reduction of $Ru(bpy)_3^{2+}$, will be discussed in detail in a later publication.

In acetonitrile a rapid reaction occurs between $Ru(bpy)_3^+$ and O_2 to give $Ru(bpy)_3^{2+}$ quantitatively[23] and presumably superoxide ion (eq 5).

$$Ru(bpy)_3^+ + O_2 \rightarrow Ru(bpy)_3^{2+} + O_2^- \qquad (5)$$

We have obtained evidence for eq 5 by flash photolysis of an acetonitrile solution containing DMA (5.8×10^{-2} M), O_2 (1.4×10^{-3} M),[24] and $Ru(bpy)_3^{2+}$ (1.1×10^{-5} M). Following flash photolysis, the absorbance due to a transient (λ_{max} 470 nm) produced during the flash decreased by a process which followed second-order, equal concentration kinetics. The difference spectrum showed that the transient was DMA^+ [15] and that it was the only spectrally observable intermediate.[15,25] No evidence for $Ru(bpy)_3^+$ was found on this time scale.

In the experiment, reactions 1 and 2 (D = DMA) occur during the flash, followed by rapid oxidation of $Ru(bpy)_3^+$ by O_2 (eq 5). The reaction observed by flash photolysis must be eq 6 ($k(22 \pm 2\ °C) = 8.5 \times 10^8\ M^{-1}\ s^{-1}$).

$$O_2^- + DMA^+ \rightarrow O_2 + DMA \qquad (6)$$

The observation of second-order, equal concentration kinetics for reactions 3 and 6 shows that eq 3, 5, and 6 are stoichiometric as written.[27] The superoxide ion is an activated form of O_2[29] and it may be possible to develop photocatalytic schemes for carrying out useful chemical reactions based on the chemistry of O_2^- or HO_2 where the reactions are driven by visible light.

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Room Temperature Generation of Radicals from Dimethylaminomalononitrile, Probably via Dimethylaminocyanocarbene

Sir:

Under basic or thermal conditions, *tert*-octylaminomalononitrile decomposes with generation of radicals and of hydrogen cyanide; α-elimination of the latter was postulated to give initially an aminocyanocarbene intermediate, but β-elimination could not be entirely ruled out.[1] It is now found that both dimethylaminomalononitrile (**1**)[2] and its monodeutero analogue decompose at ambient temperature to give the dimethylaminodicyanomethyl radical (**3**). The ESR spectrum of a neat degassed sample—taken 4 h after reaching room temperature—and its computer simulation are shown (Figure 1). Generation of the radical **3** in dilute solution requires prior heating to 140 °C for 30 min but is also achieved in the cold by UV irradiation (30 s) of a 5% solution of **1** in benzene/di-*tert*-butyl peroxide (95:5).

In the decomposition of **1**, hydrogen cyanide and the dimethylaminocyanomethyl radical (**4**) are also produced. The formation of hydrogen cyanide is shown by mass spectroscopy and by the isolation of 1-dimethylamino-2-aminomaleonitrile[3] (**7**) from the thermolysis (60 °C) product of **1**. Compound **7** results from addition of hydrogen cyanide to **1** (reaction h).[3]

Direct evidence for the generation of the less stable (see below) radical **4** is lacking since it is not detected by ESR spectroscopy; however, its generation is inferred from the isolation of dimethylglycinonitrile (**5**) from the thermolysis (60 °C) product of **1**. Compound **5** results from hydrogen abstraction by **4** according to reaction f,[3] which additionally leads to the accumulation of radical **3**.

Generation of the radicals **3** and **4** and of hydrogen cyanide also accounts for the formation of the crystalline thermolysis

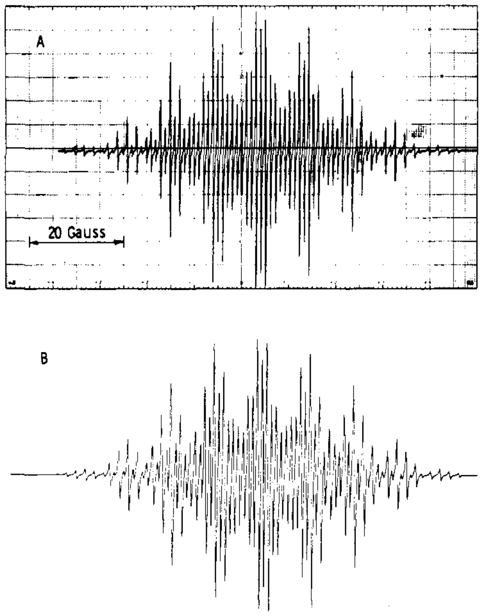
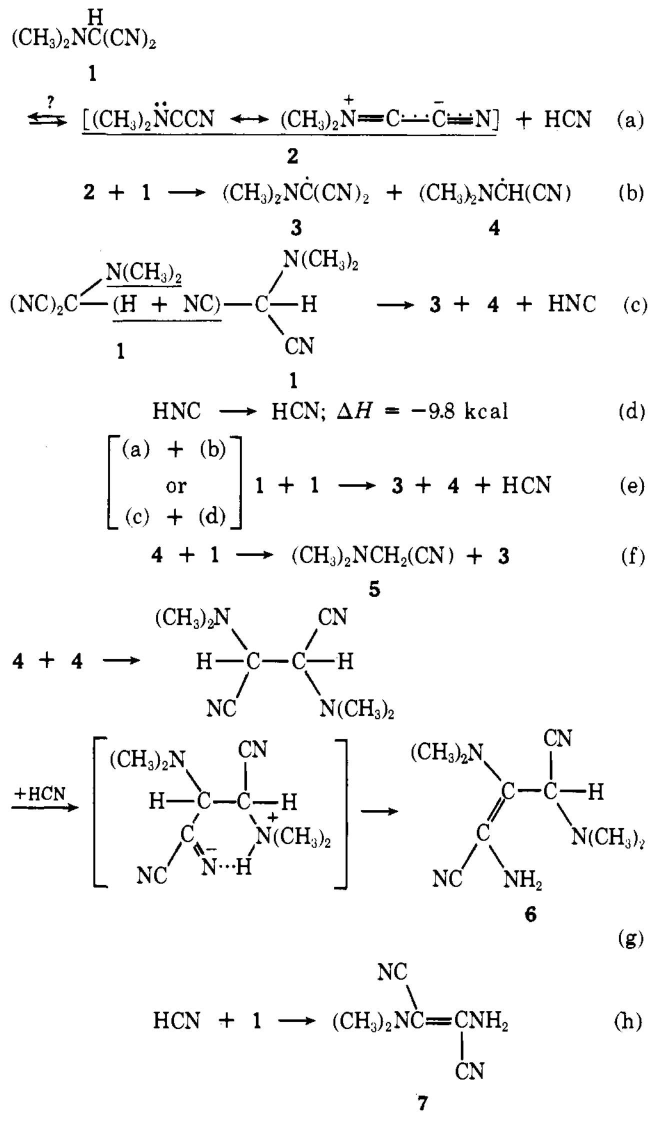


Figure 1. (A) ESR spectrum of dimethylamino–dicyanomethyl radical (**3**). Conditions, neat dimethylaminomalononitrile (**1**) 4 h at room temperature. (B) Computer simulation, *a* values: 6 H [N(C*H*$_3$)$_2$] 9.19,[a] 8.61;[b] 1 N[*N*(CH$_3$)$_2$] 10.25,[a] 9.61;[b] 2 N (C≡*N*) 1.99,[a] 2.04;[b] *g* = 2.0030. ([a]Neat **1**, line width 0.3 G. [b]5% **1** in xylene, line width 0.1 G, spectrum (not shown[3]) obtained at room temperature, after 30 min at 140 °C.)



$$\overset{\mathrm{H}}{(CH_3)_2NC(CN)_2}\ \mathbf{1}\ \overset{?}{\rightleftarrows}\ \underbrace{[(CH_3)_2\ddot{N}CCN \longleftrightarrow (CH_3)_2\overset{+}{N}{=}C{-}\overset{-}{C}{\equiv}N]}_{\mathbf{2}} + HCN \quad \text{(a)}$$

$$\mathbf{2} + \mathbf{1} \longrightarrow \underset{\mathbf{3}}{(CH_3)_2N\dot{C}(CN)_2} + \underset{\mathbf{4}}{(CH_3)_2N\dot{C}H(CN)} \quad \text{(b)}$$

$$\longrightarrow \mathbf{3} + \mathbf{4} + HNC \quad \text{(c)}$$

$$HNC \longrightarrow HCN;\ \Delta H = -9.8\ \text{kcal} \quad \text{(d)}$$

$$\left[\begin{array}{c}\text{(a)} + \text{(b)}\\ \text{or}\\ \text{(c)} + \text{(d)}\end{array}\right] \mathbf{1} + \mathbf{1} \longrightarrow \mathbf{3} + \mathbf{4} + HCN \quad \text{(e)}$$

$$\mathbf{4} + \mathbf{1} \longrightarrow \underset{\mathbf{5}}{(CH_3)_2NCH_2(CN)} + \mathbf{3} \quad \text{(f)}$$

$$\mathbf{4} + \mathbf{4} \longrightarrow \cdots \xrightarrow{+HCN} \cdots \longrightarrow \mathbf{6} \quad \text{(g)}$$

$$HCN + \mathbf{1} \longrightarrow \underset{\mathbf{7}}{(CH_3)_2N\overset{\mathrm{NC}}{C}{=}\underset{\mathrm{CN}}{C}NH_2} \quad \text{(h)}$$

product, 1-amino-2,3-bis(dimethylamino)-1,3-dicyanopropene (**6**), isolated in 19% yield. The initial step in its formation is dimerization of **3** (reaction g).[3]

Generation of the radicals **3** and **4** from **1** under such mild conditions defies conventional explanations. Breaking of C-CN bonds invariably requires a large energy expenditure (144 kcal in (CN)$_2$, 121 kcal in CH$_3$CN).[4] Therefore, only concerted processes appear feasible, in which the energy of formation of the HCN bond (129 kcal[4]) combined with the stabilization energies of the product species can in part offset the breaking of the C-H and C-CN bonds.

Charge-separated forms contribute to stabilization of the radicals **3** and **4** (merostabilization[5]), **4** being expected to be much less persistent than **3** because it is less sterically hindered and has only a single nitrile group.[6]

The hyperfine splitting constant for the aminonitrogen in radical **3** is almost twice that for the α-diethylaminoethyl radical (a(N) CH$_3\dot{C}$HN(CH$_2$CH$_3$)$_2$ = 5.2)[7a] and represents more than half of the value for the dimethylaminium radical (a(N) H$^+\dot{N}$(CH$_3$)$_2$ = 19.28).[7b] This confirms that the lone electron is appreciably delocalized and hence **3** can be seen as a carbon analogue of the nitroxides, having a similar principal resonance form, i.e., $(CH_3)_2\dot{N}^{+-}C(CN)_2$ vs. $(CH_3)_2\dot{N}^{+}O^{-}$. The stability of **3** is therefore an additional example of the analogy between O and C(CN)$_2$, which was recently pointed out by Wallenfels et al.[8]

The observed solvent effect (see legend under figure) is consistent with the expected greater contribution of charge-separated forms in a more polar medium.

Two concerted processes are conceivable: 1. Initial distortion of the Me$_2$N—C—C≡N moiety to a near linear array, followed by α-elimination of hydrogen cyanide in a symmetry allowed $\sigma^{2s} + \sigma^{2a}$ nonlinear cheletropic fragmentation[3,9] to give the linear[10] dimethylaminocyanocarbene (**2**, reaction a). The carbene, **2**, subsequently abstracts a hydrogen atom from **1** (reaction b) to give the two radicals **3** and **4**. 2. Two molecules of **1** generate the radicals **3** and **4** and a molecule of isohydrocyanic acid, which rearranges to hydrogen cyanide (reactions c and d). Route 1 is preferred for several reasons:

Reactions a + b (route 1) and c + d (route 2) give the same products (reaction e) so that as a route to the radicals **3** and **4**, a + b is energetically favored over c by Δ*H* (reaction e) = 9.8 kcal.[11]

Although route 2 avoids the intermediacy of the carbene **2**, precedence does not favor it; bimolecular generation of radicals from saturated substrates—as in reaction c—is virtually unknown.[12]

Additionally, the activation energy for reaction a is likely to be moderate or even low because aminocyanocarbenes—while fairly energetic species[10]—are expected to be uniquely stabilized relative to carbenes with only amino or cyano substituents. The electron—withdrawing and supplying substituents on the carbene carbon atom—as in **2**, allows stabilization by ylide-like, charge-separated forms[10] regardless of the multiplicity.

The absence of bisdimethylaminofumaro- or maleonitrile[3,13] among the thermolysis products of **1** indicates that simple dimerization of the carbene **2** does not occur. This is probably due to Coulombic repulsion resulting from high electron density at the carbene carbon atom.[3,10] However, polymerization of **2** via the $(CH_3)_2{}^+N{=}C{=}C{=}N^-$ form[14] or the corresponding diradical[10] may account for a large amount of polymeric dark-colored thermolysis product.

Aminocyanocarbene has been suggested as a possible intermediate in the prebiotic synthesis of polypeptides and purines.[14] Its direct formation by rearrangement of iminoacetonitrile—the hydrogen cyanide dimer—is improbable;[15] it may, however, originate from thermolysis of aminomalonitrile—the hydrogen cyanide trimer.

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Cysteine Modification and Cleavage of Proteins with 2-Methyl-N^1-benzenesulfonyl-N^4-bromoacetylquinonediimide

Sir:

The determination of protein sequence has been greatly facilitated by cleavage techniques which cause fragmentation of the macromolecular peptide chain in a specific manner.[1,2] The use of peptidases for this purpose has been supplemented in recent years by a number of chemical cleavage techniques.[3-5] Nevertheless, the majority of chemical methods proposed for this purpose (based on studies of model substrates) have not been applied successfully to proteins. We wish to report the design and development of a new, cysteine-selective modification and cleavage agent, 2-methyl-N^1-benzenesulfonyl-N^4-bromoacetylquinonediimide (**1**),[6] which has been observed to cause fragmentation of both ovalbumin and reduced bovine pancreatic ribonuclease,[7] as well as model substrates.

Incubation of these proteins in 0.1 N acetic acid solution containing 0.5% SDS and 0.001 M EDTA (ovalbumin 4.5×10^{-5} M; ribonuclease 6.7×10^{-5} M) with an excess of **1** (as a 0.08 M solution in acetone) at room temperature for 12 h followed by heating at 80 °C for 1 h resulted in chain cleavage, which was detected by SDS gel electrophoresis[8] (see Figure 1). In each case, although under these particular conditions some uncleaved protein is observed,[8] a number of lower molecular weight protein fragments are produced upon such treatment. This fragmentation is proposed to occur via the route illustrated in Scheme I.

Initial 1,4-addition of the cysteine sulfhydryl function is directed "para" relative to the 2-methyl substituent, to form an aromatic thioether (**2**).[9,10] This primary regiospecific alkylation (easily observed and monitored by UV at 305 nm) permits subsequent alkylation of the sulfur atom by the adjacent bromoacetamido function to generate a labile sulfonium

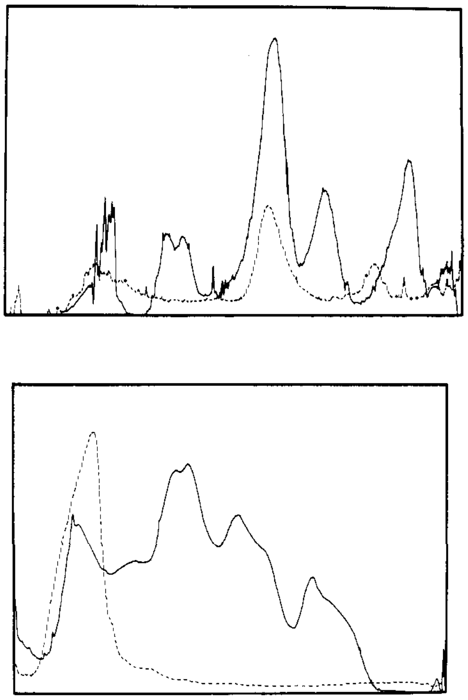

Figure 1. Superimposed densitometer tracings of polyarcylamide gels from SDS disk gel electrophoresis (1% mercaptoethanol; stained with coomassie blue and scanned at 550 nm). Gels scanned left to right—high to low molecular weight. Trace of standard reaction mixture with protein each treated in 0.1 M acetic acid 0.5% SDS, 0.001% EDTA, 12 h, r.t. 1 h, 80 °C without quinonediimide 1 (···) and with quinonediimide (—): top, partially reduced bovine pancreatic ribonuclease (Worthington); bottom, ovalbumin. The profile of ovalbumin is unchanged if the reaction is not heated after treatment with quinonediimide.

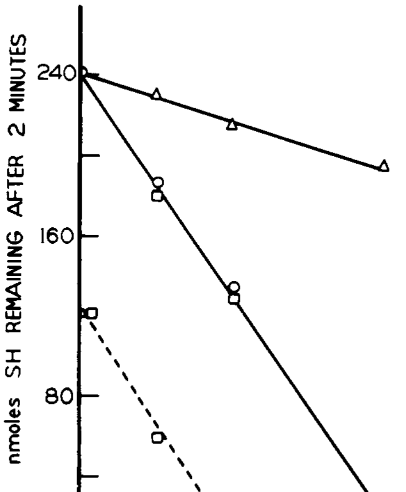

Figure 2. Loss of sulfhydryl of N-acetylcysteine (6.2×10^{-5} M) (—) and ovalbumin (1.25×10^{-5} M) (···) in 4.0 mL of 0.1 M phosphate buffer, pH 8, containing 0.5% SDS and 0.001 M (EDTA) after 2 min reaction time with 2-methyl-N'-benzenesulfonyl-N'-4-bromoacetylquinonediimide, **1** (□), or with 2-methyl-N'-benzenesulfonyl-N-4-acetylquinonediimide, **1a** (Br = H) (○), or with reduced reagent 2-methyl-4-bromoacetamidobenzenesulfonanidide (△). Sulfhydryl was determined by incubation with Ellman's reagent (DTNB) and recording absorbance at 420 nm after 20 min.