# Patterns of muonium addition to imidazoles: a model of radiation-produced hydrogen-atom reactivity with key biological subunits

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From a study of imidazole derivatives using transverse-field muon spin rotation (TF-μSR) spectroscopy, it is found that muonium adducts are formed preferentially at the 2- and 5-position, the latter predominating. It is further concluded that muonium is the true precursor of these radicals, and so this approach is valid for the study of organic molecules, of biological relevance, in which muonium acts as a radioactive hydrogen atom probe (tracer) of reactions involving free H-atoms, such as are produced during the radiolysis of aqueous (intracellular) media.

In experiments where the imidazole was present simultaneously with acetone, competitive addition was found, so that $Me_2\dot{C}$–OMu radicals were formed: evidence is presented for a specific interaction (Mu · · · N) between the radical muon and the imidazole ('pyridine-type') nitrogen atom, the strength of which varies according to the nature of substituents in the imidazole moiety.

Muonium adducts of a simpler sub-unit (the C=N functional group) are also studied.

## Introduction

Coupled with the technique of tranverse-field muon spin rotation (TF-μSR) spectroscopy,[1] 'muonium,' the bound state of a positive muon with an unpaired electron, ($\mu^+e^-$), provides a unique radioactive probe (tracer) in reactions involving hydrogen atoms. From a chemist's viewpoint, muonium is simply a very light hydrogen atom, in which the muon occupies the place of a proton, but with 1/9th of its mass: its 'trace' is a positron, which is emitted on a microsecond timescale by the radioactive decay of the muon.

Herein, our interest concerns their role as reaction probes of H-atom processes of relevance to radiation biology, in which they are produced by energy transfer from ionising radiation to aqueous, most importantly intracellular, media, where their reactions, particularly with deoxyribonucleic acid (DNA) bases, can lead to significant changes in biological activity and cell mutation.[2–4] The production of reactive radicals in the radiolysis of water may be represented by eqn. (1), where the

$$H_2O \longrightarrow (2.8)e^-_{(aq)} + (2.8)^{\bullet}OH + (0.6)H^{\bullet} \quad (1)$$

numbers in parentheses are *G*-values (molecular radiation chemical yield: number of molecules transformed or produced per 100 eV of energy absorbed).[5] Reactions of $^{\bullet}OH$ radicals with biologically important substrates have been studied by electron spin resonance (ESR) methods, using (i) reagent flow-systems[2] and (ii) *in situ* electron radiolysis;[6] in the latter case, highly alkaline solutions are used to suppress $H^{\bullet}$ formation *via* $H^+ + e^-$ [which are often also saturated with $N_2O$ to provide an additional source of $^{\bullet}OH$ radicals, *via* dissociative electron capture as in eqn. (2)]; at very high pH,

$$e^- + N_2O \xrightarrow{H^+} {}^{\bullet}OH + N_2 \quad (2)$$

the conjugate base of $^{\bullet}OH$ ($O^{\bullet-}$) is present as the predominant reactive species.

Studies of H-atom reactions are far more difficult, and highly acidic solutions are often used in order to maximise the H-atom yield, especially for kinetic studies of H-atoms using the ESR–pulse radiolysis method.[7] Certainly in regard to their biological relevance, results obtained under conditions such as these must be treated with caution. In any case, we are not aware of any reports directly relevant to the present topic, in which H-atom addition radicals are identified *in situ* during such experiments, mainly due to the very low signal-to-noise ratios obtained.[8]

By means of the TF-μSR technique[1,9] there is no such restriction on pH, and, given its extreme sensitivity, which relies on single-particle counting, reactions of $Mu^{\bullet}$ ($H^{\bullet}$ equivalent) atoms with biological constituents (or key subunits therefrom) may be studied and resulting product radicals identified.

We have chosen the imidazole unit for the present study since it is a functional group of widespread importance in biology: as part of the histidine residue, it is *inter alia*, of vital importance to the active centre of enzymes such as ribonuclease[10] and superoxide dismutases[11]–the latter being of considerable importance to the mechanisms by which living systems avoid damage by oxygen radicals;[2,12] perhaps more important to radiation biology, it is also a functional building block (subunit), of the DNA constituents adenine and guanine.[2] 'Formal' H-atom adducts of imidazole have been studied by ESR/electron–nuclear double resonance (ENDOR) methods in γ-irradiated single crystals,[13,14] but there is some ambiguity over the mechanism by which they are formed.

## Experimental

The imidazole samples were purchased from Aldrich or Fluka and used without further purification, other than the *N*-acetyl, *N*-benzoyl, *N*-benzyl, *N*-methylsulfonyl and *N*-phenylsulfonyl derivatives, the preparation of which will be reported in a study of imidazole radical cations using ESR spectroscopy.[15] Full details of the TF-μSR method were described previously;[1] for the present experiments, samples of the pure methylimidazoles (other than 4-methylimidazole which was a saturated solution in water), or solutions of the other derivatives in the solvent listed in the Table 2 were deoxygenated using 3–4 freeze–pump–thaw cycles and sealed into 35 mm outside diameter (o.d.) thin-walled Pyrex ampoules. For each experiment, the sample was maintained in an external magnetic field of 2 kG (0.2 T) and exposed to a beam of spin-polarised, positive muons, using the μE4 channel at the Paul Scherrer Institute, Villigen, Switzerland. Data analysis was made in Fourier space as previously described,[1] to reveal the polarisations (yields) and muon precession frequencies for the muonium–imidazole

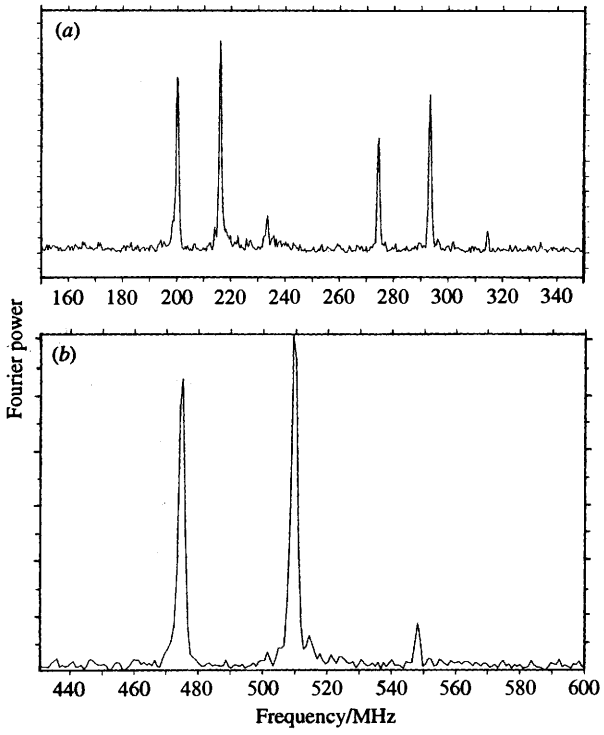



**Fig. 1** (*a*) TF-μSR spectrum of 1-methylimidazole showing the presence of three radicals; (*b*) correlation spectrum of above, revealing hyperfine frequencies for radicals in (*a*)

adducts; for the acetone–imidazole experiments (see text), the diamagnetic signal was 'removed' by frequency filtering prior to the data analysis.

Calculations were carried out on the John Moores University Vax Cluster, using the PM3 Hamiltonian,[16] as available in the MOPAC6 package.[17] The geometry was optimised using either an unrestricted Hartree–Foch (UHF) or restricted Hartree–Foch (RHF) Hamiltonian. In the single-point calculations, with the RHF approximation, a configuration interaction was allowed over an active space consisting of the two highest doubly occupied and the singly occupied together with the lowest three unoccupied levels.

## Results

### Concentrated imidazole samples

Fig. 1(*a*) shows the TF-μSR spectrum recorded from *N*-methylimidazole from which three radicals are readily identified: this is confirmed by the correlation plot[18,19] [Fig. 1(*b*)]. It is crucial to distinguish between radicals formed by muonium addition to carbon or nitrogen atom sites in the imidazole moiety. The maximum possible muon coupling for a nitrogen-centred radical, in which muonium is directly bound to the nitrogen atom, may be estimated from the isotropic proton coupling in the $NH_2^{\bullet}$ radical of −23.8G,[20] by multiplying with the muon/proton magnetic moment ratio (3.1833) and converting into MHz: this yields a figure of −212 MHz for a *spin-localised* radical, but in all probability the radical would be *delocalised*, with a smaller coupling than this. [Previous results for the isoelectronic 1-methylimidazole radical anion show two equivalent nitrogen atoms,[21] with spin populations of 0.4 on *each*, from which a muon coupling of ~ −85 MHz is expected for an N(3)–Mu imidazole adduct with a similar spin distribution.] We are therefore confident that the three radicals, given their much larger couplings (Table 1), arise from addition to the three possible carbon sites. The radical with the largest coupling is from addition to the 4-position,

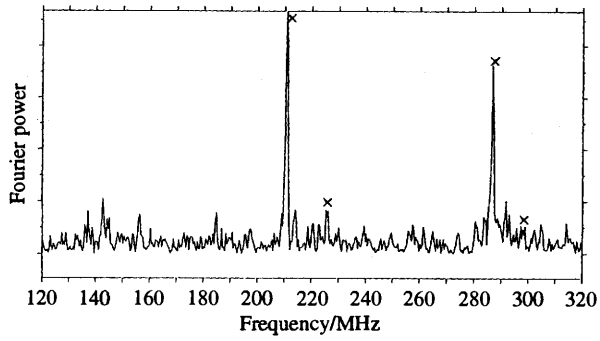



**Fig. 2** TF-μSR spectrum from 2-methylimidazole showing absence of 2–Mu adduct **II**, but increase in the 5-Mu **III**

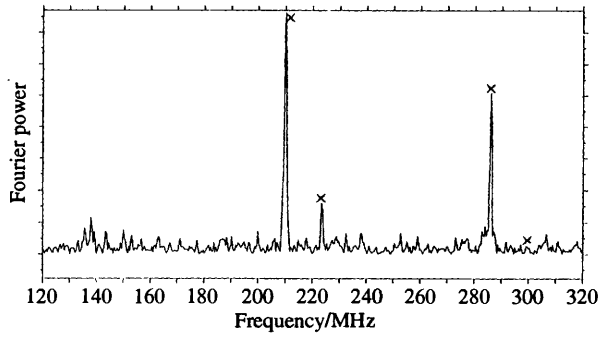



**Fig. 3** TF-μSR spectrum from 1,2-dimethylimidazole, showing changes as in Fig. 2

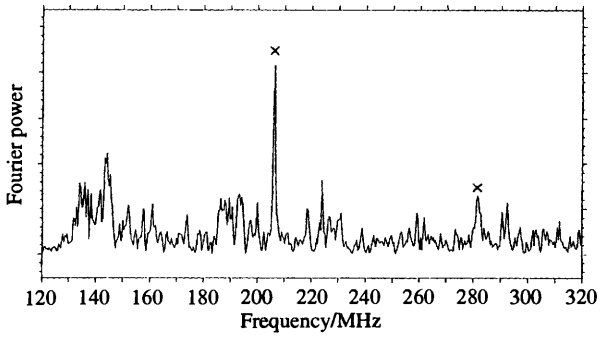



**Fig. 4** TF-μSR spectrum from 4-methylimidazole as a 30% solution in water, showing a single radical 5-Mu **III**

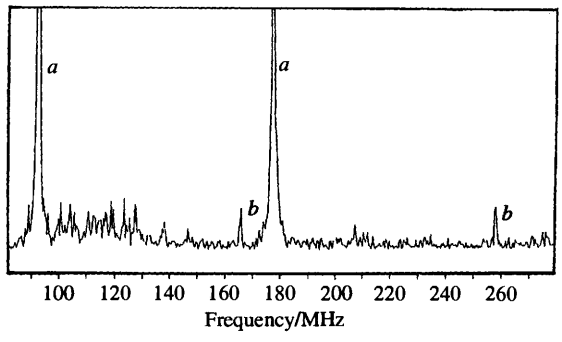



**Fig. 5** TF-μSR spectrum from 1-vinylimidazole showing the presence of (*a*) the C2-vinyl–Mu adduct, and (*b*) the 4–Mu adduct (see text)

since this is an essentially localised radical (**I**): in all cases this is formed in smallest yield. In an extensive study[22] of cyclohexadienyl radicals formed by muonium addition to 22 monosubstituted benzene derivatives, it was found that in only one case ($PhCF_3$) was the *ipso* isomer formed; in all other cases including $PhCH_3$ the substituent effectively blocked addition to the *ipso* position. By similar reasoning, a discrimination

**Table 1** Hyperfine couplings, product yields and (calculated spin-densities and energies) for muonium (hydrogen) adducts of imidazole derivatives

| Parent compound[a] | $A\mu$/MHz | Relative yield | Energy/eV | Spin Density[b] RHF | Spin Density[b] UHF |
|---|---|---|---|---|---|
| 1-Methylimidazole | | | | | |
| 2- | 474.6 | 38.3 | −905.39 | 0.032/0.041 | 0.027/0.034 |
| 4- | 548.6 | 18.3 | −905.33 | 0.053/0.051 | 0.040/0.040 |
| 5- | 509.5 | 43.4 | −905.43 | 0.045/0.038 | 0.032/0.037 |
| 1-$Me_3Si$-imidazole | | | | | |
| 2- | 445.9 | 31.7 | −1309.72 | 0.038/0.045 | 0.031/0.037 |
| 4- | 480.9 | 25.1 | −1309.72 | 0.056/0.057 | 0.043/0.044 |
| 5- | 475.8 | 43.2 | −1309.76 | 0.044/0.041 | 0.036/0.041 |
| 1,2-Dimethylimidazole | | | | | |
| 4- | 526.1 | 23.9 | −1055.06 | 0.052/0.053 | 0.040/0.043 |
| 5- | 496.0 | 76.1 | −1055.18 | 0.036/0.042 | 0.029/0.034 |
| 4-Methylimidazole | | | | | |
| 2- | | 0 | −905.70 | 0.033/0.043 | 0.027/0.036 |
| 5- | 488.1 | 100 | −905.79 | 0.038/0.043 | 0.032/0.036 |
| 2-Methylimidazole | | | | | |
| 4- | 533.4 | 22.6 | −905.69 | 0.053/0.053 | 0.043/0.045 |
| 5- | 498.3 | 77.4 | −905.79 | 0.032/0.043 | 0.030/0.035 |
| 1-Vinylimidazole | | | | | |
| 2- | | | −1023.10 | | |
| 4- | 424.2 | 19.0 | −1023.12 | | 0.043/0.044 |
| 5- | | | −1023.07 | | |
| vinyl C(1) | | | −1023.10 | | |
| Vinyl C(2) | 269.9 | 81.0 | −1023.63 | | 0.002/0.025[c] |

[a] Numbers show position of muonium (hydrogen) addition. [b] Spin-density in 1s orbitals of H-atom (methylene) pair at site of H-atom addition to imidazole ring. [c] Methyl group oriented with one H in radical plane, other two with dihedral angles of 30° with respect to singly occupied molecular orbital.

**Table 2** Couplings for muonium adducts obtained in dilute solutions (see text)

| Parent compound | $A\mu$/MHz |
|---|---|
| 1-Acetylimidazole/acetone | 469 |
| 1-Benzoylimidazole/acetone | 456 |
| 1-Phenylsulfonylimidazole | 459 |
| 1-Acetyl-2-methylimidazole | 468 |
| 1-Benzylimidazole | 474, 491, 505[a] |

[a] For assignments see text.

between the 2-Mu (**II**) and 5-Mu (**III**) adducts is provided by results for the 2-methyl and 1,2-dimethylimidazoles, in which only two radicals are observed (Figs. 2 and 3), the *ipso* adduct now being absent. [The *pairs* of frequencies in Figs. 2–5 are confirmed as each belonging to a particular radical, as shown, by means of correlation plots.[18,19]] We therefore ascribe the radical with the smallest coupling derived from each imidazole derivative (Table 1) to the 2-Mu adduct. This accords with the calculated order of spin-densities and indeed with the assignments to the 5-H and 2-H adducts of imidazole, made by Lamotte,[13,14] and with incomplete neglect of differential overlap (INDO) calculations made by Westhof and Flossmann.[23] It is very significant that, when addition to the 2-position is thus strongly disfavoured, the yield of the 5-Mu adduct nearly doubles, with only a relatively minor enhancement in the 4-Mu yield: this is consistent with *all* adducts arising through a common mechanism.

Only one radical was detected from 4-methylimidazole (Fig. 4), which we believe to be the 5-Mu isomer since the coupling is closest to that for the 5-Mu isomers than for the other derivatives; owing to the tautomeric nature of imidazoles, some 5-methylimidazole must simultaneously be present, but the equilibrium is known to disfavour this isomer,[24] and we do not find any evidence for the presence of additional radicals that could be ascribed to it.

We have published the spectrum from *N*-vinylimidazole previously[25] which shows one major radical, arising from muonium addition to the terminal carbon atom of the vinyl group; here, the assignment is obvious, because this is the most delocalised radical possible, but is supported by the UHF calculated energy (Table 1), which is far lower for this than for any of the other isomers. On careful analysis of the data, the presence of a second radical becomes apparent (Fig. 5), but with a coupling greatly reduced from those for typical ring adducts in Table 1; according to the calculations, the 4-H adduct **I** is most stable, but it is not obvious why this (or its 4-Mu equivalent) should be favoured over the 'allylic' 2-Mu/5-Mu radicals **II**/**III**. It may be relevant that in the *N*-(trimethylsilyl)imidazole (Fig. 6) the 4-Mu yield is enhanced, doubtless due to the well established power of the silicon group to delocalise spin density:[26] the vinyl group will be more efficient in this regard, and may favour the 4-Mu adduct.

### Spin distributions

The calculations (Table 1) indicate that, in their minimum-energy structures, the hydrogen atom 'pairs' at the site of H-atom addition are, in general, inequivalent: this is particularly marked for the 2- and 5-adducts. This implies that the rings are puckered, yet we see only a single radical from each position for muonium addition, which could represent a dynamic average, given that they are measured in solution at room temperature (we note that the rings are calculated to be non-planar in 'OH adducts of imidazole).[27] The rings are apparently 'flatter' for the 4-H adducts since the couplings are nearly equal for each substrate. For *N*-methylimidazole (Fig. 7) the adducts are viewed 'end-on' and it is clear that the rings are still very close to being planar [*the projections in Fig. 7 are drawn to show principally the spin density on the* $CH_2$ *unit, which means that the* $2p_z$ *spin populations on atoms to the rear of this perspective are not visible*; other perspectives, which enable a partial view of these other positions of π-spin density cause the $CH_2$ unit to appear artificially unsymmetrical, and distort the truth]. Viewed from 0.25 Å *above* the ring plane (Fig. 8), the overall π-spin density distribution may be seen, and provides a qualitative

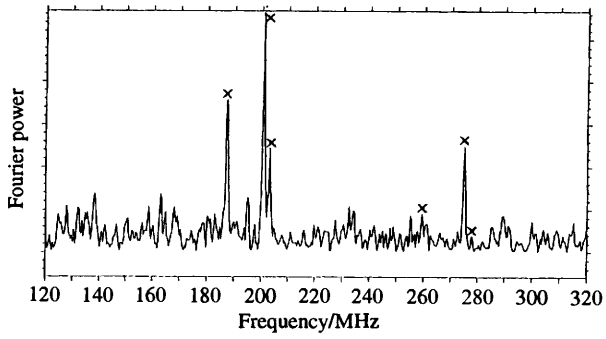



**Fig. 6** TF-μSR spectrum from 1-$Me_3Si$-imidazole as a 50% solution in diethyl ether, showing the presence of three radicals

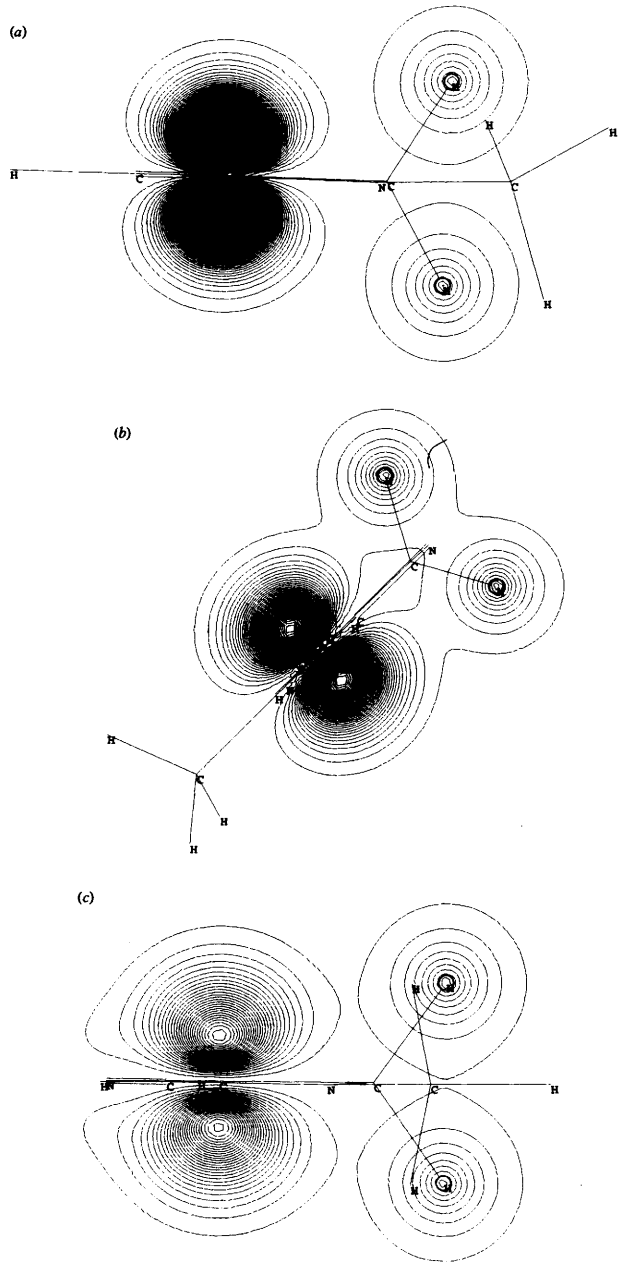



**Fig. 7** UHF-calculated π spin-density contour for 1-methylimidazole/H-atom adducts, presented to show spin at adduct $CH_2H$-atoms (spin populations on atoms to the rear of this perspective are not visible, see text): (*a*) 2-H, (*b*) 4-H, (*c*) 5-H

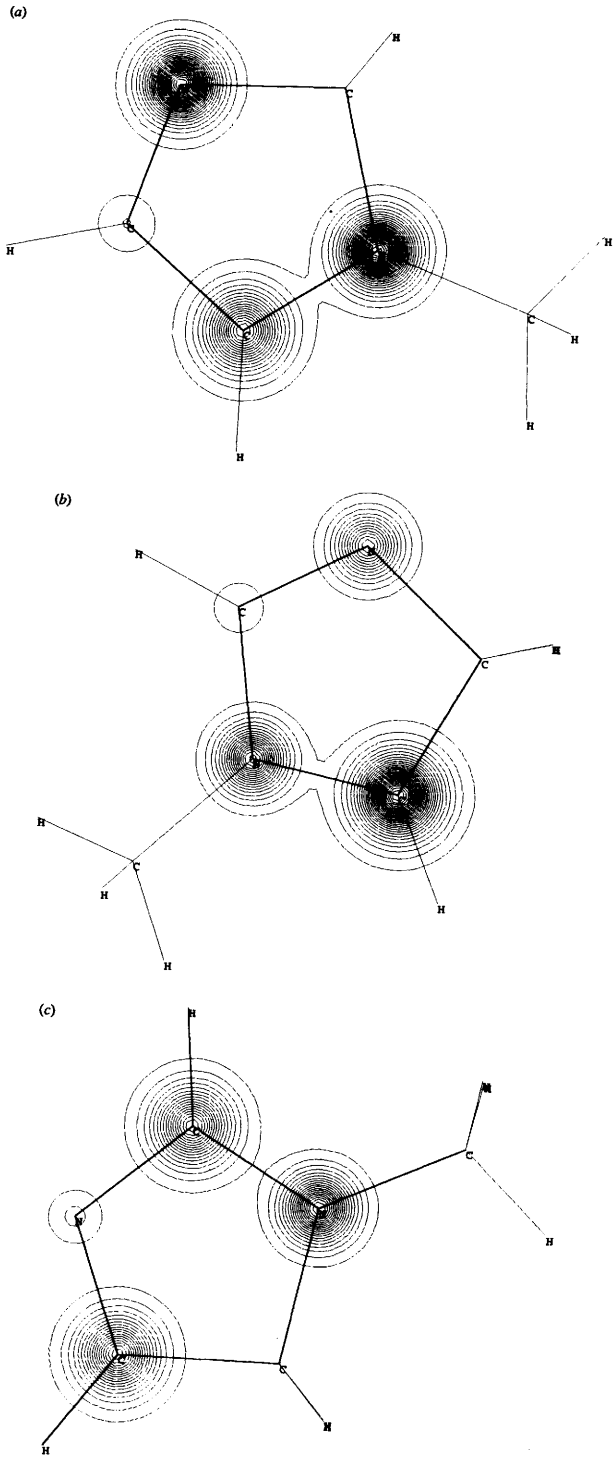



**Fig. 8** UHF-calculated π-spin density contour for 1-methylimidazole/H-atom adducts, viewed from a point 0.25 Å above the ring plane: (*a*) 2-H, (*b*) 4-H, (*c*) 5-H

explanation for the large muon couplings observed since the $CH_2$ (CHMu) group is in each case adjacent to positions of high spin population. We note that the 4-Mu adduct **I** is not quite as localised as might be expected from simple considerations, and has a significant spin population at the adjacent N atom. The 2-Mu adduct **II** is the most symmetrical molecule, and its spin distribution reflects this–the N(1)-methyl group acts as a perturbation which breaks the element of a vertical plane of symmetry which would be present in its absence.

**Imidazole solutions**

The compounds in Table 2 were run as ~30 wt% solutions in the solvents shown: accordingly, the spectra obtained were very weak † and the couplings shown were obtained from correlation plots.[18,19] For the acetone solutions, we observed a competition for addition to the solvent or to the imidazole, since $Me_2\dot{C}$–OMu radicals were also observed (next section). We were able to detect only a single imidazole adduct radical in these cases, which according to the foregoing we assign to the 5-Mu adduct: this is strongly supported by results for the *N*-acetyl and *N*-acetyl-2-methyl derivatives, for which the couplings are identical (within ~0.2%), and addition to the 2-position is not feasible in the latter case, *vide supra*.

With the *N*-benzoyl- and *N*-phenylsulfonyl-imidazoles, there is some ambiguity regarding which ring the muonium has added to; however, results for MuPhCHO adducts[29] give 463 MHz (for the *ortho*-adduct) as the only near contender. If we compare data for the 1-(trimethylsilyl)imidazole, and bear in mind that results for $Me_2NCOMe^{\bullet+}$[30] and $Me_2NSiMe_3^{\bullet+}$[26] radical cations show the C=O function is slightly more effective in delocalising spin density than is $Me_3Si$, a minor decrease (Table 2) from 476 MHz assigned to the 5-Mu adduct is reasonable. Since preliminary ('single shot') calculations indicate that addition to the imidazole ring is favoured energetically, we propose that the 5-Mu adducts are detected for the benzoyl and phenylsulfonyl derivatives.

A similar problem of assignment exists for *N*-benzylimidazole but, given the similarity in the 474 and 491 MHz couplings with those for the 2-Mu and 5-Mu radicals from the methyl derivatives (Table 1), we ascribe them similarly; the 505 MHz coupling, in contrast, is so far below that for any of the 4-Mu derivatives in Table 1, and there is no obvious reason (*i.e.*, enhanced delocalisation) for this, that we believe this to be the *m*-Mu adduct from the benzene ring–the coupling is very close to that reported previously for the *m*-Mu toluene adduct.[22]

**Competition with addition to acetone**

In competition with addition to the imidazoles as shown in Table 3 was the formation of the $Me_2\dot{C}$–OMu radical (Fig. 9), which is reasonable given the well established tendency for acetone[31–38] and other carbonyl compounds[29,37,38] to add Mu$^\bullet$ to the carbonyl oxygen atom. More important is the clear variation in the magnitude of the *muon* coupling in this radical according to the nature of the imidazole derivative that is present: we propose that this is a consequence of a specific interaction between the *basic* imidazole unit and the muon (Mu · · · N), similar to that invoked to explain the fall in *a*(Mu) in this[28] and in the benzaldehyde adduct (PhĊH–OMu)[29] in solvents of high donor power. The Mu · · · N interaction with the imidazole unit is evidently 'tuned' according to the nature of substituents present.

**Imine adducts of muonium**

The observation of one imine (C=N) muonium adduct was reported previously by Louwrier *et al.*,[39] which has a relatively small muon coupling, as might be expected by comparison with Mu–carbonyl adducts,[29,31–38] which are close structural analogues. We have managed (Table 4) to extend the series to aromatic derivatives (*e.g.* Fig. 10), and those derived from oximes, and include imine adducts here, since the C=N unit is ubiquitous and fundamental as a functional subunit in a variety of heterocyclic organic compounds, including the imidazoles, and it may later be possible to extrapolate information obtained regarding the radiation chemistry of these materials to that of more complex biological molecules. Louwrier[39] found that Mu$^\bullet$ preferred to add to the N-atom of the C=N unit, unless a more stable radical could otherwise be formed, *e.g.* in addition to propanal azine which gives EtCH(Mu)Ṅ–N=CHEt; herein N-addition would be favoured by the formation of 'benzylic' radicals (we found previously[40] that the normal tendency for Mu$^\bullet$ to add to the *carbon* atom of C=S groups was reversed in $Ph_2C$=S).

The radical $Ph_2\dot{C}$–N(H)Mu, formed from benzophenone imine, is interesting in that, compared with $Ph_2\dot{C}$–$NH_2$, a symmetry element has been destroyed by the muon substitution; this makes no difference to the electronic structure, however, since the muon coupling remains small.

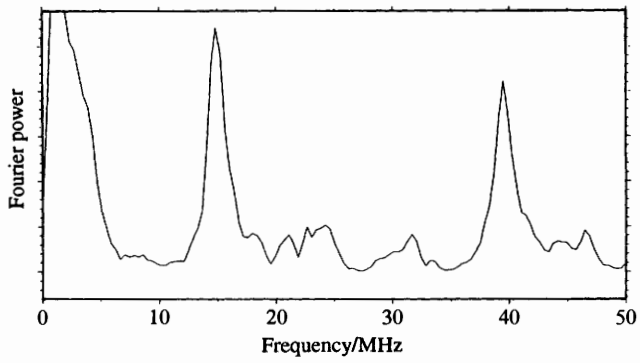



**Fig. 9** TF-μSR spectrum of the $Me_2\dot{C}$–OMu radical recorded in a mixture of acetone and 1-acetylimidazole

**Table 3** Couplings for $Me_2\dot{C}$–OMu radical in acetone solution with imidazole shown

| Imidazole | $A\mu$/MHz |
|---|---|
| 1-Methylsulfonylimidazole | 26.30 |
| 1-Acetylimidazole | 24.68 |
| 1-Acetyl-2-methylimidazole | 24.66 |
| 1-Benzoylimidazole | 24.29 |
| Pure acetone | 26.98[a] |

[a] Extrapolated from data in ref. 31.

**Table 4** Couplings for imine (C=N)-derived radicals

| Radical | $A\mu$/MHz |
|---|---|
| PhĊH–N(Et)Mu | 18.81 |
| $Ph_2\dot{C}$–N(Et)Mu | 18.86 |
| $Ph_2\dot{C}$–N(H)Mu | 25.00 |
| $Me_2\dot{C}$–N(Mu)OH | 10.54[a] |
| MeĊH–N(Mu)OH | 9.37[a] |

[a] Recorded as saturated solutions in ethanol.

## Discussion

Westhof and Flossmann[23] reported incomplete neglect of differential overlap (INDO) calculations on 2-H and 5-H atom adducts of imidazole, which are in agreement with the results for these radicals in γ-irradiated imidazole crystals reported by Lamotte.[13,14] They make further reference to a report by Kasai and McLeod[41] of a radical formed by photoelectron addition to imidazole in an argon matrix, but conclude that there is an error in assignment, since their calculations predict substantially

† This is a general problem with the TF-μSR technique, that it is necessary to form the radical on a timescale related to the muonium hyperfine frequency (4.5 GHz):[9] *i.e.* within *ca.* $10^{-10}$ s. Otherwise, there is a loss of phase coherence[28] which reduces the mean positron signal amplitude. Effects which cause rates for muonium addition to be less than this result either in reduced amplitudes (particularly of the high-frequency line) or in complete loss of signal from a Mu$^\bullet$ product radical. Here, the rate of addition is lowered by the smaller concentration of the imidazole substrate.

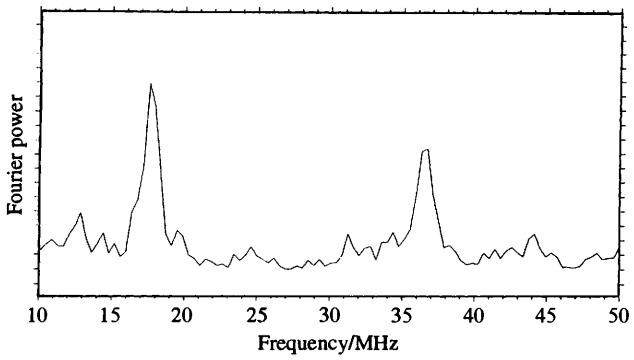


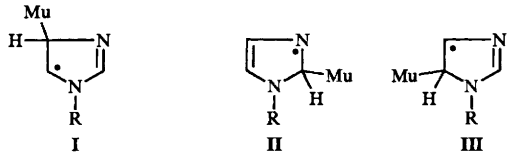

**Fig. 10** TF-μSR spectrum of the PhĊH–N(Et)Mu radical recorded in a sample of the liquie imine

larger couplings in the rearranged anion radical than were actually observed.[41] Instead, it is proposed that Kasai and McLeod have actually formed the 5-H adduct (a neutral radical), because the 50 G doublet splitting fits both with calculations and with the WH single-crystal study;[13,14] the alternative 2-H adduct, which is formed dominantly by radiolysis, is similarly eliminated. They make the salient point, based on Hückel 'Free Valence,' that there is a different centre of reactivity for neutral and anionic species: respectively, the 5-and 2-position for imidazole. This leads to the conclusion that the 2-H adduct arises from an anionic precursor state in an irradiated imidazole crystal, whereas Kasai and McLeod's result is due to *true* hydrogen-atom addition in the argon matrix. It is interesting that, on other grounds, we[21] had proposed similarly that the reactivity of γ-irradiated single crystals of imidazole was that of initially formed radical ions: specifically that protonation or deuteriation of imidazole radical anions by 'bridge' H(D) atoms occurs, giving the 2-H adduct.

**Mechanism for 'Mu' addition**

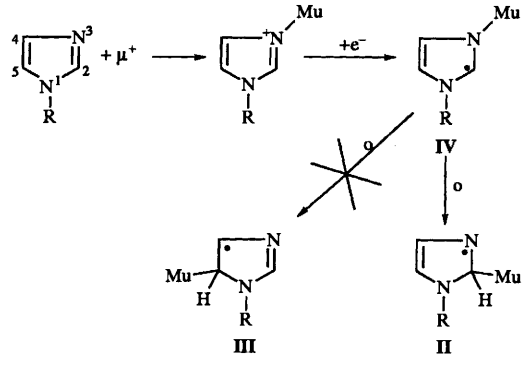
It is a matter of central importance to know the mechanistic origin of the imidazole–Mu adducts that we observe. As discussed previously for acetone,[28,38] when the substrate is polar and could readily accept either radiolytically generated electrons or muons, the actual radical precursor may not necessarily be Mu• itself, since ionic routes could be important–imidazole is known from pulse-radiolysis experiments[42] to add electrons to form the radical anion, and will easily protonate (and therefore muonate) given its $pK_a$.[24]

To explore conceptually the possibility of ionic routes, we need to consider what happens when a high-momentum (85 MeV/*c*) muon interacts with the medium: clearly, in the transfer and dissipation of so much energy, there will be extensive ionisation along the muon track–those events most relevant to chemistry will occur close to the end of the track, when all reactive species have achieved *near* thermal energies. The muon must then find itself surrounded by electrons; if it *binds* with one of these, *actual* muonium will be formed; if the electron is already associated with one particular molecule (radical anion), muon addition will lead to an *overall* muonium adduct–this is a more favourable process than electron abstraction by the muon *from* the radical anion to yield muonium. An alternative is for the muon to first add to ('protonate') a substrate molecule, which must then capture an electron to form a free radical.



I II III

We first consider the possible outcomes of these ionic routes (Scheme 1). Primary muon addition to an imidazole, followed by electron addition, would give the nitrogen-adduct **IV**; in order for this to form the radical **III**, which is predominant, this would need to rearrange on a $10^{-10}$s timescale, and *via* a 1,3-shift, which would be unprecedented. Were rearrangement to occur, it is the 2-isomer **II** that would be formed, in analogy with the apparent behaviour of anionic centres in imidazole single crystals according to ESR measurements;[13,14] ESR and μSR differ, however, in that the latter is critically dependent on the final radical being formed very rapidly (*vide supra*) a restriction that does not apply to ESR; similarly, electron addition followed by muon addition would also lead to the radical **II**.



**Scheme 1**

It is significant that the calculated relative energies of the H-atom adducts (Table 1) are in accord with the observed yields of the muonium adducts, which points to all 3 isomers originating *via* a common mechanism, *i.e.* approach by muonium to the imidazole ring from which the distribution of product radicals is dictated by their relative energies. This is further supported by the behaviour of 1-methylimidazole and 1,2-dimethylimidazole in which addition at the 2-position is strongly disfavoured; if the 2-isomer arose from an independent route involving, say, rearrangement of an ionic precursor, it is reasonable that its formation could also be inhibited; however, since we have already argued that the 5-Mu product could not be thereby formed on the necessary rapid μSR timescale, probably *no radical* would arise *via* this route–what happens though is that the yield of the 5-Mu adduct almost doubles, which is in accord with the above model where Mu approaches the imidazole and, not being able to attack the 2-position, is instead directed mainly at C-5, with a lesser increase in attack at C-4.

Examination of the spectra in Figs. 1–5 reveal that the high-frequency line for each radical is of lower intensity than its low-frequency counterpart–this effect has been noted previously in the case[28] of the muonium adducts of acetone and of 2,3-dimethylbuta-1,3-diene. An explanation was proposed relating to the mechanism for radical formation, and it was concluded that this is a consequence of muonium being the major radical precursor: dephasing of the muon polarisation occurs as a result of precession at the muonium frequency prior to radical formation, so less transfer of the polarisation occurs to the product; the effect is most pronounced on the high-field line since for this the frequency differential between the precursor and product states is greatest; we have collated the ratio of *powers* for the high/low-frequency lines for each of the radicals in Table 5 (the square roots of these ratios give the polarisation ratio). It is very telling that this power ratio follows the trend in product distributions, so that the muonium adds more slowly to positions that yield the less stable isomer. [For very large

**Table 5** Ratio of powers for low-frequency/high-frequency lines in muonium–imidazole adduct radicals

| Parent Compound | 2-Mu | 5-Mu | 4-Mu(Adduct) |
|---|---|---|---|
| 1-Methylimidazole[a] | 1.52 | 1.33 | 1.63 |
| 2-Methylimidazole[a] | | 1.27 | 1.70 |
| 1,2-Dimethylimidazole[a] | | 1.50 | 2.00 |
| 4-Methylimidazole[b] | | 3.50 | |
| 1-$Me_3Si$-Imidazole[c] | 3.70 | 2.29 | 7.78 |
| Order of addition of Mu: | medium | fast | slow |

[a] Pure compound. [b] 30% Solution in ethanol. [c] 50% Solution in diethyl ether.

hyperfine couplings, there is an effect due to the 'passband' of the spectrometer,[9] which also causes the high-frequency line to appear with reduced intensity, but, here, the polarisation ratio follows product distribution rather than frequency, so that the former is the important influence.] The effect is enhanced by dilution (Figs. 4 and 5), because the precursor lifetimes and hence relative polarisation *losses* are increased, and the high-frequency line almost disappears in some cases.

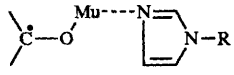
We are therefore convinced that muonium is the true precursor to these adducts, and we can make a useful comparison with hydrogen-atom chemistry, since, if Kasai's spectrum[41] is from actual H-atom addition to imidazole,[23] and is dominantly the 5-H isomer, we have a close parallel with the muonium results–additionally, we measure a significant yield of the 2-Mu adduct, and, considering the complex nature of the spectrum published by Kasai, it is entirely possible that some of the 2-H isomer could be present; indeed, this might explain some of the substructural complexity of the spectrum. The more nucleophilic nature[43] of Mu over H[7] could lead to an increase in the formation of the 2-Mu over 2-H, given the positive charge at C-2,[24,44] but the dominance of the 5-isomer in each case shows that the Mu and H reactions are of the same character; therefore Mu is a suitable H-atom probe as we have asserted.

In regard to the matter of muonium being formed dominantly, rather than other (ionic) precursors, we note the following: in the radiation *spur* the species with by far the highest electron affinity is the *muon*, likewise, an electron has by far the greatest (proton) muon affinity–the formation of *actual* muonium is then the most likely event; it follows, therefore, that in most media, the observed radicals arise from the *actual* addition of muonium, and which dominates over less favourable ionic routes.

**Nucleophilic *vs.* electrophilic character of Mu˙ and H˙ atoms**

Walker and his co-workers[43] have reported that muonium atoms are slightly nucleophilic in their addition to substituted benzenes and benzoic acids; this stands in apparent contrast with ordinary H-atoms which are slightly electrophilic.[7] It must be stressed, however, that for both Mu˙ and H˙ the degree of polar dependence on their reactivity is *very small*. In a later report,[45] these workers claim to have found that this difference in *philicity* leads to completely different reaction pathways in their addition to pyrazine, in which H-atoms add at nitrogen while Mu˙ adds to carbon; while the measured rate constants show clearly that Mu˙ adds significantly more rapidly than H˙ to pyrazine, the conditions for each are quite different since an aqueous solution was used for Mu˙ but a strongly acidic solution (pH 1) for H˙–while this is perhaps less important regarding the kinetics (since the neutral and protonated species present may be quantified from the $pK_a$s and allowances made), it is crucially important to the identification of the product radical by ESR spectroscopy. Zeldes and Livingston[46] did not form the H-adduct radical **V** from pyrazine by direct H-atom addition but *via* electron transfer from photochemically generated $Me_2\dot{C}$–OH radicals–the radical **V** being consistent with subsequent protonation of the initially formed pyrazine radical anion. Even if the reaction involved H-atom transfer from the $Me_2\dot{C}$–OH radical, it is still not a genuine reaction of H-atoms and will probably be of entirely different character; moreover, radicals **V** may be observed simply because they are the most stable, while the (more reactive) H-atom adducts are destroyed by further reaction. Thus the ESR measurements cannot be taken with sufficient assurance to state that different products are formed with Mu˙ and H˙.

**V**



**VI**

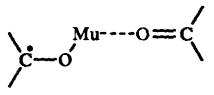
It appears more likely that the mechanism is the same for both, merely that the more nucleophilic character of Mu˙ accelerates the process. This illustrates the great problem with H-atom studies, that kinetics and product identification from *actual* H-atom reactions, under the *same* conditions, are rarely possible; the TF-μSR approach does not suffer from these disadvantages.

We note, here, a recent paper by David Walker's group, which has succeeded in detecting radicals formed by muonium addition to thymine and uracil derivatives using the Level-Crossing relaxation method.[47]

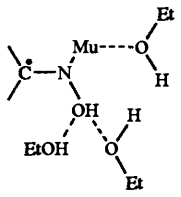
**A specific interaction between the $Me_2\dot{C}$–OMu radical and imidazoles**

Coupling constants for the title radical, formed in acetone/imidazole mixtures, are shown in Table 3, and it is clear that there is a substantial variation according to the nature of the imidazole substitution. As a starting point, we need a *base* value for $Me_2\dot{C}$–OMu in *pure* acetone, which is a problem since there are several quotes in the literature–all different, probably due to the presence of small, but varying quantities of moisture. The most detailed study of the acetone/water system is that by Buttar *et al.*;[35] extrapolation of their data for pure acetone yields a value of 26.98 MHz at 298 K–this may be compared with the values in Table 3, which are *all* lower. In analogy with results for the Mu/carbonyl adduct radicals formed in acetone/water[31–33] and benzaldehyde/solvent interactions,[29] we propose that the imidazole acts, in each case, as a donor, forming a Mu • • • N bond, as in **VI**; the bond with imidazole is evidently stronger than that with another acetone molecule **VII**. The effect is weakest with the $MeSO_2$ substituent, probably because of its strong electron-withdrawing power, but increases with the carbonyl substituents; at first sight this is surprising, but may be explained if the inductive effect is dominant in determining the binding capacity of the N(3) lone-pair. The significance of this result is that it may be possible to study H(Mu)-bonding between a C–OMu radical (as a probe of molecules with hydroxylic functions, ROH) and nucleotide building blocks; in analogy with results with our 'environmental

probe,' PhĊH–OMu,[29] which is extremely sensitive to solvent 'cybotactic' phenomena.[48]



VII



VIII

**Imine-adducts: $R_2$Ċ–NR(Mu)**

The low coupling constants shown in Table 4 tell that, as with carbonyl adducts, Ċ–OMu, the addition of muonium is not at the carbon, but the nitrogen atom. Considering the structural equity between these radicals, with appreciable π-bonding between the carbon and either oxygen[28,29,31-38] or nitrogen atoms, we propose that the muon is located close to the radical plane but executes shallow torsional oscillations which confer a positive sign to the coupling: for $Ph_2$Ċ–N(Et)Mu and PhĊH–N(Et)Mu radicals, the couplings are almost identical, but a sharp increase is found with $Ph_2$Ċ–N(H)Mu. Almost certainly, this is merely a consequence of the lower mass of the muon-bearing group in the latter case, which can vibrate with a higher mean amplitude and so sample more of the positive coupling region.[29,37,38]

Most remarkable is the fall in the coupling for the radicals derived from oximes; this cannot be a question of mass differences for the vibrating muon-bearing group, and is more reasonably attributed to H-bonding effects, given that the group now bears a hydroxy function and is in ethanol as a medium. We envisage solvent interactions as in structure **VIII** which will greatly restrict the vibrational excursions of the muon from the radical plane.

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