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Tetrahedron Letters 45 (2004) 4003–4007
Tetrahedron Letters

Application of quantum chemical calculations of ^{13}C NMR chemical shifts to quinoxaline structure determination

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Received 2 February 2004; revised 22 March 2004; accepted 30 March 2004

Abstract—Comparison of experimental and theoretical (GIAO DFT) ^{13}C NMR chemical shifts allows the reliable assignment of isomeric structures of heteroaromatic compounds. This methodology was applied to establish the structures of isomeric quinoxalines. A modern 1D NOE technique permitted independent proof of the proposed structures.

In this study, we wanted to establish unambiguously the structures of two novel isomeric quinoxalines **1** (mp = 250 °C) and **2** (mp = 320 °C) with molecular formula $C_{21}H_{13}N_5O_2$, which could have structures **A** or **B** (Fig. 1), and are analogues of biologically active quinoxalines.[1] Determination of isomeric structures is a very important and vital task in problems related to natural product chemistry, medicinal chemistry, etc. Application of X-ray diffraction analysis is sometimes limited due to unavailability of suitable crystals. Chemical methods to correlate isomeric structures based on known synthetic pathways are time consuming and not straightforward. So, reliable and easily accessible methods to establish isomeric structures are needed. NMR spectroscopy is one of the most efficient and convenient methods to get information about chemical and conformational structures of complex organic compounds in solution.[2]

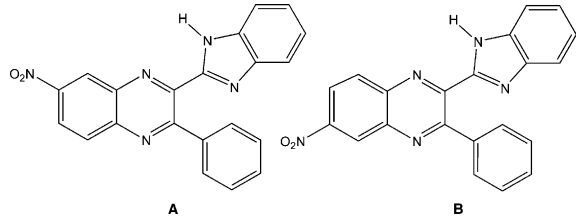

Figure 1.

Modern 1D and 2D NMR techniques allow correlation of interactions between different nuclei due to spin–spin and dipole–dipole interactions and in this way establish the structures of fragments and, in many cases, of a whole molecule.[3] However, if there is a chain of several nuclei with non 1/2 spin moment (^{12}C, ^{14}N, ^{17}O, ^{33}S) there is no longer a way to follow sequentially the whole molecule via scalar interactions. In these cases correlation techniques can only be used to deduce the structures of separate fragments. If the molecular fragments are simple and linked by one bond, empirical chemical shift increments can be exploited to predict the influence of neighboring groups and in this way to establish the overall structure.[4] However, there are many cases where fragments are bonded by two or even three bonds. In such cases simple empirical rules cannot be deduced to take into account the influence of the vicinal fragments. In recent years significant progress has been made in the application of nonempirical calculations of NMR chemical shifts of complicated molecules, and quantitative correlations between experimental and theoretical data obtained in many cases.[5] Therefore, it seems that the combination of theoretically computed NMR chemical shifts and experimental NMR data can provide a tool for structural elucidation and characterization of new compounds, in particular, if there are no well established model compounds to run structure–chemical shift correlations.

The ^1H NMR spectra of both isomers **1** and **2** consisted of signals expected for a phenyl substituent and the

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doi:10.1016/j.tetlet.2004.03.182

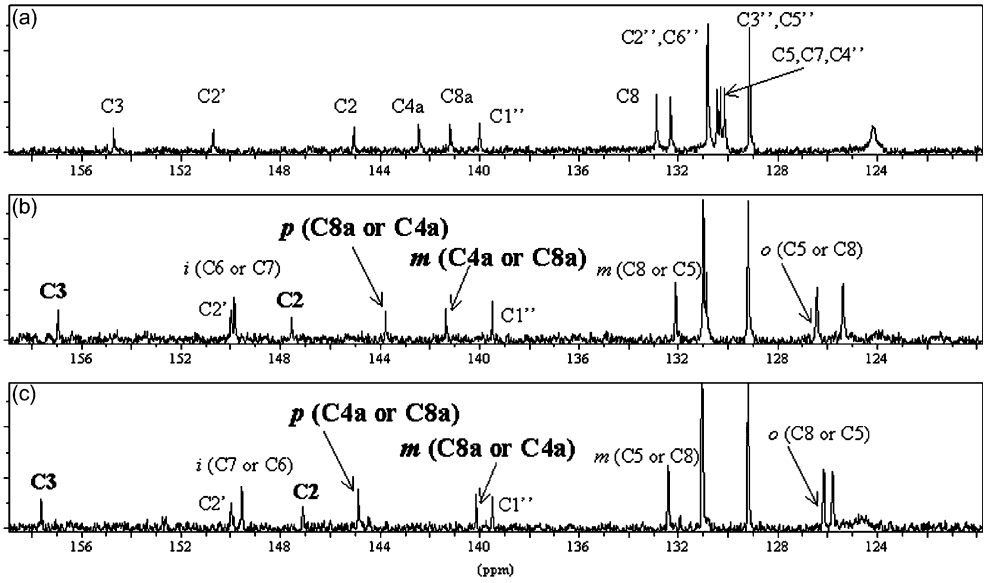
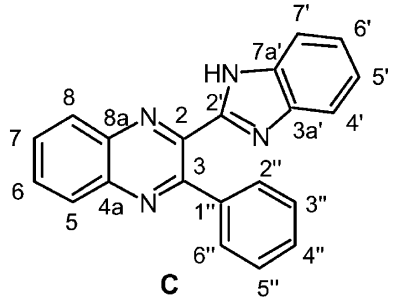


Figure 2. ^{13}C NMR spectra of compounds **C** and **1**, **2**: (a) **C**, (b) compound **1**, (c) compound **2**.[8]

benzo moieties of quinoxaline and benzimidazole. However, the ^{1}H NMR spectra of the two isomers were almost identical and could not be used even to differentiate between these structures.[6] In this respect, ^{13}C NMR chemical shifts are much more sensitive to electronic structure and it can be expected that isomers will be differentiated in their carbon NMR. Preliminary calculations of ^{13}C NMR chemical shifts strongly support this idea. To check this hypothesis the ^{13}C NMR spectra of isomers **1** and **2** were recorded (Fig. 2).[6]

Indeed, the ^{13}C NMR spectra of these isomers differed significantly. However, there are no simple and direct rules (at least empirical ones, as for example the γ-effect[4]) to determine isomeric structures. Therefore advanced methods to correlate the mutual orientation of fragments and spectra are necessary. For these calculations, the gauge-including atomic orbital (GIAO) method was used, with molecular geometry being optimized at the HF/6-31G level of theory.[5a] The calculated chemical shifts are referred to TMS. A comparison of experimental versus theoretical chemical shifts for a simpler parent molecular system **C** is shown in Figure 3.



As can be seen there is a good correlation between experimental and calculated chemical shifts. Some underestimate of the chemical shifts by theory observed

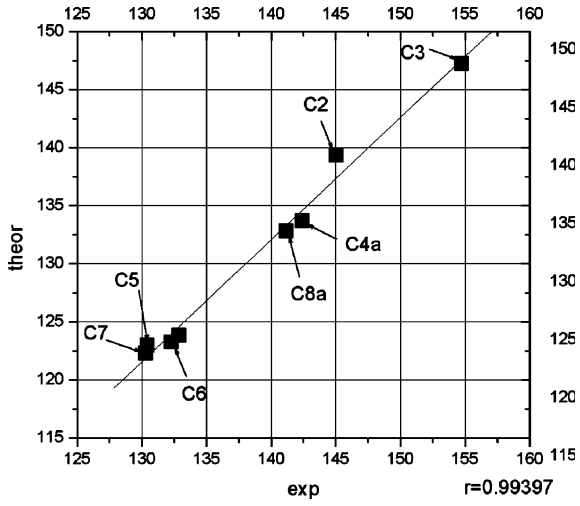


Figure 3. Correlation of calculated (GIAO B3LYP/6-31G(d)//HF/6-31G) and experimental ^{13}C NMR chemical shifts for **C**.

in this case is in accordance with previous reports for other systems and can be well attributed to the drawback of the method. It can be easily corrected for by calibration. It is of the upmost importance in this case that the sequence of signals for all carbons is well reproduced. Thus we concluded that the level of theory change to optimize geometry and the one used to calculate chemical shifts are good enough to reproduce experimentally observed values and details of structure. Therefore such an approach was used to calculate the chemical shifts of isomers **1** and **2**.

According to these calculations chemical shifts of the benzo fragment of the quinoxaline should be noticeably different for these isomers (Fig. 4). For example, C_2 and C_3 should resonate at δ 141.69 and 149.80 ppm in **A** versus 141.94 and 149.38 ppm in **B**, respectively. Similarly for C_{4a} and C_{8a}: 132.29 and 136.75 versus 135.92 and 133.17 ppm. These shift differences are large com-

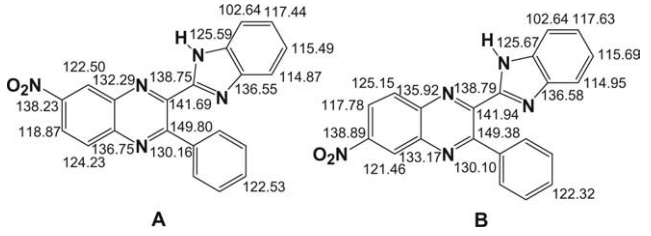


Figure 4. Calculated ^{13}C NMR chemical shifts for isomers **A** and **B**.

pared to the uncertainty in the theoretical calculation and might be used to identify the isomeric structures.

A comparison of the calculated ^{13}C NMR spectra of the two isomers demonstrates that it is the position of the NO_2 group that influences dramatically the chemical shifts of the quinoxaline carbon atoms. Moreover the chemical shifts of the quaternary carbons, adjacent to nitrogen, are the most characteristic (C_2, C_3, C_{4a} and C_{8a}). In order to attribute the isomeric structures, we compared the set of experimental chemical shifts with the theoretical data for both isomers, analyzing only the differences for the two isomers ($\Delta\delta = \delta_1 - \delta_2$ vs $\Delta\delta = \delta_A - \delta_B$) but not their absolute values (Fig. 5) as the latter are much larger. Thus, we compared the chemical shifts of C_2 (**A**) versus C_2 (**B**), C_3 (**A**) versus C_3 (**B**), because these pairs of atoms are in a similar magnetic environment (except the different positions of the NO_2 group, Fig. 4). However, for the benzo moiety of the quinoxaline, chemical shifts of C_p (**A**) versus C_p (**B**) and C_m (**A**) versus C_m (**B**) (quaternary) have to be compared in order to keep constant the contribution of the nearest NO_2 group on the chemical shift values (Fig. 4) and to reveal the different positions of the phenyl and benzimidazole groups in these isomers. The analysis of the experimental and theoretical differences showed that for the first assignment of the isomers (isomer **1** with mp = 250 °C being **A** and **2** with mp = 320 °C being **B**, Fig. 1) $\Delta\delta$ values for these carbons are not consistent (Fig. 5a) while for the reverse assignment the two sets of data are in good agreement (Fig. 5b). Thus isomer **1** corresponds to structure **B**, with the NO_2 group at the C_6 position while isomer **2** corresponds to structure **A** with this group at C_7.

Thus, the isomeric structures of the title compound have been successfully assigned via comparison of theoretical and experimental ^{13}C NMR chemical shifts. Modern 1D NOESY data[9] confirmed the structures of each compound through the direct through-space interaction of the protons as shown in Figure 6.

In these isomers such interactions and hence NOE's might be observed between H5 and the Ph protons in both isomers, but the value of the H5 chemical shift in these isomers depends dramatically on its position with respect to the NO_2 group (*meta*- or *ortho*-) and therefore can be used to determine the NO_2 position in the ring. These protons are well separated (more than 4.2 Å, Fig. 6) and therefore only a minimal NOE can be expected to be observed by regular 1D and 2D NMR.[10] Moreover due to rotation around the C_3-Ph bond, the NOE is lower. However, the DPFGNOE method may help in this case and there is a chance to observe such a weak NOE. In Figure 7 the spectra of both isomers are shown as well as the NOE spectra. An NOE is observed in isomer **A** on H2″/H6″ when proton H5 (in the *meta*

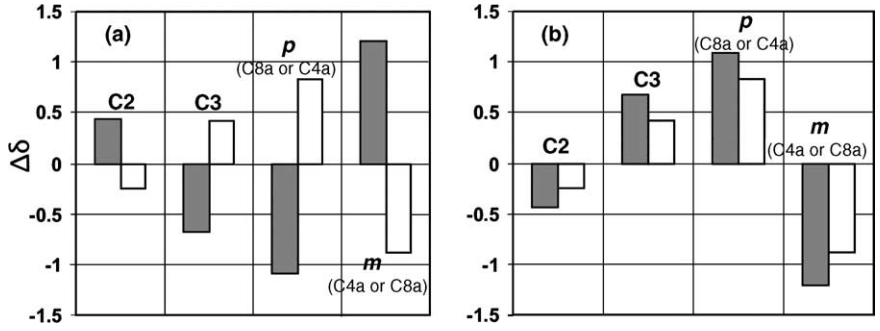


Figure 5. Comparison of calculated (□) and experimental (■) $\Delta\delta$ values (in ppm) for the two different assignments of isomeric structure: (a) ($\Delta\delta = \delta_1 - \delta_2$ vs $\Delta\delta = \delta_A - \delta_B$), (b) ($\Delta\delta = \delta_2 - \delta_1$ vs $\Delta\delta = \delta_A - \delta_B$).

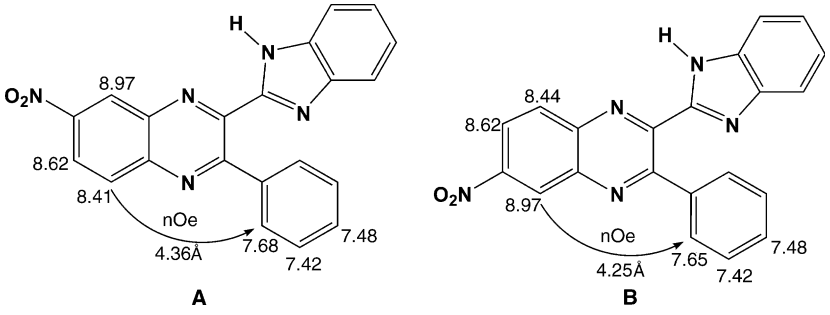


Figure 6.

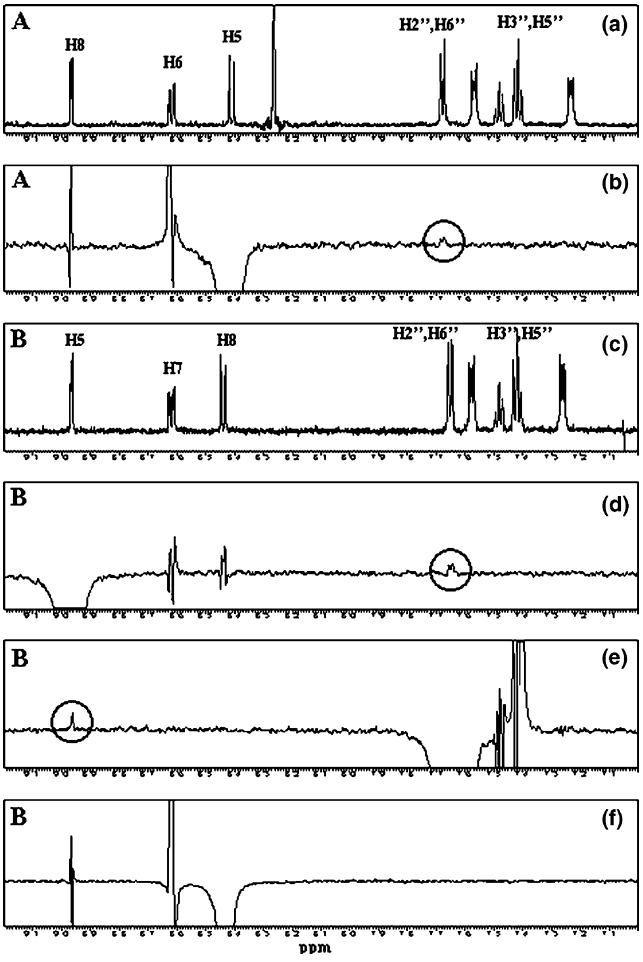


Figure 7. ^{1}H NMR spectra of isomers **A** and **B** with selective irradiation of protons. DMSO, $T = 323$ K. DPFGNOE method was used with a mixing time of 600 ms.

position to the NO_2 at δ 8.41 ppm, spectra **b** vs **a**, Fig. 7) is irradiated, while in **B** the NOE is seen only when proton H5 (*ortho*-to NO_2 at δ 8.97 ppm, spectra **d** vs **c**). At the same time if the H8 proton (*ortho*-to NO_2 at δ 8.97 ppm) in **A** and the H8 proton (*meta*-to NO_2 at δ 8.44 ppm, spectrum **f** vs **c**, Fig. 7) in **B** are irradiated, no NOE is observed on the Ph protons. Moreover, when the Ph ($H2''/H6''$) protons are irradiated (spectrum **e** vs **c**, Fig. 7), an NOE is selectively observed on the H5 proton in **A** and in **B**. These results prove unambiguously the isomeric structures of the title compounds **1** and **2** and are in full agreement with the conclusion derived from ^{13}C NMR, data.

In conclusion, identification of isomeric structures may be performed by comparison of experimental and calculated ^{13}C NMR chemical shifts. This approach was applied to determine the structure of two new quinoxalines and its validity was independently proved by the NOESY method. This proposed method is general, and may be applied to many cases.[11]

Acknowledgements

We wish to thank the CNRS for financial support.

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6. Compounds **1** and **2** were prepared by the condensation of 3-benzoylquinoxalin-2-one with 4-nitro-1,2-phenylenediamine according to Ref. 7. Complete assignments of the ^{1}H and ^{13}C NMR signals of the compounds were accomplished by 2D HSQC, HMBC, COSY experiments. **2**-(Benzimidazo-2-yl)-3-phenyl-6-nitroquinoxaline (**1**, mp =

250 °C): ^{1}H NMR (DMSO, 600 MHz, 323 K) δ 13.02 (1H, br s, NH), 8.97 (1H, d, $J = 2.6$ Hz, H-5), 8.62 (1H, dd, $J = 8.8$, 2.6 Hz, H-7), 8.44 (1H, d, $J = 9.3$ Hz, H-8), 7.65 (2H, d, $J = 7.2$ Hz, H-2″, H-6″), 7.57 (2H, dd, $J = 6.2$, 3.1 Hz, H-4′, H-7′), 7.48 (1H, m, H-4″), 7.42 (2H, t, $J = 7.5$ Hz, H-3″, H-5″), 7.26 (2H, dd, $J = 6.2$, 3.1 Hz, H-5′, H-6′); ^{13}C NMR (DMSO+CD_3OD, 100.62 MHz, 298 K) 156.97 (C3), 149.97 (C2′), 149.86 (C6), 147.56 (C2), 143.78 (*para* vs NO_2, C8a), 141.33 (*meta* vs NO_2, C4a), 139.48 (C1″), 132.10 (C8), 130.98 (C2″, C6″), 130.84 (C4″), 129.17 (C3″, C5″), 126.41 (C5), 125.35 (C7), 123.92 (br, C5′, C6′).

2-(Benzimidazo-2-yl)-3-phenyl-7-nitroquinoxaline (**2**, mp = 320 °C): ^{1}H NMR (DMSO, 600 MHz, 323 K) δ 13.16 (1H, br s, NH), 8.97 (1H, d, $J = 2.5$ Hz, H-8), 8.62 (1H, dd, $J = 9.3$, 2.7 Hz, H-6), 8.41 (1H, d, $J = 9.0$ Hz, H-5), 7.68 (2H, dt, $J = 7.1$, 1.2 Hz, H-2″, H-6″), 7.57 (2H, dd, $J = 5.9$, 3.1 Hz, H-4′, H-7′), 7.48 (1H, m, H-4″), 7.42 (2H, t, $J = 7.4$ Hz, H-3″, H-5″), 7.23 (2H, dd, $J = 5.9$, 3.1 Hz, H-5′, H-6′); ^{13}C NMR (DMSO + CD_3OD, 100.62 MHz, 298 K) δ 157.65 (C3), 149.98 (C2′), 149.56 (C7), 147.12 (C2), 144.87 (*para* vs NO_2, C4a), 140.12 (*meta* vs NO_2, C8a), 139.47 (C1″), 132.39 (C5), 131.04 (C2″, C6″, C4″), 129.17 (C3″, C5″), 126.13 (C8), 125.77 (C6). 124.66 (br, C5′, C6′), (C3a′, C7a′, C4′, C7′—are not observed due to extensive broadening).

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