Journal of Molecular Structure 1134 (2017) 576–581

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

# Limiting values of the one-bond C–H spin-spin coupling constants of the imidazole ring of histidine at high-pH

Jorge A. Vila [a, *], Harold A. Scheraga [b]

[a] IMASL-CONICET, Universidad Nacional de San Luis, Ejército de Los Andes 950, 5700 San Luis, Argentina
[b] Baker Laboratory of Chemistry, Cornell University, Ithaca, NY, USA

ARTICLE INFO

*Article history:*
Received 25 July 2016
Received in revised form
3 January 2017
Accepted 4 January 2017
Available online 8 January 2017

*Keywords:*
SSCC
One-bond spin-spin coupling constant
High-pH limiting values
Histidine tautomers
Protonated form of His
Solvent effects

ABSTRACT

Assessment of the relative amounts of the forms of the imidazole ring of Histidine (His), namely the protonated ($H^+$) and the tautomeric $N^{\varepsilon 2}$-H and $N^{\delta 1}$-H forms, respectively, is a challenging task in NMR spectroscopy. Indeed, their determination by direct observation of the $^{15}N$ and $^{13}C$ chemical shifts or the one-bond C–H, $^1J_{CH}$, Spin-Spin Coupling Constants (SSCC) requires knowledge of the "canonical" limiting values of these forms in which each one is present to the extent of 100%. In particular, at high-pH, an accurate determination of these "canonical" limiting values, at which the tautomeric forms of His coexist, is an elusive problem in NMR spectroscopy. Among different NMR-based approaches to treat this problem, we focus here on the computation, at the DFT level of theory, of the high-pH limiting value for the $^1J_{CH}$ SSCC of the imidazole ring of His. Solvent effects were considered by using the polarizable continuum model approach. The results of this computation suggest, first, that the value of $^1J_{C\varepsilon 1H} = 205 \pm 1.0$ Hz should be adopted as the canonical high-pH limiting value for this SSCC; second, the variation of $^1J_{C\varepsilon 1H}$ SSCC during tautomeric changes is minor, *i.e.*, within ±1 Hz; and, finally, the value of $^1J_{C\delta 2H}$ SSCC upon tautomeric changes is large (15 Hz) indicating that, at high-pH or for non-protonated His at any pH, the tautomeric fractions of the imidazole ring of His can be predicted accurately as a function of the observed value of $^1J_{C\delta 2H}$ SSCC.



## 1. Introduction

The role of Histidine (His) in many biological functions and activities is very well documented [1–4], and the reason for such versatility can be found in three distinctive properties of the His amino acid residue: (*i*) existence of two neutral, chemically-distinct forms ($N^{\delta 1}$–H and $N^{\varepsilon 2}$–H tautomers, also known as π and τ tautomers, respectively [5], and a charged $H^+$ form, shown in Fig. 1), with one form favored over the other by the protein environment and pH [6]; (*ii*) the only ionizable residue (the charged form) that titrates around neutral pH has a $pK^o$ of 6.6 [7] and (*iii*) appearance of a population of ~50% in all enzyme active sites [8].

Despite these well-known facts, the physical properties of neutral His are difficult to characterize experimentally [9], making a proper determination of the fractions of the tautomeric forms of the imidazole ring of His a challenging problem in NMR spectroscopy. Among the experimental methods in current use, are those based on the observed: (*a*) $^{15}N$ chemical shifts [10–12]; (*b*) $^{13}C^{\gamma}$ and $^{13}C^{\delta 2}$ chemical shifts [6,13]; and (*c*) $^1J_{CH}$ Spin-Spin Coupling Constant (SSCC) of the imidazole ring of His [13–15]. As with any experimental method, *all* these mentioned approaches possess shortcomings: (*i*) the tautomeric fractions obtained by the $^{15}N$-based method may differ significantly depending on the adopted canonical limiting values of the $^{15}N$ chemical shift [16]; (*ii*) the $^{13}C^{\gamma}$ and $^{13}C^{\delta 2}$ chemical shifts cannot always be observed. In fact, only 106 $^{13}C^{\gamma}$, versus 4703 $^{13}C^{\delta 2}$, chemical shifts of the imidazole ring of histidine have been deposited in the Biological Magnetic Resonance data Bank (BMRB) [17]. Hence, problems in the determination of the chemical shifts for these nuclei, such as that for the ground state of His 40 in the protein Im7 [14], often prevent the use of this methodology; and (*iii*) the observed one bond C–H SSCC value at the high-pH limit is ambiguous, as will be discussed below.

It should be noted, from Fig. 1, that there are *only* two one-bond C–H's, $^1J_{CH}$, SSCC's of the imidazole ring of Histidine, namely the $^{13}C^{\varepsilon 1}$–H, $^1J_{C\varepsilon 1H}$, and the $^{13}C^{\delta 2}$–H, $^1J_{C\delta 2H}$, SSCC, respectively. Absences of an accurate value for each of these SSCC's, at the high-pH limit, gives rise to two different kinds of problems as explained below.

* Corresponding author.
*E-mail address:* vila@unsl.edu.ar (J.A. Vila).

http://dx.doi.org/10.1016/j.molstruc.2017.01.022
0022-2860/

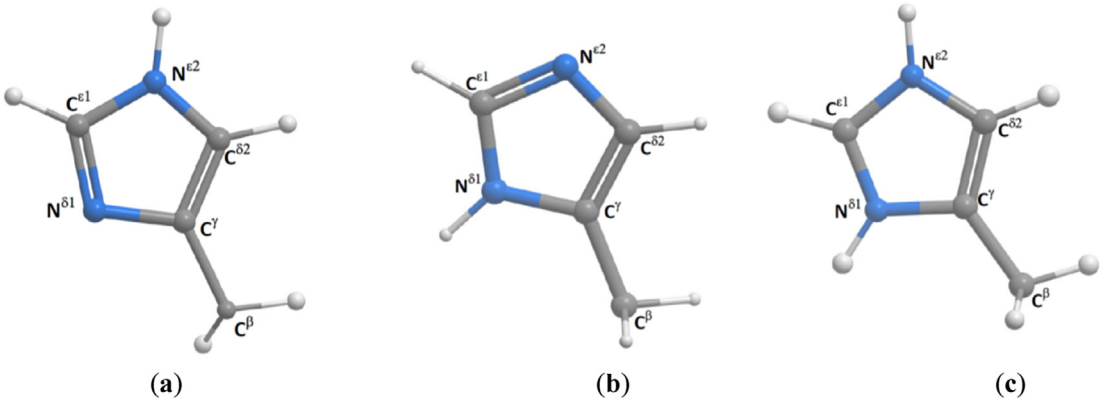


**Fig. 1.** Ball and stick representation of the forms of the imidazole ring of His, namely the: (**a**) $N^{\varepsilon 2}$-H, or $\tau$ tautomer [5], (**b**) $N^{\delta 1}$-H, or $\pi$ tautomer [5], and (**c**) $H^+$ form, respectively.

The first problem pertains to the use of $^1J_{C\varepsilon 1H}$ SSCC to determine the protonation fraction of His, e.g., to detect sparsely populated, short-lived, protein states [14]. In detail, the low-pH limiting value for $^1J_{C\varepsilon 1H}$ SSCC appears to be quite well defined (221 ± 1.0 Hz [14]), for the $^1J_{C\varepsilon 1H}$ SSCC pH-dependence of four titrating His residues (His 6, His 13, His 26, His 87) of the PLCγ SH2 protein domain [14,18]. However, the observed high-pH limit for $^1J_{C\varepsilon 1H}$ SSCC differs among *five* His residues, of the PLCγ SH2 protein domain, by up to ~6 Hz [14], i.e., four titrating His residues converge to a high-pH limiting value of 207 ± 1.0 Hz while the remaining one (His 57), which is the *only* non-titrating His residue, shows an almost flat, pH-independent, value of 203 ± 1.0 Hz [14]. The existence of two possible high-pH limiting values for $^1J_{C\varepsilon 1H}$ SSCC, namely 207 ± 1.0 Hz or 203 ± 1.0 Hz [14], is a source of ambiguity. A similar ambiguity is found for four non-titrating His residues of subtilisin BPN′ having $^1J_{C\varepsilon 1H}$ SSCC in the range of ~205 Hz–~209 Hz [19].

The second problem pertains to a potential contradiction between an assumption [15] and existing evidence about the variation of $^1J_{C\delta 2H}$ SSCC upon tautomeric change [13]. Platzer et al. [15] had proposed that the variations of $^1J_{C\delta 2H}$ SSCC should be independent of the forms of the His tautomer, as for $^1J_{C\varepsilon 1H}$ SSCC. On the other hand, there is experimental evidence for L-histidine at pH 12 in 80% d6-ethanol/20% $H_2O$ at −55 °C [13], showing the existence of a large, rather than a small change, of $^1J_{C\delta 2H}$ SSCC upon changes of the tautomeric forms.

As can be inferred from the above, a common problem, in both $^1J_{CH}$ SSCC's and the $^{15}$N-based methods, is the need for accurate knowledge of the "canonical" limiting values of the imidazole ring of His in which each form of His, namely the protonated ($H^+$) and the tautomeric $N^{\varepsilon 2}$-H and $N^{\delta 1}$-H forms, respectively, is present to the extent of 100%. In this regard, the canonical limiting values of the $^{15}$N chemical shift have already been analyzed [16] and, hence, here we will determine the high-pH limiting values for both $^1J_{CH}$ SSCC's of the imidazole ring of His. By doing this, we will be able to: (*i*) eliminate any possible ambiguity about the actual value of $^1J_{C\varepsilon 1H}$ SSCC; and, (*ii*) resolve a possible contradiction associated with the variations of $^1J_{C\delta 2H}$ SSCC upon changes in the relative amounts of the tautomeric forms.

## 2. Materials and methods

### 2.1. Calculations details

All DFT-calculations of the two $^1J_{CH}$ SSCC's, of the imidazole ring of **His** in the Ac-**His-**NMe molecule, were carried out by using the Gaussian 09 suite of programs [20]; the Keywords used in Gaussian 09 (listed here for assessing the reproducibility of the calculations) were: "NMR = Mixed", with the options "CPHF=Conv = 10" and "Int = ultrafine" [21]. Additional Keywords, such as "Readatoms", were also tested (see Results and Discussion section).

There are four contributions to the NMR coupling constants [22], namely, the Fermi Contact (**FC**), the Spin Dipolar (**SD**), the Paramagnetic Spin-Orbit (**PSO**), and the Diamagnetic Spin-Orbit (**DSO**) contribution, respectively. All four are known as the Ramsey contributions. For this reason, in each of the Tables, we have listed: (*i*) the sum ($\Sigma$) of *all* four Ramsey contributions to each DFT-computed $^1J_{CH}$ SSCC, as computed with the Gaussian 09 suite of programs; and (*ii*) the predicted values for the $^1J_{CH}$ SSCC's, listed in the last column of each Table, are obtained after adding an *ad-hoc* contribution of 5 Hz, to the $\Sigma$ term, due to the Zero Point Vibrational Contribution [23].

All the results in Table 1 correspond to gas-phase DFT calculations, while the ones in Tables 2–4 include DFT-calculations in both gas-phase and in the presence of solvent (see *Solvent Effects* section below). For the latter, all the results obtained with solvent are highlighted in italics and bold face.

### 2.2. Structural geometry of His

All the $^1J_{CH}$ SSCC calculations, at the DFT level of theory, were carried out by using the histidine geometry as defined in the Empirical Conformational Energy Program for Peptides and Proteins (ECEPP) in which their bond-lengths and bond-angles were

**Table 1**
Test of functionals for the DFT computations of $^1J_{C\varepsilon 1H}$ SSCC of the $N^{\varepsilon 2}$-H tautomer.[a]

| Functional | $\Sigma_{\{FC,SD,PSO,DSO\}}$ (Hz) | $^1J_{C\varepsilon 1H}$ (Hz) |
|---|---|---|
| **OPW91** | **199.02** | **~204** |
| B3LYP | 231.65 | ~237 |
| B3P86 | 215.54 | ~220 |
| **OPBE** | **198.10** | **~203** |
| B972 | 212.57 | ~218 |
| BP86 | 211.35 | ~216 |

[a] All gas-phase DFT calculations of $^1J_{C\varepsilon 1H}$ SSCC were carried out on Ac-His-NMe by using the Gaussian 09 suite of programs [20]; the chosen ϕ, ψ, ω, χ1 and χ2 torsional angles for His correspond to a local-minimum of the ECEPP force-field [25] in the α-helical region of the Ramachandran map: −73.563°, −35.197°, −179.856°, 66.389° and −62.607°, respectively; for each functional we used an "aug-cc-pVTZ-J" basis set [37] on *all* nuclei of the imidazole ring of His, and a "6-31G" basis set on the remaining nuclei of Ac-**His**-NMe. The total ($\Sigma$) is a sum over the four Ramsey contributions [22], as given by the output of the Gaussian 09 suite of programs [20], namely, the Fermi Contact (**FC**), the Spin Dipolar (**SD**), the Paramagnetic Spin-Orbit (**PSO**), and the Diamagnetic Spin-Orbit (**DSO**) contribution, respectively; the last column lists the predicted value for the one-bond $^1J_{C\varepsilon 1H}$ SSCC after adding 5 Hz to the $\Sigma$ term (second column), due to the Zero Point Vibrational Contribution [23].