# [1-β-MERCAPTOPROPIONIC ACID, 4-ASPARAGINE,8-D-ARGININE]-VASOPRESSIN, AN ANALOGUE WITH A HIGH AND VERY PURE ANTIDIURETIC ACTIVITY*

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By condensation of β-benzylthiopropionyl-tyrosyl-phenylalanine with asparaginyl-asparaginyl--S-benzylcysteine methyl ester, conversion of the resulting pentapeptide ester to the hydrazide and its condensation with prolyl-$N^G$-tosyl-D-arginyl-glycine amide according to the azide method, β-benzylthiopropionyl-tyrosyl-phenylalanyl-asparaginyl-asparaginyl-S-benzylcysteinyl-prolyl-$N^G$--tosyl-D-arginyl-glycine amide was prepared. Removal of protecting groups and purification afforded [Mpr[1], $Asn^4$, D-$Arg^8$]VP.** The AD value of the analogue corresponds to 10500 to 11000 IU/mg, while the UT value is negligible.

One of the first analogues prepared in this Laboratory in connection with investigations on the relationship between chemical structure and biological effects of vasopressins, was [$Asn^4$]LVP (ref.[1]) which exhibited the AD values of about 2000 IU/mg (at the dose level of $10^{-9}$ mg per the animal assayed). [$Asn^4$]LVP was also prepared by Boissonnas and coworkers[2] who claimed the AD value of about 25 IU/mg. The discrepancy proved to be mainly due to a lower steepness of the log dose *vs* activity dependence of the analogue when compared with the parent substance[1,3,4]. [$Asn^4$]. . LVP is in low overthreshold doses more active than LVP but is less active in higher doses. The low steepness of the AD regression line depreciates the attractive properties of [$Asn^4$]LVP displayed at low overthreshold doses. In the case of the clinically interesting [1-β-mercaptopropionic acid, 8-D-arginine]vasopressin[5] (DDAVP), the situation is reversed. In higher doses, the AD value of DDAVP is extraordinarily high and very specific, while in the region of low overthreshold doses, the activity is relatively low. These facts raised the question of the possibility to prepare a vasopressin analogue with a steeper (when compared with [$Asn^4$]LVP) and perhaps

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** Aside from symbols and abbreviations usual in the chemistry of peptides, the following designations were applied: Mpr, β-mercaptopropionic acid; VP, vasopressin; LVP, lysine-vasopressin; AD, antidiuretic activity; BP, pressor activity; UT, uterotonic activity; G, galactogogous activity. Unless state otherwise, the optically active amino acids in this paper are of the L-series.

even linear (when compared with DDAVP) regression line for AD by introduction of the structural modifications occurring in the aforementioned analogues into a single molecule. In order to answer this question, the synthesis of the relevant compound, namely, [Mpr[1], Asn[4], D-Arg]VP ([Asn[4]]DDAVP) was undertaken.

In the synthesis of [Asn[4]]DDAVP, β-benzylthiopropionyl-tyrosyl-phenylalanyl--asparaginyl-asparaginyl-S-benzylcysteine azide was condensed with prolyl-$N^G$-tosyl--D-arginyl-glycine amide. The hydrazide of the peptide was obtained analogously to the corresponding amino compound[5] (for some methodic modifications see Experimental). β-Benzylthiopropionyl-tyrosyl-phenylalanyl-asparaginyl-asparaginyl--S-benzylcysteinyl-prolyl-$N^G$-tosyl-D-arginyl-glycine amide was converted to [Asn[4]] . . DDAVP by means of the usual procedure[5]. The analogue was assayed on vasopressin and oxytocin effects by usual methods[6–10]. The antidiuretic activity of [Asn[4]]DDAVP corresponded to 10500–11000 IU/mg, the uterotonic activity was about 0·007 IU/mg. The analogue did not affect blood pressure of the animal assayed up to $8 . 10^{-2}$ mg and was not active in the galactogogous assay up to the same dose. [Asn[4]]DDAVP can be thus regarded as a superactive* vasopressin analogue with an almost pure antidiuretic activity. In the assays performed, the dependence of the activity on the logarithm of the dose was parallel to that of LVP and was linear. Introduction of structural modifications present in [Asn[4]]LVP and DDAVP into a single molecule thus virtually resulted in a substance, [Asn[4]]DDAVP, with a steeper and linear log dose *vs* activity dependence. In view of the AD magnitude and the course and purity of the action, the novel analogue could also be of some interest in the clinical praxis.

## EXPERIMENTAL

Melting points (uncorrected) were taken on a heated microscope stage (Kofler block). Unless stated otherwise, the analytical samples were dried over phosphorus pentoxide at 100°C and 0·1 Torr for 8 h. The physicochemical measurements and purifications were carried out in the same instruments as in the earlier papers on vasopressin.[12]

### Benzyloxycarbonylprolyl-$N^G$-tosyl-D-arginyl-glycine Amide

At −10°C, pivaloyl chloride (1·5 ml; 14·9 mmol) was added to a solution of $N^\alpha$-benzyloxycarbonyl-$N^G$-tosyl-D-arginine (6·9 g; 14·9 mmol) and triethylamine (2·1 ml; 14·9 mmol) in dimethylformamide (30 ml). The mixture was maintained at 0°C for 5 min, cooled down to −10°C and treated with a solution of glycine amide hydrobromide (2·3 g; 14·9 mmol) and triethylamine (2·1 ml; 14·9 mmol) in dimethylformamide (30 ml). The whole was kept at room temperature for 1 h and evaporated under diminished pressure. Saturated (at 18°C) aqueous sodium sulfate was added to the residue and the mixture extracted with two 50 ml portions of 4 : 1 ethyl acetate–ethanol. The extract was washed with two 25 ml portions of 2% hydrochloric acid and two

* As superactive are designated those analogues, the biological activity of which is at least one order of ten higher than that of the naturally occurring hormone.

30 ml portions of saturated aqueous sodium hydrogen carbonate, dried over anhydrous sodium sulfate, and evaporated under diminished pressure. The residue was dried *in vacuo* at 50°C to the constant weight. Yield, 5·8 g (75%) of the foamy product. The foam was dissolved in acetic acid (10 ml) and the solution was heated with 35% hydrogen bromide in acetic acid (10 ml) for 5 min at 60°C. The resulting mixture was poured with stirring into 250 ml of ether. The precipitate (hydroscopic flaskes) was collected with suction, washed on the filter with ether, and dried under diminished pressure over phosphorus pentoxide for 20 h. The dry precipitate was dissolved in methanol (5 ml) and the solution passed through a 2 × 30 cm column of Zerolite FF (base) exchanger. The effluent (250 ml; about 50 ml blank) was evaporated under diminished pressure and the residue dried at 40°C in vacuo until its weight was constant; yield, 3·54 g (74%). The residue was dissolved in dimethylformamide (15 ml), the solution treated with benzyloxycarbonyl-proline (2·3 g; 9·2 mmol) and then, at 0°C, with a solution of N,N'-dicyclohexylcarbodiimide (1·9 g; 9·2 mmol) in dimethylformamide (3 ml). The mixture was kept at 0°C for 1 h and then at room temperature for 20 h. The N,N'-dicyclohexylurea was filtered off and the filtrates evaporated under diminished pressure. The acidic and basic contaminants were removed from the residue as discribed in the isolation of the protected dipeptide amide. Yield, 5·2 g (92%). This product was subjected to decarbobenzoxylation and the resulting mixture processed analogously to the case of Z-D-Arg(Tos)-Gly-$NH_2$. Yield, 2·9 g (71%) of prolyl-$N^G$-tosyl-D-arginyl-glycine amide (foam). Amino-acid analysis: Pro 1·03, Arg 0·96, Gly 1·01. As indicated by thin-layer chromatography (1-butanol–acetic acid–water 4 : 1 : 1 and chloroform–methanol 1 : 1) and paper electrophoresis (Whatman No 3 MM, pyridine acetate buffer solution pH 5·7, 6% aqueous acetic acid, 700 V, 45 min), the solid foam did not contain more than 10—15% contaminants. The product was used directly in the further work.

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-asparaginyl-asparaginyl-S-benzylcysteine Methyl Ester

The title compound was prepared according to a reported[1] procedure from β-benzylthiopropionyl-tyrosyl-phenylalanine hydrazide[5] (4·9 g; 9·35 mmol), asparaginyl-asparaginyl-S-benzyl-cysteine methyl ester[1] (4·24 g; 9·35 mmol), hydrochloric acid (37%; 5 ml), sodium nitrite (0·645 g; 9·35 mmol) with the use of dimethylformamide (45 ml) as solvent. Recrystallisation from dimethylformamide–water yielded 5·24 g (59%) of the product; m.p. 215—218°C; $[\alpha]_D^{20}$ —26·8° (*c* 0·5, dimethylformamide). For $C_{47}H_{55}N_7O_{10}S_2 \cdot \frac{1}{2} H_2O$ (951·1) calculated: 59·35% C, 5·93% H, 10·31% N, 6·74% S; found: 59·33% C, 5·94% H, 10·37% N, 7·03% S. Amino-acid analysis: Tyr 1·01, Phe 1·10, Asp 1·88.

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-asparaginyl-asparaginyl-S-benzylcysteine Hydrazide

The title compound was prepared according to a reported procedure[1] from β-benzylthiopropionyl--tyrosyl-phenylalanyl-asparaginyl-asparaginyl-S-benzylcysteine methyl ester (5·04 g; 5·3 mmol) by the action of hydrazine hydrate (100%; 4·2 ml) in dimethylformamide (16·5 ml). Yields, 4·18 g (83%) of the crude product and 3·12 g (62%) of the recrystallised product. M.p. 237—240°C (dimethylformamide); $[\alpha]_D^{20}$ —36·4° (*c* 0·5, dimethylformamide). For $C_{46}H_{55}N_9O_9S_2$ (942·1) calculated: 58·64% C, 5·88% H, 13·38% N, 6·81% S; found: 58·43% C, 6·13% H, 13·53% N, 7·03% S.

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-asparaginyl-asparaginyl-S-benzylcysteinyl--prolyl-$N^G$-tosyl-D-arginyl-glycine Amide

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-asparaginyl-asparaginyl-S-benzylcysteine hydrazide (1·43 g; 1·52 mmol) was dissolved in a mixture of dimethylformamide (13 ml) and hydrogen chloride in dioxane (0·71 ml of a 4M solution). The solution was cooled down to −20°C and treated with amyl nitrite (175 mg; 1·52 mmol) in dimethylformamide (5 ml). The course of the reaction was checked with the use of the Griess reagent[11]. After 15 min, the mixture was adjusted with N-ethylpiperidine to pH 7 and a solution of prolyl-$N^G$-tosyl-D-arginyl-glycine amide (868 mg; 1·8 mmol) in dimethylformamide (5 ml) was added. The mixture was kept at 0°C for 15 h and at room temperature for 3 h, and evaporated under diminished pressure; 1% hydrochloric acid (50 ml) was added to the residue. The product was collected with suction, washed on the filter with water, saturated aqueous hydrogen carbonate, and water again, and dried. Yields, 2·05 g (98%) of the crude product and 1·68 g (80%) of the recrystallised product. M.p. 197–200°C (dimethylformamide–water); $[\alpha]_D^{20}$ −24·2° (*c* 0·5, dimethylformamide). For $C_{66}H_{82}N_{14}O_{14}S_3 \cdot 1\frac{1}{2}H_2O$ (1418·7) calculated: 55·87% C, 6·04% H, 13·82% N, 6·78% S; found: 55·87% C, 5·90% H, 13·81% N, 6·76% S.

[1-β-Mercaptopropionic Acid, 4-Asparagine, 8-D-Arginine]vasopressin

The title compound was prepared as usual[12] from β-benzylthiopropionyl-tyrosyl-phenylalanyl--asparaginyl-asparaginyl-S-benzylcysteinyl-prolyl-$N^G$-tosyl-D-arginyl-glycine amide (681 mg; 0·48 mmol). Yields, 258 mg of the crude and 113 mg of the purified freeze-dried material. Optical rotation: $[\alpha]_D^{25}$ −51·2° (*c* 0·12, water). The analytical sample was dried over phosphorus pentoxide at 100°C and 0·1 Torr for 10 h. Elemental analysis indicated the presence of 3 molecules of acetic acid. For $C_{45}H_{62}N_{14}O_{12}S_2 \cdot 3\,CH_3COOH$ (1235·35) calculated: 49·58% C, 6·04% H, 15·87% N; found: 49·31% C, 5·79% H, 15·71% N. Amino acid analysis: Cys 1·03, Tyr 0·90, Phe 1·07, Asp 2·06, Pro 1·05, Arg 0·90, Gly 0·98.

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