PURIFICATION OF TEN-GRAMMES AMOUNTS OF [1-β-MERCAPTOPROPIONIC ACID, 8-D-ARGININE]VASOPRESSIN (DDAVP) ON A CONTINUOUS FREE-FLOW ELECTROPHORESIS. CHEMICAL AND PHARMACOLOGICAL DATA OF DDAVP AND BY-PRODUCTS*

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By purification on a continuous free-flow electrophoresis, more than 50 g of DDAVP has been prepared. A number of data concerning composition, physicochemical properties and pharmacology of DDAVP has been obtained and statistically processed. On the basis of these data, statistically founded parameters of DDAVP have been determined. From the by-products arising in the last step of the synthesis of DDAVP, deaminopressinoic acid (*I*), the hexapeptide *II* and the DDAVP dimer *III* have been isolated. The dimer *III* exhibits a considerably high and very specific antidiuretic activity; a depressor effect is shown in the blood pressure assay in rats.

The preparation of higher-molecular-weight peptides (MW $\geqq$ 1 000) still represents a complex multistep process which is accompanied by numerous side reactions. This holds not only for syntheses carried out in the classical manner but also for preparations on polymeric supports, for the N-carboxyanhydride syntheses and the like. Whotever the synthetic method may be, the purification always plays an important role and frequently represents the key step. Of a special importance is the purification process in the rapidly growing production of peptide substances, particularly peptide hormones. The requirement of the isolation of a pure substance is in this instance accompanied by the need of economy. Some time ago, we were confronted with the problem to prepare and purify several tens of grammes of the synthetic vasopressin analogue [1-β-mercaptopropionic acid, 8-D-arginine]vasopressin[1] (DDAVP). From the purification methods considered for this case, the continuous free-flow electrophoresis[2] was selected since very good results were obtained with the technique in the laboratory praxis (*e.g.*[3,4]).

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The apparatus for a continuous free-flow electrophoresis is relatively complex but the technique indisputably exhibits many advantageous features. The separation process is continuous and relatively rapid. It proceeds under mild and exactly controlled conditions which are readily reproducible and which may be easily varied if required. Small amounts of inflammable solvents are used which are easily and with safety to handle. Considerably acidic or basic solvent systems can be used. The possibility to work in acidic media and at lower temperatures (0°C) is of an extraordinary importance with respect to the properties of vasopressins[5,6]. The separation proceeds between glass plates. Consequently, the product cannot be contaminated by extractive substances contrary to the electrophoresis on various supports, chromatography, gel filtration and the like.

The purification of a larger amount of DDAVP was of interest from several standpoints. Thus, we wanted to verify the suitability of continuous free-flow electrophoresis for this purpose. The purification made possible to investigate or reinvestigate the chemical, physicochemical, and pharmacological properties of DDAVP, to sum up the results obtained during a ten years' investigation of this analogue, and to verify and evaluate statistically the fundamental biological parameters which are useful both from the practical point of view and as a basis for a discussion of some ideas recently presented in the literature[7,8]. Finally, the purification of DDAVP made possible to accumulate and examine the by-products arising in the last step of the synthesis in a relatively small quantity and therefore not yet studied in detail.

EXPERIMENTAL

Materials, Apparatus, Methods

The material for the purification on the continuous free-flow electrophoresis was prepared from β-benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-N^G-tosyl-D-arginyl-glycine amide by the reported procedure[1]. All the batches of the starting material exhibited the m.p. value in the range of 199—201° and the optical rotation (mean ± SE) $[\alpha]_D^{25}$ equal to $-20{\cdot}5° \pm 0{\cdot}6°$ (*c* 0·5, dimethylformamide) and satisfactory elemental analyses and amino-acid analyses.

The purification operations were carried out on the Hannig[2] apparatus modified and constructed in the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague. The magnitude of plates (horizontal arrangement) is 50 cm × 50 cm, the space between plates is 0·5 mm wide and the volume of the working space is about 125 ml. The plates are placed in a box from plastics and cooled with a thermostatted air (variation of temperature, ±0·5°C at most). The electrodes are separated from the working space by ionex membranes (Nepton AR-111-A and Nepton CR-61; Ionics Inc., Massachusetts, U.S.A.). The carrier electrolyte is transported between the plates by means of a sixfold piston pump; the flow-rate through the plate is about 120 ml per 45 min. The electrode electrolyte is transported into the electrode space by means of a rotary pump. The maximum admissible voltage on electrodes is 4000 V (stabilised with accuracy better than 1%), maximum current 500 mA and maximum load of the plate 1000 W, working load 600 W. The sample is applied between the plates by means of a peri-

staltic pump with an adjustable pumping rate (1 ml/h to 128 ml/h) on five optional positions. The liquid leaving the working space is withdrawn at 48 positions into cylindric collectors and transported into storage vessels by means of siphons. The vessels are placed in a cooled hermetically closed box. The transport is effected by lowering the pressure inside the box and operated by a mechanical impulse counter. In the purification process, the earlier developed conditions were in principle used[3,4]. As the carrier and electrode electrolyte, 6% and 9% aqueous acetic acid was used. The sample, a 5% solution of the peptide mixture in 20% aqueous acetic acid, was introduced between the plates in the proximity of the anode (the inlet distance from the ionex membrane was about 8 cm) by the rate of 1 ml/h and 2 ml/h. The temperature of the circulating air was −3°C. In the course of the purification process, the voltage between electrodes was 2700 V and 3000 V (about 50 V/cm and 60 V/cm). The separation was checked by colorimetry at 275 nm and paper electrophoresis. Fractions containing the pure product were pooled and filtered; the solvent was removed by freeze-drying. The purity of thus-obtained substance was checked by paper electrophoresis and thin-layer chromatography. The substance was characterised by determination of the nitrogen content, optical rotation, UV spectrum, polarography, amino-acid analysis, and biological assays.

The countercurrent distribution was carried out in an all-glass Quickfit (Staffordshire, England) apparatus with the both upper and lower phase shift in the solvent system 2-butanol–0·05% aqueous acetic acid (1 : 1). The gel filtration and chromatography was performed on Biogel P_2 (95 × 1 cm column C_I, 3M-CH_3COOH (S_1), flow-rate 7—8 ml/h), on Biogel P_4 (90 × 1 cm column C_{II}, S_1, flow-rate 4·5 ml/h; 130 × 1·5 cm column C_{III}, S_1, flow-rate 8·5 ml/h), Sephadex G 25 (90 × 1·5 cm column C_{IV}, 1-butanol–benzene–3·5% CH_3COOH in 1·5% aqueous pyridine (2 : 1 : 3) (S_2) anchored lower phase, flow-rate 10 ml/h), and on CM-Sephadex (35 × 1 cm column C_V, concentration gradient 1M to 7M-CH_3COOH, flow-rate 20 ml/h). The operations on columns were checked by a flow colorimeter at 280 nm and by the Folin–Ciocalteau reaction. The discontinuous colorimetry was performed on a VSU-2P (Carl Zeiss, Jena, German Democratic Republic). Analytical electrophoresis was performed on paper Whatman 3 MM in S_3, 1M-CH_3COOH, and in S_4, pyridine acetate buffer solution (pH 5·7), at 20 V/cm (60 min). Thin-layer chromatography was performed on ready-for-use Silufol (Kavalier Glassworks, Votice, Czechoslovakia) silica gel sheets in the solvent systems S_5, 1-butanol–99% formic acid–water (75 : 12·3 : 12·7); S_6, 1-butanol–pyridine–acetic acid–water (30 : 20 : 6 : 12); and S_7, chloroform–methanol–water (70 : 30 : 5). Spots were detected by the chlorination process[9] and Sakaguchi reaction. The amino-acid composition of samples was determined on an automatic Amino Acid Analyzer (Developmental Workshops, Czechoslovak Academy of Sciences, Type 6020). The optical rotation was measured on a Perkin-Elmer polarimeter, Type 141. Polarographic determinations were performed with the use of the LP-7 Polarograph and the EZ 11 recorder.

The antidiuretic effect was determined by the method of Vávra and coworkers[10] and by the method of Sawyer[11] as modified by Pliška and Rychlík[12]. The blood pressure effect was determined by the method of Krejčí and coworkers[13] and the uterotonic effect was assayed according to Munsick[14] in a medium free of the Mg^{2+} ions.

Analytical

Removal of protecting groups from Mpr(BZL)-Tyr-Phe-Gln-Asn-Cys(BZL)-Pro-D-Arg(Tos)-Gly-$-NH_2$, closure of the disulfide ring, desalting, and freeze-drying afforded the crude peptide material (lyophilisate I) which was separated by continuous free-flow electrophoresis into three fractions (Fig. 1, bands A, B, and C). The pure DDAVP was usually present in band A, fractions 21—27 (2700 V) or 25—31 (3000 V). Band B (fractions 7—17) contained a mixture of neutral

and acidic substances. The fastest fractions (band C; from fraction 27 at 2700 V or from fraction 32 at 3000 V), manifesting themselves as a shoulder on the DDAVP band contained more basic substances along with DDAVP.

Yields. Reductions were performed in 20 g batches. The yields of lyophilisate I (crude product) and lyophilisate II (pure product) are shown in Table I. For some analytical data and biological activities of the particular batches see Table II. According to an approximate graphical estimation, the lyophilisate I contains about 80% of DDAVP. The mixed fractions thus contain additional 37% of the total DDAVP which can be obtained from lyophilisate I.

Analysis of band B and band C (*cf.*[15]). As shown by thin-layer chromatography in S_5, three components were present in band B. Two predominating components (*I* and *II*) were present in the ratio of about 3 : 1. The third component was present in a small amount only and was not examined in detail. The two main components were isolated in a pure state by gel filtration on Sephadex G-25 (C_{IV}, S_2). Component *I*: R_F 0·5—0·6; amino acid analysis: $CysSO_3H$ 0·97, Tyr 1·00, Phe 0·99, Glu 1·01, Asp 1·02. Component *II*: R_F 0·9; amino acid analysis: Tyr 0·9, Phe 1·00, Glu 1·00, Asp 1·00, Pro 0·70 (Cys present but not determined). Substance *II* was also isolated by countercurrent distribution (100 shifts of the upper phase, 101 shifts of the lower phase, K 6·8) followed by gel filtration on a column of Biogel P_2 (C_I, S_1). Both compounds were homogeneous on thin-layer chromatography in the solvent system S_5 (*I*, R_F 0·28; *II*, R_F 0·4) and S_7 (*I*, R_F 0·05; *II*, R_F 0·1). According to the gel filtration (Biogel P_4, C_{II}, S_1), the molecular weight of compound *I* (V_e 80 ml) is lower than that of DDAVP (V_e 60—62 ml). On the basis of amino acid analysis, gel filtration, thin-layer chromatography, paper electrophoresis and comparison with compounds prepared earlier as well as on the basis of biological assays, compound *I* was assigned the structure of deaminopressinoic acid[16] and compound *II* was ascribed the structure of deaminovasopressin-(1—7)-hexapeptide. By gel filtration on a column of Biogel P_4 (C_{III}, S_1), the homogeneous compound *III* (R_F 0·5) was isolated in addition to DDAVP (R_F 0·4) from mixed fractions of band C. The amino acid composition of compound *III* is equal to that of DDAVP: Cys 0·78, Tyr 1·08, Phe 1·05, Glu 1·00, Asp 1·05, Pro 0·97, Arg 0·93, Gly 1·05. When compared with DDAVP, compound *III* was faster on paper electrophoresis, exhibited a different mobility on thin-layer chromatography in S_6 (*III*, R_F 0·17; DDAVP, R_F 0·37), and showed a different optical rotation $[\alpha]_D^{20}$ −44·4° (*c* 0·2, 1M-CH_3COOH). Elution volumes of DDAVP and compound *III* are well comparable with those of [Tyr (Me)^2]oxytocin and its dimer obtained under similar experimental conditions[17]. On the basis of the above results, compound *III* was assigned the structure of a DDAVP dimer. A product of the same properties

TABLE I

Yields of the Lyophilisate and Peptide Material from 20·0 g of the Protected Octapeptide Amide Derivative

Lyophilisate	Yield	Peptide, material	
	g	g	%
I	7·0—10·0	5·8—8·3	38—54·5
II	3·5—5·0	2·9—4·15	19—27

TABLE II

Analytical and Physicochemical Data and Biological Activity of DDAVP Batches 1—20

Batch	% N[a]	Peptide content in lyophilisate[b]	$[\alpha]_D^{20}$[c]	AD[d]	BP[d]
1	15·55	84·8	−63·9	99·8	1·91
2	15·64	85·3	−66·7	128	0·93
3	15·72	85·7	−60·3	128	0·93
4	15·97	87·1	−66·3	96·1	—
5	16·02	87·3	−61·7	88·4	—
6	15·30	83·4	−60·0	86·4	—
7	15·80	86·1	−60·3	79·8	—
8	15·67	85·4	−65·9	84·5	—
9	15·49	84·5	−59·5	99·5	—
10	15·46	84·3	−62·0	96·7	1·31
11	15·57	84·9	−62·4	95·1	1·4
12	13·64	74·4	−61·8	120	0·5
13	13·88	75·7	−62·0	103	0·7
14	14·64	79·8	−61·5	97·7	0·4
15	15·10	82·3	−61·6	89·6	—
16	14·86	81·0	−61·4	119·6	0·3
17	14·67	79·9	−61·5	107	—
18	14·43	78·7	−61·5	103	—
19	14·30	78·0	−65·9	88	—
20	15·00	81·8	−61·0	92	1·2
Number of determinations	20	20	20	20	10
Arithmetic mean	15·14	82·52	−62·36	100·12	0·96
Standard deviation	0·69	3·74	2·18	14·07	0·51
Standard error	0·15	0·85	0·49	3·15	0·16

[a] Calculated: 18·34% N. [b] Calculated from the nitrogen content. [c] Measured in 1M-CH_3COOH; *c* 0·5 with respect to the weight of the lyophilisate. [d] In antidiuretic (AD) and blood pressor (BP) assays, the concentration of DDAVP samples was expressed by an arithmetic mean of the concentration calculated from the nitrogen content and determined polarographically.

was also obtained by chromatography on a column of CM Sephadex (C_V, concentration gradient 1M- to 7M-CH_3COOH).

Polarography. Compounds *I* to *III* afforded under usual conditions[18] well developed waves corresponding to reduction of the disulfide bridge. In acidic media (pH < 5) and at concentrations higher than $1 . 10^{-4}$M, the curves of DDAVP and the dimer *III* exhibit a low pre-wave of an adsorptional-kinetical character and corresponding to reduction of mercury salts of the two substances (the salts are formed by reaction of the —S—S— group with the mercury surface).

UV *spectrum.* Fig. 2 shows the UV spectrum of DDAVP. For a detailed discussion of the spectral behaviour of DDAVP and some analogues see ref.[19].

RESULTS AND DISCUSSION

During systematic investigations focussed on elucidation of structural features of the vasopressin molecule imparting to the substance antidiuretic and pressor effects and on preparation of analogues with an increased specificity of the antidiuretic activity,

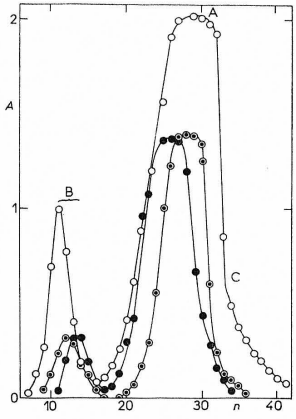

FIG. 1

Purification of DDAVP on a Continuous Free-Flow Electrophoresis

Abscissa, number of fractions; ordinate, absorbance at 275 nm. ● 2700 V, 1 ml/h (pure compound in fractions 21—27); ⊙ 3000 V, 1 ml/h (23—29); ○ 3000 V, 2 ml/h (25 to 31).

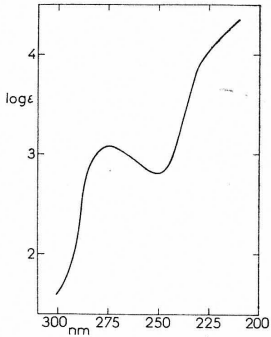

FIG. 2

The UV Spectrum of DDAVP in Water Acidified with Hydrochloric Acid to pH 3·5

it has been discovered in this Laboratory[3,20] that a change in configuration of the basic amino acid in sequence position 8 of the vasopressin molecule results in an increased specificity of the antidiuretic effect. It has been demonstrated in subsequent papers[21,22] that the effect of the configurational change in sequence position 8 is of a general character[21,22] and affords in all cases substances exhibiting a higher specificity of the antidiuretic effect when compared with the naturally occurring hormones. Furthermore, the increased specificity was shown not to be due to a decreased effect on blood pressure only but to be also affected by an increased effect on diuresis[23]. Moreover, the homologuisation experiments with purpose applied for the first time by this Laboratory to the field of peptide hormones indicate that the magnitude of the antidiuretic (and pressor) effect depends on the length of the side chain of the basic amino acid[21]; the character of this dependence differs in various homologous series of vasopressin analogues[21-25]. The substitution in sequence position 4 was shown to be of minor importance in development of vasopressin analogues with a high and specific antidiuretic effect and the idea on the importance of lipophilic changes in this position was controverted[4,23]. On the basis of results obtained in the early beginnings of our investigations, a vasopressin analogue with an extraordinarily high and very specific antidiuretic effect was designed, prepared (1964–1966) and reported[1] in 1967. Excellent properties of this analogue have been demonstrated not only in laboratory assays but also in clinical praxis[26-33], particularly in the substitution therapy of diabetes insipidus. This analogue, [1-β-mercaptopropionic acid, 8-D-arginine]vasopressin (DDAVP), is the first one from the group of "superactive"* vasopressin analogues. In the light of facts contained in our papers[1,3,4,20-25], the opinion of Manning and coworkers[7] that the trend of our papers was to hide the deep influence of the introduction of D-arginine into the sequence position 8 on the antidiuretic specificity of vasopressin analogues is incorrect.

We were well aware of the fact that we have in our hands a new, general approach to a new, very attractive group of analogues (specifically acting antidiuretics[1,3,4,20-25]). Already in the early stage of the DDAVP work we knew well the characteristic features of this analogue, the very high and very specific antidiuretic effect[1,34], the nonparallelity of the log dose – response dependence as well as its therapeutic value[26,34].** However, ten years ago the properties of DDAVP seemed so surprising that, in the new area, which DDAVP opened ("superactive analogues") and in the particular case of DDAVP we were cautious and critical. The first report on the antidiuretic activity of DDAVP is contained in the patent[34] (1966), which

* We consider the term "superactive" as justified for analogues at least by one order of ten more active than the natural substance.

** The first (preclinical) trials were accomplished (1966) by Dr V. Holeček at the III. Clinic for Internal Medicine, Charles University, Prague, with kind permission of professor J. Charvát.

gives the figure 16000 ± 200 IU/mg. This value corresponds to dose level of about 10^{-9} g which falls in the range of therapeutical doses. For the second time, the activity is mentioned in the communication[1] where the figure 870 IU/mg is given. This activity corresponds to low overthreshold doses where the regression lines of DDAVP and of the standard (lysine vasopressin) are nearly parallel and the customary evaluation and expression of the antidiuretic activity is possible. Vávra and coworkers[10] estimate the antidiuretic activity of DDAVP to about 50400 IU/mg on the basis of the calculated best-fit parallel regression lines for DDAVP and lysine vasopressin. The specificity of the antidiuretic activity is expressed by the fraction antidiuretic effect/pressor effect[34]. With DDAVP the numerator (antidiuretic activity) increases steeply with the dose whereas the denumerator (pressor activity) remains virtually unchanged. Consequently, the specificity grows with the dose too. Conceivably, such dependence as just discussed cannot be appreciated only at a single point, at the lowest value of the antidiuretic effect (which is only of theoretical significance). However, even with higher values of the pressor effect which we had to our disposal at the early stage of the work, the high specificity of the antidiuretic effect of DDAVP was evident at low overthreshold doses and was quite evident at higher doses. At any rate we quoted the specificity of the antidiuretic effect of DDAVP in the patent[34] and for reasons apparent from the preceding discussion not in the communication[1], the data of which Manning and coworkers used to calculate the specificity. We agree that the calculation of Manning and coworkers tended to obscure the fact of high specificity of the antidiuretic action of DDAVP which we demonstrated in[34]. The stated figure (1454) is comparable with the figure of Manning and coworkers[7] (2000) and surely does not tend to hide the deep influence of the D-arginine substitution in position 8 on the antidiuretic specificity.

Substances of the DDAVP type represent a special group of compounds exhibiting characteristic properties which also manifest themselves in pharmacological assays (difficulties in the test according to Pliška and Rychlík[12], frequent nonparallelities in the log dose – response dependence, protracted effects). The determination of the antidiuretic effect, evaluation of results and their presentation is thus rather difficult. The comparison of antidiuretic data obtained by various investigators must be therefore very cautious and critical in spite of identical or similar methods applied. Since 1966–1967 when the synthesis and fundamental properties of DDAVP were reported[1,34], numerous results were accumulated in repeated preparations of this analogue and subjected to statistical processing. Prior to the discussion of experimental data, attention should be drawn to deviations between the routine determination of the antidiuretic effect and the usual procedures as experienced in our laboratories. The composition of DDAVP lyophilisates varied considerably (Table II). This variation was reflected in biological assays and led to scattered biological data. In order to decrease the scattering, the concentration of samples for biological titrations was expressed as an arithmetic mean of the concentration as determined from the nitrogen

content of the lyophilisate and the concentration as determined polarographically from the solution of the sample. This procedure afforded the most consistent results. When compared with the standard, the log dose – response dependence of DDAVP was observed to exhibit a different shape[1,26]. This circumstance complicates the antidiuretic assay and makes difficult to express the results. The biological effect of the analogue cannot be expressed by a single figure. The values lacking the dose range are meaningless. In order to simplify this situation, the activity of the particular batches of the analogue was compared with that of a standard batch of DDAVP as determined against the 3rd International Standard for Oxytocic, Vasopressor, and Antidiuretic Substances. The activity of the particular batches was expressed in percentage of the standard DDAVP batch activity (its activity was considered as equal to 100%). Table II shows antidiuretic data of twenty independent preparations of DDAVP, obtained by the above mentioned method[10]. The range of results (85 to 128%) and the statistical evaluation of the whole set can be regarded as satisfactory with respect to the delicacy of the antidiuretic assay.

In the communication[1], the antidiuretic activity of DDAVP was estimated to be 870 IU/mg while Manning and coworkers[8] give the value of 955 ± 95 U/mg (USP U ± SE)* for a DDAVP lyophilisate of an also unspecified composition and without stating the dose range. The difference between the two data (85 U/mg) is felt[7] as discrepancy though the two values can be hardly compared. Manning and coworkers (*e.g.*[35,36]) characterise their final products (lyophilisates) by the amino-acid analysis only; the published data do not inform, however, on the peptide content. A comparison of biological effects of lyophilisates regardless their composition which can vary to a considerable extent (ref.[5] and Table II) is inaccurate and of a roughly informative value only. On the basis of such a comparison it is hardly possible to draw any rigorous conclusions on the relative activity of substances contained in the lyophilisates or any additional conclusions. The differences in biological activities are judged by Manning and coworkers very loosely. Thus *e.g.* between figures found for the antidiuretic activity of deaminoarginine vasopressin by Manning and coworkers[35] (1745 ± 385 U/mg) and by Huguenin and Boissonnas[37] (1300 ± 200 IU/mg) there is the discrepancy of 475 U/mg. This value is five times higher than the above discussed difference (85 U/mg) and even by about one third higher than the difference between our original value and the newest figure of Manning and coworkers[35] for DDAVP (1200 + 126 U/mg) (the composition of the lyophilisate again not given; the discrepancy between the figures of the American authors could in part be due to the difference in the composition of the lyophilisates). The above discrepancy (475 U/mg) is declared by Manning and coworkers[35] "as properties in essential agreement". Manning and coworkers do not even comment the discrepancy in the uterotonic activity of hydroxyoxytocin, 1275 ± 51 U/mg (Manning and coworkers[36])

* Based on USP Reference Standard.

and 1067 U/mg (Hope and Walti[38,39]). This discrepancy would really deserve explanation since the uterotonic activity can be determined very exactly, much more exactly than the antidiuretic activity. The pressor activity of our batches of DDAVP varied between 0·5 and 2·0 IU/mg. In our first communication[1], the value of 11 IU/mg was given; this value was determined by a somewhat different method. The discrepancy between the two data is most probably due mainly to different assay methods. This explanation is very probable since the blood pressure data obtained with samples prepared from the same starting material (the protected octapeptide) or from peptides obtained from the same starting compounds were also by about one order of ten lower. To the present knowledge, the method of synthesis excludes racemisation in sequence position 8.

The last step in the synthesis of DDAVP consists in reductive removal of protecting groups by the action of sodium in liquid ammonia[40] and oxidative closure of the disulfide ring[41]. The former reaction could be accompanied by fission of bonds, particularly of the Cys-Pro bond, and to a lesser extent, by fission of the proline ring, desulfuration of Cys, and some further side reactions. In the latter reaction, the formations of dimers and polymers can be expected. The presence of deaminopressinoic acid (*I*) isolated as one of the main components of band B, is not surprising. Somewhat unexpected was the presence of compound *II* which contains a hexapeptide sequence indicative of fission of the Pro-Arg bond.

The biological activity of deaminopressinoic acid (*I*) is very low[16]. The observed value of about 0·05 IU/mg is in accord with the reported one[16]. The biological activity of compound *II* has not been examined. From the biological standpoint, the dimer *III* was more interesting than compounds *I* and *II*. The antidiuretic activity of compound *III* was qualitatively different from that of DDAVP *i.e.* from the monomer. The action of *III* began very slowly and was of long duration. In some experiments, the overall antidiuretic effect of *III* was equal to that of the monomer. It is the first case of a commensurability of monomer effects with dimer effects in the field of neurohypophyseal hormones of the oxytocin and vasopressin type. Compound *III* did not raise the blood pressure when applied to rats. On the contrary, a marked hypotensive effect was observed in doses 0·5 to $1{\cdot}0 \cdot 10^{-1}$ mg (*per animal*). The uterotonic activity of the dimer *III* was considerably lower (0·3 IU/mg) than that of DDAVP (5·1 IU/mg).

By purification of crude DDAVP on a continuous free-flow electrophoresis, all the by-products can be removed (deaminopressinoic acid (*I*), the hexapeptide-acid *II*, and the DDAVP dimer (*III*)). The purification procedure can be used for the preparation of considerable amounts (tens to hundreds of grammes) of DDAVP. The biological effects of the by-products contained in crude DDAVP are either negligible or of such a character that they would not affect the biological properties of DDAVP-containing preparations in an undesirable manner. The analytical and

pharmacological data of numerous batches of DDAVP are in a good agreement. The differences in biological effects of DDAVP pointed out by Manning and coworkers, are regular. The lyophilisates of Manning and coworkers are not characterised in a satisfactory manner and their chemical composition is not stated. It is incorrect to compare the biological activity of lyophilisates regardless their composition. Under such conditions, the differences in biological data do not surprise. Taking into account all these facts, the known delicacy of the antidiuretic assay, the different methodics, the character of the log dose-response dependence of DDAVP (for the importance of these factors in determinations of antidiuretic data see for example ref.[42]), the data obtained in our experiments and those of Manning and coworkers are not at variance but are in an almost surprising agreement.

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