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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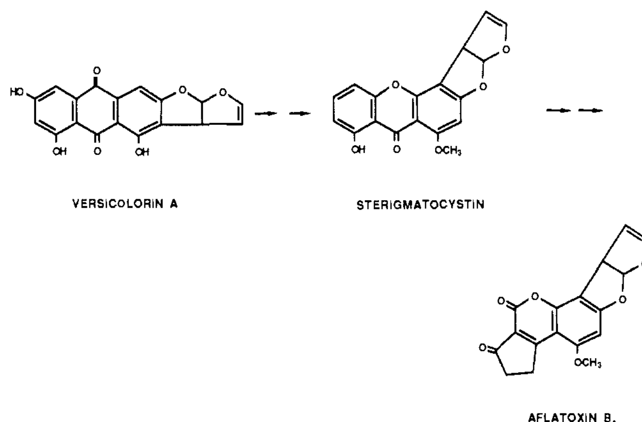
A long standing problem in the biosynthesis of polyketide derived natural products (for reviews, see ref 2 and 3) is the mechanism of removal of phenolic hydroxyl groups.[2] For example, the biochemical conversion of versicolorin A to sterigmatocystin, a sequence which forms the central segment of aflatoxin 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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Table I. Percent Mono- and Dideuteriated Chrysophanol and Emodin After Incubation of Emodin with a Crude Extract from *Pyrenochaeta terrestris* in 50% D_2O

substance recvd	incubn medium	% 1 D/mol	% 2 D/mol
chrysophanol	(a) complete (50% D_2O–buffer A)	38	15
emodin	As (a)	17	4
emodin	As (a)—NADPH	8	2
emodin	As (a), boiled extract	6	1

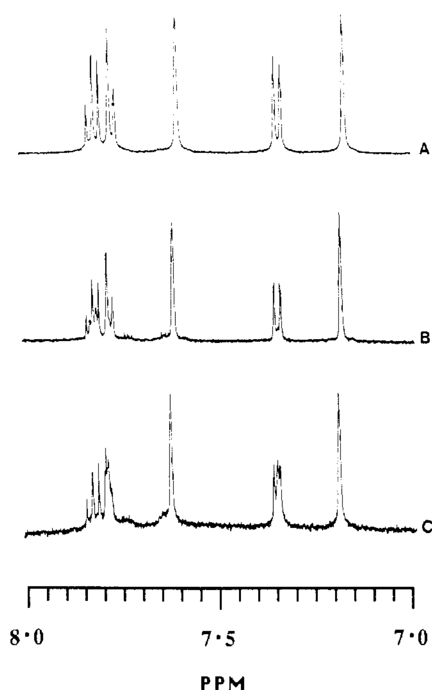
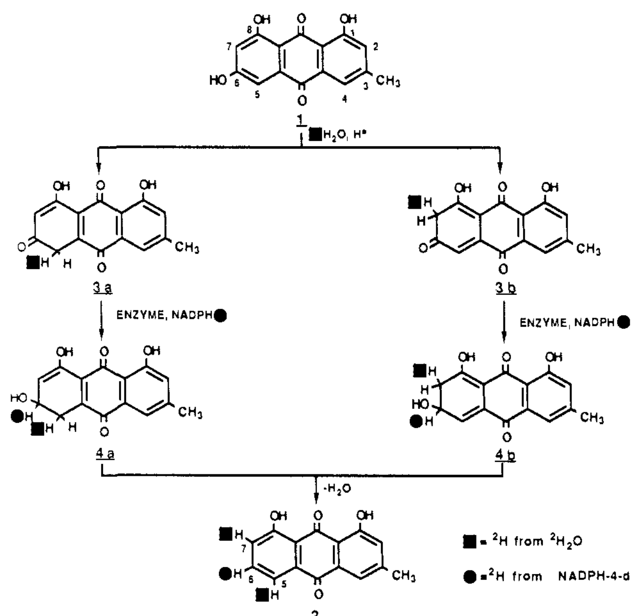


Figure 1. ^1H spectra (500 MHz) of (A) natural chrysophanol, (B) chrysophanol produced in medium containing 50% D_2O in the presence of NADPH, and (C) chrysophanol produced in medium containing NADPH-*4-d*.

Scheme I



cell-free extract in 50% D_2O buffer (Table I). In all cases, significant ^2H incorporation took place, but incorporation was highest in the presence of active enzyme (even in the absence of NADPH) indicating enzyme stabilization of the keto tautomers **3a,b**. The presence of 5% ^2H enrichment at position 6 could arise from exchange or from production of some NADPH-*4-d* in the medium. The latter mechanism was confirmed by incubation of emodin in the cell-free system containing the coupled enzyme components necessary for the generation of NADPH-*4-d*.[15] Isolation of the resultant chrysophanol and analysis by mass spectrometry showed 40% enrichment with deuterium. Inspection of the 500 MHz NMR spectrum of this specimen (Figure 1C) reveals that regiospecific deuteriation at C-6 has taken place. The sharp triplet (for H-6) at 7.83 δ is reduced in size by 40%, and the H-5 and H-7 doublets have large singlet components, indicating absence of coupling to H-6 (Scheme I). In one earlier report, NADPH has been shown to be necessary for phenolic reductions,[16] but the emodin–chrysophanol conversion is the first example of reduction of a phenolic substrate at the cell-free level, in which the cofactor NADPH has been shown to serve as the source of hydride at the C-6 position in the final product. It should also be noted that a chemical model for the reduction of 1,3,6,8-tetrahydroxynaphthalene (known to exhibit phenol–keto tautomerism) to the hydroaromatic substance scytalone is available.[17]

Further examples of this type of deoxygenation process are under study at the cell-free level in the expectation that the *absence* of a phenolic hydroxyl (e.g., in sterigmatocystin) may frequently signal operation of post-aromatic (rather than precyclic)[4-6] reduction–dehydration as demonstrated in this study, especially for polycyclic phenols.

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(15) Crude extract[8] (80 mL, 2.7 mg/mL protein) was brought to 0.75% in protamine sulfate and centrifuged. Ammonium sulfate (32 g, 63% saturation) was added to the supernatant. After centrifugation, the pellet was dialyzed against buffer A of ref 8. A solution containing 5.4 mmol of 2-propanol-*d8*, 80 units of *Thermoanaerobium brockii* alcohol dehydrogenase (Sigma A8278), and 42 μmol of $NADP^+$ in buffer A in a total volume of 7.5 mL was incubated for 1.0 h at 35 °C. $FeCl_2$, ATP, and emodin (same concentrations as in ref 8), ammonium sulfate fraction (48 mg), and buffer A were added to give a final volume of 22.5 mL. The mixture was incubated at 25 °C for 30 h. Chrysophanol was purified by preparative TLC in the usual manner[8] (yield 100 μg).

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Total Synthesis of (–)-Botryococcene

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The broadly distributed, unicellular green alga *Botryococcus braunii* (Kützing) produces a large number of linear and monocyclic irregular triterpenes ("botryococcenes") that constitute as much as 90% of the dry weight of the organism.[1] The geochemical significance of this prolific hydrocarbon source has been noted,[2] and considerable effort has been expended on its cultivation for commerical purposes.[3] The most abundant member of this

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