mental error with those obtained aerobically. (The anaerobic experiments require the strict exclusion of oxygen and thus were less convenient to perform.) The runs were not thermostated and were performed at 27 ± 2°.

Rate Law. The reactions of the 2-hydroxyalkylcobalt chelates with alkali under pseudo-first-order conditions all gave straight $\log(OD_t - OD_\infty)$ *vs.* time plots; most rate constants listed in Table II were calculated using the Guggenheim treatment.[38] All the reactions were run in water or 10% methanol in water. No observable rate differences were observed between the two solvent systems. The base-catalyzed decomposition of the 2-hydroxyalkylcobalt chelates obeyed a second-order rate law, $-d[CoR]/dt = k_2[CoR][OH]$. The concentration of OH^- was varied by a factor of 10 with no change in the observed second-order rate constants.

Hydrogen–Deuterium Exchange Experiments. The deuterium-exchange reaction between acetone-d_6 and 2-methylenetetrahydrofuran[39] and 2-dihydropyran was demonstrated as follows. Nmr tubes were charged with 0.7 ml of 2-methylenetetrahydrofuran or 2-hydropyran, respectively, and with 0.2 ml of acetone-d_6 and of 0.1 ml of acetic acid-d_1. The tubes were sealed and the exchange was followed by nmr. The disappearance of the complex spin-coupling pattern of the nmr spectrum of 2-methylenetetrahydrofuran was accompanied by the appearance of the multiplet due to partially protonated acetone at 2.1 ppm. In the case of 2-dihydropyran the disappearance of the H_2 multiplet at 4.6 ppm and collapse of the H_1 doublet at 6.3 ppm in the dihydropyran spectrum proved the substitution of hydrogen by deuterium in the 3 position of 2-dihydropyran. Again, a multiplet due to partially protonated acetone was observed at 2.1 ppm. Similar exchange experiments were carried out with neutral aluminum oxide (chromatographic quality) as the catalyst, except that the reaction was carried out at 65° in a sealed vial. For the nmr measurements the solutions were freed of Al_2O_3 by filtration.

Determination of H/D Isotop eEffect. 2-Hydroxypropylcobalamin-2-*d* was synthesized from vitamin B_{12s} and 1-chloro-2-propanol-2-d_1 and purified by phenol extraction. The rate of alkali decomposition was determined in 2 *M* aqueous NaOH at 27°.

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The Synthesis and Pharmacological Properties of [1-(δ-Mercaptovaleric acid)]-oxytocin, a Homolog of Deamino-oxytocin Containing a Twenty-Two-Membered Ring[1,2]

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Abstract: [1-(δ-Mercaptovaleric acid)]-oxytocin, a homolog of deamino-oxytocin in which the size of the disulfide ring is increased from 20 to 22 members by the formal insertion of two methylene groups at position 1, has been synthesized by the Merrifield solid phase method. This homolog does not exhibit avian vasodepressor or oxytocic activity but possesses a small inhibitory activity against the oxytocic activity of oxytocin itself. The mixed disulfide of cysteine and δ-mercaptovaleric acid has been synthesized, and its chromatographic behavior in the Beckman-Spinco amino acid analyzer has been established.

As has been shown in earlier studies,[5,6] the pharmacological activities of deamino-oxytocin, a highly active analog of oxytocin (Figure 1), are strongly dependent on the size of the disulfide ring. Thus [1-(γ-mercaptobutyric acid)]-oxytocin, a homolog of deamino-oxytocin in which the β-mercaptopropionic acid residue at position 1 is replaced by γ-mercaptobutyric acid to form a 21-membered ring, possesses approximately 3 units/mg of oxytocic activity and no detectable avian vasodepressor activity.[5] [1-(Mercaptoacetic acid)]-oxytocin, a deamino-oxytocin homolog in which a methylene group has been formally removed from the residue at position 1 to form a 19-membered ring, possesses 25 units/mg of oxytocic activity and 4 units/mg of avian vasodepressor activity.[6] Deamino-oxytocin possesses approximately 800 units/mg of oxytocic and 975 units/mg of avian vasodepressor activity.[7]

This paper deals with the synthesis of [1-(δ-mercaptovaleric acid)]-oxytocin, in which the β-mercaptopropionic acid residue of deamino-oxytocin is replaced with a δ-mercaptovaleric acid residue. Thus two methylene groups are introduced at position 1 adjacent to the disulfide bridge to form a 22-membered ring. For the synthesis of the desired deamino-oxytocin homolog, the Merrifield solid phase method was used.[8–10] The synthesis was carried out in eight cycles as described for the solid phase synthesis of deamino-oxytocin,[11] except that the glycine-resin used was in the unnitrated form and in the last cycle of the synthesis, S-benzyl-δ-mercaptovaleric acid was used instead of S-benzyl-β-mercaptopropionic acid. The cleavage of the final protected polypeptide from the resin support was achieved with

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(2) All optically active amino acid residues are of the L variety.
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ammonia in anhydrous methanol. Thin-layer chromatography showed a main fraction as well as various faster moving contaminants. Partition chromatography on Sephadex LH-20 yielded a peptide that appeared homogeneous upon thin-layer chromatography. This protected polypeptide was treated with sodium in liquid ammonia according to the method of Sifferd and du Vigneaud,[12] and the resulting dithiol was oxidized to the corresponding cyclic disulfide compound with an aqueous solution of potassium ferricyanide.[13] Ferrocyanide and excess ferricyanide ions were removed with an ion-exchange resin. The homolog was purified by partition chromatography[14] followed by gel filtration[15] on Sephadex G-25. An acid hydrolysate of the lyophilized product had the expected amino acid composition. The peak corresponding to the mixed disulfide of cysteine and δ-mercaptovaleric acid appears shortly before tyrosine in the 50° system of the Beckman-Spinco amino acid analyzer. The color value for this compound was determined by ion-exchange chromatography of the synthetically prepared mixed disulfide under the same conditions. This compound was synthesized according to the procedure of Hope, Murti, and du Vigneaud[13] for the synthesis of the mixed disulfide of cysteine and β-mercaptopropionic acid.

[1-(δ-Mercaptovaleric acid)]-oxytocin has no detectable avian vasodepressor or oxytocic activity.[16] On the other hand, it exhibits some degree of antioxytocic activity. At a molar ratio of 100:1 (homolog–oxytocin), the oxytocic response to oxytocin was inhibited approximately 50%.

Experimental Section[17]

S-Benzyl-δ-mercaptovaleric Acid. A mixture of freshly distilled α-mercaptotoluene (9.3 g) and 5-bromovaleric acid (4.5 g) in 30 ml of 4 *N* NaOH and 30 ml of absolute ethanol was stirred for 48 hr at 45°. The ethanol was removed under aspirator vacuum. To the remaining aqueous solution, 15 ml of 4 *N* NaOH and 50 ml of potassium ferricyanide (1 *N* aqueous solution) were added to oxidize excess α-mercaptotoluene. The turbid solution was washed with five 20-ml portions of ether. The aqueous layer was placed in a rotary evaporator to remove all traces of ether. The solution was then cooled in an ice bath and acidified with 6 *N* HCl to pH 2. A white precipitate was filtered off, washed with five 5-ml portions of water and dried *in vacuo* over KOH at 20°; wt 3.4 g, mp 27–28°. The product was dissolved in hexane (150 ml) at 50° and the solution was allowed to stand in the freezer overnight. The crystalline solid was filtered off, washed with three 5-ml portions of ice cold hexane and dried *in vacuo;* wt 2.3 g, mp 30–31°. The mother liquor and washings were concentrated to about 100 ml and allowed to stand overnight at −20°. The crystalline second crop was filtered off and washed with ice-cold hexane; wt 0.6 g, mp 30.5°.

Anal. Calcd for $C_{12}H_{16}O_2S$: C, 64.3; H, 7.19; S, 14.3. Found: C, 64.3; H, 7.23; S, 14.4.

S-Benzyl-δ-mercaptovaleryl-O-benzyltyrosylisoleucylglutaminyl-asparaginyl-S-benzylcysteinylprolylleucylglycyl Resin. Boc-glycine (2.63 g) and triethylamine (1.89 ml) in 35 ml of ethyl acetate were added to 10.0 g of chloromethylcopolystyrene–2% divinylbenzene. The reaction mixture was stirred at reflux temperature for 48 hr. The resin was filtered off and washed three times each with ethyl acetate, methanol, water, and again with methanol. The esterified resin was dried *in vacuo;* wt 11.08 g. Amino acid analysis of an acid hydrolysate gave 0.61 mmol of glycine/g of esterified resin. This Boc-glycyl resin (1 g) was placed in a Merrifield reaction vessel and subjected to eight 12-step cycles as described for the solid phase synthesis of deamino-oxytocin.[11] After step 12 of cycle 8 (incorporation of the S-benzyl-δ-mercaptovaleric acid), the resin was washed thoroughly with three 15-ml portions of glacial acetic acid, three 15-ml portions of absolute ethanol, three 15-ml portions of methylene chloride, and dried *in vacuo;* wt 1.7 g.

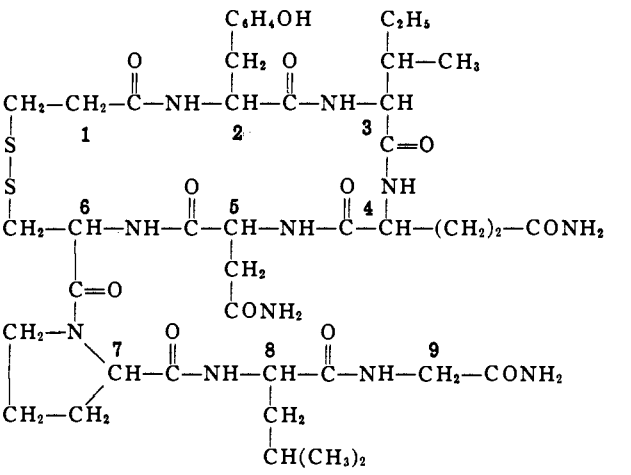

Figure 1. Structure of deamino-oxytocin with numbers indicating the position of the individual amino acid residues.

S-Benzyl-δ-mercaptovaleryl-O-benzyltyrosylisoleucylglutaminyl-asparaginyl-S-benzylcysteinylprolylleucylglycinamide. Resin compound (1.7 g) was suspended in 100 ml of anhydrous methanol for the removal of the protected polypeptide from the resin. Dry ammonia was bubbled into the stirred suspension for 2 hr at 0°. The reaction mixture was stirred overnight at the same temperature. The ammonia and methanol were removed under reduced pressure, dimethylformamide (100 ml) was added to the residue, and the suspension was stirred for 1 hr. The resin was filtered off and washed with three 10-ml portions of dimethylformamide. The filtrate and washings were combined and the solvent was evaporated on a rotary evaporator. The residue was dissolved in 10 ml of warm dimethylformamide, cooled in an ice bath, and distilled water (8 ml) was added. The turbid solution was kept at 4° overnight. The precipitate was filtered off, washed with three 5-ml portions of water and two 5-ml portions of absolute ethanol, and dried *in vacuo;* wt 603 mg, mp 232–236°. This compound (300 mg) was dissolved in the upper phase of the solvent system toluene–glacial acetic acid–water (20:20:3.5). The solution was put onto a Sephadex LH-20 column (2.83 × 56.0 cm) which had been equilibrated with the lower phase of the solvent system. Elution of the column was carried out with the upper phase. Fractions of 400 drops (9.8 ml) were collected. Aliquots of each fraction (0.2 ml) were dried *in vacuo* and the residues were dissolved in 0.1 ml of dimethylformamide. A plot of the Folin–Lowry color values[18] developed from these samples showed a main peak in the region of fractions 25–38. Isolation of the material represented by this peak gave 238 mg; mp 243–245°, $[\alpha]^{22}D$ −31.1° (*c* 0.9, dimethylformamide). Thin layer chromatography on Silica Gel G (E. Merck AG-Darmstadt) in the solvent system chloroform–methanol (8:2) showed only one spot with the chlorine–tolidine reagent.[19] A sample was dried for 5 hr over P_2O_5 at 100° *in vacuo* for elemental analysis.

Anal. Calcd for $C_{66}H_{89}N_{11}O_{12}S_2$: C, 61.3; H, 6.94; N, 11.9. Found: C, 61.2; H, 7.05; N, 11.7.

[1-(δ-Mercaptovaleric acid)]-oxytocin. The preceding protected polypeptide (225 mg) was dissolved in stirred, boiling ammonia

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(freshly distilled from sodium). The reduction was carried out by dipping a 1-ml Mohr pipet containing sodium into the solution until the blue color spread throughout the entire solution and persisted for 20–30 sec. (If the blue color persists beyond this time a few milligrams of ammonium chloride is added to oxidize the excess sodium.) The solution was concentrated to about 20 ml under reduced pressure at the water pump and then lyophilized. The residue was dissolved in 600 ml of 0.03% trifluoroacetic acid, and the pH of the solution was adjusted to 8.0 with 1% ammonium hydroxide. An excess (3.0 ml) of a 0.2 *N* solution of potassium ferricyanide was added and the solution was stirred for 15 min. AG 3-X4 resin (Bio-Rad Laboratories, Richmond, Calif.) in the trifluoroacetate form was added and stirring was continued for 15 min. The resin was removed by filtration and the solution was lyophilized. A column of Sephadex G-25 (100–200 mesh) (2.82 × 65.3 cm) in 0.2 *N* acetic acid was prepared and equilibrated with the lower phase of the solvent system 1-butanol–benzene–3.5% acetic acid in 1.5% aqueous pyridine (1:2:3). The lyophilized powder was dissolved in 12 ml of the upper phase of this solvent system, applied to the column, and eluted with the upper phase. Fractions of 400 drops (9.9 ml) were collected. The chromatogram obtained by plotting the Folin–Lowry color values of alternate fractions indicated a major peak with R_f 0.25. The contents of fractions 32–44 corresponding to this peak were pooled, concentrated under reduced pressure, and lyophilized. The lyophilized powder (50 mg) was dissolved in 10 ml of 0.2 *N* acetic acid and subjected to gel filtration on a Sephadex G-25 (200–270 mesh) column (2.82 × 62.0 cm) that had been equilibrated with 0.2 *N* acetic acid. Fractions of 9.0 ml were collected. Folin–Lowry color values of the fractions showed a single peak with a maximum at fraction 36. The fractions corresponding to this peak were pooled and lyophilized; wt 46 mg, $[\alpha]^{22}D$ +30.2° (*c* 0.7, 1 *N* acetic acid).

Anal. Calcd for $C_{45}H_{69}N_{11}O_{12}S_2$: C, 53.0; H, 6.81; N, 15.1. Found: C, 53.0; H, 6.70; N, 15.0.

A sample was hydrolyzed in 6 *N* HCl at 110° for 22 hr and analyzed[20] in the 50° system of the Beckman-Spinco amino acid analyzer (Model 116). The following molar ratios were obtained: aspartic acid, 1.02; glutamic acid, 1.04; proline, 0.97, glycine, 1.00; half-cystine, 0.46; mixed disulfide of cysteine and δ-mercaptovaleric acid, 0.58; isoleucine, 1.01; leucine, 1.01; tyrosine, 0.92; and ammonia, 2.83. Thin layer chromatography on Silica Gel G in the system 1-butanol–glacial acetic acid–water (4:1:1) showed one spot (R_f 0.36) with the chlorine–tolidine reagent.

Mixed Disulfide of δ-Mercaptovaleric Acid and Cysteine. S-Benzyl-δ-mercaptovaleric acid (1.12 g) and cysteine hydrochloride monohydrate (2.64 g) were suspended in about 150 ml of stirred, boiling ammonia and treated with sodium as described earlier. The ammonia was allowed to evaporate under reduced pressure, and the resulting residue was dissolved in 200 ml of water. Potassium ferricyanide (1 *N*) was added while the pH was maintained near 7 with 1% ammonium hydroxide. When 22 ml of the ferricyanide solution had been added, the white precipitate of cystine was collected on a filter. The filtrate was concentrated to about 70 ml, acidified with 2 *N* HCl until the pH reached 1.0, and allowed to stand in the refrigerator overnight. The white precipitate was filtered off, washed with two 2-ml portions of water, and dried *in vacuo;* wt 151 mg, mp 194–195° dec. This compound was crystallized from 25 ml of boiling water; wt 112 mg, mp 194–195° dec, $[\alpha]^{21}D$ −101.5° (*c* 1, dimethylformamide).

Anal. Calcd for $C_8H_{15}NO_4S_2$: C, 37.9; H, 5.97; N, 5.53. Found: C, 38.2; H, 6.08; N, 5.68.

A sample was analyzed in the 50° system of the Beckman-Spinco amino acid analyzer. The compound was chromatographically pure as shown by the presence of only a single peak with a position between those of leucine and tyrosine. Thin layer chromatography on Silica Gel G in the solvent system 1-butanol–acetic acid–water (4:1:1) showed only one spot (R_f 0.35) with ninhydrin or with the KCN–sodium nitroprusside reagent.

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Rate-Determining Steps in the Oxidation of Succinate Catalyzed by Succinic Dehydrogenase[1]

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Abstract: The magnitude of the kinetic isotope effect observed in the oxidation of tetradeuteriosuccinate depends on the concentration of oxidant and not on the concentration of succinate. Fumarate and dideuteriofumarate are identically effective as competitive inhibitors of succinic dehydrogenase against both succinate and tetradeuteriosuccinate We conclude (1) that the isotope effect occurs at a step prior to the attack of oxidant, presumably upon the reduction of enzyme by succinate, and (2) that this step is rate determining when the step involving attack by oxidant is not rate determining.

The oxidation of succinate catalyzed by succinic dehydrogenase proceeds by the attack of an oxidant on an enzyme–substrate complex, which can be represented probably as a complex between reduced enzyme and fumarate, rather than by attack on reduced enzyme after the dissociation of fumarate.[2,7–10] The kinetics

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