92°; $\lambda_{max}^{CHCl_3}$ 5.80 (small) and broad, strong band at 6.2 μ; λ_{max}^{EtOH} 238.5 and 335 mμ, log ϵ 4.02 and 4.29; λ_{max}^{EtOH} 253 mμ, log ϵ 3.64.

Anal. Calcd. for $C_{11}H_{10}O_4$: C, 64.07; H, 4.89; C-CH_3, 7.29. Found: C, 63.60; H, 4.82; C-CH_3, 8.13.[26]

(26) The high value may be due to small amounts of piperonylic acid carried over in the Kuhn-Roth determination.

The substance proved to be identical by mixture melting point determination and infrared comparison with a synthetic specimen (m.p. 92–93°) prepared by condensation of methyl piperonylate and acetone in ether solution in the presence of sodium.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE]

The Isoglutamine Isomer of Oxytocin: Its Synthesis and Comparison with Oxytocin[1]

BY CHARLOTTE RESSLER AND VINCENT DU VIGNEAUD

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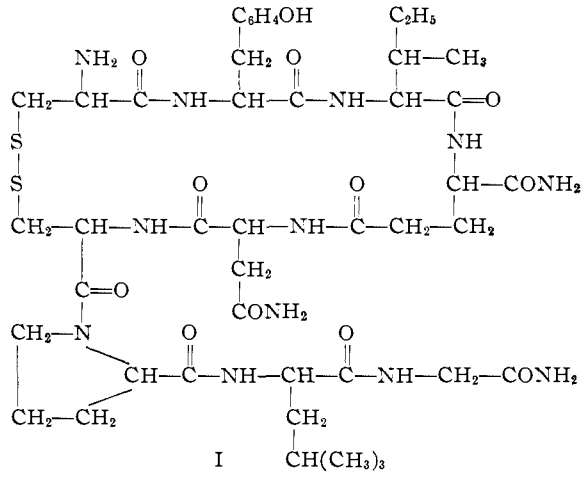
Synthesis of the isomer of oxytocin in which the glutamine residue has been substituted by isoglutamine is described. The change in the nature of the glutamic acid linkage with the consequent ring enlargement was found to result in virtually complete loss of the oxytocic and avian depressor activities of oxytocin. The *isoglutamine-oxytocin* differed from oxytocin also in some of its physical properties and exhibited a striking difference in optical rotation. However, several of the other properties of the two polypeptides were found to be indistinguishable.

The structure of oxytocin, the chief oxytocic principle of the posterior pituitary gland, has been established through its synthesis[2,3] as an octapeptide composed of a 20-membered cyclic disulfide ring branched by a tripeptide side chain, namely, the cyclic disulfide of L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-leucylglycinamide.

It may be recalled that this synthesis was based on structural studies[4] in this Laboratory on natural oxytocin and, in addition, on certain assumptions regarding the position of two amide groups and the nature of the linkages of the aspartic acid and glutamic acid residues. In proposing the structure for oxytocin[4,5] it seemed reasonable to assign the position of two amide groups to the aspartic acid and glutamic acid residues and, furthermore, it was assumed that these were present as asparaginyl and glutaminyl residues rather than as the isomeric isoasparaginyl and isoglutaminyl residues. After this structure for oxytocin had been established through synthesis, further confirmatory evidence with respect to the assumptions made was obtained by the demonstration of the presence of glutamine and asparagine in enzymatic hydrolysates of oxytocin and vasopressin.[6]

It was of considerable interest to determine whether oxytocin could be distinguished from the octapeptides isomeric with it with respect to the glutamine and/or asparagine residues. Such isomers would allow observations on the effect of a small structural change on the physical and chemical properties of oxytocin, and these results might be of interest with respect to the characterization of polypeptides and proteins. Comparison of the biological activities of oxytocin with those of its isoglutamine and/or isoasparagine isomer would provide, in addition, information bearing on the relationship of structure to the biological activities of the oxytocic hormone.

These considerations have led us to undertake the synthesis of the isoglutamine isomer of oxytocin. In this polypeptide an isoglutamine residue replaces the glutamine residue of oxytocin. It may be noted that the ring moiety of this isomer is larger by two methylene units than that present in oxytocin, and thus, *isoglutamine-oxytocin* (I) possesses a 22-membered ring.



I

The synthesis of isoglutamine-oxytocin was approached *via* the nonapeptide intermediate N-carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-isoglutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (VI), which corresponds in structure to the key intermediate used in the synthesis of oxytocin, with isoglutamine replacing the glutamine of the oxytocin intermediate. In the synthesis of oxytocin, suitable reduction of the nonapeptide intermediate followed by oxidation led to the desired 20-membered cyclic disulfide hormone. The projected synthesis of isoglutamine-oxytocin through a similar route, *i.e.*, by oxidation of the appropriate sulfhydryl nonapeptide, involved the as-

(1) This work was supported in part by a grant (H-1675) from the National Heart Institute, Public Health Service.

(2) V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, P. G. Katsoyannis and S. Gordon, THIS JOURNAL, **75**, 4879 (1953).

(3) V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts and P. G. Katsoyannis, *ibid.*, **76**, 3115 (1954).

(4) V. du Vigneaud, C. Ressler and S. Trippett, *J. Biol. Chem.*, **205**, 949 (1953).

(5) H. Tuppy, *Biochim. Biophys. Acta*, **11**, 449 (1953).

(6) H. C. Lawler, S. P. Taylor, A. M. Swan and V. du Vigneaud, *Proc. Soc. Exper. Biol. Med.*, **87**, 550 (1954).

sumption that ring closure to a 22-membered cyclic disulfide could be effected. Actually, the synthesis of the appropriate sulfhydryl nonapeptide afforded an excellent opportunity to determine whether oxidation would lead to a 22-membered cyclic disulfide. If closure to a ring of this size could not be effected, a larger disulfide ring might well be formed or a polymer might result. It was therefore vital, after oxidation of the isoglutamine sulfhydryl nonapeptide and isolation of the corresponding disulfide, to determine the molecular weight of the compound.

The synthetic reactions leading to isoglutamine-oxytocin are summarized in the accompanying diagram. Throughout the synthetic work described, the carbobenzoxy group was used to protect the amino group,[7] and the benzyl group served to protect the sulfhydryl group. The protective groups were removed by reductions with sodium in liquid ammonia.[8]

The preparation of VI was accomplished by coupling N-carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucine (V) with the hexapeptide amide L-isoglutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (IVa), which had been prepared in turn from the coupling product of carbobenzoxy-L-isoglutaminyl-L-asparagine (II) and S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (III).[9]

Carbobenzoxy-L-isoglutaminyl-L-asparagine (II) was prepared by the condensation of carbobenzoxy-L-isoglutamine with asparagine in aqueous solution by means of the mixed anhydride procedure with isobutyl chlorocarbonate,[10] modified by the use of triethylamine in place of sodium hydroxide. The protected dipeptide (II) was then coupled with III by the use of tetraethyl pyrophosphite[11] to yield the hexapeptide product carbobenzoxy-L-isoglutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (IV). In this reaction it was found advantageous to use a temperature somewhat lower than that generally employed for such couplings. IV was converted to IVa in 81% yield by treatment with sodium in liquid NH_3 followed by addition of benzyl chloride in the same medium. Close examination of IVa by the use of countercurrent distribution[12] in two different solvent systems revealed that the hexapeptide was highly homogeneous. Certain side reactions that had been noticed in the preparation of a similar peptide,[13,14] and which resulted in alteration of the asparagine residue, appeared to be minimal.

V was prepared *via* its methyl ester from N-carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosine and methyl L-isoleucinate by the N,N′-dicyclohexylcarbodiimide method.[15] Although some of the physical constants of both the ester and acid V differed from the values in the literature for these compounds obtained through several other methods, application here of one of the reported procedures[16] led to material which, after further purification, agreed closely in melting point and rotation with the methyl ester of V obtained here through the carbodiimide method.

V was coupled with IVa in aqueous tetrahydrofuran by the use of N,N′-dicyclohexylcarbodiimide and yielded the protected nonapeptide VI in 64% yield. All the protecting groups were then removed from VI by treatment with sodium in liquid NH_3, and the resulting material was oxidized to the disulfide form by aeration in aqueous solution at *p*H 6.9.

The final product was isolated by countercurrent distribution in 0.05% acetic acid–*sec*-butyl alcohol (K = 0.37) followed by distribution in 0.01 M NH_3–*sec*-butyl alcohol (K = 1.8). Only one product was found in contrast to the situation with the earlier syntheses of two other related polypeptides, namely oxytocin[3] and the cyclic disulfide of L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-cysteinamide.[13,14] Data obtained from the final distribution of the isoglutamine polypeptide indicated that the material possessed a high degree of purity.

That the compound actually possessed the desired monomeric octapeptide structure was shown by the value obtained for the molecular weight, which was within the expected range.

Isoglutamine-oxytocin was tested for some of the physiological activities associated with oxytocin. Oxytocic activity was measured on the isolated rat uterus according to the method of Burn[17] and the avian blood pressure effect was determined in the anaesthetized chicken by the method of Coon.[18] The U. S. P. Standard posterior lobe powder served as reference. Since it was desired to test the polypeptide also in its reduced form, a solution of the peptide was also assayed in the chicken soon after reduction without subsequent aeration. In addition, the reduction product was tested in the presence of added cysteine. The isoglutamine polypeptide was found to be essentially inert, any activity, if present, existing below the limits of detection of the assay procedures. Similar results were obtained with the reduced preparations. Based on the amounts of compound tested, the oxytocic activity was estimated to be below 0.01 unit per mg., and the avian vasodepressor activity below 0.05 unit per mg. In view of the high potencies of these biological effects in oxytocin (500 units per mg.) and the great sensitivity of the assay procedures,[19] the virtual absence of these activities in isoglutamine-oxytocin is striking and emphasizes the very high degree of biological specificity associated with those structural aspects of the oxytocic hormone under consideration. Fur-

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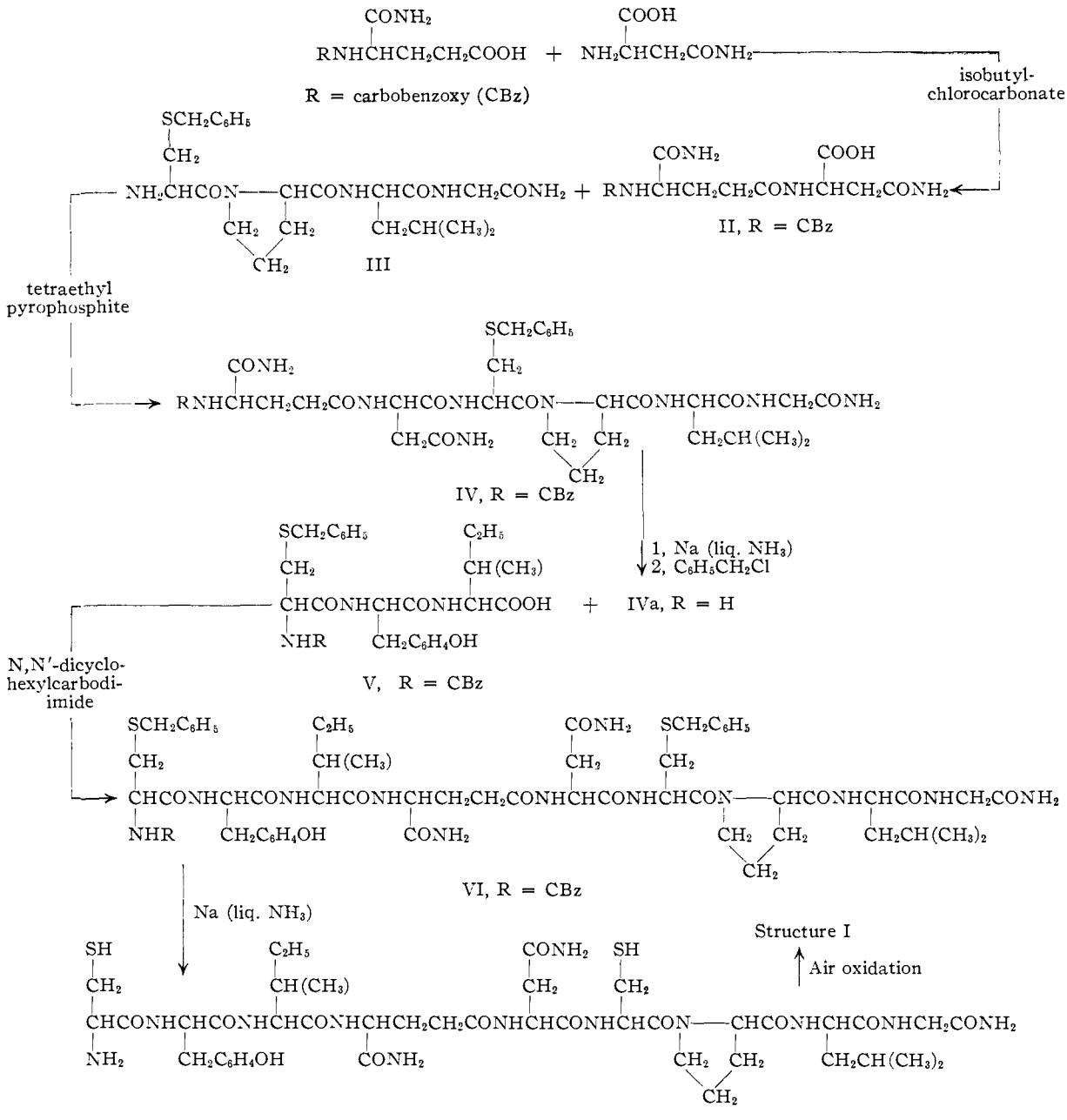
(19) H. B. van Dyke, K. Adamsons, Jr., and S. L. Engel, *Recent Progr. Hormone Research*, VII, 1, Academic Press Inc., New York 10, N. Y., 1955.



thermore, no pressor activity was detected when measured in the rat.[20]

The isoglutamine octapeptide was found to have the expected amino acid composition. Its distribution coefficient was essentially the same as that of oxytocin in the acetic acid–*sec*-butyl alcohol system, but in the ammonia–*sec*-butyl alcohol system the coefficient was somewhat higher than that of oxytocin. The physical constant that was found to differ most strikingly from oxytocin was the optical rotation. The specific rotation in water of the acetate of isoglutamine-oxytocin was approximately 59 degrees higher than that of oxytocin, as given in the Experimental section. No differences were detected in the infrared patterns of isoglutamine-oxytocin and oxytocin. Only small differences were observed when the two polypeptides were subjected to electrophoresis on paper at both *p*H 5.5 and *p*H 9.5.

These comparative data on oxytocin and its isoglutamine isomer demonstrate that small changes in the structure of a polypeptide of this degree of complexity may indeed be reflected in some of the properties. Thus, oxytocin and its isoglutamine isomer can be differentiated readily on the basis of certain differences in physical behavior as well as in biological properties, although a few of the properties of these two polypeptides are indistinguishable.

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Experimental[21]

Methyl N-Carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucinate.—Methyl L-isoleucinate hydrochloride was prepared as described[16] with the following modification.

(21) The reported melting points are corrected, capillary melting points.

The base obtained from the crude hydrochloride was not distilled but was converted to the hydrochloride by acidification of an ethereal solution with concd. HCl. The solution was evaporated to dryness, and the residue was recrystallized by dissolving it in ethyl acetate followed by the addition of ether. The hydrochloride melted at 100.5–101°; $[\alpha]^{23}D$ +26.6° (2% in water); reported[22] m.p. 98–100°; $[\alpha]^{21}D$ +26.6° (2% in water).

To a solution of 324 mg. of the hydrochloride in 2 cc. of tetrahydrofuran was added 0.25 cc. of triethylamine. The precipitated solid was removed by filtration, and the filtrate was added to a solution of 625 mg. of N-carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosine[23] in 3 cc. of tetrahydrofuran. Three hundred and forty mg. of N,N'-dicyclohexylcarbodiimide was then added, and the reaction mixture was allowed to stand at room temperature for 2.5 hours with occasional shaking. Following the addition of several drops of acetic acid, the solid was removed by filtration. The filtrate was concentrated *in vacuo*, and the residue was dissolved in 10 cc. of ethyl acetate. Removal of the solvent left a residue which started to crystallize. Precipitation was completed by the addition of ether and hexane, and the solid product was separated by filtration; wt. 0.7 g., m.p. 145.5–148°. The solid was then crystallized by stirring with warm nitromethane; wt. 0.52 g. (66%), m.p. 151–154°. A second crop of 104 mg., m.p. 146–151°, was obtained from the nitromethane liquor. When the reaction was conducted on 3 times this scale it yielded 1.5 g. melting at 154–156°.

For analysis, the methyl N-carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucinate was recrystallized twice from nitromethane as clusters of needles, m.p. 159.5–160°; $[\alpha]^{23}D$ −34.8° (*c* 15 mg. in 1 cc. of methanol); reported[16] m.p. 135°; $[\alpha]^{19.5}D$ −32.3° (*c* 2.9, methanol).

Anal. Calcd. for $C_{34}H_{41}O_7N_3S$: C, 64.2; H, 6.50; N, 6.61. Found: C, 64.4; H, 6.59; N, 6.65.

This compound was also prepared by the mixed anhydride procedure.[16] In both methods an excess of methyl L-isoleucinate was used since this procedure yielded products which were easier to purify. The ester melted at 159–159.5° after several recrystallizations from ethyl acetate–hexane and a final crystallization from nitromethane; $[\alpha]^{24}D$ −34.2° (*c* 29 mg. in 1 cc. of methanol); $[\alpha]^{24}D$ −34.7° (*c* 13.6 mg. in 1 cc. of methanol).

N-Carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucine (V).—The methyl ester (500 mg.), m.p. 156–157°, was hydrolyzed as described[16] with the exception that the reaction time was extended to 3.5 hours. The crude product obtained after acidification of the reaction mixture melted at 162–166°. However, after the product had been extracted from an ethyl acetate solution into 0.1 *N* $NaHCO_3$, acidification of the extract yielded material melting at 179–181°. After 2 recrystallizations from aqueous ethanol, 326 mg. (67%) of felty needles melting at 183–184° was obtained.

For analysis, a sample was further recrystallized, and it melted at 185–186°; $[\alpha]^{23.5}D$ −10.1° (*c* 39 mg. in 1 cc. of acetic acid); $[\alpha]^{23}D$ −31.2° (*c* 9.5 mg. in 1 cc. of acetone); reported m.p. 155°,[16] 161–163°,[24] 164–165°[25]; $[\alpha]^{23}D$ −11° ± 1 (*c* 3.84, acetic acid).[25]

Anal. Calcd. for $C_{33}H_{39}O_7N_3S$: C, 63.8; H, 6.32; N, 6.76. Found: C, 63.7; H, 6.51; N, 6.75.

Carbobenzoxy-L-isoglutaminyl-L-asparagine (II).—Carbobenzoxy-L-isoglutamine was prepared according to Bergmann and Zervas[7] by the addition of carbobenzoxy-L-glutamic acid anhydride[26] to a cooled solution of ammonia in chloroform. It was recrystallized from acetone–petroleum ether followed by ethanol–petroleum ether, after which the melting point was 172.5–173.5°; reported[7] m.p. 175°. A sample treated at room temperature with 1 *N* HBr in acetic acid for 2 hr. to remove the carbobenzoxy group was free of glutamine as determined by electrophoretic analysis on paper in borate buffer at *p*H 9.5.

A solution of 4.0 g. of carbobenzoxy-L-isoglutamine in 32 cc. of anhydrous tetrahydrofuran containing 2.0 cc. of triethylamine was cooled to −5° and 1.83 cc. of isobutylchlorocarbonate was added. The mixture was kept at this temperature for 5 minutes after which a suspension of 2.14 g. of asparagine hydrate in 16 cc. of water containing 2.0 cc. of triethylamine was added in two portions. The temperature of the reaction mixture rose to approximately 10°, and the mixture was allowed to warm spontaneously for ½ hour. It was then maintained at 25–30° for 1.5 hours. The clear solution was then decanted from a small amount of undissolved asparagine and was acidified to *p*H 3 with 1 *N* hydrochloric acid. Addition of 50 cc. of ether soon caused precipitation of the product as a white crystalline solid. The crude product weighed 2.9 g., m.p. 166–170°. It was then washed with warm absolute ethanol, and 2.6 g. of material melting at 178.5–180° remained. The material was recrystallized by dissolving it in a small volume of dimethylformamide followed by the addition of acetonitrile; wt. 2.3 g. (41%), m.p. 186.5–187.5°. A sample recrystallized for analysis melted at 187.5–188°; $[\alpha]^{21}D$ +7.28° (*c* 3.0 mg. in 1 cc. of dimethylformamide).

Anal. Calcd. for $C_{17}H_{22}O_7N_4$: C, 51.8; H, 5.62; N, 14.2; amide N, 7.10. Found: C, 52.0; H, 5.74; N, 14.3, 13.9; amide N, 7.27.

The corresponding glutaminyl compound carbobenzoxy-L-glutaminyl-L-asparagine[27] has been reported to melt at 206°.

Carbobenzoxy-L-isoglutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (IV).—Throughout this experiment anhydrous conditions were maintained. A solution of 239 mg. of S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide[9] in 1 cc. of diethyl phosphite was warmed in an oil-bath at 78°, and 0.2 cc. of tetraethyl pyrophosphite was added. After the mixture had been heated for two minutes, 197 mg. of II was added in several portions followed by 0.5 cc. of diethyl phosphite and an additional 0.1 cc. of tetraethyl pyrophosphite. The mixture was stirred in a bath maintained at 70–72° for 1.5 hours. Sometime after the protected dipeptide had dissolved, precipitation of the product as a gel occurred. After the mixture had been cooled thoroughly, the gel was collected by filtration and washed with a small portion of diethyl phosphite. The material was then washed thoroughly with water followed by dilute sodium bicarbonate solution and water and then dried. The weight of the crude product was 317 mg., m.p. 202–206°. The dried material was freed of colored impurities by washing it twice with small portions of hot absolute methanol, and 228 mg. (54%) melting at 210–213° remained. Addition of water to the diethyl phosphite solution and washings yielded a small further crop of less pure material. Similar results were obtained when the experiment was conducted on six times this scale.

For analysis, a sample was dissolved in hot aqueous methanol and allowed to reprecipitate; m.p. 212–214°.

Anal. Calcd. for $C_{40}H_{55}O_{10}N_9S$: C, 56.3; H, 6.49; N, 14.8. Found: C, 55.9; H, 6.40; N, 14.8.

L-Isoglutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (IVa).—A solution of 200 mg. of the protected hexapeptide amide IV in 50 cc. of liquid ammonia was treated at −40° under anhydrous conditions with sodium until a deep blue color lasting approximately 30 seconds was obtained. A solution of 34 mg. of benzyl chloride in toluene was added, and the mixture was stirred for 1 hour, after which 146 mg. of ammonium chloride was added, and the ammonia was removed *in vacuo*. The water-soluble residue was extracted with ether and the insoluble portion was distributed for 105 transfers in the system 0.01 *M* NH_3–*sec*-butyl alcohol. The distribution of material was followed by determining the absorption in the ultraviolet at 260 mμ and the copper-Folin color[28] at 700 mμ. One main band, with a partition coefficient of 1.3, was obtained which agreed rather well with the theoretical curve. Concentration and lyophilization of the contents of the appropriate tubes yielded 114 mg. (68%).

(22) E. L. Smith, D. H. Spackman and W. J. Polglase, *J. Biol. Chem.*, **199**, 801 (1952).

(23) This compound was obtained from its ethyl ester which was prepared by the ethylene chlorophosphite procedure (R. W. Young, K. H. Wood, R. J. Joyce and G. W. Anderson, THIS JOURNAL, **78**, 2126 (1956)). It melted at 200–202°, reported m.p. 198–200° (C. R. Harington and R. V. Pitt Rivers, *Biochem. J.*, **38**, 417 (1944)).

(24) C. W. Roberts, THIS JOURNAL, **76**, 6203 (1954).

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(26) W. J. LeQuesne and G. T. Young, *J. Chem. Soc.*, 1954 (1950).

(27) J. Rudinger, J. Honzl and M. Zaoral, *Coll. Czech. Chem. Comm.*, **21**, 202 (1956).

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

For analysis, a sample was dried at 100° and 0.1 mm. to constant weight.

Anal. Calcd. for $C_{32}H_{49}O_8N_9S$: N, 17.5; amide N, 5.84. Found: N, 17.2; amide N, 5.60.

A small second band (K = 0.11) that was copper-Folin-positive and UV-negative was also obtained, and this presumably contained unbenzylated peptide.

When the reaction was carried out on a 2.0-g. scale using a 2 mole excess of benzyl chloride, the distribution yielded a single band with a total yield of 1.6 g. A portion of a large center fraction of this material, representing an 81% yield of IVa, was redistributed in 0.5% acetic acid–*sec*-butyl alcohol for 177 transfers. The material was recovered as the acetate almost quantitatively from a band (K = 0.34) which essentially coincided with the theoretical curve.

Carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-isoglutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (VI).—To a solution of 240 mg. of N-carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucine and 250 mg. of IVa in 1.5 cc. of 75% tetrahydrofuran at +5° was added 90 mg. of N,N′-dicyclohexylcarbodiimide. The mixture was stored in the refrigerator for 19 hours. The solvent was then evaporated, and the residue was extracted with hot dimethylformamide. After the extracts had been cooled and filtered, the filtrate was evaporated under reduced pressure to a small volume and the product was precipitated with ether. The white solid was collected by filtration; wt. 400 mg., m.p. 229–231°. When washed three times with small volumes of warm methanol and dried, 296 mg. (64%) melting at 234.5–236.5° remained.

For analysis, the compound was dissolved in several drops of cold formic acid and was reprecipitated with cold water, m.p. 234.5–236.5°. The sample was dried at room temperature for 2 days at 0.2 mm. over P_2O_5, $[\alpha]^{21.7}D$ −44.1° (*c* 9.99 mg. in 1 cc. of dimethylformamide).

Anal. Calcd. for $C_{65}H_{86}O_{14}N_{12}S_2 \cdot H_2O$: C, 58.2; H, 6.62; N, 12.5; H_2O, 1.36. Found: C, 58.0; H, 6.58; N, 12.4; H_2O, 1.80.

Reduction of Protected Nonapeptide Amide (VI) and Oxidation of the Product.—Two batches VI of 200 mg. each were reduced with sodium in liquid ammonia and treated as described for the preparation of oxytocin from its corresponding nonapeptide derivative[3] with the following modifications. The dried reduced material was aerated in solution at a concentration of 0.4 mg. per cc. at *p*H 6.9 for approximately 3 hours. After standing in the ice-box overnight, the combined solutions were adjusted to *p*H 3.5 and were concentrated and lyophilized to a white powder.

Isolation of the Product.—The material was divided in the first 5 tubes of the all-glass automatic countercurrent apparatus[29] and was distributed in the system *sec*-butyl alcohol–0.05% acetic acid for 592 transfers. Determinations of weight, absorption in the ultraviolet, and copper-Folin color[28] all defined a single band traveling with a K of 0.37, which was irregularly shaped due probably to the emulsions which occurred in this area during the distribution. The material was then redistributed for 197 transfers in *sec*-butyl alcohol–0.01 M NH_3 in which the K was approximately 1.8. Spectrophotometric analysis at 275 mμ and at 700 mμ for the copper-Folin color, and comparison of the resulting curves with the theoretical curve indicated that the material had a purity estimated as over 90%. The contents of the peak were removed in fractions, and these were concentrated to a small volume. The solutions were acidified with acetic acid and finally were dried by lyophilization yielding a total of 152 mg. (50%) of a white fluffy powder; $[\alpha]^{21.5}D$ +33.0° (*c* 0.55, water), $[\alpha]^{24}D$ +24.2° (*c* 0.54, 1 N acetic acid). In comparison, oxytocin acetate possessed the negative rotations $[\alpha]^{22}D$ −26.2° (*c* 0.53, water)[3] and $[\alpha]^{22.5}D$ −23.1° (*c* 0.51, 1 N acetic acid).

A sample was hydrolyzed in 6 N HCl at 120° for 14 hours and was analyzed on the starch column by the method of Moore and Stein.[30] The following ratios were obtained: leucine, 1.0; isoleucine, 1.0; tyrosine, 0.9; proline, 0.9; glutamic acid, 1.0; aspartic acid, 1.1; glycine, 1.0; ammonia, 3.1; and cystine, 0.8.

Determination of the molecular weight of the isoglutamine polypeptide by the thermoelectric osmometer method[31] indicated a value of 852. This result corresponds with what would be expected of the cyclic octapeptide represented by Structure I, which has a calculated molecular weight of 1007.

Isoglutamine-oxytocin and oxytocin were applied separately to a doubled strip of Whatman No. 1 filter paper and subjected to electrophoretic analysis using 300 v. at 5°. Acetate buffer at *p*H 5.5 was used in one experiment which was run for 11 hours, and glycine buffer at *p*H 9.5 for 8 hours was used in another. The materials were stained with the brom phenol blue–mercuric chloride reagent.[32] The isoglutamine polypeptide traveled slightly faster than oxytocin in both buffers. However, when a mixture of the two peptides was run at *p*H 5.5 for 19 hours, separation was incomplete. A similar, small difference in mobility on paper was obtained when isoglutamine-oxytocin and oxytocin were subjected to chromatography on Whatman No. 1 filter paper for 16 hours in *n*-butyl alcohol–acetic acid–water (5:1:5).

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