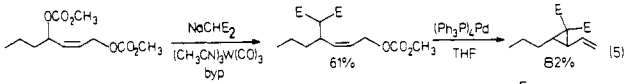
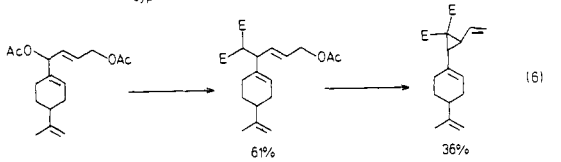
to recovered starting material. Simply inserting a single methylene group (i.e., **7**, $n = 1$) is sufficient to reduce the destablizing inductive effect to permit smooth alkylation.

The unusual ability to effect chemoselective displacements at a more substituted carbon led us to focus our efforts on the tungsten-catalyzed reaction. Equations **5** and **6** illustrate some



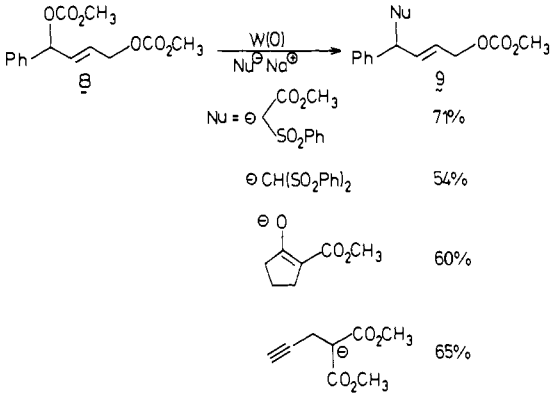
(5)



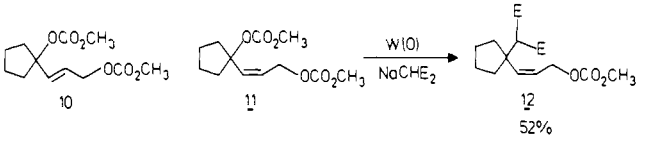
(6)

additional examples of the selective displacement and further substitution. Note the retention of olefin geometry in reactions 3 and 5 which contrasts to palladium-catalyzed reactions[9] where olefin geometry is frequently scrambled. In the disubstituted example of eq 6, the diacetate sufficed. While these last two examples employed the acetonitrile complex of tungsten, we now prefer the propionitrile complex because of its better stability and higher catalytic activity.

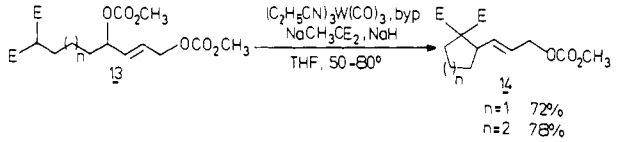
A wide range of nucleophiles has been examined by using the dicarbonate **8**. In each and every case, chemo- and regioselective alkylation occurred to give **9**.[4] The success of the acetylenic



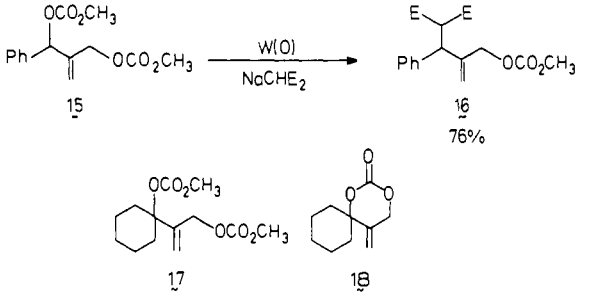
malonate stands in contrast to molybdenum-catalyzed reactions[10] whereby the presence of an acetylene prevented alkylation reactions. Steric hindrance around the olefin may be important. Whereas the *E*-olefin dicarbonate **10** only returned starting material, the *Z*-olefin **11** led to the desired alkylation product **12** (52% yield) in addition to an elimination product (37% yield).



Initial attempts to extend this chemoselective alkylation reaction to a cyclization of **13** failed—only starting material was recovered. During our preliminary examination of the W(0) reactions, we determined that the order of addition of the nucleophile and electrophile was critical. As stated earlier, the nucleophile was added *prior* to the electrophile—a requirement that cannot be met in the case of **13** which, of necessity, adds both simultaneously. This dilemma was resolved by initially reacting the tungsten complex with dimethyl sodiomethylmalonate (1 equiv with respect to substrate) and then adding the sodium salt of the cyclization substrate generated by treating **13** with sodium hydride. While, obviously, the possibility of an intermolecular alkylation arose, no such products were detected. Excellent chemo- and regioselective cyclization occurred to form the five-[11] and six-membered rings **14**,[4] $n = 1$ and 2.



Other types of dicarbonates may be envisioned to require similar chemoselectivity. Thus, **15** led smoothly to the monoalkylation product **16**.[4] On the other hand, the dicarbonate **17** or **18** failed to give any reaction whatsoever. Presumable the steric hindrance



of these latter systems precluces their ability to serve as substrates for tungsten. The demonstration that such subtle selectivity can be achieved by rational catalyst choice whereby the nature of the transition state of displacement may be altered from a S_N2 type to a S_N1 type encourages us to seek such solutions to other problems of chemoselectivity.

Acknowledgment. We thank the National Science Foundation for their generous support of our programs.

Supplementary Material Available: General procedures for the reactions **2** → **4**, **2** → **5**, **5** → **6**, and **13** → **14** (3 pages). Ordering information is given on any current masthead page.

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(11) In this case the cyclization product contained some of the transesterified dimer (carbonate related to **14**, $n = 1$).

Device for Simultaneous Measurements of Transient Raman and Absorption Spectra of Enzymic Reactions: Application to Compound I of Horseradish Peroxidase

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Application of resonance Raman (RR) spectroscopy to intermediates of enzymic reactions, is a fascinating subject since it brings about structural information essential to elucidate a catalytic mechanism,[1] but a problem arises when the intermediate is photolabile. For compound II of horseradish peroxidase (HRP) with a ferryl heme,[2] which is the second intermediate in the reaction of ferric HRP with H_2O_2, the RR spectra reported by several groups[3-6] are in agreement with each other and the

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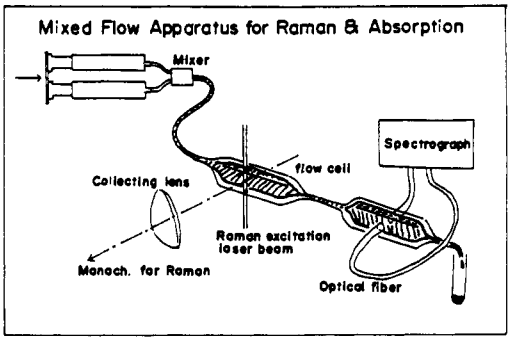
Fe^{IV}=O stretching Raman line was identified.[5a,6a] However, the RR spectra of the first intermediate, compound I, with the oxidation state higher than that of compound II by one oxidative equivalent, are controversial; we obtained its RR spectrum through cryogenic measurements,[7] but later Van Wart and Zimmer[8] claimed that the reported spectrum was of ferrous HRP, generated by photoreduction of compound I. Although compound I had been noticed to be photolabile,[9] the absorption maximum (λ_{max}) of the photoproduct reported at 416 nm[9] was quite different from λ_{max} of ferrous HRP (437 nm).[10] On the other hand, Oertling and Babcock[11] obtained the RR spectra of compound I by using a 10-ns pulse laser under the assumption that the rate of photoreduction would be much slower than 10 ns. Since the photon density in their laser pulse was ca. 10^7-fold higher than that used for the other two measurements, although the average powers were similar, a possibility of photoreduction cannot be completely excluded. To overcome this problem we constructed a device to monitor the transient Raman and absorption spectra at the same time for a flowing sample and applied it successfully to compound I of HRP.

The inset of Figure 1 illustrates the device; it consists of two glass syringes held in a copper jacket, a three-exit jet mixer, and two rectangular sprasil cells (height 5 mm, thickness 0.3 mm). The enzyme and substrate solutions in each syringe, which are kept at 5 °C by circulating thermostated water through the holder, are pushed out simultaneously by motor-driven pistons, and the mixed solution flows with the rate of 5–20 mL/min to the first cell, where Raman scattering is observed. The excitation light is focused by a cylindrical lens so as to illuminate the whole flowing sample. Since the beam width is ~50 μm, the residence time of an arbitrary molecule in the laser beam is 0.9–0.23 ms. Just after the Raman excitation, the solution passes through the other flat cell, which is sandwiched by a bundle of optical fibers; seven fibers with a 0.4-mm ϕ are arranged straightly, and the optical axis of the two bundles are adjusted with an x–y–z stage. At the other ends, the optical fibers are arranged in a hexagonal form so that the probe light could be easily focused into the optical fibers and returned to the original path in the sample room of an ordinary spectrophotometer (Hitachi 220S). The dead time after mixing is 6.6–1.7 s, and the interval between the Raman and absorption measurements is 7.9–2.0 s.

Isozyme C of HRP (Toyobo, grade I-C) with the purity parameter (A_{408}/A_{280}) of 3.2 was dissolved in 50 mM potassium phosphate buffer (pH 7.1). Raman scattering was excited at 406.7 nm with a Kr^+ ion laser (Spectra Physics, 165-03) and detected with a Reticon diode array (PAR 1420) attached to a double monochromator (Spex 1404).

Traces a and d in Figure 1 show the absorption (Soret band) and RR spectra, respectively, of the resting enzyme in the flowing cell. When 1.5 equiv of H_2O_2 was mixed with the resting HRP, the color changed from brown to green. Traces b and e are its absorption and Raman spectra, respectively. Reduction of absorbance in spectrum b with λ_{max} at 400 nm is characteristic of compound I, and there is no absorption band around 419 nm, where strong absorption is expected for compound II.[10] This strongly suggests that the sample which gave Raman spectrum e hardly contains compound II. However, it may contain a small amount of unreacted HRP with λ_{max} at 402 nm. Due to proximity of its λ_{max} to the excitation wavelength, the RR spectrum of the unreacted species is strongly resonance enhanced and its contribution to spectrum e cannot be neglected. Therefore, spectrum d was subtracted from spectrum e, in which a coefficient was adjusted so as to yield no negative peak. The resultant spectrum is depicted in Figure 2A.



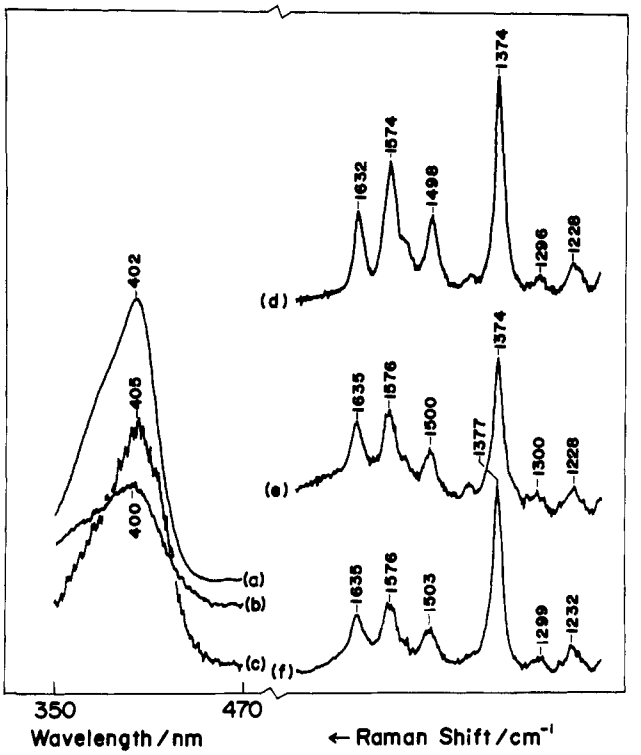


Figure 1. Absorption (a–c) and RR spectra (d–f) of HRP in the flowing cells: (a) and (d), resting HRP (200 μM); (b) and (e), compound I obtained by mixing the resting enzyme (200 μM) with H_2O_2 (300 μM); (c) and (f), compound II obtained by mixing compound I (100 μM) with ferrocyanide (100 μM). The flow rates for the spectral sets of (a) and (d), (b) and (e), and (c) and (f) are 0, 5, and 5 mL/min, respectively. Instrumental conditions for absorption measurements: scan speed, 120 nm/min; slit width, 2 nm; time constant, 0.5 s. The ordinate full scales were changed to be 0.6, 0.3, and 0.15 for spectra a, b, and c, respectively, so as to compensate the reduction of concentration upon mixing. Therefore, the three traces reflect the relative absorption intensities in practice. Instrumental conditions for Raman measurements; laser, 406.7 nm, 6 mW at sample point; accumulation time, 200 s for spectra d and f and 90 s for spectrum e. The residence time of an arbitrary molecule in a laser beam is 0.9 ms for spectra e and f, and the time interval between the Raman and absorption measurements is 7.9 s. The device used to monitor the transient Raman and absorption spectra at the same time (see text) is illustrated.

Traces c and f in Figure 1 were obtained when equimolar amounts of compound I and ferrocyanide were mixed. In contrast to its behavior at alkaline pH, compound II is not so stable at neutral pH in that it partially returns to the native state. To obtain the spectrum of compound II for this sample, spectrum d was subtracted from spectrum f in a similar way. The results are shown in Figure 2B. The frequencies of Raman lines for compound II are in reasonable agreement with those reported by others.[3,4,8] We

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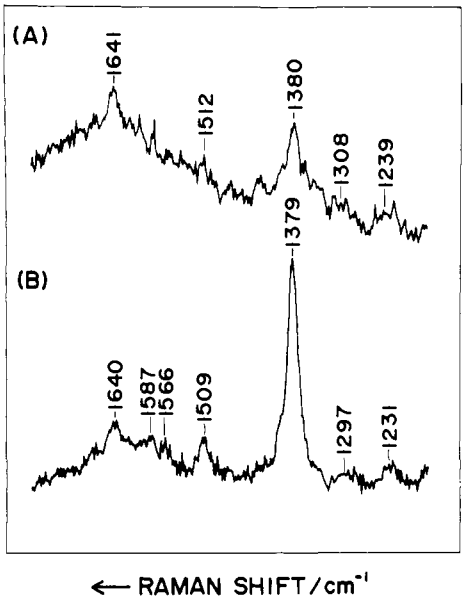

Figure 2. RR spectra of compound I (A) and compound II (B) of HRP obtained by subtracting the contribution of the ferric enzyme from the transient spectra shown in Figure 1: spectrum A = spectrum e - (0.23 × spectrum d); spectrum B = spectrum f - (0.25 × spectrum d).

note that the relative intensity of Raman lines at 1640 and 1379 cm^{-1} is distinctly different between parts A and B in Figure 2, although their frequencies are unexpectedly close.

Previously Van Wart and Zimmer[8] observed the spectrum of compound I in frozen solution with low laser power, but due to its similarity to the spectrum of compound II, they attributed their observations to a photoreduced species of compound I, that is, compound II. As noted above, the present sample does not contain compound II. If compound I was photoreduced to the ferrous state and it reacted with O_2, then compound III with $\lambda_{max} = 417$ nm might be formed.[12] However, such a possibility is ruled out from the absence of an absorption band around 417 nm in trace b in Figure 1. These considerations lead us to propose that spectrum A in Figure 2 arises from compound I of HRP and to admit that our cryogenic measurements reported previously yielded the photoreduced ferrous species.

HRP compound I has been considered to contain the a_{2u} porphyrin π-cation radical on the basis of the absorption spectra of M(OEP) derivatives (OEP = octaethylporphyrin).[13] Recently, Spiro and co-workers[14] succeeded in categorizing the RR spectra of cation radicals of M^{II}(OEP); the a_{1u} radical exhibits positive (14 to 21 cm^{-1}) and negative (-19 to -38 cm^{-1}) shifts for the ν_4 and ν_2 modes, respectively, whereas the a_{2u} radical exhibits negative (-12 to -22 cm^{-1}) and positive (20 to 23 cm^{-1}) shifts for them upon formation of a cation radical. Accordingly, it was expected that the ν_4 band around 1380 cm^{-1} of compound II exhibited a negative shift in compound I. Nevertheless, there is no prominent frequency shift between parts A and B in Figure 2. One likely reason for this insensitivity is that a clear cation-radical state as seen for M^{II}(OEP) is not generated for compound I. This would mean that the oxidative equivalent is extensively delocalized through iron and axial ligands. An alternative explanation assumes that the shifts expected for the formation of the cation radical of Fe^{IV} porphyrin are much smaller than those for M^{II}(OEP). This possibility arised from the facts that the amount of frequency shifts upon the formation of cation radicals are noticeably metal-dependent[14] and that there has been no experimental data for iron porphyrins. Thus the RR spectra cannot distinguish between the a_{1u} and a_{2u} radicals for compound I. However, it has been demonstrated that the device presented here is very useful in a general sense and rather necessary for discussing the RR spectra of a transient species of enzymic reactions.

Registry No. Peroxidase, 9003-99-0.

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A Two-Photon Study of the "Reluctant" Norrish Type I Reaction of Benzil[1]

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During the last few years the applications of pulsed lasers in industry and in research have increased dramatically. It is now recognized that in some cases laser and conventional (e.g., lamps or sunlight) irradiation can lead to different chemical consequences, even when the total energy and wavelength used may have been the same. A few studies have been concerned with the transient phenomena associated with these differences,[2-8] and occasionally the "laser vs. lamp" effects on products have been examined.[3a,7,9] There are, however, few examples where products and transient phenomena have been examined as part of the same study.[3a,7]

The laser vs. lamp differences are frequently related to the involvement, directly or indirectly, of multiphoton processes under laser irradiation. In our work we have found it desirable to identify systems where two-photon processes would take place, but where no monophotonic chemistry occurs. Compounds with these characteristics could find wide application as laser-photosensitive materials, but where no protection from normal illumination (e.g., room lights or sunlight) is necessary.

In this paper we report preliminary results on the photochemistry of benzil (Ia), which has been reported to be essentially photostable at room temperature[10] but which under conditions of laser irradiation undergoes the Norrish type I cleavage, reaction 1.

Irradiation of benzil (5×10^{-4} M in benzene) with the 308-nm pulses from an excimer laser (~5 ns, ≤50 mJ/pulse)[11] leads to

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