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DIMERIZATION OF THE TRYPTOPHYL MOIETY

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A compound isolated from solutions of N-acetyl-tryptophan methyl ester in hydrogen fluoride and trifluoracetic acid was found to be a dimer with the structure shown in Fig. 1.

Side reactions are known to occur during synthesis of peptides containing tryptophan, because tryptophan residues are acid-labile. Theodoropolous and Fruton1) suggested that on treatment of a carbobenzoxy-tryptophan peptide with hydrogen bromide in acetic acid tryptophan might be oxidized to β-oxyindolylalanine. This has not been proved, but β-mercaptoethanol2) and tryptophan3) have been successfully used in some cases to protect tryptophan residues from modification during peptide synthesis. In previous studies on the effect of anhydrous liquid hydrogen fluoride on hen egg-white lysozyme4,5) under the conditions for peptide synthesis, we found that tryptophan did not act as a scavenger, but influenced the fatal effect on the enzymatic activity of hen egg-white lysozyme, which has six tryptophan residues. Thus, we became interested in the side reactions of tryptophyl moieties under conditions for peptide synthesis, and particularly in acidic media such as hydrogen fluoride and trifluoracetic acid.

In this work, we isolated and identified a compound formed using a solution of N-acetyl-tryptophan methyl ester (Ac-Trp-OMe) in hydrogen fluoride and trifluoracetic acid, as a model of peptide synthesis.

Treatment of Ac-Trp-OMe with Hydrogen Fluoride or Trifluoracetic Acid. Solutions of Ac-Trp-OMe I in anhydrous liquid hydrogen fluoride (HF)6) and anhydrous trifluoracetic acid (TFA) were kept at 0°C for 90 min and at room temperature for 60 min, respectively and then analyzed by thin layer chromatography. Both solutions gave several yellow-greenish or blue coloured spots with Ehrlich's reagent. To isolate the reaction products, the solution was concentrated to an oily residue *in vacuo*, and this was triturated with a mixture of ethanol and ether. In a typical experiment, treatment of Ac-Trp-OMe (10 g) with TFA (60 ml) at room temperature overnight gave a crude product (4.1 g), which was recrystallized as colorless fine needles (2.8 g). The properties of the crystalline product II, isolated from solutions of compound I in HF and TFA, are shown in Table 1.

Table 1. Comparison of Ac-Trp-OMe I with Compound II

	I			II		
Mp(°C)	153-154 (Lit.7) 151)			218.3-218.8		
$[\alpha]_D^{25}$ (c 1, MeOH)	+13.2 (Lit.7) +13.2)			+133.6		
TLC (R_f)						
$CHCl_3$:Acetone (2:1)	0.50			0.30		
$CHCl_3$:CH_3CN (4:1)	0.29			0.06		
$CHCl_3$:MeOH:AcOH (95:5:5)	0.62			0.49		
UV (22°C)						
λ_{max}^{MeOH} nm(ε)				292(1.13×10^4), 284(1.19×10^4)		
λ_{min}^{MeOH} nm(ε)				289(1.09×10^4), 260(8.73×10^3)		
MS8)						
molecular ion (m/e)	260			520		
Analysis	C	H	N	C	H	N
Found:	64.87	6.16	10.90	64.37	6.15	10.99
$C_{14}H_{16}O_3N_2$:	64.60	6.20	10.76			

Determination of the Structure of Crystalline Product II. Mass spectra8) of the crystalline product II showed a molecular ion at m/e 520, which is twice as compared with that of the starting compound I. This suggested that compound II was a dimer of Ac-Trp-OMe, since it gave the same values on elemental analysis as compound I. For further examination of the structure of compound II, its PMR spectra$^{9)}$ (Fig. 1) in dimethylsulfoxide-d_6 (DMSO-d_6) with and without D_2O were compared with those of compound I. The presence of two types of $\rangle CH(\alpha)-CH_2(\beta)-$ was deduced from the results of decoupling studies: the coupling of the α-methine proton [H, m, δ 4.25-4.32] with β-methylene protons [2H, m, δ 1.95-2.16] and the coupling of the α-methine proton [H, m, δ 4.52-4.60] with β-methylene protons [2H, q, δ 3.08-3.18]. By irradiating the region [δ ~ 3.50] of the ester group, in which one proton was thought to be concealed, this concealed proton was demonstrated to be coupled with β-methylene protons in higher field and also with a proton [H, d-d, δ 4.67-4.73], which was further coupled with a exchangable proton [H, d, δ 5.97]. These results suggest that the β-carbon of the former type is attached to the 3-position of $-\overset{(3)}{\underset{|}{C}}H-\overset{(2)}{\underset{|}{C}}H-\overset{(1)}{N}H-$. On the other

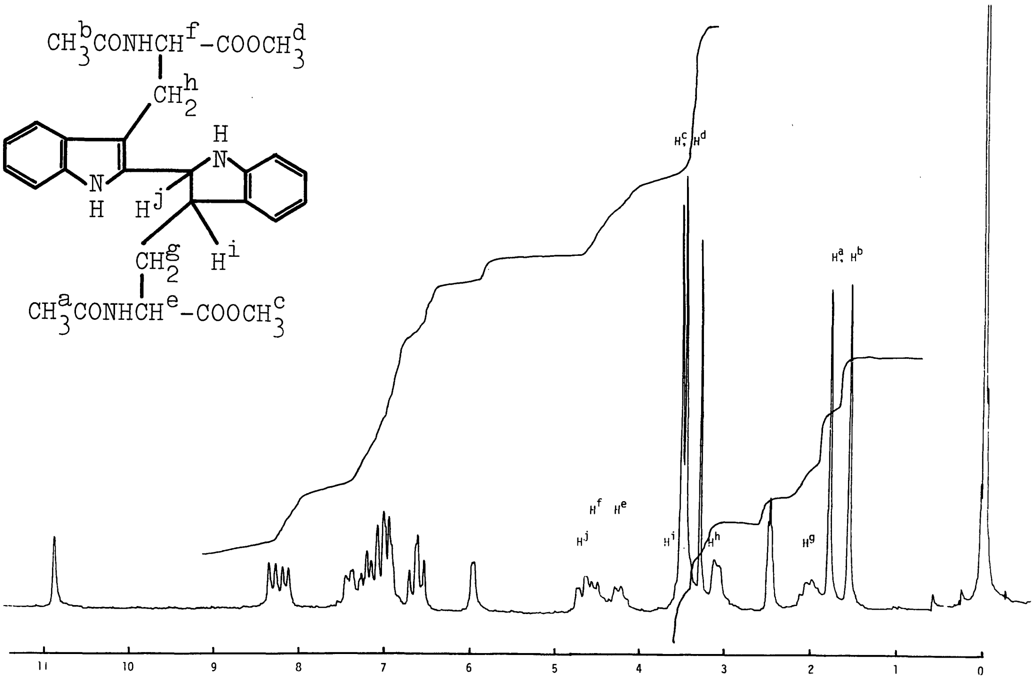

Fig. 1 NMR of compound II in DMSO-d_6 at 100 MHz

hand, the β-methylene of the latter type combines with the β-carbon of the indole ring, since β-methylene protons were coupled only with an α-methine proton and the observed chemical shifts were very similar to those of Ac-Trp-OMe.

It is concluded from these results that compound II has the structure shown in Fig. 1.

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8) A Hitachi RMU-6M Spectrometer was used with 80eV ionizing energy, 70μA emission current, and an ion source temperature of 200°C.
9) PMR spectra were recorded on a JEOL PFT-100 pulse Fourier transform NMR spectrometer locked on deuterium and equipped with FT-1A pulse control system. Chemical shifts were measured relative to the internal reference of TMS.

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