VASOPRESSIN ANALOGS MODIFIED IN POSITION 3. INFLUENCE OF CHANGES OF CONFIGURATION

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The condensation of *p*-toluenesulfonyl-S-benzylcysteinyl-tyrosine azide with D-phenylalanine methyl ester afforded *p*-toluenesulfonyl-S-benzylcysteinyl-tyrosyl-D-phenylalanine methyl ester which was subsequently converted into the hydrazide and azide and coupled with glutaminyl--asparaginyl-S-benzylcysteine methyl ester. *p*-Toluenesulfonyl-S-benzylcysteinyl-tyrosyl-D-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine methyl ester was condensed *via* the hydrazide and azide with prolyl-N$^{\gamma}$-*p*-toluenesulfonyl-α,γ-diaminobutyryl-glycine amide. After removal of the protecting groups, closure of the disulfide bridge and purification, [3-D-phenylalanine, 8-α,γ-diaminobutyric acid]vasopressin was obtained which shows an appreciable antidiuretic effect of distinct specificity.

The vasopressins and oxytocin contain two key positions, where molecules of the two hormones differ from each other, *i.e.* position 3 and 8. The nature of amino acids situated here determines the character of the hormonal effects. Previous investigations carried out in this Laboratory were devoted mainly to the latter position and revealed[1-3] the importance of the configuration and of the length of the side chain of the pertinent amino acid for the biological effects. Highly active analogs as well as groups of compounds with a very specific and a very high and very specific antidiuretic effect, respectively, were obtained in the course of these studies[4-7]. This communication deals with the second of the key positions, with position 3. As in the preceding work we decided to start with the change of configuration. We intended to determine whether this change would lead to similar or different consequences as compared to position 8. [Dab^8]VP (ref.[1,2]) was chosen as a model compound. It is readily accessible, easier than [Dap^8]VP (ref.[3]) which was also taken into consideration. [Dab^8]VP has relatively high biological activities. Antidiuretic effect of [Dab^8]VP

The symbols and abbreviations usual in peptide chemistry were used. Dab and Dap, earlier abbreviations for α,γ-diaminobutyric and α,β-diaminopropionic acid, used by us in the whole vasopressin series, were retained. Other abbreviations: VP vasopressin, LVP lysine vasopressin, AD antidiuretic effect, BP pressor effect, UT uterotonic effect.

is retained even after the configurational change in position 8 whereas this change in LVP results in an almost complete loss of activity[8]. We had no rational reason, indeed, to expect that the change of configuration in position 3 and 8 should have the same consequences; nevertheless, at this stage of the development of the problem we preferred to avoid also LVP as a model compound.

The linear protected precursor of the analog was prepared by liquid-phase synthesis following the usual scheme[9]. We condensed *p*-toluenesulfonyl-S-benzylcysteinyl--tyrosine azide with D-phenylalanine methyl ester to *p*-toluenesulfonyl-S-benzylcysteinyl-tyrosyl-D-phenylalanine methyl ester, which we converted by treatment with hydrazine hydrate into the hydrazide. We condensed the latter *via* the azide with glutaminyl-asparaginyl-S-benzylcysteine methyl ester to *p*-toluenesulfonyl--S-benzylcysteinyl-tyrosyl-D-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine methyl ester. Subsequently we prepared from the methyl ester the hydrazide and the azide by usual procedures and condensed the azide with prolyl-N$^{\gamma}$-*p*-toluenesulfonyl-α,γ-diaminobutyryl glycine amide to *p*-toluenesulfonyl-S-benzylcysteinyl-tyrosyl-D-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-N$^{\gamma}$-*p*-toluenesulfonyl-α,γ-diaminobutyryl glycine amide. The removal of the protecting groups, oxidative closure of the disulfide ring, desalting, purification, and determination of biological effects were carried out by the usual procedures[5,10-12].

[D-Phe^3, Dab^8]VP has an appreciable antidiuretic effect of distinct specificity (Table I). The change of configuration in position 3 and 8 seems to have rather similar influence on the biological properties of [Dab^8]VP. However, the antidiuretic activity of [D-Dab^8]VP and [D-Phe^3, Dab^8]VP was established by two different methods[2,10]. No definitive conclusion can therefore be drawn unless the two analogs are compared in the same assay system.

Walter explains the importance of positions 3 and 8 (and of positions 4 and 7) by postulating that they represent "corner" sites in the three-dimensional structure of the hormone[13]. The side chains of amino acids are thus most accessible to intermolecular interactions. The assumption[13] has been voiced that these side chains

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

Compound	AD	BP	UT	AD/BP
[Dab^8]VP	120 ± 30	149	3·5	0·8
[D-Dab^8]VP	120 ± 30	3·5	0·1	33
[D-Phe^3, Dab^8]VP	~30	13	0·005	~2·3

contain structural elements which provide for the specific binding of the hormone to the receptor. An obvious condition of the specific interaction, however, is the retainment of the fundamental spatial relations, of the basic "fit conformation" of the binding center of the hormone. A change in conformation in position 3 and 8 necessarily results in a significant interference with the conformation of the peptide. This leads to a selective change of biological effects, a marked decrease of the pressor effect and a far smaller change of the antidiuretic effect. This result, like the preliminary results of CD spectra measurements, suggests a local rather than an overall conformational change. If the above condition of specific interaction is valid one can speculate that positions 3 and 8 by themselves are not very important for this process.

EXPERIMENTAL

The apparatus and analytical methods used are the same as those described in the preceding communication[14].

p-Toluenesulfonyl-S-benzylcysteinyl-tyrosyl-D-phenylalanine Methyl Ester

p-Toluenesulfonyl-S-benzylcysteinyl-tyrosine hydrazide (7·0 g, 12·9 mmol) was dissolved in a mixture of 15 ml of dimethylformamide and 5·3 ml of 4·9M solution of hydrogen chloride in dioxane. The solution was cooled down to −20°C and a solution of 1·51 g (12·9 mmol) of amyl nitrite in 3 ml of dimethylformamide was added during 5 min. The mixture was cooled down to −30°C, after 20 min of standing, its pH was adjusted to *c.* 7 by N-ethylpiperidine, and a solution of D-phenylalanine methyl ester (2·3 g, 12·9 mmol) in 5 ml of dimethylformamide was added. The mixture was set aside for 20 h at 0°C and for 5 h at room temperature. The solvents were distilled off under reduced pressure. The residue was triturated three times with 3% hydrochloric acid, a saturated solution of sodium bicarbonate, washed with water on the filter, dried, and recrystallized from aqueous methanol. Yield 8·1 g (91%). After additional crystallization from the same solvents the yield was 6·7 g (75%) of a product of m.p. 166—167°C, $[\alpha]_D^{24}$ −28·2° (*c* 2·0, methanol). For $C_{36}H_{39}N_3O_7S_2$ (689·7) calculated: 62·69% C, 5·70% H, 6·11% N, 9·28% S; found: 62·84% C, 5·74% H, 6·23% N, 9·09% S.

p-Toluenesulfonyl-S-benzylcysteinyl-tyrosyl-D-phenylalanine Hydrazide

p-Toluenesulfonyl-S-benzylcysteinyl-tyrosyl-D-phenylalanine methyl ester (6·2 g, 9 mmol) was refluxed 2 h with a solution of 1 ml of hydrazine hydrate (100%) in 10 ml of methanol. Water (100 ml) was added to the mixture, the product which had separated was filtered off and recrystallized from ethanol. Yield 3·8 g (61%), m.p. 218—220°C, $[\alpha]_D^{25}$ +12·4° (*c* 0·54, dimethylformamide). For $C_{35}H_{39}N_5O_6S_2$ (689·7) calculated: 60·95% C, 5·70% H, 10·14% N, 9·28% S; found: 61·13% C, 5·76% H, 10·45% N, 9·53% S.

p-Toluenesulfonyl-S-benzylcysteinyl-tyrosyl-D-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine Methyl Ester

The compound was prepared by the same procedure as *p*-toluenesulfonyl-S-benzylcysteinyl-tyrosyl-D-phenylalanine methyl ester. From 3·0 g (4·4 mmol) of *p*-toluenesulfonyl-S-benzyl-

cysteinyl-tyrosyl-D-phenylalanine hydrazide and 2·8 g (40% excess) of glutaminyl-asparaginyl--S-benzylcysteine methyl ester, using 0·5 g (4·4 mmol) of amyl nitrite, 2·1 ml of 4·2M solution of hydrogen chloride in dioxane, and 20 ml of dimethylformamide as solvent, we obtained 4·0 g (80%) of a product twice recrystallized from acetic acid–water. M.p. 215–217°C, $[\alpha]_D^{25}$ +2·4° (*c* 1·3, dimethylformamide). For $C_{55}H_{64}N_8O_{12}S_3 \cdot \frac{1}{2}H_2O$(1134) calculated: 58·24% C, 5·76% H, 9·87%N, 8·45% S; found: 58·23% C, 5·73% H, 9·83% N, 8·14% S.

p-Toluenesulfonyl-S-benzylcysteinyl-tyrosyl-D-phenylalanyl-glutaminyl-asparaginyl--S-benzylcysteine Hydrazide

p-Toluenesulfonyl-S-benzylcysteinyl-tyrosyl-D-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine methyl ester (3·5 g, 3·1 mmol) was dissolved in 20 ml of dimethylformamide, 3·5 ml of hydrazine hydrate (100%) was added to the solution, and the mixture was set aside for 20 h at room temperature. Subsequently an excess of water was added, the product which had separated was filtered off, washed on the filter with water, and recrystallized twice from dimethylformamide–-water. Yield 2·5 g (71%), m.p. 253–255°C. For $C_{54}H_{64}N_{10}O_{11}S_3$ (1125) calculated: 57·64% C, 5·73% H, 12·44% N, 8·53% S; found: 58·10% C, 5·91% H, 12·75% N, 8·42% S.

p-Toluenesulfonyl-S-benzylcysteinyl-tyrosyl-D-phenylalanyl-glutaminyl-asparaginyl--S-benzylcysteinyl-prolyl-N$^{\gamma}$-*p*-toluenesulfonyl-α,γ-diaminobutyryl-glycine Amide

The same procedure which had been employed for the preparation of *p*-toluenesulfonyl-S-benzylcysteinyl-tyrosyl-D-phenylalanine was used. From *p*-toluenesulfonyl-S-benzyl-cysteinyl-tyrosyl--D-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine hydrazide (1·12 g, 1 mmol), prolyl-N$^{\gamma}$--*p*-toluenesulfonyl-α,γ-diaminobutyryl-glycine amide (0·53 g, 1·25 mmol), 104 mg (1 mmol) of butyl nitrite (in 2 ml of dimethylformamide) and 0·35 ml of 6·4M solution of hydrogen chloride in dioxane, using 15 ml of dimethylformamide as solvent, we obtained 1·35 g (88%) of a crude product and 0·86 g (56%) of a product recrystallized from dimethylformamide–water. M.p. 179–181°C, $[\alpha]_D^{25}$ −14·2° (*c* 0·21, dimethylformamide). For $C_{72}H_{87}N_{13}O_{16}S_4 \cdot H_2O$ (1536) calculated: 56·27% C, 5·84% H, 11·85% N; found: 56·48% C, 5·81% H, 11·61% N. Amino acid analysis: Cys(Bzl) 1·92, Tyr 0·92, Phe 1·07, Glu 1·09, Asp 1·04, Pro 0·98, Dab 0·92, Gly 1·04.

[3-D-Phenylalanine, 8-α,γ-diaminobutyric acid]vasopressin

p-Toluenesulfonyl-S-benzylcysteinyl-tyrosyl-D-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-N$^{\gamma}$-*p*-toluenesulfonyl-α,γ-diaminobutyryl-glycine amide (0·5 g) was reduced by sodium in liquid ammonia (500 ml), oxidized, desalted, and purified in the usual manner. The yield was 191 mg of the first lyophilisate (crude product) and 72 mg of the second lyophilisate (purified product). $[\alpha]_D^{22}$ −14·5° (*c* 0·22 water). For $C_{44}H_{61}N_{13}O_{12}S_2$ (1028) calculated: 17·71% N; found: 13·46% N. These values correspond to a 76% peptide content of the lyophilisate. The elemental analysis after 14 h of drying at 100°C over phosphorus pentoxide at 13 Pa corresponded to the diacetate trihydrate. For $C_{44}H_{61}N_{13}O_{12}S_2 \cdot 2\,CH_3COOH \cdot 3\,H_2O$ (1202) calculated: 47·95% C, 6·29% H, 15·14% N; found: 47·84% C, 6·03% H, 15·25% N. Amino acid analysis: Cys 1·94, Tyr 0·96, Phe 1·06, Glu 1·09, Asp 1·05, Pro 1·00, Dab 0·87, Gly 1·03. The concentration of the sample for the purpose of biological activity data was expressed as arithmetic mean value of peptide content of lyophilisate, calculated from nitrogen content and polarographic assay.

REFERENCES

1. Zaoral M., Šorm F.: This Journal *31*, 90 (1966).
2. Zaoral M., Šorm F.: This Journal *31*, 310 (1966).
3. Zaoral M., Kolc J., Šorm F.: This Journal *35*, 1716 (1970).
4. Zaoral M., Kolc J., Šorm F.: This Journal *32*, 1250 (1967).
5. Zaoral M., Brtník F., Barth T., Machová A.: This Journal *41*, 2088 (1976).
6. Zaoral M., Krchňák V., Brtník F., Machová A., Škopková J.: This Journal *44*, 2447 (1979).
7. Zaoral M., Brtník F., Flegel M., Škopková J., Machová A.: This Journal *44*, 1179 (1979).
8. Zaoral M., Kolc J., Šorm F.: This Journal *32*, 1242 (1967).
9. Zaoral M.: This Journal *30*, 1853 (1965).
10. Vávra I., Machová A., Krejčí I.: J. Pharmacol. Exp. Ther. *188*, 241 (1974).
11. Krejčí I., Kupková B., Vávra I.: Brit. J. Pharmacol. Chemother. *30*, 497 (1967).
12. Munsick R. A.: Endocrinology *66*, 451 (1960).
13. Smith C. W., Walter R.: Science *199*, 297 (1978).
14. Zaoral M., Brtník F.: This Journal *40*, 905 (1975).

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