NMR analysis confirmed that the MTPA methodology[16a] is applicable in this series.[16b] Since the R-MTPA derivative of **1** and the S derivative of **11** have identical NMR spectra[17] (which are distinct from those of S-MTPA-**1** and R-MTPA-**11**), it can be concluded that the natural material is diastereomeric with our synthetic sample of **11** which has unambiguous stereochemistry. Therefore, the precise stereostructure of uvaricin can now be formulated as that shown in **1**, and **11** can be named (+)-15,16,19,20,23,24-*hexepi*-uvaricin [or (+)-(36-*epi*)-*ent*-uvaricin since the *relative* stereochemistry of **1** and **11** differs only at C(36)].

The work described here sets the stage for syntheses and biological evaluation of (i) more complex and more potent naturally occurring members of the bis(tetrahydrofuranyl) acetogenin family,[3] (ii) other non-natural stereoisomeric uvaricin analogues, and (iii) structurally simpler analogues as part of a quest to identify the minimum pharmacophore.

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Supplementary Material Available: Experimental procedures and characterization data for all new compounds (20 pages). Ordering information is given on any current masthead page.

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(17) The C(35), C(36), and C(37) protons have identical chemical shifts in the **1**/**11**, S-MTPA-**1**/R-MTPA-**11**, and R-MTPA-**1**/S-MTPA-**11** diastereomeric pairs, suggesting that those sites are sufficiently far removed from the C(15) Mosher ester attachment site to be unaffected.

Spectral Characterization of 4-Carboxy-5,6-dihydroxy-2,4-cyclohexadienone, a Likely Component of Intermediate II in *p*-Hydroxy Benzoate Hydroxylase

Gábor Merényi*,† and Johan Lind‡

Department of Physical Chemistry and Department of Nuclear Chemistry The Royal Institute of Technology 10044 Stockholm, Sweden

Robert F. Anderson

Gray Laboratory of the Cancer Research Campaign, Post Office Box 100 Mount Vernon Hospital Northwood, Middlesex HA6 2JR, United Kingdom

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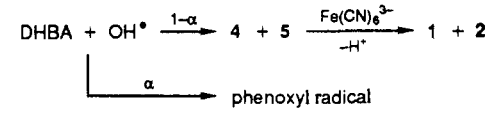
During aromatic hydroxylation by *p*-hydroxy benzoate hydroxylase or phenol hydroxylase three distinct intermediates have been observed.[1] Intermediates I and III have been identified as the corresponding 4a-hydroperoxy- and 4a-hydroxyflavins, respectively.[2] Among the several proposals for intermediate II we find a ring-opened flavin species[2,3] and a radical pair[4,5] consisting of flavin and substrate moieties. While rationalizing certain aspects[2,4-6] of intermediate II, these suggestions fail to explain the entirety of experimental observations.[7-10] An early model[11] assumed Chart I to account for aromatic hydroxylation. Although the large deuterium effect observed in phenol hydroxylase[12] for the conversion of intermediate II into III strongly supports this model, it has been abandoned on the assumption that the cyclohexadienone species in Chart I is probably transparent[13] around 400 nm and thus fails to account for the color of intermediate II. The present work will provide evidence on the contrary by producing the 2,4-cyclohexadienone of the substrate 2,4-dihydroxy benzoate (DHBA). In a modified version of the pulse-radiolytic method described in ref 4 the cyclohexadienones were produced through the reaction of the $OH^{\bullet}$ radical according to



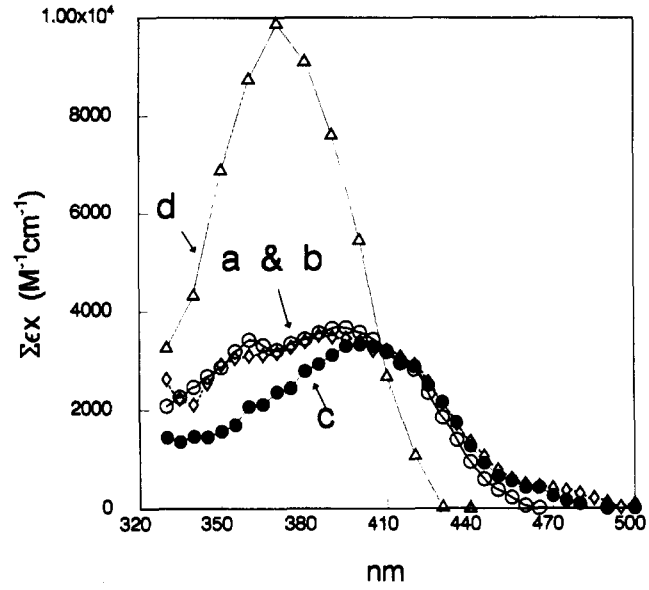


Figure 1. Composite spectra representing the cyclohexadienones **1**, **2** (a-c), and **3** (d). The spectra were obtained upon pulse irradiating N_2O saturated aqueous solutions containing 1.5×10^{-3} M 2,4-dihydroxy benzoate or 2,4,6-trihydroxy benzoate (d) and 10^{-3} M $Fe(CN)_6^{3-}$ and were corrected according to the text: (a) pH 5.75, (b) pH 8.35, (c) pH 3.75, and (d) the spectrum of **3** at pH 7.

Figure 1 presents $\sum x_i\epsilon_i$, where x_i and ϵ_i denote the fraction and extinction coefficient of the ith cyclohexadienone isomer, i.e., $\sum x_i = 1$. Figure 1 was constructed as follows: α, which varies with the substrate and somewhat with pH was determined in each case by measuring the absorbance between 470 and 500 nm where all other species are transparent. The phenoxyl radicals were unreactive toward $Fe(CN)_6^{3-}$ on the experimental time scale, while **4** and **5** were rapidly oxidized by $Fe(CN)_6^{3-}$ ($k = 3 \times 10^9$ M^{-1} s^{-1}). The spectra were recorded ca. 5 μs after the end of the pulse, by which time the concentration of cyclohexadienones amounts to $(1 - \alpha)c_0$, where c_0 is the initial concentration of $OH^{\bullet}$ radicals. This is paralleled by the conversion of the same amount of $Fe(CN)_6^{3-}$ into $Fe(CN)_6^{4-}$. Denoting by ϵ_{Ph}, $\epsilon_{Fe(III)}$, and $\epsilon_{Fe(II)}$,

† Department of Physical Chemistry.
‡ Department of Nuclear Chemistry.

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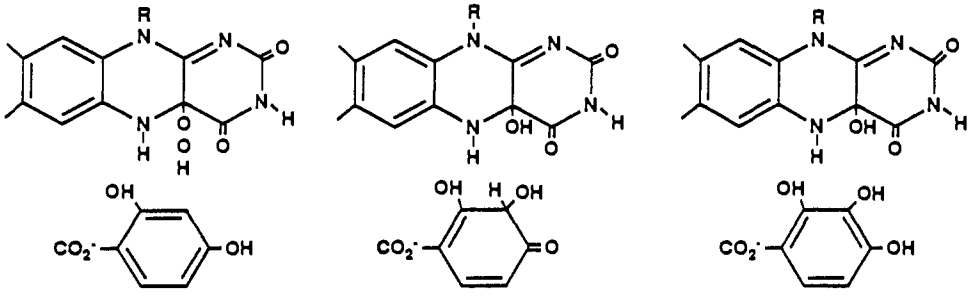
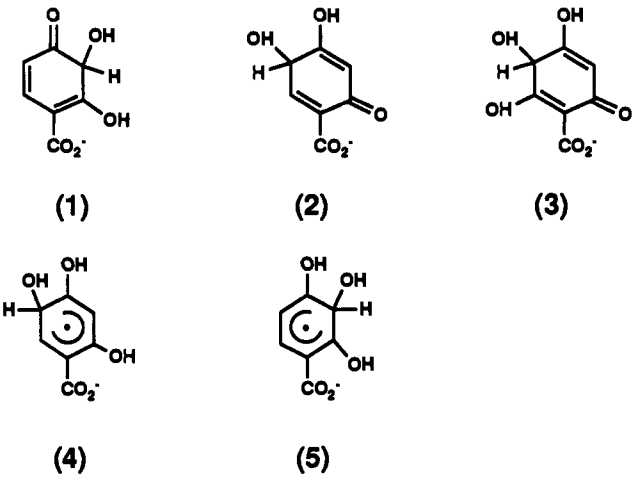
Chart I



(I) (II) (III)

Chart II



(1) (2) (3)

(4) (5)

respectively, the extinction coefficients of the phenoxyl radical, ferricyanide and ferrocyanide (all determined in separate experiments), and by OD, the measured optical densities, we obtain the relationship between Figure 1 and the experimental quantities:

$$\sum x_i \epsilon_i = (\mathrm{OD} - \alpha c_0 \epsilon_{\mathrm{Ph}} + (1-\alpha) c_0 (\epsilon_{\mathrm{Fe(III)}} - \epsilon_{\mathrm{Fe(II)}}))/(1-\alpha) c_0$$

The difference between the raw spectra and those in Figure 1 decreases with decreasing wavelength, being <30% below 400 nm. Spectra a and b are almost identical and do not change when oxygen instead of ferrocyanide is the oxidant. This confirms that the species are cyclohexadienones and that they are in the same protonation state betwen pH 6 and 8.3.

The sole detected[4] trihydroxy benzoate was 3-OH DHBA, making up but 50% of initial OH• adducts. We believe that, about 50% 5-OH DHBA may have formed but has been further oxidized. Absence of 6-OH DHBA (which should be stable), rules out 6 addition. Ascribing spectrum d to species **3**, we assign the short and long wavelength components in spectra a and b to species **2** and **1**, respectively. Since protons apparently suppress the absorbance around 360 nm, spectrum c should belong to species **1**, present with a fraction of 0.5. Thus we estimate the extinction coefficient of **1** at its peak maximum to be 6500 M^{-1} cm^{-1}. We note that both spectral size and shape of **1** are in good agreement with that of the difference spectrum between intermediate II and III.[2,14] Unlike species **1** intermediate II displays a spectral shift[14] with a pK_a around 8, similar to the pK_a = 7.8[15] of the Tyr201[16] in the enzyme. It could be that loss of a hydrogen bond between Tyr201 and **1** causes the spectrum of the latter to red-shift. Further speculation must await knowledge of the protonation characteristics of **1**.

Acknowledgment. We are indebted to the Swedish Natural Science Research Council (G.M. and J.L) and the Cancer Research Campaign (R.F.A) for financial support.

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Model Complexes for Molybdopterin-Containing Enzymes: Preparation and Crystallographic Characterization of a Molybdenum-Ene-1-perthiolate-2-thiolate (Trithiolate) Complex

Robert S. Pilato, Kenneth A. Eriksen, Mark A. Greaney, and Edward I. Stiefel*

Exxon Research and Engineering Co.
Route 22 East, Clinton Township
Annandale, New Jersey 08801

Shyamaprosad Goswami, Latonya Kilpatrick,
Thomas G. Spiro, and Edward C. Taylor*

Department of Chemistry, Princeton University
Princeton, New Jersey 08544

Arnold L. Rheingold

Department of Chemistry, University of Delaware
Newark, Delaware 19716
Received May 24, 1991

With the exception of nitrogenase, all molybdenum enzymes are believed to contain the pterin cofactor designated Moco.[1] The lack of this cofactor or its improper function is linked to human disorders including gout, sulfite allergy, and the often fatal oxidase deficiency.[2] It is generally accepted that Moco is a C(6)-substituted pterin with a four-carbon side chain to which molybdenum is bound via a 1,2-ene dithiolate linkage.[3] We seek to prepare

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