[1-β-MERCAPTOPROPIONIC ACID, 8-α-AMINO-β-GUANIDINOPROPIONIC ACID]VASOPRESSIN AND [1-β-MERCAPTOPROPIONIC ACID, 8-D-α-AMINO-β-GUANIDINOPROPIONIC ACID]VASOPRESSIN; ANALOGS SHOWING A HIGH AND SPECIFIC ANTIDIURETIC EFFECT

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β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine azide was condensed with prolyl-α-amino-β-nitroguanidinopropionyl-glycine amides (L^2; D^2) to β-benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-α-amino-β-nitroguanidinopropionyl-glycine amides (L^8; D^8) which after removal of the protecting groups in liquid hydrogen fluoride, closure of the disulfide ring, desalting, and electrophoretic purification afforded [1-β-mercaptopropionic acid, 8-α-amino-β-guanidinopropionic acid]vasopressin (*I*) and [1-β-mercaptopropionic acid, 8-D-α-amino-β-guanidinopropionic acid]vasopressin (*II*). The antidiuretic effect of *I* (*II*) is about 10% of the effect of [1-β-mercaptopropionic acid, 8-D-arginine]vasopressin (DDAVP) (87 ± 8% DDAVP), the pressor effect is 49·5 I.U./mg (2·7 I.U./mg).

The symbols and abbreviations common in peptide chemistry were used. Other abbreviations: Mpr β-mercaptopropionic acid, Dap α,β-diaminopropionic acid DapG α-amino-β-guanidinopropionic acid, LVP lysine-vasopressin, AVP arginine-vasopressin, DDAVP [1-β-mercaptopropionic acid 8-D-arginine]vasopressin, AD antidiuretic effect, BP pressor UT uterotonic effect. The designation LVP series *etc.* have the same meaning as in the preceding communications (see, *e.g.*[1]).

There were two reasons which led us to prepare [Mpr^1, $DapG^8$]VP (*I*) and [Mpr^1, D-$DapG^8$]VP (*II*): *1*) the necessity to complete the deamino-AVP series as we did recently with the deamino-LVP series in order to be able to obtain an overall picture of the antidiuretic and pressor effect in the corresponding deamino-LVP and deamino-AVP group of compounds; *2*) the interesting properties of [Mpr^1,Dap^8] VP (*V*) and [Mpr^1,D-Dap^8] VP (*VI*). These two analogs show a considerable antidiuretic effect of remarkable specifity (Table I). *V* is an analog belonging to the L-series yet it has an extraordinarily low pressor effect and thus a considerably high specificity of the AD (~77). By contrast, *VI*, an analog of the D-series, shows

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a relatively high BP and a relatively low specificity of the AD (~42), lower than that of *V*. The replacement of the amino acid of the lysine type in position 8 by an amino acid of the arginine type (this replacement will be referred to as "guanidination") strongly potentiates as a rule the biological effects of the vasopressins. We were interested in the effect of this replacement on analogs *V* and *VI* since it was justified to expect that it may lead to products of top quality both as regards the magnitude and the specificity of the antidiuretic effect.

The synthesis of the linear protected precursor of analog *II* has been described recently[2]. The corresponding all-L- peptide was prepared in the same manner. The protecting groups were removed by treatment with liquid hydrogen fluoride[3]. The closure of the disulfide ring, desalting, and purification of crude analogs, as well as the determination of biological activities were carried out by conventional procedures[4–9]. The antidiuretic effect was determined with respect to a standard batch of DDAVP. The pressor and uterotonic effect was compared with synthetic LVP and oxytocin (whose effect had been determined with respect to the IIIrd International Standard for Oxytocin and Vasopressin Compounds). The concentration of samples of *I* and *II* for the determination of biological activities was expressed as described in[10].

The guanidination of *V* and *VI* in position 8 has led to very interesting products. In the first case an increase of both the antidiuretic and pressor effect was observed (Table I). The former was increased considerably more than the latter and thus the "anomalous" character of *I* is even more pronounced than that of *V*. *I* has the highest specificity of the AD of all the analogs of the L-series prepared by us. The guanidination of *VI* led, as expected, to a considerably higher increase of the AD (more than by one order) than in the preceding case. The effect of *II* in this respect is practically the same as that of DDAVP. The pressor effect of *II* is low yet slightly higher than the effect of DDAVP. It is, however, considerably lower than the effect of *I*. We do not meet here with the anomaly observed with *V* and *VI*, where the com-

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

	[X]vasopressin	AD	BP	UT	AD/BP
I	Mpr^1, $DapG^8$	10% DDAVP	49·5	1·5	102
II	Mpr^1, D-$DapG^8$	87 ± 8% DDAVP	2·7	0·4	16 200
V	*Mpr^1, Dap^8	1 079	14	0·9	77
VI	*Mpr^1, D-Dap^8	953	22·8	1·7	42
	DDAVP	100%	0·96	5·1	52 500

* See ref.[1].

pound of the L-series has a higher specifity of the AD than its D-counterpart. The properties of *II* are commensurable with those of DDAVP. We may therefore classify *II* as belonging to the group of "superactive" vasopressin analogs with a highly specific AD.

EXPERIMENTAL

All the general experimental details including the instruments used for the measurement and purification were reported elsewhere[4].

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl--α-amino-β-nitroguanidinopropionyl-glycine Amide (*III*)

Using the procedure described in[4], 1·09 g (1·14 mmol) of β-benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine hydrazide[11] and 0·58 g (1·74 mmol) of prolyl--α-amino-β-nitroguanidinopropionyl-glycine amide hydrobromide[2] afforded 1·22 g (84%) of *III*, m.p. 195–201°C. The yield of the product recrystallized from aqueous acetic acid was 1·09 g (75%). M.p. 201–204°C, $[\alpha]_D^{20}$ −34·8° (*c* 1·0, dimethylformamide). Amino acid analysis: Tyr 0·94, Phe 1·00, Glu 1·01, Asp 1·05, Cys(Bzl) 0·96, Pro 1·02, Gly 1·03. For $C_{58}H_{73}N_{15}O_{14}S_2$ (1 268) calculated: 54·93% C, 5·80% H, 16·56% N; found: 55·36% C, 5·86% H, 16·39% N.

[1-β-Mercaptopropionic Acid, 8-α-Amino-β-guanidinopropionic Acid]vasopressin (*I*)

The protected octapeptide derivative *III* (400 mg) was stirred 30 min at room temperature with *c.* 20 ml of anhydrous hydrogen fluoride in the presence of 0·4 ml of anisol. After hydrogen fluoride had been evaporated (water pump) the residue was dried *in vacuo* (1 h water pump and 2 h oil pump). Subsequently the dry residue was dissolved in 500 ml of 2·5% acetic acid, the solution was extracted 5-times with ether free of peroxides, and the pH of the solution was adjusted to 6·75 by aqueous ammonia. The solution was oxidized by 0·01M solution of potassium ferricyanide until a yellow color stable for 30 min had developed. The solution was adjusted to pH 4·5 by acetic acid and filtered through an ion-exchange column (25 ml of Amberlite IRC 50). The column was washed with 150 ml of 0·25% acetic acid and the peptides eluted by 50% acetic acid. 40 ml of effluent (void volume 15 ml) was collected, diluted to 70 ml with distilled water, and lyophilized. The yield was 83 mg of crude product which was purified by continuous free-flow electrophoresis as described in[4]. This procedure afforded 27 mg of [Mpr[1], DapG[8]]VP (*I*) which was homogeneous when examined by paper electrophoresis (5% acetic acid, 800 V, Whatman 3 MM) and thin-layer chromatography (Silufol, n-butyl alcohol–tert-butyl alcohol–acetic acid–water, 2 : 2 : 1 : 1). The peptide content of the lyophilisate was 76% (an average value obtained polarographically and from nitrogen content), $[\alpha]_D^{20}$ −14·6° (*c* 0·05, water). Amino acid analysis: Tyr 0·96, Phe 1·08, Glu 1·08, Asp 1·09, Pro 0·91, DapG 0·72, Gly 0·90.

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

The preparation of *II* was carried out as described for *I*. A quantity of 180 mg of the protected, linear precursor afforded 58 mg of crude and 18 mg of purified lyophilisate. The peptide content of the lyophilisate was 78%, $[\alpha]_D^{20}$ −44° (*c* 0·05, water). Amino acid analysis: Tyr 0·97, Phe 1·05 Glu 1·06, Asp 1·03, Pro 0·99, DapG 0·61, Gly 0·90. The lower values for DapG obtained in both cases indicate partial decomposition of DapG during acid hydrolysis of the peptide.

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