the crystal axes. The differences in the principal directions of the $\tilde{g}$ and $\tilde{A}$ tensors reflect the low symmetry (triclinic) of the center.

The question that arises now is, where is the cobalt ion located? Owing to the method of preparation of the doped compound, it is very likely that the cobalt enters into the core and substitutes for one of the core iron ions. It is worth noting that Co^{2+} ions pseudotetrahedrally coordinated to sulfur ions have already been observed.[6-8] The most convincing evidence for our assumption is the existence of the three other species since the cobalt ion has on the whole four ways of substituting for an iron ion of the core. We reject the possibility that the cobalt ion is located in an interstitial site of the lattice (in particular no proton superhyperfine structure appears, which might be the case since the cobalt ion would then be placed closer to an organic part of the compound). We are thus dealing with a mixed-cluster $CoFe_3S_4$ which must be in the +2 oxidation state in order to preserve the electrical neutrality of the compound.

It is worthwhile to recapitulate our relevant observations: the resonance occurs in an $S = {}^1/_2$ ground state, the spin-lattice relaxation time is short, and the g values are less than 2 with an average g of 1.943. For a $CoFe_3S_4$ heteronuclear system, we cannot expect to see an EPR spectrum similar to that given by a cobalt ion pseudotetrahedrally coordinated to sulfur ions, because the cobalt is in the vicinity of the three iron ions. Indeed, the g values and the saturation behavior of the center are very different from all the previously reported data for cobalt complexes.[9] All the relevant observations we have made are consistent with a polynuclear system $(CoFe_3S_4)^{2+}$ where the metal ions are antiferromagnetically exchange-coupled to yield an $S = {}^1/_2$ ground state as in the $(Fe_4S_4)^+$ parent core.[10] The latter is encountered in reduced 4Fe-4S ferredoxins and their synthetic model compounds and we have succeeded in creating it also in the $[NBu_4]_2[Fe_4S_4(SPh)_4]$ compound itself by γ irradiation.[11] In that case, three high-spin ferrous ions ($3d^6$, $S = 2$) and one high-spin ferric ion ($3d^5$, $S = {}^5/_2$) couple together to give an $S = {}^1/_2$ ground state. For the $(CoFe_3S_4)^{2+}$ core, the $S = {}^1/_2$ ground state plausibly results from one of the two combinations of formal integral oxidation states $Co^{II} + Fe^{II} + 2Fe^{III}$ or $Co^{III} + 2Fe^{II} + Fe^{III}$ where $S = {}^3/_2$ for high-spin Co^{II} ($3d^7$) and $S = 2$ for high-spin Co^{III} ($3d^6$). But as the $(CoFe_3S_4)^{2+}$ core must be, like the $(Fe_4S_4)^+$ core, a mixed-valence system, the distinction between these two configurations is not relevant.

It would have been interesting to find tensorial pseudosymmetries associated with the structure of the Fe_4S_4 cluster to localize the cobalt ion in the core. A comparison of the principal directions of the tensors with atomic directions calculated from the X-ray crystallographic data for the pure compound do not show any clear relation either with one of the four FeS_4 coordination units or with the overall pseudosymmetry of the pure cluster. This is not surprising because the replacement of an iron ion by a cobalt ion introduces some extra distortions of the core. More information about the problem may come from the analysis of the relative orientations of the tensors of the four species. Work is in progress to attempt to extract this information.

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(6) Davison, A.; Switkes, E. S. *Inorg. Chem.* **1971**, *10*, 837.

(7) Holah, D. G.; Coucouvanis, D. *J. Am. Chem. Soc.* **1975**, *97*, 6917.

(8) Lane, R. W.; Ibers, J. A.; Frankel, R. B.; Papaefthymiou, G. C.; Holm, R. H. *J. Am. Chem. Soc.* **1977**, *99*, 84.

(9) See, e.g.: (a) Abragam, A.; Bleaney, B. *Electron Paramagnetic Resonance of Transition Ions*; Clarendon Press: Oxford, 1970. (b) Banci, L.; Bencini, A.; Benelli, C.; Gatteschi, D.; Zanchini, C. *Struct. Bonding (Berlin)* **1982**, *52*, 39. (c) Goodman, B. A.; Raynor, J. B. *Adv. Inorg. Chem. Radiochem.* **1970**, *13*, 135.

(10) See, e.g.: (a) Dickson, D. P. E.; Johnson, C. E.; Thompson, C. L.; Cammack, R.; Evans, M. C. W.; Hall, D. O.; Rao, K. K.; Weser, U. *J. Phys. (Les Ulis, Fr.)* **1974**, *35*, C6, 343. (b) Cammack, R.; Dickson, D. P. E.; Johnson, C. E. In *Iron-Sulfur Proteins*; Lovenberg, W., Ed.; Academic Press: New York, 1977; Vol. I, pp 283-300.

(11) Gloux, J.; Gloux, P.; Lamotte, B.; Rius, G. *Phys. Rev. Lett.* **1985**, *54*, 599.

(12) **Note Added in Proof**: During reviewing of this paper, an article was published by I. Moura et al. (*J. Am. Chem. Soc.* **1986**, *108*, 349) giving evidence for a $CoFe_3S_4$ cluster in a protein. The single-crystal data reported here agree well with their results.

Free-Radical-Induced Elimination of H_2S from Dithiothreitol. A Chain Reaction

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Dithiothreitol (DTT) is used in many biochemical studies because it is a powerful reducing agent, like many thiols, but it can be handeled with some advantage over other thiols, because it is not as readily oxidized by air.[1] In radiation biological and flanking radiation chemical studies it has been used quite often and its free-radical chemistry has found considerable attention.[2-6] It was originally assumed that OH radicals abstract an H atom from one of the thiol groups (cf. reaction 3) but it was reported later that OH radicals cause considerable H_2S formation and it has been concluded that, depending on pH, as much as 25-40% of the OH radicals react by abstracting carbon-bound H atoms thereby forming **4**.[5] In the present paper we show that the H_2S formation is entirely due to a chain reaction and OH attack at carbon is negligible.

In the radiolysis of N_2O-saturated aqueous DTT solutions the solvated electrons (e^-_{aq}) from reaction 1 are converted into OH radicals (reaction 2). Thus the system contains mainly OH

$$H_2O \xrightarrow[\text{radiation}]{\text{ionizing}} {}^{\bullet}OH,\ e^-_{aq},\ H^{\bullet},\ H^+,\ OH^-,\ H_2O_2,\ H_2 \qquad (1)$$

$$e^-_{aq} + N_2O + H_2O \rightarrow {}^{\bullet}OH + OH^- + N_2 \qquad (2)$$

radicals (90%g, $G(OH) = 5.4$ molecules $(100\ eV)^{-1}$) and only a small amount of H atoms (10%, $G(H) = 0.55$). These primary water radicals react with the substrate (DTT) by H-abstraction. The H atom is also known to undergo displacement reactions thereby forming H_2S (for a review see ref 7). In the present system the latter reaction only contributes about 15%.[8]

It can be seen from Table I that at pH 4 and at a given dose rate $G(H_2S)$ is independent of DTT concentration but that at a given DDT concentration $G(H_2S)$ strongly increases with decreasing dose rate. At low dose rates $G(H_2S)$ exceeds $G(OH + H)$, hence a chain reaction must prevail. If in a chain reaction a propagation process, kinetically of first order in radicals, competes with the bimolecular decay of the radicals, the yield of the chain product must increase linearly with the inverse of the square root of the steady-state concentrations of the radicals (i.e., the dose rate). Such a plot is shown in Figure 1. Extrapolation to infinite dose rate ($(\text{dose rate})^{-1/2} \rightarrow 0$) shows that practically all H_2S originates from the chain reaction. The small intercept ($G(H_2S) = 0.2$) can be accounted for by the reaction of the H atoms. We therefore conclude that OH radicals as such do not cause H_2S formation.

We propose that H_2S formation is due to an intramolecular H-abstraction of the primary thiyl radical **1** (reaction 8) followed by either H_2S elimination (reaction 10) or $HS^{\bullet}$ elimination (reaction 11). H-Abstraction of carbon-bound H atoms by thiyl radicals is not without precedence[9], and reaction 10 finds its

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(1) Cleland, W. W. *Biochemistry* **1964**, *3*, 480.

(2) Redpath, J. L. *Radiat. Res.* **1973**, *54*, 364.

(3) Chan, P. C.; Bielski, B. H. J. *J. Am. Chem. Soc.* **1973**, *95*, 5504.

(4) Lal, M. *Radiat. Phys. Chem.* **1980**, *15*, 649.

(5) Elliot, A. J.; Sopchyshyn, F. C. *Radiat. Phys. Chem.* **1982**, *19*, 417.

(6) Elliot, A. J.; Simsons, A. S.; Sopchyshyn, F. C. *Radiat. Phys. Chem.* **1984**, *23*, 377.

(7) von Sonntag, C.; Schuchmann, H.-P. In *The Chemistry of Functional Groups Supplement E*; Patai, S., Ed.; Wiley: New York, 1980; p 971.

(8) In Ar-saturated solutions at pH 1.0, $G(H_2) = 3.3$ was found for 10^{-3} M DTT solutions while the corresponding value for isopropyl alcohol solutions was 3.84. $G(H^{\bullet})$ in these systems is 3.4 and $G(H_2)_{mol} \approx 0.45$. Hence in the DTT system the H atom yields H_2 with an efficiency of ca. 85%, the remainder being expected to form H_2S.

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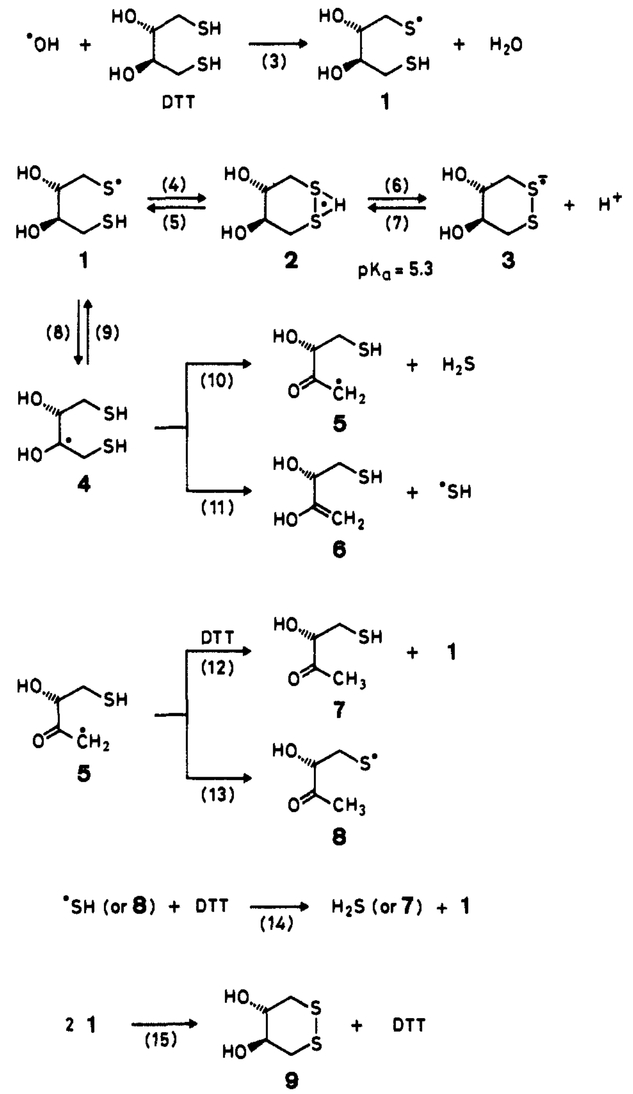

analogy in carbohydrate free-radical chemistry where the corresponding H_2O elimination is the most dominating process (for a review see ref 10). The chain continues when the carbon-centered radical **5** is reduced by DTT (reaction 12) or intramolecularly (reactions 13 and 14, k_9 is expected[11] to be around 2×10^7 M^{-1} s^{-1}). Reduction of carbon-centered radicals by thiols is generally quite fast,[7] although radicals of the type $R\text{-}CO\text{-}CH_2^{\bullet}$ tend to react more slowly.[12] The products of this chain reaction are H_2S and **7**.

An alternative to reaction 10 is the homolytic $HS^{\bullet}$ elimination (reaction 11). Such reactions are known to be rapid.[13] The product of reaction 11 is **6**, the enol form of **7** (**6** → **7**). In this alternative mechanism the chain continues when $^{\bullet}SH$ reacts with DTT (reaction 14).

Evidence that the second proposed chain product **7** is indeed formed comes from the fact that periodate oxidation yields acetic acid in amounts equal to the H_2S yield. Termination of the chain reaction is mainly by the disproportionation of **1** which yields **9** (oxidized DTT) and DTT (reaction 9, $2k_{15} = 1.7 \times 10^9$ M^{-1} s^{-1} at pH 4,[6] $G(\mathbf{9}) \approx {}^1/_2\, G({}^{\bullet}OH + H^{\bullet}) \approx 3.5 \pm 0.5$.[14]

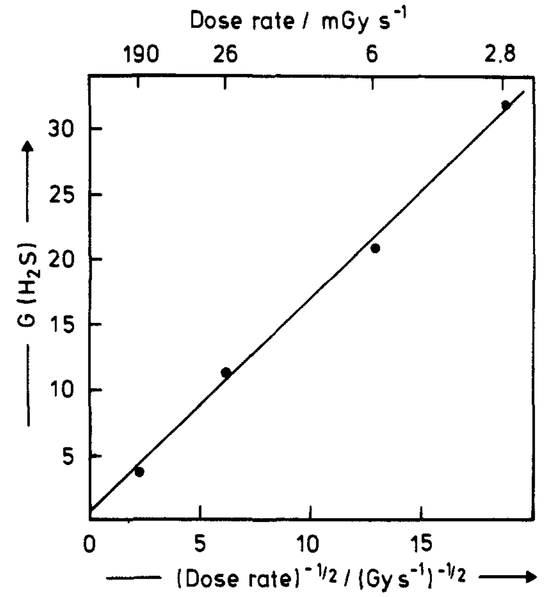

Figure 1. $G(H_2S)$ from the radiolysis of aqueous solutions of dithiothreitol (10^{-3} M) at pH 4 plotted vs. the inverse square root of the dose rate.

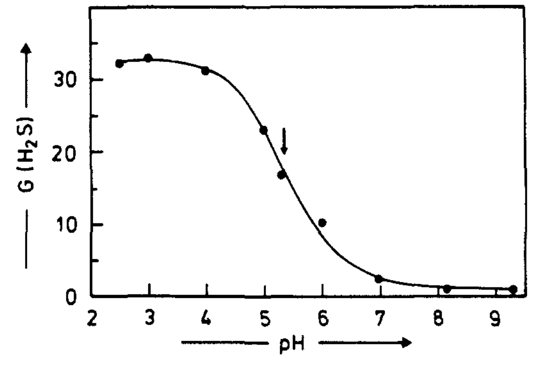

Figure 2. $G(H_2S)$ from the radiolysis of aqueous solutions of dithiothreitol (10^{-3} M) plotted vs. the pH. Dose rate 2.8×10^{-3} Gy s^{-1}.

It is well documented that the DTT radical has a pK_a value about 5.3 (equilibrium (6)/(7)).[3] Hence it is of interest to know whether it is the radical **1** or the disulfide radical anion **3** which propagates the chain. In figure 2 the pH dependence of $G(H_2S)$ is shown. It can be seen that at pH 9, where the disulfide radical anion **3** predominates, H_2S formation drops to a low value which is independent of dose rate (data not shown). One recalls that some H_2S is always formed due to the action of the H atoms. The inflection point in Figure 2 is at pH 5.3 (see arrow), this coincides with the pK_a value of the radical and we conclude that the chain reaction is only possible with the neutral radical **2**. The independence of the chain length on DTT concentration (Table I) is an indication that this reaction must proceed intramolecularly rather than intermolecularly. In agreement with this interpretation is our observation that 2-mercaptoethanol does not yield any H_2S under conditions where DTT afforded H_2S with a G value of 32. 2-Mercaptoethanol has the same structural elements as DTT, but being only "one-half" of the DDT molecule an intramolecular H-abstraction as depicted by reaction 8 is not possible. From the known rate constant of the bimolecular decay of the DTT radicals

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[9] Huyser, E. S.; Kellogg, R. M. *J. Org. Chem.* **1966**, *31*, 3366. Morita, M.; Sasai, K.; Tajima, M.; Fujimaki, M. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 2257. Thiyl radicals also undergo H-abstraction from *cis*- and *trans*-dimethyltetrahydrofuran, a reaction which leads to cis ⇄ trans isomerisation via a chain reaction: Akhlaq, M. S.; Schuchmann, H.-P.; von Sonntag, C., unpublished results.

[10] von Sonntag, C. *Adv. Carbohydr. Chem. Biochem.* **1980**, *37*, 7.

[11] At pH 6 the radical $HOCH_2CH_2S^{\bullet}$ reacts with DTT by H-abstraction ($k = 2 \times 10^7$ M^{-1} s^{-1}) as monitored by the formation of the DTT radical anion **3** at 400 nm (2-mercaptoethanol 5×10^{-2} M, DTT 10^{-3} M, N_2O saturated, phosphate buffered).

[12] Tamba, M.; Quintiliani, M. *Radiat. Phys. Chem.* **1984**, *23*, 259.

[13] Collin, G. J.; Perrin, P. M.; Garneau, F. X. *Can. J. Chem.* **1974**, *52*, 2337.

[14] H_2S was determined by passing for 1 h a slow stream of Ar through the acid solution collecting H_2S in a phosphate-buffered (pH 8.5) solution containing 5,5′-dithiobis(2-nitrobenzoic acid). The 2-nitro-5-sulfidobenzoic acid was measured (ϵ(412 nm) 13 600 M^{-1} cm^{-1}) taking into account that 1 mol of H_2S yields 2 mol of the reagent thiolate. Acetic acid was determined by ion chromatography (Dionex 2010i HPIC-AS 4, eluent 5×10^{-4} M $NaHCO_3$) and also by gas chromatography (glas capillary column coated with Carbowax 20 M which has been cross-linked by γ-ray to avoid destruction by water). Oxidized DTT **9** was measured photometrically at 283 nm where it has an absorption maximum (ϵ(283) 270 M^{-1} cm^{-1}).

Table I. $G(H_2S)$ in the γ-Radiolysis of Dithiothreitol at pH 4 and 20 ± 1 °C at Different Dose Rates[a]

[DTT], M	1.9×10^{-1}, Gy s^{-1}	2.6×10^{-2}, Gy s^{-1}	2.8×10^{-3}, Gy s^{-1}
5×10^{-4}		12.2	31.6
1×10^{-3}	3.6	11.2	32.3
3×10^{-3}		11.3	31.4
6×10^{-3}		11.6	32.3
8×10^{-3}		11.7	31.1
1×10^{-2}		10.8	31.0

[a] At the highest dose rate the dose did not exceed 55 Gy, at the lowest dose rate 10 Gy.

($2k_{15} = 1.7 \times 10^9$ M^{-1} s^{-1} at pH 4)[6] and the *G* values given above, a rate constant of k(propagation) = 8.5 s^{-1} can be calculated for the rate-limiting step. The present results do not allow us to assign this to one distinct elementary step: Eliimination of H_2S or $HS^{\bullet}$ (reactions 10 or 11) must compete with the reverse of reaction 8 (reaction 9; $k_9 \geq 10^8$ s^{-1}).[15] Thus the H_2S ($HS^{\bullet}$) elimination occurs from the quasi-equilibrium (8)/(9) which itself is connected with another quasi-equilibrium (equilibrium (4)/(5)), the equilibrium between the free thiyl radical **1** and its three-electron-bonded ring-closed[16] isomer **2**. In the equilibrium between the free thiyl radical **1** and its three-electron-bonded ring-closed[16] isomer **2** (equilibrium (14)/(15)),[17] it is to be expected that it is the free thiyl radical **1** rather than the ring-closed isomer **2** which undergoes the intramolecular H-abstraction reaction.

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(15) α-Hydroxyalkyl radicals react with DTT in their bimolecular reaction with $k \geq 10^8$ M^{-1} s^{-1} and we estimate that the intramolecular rate constant k_9 should not be lower than 10^8 s^{-1}.

(16) For three-electron bonded systems in sulfur free-radical chemistry see ref 7 and: Asmus, K.-D. *Acc. Chem. Res.* **1979**, *12*, 436. In contrast to the thiyl radical derived from 2-mercaptoethanol ($HOCH_2CH_2S^{\bullet}$) which does not readily reduce tetranitromethane, the DTT radical at pH 4 is rapidly ($k = 2.5 \times 10^8$ M^{-1} s^{-1}) oxidized which is an indication that the reducing three-electron-bonded species **2** must predominate.

(17) The rate constant k_{14} has been measured by monitoring the buildup of 2 (ϵ(380 nm) = 430 ± 20) at relatively high DTT concentration (10^{-3} M) and at pH 4 and was found to be 1.5×10^6 s^{-1}. Under these conditions reaction 1 is much faster ($k_1[\mathrm{DTT}] = 2 \times 10^7$ s^{-1}). A value for k_5 is not yet available, but it is reasonable to assume that it is much lower than k_4 (cf. equilibria between disulphides and thiyl radicals: Bonifacic, M.; Asmus, K.-D. *J. Phys. Chem.* **1984**, *88*, 6286.) Radical **2** has been presented as a three-electron-bonded species, but it cannot yet be excluded that the H atom resides on one of the sulfur atoms.

A Doubly Pyramidalized Olefin: Matrix Isolation Spectroscopy of Tricyclo[3.3.2.0^{3,7}]dec-3(7)-ene

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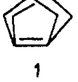
Strong distortions of the C=C double bond from planarity should lead to olefins with unusual properties. Considerable attention has been paid to twisting distortions[2] (e.g., adamantene[3]), relatively less to pure pyramidalization.[4] Tricyclo[3.3.2.0^{3,7}]dec-3(7)-ene (**1**)[5] and its benzo derivative **2**[6] have both been generated pyrolytically and trapped chemically. The recent preparation of β-lactone **3** as a precursor for **1**[5] has now permitted the direct observation of this olefin.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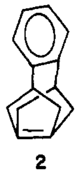
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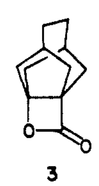
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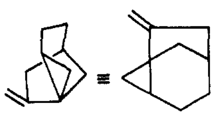
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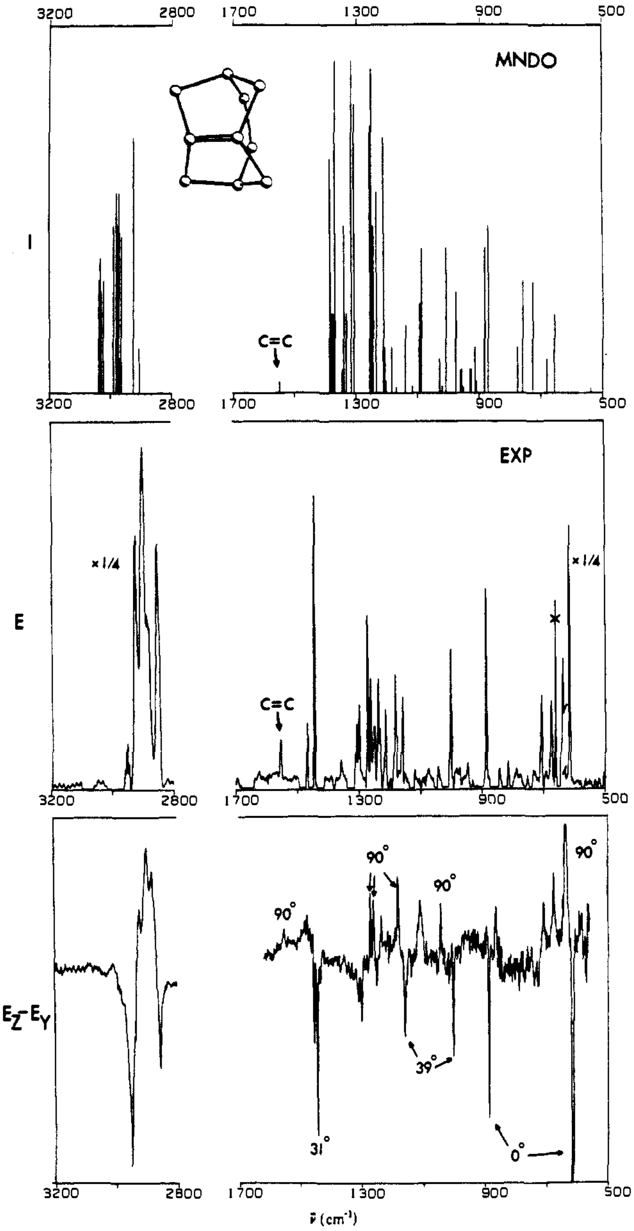

Figure 1. IR spectra of **1**: (top) calculated (MNDO, correction factor 0.915); (center and bottom) measured in argon matrix at 10 K (CO_2 absorption crossed out). The center spectrum was obtained as a difference of spectra recorded before and after photodestruction of **1** at 248 nm. The bottom spectrum is a difference of spectra polarized parallel (E_Z) and perpendicular (E_Y) to the electric vector direction of the polarized 248-nm laser light after partial photodestruction of **1** (see text).

Flow pyrolysis (450–650 °C) of **3**, either neat or diluted with excess argon, and deposition onto a 10 K window yielded CO_2 and a material to which we assign structure **1**. Also detected were

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(1) (a) University of Utah. (b) University of Washington.

(2) Greenberg, A.; Liebman, J. F. *Strained Organic Molecules*; Academic Press: New York, 1978.

(3) Conlin, R. T.; Miller, R. D.; Michl, J. *J. Am. Chem. Soc.* **1979**, *101*, 7637.

(4) Szeimies, G. In *Reactive Intermediates*; Abramovitch, R. A., Ed. Plenum Press: New York, 1983; Vol. 3, p 299. Watson, W. H.; Galloy, J.; Bartlett, P. D.; Roof, A. A. M. *J. Am. Chem. Soc.* **1981**, *103*, 2022. See also: Böhm, M. C.; Carr, R. V. C.; Gleiter, R.; Paquette, L. A. *J. Am. Chem. Soc.* **1980**, *102*, 7218.

(5) Renzoni, G. E.; Yin, T.-K.; Miyake, F.; Borden, W. T. *Tetrahedron* **1986**, *42*, 1581.

(6) Greenhouse, R.; Borden, W. T.; Hirotsu, K.; Clardy, J. *J. Am. Chem. Soc.* **1977**, *99*, 1664.

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