Preparation of 5-Hydroxy[*ar*-^{3}H]tryptophans

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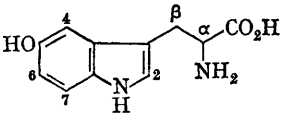
5-HYDROXYTRYPTOPHAN (I), labelled with tritium in the aromatic nucleus but not in the side-chain, was required for studies[1] on the metabolism of phenolic arylethylamines. Acid-catalysed exchange in tritiated water appeared to be the most suitable method of preparation. Daly and Witkop[2] have recently described the exchange of 5-hydroxytryptophan at the 4- and 4,6-positions in acidic solution. We now report conditions which permit selective labelling at each position in the aromatic nucleus.

The n.m.r. spectrum of 5-hydroxytryptophan was measured in D_2O containing one mole of DCl. Bu^{t}OH (τ 8·77) was used as an internal standard. First-order analysis (see Table) allowed unambiguous identification of signals produced by each aromatic proton. Exchange of the 4-proton for deuterium was essentially complete in 5 min. at 100°. After 30 min. heating, appreciable (*ca.* 40%) exchange at position 6 was also observed. In 1 M-DCl, exchange at position 4 was complete after 45 min. at room temperature. Heating at 100° for 3 min. and 19 min. gave, respectively, the 4,6- and 2,4,6-deuterated derivatives. No further exchange had occurred after 40 min. at 100°. In 4 M-DCl, the exchange at position 4 was too rapid to follow at the spectrometer temperture(40°). After 45 min. at room temperature the 4,6-dideuterated derivative was obtained, whereas at 100° the exchange of all the nuclear protons was complete in 1 hr. No exchange of the α (τ 5·73) and β (τ 6·70) protons was observed in any of these experiments.



(I)

These results establish the qualitative rate order, $4 > 6 > 2 > 7 \gg \alpha,\beta$. 5-Hydroxy[*ar*-^{3}H]tryptophan was prepared by exchange in 4 M-tritiated hydrochloric acid at 100° for 1 hr. The labelled amino-acid crystallised out when the pH of the cooled solution was adjusted to the isoelectric point by addition of solid sodium hydrogen carbonate.

N.m.r. spectrum of 5-hydroxytryptophan hydrochloride in D_2O

Proton	4	6	2	7
τ	2·94	3·16	2·76	2·65
J (c./sec.)	2·3	8·7, 2·3	singlet	8·7

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[1] J. Arendt, S. F. Contractor, and M. Sandler, *Biochem. Pharmacol.*, 1967, **16**, 43.
[2] J. W. Daly and B. Witkop, *J. Amer. Chem. Soc.*, 1967, **89**, 1032.