of the added label. Very recently, 3-amino-5-hydroxybenzoic acid has been shown to be incorporated into mitomycin[15] and the ansamycin antibiotics actamycin[16] and rifamycin S[17] and, most pertinently, to be a part of the starter unit for rifamycin S.[18] A decision as to whether 3-amino-5-hydroxybenzoic acid is also a precursor of *m*-aminobenzoic acid must await the outcome of additional experiments.

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(15) Anderson, M. G.; Kibby, J. J.; Rickards, R. W.; Rothschild, J. M. *J. Chem. Soc., Chem. Commun.* **1980**, 1277–1278.

(16) Kibby, J. J.; McDonald, I. A.; Rickards, R. W. *J. Chem. Soc., Chem. Commun.* **1980**, 768–769.

(17) Ghisalba, O.; Nüesch, J. *J. Antibiot.* **1981**, *34*, 64–71.

(18) Ghisalba, O.; Fuhrer, H.; Richter, W. J.; Moss, S. *J. Antibiot.* **1981**, *34*, 58–63.

Electron Spin Resonance–High-Performance Liquid Chromatography Study of Organometallic Free Radical Reactions: Separation and Characterization of the Rhenium Carbonyl-*o*-Quinone Radical Complex

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The successful use of high-performance liquid chromatography (HPLC) to separate nitroxide and other organic oxygen- and nitrogen-centered radicals has recently been reported.[1,2] Because of the wide potential applications of HPLC to chemical, biochemical, and organometallic studies and the established technique of ESR in characterizing free radicals and paramagnetic intermediates involved in chemical reactions, we have currently initiated a series of investigations of organometallic free radical reactions in solution by using an integral general-purpose HPLC–ESR apparatus recently assembled in our laboratory. We present here our first successful application of this HPLC–ESR technique to the separation and characterization of an interesting organometallic radical complex, the rhenium carbonyl-3,5-di-*tert*-butyl-*o*-quinone complex which is readily formed during the photolysis of a benzene solution containing both the parent quinone and $Re_2(CO)_{10}$. The conclusive results shed some light on the primary photochemical process between the rhenium carbonyl and the quinone, but the demonstrated usefulness of the HPLC–ESR technique in organometallic free radical studies should be of wider general interest.

The principal components of the integral HPLC–ESR apparatus consist of a positive-displacement pump coupled to a homemade pulse dampener and a Valco sample-injection six-port valve. All columns were packed in our laboratory; the general-purpose column was packed with Whatman Partisil-10 and measured 3 mm × 60 mm. The components were mounted on a platform next to the ESR bridge of a Varian V4500 X-band ESR spectrometer with 100-kHz field modulation. The connection between the HPLC column and the ESR cavity is made by Teflon tubing which is coiled around a quartz tube inserted into the cavity. A standard radical sample placed in a capillary tube is inserted into the quartz tube in the cavity without disturbing the Teflon sample coil. The magnetic field is manually set by the standard sample and locked in by a Varian F-8A fluxmeter. The standard is then removed before the HPLC operation begins. A Narda GaAs microwave preamplifier was installed at the signal front end to enhance the detection sensitivity. Normally the HPLC–ESR apparatus is run in a dual-detector configuration with the sample solution routed from the ESR cavity directly into either a Varian SF330 double-beam spectrofluorometer or a Hilgawatt infrared spectrophotometer.

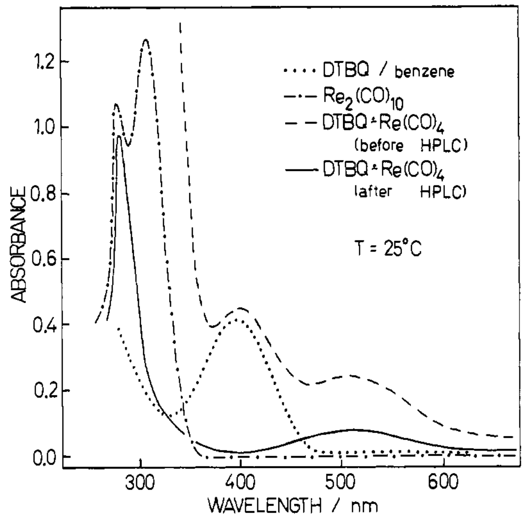
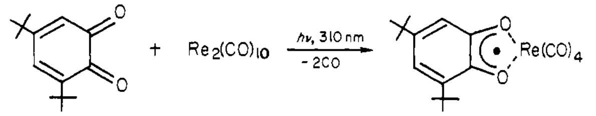


Figure 1. Electronic absorption spectra of DTBQ, $Re_2(CO)_{10}$, and the $\mathrm{DTBQ}\dot{-}\mathrm{Re(CO)_4}$ radical complex in benzene, before and after HPLC separation. The band at 278 nm is probably due to Re → CO charge transfer transition,[3] which should remain before and after the complexation with DTBQ.

When a green benzene solution containing $Re_2(CO)_{10}$ and 3,5-di-*tert*-butyl-*o*-benzoquinone (DTBQ) was irradiated by a 200-W mercury superpressure lamp equipped with a monochromator set at 310 nm and the solution was continuously degassed by nitrogen, the color of the solution was changed to red due to the formation of the $\mathrm{DTBQ}\dot{-}\mathrm{Re(CO)_4}$ radical complex.



(1)

HPLC was then used to separate the radical complex from the reactants and any other secondary products/radicals. The eluent of the column was monitored by ESR as well as by either IR or UV–vis absorption/emission spectrophotometry. Benzene was used as the eluting solvent. Typically and at a flow rate of 0.6 mL/min, the retention time for the $\mathrm{DTBQ}\dot{-}\mathrm{Re(CO)_4}$ radical complex is 130 s while that of the parent quinone is 410 s, as monitored by ESR and visible absorption, respectively. Evaporation of the eluent containing the radicals only leaves a paramagnetic solid, which is confirmed by ESR as the $\mathrm{DTBQ}\dot{-}\mathrm{Re(CO)_4}$ complex.

The electronic absorption spectra shown in Figure 1 illustrate the potential of HPLC to separate the organometallic radical complex from the parent compounds. The visible band of the quinone (λ_{max} 395 nm) is shifted upon complexation with the rhenium carbonyl to lower energy (λ_{max} 505 nm). This is expected as the quinone π electrons are delocalized onto the rhenium carbonyl moiety. The $\sigma_b \rightarrow \sigma^*$ band[3] of the $Re_2(CO)_{10}$ (λ_{max} ~308 nm) is not present in the complex, as expected.

The emission spectrum of the $\mathrm{DTBQ}\dot{-}\mathrm{Re(CO)_4}$ radical complex was recorded in benzene at 25 °C. It shows a mirror image relationship to the absorption spectrum, having band centers at

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(1) T. Reich, K. S. Chen, and J. K. S. Wan, *J. Chromatogr.*, **206**, 139 (1981).

(2) K. Makino, *J. Phys. Chem.*, **84**, 1012, 1016 (1980); K. Makino, N. Suzuki, F. Moriya, S. Rokushika, and H. Hatano, *Anal. Lett., Part A*, **13**, 301 (1980).

(3) M. S. Wrighton and D. S. Ginley, *J. Am. Chem. Soc.*, **97**, 2065 (1975).

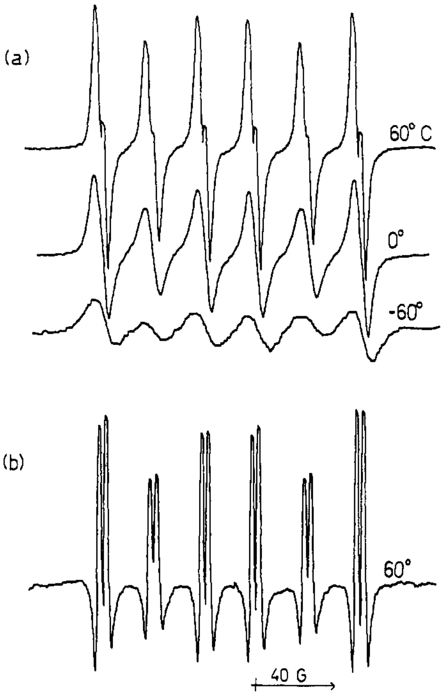

Figure 2. (a) First-derivative ESR spectra of the DTBQ$^{\cdot-}$Re(CO)$_4$ radical complexes in toluene at various temperatures; (b) second-derivative ESR spectrum of the same radical complex at 60 °C.

345 and 705 nm. This suggests that the planarity of the quinone moiety has been retained in the radical complex. As a comparison the emission spectrum of the parent DTBQ has band centers at 335 and 665 nm.

Comparison of the IR spectrum of the DTBQ$^{\cdot-}$Re(CO)$_4$ radical complex with those of the starting compounds (in KBr pellets) suggests that the carbonyl groups of the *o*-quinone have been drastically altered, whereas the carbonyl groups originally complexed to the rhenium retain much of their original character upon radical formation. This is inferred from the observation that the carbonyl stretch of the quinone at 1650 cm^{-1} completely disappears from the IR spectrum of the radical, while the carbonyl absorption region of Re$_2$(CO)$_{10}$ at ~2000 cm^{-1} changes very little. The C–H stretching region is very similar in both the parent quinone and the rhenium–quinone radical complex, which supports the assignment of the DTBQ$^{\cdot-}$Re(CO)$_4$ radical as having a planar quinone moiety with the Re complexing symmetrically to the two quinone oxygen atoms.

The first-derivative ESR spectrum of the radical complex at various temperatures is shown in Figure 2. The second-derivative spectrum at 60 °C, also shown in Figure 2, clearly exhibits the proton hyperfine splitting of 3.46 G, which by analogy to the uranyl–DTBQ complex[4] is assigned to the proton between the two *tert*-butyl groups on the quinone ring. The *g* factor of the DTBQ$^{\cdot-}$Re(CO)$_4$ radical complex was measured to be 2.003. The "average" Re hyperfine coupling constant is 28.2 G; the two isotopes of ^{185}Re (37.05%, $I = {}^5/_2$) and ^{187}Re (62.93%, $I = {}^5/_2$) could not be resolved, as their magnetic moments differ only by 1%. The low *g* factor of the radical complex is expected and is probably due to the large spin–orbit coupling of the Re atom.

A number of related diketone–rhenium carbonyl radical complexes have also been prepared and studied in our laboratory. These include the furil–rhenium carbonyl, the *o*-naphthoquinone–rhenium carbonyl, the tetrachloro-*o*-quinone–rhenium carbonyl, the tetrabromo-*o*-quinone–rhenium carbonyl, and the di-*tert*-butyl azodicarboxylate–rhenium carbonyl radical complexes. Two unusual features common to the ESR spectra of the rhenium radical complexes are the symmetrical broadening of the pair of lines corresponding to $m_I = {}^3/_2$ and the slightly unequal spacings among the rhenium lines, with the splittings at the high-field half being about 5% greater than the corresponding low-field half. These features are due to second-order effects, including both nuclear hyperfine and quadrupole coupling effects. A detailed ESR investigation of the rhenium–quinone radical complexes and other group 7B metal–ketone radical complexes is in progress.

The unique property of the DTBQ$^{\cdot-}$Re(CO)$_4$ (and other related ketone–rhenium complexes) radical is its extraordinary thermal stability. Even at high temperatures up to 80 °C, the radical complexes do not decay significantly. Planned experiments include both X-ray and ^{13}C NMR characterization of these interesting quinone–rhenium radical complexes.

Finally, the results also shed some light on the mechanism of the photoreaction of quinone and the rhenium carbonyl. If the benzene solution containing both reactants was irradiated at the n,π^* band of the *o*-quinone at 395 nm, no reaction was observed. However, if the photolysis was carried out at 310 nm, which is associated with the $\sigma_b \rightarrow \sigma^*$ band of the Re$_2$(CO)$_{10}$, the solution immediately turned red with the formation of the DTBQ$^{\cdot-}$Re(CO)$_4$ radical complex. This is consistent with the following reaction mechanism:

$$Re_2(CO)_{10} \xrightarrow{h\nu,\ 310\ nm} 2{\cdot}Re(CO)_5 \tag{2}$$

$$\cdot Re(CO)_5 + DTBQ \rightarrow DTBQ^{\cdot-}Re(CO)_4 + CO \tag{3}$$

The escape of CO from the solution during photolysis was also observable. In the present system, a photochemical charge transfer mechanism between Re$_2$(CO)$_{10}$ and DTBQ is unlikely, as quinone is not the photoactive reactant here and the quinone radical anion was not observed during the reaction.

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(4) D. A. Hutchinson, K. S. Chen, J. Russell, and J. K. S. Wan, *J. Chem. Phys.*, **73**, 1862 (1980).

An NMR Technique for Tracing Out the Carbon Skeleton of an Organic Molecule

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We propose a new method of establishing the connectivity of the carbon skeleton of an organic molecule, the first and most important step in a complete structural determination. The technique is based on an earlier NMR experiment[1,2] for studying carbon-13–carbon-13 spin–spin coupling which uses the momentary creation of double-quantum coherence[3-5] to suppress the strong signals from isolated carbon-13 spins and reveal the weak carbon-13 satellite spectrum. If the magnitudes of the coupling constants are sufficiently well differentiated, they may be assigned to specific pairs of carbon resonances simply by picking out the repeated splittings. Adjacent carbon sites are thus identified directly. However, in many cases the molecular framework is too complex for this simple method to be applicable—each carbon site can have up to four directly bonded neighbors, and the carbon–carbon splittings may be close in magnitude or not clearly resolved. Consequently an *independent* method of assignment is needed.

[†] On leave from the Delft University of Technology, Delft, The Netherlands.

(1) Bax, A.; Freeman, R.; Kempsell, S. P. *J. Am. Chem. Soc.* **1980**, *102*, 4845.
(2) Bax, A.; Freeman, R.; Kempsell, S. P. *J. Magn. Reson.* **1980**, *41*, 349.
(3) Hatanaka, H.; Terao, T.; Hashi, T. *J. Phys. Soc. Jpn.* **1975**, *39*, 835.
(4) Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229.
(5) Vega, S.; Pines, A. *J. Chem. Phys.* **1977**, *66*, 5624.

0002-7863/81/1503-2102$01.25/0 © 1981 American Chemical Society