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^{15}N NMR Investigation of Azo–Hydrazone Acid–Base Equilibria of FD & C Yellow No. 5 (Tartrazine) and Two Analogs*‡

Sandra J. Bell, Eugene P. Mazzola

Center for Food Safety and Applied Nutrition, US Food and Drug Administration, Washington, DC 20204, USA

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Bruce Coxon

Center for Analytical Chemistry, National Institute of Standards and Technology, Gaithersburg, Maryland 20899, USA

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ABSTRACT

FD & C Yellow No. 5 and two analogs have been characterized by ^{15}N NMR at several pH values. ^{15}N chemical shift data indicate the existence of azo–hydrazone, acid–base equilibria and suggest that these compounds are present in the hydrazone form at pH 7 and the azo form at pH 12. Approximately equal concentrations of these two species are observed at pH 10·3.

1 INTRODUCTION

FD&C Yellow No. 5 (I) is a pyrazolone colorant used world-wide in foods, drugs and cosmetics.[1] Its implication as an allergen has led to product

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‡ All correspondence should be addressed to Dr Eugene P. Mazzola, Division of Contaminants Chemistry (HFF-423), USFDA, Center for Food Safety and Applied Nutrition, 200 C Street S.W., Washington, DC 20204, USA.

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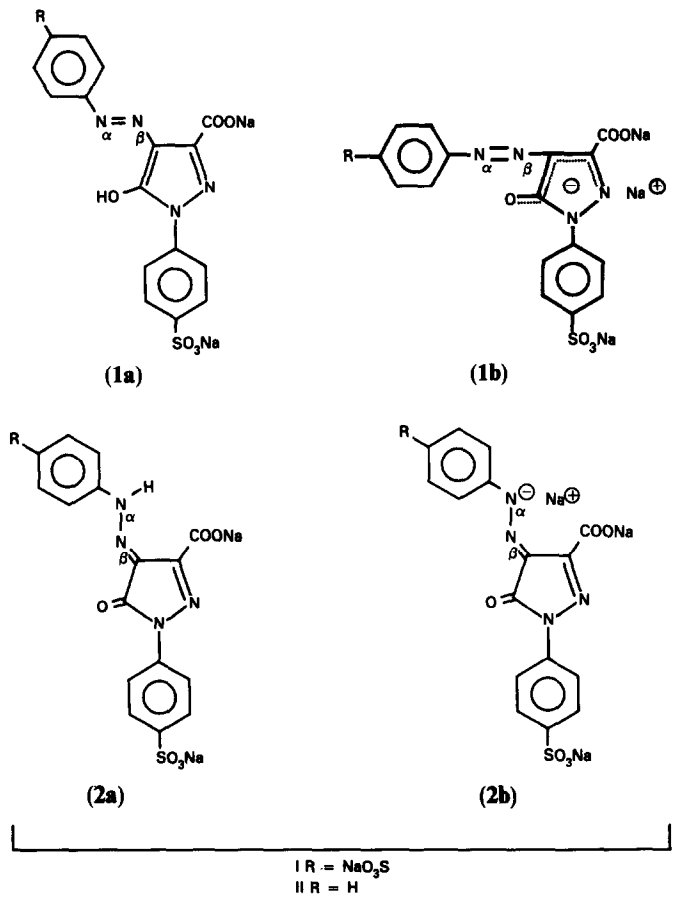
labeling requirements and has aroused interest in the biological activity of both the dye and its manufacturing impurities and decomposition products.

While pyrazolone compounds have been the subject of considerable research concerning their azo–hydrazone tautomerism in various organic solvents,[2] their water-soluble analogs have received very little attention. This neglect is not surprising considering (1) that much of the characterization of pyrazolone tautomers has been by IR spectroscopy and (2) the difficulty of obtaining IR spectra of aqueous solutions. Alternatively, some authors have used proton–nitrogen couplings (observing ^{1}H NMR) as an indication of azo–hydrazone tautomerism.[2] However, the technique is not applicable to aqueous solutions due to the labile nature of the proton in question. Nevertheless, ambiguities do exist regarding the structural nature of water-soluble pyrazolones with changes in solution pH. Compound (I) is, for instance, nominally classified as an 'azo' dye.[3] Thus, its acid–base equilibrium can be expressed as a proton-transfer process involving the

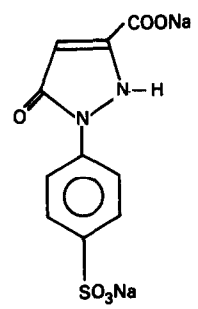


5-hydroxyl group (**1a–1b**). However, pyrazolone azo dyes are frequently represented as hydrazones,[4] common tautomeric forms for organic-soluble azo compounds which possess *ortho* keto–enol groups.[2] The corresponding acid–base equilibrium is depicted as a transfer process involving the α-nitrogen proton (**2a–2b**).

^{15}N NMR is ideally suited for the investigation of these dyes at various pH values because it provides a direct probe of the critical nitrogen atoms which may be involved in deprotonation as well as in azo–hydrazone equilibrium. To this end, ^{15}N NMR spectra have been obtained at critical pH values for **I**, its β-^{15}N-labeled isotopomer ([β-^{15}N]-**I**) and an α-^{15}N-labeled aniline analog (**II**). In these compounds, note that the nitrogen atom which is directly attached to the pyrazole ring is designated 'β' and the adjacent nitrogen 'α'. These data together with those from acid–base titrations and visible spectra permit **I** to be characterized in acid, neutral and basic solution.

2 EXPERIMENTAL

4,5-Dihydro-5-oxo-1-(4-sulfophenyl)-4-(4-sulfophenyl)azo-1*H*-pyrazole-3-carboxylic acid, trisodium salt (**I**) was obtained from Hilton-Davis‡ and used without further purification. Its ^{15}N-labeled analog (**II**) was prepared by diazotization of [^{15}N]aniline (97 at.% ^{15}N, Merck) and coupling to pyrazolone T (**III**, Hilton-Davis).[5] The β-^{15}N-labeled isotopomer ([β-^{15}N]-**I**) was prepared by diazotization of 4-amino-benzenesulfonic acid (Fisher) with $Na^{15}NO_2$ (95 at.% ^{15}N, Merck) and coupling to **III**.



III

‡ Certain commercial equipment, instruments or materials are identified in this paper to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology or the Food and Drug Administration, and it does not imply that the materials or equipment identified are necessarily the best available for the purpose.

^{15}N NMR spectra of **I** and **II** were recorded at 40·6 MHz, at various pH values, by use of a Bruker Instruments WM-400 spectrometer. Minimum amounts of D_2O were added to the aqueous solutions for locking purposes. Proton-decoupled spectra, described by 8192 data points (real part), were obtained at 304 K with two-level, broad-band irradiation at 400 MHz. Pulse widths of 22 μs were employed, which correspond to tip angles of 45° with 15-mm sample tubes. Spectral widths of 29·4 kHz were used, corresponding to acquisition times of *c.* 0·28 s. A pulse recycle time of 5·28 s was used, and the data were subjected to a 2-Hz line broadening. Aqueous, saturated $^{15}NH_4{}^{15}NO_3$solution was employed as an external standard, and chemical shifts are reported relative to the $^{15}NO_3^-$ resonance.

Titrations were carried out on a 300-mg sample of **I** with 0·1N HCl using a Radiometer RT5622 automatic titrator. The pH of the starting solution was 12, and the final value was 2.

3 RESULTS AND DISCUSSION

The pH values at which ^{15}N NMR spectra of **I** and **II** were recorded were selected on the basis of titration results, visible spectra and preliminary ^{13}C NMR spectra. Compound **I** exhibited equivalence points at pH 5·2 and 10·3 and had a pK_a value of 8·9 (the carboxylate and the two sulfonate groups are not normally titrated). These data, together with visible spectra taken over a wide pH range, indicate that **I** exists as a tetra-anion at pH 10·3 and as a tri-anion at pH 5·2. ^{13}C NMR spectra showed broadening of most signals between pH 9 and 11, indicating the existence of one or more intermediate (on the NMR time-scale) equilibrium processes.[6] Such processes include interconversion between *syn*- and *anti*-hydrazone species and rotation about the C_4—N_β bond in *trans*-azo conformers. Maximum line broadening was observed at pH 10·3. These experimental results suggested that ^{15}N NMR spectra be determined (1) at, or near, the equivalence points and away from the pK_a value of 8·9 to minimize complications arising from multiple charged species (viz., two or more sets of resonance lines) and (2) at the point of maximum ^{13}C spectral line broadening (pH 10·3). Accordingly, the following pH values were chosen: (a) pH 7, to be below the pyrazolone NH/N^- or OH/O^- pK_a of 8·9, but not acidic to avoid solubility problems, (b) pH 10·3, which is both the second equivalence point and the pH at which maximum ^{13}C spectral line-broadening effects are observed, and (c) pH 12, to be sufficiently removed from both the pK_a and the location of greatest ^{13}C spectral line broadening. Table 1 presents ^{15}N NMR chemical shift data for compounds **I** and **II**.

Cursory examination of these NMR data reveals that the spectra of **I** and

TABLE 1
^{15}N Chemical Shifts of Compounds I and II at pH 7, 10·3 and 12[a]

pH	I, N_α	I, N_β	II, $^{15}N_\alpha$	I, $^{15}N_\beta$
7	—[b]	—[b]	−168·9	−11·2
10·3	—[b]	—[b]	−168·8,[c] 51·3[c]	96·2,[c] −10[c]
12	40·5	97·5	52·9	97·0

[a] Referenced to $NH_4{}^{15}NO_3$.
[b] Not determined because of limited solubility.
[c] Broad signal.

II undergo significant change from pH 7 to 12. Comparison of the chemical shift values with literature data affords the following conclusions. At pH 7 the 'azo' dyes **I** and **II** exist in the hydrazone form, here depicted in the *anti*-configuration (**2a**). Chemical shifts of −11·3 and −14·2 ppm,[7] −14·1 and −26 ppm[8] and −17·3 ppm[9] have been reported for hydrazone imine-nitrogen atoms (relative to $NH_4{}^{15}NO_3$) and are similar to the value of −11·2 ppm found for [β-^{15}N]-**I**. In addition, chemical shift values of −195·7 ppm[7] and −205·2ppm[9] have been reported for hydrazone amine-nitrogens vs a −168·9-ppm signal observed for **II**. Although agreement with the literature chemical shifts for hydrazone amine-nitrogens is not as good as that obtained for the imine-nitrogen, this is not unexpected. The partially dissociated hydrazone amine-nitrogen should be more susceptible to solvent effects. Hydrogen bonding, in particular, has a great effect on nitrogen chemical shifts.[10] Both of the literature chemical shift values cited above for hydrazone amine-nitrogens are due to strongly hydrogen-bonded nitrogen atoms in organic solvents, while that for **II** is for neutral aqueous solution. The more deshielded resonance observed for the hydrazone amine-nitrogen of **II** (−168·9 ppm) suggests that it is less strongly hydrogen bonded than the nitrogens of the literature reference compounds.[10]

^{15}N NMR chemical shifts determined at pH 12 are, however, markedly different from those at pH 7. The α-nitrogen signal of **II** is deshielded by *c.* 220 ppm (from −168·9 to 52·9 ppm), while that of the β-nitrogen is shifted downfield by nearly 110 ppm (from −11·2 ppm in [β-^{15}N]-**I** to 97·5 ppm in **I**). The former chemical shift difference is well beyond that expected for deprotonation of an aromatic amine.[10] The magnitudes of these downfield shifts indicate that a profound change occurs in the chemical nature of the α- and β-nitrogens of **I** and **II** upon basification. The chemical shift values are similar to those reported for the azo nitrogen atoms of 2-hydroxy-*tert*-butylazobenzene (69·4 and 110·2 ppm).[9] These data suggest that compounds **I** and **II** exist in the azo form (**1b**) at pH 12.

At pH 10·3, broadened signals are found for the α-nitrogen of **II** at 51·3 and −168·8 ppm. This observation, together with that of greatly broadened ^{13}C NMR resonances over the pH range 10–10·5, is indicative of an azo–hydrazone equilibrium for **I** and **II** involving species such as **1b** and **2b**, among others. On the basis of the slight displacement of these resonance lines from the azo (52·9 ppm) and hydrazone-amine (−168·9 ppm) chemical shift values, approximately equal concentrations of the azo and hydrazone species are concluded to be present and interconverting at an intermediate rate on the NMR time-scale.[6] In this regard, Lycka and co-workers have reported analogous pairs of ^{15}N NMR resonances for an equilibrium mixture of azo and hydrazone tautomers for 1-phenylazo-2-naphthol (36·7 and −104 ppm)[9] and 2-phenylazo-1-naphthol (26·2 and −133·4 ppm).[11]

4 CONCLUSION

The ^{15}N NMR chemical shift data indicate that FD&C Yellow No. 5 and its analogs, **I** and **II**, are involved in azo–hydrazone, acid–base equilibria in aqueous solution. These compounds occur almost exclusively in the hydrazone form at pH 7 (physiologic pH) and predominantly in the azo form at pH 12. At the equivalence point of pH 5·2, **I** exists as a tri-anionic hydrazone. As the solution pH is raised, the hydrazone amine-nitrogen deprotonates until the pK_a is reached at pH 8·9. Deprotonation continues above pH 8·9, and **I** begins to undergo reversible conversion to the azo form. At the equivalence point of pH 10·3, **I** exists as a tetra-anion, approximately half in the hydrazone form and half in the emerging azo form. Above pH 10·3, **I** continues its conversion to the predominant, high-pH azo form.

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