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The deamino carba analogs of [Phe^2, Orn^8]vasotocin

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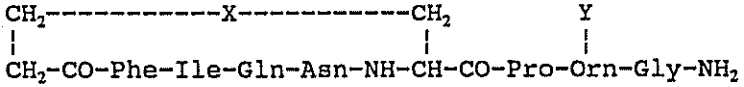
Synthesis of analogs with a selective and at the same time sufficiently high effect is important not only in elucidation of the mechanism of action of vasopressin but also in clinical practice [1]. A study of structure-activity relationship showed that the pressor effect is enhanced by introduction of (i) phenylalanine into position 2 (in combination with lysine or its homologue in position 8 only), (ii) isoleucine into position 3 (vasotocin analogs) and (iii) ornithine into position 8. The [Phe^2, Orn^8]oxytocin [2] is one of the most specific pressor analogs described up to now, even if its pressor activity is lower than that of the mother compound. This work aimed at finding whether the high selectivity will be preserved if the disulfide bridge will be substituted for the carba-1 or carba-6 bridge and the amino group in position 1 will be removed. It is known that removal of the amino group in position 1 affords analogs with considerable pressor activity; however, also other activities are enhanced. Similarly carba-1 analogs exhibit high pressor activity but relatively low selectivity.

As a consequence, the following analogs of deamino carba-1 and carba-6 oxytocin with phenylalanine in position 2 and ornithine in position 8 were prepared: [Phe^2, Orn^8]deamino-carba-1-oxytocin (**I**) and [Phe^2, Orn^8]deamino-carba-6-oxytocin (**II**). Both analogs were synthesized in Prague and Malmö, using slightly different methods, and tested in both laboratories (Scheme 1).

In the first case, the synthesis was performed by solid-phase methodology on benzhydrylamine resin with linker and Fmoc-technique. The 4-(α-Fmoc-amino-2', 4'-dimethoxybenzyl)phenoxyacetic acid linkage [3] was connected via glycine on benzhydrylamine resin. As key intermediary products pentafluorophenyl esters of N$^{\alpha}$-Fmoc-S-(3-*tert*-butyloxycarbonylpropyl)cysteine and N$^{\alpha}$-Fmoc-S-(2-*tert*-butyloxycarbonylethyl)homocysteine [4] were used. For the side-chain protection of ornithine a Z-group was used. Protected amino acids were coupled by DIC and



I	X = CH_2-S	Y = H	II	X = S-CH_2	Y = H
Ia	X = CH_2-S	Y = Z	IIa	X = S-CH_2	Y = Z
Ib	X = CH_2-SO	Y = Z	IIb	X = SO-CH_2	Y = Z

Scheme 1.

Table 1 *Biological activities (rat) of new vasotocin analogs (IU/mg)*

Compounds	Uterotonic		Pressor
	In vitro	In situ	
I	~6.0 / pA_2 = 7.7	86.1	217
II	~1.0 / pA_2 = 7.6	81.3	225
IIa	~31.7[a] / pA_2 = 6.5	85.5	1.7
IIb	~15.0[a] / pA_2 = 6.8	81.4	1.8

[a] Maximal contractions do not reach the maximal value evoked by oxytocin.

HOBt or the pentafluorophenyl active ester method in NMP. Bu^t was cleaved simultaneously with the cleavage of the peptide from the resin by TFA. Under these conditions partial splitting of the Z-protecting group from the side chain of ornithine occurred.

The mixture was therefore purified by continuous free-flow electrophoresis before cyclization. The cyclization was performed in DMF by diphenylphosphoryl-azide and dipotassium hydrogen phosphate [5] and cyclic protected analogs were isolated and purified by HPLC. The side chain protecting group was removed by liquid HF with anisole and analogs were again purified by HPLC. Besides, analogs with the δ-amino group of Orn protected by the Z-group and its corresponding sulfoxides were isolated and in the case of the carba-6 modification tested too. In the second case solid-phase methodology on PAM resin was used. Protected amino acids were coupled by HBTU and HOBt in NMP [6]. The cyclization was performed on the resin with TBTU, HOBt and DIEA in NMP [7]. Removal of peptide from resin was performed by ammonium in methanol.

Biological activities of the analogs are given in Table 1. Both deprotected analogs were found to be high uterotonic in vivo, pressor and antidiuretic agonists. Surprisingly, in uterotonic in vitro test they are partial agonists. The analog with protected ornithine and its corresponding sulfoxide have preserved high uterotonic in vivo activity as opposed to the pressor activity that was strongly decreased. Antidiuretic activity of analogs **I** and **II** measured in the Burn test (conscious rats) is in the order of that of LVP, that of carba-6 analog being slightly higher. Analysing the results of in vivo tests, we will find that the new analogs show no selectivity. The high antidiuretic activity in conscious rats after s.c. administration seems to be due to deamination and carba modification because both of these modifications enhance prolongation of antidiuresis which reflects itself in the Burn test.

References

1. Jošt, K., Lebl, M. and Brtník, F. (Eds.) CRC Handbook of Neurohypophyseal Hormone Analogs, CRC Press, Boca Raton, FL, 1987.
2. Huguenin, R.L., Helv. Chim. Acta, 47(1964)1934.
3. Atherton, E. and Sheppard, R.C., Solid Phase Peptide Synthesis, A Practical Approach, IRL Press, Oxford, 1989, pp. 66, 73.
4. Procházka, Z., Slaninová, J., Barth, T., Stierandová, A., Trojnar, J., Melin, P. and Lebl, M., Coll. Czech. Chem. Commun., 57(1992)1335.
5. Brady, S.F., Varga, S.L., Freidinger, R.M., Schwenk, D.A., Mendlowski, M., Holly, F.W. and Veber, D., J. Org. Chem., 44(1979)3101.
6. Dourtoglou, V. and Gross, B., Synthesis, (1984)572.
7. Knorr, R., Trzeciak, A., Bannwarth, W. and Gillesen, D., Tetrahedron Lett., 30(1989)1927.