much further than to its direct coupling partner (H2'). For longer times, the magnetization propagates further away from the starting point (the anomeric hydrogen in this case) into the H5',5'' direction (Figure 2b,c). Spectral assignments are in agreement with those proposed by Doornbos et al.,[8] who based their results on 360-MHz double-resonance experiments and on comparison with spectra of the dinucleotide A2'-P-5'A.

The actual pulse sequence used in the present work is as follows: $180°_{sel}$ (on/off), $90°_x$, $(SL_y60°_{-y}300°_ySL_{-y}60°_y300°_{-y})_n$, Acq(±). The sequence between parentheses is the actual propagation scheme, where SL_y and SL_{-y} denote spin lock along the y and $-y$ axis, respectively. As explained elsewhere,[3,6] the duration τ of the spin lock period SL_y (and SL_{-y}) should obey

$$\tau < \nu/2\Delta^2$$

where ν is the nominal rf field strength during the spin lock, and Δ is the largest offset of a resonance of interest to the carrier frequency. The carrier frequency is placed at the center of the region of interest to minimize the value of Δ. The extra pulse pair, $60°_{-y}300°_y$ (and its inverse) is a composite pulse that rotates magnetization from the $+y$ effective rf field to the $-y$ effective rf field direction. This limits the loss of spin-locked magnetization during phase alternation of the spin-lock field to less than 1% for Δ/ν ratios of up to 0.3. This permits the use of rather weak rf fields (2–10 kHz) for most cases of interest (covering 1200–6000-Hz spectral width). Therefore, several watts of rf power is generally sufficient to induce effective spin propagation. Considering the rf duty cycle over the entire experiment ($<\sim$10%), this is well within the safe range for most commercial high-resolution spectrometers. On some spectrometers, the low-power mode provides a sufficiently strong rf field; on our spectrometer a ENI Model 420 L rf power amplifier was used to further amplify the low-power mode rf. The ^1H heteronuclear decoupler amplifier can also be used for this purpose. Use of the high-power observe amplifier for generating the spin-lock field should be discouraged since this may lead to probe or attenuator damage.

The spin propagation method is not restricted to systems that consist of well-defined subunits that are not mutually coupled (peptides, oligonucleotides, oligosaccharides) but can also be used in very complex coupling networks as often found in steroids and alkaloids. In these cases one can use the propagation time dependence to assign hydrogen resonances that are an increasing number of bonds removed from the starting point since the relay of magnetization proceeds sequentially between coupled pairs of spins, with each step occurring at a rate determined by the coupling constant. It should be pointed out that although the subspectra appear to be absorptive, there is also antiphase dispersive character within the multiplet.[2,9] This partial antiphase dispersive nature of the individual multiplet components can lead to errors if one attempts to measure precise coupling constants from such subspectra. Computer simulations and experimental results suggest that for systems containing four or more nonequivalent hydrogens, the dispersive components tend to disappear for long propagation times, and the multiplets approach their "natural shape". Currently, we are investigating whether it is possible to measure accurate J values from the time dependence of the propagation rather than from the multiplet splittings.

Cross-relaxation among spin-locked magnetization components[10] also forms a transfer mechanism. However, this effect is relatively small and the resulting resonances in the difference spectrum are of negative sign.[7]

The one-dimensional propagation method provides a very simple and fast way to obtain spectral assignment in complex overlapping spectral regions. The major limitation is the decay of magnetization, with time constant $T_{1\rho}$, which approximately equals T_2 and which prohibits the use of long propagation times for macromolecules. New propagation schemes, based on composite pulse decoupling cycles,[11] may alleviate this problem.[12]

Acknowledgment. We are indebted to Rolf Tschudin for continuous technical support. The trinucleotide was kindly provided by Dr. Paul Torrence.

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Synthesis of 9-Decarboxymethoxatin. Metal Complexation of Methoxatin as a Possible Requirement for Its Biological Activity

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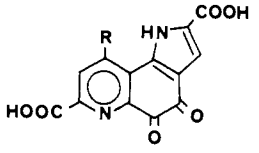
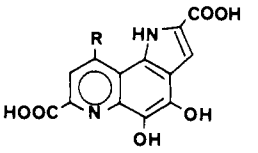
The pyrroloquinoline quinone methoxatin[1-3] ($\mathbf{1}_{ox}$) is the cofactor for certain non-flavin or nicotinamide dependent bacterial dehydrogenases known as quinoenzymes. The Cu^{II} requiring bovine serum amine oxidase[4] has recently been reported to be a quinoenzyme. Substantiation of this claim would almost certainly amount to the finding that $\mathbf{1}_{ox}$ is a human cofactor and, therefore, plausibly a vitamin.



$\mathbf{1}_{ox}$, R = COOH
$\mathbf{2}_{ox}$, R = H



$\mathbf{1}_{red}$, R = COOH
$\mathbf{2}_{red}$, R = H

It has gone without notice that structural features of $\mathbf{1}_{ox}$ are shared by known chelating agents. With attention to the 7-carboxyl group, $\mathbf{1}_{ox}$ may be viewed as a derivative of the metal complexing agent[5] α-picolinic acid. Two-electron reduction of $\mathbf{1}_{ox}$ by substrate provides the dihydrodiol $\mathbf{1}_{red}$. The latter is both a derivative by substitution and annelation of α-picolinic acid and the metal complexing agent[6] 8-hydroxyquinoline. Perhaps these features are germane to the mechanism of metal-requiring quinoenzymes.

Methoxatin must still be considered as a rather rare and generally unobtainable compound. In order to ascertain the structural requirements for biological activity and study the chemistry[7-9] and in particular the metal-binding properties of $\mathbf{1}_{ox}$ and $\mathbf{1}_{red}$ we have designed a synthesis of an easily attainable analogue, 9-decarboxymethoxatin ($\mathbf{2}_{ox}$).

8-Hydroxyquinoline was converted to 2-cyano-8-hydroxyquinoline (**3**) in three steps by a literature route.[10] Nitration of **3** in concentrated nitric–acetic acid at 20 °C produced a mixture of the 5- and 7-nitro isomers from which the 5-nitro isomer[11] **4**

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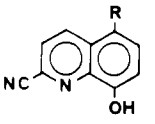
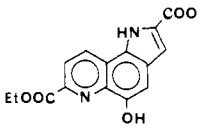


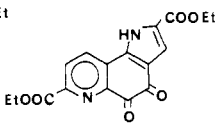
3, R = H
4, R = NO_2
5, R = NH_2

6, R = NH–N=C(CH$_3$)–COOEt

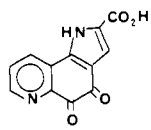
could be separated by recrystallization from acetone–ethanol, mp >230 °C sublimes (37%). Hydrogenation of **4** in ethanol over 10% Pd/C followed by filtration into 1 N HCl and evaporation afforded the amine hydrochloride[11] **5** (93%). Treatment of **5** with 1 equiv of aqueous HCl and sodium nitrite (5 °C for 10 min) yielded the diazonium salt which was added to a stirred ethanolic solution of 1.2 equiv of ethyl α-methylacetoacetate and potassium hydroxide at 0 °C. After 18 h at 4 °C **6**[11] was isolated by ether extraction (18%). Cyclization and transformation of the nitrile function to an ester function (Pinner synthesis) to yield the indole[11] **7** was carried out in one step by stirring a solution of **6** in saturated



7



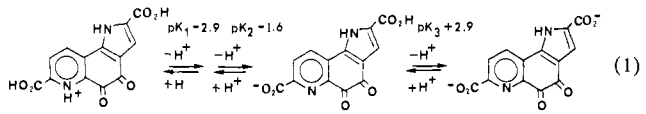
8



9_{ox}

ethanolic HCl for 3 days followed by evaporation and addition of water (77%). Oxidation of the *o*-quinone **8** was accomplished by adding an aqueous solution of ceric ammonium nitrate (5.5 equiv) to a suspension of **7** in acetonitrile at 5 °C. Evaporation to low volume and addition of water yielded the crude *o*-quinone (51%). Recrystallization from acetonitrile afforded **8**[12] as bright-orange flakes mp 314–316 °C (darkens >300 °C). TLC (silica gel with methylene chloride–ethanol 9:1) shows single spot R_f 0.74; UV_{max} (CH_3CN) 220 (ε 13 000), 242 (ε 15 500), 275 (ε 25 200), 308 sh (ε 15 800), 318 sh (ε 16 700) 329 nm (ε 17 600). Hydrolysis of **8** was accomplished by stirring in concentrated hydrochloric acid at 100 °C for 20 h to precipitate 9-decarboxymethoxatin[13] ($\mathbf{2}_{ox}$) as an orange-brown solid after filtration, washing with water and acetone, and drying (86%). TLC (reverse phase with water–ethanol–triethylamine 70:30:1) shows single spot R_f 0.9; UV_{max} (H_2O) 243 (ε 20 900), 275 (ε 30 600), 315 nm (ε 17 700).

The macroscopic pK_a values of $\mathbf{2}_{ox}$ (eq 1) were determined by

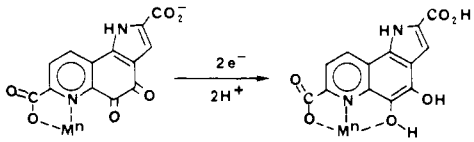


(1)

spectral titration between H_0 = −4.4 (315 nm) and pH 4.29 (315 and 275 nm).

Equilibrium complexing of Cd^{2+} with $\mathbf{2}_{ox}$ and $\mathbf{9}_{ox}$ was studied at pH 4.0 (μ = 1.0 with $NaClO_4$) by the procedure of Walker.[14] A plot of ln $(A_0 - A_t)/(A_t - A_\infty)$ vs. ln $[Cd^{2+}]$ provided stoichiometry of 1/1 as slope and $K = 1.9 \times 10^4$ M^{-1} (330 and 315 nm) as intercept. This may be compared to $K = 3.5 \times 10^7$ M^{-1} for 1:1 complexing of Cd^{2+} by α-picolinic acid.[5] No complexation of Cd^{2+} by $\mathbf{9}_{ox}$ could be detected. Thus, although the pyridine nitrogen and C(7) carboxyl functions of $\mathbf{2}_{ox}$ are only very weakly basic, $\mathbf{2}_{ox}$ is a reasonable metal-complexing agent. By using the same plotting technique to determine the binding of Cu^{2+} to $\mathbf{2}_{ox}$, there was obtained a slope of 1.5/1, interpretable as the formation of a $(\mathbf{2}_{ox})_2(Cu^{2+})_3$ complex ($K = 4 \times 10^7$ M^{-4}).

Examination of Stuart–Briegleb molecular models and considerations of functional group basicity assures us that $\mathbf{1}_{red}$ and $\mathbf{2}_{red}$ will behave as strong tridentate metal-complexing agents. Since metal ion binding to $\mathbf{1}_{red}$ and $\mathbf{2}_{red}$ should be much greater than to $\mathbf{1}_{ox}$ and $\mathbf{2}_{ox}$, metal ions may well play a catalytic role in the reduction of these quinones (eq 2). Availability of $\mathbf{2}_{ox}$ in



(2)

reagent quantity has made it possible to undertake our current studies to determine: (i) the complexing constants of various metal ions with $\mathbf{2}_{ox}$ and $\mathbf{2}_{red}$; (ii) modes of interaction of metal ions with the radical $\mathbf{2}_{rad}$; (iii) the influence of metal ion binding upon the electrochemical potentials and rates of stepwise 1e$^-$ reduction of $\mathbf{2}_{ox}$ to $\mathbf{2}_{rad}$ and $\mathbf{2}_{red}$. The results of these studies will be reported as full papers.

Acknowledgment. This work has been supported by a grant from the National Institutes of Health. E.J.R. expresses gratitude to the University of California for a Graduate Opportunity Fellowship.

(11) ^{1}H NMR, IR, and mass spectral data were obtained for each intermediate and were in full agreement with the postulated structure.

(12) Additional data for **8** are as follows: ^{1}H NMR (Me_2SO-d_6) δ 1.34 (3 H, t, CH_3), 1.36 (3 H, t, CH_3), 4.33 (2 H, q, CH_2), 4.42 (2 H, q, CH_2), 7.19 (1 H, s, H_3), 8.27 (1 H, d, H_8), 8.85 (1 H, d, H_9), 13.42 (1 H, s, NH) exchangeable. Anal. Calcd for $C_{17}H_{14}N_2O_6$: C, 59.65; H, 4.09; N, 8.19. Found: C, 59.80; H, 4.15; N, 8.45; IR_{max} 1660, 1720, 3500 cm^{-1}.

(13) Additional data for **2** are as follows: ^{1}H NMR (Me_2SO-d_6) δ 7.15 (1 H, s, H_3), 8.24 (1 H, d, H_8), 8.22 (1 H, d, H_9), 13.35 (1 H, s, NH) exchangeable. Anal. Calcd for $C_{13}H_6N_2O_6 \cdot {}^1/_2H_2O$: C, 52.88; H, 2.37; N, 9.49. Found: C, 53.03; H, 2.55; N, 9.38. IR_{max} 1600, 1700 cm^{-1}.

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The Valence σ Ionization in Systems with Multiple Metal–Metal Bonds

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Chemical systems containing multiple bonds between metal atoms have provided many theoretical and synthetic challenges.[1] Photoelectron spectroscopy (PES) has played an important role in the experimental investigation of their electronic structure,[2] particularly for the π- and δ-type bonds.[3-8] However, an ionization associated with a σ bond has not been firmly identified, although a sharp "extra" ionization (fwhm 0.3 eV) in the PES of W_2-

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