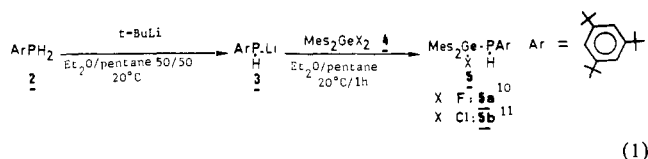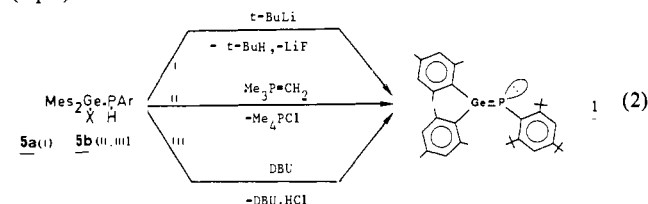to dimesityldihalogermanes[9] **4** (eq 1).

(1)

Dehydrohalogenation of **5** was accomplished using different strong bases: (i) *tert*-butyllithium, (ii) trimethylmethylene-phosphorane, and (iii) 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU)[12] (eq 2).
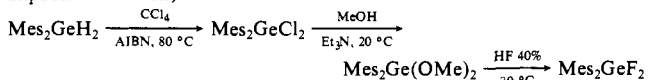

(2)

(i) An equivalent of *t*-BuLi was added to an equivalent of **5a** in ethereal solution at −15 °C, under an argon atmosphere. An orange color developed immediately; ^{31}P NMR investigation of the solution showed the nearly quantitative formation of **1** [δ [31]P +175.4 (H$_3$PO$_4$)]. Centrifugation of lithium fluoride and removal of ether/pentane solvents under vacuum afforded an air-sensitive crude material. Recrystallization of crude **1** from pentane gave pure orange crystals of **1**.[13]

(ii) An equimolar quantity of trimethylmethylenephosphorane (Me$_3$P=CH$_2$) (in pentane) was added to a solution of **5b** in the same solvent at 20 °C. The reaction mixture was filtered to remove Me$_4$PCl. ^{31}P NMR analysis of the filtrate showed the formation of **1** (30%) and **6** (35%) and the presence of unreacted starting material **5b** (30%). The adduct **6**[14] presumably results from the addition of trimethylmethylenephosphorane to **1** (see further).

(iii) Addition of 1 equiv of DBU to **5b** in pentane solution afforded **1** in small amount (~15%); the yield of **1** cannot be improved even with a large excess of DBU.

The structure of **1** was corroborated by its chemical behavior. A preliminary investigation reveals the expected polarity[15] of the

Ge=P bond with germanium as the more positive partner.

Reactivity of **1** is very high particularly toward compounds with active hydrogens, e.g., water, methanol, hydrogen chloride, and trimethylmethylenephosphorane, which add to the germanium-phosphorus double bond; addition of these reagents to an orange solution of **1** in pentane at room temperature resulted in immediate decoloration of reaction mixture and afforded respectively the adducts **7**,[16] **8**,[17] **5b**,[11] and **6**[14] in nearly quantitative yields (eq 3).

(3)

Germaphosphene **1** is the first isolated compound with a double bond between germanium and phosphorus. Further investigations of its chemical and spectroscopic properties are now in progress.

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(16) **7**: ^{1}H NMR (C$_6$D$_6$) δ 1.21 (s, 9 H, *p-t*-Bu), 1.40 (s, 18 H, *o-t*-Bu), 2.05 (s, 6 H, *p*-Me), 2.22 (s, 12 H, *o*-Me), 6.65 (br s, 4 H, Ar Mes); ^{31}P NMR (C$_6$D$_6$) δ −105.6 ([1]J$_{PH}$ 209 Hz); mass spectrum (desorption), *m/e* 606 (M).

(17) **8**: ^{1}H NMR (C$_6$D$_6$) δ 1.20 (s, 9 H, *p-t*-Bu), 1.38 (s, 18 H, *o-t*-Bu), 2.00 (s, 6 H, *p*-Me), 2.13 (s, 12 H, *o*-Me), 6.60 (br s, 4 H Ar Mes); ^{31}P NMR (C$_6$D$_6$) δ −107.0 ([1]J$_{PH}$ = 212 Hz); mass spectrum (EI), *m/e* 619 (M).

Resonance Raman Studies of the Photoreduction of Horseradish Peroxidase Compounds I and II

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Horseradish peroxidase (HRP) (EC 1.11.1.7) catalyzes the oxidation of substrates by hydrogen peroxide without the formation of ternary complexes.[1-3] Instead, the enzyme is first oxidized by peroxide to form an intermediate referred to as compound I which is deficient from the resting enzyme by two electrons. Resting HRP contains high-spin, five-coordinate ferriheme. The most widely held view is that, on oxidation to compound I, one electron is removed from the iron atom and one oxygen atom of peroxide is retained to form an oxyferryl ion, while the second electron is abstracted from the porphyrin ring to form a π-cation radical. Compound I is catalytically active and its porphyrin radical abstracts one electron from the substrate to form a second intermediate, called compound II, which is subsequently reduced back to the resting enzyme by one-electron oxidation of a second molecule of substrate.

Resonance Raman (RR) spectroscopy has proven to be a valuable tool for the study of heme proteins[4] and has been applied to HRP and its intermediates.[5-14] Compound I has long been

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(9) Mes$_2$GeCl$_2$ and Mes$_2$GeF$_2$ have been obtained as followed (Riviere, P., unpublished results):

$$\text{Mes}_2\text{GeH}_2 \xrightarrow[\text{AIBN, 80 °C}]{\text{CCl}_4} \text{Mes}_2\text{GeCl}_2 \xrightarrow[\text{Et}_3\text{N, 20 °C}]{\text{MeOH}}$$
$$\text{Mes}_2\text{Ge(OMe)}_2 \xrightarrow[\text{20 °C}]{\text{HF 40\%}} \text{Mes}_2\text{GeF}_2$$

(10) **5a**: ^{1}H NMR (60 MHz, C$_6$D$_6$) δ 1.45 (s, 9 H, para *t*-Bu) 1.60 (s, 18 H, ortho *t*-Bu), 2.13 (s, 6 H, *p*-Me), 2.30 (s, 12 H, *o*-Me), 6.70 (br s, 4 H Ar Mes); ^{31}P NMR (36.4 MHz, C$_6$D$_6$) δ −110.6 ([1]J$_{PH}$ = 209, [2]J$_{PF}$ = 9 Hz); IR (pentane) ν(PH) 2330 cm^{-1}. Anal. Calcd for C$_{36}$H$_{52}$FGeP: C, 71.19; H, 8.63; F, 3.13. Found: C, 71.30; H, 8.84; F, 3.40.

(11) **5b**: ^{1}H NMR (60 MHz, C$_6$D$_6$) δ 1.38 (s, 9 H, *p-t*-Bu), 1.50 (s, 18 H, *o-t*-Bu), 2.05 (s, 6 H, *p*-Me), 2.35 (s, 12 H, *o*-Me), 6.69 (br s, 4 H Ar Mes); ^{31}P NMR (C$_6$D$_6$) δ −90.9 ([1]J$_{PH}$ = 215 Hz); IR (pentane) ν(PH) 2360 cm^{-1}. Anal. Calcd for C$_{36}$H$_{52}$ClGeP: C, 69.31; H, 8.40; Cl, 5.68. Found: C, 69.08; H, 8.57; Cl, 5.82.

(12) Attempts to dehydrochlorinate **5b** by amines such as trimethylamine, triethylamine, or 1,4-diazabicyclo[2.2.2]octane were unsuccessful.
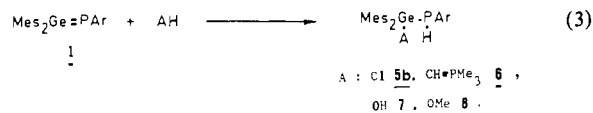
(13) **1** presents as highly air-sensitive orange crystals (mp 155–160 °C, sealed tube) soluble in usual organic solvents and stable at room temperature. ^{1}H NMR (C$_6$D$_6$) δ 1.38 (s, 9 H, *p*-Me), 1.77 (s, 18 H, *o-t*-Bu), 1.98 (s, 6 H, *p*-Me), 2.12 (s, 6 H, *o*-Me of Mes'), 2.60 (s, 6 H, *o*-Me of Mes''), 6.58 and 6.73 (two br s, 4 H Ar Mes), 7.47 (d, 2 H, Ar Ar, [4]J$_{PH}$ = 6.0 Hz). Mesityl groups are nonequivalent (no rotation about Ge=P bond). Mes'' is probably the mesityl group cis with the lone pair on phosphorus; similar deshielding has already been observed in 2-germaphospholanes (Couret, C.; Escudie, J.; Satge, J.; Redoules, G. *C. R. Acad. Sci. Ser. 3* **1974**, *279*, 225–228). ^{31}P NMR (C$_6$D$_6$) δ +175.4; mass spectrum (EI), *m/e* 588 (M).

(14) **6**: ^{1}H NMR (60 MHz, C$_6$D$_6$) δ 0.20 (m, 1 H, CHGe), 1.0 (d, 9 H, [2]J$_{PH}$ = 13.0 Hz, PMe$_3$), 1.32 (s, 9 H, *p-t*-Bu), 1.53 (s, 18 H, *o-t*-Bu); 2.13 (s, 6 H, *p*-Me), 2.47 (s, 12 H, *o*-Me), 6.77 (br s, 4 H, Ar Mes); ^{31}P NMR (C$_6$D$_6$) δ −5.5 (PMe), −81.2 (PAr, [1]J$_{PH}$ = 206 Hz); mass spectrum (EI), *m/e* 677 (M).

(15) The reactivity of the Ge=P double bond is in good agreement with the polarity predictions arising from calculations made in collaboration with J. C. Barthelat (Laboratoire de Physique Quantique, Toulouse); this theoretical investigation has now been achieved and submitted to publication.

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3380 *J. Am. Chem. Soc., Vol. 107, No. 11, 1985*
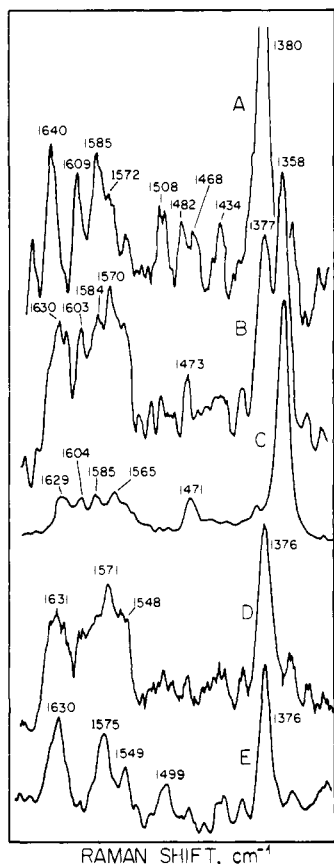
Communications to the Editor

Figure 1. RR spectra of various HRP species. All spectra are of frozen aqueous solutions (0.5 mM) at −5 °C excited at 457.9 nm and recorded with a spectral band-pass of 8 cm⁻¹ and an acquisition time of 15 min: (A) compound II, 20 mW; (B) compound II, spectrum acquired after 1 h of irradiation with 100 mW; (C) ferro-HRP, 30 mW; (D) spectrum B minus spectrum C; (E) ferri-HRP, 40 mW.

recognized to be photolabile,[15-17] making its RR spectrum difficult to obtain. In the present study, it is demonstrated that frozen samples of both compounds I and II undergo photoreduction on laser irradiation. Previously reported RR experiments[14] on frozen compound I are reinterpretted and shown not to yield the spectrum of compond I, but rather that of its photoproducts.

HRP (Sigma, Type VI) was purified as described earlier.[13] All enzyme species were examined in 20 mM phosphate buffer, pH 6.8, except for compound I, which was prepared in 20 mM borate, pH 8.7. Ferro-HRP was prepared by reduction of an oxygen-free solution of ferri-HRP with a tenfold excess of sodium dithionite. Compound I was produced by addition of equimolar hydrogen peroxide to ferri-HRP and compound II prepared by addition of a twofold molar excess of ascorbate to a solution of compound I. The purity of the product in each reaction was confirmed from its optical spectrum recorded at 4 °C. The RR spectra were recorded on a SPEX instrument using a cooled, spinning sample cell and an argon ion laser for excitation.[13] The data were stored in digital form on disks. Curve smoothing, background subtraction, and subtractions of individual spectra were performed with a SPEX SCAMP.

Our RR experiments on HRP compounds I and II show that the species formed on laser irradiation depend markedly on the excitation wavelength, incident power, and also the physical state of the sample. For compound II in solution, excitation at low and
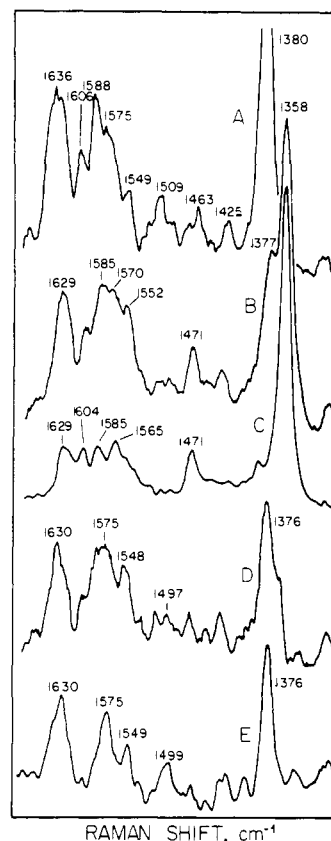
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Figure 2. RR spectra of various HRP species. All spectra are of frozen aqueous solutions (0.5 mM) at −5 °C excited at 457.9 nm and recorded with a spectral band-pass of 8 cm⁻¹ and an acquisition time of 15 min: (A) compound I, 10 mW; (B) compound I, 100 mW; (C) ferro-HRP, 30 mW; (D) spectrum B minus spectrum C; (E) ferri-HRP, 40 mW.

moderate powers with either 514.5- or 457.9-nm light produces RR scattering attributable to compound II itself.[7-9,12] The same holds true for frozen compound II using 514.5-nm excitation (20–100-mW power). However, RR excitation of frozen compound II with 457.9-nm light gives spectra that depend markedly on the incident power and irradiation time. The spectrum in Figure 1A was obtained with 20 mW of 457.9-nm laser light and agrees well with that obtained for solution-phase compound II excited at this wavelength. In particular, compound II displays a characteristic band due to mode ν_4 at 1380 cm⁻¹.

When frozen compound II is irradiated at this wavelength with 100 mW, time-dependent changes in the RR spectrum are observed. After 1 h, the changes are complete and spectrum 1B is obtained which displays ν_4 bands at 1377 and 1358 cm⁻¹. These bands are characteristic of ferri-HRP and ferro-HRP, respectively. Figure 1C shows a spectrum of frozen ferro-HRP with band ν_4 scaled to be equal in height to that of the 1358-cm⁻¹ band in spectrum 1B. Subtraction of spectrum 1C from 1B gives spectrum 1D, which is nearly identical with that of frozen ferri-HRP shown in Figure 1E. If the frozen photolyzed sample that gave rise to spectrum 1B is allowed to come to room temperature, the ferro-HRP is oxidized to ferri-HRP by atmospheric oxygen and the spectrum of ferri-HRP alone is obtained.

Similar experiments have been carried out with compound I. Irradiation of compound I in solution with either 514.5- or 457.9-nm laser light at low or high powers results in its photoreduction to a species whose RR spectrum is almost identical with that of compound II.[7,8] Irradiation of frozen compound I at 457.9 nm at low (20 mW) power gives the spectrum in Figure 2A, which is very similar to that of compound II. Prolonged irradiation at low power or immediate excitation at higher (100 mW) power gives spectrum 2B, which is very similar to that obtained on irradiation of frozen compound II for longer periods of time (Figure 1B). Accordingly, it is shown in Figure 2C–E that

spectrum 2B is a superposition of the spectra of ferri-HRP and ferro-HRP. Thus, irradiation of frozen compound I with 457.9-nm light results in photoreduction first to compound II and ultimately to a mixture of ferri-HRP and ferro-HRP. However, the reaction to produce the two latter species occurs much more rapidly when compouned I is the starting species compared to compound II.

Our finding that B-band excitation of frozen compound I causes extensive photoreduction necessitates a reinterpretation of previous RR experiments. Teraoka and associates[14] have reported RR spectra of frozen compound I obtained with excitation even closer to the B band (441.6 nm). These authors observed two ν_4 bands in the frozen samples at 1381 and 1359 cm^{-1} and a single band at 1377 cm^{-1} after warming the sample to room temperature. The band at 1359 cm^{-1} was attributed to compound I. However, on the basis of the data presented here, the ν_4 bands observed at 1381 and 1359 cm^{-1} in the frozen sample were due to compound II and ferro-HRP, respectively, while that at 1377 cm^{-1} in the warmed sample was due to ferri-HRP. The ν_2 band attributed to compound I at 1590 cm^{-1} arises from compound II and the ν_{10} band at 1636 cm^{-1} is the superposition of that of compound II at 1640 cm^{-1} and that of the peripheral vinyl $\nu(C=C)$ band of ferro-HRP at 1629 cm^{-1}. The iron–His stretching band reported for compound I at 248 cm^{-1} arises instead from that of ferro-HRP. Thus, all of the spectral features attributed to compound I can be accounted for by the photoreduction products described here. Therefore, cryogenic stabilization of compound I is not sufficient to overcome its photolability[15] and RR arguments to support its structure must wait until a method is found of obtaining its spectrum unequivocally. Clark and associates have observed a similar photoreduction of ferrylmyoglobin to ferromyoglobin[18] and recently Kitagawa and associates have reported that HRP compound II is photolabile in solution when excited at 406.7 nm.[19] Thus, photolability is apparently a general property of oxidized forms of heme proteins.

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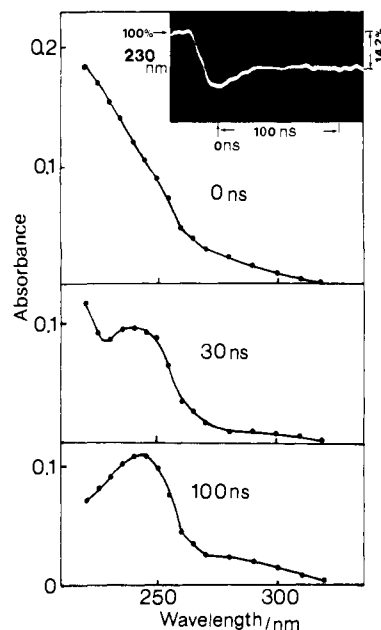
Figure 1. Time-resolved absorption spectra of phenyl radical produced by photolysis of 2.0 torr of chlorobenzene at 193 nm in the presence of 800 torr of nitrogen. A typical oscillogram of the transient absorption at 230 nm is inserted.
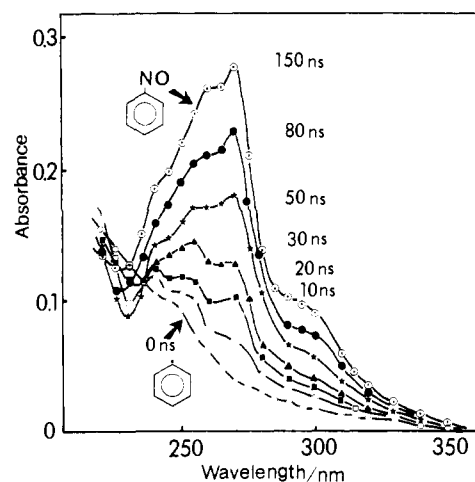

Figure 2. Time-resolved absorption spectra of 3 torr of chlorobenzene in the presence of 20 torr of nitrogen monoxide and 800 torr of nitrogen.

Observation of the Ultraviolet Absorption Spectrum of Phenyl Radical in the Gas Phase

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Although the phenyl radical is the simplest aromatic radical, its UV absorption spectrum has not yet been established. According to the pioneering works with flash photolysis by Porter and Ward, phenyl radicals have weak absorption bands around 430–530 nm, which are assigned to the n ← π transitions.[1] No other absorption band was found between 290 and 700 nm by them. From ESR studies, the phenyl radical is known to be an n-radical but not a π-radical[2,3] and has almost the same π-electronic system as the parent molecule. It is, therefore, expected that some absorption bands corresponding to the B_{2u} and/or B_{1u} states of benzene exist in the UV region around 250 nm. By pulse-radiolysis technique weak absorptions were found around 255–260 nm in solution and were suggested to be those of phenyl radicals,[4,5] but those spectra were obtained in an indirect way:

a difference spectrum of individual measurements in the presence and absence of radical scavenger, N_2O, in halogenated benzene solutions.

In this paper, we report for the first time the UV absorption spectrum of phenyl radical in the gas phase directly obtained by nanosecond laser photolysis.[6] Figure 1 shows time-resolved absorption spectra upon excitation of chlorobenzene to the S_3 state at 193 nm. In the photochemistry of chlorobenzene, the quantum yield of phenyl radical has been reported to be close to unity at 184.9 nm with a decomposition rate more than 10^{13} s^{-1}.[9] The formation of phenyl radical is expected to be completed within the exciting pulse width (12 ns). Therefore, the absorption spectrum immediately after excitation must be due to phenyl radical.

In the presence of nitrogen monoxide as a scavenger, a characteristic absorption of nitrosobenzene appears by the reaction

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