sive kinetic studies on this and other thiaether complexes[9,13] rule out the presence of dimeric copper species, and the identical kinetic behavior observed by monitoring either at 600 or 400 nm provides strong evidence that a single species gives rise to both absorption bands.

In agreement with the relatively high affinity of Cu(I) for thiaether sulfur donors,[16] Cu(II) in the thiaether complexes has been found to be readily reduced to Cu(I) in a reversible manner. Preliminary cyclic voltammetric measurements on the 13-ane-S_4 and 14-ane-S_4 complexes (presumed to represent nonplanar and planar sulfur coordination, respectively) indicate a reduction potential in the vicinity of +0.7 V (vs. SHE in 80% methanol) which is strikingly similar to the Cu(II)-Cu(I) potentials which have been observed in the blue copper proteins.[7]

In the recent studies identifying the presence of one or more Cu(II)-S bonds in the blue copper proteins,[6-8] the reporting investigators have suggested that the sulfhydryl moiety of a cysteine residue is involved, presumably because thiaether sulfurs have generally been assumed to have little affinity for Cu(II).[16-18] However, the present work strongly suggests that *the thiaether sulfurs of methionine groups* (which generally equal or exceed the number of cysteine residues)[19] *represent the ligating S-donor atom(s) in blue copper proteins.* This would then account for the observation that Cu(II) is not automatically reduced to Cu(I) as would be anticipated if sulfhydryl coordination were involved.

Although the thiaether complexes lack the nitrogen- and/or oxygen-donor atoms which are presumed to comprise part of copper's inner coordination sphere in the blue copper proteins, the existence of both the intense 600-nm absorption band and the positive reduction potentials characteristic of blue copper proteins should make them promising models for further studies on the importance of copper-sulfur interactions in these and other copper-containing proteins.

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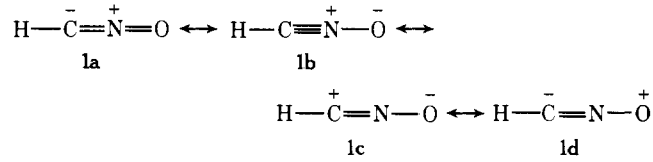
Concerted 1,3-Dipolar Addition of Fulminic Acid to Acetylene and Ethylene. An ab Initio Molecular Orbital Study

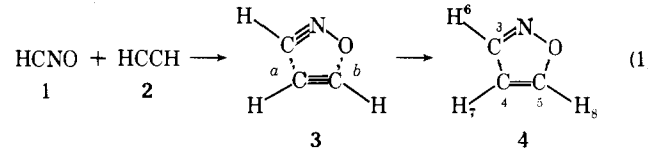
Sir:

The thermal addition of 1,3-dipoles to unsaturated molecules[1] is one of the textbook examples for the application of orbital symmetry rules.[2] In valence bond terms, 1,3-dipoles are written as a superposition of dipolar structures such as **1a–d.** The molecular orbital description of 1,3-dipoles[3] re-



1a **1b** **1c** **1d**

veals that these compounds are isoconjugate with the allyl anion, i.e., possess 4π electrons which are delocalized over three atoms. In some 1,3-dipoles, e.g., in fulminic acid (**1**) and in nitrile oxides RCNO there is a second, orthogonal π system, also containing four electrons. The addition of a 1,3-dipole to an unsaturated molecule (the dipolarophile) can then be formulated as a ($_{\pi}4_s + {}_{\pi}2_s$) process and is therefore expected to proceed in a concerted, stereospecific manner.[2] In accordance with this prediction, the experimental data are generally interpreted as being fully compatible only with a transition state, **3**, in which both new σ bonds *a* and *b* between dipole (e.g., fulminic acid) and dipolarophile (e.g., acetylene **2**) are formed to a similar extent.[3]



1 **2** **3** **4** (1)

There have been numerous attempts to rationalize relative reactivities of different dipoles and dipolarophiles, and the direction of addition in unsymmetrically substituted systems, on the basis of simple molecular orbital theory.[4] These calculations usually involve rather drastic assumptions concerning the geometry of the transition state. Since transition states are not observable experimentally, these assumptions can be checked only theoretically, by more so-

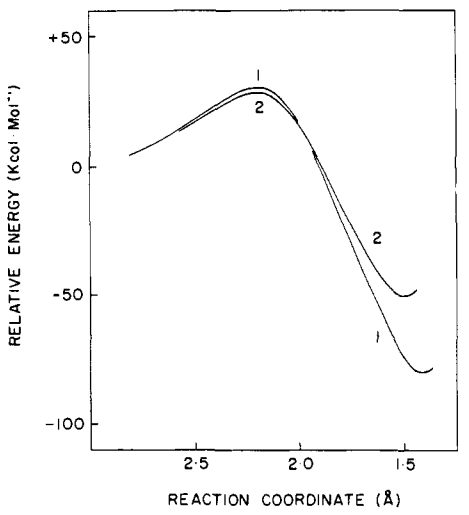


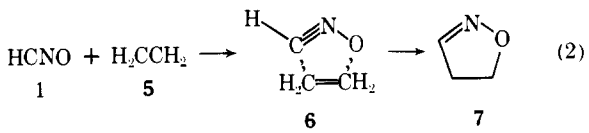
Figure 1. Reaction profiles (4-31G) for the 1,3-dipolar addition of fulminic acid to acetylene (1) and ethylene (2). The reaction coordinate is defined in the text. Each curve is based on energies at eight values of the reaction coordinate.

Table I. Optimum Geometries[a] (STO-3G) for Transition States

Variable	3	6
O–N	1.300	1.308
N–C	1.200	1.205
C3–C4 (= *a*)	2.309	2.353
C4–C5	1.196	1.360
C5–O (= *b*)	2.091	2.047
C3–H6	1.081	1.083
C4–H7	1.066	1.080
C5–H8	1.068	1.084
O–N–C3	136.1	133.5
N–C3–C4	94.5	98.7
C3–C4–C5	101.7	95.1
C4–C5–O	107.8	110.1
N–C3–H6	143.3	140.7
C4–C3–H6	122.1	120.7
C3–C4–H7	95.6	100.7
C5–C4–H7	161.4	122.0
C4–C5–H8	163.7	121.2
O–C5–H8	88.4	90.7

[a] Bond lengths X–Y in Å, bond angles X–Y–Z in degrees. The atom numbering for **6** is the same as for 3; H7 and H8 are the methylene hydrogens above the CCC plane. Data for methylene hydrogens below the plane are not shown.

phisticated computations. It is the purpose of this communication to report the results of an ab initio molecular orbital study of two of the simplest 1,3-dipolar reactions, the addition of fulminic acid to acetylene (eq 1) and to ethylene (eq 2).



(2)

All calculations[6] were carried out in the framework of single determinant SCF-MO theory for closed shells.[7] The reaction path was constructed by varying an assumed reaction coordinate $R = (a + b)/2$ and optimizing the remaining geometrical variables (one of which, $(a - b)/2$, measures the "concertedness" of the reaction) for each value of R. For the optimization, the minimal STO-3G basis set[8] was employed. Points on the reaction path were then recalculated using the split-valence 4-31G basis set[9] to get a more reliable estimate for the energy differences involved.[10]

The resulting energy profile for reaction 1 (see Figure 1) shows a single maximum at $R = 2.20$ Å, corresponding to a transition state **3** with $a = 2.31$ Å and $b = 2.09$ Å. The other variables are shown in Table I. For the product isoxazole **4**, the optimum values are $a = 1.44$ Å and $b = 1.38$ Å. Since the ratio a/b is only slightly higher in the transition state than in the product, the computed reaction path must clearly be labeled as "synchronous". For reaction 2, the transition state is found at about $R = 2.20$ Å with $a = 2.35$ Å and $b = 2.05$ Å. For both reactions, the theoretical results support the concerted mechanism[3] rather than Firestone's diradical hypothesis.[13] The best geometries along the reaction paths were found to have an essentially planar heavy atom skeleton, although this was not an imposed constraint. This is perhaps not very surprising, since the products **4** and **7** are known[14] to have C_s symmetry and the reactions via C_s transition states **3** and **6** are allowed according to the Woodward-Hoffmann selection rules.[2] The calculated (4-31G) exothermicities of reactions 1 and 2 are 80.9 and 50.1 kcal/mol, respectively. The theoretical barrier heights are 30.4 kcal/mol (reaction 1) and 28.8 kcal/mol (reaction 2). No experimental data are available for direct comparison. Although the calculated difference of barrier heights is small, it is consistent with experimental data for substituted compounds.[5]

In the Dewar-Zimmerman terminology,[15] the transition state of a concerted 1,3-dipolar cycloaddition is aromatic. From the five in-plane p orbitals which form one of the orthogonal π systems of **1** and **2**, and the π system of **5**, a cyclic delocalized system (indicated by dotted lines in **3** and **6**) can be constructed, which houses six electrons. The developing (out-of-plane) aromatic π system of **4** should provide additional stabilization for the planar transition state **3**. It has been argued[3] that this additional stabilization, which is absent in transition state **6** (the product, 2-isoxazoline, **7**, not being aromatic), should lead to a higher dipolarophile activity of alkynes compared with alkenes. The experimental observation that alkynes do not add faster than alkenes to 1,3-dipoles has consequently been taken as evidence against a planar transition state for the 1,3-dipolar addition.[3] The ab initio results which predict planar transition states do not support this rationalization but do suggest an alternative rationalization of the experimental results. The Hammond postulate[16] predicts that because of the high exothermicity of reaction 1, the transition state should resemble reactants rather than products. The calculated geometry for **3** with long bonds a and b is consistent with this argument. As a consequence, the overlap of p_π orbitals across the bonds a and b is small, 0.033 and 0.027, respectively, compared with 0.199 and 0.148 in **4** (STO-3G). The resultant conjugative stabilization of **3** is therefore expected to be very small and can be outweighed by other effects.

Further details will be presented in a full report.

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Cyanogen Bromide as a Cleavage Procedure in Solid Phase Peptide Synthesis

Sir:

One disadvantage of the Merrifield solid phase method has been the vigorous conditions used for removal of the completed peptide from the support.[1] Several workers have attempted to solve this problem by attachment of the peptide to the support with a stable linkage which can be made labile at the end of the synthesis to allow facile removal of the product. Despite the attractiveness of this approach, several practical details prevent its routine use, e.g., preparation of the solid support often requires long syntheses,[2,3] or activation of the stable linkage can involve destructive reaction conditions.[4-7]

Cyanogen bromide has several ideal properties for use as a specific agent for the cleavage of peptides and has found wide use in the determination of the amino acid sequence of proteins. Cyanogen bromide was shown to react specifically with methionine, with cleavage of the peptide chain in high yield at the carboxyl end of methionine.[8] Furthermore, the by-products and excess reagent are volatile and readily removed from the product. For these reasons, we propose the use of cyanogen bromide for cleavage of the peptide from the resin at the completion of a solid phase synthesis. In Figure 1 the proposed strategy is outlined in Scheme A, in which the desired peptide is synthesised with methionine added to the C-terminus. At the end of the synthesis, the peptide-resin is treated with cyanogen bromide and the peptide with homoserine at the C-terminus is released from the resin. The homoserine is then removed with carboxypeptidase A yielding the desired peptide. Carboxypeptidase A readily cleaves the peptide bond adjacent to a C-terminal homoserine, but not adjacent to lysine or arginine.[9]

The mild conditions used in this new cleavage procedure should allow the preparation of acid sensitive peptides that cannot be readily obtained by the normal cleavage methods, e.g., HBr-trifluoroacetic acid or anhydrous HF. Also the method has allowed the preparation of protected peptide fragments which can be used in further synthetic operations. Furthermore, the simplicity of the new cleavage procedure allows it to be carried out in the vessel used for the synthesis. The cleavage reaction can now be incorporated into an automated synthetic program.

The cleavage reaction was found to occur more readily if the methionine residue was at least two amino acids removed from the point of attachment to the resin.[10] This result is presumably due to a decrease in steric hindrance at a greater distance from the interior of the resin. Before the desired peptide was synthesized, therefore, the sequence methionylglycylglycine was added to the resin. After the peptide had been assembled using the normal solid phase

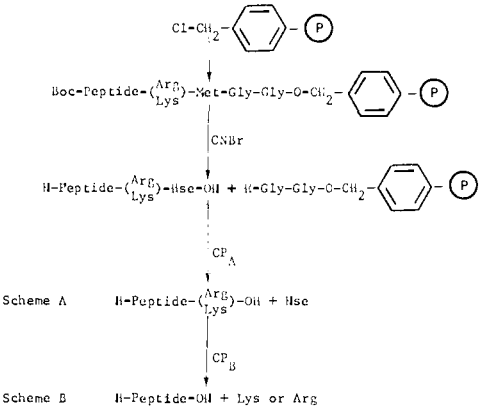

Figure 1. The strategy for the use of cyanogen bromide as a cleavage reagent. Carboxypeptidase A and B are represented by CP_A and CP_B, respectively.

Table I. Yields of Protected Amino Acids and Peptides Prepared by Cyanogen Bromide and Carboxypeptidase A Cleavage

Peptide	CNBr cleavage[a,b] yield mol %	Carboxypeptidase A[b] cleavage yield mol %
Glu(OBzl)-Arg(NO_2)[c,d]	90	83
Arg(NO_2)-Arg(NO_2)[c,e]	65	90
H-Ser(Bzl)-Thr(Bzl)-Ile-Glu(OBzl)-Glu(OBzl)-Arg(NO_2)-OH[c]	55	80
Arg(NO_2)[c,f]	95	98
Ala-Lys(Z)[c,g]	64	95
[a]Glu(OBzl)[c,h]	98	98

[a] The yield is based on the amount of methionine present in the peptide–resin. The peptide–resin was hydrolyzed with HCl–propionic acid at 130° for 2 hr,[12] and the methionine was quantitated by amino acid analysis. [b] The amount of peptide was determined by amino acid analysis.[16] [c] The products had amino acid analysis which were consistent with the structures shown. [d] Mp 208–211° dec. [e] This peptide was identified by conversion to Arg-Arg, mp 287–289° (lit. mp 289–290°).[17] [f] Mp 250–253° (lit. mp 255° dec).[18] [g] The hydrogenation product Ala-Lys was identified by comparison with an authentic sample supplied by Bachem. [h] Mp 171–173° (lit. mp 169–170°).[19]

method,[11] the cleavage was carried out by the addition of cyanogen bromide dissolved in propionic acid. The choice of propionic acid was promoted by its use, in combination with hydrochloric acid, for hydrolysis of peptide-resin samples for amino acid analysis.[12] Propionic acid was found to be superior to acetic acid, and formic acid for the cleavage reaction. A large excess of cyanogen bromide (50-fold) and long reaction times (20-40 hr) were used to ensure a high cleavage yield.[13]

As is shown in Table I, several peptides have been cleaved from the resin in high yield. In each case, the crude products had satisfactory amino acid analyses and showed only one major component on gel filtration, high voltage electrophoresis, and thin layer chromatography (TLC). Treatment of these peptides with carboxypeptidase A released only homoserine, and the desired peptide was then isolated by gel filtration. The resistance to cleavage of the protected forms of Arg and Lys may possibly be explained by retained charge (Arg), and by steric interaction between the hydrophobic pocket of carboxypepidase and the increased bulk of the protected side chain (Lys).

The BOC group was found to be labile in the presence of propionic acid and cyanogen bromide, but side chain protecting groups such as the γ-benzyl ester of glutamic acid, carbobenzoxy group of lysine, and N^G-nitro group of arginine are all retained. Although the cleaved peptide had lost the BOC group, either the NH_2- or -COOH function can