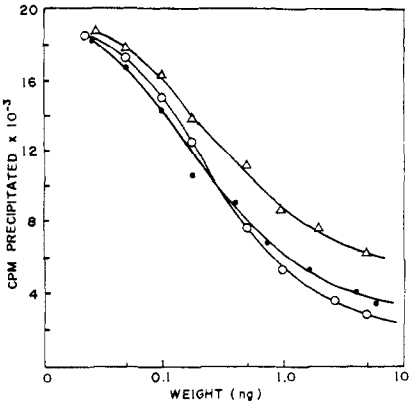


Figure 2. Double antibody immunoassays: (●) natural bovine C-peptide; (○) synthetic peptide I; and (△) synthetic peptide II. $^{125}$I synthetic tyrosinated bovine connecting peptide was used as a tracer.

almost identically with natural bovine connecting peptide, while II failed to displace the tracer completely (Figure 2). Accurate evaluation of the cross-reactivity of II was difficult because of nonparallelism in the displacement curves of the synthetic peptide and the natural bovine proinsulin or connecting peptide standard. Formyl protection of lysine residue at position 59 was shown to have no effect on the immunological reactivity of the connecting peptide. Both peptide III and IV, which were used as the amino components in the fragment condensations, showed no cross-reaction with bovine proinsulin or the connecting peptide. Detailed immunological evaluation of the synthetic bovine connecting peptide and the related peptides will be published elsewhere.

It was clearly shown that full cross-reactivity of the bovine connecting peptide is obtained with the revised amino acid sequence. The distinct difference of immunological reactivity observed which was caused by an inversion of residues 50 and 52 seems to indicate that the 47–60 sequence is indeed involved in the immunological activity tested. The fact that synthetic peptide III was immunologically inactive could mean that only part of the antigenic determinant resides in the 47–60 sequence or that the whole 31–60 sequence is necessary for the determinant to acquire the conformation in which it is immunologically active with the particular antisera used. In addition, the present demonstration shows that the structural change in the sequence in which the antigenic determinant is located can be detected by the immunological technique when homogeneous synthetic peptides of definite structure are used. Further synthetic studies on the antigenic determinant of bovine connecting peptide are now under way.

The syntheses of I and II were accomplished by the Rudinger azide modification.[15] For the synthesis of I, the azide derived from Z-Arg($NO_2$)-Arg($H^+$)-Glu-Val-Glu-Gly-Pro-Gln-Val-Gly-Ala-Leu-Glu($OBu^t$)-Leu-Ala-Gly-$N_2H_2$-Boc acetate trihydrate (V) [mp 228–230°; $[\alpha]^{25}D$ −35.1° (*c* 1.1 DMSO); $R_f^{I}$ 0.45; $R_f^{II}$ 0.75; amino acid ratios in acid hydrolysate, Arg + $Orn_{1.89}Glu_{4.04}Pro_{0.96}Gly_{2.91}Ala_{2.01}Val_{2.09}Leu_{1.98}$. *Anal.* Found: C 50.4; H, 7.2; N, 17.0] was coupled with III [$[\alpha]^{28}D$ −112.0° (*c* 1.0, 10% acetic acid); $R_f^{I}$ 0.02; $R_f^{II}$ 0.30; amino acid ratios in acid hydrolysate, $Lys_{0.94}Arg_{0.97}Glu_{1.92}Pro_{2.91}Gly_{5.18}Ala_{0.98}Leu_{1.09}$; peptide content 88%], which had been obtained by catalytic hydrogenolysis, followed by gel filtration on Sephadex G-10, of Z-Gly-Pro-Gly-Ala-Gly-Gly-Leu-Glu-Gly-Pro-Pro-Gln-Lys(F)-Arg($H^+$)-OH acetate pentahydrate [mp 184–192°; $[\alpha]^{28}D$ −44.0° (*c* 1.0, DMF); $R_f^{I}$ 0.19; $R_f^{II}$ 0.59; amino acid ratios in acid hydrolysate, $Lys_{0.91}Arg_{0.93}Glu_{1.86}Pro_{3.09}Gly_{5.18}Ala_{1.06}Leu_{0.98}$. *Anal.* Found: C, 49.1; H, 7.0; N, 16.8].

The resulting crude protected triacontapeptide was hydrogenated to give a crude preparation of I, which was purified by chromatography on CM-Sephadex C-25 using ammonium acetate buffer as an eluent and desalted by gel filtration on Sephadex G-25 [$[\alpha]^{28}D$ −100.0° (*c* 1.0, 10% acetic acid); $R_f^{I}$ 0.08; $R_f^{II}$ 0.54; amino acid ratios in acid hydrolysate, $Lys_{0.96}Arg_{3.09}Glu_{5.70}Pro_{4.22}Gly_{8.21}Ala_{3.09}Val_{1.85}Leu_{2.88}NH_{3(2.41)}$; peptide content 87%].

The synthesis of II was carried out in the same manner as described above. The azide derived from V was coupled with IV [$[\alpha]^{25}D$ −117.5° (*c* 0.3, 10% acetic acid); $R_f^{I}$ 0.05; $R_f^{II}$ 0.28; amino acid ratios in acid hydrolysate, $Lys_{0.96}Arg_{0.92}Glu_{1.92}Pro_{3.08}Gly_{5.16}Ala_{1.07}Leu_{0.99}$; peptide content 91%], which was prepared by hydrogenation of Z-Gly-Pro-Gly-Gly-Gly-Ala-Leu-Glu-Gly-Pro-Pro-Gln-Lys(F)-Arg($H^+$)-OH acetate trihydrate [mp 166–168°; $[\alpha]^{28}D$ −51.8° (*c* 1.0, DMF); $R_f^{I}$ 0.17; $R_f^{II}$ 0.53; amino acid ratios in acid hydrolysate, $Lys_{0.96}Arg_{0.92}Glu_{1.94}Pro_{3.08}Gly_{5.16}Ala_{1.02}Leu_{0.99}$. *Anal.* Found: C, 50.5; H, 7.2; N, 16.2]. The resulting mixture of crude materials was hydrogenated, and in the manner as described for I pure triacontapeptide II was isolated [$[\alpha]^{28}D$ −104.9° (*c* 0.5, 10% acetic acid); $R_f^{I}$ 0.09; $R_f^{II}$ 0.55; amino acid ratios in acid hydrolysate, $Lys_{0.94}Arg_{2.91}Glu_{6.07}Pro_{3.89}Gly_{8.10}Ala_{3.15}Val_{2.03}Leu_{2.91}NH_{3(2.46)}$; peptide content 90%].

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## An Electron Spin Resonance Study of Some Group IVb Organometallic Peroxy Radicals

*Sir:*

Electron spin resonance spectroscopy (esr) has proved to be a useful technique for the study of the chain carrying peroxy radicals (ROO·) involved in the autoxidation of organic compounds.[1] For example, absolute

(1) J. A. Howard, *Advan. Free-Radical Chem.*, **4**, 49 (1972), and references cited therein.

values of the rate constants, $2k_t$, for the chain termination step

$$\mathrm{ROO\cdot} + \mathrm{ROO\cdot} \xrightarrow{2k_t} \text{molecular products} \quad (1)$$

and changes in the enthalpy and entropy for the equilibrium process

$$\mathrm{ROO\cdot} + \mathrm{ROO\cdot} \rightleftharpoons \mathrm{ROOOOR} \quad (2)$$

have been determined by this technique. These studies have, however, been almost entirely confined to hydrocarbon peroxy radicals although FOO·,[2] HOO·,[3] and some phosphoranylperoxy[4a] and arsanylperoxy[4b] radicals have received some attention.

In this communication we report an esr investigation of some group IVb organometallic peroxy radicals of the structure $(CH_3)_3MOO\cdot$, where M = Si, Ge, Sn, or Pb. The identification of these radicals and a knowledge of their stabilities are of fundamental importance to the development of a better understanding of the autoxidation of organosilicon, -germanium, -tin, and -lead compounds.

The organometallic radicals $(CH_3)_3M\cdot$ have been prepared and identified by esr either by liquid-phase photolyses of di-*tert*-butyl peroxide in the presence of the hydride[5,6]

$$t\text{-BuOOBu-}t \xrightarrow{h\nu} 2t\text{-BuO}\cdot$$

$$t\text{-BuO}\cdot + (CH_3)_3MH \longrightarrow t\text{-BuOH} + (CH_3)_3M\cdot$$

or by reaction of the trimethylorganometallic halide with sodium in a rotating cryostat.[7,8] When these reactions were carried out in the presence of oxygen, the appropriate peroxy radicals were produced by the reaction

$$(CH_3)_3M\cdot + O_2 \longrightarrow (CH_3)_3MOO\cdot$$

The principal $g$ factors for these radicals are given in Table I together with those for *tert*-butylperoxy radicals.

With the exception of silicon, the average $g$ factors for the radicals $(CH_3)_3MOO\cdot$ in the solid phase and the isotropic values in solution increase with increasing atomic number of M. This trend is similar to that found for the group IVb metal centered radicals $(CH_3)_3M\cdot$ in which the unpaired electron is located mainly in a valence shell p orbital: $g_{iso}(M = C) = 2.0029$,[9] $g(Si) = 2.0031$,[5] $g(Ge) = 2.0104$,[6] $g(Sn) = 2.0163$,[6,8] $g(Pb) = 2.0389$.[8]

The origin of the deviation of the principal $g$ factors in $\pi$-electron radicals from the free spin value (2.0023) is reasonably well understood.[10] The $g$ shifts are associated with spin–orbit interactions between the ground state and excited states of the radical and $\Delta g \sim 2\zeta/\nu_1$ (where $\zeta$ is the spin–orbit coupling constant and $\nu_1$ is the energy difference between the ground and excited states).

**Table I.** Esr Parameters of $(CH_3)_3MOO\cdot$ in the Solid Phase and in Solution[a]

| M | Solvent or matrix | Solid phase $g_{xx}$ | $g_{yy}$ | $g_{zz}$ | $g_{av}$[b] | Solution $g_{iso}$ |
|---|---|---|---|---|---|---|
| C | $C_6D_6$ | 2.0027 | 2.0086 | 2.0395 | 2.0169 | 2.0154 |
| Si | Cyclopropane | 2.0022 | 2.0079 | 2.0587 | 2.0229 | |
| | | | | | | 2.0277[c] |
| Ge | Cyclopropane | 2.0024 | 2.0083 | 2.0530 | 2.0212 | 2.024 |
| | $C_6D_6$ | 2.0023 | 2.0083 | 2.0535 | 2.0214 | |
| Sn | Cyclopropane | 2.0021 | 2.0083 | 2.0600 | 2.0235 | 2.024 |
| | $C_6D_6$ | 2.0026 | 2.0082 | 2.0602 | 2.0237 | |
| Pb | Cyclopropane | 2.0011 | 2.0078 | 2.0772 | 2.0287 | 2.034 |
| | $C_6D_6$ | 2.0007 | 2.0078 | 2.0798 | 2.0294 | |

[a] Peroxy radicals prepared in the rotating cryostat were trapped in either $C_6D_6$ or cyclopropane and at −196° gave powder spectra. The spectra of the radicals prepared in solution were isotropic. $g$ factors were calculated by comparison with the value of 2.0036 for $\alpha,\alpha'$-diphenyl-$\beta$-picrylhydrazyl. [b] $g_{av} = {}^1/_3(g_{xx} + g_{yy} + g_{zz})$. [c] $(C_6H_5)_3SiOO\cdot$.

For organic peroxy radicals the unpaired electron is located in a $\pi$ orbital centered almost completely on the two oxygen atoms, and as a result $g$ shifts are much larger than those of the corresponding carbon-centered radicals. The even larger $g$ shifts observed for the $(CH_3)_3MOO\cdot$ radicals may indicate that a significant fraction of the spin density is associated with the central metal atom, M, although in no case could hyperfine structure due to M (*e.g.*, $^{117}Sn$, $I = {}^1/_2$, 7.5% abundance) be observed. However, it is also feasible that the presence of low-lying d orbitals in the valence shell of the central atom may alter the separation between the ground and excited states, which will also contribute to the observed $g$ shifts. The anomalous $g$ shift in the silylperoxy radical may be caused by a combination of these effects.

The discrepancies in the values of the average $g$ factors calculated from the spectra of the trapped radicals and those of the isotropic $g$ factors in solution are rather greater than are usually obtained. The reason for these differences is not clear but may well be due to incomplete rotational averaging of the spectra in solution.

Both trimethylsilylperoxy and trimethylgermylperoxy radicals prepared in the cryostat in cyclopropane disappeared immediately when the solvent melted (∼−127°) and did not reappear upon warming the samples to −40°. Photolysis of di-*tert*-butyl peroxide in oxygen-saturated trimethylsilane did not give a detectable concentration of peroxy radicals, whereas the same experiment with trimethylgermane gave a peroxy radical that was stable from −60 to −100°. For silicon these results imply that trimethylsilylperoxy radicals terminate with a high rate constant even at low temperatures. The stabilities of trimethylgermylperoxy radicals prepared by the two methods were quite different even though the radicals had the same $g$ factor (see Table I). It is clear that a careful kinetic study of organogermylperoxy radicals is needed to elucidate this anomaly.

Rates of decay of trimethylstannylperoxy radicals were second order in the radical concentration, and there was no evidence for the existence of a reversible

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radical–dimer equilibrium process analogous to reaction 2. Absolute values of the termination rate constant for this radical obeyed the equation

$$2k_t = 10^{12}e^{-8000/RT}\ M^{-1}\ \text{sec}^{-1}$$

with the activation energy in cal mol$^{-1}$.

Trimethylplumbylperoxy radicals prepared in the cryostat in cyclopropane were stable up to the boiling point of the solvent, and there was again no evidence for a radical–dimer equilibrium.

From this rather cursory kinetic investigation, it would appear that radical stabilities decrease in the order $(CH_3)_3PbOO\cdot > (CH_3)_3SnOO\cdot > (CH_3)_3SiOO\cdot$ with the position of $(CH_3)_3GeOO\cdot$ in some doubt. A more comprehensive study of the kinetics of the self-reaction of group IVb organometallic peroxy radicals is being carried out and will be presented in a subsequent paper.

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## Reactions of Coordinated Nucleophiles. Formation of an Amide from a Nitrile

*Sir:*

The extraordinary reactivity of coordinated hydroxide ion and water for intramolecular hydrolysis has been established for coordinated glycine esters[1] and amides.[2] This communication deals with a related addition process at an unsaturated C atom, namely the hydration of a nitrile to an amide.

The treatment of the kinetically robust and purple complex *cis*-$[Co(en)_2(NH_2CH_2CN)Br]^{2+}$ [3] with $Hg_{aq}^{2+}$ rapidly gave an orange solution of $[Co(en)_2glyNH_2]^{3+}$ (~95%). Spectrophotometric studies showed that the rate was first order in $[Hg^{2+}]$ and was independent of $[H^+]$ between 0.09 and 0.89 *M* $HClO_4$ ($k$ = 0.14 $M^{-1}$ sec$^{-1}$, 25°, $\mu$ = 1.2–1.6 *M*, $ClO_4^-$ = 1.1 *M* at 560, 470, and 280 nm). The visible spectrum of the product agreed with that of authentic $[Co(en)_2glyNH_2](NO_3)_2ClO_4\cdot 2H_2O$[4] ($\epsilon_{487}$ = 104 $M^{-1}$ cm$^{-1}$, 25°, 0.5 *M* $Hg(ClO_4)_2$, 0.1 *M* $HClO_4$) as did the ion-exchange behavior (Dowex 50W-X2, $NaClO_4$ eluent). Two fractions of the product were isolated in the manner described for the authentic material.[4]

*Anal.* Calcd for $[Co(en)_2(gly\text{-}NH_2)](NO_3)_2ClO_4\cdot 2H_2O$: C, 14.05; H, 5.11; N, 21.86. Found: C, 13.8; 14.1; H, 4.8, 5.1; N, 21.7, 21.7.

The product was also characterized by its pmr spectrum and optical rotatory power. The chelated amide was generated *in situ* using $Hg(ClO_4)_2$ (1 *M* in 0.1 *M* $DClO_4$, 0.3 *M* in complex). Under these conditions $Br^-$ removal was complete in <3 min and the first pmr spectrum (~5 min) agreed with that for the authentic amide under the same conditions, and remained unchanged for several hours. Similarly the rotatory dispersion curve of $(-)_{589}$-$[Co(en)_2(NH_2CH_2CN)Br](ClO_4)_2$ ($[M]_{589}$ −580°, $[M]_{436}$ +1250° in 0.1 *M* $HClO_4$) in $Hg(ClO_4)_2$ (1.0 *M*, $HClO_4$ 0.1 *M*) also agreed (within 5%) with that of the amide complex under the same conditions ($[M]_{465}$ +5860°). The rate constants obtained polarimetrically also agreed with the spectrophotometric data. Any question of a coincidence of properties between the chelated amide and aquonitrile complexes was removed by tlc experiments. The free nitrile was separated from ethylenediamine and glycinamide on silica gel plates using *n*-butyl alcohol–HCl (12 *M*)–$H_2O$ (8:1:1) as eluent. With the cobalt complexes, the ligands were released by reducing Co(III) to Co(II) with $NaBH_4$ in acidic solution; $[Co(en)_2(NH_2CH_2CN)Br]^{2+}$ gave a positive test for nitrile under these conditions but the reaction product showed no nitrile. Both experiments were conducted with suitable blanks containing free nitrile and amide. Clearly the amide is produced rapidly after $Br^-$ is removed. The possibility that *cis*-$[Co(en)_2(glyNH_2)(H_2O)]^{3+}$ was initially formed was also excluded. In 2 *M* HBr, no *cis*-$[Co(en)_2(glyNH_2)Br]^{2+}$ was formed after 6 days at 25°. Further, it is known that *cis*-$[Co(en)_2(glyNH_2)(H_2O)]^{3+}$ forms $[Co(en)_2(gly)]^{2+}$ ($t_{1/2}$ ~ 120 sec at 25°) and not chelated $[Co(en)_2glyNH_2]^{3+}$.

Since the actual chelation step was not observed with the aminoacetonitrile complex the analogous aminopropionitrile complex was examined. The expectation was that formation of the larger chelate would be a slower process. Preliminary kinetic studies in the presence of 0.1 *M* $Hg^{2+}$ show that there is a slow reaction ($t_{1/2}$ ~108 min, 25°, $[H^+]$ = 0.1 *M*) following the rapid removal of $Br^-$. This process shows an inverse acid dependence and a term in $[Hg^{2+}]^1$ concentration. The observed spectral change is characteristic of a cis aquo complex going to a chelated product ($\epsilon_{488}$ ~80 to $\epsilon_{492}$ 123, 0.1 *M* $Hg^{2+}$, 0.1 *M* $H^+$). Ion exchange analysis indicates that the final product has a 3+ charge and the visible spectrum is characteristic of the type of product expected ($\epsilon_{max}$ at 492 and 351 nm). The products at various stages of the reaction were identified by tlc. When the initial product from the $Hg^{2+}$-catalyzed reaction was analyzed, no amide was found, only nitrile. However, after several days the product contained only amide and no nitrile. Heating this product with HBr gave no bromo complex which also indicates the chelate had formed. In concentrated HBr, however, the bromonitrile complex was slowly converted to the bromomonodentate-β-alaninamide complex (~2 days for completion).

The order of events for the overall process for both complexes appears to be the following: (1) $Hg^{2+}$-assisted removal of $Br^-$; (2) rapid entry of solvent into the vacant coordination site; (3) $Hg^{2+}$ assisted nucleophilic attack by bound $OH^-$ at the nitrile to give the chelated amide.

Subsequently the *cis*-aquoaminopropionitrile complex was prepared and its kinetic behavior is in agreement with the data obtained from the cis bromo complex $[Co(en)_2(NH_2CH_2CH_2CN)(H_2O)](ClO_4)(NO_3)_2$. *Anal.*

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