[1-β-MERCAPTOPROPIONIC ACID, 8-α-AMINO-β-GLYCYLAMINOPROPIONIC ACID]VASOPRESSIN. A VASOPRESSIN ANALOG WITH AN ISOPEPTIDE STRUCTURE IN ITS MOLECULE

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[1-β-Mercaptopropionic acid, 8-α-amino-β-glycylaminopropionic acid]vasopressin (*II*) was prepared by solid-phase peptide synthesis. *II* has an antidiuretic effect of considerable magnitude (435 I.U./mg) and specificity.

The difficult accessibility of homologs of basic amino acids used by us[1,2] in studies on the relations between chemical structure and biological activities of vasopressins has led us to replace these homologs, at least in orienting experiments, by analogs which are more readily available. There are many possibilities in this respect. Perspective appeared isopeptides prepared from an appropriate basic or acidic amino acid to which subsequently a side chain is linked by the peptide bond. The advantages of isopeptides are obvious. They are readily accessible, they enable the preparation of various types of analogs, and they do not introduce anomalous structural elements into the molecule. On the other hand, the carboxamide group alters the spatial arrangement of the side chain and its conformational stability, polarity, and enables other inter- and intramolecular interactions to take place which can both increase the stability of the parent molecule and also modify its binding to other systems including the receptor of the target tissue. The replacement of the basic amino acid by an isopeptide analog will necessarily influence the biological effects. In order to verify the passableness of this line of approach we synthetized an isopeptide analog of lysine-vasopressin, [1-β-mercaptopropionic acid, 8-α-amino-β-glycylaminopropionic acid]vasopressin (*II*).

II was prepared by solid-phase synthesis[3-5] essentially according to the scheme developed earlier[6]. A few minor modifications are described in the experimental

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section. When synthetizing the iso component we condensed first α-benzyloxycarbonylamino-β-tert-butyloxycarbonylaminopropionic acid with glycine esterified to the resin. We split off the tert-butyloxycarbonyl residue by trifluoroacetic acid in dichloromethane and acylated the β-amino group by *p*-toluenesulfonylglycine chloride. In orienting experiments the possibility of acylation by *p*-toluenesulfonylglycine in the presence of N,N'-dicyclohexylcarbodiimide and of N-hydroxybenzotriazole[7] was explored; however, the reaction did not proceed and the growth of peptide chains from both possible reaction centers was observed. The analog thus formed and its properties will be dealt with later. The subsequent steps of synthesis were carried out in the standard manner. After completion of the synthesis the peptide was split off from the resin by ammonolysis[8,9]. The transformation of the protected linear peptide to the analog and its biological testing were effected by conventional procedures[10–13].

Compound *II* has a considerable antidiuretic effect, which is approximately by 1/3 higher as compared to the corresponding LVP* analog (Table I), a low pressor activity, and therefore a considerable specificity of the antidiuretic effect. The introduction of the isopeptide lysine analog into position 8 of lysine-vasopressin profoundly affected its biological properties. We ascribe the changes observed to the combined influence of delocalization of the ω-amino group and to an increase in polarity (*cf.*[14]). The results obtained in this study do not allow us to judge to what degree participate on these changes the stabilization of the side chain, other intra- and intermolecular interactions or alternatively the increase in the strength of the bond between the analog and the receptor. The result of the replacement of lysine in LVP by its isoanalog seems to be interesting; it points to new possibilities of examination of the structure-activity relationship and of designing new vasopressin analogs and new analogs of biologically active peptides in general.

TABLE I
Biological Activity (I.U./mg) of Vasopressin Analogs

Compound	AD	BP	UT	AD/BP
[Mpr^1]LVP[20]	301 ± 11	126 ± 2	12 ± 0·5	2·4
[Mpr^1, Dap(Gly)^8]VP	435	5·75	0·2—0·3	76

* The symbols and abbreviations usual in peptide chemistry were used. Other abbreviations: Dap α,β-diaminopropionic acid, LVP lysine-vasopressin, AD antidiuretic effect, BP pressor effect, UT uterotonic effect.

EXPERIMENTAL

All the general experimental details including the description of apparatus used for the measurement and purification have been reported before[15]. Thin-layer chromatography was carried out in the system n-butyl alcohol–tert-butyl alcohol–acetic acid–water (2 : 2 : 1 : 1) on silica gel layers (Silufol, Kavalier, Votice).

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl--prolyl-α-amino-β-(*p*-toluenesulfonylglycyl)aminopropionyl-glycine Amide (*I*)

The synthesis was carried out on chloromethylated polystyrene resin cross-linked by 2% of divinylbenzene (Calbiochem, Los Angeles, U.S.A.); the chlorine content was 0·96 mmol/g of resin. After esterification[16] with Bcc-Gly-OH the resin contained 0·35 mmol of Gly/g. The synthesis was carried out with 4·3 g of esterified resin in a synthetizer operated manually. All the α-amino groups of the amino acids used were protected by a tert-butyloxycarbonyl group, with the exception of glycine in the side chain (Tos) and α,β-diaminopropionic acid (Z-Dap(Boc)-OH) (ref.[17]). The synthesis of the tert-butyloxycarbonylamino acids was carried out according to[18]. The synthesis of the linear protected precursor I was effected by following the scheme described earlier[6] (program No 6) with two modifications. The coupling of Tos-Gly-OH was performed by Tos-Gly-Cl: 0·625 ml (4·5 mmol) of triethylamine and a solution of 1·11 g (4·5 mmol) of Tos--Gly-Cl in 10 ml of dichloromethane were added to a suspension of the resin in 40 ml of dichloromethane. The mixture was allowed to react with stirring 30 min. The resin was then washed three times with dichloromethane (50 ml) and the coupling was repeated under identical conditions. The benzyloxycarbonyl residue was split off by repeated treatment with 10% solution of hydrogen bromide in acetic acid (50 ml) (1st treatment 5 min, 2nd treatment 15 min). The resin was washed with five 50-ml portions of methanol before neutralization. After completion of the synthesis the product was split off from the resin by ammonolysis[8,9]. The yield of the crude product was 1·31 g (62·5%). The yield after double crystallization from the mixture ethanol–water was 0·65 g (31%) (based on the first amino acid esterified to the resin). M.p. 211—213°C, $[\alpha]_D^{20}$ —12·5° (*c* 0·5, dimethylformamide). The product was chromatographically homogeneous. For $C_{66}H_{81}N_{13}O_{15}S_3.H_2O$ (1411) calculated: 56·19% C, 5·93% H, 12·90% N; found: 56·22% C, 5·86% H, 13·12% N. Amino acid composition: Cys(Bzl) 1·08, Tyr 1·01, Phe 1·08, Glu 1·00, Asp 1·02, Pro 0·91, Dap 1·07, Gly 1·86.

[1-β-Mercaptopropionic Acid, 8-α-Amino-β-glycylaminopropionic Acid]vasopressin (*II*)

Protected peptide *I* (300 mg) afforded after reduction by sodium in liquid ammonia, oxidation by potassium ferricyanide, desalting on an Amberlite IRC-50 column and lyophilization 97 mg of the first lyophilisate (crude product). After purification by continuous free-flow electrophoresis 17 mg of chromatographically and electrophoretically pure second lyophilisate was obtained. The latter contained according to elemental analysis and polarographic assay[19] 81% of peptide (arithmetic mean). $[\alpha]_D^{20}$ —79·3° (*c* 0·1, 1M-CH_3COOH). Amino acid composition: Tyr 1·05, Phe 1·09, Glu 0·98, Asp 0·98, Pro 1·04, Dap 0·95, Gly 1·88. The elemental analysis of the product dried over phosphorus pentoxide at 100°C and 10 Pa corresponded to the monoacetate. For $C_{45}H_{61}N_{13}O_{13}S_2.CH_3COOH$ (1116) calculated: 50·57% C, 5·87% H, 16·13% N; found: 50·33% C, 5·69% H, 16·43% N.

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