[1-(ω-Mercaptoundecanoic acid)]-oxytocin, a 28-Membered Ring Homolog of Deamino-oxytocin[1,2]

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Abstract: [1-(ω-Mercaptoundecanoic acid)]-oxytocin, a homolog of the highly active deamino-oxytocin ([1-(β-mercaptopropionic acid)]-oxytocin) in which the size of the disulfide ring is increased from 20 to 28 members, has been synthesized by the use of the Merrifield solid phase method. This homolog possesses no detectable oxytocic or avian vasodepressor activity, but unlike [1-(δ-mercaptovaleric acid)]-oxytocin, the 22-membered ring homolog of deamino-oxytocin, it does not possess antioxytocic activity.

It has been shown that the enlargement of the 20-membered ring of deamino-oxytocin (Figure 1) to 21 members by the formal insertion of one additional methylene group at position 1 brings about a complete loss of the avian vasodepressor activity and reduces the oxytocic potency to about 3 units/mg.[5] Deamino-oxytocin itself possesses approximately 800 units/mg of oxytocic and 975 units/mg of avian vasodepressor activity.[6] Furthermore, [1-(δ-mercaptovaleric acid)]-oxytocin, the 22-membered ring homolog of deamino-oxytocin, possesses no detectable oxytocic or avian vasodepressor activity, but instead shows a small inhibitory activity against the oxytocic activity of oxytocin itself.[7] In order to investigate whether any enhancement of the antioxytocic activity would result from considerable enlargement of the ring size, the synthesis of [1-ω-mercaptoundecanoic acid)]-oxytocin, a 28-membered ring homolog of deamino-oxytocin, was undertaken.

The Merrifield solid phase method[8-10] was used for the synthesis of the desired homolog. The stepwise synthesis was carried out in eight cycles as described for the solid phase synthesis of [1-(δ-mercaptovaleric acid)]-oxytocin.[7] In the last cycle of the synthesis S-benzyl-ω-mercaptoundecanoic acid, instead of S-benzyl-δ-mercaptovaleric acid, was coupled to the octapeptide resin. The final protected polypeptide resin was treated with ammonia in anhydrous methanol to yield the corresponding crude polypeptide amide. Partition chromatography on Sephadex LH-20 gave a main fraction with the expected amino acid composition and one faster moving fraction. The main fraction appeared homogeneous upon thin layer chromatography. The purified protected polypeptide amide was reduced with sodium in liquid ammonia by the method of Sifferd and du Vigneaud[11] to remove the benzyl groups, and the sulfhydryl compound so produced was oxidized to the cyclic disulfide form by aeration. Purification of the [1-(ω-mercaptoundecanoic acid)]-oxytocin was accomplished by partition chromatography on Sephadex G-25[12] followed by gel filtration on Sephadex LH-20.

When the [1-(ω-mercaptoundecanoic acid)]-oxytocin was subjected to bioassay,[13] it was found to exhibit no oxytocic or avian vasodepressor activity and to possess no inhibitory activity against the oxytocic activity of oxytocin.

Experimental Section[14]

S-Benzyl-ω-mercaptoundecanoic Acid. ω-Bromoundecanoic acid (6.6 g) was dissolved in a mixture of 200 ml of 1 *N* NaOH and absolute ethanol (1:1). Redistilled α-mercaptotoluene (4.6 g) was added to the stirred solution and stirring was continued overnight. The resulting suspension of white solid was cooled to 0°, and 15 ml of 1 *N* aqueous potassium ferricyanide was added to oxidize the excess α-mercaptotoluene to its disulfide. The mixture of the sodium salt of the S-benzyl-ω-mercaptoundecanoic acid and the disulfide was filtered off, washed with three 10-ml portions of water, and dried *in vacuo*. The mixture was then triturated in a mortar with 30 ml of ether to remove the disulfide. The sodium salt was filtered off, washed with two 50-ml portions of ether, dried, and then suspended in 500 ml of water and acidified with 2 *N* HCl to pH 2. The resulting precipitate was filtered off, washed with three 50-ml portions of water, and dried *in vacuo;* wt 7.3 g. The product was dissolved in 300 ml of hexane at 60°, and the solution was filtered and kept at −20° for 4 hr. The crystals were filtered off, washed with two 10-ml portions of cold hexane, and dried *in vacuo;* wt 6.8 g, mp 50–54°. After recrystallization from 250 ml of hexane, 6.2 g, mp 53–55°, was obtained.

Anal. Calcd for $C_{18}H_{28}O_2S$: C, 70.1; H, 9.15; S, 10.4. Found: C, 70.1; H, 9.22; S, 10.4.

S-Benzyl-ω-mercaptoundecanoyl-O-benzyltyrosylisoleucylglutaminylasparaginyl-S-benzylcysteinylprolylleucylglycinamide. Chloromethylcopolystyrene–2% divinylbenzene (0.72 mmol of Cl/g of resin) was esterified with Boc-glycine by the procedure reported earlier.[15] Amino acid analysis of an acid hydrolysate (dioxane–12 *N* HCl, 1:1) gave 0.17 mmol of glycine/g of esteri-

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(2) All optically active amino acid residues are of the L variety.

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(13) Avian vasodepressor assays were performed on conscious chickens according to the procedure employed by R. A. Munsick, W. H. Sawyer, and H. B. Van Dyke, *Endocrinology*, **66**, 860 (1960). Oxytocic assays were performed on isolated uteri from rats in natural estrus according to the method of P. Holton (*Brit. J. Pharmacol.*, **3**, 328 (1948)), as modified by R. A. Munsick (*Endocrinology*, **66**, 451 (1960)), with the use of magnesium-free van Dyke–Hastings solution as the bathing fluid. Because of the very low solubility of the homolog in aqueous solutions, samples for bioassay were dissolved in glycerol and tested against solutions of oxytocin in glycerol, which showed approximately the same potency as equivalent solutions in saline.

(14) Melting points were done in capillary tubes and are uncorrected.

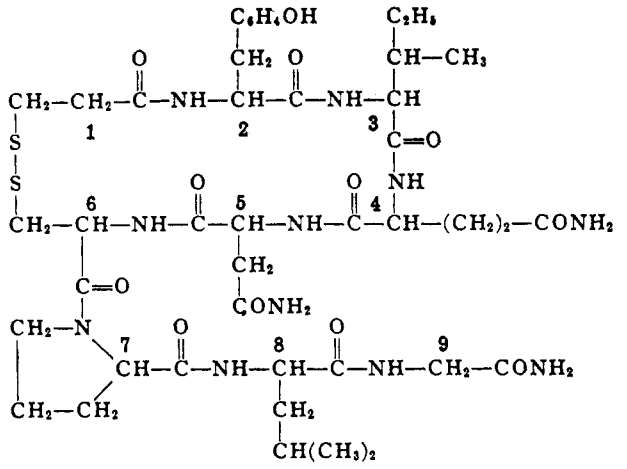

Figure 1. Structure of deamino-oxytocin with numbers indicating the position of the individual amino acid residues.

fied resin. This Boc-glycyl resin (7.0 g) was placed in a Merrifield reaction vessel and subjected to eight 12-step cycles as described in an earlier communication.[15] Following the incorporation of the S-benzyl-ω-mercaptoundecanoic acid, the protected polypeptide resin compound was washed with glacial acetic acid, absolute ethanol, and methylene chloride (three 15-ml portions each), and dried *in vacuo*. It was then treated with ammonia in anhydrous methanol as previously described for the synthesis of the comparable intermediate in the synthesis of [1-(δ-mercaptovaleric acid)]-oxytocin[7] to give 976 mg of an off-white powder; mp 234–239°. The polypeptide amide (400 mg) was dissolved in the upper phase of the solvent system *n*-hexane–toluene–glacial acetic acid–water (50:150:200:35) and applied to a Sephadex LH-20 column (2.83 × 55.8 cm) which had been equilibrated with the lower and upper phases. The column was eluted with the upper phase and fractions of 9.7 ml were collected. Aliquots of alternate fractions were dried *in vacuo* and the residues were dissolved in dimethylformamide. Plotting of the Folin–Lowry color values[16] of these aliquots showed a main peak in the region of fractions 26–40. The contents of these fractions were pooled, dried *in vacuo*, and dissolved in 10 ml of glacial acetic acid. Water (100 ml) was added and the resulting precipitate was filtered off and dried *in vacuo*; wt 312 mg, mp 245–248°, $[\alpha]^{22}D$ −36.2° (*c* 0.8, dimethylformamide). A sample was dried for 5 hr over P_2O_5 *in vacuo* at 100° for elemental analysis.

Anal. Calcd for $C_{72}H_{101}O_{12}N_{11}S_2$: C, 62.8; H, 7.39; N, 11.2. Found: C, 62.8; H, 7.47; N, 11.0.

A sample was hydrolyzed in 6 *N* HCl at 110° for 22 hr and analyzed[17] in the Beckman–Spinco amino acid analyzer. The following molar ratios of amino acids and ammonia were obtained: aspartic acid, 0.99; glutamic acid, 1.08; proline, 0.98; glycine, 1.00; isoleucine, 1.02; leucine, 1.01; tyrosine, 0.81; S-benzylcysteine, 0.86; and ammonia, 3.10.

Thin layer chromatography of the protected polypeptide amide on silica gel G (E. Merck AG-Darmstadt) in chloroform–methanol (8:2) showed a single spot (chlorine–tolidine reagent).[18]

[1-(ω-Mercaptoundecanoic acid)]-oxytocin. The protected polypeptide amide (280 mg) was suspended in boiling liquid ammonia (200 ml). The reduction with sodium was carried out in the same manner as described earlier.[7] The clear solution was concentrated at the water pump to about 20 ml and then lyophilized. The powder was dissolved in 0.03% trifluoroacetic acid (1400 ml) and the solution was adjusted to pH 6.9 with 0.1 *N* ammonium hydroxide. A slow stream of oxygen was passed through the solution overnight while the pH of the solution was kept at 6.9. After 24 hr a sample of the solution showed a negative nitroprusside reaction. The cloudy solution was concentrated in a flash evaporator to about 100 ml and lyophilized. The residue was dissolved in the upper phase of the solvent system hexane–glacial acetic acid–benzene–water (1:10:10:2) and put onto a Sephadex G-25 (100–200 mesh) column (2.82 × 59.8 cm) which had been equilibrated against the lower and upper phases of the solvent system. Chromatography was carried out by elution with the upper phase. The chromatogram obtained by plotting the Folin–Lowry color values of alternate fractions showed two peaks. Isolation of the material represented by these peaks gave 71 mg from the main fraction and 31 mg from the faster moving second fraction which was shown to consist of polymeric material. Thin layer chromatography in chloroform–methanol (8:2) showed a single spot for the main fraction and several spots for the second fraction. The main fraction (68 mg) was dissolved in 5 ml of dimethylformamide and applied to a Sephadex LH-20 column (2.82 × 48.0 cm) that had been equilibrated with dimethylformamide. The column was eluted with dimethylformamide, and fractions of 6.5 ml were collected and subjected to Folin–Lowry color analysis. The material isolated from the fractions corresponding to the single symmetrical peak with a maximum at fraction 14 was precipitated from 5 ml of dimethylformamide by addition of 100 ml of water, centrifuged, and dried *in vacuo*; wt 61 mg, $[\alpha]^{22}D$ −45° (*c* 0.8, dimethylformamide).

Anal. Calcd for $C_{51}H_{81}O_{12}N_{11}S_2$: C, 55.5; H, 7.39; N, 14.0. Found: C, 55.3; H, 7.26; N, 13.9.

Thin layer chromatography on silica gel G showed a single spot in chloroform–methanol (8:2) and in 1-butanol–acetic acid–water (4:1:1).

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