Hydrogen-Deuterium Exchange in Tryptophan

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A solution of tryptophan (T) in anhydrous or aqueous CF_3COOH at room temperature undergoes no significant changes within 3—4 days as seen by a proton magnetic resonance (PMR) spectrum. This is drastically changed for solutions of (T) in 0—25 % (vol./vol.) CF_3COOD + D_2O. Exchange of hydrogen by deuterium starts at once, continuing through 2—3 days towards an equilibrium.

Most of our PMR spectra (referred to tetramethylsilane (TMS) as internal standard) were recorded at 60 $Mcsec^{-1}$ instrumental frequency, a few also at 220 $Mcsec^{-1}$. As expected, they show that the COOH and the NH_2^+ and NH_3^+ protons exchange instantaneously, while the protons of the $-CH_2CH <$ group remain unexchanged. The "aromatic" protons 2, 4, 5, 6, and 7 (Fig. 1.) exchange with fairly different rates. We intend to indicate qualitatively the relative rates of exchange of these protons.

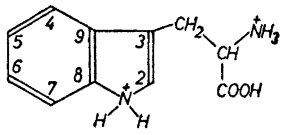

Fig. 1. Tryptophan in CF_3COOH. "Aromatic" hydrogen atoms at positions 2, 4, 5, 6, and 7.

In the PMR spectrum (60 $Mcsec^{-1}$) of (T) in CF_3COOH the H(2) signal is at once identified at 7.724 ppm, since it is the only sharp singlet occurring, owing its non-multiplet character to the rapid chemical exchange of NH_2^+ protons with CF_3COOH protons. In the spectrum of a freshly prepared solution of (T) (50 mg) in CF_3COOD (500 μl) the H(2) signal decreases rapidly while a second signal immediately starts to increase, only to end as a singlet at 7.575 ppm. This signal must be generated by either H(4) or H(7) as a result of rapid H(5), respectively H(6), exchange since rapid exchange of H(4) and/or H(7) is not going to produce any singlet. The location of the increasing singlet at the low-field end of the spectrum suggests that it must be assigned to H(7) which is geometrically closer to NH_2^+ than H(4). Therefore, H(6) must be a rapidly exchanging proton while H(5) is exchanged much slower. The sharpness of the H(7) resonance also shows that the *meta* coupling J_{57} must be small, of the order of 1 $csec^{-1}$. Next, a freshly prepared solution of 75 mg 90 % deuterated (T) (not deuterated in the $-CH_2CH <$ group) dissolved in 250 μl CF_3COOH and 250 μl CF_3COOD, was investigated. Mono-deuterated species dominate this mixture in the beginning. All five chemical shifts of

Table 1. Chemical shifts, relative to internal TMS, of H(2), H(4), H(5), H(6), and H(7) of tryptophan dissolved in CF_3COOD, at 60 and 220 $Mcsec^{-1}$ instrumental frequencies. Exchange rates are given qualitatively.

Proton	60 $Mcsec^{-1}$		220 $Mcsec^{-1}$		Exchange rates
	cps	ppm	cps	ppm	
H(2)	434.8	7.247			fast
H(4)	445.2	7.420	1633.0	7.423	slow
H(5)	437.4	7.290	1603.0	7.286	slow
H(6)	432.0	7.200			fast
H(7)	454.5	7.575	1667.7	7.580	slow

Table 1 could be read directly. In 15—45 min (room temperature) the H(2) peak, already identified, increases markedly. A second peak, at 7.200 ppm, behaves similarly. Therefore, it must be due to H(6). Of the remaining three peaks (increasing only slowly) at 7.575, 7.420, and 7.290 ppm, the line at 7.575 ppm has already been identified above as originating from H(7). The line at 7.420 ppm conserves its pronounced singlet character contrary to the signals at 7.575 and 7.290 ppm. Since the rapid introduction of hydrogen in the 6-position provokes multiplet structure for the H(7) and H(5) resonances, the line at 7.420 ppm must be assigned to H(4). The pronounced singlet character of this line under conditions where we know that H is present in the 6-position shows that the *meta* coupling, J_{46}, is small. Thus, both *meta*-couplings are small. The derived chemical shifts of protons 2, 4, 5, 6, and 7 are collected in

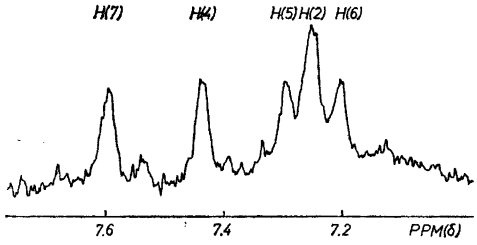

Fig. 2. Time averaged PMR spectrum (50 scans), in the region 7.0–7.8 ppm/TMS, of tryptophan dissolved in CF_3COOD at equilibrium. The ratio of exchangeable H to D is 0.25. The broad unresolved band at *ca.* 7.1 ppm is due to remaining NH_3^+ protons.

Table 1 together with "fast" or "slow" for the H → D exchange rate in CF_3COOH at room temperature.

These conclusions agree completely with a PMR spectrum of (T) in CF_3COOD taken by us at 220 Mcsec^{-1}. This spectrum was recorded at a stage where signals from the rapidly exchanging protons 2 and 6 are almost absent. Only H(4), H(5), and H(7) resonances occur. J_{57} can again be seen to be insignificant (*ca.* 1 csec^{-1}). J_{45} is derived to be 8.2 csec^{-1}. J_{67} (from "backward exchange" experiments) likewise is 8.2 csec^{-1}. More indirectly, we conclude $J_{56} = 6.5 \pm 0.5$ csec^{-1}.

The assignment of Table 1 also agrees with the observed intensity distribution in a spectrum of an equilibrium mixture of (T) and CF_3COOD (the [H]/[D] ratio of the mixture being 25 %), recorded after three days at room temperature. Here, the H(4) and H(7) peaks must appear with equal intensity (1.0 on an arbitrary scale), both stronger than the equally intense signals from H(5) and H(6) (0.8). The H(2) resonance must dominate (1.3). Fig. 2 shows this to be the case.

When the H_2O/CF_3COOH (vol./vol.) % > 50 no exchange takes place. This has the important consequence that the tryptophan residue can be deuterium- or tritium-marked directly in the polypeptide chain at high acidity and afterwards transferred to neutral medium, conserving its nuclear labels. Under similar conditions the phenyl ring hydrogen atoms of the amino acid residues phenylalanine and tyrosine, both generally occurring in polypeptides, are not exchanged by deuterium.

Received July 3, 1967.

Über einige Derivate des Trichlormethylthiolcarbamats

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Das Trichlormethylthiocyanat (*I*) ist schon seit längerem bekannt,[1] jedoch finden sich über seine Reaktionen in der Literatur keine Angaben. Wie wir feststellen konnten, lassen sich Anlagerungsreaktionen an die Kohlenstoff-Stickstoff-Dreifachbindung von *I* durchführen, wenn auch die Reaktivität im Vergleich zu anderen Thiocyanaten [2] herabgesetzt ist. In 95 %-iger Schwefelsäure lagert *I* nach (1) Wasser an. Die Reaktionsdauer ist für die Ausbeute kritisch. Die letztere beträgt nach 0,5

$$CCl_3SCN + H_2O \xrightarrow[0-5^\circ]{H_2SO_4} \underset{I}{} \qquad (1)$$

$$CCl_3-S-\overset{\overset{O}{\|}}{C}-NH_2$$

II

Stunden 1 %, nach 17 Stunden 14 % und nach 44 Stunden 1 %. In Polyphosphorsäure als Reaktionsmedium gelingt die Wasseranlagerung an *I* nicht.

II lässt sich im Eintopfverfahren am Stickstoff nach (2) mit Aldehyden kondensieren bzw. mit sekundären Alkoholen nach (3) N-alkylieren (vgl. Ref. 2).

$$2\ CCl_3SCN + RCHO + H_2O \xrightarrow[0-10^\circ]{H_2SO_4} \underset{I}{}$$

$$CCl_3-S-\overset{\overset{O}{\|}}{C}-NH-\underset{\underset{R}{|}}{CH}-NH-\overset{\overset{O}{\|}}{C}-S-CCl_3 \qquad (2)$$

III

$$CCl_3SCN + RR'CHOH \xrightarrow[-5-5^\circ]{H_2SO_4} \underset{I}{} \qquad (3)$$