Definitive Assignment of ^{13}C NMR Signals in Tryptophan and Related Molecules

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Selective photodeuteration of carboxyltryptamines at C-4 allowed to settle the controversy about the ^{13}C nmr assignment of C-4, C-5 and C-6, which for tryptophan are definitively assigned at 118.4, 118.2 and 120.6 ppm, respectively.

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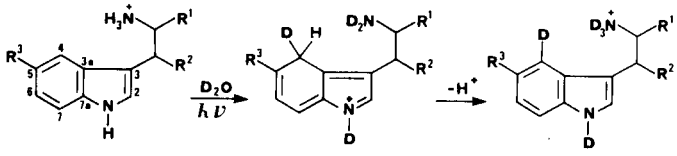
In connection with synthetic studies on therapeutically valuable tryptophan derivatives [1] we became interested in studying their ^{13}C nmr spectra, since previous studies [2-16] showed inconsistency in the assignment of the signals arising from carbons 4, 5 and 6. In these studies the basis for most of the assignments was provided by shift comparison with the spectrum of the parent indole [17], which had been assigned with the help of selective decoupling experiments and off-resonance splittings. A further assignment of tryptophan methyl ester [5] was arbitrarily extrapolated from 5-deuteroindole and their derivatives [18]. More recently, data derived for C-13 enriched tryptophan (C3) [6] and INDO calculations [7] were published. It should be noted that the order of increasing chemical shifts for C-4, C-5 and C-6 culled from published data are widespread, as shown by the following sets: C4 < C5 < C6 [15], C6 < C5 < C4 [2,14], C6 < C4 < C5 [8], in perdeuterodimethyl sulfoxide; C4 < C5 < C6 [6], C4 < C6 < C5 [4,9], C6 < C5 < C4 [2,12] in deuterium oxide; C4 < C5 < C6 [7] in water and C4 < C5 < C6 [5,13,15] in deuterochloroform. Similarly, for 5-hydroxyindole and derivatives [7,19-23] an analogous situation is recognized for the signal owing to carbons 4 and 6. Conclusions drawn from such data are often uncertain, as shown by various "unambiguous" assignments for tryptophan and related compounds.

We now report an independent assignment of three carboxyltryptamines to settle the controversy using 4-deuterio derivatives. Selective deuteration is often a laborious procedure. However photochemical hydrogen-deuterium exchange has been shown to be a highly selective and very simple procedure in these systems. Photosubstitution of the C-4 hydrogen of tryptophan has been suggested [8] to follow a mechanism involving intramolecular proton transfer from the α-ammonium group. This process plays an important role in nonradiative decay of singlet tryptophan at neutral *p*H. Such isotope incorporation was achieved by external photodeuteration with Pyrex-filtered uv light from a 400 Watt high pressure mercury lamp. In our experiments, immersion irradiation of a degassed solution of the corresponding carboxyltryptamine in deuterated water using a low-pressure 110-Watt mercury lamp provided 4-deuterotryptophan, 3-amino-2-(4-deuterio-3-indolyl)propionic acid and 3-amino-2-(4-deuterio-5-methoxy-3-indolyl)propionic acid (Scheme 1).

Scheme 1

	R^1	R^2	R^3
1	CO_2^-	H	H
2	H	CO_2^-	H
3	H	CO_2^-	OCH_3



It is noted that 5-deuteroindol has been investigated for signal assignments [18]. However, in this case the C-4 signal can not be unambiguously identified and distinguished from that of C-6 signal, since these carbons are both *ortho* to the deuterated site and underwent similar deuterium isotope shifts. In practice, the signal of a deuterated carbon almost disappears while adjacent carbons exhibit an upfield isotope shift of a few tenths of a ppm [24]. Moreover the carbon β to the site of deuteration looses the long-range coupling constant ($^3J_{CH}$). These effects make a 4-deuteroindole derivative a more suitable compound.

Comparison of the spectra of tryptophan (**1**) with those of the 4-deuterio derivative is depicted in Figure 1. The C-4 resonance was assigned in the ^1H-decoupled spectrum from the disappearance of the signal at 118.4 ppm. C-6 assignment is evident in the gated-decoupling experiment, since the original double doublet ($^1J_{CH}$ = 158.4 Hz; $^3J_{CH}$ = 7.1 Hz) at 120.6 ppm looses the three bond long-range coupling upon deuteration at C-4. The remaining controversial assignment is that of C-5, which appears at 118.2

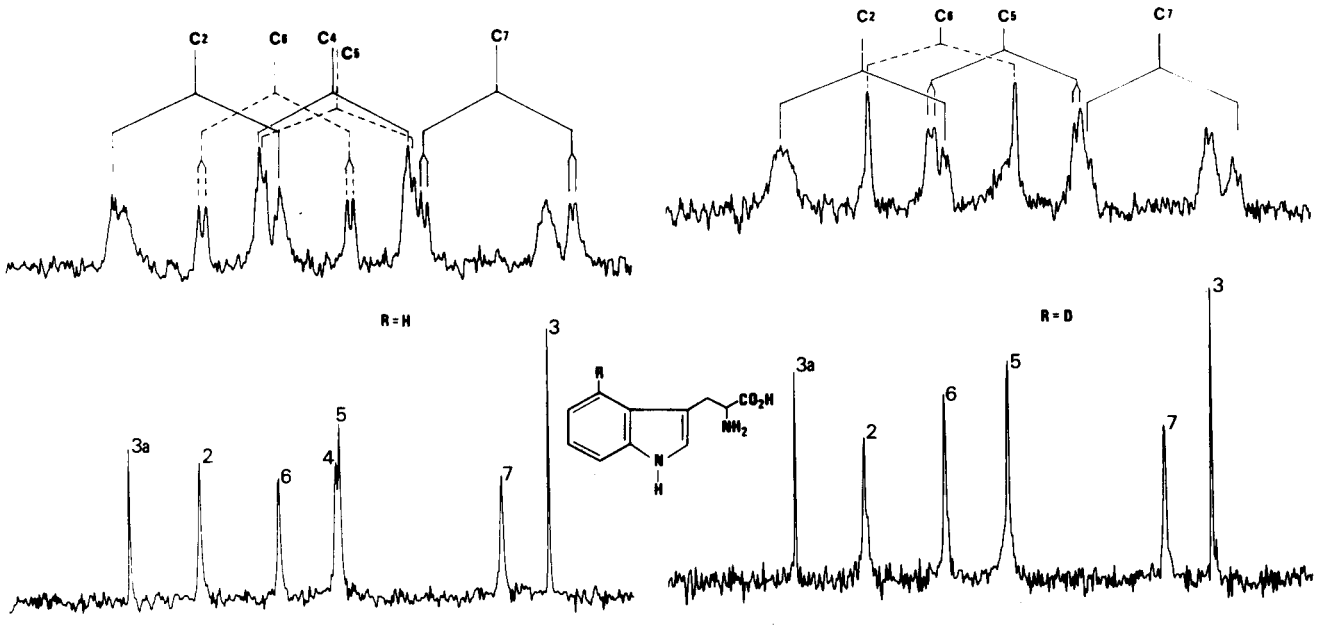


Figure 1. The 130 to 105 ppm region of the ^{1}H-decoupled and ^{1}H-coupled ^{13}C nmr spectra of tryptophan and 4-deuterotryptophan.

Table I

C-13 NMR Spectra [a] of Carboxyltryptamines

Compound No.	Chemical Shift C2	C3	C3a	C4	C5	C6	C7	C7a
1 [b]	124.2 (d)	109.4 (s)	127.3 (s)	118.4 (d)	118.2 (d)	120.6 (d)	111.4 (d)	136.3 (s)
2 [c]	123.3 (d)	112.5 (s)	126.8 (s)	118.9 (d)	118.4 (d)	120.9 (d)	111.5 (d)	136.3 (s)
3 [d]	123.8 (d)	112.3 (s)	127.0 (s)	101.2 (d)	152.9 (s)	110.8 (d)	111.9 (d)	131.4 (s)

[a] Solutions in DMSO-d_6, δ in ppm. C=O, CH, CH_2. [b] 170.7, 54.8, 27.2. [c] 175.1, 42.4, 42.0. [d] 175.6, 42.8, 42.2.

Coupling Constant J (Hz) of Protonated Aromatic C

Compound No.	C2	C4	C5	C6	C7
1	^1J_d = 180.1	^1J_d = 159.7	^1J_d = 159.7	^1J_d = 158.4	^1J_d = 158.8
	o	o	o	^3J_d = 7.1	^3J_d = 6.3
2	^1J_d = 180.5	^1J_d = 159.8	^1J_d = 159.0	^1J_d = 159.2	^1J_d = 160.2
	o	^3J_d = 7.5	^3J_d = 7.3	^3J_d = 8.4	^3J_d = 7.2
3	^1J_d = 180.2	^1J_d = 159.5	s	^1J_d = 158.9	^1J_d = 160.5
	^3J_d = 4.4	^3J_d = 4.9	^3J_q = 8.0 ^3J_d = 3.9	^3J_d = 4.6	—

s = singlet, d = doublet, q = quartet, o = overlap.

ppm in the spectrum of the non-deuterated molecule. It underwent the typical [24] two-bond upfield shift to 118.0 ppm in the deuterated sample.

Deuterium incorporation in 3-amino-2-(3-indolyl)propionic acid (**2**) was achieved to approximately 50%. Under these conditions, the C-4 and C-5 resonances exhibited in the ^{1}H-decoupled spectrum the signals for both the protonated and deuterated species. C-5 appears as a double signal at 118.3 ppm for the deuterated sample and at 118.4 ppm for the undeuterated compound. The C-4 signal showed the spectral changes associated with C-D coupling [25] and single signal for the undeuterated compound at 118.9 ppm. The absorption at 120.9 ppm was assigned to C-6 from the observed collapsing of the signal from a dou-

ble doublet ($^1J_{CH}$ = 159.2 Hz; $^3J_{CH}$ = 8.4 Hz) to a doublet in the partially deuterated compound.

The ^{1}H-decoupled spectrum of 3-amino-2-(5-methoxy-3-indolyl)propionic acid (**3**) showed the two controversial resonances at 101.2 ppm and 110.8 ppm resolved as a double doublet ($^1J_{CH}$ = 159.5 Hz; $^3J_{CH}$ = 4.9 Hz and $^1J_{CH}$ = 158.9 Hz; $^3J_{CH}$ = 4.6 Hz), respectively. The C-6 resonance was assigned to absorption at 110.8 ppm by comparison with the deuterated compound, since this signal collapsed into a doublet in the deuterated derivative. It follows that the signal at 101.2 ppm could be assigned directly to C-4.

The unequivocal results obtained by these methods allow the correction of all earlier controversial resonance assignments for tryptophan in the literature. In turn, the assignments for a number of related molecules have to be revised.

EXPERIMENTAL

Deuterium incorporation was monitored by ^{1}H nmr spectra on a Varian Associates EM-390, the extent of deuterium labeling being determined by integration of the signals in the aromatic region. The ^{13}C nmr spectra were obtained at 25.16 MHz on a Varian Associates XL-100A-FT-16K using perdeuterodimethyl sulfoxide as the solvent and tetramethylsilane as internal standard. An observation pulse of 40° and a pulse repetition of 2 seconds were used to acquire 8 K data points, which were Fourier transformed providing a digital resolution of 1.25 Hertz per point. L-tryptophan (**1**) was obtained from commercial sources (Sigma Chemical Co.), 3-amino-2-(3-indolyl)propanoic acid (**2**) and 3-amino-2-(5-methoxy-3-indolyl)propanoic acid (**3**) were synthesized according to the literature [26]. The physical data of the three indoles are in agreement with those reported: Tryptophan (**1**) shows mp 280-285°, lit [27] mp 283-285°; 3-amino-2-(3-indolyl)propionic acid (**2**) shows mp 245-246° dec, lit [1] mp 246° dec and 3-amino-2-(5-methoxy-3-indolyl)propionic acid (**3**) shows mp 205-207° dec, lit [1] mp 205-207° dec. Deuterium oxide (99.8%) was obtained from Aldrich Chemical Co.

General Photodeuteration Procedure.

A solution of the corresponding carboxyltryptamine (**1**, **2** or **3**) in 10 ml of deuterium oxide (~50 mmolar) was degassed by bubbling argon and the system kept under a slight argon pressure throughout the irradiation with an immersion low-pressure mercury lamp at 253.7 nm for 4 hours. The solution was concentrated on a rotary evaporator and the crude product was recrystallized from water (*protio*), washed with cold ethanol and dried. Using these conditions, deuterium incorporation was greater then 90% for compounds **1** and **3** and approximately 50% for **2**; ^{1}H nmr **1** 4-d_1 (perdeuterodimethyl sulfoxide-deuterium oxide): 6.9-7.2 (H-5, H-6), 7.24 (s, H-2), 7.40 (q, ^3J = 7.2 Hz; ^4J = 2.1 Hz, H-7), 7.64 (H-4, not observed); **2** 4-d_1 (perdeuteroacetic acid): 7.0-7.3 (H-5, H-6), 7.42 (s, H-2), 7.48 (H-7), 7.68 (weak broad, H-4); **3** 4-d_1 (perdeuteroacetic acid): 6.95 (d, ^3J = 9.0 Hz, H-6), 7.22 (weak d, ^4J = 2.4 Hz, H-4), 7.41 (s, H-2) and 7.42 ppm (d, ^3J = 9.0 Hz, H-7).

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