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Nuclear Magnetic Resonance Investigations of the Azo–Hydrazone Tautomerism of Azoreactive Dye Chromophores

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High-field ^{1}H and ^{13}C NMR methods (e.g. ^{1}H{^{1}H} and ^{13}C{^{1}H} NOE spectroscopy) were used to examine the tautomeric characteristics, particularly with regard to the question of azo–hydrazone tautomerism, of two azo dyes. Full ^{1}H and ^{13}C NMR signal assignments are given for both molecules. The NMR results indicate that in DMSO solution the tautomeric equilibria are shifted predominantly toward the hydrazone from II in both compounds. The presence of a fast II $\rightleftharpoons$ III interconversion could also be plausibly assumed. © 1997 by John Wiley & Sons, Ltd.

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INTRODUCTION

A recent paper by Mazzola *et al.*[1] reported on ^{1}H, ^{13}C and ^{15}N NMR investigations of the azo–hydrazone acid–base equilibrium of the dye FD&C Yellow No. 6 (Y6). One of their conclusions was that in D_2O at acidic to moderately basic pH values the hydrazone tautomer prevails. This finding prompted us to publish our results concerning the tautomerism of model compounds **1** and **2** (Scheme 1), which were studied by ^{1}H and ^{13}C NMR as part of a program aimed at finding a correlation between the structure and the fading characteristics of dyes. Our approach to the problem was different from that used by Mazzola *et al.*[1] In particular, we used DMSO-d_6 as a solvent in order to observe exchangeable resonances, and our investigations relied heavily on ^{1}H{^{1}H} and ^{13}C{^{1}H} nuclear Overhauser effect (NOE) measurements. In this paper we give full ^{1}H and ^{13}C NMR signal assignments using modern high-field NMR methods for **1** and **2**. It is also shown that in DMSO solution the tautomeric equilibria are shifted predominantly towards form **II** (hydrazone) in both **1** and **2**. The presence of a fast **II** $\rightleftharpoons$ **III** interconversion could be indirectly inferred from exchange phenomena observed in the presence of D_2O. However, for both compounds the population of **III** must be small. Related issues, such as the investigation of possible solvent and pH effects as required by research into the detailed nature and dynamics of these complex equilibria, are not addressed here.

Our results accord with those of Mazzola *et al.*[1] and indicate that for related structures the hydrazone form is the predominant tautomer over a wide range of pH values. Further, these results highlight the versatility and power of modern NMR spectroscopy in structural investigations.

RESULTS AND DISCUSSION

The plausible tautomeric forms of **1** and **2** are shown in Scheme 1 (tautomers **II** and **III** represent the hydrazone, while tautomer **I** is the azo form). Note that while a direct equilibrium between $\mathbf{1}_{\mathrm{II}}$ and $\mathbf{1}_{\mathrm{IV}}$ involves an intramolecular proton transfer between the C(4)NH_2 and C-6 carbonyl within $\mathbf{1}_{\mathrm{II}}$, a direct $\mathbf{2}_{\mathrm{II}} \rightleftharpoons \mathbf{2}_{\mathrm{IV}}$ equilibrium would entail an intermolecular process and, therefore, seems less likely.

For both compounds the NMR spectra (room temperature, DMSO solution) exhibited only one set of signals and, as a first approximation, this allows for two possible scenarios: (a) interconversion between some (or all) of the relevant tautomeric pairs is slow on the chemical shift time-scale, but only one predominant

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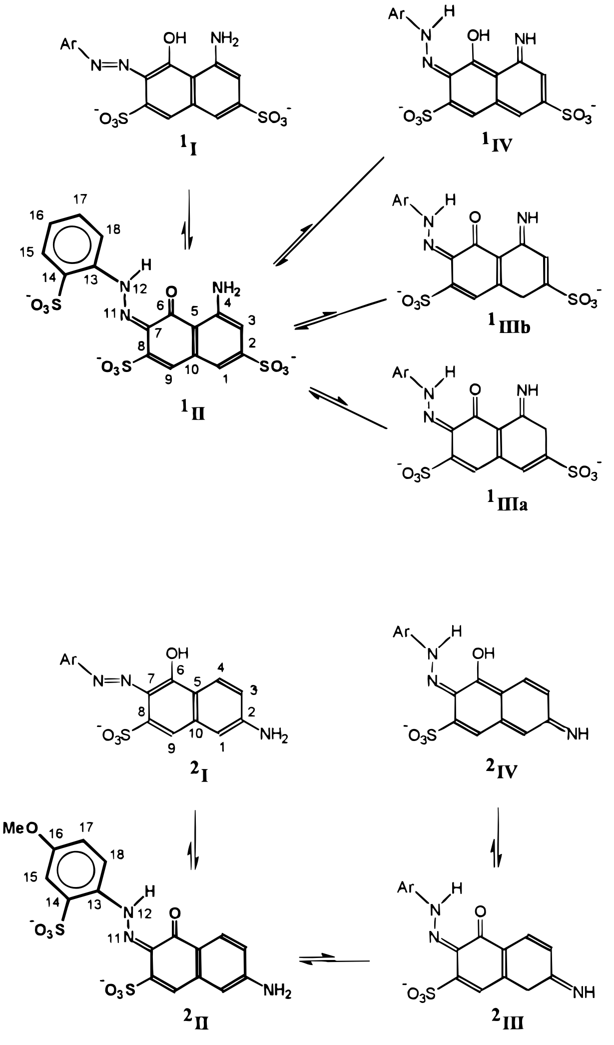


Scheme 1

tautomeric form is present with all others having negligible populations; and (b) tautomeric exchange is fast on the δ time-scale, and an averaged state is observed which reflects either a single dominant tautomeric form or the average of significantly populated tautomers involving any of the species in Scheme 1.

It is noted that **1** and **2** had been prepared by a method involving salting-out with sodium chloride. While both compounds contain an excess of sodium chloride, the pH values measured in 1% aqueous solutions were 3.20 for **1** and 4.26 for **2**, respectively. Neutralization of the SO_3H groups must therefore be regarded as partial. (For convenience, all sulfonic groups have been represented as SO_3^- in Schemes 1 and 2.) Further, the question of intra- and intermolecular salt formation involving the basic NH_2 nitrogen and one of the acidic SO_3H functions must be considered. Although the amino group in both molecules will be referred to and represented below as NH_2, clearly it must be viewed as an $NH_2 \rightleftharpoons {}^+NH_3$ system.

NMR signal assignments

All ^1H and ^{13}C assignments were established by consideration of the observed networks of scalar and dipolar connectivities. Two- and three-bond H–H connections were established from DQFCOSY, long-range H–H connections from COSYLR, one bond C–H connections from HSQC and long-range C–H connections from HMBC experiments. Owing to their particular importance in the present investigations, the presence of long-range H–H couplings was also verified by simple homonuclear spin-decoupling experiments. Dipolar (NOE) connectivities were established by NOESY (mixing times were varied from 0.1 s to 1 s) and also $^1H\{^1H\}$ and $^{13}C\{^1H\}$ NOE-difference experiments.

One notable aspect of the NOE investigations is that at room temperature all homonuclear enhancements turned out to be negative for both **1** and **2**. Clearly, the size of these molecules suggests that overall molecular tumbling rates should fall into the region of extreme narrowing and, therefore, H–H NOEs should be positive. The observed negative effects indicate that the molecules probably undergo autoaggregation due to intermolecular salt formation, thus increasing the effective rotational correlation times and bringing the molecules into the spin-diffusion region. [At higher temperatures (70–90 °C), faster tumbling rates resulted in the negative enhancements becoming much less pronounced, with some enhancements even becoming positive; this is an indication of the system being near to the zero-crossing region between positive and negative enhancements.] H–H NOE connectivities could, therefore, be conveniently and reliably studied by NOESY experiments at 30 °C at 500 MHz. Variable mixing-time experiments showed that mixing times of 0.8–1 s gave readily detectable NOE cross peaks without any appreciable spin-diffusion effects.

Another significant consequence of the long rotational correlation times associated with **1** and **2** is that NOEs into carbons become much decreased (*ca.* + 4% in the slow-tumbling region as opposed to *ca.* + 200% in the extreme narrowing region).[2] In such a case $^{13}C\{^1H\}$ NOEs can, in principle, be more readily measured at lower field strengths where the system is closer to the fast tumbling limit. Indeed, $^{13}C\{^1H\}$ NOE-difference experiments run with 75 MHz carbon detection at 24 °C gave ^{13}C NOEs whose quality of detection was superior to those measured at 125 MHz at 30 °C. (At 125 MHz the magnitude of $^{13}C\{^1H\}$ NOEs could be increased by elevating the temperature to 70 or 90 °C, but the lower field experiments still proved to be more sensitive.)

The assignment of all CH proton and carbon signals was straightforward from the measured H–H (DQFCOSY and COSYLR) and one-bond C–H (HSQC) scalar correlations and also the observed H–H NOE connectivities (NOESY) without making any assumptions about the tautomeric characteristics at this point. ^1H and ^{13}C assignments, together with the obtained scalar and NOE connections, are listed in Tables 1 and 2. (The measured two- and three-bond H–H connections and one-bond C–H connections correlate with the molecular frameworks in a trivial way and are, therefore, not depicted in the tables).

With regard to the NH and OH proton resonances, a very low-field and relatively sharp signal was assigned to N(12)H as discussed below. A broad resonance, centered at δ 3.68 in **1** and δ 3.54 in **2**, was established by

Table 1. ^{1}H **chemical shifts (30 °C), scalar H,H couplings, long-range H–H connectivities and dipolar H–H and C–H connections in compounds 1 and 2**[a]

			1		
Proton	δ_H (ppm)	$J_{H,H}$ (Hz)	NOESY	COSYLR	C{H} NOE
H-1	6.99 d[b]	$^{4}J_{1,3} = 1.4$	H-9	H-3, H-9	
H-3	7.13 d[b]		NH_2	H-1	
H-9	7.38 s[b]	$^{4}J_{1,9} < 1$	H-1	H-1	
H-12	15.47 s[b]	$^{4}J_{12,18} < 1$ $^{5}J_{12,17} < 1$	H-18, H-15	H-18, H-17	C-13, C-6, C-7
H-15	7.76 dd	$^{3}J_{15,16} = 7.7$ $^{4}J_{15,17} = 1.4$	H-16, H-12	H-17	C-14
H-16	7.14 ddd		H-15, H-17	H-18	
H-17	7.44 ddd		H-16, H-18	H-15, H-12	
H-18	8.29 dd	$^{3}J_{18,17} = 8.3$ $^{4}J_{18,16} = 1.2$	H-12	H-12, H-16	C-13
NH_2	3.68 br, s		H-3		C-4
			2		
Proton	δ_H (ppm)	$J_{H,H}$ (Hz)	NOESY	COSYLR	C{H} NOE
H-1	6.73 d[b]	$^{4}J_{1,3} = 1.7$	H-9, NH_2	H-3, H-9	
H-3	6.76 dd		H-4, NH_2	H-1	
H-4	8.10 d[b]	$^{3}J_{3,4} = 8.6$	H-3, H-12	H-9	C-5, C-6
H-9	7.33 s[b]	$^{4}J_{9,1} < 1$ $^{5}J_{9,4} < 1$	H-1	H-4, H-1	
H-12	15.97 s[b]	$^{4}J_{12,18} < 1$ $^{5}J_{12,17} < 1$	H-18, H-4	H-18, H-17	C-13, C-6, C-7
H-15	7.35 d	$^{4}J_{15,17} = 2.9$	OMe	H-17, H-18	C-14, C-16
H-17	7.07 dd		OMe, H-18	H-15, H-12	C-16
H-18	8.22 d	$^{3}J_{18,17} = 9.0$	H-12, H-17	H-12, H-15	C-13
OMe	3.84 s[b]	$^{5}J_{OMe,15} < 1$ $^{5}J_{OMe,17} < 1$	H-15, H-17	H-15, H-17	
NH_2	3.54 br, s		H-1, H-3		C-2

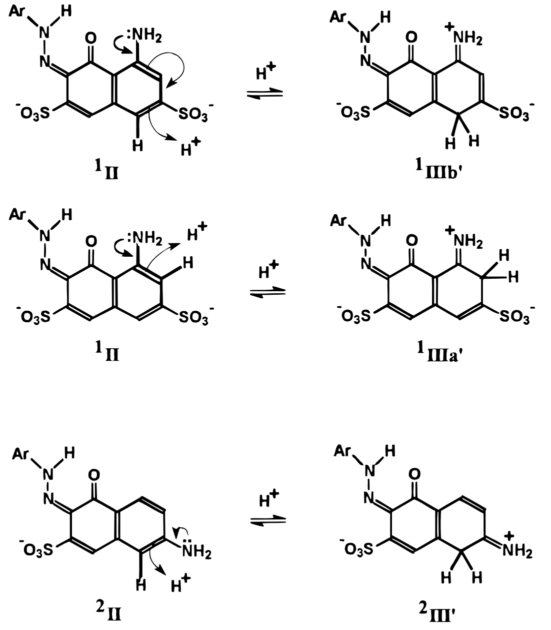
[a] Small characters indicate weak interactions manifested in cross peaks whose intensity is significantly less than that of those represented by normal-sized characters.

[b] Small, slightly resolved or unresolved long-range couplings are present.

Table 2. ^{13}C **chemical shifts (30 °C) and long-range C–H correlations (for quaternary carbons only) for compounds 1 and 2**[a]

		1		2
Carbon	δ_C (ppm)	HMBC	δ_C (ppm)	HMBC
C-1	113.4		110.4	
C-2	143.0	H-9	154.1	H-4
C-3	112.1		114.3	
C-4	152.7	H-3	129.8	
C-5	112.3	H-1, H-3, H-9	120.3	H-1, H-3, H-9
C-6	180.8	H-9, H-3, H-1	176.1	H-4, H-9, H-1, H-3
C-7	129.3	H-9, H-12	128.4	H-9, H-12
C-8	153.0	H-9, H-1		143.5, H-9
C-9	122.9		121.1	
C-10	136.5	H-1, H-9	138.0	H-4, H-9
C-13	139.9	H-15, H-17, H-18, H-12	133.4	H-15, H-17, H-18, H-12
C-14	134.3	H-16, H-18	135.3	H-18, H-15
C-15	127.1		111.7	
C-16	123.1		155.7	H-18, OMe
C-17	130.3		116.9	
C-18	117.3		118.9	
			55.6 (OMe)	

[a] Small characters indicate weak interactions manifested in cross peaks whose intensity is significantly less than that of those represented by normal-sized characters.



Scheme 2

$^1H\{^1H\}$ and $^{13}C\{^1H\}$ NOE experiments (see tables) to belong to the NH_2 protons.

Assignment of the sp^2 quaternary carbons required the concerted use of observed long-range H–H and C–H scalar connectivities and H–H and C–H NOEs and also the simultaneous consideration of tautomeric possibilities. Various correlations depicted in the tables verify the established assignments in a self-explanatory manner and, therefore, this aspect of the investigations will not be discussed further.

Tautomeric characteristics

Several pieces of NMR spectroscopic information indicate that in **1** and **2** the dominant tautomeric form in DMSO is **II**, as follows.

(a) A very low-field exchangeable resonance (δ 15.47 for **1** and δ 15.97 for **2**) of one proton intensity is present in both ^1H spectra and is conspicuously distinct from other NH and OH signals which resonate at much higher field. This proton shows long-range scalar connections with H-18 and H-17, thus it must be situated not more than 4–5 bonds away from the latter protons. The resonance in question must, therefore, be assigned to N(12)H with the assumption of **II** being the dominant tautomeric entity. In accordance with this, the large chemical shift of N(12)H can be rationalized in terms of N(12)H being hydrogen bonded to the C-6 carbonyl as facilitated by the depicted *anti* configuration of the hydrazone species (Scheme 1); the deshielding effect of the neighboring aromatic ring may also contribute to the large chemical shift of N(12)H. The fact that N(12)H gives NOE into C-6, but not to C-8, underlines the predominance of the *anti* hydrazone geometry as opposed to a *syn* configuration.

(b) One of the quaternary carbons resonates at δ 108.8 in **1** and δ 176.1 in **2**; this large chemical shift can only be ascribed to the C(6)=O carbon in tautomer **II**. Further, the presence and the location of this carbonyl are both consistent with the observed $N(12)H \rightarrow C{=}O$ NOE in **1** and **2**, and also with the $H\text{-}4 \rightarrow C{=}O$ NOE in **2**.

(c) In the bicyclic ring system the long-range C–H correlations observed in an HMBC experiment (see Table 2 and the Experimental section) show deviations from those expected for the usual pattern of long-range C–H couplings characteristic of an aromatic system ($^3J_{C,H} \approx 8$ Hz, $^2J_{C,H} \approx 2$ Hz). Again, this is in line with a preferential tautomer **II** rather than **I**.

(d) Upon addition of D_2O to the solutions, the NH and OH protons exchanged quickly with deuterium. During the course of these investigations we observed that in **2** H-1 also exchanged, more slowly but spontaneously, with D. This exchange process may be explained by the fact that the vinylog position of the C-6 carbonyl enhances the polarity of the C(1)—H bond, and thus promotes a hydrogen transfer between H-1 and the NH_2 protons, facilitated by a $\mathbf{2}_{II} \rightleftharpoons \mathbf{2}_{III}$ tautomeric equilibrium as depicted in Scheme 1. In **1** the exchange process did not appear to proceed spontaneously but was promoted by the addition of a catalytic amount of trifluoroacetic acid, and both H-1 and H-3 exchanged with deuterium. In this case the presence of the electron-donating SO_3^- in the α-position with respect to C-1 and C-3 diminishes the polarity of the C(1)—H and C(3)—H bonds as compared with **2**. The presence of TFA shifts the $SO_3^- \rightleftharpoons SO_3H$ equilibrium towards the acidic form, thus transform the electron-donating group into an electron-withdrawing one and enhancing again the polarity of the neighboring C—H bonds. The exchangeable nature of H-3 and H-1 may again be envisaged to involve the NH_2 group and can, in analogy with **2**, stem from the $\mathbf{1}_{II} \rightleftharpoons \mathbf{1}_{IIIa}$, $\mathbf{1}_{II} \rightleftharpoons \mathbf{1}_{IIIb}$ equilibria, respectively.

However, we cannot exclude the possibility of an aromatic electrophile substitution being responsible for the observed $H \rightleftharpoons D$ exchange (Scheme 2).

EXPERIMENTAL

The dyes studied were synthesized and donated by Sumimoto Chemical (Japan).

NMR measurements were carried out primarily on a Varian UNITYplus-500 spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C) in DMSO-d_6 solution, mostly at 30 °C. Chemical shifts are given relative to $\delta_{TMS} = 0.00$ ppm. Two-dimensional (2D) chemical shift correlation experiments [DQFCOSY, long-range COSY (COSYLR), HSQC, HMBC (optimized for 8 Hz long-range C–H couplings), NOESY] were recorded by using the standard spectrometer software package and utilizing its pulsed field gradient facility. In the NOESY experiments mixing times were varied from 0.1 to 1 s. The $^1H\{^1H\}$ and $^{13}C\{^1H\}$ NOE-difference experiments were run in non-degassed samples typically with 3 s ($^1H\{^1H\}$) and 6 s ($^{13}C\{^1H\}$) preirradiation times. For the selective $^{13}C\{^1H\}$ NOE measurements a laboratory written pulse sequence was used, employing the same basic principles as described by Sánchez-Ferrando.[3] All the observed C–H NOE connections were further verified by $^{13}C\{^{1}H\}$ NOE-difference experiments using a Varian VXR-300 NMR spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C) at 24 °C.

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REFERENCES

1. E. P. Mazzola, S. A. Turujman, S. J. Bell, J. N. Barrows, J. E. Bailey, Jr, E. G. Mouchahior, S. Prescott, M. D. Archer, M. Oliver, W. F. Reynolds and K. W. Nielsen, *Tetrahedron* **52**, 5691 (1996).
2. D. Neuhaus and M. P. Williamson, *The Nuclear Overhauser Effect in Structural and Conformational Analysis*. VCH, New York (1989).
3. F. Sánchez-Ferrando, *Magn. Reson. Chem.* **23**, 185 (1985).