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Factor Analysis of Deuterium Isotope Effects on ^{13}C NMR Chemical Shifts in Schiff Bases

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Abstract: We have analyzed deuterium isotope effects on ^{13}C chemical shifts in a series of *o*-hydroxy Schiff bases by applying factor analysis. Two orthogonal factors were obtained that explain about 80 and 10% of the variance of the data. The numerical values of these factors can be related to ^{1}H NMR chemical shifts of the proton involved in the intramolecular bonds δ(XH) (X = O or N). Such a relation allows one to identify clusters of compounds with different tautomeric forms of hydrogen bonding. Application of a similar approach to solution ^{13}C NMR chemical shifts produces three important factors, which have a different structure to factors describing isotope effects. This illustrates well the different nature of chemical shifts and isotope effects. The three factors explain about 54, 15, and 13% of variance. They can be rationalized and are strongly related to the electronic properties and location of substituents.

Keywords: hydrogen bonding · isotope effects · NMR spectroscopy · Schiff bases · tautomerism

Introduction

Isotopic substitution is a powerful and widely applicable NMR technique providing information about the shape of molecular potential energy surfaces.[1–3] After substitution of one isotope for another, the shape of the potential-energy surface does not change according to the Born–Oppenheimer approximation. Although the binding forces remain the same, the masses of the isotopic atoms change. Consequently, isotopic substitution affects the vibrational frequencies and the zero-point energy.

Replacement of hydrogen by deuterium in hydrogen-bonded systems produces intrinsic and equilibrium isotope effects.[2,4] The intrinsic isotope effects on chemical shifts are induced by changes in molecular geometries, whereas the equilibrium isotope effects appear when isotopic substitution modifies the chemical (conformational, tautomeric, etc.) equilibrium of a given molecule.

The intrinsic isotope effects are strictly connected with the molecular vibrational wavefunctions[5] and, precisely, with the anharmonicity of potential of the X–H bond. Because of the anharmonicity, the positions of the maxima of probability of finding protium and deuterium significantly differ. As a result, the average bond length for deuterium becomes shorter than for protium. Such an effect usually causes an increase in electron density in the neighboring bonds, some shielding of the neighboring nuclei and, in consequence, a decrease in chemical shifts.[1,6,7] The magnitudes of the intrinsic effects are most pronounced at the site of deuteration and generally fall off quite rapidly as a function of bond separation between the substitution site and the observation site. In principle, the intrinsic isotope effects are independent of temperature as long as vibrational excitation and other temperature-dependent changes of the nuclear geometry are negligible.[8]

The equilibrium isotope effects on chemical shifts arise from isotopic fractionation between different sites[9–12] that interconvert rapidly on the NMR timescale. For example, a two-state equilibrium of tautomers rapidly interconverting

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Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author: Appendix on factor analysis as well as numerical values of the isotope effects and chemical shifts for all compounds studied and numerical values of all factors (F*n* and CSF*n*).

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by intramolecular proton transfer between electronegative atoms A and B from the same molecule is described (when charges are neglected) by Equation (1).

$$AH + B \rightleftharpoons A + HB \quad (1)$$

In such a case, no separate NMR signals for an atom X belonging to the tautomer A or AH [δX_{AH} and δX_A] are expected. The observed chemical shifts are averaged [Eq. (2)].

$$\delta X_{obs} = x_{AH}\delta X_{AH} + (1-x_{AH})\delta X_A \quad (2)$$

The equilibrium effect on atom X, $\Delta X(D)_{eq}$, appears when K^H and K^D differ[9] as a consequence of a difference in zero-point energies for the two X–H bond potentials, and is described by Equation (3):

$$\Delta X(D)_{eq} = \Delta_x[\delta X^H_{AH} - \delta X^H_A]$$
$$\Delta_x = [x_{AH} - x_{AD}] \quad (3)$$

where Δ_x is the change in the molar fraction on deuteration. Consequently, two major factors influence the equilibrium isotope effects on the chemical shift: a large perturbation of the equilibrium, that is, large Δ_x, and a significant difference in chemical shifts for a given atom in two different tautomeric forms. It is difficult to ascertain in equilibrating systems whether the isotopic effects on chemical shifts are caused by the intrinsic or equilibrium effect, or both of them. Only the sum of the intrinsic and the equilibrium effects is observed [Eq. (4)].

$$\Delta X(D)_{obs} = \Delta X(D)_{int} + \Delta_x[\delta X^H_{AH} - \delta X^H_A] \quad (4)$$

Abstract in Polish: *Analiza czynnikowa deuterowego efektu izotopowego na przesunięcia chemiczne jąder atomów węgla ^{13}C wykonana dla serii orto-hydroxy zasad Schiffa pozwoliła zidentyfikowć 2 ortogonalne czynniki wyjaśniające ok. 80% and 10% wariancji danych. Wartości numeryczne uzyskanych czynników są w nieliniowy sposób związane z przesunięciami chemicznymi δ(XH) (X=O or N) protonu (^1H NMR) zaangażowanego w wewnątrzcząsteczkowe wiązanie wodorowe. Taka zależność pozwala zidentyfikować klastry pochodnych zasad Schiffa z wiązaniem wodorowym w różnych formach tautomerycznych. Podobna procedura zastosowana do przesunięć chemicznych jąder węgla (^{13}C NMR) pozwala uzyskać trzy istotne czynniki różniące się swoją budową od czynników uzyskanych dla efektów izotopowych. Takie wyniki dobrze ilustrują różną naturę obu zjawisk. Wszystkie trzy czynniki wyjaśniają ok. 54%, 15% i 13% wariancji zbioru przesunięć chemicznych. Czynniki te moga być zinterpretowane poprzez właściwości elektronowe oraz położenie podstawników.*

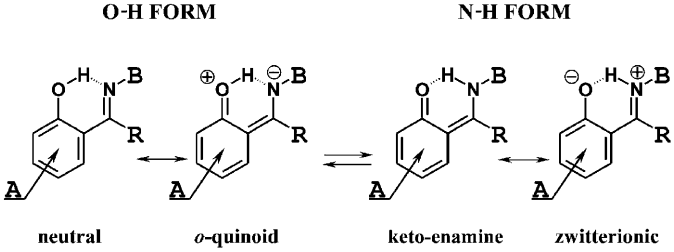
The secondary isotope effects are defined by Equation (5):

$${}^n\Delta X(D) = \delta X^H - \delta X^D \quad (5)$$

where n is the number of bonds between X and deuterium, and δX^H and δX^D are chemical shifts of the observed nucleus X in the molecules substituted with protium (H) and deuterium (D).[13]

Deuterium isotope effects on chemical shifts have been shown to be useful in describing hydrogen-bonded systems.[2,9–38] Hydrogen bonding increases the anharmonicity of the X–H bond, and indeed $^2\Delta X(D)$ has been found to correlate well with δH for various classes of compounds.[15–19,21] Such correlation falls off when equilibrium effects become significant. Hansen and Bolvig proposed using the $^2\Delta X(D)$ versus $^4\Delta X(D)$ relation to distinguish between localized and tautomeric systems.[24] A characteristic S-shaped correlation between deuterium isotope effects and mole fraction was found for several equilibrium systems.[24,27] The primary isotope effects were used to distinguish between the double- and single-minimum proton potentials.[37–39]

We have concentrated our attention on *ortho*-hydroxy Schiff bases that form intramolecular resonance-assisted hydrogen bonds (RAHBs)[40,41] and are excellent examples to study isotope effects. These compounds exhibit some very interesting properties, such as thermochromism and photochromism, usually attributed to intramolecular proton transfer.[42] Possible tautomeric and resonance structures for the *ortho*-hydroxy Schiff bases are illustrated in Scheme 1.



Scheme 1. Resonance and tautomeric forms of *ortho*-hydroxy Schiff bases.

It was demonstrated in some model Schiff bases[43] that the OH tautomer exists mainly in the neutral form (90%), whereas the NH tautomer is a mixture of the keto-enamine and zwitterionic forms in a ratio of 4:6.

The shape of the potential-energy function describing proton transfer in Schiff bases is influenced by electronic and steric factors. Among the electronic factors, the difference in the acidity of the oxygen atom and the basicity of the nitrogen atom ΔpK_a, and strong π-electron interactions between these centers play the most important roles. The π-donating/withdrawing ability of substituents attached to Schiff fragments and the substitution site are the most im-

portant factors that control the electronic situation in these molecules. Steric effects in Schiff bases were first reported in reference [44]. The authors showed that any substituent (alkyl or aryl) at the Cα atom imposes an external squeezing of the hydrogen bond and efficiently reduces the O···N distance.[44–48] It was also demonstrated that steric interactions of the substituent at position C6 (see Scheme 2) shorten and strengthen the hydrogen bond.[49]

Such factors as solvent and temperature also have an important influence on tautomeric equilibrium. The solvent can influence this equilibrium not only because of its polarity (proton transfer is promoted by an increase in solvent polarity), but also because of hydrogen-bonding ability.[50] Shielding of donor–acceptor centers from solvent molecules by N-aliphatic chains should also be taken into account.[51] In the OH form, hydrogen bonding strengthens with decreasing temperature due to an increasing fraction of the *o*-quinoid form. For a hydrogen bond with prevailing NH character, a decrease in temperature leads to weakening of the hydrogen bond and supports increased keto-enamine character of the structure.

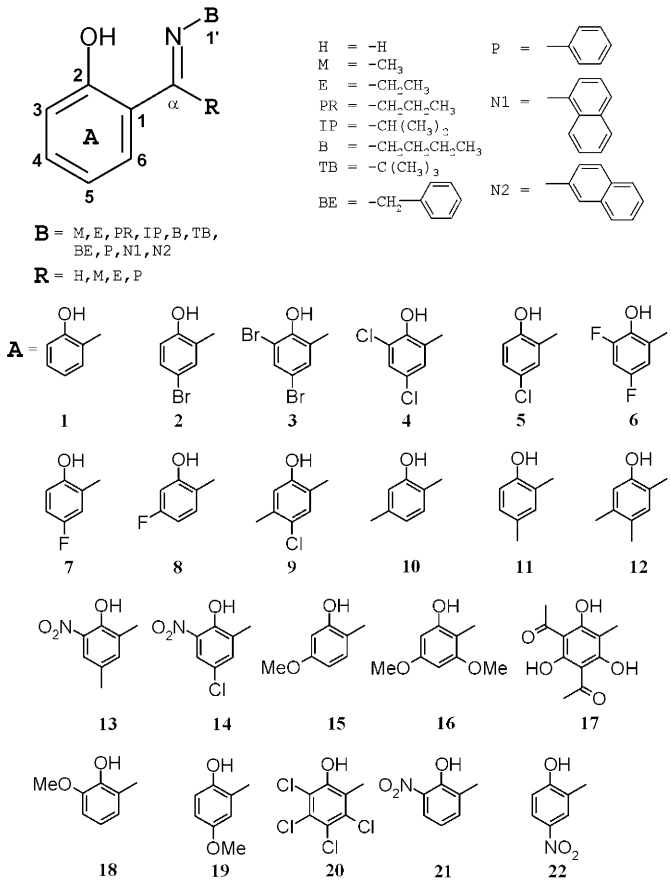
There are many reports[18,20,22,27,28,52] describing relations between primary and secondary effects and some other measured properties, such as chemical shifts, coupling constants, distance between donor and acceptor in H bonds, hydrogen-bond energy, and mole fractions. On the other hand, there are only a few reports[12,14,23] exploring primary and secondary effects up to very long range[17,53] by means of correlation analysis. The compounds investigated are chosen to cover all the above-mentioned aspects. In addition, unusual substituent patterns and multiple substitution are included to ensure that additivity effects are no longer valid and thereby lead to new electronic situations.

Here, we propose a general approach for all secondary isotope effects appearing in a molecular fragment present in a series of similar compounds. We perform a statistical factor analysis of the deuterium secondary isotope effects on ^{13}C chemical shifts to find major factors describing the response of the system upon deuteration of the O···H/D··N hydrogen bond. We also compare the structure of factors describing the isotope effects to factors obtained for ^{13}C chemical shifts for the same systems.

Results and Discussion

We measured deuterium isotope effects on ^{13}C chemical shifts $^{n}\Delta C(XD)$ (X = O or N) for 56 Schiff bases. Their formulae and numbering scheme are shown in Scheme 2. Data for the seventeen Schiff bases with hydrogen at the Cα atom (without R substituent) were already published by Rozwadowski et al.[27] The data for $R = CH_3$ are taken from Refs. [54,55]. For most of the Schiff bases, the isotope effects were measured at least at two different temperatures. From a statistical point of view, this sample of compounds is large enough and has a sufficiently wide variation of substituents to allow statistical treatment of the data and application of factor analysis.

The secondary isotope effects observed on the C1, C2, C3, C4, C5, and C1′ atoms have both positive and negative



1EBE, 1HM, 1MBE, 1MM, 1PBE, 1PIP, 2HM, 2ME, 2MM, 3HM, 3MBE, 3MM, 3MN1, 3MN2, 4HM, 4MBE, 4MM, 4MPR, 5HM, 5MM, 5MPR, 6MIP, 6MM, 7MM, 8MIP, 8MM, 9ME, 9MPR, 9PE, 9PM, 10MM, 10PM, 11HM, 11MM, 12MBE, 13ME, 13MM, 13MPR, 14MB, 14ME, 14MP, 14MPR, 15HBE, 15HM, 15HTB, 15MIP, 16HM, 16HTB, 16MM, 17MBE, 18HM, 19HBE, 19HM, 20HM, 21HPR, 22HPR

Scheme 2. The numbering scheme and definition of substituents A, B, and R with enumeration of all abbreviations for the Schiff bases studied. A stands for the whole aromatic ring together with substituents, and in the abbreviations is defined by a integer in the range from 1 to 22, B is a substituent at the nitrogen atom, and R is a substituent at Cα. The order of substituents in the abbreviations is ARB.

signs. The values of $^{n}\Delta$C6(XD) have always negative signs, whereas those for Cα, $^{n}\Delta$Cα(XD), are always positive. The $^{n}\Delta$C2(XD) variable shows the largest variation, while $^{n}\Delta$C6(XD) has the lowest, in the range from −965.7 up to 1243.1 ppm, and from −337.0 to 0.0 ppm, respectively. When it is obvious from the context, we will abbreviate the symbol of the isotope effect on a carbon atom *m*, $^{n}\Delta$C*m*(XD), just by the symbol of this atom C*m*.

Table 1. Correlation coefficients for ^{13}C isotope effect variables and ^{13}C chemical shifts variables for the *ortho*-hydroxy Schiff bases. All correlations written in bold are significant from a statistical point of view at the significance level $\alpha = 0.05$ for the number of data points $N = 153$ and 111, respectively.

Isotope effect	C2	**−0.92**						
	C3	**−0.93**	**0.94**					
	C4	**−0.84**	**0.94**	**0.88**				
	C5	**0.92**	**−0.93**	**−0.94**	**−0.92**			
	C6	**−0.46**	**0.55**	**0.53**	**0.60**	**−0.50**		
	Cα	**−0.74**	**0.65**	**0.67**	**0.68**	**−0.80**	**0.37**	
	C1′	**0.92**	**−0.97**	**−0.92**	**−0.91**	**0.91**	**−0.48**	**−0.62**
		C1	C2	C3	C4	C5	C6	Cα
Chemical shift	C1′	0.18	**−0.21**	−0.07	−0.06	0.04	−0.03	−0.19
	Cα	**0.22**	0.07	**0.28**	−0.09	0.04	−0.01	
	C6	**−0.73**	**0.76**	**−0.39**	**0.68**	**−0.71**		
	C5	**0.52**	**−0.68**	**0.46**	**−0.74**			
	C4	**−0.76**	**0.82**	**−0.74**				
	C3	**0.57**	**−0.50**					
	C2	**−0.78**						

Six out of eight secondary isotope effect variables, that is, C1, C2, C3, C4, C5, and C1′, are highly intercorrelated (Table 1). In particular, when the secondary isotope effects on the C1, C5, and C1′ atoms increase, then the secondary isotope effects on C2, C3, and C4 decrease. Figure 1 presents a graphical visualization of some of these correlations. The scatter of data points around the regression line is not random, as it should be in the case of a perfect correlation. It is possible to distinguish certain clusters of Schiff bases with common substituent groups (which are written in bold) with slightly different slopes than that of the final regression line. One can see that the main grouping factor in Figure 1a is size and type of substituent at C1′. Apparently (Figure 1b), the strongly electron-withdrawing nitro group at C3 magnifies the isotope effects at the neighboring nucleus C4.

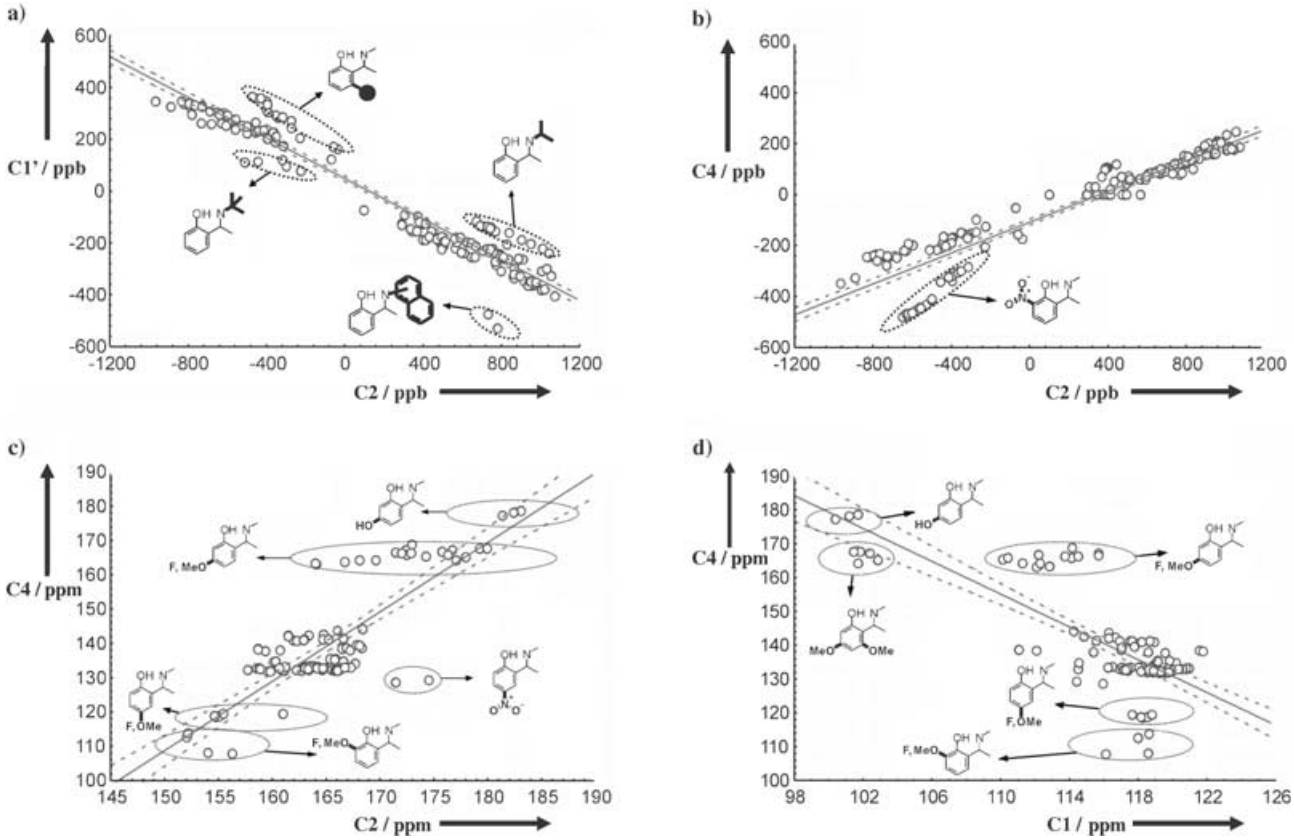


Figure 1. Graphical visualization of selected correlations between particular carbon positions: a) isotope effects C1′ versus C2, b) isotope effects C4 versus C2, c) chemical shifts C4 versus C2, d) chemical shifts C4 versus C1. Subgroups of Schiff bases with common groups are indicated. Number of data points $N = 154$ for isotope effects and $N = 111$ for chemical shifts.

We applied statistical factor analysis to detect structure in the relationships among secondary isotope effect variables. At the beginning, a principal-components analysis was performed. Only cases that did not contain any missing data for any of the variables were selected for the analysis. As a result, we obtained a new set of variables (called principal components or unrotated factors) that are linear combinations of the original variables. They are also orthogonal. Such linear combinations explain most of the variation of the data to the maximum possible extent. The variance accounted for by successive factors is given in Table 2, which also contains correlations between the original isotope-effect variables and the first three factors. These correlation coefficients are also called factor loadings.

From solution of the principal-component analysis, we chose only the first two factors (F1, F2) on the basis of Cattell's scree test (see line graph of the eigenvalues, Figure 2 a).[56]

In the next step of the analysis, we rotated the factor loadings to find a physicochemical interpretation of the results. Various rotational strategies could be used to obtain a clear pattern of loadings. The standard computational method of rotation is called the varimax rotation.[56] Another is quartimax rotation of the normalized factor loadings, which was used by us in our analysis.[57] Factor loadings after quartimax normalized rotation and factor score coefficients (i.e., the weights) are listed in Table 2.

We finally found two major factors describing the secondary isotope effects on ^{13}C chemical shifts in Schiff bases. Factor 1 (F1) consists of large loadings on almost all variables, that is, C1, C2, C3, C4, C5, Cα, and C1′. Factor 2 (F2) has—in a first approximation—a high loading only for C6. The structure of the F1, especially the signs of the factor loadings, shows how the variables depend on one another. With decreasing values of C1, C5, and C1′, the values of C2, C3, C4, and Cα increase. Moreover, the sign of F1 is associated with the signs of C1, C2, C3, C4, C5, and C1′. In the case of positive values of F1, one can observe negative isotope effects on atoms C1, C5, and C1′ and positive ones on atoms C2, C3 and C4. The opposite is true for negative values of F1. The structure of factors F1 and F2 is schematically depicted in Figure 3 a.

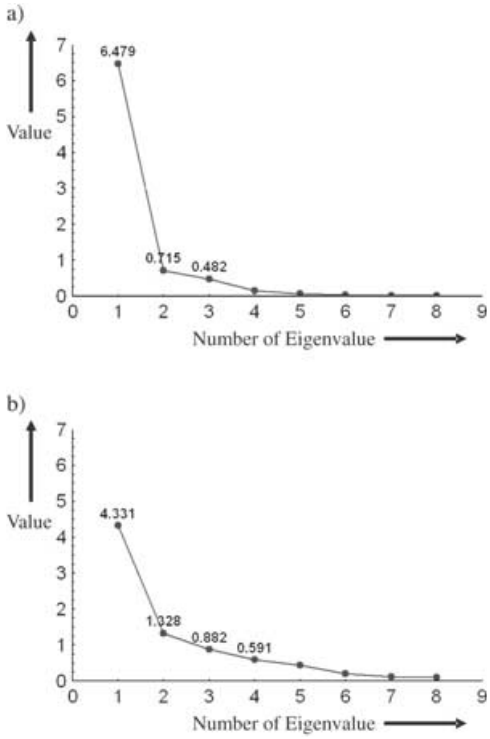


Figure 2. Plot of eigenvalues for a) isotope effect and b) chemical shift variables.

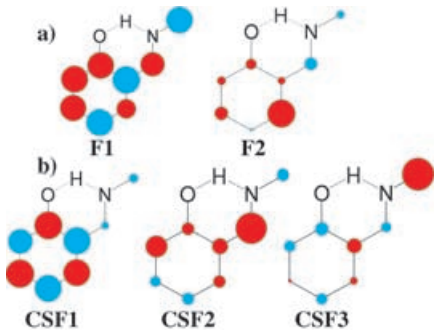


Figure 3. Graphic representation of factor loadings for a) isotope effect factors F1 and F2 and b) chemical shift factors CSF1, CSF2, and CSF3. The area of the circles is proportional to the magnitude of the factor loadings. Red stands for positive and blue for negative contributions.

Table 2. Factor analysis of isotope effects. Number of data points $N = 153$. Factor loadings numerically larger than 0.75 are in bold.

	Factor loadings					Factor score coefficients	
	Unrotated			Quartimax-normalized		Quartimax-normalized	
Variable	F1	F2	F3	F1	F2	F1	F2
C1	**−0.95**	0.15	0.03	**−0.96**	0.04	−0.17	0.19
C2	**0.97**	0.00	−0.18	**0.96**	0.12	0.15	0.02
C3	**0.96**	−0.04	−0.13	**0.96**	0.07	0.15	−0.04
C4	**0.95**	0.08	−0.06	**0.93**	0.19	0.13	0.13
C5	**−0.98**	0.12	−0.06	**−0.98**	−0.00	−0.17	0.14
C6	0.60	**0.78**	0.16	0.50	**0.85**	−0.04	1.10
Cα	**0.77**	−0.24	0.59	**0.79**	−0.15	0.16	−0.32
C1′	**−0.95**	0.06	0.24	**−0.95**	−0.05	−0.16	0.07
Expl. var.	6.479	0.715	0.482	6.399	0.795	–	–
% of Total	81	9	6	80	10	–	–

The first dominating factor F1 is related to about 80% of the variance of the data, and it affects most of the carbon positions of Schiff bases. Factor F2, being orthogonal to F1, explains only up to 10% of the variance. For the data collected at one temperature (250 or 220 K), the structure of both these factors remains the same.

To obtain more information about these two factors, we correlated them with the δXH chemical shift of the proton participating in the hydrogen bonding. There is no linear correlation between F1 and δXH at all. However, a nonrandom relationship between F1 and δXH is present (Figure 4a). We propose the following interpretation of this relationship. The sign of the factor F1 could be associated with the position of the proton in this crucial O···H···N hydrogen bond. These compounds, in which the proton is localized at the oxygen atom, or for which the OH form is dominant, have positive values of F1. The opposite is true for the compounds with dominant NH form.

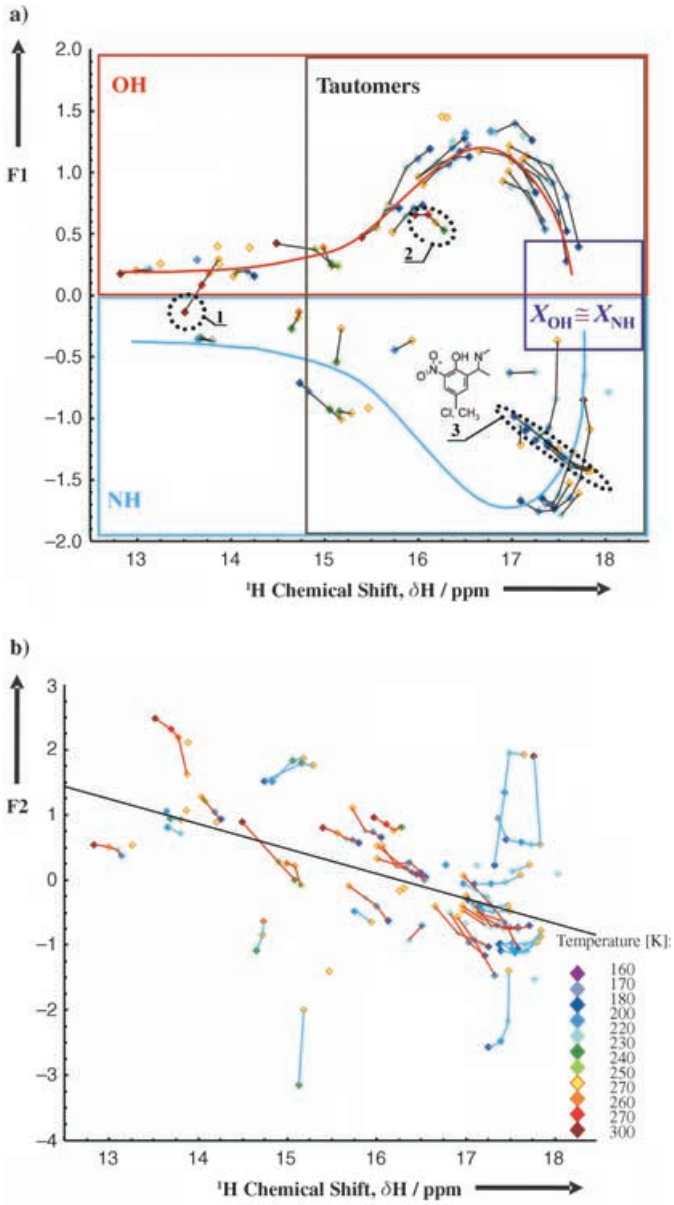


Figure 4. Dependence of the factors a) F1 and b) F2 on the proton chemical shift δXH.

An increase in the δXH chemical shift indicates that the proton becomes more and more unshielded. This is possible when the proton becomes more or less equally affected by the donor and acceptor atoms. Such a situation is realized in strong hydrogen bonds. We postulate then that on going from left to right in Figure 4a, one has first a localized hydrogen bond with the OH form for $F1 > 0$ and the NH form for $F1 < 0$. Then there is an equilibrium of two tautomers with the OH form dominating for $F1 > 0$ and the NH form dominating for $F1 < 0$. Finally, there are very strong hydrogen bonds with the molar fraction x_{OH}:x_{NH} close to 1:1 or with the proton vibrating close to the center of the bond. This interpretation is consistent with the fact that the equilibrium effect should not be observed for a situation with an equilibrium constant close to 1.[24] The factor F1 has the largest values for systems with tautomeric equilibrium and, quite surprising, has a maximum of δXH between 16 and 17 ppm. Apparently, there is a limiting value of the proton chemical shift (ca. 17.7 ppm) for the Schiff base derivatives analyzed in this work.

There are different temperature effects for particular tautomeric forms of *ortho*-hydroxy Schiff bases. The influence of temperature on the isotope effects is illustrated by different colors of data points. Lines join data points for the same compound measured at different temperatures. A decrease in temperature is associated with a positive shift of δXH for the OH form and a negative one for the NH form. This results from the strength of hydrogen bonding[48,58] or a shift of the equilibrium, or both.

Additionally, Figure 4a (and even more so, 4b) stresses the lack of equivalence of the O–H and N–H accepting sites. Oxygen is a stronger donor than nitrogen and this is one reason why the scatter of data points for the OH form seems to be smaller than for the NH form. The other justification of the above could be an influence of steric effects of the groups linked to the N atom.

There are a number of outliers in this plot, for example, data point 1 for 1HM has probably a wrong value. We would expect a small positive value of F1 for this compound at 300 K. However, the higher the temperature the larger the errors associated with measurements, and this influences the numerical value. The data points 2 were collected for 1PIP dissolved in C_5D_5N. Data measured for the same compound in other solvents follow the general trends, but pyridine must interact in a qualitatively different manner, significantly perturbing the hydrogen bond in 1PIP. One would expect some higher values of δXH for this kind of temperature dependence. Third, the series of data points for 13MM, 13ME, 13MPR, 14ME, 14MPR denoted by number 3 in Figure 4a, is characteristic for nitro derivatives defined by the neighboring formula. Apparently, the nitro group at C3 deshields the XH proton strongly. Furthermore, the nitro group is twisted out of the ring plane. This twist will change

with changing temperature and probably lead to an irregular change in δXH.

A weak, but statistically significant, correlation between F2 and δXH ($r=-0.51$, $p=0.00$) is illustrated in Figure 4b. Closer inspection of this correlation shows that it is present mainly for Schiff bases with positive F1 (red lines; $r=-0.77$, $p=0.00$), that is, for the OH form. F2 decreases for the systems with the strongest hydrogen bonds. F2 also decreases with decreasing temperature, with the exception of compounds with very high δXH, for which some kind of minimum is achieved at the lower temperatures. It is noteworthy that for some compounds with $F1<0$ (blue lines), the values of F2 change rapidly with temperature.

Both F1 and F2 could possibly be interpreted in terms of the equilibrium (F1) and intrinsic (F2) isotope effects or as a sum (F1) and difference (F2) of these effects. Nevertheless, they allow one to classify Schiff bases into groups according to the properties of their hydrogen bonds.

Factor analysis of ^{13}C NMR chemical shifts: To verify whether factor analysis is able to differentiate between isotope effects and chemical shifts, we also analyzed ^{13}C NMR chemical shift data for the same set of compounds. As a result, we have obtained different numbers and definitions of factors for both phenomena.

First, the ^{13}C chemical shift variables are far less intercorrelated than the isotope-effect data. All correlation coefficients between chemical shift variables are given in Table 1, and in Figure 1c,d we present details of two examples. These are relations between chemical shifts of nuclei C2 and C4 and between C1 and C4. One can easily see some influence of substituent effects at particular positions, both in the aromatic ring as well as at Cα and C1′. Data are grouped in layers characteristic of a particular type of substitution. One can also cross-correlate chemical shifts of some other nuclei. However, this common practice is not our goal. We believe that it is the whole molecular fragment that responds to different electronic and steric perturbations, and it should be considered as an entire moiety.

Factor analysis of the ^{13}C chemical shift data suggests three major factors (hereafter abbreviated CSF1, CSF2, and CSF3; see Table 3 and Figure 3b) related to about 54, 15, and 13% of the variance of the chemical shift data, respectively. Again, we checked that there is no influence of temperature on the structure of these three factors.

The first factor, CSF1, consists of chemical shifts at the aromatic ring carbons C1, C2, C3, C4, C5, and C6. The neighboring carbon atoms contribute to this factor in the opposite manner (their contributions have different signs). In other words, with decreasing chemical shifts of the atoms C1, C2, and C3, the chemical shifts of atoms C4, C5, and C6 increase and vice versa. Apparently, substituent effects of all groups (the OH group in particular) attached to the aromatic ring influence the definition of this factor. Numerical values of this factor describe how uniform the electron density distribution of the aromatic ring is and could also be related to the aromaticity of the ring. However, the chemical shift is not only related to the distribution of π electrons, and it is not a property of the ground state alone. About 70% of the values of this factor are grouped close to 0.25, as can be seen in Figure 5a. Only F- and MeO-substituted compounds differ from the compounds with other substituents. An MeO group in the *ortho* and/or *para* position(s) relative to the OH group increases the value of the CSF1 factor close to 2. This can be associated with deshielding of the C1, C3, and C5 nuclei relative to the other nuclei. Surprisingly, a similar effect is observed for fluoro substituent(s) attached at the same positions. However, the fluoro substituent is known to behave in an unexpected way.[59,60] It is strongly electron accepting and relatively strongly π-electron donating. When F or MeO groups are in the *meta* position(s), CSF1 changes its sign. Again, this can easily be rationalized as above.

The CSF2 factor consists of the chemical shift at Cα with a small contribution of the chemical shift at C3. It takes on negative values for those compounds that have a H atom at Cα (with the smallest values for 16HTB and 15HTB). The second cluster of data is formed by compounds with any non-H substituent at Cα. For this factor, it is difficult to see a clustering of data as a function of substitution of the aromatic ring (see Figure 5b). The numerical values of CSF2 are larger for the second cluster. In general, any C substituent at Cα increases the chemical shift of the hydrogen-bonded proton, which could be associated with steric effects of the C substituents.[44–48]

Table 3. Factor analysis of chemical shifts. Number of data points $N=111$. Factor loadings larger than 0.75 are in bold.

	Factor loadings							Factor score coefficients		
	Unrotated				Quartimax-normalized			Quartimax-normalized		
Variable	CSF1	CSF2	CSF3	CSF4	CSF1	CSF2	CSF3	CSF1	CSF2	CSF3
C1	**−0.87**	−0.02	−0.23	−0.14	**0.85**	0.23	0.21	0.176	0.185	0.206
C2	**0.90**	−0.24	0.03	0.03	**−0.90**	0.14	−0.16	−0.216	0.150	−0.090
C3	−0.71	−0.39	−0.12	0.53	0.68	0.44	−0.11	0.140	0.328	−0.062
C4	**0.93**	0.05	−0.04	−0.21	**−0.93**	−0.09	0.01	−0.217	−0.021	0.045
C5	**−0.81**	0.06	0.24	−0.10	**0.83**	−0.12	−0.11	0.211	−0.178	−0.177
C6	**0.84**	−0.12	−0.20	0.36	**−0.86**	0.14	0.05	−0.218	0.192	0.121
Cα	−0.13	**−0.79**	−0.48	−0.31	0.05	**0.93**	−0.09	−0.042	0.805	0.073
C1′	−0.12	0.68	−0.70	0.04	0.08	−0.12	**0.97**	−0.026	0.066	0.941
Expl. var.	4.331	1.328	0.882	0.591	4.298	1.189	1.054	–	–	–
% of total	54	17	11	7	54	15	13	–	–	–

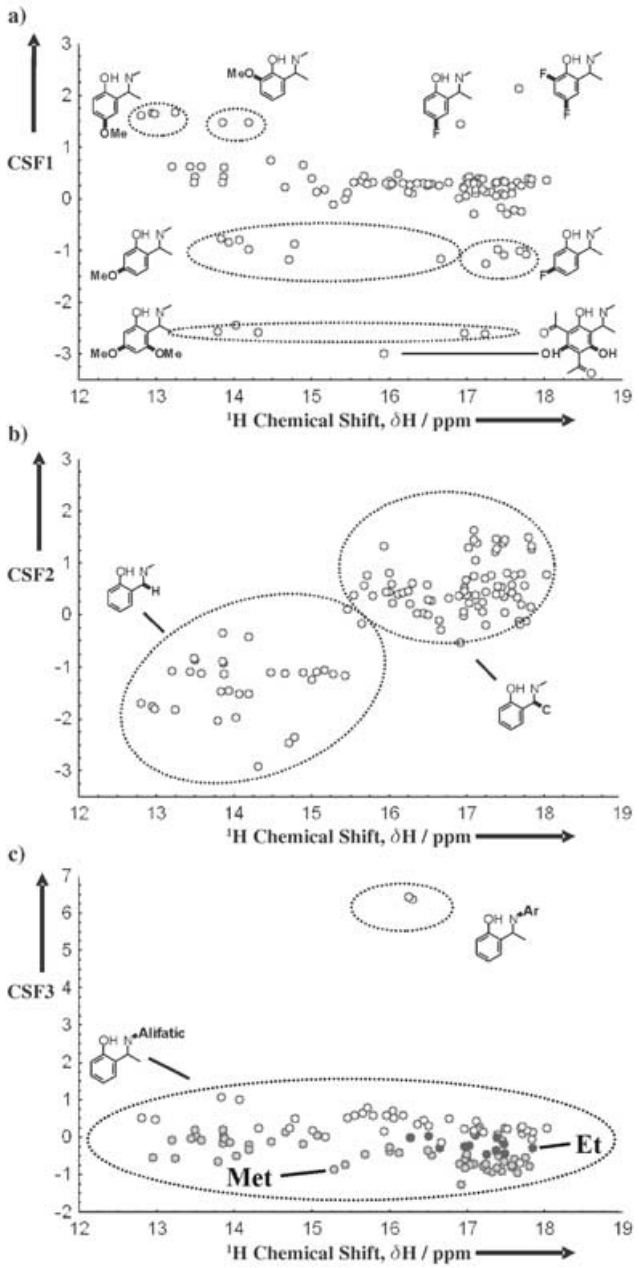


Figure 5. Dependence of the factors a) CSF1, b) CSF2, and c) CSF3 on the proton chemical shift δXH.

The third factor (CSF3) is defined by the chemical shift at C1′. It clearly divides all data points into two categories: those for which C1′ is an aromatic C atom (only two compounds: 3MN1 and 3MN2), and those in which C1′ is aliphatic. There is a trend in the latter group of compounds relating the CSF3 values and the size or electronic properties of substituents. The CSF3 factor increases in the following order of substituents: Me < Et < Pr, Bz, *t*Bu, *i*Pr (grey, black, and open-circle data points in Figure 5c).

Additionally, it appears that no correlation is found between factors describing isotope effects and those related to ^{13}C NMR chemical shifts. Interestingly, a dependence between the ^{13}C NMR chemical shift at C2 and the isotope effects described by F1 (Figure 6) can be seen. Two different clusters of data are present. For the first group, the positive

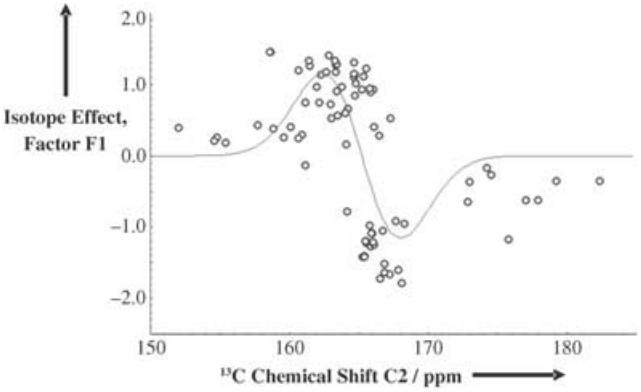


Figure 6. Dependence of the isotope effect factor F1 on the ^{13}C chemical shift of nucleus C2. (F1 = 0.7(1)(165.2(2)−C2)exp(−(165.2(2)−C2)^2/16(4); R = 0.69).

F1 values are associated with ^{13}C chemical shifts of C2 less than 165 ppm, whereas for the second group, negative F1 values correspond to chemical shifts larger than 165 ppm. So there seems to be a limiting value of ^{13}C chemical shifts of C2 (ca. 165 ppm), which divides all Schiff bases studied into groups in which the OH or NH forms of hydrogen bonding dominate.

Conclusion

By applying factor analysis to isotope effects on ^{13}C NMR solution data, we have been able to obtain two orthogonal factors that describe the total isotope effects on the Schiff fragment. The first factor explains most of the data variance and is defined by contributions from all carbon atoms in the Schiff fragment with exception of C6. The isotope effect at C6 is the second factor and explains a little less than 10% of variance. The numerical values of these factors can be related to ^{1}H NMR chemical shifts of the proton participating in hydrogen bonding. Such relations allow clusters of compounds with different forms of hydrogen bonding, and which are under the influence of particularly strong electronic effects, to be identified. The relationships we found hold for Schiff bases that exist in tautomeric equilibria in solution, and for those existing exclusively in one form.

Applying a similar approach to ^{13}C NMR chemical shifts of the same set of compounds produced three important factors that have a completely different structure than those associated with isotope effects. There is no correlation between the two groups of factors. All three factors can easily be rationalized and are strongly related to the electronic properties and location of substituents.

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