The potencies of these compounds are compared with the potencies of oxytocin and deamino-oxytocin in Table I. It can be seen from Table I that the replacement of one sulfur atom by one selenium atom[23] yields highly active isologs. With respect to the "oxytocin-like" activities, *i.e.*, the avian depressor, oxytocic, and milk-ejecting activities, the selenium-containing isologs exhibited somewhat lower potencies than the corresponding disulfide-containing peptides, oxytocin and deamino-oxytocin. This reduction in biological activity appears to be due at least in part to an increased tendency of the hemiseleno isologs, as compared with the corresponding disulfide isologs, to form aggregates of higher molecular weight.[19] Furthermore, it was found that the isologs in which selenium replaces sulfur in position 1, as compared with the corresponding 6 isomers, had a greater tendency to decompose and polymerize during oxidative closure of the 20-membered ring and during lyophilization. This may explain the slightly lower values of "oxytocin-like" activities recorded for the 1-seleno isologs as compared with the 6-seleno isologs. One exception is the higher oxytocic activity of deamino-1-seleno-oxytocin relative to that of deamino-6-seleno-oxytocin, a finding which may be related to the oxytocic potentiat-

(18) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

(19) For example, when 1-seleno-oxytocin is purified by countercurrent distribution in the solvent system BuOH–benzene–pyridine–0.1% AcOH (6:2:1:9) and BuOH–PrOH–0.5% AcOH containing 0.1% pyridine (6:1:8) and then lyophilized and redistributed in the latter system, a considerable amount of material with an approximate K value of 0.19 (for 1- and 6-seleno-oxytocin K values between 0.48 and 0.53 were found) is formed as revealed by the Folin–Lowry color reaction.

(20) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, 30, 1190 (1958).

(21) I. Rosenfeld, *Federation Proc.*, 20, 10 (1961).

(22) Similarly, a mixed disulfide interchange is observed during the acid hydrolysis of deamino-oxytocin analogs.[15]

(23) Previously Frank[13] reported the synthesis of diseleno-oxytocin. The last compound in his series of syntheses which was chemically characterized was the protected nonapeptide amide. The protecting groups of this intermediate were removed with sodium in liquid ammonia, and the resulting diseleno-oxytoceine was oxidized with air. Unfortunately, the diseleno-oxytocin, which undoubtedly had formed, was neither chemically nor pharmacologically characterized in the paper so that no comparison of this compound with oxytocin or our seleno analogs is possible. A preliminary account of the synthesis and pharmacological evaluation of deamino-diseleno-oxytocin has been reported: R. Walter and V. du Vigneaud, *J. Am. Chem. Soc.*, **88**, 1331 (1966). It was found that this isolog, in which both sulfur atoms were replaced with selenium, had an even higher avian vasodepressor potency than the deamino-hemiseleno isologs reported here.

ing effect peculiar to this isolog. When introduced into the bathing medium of the isolated rat uterus, deamino-1-seleno-oxytocin potentiated the responses to subsequent injections of the isolog as well as to the reference standard.

In the case of rat pressor activity practically no difference between the seleno and the sulfur isologs is detected. The antidiuretic potencies of 6-seleno-oxytocin and deamino-6-seleno-oxytocin are comparable to those of oxytocin and deamino-oxytocin; somewhat higher values are found for 1-seleno-oxytocin and deamino-1-seleno-oxytocin in this assay.

The biological data also show that in the case of 1-seleno-oxytocin as well as in the case of 6-seleno-oxytocin, the replacement of the free amino group by hydrogen enhances the oxytocic, avian vasodepressor, and rat antidiuretic activities and lowers the pressor activity as it does in the case of oxytocin.[24]

Apparently, subtle differences, both chemical and pharmacological, are introduced into oxytocin and deamino-oxytocin by the replacement of one sulfur atom by a selenium atom. In addition, the biological data indicate a nonequivalence of the selenium substitutions in positions 1 and 6.

Experimental Section[25]

Resolution of N-Acetyl-Se-benzyl-DL-selenocysteine with Hog Acylase I. N-Acetyl-Se-benzyl-DL-selenocysteine[9] (15.0 g) was suspended in 800 ml of water and after the pH was adjusted to 7.5 with concentrated ammonium hydroxide, the volume was increased to 1000 ml by the addition of water. The temperature of the solution was brought to 38° and 2.0 g of hog acylase I was added. Overnight Se-benzyl-L-seleno-cysteine crystallized from the reaction mixture. After 24 hr of incubation an additional 0.5 g of acylase I was added and 24 hr later the pH of the reaction mixture was adjusted with AcOH to 5. Caprylic alcohol (1 ml) was added to the digest, which was subsequently concentrated under reduced pressure to 200 ml. The precipitate, consisting of Se-benzyl-L-selenocysteine and coagulated protein, was collected, repeatedly washed with cold water, and extracted with two 100-ml portions of 2 *N* hydrochloric acid at 70° in the presence of charcoal. The pH of the combined hydrochloric acid filtrates was brought to 6 by the addition of 25% ammonium hydroxide solution, which induced the crystallization of Se-benzyl-L-selenocysteine. The crystalline material, which increased upon chilling of the mixture, was filtered off and washed successively with ice-cold water, ethanol, and ether, yield 2.0 g, mp 183–184°, $[\alpha]^{24}D$ +35.2° (*c* 2, 1 *N* sodium hydroxide) [lit.[10] mp 191–192°, $[\alpha]^{23}D$ +35.6° (*c* 2, 1 *N* sodium hydroxide)].

Anal. Calcd for $C_{10}H_{13}NO_2Se$: C, 46.5; H, 5.08; N, 5.43. Found: C, 46.8; H, 5.22; N, 5.43.

N-Carbobenzoxy-Se-benzyl-L-selenocysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide. A sample of the protected octapeptide (0.5 g), N-carbobenzoxy-O-benzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide,[26] suspended in 10 ml of freshly distilled trifluoroethanol, was treated for 15 min with dry hydrogen bromide, and the resulting light brown solution was allowed to stand for an additional 30 min at room temperature. After removal of the solvent the residue was washed with six 100-ml portions of ether and then dissolved in 50 ml of dry methanol. This methanolic solution was passed through a Rexyn RG1(OH) column which was subsequently washed with 80 ml of methanol. The eluate and washings were removed under reduced pressure, and the resulting solid was dissolved together with 0.5 g of N-carbobenzoxy-Se-benzyl-L-selenocysteine in 8 ml of DMF. The solution was cooled with an ice bath, and 0.3 g of DCC was added. During the next 3 hr, the mixture was allowed to warm up to room temperature and stirring was continued for 2 days. Thereupon, 50 ml of ethyl acetate was added to the reaction mixture; the precipitate thus formed was collected and washed with two 30-ml portions of ethanol–ethyl acetate (9:1) to eliminate the N,N′-dicyclohexylurea. After being dried over P_2O_5 *in vacuo* overnight the material weighed 0.47 g, mp 246–248° dec, $[\alpha]^{24}D$ −60.0° (*c* 1, 97% formic acid).

Anal. Calcd for $C_{65}H_{86}N_{12}O_{14}SSe$: C, 57.0; H, 6.33; N, 12.3. Found: C, 57.0; H, 6.38; N, 12.1.

1-Seleno-oxytocin. The nonapeptide (0.4 g) described in the foregoing paragraph was dissolved in 400 ml of anhydrous liquid ammonia and treated at the boiling point with sodium until a faint blue color enveloped the solution which persisted for about 10 sec. One drop of glacial AcOH was added, and the ammonia was evaporated *in vacuo*, the last 50 ml being removed by lyophilization. The residue was dissolved in 400 ml of 0.1% AcOH, and after adjustment of the pH to 6.8, the solution was titrated with 40 ml of 0.01 *M* ferricyanide solution. The ferrocyanide and ferricyanide ions were removed by passage through a column of ion-exchange resin AG3X4 in the chloride form. The solution was concentrated at a temperature below 20° to a volume of approximately 20 ml, placed in the first six tubes of a countercurrent distribution machine, and subjected to a total of 325 transfers in the solvent system BuOH–benzene–pyridine–0.1% AcOH (6:2:1:9). Determination of Folin–Lowry color values indicated a peak with a *K* value of approximately 0.63, which is identical with the *K* value reported for oxytocin,[17] accompanied by one larger, more slowly moving peak. High avian depressor activity was associated with the peak having the *K* value of 0.63. The contents of 30 tubes representing the middle portion of this peak were pooled, evaporated in a flash evaporator to a volume of approximately 40 ml, and lyophilized to give 200 mg of fluffy, white product. This material was subjected to a second countercurrent distribution in the solvent system BuOH–PrOH–0.5% AcOH containing 0.1% pyridine (6:1:8). After 360 transfers a separation into two peaks, a faster moving major peak with a *K* value of 0.53 and a smaller one with a *K* value of 0.19, had been accomplished, as determined by the Folin–Lowry color reaction. Another preparation was purified twice by countercurrent distribution in the latter solvent system; however, the peak having a *K* value of 0.19 persisted with approximately the same intensity. This material must form during the isolation of the hormone isolog.[19] The contents of 20 tubes of the central portion of the major peak were combined, concentrated under reduced pressure to approximately 100 ml, and lyophilized, giving 0.132 g of a fluffy powder, $[\alpha]^{20}D$ −13.0° (*c* 1, 1 *N* AcOH).

Anal. Calcd for $C_{43}H_{66}N_{12}O_{12}SSe$: C, 49.0; H, 6.31; N, 15.9. Found: C, 48.9; H, 6.37; N, 16.0.

Upon chromatography on paper and gel filtration 1-seleno-oxytocin, R_f 0.57 (S_1), R_f 0.50 (S_2), was indistinguishable from oxytocin, R_f 0.59 (S_1),[11] R_f 0.50 (S_2). A sample of the isolog was hydrolyzed[27] and analyzed on an amino acid analyzer for ninhydrin-active materials. The following ratios were obtained, glycine being taken as 1.0: $Asp_{1.0}Glu_{1.0}Pro_{1.0}Gly_{1.0}Cys_{0.4}Ile_{1.0}Tyr_{0.7}NH_{3\ 3.0}$. In addition, selenocystine[28] and 1,6-diamino-3-thio-4-selenohexane-

(24) W. Y. Chan and V. du Vigneaud, *Endocrinology*, 71, 977 (1962).

(25) All melting points (mp) were determined with a Thomas-Hoover capillary melting-point apparatus and are corrected. Optical rotations were determined with a Carl Zeiss Kreispolarimeter, 0.01°, or a Carl Zeiss photoelectric precision polarimeter, 0.005°. Descending chromatography was performed on Whatman No. 1 paper with BuOH–AcOH–water (4:1:5, v/v/v, upper phase) (S_1) and BuOH–AcOH–water (5:1:5, v/v/v, upper phase) (S_2) as solvents at concentrations of 25, 50, and 100 μg of the hormones. Gel filtrations were carried out on a Sephadex G-25 column (0.9 × 98.0 cm) with 0.2 *N* AcOH. The peptides were detected according to the procedure by H. Zahn and E. Rexroth, *Z. Anal. Chem.*, **148**, 181 (1955). The samples for elementary analysis were dried for 12 hr at room temperature over P_2O_5 *in vacuo* and analyzed by Galbraith Laboratories, Knoxville, Tenn.

(26) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).

(27) The samples were hydrolyzed in 6 *N* hydrochloric acid *in vacuo* at 110° for 22 hr. After the hydrolysates were evaporated to dryness, the residues were dissolved in a citrate buffer (pH 2.2) and placed onto the column of a Beckman-Spinco amino acid analyzer Model 120B, as modified through the application of Beckman Custom Research Resin using a flow rate of 50 cc/hr of buffer, 25 cc/hr of ninhydrin, and a 2-sec print time. The analyses were carried out at a temperature of 52.5°.

(28) The column was initiated with a pH 3.28 citrate buffer and a 0.2 *N* sodium ion concentration, changing to a pH 4.25 citrate buffer after 96 ml had been passed onto the column. The buffer change appeared on the chromatogram after 142 ml of buffer. Under these conditions, methionine appeared on the chromatogram after 150 ml, isoleucine after 158 ml, and leucine after 163 ml of buffer. The DL-selenocystine appeared on the chromatogram after 149 ml of buffer had passed onto the column. The position of DL-selenocystine, secured in a test run with isoleucine as reference, agreed with that previously reported [K. P. McConnell and C. H. Wabnitz, *Biochim. Biophys. Acta*, **86**, 182 (1964), and J. L. Martin and L. M. Cummins, *Anal. Biochem.*, **15**, 530 (1966)].

1,6-dicarboxylic acid[29] were detected.

Se-Benzyl-β-selenopropionic Acid. To an ice-cold mixture of 4.46 g of benzylselenol[30] and 2.0 ml of acrylic acid 4.5 ml of piperidine was added. In the course of a few minutes the exergonic reaction yielded a yellowish solid. After a period of 12 hr at room temperature this material was dissolved in 50 ml of 9% hydrochloric acid and extracted with 100 ml of ether. To separate the unwanted dibenzyl diselenide the product was extracted with three 30-ml portions of a saturated aqueous sodium bicarbonate solution, which was immediately acidified with concentrated hydrochloric acid to congo red and extracted with two 50-ml portions of ether. The ethereal solution was twice washed with 25-ml portions of a saturated sodium chloride solution and subsequently dried over anhydrous sodium sulfate. Upon evaporation of the solvent yellowish crystals were obtained which were recrystallized from an ether–petroleum ether (bp 60–90°) mixture to yield 5.6 g of white, odorless crystals with mp 74–76°.

Anal. Calcd for $C_{10}H_{12}O_2Se$: C, 49.4; H, 4.98. Found: C, 49.4; H, 4.98.

***p*-Nitrophenyl Se-Benzyl-β-selenopropionate.** Se-Benzyl-β-selenopropionic acid (2.0 g) and *p*-nitrophenol (1.3 g) were dissolved in 30 ml of ethyl acetate. The solution was cooled to 0° and stirred while DCC (1.7 g) was added. N,N′-Dicyclohexylurea started immediately to precipitate. The stirring was continued for 30 min at 0° and then for 12 hr at room temperature whereupon five drops of glacial AcOH were added. An hour later the N,N′-dicyclohexylurea was filtered off and washed with 10 ml of ethyl acetate. The filtrate and the washings were concentrated and passed through a silica gel column (1.0 × 8.0 cm). Upon evaporation of the solvent a yellowish oil resulted which started to crystallize when kept in the refrigerator overnight. After the addition of 10 ml of a methanol–petroleum ether (bp 60–90°) mixture (1:1) the crystallization proceeded faster. The crystals were filtered off in the cold room and washed with the methanol–petroleum ether mixture, giving 2.06 g, mp 38–39°.

Anal. Calcd for $C_{16}H_{15}NO_4Se$: C, 52.8; H, 4.15; N, 3.85. Found: C, 52.7; H, 4.31; N, 3.76.

Se-Benzyl-β-selenopropionyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide. The octapeptide, N-carbobenzoxy-O-benzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide, was suspended in 15 ml of freshly distilled trifluoroethanol and treated for 15 min with dry hydrogen bromide gas at room temperature. The reaction mixture was kept at room temperature for an additional 30 min before the solvent was evaporated *in vacuo*. The resulting hydrobromide salt of the octapeptide was washed once with ether, then dissolved in 3 ml of dry methanol and precipitated with 50 ml of ether. The solid material was collected by quick filtration and dissolved in 3 ml of DMF at 0°. Following the adjustment of the pH to 7.5 by dropwise addition of triethylamine, the mixture was allowed to react with 0.43 g of *p*-nitrophenyl Se-benzyl-β-selenopropionate. Three days later, 20 ml of water was added to the reaction mixture, and the solid material was collected and purified with two 30-ml portions of ethanol–ethyl acetate (9:1). When it had been dried over P_2O_5 *in vacuo* at room temperature for 12 hr the product weighed 0.87 g, mp 239–241° dec, $[\alpha]^{20}D$ −38.4° (*c* 1, DMF).

Anal. Calcd for $C_{57}H_{79}N_{11}O_{12}SSe$: C, 56.0; H, 6.52; N, 12.6. Found. C, 55.9; H, 6.70; N, 12.4.

Deamino-1-seleno-oxytocin. A solution of 0.4 g of the protected polypeptide described in the preceding section in approximately 300 ml of boiling ammonia was treated with a slight excess of the theoretical amount of sodium required for the debenzylation. The solution was evaporated *in vacuo* to about 50 ml and lyophilized. The resulting fluffy residue was dissolved in 100 ml of 0.01% AcOH. Following adjustment of the pH of the solution to 6.8 and oxidation with 48 ml of 0.01 *M* aqueous ferricyanide, the mixture was passed through a column of AG3X4 resin. The column was washed with 200 ml of distilled water. The eluate and washings were concentrated *in vacuo* to about 18 ml, and this solution was placed into the first six tubes of a countercurrent distribution machine. For purification the material was subjected to a total of 400 transfers in the solvent system BuOH–benzene–0.05% AcOH (3:2:5). Folin–Lowry color determination revealed a peak with a *K* value of 1.01. The contents of 40 tubes representing the central portion of the peak were combined, concentrated to approximately 80 ml, and lyophilized to give 0.018 g of deamino-1-seleno-oxytocin, $[\alpha]^{24}D$ −46.4° (*c* 0.5, glacial AcOH).

Anal. Calcd for $C_{43}H_{65}N_{11}O_{12}SSe$: C, 49.7; H, 6.31; N, 14.8. Found: C, 49.7; H, 6.36; N, 14.5.

Upon paper chromatography deamino-1-seleno-oxytocin gave a spot at R_f 0.77 (S_1) and R_f 0.75 (S_2) (at a concentration of 50 μg or higher a very small faint second spot at R_f 0.28 was detected). Deamino-oxytocin exhibited under identical conditions R_f 0.78 (S_1)[15] and R_f 0.75 (S_2). The deamino-1-seleno-oxytocin was also indistinguishable from deamino-oxytocin upon gel filtration. Amino acid analysis gave the following molar ratios, glycine being taken as 1.0: $Asp_{1.0}Glu_{1.0}Pro_{1.1}Gly_{1.0}Cys_{0.3}Ile_{1.0}Leu_{1.0}Tyr_{0.8}NH_{3\ 3.3}$. In addition, the peak of 1-amino-3-thio-4-selenohexane-1,6-dicarboxylic acid was recorded.[31]

***p*-Nitrophenyl N-Carbobenzoxy-Se-benzyl-DL-selenocysteinate.** N-Carbobenzoxy-Se-benzyl-DL-selenocysteine (7.1 g)[32] and *p*-nitrophenol (2.86 g) were dissolved in dry THF (70 ml). The solution was cooled to 0° and stirred while DCC (3.75 g) was added. Stirring was continued for 30 min at 0° and then overnight at room temperature. Unreacted DCC was destroyed by the addition of 1 ml of 1 *N* hydrochloric acid. The N,N′-dicyclohexylurea was filtered off 15 min after the addition of the acid and washed with a few milliliters of THF. The combined filtrate and washings were evaporated *in vacuo*. The resulting oil crystallized from ethyl acetate–petroleum ether (bp 30–60°), giving 6.3 g, mp 96–98°. A sample was twice recrystallized from the ethyl acetate–petroleum ether mixture, mp 98–99°.

Anal. Calcd for $C_{24}H_{22}N_2O_6Se$: C, 56.1; H, 4.32; N, 5.45. Found: C, 56.2; H, 4.38; N, 5.41.

N-Carbobenzoxy-Se-benzyl-L-selenocysteinyl-L-prolyl-L-leucylglycinamide. To an ice-cold solution of 7.4 g of L-prolyl-L-leucylglycinamide[11] in 22 ml of DMF 13.4 g of *p*-nitrophenyl N-carbobenzoxy-Se-benzyl-DL-selenocysteinate was added. The reaction mixture, after being stirred for 3 hr at 0° and 3 days at room temperature, was dissolved in 500 ml of ethyl acetate. The resulting solution was extracted with ten 100-ml portions of ice-cold 1 *N* ammonium hydroxide, two 100-ml portions of 1 *N* hydrochloric acid, and two 100-ml portions of water and then dried with anhydrous sodium sulfate. The drying agent was removed, and the filtrate was concentrated to approximately 160 ml and seeded with N-carbobenzoxy-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide. Ether (40 ml) was added in small portions over the next 2 days. After 7 days the crystalline, protected L-tetrapeptide amide was filtered off and recrystallized from aqueous methanol, yield 6.67 g (78% of the yield of the L-diastereoisomer), mp 162–163°, $[\alpha]^{18}D$ −52.3° (*c* 2, DMF) [lit.[13] mp 157–159°, $[\alpha]^{25}D$ −48.1° (*c* 0.68, DMF)]. Upon descending paper chromatography in the solvent system $CHCl_3$–C_6H_{12} (7:3) (2-PrOH–water, 2:1) one spot with an R_f of 0.87 [0.88] was obtained (for the corresponding sulfur-containing tetrapeptide an identical R_f was recorded). In addition, thin layer chromatography in the system $CHCl_3$–methanol (9:1) gave only one spot.

Anal. Calcd for $C_{31}H_{41}N_3O_6Se$: C, 56.5; H, 6.27; N, 10.6. Found: C, 56.3; H, 6.20; N, 10.5.

N-Carbobenzoxy-L-asparaginyl-Se-benzyl-L-selenocysteinyl-L-prolyl-L-leucylglycinamide. A solution of 5.0 g of the protected tetrapeptide in 20 ml of glacial AcOH was treated with 20 ml of 4 *N* HBr in glacial AcOH. After 1 hr at room temperature the solution was poured into 400 ml of cold, anhydrous ether. The precipitated hydrobromide was washed by decantation with three

(29) In addition to the two peaks representing selenocystine and cystine, a third peak appeared on the chromatogram after 143 ml of buffer. That this peak represents the selenenyl thiolate of selenocysteine and cysteine was established when an air-oxidized sample (at room temperature) of an equimolar mixture of selenocystine and cysteine hydrochloride monohydrate was chromatographed on the amino acid analyzer under identical experimental conditions for amino acid content. Furthermore, when mixtures of selenocystine and cystine were analyzed after being subjected to conditions used for the hydrolysis of 1- and 6-seleno-oxytocin, the recoveries of selenocystine and cystine were found to be low as compared to the recovery of an identical mixture not subjected to the acid treatment, indicating that considerable destruction had occurred under the hydrolytic conditions.[21] The nature of the products which originated during acid hydrolysis is presently under investigation. Therefore, only the positions of the selenium-containing amino acids in the chromatograms are reported in this paper and not the recoveries.

(30) E. P. Painter, *J. Am. Chem. Soc.*, **69**, 229 (1947).

(31) This dicarboxylic acid appears on the chromatogram after 146 ml under identical experimental conditions described in ref 27 and 28.

(32) J. Janicki, J. Skupin, and B. Zagalak, *Roczniki Chem.*, **36**, 353 (1962).

400-ml portions of ether. After being dried *in vacuo* over KOH and P_2O_5 overnight, the hydrobromide was dissolved in 200 ml of methanol and passed through a column of the ion-exchange resin Rexyn RG1(OH) (20-ml resin volume). The column was washed with 100 ml of methanol, and the solid obtained after evaporation of the solvent from the eluate and washings was dissolved in 7 ml of DMF. The solution was cooled to 0° and 3.2 g of *p*-nitrophenyl N-carbobenzoxy-L-asparaginate was added. The reaction mixture solidified overnight. After storage for 2 days at room temperature the product was collected and washed nine times with 60-ml portions of an ethyl acetate–ethanol mixture (3:2). The material was dried to constant weight over P_2O_5 *in vacuo* at room temperature to give 4.0 g, mp 209–211°, $[\alpha]^{20}D$ −54.0° (*c* 1, DMF) (lit.[13] mp 205–207°).

Anal. Calcd for $C_{35}H_{47}N_7O_8Se$: C, 54.4; H, 6.13; N, 12.7. Found: C, 54.1; H, 6.15; N, 12.9.

N-Carbobenzoxy-L-glutaminyl-L-asparaginyl-Se-benzyl-L-selenocysteinyl-L-prolyl-L-leucylglycinamide. The protected pentapeptide (3.9 g) was decarbobenzoxylated and isolated according to the procedure described in the preceding paragraph. The methanolic solution (300 ml) of the hydrobromide was passed through a Rexyn RG1(OH) column (28 ml). After removal of the solvent the residue was dissolved in 10 ml of DMF and allowed to react with 2.2 g of *p*-nitrophenyl N-carbobenzoxy-L-glutaminate. The reaction mixture solidified during the next 12 hr. After 2 days the solid material was collected and washed with eight 80-ml portions of an ethyl acetate–ethanol mixture (4:1). After being dried *in vacuo* at room temperature over P_2O_5, the material weighed 4.2 g, mp 215–219° dec, $[\alpha]^{20}D$ −57.0° (*c* 1, DMF). The material was dissolved in a boiling mixture of THF–water (1:1). The peptide amide which reprecipitated upon cooling was filtered off and dried *in vacuo* over P_2O_5, yielding 3.9 g, mp 222–224°, $[\alpha]^{20}D$ −56.5° (*c* 1, DMF) [lit.[13] mp 206–210°, $[\alpha]^{24}D$ −52.0° (*c* 1, DMF)].

Anal. Calcd for $C_{40}H_{55}N_9O_{10}Se$: C, 53.3; H, 6.15; N, 14.0. Found: C, 53.6; H, 6.42; N, 13.8.

N-Carbobenzoxy-L-isoleucyl-L-glutaminyl-L-asparaginyl-Se-benzyl-L-selenocysteinyl-L-prolyl-L-leucylglycinamide. The protected hexapeptide (3.8 g) was decarbobenzoxylated and isolated according to the procedure described in the preceding paragraph. The salt was dissolved in 400 ml of methanol and passed through a Rexyn RG1(OH) column (25 ml) which was afterwards washed with 400 ml of methanol. The solid obtained after removal of the solvent from the eluate and the washings was dissolved in 20 ml of DMF. To the ice-cold solution 1.8 g of *p*-nitrophenyl N-carbobenzoxy-L-isoleucinate was added. The reaction mixture which solidified during the next 2 days was collected and washed with five 100-ml portions of an ethanol–ethyl acetate mixture (4:1). After being dried *in vacuo* the product weighed 3.3 g, mp 247–249° dec, $[\alpha]^{20}D$ −46.2° (*c* 2, DMF). A sample was dissolved in a boiling mixture of THF–water (1:1) and reprecipitated by cooling, mp 248–250° dec (lit.[13] mp 248°).

Anal. Calcd for $C_{46}H_{66}N_{10}O_{11}Se$: C, 54.5; H, 6.56; N, 13.8. Found: C, 54.3; H, 6.63; N, 13.6.

N-Carbobenzoxy-O-benzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-Se-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide. The hydrobromide of the heptapeptide (3.2 g), obtained after decarbobenzoxylation according to the procedure described in the preceding paragraph, was dissolved in 300 ml of methanol, and the solution was passed through a Rexyn RG1(OH) column (24 ml). The column was washed with 300 ml of methanol. After the removal of the eluate and washings the amine was dissolved in 18 ml of DMF, cooled to 0°, and allowed to react with 1.83 g of *p*-nitrophenyl N-carbobenzoxy-O-benzyl-L-tyrosinate. After 3 days the reaction mixture was collected and washed with four 100-ml portions of ethanol–ethyl acetate (4:1). The material was then dried to constant weight *in vacuo* at room temperature to give 3.3 g, mp 249–251° dec, $[\alpha]^{21}D$ −39.7° (*c* 1, DMF). Further purification with a THF–water mixture (1:1) as described in the previous paragraph did not change the physical constants.

Anal. Calcd for $C_{62}H_{81}N_{11}O_{13}Se$: C, 58.8; H, 6.44; N, 12.2. Found: C, 58.6; H, 6.61; N, 12.4.

N-Carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-Se-benzyl-L-selenocysteinyl-L-prolyl-L-leucylglycinamide. A suspension of protected octapeptide (0.5 g) in 10 ml of trifluoroethanol, treated for 15 min with dry hydrogen bromide gas, was kept for 30 min at room temperature. The residue which resulted upon evaporation of the solvent under reduced pressure was washed four times with 100-ml portions of ether and then dissolved in 50 ml of methanol. The solution was passed through a Rexyn RG1(OH) column (8 ml), and the column was washed with 50 ml of methanol. The solvent was removed under reduced pressure, and the resulting solid was dissolved in 4 ml of DMF. To the ice-cold solution 0.2 g of *p*-nitrophenyl N-carbobenzoxy-S-benzyl-L-cysteinate was added. The product solidified partially during the next day. The precipitation of the peptide was completed by addition of 50 ml of ethyl acetate after 2 days. The solid was filtered off and washed with three 50-ml portions of ethanol. The material was then dried to constant weight over P_2O_5 *in vacuo* at room temperature, giving 0.2 g, mp 242–245° dec, $[\alpha]^{21}D$ −51.4° (*c* 1, DMF).

Anal. Calcd for $C_{65}H_{86}N_{12}O_{14}SSe$: C, 57.0; H, 6.33; N, 12.3. Found: C, 56.8; H, 6.41; N, 12.2.

6-Seleno-oxytocin. A sample of the preceding protected nonapeptide (0.09 g) was dissolved in approximately 100 ml of liquid ammonia freshly distilled from sodium. The protected peptide was treated with sodium at the boiling point of the liquid ammonia until a blue color persisted in the solution for 20 sec. The ammonia solution was subsequently concentrated and the final 50 ml was removed by lyophilization. The fluffy residue was then dissolved in 100 ml of 0.1% AcOH. The pH of the solution was adjusted to 6.9 with 1 *N* ammonium hydroxide and oxidized by titration with a slight excess of a 0.01 *M* aqueous potassium ferricyanide solution. Ferrocyanide and ferricyanide ions were removed by passage of the oxidized solution through a column of AG3X4 resin in the chloride form. After being concentrated to a volume of 25 ml, the solution was subjected to countercurrent distribution in the solvent system BuOH–PrOH–0.5% aqueous AcOH containing 0.1% pyridine (6:1:8). After 500 transfers, a separation into two peaks, a faster moving major peak with a *K* of 0.52 and a slower moving peak with a *K* of 0.20, had been accomplished as detected by the Folin–Lowry color reaction. The contents of 20 tubes, representing the middle portion of a peak with the *K* value of 0.52, were pooled. The volume of the resulting solution was tripled by the addition of distilled water and then reduced *in vacuo* to approximately 50 ml, which was removed by lyophilization, yield 0.018 g, $[\alpha]^{20.5}D$ −10.5° (*c* 0.5, 1 *N* AcOH).

Anal. Calcd for $C_{43}H_{66}N_{12}O_{12}SSe$: C, 49.0; H, 6.31; N, 15.9. Found: C, 48.7; H, 6.48; N, 15.6.

The 6-seleno-oxytocin was indistinguishable from oxytocin and 1-seleno-oxytocin upon thin layer chromatography on silica gel in the upper phase of the solvent system BuOH–AcOH–water (4:1:5, ascending), paper chromatography (R_f 0.59 (S_1), R_f 0.50 (S_2)), and gel filtration. A sample was hydrolyzed and analyzed for ninhydrin-active materials. The following molar ratios were obtained, glycine being taken as 1.0: $Asp_{1.0}Glu_{1.0}Pro_{1.0}Gly_{1.0}$-$Cys_{0.3}Ile_{1.0}Leu_{1.0}Tyr_{0.9}NH_{3\ 3.3}$. In addition, the presence of selenocystine and 1,6-diamino-3-thio-4-selenohexane-1,6-dicarboxylic acid was detected.[29]

S-Benzyl-β-propionyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-Se-benzyl-L-selenocysteinyl-L-prolyl-L-leucylglycinamide. The preceding octapeptide (0.5 g) was suspended in 10 ml of freshly distilled trifluoroethanol, and subsequent treatment for 15 min with dry hydrogen bromide gas at room temperature brought about a clear solution. The reaction mixture was kept for an additional 20 min at room temperature before the solvent was evaporated. The resulting hydrobromide salt of the octapeptide was washed with three 50-ml portions of ether, dissolved in 3 ml of methanol, and then reprecipitated with 50 ml of ether. The solid material was collected by quick filtration and dissolved in 3 ml of ice-cold DMF. Following the adjustment of pH to 7.5 by dropwise addition of triethylamine, the mixture was allowed to react with 0.138 g of *p*-nitrophenyl S-benzyl-β-mercaptopropionate. Three days later, 20 ml of water was added to the reaction mixture, and the solid material collected was further purified by washing with ethanol and ethyl acetate. After drying over P_2O_5 *in vacuo* for 12 hr at room temperature the product weighed 0.3 g, mp 244–248° dec, $[\alpha]^{20}D$ −45.0° (*c* 1, DMF).

Anal. Calcd for $C_{57}H_{79}N_{11}O_{12}SSe$: C, 56.0; H, 6.52; N, 12.6. Found: C, 55.8; H, 6.54; N, 12.8.

Deamino-6-seleno-oxytocin. The debenzylation of the dibenzyldeamino-6-seleno-oxytoceine (0.1 g) was performed with sodium in 100 ml of liquid ammonia freshly distilled from sodium. The blue color persisted for 30 sec. The solution was concentrated, and the last 50 ml of liquid ammonia was lyophilized. The remaining white residue was taken up with 100 ml of 0.1% AcOH. The pH was adjusted to 6.9 with 1 *N* ammonium hydroxide, and the resulting solution was titrated with 6 ml of 0.01 *M* potassium ferricyanide solution. This oxidized solution was passed through a column of AG3X4 resin in the chloride form and concentrated in a flash evaporator at 20° to a volume of approximately 20 ml. It was then subjected to countercurrent distribution for a total of 520

transfers in the solvent system BuOH–benzene–0.05% AcOH (3:2:5). A symmetrical peak with a K value of 1.04 was obtained as determined by measurement of the Folin–Lowry color values, and the avian depressor activity was associated with the material of this peak. The contents of 20 tubes, representing the middle portion of this peak, were pooled, concentrated, and lyophilized to yield 0.029 g of the deamino-6-seleno-oxytocin, $[\alpha]^{21}D$ −54.0° (c 1, 1 N AcOH).

Anal. Calcd for $C_{48}H_{65}N_{11}O_{12}SSe$: C, 49.7; H, 6.31; N, 14.8. Found: C, 49.6; H, 6.50; N, 14.6.

The deamino-6-seleno-oxytocin was indistinguishable from deamino-oxytocin and from deamino-1-seleno-oxytocin upon thin layer chromatography, paper chromatography (R_f 0.78 (S_1),[15] R_f 0.75 (S_2)), and gel filtration. The ninhydrin-active substances of the acid hydrolysate of a sample of deamino-6-seleno-oxytocin showed the following molar ratios, with glycine taken as 1.0: $Asp_{1.0}Glu_{1.0}Pro_{1.0}Gly_{1.0}Ile_{1.0}Leu_{1.0}Tyr_{1.0}NH_{3\ 3.1}$. In addition, selenocystine and 1-amino-3-seleno-4-thiohexane-1,6-dicarboxylic acid were found.[33]

Bioassay Methods. Avian vasodepressor assays were performed on conscious chickens according to the procedure employed by Munsick, Sawyer, and van Dyke.[34] Oxytocic assays were performed on isolated uteri from rats in natural estrus according to the method of Holton[35] modified by Munsick[36] with the use of magnesium-free van Dyke–Hastings solution as the bathing fluid. Rat pressor assays were carried out on atropinized, urethane-anesthetized male rats as described in the United States Pharmacopeia.[37] Assays for antidiuretic activity were performed on anesthetized, hydrated male rats according to the method of Jeffers, Livezey, and Austin[38] as modified by Sawyer.[39] The milk-ejecting activity in anesthetized, lactating rabbits was determined by the method described by van Dyke, Adamsons, and Engel[40] as modified by Chan.[41] In all of these bioassays the four-point design was used; the biological activities were measured against the USP Posterior Pituitary Reference Standard.

Acknowledgments. R. W. gratefully acknowledges the guidance and interest to Drs. Vincent du Vigneaud (in whose laboratory this work was initiated) and I. L. Schwartz (in whose laboratory this work was continued). We are indebted to Dr. D. Yamashiro for the supply of the C-terminal protected octapeptide of oxytocin; to Dr. Theodore Mahowald, Mrs. Caroline Holzhauser, and Mr. David H. Schlesinger for amino acid analyses; and to Miss Margitta Wahrenburg, Mrs. Maxine Goldberg, and Miss Gloria Bisesi for the bioassays. We also wish to thank Miss Patricia Herling and Mr. Louis Trauth for technical assistance.

(33) The position of the latter amino acid was found at 147.5 ml of buffer under the same conditions as described in ref 27 and 28.

(34) R. A. Munsick, W. H. Sawyer, and H. B. van Dyke, *Endocrinology*, **66**, 860 (1960).

(35) P. Holton, *Brit. J. Pharmacol.*, **3**, 328 (1948).

(36) R. A. Munsick, *Endocrinology*, **66**, 451 (1960).

(37) "The Pharmacopeia of the United States of America," 17th revised ed, Mack Printing Co., Easton, Pa., 1965, p 749.

(38) W. A. Jeffers, M. M. Livezey, and J. H. Austin, *Proc. Soc. Exptl. Biol. Med.*, **50**, 184 (1942).

(39) W. H. Sawyer, *Endocrinology*, **63**, 694 (1958).

(40) H. B. van Dyke, K. Adamsons, Jr., and S. L. Engel, *Recent Progr. Hormone Res.*, **11**, 1 (1955).

(41) W. Y. Chan, *J. Pharmacol. Exptl. Therap.*, **147**, 48 (1965).

Communications to the Editor

Hydrochlorination of Bornylene and 1-Methylnorbornene. Absence of a Significant Directive Influence of the 1-Methyl Substituent in a Carbonium Ion Reaction

Sir:

Addition of hydrogen chloride to bornylene, under typical electrophilic conditions, results in the formation of *exo* chlorides, in spite of the steric influence of the 7,7-dimethyl substituents. Hydrochlorination of 1-methylnorbornene also proceeds to the *exo* products. In both cases, protonation of the double bond occurs approximately equally at the C-2 and C-3 positions, thus failing to show the large directive influence of the 1-methyl substituent anticipated for a σ-bridged intermediate.

In the past heavy reliance has been placed on data from solvolytic reactions to support the proposal for the σ-bridged norbornyl cation.[1] However, there is growing evidence that solvolytic reactions, even with the 2-octyl arenesulfonates in highly aqueous dioxane,[2] proceed to ion pairs, rather than to the free carbonium ions usually written as the reaction intermediate.[3] Consequently, the possibility exists that the complete loss of optical activity or the complete scrambling of the tag observed in the solvolysis of norbornyl derivatives may be the result of a relatively long life for the *exo* ion pair, which gives it adequate time to equilibrate completely, rather than the result of the formation of a σ-bridged intermediate, such as has been so long accepted as an explanation for the observed racemization.[4]

It would appear desirable to examine other representative carbonium ion reactions[5] in order to establish whether those characteristics which are now used to support a σ-bridged structure for the norbornyl cation are general, or whether they are essentially restricted to solvolytic reactions.

Polar hydrochlorination of olefins is a typical electrophilic reaction, one which has long been considered to involve carbonium ions as intermediates.[6,7] The successful adaptation of the automatic hydrogenator[8] to hydrochlorinations[9,10] encouraged us to undertake a systematic study of the influence of structure on the products obtained in the hydrochlorination of representative bicyclic olefins. The present study was devoted to examining the question of whether a 1-methyl sub-

(1) For a recent review see G. D. Sargent, *Quart. Rev.* (London), 20, 301 (1966).

(2) H. Weiner and R. A. Sneen, *J. Am. Chem. Soc.*, **87**, 292 (1965).

(3) For a review see C. A. Bunton, "Nucleophilic Substitution at a Saturated Carbon Atom," Elsevier Publishing Co., New York, N. Y., 1963, pp 135–148.

(4) S. Winstein and D. Trifan, *J. Am. Chem. Soc.*, 74, 1154 (1952).

(5) E. J. Corey, *et al.*, *ibid.*, **85**, 165, 169 (1963).

(6) P. B. D. de la Mare and R. Bolton, "Electrophilic Additions to Unsaturated Systems," Elsevier Publishing Co., New York, N. Y., 1966.

(7) M. J. S. Dewar and R. C. Fahey, *J. Am. Chem. Soc.*, **85**, 2245, 2248, 3645 (1963); *Angew. Chem. Intern. Ed. Engl.*, **3**, 245 (1964).

(8) C. A. Brown and H. C. Brown, *J. Org. Chem.*, **31**, 3898 (1966).

(9) H. C. Brown and M.-H. Rei, *ibid.*, **31**, 1090 (1966).

(10) H. C. Brown and K.-T. Liu, *J. Am. Chem. Soc.*, **89**, 466 (1967).