SYNTHESIS, TRITIATION AND BIOLOGICAL STUDIES WITH [2-O-METHYL-TYROSINE, 7-(3,4-DEHYDROPROLINE]DEAMINO-1-CARBA-OXYTOCIN

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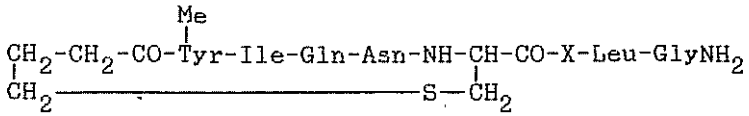
Introduction

Recognition of biological properties of Carbetocin [2-O-methyl-tyrosine]deamino-1-carba-oxytocin (I) (1-3) resulted in the introduction of this compound into veterinary practice as a drug with selective uterotonic and milk-ejecting action (Depotocin-Spofa and Decomoton-Nordvacc) In order to elucidate further properties, metabolism and distribution of Carbetocin we decided to prepare its radioactively labelled form.

Van Nispen at al. (4) described unsuccessful attempts of tritiation of dehydroproline containing oxytocin analogues. We tried to apply this principle of labelling in the case of mono-carba analogue in the hope that the sulfide containing compound may behave differently then disulfide-containing one.



I, X=Pro; II, X=3,4-dehydroproline; III, X=3,4-($^3H_2$)proline

Results

Preparation of Compound II. The synthesis of dehydroproline containing Carbetocin was carried out on a p-methylbenzhydrylamine resin (0.4 g, 0.8 mmol NH_2/g). After the coupling of the carboxyterminal amino acid (Boc-Gly), Boc-Leu, Boc-$\Delta^{3,4}$Pro, Fmoc-Cys($C_3H_6COOBu^t$), Fmoc-Asn, Fmoc-Ile and Boc-Tyr have been coupled successively employing a standard protocol. Boc-protecting group has been removed with trifluoroacetic acid, the resin neutralized with a triethylamine solution and the peptide cyclized by the action of 10 equivalents of DCCI and HOBt in 2,2,2-trifluoroethanol. The peptide was cleaved with liquid hydrogen fluoride (20 ml), anisole (1 ml) and ethandithiole (0.5 ml) mixture (1 h, 0°C). Product was purified by gel-filtration (Bio-gel P4) and RP-HPLC.

FAB MS: Part of the purified product (0.2 mg) was hydrogenated on freshly prepared palladium in dimethylacetamide and the product undistinguishable (HPLC, TLC, uterotonic test) from the analogue I was obtained. Attempts for hydrogenation on Pd/C (5%, 10%) in various solvents were unsuccessful.

Biological properties of the compound II have been compared with those of Carbetocin (I). All the activities tested were significantly enhanced (Table 1). This is in accordance with previously found importance of lipophilicity enhancement in this position for increased activity (for review see 5).

Table 1. Biological Properties of Carbetocin and its Derivative with 3,4-Dehydroproline in Position 7.

Compound	Activity (IU/mg) Rat uterus	Rat mammary gland
I	17.1[a]	45.0[b] 35.0[c]
II	36.2	169.3 91.0

a-in vitro; b,c-in vivo

Preparation of compound III. PdO (19.0 mg) was reduced with H_2 in methanol which was subsequently evaporated. Compound II (0.5 mg) and dimethylacetamide (1 ml) were added to the catalyst. The mixture was reduced with gaseous tritium (60%, 2.5 h, 84 kPa). After lyophilization the resulted product was further fractionated by means of HPLC (Separon SX C18 column 250x8mm, 53% aqueous methanol). The yield reached 140 MBq (4 mCi). Biological tests on isolated rat uterus and on rat uterus in vivo proved, that by means of the mentioned procedure we obtained approximately 150 ug (0.15 umol) of biologically active tritiated [2-O-methyltyrosine]deamino-1-carba-oxytocin, the specific activity of which was 27 Ci/mmol.
The obtained compound was used in the studies on the penetration of Carbetocin between lactating and suckling rats by milk and in the studies of the metabolic fate of Carbetocin.

References

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