Notes

Solid-Phase Synthesis and Pressor Potency of [1-Deamino-9-ethylenediamine]-lysine-vasopressin†

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In connection with other work on lysine-vasopressin (LVP) and its deamino analog, we wished to have the analog of 1-deamino-lysine-vasopressin (deamino-LVP) in which the terminal glycinamide residue is replaced by that of ethylenediamine. LVP has the following structure, in which the numbers indicate the positions of the individual amino acid residues.

Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Lys-Gly-NH_2
1 2 3 4 5 6 7 8 9

It is to be noted that [1-deamino-9-ethylenediamine]-LVP, like LVP, possesses two amino groups per molecule. For the synthesis of the desired analog, the protected polypeptide-resin β-mercaptopropionyl(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Z)-resin was prepared by the Merrifield solid-phase method[1-3] using nitrophenyl esters[4] for the successive incorporation of all residues except Boc-proline, which was incorporated through the action of DCI in the presence of 1-hydroxybenzotriazole.[5] The final protected polypeptide-resin was treated with anhydrous ethylenediamine to yield β-mercaptopropionyl(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Z)-$NHCH_2CH_2NH_2$. This compound was treated with sodium in boiling NH_3[6] and the resulting disulfhydryl compound was oxidized in dilute aqueous solution with $K_3Fe(CN)_6$.[7] The [1-deamino-9-ethylenediamine]-LVP thus obtained was purified by partition chromatography[8,9] on Sephadex G-25 as described in the Experimental Section. A rat pressor potency[10] of less than 0.05 unit/mg was observed for the purified analog as compared to *ca.* 126 units/mg for deamino-LVP.[11] Zaoral, *et al.*, who synthesized [9-ethylenediamine]-LVP, found it to have a pressor potency of 0.002 unit/mg in comparison to 243 units/mg for their preparation of LVP.[12] These authors also found that the antidiuretic potency of their analog was two orders of magnitude less than that of LVP.

Experimental Section‡

Boc-Lys(Z)-resin. Chloromethylated copolystyrene–2% divinylbenzene (5.0 g, containing 1.9 mmol of Cl/g of resin), Boc-Lys(Z)-OH (3.76 g), and $N(Et)_3$ (0.88 g) in 15 ml of absolute EtOH were held at reflux for 46 hr. The esterified resin was washed with 95% EtOH and dried *in vacuo*, yield 6.7 g. A sample of the esterified resin was deprotected with 1 *N* HCl in AcOH and hydrolyzed in dioxane–1 2 *N* HCl (1:1) at 110° for 22 hr. Amino acid analysis[13] of the hydrolysate showed the product to contain 0.39 mmol of lysine/g of esterified resin.

β-Mercaptopropionyl(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Z)-resin. A portion of the Boc-Lys(Z)-resin (4.0 g) was washed with three 40-ml portions of AcOH. The following cycle of deprotection, neutralization, and coupling, interspersed with the indicated wash sequences, was carried out for the successive incorporation of each residue. (1) Boc groups were cleaved with 40 ml of 1 *N* HCl in AcOH for 25 min at room temperature, except that the cleavage of Boc groups from Gln was mediated by 40 ml of F_3CCOOH.[14] After cleavage, the resin was washed with three 40-ml portions each of AcOH, absolute EtOH, and DMF. (2) Hydrochlorides were neutralized with 1 ml of $N(Et)_3$ in 40 ml of DMF for 10 min at room temperature and the resin was washed with three 40-ml portions of DMF. (3) The appropriate nitrophenyl ester (1.56 mmol) dissolved in 40 ml of DMF was added to the neutralized polypeptide-resin. After 5 hr of shaking, 1-hydroxybenzotriazole (0.1 g) was added and shaking was continued for another 16 hr before washing with three 40-ml portions of DMF. For incorporation of the Boc-proline residue, Boc-proline (7.02 mmol) and 1-hydroxybenzotriazole (7.02 mmol) dissolved in 30 ml of DMF were added to the resin and the suspension was cooled to −10°. To this mixture was added DCI (7.02 mmol) in 20 ml of DMF at −10°. The suspension was allowed to come to room temperature and was shaken overnight before the washing with DMF. Steps 2 and 3 were repeated for each residue incorporated.[15]

After the second coupling step in each elongation cycle, a small aliquot of resin was withdrawn from the reaction vessel, washed with absolute EtOH, and subjected to a ninhydrin assay for free amino groups.[16] In every case a negative reaction was obtained except after the addition of Boc-phenylalanine, when a faint color was observed. In this case the resin was washed with three 40-ml portions of DMF, treated with 3-nitrophthalic anhydride[17] (1.0 g) and $N(Et)_3$ (1.0 ml) in 40 ml of DMF for 30 min, and finally washed with DMF as usual before the deprotection phase of the next peptide elongation cycle.

Following incorporation of the *S*-benzyl-β-mercaptopropionyl residue, the protected polypeptide-resin was washed successively with three 40-ml portions each of DMF, AcOH, absolute EtOH, and ether. The product was dried *in vacuo* over NaOH, yield 5.2 g.

β-Mercaptopropionyl(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Z)-$NHCH_2CH_2NH_2$. A portion (2.0 g) of the protected polypeptide-resin, dried at 80°, was suspended in a mixture of absolute MeOH (10 ml) and anhydrous ethylenediamine (10 ml).§ The mixture was stirred at room temperature for 20 hr with protection from atmospheric moisture. The mixture was then taken to dryness by rotary evaporation and the residue was triturated thoroughly with DMF. The DMF suspension was filtered and the filtrate was concentrated to 10 ml by rotary evaporation. The concentrated solution was added dropwise through a glass wool filter into a stirred bath (100 ml) of 1% AcOH in absolute EtOH to form a clear solution from which a precipitate began to separate after a few minutes. The precipitate was dissolved in 5 ml of AcOH and reprecipitated by dropwise addition to absolute EtOH. The precipitate was collected by filtration, washed with absolute EtOH and ether, and then dried *in vacuo*: yield 390 mg; $[\alpha]^{23}D$ −32.7° (*c* 0.6, AcOH). Amino acid analysis of an acid hydrolysate (6 *N* HCl, 22 hr, 110°) gave the following molar ratios: Cys(Bzl) 1.0, Lys 1.0, NH_3 2.1, Asp 1.0, Glu 1.0, Pro 1.1, Tyr 0.9, and Phe 1.0. *Anal.* ($C_{75}H_{92}N_{12}O_{13}S_2 \cdot C_2H_4O_2$) C, H, N.

[1-Deamino-9-ethylenediamine]-lysine-vasopressin. The partially protected polypeptide (133 mg) was treated with Na in boiling NH_3, cyclized in aqueous solution at pH 6.8 with $K_3FE(CN)_6$, and freed of salts according to previously published procedures.[18] Lyophilization of the desalted solution yielded 89.8 mg of white material. The lyophilized product (85 mg) was subjected to partition chromatography[8,9] on a Sephadex G-25 (100–200 mesh) column in the system *n*-BuOH–EtOH–3.5% AcOH in 1.5% aqueous pyridine (7:2:9). Effluent fractions (7.0 ml) were collected and assayed by the Folin–Lowry method[19] for peptide content. The resulting chromatogram showed a major peak centered at R_f 0.15, which was preceeded by a smaller peak at R_f 0.32. [1-Deamino-9-ethylenediamine]-lysine-vasopressin was isolated from the major peak: yield 35.5 mg; $[\alpha]^{23}D$ −85.8° (*c* 0.45, 1 *N* AcOH). Amino acid analysis of an acid

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‡Melting points were done in capillary tubes and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for the elements were within ±0.4% of the theoretical values.

§Ethylenediamine (98%) was stored for 2 days over sodium metal shavings, then refluxed briefly, and distilled from the sodium, bp 115–115.5°.

hydrolysate (6 *N* HCl, 20 hr, 110°) gave the following molar ratios: Lys 1.0, NH_3 1.8, Asp 1.1, Glu 1.1, Pro 1.0, Cys 0.8, mixed disulfide[7] of Cys and β-mercaptopropionic acid 0.3, Tyr 1.0, and Phe 1.0. *Anal.* ($C_{46}H_{66}N_{12}O_{11}S_2 \cdot C_2H_4O_2 \cdot 2H_2O$) C, H, N.

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A Bis-*N,O*-diacetylhydroxylamine Analog of Diaminodiphenyl Sulfone Possessing Antimalarial Activity

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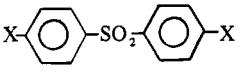
4,4′-Bis(*N,O*-diacetylhydroxylamino)diphenyl sulfone (TAHDS) was prepared and found to have good prophylactic activity against *Plasmodium berghei*. It is significantly more active than is 4,4′-diaminodiphenyl sulfone (DDS). 4,4′-Dihydroxylaminodiphenyl sulfone (DHDS), a possible metabolite of DDS, was prepared and found to be essentially inactive. The antimalarial activity of TAHDS does not appear to be the result of hydrolysis or metabolic conversion to DDS. TAHDS also possesses antileprotic activity.

4,4′-Diaminodiphenyl sulfone (DDS), used extensively for the treatment of leprosy[1] and of dermatitis herpetiformis,[2] has, since 1941, been known to possess antimalarial activity also.[3] No great interest in its antimalarial activity was aroused until 1960 when it was reported that leprotic patients undergoing treatment with DDS were protected from *falciparum* and to some extent *quartan* malaria.[4] Subsequent work by Elslager and coworkers[5] led to the development of repository combinations of DDS derivatives with cycloguanil or pyrimethamine.

The current regimen for prevention of chloroquine-resistant *falciparum* malaria is administration of DDS along with the standard antimalarial drugs chloroquine and primaquine. However, the use of DDS is undesirable due to the fact that it has a very short lifetime as a consequence of rapid metabolism, it causes methemoglobinemea,[6,7] and, particularly in combination with primaquine, DDS causes hemolysis.[8] The high doses of DDS required to compensate for rapid elimination from the body generates higher initial blood levels than are needed to suppress malaria and causes toxicity. Acylated derivatives, especially the *N,N′*-diacetyl DDS (DADDS), are reported to be longer acting, presumably as a consequence of slow enzymatic liberation of DDS. The dependence on enzymatic deacetylation results in erratic activity, and the rapid excretion of liberated DDS still results in low blood sulfone levels.

As part of studies aimed at determining whether a metabolite might be responsible for the antimalarial and/or the methemoglobinemia activity of DDS, we were interested in preparing 4,4′-dihydroxylaminodiphenyl sulfone. In the course of preparing this highly labile molecule for testing, a stable acetylated hydroxylamino derivative was also prepared which proved to be active against *Plasmodium berghei*.



DDS, X = $-NH_2$
DADDS, X = $-NHAc$
DHDS, X = $-NHOH$
TAHDS, X = $-N(OAc)Ac$

For the preparation of 4,4′-dihydroxylaminodiphenyl sulfone (DHDS), we investigated a procedure similar to that reported to give 2,2′-dihydroxylaminodiphenyl sulfone by catalytic reduction of the corresponding nitro compound.[9] However, attempts to reduce 4,4′-di(nitrophenyl) sulfone in this manner led only to complex mixtures of incompletely reduced labile materials. Matsukawa and coworkers[10] reported the preparation of DHDS, mp 170° dec, by reduction of the dinitro compound with Zn and aqueous EtOH. Repeated attempts to reproduce this reaction failed. Attempted preparation using other catalysts or reagents which normally reduce nitro compounds (Al and NaOH; Al-Hg and NaOH; Na_2S)[11] gave either no reaction, mixtures of high-melting solids, or the diamine.

Study of the Matsukawa procedure led to our finding that the reduction was catalyzed by traces of AcOH.† Using AcOH, coupled with a short reaction time to minimize thermally induced rearrangements, reproducibly yielded DHDS, mp 184–186° dec. This compound was found to be

†Although there are many reports of the successful application of Zn–NH_4Cl–EtOH reduction of nitro compounds to hydroxylamines, there are also many reports of frustratingly erratic behavior[11] and the significant variable has never been identified. We have found that, at least in the present reduction, the presence of a small amount of AcOH is essential. In its absence no reaction occurred; if too much was present, reduction continued all the way to the diamine. The presence of the small amount of AcOH repeatedly gave reproducible reductions.