in a site specific manner,[8] the ability to prepare duplexes containing isotopically labeled abasic sites will allow detailed investigation of their structural properties as well as their enzymatic and chemical reactivities.[18]

Acknowledgment. This research was supported by NIH GM-34573 to J.A.G. and P.H.B.

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FT ESR Study of Photoinduced Electron Transfer

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With Fourier transform ESR (FT ESR) well resolved spectra of organic free radicals can be obtained even if the free induction decay, following a $\pi/2$ microwave pulse, is as short as 1 μs.[1] This makes the technique particularly suitable for the study of short-lived radicals. Results are presented of a FT ESR study of a transient free radical generated in a reversible photoinduced electron transfer reaction. The reaction and spin dynamics have been monitored from the time the radical is generated with nanosecond time resolution. The technique provides data complementary to those provided by flash photolysis.

Figure 1 shows a series of FT ESR spectra of the duroquinone anion radical (DQ^-). The radical was generated by electron transfer from photoexcited zinc tetraphenylporphyrin (ZnTPP, 5×10^{-4} M) to duroquinone (5×10^{-3} M) in ethanol at 245 K.[2] Samples were degassed on a vacuum line by repeated freeze–pump cycles. A Lambda Physik FL2001 dye laser (Rhodamine B, 600 nm, 2 mJ) pumped by a Lambda Physik EMG103MSC excimer laser (pulse width 15 ns, rate 40 Hz) was used as excitation source. Sample volume exposed to the laser beam was approximately 0.1 mL. The FT ESR spectrometer has been described briefly in ref. 1. The spectra represent Fourier transforms of the sum of 10000 (10 μs long) FID's (data acquisition time 4 min) detected in sequential quadrature mode.[1]

Under steady-state conditions, the ESR spectrum of the DQ^- anion radical consists of 13 lines with a binomial intensity distribution and hyperfine splitting of 1.9 G.[4] A maximum of ten hyperfine peaks can be discerned in the spectra presented here. Figure 1 shows that a variation in time delay between laser and microwave pulses affects the overall signal amplitude. The delay also determines the relative intensities and phase of the hyperfine lines. Four distinct time domains can be identified: **[0–1 μs].** The overall signal amplitude increases with increasing time delay. *In this time period there is also an evolution of the population distribution over available spin states.* At 10 ns delay all hyperfine lines are in absorption, as the delay grows low field peaks become emissive. **[1–10 μs].** Relative peak intensities stay constant, while the overall signal intensity drops by a factor of 2 to 3. **[10–80 μs].** The decrease in overall signal amplitude is accompanied by a change of emission peaks into absorption peaks. **[>80 μs].** No further change in relative amplitudes of hyperfine lines is observed. With a delay of 2 ms a weak DQ^- spectrum can still be recorded.

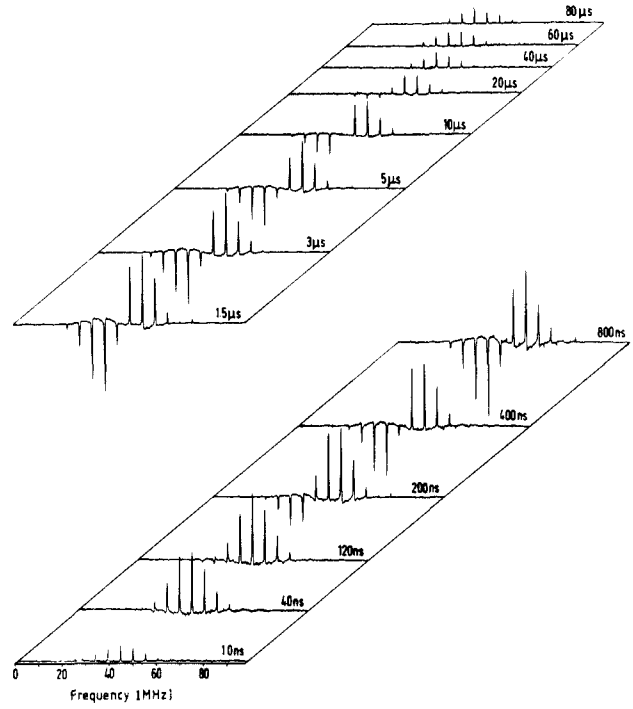

Figure 1. Quadrature-detected FT ESR spectra of photogenerated DQ^- as function of time delay between exciting laser pulse and $\pi/2$ (20 ns) microwave pulse. Absorption peaks point up, emission peaks down. Sample temperature 245 K.

A complete analysis of the data will be presented elsewhere; here a qualitative interpretation will be given. The initial signal growth reflects the kinetics of electron transfer from photoexcited ZnTPP to DQ. The DQ^- concentration reaches a maximum within 1 μs; this is consistent with a reaction rate that is close to diffusion controlled. The change in relative intensities of hyperfine lines in the 0–1 μs domain is due to competition between triplet (TM) and radical pair (RP) spin polarization mechanisms.[5] Spin selective intersystem crossing in photoexcited ZnTPP generates spin polarized triplets.[6] DQ^- and $ZnTPP^+$ formed before spin lattice relaxation obliterates the triplet spin polarization will give enhanced absorption ESR signals. The RP mechanism generates a DQ^- ESR spectrum in which nine low field peaks are in emission and the others in absorption.[7] Both mechanisms are operative, but, as the reaction proceeds, the TM will diminish in relative importance because of triplet spin lattice relaxation ($T_1 \approx 30$ ns). As a consequence, the TM dominated spectrum (10 ns delay) is gradually replaced by a RP dominated spectrum. The signal decay observed from 1 to 10 μs is due to back electron transfer. It is estimated that the initial DQ^- ($ZnTPP^+$) concentration is $\approx 5 \times 10^{-5}$ M. This gives a back electron transfer rate constant of $\approx 2 \times 10^9$ M^{-1} s^{-1}. With longer delays, spin lattice relaxation ($T_1 \approx 10^{-5}$ s) thermalizes the populations. Due to reduction of $ZnTPP^+$ in a side reaction competing with back electron transfer, complete regeneration of DQ also involves a disproportionation reaction.[10] This accounts for the fact that a weak DQ^- signal can be observed milliseconds after the laser pulse.

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The study demonstrates that radical formation and decay can be studied with nanosecond time resolution by using FT ESR. This makes it possibile to monitor reactions that proceed at close to diffusion-controlled rates. Spin polarization effects give information on reaction mechanisms. The ability to probe the spin state within nanoseconds of radical formation may make it possible to study development of RP generated spin polarization and get information on the electronic state of the transient ion pair formed in the electron-transfer reaction. It is noteworthy that spectra obtained with delays less than 1 μs exhibit unusual phase effects that may have their origin in the mechanism of spin state development.[11,12] Well-resolved (line width < 100 kHz) spectra with good signal-to-noise can be obtained. This facilitates identification of free-radical products. The method is superior to time-resolved ESR with use of a CW microwave source as well as spin echo ESR measurements in terms of sensitivity, spectral resolution, and time resolution.[13] Lifetime broadening will affect spectral resolution significantly for radical lifetimes <1 μs. In that case kinetic data can be obtained by measuring the time evolution of the FID amplitude.

ESR studies of photoinduced electron transfer of porphyrins (or chlorophylls) to quinones have lacked the time resolution required for studies of forward and back reactions. The time resolution of the FT ESR measurements is similar to that of flash photolysis measurements, and the results appear to be in general agreement.[10]

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Deoxygenation of Phenolic Natural Products. Enzymatic Conversion of Emodin to Chrysophanol

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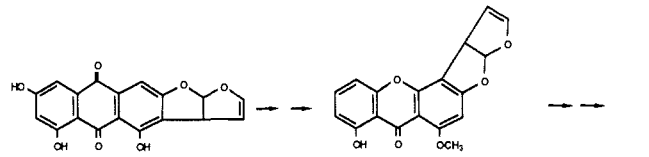
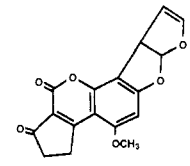
A long standing problem in the biosynthesis of polyketide derived natural products (for reviews, see ref 2 and 3) is the mechanism of removal of phenolic hydroxyl groups.[2] For example, the biochemical conversion of versicolorin A to sterigmatocystin, a sequence which forms the central segment of aflatoxin biosynthesis,[4] involves the reductive removal of the 6-hydroxyl group of versicolorin A (or a related intermediate) by a mechanism which remains cryptic. In contrast, the appearance of deoxygenated aromatic compounds in nature is usually interpreted as the result





of reductive dehydration of a linear polyketide enzyme complex, as has been experimentally demonstrated at the cell free level for fatty acid[5] and 6-methylsalicylic acid (6-MSA) biosynthesis,[6,7] although evidence for post aromatic deoxygenation has appeared recently.[8,9] Thus, preliminary work[8] showed that a specimen of the anthraquinone emodin (**1**) generally labeled with ^3H is converted by a crude cell-free system from *Pyrenochaeta terrestris* to its 6-deoxy derivative chrysophanol (**2**) indicating that deoxygenated metabolites cyanodontin and the secalonic acids produced by the organism[10,11] are derived from emodin (**1**) via chrysophanol (**2**). In this study, the requirement for the cofactor NADPH was suggested, but the mechanism of the reaction was not rigorously defined. In this communication, we present experimental evidence for cell-free enzymatic reduction of the resorcinol ring in emodin (**1**) to chrysophanol (**2**) mediated by NADPH as cofactor, i.e., the *aromatic* counterpart of deoxygenation in fatty acid and polyketide biosynthesis.

When emodin was incubated in a cell-free medium containing 50% D_2O,[12] mass spectral analysis[13] of the resultant chrysophanol specimen revealed the presence of non-, mono-, and dideuteriated species in the ratio 1:0.8:0.3 (Table I). The centers of deuteriation were determined by ^1H NMR spectroscopy.[14] As is seen in Figure 1A, natural chrysophanol shows singlets at 7.20 δ and 7.63 δ for H-2 and H-4, doublets at 7.80 δ and 7.36 δ for H-5 and H-7, and a triplet at 7.83 δ for H-6. The deuteriated chrysophanol specimen showed relative ^1H intensities in accord with deuterium substitution at positions 5 (13%), 6 (5%), and 7 (25%) (Figure 1B). A mechanism which would account for deuterium enrichment at positions 5 and 7 is shown in Scheme I, involving NADPH reduction of the keto tautomers of emodin (**3a,b** to **4a,b**). Further evidence for phenol-keto tautomerism was provided by mass spectral analysis of emodin recovered from incubation of the

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(12) *P. terrestris* culture methods were as described in ref 10. Preparation of enzyme extracts was as described in ref 8. Enzymatic incubation was performed as in ref 8 except that 50% D_2O buffer was used. Chrysophanol and emodin were extracted from the incubation medium and purified by using silica gel TLC as described in ref 8. The extent and position of deuteriation were determined by EI-MS and NMR spectroscopy, respectively. Chrysophanol from two 24-h incubations (total yield 300 μg) was used for measurement of NMR spectra.
(13) Mass spectra were recorded on a Hewlett Packard 5995B/C GC-MS spectrometer with use of a direct insertion probe by selective ion measurement. The intensities of the m + 1 and m + 2 peaks were corrected for natural abundance ^{13}C, ^{17}O, and ^{18}O.
(14) ^1H NMR spectra were recorded at 500.13 MHz on a Bruker AM 500 instrument. Samples were in acetone-*d6* in 5-mm tubes, 32-K data points, 1-s pulse delay, 45° pulse.

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