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ASSIGNMENT OF β-PROTON RESONANCES OF L-HISTIDINE BY STEREOSELECTIVE DEUTERIUM SUBSTITUTION

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The 270-MHz ^{1}H NMR spectrum of the mixture of deuterated histidines was compared at pD 8.2 with that of L-histidine. It was seen that the lower-field β-proton resonance disappeared in the deuterated species, in which one of the β-protons was selectively replaced with a deuterium. Accordingly, at this pD, the lower-field β-proton of L-histidine is assignable to pro-R and the higher-field one to pro-S.

For the conformational analysis of α-amino acids and peptides in solutions by ^{1}H NMR spectroscopy, it is often necessary to discriminate the chemical shifts and/or the coupling constants arising from the prochiral groups, such as methylene or geminal methyls. Stereospecific deuterium substitution has been proved to be useful for this purpose.[1-3] In the present study, the 270-MHz ^{1}H NMR spectra of L-histidine (His) and of the deuterated DL-His are to be compared in order to assign the β-proton resonances.

As has been described previously,[4] deuterated DL-His was prepared by the Pd-catalyzed reduction of the precursor azlactone using deuterium gas. Due to appreciable extent of the hydrogen contamination during the deuteration procedure, the obtained His therefore has the following several deuterated species together with a small amount of non-deuterated DL-His: (A), {(2S,3R);(2R,3S)}-[2,3-D_2]-; (B), {(2S,3R);(2R,3S)}-[3-D]- ; (C), (2S;2R)-[2-D]His.[4,5] As ^{1}H NMR spectra for the enantiomeric pairs are identical to each other, this mixture can be used for the assignment of the β-protons.

The theoretical spectra for A ~ C calculated using the parameters obtained from the analysis of the spectrum for L-His at pD 8.2 (uncorrected meter reading) are shown in Figure 1. Note that the deuterium-proton spin couplings are not included in these hypothetical spectra, because those couplings were not in fact resolved under the experimental conditions. This is undoubtedly due to the short relaxation times of the deuterium nuclei, which cause the resonances fairly broad (Figure 2, top and middle).

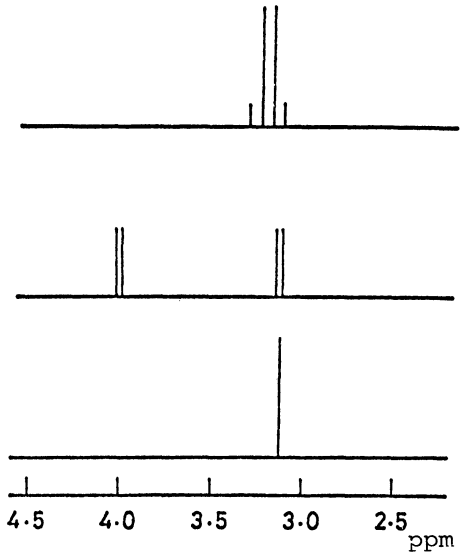

Figure 1. Schematic representation of the hypothetical spectra for the three isotopic isomers of deuterated DL-His: (bottom) {(2S,3R);(2R,3S)}-[2,3-D_2]-; (middle) {(2S,3R);(2R,3S)}-[3-D]-; (top) (2S;2R)-[2-D]His.

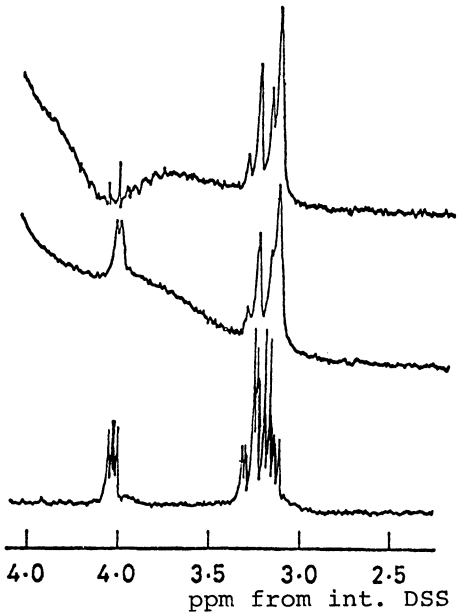

Figure 2. 270-MHz ^1H NMR spectra (D_2O, pD 8.2, 23 °C): (bottom) L-His; (middle) deuterated DL-His; (top) deuterated DL-His with a double irradiation at 4.02 ppm (α-proton). Bruker WH-270, Fourier transform mode.

The observed spectrum for the deuterated His at pD 8.2 shown in Figure 2 (middle) resembles closely to the superposition of three hypothetical spectra at a ratio of about unity. To confirm further this interpretation of the experimental spectrum in terms of the isotopically isomeric mixture, the following double resonance experiment was carried out.

On irradiating the α-proton at 4.02 ppm (partially resolved doublet) in the spectrum for the deuterated His, the higher-field region of the β-proton (~3.13 ppm) became sharper, while virtually no effect was observed in the lower-field region (Figure 2, top). As the deuterated His contains species B as the major source for the α-proton resonance, and non-deuterated DL-His exists apparently very little, the result confirms the previous view [5] indicating that the deuteration proceeds stereoselectively, although the isotopic purity decreases during the reaction.

In conclusion, by comparing the spectra of L-His and the deuterated DL-His (Figure 2, bottom and middle), the lower-field β-proton at pD 8.2 should be assigned to pro-R (for L-His where C-2 is S) or to pro-S (for D-His where C-2 is R), and the higher-field one should be assigned to pro-S (for L-His) or to pro-R (for D-His).

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