The Synthesis and Some Pharmacological Properties of Tocinoic Acid and Deaminotocinoic Acid[1a,b]

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Received December 17, 1971

Abstract: Tocinoic acid, the 20-membered cyclic disulfide ring of cysteinyltyrosylisoleucylglutaminylasparaginylcysteine, which possesses the tocin ring of oxytocin, was synthesized by the stepwise *p*-nitrophenyl ester method. All protecting groups of the protected intermediate Z-Cys(Bzl)-Tyr-Ile-Gln-Asn-Cys(Bzl)-ONB were removed on addition of this peptide to Na in NH_3. The reduced peptide was converted to purified tocinoic acid by oxidation, followed by partition chromatography and gel filtration. Deaminotocinoic acid was synthesized by similar methods. No C-terminal carboxamide derivatives could be detected. Tocinoic acid possessed 0.2–0.3 unit/mg of oxytocic activity and no detectable avian vasodepressor activity, while deaminotocinoic acid had 3.7 ± 0.3 units/mg of oxytocic activity and no detectable avian vasodepressor activity. Deaminotocinoic acid possessed a slight inhibitory effect on the avian vasodepressor activity of synthetic oxytocin.

Oxytocin (Figure 1) possesses a 20-membered disulfide containing ring and a tripeptide side chain. The question of the intrinsic activity associated with the ring structure was first investigated by Ressler, who synthesized the cyclic pentapeptide amide corresponding to the ring of oxytocin[4] and found that the compound possessed low but significant oxytocic and milk ejecting activities but no avian vasodepressor (AVD) activity. In a recent paper[5] a new synthesis of the cyclic pentapeptide amide of oxytocin was presented and the name tocinamide (Figure 2) was suggested for this compound. Tocinamide was also recently synthesized by Kaurov, Martynov, and Popernatskii by a different method.[6] In the report by Hruby, Ferger, and du Vigneaud[5] a synthesis of deaminotocinamide ([1-β-mercaptopropionic acid]tocinamide) (Figure 2) was also reported, and the latter compound was found to possess 34.2 ± 3.0 units/mg of oxytocic activity, about ten times the potency of tocinamide. As with tocinamide, the deamino analog had no AVD activity.

In other studies, four ring compounds corresponding to the vasopressins and the deaminovasopressins were synthesized, namely, pressinamide,[7,8] pressinoic acid,[7] deaminopressinamide,[7] and deaminopressinoic acid[7] (Figure 2). None of these compounds showed any pressor or avian vasodepressor activity, and except for pressinoic acid each exhibited a slight degree of oxytocic activity (0.05–0.5 unit/mg). No inhibitory activities were noted for these compounds in the assay systems studied.[7]

Recently it has been reported that tocinoic acid (Figure 2) is a powerful inhibitor of the release of melanocyte stimulating hormone from the rat pituitary *in vitro*.[9,10] We report here the synthesis of this compound and its deamino analog, deaminotocinoic acid (Figure 2), and some of the other pharmacological properties of these compounds.

In the synthesis of tocinamide and deaminotocinamide,[5] we utilized the intermediates Z-Cys(Bzl)-Tyr-Ile-Gln-Asn-Cys(Bzl)-ONB (**1**) (ONB signifies *p*-nitrobenzyl ester) and S-Bzl-β-mercaptopropionyl-Tyr-Ile-Gln-Asn-Cys(Bzl)-ONB (**2**). The same intermediates are used in the synthesis reported here. However, an important modification which provides a more efficient synthesis of the tetrapeptide precursor is employed, and a modified methodology for removal of the protecting groups from the hexapeptide intermediates is utilized to ensure that a carboxyl group is obtained in position 6 instead of the carboxamide group obtained earlier.

In the previous synthesis of the desired intermediates,[5] the protected tripeptide Z-Gln-Asn-Cys(Bzl)-ONB was treated with HBr–HOAc and, after neutralization, the next amino acid residue, Ile, was added by the use of Z-Ile-ONp. However, the yield was always very low,[11] and hence we have prepared Boc-Gln-Asn-Cys(Bzl)-ONB instead. This derivative was treated with trifluoroacetic acid (TFA) to remove the Boc protecting group and then condensed with Z-Ile-ONp. In this case the yield of tetrapeptide was excellent (see the Experimental Section).

It may be recalled that in the synthesis of the cyclic amides tocinamide and deaminotocinamide, the hexapeptide esters **1** and **2** were converted to the correspond-

(1) (a) This work was supported in part by Grant No. HL-11680 (VduV) from the National Heart and Lung Institute, U. S. Public Health Service, and in part by Grant No. AM-14062 (VJH) from the U. S. Public Health Service and Grant No. GB-8347 (VJH) from the National Science Foundation. (b) All optically active amino acids are of the L variety. The symbols for the amino acid residues follow the tentative rules of the IUPAC–IUB Commission on Biochemical Nomenclature.

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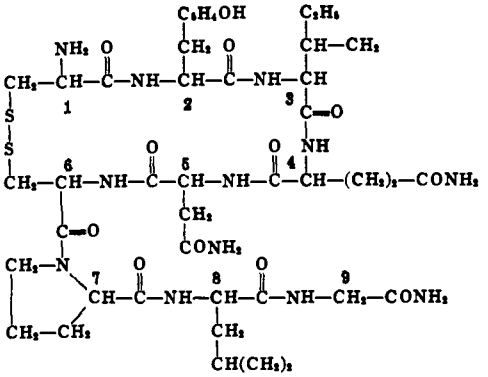

Figure 1. Structure of oxytocin, with numbers indicating the position of the individual amino acid residues.

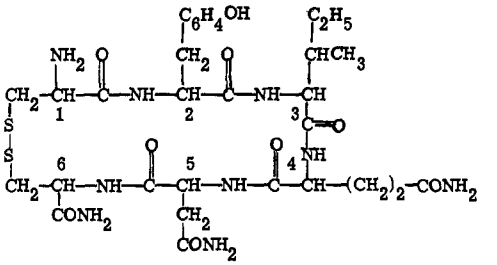

Figure 2. Structure of tocinamide, with the numbers indicating the position of individual amino acid residues. In deaminotocinamide the NH_2 at position 1 is replaced with H. In tocinoic acid the $CONH_2$ at position 6 is replaced with COOH. In deaminotocinoic acid the NH_2 at position 1 is replaced with H and the $CONH_2$ at position 6 is replaced with COOH. The corresponding compounds of the pressin ring series have Phe in place of Ile at position 3.

ing carboxamide compounds by liquid NH_3[5] before cleavage of the Z and Bzl groups by addition of Na to the NH_3 solution.[12] To minimize this C-terminal carboxamide formation in the preparation of tocinoic acid and deaminotocinoic acid, an excess of Na was dissolved in liquid NH_3 and then **1** or **2** was added. The linear disulfhydryl intermediates resulting from cleavage of the protecting groups were converted to the desired cyclic disulfide compounds by 0.01 *N* $K_3Fe(CN)_6$.[13]

When the solvent system (1-BuOH–C_6H_6–3.5% HOAc in 1.5% aqueous pyridine (2:1:3))[5] used for the purification of deaminotocinamide by partition chromatography[14] was applied to the purification of deaminotocinoic acid, no peptide material was eluted at the position for deaminotocinamide (R_f 0.21). In this solvent system deaminotocinoic acid was not eluted ($R_f < 0.1$), and was removed from the column by 1 *N* pyridine. Deaminotocinoic acid was purified by partition chromatography in the solvent system 1-BuOH–EtOH–pyridine–HOAc–H_2O (5:1:1:0.15:7), followed by gel filtration.[15] Tocinoic acid was purified by partition chromatography in the solvent system 1-BuOH–EtOH–pyridine–HOAc–H_2O (4:1:1:0.4:6.4) and by gel filtration.

The highly purified compounds were tested for oxytocic and avian vasodepressor activities. The four-point assay design[16] was used for the bioassays whenever measurable activity was encountered, and the U.S.P. posterior pituitary reference standard was used throughout. Oxytocic assays were performed on isolated uteri from rats in natural estrus according to the method of Holton,[17] as modified by Munsick,[18] with the use of magnesium-free van Dyke–Hastings solution as the bathing fluid. Avian vasodepressor assays were performed by the method of Coon,[19] as described in the U. S. Pharmacopeia,[20] using conscious chickens as suggested by Munsick, Sawyer, and van Dyke.[21] Tocinoic acid and deaminotocinoic acid were found to possess 0.2–0.3 unit/mg and 3.7 ± 0.3 units/mg of oxytocic activity, respectively. Neither tocinoic acid nor deaminotocinoic acid inhibited the oxytocic response to U.S.P. standard. Neither compound had any AVD activity. However, at a weight ratio of 2000:1, deaminotocinoic acid was found to inhibit the AVD response to synthetic oxytocin by 60%.

It may be recalled that tocinamide and deaminotocinamide were found to possess 3.2 ± 0.2 and 34.2 ± 3.0 units/mg of oxytocic activity, respectively.[5] Thus tocinamide has roughly ten times the oxytocic potency of tocinoic acid, and deaminotocinamide likewise has about ten times the potency of deaminotocinoic acid. Furthermore, deaminotocinoic acid and deaminotocinamide have roughly ten times the potency of the corresponding tocin ring analogs, respectively.

Clearly the pentapeptide ring moiety of oxytocin possesses intrinsic oxytocic activity, whether there is a carboxyl or a carboxamide group at position 6, and this activity can be dramatically increased by formal replacement of the free amino group at position 1 with hydrogen. On the other hand, except for the very weak inhibitory activity noted for deaminotocinoic acid, the tocin ring is apparently not sufficient for AVD activity.

Experimental Section[22]

Boc-Gln-Asn-Cys(Bzl)-ONB. A slurry of 1.82 g (3 mmol) of Z-Asn-Cys(Bzl)-ONB[5] in 20 ml of anhydrous HOAc was stirred with 19 ml of 5.4 *N* HBr–HOAc at room temperature for 50 min. The resulting hydrobromide salt was precipitated out with Et_2O (250 ml), filtered off, washed with three 40-ml portions of Et_2O, and dried *in vacuo* for 2 hr. The salt was dissolved in 12 ml of anhydrous dimethylformamide (DMF), and neutralized to pH 7 with *N*-methylmorpholine. Boc-Gln-ONp (1.47 g, 3.5 mmol) was added, and the mixture was stirred for 40 hr at room temperature. The mixture was triturated with 1 ml of H_2O in 100 ml of EtOAc. The gelatinous precipitate was filtered off and washed with two 15-ml portions of EtOAc, two 15-ml portions of EtOH, 15 ml of EtOAc, and two 15-ml portions of Et_2O. The product was reprecipitated from EtOH–DMF (10:3), filtered off, washed with four 15-ml portions of EtOH, and dried *in vacuo:* wt 1.82 g (88%); mp 175–177° dec; $[\alpha]^{23}D$ −19.7° (*c* 1.0, DMF). *Anal.* Calcd for $C_{31}H_{40}N_8O_{10}S$: C, 54.06; H, 5.84; N, 12.20. Found: C, 53.69; H, 5.70; N, 12.18.

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Z-Ile-Gln-Asn-Cys(Bzl)-ONB. A mixture of 1.38 g (2 mmol) of Boc-Gln-Asn-Cys(Bzl)-ONB and 1.5 ml of anisole was stirred for 30 min with 17.5 ml of trifluoroacetic acid (TFA). The salt was precipitated with Et_2O (250 ml), filtered off, washed with three 30-ml portions of Et_2O, and dried *in vacuo*. The white powder was dissolved in 11 ml of anhydrous DMF and neutralized to pH 7 with *N*-methylmorpholine. Z-Ile-ONp (0.90 g, 2.5 mmol) was added, and the mixture was stirred for 20 hr at room temperature. The slurry was triturated with 2 ml of H_2O and 75 ml of EtOAc. The precipitate was filtered off and washed with 15 ml of EtOAc, 15 ml of EtOH, two 15-ml portions of EtOH–H_2O (1:1), 15 ml of EtOH, and two 15-ml portions of Et_2O. The product was dried *in vacuo:* wt 1.14 g (68%); mp 241–243° dec (lit.[5] mp 240–241.5° dec). This sample was found to be identical by infrared and mixture melting point comparison with the sample prepared earlier.[5]

Tocinoic Acid. A solution of about 0.6 mmol of Na in anhydrous liquid NH_3 (125 ml, freshly distilled from Na) was brought near boiling with stirring, and 65 mg of finely powdered Z-Cys(Bzl)-Tyr-Ile-Gln-Asn-Cys(Bzl)-ONB[5] was added. The solution was stirred for 4–5 min and the blue color was maintained throughout by addition of small amounts of Na. The excess Na was destroyed by TFA and the NH_3 removed by evaporation and lyophilization. The residue was dissolved in 150 ml of 0.1% aqueous HOAc. The pH of the solution was adjusted to 8.5 with 2 *N* NH_3, and the sulfhydryl compound was oxidized with 12 ml of 0.01 *N* $K_3Fe(CN)_6$. After 30 min, the pH was adjusted to 5 with dilute HOAc, and 4 ml of Rexyn 203 (Cl^- cycle) (Fisher Scientific Co.) was added to remove ferro- and excess ferricyanide ions. The suspension was stirred for 15 min, the resin was filtered off and washed with three 15-ml portions of 10% HOAc, and the combined filtrates were lyophilized. The residue was dissolved in 6 ml of the upper phase and 2 ml of the lower phase of the solvent system 1-BuOH–EtOH–pyridine–HOAc–H_2O (4:1:1:0.4:6.4) and subjected to partition chromatography by the method of Yamashiro[14] on a 2.2 × 61 cm column of Sephadex G-25 (100–200 mesh) at a flow rate of 17 ml/hr. The peptide material was detected by the Folin–Lowry method[23] and that part comprising the major peak at R_f 0.23 was isolated by lyophilization: wt 21.6 mg. This product was dissolved in 3 ml of 0.2 *N* HOAc and further purified by gel filtration on a 2.8 × 65 cm column of Sephadex G-25 (200–270 mesh) equilibrated with 0.2 *N* HOAc. The compound emerged as a single sharp peak at 93% of the total column volume and was isolated by lyophilization: wt 16.2 mg; $[\alpha]^{22}D$ −7.0° (*c* 0.5, 1 *N* HOAc). The peptide gave a single spot on tlc in system A. Amino acid analysis[24] following 24-hr hydrolysis in 6 *N* HCl at 110° gave the following molar ratios: Asp, 1.0; Glu, 1.0; Cys, 2.0; Ile, 1.1; Tyr, 0.87; NH_3, 2.0. *Anal.* Calcd for $C_{30}H_{44}N_8O_{10}S_2$: C, 48.64; H, 5.98; N, 15.13. Found: C, 48.35; H, 6.12; N, 14.95.

Deaminotocinoic Acid. A sample of 155 mg (0.15 mmol) of *S*-benzyl-β-mercaptopropionyl-Tyr-Ile-Gln-Asn-Cys(Bzl)-ONB[5] was treated with a solution of excess Na in 200 ml of liquid NH_3 as described previously. The lyophilized product was dissolved in 400 ml of 0.1% HOAc and oxidized at pH 8.3 with 30 ml of 0.01 *N* $K_3Fe(CN)_6$. The ferro- and excess ferricyanide ions were removed with 6 ml of Rexyn 203 (Cl^- cycle), and the resulting solution was lyophilized. The residue was dissolved in 5 ml of the upper phase and 2 ml of the lower phase of the solvent system 1-BuOH–EtOH–pyridine–HOAc–H_2O (5:1:1:0.15:7) and subjected to partition chromatography on a 2.9 × 63 cm column of Sephadex G-25 (100–200 mesh). The major peak of peptide material was eluted at R_f 0.40. The product isolated from this peak (40.6 mg) was subjected to gel filtration on a 2.9 × 65 cm column of Sephadex G-25 (200–270 mesh) in 0.2 *N* HOAc and eluted at 100% of column volume: wt 25 mg; $[\alpha]^{22}D$ −57.0° (*c* 0.5, 1 *N* HOAc). The peptide showed a single spot on tlc in system A. Amino acid analysis[24] following 24-hr hydrolysis in 6 *N* HCl gave the following molar ratios: Asp, 1.0; Glu, 1.0; Ile, 1.0; Tyr, 0.85; NH_3, 2.2; Cys, 0.27; mixed disulfide of Cys and β-mercaptopropionic acid, 0.78. *Anal.* Calcd for $C_{30}H_{43}N_7O_{10}S_2 \cdot H_2O$: C, 48.44; H, 6.10; N, 13.18. Found: C, 48.81; N, 5.91; N, 13.06.

Acknowledgment. The authors thank Mrs. Janet Huisjen and Mrs. Eileen Suffern for the oxytocic and AVD bioassays performed in the laboratories of Dr. Louis L. Nangeroni of the New York State Veterinary College at Cornell University. We thank Mr. Kenneth Ehler for performing the amino acid analyses.

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Synthesis and Properties of the Dinucleoside Monophosphate of Adenine 8-Thiocyclonucleoside

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Abstract: The dinucleoside monophosphate (A^spA^s) of 8,2′-anhydro-8-mercapto-9-(β-D-arabinofuranosyl)-adenine was synthesized; its properties were investigated by ultraviolet absorption, circular dichroism, and proton magnetic resonance. Comparison was made with diadenosine monophosphate (ApA) in these properties. The results indicate that the conformation of A^spA^s has two special characteristics: (1) it is a left-handed stack with considerable base–base overlap, and (2) it is relatively stable against thermal perturbation. Also, A^spA^s is very resistant to both venom and spleen phosphodiesterases and does not form a complex with poly U.

The importance of the torsion angle (ϕ_{cn})—which defines the geometrical relationship between the base and the furanose—to the nucleoside conformation has been generally recognized.[2-4] The influence of the

(1) (a) Osaka University; (b) research done in Osaka University was supported by a grant-in-aid for scientific research from the Ministry of Education of Japan; (c) Johns Hopkins University; (d) research done in Johns Hopkins University was supported in part by a grant from the National Science Foundation (GB-8500) and a grant from the National Institutes of Health (GM 16066-04).

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