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PN 1183

The synthesis of Ser⁴–Ile⁸–oxytocin (Isotocin)

The oxytocic principle from the pituitaries of the teleost fishes *Pollachius virens L.*, *Merluccius merluccius L.* and *Gadus luscus L.* was isolated by ACHER and coworkers[1]. They proposed for the new hormone the nonapeptide structure having the following amino acid sequence

Cys–Tyr–Ile–Ser–Asp(NH_2)–Cys–Pro–Ile–Gly–NH_2 (= Ser⁴–Ile⁸–oxytocin = Isotocin).

We have synthesized this nonapeptide in the manner outlined in Fig. 1*.

N-Carbobenzoxy-L-isoleucyl-L-serine methyl ester (I) was prepared from *N*-carbobenzoxy-L-isoleucine[2] and methyl L-serinate[3] by the mixed anhydride procedure (yield 62%; m.p. 180.5–181.5° (ethyl acetate–petroleum ether); $[\alpha]_D^{24} = +3.9°$ (in dimethylformamide). Calcd. for $C_{18}H_{26}N_2O_6$: C, 59.00; H, 7.16; N, 7.64%. Found: C, 59.15; H, 7.05; N, 7.75%). Catalytic hydrogenation of the protected dipeptide I in methanol–conc. HCl gave the hydrochloride of L-isoleucyl-L-serine methyl ester (II) (yield 88%; m.p. 203–204° (decomp.) (methanol–acetone–ether); $[\alpha]_D^{24} = +12.6°$ (in methanol). Calcd. for $C_{10}H_{20}N_2O_4 \cdot HCl$: C, 44.70; H, 7.88; N, 10.42; Cl, 13.19%. Found: C, 44.61; H, 7.61; N, 10.26; Cl, 13.28%). The coupling of *S*-benzyl-*N*-tosyl-L-cysteinyl-L-tyrosine[4] with II according to the *N*,*N'*-dicyclohexyl-carbodiimide method in dimethylformamide–acetonitrile gave *S*-benzyl-*N*-tosyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-serine methyl ester (III) (yield 45%; m.p. 219–221° (methanol); $[\alpha]_D^{23} = -16.2°$ (in pyridine). Calcd. for $C_{36}H_{46}N_4O_9S_2$: C, 58.20; H, 6.25; N, 7.53; S, 8.64%. Found: C, 58.28; H, 6.27; N, 7.71; S, 8.64%), which was converted to the corresponding hydrazide IV (yield 61%; m.p. 226–229° (decomp.) (dimethylformamide–acetonitrile); $[\alpha]_D^{24} = -69.2°$ (in formic acid). Calcd. for $C_{35}H_{46}N_6O_8S_2$: C, 56.58; H, 6.25; N, 11.32; S, 8.64%. Found: C, 56.50; H, 6.26; N, 11.40; S, 8.57%). Hydrazinolysis of *N*-carbobenzoxy-L-asparaginyl-*S*-benzyl-L-cysteine methyl ester[5] afforded *N*-carbobenzoxy-L-asparaginyl-*S*-benzyl-L-cysteine hydrazide (V) (yield 70%; m.p. 214.5–215.5° (decomp.) (dimethylformamide–acetonitrile); $[\alpha]_D^{25} = -29.7°$ (in dimethylformamide). Calcd. for $C_{22}H_{27}N_5O_5S$: C, 55.79; H, 5.75; N, 14.79; S, 6.77%. Found: C, 55.79; H, 5.61; N, 14.89; S, 6.83%). Reaction of the hydrazide V with $NaNO_2$ in dimethylformamide–1 *N* HCl gave the azide, which was condensed with L-prolyl-L-isoleucylglycinamide[2] in dimethylformamide to obtain *N*-carbobenzoxy-L-asparaginyl-L-cysteinyl-L-prolyl-L-isoleucylglycin-

* All of the intermediates with the exception of the protected nonapeptide VIII, which contained a minor impurity, were found to be homogeneous by thin-layer chromatography using several solvent systems.

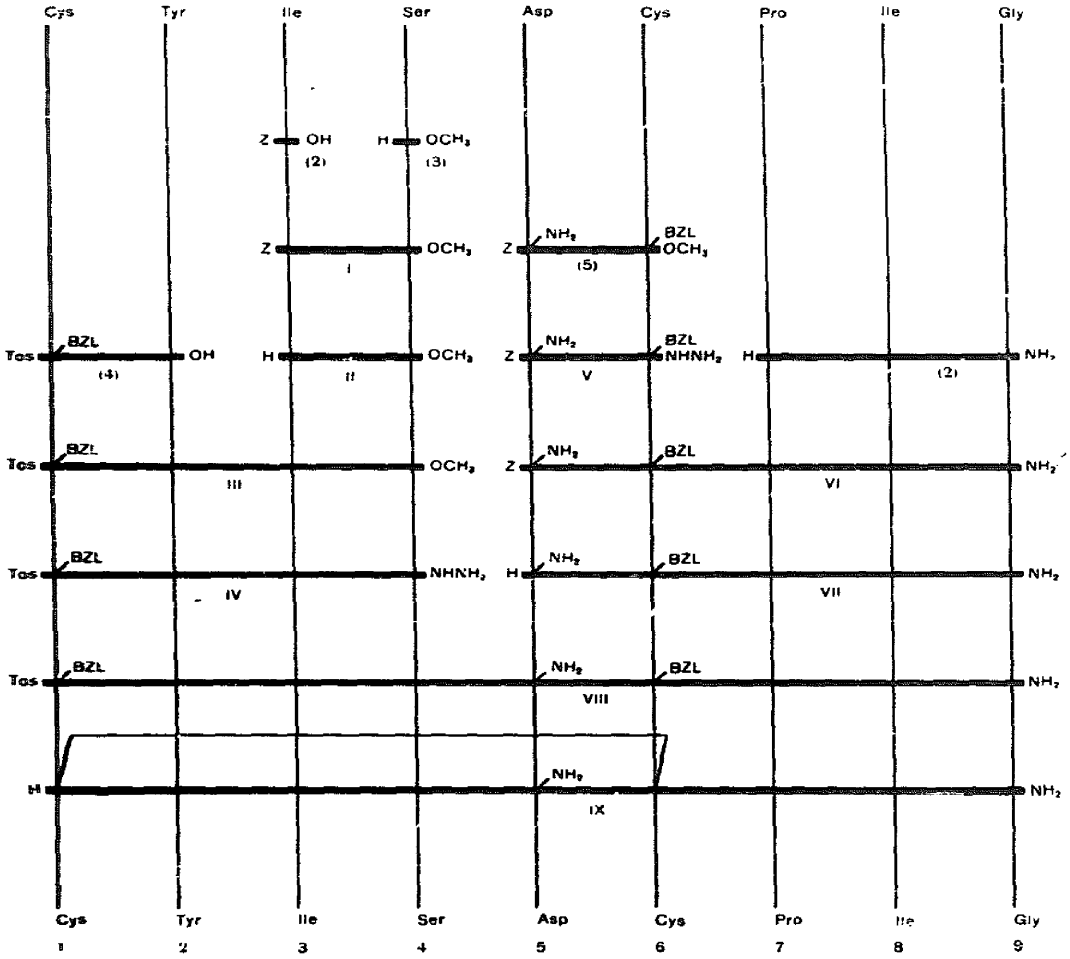

Fig. 1. Scheme for the synthesis of Ser^4-Ile^8-oxytocin. Z-, carbobenzoxy-; Tos-, tosyl-; BZL-, benzyl-.

amide (VI) (yield 45 %; m.p. 233–235° (decomp.) (dimethylformamide–acetonitrile); $[\alpha]_D^{25} = -79.3°$ (in glacial acetic acid). Calcd. for $C_{35}H_{47}N_7O_8S$: C, 57.91; H, 6.53; N, 13.51; S, 4.42 %. Found: C, 57.85; H, 6.43; N, 13.76; S, 4.48 %).

After removal of the carbobenzoxy-group of VI with HBr in glacial acetic acid the resulting hydrobromide was converted to the free pentapeptide amide VII on a Dowex-21K column and the latter coupled with the protected tetrapeptide azide, obtained from *S*-benzyl-*N*-tosyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-serine hydrazide (IV), to yield *S*-benzyl-*N*-tosyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-seryl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-isoleucylglycinamide (VIII) (yield 32 %; m.p. 225.5–228.5° (decomp.) (dimethylformamide–acetonitrile and dimethylformamide–methanol); $[\alpha]_D^{24} = -22.7°$ (in dimethylformamide). Calcd. for $C_{62}H_{83}N_{11}O_{14}S_3$: C, 57.17; H, 6.42; N, 11.83; S, 7.38 %. Found: C, 57.20; H, 6.55; N, 11.65; S, 7.39 %). The protected nonapeptide amide VIII was then treated with sodium in liquid ammonia and the resulting Ser^4-Ile^8-oxytoceine oxidized by aeration in a

dilute aqueous solution at pH 6.6–6.8. The crude nonapeptide amide IX was purified by counter-current distribution in the system 2-butanol–0.017 *N* acetic acid[6]. Redistribution of Ser[4]–Ile[8]–oxytocin (IX) in the same solvent system gave a material having at 25° a partition coefficient of 0.53. The experimental curve, obtained from the Folin color values[7], coincided with the theoretical curve. The purified product showed a correct quantitative amino acid composition and possessed an oxytocic activity of approx. 130 I.U./mg pure peptide, when assayed on the isolated rat uterus[8].

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Note added in proof: Since this paper was submitted for publication, GUTTMANN *et al.*[9] described in a brief report a synthesis and some pharmacological effects of Ser[4]–Ile[8]–oxytocin. According to the authors the peptide possesses an oxytocic activity on the isolated rat uterus[8] of 150 ± 12 I.U./mg. (Received November 29th, 1962)

PN 1189

On the interaction of insulin with raney nickel

In a former survey[1] we studied the interaction of methionine with Raney nickel, establishing a quantitative removal of sulphur and the formation of methane and α-aminobutyric acid. On this basis a method was developed for the determination of methionine by determination of the quantity of methane by an infrared spectrometric procedure.

In the present survey, the behaviour to Raney nickel of products with a peptide structure and a higher molecular weight has been studied. We took note of the observations made by COOLEY AND WOOD[2], namely that the treatment of egg albumin with Raney nickel results in an almost complete removal of sulphur without a noticeable fragmentation of the protein molecule. In order to follow with greater ease and assurance the interaction of proteins with Raney nickel we selected insulin since its chemical structure is known. We were further inclined to use this protein because of various data in the literature on the reactivity of its disulphide bonds under differing conditions. Thus CECIL AND LOENING[3] have studied the interaction of the disulphide groups of insulin with sodium sulphite, establishing that the intra-chain disulphide