THE REACTION OF DIMETHYL(2-HYDROXY-5-NITROBENZYL)SULFONIUM BROMIDE WITH *N*-ACETYL-L-TRYPTOPHAN AMIDE

C.P. HEINRICH, S. ADAM and W. ARNOLD
Research Department of F. Hoffmann-La Roche & Co., Ltd., 4002 Basel, Switzerland

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1. Introduction

In the course of our studies on the thiamine diphosphate binding site in transketolase from baker's yeast, tryptophan was found to be involved in coenzyme binding. Using the newly synthesized water-soluble tryptophan reagent dimethyl(2-hydroxy-5-nitrobenzyl)sulfonium bromide (II) [1] it was possible to modify the tryptophan at the active site of the enzyme [2]. The tryptophan modification could be followed continuously by magnetocircular dichroism measurements [3]. In order to obtain more detailed information on the chemistry of the modification reaction, we have studied the reaction between the sulfonium bromide II and *N*-acetyl-L-tryptophan amide (I) as a model system for the tryptophan moiety in a peptide backbone.

There are reports on the reaction of 2-hydroxy-5-nitrobenzyl bromide with tryptophan and some indole derivatives which have demonstrated a very complicated reaction, because the indole ring system can be attacked preferentially at position 3, but 2- and 1-substitutions are also found [4–7]. In addition, disubstitution as well as different kinds of ring closures can occur [5–7].

Even more complex alkylations had to be expected for the dimethyl sulfonium reagent II, since methylation reactions have to be taken into account. Although II has been successfully used in the modification of tryptophan, small peptides and some proteins [1, 2], the reaction products have not yet been characterized.

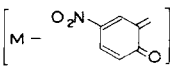
2. Materials and methods

PMR spectra were run on a Varian HA 100 spectrometer in dimethylsulfoxide(d_6) with tetramethylsilane as internal standard. Mass spectra were obtained on an A.E.I. MS-spectrometer by use of the direct insertion technique. TLC was performed on silica gel G (Merck).

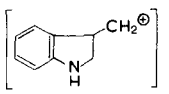
2.1. *Reaction of N-acetyl-L-tryptophan amide (I) with dimethyl-(2-hydroxy-5-nitrobenzyl)sulfonium bromide (II)*

1 mmole of I dissolved in 20 ml of an aqueous solution of ethanol (1:1, v/v) was allowed to react at 25°C with 1.2 mmoles of II for 60 min. A pH of 4.7 was kept constant by addition of methanolic sodium acetate. At least 10 different products were separated by thin-layer chromatography. The reaction mixture was extracted 3–5 times with ether and evaporated to dryness. The residue was dissolved in 0.5 ml of ethanol and subjected to preparative TLC (silica gel plates, 20 × 20 cm of 0.5 mm thickness). The solvent system was ethanol (10%) in chloroform. The plates were eluted 6 times and dried after each elution.

The compound having the same chromatographic mobility as I in the TLC system described was obtained as a crystalline product (mp 158°C dec. after 2 recrystallisations from MeOH) in 10% (40 mg) yield. Structure VI was assigned to this compound.



Scheme 1.



Scheme 2.

The mass spectrum of VI (source 145°C, 70 eV, 100 μA) shows a molecular peak at m/e = 396 (2%) [M] and peaks at 245 (12%) (scheme 1) 186 (35%) [245 – CH_3CONH_2] ; 130 (100%) (scheme 2).

2.2. *PMR spectrum of VI in DMSO-d_6, δ-values in ppm, internal TMS = 0 ppm*

1.79, s, -$COCH_3$; ~ 2.0 and ~ 2.28, AB-part of ABX-spectrum, J_{AB} ~ 13 Hz, J_{AX} ~ 13 Hz, J_{BX} ~ 5 Hz, 4-CH_2; 2.96, center of AB-spectrum, J_{AB} ~ 13 Hz, aryl-CH_2; ~ 4.06, m, X-part of above-mentioned ABX-spectrum, further coupling with NH of ~ 9 Hz, which disappears after D_2O-addition; 5.03, dd, J ~ 3 Hz, J ~ 1 Hz, s after D_2O-addition, 9a-proton; 6.01, slightly broadened, disappears after D_2O-addition, –NH–, assumed to be 9-proton; 6.47–6.73, m, 2 aromatic H; 6.9–7.1, m, 3 aromatic H, with H_a, d, J = 9 Hz at ~ 6.97; 7.59, d, J = 3 Hz, H_c; 7.79, d, J ~ 3 Hz, disappears after D_2O-addition, –NH–, assumed to be 1-

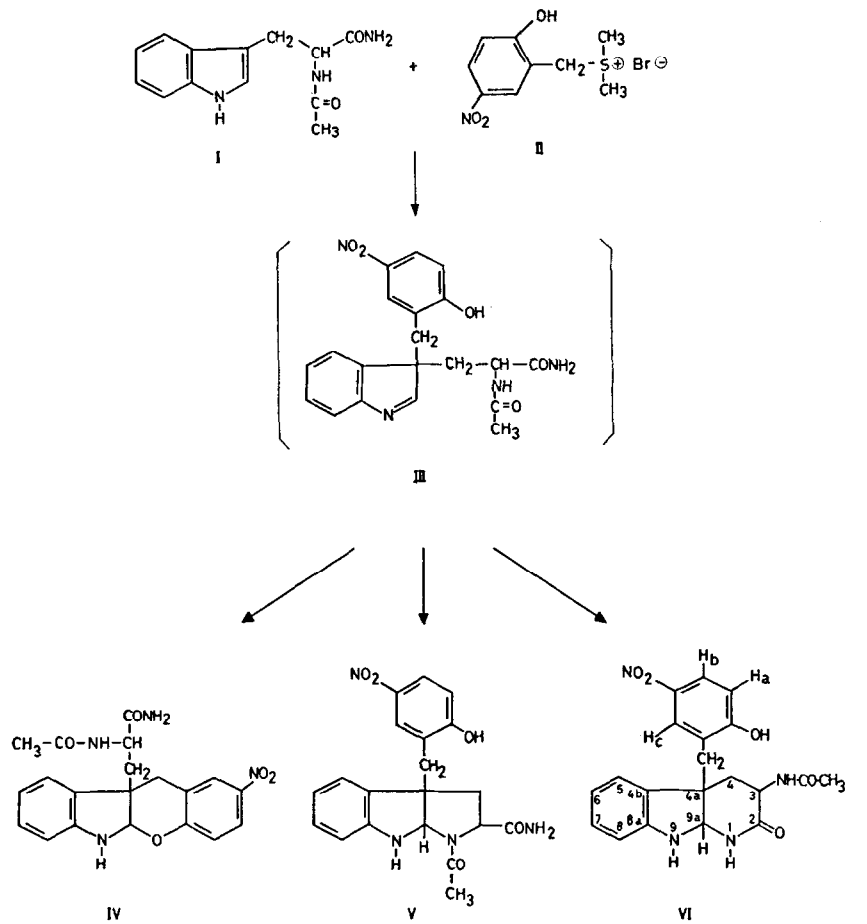


Fig. 1.

NH; 7.85, d, $J \sim 9$Hz, disappears after D_2O-addition, –NH–Ac; 7.96, dd, $J \sim 9$ Hz, $J \sim 3$ Hz, H_b; ~ 10.9, very broad, disappears after D_2O-addition, phenolic-OH.

3. Results and discussion

When I was allowed to react with II at a constant pH of 4.7, at least 10 different reaction products could be detected. Four of them were isolated. All exhibited the same molecular ion peak (*m/e*, 396), indicating monosubstitution by the 2-hydroxy-5-nitrobenzyl (HNB) group. Whereas the work on the elucidation of the structures of three of the above-mentioned compounds is still going on, we are able to report the structure of VI, the most stable and abundant one. The optical density at 410 nm of an alkaline solution of a definite amount of VI clearly demonstrated that one mole of HNB per mole of I was incorporated. An extinction coefficient of 18 000 M^{-1} cm^{-1} for HNB was used [8].

Fig. 1 shows the various ring closures of III as an assumed intermediate in the reaction of I with II. Structure IV was expected according to Spande et al. [7], where 3-methyl-indole reacted with 2-hydroxy-5-nitrobenzyl bromide, whereas reaction products of type V were formed when tryptophan [5], tryptophan ethyl ester [6] and *N*-acetyl-tryptamine [7] were allowed to react with 2-hydroxy-5-nitrobenzyl bromide. However, in the case of *N*-acetyl-tryptophan amide (I) there exists a further possibility of cyclization leading to the 2-piperidone ring system (VI). Evidence for structure VI was obtained from the PMR spectrum (see Materials and methods). Special features of the PMR spectrum which support structure VI and eliminate III, IV and V, are the signals of three NH-groups which are coupled to CH, and the shift and signal pattern of the 9a proton. The shift of about 5 ppm compared with the value of ca. 5.8 ppm for the corresponding proton in a compound of type V described by Spande et al. [7] proves the absence of a vicinal *N*-acetyl group with its deshielding effect. Furthermore, the signal pattern of the 9a-proton (doublet of doublets) and its change after D_2O addition (sharp singlet) proves that it is linked to two NH-groups. Also the absence of the typical signals of a $-CONH_2$ group excludes V unambiguously, while the presence of the broad phenolic OH-signal excludes structure IV.

To our knowledge, it is the first time that the reaction described leads to the formation of a 2-piperidone ring system.

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