Synthesis of an Analogue of Desamino-lysine-vasopressin Containing No Disulfide Bond

Shumpei SAKAKIBARA and Satoe HASE

Peptide Center, Institute for Protein Research, Osaka University, Kita-ku, Osaka

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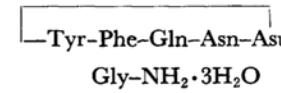
Jošt and Rudinger synthesized two analogues of desamino-oxytocin which have no disulfide bond, and they demonstrated for the first time that the disulfide bond in oxytocin is important as a structural element but is not necessary for biological activity.[1] The present communication will report the synthesis of an analogue of desamino-lysine-vasopressin which has an ethylene linkage in place of the disulfide bond.

The following new compounds were synthesized as starting materials by conventional methods:*1 Z-Lys(Pht)-ONp, mp 99—100°C, $[\alpha]_D^{22}$ −16.0° (*c* 4, DMF); bis-[Z-Asu(OBu^t)-OH] piperazine salt which was derived from Z-Asu-OH,[2] mp 130—131°C, $[\alpha]_D^{28}$ −2.7° (*c* 2, AcOH); Z-Asn-OSu, mp 129—130°C, $[\alpha]_D^{24}$ −27.5° (*c* 2, DMF); Aoc-Phe-OSu, 130—131°C, $[\alpha]_D^{18}$ −52.0° (*c* 2, DMF); Aoc-Tyr(Bu^t)-OSu, mp 112—113°C, $[\alpha]_D^{23}$ −43.4° (*c* 2, DMF).

The synthesis of the protected peptide was started from glycine amide by the step-by-step elongation method using the respective acylamino acid active esters. Carbobenzoxy (Z) groups were removed by catalytic hydrogenation. *t*-Amyloxycarbonyl (Aoc) groups were removed by treatment with trifluoroacetic acid. Thus, the following intermediates were synthesized: Z-Lys(Pht)-Gly-NH_2, mp 215—217°C, $[\alpha]_D^{22}$ −2.2° (*c* 2.1, DMF); Z-Pro-Lys(Pht)-Gly-NH_2, mp 173—175°C, $[\alpha]_D^{22}$ −36.4° (*c* 2.1, DMF); Z-Asu(OBu^t)-Pro-Lys(Pht)-Gly-NH_2, mp 112—115°C, $[\alpha]_D^{18}$ −37.5° (*c* 2, DMF); Z-Asn-Asu-(OBu^t)-Pro-Lys(Pht)-Gly-NH_2, mp 188—190°C, $[\alpha]_D^{19}$ −38.0° (*c* 2, DMF); Z-Gln-Asn-Asu-(OBu^t)-Pro-Lys(Pht)-Gly-NH_2, mp 189—191°C, $[\alpha]_D^{19}$ −36.9° (*c* 1.6, DMF); Aoc-Phe-Gln-Asn-Asu(OBu^t)-Pro-Lys(Pht)-Gly-NH_2, mp 188—190°C (decomp.), $[\alpha]_D^{22}$ −35.4° (*c* 1.5, DMF); Aoc-Tyr(Bu^t)-Phe-Gln-Asn-Asu-Pro-Lys(Pht)-Gly-NH_2 (I), mp 224—226°C (decomp.), $[\alpha]_D^{25}$ −41.9° (*c* 1, AcOH). Found: C, 59.08; H, 6.75; N, 12.76%. Calcd for $C_{66}H_{90}O_{17}N_{12}\cdot H_2O$: C, 59.09; H, 6.91; N, 12.53%. The product, I, was converted to the *p*-nitrophenyl ester at the ω-position of the Asu-residue with *p*-nitrophenyl trifluoroacetate;[3] mp 217—220°C (decomp.), $[\alpha]_D^{25}$ −29.2° (*c* 1, DMF). The Aoc and *t*-butyl ether groups were removed with trifluoroacetic acid, and the product was treated in pyridine at 50°C (1 mmol/*l*). Then, the phthalyl group was removed with hydrazine acetate in DMF. The final product was purified by column chromatography on CM-Sephadex C-25, and lyophilized; yield 22% (calcd from I). $[\alpha]_D^{27}$ −80° (*c* 0.44, 1 N AcOH). Found: C, 54.30; H, 6.85; N, 15.89%. Calcd for $C_{48}H_{68}O_{12}N_{12}\cdot 3H_2O$: C, 54.43; H, 7. 04; N, 15.87%. Amino acid analysis: $Tyr_{0.98}$, $Phe_{1.05}$, $Glu_{0.96}$, $Asp_{1.00}$, $Asu_{1.00}$, $Pro_{1.06}$, $Lys_{1.03}$, $Gly_{0.97}$. These analytical data support the following structure for this material:



(II)

Paper chromatography showed that this material was homogeneous: R_f 0.33 (*n*-BuOH : AcOH : H_2O=4 : 1 : 1), R_f 0.57 (Pyridine : AcOH : H_2O= 50 : 35 : 15).

The material II showed distinct pressor and antidiuretic activities, as is shown in Table 1.

TABLE I. BIOLOGICAL ACTIVITY OF COMPOUND II

Compound	Pressor (Rat)	Antidiuretic (Rat)
II	10.4 U/mg	7.8 U/mg
Desamino-Lys-vasopressin[a]	126	301

a) R. D. Kimbrough, Jr., W. D. Cash, L. A. Branda, W. Y. Chan and V. du Vigneaud, *J. Biol. Chem.*, **238**, 1411 (1963).

Thus, it was shown that the disulfide bond in vasopressin was replaceable by a stable ethylene bridge without any essential loss of biological activity.

1) K. Jošt and J. Rudinger, *Coll. Czech. Chem. Commun.*, **32** 1229 (1967).

*1 The tentatively proposed rules by the IUPAC-IBC were followed in the use of abbreviations: *J. Biol. Chem.*, **241**, 2491 (1966). DMF=dimethylformamide. Asu=α-aminosuberic acid. -OSu=*N*-hydroxysuccinimide ester. The amino acids used were in the L-form.

2) S. Hase, R. Kiyoi and S. Sakakibara, This Bulletin, **41**, 1266 (1968).

3) S. Sakakibara and N. Inukai, *ibid.*, **38**, 1979 (1965).