# $^{13}$C Chemical Shielding Tensors in Ampicillin and Penicillin-V: A Theoretical Study†

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The principal components of the $^{13}$C shielding tensors in two antibiotics, ampicillin and penicillin-V, are calculated using the coupled Hartree−Fock gauge-including atomic orbital (CHF-GIAO) method as well as using a hybrid density functional scheme. Calculated results are compared with solid state experimental nuclear magnetic resonance data. Using the known X-ray structures of these antibiotics, it is demonstrated that the computed shieldings compare favorably with experiment such that, in some cases, calculations can now be utilized in assigning shielding tensor data.

## Introduction

Penicillin-V and ampicillin, considered as $\beta$-lactam antibiotics, both have a thiazolidine ring. The conformation of this ring is believed to influence the biological activity of these drugs.[1] X-ray structures of both penicillin-V and ampicillin are already available.[2,3] Furthermore, isotropic $^{13}$C nuclear magnetic resonance (NMR) chemical shifts have been shown to correlate strongly with the thiazolidine conformation.[4] Since the shielding tensor offers additional and independent pieces of information, Antzutkin and co-workers[5] recently applied the two-dimensional phase adjusted spinning sideband (2D-PASS)[6] experiment in measuring the $^{13}$C shielding tensors in ampicillin and penicillin-V. Based on these novel data, speculations were made with regard to the dependence of the shielding anisotropy on the thiazolidine ring conformation. In addition, new questions have surfaced with regard to the assignments of the methyl $^{13}$C resonances in ampicillin.

For the above reasons, it is hoped that ab initio calculations of the shielding will be of great aid in clarifying the assignments as well as the correlation seen between the shielding tensors and molecular structure. Methods of computing shielding have reached a stage at which the quality of the calculated numbers is already approaching the precision of the experiment.[7] For example, the $^{13}$C shielding tensor components (expressed in the icosahedral representation[8] which allows for both magnitude and orientation to be evaluated) of all the C sites in zwitterionic L-threonine can be predicted within an error of only 4 ppm.[9] At this level of accuracy, an ab initio shielding computation can easily serve as an additional assignment tool for interpreting solid state NMR data.

The five-membered (S-1, C-2, C-3, N-4, C-5) thiazolidine ring is nonplanar and can exist in two distinct conformations (shown in Figure 1), called C-3 or S-1. As evident from the figure, the notation indicates which atom in the ring is significantly removed from the plane of the other four atoms. X-ray studies show that penicillin-V assumes the C-3 conformation (the C-3 site carries the carboxyl group) while ampicillin takes the S-1 geometry. The $^{13}$C sites that have been of interest to NMR spectroscopists are the methyl (2$\alpha$-Me and 2$\beta$-Me) groups attached to the C-2 position of the ring (The $\alpha$-methyl substituent is always on the same side as the carboxyl group on C-3). Not shown in Figure 1 is the carbonyl group (C-7) of the $\beta$-lactam ring which is attached to the N-4 position of the thiazolidine ring. This carbonyl group becomes 1 Å closer (from 4.5 to 3.5 Å) to the 2-$\beta$-methyl substituent as one goes from the S-1 to the C-3 conformation. Clayden et al.[4] have attributed the observed deshielding of the $^{13}$C resonance of 2$\beta$-Me in penicillin-V to this closer separation. Due to this apparent correlation, the $^{13}$C chemical shift of 2$\beta$-Me can effectively serve as an indicator for the conformation of the thiazolidine ring. Unfortunately, the assignment for the resonances of the methyl groups in ampicillin has been recently challenged by Antzutkin et al.[5] The revision has been prompted by a need to preserve the chemical shielding anisotropy (CSA) of the methyl $^{13}$C sites upon changing the thiazolidine ring conformation, an assumption, as noted by Antzutkin et al.,[5] that is not beyond scrutiny.

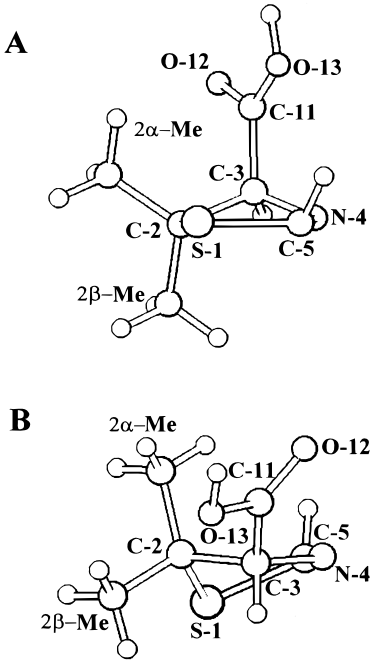


**Figure 1.** Possible conformations of the thiazolidine ring: (**A**) C-3 conformation as exemplified by penicillin-V; (**B**) S-1 conformation as observed in ampicillin.

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† Dedicated to the memory of Professor Leonard Kotin (1932−1999).

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After all, shielding as a tensor quantity can also be sensitive to molecular conformation.

This paper will make use of presently available methodologies for shielding computations. With considerable progress in both hardware and software, it is now possible to perform shielding calculations on molecules as large as these antibiotics even with large triple-$\zeta$ quality basis sets. Both Hartree−Fock and density functional methods will be explored. The results of these calculations will be compared to experiment to evaluate the adequacy of the theory in assigning the solid state NMR spectra. Favorable comparison between theory and experiment will indicate that the trends seen in the shielding tensor data are primarily due to the conformation of a single molecule and not directly from intermolecular effects that arise from the packing of the molecules in a crystal.

## Computational Details

Before comparing theoretical and experimental shielding tensor quantities, it is important that the same convention describing CSA quantities be applied to both. We have chosen to follow the convention[10] that is convenient to theoreticians as it pertains to quantities readily taken from the output of quantum mechanical calculations. First, the principal components of the shielding tensor are defined as follows:

$$\sigma_{11} < \sigma_{22} < \sigma_{33} \tag{1}$$

Since the NMR chemical shift goes in an opposite direction to shielding, then the following relationship holds for the chemical shift tensor components:

$$\delta_{33} < \delta_{22} < \delta_{11} \tag{2}$$

The isotropic shielding ($\sigma_{iso}$) and chemical shift ($\delta_{iso}$) are simple averages of their respective principal components. The tensor can also be described by the span ($\Omega$), anisotropy ($\delta_{aniso}$) and skew ($\kappa$) parameters, which are defined as follows:

$$\Omega = \sigma_{33} - \sigma_{11} = \delta_{11} - \delta_{33} \tag{3}$$

$$\delta_{aniso} = \sigma_{33} - ((\sigma_{11} + \sigma_{22})/2) = ((\delta_{11} + \delta_{22})/2) - \delta_{33} \tag{4}$$

$$\kappa = 3(\sigma_{iso} - \sigma_{22})/\Omega = 3(\delta_{22} - \delta_{iso})/\Omega \tag{5}$$

Thus, before comparing experimental values, the data reported by Antzutkin et al.[5] were converted first to follow the above definitions. The use of the above parameters allows for a direct comparison between theoretical and experimental values without the additional concern of chemical shift referencing.

Ab initio shielding calculations are very sensitive to the positions of hydrogens in a molecule. Since X-ray structures are known to provide inadequate or inaccurate hydrogen positions for shielding computations, a partial geometry optimization of the proton positions in the given X-ray structure is performed at the B3LYP (a hybrid method which makes use of the Becke exchange functional[11] mixed with Hartree−Fock contributions and the correlation functionals of Lee, Yang, and Parr[12]) level of theory with a 6-31G** basis set prior to the shielding computation. The shielding is calculated via the gauge-including atomic orbital (GIAO) method[13,14] with a 6-311++G-(3d,2p) basis set. The shielding computations are performed at two levels of theory, restricted Hartree−Fock (RHF) and B3LYP. For a valid comparison of these two different levels of theory, it is necessary to use large basis sets as basis set deficiencies are known to cause fortuitous cancellation of errors,

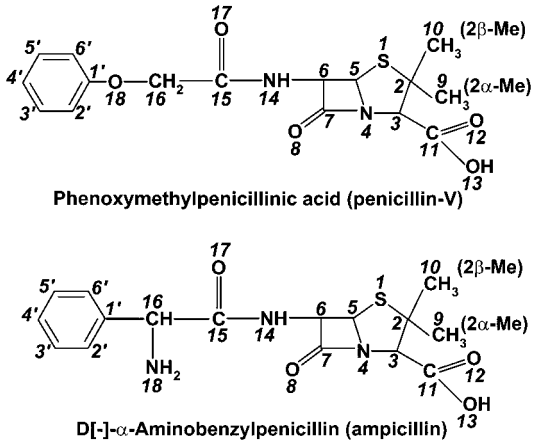


**Figure 2.** The structures of penicillin-V (top) and ampicillin (bottom) with the numbering and labeling of atoms used in the text.

**TABLE 1: Calculated $^{13}$C Shielding Tensor Components (ppm) for Penicillin-V**

| | RHF | | | | B3LYP | | | |
|---|---|---|---|---|---|---|---|---|
| carbon no. | $\sigma_{iso}$ | $\sigma_{11}$ | $\sigma_{22}$ | $\sigma_{33}$ | $\sigma_{iso}$ | $\sigma_{11}$ | $\sigma_{22}$ | $\sigma_{33}$ |
| 2α-methyl | 164.4 | 146.1 | 162.8 | 184.4 | 151.7 | 129.9 | 149.5 | 175.5 |
| 2β-methyl | 162.5 | 140.9 | 158.8 | 187.7 | 148.9 | 123.9 | 144.2 | 178.6 |
| 16 | 125.5 | 104.2 | 110.4 | 162.0 | 108.8 | 85.1 | 93.6 | 147.7 |
| 2 | 131.3 | 88.6 | 145.7 | 159.6 | 104.2 | 54.7 | 119.3 | 138.6 |
| 3 | 126.4 | 109.2 | 131.5 | 138.5 | 104.8 | 81.3 | 112.3 | 120.8 |
| 5 | 125.4 | 93.6 | 124.2 | 158.4 | 101.0 | 61.9 | 102.9 | 138.2 |
| 6 | 137.0 | 116.2 | 140.0 | 154.9 | 119.4 | 94.8 | 122.0 | 141.4 |
| 6′ | 67.9 | −36.5 | 61.2 | 179.1 | 58.6 | −39.4 | 50.9 | 164.3 |
| 5′ | 48.5 | −73.1 | 35.0 | 183.4 | 42.7 | −69.6 | 29.3 | 168.3 |
| 4′ | 71.5 | −39.0 | 67.6 | 185.9 | 60.3 | −45.8 | 54.6 | 172.2 |
| 3′ | 49.0 | −73.2 | 36.6 | 183.7 | 44.1 | −67.3 | 31.0 | 168.5 |
| 2′ | 62.4 | −43.5 | 61.3 | 169.3 | 53.5 | −43.9 | 50.8 | 153.5 |
| 1′ | 16.3 | −89.8 | 17.1 | 121.5 | 5.3 | −80.0 | −2.4 | 98.3 |
| 15 | −8.5 | −123.3 | −4.6 | 102.5 | −7.0 | −109.5 | 6.8 | 81.8 |
| 11 | −18.1 | −152.5 | −0.6 | 98.9 | −16.9 | −134.7 | 3.9 | 80.1 |
| 7 | 3.5 | −116.7 | 55.9 | 71.3 | −0.9 | −109.3 | 51.4 | 55.2 |

thereby causing wrong conclusions to be drawn. With a large basis set, the differences seen between the results obtained using RHF and B3LYP can therefore be solely attributed to the difference in the level of theory. Thus, penicillin is given 956 basis functions, while ampicillin receives 966 basis functions. Both geometry optimization and shielding computation are performed using a parallel version of Gaussian98.[15] Starting with the X-ray structure, partial geometry optimization of the proton positions takes about 1 day. On the other hand, the shielding calculation takes about 4 days. All computations were performed on an Origin 2000 workstation equipped with four processors (Silicon Graphics, Inc.). Shielding computations were also performed at the B3LYP level of theory with a smaller basis set, 6-31+G*. These shielding calculations take less than 8 h on the Origin 2000 workstation. Results from these "smaller basis set" computations considerably differ from those of 6-311++G(3d,2p), but upon comparison with experimental data, they are still generally superior than the RHF results, and are as nearly as good as the B3LYP/6-311++G(3d,2p) calculations.

## Results and Discussion

The non-hydrogen atoms in ampicillin and penicillin-V are labeled according to Figure 2. The results of the shielding computations are presented in Table 1 (penicillin-V) and Table 2 (ampicillin). Calculated results and comparison with experiment of $^{13}$C CSA parameters for penicillin-V are presented in Table 3. The corresponding values for ampicillin are displayed in Table 4. Figure 3 shows a comparison between calculated

**TABLE 2: Calculated $^{13}$C Shielding Tensor Components (ppm) for Ampicillin**

| carbon no. | $\sigma_{iso}$ | $\sigma_{11}$ | $\sigma_{22}$ | $\sigma_{33}$ | $\sigma_{iso}$ | $\sigma_{11}$ | $\sigma_{22}$ | $\sigma_{33}$ |
|---|---|---|---|---|---|---|---|---|
| 2α-methyl | 167.3 | 146.9 | 166.6 | 188.5 | 154.1 | 131.0 | 152.6 | 178.7 |
| 2$\beta$-methyl | 168.5 | 147.1 | 166.7 | 191.6 | 155.4 | 131.7 | 152.3 | 182.2 |
| 16 | 136.7 | 123.5 | 135.6 | 151.2 | 119.3 | 104.6 | 119.6 | 133.8 |
| 2 | 133.7 | 92.7 | 135.1 | 173.3 | 108.3 | 62.4 | 109.0 | 153.5 |
| 3 | 121.5 | 94.7 | 128.8 | 140.8 | 97.0 | 64.5 | 107.9 | 118.7 |
| 5 | 137.0 | 105.0 | 141.5 | 164.6 | 115.7 | 79.4 | 120.2 | 147.5 |
| 6 | 141.0 | 112.5 | 147.8 | 162.5 | 123.7 | 92.9 | 129.4 | 148.8 |
| 6′ | 56.3 | −58.5 | 53.3 | 174.1 | 48.3 | −57.1 | 44.2 | 157.7 |
| 5′ | 52.0 | −66.1 | 34.3 | 188.0 | 44.1 | −66.9 | 24.7 | 174.4 |
| 4′ | 50.8 | −69.2 | 32.4 | 189.2 | 43.4 | −68.6 | 24.3 | 174.4 |
| 3′ | 52.4 | −67.7 | 36.0 | 188.9 | 42.9 | −70.1 | 24.8 | 173.9 |
| 2′ | 63.7 | −51.3 | 53.1 | 189.4 | 56.9 | −49.9 | 45.5 | 174.9 |
| 1′ | 57.6 | −48.6 | 37.6 | 183.8 | 45.5 | −49.4 | 21.0 | 165.0 |
| 15 | 23.8 | −65.0 | 30.5 | 105.8 | 20.8 | −61.9 | 38.5 | 85.8 |
| 11 | 26.2 | −67.0 | 61.3 | 84.3 | 20.3 | −69.7 | 59.8 | 70.7 |
| 7 | 17.1 | −88.3 | 53.4 | 86.2 | 14.4 | −85.3 | 57.9 | 70.5 |

**TABLE 3: Calculated[a] and Experimental[b] CSA Parameters[c] for Penicillin-V**

| carbon no. | | anisotropy, $\sigma_{aniso}$ (ppm) | span, Ω (ppm) | skew, $\kappa$ |
|---|---|---|---|---|
| 2α-methyl | expt | 33.0 | 42.2 | 0.128 |
| | RHF | 30.0 | 38.3 | 0.125 |
| | B3LYP | 35.8 | 45.6 | 0.145 |
| 2$\beta$-methyl | expt | 44.4 | 53.7 | 0.307 |
| | RHF | 37.8 | 46.8 | 0.237 |
| | B3LYP | 44.5 | 54.7 | 0.258 |
| 2 | expt | 40.7 | 65.8 | −0.529 |
| | RHF | 42.4 | 71.0 | −0.608 |
| | B3LYP | 51.5 | 83.9 | −0.540 |
| 6 | expt | 48.8 | 72.6 | −0.314 |
| | RHF | 26.8 | 38.7 | −0.233 |
| | B3LYP | 33.0 | 46.6 | −0.167 |
| 6′ | expt | 151.5 | 181.3 | 0.342 |
| | RHF | 166.8 | 215.6 | 0.093 |
| | B3LYP | 158.6 | 203.7 | 0.113 |
| 5′ | expt | 177.8 | 217.4 | 0.270 |
| | RHF | 202.5 | 256.5 | 0.158 |
| | B3LYP | 188.5 | 237.9 | 0.169 |
| 4′ | expt | 174.5 | 216.3 | 0.226 |
| | RHF | 171.6 | 224.9 | 0.052 |
| | B3LYP | 167.8 | 218.0 | 0.078 |
| 3′ | expt | 177.8 | 217.4 | 0.270 |
| | RHF | 202.0 | 256.9 | 0.145 |
| | B3LYP | 186.7 | 235.8 | 0.167 |
| 2′ | expt | 143.1 | 176.0 | 0.252 |
| | RHF | 160.4 | 212.8 | 0.016 |
| | B3LYP | 150.0 | 197.4 | 0.041 |
| 1′ | expt | 135.9 | 175.3 | 0.101 |
| | RHF | 157.9 | 211.3 | −0.011 |
| | B3LYP | 139.5 | 178.3 | −0.130 |
| 15 | expt | 117.6 | 161.8 | −0.093 |
| | RHF | 166.4 | 225.8 | −0.052 |
| | B3LYP | 133.2 | 191.3 | −0.216 |
| 11 | expt | 90.5 | 151.6 | −0.613 |
| | RHF | 175.5 | 251.4 | −0.208 |
| | B3LYP | 145.5 | 214.8 | −0.291 |
| 7 | expt | 101.6 | 160.4 | −0.468 |
| | RHF | 101.7 | 188.0 | −0.836 |
| | B3LYP | 84.2 | 164.5 | −0.954 |

[a] This work. [b] Experimental data taken from ref 5. [c] See text for definitions.

isotropic shieldings and experimental chemical shifts, while Figure 4 is a comparison of theoretical and experimental principal components. The parameters describing the best-fit line obtained upon comparing calculated shieldings and experimental shifts are given in Table 5. It is apparent that the hybrid density functional method, B3LYP, performs better than coupled Hartree−Fock (RHF). For both isotropic and principal values,

**TABLE 4: Calculated[a] and Experimental[b] CSA Parameters[c] for Ampicillin**

| carbon no. | | anisotropy, $\sigma_{aniso}$ (ppm) | span, Ω (ppm) | skew, $\kappa$ |
|---|---|---|---|---|
| 2α-methyl | expt | 34.4 | 45.1 | 0.047 |
| | RHF | 31.8 | 41.6 | 0.050 |
| | B3LYP | 36.9 | 47.7 | 0.094 |
| 2$\beta$-methyl | expt | 42.9 | 50.9 | 0.371 |
| | RHF | 34.7 | 44.5 | 0.121 |
| | B3LYP | 40.2 | 50.5 | 0.184 |
| 2 | expt | 55.5 | 79.0 | −0.190 |
| | RHF | 59.4 | 80.6 | −0.052 |
| | B3LYP | 67.8 | 91.1 | −0.023 |
| 3 | expt | 47.4 | 56.9 | 0.332 |
| | RHF | 29.1 | 46.1 | −0.475 |
| | B3LYP | 32.5 | 54.2 | −0.603 |
| 5 | expt | 45.5 | 66.8 | −0.278 |
| | RHF | 41.4 | 59.6 | −0.227 |
| | B3LYP | 47.7 | 68.1 | −0.198 |
| 6′ | expt | 175.4 | 212.8 | 0.296 |
| | RHF | 176.7 | 232.6 | 0.039 |
| | B3LYP | 164.2 | 214.8 | 0.057 |
| 5′ | expt | 173.0 | 214.5 | 0.225 |
| | RHF | 203.9 | 254.1 | 0.209 |
| | B3LYP | 195.5 | 241.3 | 0.241 |
| 4′ | expt | 182.9 | 224.3 | 0.261 |
| | RHF | 207.6 | 258.4 | 0.214 |
| | B3LYP | 196.6 | 243.0 | 0.236 |
| 3′ | expt | 173.0 | 214.5 | 0.225 |
| | RHF | 204.8 | 256.6 | 0.192 |
| | B3LYP | 196.5 | 244.0 | 0.223 |
| 2′ | expt | 156.9 | 198.2 | 0.166 |
| | RHF | 188.5 | 240.7 | 0.132 |
| | B3LYP | 177.1 | 224.8 | 0.152 |
| 1′ | expt | 173.3 | 208.5 | 0.324 |
| | RHF | 189.3 | 232.4 | 0.258 |
| | B3LYP | 179.2 | 214.4 | 0.343 |
| 15 | expt | 115.7 | 166.2 | −0.217 |
| | RHF | 123.0 | 170.8 | −0.118 |
| | B3LYP | 97.5 | 147.7 | −0.360 |
| 11 | expt | 97.2 | 131.9 | −0.052 |
| | RHF | 87.2 | 151.3 | −0.696 |
| | B3LYP | 75.7 | 140.4 | −0.844 |
| 7 | expt | 107.0 | 164.5 | −0.399 |
| | RHF | 103.6 | 174.5 | −0.624 |
| | B3LYP | 84.1 | 155.8 | −0.838 |

[a] This work. [b] Experimental data taken from ref 5. [c] See text for definitions.

the slopes obtained from the B3LYP calculations are closer to the ideal value of −1. The same conclusion is drawn after an initial inspection of the calculated CSA parameters. B3LYP yields values closer to experiment for all three parameters: anisotropy, span, and skew. Figures 3 and 4 indicate that the outlying points belong to highly deshielded species (Figure 3) and the two principal components, $\sigma_{11}$ and $\sigma_{22}$ (Figure 4). This observation clearly points out the dependence of the quality of the calculation on the type of carbon whose shielding is being calculated. The most deshielded $^{13}$C sites in these antibiotics belong to the carbonyl group (with the exception of 1′ in penicillin-V, an aromatic C attached to an O atom). Therefore, in evaluating the quality of the ab initio calculations, it will be wise to make a distinction between different types of C sites.

**Carbonyl and Carboxyl Groups (7, 11, 15).** The calculated isotropic shieldings for these sites are not sensitive to the level of theory. The B3LYP values generally differ from those obtained through RHF by about 2−4 ppm. The principal components, however, are very sensitive to the choice of method. As shown in Tables 3 and 4, both anisotropy and span change significantly as one goes from RHF to B3LYP. These dramatic changes do not manifest in the isotropic values since

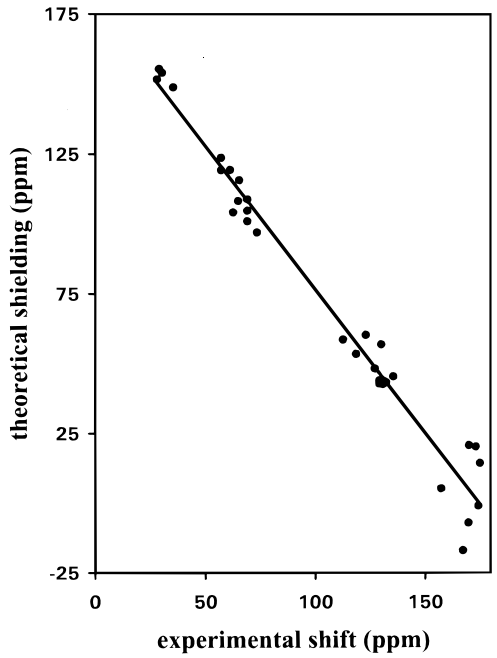


**Figure 3.** Comparison between theoretical (B3LYP/6-311++G-(3d,2p)) shieldings and experimental shifts for the $^{13}C$ sites in penicillin-V and ampicillin.

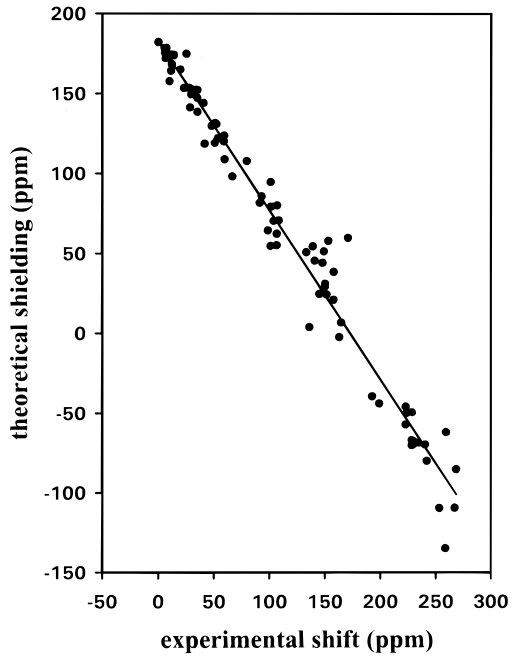


**Figure 4.** Comparison between theoretical (B3LYP/6-311++G-(3d,2p)) shielding and experimental shift components for the $^{13}C$ sites in penicillin-V and ampicillin.

**TABLE 5: Comparison between Theoretical Shieldings and Experimental Shifts**

| | | slope | intercept (ppm) | regression coeff, $R^2$ | rmsd (ppm) |
|---|---|---|---|---|---|
| isotropic | RHF | −1.14 | 203.5 | 0.97 | 9.2 |
| | B3LYP | −1.02 | 179.0 | 0.97 | 8.7 |
| principal components | RHF | −1.15 | 203.9 | 0.97 | 15.9 |
| | B3LYP | −1.06 | 183.1 | 0.97 | 14.4 |

they involve an increase in $\sigma_{11}$ and a simultaneous decrease in $\sigma_{33}$ (see Tables 1 and 2). The changes in the two components cancel each other such that the isotropic value is only slightly varied. The span of the tensor, on the other hand, is clearly lower (and closer to experiment) with the B3LYP method. Thus, to evaluate contributions from electron correlation, it is clearly not sufficient to examine only the isotropic values. Examining the principal components allows for a more detailed analysis of electron correlation contributions to shielding. Based on the calculations, the principal components of the carbonyl carbon shielding that are sensitive to electron correlation lie perpendicular to the C═O bond, with the most deshielded component $\sigma_{11}$ residing on the $sp^2$ plane and $\sigma_{33}$ lying normal to this plane. Even with the B3LYP method, agreement between theory and experiment is still poor, a discrepancy that is evident not only with the principal components but also with isotropic values. In fact, if one removes the isotropic shifts of the carbonyl sites from the comparison between theory and experiment, the root-mean-square deviation (rmsd) is significantly reduced to 4 ppm for both RHF and B3LYP calculations. Since the error seems to be present in both methods, it may not be simply attributed to the level of theory. Previous calculations on the zwitterionic amino acids L-threonine and L-tyrosine showed that the isotropic shielding and principal components of a carboxyl site are dramatically influenced by hydrogen bonding.[9] For carboxyl sites, the two components, $\sigma_{11}$ and $\sigma_{22}$, are very sensitive to hydrogen bonding. Hydrogen bonding causes the least shielded component $\sigma_{11}$ to increase while decreasing $\sigma_{22}$. On the other hand, theoretical studies on the shielding of carbonyl carbon sites in model peptides show that the $\sigma_{22}$ component is most susceptible to hydrogen bonding.[15] The above trends manifest in the CSA parameters as shown in Tables 3 and 4. Since the calculations in this present paper only employ a single molecule, hydrogen bonding effects are completely neglected. Consequently, the spans for C-11 of both penicillin-V and ampicillin are overestimated since the component $\sigma_{11}$ is underestimated. Moreover, errors in $\sigma_{22}$ will appear in the skew parameter, $\kappa$. The skew of a tensor essentially describes the shape of the tensor, which is intimately related to the position of $\sigma_{22}$ in the pattern of the tensor. As seen in Tables 3 and 4, both RHF and B3LYP perform poorly in reproducing the skew for C-7, C-11, and C-15. For the carbonyl sites, C-7 and C-15, the calculated B3LYP skews are more negative than the experimental values. This is to be expected, since without hydrogen bonding, $\sigma_{22}$ will be overestimated which makes $\kappa$ more negative. For the carboxyl site, C-11, the error in the skew comes from two sources, $\sigma_{22}$ and the span. Due to the exclusion of hydrogen bonding, the span is overestimated, which effectively reduces the absolute magnitude of the skew. This happens to be the case for the carboxyl site (C-11) of penicillin-V.

**Aromatic Carbons (1′−6′).** The value of the most shielded component for this type of carbon is overestimated in RHF. As a result, the span is overestimated for all the aromatic carbons in both antibiotics. Significant improvement is achieved with the B3LYP method, indicating that electron correlation gives significant contributions to the most shielded component, $\sigma_{33}$. This component lies normal to the aromatic plane. Even with B3LYP, the span is still overestimated by about 20 ppm in some cases (about 10% of the experimental values). Although the CSA parameters are reproduced in a semiquantitative manner, the calculations are unable to predict the relative ordering in terms of isotropic shieldings of the aromatic carbons. In ampicillin, for example, the experimental isotropic chemical shifts of the aromatic carbons encompass a range of only 8 ppm, which is below the rmsd listed in Table 5. Thus, at this level of theory and quality of X-ray structure, it is not yet possible to rely on ab initio calculations in assigning isotropic chemical shifts of aromatic carbons.

**Carbons in Nonaromatic Heterocycles (2, 3, 5, 6).** Some of these sites are not completely resolved in the 2D-PASS spectra, and their tensors have not yet been reported.[5] From the B3LYP calculations, the C-6 site of ampicillin has an isotropic shielding of 123.7 ppm (experimental shift = 57 ppm) and an anisotropy of 38 ppm. The calculated shielding for the C-6 site of penicillin-V is 119.4 ppm (experimental shift = 61.1 ppm). The difference in the calculated shieldings between the C-6 sites of penicillin-V and ampicillin compares fairly well with experiment. This excellent agreement, however, is only fortuitous. As in the aromatic carbons, the ab initio calculations fail to predict the relative ordering of sites in terms of shielding. For example, the calculations suggest that in ampicillin C-5 is more shielded than C-2 by about 7 ppm when the experiment indicates that C-2 is more shielded compared to C-5 (by about 0.5 ppm). After closely examining the CSA parameters for these sites, no obvious trend in the discrepancies is evident. Thus, it is very likely that the poor prediction of the isotropic shieldings for these sites may be due to the quality of the structure employed in the computation. C-2 is the only site of this kind whose tensor has been measured for both penicillin-V and ampicillin. In qualitative agreement with experiment, calculations show that the anisotropy of the C-2 shielding tensor is 16.3 ppm lower in penicillin-V than in ampicillin. Experimentally, the skew for this site also dramatically changes from penicillin-V to ampicillin, which is nicely reproduced by this theoretical work. Thus, although not perfect, ab initio calculations can assist in investigating the effect of the ring conformation on the $^{13}$C shielding tensor. One trend worth noting is that, unlike in unsaturated carbons (aromatic and carbonyl), inclusion of electron correlation increases the span of shielding tensors in saturated carbons. In addition, the isotropic shielding for these sites changes significantly upon inclusion of electron correlation.

**Methyl Substituents (2-$\alpha$, 2-$\beta$).** These sites are of great interest since their isotropic chemical shifts display a strong correlation with the conformation of the thiazolidine ring. Similar to the saturated carbons in the heterocycles, B3LYP also produces a higher anisotropy and span than RHF does. Also, as for the rest of the carbons, B3LYP values are closer to experiment. The anisotropy is smallest for methyl groups, but B3LYP seems to be able to predict the anisotropy and span within 2 ppm or less (about 5% of experimental value). The excellent agreement seen between the calculated and experimental shielding tensor components provides confidence in the calculated isotropic chemical shifts. Therefore, based on both RHF and B3LYP results, the changes made by Antzutkin et al.[5] on the previous assignments of the methyl resonances of Clayden et al.[4] are correct. Furthermore, as speculated by Antzutkin et al.,[5] the anisotropy and span are essentially preserved between penicillin-V and ampicillin.

The conformation of the thiazolidine ring can be expected to influence the shielding tensors of C-2, C-3, C-5, C-6, C-7, C-11, and the methyl groups. In Table 6, the differences between the CSA parameters of C-2 and the methyl groups of the two antibiotics are shown. The values pertaining to C-7 and C-11 are not shown because of the poor agreement between theory and experiment. These are carbonyl and carboxyl carbons, respectively, and as discussed above, the shielding tensors at these sites are very sensitive to hydrogen bonding. Nevertheless, Table 6 shows that ab initio calculations are capable of reproducing the trends in CSA parameters caused by the change in conformation of the thiazolidine ring. This comparison provides additional confidence in the calculated shielding tensors of saturated carbons. Lastly, it is interesting to note that RHF likewise nicely reproduces the differences in the shielding caused by the change in ring conformation, indicating that the conformational dependence of the $^{13}$C shielding tensors is already accounted for at the Hartree−Fock level of theory.

**TABLE 6: Calculated[a] and Experimental[b] Differences[c] in CSA Parameters[d] between Penicillin-V and Ampicillin**

| carbon no. | | anisotropy, $\sigma_{aniso}$ (ppm) | span, $\Omega$ (ppm) | skew, $\kappa$ |
|---|---|---|---|---|
| 2$\alpha$-methyl | expt | −1.4 | −2.9 | 0.081 |
| | RHF | −1.8 | −3.3 | 0.075 |
| | B3LYP | −1.1 | −2.1 | 0.051 |
| 2$\beta$-methyl | expt | 1.5 | 2.8 | −0.064 |
| | RHF | 3.1 | 2.3 | 0.116 |
| | B3LYP | 4.3 | 4.2 | 0.074 |
| 2 | expt | −14.8 | −13.2 | −0.339 |
| | RHF | −17.0 | −9.6 | −0.556 |
| | B3LYP | −16.3 | −7.2 | −0.517 |

[a] This work. [b] Experimental data taken from ref 5. [c] The above differences were obtained by subtracting a given CSA parameter of ampicillin (Table 4) from its corresponding parameter in penicillin-V (Table 3). [d] See text for definitions.

Future studies will focus on the specific factor(s) responsible for the $^{13}$C shielding trends in $\beta$-lactam antibiotics. Since only two of these antibiotics have their $^{13}$C shielding tensors fully characterized, additional data will be required in order to draw generalizations.

## Conclusions

This work examined the use of present shielding computational methodologies in understanding shielding tensors of carbon sites in the antibiotics penicillin-V and ampicillin. Upon evaluation of trends seen for each type of carbon site in these molecules, insights on the limitations as well as the capabilities of ab initio calculations of NMR shieldings are drawn. For example, it is evident that electron correlation affects the principal components of the shielding tensor differently. The calculations also support the recent reassignment of the methyl resonances in ampicillin. Ab initio calculations have not yet reached the accuracy required to correctly assign $^{13}$C resonances. However, using the full tensor information as summarized by the anisotropy, span, and skew, in addition to the isotropic value, ab initio calculations can provide valuable assistance in assigning sideband manifolds and powder patterns in solid state NMR spectroscopy.

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