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8-Alanine-oxytocin, 8-Alanine-oxypressin, and Their Deamino Analogs. Their Synthesis and Some of Their Pharmacological Properties*

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ABSTRACT: 8-Alanine-oxytocin, 8-alanine-oxypressin, and their 1-deamino analogs have been synthesized and tested for some of the pharmacological activities characteristic of the neurohypophysial hormones. The analogs were prepared through the use of the stepwise *p*-nitrophenyl ester method as employed in the synthesis of oxytocin and deamino-oxytocin. L-Prolyl-L-alanylglycinamide served as the starting material for all four analogs. In each case the analog was isolated in highly purified form by countercurrent distribution. Upon bioassay the following mean potencies and standard errors were found for 8-alanine-oxytocin: 240 ± 25 units/mg of avian vasodepressor, 166 ± 6 units/mg of oxytocic, and 13 ± 1 units/mg of rat pressor activity. The corresponding values for 8-alanine-oxypressin were found to be 38 ± 2, 15 ± 0.6, and 21 ± 1 units/mg; for 1-deamino-8-alanine-oxytocin 453 ± 6, 314 ± 10, and 8 ± 0.4 units/mg; and for 1-deamino-8-alanine-oxypressin 47 ± 2, 25 ± 0.6, and 4.6 ± 0.1 units/mg.

In the course of extensive studies of the relationship between structure and biological activity of oxytocin (Figure 1) attention has been repeatedly focused on replacements of the leucine residue in the 8 position by other amino acid residues. Analogs in which a neutral amino acid residue, *e.g.*, isoleucine, valine (Jaquenoud and Boissonnas, 1961), or citrulline (Bodanszky and Birkhimer, 1962), was substituted for the leucine residue in oxytocin were all found to possess high oxytocic and avian vasodepressor activities. In view of the high potencies of these analogs it was decided to investigate the effect on these activities of a reduction in the length of the side chain of the amino acid residue in the 8 position of the oxytocin molecule. We therefore synthesized 8-alanine-oxytocin and 1-deamino-8-alanine-oxytocin, analogs of oxytocin and 1-deamino-oxytocin which contain an alanine residue in place of the leucine residue in the 8 position.

Another facet of this study was the evaluation of

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TABLE I: Biological Activities of 8-Alanine-oxytocin, 8-Alanine-oxypressin, and Their 1-Deamino Analogs.

Compound	Mean Potencies (units/mg) and Standard Errors		
	Depressor (fowl)	Oxytocic (rat)	Pressor (rat)
Oxytocin	507 ± 15[a]	546 ± 18[b]	3.1 ± 0.1[a]
1-Deamino-oxytocin	975 ± 24[c]	803 ± 36[c]	1.14 ± 0.6[c]
Oxypressin	~45[d]	~20[d]	~3[d]
8-Alanine-oxytocin	240 ± 25[e]	166 ± 6[e]	13 ± 1[e]
1-Deamino-8-alanine-oxytocin	453 ± 6	314 ± 10	8 ± 0.4
8-Alanine-oxypressin	38 ± 2	15 ± 0.6	21 ± 1
1-Deamino-8-alanine-oxypressin	47 ± 2	25 ± 0.6	4.6 ± 0.1

[a] Value reported by Chan and du Vigneaud (1962). [b] Value reported by Chan *et al.* (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 without regard for stage of the estrous cycle was found to be 486 ± 5 units/mg (Chan and du Vigneaud, 1962). [c] Value reported by Ferrier *et al.* (1965). [d] Value reported by Katsoyannis (1957). [e] Jaquenoud (1965) reported 135 ± 13 units/mg of avian vasodepressor, 141 ± 17 units/mg of oxytocic, and 11.6 ± 0.6 units/mg of pressor activity.

the pharmacological changes brought about by replacement of the leucine residue by an alanine residue in the 8 position of oxypressin. The synthesis of 8-alanine-oxypressin (3-phenylalanine-8-alanine-oxytocin) and its 1-deamino analog was therefore undertaken. It may be recalled that oxypressin and arginine-vasotocin are two hybrid analogs of oxytocin and vasopressin. The former contains the ring of vasopressin and the side chain of oxytocin and was designated oxypressin (Katsoyannis, 1957); the latter contains the ring of oxytocin and the side chain of arginine-vasopressin and was therefore designated arginine-vasotocin (Katsoyannis and du Vigneaud, 1958). It may be noted that for convenience the ring of oxytocin may be referred to as the *tocin* ring and that of vasopressin as the *pressin* ring. In the latter the isoleucine residue of the tocin ring (Figure 1) is replaced by that of phenylalanine.

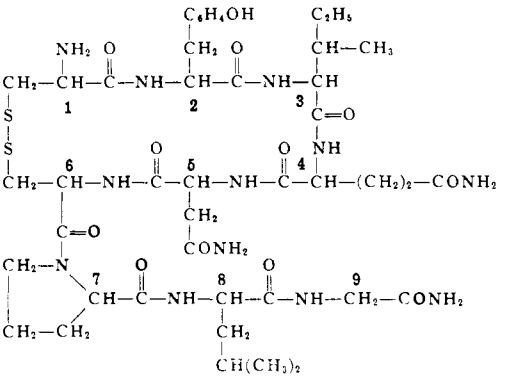

FIGURE 1: Structure of oxytocin with *numbers* indicating the position of the individual amino acid residues.

After the preparative work for this study was completed, Jaquenoud (1965) reported the syntheses and pharmacological properties of 8-glycine-, 8-alanine-, and 8-aminobutyric acid-oxytocin. Our method of synthesis for 8-alanine-oxytocin differs in several respects from that reported by Jaquenoud. Furthermore, the potencies exhibited by our preparation of the analog with respect to oxytocic and avian vasodepressor activities differ from those reported by Jaquenoud for his preparation (Table I).

The carbobenzoxy-L-prolyl-L-alanylglycinamide, obtained by a synthetic route different from that previously employed (Heyns and Legler, 1961), served as the starting material for our syntheses of the structurally modified oxytocin, oxypressin, and their 1-deamino analogs. The free tripeptide amide, obtained after cleavage of the carbobenzoxy group by catalytic hydrogenation in methanol, was lengthened by the *p*-nitrophenyl ester procedure (Bodanszky, 1955) as employed in the stepwise synthesis of oxytocin (Bodanszky and du Vigneaud, 1959).

For the syntheses of 8-alanine-oxytocin and 8-alanine-oxypressin the protected nonapeptides, *N*-carbobenzoxy-*S*-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-alanylglycinamide and *N*-carbobenzoxy-*S*-benzyl-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-alanylglycinamide, were reduced with sodium in liquid ammonia according to the method of Sifferd and du Vigneaud (1935), as employed in the synthesis of oxytocin (du Vigneaud *et al.*, 1953), to remove the benzyl and carbobenzoxy groups. Following removal of the ammonia the disulfhydryl compounds were oxidized to the cyclic octapeptides at pH 6.8 by treatment with potassium ferricyanide (Hope *et al.*, 1962). The ferricyanide and ferrocyanide ions were removed with the cation-exchange resin AG3X4 (Bio-Rad). Purification of the hormone analogs was effected by subjection of the solutions

containing the crude products to countercurrent distribution (Craig *et al.*, 1951). After isolation the hormones were subjected to a second countercurrent distribution. The solvent systems as given in the Experimental Section for these distributions provided for each hormone analog one small and one large partition coefficient. Following purification the analogs gave satisfactory elemental and amino acid analyses. A single sharp peak was obtained for each analog upon gel filtration on Sephadex G-25.

For the preparation of the deamino analogs of 8-alanine-oxytocin and 8-alanine-oxypressin the key intermediates, *S*-benzyl-β-mercaptopropionyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-alanylglycinamide and *S*-benzyl-β-mercaptopropionyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-alanylglycinamide, were debenzylated with sodium in liquid ammonia, oxidized, and deionized in a manner similar to that described in the preceding paragraph for the hormone analogs. One countercurrent distribution was found to suffice for the purification of the deamino analogs, as indicated by elemental and amino acid analyses and gel filtrations on Sephadex.

The 8-alanine-oxytocin, 8-alanine-oxypressin, and their 1-deamino analogs were assayed for some of the biological activities characteristic of the posterior pituitary hormones. For all bioassays a four-point design was used. Avian vasodepressor assays were performed on conscious chickens according to the procedure employed by Munsick *et al.* (1960). Oxytocic assays were performed on uteri from rats in natural estrus according to the method of Holton (1948) with the use of magnesium-free van Dyke–Hastings solution as employed by Munsick (1960). Rat pressor assays were carried out on urethan-anesthetized male rats as described in the United States Pharmacopeia (1965). All potencies were measured against the USP posterior pituitary reference standard. The potencies of 8-alanine-oxytocin, 8-alanine-oxypressin, and their deamino analogs are enumerated in Table I along with those of oxytocin, oxypressin, and deamino-oxytocin.

The data in Table I reveal that the replacement of the leucine residue by the alanine residue in oxytocin or oxypressin results in analogs which retain a high degree of pharmacological potency. The results also show that the replacement of the free amino group of 8-alanine-oxytocin and 8-alanine-oxypressin by hydrogen enhances the oxytocic and avian vasodepressor activity but lowers the pressor activity, just as in the case of oxytocin (Chan and du Vigneaud, 1962). The avian vasodepressor and the oxytocic activity are markedly reduced in going from 8-alanine-oxytocin to 8-alanine-oxypressin, that is, when the tocin ring is supplanted by the pressin ring. This finding resembles a similar diminution of the avian vasodepressor and oxytocic activities in going from oxytocin to oxypressin (Katsoyannis, 1957).

Experimental Section[1]

Carbobenzoxy-L-prolyl-L-alanine. Carbobenzoxy-L-proline (12.5 g) was dissolved in freshly distilled tetrahydrofuran (70 ml). Immediately upon addition of triethylamine (7.5 ml) and cooling of the solution to −10° isobutyl chloroformate (6.6 ml) was added. The mixture was continually stirred at −10° for 20 min. After this period a solution of L-alanine (6.8 g) and triethylamine (12.6 ml) in water (50 ml) was added. Vigorous stirring was continued for 1.5 hr without further cooling. The reaction mixture was acidified by the slow addition of concentrated hydrochloric acid. The tetrahydrofuran was removed *in vacuo* and the resulting solid–water mixture was extracted three times with 200-ml portions of chloroform. After being dried with anhydrous sodium sulfate the chloroform solution was evaporated *in vacuo*, yielding a solid material (14.2 g). The product crystallized from 30 ml of acetic acid and 400 ml of water in short white needles; wt 10.0 g, mp 161–162°, $[\alpha]_D^{20}$ −59.6° (*c* 2, absolute ethanol).

Anal. Calcd for $C_{16}H_{22}N_2O_5$: C, 60.0; H, 6.29; N, 8.80. Found: C, 59.9; H, 6.31; N, 8.70.

Methyl Carbobenzoxy-L-prolyl-L-alanylglycinate. Carbobenzoxy-L-prolyl-L-alanine (16.0 g) was dissolved in freshly distilled tetrahydrofuran (70 ml). Following addition of triethylamine (7.5 ml) and cooling of the solution to −10° isobutyl chloroformate (6.6 ml) was added all at once. The mixture was then stirred for 20 min at −10°, after which a solution of methyl glycinate hydrochloride (7.5 g) and triethylamine (12 ml) in water (30 ml) was added. Stirring was continued for 1.5 hr without further cooling. Water (100 ml) was added and the mixture was acidified by slow addition of concentrated hydrochloric acid. The removal of the tetrahydrofuran *in vacuo* resulted in a solid–water mixture, from which the solid was extracted with four 50-ml portions of chloroform. This organic layer was successively washed with 50-ml portions of water, 5% sodium bicarbonate solution, and water. The solid obtained upon evaporation of the solvent was dissolved in ethanol and reprecipitated with petroleum ether (bp 60–110°); wt 12.0 g. A sample was crystallized from an ethanol–hexane mixture, mp 133–134.5°, $[\alpha]_D^{20}$ −81.0° (*c* 2, absolute ethanol).

Anal. Calcd for $C_{19}H_{25}N_3O_6$: C, 58.3; H, 6.44; N, 10.7. Found: C, 58.2; H, 6.54; N, 10.6.

Carbobenzoxy-L-prolyl-L-alanylglycinamide. To 300 ml of absolute ethanol, saturated with dry ammonia at 0°, 21.0 g of methyl carbobenzoxy-L-prolyl-L-alanylglycinate was added. During the next 2 days, following repeated treatment of the mixture with dry ammonia (five times for 15 min at 0°), the original solid went into solution and the amide started to precipitate. The reaction mixture was kept for 7 days at room temperature. The solvent was removed under

[1] All melting points are capillary melting points and are corrected.

reduced pressure, and the resulting solid material was crystallized from ethanol as long needles; wt 17.3 g, mp 195–196°, $[\alpha]_D^{20}$ −39.8 (*c* 3, dimethylformamide) and −95.0° (*c* 1.2, water); [lit. (Heyns and Legler, 1961) mp 191–192°, $[\alpha]_D^{20}$ −95.0° (*c* 1.2, water); lit. (Jaquenoud, 1965) mp 191–192°, $[\alpha]_D^{20}$ −90.0 ± 2.5° (*c* 1.2, water)].

Anal. Calcd for $C_{18}H_{24}N_4O_5$: C, 57.4; H, 6.42; N, 15.0. Found: C, 57.6; H, 6.50; N, 14.9.

*N-Carbobenzoxy-S-benzyl-*L*-cysteinyl-*L*-prolyl-*L*-alanylglycinamide.* The mixture of 16.3 g of carbobenzoxy-L-prolyl-L-alanylglycinamide and 1.76 g of 10% palladium on charcoal catalyst in 180 ml of methanol was treated with hydrogen for 2.5 hr at 50° to effect decarbobenzoxylation. After removal of catalyst and solvent the reduction product was dissolved in 9 ml of dimethylformamide. The *p*-nitrophenyl *N*-carbobenzoxy-*S*-benzyl-L-cysteinate (22.2 g) was added to the solution as it was being stirred. The product solidified overnight. The next day the material was collected and purified with ethyl acetate containing a small amount of ethanol. After being dried *in vacuo* over P_2O_5 at room temperature, the product weighed 15.0 g, mp 147–149°, $[\alpha]_D^{25}$ −47.9° (*c* 2, dimethylformamide) [lit. (Jaquenoud, 1965) mp 144°, $[\alpha]_D^{22}$ −46.5 ± 1° (*c* 2, dimethylformamide)]. A sample was crystallized from a dimethylformamide–ethyl acetate–ether mixture. The physical constants of the crystalline material were the same as those recorded for the amorphous solid.

Anal. Calcd for $C_{28}H_{35}N_5O_6S$: C, 59.1; H, 6.19; N, 12.3. Found: C, 59.4; H, 6.24; N, 12.5.

*Carbobenzoxy-*L*-asparaginyl-S-benzyl-*L*-cysteinyl-*L*-prolyl-*L*-alanylglycinamide.* A solution of 14.4 g of *N*-carbobenzoxy-*S*-benzyl-L-cysteinyl-L-prolyl-L-alanylglycinamide in 43 ml of glacial acetic acid was treated with 58 ml of 5 N HBr solution in glacial acetic acid. After 75 min at room temperature the solution was poured into 600 ml of cold dry ether. The precipitated hydrobromide of the free base was washed by decantation with three 300-ml portions of cold ether. After being dried *in vacuo* over KOH and P_2O_5, the hydrobromide was dissolved in 400 ml of dry methanol and passed through a column of ion-exchange resin Rexyn RGl-(OH) (Fisher). The column was then washed with 300 ml of methanol. The solid obtained after evaporation of the solvent from the eluate and the washings was dried for 3 hr *in vacuo* and then dissolved in 12 ml of dimethylformamide. To the cooled solution 11.0 g of *p*-nitrophenyl carbobenzoxy-L-asparaginate was added. After 2 days 100 ml of ethyl acetate was added to the mixture, and the solid was collected and treated four times with 100-ml portions of ethyl acetate–ethanol. The material was then dried to constant weight over P_2O_5 *in vacuo* at 100°; wt 15.6, mp 237–238°. An analytical sample was dissolved in dimethylformamide and reprecipitated with ethanol, mp 237–239°, $[\alpha]_D^{20}$ −47.2° (*c* 1, dimethylformamide) [lit. (Jaquenoud, 1965) mp 239°, $[\alpha]_D^{22}$ −43.0 ± 1° (*c* 1, dimethylformamide)].

Anal. Calcd for $C_{32}H_{41}N_7O_8S$: C, 56.2; H, 6.04; N, 14.3. Found: C, 56.2; H, 5.94; N, 14.1.

*Carbobenzoxy-*L*-glutaminyl-L-asparaginyl-S-benzyl-*L*-cysteinyl-L-prolyl-L-alanylglycinamide.* A solution of the protected pentapeptide (15.3 g) in 50 ml of glacial acetic acid was treated with 60 ml of 5 N HBr in glacial acetic acid. After 2 hr the mixture was added dropwise to 500 ml of cooled ether. The precipitated hydrobromide of the free base was washed five times with 300-ml portions of ether, dried *in vacuo* for 2 hr, and dissolved in 400 ml of methanol. The solution was passed through a Rexyn RGl(OH) column. After removal of the solvent the residue was taken up in 13 ml of dimethylformamide and allowed to react with 9.7 g of *p*-nitrophenyl carbobenzoxy-L-glutaminate. Two days later the precipitate was collected, washed with an ethyl acetate–ethanol mixture as described in the preceding paragraph, and then dried *in vacuo* over P_2O_5 at 100°. The product weighed 16.6 g, mp 240–242°, $[\alpha]_D^{23}$ −46.6° (*c* 1.5, dimethylformamide) [lit. (Jaquenoud, 1965) mp 224°, $[\alpha]_D^{22}$ −39 ± 1° (*c* 1, dimethylformamide)]. The analytical sample was dissolved in dimethylformamide, reprecipitated with ethanol, and again dried overnight *in vacuo* over P_2O_5 at 100°. The resulting product showed no change in physical constants.

Anal. Calcd for $C_{37}H_{49}N_9O_{10}S$: C, 54.7; H, 6.08; N, 15.5. Found: C, 54.7; H, 5.96; N, 15.3.

*Carbobenzoxy-*L*-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-alanylglycinamide.* The protected hexapeptide (8.0 g) was dissolved in 40 ml of glacial acetic acid and treated with 48 ml of 5 N HBr in glacial acetic acid for 2 hr. The hydrobromide of the free hexapeptide was isolated, dried, and dissolved in 400 ml of dry methanol according to the procedures already described. The solution was passed through a column of Rexyn RGl(OH). After removal of the solvent from the eluate and washings the residue was dried for several hr over P_2O_5 and KOH *in vacuo*. The free hexapeptide was suspended in 20 ml of dimethylformamide and allowed to react with 4.2 g of *p*-nitrophenyl carbobenzoxy-L-isoleucinate. The mixture solidified within 15 min. After 2 days at room temperature the product was purified as heretofore described with an ethanol–ethyl acetate mixture (9:1, v/v) and dried *in vacuo* over P_2O_5 at 100°; wt 6.5 g, mp 233–234°, $[\alpha]^{20}D$ −42.1° (*c* 1.5, dimethylformamide). For elementary analysis the protected heptapeptide was twice dissolved in dimethylformamide and reprecipitated by addition of ethanol, mp 234–236° dec, $[\alpha]_D^{20}$ −41.6° (*c* 1.5, dimethylformamide).

Anal. Calcd for $C_{43}H_{60}N_{10}O_{12}S$: C, 54.9; H, 6.43; N, 14.9. Found: C, 55.1; H, 6.51; N, 14.8.

*N-Carbobenzoxy-O-benzyl-*L*-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-alanylglycinamide.* The protected heptapeptide (6.2 g) was dissolved in 30 ml of glacial acetic acid and 35 ml of 5 N HBr in glacial acetic acid was added. After 1.5 hr the hydrobromide of the free heptapeptide was isolated, dried, dissolved in 300 ml of dry methanol, and passed through a

column of Rexyn RGl(OH) according to the procedure already described. Following the removal of the solvent from the eluate and washings the residue was dried for several hours and allowed to react with 3.8 g of *p*-nitrophenyl *N*-carbobenzoxy-*O*-benzyl-L-tyrosinate. The reaction product was kept at room temperature for 2 days and then purified with ethanol and ethyl acetate. Finally, the material was dissolved in dimethylformamide, reprecipitated with ethanol, and dried at 100° *in vacuo* over P_2O_5. The product weighed 6.44 g, mp 242–245° dec (the material started to soften at 236°); $[\alpha]_D^{20}$ −34.7° (*c* 2, dimethylformamide). A small amount of the protected octapeptide was dissolved in dimethylformamide and again precipitated by addition of ethanol, mp 244–246° dec (the material started to soften at 239°), $[\alpha]_D^{20}$ −34.0° (*c* 1.5, dimethylformamide).

Anal. Calcd for $C_{59}H_{75}N_{11}O_{18}S$: C, 60.1; H, 6.41; N, 13.1. Found: C, 60.0; H, 6.43; N, 12.8.

*N-Carbobenzoxy-S-benzyl-*L*-cysteinyl-*L*-tyrosyl-*L*-isoleucyl-*L*-glutaminyl-*L*-asparaginyl-S-benzyl-*L*-cysteinyl-*L*-prolyl-*L*-alanylglycinamide.* METHOD A. The protected octapeptide (1.0 g) was dissolved in 10 ml of glacial acetic acid. Subsequently the solution was treated for 1.5 hr with 5 ml of 5 N HBr in glacial acetic acid. The hydrobromide of the free octapeptide was then isolated, dried, dissolved in 200 ml of dry methanol, and passed through a Rexyn RGl(OH) column according to the procedure already described. After removal of the methanol from the eluate and washings the residue was dried over NaOH *in vacuo* at room temperature and then allowed to react with 440 mg of *p*-nitrophenyl *N*-carbobenzoxy-*S*-benzyl-L-cysteinate in 3 ml of dimethylformamide. After 3 days the reaction product was collected and purified by repeated washings with ethanol. When it had been dried over P_2O_5 at 100° for 15 hr, the material weighed 574 mg, mp 244–247° dec, $[\alpha]_D^{20}$ −46.4° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{62}H_{80}N_{12}O_{14}S_2$: C, 58.1; H, 6.29; N, 13.1. Found: C, 58.0; H, 6.31; N, 13.1.

METHOD B. A suspension of protected octapeptide (500 mg) in 10 ml of freshly distilled trifluoroethanol was treated for 30 min with dry hydrogen bromide at room temperature. The brownish residue, which resulted upon evaporation of the solvent under reduced pressure, was washed three times with 100-ml portions of ether and then dissolved in 3 ml of dimethylformamide. Following adjustment of the pH of the solution to 7.5 by dropwise addition of triethylamine the mixture was allowed to react with 220 mg of *p*-nitrophenyl *N*-carbobenzoxy-*S*-benzyl-L-cysteinate. After 36 hr 20 ml of water was added to the reaction mixture and the solid material was collected and purified by repeated washing with ethanol and ethyl acetate. When the material had been dried over P_2O_5 at 100° for 15 hr, the product weighed 391 mg, mp 245–248° dec, $[\alpha]_D^{20}$ −45.0° (*c* 1, dimethylformamide).

8-Alanine-oxytocin. The protected nonapeptide (100 mg) was dissolved in 200 ml of liquid ammonia freshly distilled from sodium. The protected nonapeptide was treated with sodium at the boiling point of liquid ammonia until a blue color persisted for 20 sec. The ammonia solution was subsequently concentrated to 50 ml and the rest of the ammonia was removed by lyophilization. The white residue was then dissolved in 100 ml of 0.1% acetic acid. After adjustment of the pH to 6.8 with NH_4OH the solution was titrated with a total of 12.5 ml of 0.01 M aqueous potassium ferricyanide. Ferrocyanide and ferricyanide ions were next removed by passing the oxidized solution through a column of AG3X4 resin in the chloride form. The solution, concentrated to a volume of 12 ml, was placed in the first four tubes of a Craig countercurrent distribution machine and subjected to a total of 800 transfers in the solvent system butanol–benzene–pyridine–0.01% acetic acid (6:1:1:8). Folin–Lowry color values (Lowry *et al.*, 1951) indicated that the main peak traveled with a partition coefficient (*K*) of 0.14. The contents of tubes 80–110 were pooled and the volume of the solvent was then reduced in a flash evaporator to approximately 20 ml. Two 100-ml portions of water were added to this solution, which was again concentrated to 20 ml and finally lyophilized. The material (34.0 mg) was dissolved in 6 ml of the lower phase of the system butanol–pyridine–0.1% acetic acid (5:3.5:12) and subjected to countercurrent distribution (455 transfers). Folin–Lowry color values revealed only one peak with a *K* value of 1.16. The contents of tubes 230–260 were pooled, and the solution was concentrated to approximately 20 ml. Water (110 ml) was added to the solution, and the volume was reduced to about 20 ml. This procedure was repeated twice and the solution was then lyophilized, yielding 29.0 mg of 8-alanine-oxytocin, $[\alpha]_D^{20}$ −25.7° (*c* 1, 1 N acetic acid). On electrophoresis in a pyridine–acetate buffer (pH 5.84) a sample of the compound traveled as a single round spot to the cathode. For elemental analysis a sample was dried at 100° over P_2O_5 *in vacuo* for 6 hr and a loss in weight of 8.9% was observed.

Anal. Calcd for $C_{40}H_{60}N_{12}O_{12}S_2$: C, 49.8; H, 6.27; N, 17.4. Found: C, 49.8; H, 6.34; N, 17.1.

A sample was hydrolyzed in 6 N hydrochloric acid at 110° for 22 hr and subsequently analyzed (by the method of Spackman *et al.*, 1958) in the 30–50° system on a Beckman-Spinco amino acid analyzer. The value of glutamic acid was taken as 1.0 and the following molar ratios of amino acids and ammonia were obtained: aspartic acid, 1.0; glutamic acid, 1.0; proline, 1.0; glycine, 0.95; alanine, 1.0; cystine, 1.0; isoleucine, 1.0; tyrosine, 0.9; and ammonia, 3.0.

*S-Benzyl-β-mercaptopropionyl-*L*-tyrosyl-*L*-isoleucyl-*L*-glutaminyl-*L*-asparaginyl-S-benzyl-*L*-cysteinyl-*L*-prolyl-*L*-alanylglycinamide.* The hydrobromide of the free octapeptide was obtained from 1.0 g of protected octapeptide according to the procedure described for the preparation of the protected nonapeptide intermediate used in synthesizing 8-alanine-oxytocin, method A. The salt was dried for several hours *in vacuo*, dissolved in 3 ml of dimethylformamide, cooled to 0°, and titrated with triethylamine to a pH

of 7.5 as indicated by aqueous Gramercy Universal indicator (Fisher). Then *p*-nitrophenyl *S*-benzyl-β-mercaptopropionate (320 mg) was added. In the course of 4 hr the product separated in a gelatinous form. Two days later, following the addition of 100 ml of ethyl acetate, the solid material was collected and washed with four 50-ml portions of ethanol. After being dried *in vacuo* at 100° over P_2O_5 for 16 hr, the product weighed 700 mg, mp 240–241° dec, $[\alpha]^{19}D$ −35.0° (*c* 1, dimethylformamide).

Anal. Calcd for $C_{54}H_{78}N_{11}O_{12}S_2$: C, 57.3; H, 6.50; N, 13.6. Found: C, 57.3; H, 6.52; N, 13.5.

1-Deamino-8-alanine-oxytocin. The debenzylation of the preceding protected polypeptide (200 mg) was performed with sodium in 200 ml of liquid ammonia freshly distilled from sodium. The blue color persisted for 20 sec. Following concentration of the solution and removal of the remaining 50 ml of ammonia by lyophilization, the residual white powder was dissolved in 200 ml of 0.1% acetic acid. The pH was then adjusted to 6.8 with NH_4OH, and the solution was titrated with 14.3 ml of 0.01 M aqueous potassium ferricyanide. The mixture was passed through a column of AG3X4 resin in the chloride form and concentrated in a flash evaporator at 20° to a volume of 15 ml. Then it was placed in the first five tubes of a countercurrent distribution machine and subjected to 700 transfers in the solvent system butanol–propanol–benzene–0.05% acetic acid (3:2:1:6). Material with a *K* value of 0.52 possessing avian vasodepressor activity appeared in a symmetrical peak as determined by plotting the Folin–Lowry color values of aliquots. The contents of tubes 215–265 were pooled, concentrated, and lyophilized to yield 71.0 mg of 1-deamino-8-alanine-oxytocin, $[\alpha]_D^{20.5}$ −107° (*c* 1, 1 N acetic acid). For analysis a sample was dried *in vacuo* at 100° over P_2O_5 for 6 hr. A loss in weight of 4.2% was observed.

Anal. Calcd for $C_{40}H_{59}N_{11}O_{12}S_2$: C, 50.5; H, 6.31; N, 16.1. Found: C, 50.3; H, 6.35; N, 15.8.

A sample was hydrolyzed in 6 N hydrochloric acid at 110° for 22 hr and then analyzed in the 50–50° system on a Beckman-Spinco amino acid analyzer. The following molar ratios of amino acids and ammonia were obtained, with the value of glutamic acid taken as 1.0: aspartic acid, 1.0; glutamic acid, 1.0; proline, 1.1; glycine, 1.0; alanine, 1.0; isoleucine, 1.0; tyrosine, 1.0; and ammonia, 3.1. In addition, cystine (0.25) and the mixed disulfide of cysteine and β-mercaptopropionic acid (0.43) were present.

Carbobenzoxy-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-alanylglycinamide. Carbobenzoxy-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-alanylglycinamide (4.0 g) was dissolved in 12 ml of glacial acetic acid and treated with 10 ml of 5 N HBr in glacial acetic acid. After 2 hr the hydrobromide of the free hexapeptide was isolated, dried, and dissolved in 100 ml of dry methanol according to the procedures already described. The solution was passed through a resin column of Rexyn RGI(OH), whereupon the free hexapeptide started to crystallize in fine white needles from the eluate. To complete crystallization the mixture was kept for 2 hr at 0°, and the crystals were then separated by filtration; yield 2.7 g, mp 205° $[\alpha]_D^{20}$ −50.4° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{29}H_{43}N_9O_8S$: N, 18.6. Found: N, 18.8.

To a solution of the crystalline free hexapeptide (2.63 g) in 20 ml of dimethylformamide, *p*-nitrophenyl carbobenzoxy-L-phenylalaninate (2.2 g) was added. After 2 days the solid material was isolated and purified with ethanol–ethyl acetate by the procedure already described. The product, after being dried *in vacuo* over P_2O_5 at 100° weighed 3.5 g; mp 248–249° dec, $[\alpha]_D^{21}$ −41.3° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{46}H_{58}N_{10}O_{11}S$: C, 57.2; H, 6.16; N, 14.8. Found: C, 57.4; H, 6.11; N, 14.7.

N-Carbobenzoxy-O-benzyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-alanylglycinamide. The protected heptapeptide (3.2 g) was dissolved in 20 ml of glacial acetic acid and treated for 2 hr with 15 ml of 5 N HBr in glacial acetic acid. The free heptapeptide was isolated, dried, and dissolved in 200 ml of dry methanol according to the procedure already described. The solution was passed through a column of Rexyn RGI(OH) resin. Following the removal of the solvent from the eluate and washings the residue was dried for 2 hr *in vacuo*. The material was then dissolved in 20 ml of dimethylformamide, *p*-nitrophenyl *N*-carbobenzoxy-*O*-benzyl-L-tyrosinate (1.86 g) was added, and the mixture was allowed to stand for 2 days at room temperature. The solid was collected and washed with five 50-ml portions of ethanol. Finally, the product was dissolved in a boiling mixture of tetrahydrofuran–water (1:1) and reprecipitated by cooling to 5°; wt 3.44 g, mp 257–258° dec, $[\alpha]_D^{17}$ −39.3° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{62}H_{73}N_{11}O_{13}S$: C, 61.4; H, 6.07; N, 12.7. Found: C, 61.2; H, 5.84; N, 12.8.

N-Carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-alanylglycinamide. A suspension of protected octapeptide (1.0 g) in 25 ml of freshly distilled trifluoroethanol was treated with dry hydrogen bromide at room temperature. The reaction was terminated 25 min later when complete solution had occurred, and the solvent was evaporated *in vacuo*. The yellowish residue was washed three times with 50-ml portions of ether and then dissolved in 5 ml of dimethylformamide. After the pH was adjusted to 8 by dropwise addition of triethylamine, the mixture was cooled with ice water and allowed to react with 425 mg of *p*-nitrophenyl *N*-carbobenzoxy-*S*-benzyl-L-cysteinate. The material was kept for the next 2 days without further cooling. The product was isolated and then purified as just described with water followed by an ethanol–ethyl acetate mixture; wt 834 mg, mp 251–253° dec, $[\alpha]_D^{20.5}$ −47.8° (*c* 1, dimethylformamide).

Anal. Calcd for $C_{68}H_{84}N_{12}O_{14}S_2$: C, 60.2; H, 6.24; N, 12.4. Found: C, 60.1; H, 6.37; N, 12.6.

8-Alanine-oxypressin. The procedure for the reduction with sodium in liquid ammonia of 100 mg of protected nonapeptide and for the subsequent oxidation by titration with potassium ferricyanide was the same as in the preparation of 8-alanine-oxytocin. The oxidized solution, after removal of ferrocyanide and ferricyanide ions by means of resin AG3X4, was concentrated to a volume of 15 ml, placed into the first five tubes of the countercurrent distribution machine, and submitted to a total of 400 transfers in butanol–pyridine–0.1% acetic acid (5:3.5:12). One peak with a K value of 1.38 was detected upon determination of the Folin–Lowry color values. Concentration and lyophilization of the contents of tubes 220–244 gave 36.0 mg of a white powder. On redistribution for a total of 500 transfers in butanol–benzene–pyridine–0.1% acetic acid (6:1:1:8) the 8-alanine-oxypressin traveled as one peak with a K value of 0.32. From the contents of 34 tubes of the central portion of this peak 25.0 mg of 8-alanine-oxypressin was isolated by concentration and lyophilization; $[\alpha]_D^{20}$ −38.0° (*c* 0.75, 1 N acetic acid). Upon gel filtration of a sample of this product on a Sephadex G-25 column (0.9 × 120 cm) in 0.2 N acetic acid the sample emerged as a single sharp peak as detected by measurement of Folin–Lowry color values. For elemental analysis the product was dried *in vacuo* at 100° over P_2O_5. A weight loss of 8.5% was observed.

Anal. Calcd for $C_{43}H_{58}N_{12}O_{12}S_2$: C, 51.7; H, 5.85; N, 16.8. Found: C, 51.5; H, 5.95; N, 16.6.

A sample was hydrolyzed in 6 N hydrochloric acid at 110° for 22 hr and then placed on an amino acid analyzer. The following molar ratios of amino acids and ammonia were obtained, with the value of glutamic acid taken as 1.0: aspartic acid, 1.0; glutamic acid, 1.0; proline, 1.0; glycine, 1.0; alanine, 1.0; cystine, 1.0; tyrosine, 0.8; phenylalanine, 1.0; and ammonia, 3.0.

S-Benzyl-β-mercaptopropionyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-alanylglycinamide. The hydrobromide of L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-alanylglycinamide was obtained from 1.0 g of protected octapeptide as described under the preparation of the protected nonapeptide intermediate for the synthesis of 8-alanine-oxytocin, method B. The octapeptide hydrobromide was dissolved in 5 ml of dimethylformamide, the pH of the solution was adjusted to 7.5, and 277 mg of *p*-nitrophenyl *S*-benzyl-β-mercaptopropionate was added. The product separated overnight in a gelatinous form. Two days later, following the addition of 50 ml of water to the solution, the solid material was collected and extensively washed with an ethanol–ethyl acetate mixture. Finally, the solid was dissolved in a hot tetrahydrofuran–water mixture (1:1) and reprecipitated upon cooling. After being dried *in vacuo* overnight at 100° over P_2O_5 the product weighed 680 mg, mp 225–227° (material softens at 223°), $[\alpha]_D^{20}$ −42.5° (*c* 1, dimethylformamide).

Anal. Calcd for $C_{60}H_{77}N_{11}O_{12}S_2$: C, 59.6; H, 6.42; N, 12.8. Found: C, 59.5; H, 6.31; N, 12.9.

1-Deamino-8-alanine-oxypressin. The procedure for the reduction of 100 mg of the preceding protected polypeptide with sodium in liquid ammonia, the oxidation of the disulfhydryl peptide by titration with potassium ferricyanide, and the subsequent deionization of the solution with AG3X4 resin was the same as in the preparation of 1-deamino-8-alanine-oxytocin. The solution was concentrated to a volume of 15 ml, placed in the first five tubes of the countercurrent distribution machine, and submitted to a total of 400 transfers in the solvent system butanol–propanol–benzene–0.5% acetic acid (3:2:1:6). One peak with a K value of 1.07 was detected upon determination of the Folin–Lowry color values. Concentration and lyophilization of the contents of 35 tubes from the central portion of this peak gave 38 mg of 1-deamino-8-alanine-oxypressin, $[\alpha]_D^{20}$ −106.8° (*c* 1, 1 N acetic acid). Upon gel filtration of 1 mg of this product on a Sephadex G-25 column (0.9 × 120.0 cm) in 0.2 N acetic acid the sample emerged in a single peak as detected by measurement of Folin–Lowry color values. For elemental analysis the product was dried at 100° over P_2O_5 *in vacuo* for 6 hr and a weight loss of 2.9% was observed.

Anal. Calcd for $C_{43}H_{57}N_{11}O_{12}S_2$: C, 52.5; H, 5.84; N, 15.7. Found: C, 52.5; H, 6.01; N, 15.5.

A sample was hydrolyzed in 6 N hydrochloric acid at 110° for 22 hr and then analyzed for amino acid content on the amino acid analyzer. The following molar ratios of amino acids and ammonia were obtained, with the value of glutamic acid taken as 1.0: aspartic acid, 1.0; glutamic acid, 1.0; proline, 1.0; glycine, 1.0; alanine, 1.0; tyrosine, 1.0; phenylalanine, 1.0; and ammonia, 2.9. In addition, cystine (0.27) and the mixed disulfide of cysteine and β-mercaptopropionic acid (0.37) were present.

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