cross-linked DNA.[9] The desired bands were cut from the gel, isolated by electroelution, treated with piperidine (90 °C, 30 min), and resubmitted to PAGE analysis. Cross-linked material 5′-end-labeled on the shorter strand afforded a major cut at the guanine at position 12 (G_{12}) when compared to a Maxam and Gilbert G-specific lane.[10] Likewise, cross-linked material 5′-end-labeled on the longer strand afforded cleavage at the A_{17} residue, corresponding to a three base pair bisalkylation between G and A residues in the sequence

5′-GCT-3′
3′-CGA-5′

Conversion of isolated cross-link to a major cleavage site on each strand suggests that the covalent bond formation occurs at the piperidine-labile locations.[11,12]

Inosine (I) and the modified nucleoside 7-deazaguanine (Z) were synthetically incorporated to evaluate potential base alkylation sites. Substitution of the G_{12} residue involved in the G-to-A cross-link in **1** with 7-deazaguanine **2** resulted in no cross-link formation. No piperidine-sensitive scission was detected at this base even in single-stranded experiments. Cross-links were generated upon replacement of G_{18} with 7-deazaguanine **3**, but were not observed with either T (**6**) or A (**7**) substitution at this base. Substitution of inosine for either the G_{12} or G_{18} residue afforded cross-links (**4**, **5**). However, yields were lower for **4**, consistent with the decreased nucleophilicity of the N7 atom of inosine.[13] Substitution of the A_{17} residue with G afforded G-to-G bisalkylations in the sequences

5′-GCC-3′ 3′-CGG-5′	5′-GAC-3′ 3′-CTG-5′
8	**9**

Attempts to induce G-to-G cross-links involving adjacent base pairs failed (**10**).[14]

All G residues in either *single* strand of **1** are alkylated by carzinophilin at various levels of efficiency, whereas all A residues (including A_{17}) are inert (data not shown). Given the differences in nucleophilicity of N7 of A and G, these results support a model for cross-link formation in which the first alkylation at G_{12} directs the second alkylation at A_{17} (Figure 1). The reaction at the usually inert A_{17} residue is thus template directed, and the kinetics of alkylation are governed by the high concentration of electrophile at the second alkylation site. For **8** and **9**, G-to-G cross-links could lead to a mixture of diastereomeric products. Within this framework, involvement of N7 of G_{12} is supported by the results obtained with the modified duplexes **2–5**.[15] The greater propensity for depurination at the A_{17} residue (vs G_{12}) after cross-link formation in **1** is consistent with the lower stability of N7 alkylations at this base.[16] Finally, formation of cross-links in **1**, **3–5**, **8**, and **9** at purine residues located in the 5′ direction of the three base pair sequence leads us to the preliminary conclusion that this novel natural product reacts in the major groove. Further characterization of the drug–DNA complex will be determined when suitable amounts of material become available.

Acknowledgment. We wish to thank the National Science Foundation (CHE-8858059) and the Office of Naval Research (N00014-88-K-0544) for financial support of this work. We also thank Dr. T. Fukuyama and Dr. H. Nakano (Kyowa Hakko Kogyo) for procuring a sample of carzinophilin, Dr. T. Doyle (Bristol-Myers-Squibb) for a sample of azinomycin B, and Professor David S. Sigman for helpful discussions. R.W.A. is an Alfred P. Sloan and Ely Lilly Fellow.

Supplementary Material Available: Experimental details and polyacrylamide gels showing cross-linking experiments for DNAs **1–10** (7 pages). Ordering information is given on any current masthead page.

---

[9] DNA–CZ complexes were treated with 0.2 N NaOH in order to prevent depurination of cross-links when submitted to PAGE analysis. Cross-linked DNA which had not been exposed to base (obtained in low yield) could be electroeluted from gel and converted to material of identical migration to that which had been treated with NaOH prior to gel analysis. Stabilization of N7 alkylations under similar conditions has been previously reported: Kohn, K. W.; Spears, C. L. *Biochim. Biophys. Acta* **1967**, *145*, 743. For a recent demonstration of nitrogen mustard cross-link stabilization using base to elucidate the novel 5′-GXC-3′ sequence selectivity of mechloroethylenamine, see: Ojwang, J. O.; Grueneberg, D. A.; Loechler, E. L. *Cancer Res.* **1989**, 6529.

[10] Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 499.

[11] Alkylations (<20%) were also detected by piperidine treatment of isolated cross-linked DNA at the G residues of the second potential cross-link site

5′-CG-3′
3′-GC-5′

Non-piperidine-labile alkylations could be difficult to detect using the base treatment strategy. However, PAGE analysis of a 3′-end-labeled isolated cross-link suggests no molecular weight change of fragments obtained upon piperidine treatment.

[12] Specific base (G and A) and groove (major) locations of the cross-links were independently confirmed by subjecting the isolated cross-linked duplexes to PAGE analysis after treatment with the chemical nuclease reagent (1,10-phenanthroline)copper (OP-Cu): Sigman, D. S.; Graham, D. R.; DAurora, V.; Stern, A. M. *J. Biol. Chem.* **1979**, *254*, 12269. Ferrous EDTA has also been used to confirm cross-link location in other systems: Weidner, M. F.; Millard, J. T.; Hopkins, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 9270. Millard, J. T.; Weidner, M. F.; Raucher, S.; Hopkins, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 3637.

[13] Belikova, A. M.; Grineva, N. I.; Karpova, G. G. *Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk* **1972**, *4*, 110. Suzuki, Y. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 2469.

[14] Reaction of carzinophilin with **10** afforded only G monoalkylations, precluding adjacent base pair cross-links. However, formation of cross-links in **8** could involve both adjacent and three base pair G residues.

[15] The absence of alkylation or cross-links for **2** can be interpreted as resulting from a modification in the electron density of the N3 atom as a consequence of replacement of N7 with carbon, precluding alkylation at the N3 position. The N7 nitrogen could also be crucial to binding or activation of the drug–DNA complex.

[16] Drinkwater, N. R.; Miller, E. C.; Miller, J. A. *Biochemistry* **1980**, *19*, 5087.

A Variable-Temperature Study of the Quenching of Benzophenone Triplet by Oxygen: Involvement of Exciplexes

Andrew J. McLean and Michael A. J. Rodgers*

Center for Photochemical Sciences
Bowling Green State University
Bowling Green, Ohio 43402

Received December 27, 1991

Throughout the last three decades, the chemistry of the high-energy triplet states of aromatic ketones has occupied the attention of photochemists.[1] Benzophenone (Bp) is typical of these species, and its relatively high T_1 energy (69 kcal/mol) causes it to be used as a photosensitizer for other molecular T_1 states. With energy acceptors such as aromatic hydrocarbons, it is generally agreed that the energy transfer proceeds with 100% efficiency. This is decidedly not the case, however, when molecular oxygen is the energy acceptor;[2] the fraction of triplet Bp(T_1) quenching processes that lead to singlet oxygen formation (S_Δ) is close to 0.3.[2c] This nonunity value of S_Δ is not confined to the case of benzophenone triplets; very few singlet oxygen sensitizers show $S_\Delta = 1$.[3] This variance possibly arises in part from the variety of the electron

---

[1] Many instances can be found in the following: Turro, N. J. *Modern Molecular Photochemistry*; Benjamin/Cummings: Menlo Park, CA, 1978.

[2] (a) Gorman, A. A.; Lovering, G.; Rodgers, M. A. J. *J. Am. Chem. Soc.* **1978**, *100*, 4527. (b) Garner, A.; Wilkinson, F. *Singlet Oxygen*; Ranby, B., Rabek, J. F., Eds.; Wiley: New York, 1978; p 48. (c) Gorman, A. A.; Hamblett, I.; Rodgers, M. A. J. *J. Am. Chem. Soc.* **1984**, *106*, 4679.

[3] For comprehensive tables of S_Δ values, see: (a) Saltiel, J.; Atwater, B. W. *Advances in Photochemistry*; Volman, D. H., Hammond, G. S., Gollnick, K., Eds.; Wiley: New York, 1988; Vol. 14, p 1. (b) Gorman, A. A.; Rodgers, M. A. J. *Handbook of Organic Photochemistry*; Scaiano, J. C., Ed.; CRC Press: Boca Raton, FL, 1989; Vol. II, p 229.

spin interactions during the encounter, viz., eqs 1–3.

$$Bp(T_1) + O_2(^3\Sigma_g^-) \rightleftharpoons {}^1(Bp{:}O_2)^* \rightarrow Bp(S_0) + O_2(^1\Delta_g) \quad (1)$$

$$\rightleftharpoons {}^3(Bp{:}O_2)^* \rightarrow Bp(S_0) + O_2(^3\Sigma_g^-) \quad (2)$$

$$\rightleftharpoons {}^5(Bp{:}O_2)^* \quad (3)$$

That $S_\Delta = 0.3$ allows the conclusion that reaction 1 is not alone in $Bp(T_1)$ deactivation despite the bimolecular rate constant for quenching by $O_2(^3\Sigma_g^-)$ ($k^T_{ox} = (2\text{–}3) \times 10^9$ M^{-1} s^{-1})[2a,4] being only one-ninth of the diffusion-controlled value. Mechanisms involving charge-transfer intermediates[4] and unstable chemical products (trioxetanes)[5] have been invoked to rationalize these apparently contradictory observations.

An interesting possibility which has been discussed but not demonstrated is the intermediacy of an exciplex state between sensitizer triplet and oxygen.[3a,4a] The above scheme allows the putative existence of exciplexes of singlet, triplet, and quintet multiplicities, and intersystem crossing between channels 1 and 2 could lead to a range of S_Δ values and k^T_{ox} of approximately $^1/_9 k_d$. However, to our knowledge, definitive evidence for exciplex involvement has not been presented heretofore. In this communication we present such evidence.

We have measured the temperature dependence of k^T_{ox} for $Bp(T_1)$ in toluene solution using laser flash photolysis[6] (355-nm excitation). The decay of Bp triplet was monitored at 530 nm as a function of O_2 concentration[9] and temperature. Under argon saturation the decay profiles showed mixed first- and second-order kinetics since $Bp(T_1)$ decays in part by hydrogen abstraction, and the product ketyl radical also absorbs at 530 nm and decays by second-order kinetics. First-order decay at 530 nm was observed in the presence of oxygen. Plots of the observed first-order rate constants (k') versus oxygen concentration at selected temperatures are shown in Figure 1. Using the values of k^T_{ox} so determined, the plot in Figure 2 was obtained.

The following points can be inferred from Figure 2.

(a) The largely non-Arrhenius form of the plot is a clear indication that quenching of $Bp(T_1)$ is not an elementary reaction. The positive slope at high temperature is consistent with an intermediate with a finite binding energy, i.e., an exciplex.[11]

(b) At temperatures lower than −60 °C, the rate of oxygen quenching is determined by diffusional parameters; the activation energy corresponds to that of viscous flow of the solvent,[12] and exciplex formation is irreversible. Assuming $k_d = 3 \times 10^{10}$ M^{-1} s^{-1} at room temperature and $E_d = 2.6$ kcal/mol, a diffusion-control pre-exponential factor of 2.4×10^{12} M^{-1} s^{-1} can be derived. The observed value of 7.8×10^{11} M^{-1} s^{-1} is approximately one-third of this and may reflect quenching via both singlet and triplet exciplexes that are approaching a spin-statistical distribution upon reactant encounters.

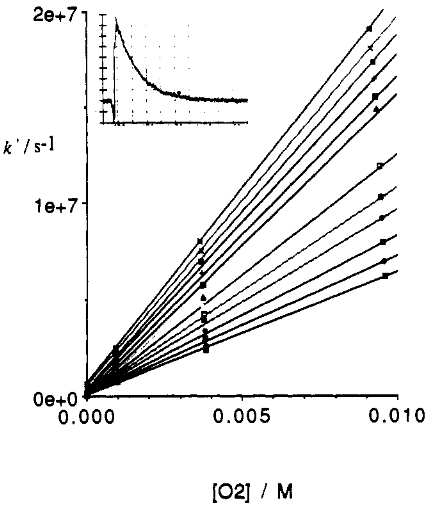

Figure 1. Plots of k' vs oxygen concentration at different temperatures. The slopes increase with increasing temperature over the range 185 → 238 K. Inset: $Bp(T_1)$ decay profile at 200 K monitored at 530 nm, $[O_2]$ = 3.83 mM; horizontal, 200 ns/division, vertical, 0.0013 absorbance unit/division.

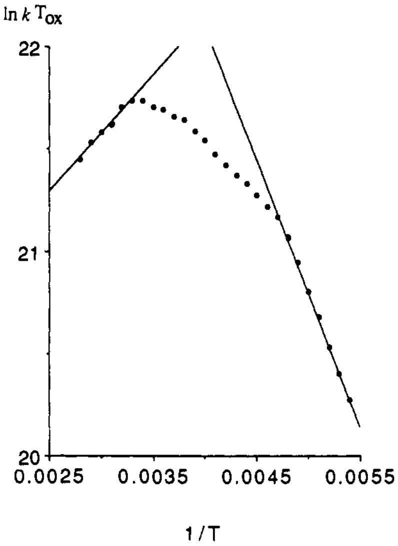

Figure 2. Arrhenius plot for triplet benzophenone quenching by oxygen.

(c) At room temperature, k^T_{ox} (2.7×10^9 M^{-1} s^{-1}) reflects a balance between diffusion and pre-equilibrium control that favors the pre-equilibrium case, i.e., where product formation becomes rate limiting due to unfavorable entropic factors, and exciplex formation is rapid and reversible.

(d) It should be noted that both singlet and triplet exciplexes can be contributing to the overall effects observed. The observation of a low-temperature limiting slope corresponding to viscous flow of the solvent and a pre-exponential factor of around one-third that of diffusion indicates that both exciplexes are formed irreversibly and that S_Δ has to be less than unity in this region. With increasing temperature the singlet and triplet exciplexes may reach pre-equilibrium at different temperatures, although the observation

---

(4) (a) Garner, A.; Wilkinson, F. *Chem. Phys. Lett.* **1977**, *45*, 432. (b) McLean, A. J.; Truscott, T. G. *J. Chem. Soc., Faraday Trans.* **1990**, *86*, 2671.

(5) Gorman, A. A.; Rodgers, M. A. J. *J. Am. Chem. Soc.* **1986**, *108*, 5074.

(6) The computer-assisted laser flash photolysis–kinetic spectrophotometry instrument has been described.[7] A variable-temperature unit similar to that of Wardman[8] was constructed.

(7) Firey, P. A.; Ford, W. E.; Sounik, J. R.; Kenney, M. E.; Rodgers, M. A. J. *J. Am. Chem. Soc.* **1988**, *110*, 7626.

(8) Wardman, P. J. *J. Phys. E* **1972**, *5*, 17. The variable-temperature cell consists of a quartz cell held against an aluminum heat sink. The temperature was controlled by balancing the flow rate of precooled N_2 gas through the block and the rate of heating by a pair of cartridge heaters sunk into the aluminum block.

(9) The oxygen concentrations were calculated assuming a concentration of 8.55 mM in oxygen-saturated toluene at room temperature[10a] and assuming that Henry's Law holds for the 10, 40, and 100% oxygen concentration values employed. All solutions were saturated with the O_2/N_2 mixture for at least 0.5 h at room temperature prior to the experiment. The samples were introduced into the variable-temperature cell by gas pressure, the pressure was then reduced, and the solution temperature was changed by balancing the flow of precooled N_2 and the rate of heating by the cartridge heaters. The variation in concentration due to solvent density changes was determined using 1.07×10^{-3} K^{-1} as the solvent expansion coefficient.[10b] In ref 10c, an expression is given for the variation of O_2 solubility in toluene over the temperature range 313–293 K. Using this expression to determine O_2 concentration produced no significant differences in the temperature dependence of k^T_{ox}. Under the conditions used in this study, where equilibrium between the vapor and solution phases only exists over the initial saturation procedure, only the correction for solvent density changes was employed in calculating O_2 concentration.

(10) (a) Emmerich, W.; Battino, R. *Chem. Rev.* **1973**, *73*, 1. (b) Scaiano, J. C. In ref 3b, p 343. (c) *Oxygen and Ozone*; Battino, R., Ed.; Solubility Data Series R, Vol. 7; Pergamon: Oxford, 1981; p 257.

(11) (a) Giering, L.; Berger, M.; Steel, C. *J. Am. Chem. Soc.* **1974**, *96*, 953. (b) Winnik, M. A.; Maharaj, U. *J. Am. Chem. Soc.* **1981**, *103*, 2328. (c) Gorman, A. A.; Hamblett, I.; Lambert, C.; Spencer, B.; Standen, M. C. *J. Am. Chem. Soc.* **1988**, *110*, 8053.

(12) Saltiel, J.; Shannon, P. T.; Zafirou, O. C.; Uriarte, A. K. *J. Am. Chem. Soc.* **1980**, *102*, 6799.

of a high-temperature limiting slope indicates both have reached pre-equilibrium where $T > 25$ °C. To what degree S_Δ may vary with temperature is dependent on the relative activation energies of at least four rate constants.

We believe that this is the first direct evidence of exciplex involvement in triplet state quenching by oxygen, and we are currently investigating the possibility that exciplexes are associated with oxygen quenching of triplet states of widely varying structure, energy, and S_Δ values.

Acknowledgment. This work is supported in part by NIH Grant GM 24235 and by the Center for Photochemical Sciences, Bowling Green State University, Bowling Green, OH.

Registry No. Benzophenone, 119-61-9; oxygen, 7782-44-7.

Photoelectron Spectrum of *o*-Benzyne. Ionization Potentials as a Measure of Singlet-Triplet Gaps

Xu Zhang and Peter Chen*[,1]

Mallinckrodt Chemical Laboratory
Harvard University, Cambridge, Massachusetts 02138

Received December 16, 1991

We report the experimental determination of ionization potentials (IP) for *o*-benzyne (*o*-C_6H_4). While *o*-C_6H_4 had been the subject of several studies[2,3] aimed at determining its IP, there nevertheless remained no clear, reliable number. In this paper, we report the photoelectron spectrum of *o*-C_6H_4 and demonstrate, for geometrically-constrained singlet biradicals (or biradical-like species), a relationship between ionization potentials and the singlet-triplet gap (Δ_{ST}) which we had recently shown for singlet ground-state carbenes.[4] We confirm the predicted identity:

$$\mathrm{IP}_\sigma[o\text{-}C_6H_4] - \mathrm{IP}_\sigma[C_6H_5] \approx \Delta_{ST}[o\text{-}C_6H_4] \qquad (1)$$

This relationship, derived from valence bond (VB) promotion energy arguments,[4-6] can be applied to the prediction of singlet-triplet gaps and ionization potentials for *m*- and *p*-benzyne.

The time-of-flight (TOF) mass and photoelectron spectrometers have been previously described.[4,6-8] Laser light at 118.2 nm (10.49 eV) was produced by frequency-tripling the Nd^{3+}-YAG third harmonic in xenon. *o*-C_6H_4 was produced by supersonic jet flash pyrolysis of benzocyclobutene-1,2-dione,[9] synthesized by the procedure of South and Liebeskind.[10] Photoionization of the benzocyclobutenedione with 10.49-eV photons gave only mass peaks at $m/e = 132$ (M^+) and $m/e = 104$ ($M^+ - CO$). Pyrolysis of <1 Torr partial pressure of the dione, seeded in 1500 Torr of helium (1300 °C by optical pyrometry, 50–100 μs contact time), gives quantitative, clean conversion to *o*-C_6H_4 with *no secondary pyrolysis or detectable bimolecular reactions* and no other detectable products (IP[CO] > 10.49 eV) in the photoionization mass spectrum of the pyrolysate. Because $m/e = 76$ is the only large peak in the mass spectrum of the pyrolyzed benzocyclobutenedione, all of the peaks observed in a photoelectron spectrum (Figure 1) taken under the same conditions can be assigned to *o*-C_6H_4. At higher temperatures, a peak at $m/e = 50$ appears in the mass spectrum, accompanied by sharp peaks at ~1.25 eV kinetic energy in the photoelectron spectrum.

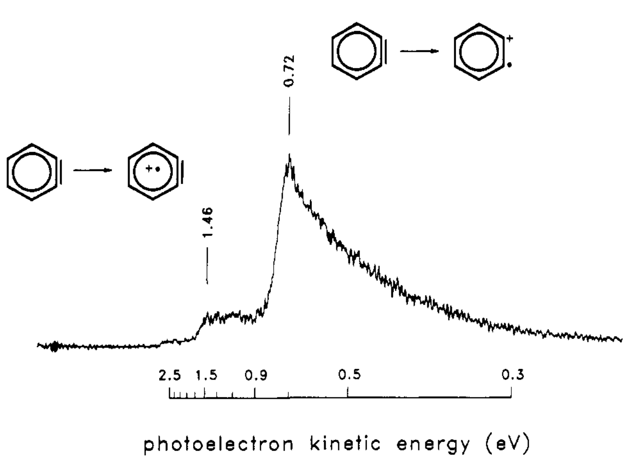

Figure 1. Time-of-flight photoelectron spectra (10.49 eV) of *o*-benzyne. Subtraction of the photoelectron kinetic energies from the 10.49-eV photon energy gives an ionization potential. The two marked ionization potentials come from removal of an electron from the out-of-plane and in-plane molecular orbitals, respectively (Koopmans' theorem). Splitting of the first band, assigned to $\mathrm{IP}_\pi[o\text{-}C_6H_4]$, is unresolved and may account for the broadness of that band.

The out-of-plane π-system in the phenyl radical, C_6H_5, was reported[11] to be almost unperturbed from that in benzene, as indicated by an observed threshold in the photoionization efficiency curve for C_6H_5, which we designated IP_π, close to $\mathrm{IP}[C_6H_6] = 9.24$ eV. Removal of an electron from the in-plane, nonbonding radical orbital of C_6H_5 gave a lower energy threshold, which we designated IP_σ, reported[11] to be $\mathrm{IP}_\sigma = 8.1 \pm 0.1$ eV. A simple Koopmans' theorem interpretation of Figure 1 finds, analogously, two ionization potentials for *o*-C_6H_4, corresponding to removal of an electron from out-of-plane and in-plane orbitals; $\mathrm{IP}_\pi[o\text{-}C_6H_4] = 9.03 \pm 0.05$ eV and $\mathrm{IP}_\sigma[o\text{-}C_6H_4] = 9.77 \pm 0.03$ eV. We assume, in our interpretation, that the splitting of the degenerate π-system HOMO in benzene, upon going from D_{6h} to C_{2v}, is small and results only in a broadening of the band associated with $\mathrm{IP}_\pi[o\text{-}C_6H_4]$. The relative ordering of ion states is consistent with both semiempirical[3] and ab initio[12] calculations for the ionization of *o*-benzyne.

That the VB promotion energy picture correctly describes *o*-C_6H_4 is seen below. We had noted[4] that $\Delta H_f[o\text{-}C_6H_4]$ differed from a simple bond additivity estimate (via cleavage of two C–H bonds in benzene) by VB promotion energy, which was approximately $\Delta_{ST}[o\text{-}C_6H_4]$. Recent reports[13-15] of $\Delta H_f[o\text{-}C_6H_4] = 106 \pm 3$ kcal/mol agree well with an estimate made assuming bond additivity, corrected by $\Delta_{ST}[o\text{-}C_6H_4]$:

$$\begin{aligned}\Delta H_f[o\text{-}C_6H_4] &\approx \Delta H_f[C_6H_6] + (2\mathrm{BDE}[C_6H_6]) - \\ &\qquad (2\Delta H_f[H^\bullet]) - \Delta_{ST}[o\text{-}C_6H_4] \\ &= 20 + (2 \times (113 \pm 3)^{\mathrm{ref16}}) - (2 \times 52) - \\ &\qquad (38 \pm 0.7)^{\mathrm{ref17}} = 104 \pm 4\ \mathrm{kcal/mol} \qquad (2)\end{aligned}$$

This is an especially favorable case because $\Delta_{ST}[o\text{-}C_6H_4]$ had been

---

[1] NSF Presidential Young Investigator, David and Lucile Packard Fellow, Camille and Henry Dreyfus Teacher–Scholar, and Alfred P. Sloan Research Fellow.

[2] Genzel, H.; Osberhaus, O. Z. *Naturforsch.* **1967**, *22a*, 331. Grutzmacher, H. F.; Lohmann, J. *Ann.* **1967**, *705*, 81.

[3] Dewar, M. J. S.; Tien, T. P. *J. Chem. Soc., Chem. Commun.* **1985**, 1243.

[4] Clauberg, H.; Minsek, D. W.; Chen, P. *J. Am. Chem. Soc.* **1992**, *114*, 99.

[5] Carter, E. A.; Goddard, W. A., III *J. Phys. Chem.* **1986**, *90*, 998. See also the discussion of the valence state: Murrell, J. N.; Kettle, S. F. A.; Tedder, J. M. *The Chemical Bond*, 2nd ed.; John Wiley & Sons: New York, 1985; pp 247–249.

[6] Clauberg, H.; Chen, P. *J. Am. Chem. Soc.* **1991**, *113*, 1445.

[7] Minsek, D. W.; Chen, P. *J. Phys. Chem.* **1990**, *94*, 8399.

[8] Blush, J. A.; Park, J.; Chen, P. *J. Am. Chem. Soc.* **1989**, *111*, 8951.

[9] Brown, R. F. C.; Browne, N. R.; Coulston, K. J.; Danen, L. B.; Eastwood, F. W.; Irvine, M. J.; Pullin, A. D. E. *Tetrahedron Lett.* **1986**, 1075. Brown, R. D.; Godfrey, P. D.; Rodler, M. *J. Am. Chem. Soc.* **1986**, *108*, 1296.

[10] South, M. S.; Liebeskind, L. S. *J. Org. Chem.* **1982**, *47*, 3815.

[11] Sergeev, Yu. L.; Akopyan, M. E.; Vilesov, F. I. *Opt. Spek.* **1972**, *32*, 230.

[12] Hillier, I. H.; Vincent, M. A.; Guest, M. F.; von Niessen, W. *Chem. Phys. Lett.* **1987**, *134*, 403.

[13] Guo, Y.; Grabowski, J. J. *J. Am. Chem. Soc.* **1991**, *113*, 5923.

[14] Wenthold, P. G.; Paulino, J. A.; Squires, R. R. *J. Am. Chem. Soc.* **1991**, *113*, 7414.

[15] Riveros, J. M.; Ingemann, S.; Nibbering, N. M. M. *J. Am. Chem. Soc.* **1991**, *113*, 1053.

[16] Robaugh, D.; Tsang, W. *J. Phys. Chem.* **1986**, *90*, 5363.

[17] Leopold, D. G.; Miller, A. E. S.; Lineberger, W. C. *J. Am. Chem. Soc.* **1986**, *108*, 1379.

0002-7863/92/1514-3147$03.00/0