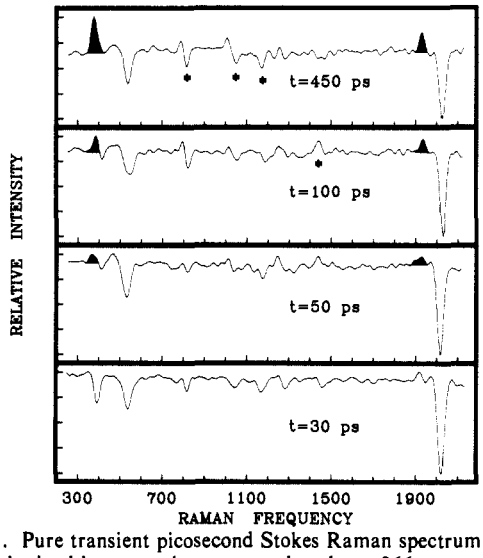

Figure 1. Pure transient picosecond Stokes Raman spectrum in cyclohexane obtained by two-pulse pump and probe at 266 nm as described in the text. Time delay between pump and probe lasers is given separately in each frame. The colored-in bands are those assigned to solvent-coordinated $Cr(CO)_5$. Asterisks are used to denote noise due to Raman bands of the solvent molecules which have been subtracted out of each spectrum. Laser intensity was 20 μJ/pulse at 2 kHz in a 0.2-mm beam waist. This laser intensity is 2 times less than that used in the previous transient infrared experiments.[4,5] Concentration is 10 mM. Frequency is in units of cm^{-1}.

cm^{-1} indicate that the transient is a metal carbonyl complex, which is assigned to $Cr(CO)_5$. The bands marked with asterisks are the result of noise generated by the spectrum differencing technique at the frequencies of the cyclohexane solvent bands. The negative peaks are ground-state $Cr(CO)_6$ bands which appear in the transient spectrum as a result of population bleaching. The ground-state 383-cm^{-1} metal-CO stretch of $Cr(CO)_6$ appears as a bleach in the 30-ps spectrum and gradually fills in at later times due to the growth of the $Cr(CO)_5$ transient band at 381 cm^{-1}.

The dynamics of vibrational cooling can be investigated by comparing the Stokes and anti-Stokes band intensities. Figure 2 illustrates the *transient* anti-Stokes spectrum in the region of the 381-cm^{-1} band assigned to $Cr(CO)_5$. The results indicate that the anti-Stokes spectrum from the hot vibrational state decays in 100 ps. The observation that the anti-Stokes and Stokes spectra have complementary dynamics is consistent with vibrational relaxation. The appearance of thermally equilibrated $Cr(CO)_5$ is therefore believed to represent the time required for the photoproduct to approach thermal equilibrium with the solvent. This conclusion clearly demonstrates the importance of nonequilibrium vibrational energy in condensed-phase photochemistry and provides an alternative explanation for the dynamics observed in the transient infrared[4,5] experiments.

It is interesting to note that the time scale we observe for complete vibrational relaxation is approximately the same as that observed[11] for the CO stretching vibration in $Cr(CO)_6$. In that experiment, a relaxation time of 145 ± 25 ps was observed in *n*-hexane. Similar rates would be expected in these two experiments if the latter dynamics represent the time required for energy randomization followed by vibrational relaxation through the entire manifold of vibrational levels. There is no direct way to compare our results to the faster dynamics attributed to vibrational decay in the transient absorption[2,6] experiments. It is likely that the latter results represent initial decay from upper vibrational levels which are difficult to characterize by using electronic absorption spectroscopy.

In summary, results are presented that demonstrate the importance of vibrational energy in the photodissociation of $Cr(CO)_6$. A single Stokes transient is observed to appear at a rate similar to the decay rate of a species with a vibrationally hot anti-Stokes spectrum. The probe wavelength (266 nm) should[14] be equally sensitive to detection of both naked $Cr(CO)_5$ and $Cr(CO)_5{\cdot}C_6H_{12}$. Even so, we do not find a thermally equilibrated precursor to the

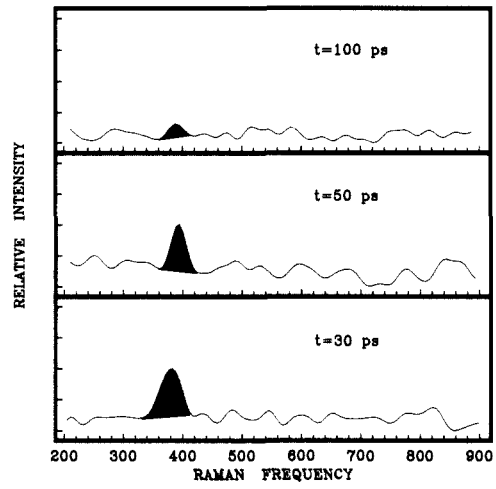

Figure 2. Transient anti-Stokes Raman spectrum obtained under similar conditions to those given in Figure 1. Time delay between pump and probe pulses is given separately in each frame. Ground-state bands have been subtracted out of the spectrum as described in the text. Spectra are normalized to the intensity of the ground-state chromium band at 532 cm^{-1} in the unsubtracted spectrum. Frequency is in units of cm^{-1}.

final product. This suggests that one of the following two reaction schemes[15] is operative. Here # indicates nonthermal vibrational energy and ·S solvent coordination.

$$Cr(CO)_5\# \xrightarrow{100\ ps} Cr(CO)_5 \xrightarrow{fast} Cr(CO)_5{\cdot}S \quad (1)$$

$$Cr(CO)_5\# \xrightarrow{fast} Cr(CO)_5\#{\cdot}S \xrightarrow{100ps} Cr(CO)_5{\cdot}S \quad (2)$$

In both mechanisms, vibrational relaxation is the rate-limiting step and is found to be remarkably long, requiring over 100 ps for complete thermalization.

(15) The anti-Stokes and Stokes vibrational frequencies for the transient species are identical and therefore suggest that both can be assigned to the solvated pentacarbonyl indicating that eq 2 is correct. However, we do not know how much solvent coordination will shift the Raman frequencies and cannot rule out eq 1.

Identification of a Unique Glutathione Conjugate of Trichloroacrolein Using Heteronuclear Multiple Quantum Coherence ^{13}C Nuclear Magnetic Resonance Spectroscopy

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The proton-detected heteronuclear multiple quantum coherence (HMQC) NMR experiment is a unique spectroscopic technique that permits the assignment of proton-heteroatom connectivity in a variety of nuclear environments.[1] Proton-detected HMQC methods, typically used for two-dimensional applications,[2] provide significant increases in sensitivity over conventional ^{13}C- and ^{15}N-detected experiments. We describe here a one-dimensional application of the proton-detected HMQC experiment for the assignment of proton-carbon connectivity in a xenobiotic animal metabolite. This method offers a powerful approach to metabolite

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(2) Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565.

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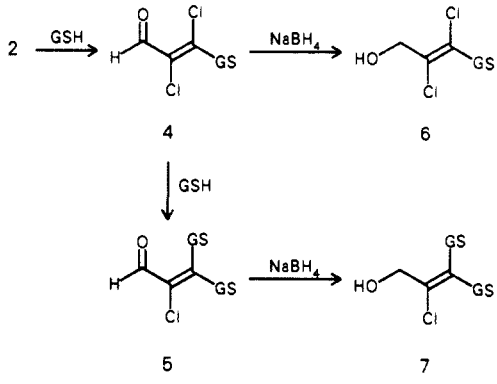
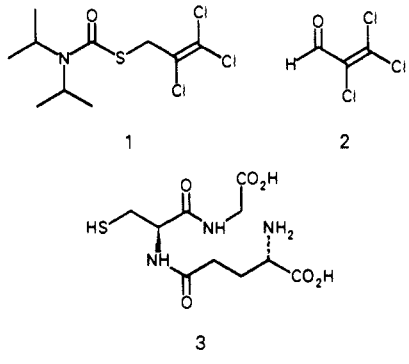
Scheme I



structure assignment in situations where ordinary proton NMR spectra are difficult to interpret and where alternative analytical methods are unavailable.

Triallate (**1**) is an established thiocarbamate herbicide used for the control of wild oats in crops. Although the xenobiotic metabolism of triallate was postulated some time ago to involve trichloroacrolein (**2**) as a reactive metabolic intermediate,[3] the latter has not been isolated in either live animal or in vitro experiments.[4] We thought that confirmatory evidence for the intermediacy of **2** might be obtained by identifying metabolites arising via its reaction with biological nucleophiles such as glutathione (**3**). Use of the HMQC ^{13}C NMR experiment has enabled us to realize this objective.



Acrolein undergoes 1,4-addition with glutathione (GSH) and other sulfur nucleophiles,[5] and we considered that **2** should undergo analogous addition–elimination reactions with glutathione. When a solution of GSH (0.1 mM) in water at pH 8.5 was treated with synthetic **2** (0.5 molar equiv),[6] the mono and bis GSH adducts **4** and **5** were rapidly formed (Scheme I), as observed by ^1H NMR. These aldehyde adducts were reduced to the allylic alcohols **6** and **7** by workup with sodium borohydride, and the latter were isolated by reverse-phase HPLC (Beckman Ultrasphere ODS, linear gradient of acetonitrile/1% acetic acid, UV detection at 254 nm). The structures of **6** and **7** were confirmed by fast atom bombardment (FAB) mass spectroscopy in a glycerol matrix.[7] The 500-MHz ^1H NMR spectrum of the bis GSH adduct **7** is illustrated for the chemical shift region of 3.6–5.6 ppm (Figure 1a), where proton resonances arising from both the GSH[8] and allylic alcohol moieties are observed.

The metabolic production of trichloroacrolein (**2**) from triallate is likely to occur via cytochrome P-450 oxidation at the allylic

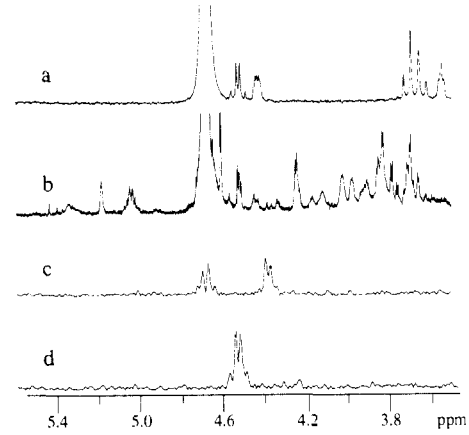
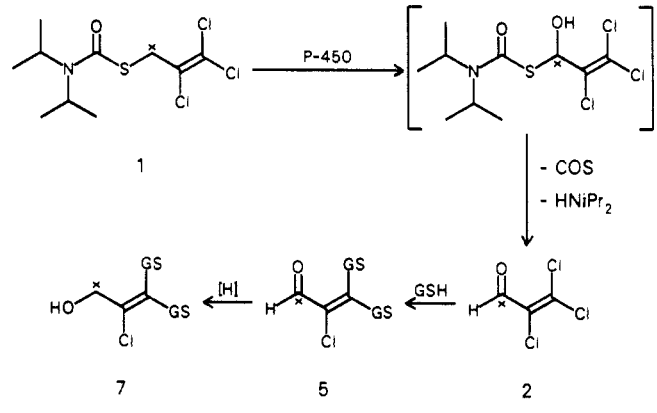


Figure 1. Comparison of 500-MHz proton NMR spectra of (a) the synthetic standard **7**, number of transients = 1024; (b) the isolated in vitro fraction containing ^{13}C-labeled metabolite **7**, number of transients = 8192; (c) the same metabolite fraction detected in the one-dimensional ^{13}C-coupled HMQC experiment, number of transients = 8192; and (d) the same metabolite fraction detected in the WALTZ-decoupled one-dimensional HMQC experiment, number of transients = 5120. Spectra a and b were acquired by using a standard single-pulse sequence and are displayed in a phase-sensitive mode. Spectra c and d were acquired by using the pulse sequence of Bax et al.[1b] (acquisition time t_2 = 0.25 s; recycle delay = 1.5 s; evolution time = 0 s; delay time $\Delta = 1/(2J_{CH})$, J_{CH} = 140 Hz) and are displayed in an absorption mode.

Scheme II



position (Scheme II).[3] To test this hypothesis and also trap generated **2**, we incubated triallate under standard conditions with a liver microsomal P-450 enzyme preparation obtained from uninduced male Sprague–Dawley rats, fortified with glutathione and NADPH.[9] The triallate used for this experiment was labeled in the allylic position with ^{13}C (90% enrichment) to permit NMR spectroscopic analysis, and with ^{14}C (5% enrichment, specific activity 1.79 mCi/mmol) to facilitate reaction analysis and product separation by HPLC with radioactivity detection. Reactions were quenched by precipitating proteins and lipids with methanol, followed by centrifugation.

The major metabolite fraction produced in such in vitro reactions corresponded by HPLC retention time to the authentic allylic alcohol **7**. However, FAB mass spectrometric analysis of this HPLC-purified fraction was unsuccessful because of coeluting reaction matrix components.[10] The simple proton NMR spectrum of this chromatographed material (45 μg) was consistent with the presence of glutathione, but was obscured by numerous extraneous peaks (Figure 1b). Attempts to suppress the HOD resonance at 4.76 ppm also saturated adjacent peaks of interest. The ^1H–^{13}C coupling pattern expected for this isotopically labeled sample thus could not be observed by means of a simple proton NMR spectrum.

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(7) FAB MS identification of GSH conjugates is described in the following: (a) Fujiwara, H.; Wratten, S. J. In *Applications of New Mass Spectrometry Techniques in Pesticide Chemistry*; Rosen, J. D., Ed.; Wiley: New York, 1987; p 128. (b) Pallante, S. L.; Lisek, C. A.; Dulik, D. M.; Fenselau, C. *Drug Metab. Dispos.* **1986**, *13*, 313.
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(9) Feng, P. C. C.; Patanella, J. E. *Pestic. Biochem. Physiol.* **1988**, *31*, 84.
(10) For a discussion of matrix interference effects in FAB MS, see: Hoffmann, K.; Baillie, T. A. *Biomed. Mass Spectrom.* **1988**, *15*, 637.

We have found that the HMQC NMR technique in a one-dimensional format[11] can be used to visualize the methylene protons directly bonded to the labeled allylic ^{13}C carbon atom of metabolite 7, without interference from matrix resonances arising from protons attached to ^{12}C and natural abundance ^{13}C carbon atoms. The same metabolite sample as illustrated in Figure 1b was analyzed with a Varian VXR 500 FT NMR spectrometer equipped with an indirect-detection probe. Acquisition of the HMQC carbon-coupled proton spectrum (Figure 1c) was completed in 6 h, much less than the time required for a conventional proton-coupled ^{13}C NMR spectrum. The methylene protons attached to the ^{13}C-labeled carbon of 7 appear as a doublet of multiplets centered about 4.53 ppm (J_{CH} = 148.5 Hz), while the HOD and other extraneous peaks are completely filtered out. Acquisition with WALTZ carbon decoupling eliminated one-bond heteronuclear coupling and provided the expected single multiplet at 4.53 ppm (Figure 1d). These spectra unambiguously confirm the structure of the isotopically labeled metabolite 7, enabling us to demonstrate that trichloroacrolein (2) is generated metabolically from triallate (1). Further studies elucidating the in vitro and in vivo metabolic pathways of triallate xenobiotic metabolism are in progress and will be reported in detail elsewhere.

In conclusion, we have shown that the proton-detected HMQC ^{13}C NMR experiment can be used to elucidate metabolite structures on a sample scale previously unattainable by conventional NMR techniques. This method provides important data on proton connectivity and heteronuclear coupling in the context of a simple experimental design which has the advantages of matrix transparency as well as greatly enhanced sensitivity relative to ordinary ^{13}C NMR spectroscopy. We anticipate that the HMQC ^{13}C NMR experiment will complement mass spectrometry as a major research tool in metabolism chemistry.

Acknowledgment. We thank Dr. Sastry Kunda for providing us with samples of isotopically labeled triallate.

(11) Applied with a nonincremental evolution time of 0.

Direct Selective Acylation of an Unactivated C-H Bond in a Caged Hydrocarbon. Approach to Systems for C-H Bond Functionalization That Proceed Catalytically and Selectively at High Substrate Conversion

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Homogeneous or heterogeneous systems that effect the replacement of unactivated C-H bonds catalytically and with high selectivity at high conversion of substrate are virtually unknown. In nearly all systems that effect oxidative functionalization of unactivated C-H bonds, the products are more reactive than the substrate.[1-11] We report here a systematic exploitation of the relative rates of photooxidation and quenching of the excited state of a complex, $W_{10}O_{32}^{4-}$, by different organic functions to effect the replacement of unactivated C-H bonds with C-C bonds in high selectivity at a reasonable conversion of substrate. Since derivatives of caged hydrocarbons are of current interest as medicinals[12] and energetic materials,[13] we chose the compound heptacyclo[6.6.0.0^{2,6}.0^{3,13}.0^{4,11}.0^{5,9}.0^{10,14}]tetradecane, commonly referred to as HCTD (1), as the substrate. Direct functionalization of this strained polycyclic hydrocarbon represents a formidable challenge as its C-H bonds are stronger than those in acyclic alkanes and its carbocyclic skeleton is susceptible to oxidative degradation. Although stoichiometric oxidation of 1 to a mixture of alcohols has recently been achieved using $Pb(OAc)_4$,[14] other attempts thus far to effect a clean direct functionalization of 1 in our laboratory and elsewhere using conventional methods have failed.[15]

Irradiation of acetonitrile solutions of 1 containing Na^+ or n-Bu_4N^+ salts of decatungstate, $W_{10}O_{32}^{4-}$, under Ar at 15 °C leads

Table I. Photochemical Functionalization of a Caged Hydrocarbon, 1, by $Na_4W_{10}O_{32}$ and $Q_4W_{10}O_{32}$ under Anaerobic and Aerobic Conditions

	polyoxometalate[a]	% conversion[b]	product (selectivity)[c]
	I. Single Irradiation, Anaerobic Conditions[d]		
1	$Na_4W_{10}O_{32}$	53	**2** (81)
2	$Q_4W_{10}O_{32}$	24	**2** (84)
	II. Irradiation, Anaerobic Conditions/Dark O_2 Reoxidation Cycles[e]		
3[f]	$Na_4W_{10}O_{32}$	62	**2** (77)
4[f]	$Q_4W_{10}O_{32}$	38	**2** (88)

[a] Q = n-Bu_4N^+. [b] (Moles of 1 consumed/moles of 1 before reaction) × 100. [c] Selectivity defined as moles of 2 produced/moles of all detectable HCTD-derived products. [d] Reaction conditions: 10 mL of a slightly wet acetonitrile solution 27 mM in 1 and 5.4 mM in polyoxometalate catalyst with 3 mg of Pt under argon at ~15 °C was irradiated with a 550-W Hg lamp (λ > 280 Pyrex cutoff) for x h; products identified and quantified by gas chromatography and GC/MS. Reaction 1: x = 128 h; catalyst partially soluble. Reaction 2: 112 h. [e] Reaction conditions were the same as those for part I. Irradiation was terminated at 16-h intervals. The reactions were sequentially (a) placed under air to reoxidize the catalyst, (b) degassed and placed under argon, and then (c) irradiated again. [f] Reactions 3 and 4 run for seven 16-h cycles, total time 112 h; reaction 3 catalyst partially soluble.

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(2) References 3-11 are recent reviews or representative papers on several types of alkane activation or functionalization systems.

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