hydroisoquinolyl)-carbinol was added in portions to a stirred solution of 0.3 g. of lithium aluminum hydride in 60 ml. of 1,2-dimethoxyethane at room temperature. After the exothermic reaction subsided the mixture was heated under reflux for 30 minutes, then cooled and decomposed with 15 ml. of wet ether. Filtration and evaporation of the filtrate to dryness gave 1 g. of a light-colored oil, which crystallized from a 2:1 cyclohexane–benzene mixture as the crude base (0.65 g., 69%) m.p. 108°. Treatment in benzene solution with hydrogen chloride and recrystallization of the product from isopropyl alcohol gave 3-skatyl-1,2,3,4-tetrahydroisoquinoline hydrochloride hemihydrate, m.p. 205–207°.

Anal. Calcd. for $C_{18}H_{18}ClN_2 \cdot 1/_2H_2O$: C, 71.29; H, 6.48. Found: C, 71.22; H, 6.78.

The anhydrous hydrochloride was obtained by drying a sample still wet with isopropyl alcohol at 150° (10^{-3} mm.). It melted at 209–211°.

Anal. Calcd. for $C_{18}H_{19}ClN_2$: C, 72.35; H, 6.42; N, 9.38. Found: C, 72.04; H, 6.46; N, 8.87.

3-Skatyl-2-methyl-1,2,3,4-tetrahydroisoquinoline Hydrochloride (IX, R = Me).—(a) One-half milliliter of chloral was added at room temperature to a suspension of 1.2 g. of 3-indolyl-3′-(1′,2′,3′,4′-tetrahydroisoquinolyl)-carbinol (VIII) in 10 ml. of chloroform and 30 ml. of 1,2-dimethoxyethane, and the mixture was heated under reflux for 45 minutes. Then another 0.5 ml. of chloral was added and the resulting solution was kept under reflux for one additional hour. The solution was evaporated to dryness *in vacuo* and the residue crystallized from 20 ml. of chloroform, yielding 0.490 g. of 3-skatyl-2-formyl-1′,2′,3′,4′-tetrahydroisoquinoline sesquisolvate, m.p. 175–176°. By evaporation of the mother liquor to dryness and crystallization of the residue from methylene dichloride a further amount of the solid was obtained (0.460 g., bringing the total yield to 0.950 g.).

Anal. Calcd. for $C_{19}H_{18}N_2O \cdot 1^1/_2\ CHCl_3$: C, 50.72; H, 4.05; N, 5.77. Found: C, 50.28; H, 3.99; N, 5.79.

Attempted recrystallizations gave products which contained varying amounts of chloroform.

(b) A solution of 0.85 g. of the above formyl derivative in 50 ml. of 1,2-dimethoxyethane was added at reflux to a stirred solution of 0.5 g. of lithium aluminum hydride in 30 ml. of dimethoxyethane over a period of 5 min. The mixture was kept under reflux for 6 hours, then cooled and decomposed with 2 ml. of water. The inorganic solid was washed several times with methylene dichloride, the washings were combined with the original organic phase, and the solvents evaporated *in vacuo*. The residual gum was dissolved in benzene and ethereal hydrogen chloride was added, causing the precipitation of 0.55 g. (41.0% yield based on VIII) of 3-skatyl-2-methyl-1,2,3,4-tetrahydroisoquinoline hydrochloride, as a creamy solid, m.p. 135°. Reprecipitation from a mixture of isopropyl alcohol–acetone–ether raised the melting point to 137°.

Anal. Calcd. for $C_{19}H_{21}ClN_2$: C, 72.95; H, 6.77; N, 8.96. Found: C, 72.87; H, 6.88; N, 8.59.

2,3-Benzo-7,8-(2′,3′-indolo)-tetrahydroquinolizine (X).—To 2.8 g. of a crude oil, containing the free base of IX (R = H), obtained in the above-described manner by reduction of 2.78 g. (0.01 mole) of VIII, a mixture of 0.5 ml. of formic acid and 1 ml. of 37% formalin was added at −10°. The mixture was warmed to 40°, when an exothermic reaction could be noted. After standing at room temperature for 16 hours, a little methanol was added and the solution was poured into aqueous sodium hydroxide, precipitating 2.3 g. of a solid, m.p. 120–125°. Treatment with a small amount of methanol left behind 0.71 g. (25.9% yield based on two stages) of 2,3-benzo-7,8-(2′,3′-indolo)-tetrahydroquinolizine as an insoluble colorless solid, m.p. 240°. Crystallization from benzene–acetone mixture (containing only a trace of benzene) gave spherical clusters, m.p. 240°.

Anal. Calcd. for $C_{19}H_{18}N_2$: C, 83.17; H, 6.61; N, 10.21. Found: C, 83.40; H, 6.63; N, 9.81.

Methochloride of X.—One gram of X was suspended in 100 ml. of methanol, and gaseous methyl chloride was added during 2 hours at room temperature and then for a further 6 hours at reflux temperature, by which time all the solid had dissolved. The solution was concentrated to a volume of 20 ml., a little ether was added, and after keeping overnight at 0°, 0.1 g. of the original base, m.p. 235–240°, was filtered off. The filtrate was evaporated to dryness under reduced pressure, and the residue solidified by trituration with ether containing a little methanol, yielding 0.95 g. of the crude methochloride, m.p. 220–226°. Recrystallization from a mixture of 12 ml. of methanol, 15 ml. of isopropyl alcohol and a little ether gave 0.8 g. of the methochloride, m.p. 263–265° after softening at 261°. The analytical sample was dried at 100° (1 mm.) for 2 hours.

Anal. Calcd. for $C_{20}H_{21}ClN_2 \cdot 1/_4H_2O$: C, 72.93; H, 6.58. Found: C, 72.96; H, 6.78.

A sample was allowed to come to equilibrium with atmospheric moisture.

Anal. Calcd. for $C_{20}H_{21}ClN_2 \cdot 1^2/_3H_2O$: C, 67.69; H, 6.90; N, 7.90; Cl, 9.99. Found: C, 67.85; H, 7.04; N, 7.87; Cl, 9.61.

Hydrochloride of X.—Dry hydrogen chloride was added to a solution of 100 mg. of X in 100 ml. of benzene and 20 ml. of isopropyl alcohol. The solution was evaporated to dryness and the solid residue was redissolved in benzene containing a trace of methanol. Dry ether was added to cloudiness and after standing at 0° overnight a jelly precipitated. It became crystalline when benzene was replaced with ether as a washing solvent. Filtration gave 110 mg. of the hydrochloride hemihydrate, m.p. 185° after softening at 177°.

Anal. Calcd. for $C_{19}H_{19}ClN_2 \cdot 1/_2H_2O$: C, 71.36; H, 6.30; N, 8.76. Found: C, 71.78; H, 6.49; N, 8.76.

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

Isoasparagine-oxytocin: The Isoasparagine Isomer of Oxytocin[1]

BY WILSON B. LUTZ, CHARLOTTE RESSLER, DONALD E. NETTLETON, JR., AND VINCENT DU VIGNEAUD

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The synthesis of *isoasparagine-oxytocin*, a cyclic polypeptide isomeric with oxytocin with respect to the asparagine residue, is presented. The oxytocic, avian vasodepressor and pressor activities of oxytocin were not detected in the isomer. Isoasparagine-oxytocin was compared with oxytocin also with respect to physical properties, several of which were found to be the same for the two polypeptides. It afforded a crystalline flavianate derivative. This synthesis, besides yielding information on the relationship of structure to biological activity and other properties in the posterior pituitary hormones, shows that intramolecular closure of an appropriate disulfhydryl intermediate to a 21-membered disulfide polypeptide ring can occur with facility.

In proposing the structure[2,3] for oxytocin, the chief oxytocic principle of the posterior pituitary gland, as the cyclic disulfide of L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-leucylglycinamide, several assumptions were made. These involved the position of

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

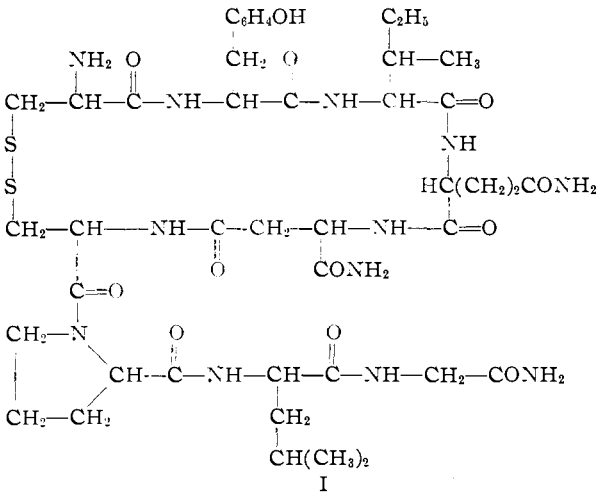
(2) V. du Vigneaud, C. Ressler and S. Trippett, *J. Biol. Chem.*, **205**, 949 (1953).

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

two amide groups and the nature of the linkages of the aspartic acid and glutamic acid residues. It seemed reasonable to assign two of the amide groups to the aspartic acid and glutamic acid residues. Furthermore, it was assumed that the latter residues were present as asparaginyl and glutaminyl residues rather than as the isomeric isoasparaginyl and isoglutaminyl residues. The proposed structure was established through synthesis[4,5]; later, further confirmatory evidence with respect to the assumptions made was provided by demonstrating the presence of asparagine and glutamine in enzymatic hydrolysates of oxytocin.[6]

The octapeptides isomeric with oxytocin with respect to the asparagine and/or glutamine residues would possess cyclic disulfide ring moieties which are larger than the ring in oxytocin by one or more methylene units. Thus, *isoglutamine-oxytocin*[7] possesses a 22-membered ring, and *isoasparagine-oxytocin* (I) would possess a 21-membered one. Furthermore, the carboxamide groups concerned, which in oxytocin are separated from the ring by one and two methylene units, are adjacent to the ring when they are part of the isomeric residues.

Although the synthetic and the later degradative work showed that oxytocin possesses a glutamine and an asparagine residue, our interest was aroused as to how the presence of an isoglutamine or isoasparagine residue would influence biological activity, and what effect such a structural modification would have on its physical properties. To determine the consequences of such structural changes, synthesis of the isoglutamine and isoasparagine isomers of oxytocin was undertaken. The synthesis of isoglutamine-oxytocin has already been presented[7] and that of the isoasparagine isomer (I) is described in the present paper.



I

The synthesis of isoasparagine-oxytocin paralleled that of oxytocin, in which a disulfhydryl nonapeptide served as the key intermediate. Oxidation of this by aeration in dilute aqueous medium led through ring closure in the case of oxytocin to the desired 20-membered cyclic disulfide. One of the chief questions involved in the present synthesis was whether oxidative cyclization of the corresponding isoasparagine-containing intermediate to a 21-membered ring would be possible. Studies aimed at evaluating the relative ease of closure of disulfhydryl peptides to intramolecular disulfide rings of various sizes are in progress also in another laboratory.[8] Several peptides possessing cyclic intramolecular disulfide rings larger than that of oxytocin have been reported recently, including a 23-membered ring related to oxytocin[9] and a 26-membered glycine, lysine and cystine-containing ring,[10] in addition to the 22-membered ring of isoglutamine-oxytocin.[7]

The chemical reactions of isoasparagine-containing compounds are of interest also because, to our knowledge, no isoasparagine-containing peptides have heretofore been reported in the literature. Moreover, in coupling reactions involving derivatives of normal asparagine with amino acids or peptides, difficulty was sometimes encountered, frequently with the formation of undesirable by-products.[11-13]

Because of the possibility of difficulty with the isoasparagine-S-benzyl-L-cysteine coupling, the synthetic route shown in the accompanying diagram was chosen, in which this coupling occurs at an early stage.

Preparation of the starting material, carbobenzoxy-L-isoasparagine (II), in reasonable yield and purity presented some difficulty. II had been prepared previously by Bergmann and Zervas[14] through the reaction of carbobenzoxy-L-aspartic anhydride with aqueous ammonia. This led to a mixture of II and carbobenzoxy-L-asparagine from which they isolated II with a melting point of 164°. A product melting somewhat higher (167–169°) has been reported from the reaction of the α-ethyl ester of carbobenzoxy-L-aspartic acid with ammonia.[15] A modification of the former route was used here, and this has now given carbobenzoxy-L-isoasparagine melting above 167°.

The required carbobenzoxy-L-aspartic anhydride was prepared through the reaction of carbobenzoxy-L-aspartic acid with acetic anhydride,[14,16,17] using a procedure which was similar to the Fischer and Whetstone[16] modification of the method of Miller and co-workers.[17] The reaction of the anhydride with ammonia was carried out under various conditions. Although high initial yields of the mixed isomers usually were obtained, the purification needed to free the carbo-

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

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

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

(7) C. Ressler and V. du Vigneaud, THIS JOURNAL, **79**, 4511 (1957).

(8) G. S. Heaton, H. N. Rydon and J. A. Schofield, *J. Chem. Soc.*, 3157 (1956).

(9) St. Guttmann, P.-A. Jaquenoud, R. A. Boissonnas, H. Konzett and B. Berde, *Naturwiss.*, **44**, 632 (1957).

(10) W. Lautsch and G. Schulz, *ibid.*, **45**, 58 (1958).

(11) C. Ressler, THIS JOURNAL, **78**, 5956 (1956).

(12) C. Ressler, *Proc. Soc. Exptl. Biol. Med.*, **92**, 725 (1956).

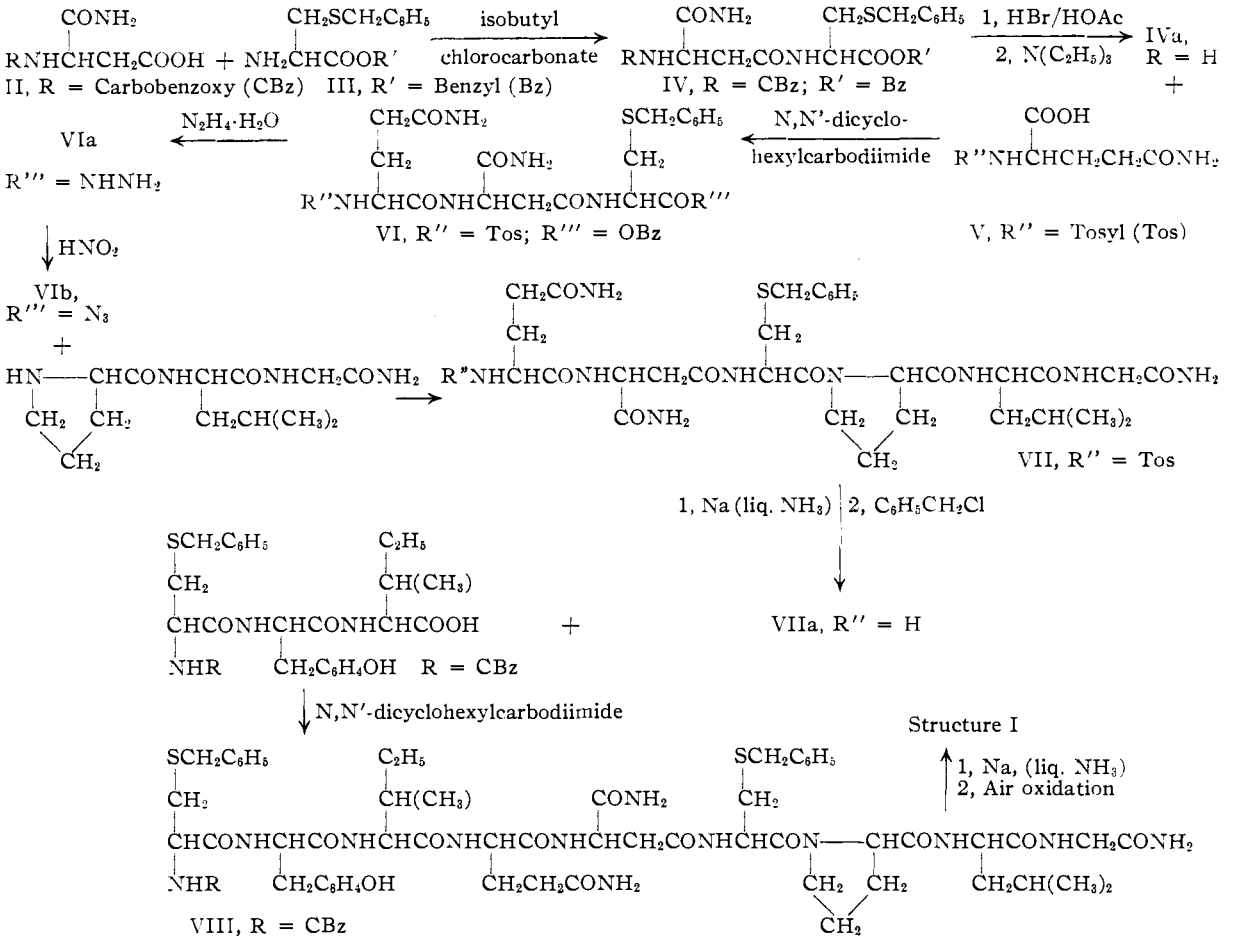
(13) D. T. Gish, P. G. Katsoyannis, G. P. Hess and R. J. Stedman, THIS JOURNAL, **78**, 5954 (1956).

(14) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(15) W. J. Le Quesne and G. T. Young, *J. Chem. Soc.*, 24 (1952).

(16) R. F. Fischer and R. R. Whetstone, THIS JOURNAL, **77**, 750 (1955).

(17) G. L. Miller, O. K. Behrens and V. du Vigneaud, *J. Biol. Chem.*, **140**, 411 (1941).



benzoxy-L-isoasparagine from the normal compound involved considerable losses in each case and generally led to over-all yields from carbobenzoxy-L-aspartic acid which were no greater than 30%. A method using dilute aqueous ammonia at 0°, which had the advantage of ease of workup, was ultimately adopted. After three or four recrystallizations of the crude product, carbobenzoxy-L-isoasparagine was obtained, which, after removal of the carbobenzoxy group by catalytic hydrogenation, was found to be chromatographically free of asparagine and aspartic acid.

Carbobenzoxy-L-isoasparagine was coupled with S-benzyl-L-cysteine benzyl ester by the mixed anhydride procedure using isobutyl chlorocarbonate.[18] The protected dipeptide product, carbobenzoxy-L-isoasparaginyl-S-benzyl-L-cysteine benzyl ester (IV), was obtained in 80% yield in a high state of purity. This was decarbobenzoxylated with hydrogen bromide in acetic acid, and the dipeptide ester IVa thereby obtained was coupled with tosyl-L-glutamine (V)[19] by means of N,N'-dicyclohexylcarbodiimide.[20] The resulting protected tripeptide VI was converted smoothly in boiling methanol to the hydrazide VIa. This was coupled *via* the azide with L-prolyl-L-leucylglycinamide[21] to give moderate yields of the protected hexapeptide tosyl-L-glutaminyl-L-isoasparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (VII). The latter was converted to the S-benzyl hexapeptide VIIa by the use of sodium in liquid ammonia, followed by rebenzylation in the same medium,[22,23] using a modified procedure that is described in detail later. VIIa was purified by countercurrent distribution.[24] It was then coupled with N-carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucine[7] by the carbodiimide procedure and yielded the protected nonapeptide VIII. Conversion of the latter to isoasparagine-oxytocin was effected by the sodium–liquid ammonia procedure[5,25] followed by aeration of the reduced material in aqueous solution at *p*H 6.9.

The product was isolated by countercurrent dis-

(18) J. R. Vaughan, Jr., THIS JOURNAL, **73**, 3547 (1951).

(19) J. M. Swan and V. du Vigneaud, *ibid.*, **76**, 3110 (1954).

(20) J. C. Sheehan and G. P. Hess, *ibid.*, **77**, 1067 (1955).

(21) Prepared by catalytic hydrogenolysis using Pd black in neutral ethanol of the N-carbobenzoxy tripeptide; for the latter, see C. Ressler and V. du Vigneaud, *ibid.*, **76**, 3107 (1954). The authors wish to thank Dr. J. R. Dice and Dr. E. Nicolaides of Parke, Davis and Company for suggesting the decarbobenzoxylation of this tripeptide without the addition of acid. The crystalline residue left after the removal of the catalyst and solvent was recrystallized from methanol–ethyl acetate and dried *in vacuo*; m.p. 121–125.5°; reported for the hemihydrate, m.p. 122–123° (M. Zaoral and J. Rudinger, *Coll. Czechoslov. Chem. Communs.*, **20**, 1183 (1955)). This material was used without further drying.

(22) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).

(23) V. du Vigneaud and O. K. Behrens, *ibid.*, **117**, 27 (1937).

(24) L. C. Craig, *Anal. Chem.*, **22**, 1346 (1950); L. C. Craig, W. Hausmann, E. H. Ahrens, Jr., and E. J. Harfenist, *ibid.*, **23**, 1236 (1951).

(25) S. Gordon and V. du Vigneaud, *Proc. Soc. Exptl. Biol. Med.*, **84**, 723 (1953).

tribution in 0.05% acetic acid–*sec*-butyl alcohol. One major component was found. This was present in a band that was somewhat broader than the theoretical curve at the trailing edge. The material in this region was redistributed in a second system and led to purified product, giving a total yield of 62% from VIII.

The distribution coefficients of the product were similar to those of oxytocin in the two systems used. The value obtained for the molecular weight[26] was within the range expected for the desired monomeric octapeptide structure I. The isoasparagine polypeptide had the expected amino acid and ammonia composition. It behaved as a single component on paper electrophoresis. A mixture of it with oxytocin was separable at *p*H 5.5, with the former traveling a little more slowly.

Isoasparagine-oxytocin, like isoglutamine-oxytocin, differed markedly in optical rotation from oxytocin. Of the physical constants that might be used to characterize these isomeric cyclic polypeptides, optical rotation has so far been found the most definitive.[7]

Isoasparagine-oxytocin was tested for some of the physiological activities associated with oxytocin. Oxytocic activity was tested using the isolated rat uterus according to the method of Burn.[27] The avian blood pressure effect was tested in the anesthetized chicken using the method of Coon.[28] Pressor activity was tested in the rat.[29] The U. S. P. Posterior Pituitary Reference Standard served as reference in each case. No activity was detected in any of these determinations under the conditions used. These results recall those obtained with the isoglutamine isomer of oxytocin, in which these biological activities were also not detected. Recently, isoglutamine-oxytocin has been observed to inhibit significantly the pressor action of arginine-vasopressin or of the U. S. P. Posterior Pituitary Reference Standard.[30] It is of interest that this effect has not been found with the isoasparagine polypeptide.

In connection with the conversion of VII to VIIa, an attempt was made to define more clearly the conditions under which the thiol group of the cysteine residue can be benzylated in liquid ammonia. In synthetic work with sulfur-containing peptides the sulfhydryl group is frequently protected by a benzyl group while the end amino group is protected by a tosyl group. For subsequent reactions it is often desired to remove only the tosyl group. The procedure generally employed involves removal of both protecting groups with sodium in liquid ammonia followed by rebenzylation of the thiol group by the addition of benzyl chloride in the same reaction medium. A solution of the peptide in liquid ammonia is treated at its boiling point with sodium until a lasting blue color results. Usually ammonium chloride or acetic acid is added in small amounts until the blue color is just discharged. Then benzyl chloride is added and allowed to react for an appropriate length of time. Acid equivalent to the sodium employed is added, after which the ammonia is allowed to evaporate. Occasionally, use of the foregoing procedure has led to low yields.

Some of the difficulty encountered might be attributable to the fact that precipitates are frequently formed when certain peptides are treated with sodium in liquid NH_3. In such cases, the precipitates may react incompletely with the benzyl chloride. It has been observed in this Laboratory that such precipitates are dissolved on the addition of NH_4Cl. Addition of NH_4Cl would furthermore tend to minimize the possibility of benzylating sites other than the sulfur.

To test the reactivity of the sulfhydryl group in the presence of excess ammonium ion, a sample of L-cystine was treated in the usual manner with sodium. Ammonium chloride was added to the reaction mixture prior to the benzyl chloride. This modification led to recrystallized S-benzyl-L-cysteine in a yield almost identical with that obtained here using the conventional method. As has been mentioned already, the modified procedure was used to convert the tosyl S-benzyl hexapeptide VII to the S-benzyl hexapeptide VIIa. It is recommended for consideration for use with peptides in which the standard procedure gives low yields.

As part of this work it was also found that the readily available L-cysteine hydrochloride can be benzylated directly in liquid ammonia with benzyl chloride, and this has led to a convenient synthesis of the useful peptide intermediate S-benzyl-L-cysteine.

Experimental[31]

Carbobenzoxy-L-aspartic Acid.—The procedure of Abderhalden and Pitschak[32] was used with the following modification. Instead of extracting the product with ether, the carbobenzoxy-L-aspartic acid was allowed to crystallize directly from the acidified reaction mixture. After one hour the milky suspension that initially formed began to crystallize. After being allowed to stand in the cold overnight, the solid was collected and washed several times with small amounts of water. The product was dried over $CaCl_2$; wt. 51.1 g. (75%), m.p. 116–117°, reported[32] m.p. 112–115°.

Carbobenzoxy-L-aspartic Anhydride.—A mixture of 40 g. of carbobenzoxy-L-aspartic acid and 88 ml. of acetic anhydride in a 500-ml. r.b. flask was shaken intermittently for 50 min. After an additional $1^2/_8$ hours, the solution was freed *in vacuo* of acetic anhydride and acetic acid, using a bath below 38°. Gelation occurred when most of the volatile material had been removed. Dry dioxane (50 ml.) then was added, and it was evaporated until gelation again occurred. The procedure was repeated using dry xylene. The product was dried finally on the lyophilizer. The practically odorless solid was stored *in vacuo* over soda lime for 36 hours; wt. 35.5 g. (95%), m.p. 101–106°. It was used without purification in the next step.

Carbobenzoxy-L-isoasparagine (II).—Carbobenzoxy-L-aspartic anhydride (25.5 g.) was added during 10 minutes to a rapidly stirred mixture of 27 ml. of concd. ammonia and 190 ml. of water, kept slightly below 0° by the use of an ice-salt bath. The mixture was stirred for 80 min. at 0–3°. It was then allowed to warm to 18°, when the white solid

(26) The authors are indebted to Dr. J. R. Rachele of this Laboratory for the molecular weight determination.

(27) J. H. Burn, D. J. Finney and L. G. Goodwin, "Biological Standardization," 2nd Ed., Oxford University Press, 1950, p. 180.

(28) J. M. Coon, *Arch. Intern. pharmacodynamie*, **62**, 79 (1939).

(29) K. M. Lindquist and L. S. Rowe, *Drug Standards*, **23**, 153 (1955).

(30) C. Ressler and J. R. Rachele, *Proc. Soc. Expll. Biol. Med.*, **98**, 170 (1958).

(31) Melting points were taken in capillaries and are corrected. Substances melting above 150° were placed in the bath 10–15° below the m.p. For the flavianate of isoasparagine-oxytocin, a hot-stage micro m.p. determination was made.

(32) E. Abderhalden and G. Pitschak, *Z. physiol. Chem.*, **265**, 31 (1940).

suspension simultaneously dissolved. The solution was filtered through Celite. Formic acid (15 ml.) was added to the clear, cooled filtrate, and the mixture was allowed to stand at room temperature for 1.5 hours. The precipitated solid was collected by filtration, washed with water and pressed as dry as possible on the filter. The product was dried *in vacuo* over $CaCl_2$ and soda lime; wt. 25.2 g. (92%), m.p. 152–161°. An additional crop of 1.5 g., m.p. 156.5–157.5°, was obtained from the mother liquors after further acidification and cooling.

Both crops were combined and dissolved in 250 ml. of formic acid at 36°. The solution was filtered through the Celite, and the filtrate was diluted with 200 ml. of water. After 2.5 hr. at 0°, the product was collected, washed with cold 50% formic acid and finally with cold water. It was dried over $CaCl_2$ and soda lime *in vacuo;* wt. 18.5 g. (68%), m.p. 162–165°. A second recrystallization, by solution of the material in 150 ml. of formic acid and dilution with 75 ml. of water, and working up as before, yielded 11.1 g. (41%) of white crystals, m.p. 164.5–167°. A further recrystallization from formic acid–water gave 8.6 g. (32%), m.p. 167.5–169.5°. To obtain product of highest purity, one or two additional recrystallizations were necessary. The best samples, when inserted in a bath at 155° and heated at the rate of *ca.* 3° per min., melted with slight decomposition at 169.5–172°; $[\alpha]^{24}D$ −27.1° (*c* 4, DMF); $[\alpha]^{23}D$ −27.2° (*c* 1, DMF); $[\alpha]^{21.5}D$ +4.81° (*c* 1.66, acetic acid), reported m.p. 167–169°,[15] 164°[14]; $[\alpha]^{18}D$ +6.9° (*c* 1.66, acetic acid).[14]

A sample melting at 168.5–170° was decarbobenzoxylated catalytically and the product was chromatographed on paper using the system *n*-butyl alcohol–water–acetic acid (4:5:2). The chromatogram was developed with ninhydrin. Reference samples of L-isoasparagine (purple ninhydrin spot, R_f = 0.11) and L-asparagine (brown spot, R_f = 0.081) were used for comparison. The presence of asparagine only was indicated. In a separate chromatogram using 95% ethanol–H_2O–concd. NH_3 (80:15:5).[33] aspartic acid was shown to be absent.

Material obtained from the mother liquors melted over the range 150–158°. Similar analysis of this product after decarbobenzoxylation showed the presence of both isoasparagine and asparagine with a preponderance of the former. Examination of rotation data led to the same conclusion. Recrystallization of the low melting material led to little change in melting point or yielded only small amounts of carbobenzoxy-L-isoasparagine.

The amidation of carbobenzoxy-L-aspartic anhydride was also carried out using concentrations of ammonia that varied from liquid ammonia to dilute aqueous NH_3 and temperatures between −50° and room temperature. The use of purified anhydride was also tried. These modifications, however, were of no significant advantage.

S-Benzyl-L-cysteine Benzyl Ester Benzenesulfonate.—The procedure used was similar to that of Hooper and coworkers[34] for preparing the toluenesulfonate, which is a modification of the general procedures of Miller and Waelsch[35] and Cipera and Nicholls.[36] The following procedure in which the formed water is removed as an azeotope with benzene has given the product with the highest yield and melting point. To a 1-l. flask containing 27 g. of S-benzyl-L-cysteine was added 60 ml. of benzyl alcohol. The mixture was warmed until complete solution occurred, when 45 g. of benzenesulfonic acid and 600 ml. of benzene were added. The two-phase system was heated under reflux for one hour using a water separator. The mixture was then allowed to distil freely for 15 min. It was then distilled under reduced pressure with gentle warming. The residue crystallized before all the benzene had been removed. The solid was suspended in ether, filtered, washed thoroughly with ether and finally dried; wt. 58.1 g. (99%), m.p. 135.5–136.5°.

Recrystallization from ethanol–ether gave material in 83% yield which melted at 137.5–138.5°, $[\alpha]^{22}D$ −19.0° (*c* 1, 95% ethanol).

Anal. Calcd. for $C_{25}H_{25}O_5NS$: C, 60.1; H, 5.48; N, 3.02. Found: C, 60.0; H, 5.56; N, 3.05.

(33) This chromatography was carried out by Dr. R. J. Stedman.

(34) K. C. Hooper, H. N. Rydon, J. A. Schofield and G. S. Heaton, *J. Chem. Soc.*, 3148 (1956).

(35) H. K. Miller and H. Waelsch, THIS JOURNAL, **74**, 1092 (1952).

(36) J. D. Cipera and R. V. V. Nicholls, *Chem. and Ind.*, 16 (1955).

Carbobenzoxy-L-isoasparaginyl-S-benzyl-L-cysteine Benzyl Ester (IV).—In a 200-ml., three-necked flask equipped with a nichrome wire stirrer and a calcium chloride tube was placed 2.66 g. of carbobenzoxy-L-isoasparagine, m.p. 167.5–170°, 60 ml. of dry, freshly distilled tetrahydrofuran (THF) and 1.62 ml. of triethylamine freshly distilled from sodium. The mixture was stirred at room temperature for one hour, when a little solid still remained undissolved. It was then cooled to −5° and treated with 1.44 g. of isobutyl chlorocarbonate. Anhydride formation was allowed to proceed for 8 min. A solution of S-benzyl-L-cysteine benzyl ester was prepared by suspending 4.83 g. of the benzenesulfonate in 46 ml. of THF containing 1.09 g. of triethylamine. After one hour at room temperature this mixture was cooled to 0° and added to the solution of the anhydride. The reaction mixture rapidly became thick and was stirred for 3 hours at 0°. Two hundred and fifty ml. of ether was then added to complete the precipitation of the product. The solid was collected, washed well with ether and dried *in vacuo*. It was freed of triethylamine salts and unreacted ester by dispersing it in water and adjusting the solution to *p*H 1 with 6 *N* HCl. The mixture was stirred for 5 min., and the product was collected and washed well with water. The material was then redispersed in water, the *p*H was adjusted to 9 with 3 *N* ammonia, and the mixture was stirred briefly. The product was again collected, washed with water until the filtrates were neutral and dried *in vacuo* over calcium chloride; wt. 4.5 g. (82%), m.p 183.5–184.5°.

For analysis a sample was recrystallized from acetonitrile. It melted at 183.5–184.5°; $[\alpha]^{21}D$ −18.7° (*c* 2, acetic acid).

Anal. Calcd. for $C_{29}H_{31}O_6N_3S$: C, 63.4; H, 5.69; S, 5.83. Found: C, 63.4; H, 5.72; S, 5.77.

L-Isoasparaginyl-S-benzyl-L-cysteine Benzyl Ester Hydrobromide (HBr Salt of IVa).—A solution of 11.4 g. of hydrogen bromide in 25 ml. of acetic acid was added to a suspension of 9.0 g. of the protected dipeptide in 50 ml. of acetic acid. The mixture was allowed to stand for 30 min. at room temperature. The yellow solution was then frozen in a Dry Ice–acetone mixture and lyophilized to dryness. The tan solid that remained was slurried with warm ethanol, and a change in crystalline form occurred. After solution was effected by further warming, dry ether was added to the point of turbidity, and the product crystallized out. The white needles were collected, washed with 1:1 ethanol–ether and dried; wt., 6.9 g. (78%), m.p. 176–178°. A sample recrystallized from ethanol melted at 177–178°; $[\alpha]^{22}D$ −24.8° (*c* 0.6, DMF).

Anal. Calcd. for $C_{21}H_{25}O_4N_3S \cdot HBr \cdot C_2H_5OH$: C, 50.9; H, 5.96; N, 7.73; Br, 14.7. Found: C, 50.8; H, 6.02; N, 7.79; Br, 14.9.

Tosyl-L-glutaminyl-L-isoasparaginyl-S-benzyl-L-cysteine Benzyl Ester (VI).—A suspension of 1.98 g. of the hydrobromide of IVa in 20 ml. of dry freshly distilled dioxane containing 0.56 ml. of triethylamine was stirred at room temperature for 30 min. and at 10° for 10 min. The solution containing the free dipeptide ester was filtered from the precipitated triethylamine hydrobromide directly into a flask containing a solution of 1.23 g. of tosyl-L-glutamine (V) in 25 ml. of dry dioxane. N,N′-Dicyclohexylcarbodiimide (0.82 g.) was added, the air was replaced by nitrogen, and the mixture was stirred for 1 hr. and stored overnight at 5°. The semi-solid reaction mixture was diluted slowly with 4 ml. of 2 *N* HCl with vigorous stirring. The mixture was diluted with 30 ml. of water, and the slurry was poured into a beaker containing 500 ml. of water. After 15 min. the product was collected and was washed well with water. The filter cake was suspended in water, and the *p*H was adjusted to 9 with triethylamine. After 15 min. of stirring, the precipitate was collected, washed with water, redispersed in water and was then again collected and washed. The dried solid was ground in a mortar with 200 ml. of ethanol for 15 min. to dissolve the dicyclohexylurea. The product was filtered and the filter cake was washed thoroughly with ethanol and finally with a little ether. The white solid was dried *in vacuo;* wt., 2.18 g. (86%), m.p. 202–204.5°.

An analytical specimen was prepared by several recrystallizations from 40% aqueous THF. The solid was dissolved in warm 80% THF, and the solution was diluted slowly with an equal volume of water. The white needles melted at 205.5–207° with softening above 198°; $[\alpha]^{21.5}D$ +35.5° (*c* 1.8, acetic acid).

Anal. Calcd. for $C_{33}H_{39}O_8N_5S_2$: C, 56.8; H, 5.63; N, 10.0. Found: C, 57.0; H, 5.84; N, 9.8.

Tosyl-L-glutaminyl-L-isoasparaginyl-S-benzyl-L-cysteine Hydrazide (VIa).—Two grams of tosyl tripeptide ester VI, m.p. 202–204.5°, was ground in a mortar with methanol and transferred to a 100-ml. r.b. flask. The total volume of solvent used was 65 ml. The mixture was stirred vigorously by means of a magnetic stirrer and was brought to reflux. After 3 min. the solid had partly dissolved, and 2.5 ml. of hydrazine hydrate was added. Refluxing and stirring were continued for 25 min. The reaction mixture which contained crystalline hydrazide was allowed to cool for 1 hr., and the product was then collected by filtration, washed with methanol and dried overnight *in vacuo;* wt. 1.6 g. (88%), m.p. 237–241.5° dec. Longer reaction times and higher concentrations of hydrazine hydrate resulted in lower yields of products, which also had much lower melting points.

An analytical sample was prepared by two recrystallizations of a sample from 70–75% methanol. The white needles melted at 240–241° dec.

Anal. Calcd. for $C_{26}H_{35}O_7N_7S_2$: C, 50.2; H, 5.68; S, 10.5. Found: C, 50.0; H, 5.73; S, 10.3.

Tosyl-L-glutaminyl-L-isoasparaginyl-S-benzyl-L-cysteine Azide (VIb).[37]—The tosyl tripeptide hydrazide, 2.58 g., was ground in a mortar with about half of a mixture containing 45 ml. of acetic acid and 7.5 ml. of 2 *N* HCl. A small amount of insoluble material was filtered off, using the remainder of the acid mixture as a rinse. The filtrate was cooled to −12°, and a solution of 289 mg. of sodium nitrite in 4 ml. of water was added dropwise during 5 min. with rapid stirring. After 7 min. at this temperature 190 ml. of ice-water was added slowly, which precipitated the azide as an easily filterable white solid. The remaining operations were carried out in the cold room at 5°. The precipitated azide was filtered, washed with cold water, suspended in 100 ml. of water, and the product was again collected. The filter cake was washed successively with water (2×), 0.3 *M* potassium bicarbonate (2×) and water (5×), and was then pressed as dry as possible using a rubber dam. The azide was dried over repeated changes of P_2O_5 *in vacuo*. It weighed 2.56 g. (98%) and melted when plunged into a bath at 140°. On slow heating starting at room temperature, it melted at 180–220° dec. It was used without further handling in the next step.

Tosyl-L-glutaminyl-L-isoasparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (VII).—A solution of 2.56 g. of the tripeptide azide VIb and 1.24 g. of L-prolyl-L-leucylglycinamide[21] in 10 ml. of dry dimethylformamide was stirred magnetically in a 100-ml. r.b. flask for 12 hours in an atmosphere of nitrogen. The gel which formed was broken up, and 0.45 ml. of triethylamine and some additional DMF were added. After a total reaction time of 36 hours the gelatinous mass was slowly diluted with vigorous stirring with ethyl acetate to a volume of about 80 ml. during the course of 2 hr. The product was collected, washed with ethyl acetate, redispersed in 80 ml. of ethyl acetate, stirred for 1 hr. and again collected; wt. 3.2 g. (90%). This crude product was dissolved in 20 ml. of formic acid, and the solution was diluted slowly with 40 ml. of water; wt. 2.21 g. (60%) of a white amorphous solid, m.p. 180–190°. It was purified further by diluting a solution of it in hot 50% aqueous pyridine with 40 ml. of water and allowing the filtered solution to stand overnight at 5°. The gelatinous solid was collected and washed with water. After it had been dried over phosphorus pentoxide, it weighed 1.86 g. and melted at 193.5–200° dec.

It was possible with considerable difficulty to crystallize a small amount of the compound from 75% ethanol in the form of small white needles, m.p. 203–206°. Usually the substance separated in amorphous form from this solvent despite seeding and variations in concentration, temperature, cooling rate, etc. With this seed, however, it was possible to induce crystallization from 50% pyridine, one sample of which melted at 199–202°; $[\alpha]^{21}D$ −73.6° (*c* 1.65, acetic acid).

For analysis, a sample obtained from a separate run was dissolved in hot methanol. On cooling, the product separated as small amorphous pebbles which melted at 201.5–205° dec.

Anal. Calcd. for $C_{39}H_{55}O_{10}N_9S_2 \cdot H_2O$: C, 52.5; H, 6.44; N, 14.1; H_2O, 2.02. Found: C, 52.8; H, 6.38; N, 13.9; H_2O, 2.16.

Material melting at 194–203° dec. was employed for the next step.

L-Glutaminyl-L-isoasparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (VIIa).—The protected hexapeptide, 1.43 g., was dissolved in 50 ml. of liquid NH_3 (distilled over sodium) in a flask fitted with a drying tube, magnetic stirrer and side-arm. The reaction mixture was held at its boiling point, and sodium was added through the side-arm. First a brown precipitate appeared, which gradually became white and was finally obscured by a deep blue color throughout the solution that persisted for 4–5 min. The sodium used amounted to *ca.* 480 mg. Ammonium chloride (419 mg.) was added, which discharged the blue color and effected solution of the precipitate. Benzyl chloride (0.57 ml.) was then added, followed 0.5 hr. later by 350 mg. of NH_4Cl and 1 hr. later by an additional 350 mg. of NH_4Cl. The ammonia was then allowed to evaporate.

The residual white solid was stirred with 35 ml. of acetic acid for 8 min. Most of the inorganic salt remained undissolved and was filtered off. The filtrate was diluted with approximately 500 ml. of ether to precipitate the product as its acetate. The white solid was collected and washed with ether.

This material was purified by countercurrent distribution. The solvent system *sec*-butyl alcohol–0.5% acetic acid was used first. The distribution of the material was followed by means of the ultraviolet absorption at 260 mμ and the copper-Folin color[38] at 700 mμ. After 100 transfers most of the material was concentrated in one large, somewhat broad peak (K = 0.45). This material was isolated and redistributed for 101 transfers using the system *sec*-butyl alcohol–0.1% ammonia. Most of the material was now present in a band with a K of 1.4. Some material was also present as slower-moving material (K = 0.3). Concentration and lyophilization of the contents of the tubes in the band (K = 1.4) yielded 0.8 g. (62%) of a white powder.

Another sample was treated similarly, using 500 mg. of VII and 0.3 ml. of benzyl chloride. After evaporation of the NH_3, the residual solid was distributed directly in the butanol–NH_3 system for 102 transfers. A band with a K of 1.43 was obtained, which yielded 395 mg. of material. Starch column analysis by the method of Moore and Stein[39] on a sample of this material, which had been hydrolyzed in 6 *N* HCl for 18 hr. at 120°, yielded the following molar ratios: glutamic acid, 0.9; aspartic acid, 1.1; S-benzylcysteine, 0.8; proline, 0.7; leucine, 1.0; glycine, 0.9; and ammonia, 2.7.

Since the final distributions were performed in a basic medium, the hexapeptide VIIa was isolated as the base and was therefore used directly after isolation in the next coupling reaction.

N-Carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-isoasparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (VIII).—To a solution of 200 mg. of VIIa and 200 mg. of N-carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucine in 1.05 ml. of 90% THF was added 115 mg. of N,N′-dicyclohexylcarbodiimide. The air in the flask was replaced by nitrogen, and the flask was stoppered and swirled intermittently for several hours. Separation of the dicyclohexylurea began within a few seconds after addition of the carbodiimide. After a total reaction time of 20 hr. the solvent was removed, first at 18 mm. and finally at 1 mm. The resulting cake was triturated well with 13 ml. of warm ethanol. The sparingly soluble protected nonapeptide was collected by filtration and washed with ethanol; wt. 268 mg. (73%), m.p. 251–254° with softening above 246°. The solid was dissolved in 3.5 ml. of formic acid and the solution was filtered using 1.5 ml. of formic acid as wash. The filtrate was then slowly diluted with 10 ml. of water. The resulting amorphous product was collected, washed with water and dried over P_2O_5 and KOH. The product now melted somewhat lower at 245–250°.

For analysis, 83 mg. of this material was dissolved in 11 ml. of warm 85% THF, and the solution was diluted gradually with 15 ml. of water. The resulting gelatinous solid was stored at 5° for 1.5 hr., separated by centrifugation and

(37) The procedure employed here is similar to that used for an analogous compound, R. A. Boissonnas, St. Guttmann, P.-A. Jaquenoud and J.-P. Waller, *Helv. Chim. Acta*, **38**, 1491 (1955).

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

(39) S. Moore and W. H. Stein, *ibid.*, **178**, 53 (1949).

washed at the centrifuge several times with 85% THF. The gel was transformed into a much denser and more easily handled precipitate by freezing it at −78°. Centrifugation then separated much additional solvent. The product was dried over P_2O_5 and finally at 100° for 4 hr. *in vacuo*, m.p. 252–254.5°.

Anal. Calcd. for $C_{65}H_{86}O_{14}N_{12}S_2$: C, 59.0; H, 6.55; N, 12.7; N amide, 3.17. Found: C, 58.8; H, 6.62; N, 11.9; N amide, 3.11.

Reduction of Protected Nonapeptide (VIII) and Oxidation of the Product.—VIII in two batches of 150 mg. each was reduced with sodium in liquid NH_3 and treated as described for the preparation of isoglutamine-oxytocin.[7] The combined aqueous solutions were acidified to *p*H 3.5 with acetic acid and were concentrated and lyophilized to a white powder.

Isolation of Isoasparagine-oxytocin (I).—The material was subjected to countercurrent distribution in the system *sec*-butyl alcohol–0.05% acetic acid for 275 transfers. The distribution of material was determined by following the ultraviolet absorption at 275 mμ and the copper-Folin color at 700 mμ. The curves so obtained agreed closely with each other and revealed one major component (K = 0.37), which was present within Tubes 52–95. Some slower moving material (K = 0.16–0.25) which distributed diffusely was also present. The leading edge of the faster moving band coincided with the theoretical curve for the material, and the contents of Tubes 70–93 in this region were concentrated at room temperature to a small volume and dried by lyophilization. One hundred and five mg. of a white fluffy powder was obtained; $[\alpha]^{21.5}D$ −49.2° (*c* 0.53, water), $[\alpha]^{22}D$ −65.1° (*c* 0.52, 1 *N* acetic acid).

The contents of Tubes 57–69 in the trailing edge of the band (K = 0.37), which was somewhat broader than the theoretical curve, were concentrated and dried, and this material (64 mg.) was then redistributed using the solvent system *sec*-butyl alcohol–0.01 *M* NH_3 through 125 transfers. Determination of the absorption at 275 mμ indicated that considerable purification was achieved by use of this solvent system. One band (K = 1.58) which agreed well with the theoretical curve was obtained, along with a well-separated trailing edge. The contents of the band were concentrated to a small volume, acidified with dilute acetic acid and dried by lyophilization.

To obtain further information concerning the extent of purification achieved by the use of the ammonia system, this material was redistributed in the original butyl alcohol–acetic acid system. After 150 transfers the absorption curve at 275 mμ indicated that the substance was now essentially homogeneous. The material, $[\alpha]^{21}D$ −63.3° (*c* 0.51, 1 *N* acetic acid), was recovered from the latter two distributions in 71% over-all yield, and the total yield of purified material from the protected nonapeptide VIII was therefore 62%.

For analysis, material that had been distributed and finally lyophilized from dilute acetic acid was dried to constant weight at 100° and 0.2 mm. The loss in weight was 8.8%.

Anal. Calcd. for $C_{43}H_{66}O_{12}N_{12}S_2 \cdot C_2H_4O_2$: C, 50.6; H, 6.61; N, 15.8. Calcd. for $C_{43}H_{66}O_{12}N_{12}S_2$: C, 51.3; H, 6.61; N. 16.7. Found: C, 51.5; H, 6.75; N, 16.2 (cor. for 0.9% ash).

The results suggest that acetic acid may have been lost during the drying process and recall a similar observation made with the acetate of isoglutamine-oxytocin.[30]

A sample was hydrolyzed in 6 *N* HCl at 120° for 14.5 hr., and the hydrolysate was analyzed by the starch column procedure. The following molar ratios were obtained: leucine, 1.0; isoleucine, 1.0; tyrosine, 0.8; proline, 0.8; glutamic acid, 1.0; aspartic acid, 1.1; glycine, 1.0; ammonia, 3.1; and cystine, 1.0.

The molecular weight of the isoasparagine polypeptide was determined by the thermoelectric osmometer method.[36,40] A value of 1170 (cor. for moisture) was obtained, which compares reasonably with the value of 1067 expected for the acetate of Structure I.

Isoasparagine-oxytocin traveled somewhat more slowly than oxytocin when they were subjected to electrophoretic analysis in the following manner. The two peptides were placed on a strip of Whatman No. 3 MM filter paper, and 300 volts were applied for 16 hours at 5°, using a pyridine–acetate buffer at *p*H 5.5. The materials were stained with the brom phenol blue–mercuric chloride reagent.[41] A mixture of the two substances was separable under these conditions.

The isoasparagine polypeptide yielded a crystalline flavianate. A solution of 4 mg. of the peptide in 0.1 ml. of water and 0.12 ml. of a 5% flavianic acid solution was allowed to stand in the refrigerator. Highly refractive plates slowly deposited. The crystals were separated by centrifugal filtration and were dried in the cold in an evacuated desiccator; wt. 1.4 mg. The dried crystals, although still highly anisotropic, no longer possessed a well-defined form. They decomposed gradually above approximately 220°.

Benzylation of Cysteine in Liquid Ammonia under Various Conditions. A. From L-Cystine.—In each of two 100-ml. flasks containing 40 ml. of liquid NH_3 that had been distilled from sodium was dissolved 2.4 g. (0.1 m.) of L-cystine. The solutions were kept at the boiling point, and sodium was added in portions until a deep blue color appeared which lasted at least 1 min. To one flask was added 4.28 g. (1.5 equivalents based on the sodium used) of ammonium chloride. To the other only a few crystals of ammonium chloride were added, so that the blue color was just discharged. Both reaction mixtures were then treated with 2.3 ml. (0.26 m.) of benzyl chloride. The NH_3 was then allowed to evaporate overnight. Traces of NH_3 were removed *in vacuo*. The residual solids were each dissolved in a minimum quantity of water, and the solutions were extracted with ether and acidified to *p*H 5–6 with 6 *N* HCl. The resulting crystalline precipitates were collected, washed and dried. Each was recrystallized once from hot water. Benzylation in the presence of excess ammonium chloride yielded 2.55 g. (60%); m.p. 221–222.5° dec. The conventional method yielded 2.71 g. (64%); m.p. 216–218° dec.

B. From L-Cysteine Hydrochloride.—Approximately 40 ml. of liquid NH_3 was condensed in a cooled 100 ml. r.b. flask containing 1.58 g. (10 mmoles) of L-cysteine hydrochloride and 1.15 ml. (10 mmoles) of benzyl chloride. The mixture was stirred magnetically. The cysteine hydrochloride soon dissolved, and a light pink solution resulted. The mixture was allowed to stand overnight during which time the NH_3 evaporated. The residue was worked up as described above. The product weighed 1.29 g. (61%) and melted at 222.5–227° with softening above 217°, $[\alpha]^{26}D$ + 24.5° (*c* 1, 1 *M* NaOH[42]); reported[43] m.p. 216–218° dec., $[\alpha]^{26.5}D$ +23.5° (*c* 1, 1 *M* NaOH).

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(40) E. J. Baldes, *Biodynamica*, No. 46, 1 (1939).

(41) E. L. Durrum, THIS JOURNAL, **72**, 2943 (1950).

(42) The rotation of this material is highly temperature-dependent.

(43) J. L. Wood and V. du Vigneaud, *J. Biol. Chem.*, **130**, 103 (1939).