was located in the 0.03 *M* acetic acid eluates by absorbancy measurements at 280 nm and tlc. Fractions containing homogenous material were pooled, the solvents were evaporated, and the residue was lyophilized from 5% acetic acid: fluffy colorless powder; 290 mg; R_f^I 0.1; R_f^{III} 0.6 (see Figure 4 for tlc); amino acid ratios in acid hydrolysate, $Tyr_{3.7}Glu_{4.1}Leu_{1.0}His_{1.9}Asp_{4.4}Gly_{3.1}Thr_{1.0}Val_{1.0}Ser_{1.9}Pro_{1.0}Lys_{1.0}$ (77%); amino acid ratios in AP-M digest, $Tyr_{4.0}$ (Glu + Asn + Ser)$_{5.8}Leu_{1.1}His_{1.9}Glu_{2.1}Asp_{1.0}Gly_{3.0}Thr_{1.0}Val_{1.1}Pro_{1.1}Formlys_{1.1}$γ-O-*tert*-$BuGlu_{1.0}$ (81%); ratios of "diagnostic" amino acid residues Leu, Val/Pro, Formlys = 1.0.

Preparation of Fragment BCD (Positions 12–47). The trifluoroacetate salt of fragment **B** hydrazide (104 mg) was dissolved in DMSO (1 ml) and DMF (1 ml) was added to the clear solution. The solution was cooled at −10°, then 6.91 *N* hydrogen chloride in dioxane diluted 1:10 with DMF (0.58 ml) was added followed by 10% *tert*-butyl nitrite in DMF (0.11 ml). The solution was stirred at −10 to −15° for 15 min and was then cooled at −25°. A 10% solution of TEA in DMF (0.78 ml) was then added and the mixture was stirred at −25° for 5 min. A solution of fragment CD *tert*-butoxycarbonylhydrazide (75 mg) in DMF (2 ml) and 10% TEA in DMF (0.11 ml) was then added to the suspension which was stirred at −4° for 66 hr. TEA (10% in DMF) was added from time to time to adjust the pH of the mixture to 7.5–8.0. The reaction mixture was diluted with 100 ml of 1-butanol–MeOH–water and the solution was added to an AG 1-X2 column (1.9 × 11 cm) which was eluted with 1-butanol–MeOH–water (50 ml), 1-butanol–MeOH–0.01 *M* acetic acid (150 ml), 1-butanol-MeOH–0.03 *M* acetic acid (150 ml), 1-butanol–MeOH–0.06 *M* acetic acid (250 ml), 1-butanol–MeOH–0.09 *M* acetic acid (150 ml), and 1-butanol–MeOH–0.12 *M* acetic acid (450 ml). The desired product was located in the 0.12 *M* acetic acid eluates by absorbancy measurements at 280 nm and tlc. Fractions containing homogeneous material were pooled and evaporated to dryness, and the residue was lyophilized from 50% acetic acid: 49 mg (48%); R_f^I 0.4; R_f^{III} 0.6; amino acid ratios in acid hydrolysate, $Ser_{6.0}Asp_{5.4}Val_{2.0}Thr_{1.9}Ala_{2.9}Glu_{5.2}Gly_{4.1}Tyr_{3.8}Leu_{1.0}His_{1.8}Pro_{1.5}Lys_{0.9}$ (94%); ratios of "diagnostic" amino acid residues Ala/Leu, Lys = 3.05 (see Figure 4 for tlc).

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Synthesis and Pharmacological Properties of Deaminotocinamide and a New Synthesis of Tocinamide[1-3]

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Abstract: Deaminotocinamide (the 20-membered disulfide pentapeptide amide ring of deamino-oxytocin) and tocinamide (the corresponding disulfide pentapeptide amide ring of oxytocin) were synthesized by the stepwise *p*-nitrophenyl ester method using the *p*-nitrobenzyl ester for C-terminal carboxyl protection. *p*-Nitrobenzyl *S*-benzyl-β-mercaptopropionyltyrosylisoleucylglutaminylasparaginyl-*S*-benzylcysteinate and *p*-nitrobenzyl *N*-benzyloxycarbonyl-*S*-benzylcysteinyltyrosylisoleucylglutaminylasparaginyl-*S*-benzylcysteinate were converted to the corresponding C-terminal amide compounds in liquid ammonia. The polypeptide amides were then converted to the corresponding ring compounds by treatment with sodium in liquid ammonia followed by oxidation and purification. Deaminotocinamide was found to possess 34.2 ± 3.0 units/mg of oxytocic activity, but no detectable avian vasodepressor activity. Tocinamide possessed 3.2 ± 0.2 units/mg of oxytocic activity, but no detectable avian vasodepressor activity.

The synthesis of oxytocin (Figure 1) established the presence of a 20-membered disulfide ring in the hormone.[6] To investigate whether the ring structure itself of oxytocin possesses intrinsic biological activity, Ressler[1] synthesized the cyclic disulfide of cysteinyltyrosylisoleucylglutaminylasparaginylcysteinamide.[7] This cyclic pentapeptide amide, tocinamide,[1] was found by Ressler to have low but significant oxytocic (3.3 units/mg) and milk-ejecting (0.5 unit/mg) potencies, but no detectable avian vasodepressor activity, whereas oxytocin possesses 546 ± 18 units/mg oxytocic,[8] 410 ± 16 units/mg milk-ejecting,[9] and 507 ± 23 units/mg avian vasodepressor[9] potencies.

It may be recalled that deamino-oxytocin possesses 803 ± 36 units/mg of oxytocic activity and 975 ± 24 units/mg of avian vasodepressor activity.[10] This paper presents the synthesis and some of the pharmacological activities of the deamino analog of the cyclic

(1) *Tocinamide* refers here to the cyclic disulfide of cysteinyltyrosylisoleucylglutaminylasparaginylcysteinamide originally synthesized by C. Ressler (*Proc. Soc. Exp. Biol. Med.*, **92**, 725 (1956)). It represents the amide of the cyclic moiety of oxytocin. Since this compound and various analogs thereof will be receiving considerable attention in the future for various chemical, biological, and particularly physical studies, we suggest referring to it as *tocinamide* and the free acid as *tocinoic acid*. The deamino analogs would thus be *deaminotocinamide* and *deaminotocinoic acid*. Likewise, the corresponding compounds in the vasopressin series would be called *pressinamide* and *pressinoic acid*, respectively. It may be recalled that in a previous communication (R. Walter and V. du Vigneaud, *Biochemistry*, **5**, 3720 (1966)) the ring of oxytocin has been referred to as the tocin ring and that of the vasopressins as the pressin ring.

(2) This work was supported in part by Grant No. HE-11680 (V. du V.) from the National Heart Institute, U. S. Public Health Service, and in part by Grant No. GB-11781 (V. J. H.) from the National Science Foundation.

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

(4) The University of Arizona.

(5) Cornell University.

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

(7) A synthesis of this amide by a method different from that reported by Ressler has recently appeared: O. A. Kaurov, V. F. Martynov, and O. A. Popernatskii. *Zh. Obsh. Khim.*, **40**, 904 (1970).

(8) W. Y. Chan, M. O'Connell, and S. R. Pomeroy, *Endocrinology*, **72**, 279 (1963).

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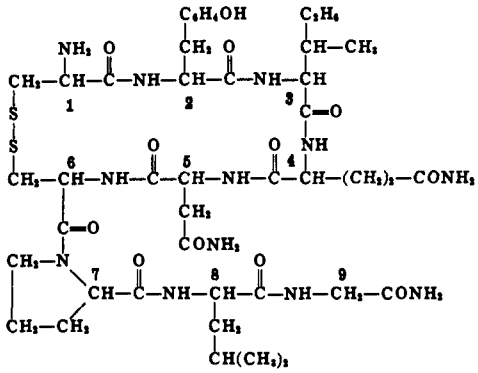

Figure 1. Structure of oxytocin, with numbers indicating the position of the individual amino acid residues.

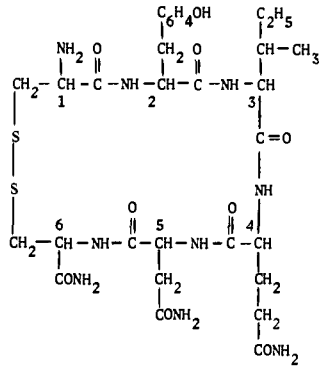

Figure 2. Structure of tocinamide, with numbers indicating the position of the individual amino acid residues.

pentapeptide mentioned above. In addition, a new synthesis of the cyclic pentapeptide amide is presented.

The protected polypeptide precursor used by Ressler was prepared by the condensation of *N*-benzyloxycarbonyl-*S*-benzylcysteinyltyrosine with isoleucylglutaminylasparaginyl-*S*-benzylcysteinamide. The present syntheses utilize protected polypeptide precursors made by the stepwise *p*-nitrophenyl ester method[11] as used in the synthesis of oxytocin,[12,13] but starting with *p*-nitrobenzyl *S*-benzylcysteinate *p*-toluenesulfonate. For the synthesis of deaminotocinamide, the *p*-nitrobenzyl *S*-benzyl-*β*-mercaptopropionyltyrosylisoleucylglutaminylasparaginyl-*S*-benzylcysteinate which we prepared was converted to the corresponding protected peptide amide in refluxing liquid ammonia. The amide was treated with sodium in liquid ammonia[14] to cleave the benzyloxycarbonyl and *S*-benzyl protecting groups, and the resulting disulfhydryl compound was oxidized in aqueous solution with potassium ferricyanide.[15] Deaminotocinamide was obtained in purified form by partition chromatography[16] and gel filtration[17] on Sephadex G-25 with the appropriate solvent systems (see Experimental Section). Tocinamide was prepared

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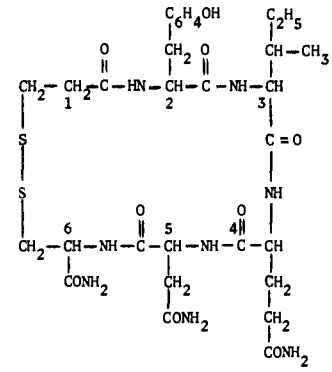

Figure 3. Structure of deaminotocinamide, with numbers indicating the position of the individual amino acid residues.

by an analogous series of steps from *p*-nitrobenzyl *N*-benzyloxycarbonyl-*S*-benzylcysteinyltyrosylisoleucylglutaminylasparaginyl-*S*-benzylcysteinate. Both analogs were then subjected to bioassay for oxytocic and avian vasodepressor activities.

The four-point assay design[18] was used for all bioassays, and the U.S.P. posterior pituitary reference standard was used throughout. Oxytocic assays were performed on isolated uteri from rats in neutral estrus according to the method of Holton,[19] as modified by Munsick,[20] with the use of magnesium-free van Dyke–Hastings solution as the bathing fluid. Avian vasodepressor assays were performed on conscious chickens according to the procedure employed by Munsick, Sawyer, and van Dyke.[21]

Our tocinamide was found to possess no detectable avian vasodepressor activity, and 3.2 ± 0.2 units/mg of oxytocic activity, in agreement with the results of Ressler. The deaminotocinamide likewise was found to possess no detectable avian vasodepressor activity, but 34.2 ± 3.0 units/mg of oxytocic activity.

The oxytocic potency of deaminotocinamide is particularly remarkable compared with that of tocinamide. The formal replacement of the amino group in the 1 position of oxytocin with hydrogen to give deamino-oxytocin brought about a 50% increase in the oxytocic potency, whereas the corresponding change from tocinamide to deaminotocinamide has resulted in a tenfold increase.

Experimental Section[22]

***p*-Nitrobenzyl *S*-Benzylcysteinate *p*-Toluenesulfonate.** A mixture of 14.0 g (66 mmol) of *S*-benzylcysteine, 14.3 g (75 mmol) of *p*-toluenesulfonic acid monohydrate, and 14.6 g (95 mmol) of *p*-nitrobenzyl alcohol was dissolved with heating in 60 ml of benzene and refluxed for 4.5 hr while water was removed by azeotropic distillation using a Dean–Stark moisture receiver (Kontes Glass Co.). The reaction mixture (now in 25 ml of benzene) solidified on standing overnight at room temperature. The solid mass was triturated with 200 ml of ether, filtered, washed with ether, and dried, then dissolved in 210 ml of boiling chloroform, cooled to room temperature, and crystallized as fine needles by gradual addition of ether. Recrystallization from 350 ml of absolute

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(19) P. Holton, *Brit. J. Pharmacol. Chemother.*, **3**, 328 (1948).
(20) R. A. Munsick, *Endocrinology*, **66**, 451 (1960).
(21) R. A. Munsick, W. H. Sawyer, and H. B. van Dyke, *ibid.*, **66**, 860 (1960).
(22) All melting points were determined in capillary tubes and are corrected. Elemental analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich.

ethanol gave 15.7 g of white needles: mp 167–169°; $[\alpha]^{22}D$ −16.6° (*c* 1.0, dimethylformamide).

Anal. Calcd for $C_{24}H_{26}N_2O_7S_2$: C, 55.6; H, 5.05; N, 5.40. Found: C, 55.4; H, 5.08; N, 5.57.

***p*-Nitrobenzyl *N*-Benzyloxycarbonylasparaginyl-*S*-benzylcysteinate.** A stirred solution of 5.19 g (10 mmol) of *p*-nitrobenzyl *S*-benzylcysteinate *p*-toluenesulfonate in 30 ml of anhydrous dimethylformamide was cooled to 0°, and 1.01 g (10 mmol) of *N*-methylmorpholine was added dropwise over a 10-min period. Some precipitation occurred during a further 10 min of stirring at 0°. *p*-Nitrophenyl *N*-benzyloxycarbonylasparaginate (4.36 g, 11 mmol) was added to the slurry, and the mixture was stirred for 22 hr at room temperature.

After treatment with 50 ml of deionized water, the mixture was cooled at 3° for a few hours. The resulting precipitate was filtered off, washed with two 20-ml portions of ethanol–water (1:1), 10 ml of ethanol, and two 20-ml portions of ether, and dried *in vacuo*. The product (3.88 g) was crystallized from ethyl acetate: 3.72 g; mp 177–178.5°; $[\alpha]^{25}D$ −29.8° (*c* 1.0, dimethylformamide).

Anal. Calcd for $C_{29}H_{30}N_4O_8S$: C, 58.6; H, 5.08; N, 9.42. Found: C, 58.6; H, 5.09; N, 9.41.

***p*-Nitrobenzyl *N*-Benzyloxycarbonylglutaminylasparaginyl-*S*-benzylcysteinate.** A slurry of 4.16 g (7.0 mmol) of *p*-nitrobenzyl *N*-benzyloxycarbonylasparaginyl-*S*-benzylcysteinate in 55 ml of anhydrous acetic acid at room temperature was treated with 50 ml of 5.4 *N* hydrogen bromide in acetic acid. The resulting solution was stirred for 1 hr at room temperature, and ether (400 ml) was added. The precipitate was filtered off, washed with two 100-ml portions of ether, and dried *in vacuo*. The salt was dissolved in 30 ml of anhydrous dimethylformamide and neutralized to pH 6.5 with *N*-methylmorpholine. Then *p*-nitrophenyl *N*-benzyloxycarbonylglutaminate (3.21 g, 8 mmol) was added, and the slurry was stirred overnight at room temperature. Ethyl acetate (200 ml) and a little water were added, and the mixture was cooled. The solid product was filtered off and washed with two 30-ml portions of ethyl acetate, two 30-ml portions of 95% ethanol, 50 ml of ethyl acetate, and two 30-ml portions of ether. The product was dried *in vacuo* to give a white powder: 3.54 g; mp 232–234° dec; $[\alpha]^{21.5}D$ −26.6° (*c* 1.0, dimethylformamide). A sample was prepared for elemental analysis by reprecipitation from dimethylformamide with water.

Anal. Calcd for $C_{34}H_{38}N_6O_{10}S$: C, 56.5; H, 5.30; N, 11.6. Found: C, 56.4; H, 5.38; N, 11.5.

***p*-Nitrobenzyl *N*-Benzyloxycarbonylisoleucylglutaminylasparaginyl-*S*-benzylcysteinate.** This protected polypeptide ester was prepared from *p*-nitrobenzyl *N*-benzyloxycarbonylglutaminylasparaginyl-*S*-benzylcysteinate (2.82 g, 4.0 mmol) and *p*-nitrophenyl *N*-benzyloxycarbonylisoleucinate (1.79 g, 4.5 mmol) in the manner described in the preceding section: yield 1.17 g; mp 236–238° dec. A sample was prepared for elemental analysis by reprecipitation from dimethylformamide with water: mp 240–241.5° dec; $[\alpha]^{21.5}D$ −23.1° (*c* 0.9, dimethylformamide).

Anal. Calcd for $C_{40}H_{49}N_7O_{11}S$: C, 57.5; H, 5.91; N, 11.7. Found: C, 57.1; H, 5.92; N, 11.6.

***p*-Nitrobenzyl *N*-Benzyloxycarbonyl-*O*-benzyltyrosylisoleucylglutaminylasparaginyl-*S*-benzylcysteinate.** This compound was prepared from *p*-nitrobenzyl *N*-benzyloxycarbonylisoleucylglutaminylasparaginyl-*S*-benzylcysteinate (1.09 g, 1.2 mmol) and *p*-nitrophenyl *N*-benzyloxycarbonyl-*O*-benzyltyrosinate (0.74 g, 1.4 mmol) in the usual manner: yield 0.94 g; mp 245–247° dec. A sample was prepared for elemental analysis by reprecipitation from dimethylformamide with water: mp 247–248°; $[\alpha]^{21}D$ −18.3° (*c* 1.0, dimethylformamide).

Anal. Calcd for $C_{56}H_{64}N_8O_{13}S$: C, 61.8; H, 5.92; N, 10.2. Found: C, 61.8; H, 5.92; N, 10.2.

***p*-Nitrobenzyl *S*-Benzyl-β-mercaptopropionyltyrosylisoleucylglutaminylasparaginyl-*S*-benzylcysteinate.** This compound was prepared from *p*-nitrobenzyl *N*-benzyloxycarbonyl-*O*-benzyltyrosylisoleucylglutaminylasparaginyl-*S*-benzylcysteinate (1.23 g, 1.0 mmol) and *p*-nitrophenyl *S*-benzyl-β-mercaptopropionate[15] (0.429 g, 1.38 mmol) in the usual manner: 1.05 g; mp 237–239 dec. For elemental analysis the compound was reprecipitated from dimethylformamide with water: mp 239.5–241.5° dec; $[\alpha]^{21.5}D$ −23.2° (*c* 1.0, dimethylformamide).

Anal. Calcd for $C_{51}H_{62}N_8O_{12}S_2$: C, 58.7; H, 5.99; N, 10.7. Found: C, 58.6; H, 6.01; N, 10.7.

***S*-Benzyl-β-mercaptopropionyltyrosylisoleucylglutaminylasparaginyl-*S*-benzylcysteinamide.** A solution of *p*-nitrobenzyl *S*-benzyl-β-mercaptopropionyltyrosylisoleucylglutaminylasparaginyl-*S*-benzylcysteinate (87 mg, 0.083 mmol) in 50 ml of anhydrous ammonia (freshly distilled from sodium) was refluxed for 1.5 hr. The ammonia was removed under reduced pressure, and the dry residue weighed 73 mg after washing with three 15-ml portions of ether, two 10-ml portions of ethanol, two 10-ml portions of ethyl acetate, and two 10-ml portions of ether.

A solution of 20 mg of the crude product in 2 ml of dimethylformamide was filtered, and water was added slowly until precipitation was complete. The precipitate was removed by centrifugation, washed twice by centrifugation from 10 ml of water, and lyophilized: 9.0 mg; mp 249–251° dec; $[\alpha]^{22}D$ −27.6° (*c* 0.5, dimethylformamide).

Anal. Calcd for $C_{44}H_{58}N_8O_9S_2$: C, 58.3; H, 6.4; N, 12.4. Found: C, 58.0; H, 6.4; N, 12.2.

A sample was hydrolyzed for 20 hr in 6 *N* HCl at 110° and was analyzed[23] on a Beckman Model 116 amino acid analyzer. The following molar ratios were obtained: aspartic acid, 1.00; glutamic acid, 1.00; isoleucine, 0.99; tyrosine, 0.90; *S*-benzylcysteine, 1.16; and ammonia, 3.19.

Deaminotocinamide. A solution of crude *S*-benzyl-β-mercaptopropionyltyrosylisoleucylglutaminylasparaginyl-*S*-benzylcysteinamide (48 mg, 0.053 mmol) in 50 ml of anhydrous ammonia (freshly distilled from sodium) was treated at the boiling point with sodium until a blue coloration persisted for 30 sec. The remaining color was discharged with ammonium chloride, and the ammonia was evaporated at reduced pressure. The residue was dissolved in 100 ml of 0.1% aqueous trifluoroacetic acid. The solution was adjusted to pH 6.8 with 2 *N* ammonium hydroxide and stirred with an excess of 0.01 *M* potassium ferricyanide for 15 min. The pH was then maintained at approximately 6.5 until the reaction was over. The solution was stirred for 15 min with 7.5 ml of AG 3-X4 resin (trifluoroacetate cycle) (Bio-Rad Lab., Richmond, Calif.) to remove ferrocyanide and ferricyanide ions. The resin was filtered off, and the solution lyophilized.

The lyophilized powder was dissolved in 2.5 ml of the upper phase of the solvent system 1-butanol–benzene–3.5% aqueous acetic acid in 1.5% aqueous pyridine (2:1:3) and applied to a 1.38 × 75-cm column of Sephadex G-25 (100–200 mesh) that had been equilibrated with lower phase.[16] The column was eluted with the upper phase, and 213 2.8-ml fractions were collected. The fractions corresponding to the major peak with R_f 0.21 (as determined by Folin–Lowry[24] color values) were pooled, twice the volume of distilled water was added, and the resulting mixture was concentrated under reduced pressure and lyophilized.

The deaminotocinoic acid was further purified by gel filtration on Sephadex G-25 (200–270 mesh). The lyophilized powder was dissolved in 0.5 ml of 0.2 *N* acetic acid, placed on a 1.24 × 191 cm column, and eluted with the same solvent in 2.6-ml fractions. A single peak with a maximum at tube 86 (as determined by absorption at 275 mμ) was obtained. The fractions corresponding to this peak were pooled and lyophilized to give 7.5 mg of a white powder: $[\alpha]^{23}D$ −67.4° (*c* 0.56, 1 *N* acetic acid).

Anal. Calcd for $C_{30}H_{44}N_8O_9S_2$: C, 49.7; H, 6.1; N, 15.5. Found: C, 49.5; H, 6.2; N, 15.0.

Amino acid analysis gave the following molar ratios: aspartic acid, 1.00; glutamic acid, 1.05; half-cystine, 0.46; isoleucine, 0.99; tyrosine, 0.91; mixed disulfide of cysteine and β-mercaptopropionic acid, 0.54; and ammonia, 2.84.

***p*-Nitrobenzyl *N*-Benzyloxycarbonyl-*S*-benzylcysteinyltyrosylisoleucylglutaminylasparaginyl-*S*-benzylcysteinate.** This protected hexapeptide ester was prepared from *p*-nitrobenzyl *N*-benzyloxycarbonyl-*O*-benzyltyrosylisoleucylglutaminylasparaginyl-*S*-benzylcysteinate (871 mg, 0.8 mmol) and *p*-nitrophenyl *N*-benzyloxycarbonyl-*S*-benzylcysteinate (420 mg, 0.9 mmol) in the usual manner: 671 mg; mp 248–249.5°. A sample was prepared for elemental analysis by reprecipitation from dimethylformamide with water: mp 252–253° dec; $[\alpha]^{21.5}D$ −32.3° (*c* 1.0, dimethylformamide).

Anal. Calcd for $C_{59}H_{69}N_9O_{14}S_2$: C, 59.4; H, 5.38; N, 10.6. Found: C, 59.1; H, 5.87; N, 10.3.

Tocinamide. The preceding protected polypeptide nitrobenzyl ester (99 mg) was refluxed in 50 ml of anhydrous ammonia for 30 min, and the resulting solution was evaporated to dryness. The residue was extracted several times with ether and dried. Part of the 75-mg residue (50 mg) was dissolved in 1.2 ml of dimethyl-

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

formamide containing 1% acetic acid and subjected to gel filtration on a 0.95 × 59 cm column of Sephadex LH-20 in the same solvent mixture. Sixty 1-ml fractions were collected and examined at 275 mμ. A major peak was observed at 39% of column volume, and its component fractions were pooled and lyophilized. The resulting material (39 mg), which gave the expected amino acid and ammonia analyses for *N*-benzyloxycarbonyl-*S*-benzylcysteinyltyrosylisoleucylglutaminylasparaginyl-*S*-benzylcysteinamide, was treated with sodium in liquid ammonia, oxidized with potassium ferricyanide, and isolated in a manner analogous to the same steps in the synthesis of deaminotocinamide. The crude product was subjected to partition chromatography in the solvent system 1-butanol–ethanol–pyridine–acetic acid–water (4:1:1:0.4:6.4) and eluted with the upper phase with an R_f of 0.26. The material solated from this peak was subjected to gel filtration on Sephadex G-25 in 0.2 *N* acetic acid and isolated by lyophilization. The hygroscopic material became light and fluffy after relyophilization from 2 ml of 0.2 *N* acetic acid: 7.5 mg; $[\alpha]^{23}D$ −4.9° (*c* 0.5, 1 *N* acetic acid).

Anal. Calcd for $C_{80}H_{44}N_8O_{12}S_2 \cdot C_2H_4O_2$: C, 48.0; H, 6.18; N, 15.8. Found: C, 47.7; H, 5.92; N, 16.2.

Amino acid analysis gave the following molar ratios: aspartic acid, 1.00; glutamic acid, 1.00; cystine, 0.92; isoleucine, 0.99; tyrosine, 0.94; and ammonia, 2.98.

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On the Specificity of Creatine Kinase. New Glycocyamines and Glycocyamine Analogs Related to Creatine

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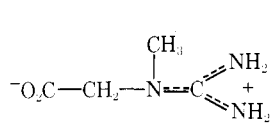
Abstract: The specificity of rabbit muscle creatine kinase (adenosine triphosphate–creatine phosphotransferase, EC 2.7.3.2) for a series of new synthetic analogs of creatine has been investigated. Two of these analogs, 1-carboxymethyl-2-iminoimidazolidine and *N*-methyl-*N*-amidinoaminomethylphosphinic acid, are more reactive (31 and 13% as reactive as creatine, respectively) as substrates in the creatine kinase reaction than any analogs of creatine reported to date. New synthetic routes to substituted glycocyamines have been developed as well as some improvements made on existing synthetic procedures. Earlier synthetic difficulties are discussed in terms of solubility properties of the glycocyamines and their ease of cyclization to glycocyamidines. In the presence of the enzyme, adenosine triphosphate has been shown to phosphorylate the highly reactive analog 1-carboxymethyl-2-iminoimidazolidine on the primary amino group to give 1-carboxymethyl-2-(phosphonoimino)imidazolidine. This result and the other specificity results are discussed in terms of bulk tolerance and geometrical requirements at the active site of the enzyme for optimal activity of the creatine analogs.

Creatine (*N*-methylglycocyamine (**1**)) is found in relatively large quantities in the muscles of vertebrates[2] as its phosphorylated derivative *N*-(phosphonoamidino)sarcosine (phosphocreatine **2**). Phosphocreatine is thought to be a storage form of energy made available for sustained muscular contraction[3,4] by its reversible reaction with adenosine diphosphate (ADP) to form adenosine triphosphate (ATP) and creatine, a reaction catalyzed by the enzyme creatine kinase (ATP–creatine phosphotransferase).[5] Creatine kinase constitutes an important fraction of the protein of vertebrate muscle.[6] The enzyme is currently of unusual medical interest since it has been noticed that creatine kinase levels in human serum rise dramatically following cellular damage, particularly after myocardial infarction.[7]



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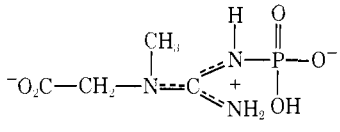

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In the past only a few glycocyamines related to creatine have been prepared. For example, syntheses of creatine itself,[8-11] glycocyamine,[9-14] *N*-ethylglycocyamine,[15] DL-*N*-amidinoalanine[9,16] and DL-*N*-amidinoproline[16-18] have been reported. Of these, only glycocyamine[19] and *N*-ethylglycocyamine[20] have been reported to be active as substrates for creatine kinase.[2]

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