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

A Method of Synthesis of Long Peptide Chains Using a Synthesis of Oxytocin as an Example[1,2]

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p-Nitrophenyl carbobenzoxy-L-leucinate was caused to react with ethyl glycinate. The carbobenzoxy group was removed from the dipeptide derivative thus obtained and the free base brought into reaction with *p*-nitrophenyl carbobenzoxy-L-prolinate. In the same manner the peptide chain was lengthened by one amino acid at each step until the protected nonapeptide. N-carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide was synthesized. Reduction of this protected nonapeptide followed by aeration gave oxytocin which was isolated in highly purified form.

The synthesis of oxytocin was first accomplished in this Laboratory.[3] Later several routes were reported,[4-7] all utilizing the same nonapeptide intermediate as used in the original synthesis but in some cases with different protecting groups. Recently an improved synthesis of oxytocin was published by the present authors.[8] The experiments described in this paper aimed not only at further improvements in the preparation of this hormone but were conducted particularly with the purpose of finding means applicable to the synthesis of longer peptide chains.

In the synthesis of long peptide chains it is obvious that there are practical difficulties connected with the high number of synthetic steps required. Thus, only methods giving high yields of essentially pure products are desirable; otherwise large amounts of starting materials would be necessary. A further basic feature of a method suitable for general use in building up longer chains is the maintenance of optical purity during the peptide bond-forming reaction. If one does not use a method giving high yields of essentially pure products which have not been subjected to racemization during the synthesis, it is quite obvious that one must stop to purify the intermediates by time-consuming methods.

All the mixed anhydride methods were eliminated from our considerations as they usually lead to two acylation products.[9] Nor were methods using activation of the amino component considered to be practical as they are based, as are the mixed anhydride methods, on the competitive action of two different acyl radicals. The azide method, although exceptional in avoiding racemization,[10] can produce a number of undesirable by-products.[11] The preparation of hydrazides especially those of peptides with several amino acids is not always simple and can be connected with hydrazinolysis of the peptide bonds.[12] Furthermore, the low solubility of some acyl peptide hydrazides makes the preparation of the corresponding azides a rather difficult task. The use of acid chlorides is impeded by the lability of tosylamino chlorides under alkaline conditions.[13] On the other hand, carbobenzoxy acids can be transformed into their chlorides only by the action of phosphorus pentachloride, and this results in the formation of phosphorus-containing by-products in the subsequent step. The very convenient and popular new method of peptide synthesis, the carbodiimide method,[14] was criticized recently.[15] As it can cause the formation of ureids,[16] dehydration[17] and racemization it can be used only within certain limits.

After consideration of these various possibilities, the active esters of acylamino acids were chosen as the most suitable agents. Among them the nitrophenyl esters were selected on account of the availability of the phenol component, the ease of removal of the nitrophenol liberated during the reaction and, last but not least, the reasonable speed at which the aminolysis reaction proceeds, even at room temperature and without a catalyst. Thus yields better than 90% can be obtained in a few hours. All the *p*-nitrophenyl esters of acylamino acids prepared for the experiments presented below are stable solids with excellent crystallization properties. That they don't ordinarily react with hydroxyl groups is best demonstrated by the fact that they are usually recrystallized from boiling alcohol.

Aminolysis of nitrophenyl esters has been applied earlier for peptide bond formation by one of the present authors.[18] A modification in the prepa-

(1) This work was supported in part by a grant from the National Heart Institute, U. S. Public Health Service, Grant No. H-1675.

(2) A preliminary report on these experiments has appeared, *Nature*, **183**, 1324 (1959).

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(4) R. A. Boissonas, St. Guttmann, P.-A. Jaquenoud and J.-P. Waller, *Helv. Chim. Acta*, **38**, 1491 (1955).

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(6) M. Bodanszky, M. Szelke, E. Tomorkeny and E. Weisz, unpublished work; *cf.* L. Gyermek and Gy. Fekete, *Experientia*, **11**, 238 (1955).

(7) L. Velluz, G. Amiard, V. Bartos, B. Goffinet and R. Heymes, *Bull. soc. chim. France*, 1464 (1956).

(8) M. Bodanszky and V. du Vigneaud, THIS JOURNAL, **81**, 2504 (1959).

(9) A. R. Emery and V. Gold, *J. Chem. Soc.*, 1443 (1950).

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(15) H. Schwartz and F. M. Bumpus, *ibid.*, **81**, 890 (1959).

(16) H. G. Khorana, *Chemistry & Industry*, 1087 (1955).

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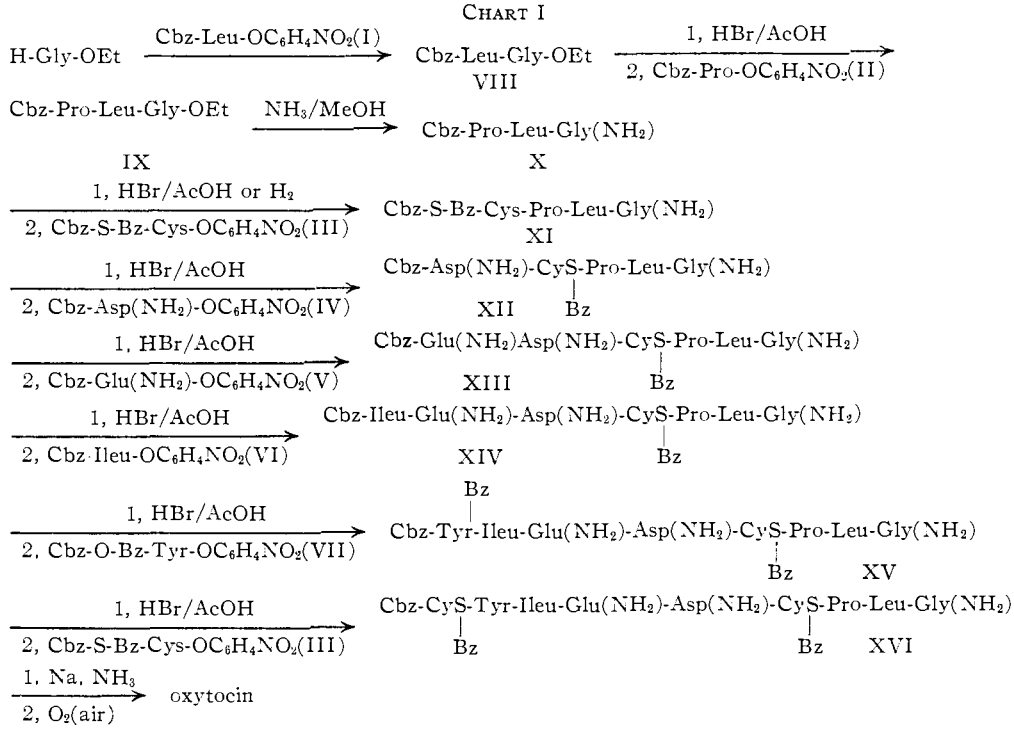
(18) M. Bodanszky, *Nature*, **175**, 685 (1955); M. Bodanszky, *Acta Chim. Hung.*, **10**, 335 (1957); M. Bodanszky, M. Szelke, E. Tomorkeny and E. Weisz, *Chemistry & Industry*, 1517 (1955); M. Bodanszky, M. Szelke, E. Tomorkeny and E. Weisz, *Acta Chim. Hung.*, **11**, 179 (1957).

CHART I



ration of such esters was introduced by Schwyzer and co-workers.[19] Recently, however, Elliot and Russel[20] proposed that dicyclohexylcarbodiimide should be used for the esterification of acylamino acids with *p*-nitrophenol. This suggestion was found to be very helpful and all the active esters used in this study were prepared by this method. However, instead of using the reaction mixture after the esterification all the active esters were isolated and used only after recrystallization.

In the case of carbobenzoxy-L-asparagine, as was expected, dehydration occurred due to the action of the carbodiimide.[17] Nevertheless, the reaction product could be freed of the contaminating dehydration product after a few recrystallizations. The thus purified *p*-nitrophenyl ester of carbobenzoxy-L-asparagine gave on reaction with methyl S-benzyl-L-cysteinate[17] only one product in good yield and high purity. Therefore this seems to be the most clearcut method for the preparation of asparaginyl peptides. In more general terms: considering the large number of steps necessary for the synthesis of a longer peptide chain, it is desirable to apply all possible purification on intermediates, which are not part of the peptide chain, and in this way limit the number of steps or operations which concern the already existing part of the peptide.

In order to avoid racemization, use was made of the experience that protecting groups, such as the carbobenzoxy group, protect the amino group to which they are attached not only against undesirable acylations but also against racemization during the peptide bond-forming reaction.[10] This last protection is not effective if the protecting group and the carboxyl group are not on the same amino acid, *i.e.*, in protected peptides.[21] Therefore in contrast with earlier syntheses of oxytocin,[3-7] in which peptides were linked together, in the present method amino acids (nitrophenyl esters) were joined one by one to the already prepared part of the chain. This stepwise procedure along with the use of nitrophenyl esters form the main characteristics of the present synthesis of oxytocin.[22,23] The synthesis itself is summarized in Chart I.

Experimental

p-Nitrophenyl Esters of Carbobenzoxy Amino Acids.—To a 0.2–0.5 molar solution of the carbobenzoxy amino acid in ethyl acetate, *p*-nitrophenol was added in about 20% excess of the calculated amount. The calculated amount of dicyclohexylcarbodiimide was added to the solution at 0°. After 0.5 hour the mixture was allowed to come to room temperature and was held there for 1 hour. The N,N′-dicyclohexylurea which separated was filtered off and washed with ethyl acetate. The combined filtrate and washings were evaporated to dryness and the crystalline residue was dissolved in hot ethanol and the solution was cooled. The nitrophenyl esters separated in high purity. Details of preparation and properties of the individual nitrophenyl esters are summarized in Table I.

In the preparation of the esters of carbobenzoxy-L-glutamine and carbobenzoxy-L-asparagine, dimethylformamide was used as the solvent instead of ethyl acetate. In these

(19) B. Iselin, W. Rittel, P. Sieber and R. Schwyzer, *Helv. Chim. Acta*, **46**, 373 (1957); *cf.* also J. A. Farrington, G. W. Kenner and J. M. Turner, *Chemistry & Industry*, 601 (1955).

(20) D. F. Elliot and D. W. Russel, *Biochem. J.*, **66**, 49P (1957).

(21) J. R. Vaughan, Jr., THIS JOURNAL, **74**, 6137 (1952).

(22) Although the tetrapeptide intermediate S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide was already prepared by a stepwise synthesis with satisfactory yield and optically pure, this part of the hormone was synthesized again by the present method. This was done to demonstrate the general usefulness of the nitrophenyl ester method. If such esters are readily available, the number of steps which are necessary for the main line of synthesis is substantially reduced.

(23) HBr in acetic acid was used rather than H_2 for the removal of the carbobenzoxy groups, on account of the S-benzyl groups. Addition of ether to the reaction mixture causes the precipitation of a product containing not one but several equivalents of HBr. Apparently the amide nitrogens in acetic acid in the presence of excess HBr are basic enough to form salts with HBr.

TABLE I

p-NITROPHENYL ESTERS OF CARBOBENZOXY AMINO ACIDS

p-Nitrophenyl ester of	Yield, %	M.p., °C. cor.	$[\alpha]^{20}D$ *c* 2, dimethylformamide	Analyses, % Calculated C	H	N	Found C	H	N
Cbz-L-leucine (I)[19]	86	95	−33.5°	62.2	5.74	7.25	62.0	5.80	7.19
Cbz-L-proline (II)	89	94–96	−68	61.6	4.90	7.565	61.6	4.98	7.43
Cbz-S-benzyl-L-cysteine (III)[18,19]	90	93–94	−43	61.8	4.75	6.01	61.9	4.91	5.91
Cbz-L-asparagine (IV)	43	165–166	−31.5	55.8	4.42	10.8	55.7	4.48	10.8
Cbz-L-glutamine (V)	59	155–156	−24	56.85	4.77	10.5	56.8	4.87	10.4
Cbz-L-isoleucine (VI)	85	60–62	−15.5	62.2	5.74	7.25	62.1	5.78	7.22
Cbz-O-benzyl-L-tyrosine (VII)	90	148–150	− 9	68.4	4.98	5.32	68.4	4.98	5.30

cases the product separated in crude but crystalline form on dilution with water. From mother liquors obtained on recrystallization of *p*-nitrophenyl carbobenzoxy-L-asparaginate a second product separated which showed higher levorotation and lower melting point than the main crop of crystals. Analysis indicated that it was the (impure) dehydration product. On the other hand, no dehydration was observed when the ester once formed was exposed to the action of dicyclohexylcarbodiimide. For the separation of *p*-nitrophenyl carbobenzoxy-L-asparaginate from the dehydration product, crystallization from dimethylformamide by addition of water was found to be effective.

Methyl Carbobenzoxy-L-asparaginyl-S-benzyl-L-cysteinate.—*p*-Nitrophenyl carbobenzoxy-L-asparaginate (0.78 g.) and methyl S-benzyl-L-cysteinate hydrochloride (0.66 g.) were dissolved in dimethylformamide (2 ml.). On addition of triethylamine (0.38 ml.) the HCl salt of the latter separated. Some evolution of heat was observed and the reaction mixture soon turned into a solid mass of crystals. After about 1 hour this mass was triturated with water (45 ml.), filtered off, washed with water and acetone; wt. 0.76 g. (80%), m.p. 199–201° (lit. 199–200°,[17] 196°,[4] 194–195°[5]), $[\alpha]^{21}D$ −31.5° (*c* 1, pyridine) (lit.[4] −31.5°, −31.9°, *c* 2.4, pyridine). In the determination of the specific rotation the lower concentration was used on account of the reduced solubility of the pure product.

Ethyl Carbobenzoxy-L-leucylglycinate (VIII).—Compound I (7.74 g.) was added to a solution prepared from ethyl glycinate hydrochloride (3.36 g.) and triethylamine (3.5 ml.) in chloroform (20 ml.). The clear yellow solution was allowed to stand at room temperature overnight. After removal of the solvent *in vacuo*, ethyl acetate and water were added to the residue. The organic layer was washed with water, *N* NH_4OH, water, *N* HCl and water again. On removal of the ethyl acetate a crystalline residue remained; wt. 6.65 g., m.p. 102–103°. Recrystallization from dilute ethanol gave 5.5 g. (73.5%), m.p. 104–105.5°, $[\alpha]^{22}D$ −27.1° (*c* 5, ethanol), and a second crop, 1.35 g. (19.3%), m.p. 100–104°, $[\alpha]^{22}D$ −26.8° (*c* 5, ethanol); lit.,[24] m.p. 105–106°, $[\alpha]^{24}D$ −25.6° (*c* 5, ethanol).

Ethyl Carbobenzoxy-L-prolyl-L-leucylglycinate (IX).—Compound VIII (7.0 g.) was dissolved in 2 *N* HBr in acetic acid (25 ml.). After 30 minutes the solution was warmed to 45° for a few minutes, cooled and dry ether (450 ml.) was added. The oil which separated was washed with ether by decantation, dissolved in chloroform (40 ml.), and triethyl amine (6 ml.) was added followed by II (7.4 g.). After standing overnight at room temperature the solution was diluted with more chloroform (150 ml.), extracted with *N* NH_4OH, water, *N* HCl and again with water. Evaporation to dryness left a crystalline residue (8.5 g.), m.p. 146–148°. Recrystallization from ethyl acetate gave 6.8 g. (76%) m.p. 151–152°, $[\alpha]^{23}D$ −82.6° (*c* 2.5, ethanol) and 0.97 g. (11%), m.p. 148–149°, $[\alpha]^{23}D$ −81° (*c* 2.5, ethanol); lit. m.p. 148–149°,[3] 150–151°,[25] $[\alpha]D$ −79.8° (*c* 2.5, ethanol).[3]

Carbobenzoxy-L-prolyl-L-leucylglycinamide (X) was prepared essentially according to Ressler and du Vigneaud.[3] IX (89.5 g.) was dissolved in methanol (1500 ml.) and NH_3 was introduced at 0° for 2 hours. After standing at room temperature for 5 hours the solvent was removed leaving a residue, m.p. 161–163°, which after washing with ethyl acetate (600 ml.) weighed 76.5 g. (91.5%), m.p. 162–164°; lit. m.p. 163–163.5°,[3] 162–163°.[25]

N-Carbobenzoxy-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (XI) was prepared as reported earlier[8]; the yield varied from 82–90%.

Carbobenzoxy-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucyl-glycinamide (XII). A.—S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide[3,25] (0.80 g.) was dissolved in ethyl acetate (20 ml.) and IV (0.80 g.) was added to the solution. The suspension was stirred at room temperature. After two days the precipitate was filtered, washed with ethyl acetate (15 ml.) and ethanol (10 ml.); wt. 1.16 g. (100%), m.p. 212–213° dec. at 214°, $[\alpha]^{22}D$ −59.4° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{35}H_{47}N_7O_8S$: C, 57.9; H, 6.53; N, 13.5. Found: C, 57.7; H, 6.62; N, 13.4.

B.—Compound XI (12.2 g.) was suspended in acetic acid (25 ml.) and treated with 4 *N* HBr in acetic acid (50 ml.). After one hour at room temperature dry ether (400 ml.) was added, the precipitate which formed was washed with ether and dissolved in methanol (150 ml.). Amberlite IRA-400 (in OH cycle) was added until the reaction for bromide ion became negative. The resin was filtered off and washed with methanol. The filtrate was evaporated to dryness leaving a residue (10.6 g.) which in turn was dissolved in ethyl acetate (200 ml.); IV (7.74 g.) was added to the solution and the suspension was stirred at room temperature for two days. The solid was washed on the filter with ethyl acetate (200 ml.) and ethanol (50 ml.); wt. 13.5 g. (93%), m.p. 213–214° dec., $[\alpha]^{21}D$ −60° (*c* 1, dimethylformamide).

Carbobenzoxy-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (XIII). A.—Compound XII (2.9 g.) was dissolved in acetic acid (30 ml.) and 4 *N* HBr in acetic acid (30 ml.) was added to the solution. After one hour at room temperature dry ether (450 ml.) was added. The precipitate was washed with ether and dried over $CaCl_2$ and NaOH; wt. 3.8 g. The calculated amount for one HBr per molecule would be only 2.7 g. This solid was dissolved in dimethylformamide (9 ml.), triethylamine (2.6 ml.) was added followed by V (1.7 g.). After 18 hours at room temperature the solid mass was mixed with ethyl acetate (75 ml.), washed on the filter with ethyl acetate (75 ml.), ethanol (100 ml.) and again with ethyl acetate (100 ml.); wt. after drying 3.15 g. (92.5%), m.p. 233–234° dec., $[\alpha]^{21}D$ −54° (*c* 1, dimethylformamide).

B.—Compound XII (5.8 g.) was treated with HBr in acetic acid as described under section A. The HBr salt was dissolved in methanol (200 ml.) and treated with Amberlite IRA-400 (in OH cycle). The residue which remained on removal of the methanol was dissolved in dimethylformamide (12 ml.) and V (3.5 g.) was added. After 15 minutes the separation of a crystalline solid from the clear yellow solution could be observed. Isolation of the product was carried out as described under A; wt. 6.3 g. (93%), m.p. 233–236° dec., $[\alpha]^{21}D$ −55° (*c* 1, dimethylformamide). This product was used in the next step.

For analysis 200 mg. was recrystallized twice from 80% ethanol giving 104 mg. of a product, m.p. 247–248° dec.; lit. m.p. 209°,[4] 209–210°.[5]

Anal. Calcd. for $C_{40}H_{55}O_{10}N_9S$: C, 56.25; H, 6.49; N, 14.8. Found: C, 55.86; H, 6.52; N, 14.6.

Carbobenzoxy-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (XIV). A.—Compound XIII (7.75 g.) was dissolved in acetic acid (55 ml.) and 4 *N* HBr in acetic acid (65 ml.) was added. After two hours at room temperature dry ether (400 ml.) was added and the precipitate was washed with ether. After short

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(25) M. Zaoral and J. Rudinger, *Chem. Listy*, **49**, 745 (1955); M. Zaoral and J. Rudinger, *Coll. Czech. Chem. Communs.*, **20**, 1183 (1955).

drying over $CaCl_2$ and NaOH the solid weighed 11.75 g. It was dissolved in dimethylformamide (20 ml.) and triethylamine (6.7 ml.) was added followed by VI (3.86 g.). Soon a solid started to separate. After 18 hours at room temperature the semi-solid mass was triturated with ethyl acetate (300 ml.), washed on the filter with ethanol (200 ml.) and with ethyl acetate (100 ml.); wt. 8.0 g. (92%), m.p. 227–229° dec.

B.—Compound XIII (7.75 g.) was treated with HBr in acetic acid as described above. The resulting hydrobromide was dissolved in methanol (200 ml.) and treated with Amberlite IRA-400 (in OH cycle). Removal of the solvent left a residue (8.3 g. probably not completely dry), which was dissolved in dimethylformamide (20 ml.) and VI (3.86 g.) was added. Isolation of the product was carried out as described under A; wt. 7.8 g. (90%), m.p. 233–235° dec., $[\alpha]^{20}D$ −50° (*c* 1, dimethylformamide). This product was used in the next step. A sample (0.20 g.) was recrystallized from 28 ml. of 80% hot ethanol; 0.14 g. was recovered, m.p. 241–243° dec.

Anal. Calcd. for $C_{46}H_{66}O_{12}N_{10}S$: C, 57.1; H, 6.88; N, 14.5. Found: C, 56.9; H, 6.96; N, 14.4.

N-Carbobenzoxy-O-benzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (XV). A.—Compound XIV (9.7 g.) dissolved in acetic acid (50 ml.) was treated with HBr in acetic acid (3.3 *N*, 50 ml.). After 1.5 hours at room temperature dry ether (400 ml.) was added. The solid which separated was filtered off and washed with ether. It was dissolved in dimethylformamide (85 ml.) and triethylamine (9 ml.) was added to the filtrate followed by VII (6.0 g.). After 20 hours at room temperature ethyl acetate (450 ml.) was added to the thick mass, the solids were washed on the filter with ethyl acetate (100 ml.), ethanol (400 ml.) and with ethyl acetate (150 ml.) again; wt. 11.3 g. (92.5%), m.p. 230–234° dec.

B.—Compound XIV (1.3 g.) was treated with HBr in acetic acid as described in section A. The HBr was removed from the resulting salt with the anion exchange resin as described with similar compounds. Only 0.92 g. (82%) of the base was recovered, probably on account of its low solubility in methanol. This amount of the free heptapeptide amide was dissolved in dimethylformamide (10 ml.) and VII (0.70 g.) was added. After isolation in the usual manner 1.35 g. of a white powder was obtained, m.p. 245–247° dec., $[\alpha]^{20}D$ −41° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{62}H_{81}O_{13}N_{11}S$: C, 61.0; H, 6.69; N, 12.6. Found: C, 60.8; H, 6.75; N, 12.5.

N-Carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (XVI).[26]—Compound XV (7.3 g.) was suspended in acetic acid (40 ml.) and HBr in acetic acid (3.3 *N*, 60 ml.) was added. After two hours at room temperature dry ether was added to the orange solution, the precipitated hydrobromide filtered and washed with ether. After short drying over $CaCl_2$ and NaOH the hydrobromide was dissolved in dimethylformamide (60 ml.) and triethylamine (5.6 ml.) was added followed by III (3.35 g.). After a day at room temperature the reaction mixture turned into a thick mass which after a further day was mixed with ethyl acetate (400 ml.). The precipitate was washed on the filter with more ethyl acetate (200 ml.), with ethanol (400 ml.) and again with ethyl acetate (50 ml.); wt. 7.3 g. (92%), m.p. 237–241°.

If the octapeptide with the free amino group was liberated from its hydrobromide, considerable loss was observed, probably due to the low solubility of the base in methanol and incomplete removal from the anion exchange resin. On the other hand, from the base obtained, on reaction with III, a quantitative yield of XVI was obtained, m.p. 245–248° dec., $[\alpha]^{20}D$ −50.5° (*c* 1, dimethylformamide) $[\alpha]^{20}D$ −64.5° (*c* 2.5, acetic acid); lit. m.p. 224–245°,[3] 241°,[4] 243–245°,[8] $[\alpha]^{20}D$ −51.5° (*c* 2.5, acetic acid),[4] $[\alpha]^{22}D$ −43° (*c* 2, dimethylformamide).[8]

Anal. Calcd. for $C_{65}H_{86}N_{12}O_{14}S_2$: C, 59.0; H, 6.55; N, 12.7. Found: C, 58.6; H, 6.52; N, 12.6.

Oxytocin (XVII).—Compound XVI was reduced with sodium in liquid ammonia as described earlier. The total avian depressor activity obtained from 50 mg. of XVI varied from 12,500 to 15,000 units. Calculated on the basis that the activity of pure oxytocin is 500 units/mg. the maximum obtainable activity would be 19,000 units.

Reduction on a larger scale (1.3 g. of XVI) led to less favorable results: a total of 168,000 units was obtained. Isolation of highly purified oxytocin was accomplished as described earlier.[8]

A series of assays[27] on the isolated material gave values of 500, 440, 500, 560, 500 U./mg.

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(26) Treatment with HBr in acetic acid removed the O-benzyl group from the octapeptide intermediate.

(27) Assayed by direct comparison with the standard solution.

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[CONTRIBUTION FROM THE RESEARCH DIVISION OF THE CLEVELAND CLINIC FOUNDATION AND THE FRANK E. BUNTS EDUCATIONAL INSTITUTE]

The Use of p-Nitrobenzyl Esters in Peptide Synthesis[1]

BY HANS SCHWARZ[2] AND KIKUO ARAKAWA[3]

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The *p*-nitrobenzyl esters of some amino acids and peptides were prepared in good yields and without racemization. They proved to be more stable to cleavage by dry hydrobromic acid than the corresponding unsubstituted benzyl esters. This fact was shown to facilitate the preferential removal of a protecting carbobenzoxy group, and to increase yield and purity of the resulting ester hydrobromide. The *p*-nitrobenzyl group was readily removed by catalytic hydrogenation.

The use of carbobenzoxy[4] groups combined with benzyl esters as protective groups in peptide syntheses has several advantages.[5] Catalytic reduction removes both protective groups in one operation, eliminating alkaline hydrolysis. Carbobenzoxy, on the other hand, is removed preferentially by cleavage with dry hydrobromic acid.[6] Benzyl esters are quite stable to this treatment, but small amounts of ester are always hydrolyzed even if the reaction time is kept to a minimum.[7] The result is a mixture of a benzyl ester hydrobromide and a small amount of the corresponding peptide. Their separation is often quite easy, but may amount to

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(2) Sandoz Pharmaceuticals, Hanover, N. J.

(3) Research Fellow of the Frank E. Bunts Educational Institute.

(4) Carbobenzoxy = benzyloxycarbonyl = cbzo.

(5) For references see M. Goodman and G. W. Kenner, *Advances in Protein Chem.*, **12**, 465 (1957).

(6) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).

(7) G. W. Anderson, J. Blodinger and A. D. Welcher, THIS JOURNAL, **74**, 5309 (1952).