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Structural dependence of isotope effects in ^{1}H and ^{13}C nuclear magnetic resonance spectra of the *trans*-N-benzylideneaniline imino group

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Abstract

Deuterium- and ^{15}N-induced isotope effects on ^{1}H and ^{13}C chemical shifts of the imino moiety of 16 *trans*-N-benzylideneaniline (tBA) isotopomers were determined and analysed. These effects appear to be a sensitive probe of molecular conformation and its changes. All determined deuterium isotope effects on the imino-proton are small and negative and are spread in the range from -0.88 ppb in 4-^{2}H-tBA to -4.97 ppb in $^{2}H_{10}$-tBA. The effects on the imino-carbon are either positive or negative and have much larger values. Negative sign of isotope effects is primarily governed by lone-pairs, while magnitude and non-additivity depend on the site of isotopic substitution, molecular conformation and distribution of rotamer populations. All ^{15}N isotope effects on both ^{1}H and ^{13}C chemical shifts are positive. A comparison of isotope effects in tBA with those in related isoelectronic compounds is made.

Keywords: NMR; Isotope effects; Conformation; *Trans*-N-benzylideneaniline

1. Introduction

Schiff bases belong to an important class of biologically active molecules, e.g. they play a main role in the process of vision [1,2]. *Trans*-N-benzylideneaniline (tBA, **1**) represents a model aromatic Schiff base. Its conformation [3] is a result of a balance between steric interactions (involving *ortho*-α hydrogens and lone-pair electrons) and conjugation effects, leading to a non-planar atom arrangement (Scheme 1).

Vibrational spectra of **1** and its deuterium labelled isotopomers have shown significant changes of the imino group vibrational modes, indicating their high sensitivity to isotopic substitution [3c]. We have recently shown [4] that the deuterium substitution in molecules of the Ph-Z-

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Ph type, where Ph refers to phenyl and Z to a bridging group (C=C, C=O, C=N, N=N, etc.), affects NMR parameters throughout the molecule. Thus, deuterium causes perturbations in ^{13}C nuclear shielding up to ten bonds away [4]. Besides, some isotope effects are interesting because of their dependence on structural parameters, such as torsional angles [5] and lone-pair interaction [4e]. For a series of isotopically labelled **1** investigated here (Scheme 1), it is shown that long range deuterium isotope effects and their non-additivity on ^{1}H and ^{13}C chemical shifts of the imino group are a sensitive probe of molecular geometry.

2. Experimental

2.1. Preparation of isotopomers

Trans-N-benzylideneaniline isotopomers (tBA) (**2**–**16**) were prepared by the addition of specifically deuteriated anilines to corresponding benzaldehydes [6]. The isotopomers were purified by recrystallization from 85% ethanol. The melting point of all synthesised compounds was 50–52°.

Benzaldehyde (Kemika), aniline (Fluka), α-^{2}H-benzaldehyde (Merck), $^{2}H_5$-benzaldehyde (Merck) and $^{2}H_5$-aniline (Campro Scientific) were commercial products. 2-, 3- and 4-^{2}H-aniline were synthesised by catalytic hydrogenolysis of 2-, 3- and 4-bromoaniline, respectively, with 5% palladium on charcoal and deuterium gas in tetrahydrofuran [7]. 2-, 3- and 4-^{2}H-benzaldehydes were prepared by the following reactions: 2-, 3- and 4-^{2}H-toluenes were mixed with N-bromosuccinimide and dibenzoylperoxide to give 2-, 3- and 4-^{2}H-benzylbromides, respectively. Benzylbromides were then allowed to react with hexamethylenetetramine in a mixture of acetic acid and water [8]. 2-, 3- and 4-^{2}H-toluenes were synthesised from respective bromotoluenes via the Grignard reaction with magnesium activated using 1,2-dibromoethane as entrainer [9]. The reaction mixture was quenched with $^{2}H_2O$.

The deuterium contents, determined with an Extrel FTMS 2001 DD mass spectrometer, were the following: **2** (90% $^{2}H_1$), **3** (90% $^{2}H_1$), **4** (90% $^{2}H_1$), **5** (97% $^{2}H_1$), **6** (95% $^{2}H_1$), **7** (91% $^{2}H_1$), **8** (97% $^{2}H_1$), **9** (79% $^{2}H_2$), **10** (97% $^{2}H_5$), **11** (96% $^{2}H_5$), **12** (92% $^{2}H_6$), **13** (85% $^{2}H_6$), **14** (92% $^{2}H_{10}$), **15** (89% $^{2}H_{11}$) and **16** (99% ^{15}N).

2.2. NMR measurements

NMR spectra were recorded with Varian Gemini 300, Bruker AM-360 and Varian Unity Inova 600 spectrometers, operating at 75.4, 90.6 and 150.9 MHz for the ^{13}C resonance, respectively. Samples were measured at 294 K in 5 mm tubes. Variable temperature measurements were performed in the temperature range of 274–324 K. Sample concentrations were 0.15–0.20 M. Deuterium from the solvent was used as the lock signal and TMS as the internal standard. Narrow region spectra with spectral widths of 500–2000 Hz were zero-filled to 64 K, thus obtaining a digital resolution better than 0.03 Hz per point (i.e. ± 0.2 ppb for ^{13}C at 150.9 MHz) after Fourier transformation.

Isotope effects were determined as a difference between chemical shifts of the light and heavy isotopomer ($\Delta = \delta_L$-δ_H), expressed in parts per billion (ppb) units. Standard deviations are given in Table 1. For non-additivity estimations total errors were obtained by summing up the individual mean square errors of the corresponding effects.

3. Results and discussion

3.1. Deuterium isotope effects

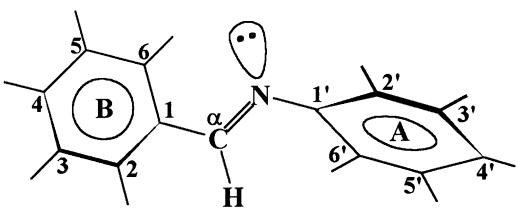
^{1}H and ^{13}C NMR spectra (Figs. 1 and 2) of the imino group ($-C_\alpha H_\alpha = N_\alpha -$) in deuteriated isotopomers of **1** reveal a number of interesting isotope effects (Table 1). All observed deuterium effects at H-α are negative, i.e. deshielding, while the effects at C-α are either positive (shielding) or negative (Table 1). The effects at H-α are much smaller than those at C-α, primarily due to the relatively narrow ^{1}H chemical shift range. From this reason, intrinsic isotope effects on ^{1}H chemical shifts have rarely been reported in the literature [10]. In determination of such effects (Fig. 1), high precision measurements are required.

Table 1
^{2}H and ^{15}N isotope effects, Δ (ppb), on ^{13}C-α and ^{1}H-α chemical shifts in the imino group of *trans*-N-benzylideneaniline isotopomers

Isotopomer	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Atom	Δ (ppb)														
H-α[a,b]	−0.88	unres.	−1.12	0.00	−1.00	0.00		−0.97	−4.31	−1.16		−4.10	−4.97		0.97
C-α[c,d]	−6.50	7.00	24.30	1.00	−4.50	7.00	260.0	0.00	31.50	8.00	291.0		28.00	287.0	25.70

[a] SD is better than 0.07 ppb for the effects at H-α; [b] solvent was acetone-$^{2}H_6$; [c] SD is better than 1.0 ppb for the one-bond effects and better than 0.5 ppb for the other effects at C-α; [d] solvent was chloroform-$^{2}H_1$. unres. = unresolved.

Deuterium isotope effects at H-α in tBA do not exceed -5.0 ppb in magnitude. In isoelectronic *cis*- and *trans*-stilbenes these effects are up to 3.3 and 8.57 ppb, respectively [11]. Since deuterium isotope effects at H-α in *cis*- and *trans*-stilbenes are positive [11], one can rationalise the negative sign in tBA by the inductive and steric influence of the nitrogen lone pair electrons. The shortest possible deuterium isotope effect at H-α, is the one over four-bonds, $^4\Delta$, observed in isotopomer **4**, amounting to -1.12 ppb (Table 1). The magnitude of isotope effect depends primarily on the distance between the isotopic and the observed nucleus [12]. Correspondingly, $^5\Delta$ in isotopomer **6** is -1.00 ppb and $^6\Delta$ in isotopomer **2** is only -0.88 ppb, the latter being also the longest range isotope effect observed in tBA at H-α (Fig. 1b). $^7\Delta$ in isotopomer **3** (4′-^{2}H-tBA) was not revealed, but



1

2 = 4-^{2}H (*p*-^{2}H)

3 = 4'-^{2}H (*p*'-^{2}H)

4 = 2-^{2}H (*o*-^{2}H)

5 = 2'-^{2}H (*o*'-^{2}H)

6 = 3-^{2}H (*m*-^{2}H)

7 = 3'-^{2}H (*m*'-^{2}H)

8 = α-^{2}H

9 = 4,4'-^{2}H$_2$

10 = ^{2}H$_{5(B)}$

11= ^{2}H$_{5(A)}$

12= ^{2}H$_{5(B)}$, α-^{2}H

13 = ^{2}H$_{5(B)}$, 4'-^{2}H

14 = ^{2}H$_{10}$

15 = ^{2}H$_{11}$

16 = ^{15}N

B = benzylidene ring, A = aniline ring

Scheme 1.

significant line-broadening was detected in ^{1}H NMR spectrum of the mixture of **3** and **1**. In isotopomers **5** and **7**, having attached a deuterium atom at *o*′- and *m*′-positions, respectively, no long range deuterium effect on H-α chemical shift was observed. However, shielding effects were found at the imino-carbon C-α (Fig. 2).

Long range effects on ^{1}H shifts, i.e. those transmitted over more than three bonds from the isotopic site, were previously detected only in a few instances: in some rigid molecules [13] and in biomolecules such as protoporphyrins [14] and macrolide antibiotics [15]. In the case of rigid systems, the existence of long range deuterium effects on ^{1}H shifts was attributed to a through-space interaction between ^{1}H and ^{2}H, while in macrolides these effects were assumed to be a consequence of changes in hydrogen-bonding upon isotopic substitution. Generally, isotope effects on NMR parameters are of a rotational–vibrational origin, i.e. they arise from different averaged rotational–vibrational geometries of isotopomers [16].

Deuterium isotope effects in isotopomers **9**–**15** are cumulative or total since more than one deuterium is present in the molecule. Comparing pentadeuterio-isotopomers **10** and **11** (Table 1), one can see that the total deuterium isotope effect on H-α chemical shifts is much larger in the former (-4.31 ppb) than in the latter (-1.16 ppb), which reflects different conformations of aniline and benzylidene moiety relative to the azomethine group plane (Scheme 1). As a matter of fact, all effects from deuterium in the aniline ring of isotopomers **3**, **5**, **7**, **9** and **11** are smaller than those from deuterium in the benzylidene ring of isotopomers **2**, **4**, **6**, **8** and **10**. Although the effects in the former set of isotopomers are transmitted through a longer pathway (one bond more) than those in the latter series, previous investigations in related molecules showed the predominant role of conformation in transmission of isotope effects. Our ab initio calculations predict a torsional $C_2{=}C_1{-}N{=}C_\alpha$ angle in tBA of 44.6°, while $C_2{=}C_1{-}C_\alpha{=}N$ is close to zero [5]. Therefore, the decrease in magnitude of isotope effects transmitted from the aniline ring should also be related to larger torsional angle.

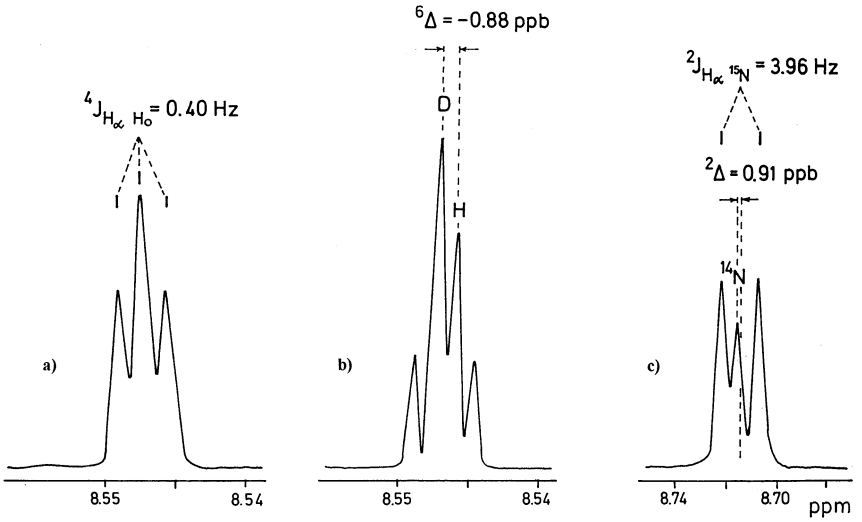


Fig. 1. Isotope effects at H-α in isotopomers of *trans*-N-benzylideneaniline. (a) H-α region of the 360 MHz proton spectrum of isotopomer **2** (4-^{2}H); (b) the same spectrum with the addition of **1**; and (c) H-α region of the 300 MHz proton spectrum of isotopomers **16** (^{15}N) and **1**.

3.2. ^{15}N isotope effects

^{15}N isotope effects at both H-α and C-α are positive, but the former is much smaller than the latter (Table 1). This is due to the two main reasons. Firstly, the effect at H-α is transmitted over two bonds, while that observed at C-α is transmitted over one bond. Secondly, ^{1}H chemical shift range is significantly smaller than that of ^{13}C. Both ^{15}N effects are much smaller than deuterium effects (Table 1), owing to the lower ^{15}N/^{14}N mass ratio.

3.3. Additivity

One of the main characteristics of isotope effects in NMR spectra is their additivity. In flexible molecules such as those containing phenyl rotors, some apparent deviations have recently been reported [4e,12]. The deviation from the additivity rule of deuterium isotope effects on ^{1}H shifts in tBA isotopomers appears to be more significant than that in isotopomers of isoelectronic molecules of *cis*- and *trans*-stilbene [11]. The sum of the effects measured in pentadeuterio-isotopomers **10** and **11** is -5.47 ppb (standard deviation is 0.14 ppb), while -4.97 ppb (standard deviation is 0.07 ppb) was determined in **14** ($^2H_{10}$). This deviation of 0.50 ± 0.21 ppb is small but significant. Non-additivity indicates that beside intrinsic isotope effects, equilibrium isotope effects are also present, which is supported by variable temperature measurements. A decrease in magnitude of isotope effect in the 50° temperature range is observed with decreasing the temperature.

The non-additivity of deuterium isotope effects on ^{13}C chemical shifts in tBA have been reported recently [4e]. With two new isotopomers discussed here, *o'*-^{2}H- (**5**) and *m'*-^{2}H-tBA (**7**), one can recognise an appreciable deviation from additivity rule at C-α, amounting to 29.1 ± 6.5 ppb. This value was calculated as follows. By summing up individual contributions of mono-deuteriated isotopomers **2**, **3**, **4**, **5**, **6** and **7**, the value of 316.1 ± 5.5 ppb was obtained. However, a much smaller effect of 287.0 ± 1.0 ppb was observed in 15 ($^2H_{11}$). The difference between these two give the above estimated non-additivity. As previously pointed out [4e,f] this should be due to slight conformational changes arising from shorter C–^{2}H than C–^{1}H bond lengths, which in the case of the deuteriated phenyl rings, releases a strain between the *ortho* and α positions, thus affecting rotational

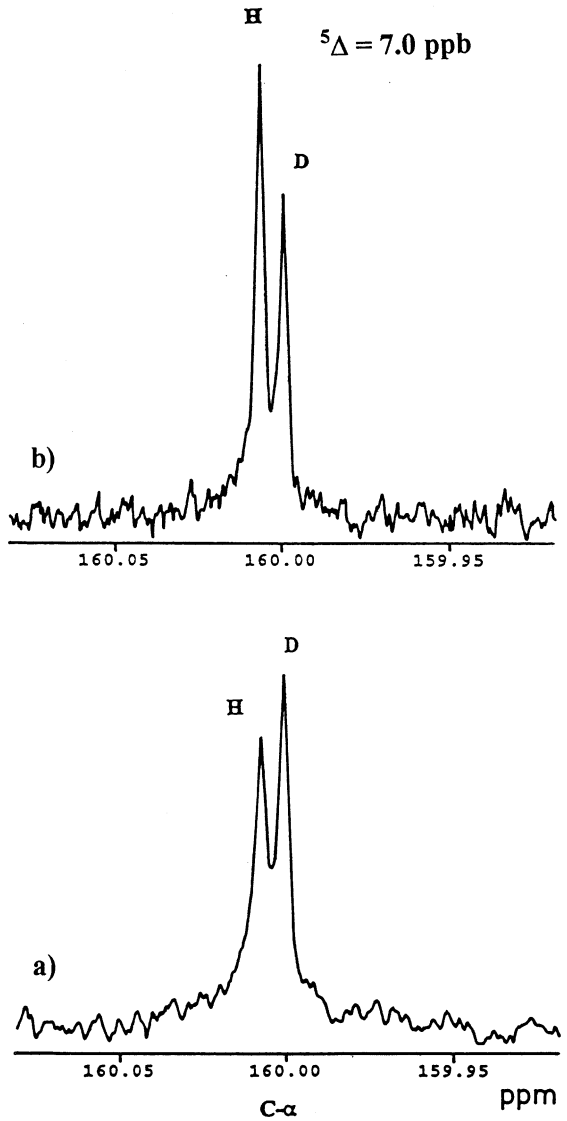


Fig. 2. Isotope effect over five bonds at C-α in isotopomer **7** (3′-^{2}H-tBA) measured at 150.9 MHz. (a) 2:1 mixture of isotopomers **7** and **1**; and (b) 1:1 mixture of the same isotopomers.

properties of benzylidene (B) and aniline (A) rings. This brings about a redistribution of rotamer populations, which affects nuclear shieldings.

4. Conclusions

Isotope effects in the imino group of *trans*-N-benzylideneaniline reflect conformational changes upon isotopic substitution and show a sensitivity to steric and lone-pair interactions. The non-additivity in polydeuteriated isotopomers arises from a redistribution of rotamer populations due to different rotational properties of undeuteriated and perdeuteriated phenyl rotors, as was previously found for benzophenones [12] and biphenyls [17]. Therefore, isotope effects in the imino moiety could serve as a probe for studying Schiff bases, both experimentally and theoretically.

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