Utilization of Deuterium Labelling and ^{13}C NMR Spectroscopy in the Investigation of the Condensation of 1-Methyl-5-nitroimidazole-2-aldehyde and 2-Amino-4-methylpyrimidine

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The product obtained condensing 1-methyl-5-nitroimidazole-2-aldehyde with 2-amino-4-trideuteriomethylpyrimidine and showing total deuterium incorporation at one of the olefinic carbons and partial deuterium incorporation para to the amino group, served to assign ^{13}C and ^{1}H resonances in the condensation products **3**. The nature of the species undergoing condensation has been inferred from labelling experiments and from ^{1}H and ^{13}C nmr spectra of precursors and products in dimethylsulfoxide and trifluoroacetic acid.

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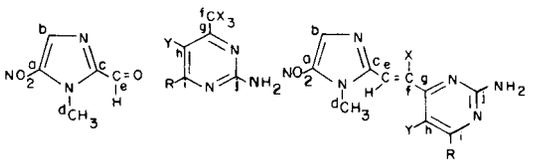
1-Methyl-5-nitroimidazole-2-aldehyde (**1**) condenses, under acidic conditions, with 2-amino-4-methylpyrimidine (**2a**) to produce the disubstituted olefin **3a**, known as "Azanidazole" (1), a potent antiprotozoal particularly active against *Trichomonas Vaginalis* (2). Aquisition of ^{13}C nmr data of biologically active molecules is relevant, not only for complementing proton nmr structural assignments, but also as they may serve for correlative analysis within a homogeneous set of similar substrates (3). Additive increments and Hammett correlations have been used successfully to interpret proton chemical shifts of other antiparasitic 2-substituted nitroimidazoles (4). Furthermore, in aromatic and heteroaromatic systems, ^{13}C shifts are suggestive of charge distribution resulting from mesomeric delocalization and from polar-inductive effects induced by substituents (5).

We report herein ^{13}C assignments in Azanidazole **3a** and its precursors: to do this we needed a sample of **3a** specifically deuterium labelled at one of the olefinic carbons. Although the synthesis of this compound was specially designed for ^{13}C peak assignment, it offered us the additional opportunity for considering more closely the mechanism of condensation of **1** and **2a**.

^{13}C assignments were first based on decoupled and off-resonance spectra of **3a** and of its methyl homologue **3b**, and on comparison of their spectra with those of precursors **1** and **2**.

Because of the sparing solubility of compounds **3** in common solvents, we took advantage of their basic properties recording their nmr spectra as trifluoroacetates in TFA (trifluoroacetic acid). Arbitrary lettering of carbons common for substrates **1-3** is reported within the formulas. ^{1}H and ^{13}C nmr parameters are reported in Tables I and II

Scheme 1



1 2 3

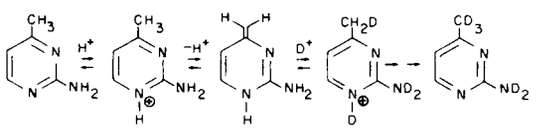
a, X = Y = R = H

b, X = Y = H, R = CH_3

c, X = D, Y = R = H

d, X = Y = D, R = H

Scheme 2



2a 4 2c

respectively; ^{13}C multiplicity of compound **3a** assigns singlets at 168.0, 155.7, 143.7 and 139.7 to quaternary carbons, doublets at 148.3, 123.3 and 111.5 ppm to aromatic C-H carbons, and finally multiplets at 139.3 and 118.7 ppm to olefinic carbons. The fact that the 148.3 ppm peak in **3a** moves downfield to 163.3 ppm in **3b** assigns this resonance to the *i*th carbon. Comparison of peaks in **3a** with those of precursors **1** and **2a** reveals that ring carbons, both in the pyrimidine and the imidazole rings move but little: a notable, and expected, exception is the *g*th carbon which, on changing the methyl in **2a** for an olefinic

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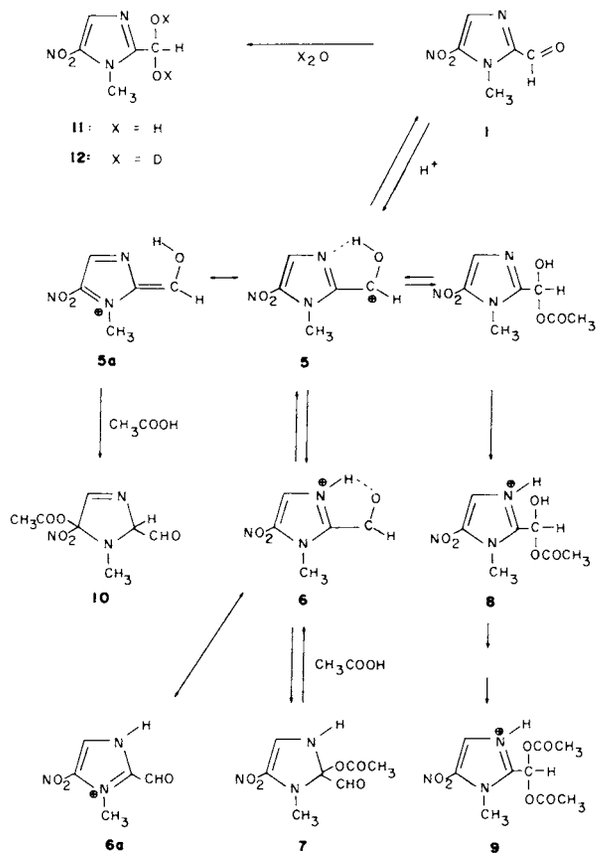
Scheme 3



carbon in **3a**, moves upfield by *ca* 7 ppm. Finally, considerable help in peak assignment of the aminopyrimidine moiety came from the comparison of our own shifts with those reported for a vast number of these derivatives (6).

Availability of the deuterated olefin **3c** would have allowed the unambiguous assignment of the 139.29 and 118.72 ppm peaks to either one of the olefinic carbons *e* and *f*. Quantitative and specific deuterium incorporation in the methyl group of 2-amino-4-methylpyrimidine occurred on boiling **2a** with *O*-deuterioacetic acid. To avoid the loss of the label, condensation of **2c** with **1** was performed, under conditions strictly analogous to those used for the protio compound, in a mixture of *O*-deuterioacetic acid and dideuteriosulfuric acid. Work up of the reaction mixture led to the isolation of a product which, besides total deuterium incorporation at the *f*th position, showed also substantial deuterium incorporation (50%) at the *h*th position, resulting thus a mixture of **3c** and **3d**. To prove that deuterium incorporation at the *h*th position occurred because of the presence of the dideuteriosulfuric acid component used in the condensation conditions, 2-amino-4-methylpyrimidine **2a** was submitted to deuterium exchange in *O*-deuterioacetic acid in the presence of dideuteriosulfuric acid, under conditions strictly analogous to those used in the condensation. In fact, besides total deuteration of the methyl group, substantial (60%) deuterium incorporation occurred at the *h*th position, *para* to the amino group.

Relative to the protio compound **3a**, the mixture of **3c** and **3d** revealed to be quite illuminating for assigning the proton and ^{13}C olefinic resonances. In fact in the proton spectrum the AB quartet due to H-*i*, H-*h* of the pyrimidine moiety is considerably decreased in intensity showing the simultaneous emergence of a singlet at 8.47 ppm (H-*i*); the AB quartet due to the olefinic system instead collapsed into a singlet at 8.14 ppm (H-*e*). Correspondingly, in the ^{13}C nmr spectrum of the same deuterated mixture, the peak at 139.3 ppm appeared broadened and of lower relative intensity than in **3a**: the assignment of this peak to carbon *f*, bearing the deuterium, is straightforward. Finally, the peak at 118.7 ppm, present as a multiplet in the off-resonance spectrum of **3a**, reduces to a clean doublet in the off-resonance of **3c** + **3d**: accordingly, this resonance is assigned to carbon *e*.

2-Amino-4-methylpyrimidine **2a** was previously observed to undergo deuteration of the methyl group at room temperature, both under basic and mineral acid catalysis (7): although no incorporation of deuterium in the aromatic ring was detected under the mild reaction conditions reported, this process is expected to occur under the more vigorous conditions we used, in analogy with the classical experiment of *para*-deuteration of aniline (8).

In analogy with 2-aminopyrimidine (9), 2-amino-4-methylpyrimidine is expected to undergo protonation on ring nitrogens: the deprotonation step in the acid-base equilibrium can occur also from the methyl hydrogens to originate the methylene base **4**. Results so far obtained indicate the methylene base **4** as the most likely nucleophilic intermediate both in the deuteration of **2a** to **2c** and in the condensation of **2a** with aldehyde **1** and **3a**. Furthermore, the deuteration of **2a** in *O*-deuterioacetic acid reveals that the organic acid catalysis is sufficient to promote the formation of **4** as a tautomer in the acid-base equilibrium. If indeed the aldehyde **1** is the true electrophilic partner of methylene base **4**, a synthetic approach to the deuteriocompound **3c**, free from **3d** as a contaminant, could take advantage of performing the condensation under organic acid catalysis only, without the simultaneous interfering deuteration para to the amino group of **2a**. We found however that the reaction of **1** with **2a** in acetic acid failed to produce any condensation product, even after a long reaction time. This result is interesting since it shows that the aldehyde **1** cannot be the reacting

Table I

^{1}H NMR Parameters (a)

Compound	Solvent	*b*	*d*	*e*	*f*	*g*	*h*	*i*
1	DMSO	8.26	4.23	9.85				
6	TFA	8.55	4.63	10.12				
6	H_2SO_4	8.80	4.80	10.20				
6	$H_2SO_4 + CH_3COOH$	8.90	4.80	10.35				
2a	DMSO					2.22	6.45	8.10
2a	TFA					2.71	7.06	8.44
3a	DMSO	8.16	3.98	7.48	7.70		6.94	8.28
3a	TFA	8.57	4.43	7.95	8.14		7.32	8.47
3b	DMSO	8.21	4.05	7.47	7.69		6.85	2.30
3b	TFA	8.52	4.40	7.85	8.05		7.07	2.63
11	DMSO	7.95	4.10	5.95				

(a) Spectra were recorded using 1*M* solutions. Chemical shifts are measured in ppm relative to TMS as internal reference in TFA and DMSO solutions, as external reference in sulfuric acid and sulfuric acid-acetic acid solutions.

Table II

^{13}C NMR Parameters (a)

Compound	Solvent	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*
1	DMSO (b)	143.1	132.1	141.2	34.8	183.7					
1	CH_3COOH	143.4	131.8	141.4	34.3	183.6					
6	TFA	140.0 s	125.5 d	138.9 s	35.6 q	175.9 d					
6	H_2SO_4	140.1	124.6	137.8	37.4	175.3					
6	$H_2SO_4 + CH_3COOH$	139.8	124.1	138.6	36.4	(c)					
2a	TFA						21.9	175.1	111.5	152.2	155.4
3a	TFA	143.7 s	123.3 d	139.7 s	35.3 q	118.7 m	139.3 m	168.0 s	111.5 d	148.3 d	155.7 s
3b	TFA	143.7	123.3	139.6	35.2	118.4	139.4	166.3	111.8	163.3	155.9
11	DMSO	(d)	130.8 d	152.3 s	33.5 q	86.1 d					

(a) Spectra were recorded using 1*M* solutions. Chemical shifts are measured in ppm relative to TMS as external reference. Assignments to carbon *a* and *c* may be interchanged: s, d, q, m refer to multiplicities in the off-resonance spectrum (singlet, doublet, quartet, multiplet respectively). (b) Chemical shifts are relative to TMS as internal reference. (c) Covered by solvent peaks. (d) Uncertain.

electrophile toward the methylene base **4**. The occurrence of the condensation of **1** and **2a** only in the presence of a strong mineral acid suggested that, instead of the aldehyde **1**, its conjugate cation is involved as the reacting electrophile in the condensation. To support this hypothesis we investigated what species are formed from **1** in a series of media, some of which were subsequently used to carry out the condensation with **2a**. Results are reported below, while nmr data are included in Tables I and II.

(i) Aldehyde **1** shows, apart from slight solvent effects, almost identical ^{13}C shifts both in dimethylsulfoxide and in acetic acid: thus in this latter solvent there is no appreciable protonation. (ii) In contrast, aldehyde **1**, both in TFA and in sulfuric acid, shows ^{1}H and ^{13}C spectra (Tables I and II) which are different from those in dimethylsulfoxide and in acetic acid and which are attributable to its conjugate cation. Because of its ambident nature, aldehyde **1** might be expected to originate two different cations, **5** and **6**, both stabilized by delocalization and possibly equilibrating rapidly. Available nmr evidence suggests that protonation occurs predominantly at N(2) of **1** to give the azolium ion **6**, although it cannot be excluded that minor amounts of **5** are also present. In fact all protons of **1** are shifted downfield on protonation and the formyl proton H-*e* moves but little. All carbons of **1**, with the exception of C-*d*, move upfield in the protonated species, a behaviour which is in line with that of the unsubstituted imidazole (10). It seems difficult to anticipate what the effects for *O*-protonation of **1** would be on ^{1}H and ^{13}C nmr shifts: the ^{13}C downfield displacement expected for the carbonium ion character of C-*e* in **5** would be certainly moderated by the delocalization of the charge onto the heterocycle. (iii) Aldehyde **1** in acetic acid and in the presence of sulfuric acid (under conditions strictly analogous to those of the condensation with **2a**) gives rise to complex ^{1}H and ^{13}C nmr spectra which indicate, besides the disappearance of the unprotonated starting material, the presence of at least three species, one of which (Tables I and II) is certainly the cation **6**. It should be further noted that this solution, upon quenching with a large amount of water, does not regenerate quantitatively the

starting aldehyde only but also sizeable amounts of decomposition products. For this reason a more detailed analysis of the species present in acetic acid-sulfuric acid is delayed at the end of the paragraph. (iv) The olefin **3a** is formed in high yields when aldehyde **1** is reacted with **2a** in TFA solution.

Since the nmr experiments clearly showed that under TFA conditions aldehyde **1** is protonated to its conjugate cation, obtainment of **3a** under analogous conditions unambiguously proves that acidic catalysis is necessary not only to generate the methylene base **4** but also to generate the conjugate cation of aldehyde **1**, which results to be the true electrophile in the condensation. Therefore, the acid catalysis must be provided by an acid strong enough to originate both species: sulfuric acid in acetic acid, and trifluoroacetic acid appear to do so, while acetic acid alone, promoting the formation of **4** only, is inefficient for the condensation.

Although the two byproducts accompanying the cation **6** in sulfuric acid-acetic acid are not involved in the mechanism of the condensation of **1** with **2a**, we wish to refer on some further observations made during an ancillary investigation aimed toward the elucidation of their structure. This investigation was hampered by two facts: the species present in the original sulfuric acid-acetic acid medium further evolved upon quenching of the solution, and in the ^{13}C nmr spectrum of the original solution, on the other hand, the large peak of the C=O absorption of the acetic acid cosolvent at 175 ppm is hiding any peak eventually attributable to aldehyde species. For this reason, the structural assignments to the products accompanying the cation **6** will be based merely on circumstantial evidence and therefore should be considered tentatively only. Peaks at 149, 146, 122.1, 121.6, 75.9, 74.2 and 36.1 ppm are left in the ^{13}C nmr spectrum of **1** in sulfuric acid-acetic acid solution after substraction of the peaks assigned to cation **6** and reported in Table II. In the undecoupled spectrum peaks at 149 and 146 ppm appear as doublets ($^1J_{C-H}$ = 213 Hz) as well as peaks at 75.9 and 74.2 ppm ($^1J_{C-H}$ = 172 Hz). The ^1H nmr spectrum of the same solution shows the formyl proton at 10.2 ppm and other broad singlets at 8.92, 8.75, 7.04, 6.7, 4.78 and 4.54 ppm respectively. All these data suggest the following considerations: (i) The two products accompanying the cation **6** should be rather close in structure, judging from the similarity of shifts of the pair of peaks in the 145, 120 and 75 ppm, and in the 8 and 6.7-7 ppm regions, in the ^{13}C and ^1H nmr spectra respectively. (ii) Peaks in the 145 and 120 ppm, and in the 8 ppm regions (^{13}C and ^1H respectively) indicate that in these products the imidazole framework has been preserved. (iii) Peaks in the 75 ppm and 6.7-7 ppm regions (^{13}C and ^1H respectively) suggest however the formation of new sp^3 C-H carbons. Structures **8** and **9**, arising from sequential acetic acid nucleophilic additions and substitutions on the *O*-protonated cation **5** and on subsequent cationic intermediates derived therefrom, agree with the above data and considerations better than structures **7** and **10** arising from nucleophilic additions on **6** and on **5a** respectively. Peaks in the 75 and 6.7-7 ppm regions (^{13}C and ^1H respectively) appear at fields strikingly similar to those of C-*e* (86.1 ppm) and H-*e* (5.9 ppm) of product **11**, once the effect of protonation on ^{13}C and ^1H nmr shifts is taken into account (^1H downfield and ^{13}C upfield displacements respectively). Also the $^1J_{C-H}$ coupling constants of C-*e* of **11** ($^1J_{C-H}$ = 163 Hz) and of the 75.9 and 74.2 peaks of the by products are close in size. Such similarities between product **11** and by products accompanying cation **6** in sulfuric acid-acetic acid solution are suggestive of comparable environment in the two cases, thus supporting the assignment of the 75 and 6.7-7 ppm peaks to C-*e* and H-*e* of products **8** and **9**. Formation of product **11** was unexpectedly observed in dimethylsulfoxide: while spectra of **1** in anhydrous dimethylsulfoxide are perfectly stable for a long time, those in the presence of water change with time: after two days the ^1H nmr spectrum of a 5% solution of **1** in dimethylsulfoxide with 20% of water shows (Table I) that the original formyl proton is present only for 25%, simultaneous with the emergence of other peaks; in particular a doublet at 6.95 ppm and a triplet at 5.9 ppm appear after some time. These data can be easily explained by assuming that water adds to the carbonyl group of **1** to give the hydrated form **11**. Disappearance of peak multiplicity upon treatment of **1** with heavy water to give **12** is then straightforward. The formation of **11** has been analogously followed by ^{13}C nmr spectroscopy.

Hydration of the carbonyl group in **1** to give **11** is quite easily explained since this process can relieve the electronic deficiency present on a heterocycle certainly not particularly electron-rich on its own and further substituted by two powerful electron-withdrawing groups. In this context it is then logical to assume that the conjugate cations of **1** will be even more susceptible to nucleophilic attacks: although in sulfuric acid-acetic acid solution no direct observation of the cation **5** is avialble, it should be admitted that the origin of products **8** and **9** is best accounted for through its intermediacy.

EXPERIMENTAL

Condensation Reactions.

Conditions described for the preparation of **3a** (2) starting from 5-nitroimidazole-2-aldehyde (**1**) (11) and the 2-amino-4-methylpyrimidine (**2a**) were analogously used for the preparation of (*E*)-2-amino-4-[2-(1-methyl-5-nitroimidazol-2-yl)vinyl]-6-methylpyrimidine (**3b**) (mp 284-286°) starting from **1** and **2b**.

Anal. Calcd. for $C_{11}H_{12}N_6O_2$: C, 50.76; H, 4.65; N, 32.29. Found: C, 50.23; H, 4.73; N, 31.98.

The same conditions were also used in the reaction of **1** with 2-amino-4-trideuteriomethylpyrimidine (**2c**) in *O*-deuterioacetic acid and dideuteriosulfuric acid to give a mixture of **3c** and **3d**.

No condensation product **3a** was detected by tlc when a mixture of aldehyde **1** (1.29 g, 8.32 mmoles) and pyrimidine **2a** (1 g, 9.2 mmoles) in glacial acetic acid (11.6 ml) was heated at 70° for 8 hours.

Aldehyde **1** (900 mg, 5.8 mmoles) and amine **2a** (700 mg, 6.4 mmoles) in TFA (5 ml), 60° for 3 hours gave, after usual work up, the condensation product **3a** (60%).

Deuteration of 2-Amino-4-methylpyrmidine **2a**. (a) In *O*-Deuterioacetic Acid.

2-Amino-4-methylpyrimidine (1 g, 9.2 mmoles) is added to the solution obtained by hydrolyzing acetic anhydride (23.48 g, 0.23 mole) with heavy water (9.2 g, 0.46 mole), and the mixture refluxed for 5 hours. The residue obtained upon evaporation of the solvent gave **2c** (99% deuteration of the methyl, no deuteration of ring protons, analysis by ^{1}H nmr).

(b) In *O*-Deuterioacetic Acid and Dideuteriosulfuric Acid.

To a warm (70°) solution of 2-amino-4-methylpyrimidine **2a** (0.51 g, 4 mmoles) in freshly prepared *O*-deuterioacetic acid (4 ml), dideuteriosulfuric acid (0.967 g) dissolved in *O*-deuterioacetic acid (2 ml) was added, and the temperature kept at 70° for 4 hours. The solvent was evaporated, the residue taken up with deuterium oxide, the solution made basic with anhydrous potassium carbonate and rapidly extracted with chloroform. The residue shows (^{1}H nmr) total deuterium incorporation at the methyl group and partial (60%) incorporation at position 5 of the ring.

NMR Spectra.

^{1}H nmr spectra were recorded on a Varian EM-390 instrument at 90 MHz. For sulfuric acid and sulfuric acid-acetic acid solutions the external capillary locking technique was employed. ^{13}C nmr spectra were recorded on a Varian XL-100 instrument operating at 25.2 MHz using 12 mm tubes containing an internal 5 mm tube of hexadeuterioacetone and TMS as a reference. Data were accumulated using 8192 data points, a spectral width of 6000 Hz, and a pulse angle of 45°. Off-resonance spectra were obtained with continuous wave decoupling by irradiating at the proton frequency of acetone increased by 800 Hz (decoupler offset: 46500).

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