^{4}PHE–^{6}VAL–ANTAMANIDE, AN ANTITOXIC CYCLODECAPEPTIDE WITH C_2 SYMMETRY*

Th. WIELAND, A. v. DUNGEN and Ch. BIRR

Max-Planck-Institut for Medical Research, Department of Chemistry, Heidelberg, Germany

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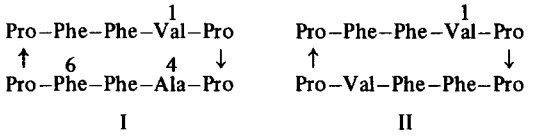
1. Introduction

In the course of our investigations on structure–activity correlations of analogues of antamanide (I) [1, 2], an antitoxic principle from the toadstool *Amanita phalloides* [3], we have also synthesized ^{4}Phe–^{6}Val–antamanide (II).



I II

The cyclopeptide II, in contrast to I, has C_2 symmetry and was expected to lend itself to a less difficult structure analysis than I. However, cyclopeptide II is of interest only if it also has antitoxic activity against phallotoxins. It is also possible to obtain an achiral cyclodecapeptide by substitution in I of ^{1}Val by Ala and an exchange of amino acid 4 ⇌ 6. However, the ^{1}Ala variant of I previously proved less than 5% as active as I, whereas substitution of ^{4}Ala by Val resulted in practically no loss of activity [3].

2. Materials and methods

The cyclodecapeptide II was synthesized by dimerizing cyclization of the pentapeptide III, Val–Pro–Pro–Phe–Phe, using the *p*-nitrophenylester of III [4] or dicylclohexylcarbodiimide (DCCI) [5] and hydroxysuccinimide [6]. Both methods gave only poor yields of II, and in addition the monomeric cyclization product IV, cyclo-Val–Pro–Pro–Phe–Phe.

Pentapeptide III was built up by stepwise condensation with DCCI on a solid phase from Boc-amino acids according to Merrifield [7] using an automated Peptide Synthesizer (Schwarz, BioResearch [1]). 10 g polymer, esterified with 1.57 mmoles/g Boc–Phe, was treated according to [1] successively with Boc-Phe, where Boc-Pro, Boc-Pro, and Boc-Val to give 15 g Boc–pentapeptide polymer. The Boc-derivative of III was removed from the resin by transesterification with 500 ml methanol, containing 58 g of ethyl-diisopropylamine [8]. The methylester Boc–Val–Pro–Pro–Phe–Phe–OMe (TLC on silica gel R_f 0.82 in chloroform–methanol–water (65:25:4), two slower moving impurities) was saponified in 20 ml dimethylformamide by adding 1 N aqueous NaOH at pH 10.9 in an autotitrator. After addition of 40 ml H_2O and acidification with citric acid, the Boc–pentapeptide was extracted into ethylacetate and, after H_2O-washing, drying the solution and evaporation in vacuo, was obtained as a solid of 4.9 g (44.3%), R_f 0.57, amino acid analysis: $Val_{1.0}$ $Pro_{2.05}$ $Phe_{2.5}$.

The nitrophenylester of pentapeptide III was obtained from 2.45 g of the Boc–pentapeptide and 1.85 g *p*-nitrophenol in 20 ml ethylacetate with 0.789 g DCCI at −10° for 3 hr and 20° for further 15 hr, filtering off the dicyclohexylurea formed, and evaporation in vacuo; R_f 0.60 (+ traces of the urea). In order to remove the Boc-residue, the substance was stirred in 45 ml trifluoroacetic acid (TFA) at 20° for 4 hr, and after evaporation in vacuo, dissolved in 20 ml dry tetrahydrofurane and precipitated by adding 100 ml of ether. Purification by chromotography on Sephadex LH-20 (5 × 180 cm) with methanol as a solvent yielded 1.9 g (65%) of the TFA salt of

* Part VIII of a series. Previous communication ref. [1].

Val–Phe–Phe–Pro–Pro–OPNP; R_f 0.71 (+ 2 weak spots of slower moving impurities).

Dimerizing cyclization

a) *p*-Nitrophenylester [4]. 1.9 g of the TFA salt of Val–Pro–Pro–Phe–Phe–OPNP was dissolved in 20 ml dimethylformamide and slowly added to 430 ml pyridine at 55°. After standing for further 3 hr at 55° and overnight at 20°, the mixture was evaporated, dissolved in methylenechloride, excess *p*-nitrophenol extracted with $NaHCO_3$ solution and the dried residue chromatographed on Sephadex LH-20 (5 × 180 cm) with methanol as solvent: the yield obtained was 40 mg of II as first and 27 mg of IV as second fraction.

b) 2.43 g Boc-derivative of III was treated with 30 ml TFA for 2 hr at 20°. The TFA-salt of III (R_f 0.41, single spot) obtained after evaporation was subjected to cyclization as described for a decapeptide in [6]. After chromatography as in a) 38 mg of II and 78 mg of IV were obtained.

3. Results

The ^{4}Phe–^{6}Val–antamamide (II) preparations obtained under a) and b) were crystallized by slowly adding water to their solution in acetone. Cyclopeptide II had an R_f of 0.68 by TLC (2-butanol–2 N NH_4OH, 100:44), m.p. 162–167°, m/e = 1174 by mass spectrometry and showed the correct amino acid composition. The substance at a dose of 1.5–2 mg/kg had a full protecting activity against 5 mg phalloidin per kg body weight of the white mouse as compared with 0.5 mg per kg of antamanide (I).

Cyclopentapeptide IV had an R_f of 0.62 (same solvent), m.p. 147–150°, the correct amino acid composition, m/e = 587 and *no* protecting action at doses as high as 20 mg per kg.

The optical rotatory dispersion (ORD) curve of II has a different shape in methanol or in dioxane respectively, similarly to antamanide [9]. In a study of several 1,4 substituted analogues [2] Lapatsanis observed a parallelism between the maximal negative rotatory value in dioxane at 240 nm and the antitoxic activity e.g. antamanide (I) showing [M] = -8.5×10^4 and the inert ^{1}Gly^{4}Gly-analogue showing [M] = -2.0×10^4 [10]. The new analogue II had [M] = -8.7×10^4; it also shows affinity for Na ions as apparent from ORD curves with and without Na^+ in methanol similarly to antamanide [9].

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