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38. Isolation and Structure Analysis of a Photoproduct of the New Photoaffinity Label *p*-Nitrophenylalanine

Preliminary Communication

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Summary

The isolation and structural analysis of a photoproduct of Ac · *p*-nitrophenylalanine ethyl ester is described and discussed. Nitrophenylalanine is proposed as a hydrogen fluoride stable photoaffinity label.

In previous work [1] [2] the amino acid L-*p*-Nitrophenylalanine has been found to be photolabile and described as a new photoaffinity label besides the already known *p*-Azidophenylalanine [3]. This unexpected behaviour of the nitro compound led to further photochemical investigation about photoproducts and mechanisms.

This photolabel has become of importance because of its stability against HF-treatment with the *Sakakibara* technique [4], whereas *p*-Azidophenylalanine is quickly

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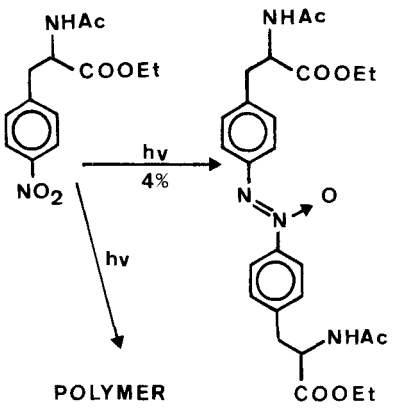
decomposed [5]. To overcome this disadvantage especially in solid phase synthesis of peptide hormone analogs for photoaffinity labelling, *p*-Nitrophenylalanine will be used in standard syntheses.

With thin layer chromatography (TLC.), it has been shown that the photoproducts of *p*-Nitrophenylalanine are independent of the photolytic wavelength between 300 and 365 nm. Comparison with *p*-azidophenylalanine-photolysis showed in TLC. no substances with the same Rf-values; therefore, it can be assumed that the *p*-nitrophenylalanine photolysis does not have the same nitrene intermediate as *p*-azidophenylalanine [6] [7].

All photolysis experiments in aqueous media using totally or partially unprotected *p*-nitrophenylalanine led to no identifiable products, always tar-like residues resulted resisting to further work up. This indicates a radical mechanism upon photolysis.

Irradiation of N-acetyl-*p*-nitrophenylalanineethyl ester in water ethanol led in poor yield (4%) to a crystalline product which has been identified with combined chemical and spectroscopic techniques as *p*, *p'*-azoxy-di-(N-acetyl-phenylalanine-ethylester).

Nitroaromatic compounds give in alcohol in presence of alkali also azoxy-derivatives [8]. The present product is therefore rather a product of photoreduction. The analogous azo- and the mononeric nitroso- and amino-phenylalanine derivatives could not be detected.



Experimental Part

Preparative photolysis has been performed in 250 ml irradiation vessel equipped with a *Osram* HQA 80W mercury source and pyrex glass filtering at 14° under nitrogen. Photolysis for TLC.-assays have been performed analogous to [1] [2].

1.0 g L-NAc · (*p*-NO_2)Phe · OEt (Mol.-Wt. 280, 3.57 mmol) were irradiated during 20 h in 200 ml water/ethanol 7:3. After evaporation i.V. the residue was passed through a prepacked silicagel column (*Merck*, Kieselgel 60, Type C) with a chloroform/methanol gradient 0→15%. After a second identical chromatography, the product has been recrystallized 3 times from water/ethanol. Yield was 41 mg (4%), yellowish needles. Hydrogenation with Pd/C yields NAc · (*p*-NH_2)Phe · OEt [2], control with TLC. and 60-MHz-^{1}H-NMR.

Structure analysis and characterization of the photoproduct. Rf Educt 0.46, product 0.20 (*Merck*, precoated TLC. plates, Silicagel F 254, solvent chloroform/methanol 20:1). – M.p. (3 × recrystallized,

open capillary, uncorrected): 195.8°. – *Tollens* test for –N–O: positive. – $[\alpha]^{20}$ (*Perkin-Elmer* 141 Polarimeter, in parentheses the ware-lenght in nm): 184° (365), 184° (436), 71.6° (546), 58.8° (578), 55.0° (589). – UV. (*Beckman* Acta V, recorded in EtOH): λmax 332 nm, (ε=6,260) (aromatic azoxy-compounds have λmax at 323 nm, aromatic N-nitrosamines at 295 nm). – IR. (*Beckman* IR 33, Nujol): no additional or different characteristic bands were detectable compared to the educt. – ^{1}H-NMR. (*Varian* HA 220, Mr. *A. Bundi*, Biophysik ETH Zürich, 220 MHz, recorded in $CDCl_3$, TMS-lock; Chemical shift δ in ppm, *s*=Singlet, *d*=Doublet, *t*=Triplet, q=Quartet, *m*=Multiplet, *J*=coupling constants (in Hz)): 8.05 (*d*, *J*=9, 2H); 7.95 (*d*, *J*=8, 2H); 7.15 (*d*, *J*=8, 2H), and 7.10 (*d*, *J*=9, 2H) (2×AA'BB'-System, aromatic H's); 6.10 (*t*, 2H, amid-H); 4.80 (*q*, 2H, H–C(α); 4.10 (*q*, *J*=7, 4H, O–CH_2); 3.10 (*m*, 4H, 4H–C(β); 1.95 (*s*, 6H, $^2COH_3$); 1.20 (*t*, *J*=7, 6H, O–CH_2CH_3). – ^{13}C-NMR. (*Varian* XL 100, Mr. *Ch. Grathwohl*, Biophysik ETH Zürich; recording in chloroform with TMS as internal standard; H-broad/band decoupling and *Fourier* transformation; chemical shift δ is indicated in Hz): 4551.2/4546.3 (*d*, 2C, $COCH_3$); 4504.7 (2C *C*OOEt); 3941.5, 3832.5, 3775.0 and 3708.6 (4C, C(1), C(1'), C(4), C(4')); 3500.5, 3401.2, and 3316.6 (8C, C(2), C(2'), C(3), C(3'), C(5), C(5'), C(6) and C(6')); 1792.1/1788.9 (*d*, 2C, 2 $COOCH_2CH_3$); 1570.8 (2C(α); 1188.7/1183.4 (*d*, 2C, 2C(β); 820.0 (2C, 2 $COCH_3$); 592.7 (2C, 2 $COOCH_2CH_3$). – MS. (*Hitachi-Perkin-Elmer* RMU, PD Dr. *J. Seibl*, ETH Zürich): 512/513 (513 is $^{12}C_{25}+^{13}C$, M^+).

$C_{26}H_{32}N_4O_7$ (512) Calc. C 60.92 H 6.29 N 10.93% Found C 60.71 H 6.33 N 10.81%

(The elemental analysis was executed by Mr. *W. Manser*, ETH Zürich).

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