completes another route for the total synthesis of these natural products.

Further details will be reported in future papers for these transformations.

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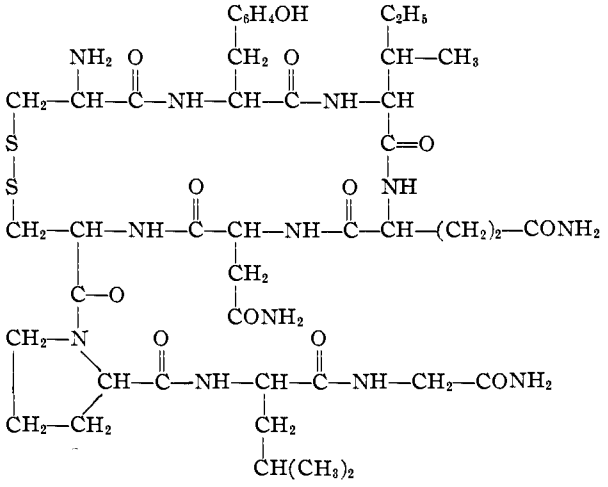
THE SYNTHESIS OF AN OCTAPEPTIDE AMIDE WITH THE HORMONAL ACTIVITY OF OXYTOCIN

Sir:

Highly purified preparations of oxytocin, the principal uterine-contracting and milk-ejecting hormone of the posterior pituitary, have been obtained in this laboratory, which upon hydrolysis gave 1 equivalent each of leucine, isoleucine, tyrosine, proline, glutamic acid, aspartic acid, glycine and cystine, and 3 equivalents of ammonia.[1,2,3,4] The active principle appeared to be a polypeptide of molecular weight approximately 1000.[3,5] Degradative studies indicated some type of cyclic disulfide.[6,7] On the basis of further degradative studies[5,8,9,10] along with the assumption that glutamine and asparagine residues were present rather than their isomers, the following structure was postulated[10] for oxytocin, the amino acids having the L configuration.



It was known from the work of Sealock and du Vigneaud[11] that oxytocin could be reduced and reoxidized without appreciable inactivation and that treatment of the reduced material with benzyl chloride resulted in loss of activity. If oxytocin could be regenerated from benzylated oxytocin and if the proposed structure be correct, a total synthesis of the hormone should follow from the preparation of the nonapeptide derivative, N-carbobenzoxy - S - benzyl - L - cysteinyl - L - tyrosyl - L-isoleucyl - L - glutaminyl - L - asparaginyl - S - benzyl-L-cysteinyl-L-prolyl-L-leucylglycine amide (I).

The preparation of S,S′-dibenzyloxytocin from the natural hormone and its possible reconversion to oxytocin were therefore explored. Oxytocin, with sodium in liquid ammonia, followed by benzyl chloride, has given the desired benzyl derivative from which the hormone can be regenerated by debenzylation by the sodium–liquid ammonia method[12] followed by oxidation with air.

Synthesis of I was accomplished by coupling N-carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosine (II) with the heptapeptide amide L-isoleucyl-L-glutaminyl - L - asparaginyl - S - benzyl - L - cysteinyl-L-prolyl-L-leucylglycine amide (V), prepared in turn from tosyl-L-isoleucyl-L-glutaminyl-L-asparagine (IV), and the tetrapeptide amide, S-benzyl-L-cysteinyl-L-propyl-L-leucylglycine amide (III).

Ethyl L-leucylglycinate was condensed with carbobenzoxy-L-proline by the isovaleryl mixed anhydride procedure[13] to give ethyl carbobenzoxy-L-prolyl-L-leucylglycinate, m.p. 148–149°, $[\alpha]^{22.5}D$ −79.8° (*c* 2.5, ethanol) (calcd. for $C_{23}H_{33}O_6N_3$: C, 61.7; H, 7.43; N, 9.39. Found: C, 61.8; H, 7.65; N, 9.24). The latter was reduced catalytically and then coupled with biscarbobenzoxy-L-cystinyl bischloride. The saponified product was reduced and benzylated in liquid ammonia to give S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycine, which was esterified to the corresponding benzyl ester hydrochloride, m.p. 193–194° dec. (calcd. for $C_{30}H_{41}O_5N_4SCl$: N, 9.26; S, 5.30. Found: N, 9.17; S, 5.36). Treatment of the benzyl ester with methanolic ammonia gave tetrapeptide amide III.

1-Tosylpyrrolid-5-one-2-carboxyl chloride, from tosyl-L-glutamic acid and phosphorus pentachloride, was coupled with L-asparagine and the resulting N-(1′-tosylpyrrolid-5′-one-2′-carbonyl)-L-asparagine, m.p. 150–151° (calcd. for $C_{16}H_{19}O_7N_3S$: C, 48.4; H, 4.82; N, 10.6. Found: C, 47.9; H, 5.04; N, 10.4), was treated with aqueous ammonia. After removal of the tosyl group from the tosyl-L-glutaminyl-L-asparagine, m.p. 197–198° (calcd. for $C_{16}H_{22}O_7N_4S$: C, 46.4; H, 5.35; N, 13.5; amide N, 6.7. Found: C, 46.3; H, 5.55; N, 13.2; amide N, 6.6), by sodium in liquid ammonia,[14] the dipeptide, m.p. 210–211° dec., $[\alpha]^{21}D$ +17.1° (*c* 1.5, water) (calcd. for $C_9H_{16}O_5N_4$: C, 41.5; H, 6.20; N, 21.5. Found: C, 41.2; H, 6.60; N, 21.3) was coupled with tosyl-L-isoleucyl chloride to give tosyl-L-isoleucyl-L-glutaminyl-L-asparagine (IV), m.p. 225–226°, $[\alpha]^{22}D$ −28.9° (*c* 1.76, 0.5 *N* $KHCO_3$) (calcd. for $C_{22}H_{33}$-

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O_8N_6S: C, 50.1; H, 6.30; N, 13.3, equiv. wt. 527. Found: C, 49.4; H, 6.55; N, 13.2: equiv. wt., 526). This new approach to glutaminyl peptides has also afforded an excellent synthesis of glutamine.

III was condensed with IV by the pyrophosphite method[15] to give tosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl - S - benzyl - L - cysteinyl - L - prolyl-L-leucylglycine amide, m.p. 235–236° dec. (calcd. for $C_{45}H_{66}O_{11}N_{10}S_2$: C, 54.8; H, 6.74; N, 14.2. Found: C, 54.4; H, 6.85; N, 13.8). The latter was treated with sodium in liquid ammonia followed by benzyl chloride to give V which was condensed similarly with protected dipeptide II[16,17] to give the desired nonapeptide derivative (I). Three hundred sixty milligrams of the latter in several batches was treated with sodium in liquid ammonia followed by aeration in dilute aqueous solution at *p*H 6.5. The combined product was assayed by the chicken depressor method,[18,19] and possessed a total of approximately 29,000 units of activity. The material was subjected to countercurrent distribution in *s*-butyl alcohol and acetic acid. The activity was found to be concentrated in a single peak ($K = 0.34$). The material in the peak tube was not distinguishable in potency from one of our best preparations of natural oxytocin, when these were assayed repeatedly against one another. The batched material as isolated had a somewhat lower activity.

The synthetic material possessed the expected oxytocic activity on the isolated rat uterus. Furthermore, the synthetic product was fully effective in stimulating labor in the human.[20] The synthetic material likewise possessed milk-ejecting activity in the human; approximately 1 γ of either the synthetic or natural material given intravenously to patients induced milk ejection in 20–30 seconds.[20]

The synthetic material possessed the specific rotation $[\alpha]^{21.5}D$ $-26.1 \pm 1.0°$ (*c* 0.53, water) compared to $[\alpha]^{22}D$ $-26.2°$ (*c* 0.53, water) for natural oxytocin. It formed an active flavianate derivative[4] identical in melting point and crystalline form (fine, silky needles) with that obtained from natural oxytocin. The amino acid composition of the synthetic material after hydrolysis as determined with the starch column,[21] expressed as molar ratios, was: leucine 1.00, isoleucine 1.00, tyrosine 0.83, proline 0.92, glutamic acid 0.91, aspartic acid 0.93, glycine 0.98, cystine 0.87 and ammonia 3.04. The distribution coefficients in both *s*-butyl alcohol–acetic acid and *s*-butyl alcohol–ammonia, and the electrophoretic mobilities on paper (*p*H 4.6 and *p*H 10.7) were the same for both the synthetic material and natural oxytocin. No differences were detected in the infrared patterns.

If the synthetic product truly represents oxytocin, which it does in so far as we have been able to ascertain, this would constitute the first synthesis of a polypeptide hormone. What effect slight changes in the structure of a compound of such complexity might have on chemical, physical and biological properties must be investigated.

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(23) Fulbright Scholar on Smith Mundt grant-in-aid, on leave from Wool Textile Research Laboratory, C.S.I.R.O., Australia.

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ENZYMATIC CLEAVAGE OF GLYCINAMIDE FROM VASOPRESSIN AND A PROPOSED STRUCTURE FOR THIS PRESSOR-ANTIDIURETIC HORMONE OF THE POSTERIOR PITUITARY

Sir:

The partial hydrolysis of performic acid-oxidized arginine-vasopressin and the determination of the structure of the resultant peptides has led to the postulation by Popenoe and du Vigneaud[1] of the sequence Cysteic.Tyr.Phe.Glu.Asp.Cysteic.[Pro, Arg, Gly],[2] for the oxidized hormone and the probable sequence CyS.Tyr.Phe.Glu.Asp.CyS.[Pro,Arg,Gly] for vasopressin itself. Additional data were obtained by application of the Edman degradation to performic acid-oxidized vasopressin and by other degradative reactions.[3]

We have now obtained evidence, through enzymatic cleavage of vasopressin, that glycinamide is terminal and arginine is in the penultimate position, making possible the complete assignment of the sequence of amino acids in vasopressin. Assuming that the glutamic and aspartic acids occur as glutamine and asparagine residues (accounting for the other two of the three moles of ammonia in hydrolysates of vasopressin) we would therefore propose the following structure for arginine-vasopressin.

The enzymatic cleavage was obtained by incubation of arginine-vasopressin with trypsin for 6 hours at 38° and *p*H 7. The resultant fractions were separated and identified by two-dimensional paper chromatography. Two spots were obtained, one of which was identical with an authentic sample of glycinamide in its behavior on paper chroma-

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(2) The convention is followed that when peptides of known structure are referred to, the residues are joined by a period and that in peptides of unknown sequence, the residues appear within brackets and are separated by commas. The amino acid appearing at the left in a known sequence is that bearing a free amino group.

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