Table I. Photoisomerization of Aryl Dienes (**2–6**) in Hexane[a,b]

compd	% conv[c]	% product dist 2-cis	% product dist 4-cis
2	5[d,e]	70	30
3	22[d]	3	97
4	24[d,f]	5	95
5	26[d,f]	<2	>98
6	15[d]	6	94

[a] Degassed solutions of all-trans isomers of aryl dienes in hexane (~0.01 M) were irradiated in Pyrex tubes for 3–25 min with a Hanovia 550-W medium-pressure Hg lamp. [b] For compound **3**, a 0–54 Corning glass filter was used. [c] Higher conversions reported for accurate determination of minor components. [d] Analyzed by HPLC on an Altex 5-μm silica gel column. An HP-1040A diode array detector system was connected in conjunction with a single-wavelength detector. Data corrected for absorbance at detecting wavelength. [e] At higher conversions comparable amounts of dicis isomer are produced. [f] Analysis by ^{19}F NMR.

compounds (i.e., Z being highly electron withdrawing) could safely rule out participation of structure **7c**. Hence, for compound **2** the ester group has apparently added sufficient stabilization to the transition state going to the Dauben intermediate **7a**, making its formation competitive with that of **7b**, which is otherwise thought to be more favored, e.g., in the hydrocarbon series.[1] But, with the addition of highly electron withdrawing fluorine substituent at either or both termini of the allyl anion (**4–6**) or the replacement of the ester group by the more electropositive aldehyde group (**3**),[6] formation of the Dauben intermediate apparently becomes competitively the dominant one.

We stress in the above discussion the importance of *relative ease of formation of the zwitterionic intermediates* and *not* the relative stability of the intermediates in determining product distributions. The difference is nontrivial when one considers that the direction of photoisomerization is determined by competitive twisting of double bonds of the Franck–Condon species while relative stability of the zwitterionic species is a thermodynamic parameter which may or may not have a significant effect on a nonreversible kinetic process cascading down the excited singlet potential surface. The polar effect being detected in this work must be a manifestation of the developing polar character[2b] of the structure corresponding to the barriers of twisting the planar excited diene. Taking this into consideration, it is then not surprising that other effects such as steric and medium effects could readily become a more dominant kinetic factor in determining the direction of twisting of the planar diene structure as revealed by an earlier study of the polyenes from this laboratory.[4] Consistent with this explanation is our added observation that a methyl substituent at the β-carbon (3-methyl-**4**) reverses the regioselectivity, making isomerization at the α,β center 4 times more favored than at the γ,δ center. For the nonpolar pentadiene system,[1] the regioselective chemistry could simply be reflecting the different masses at the two reaction centers.

We might add that the current proposal of selective formation of the Dauben intermediate in photoisomerization of negatively substituted dienes appears to be consistent with other examples of regioselective isomerization of polyenes in the literature. Hence, it was reported that removal of the 13-methyl group (hence absence of steric repulsion) in retinal results in elimination of the 13-cis isomer in photoisomerization of the all-trans isomer[7] (hence selective formation of the allyl (anion)–heptatrienyl (cation) intermediates while the same isomer is the major product in the parent retinal.[8] Also, consistent with the involvement of such intermediates is the observation that fluorine substituents in the retinal analogues appear to cause a noticeable preference for isomerization at a site adjacent to the substituted double bond.[9]

In summary, we believe that the current systems involving more polar substituents more convincingly reveal the fact that the polar character of the excited state plays a significant role in directing position of isomerization. Selective formation of the two possible intermediates is also dependent on the nature of the substituent on the chromophore. Furthermore, it should be noted that photocyclization is a different reaction where symmetry restriction could forbid possible reaction from one, but not the other, zwitterionic intermediate. Hence, detection of a specific reaction does not ensure that the corresponding intermediate is lower in energy. No such restrictions are present in the geometric isomerization reaction. Therefore, one must be cautious in applying any interpretation derived from the isomerization reaction to the more restricted cyclization process.

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Supplementary Material Available: ^1H and ^{19}F NMR data of isomers of compounds **2–6** (1 page). Ordering information is given on any current masthead page.

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Water Suppression in Two-Dimensional Spin-Locked Nuclear Magnetic Resonance Experiments Using a Novel Phase-Cycling Procedure

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A prerequisite for the determination of three-dimensional structures of proteins by NMR is the sequential assignment of proton resonances by means of two-dimensional techniques that rely on through-bond and through-space connectivities.[1] For through-bond connectivity, the power of the recently introduced homonuclear Hartmann–Hahn (HOHAHA) techniques[2-6] has been clearly demonstrated.[7-11] Because all the NOE's used for assignment purposes involve the NH protons[1] (with the exception of $C^\alpha H(i)$–$C^\beta H(i+3)$ NOE's), it is essential to correlate the NH

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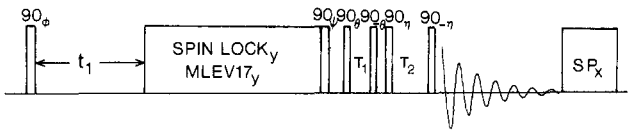

Figure 1. Pulse scheme for spin-locked experiments in H_2O. In addition to the conventional spin-locked experiments, this experiment contains a 90_ψ flip-back pulse, followed by a "1–1 echo" read sequence. To ensure identical amounts of z magnetization at the start of every sequence, a long pulse (SP) is applied after data acquisition. The phases are cycled as follows: $\phi = x, y, -x, -y$; $\psi = -x, -x, x, x$; $\theta = x$; $\eta = x, x, x, x, y, y, y, y, -x, -x, -x, -x, -y, -y, -y, -y$; acquisition $= x, x, x, x, -x, -x, -x, -x, -x, x, x, x, x, -x, -x, -x, -x$. After 16 scans, the phases θ and η and the receiver phase are incremented by 180°. Data acquired in odd- and even-numbered scans are stored in separate locations. The complete 32-step cycle is repeated four times, with all phases except that of the saturation pulse (SP) incremented by 90° in the CYCLOPS manner. Neglecting pulse width, the 1–1 echo sequence gives maximum excitation at an offset δ for $\tau_2 = 2\tau_1 = 1/4\delta$.

resonance of a given residue with its $C^\alpha H$ and side-chain proton resonances. To achieve this it is necessary to work in H_2O to observe the exchangeable NH protons. To date, HOHAHA applications to proteins in H_2O have involved the use of H_2O presaturation. Presaturation, however, abolishes signals of NH resonances that exchange rapidly with water protons and it also eliminates correlations to many of the C^α protons that resonate in the vicinity of the H_2O resonance. In this paper we describe a novel alternative approach that does not require presaturation of the H_2O resonance and that provides high-quality spectra, displaying connectivities o C^α protons even if they resonate at exactly the H_2O frequency.

The pulse scheme we propose for both the HOHAHA and the spin-locked NOE (known as CAMELSPIN[12] or ROESY[13]) experiments is shown in Figure 1. This scheme differs from the standard scheme by the addition of a 90° pulse at the end of the mixing period that flips the spin-locked magnetization back to the z axis, followed by a "read sequence" that gives zero excitation at the H_2O frequency. We employ a so-called "1–1 echo"[14] as a read sequence, which gives a perfectly flat base line with no need for linear or higher order phase correction. With regular phase cycling (i.e., if the phase of the first 90_ϕ pulse is cycled independently from the phases of the mixing pulses) it is found that in one-quarter of the individual scans the H_2O suppression is extremely poor. This is caused by the fact that, using regular phase cycling of the 90_ϕ pulse, the H_2O resonance is inverted after the 90_ψ flip-back pulse in one out of every four scans. The small amount of residual transverse H_2O magnetization is then sufficient to start the stimulated emission process, commonly known as radiation damping,[15] which creates an intense H_2O signal during data acquisition. As a result, this experiment does not produce satisfactory results on high-field NMR spectrometers with probes that have an average or better Q factor (i.e., an average or better S/N ratio). The radiation damping process can be avoided by changing the phase cycle of the 90_ψ flip-back pulse in such a way that the on-resonance H_2O signal never gets inverted. This means that in contrast to all previously proposed 2D NMR schemes, the phase cycling of the preparation pulse and the mixing period cannot be done independently. The programming of the phase cycle is most easily accomplished when data are acquired in the hypercomplex format[16] and is given in the legend of Figure 1.

Two examples of the new method are presented in Figure 2 for a sample of 11 mM BPTI in 90% H_2O at 42 °C, pH 6.6. Spectra have been recorded on an NT-500 spectrometer equipped with a Cryomagnet Systems probe head. Figure 2A shows part of the

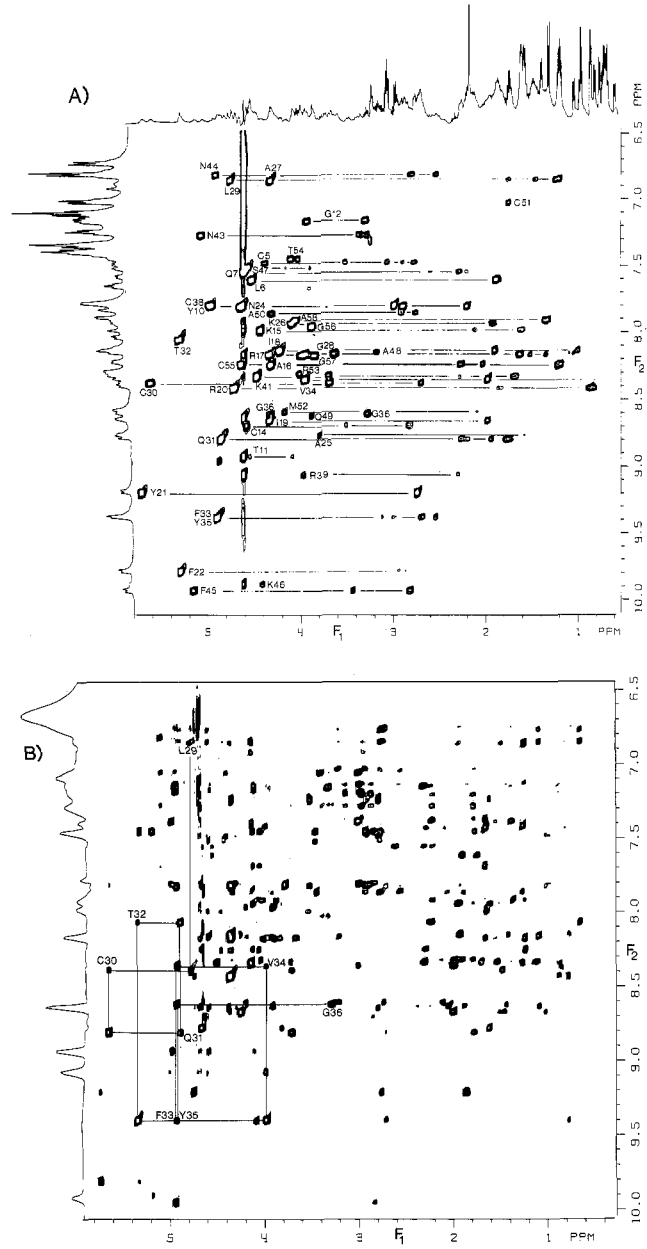

Figure 2. NH/aromatic (F_2 axis)–$C^\alpha H$/aliphatic (F_1 axis) region of the phase-sensitive 2D spectra of an 11 mM solution of BPTI in 90% H_2O/10% D_2O, pH 6.6, 42 °C, 100 mM NaCl. (A) 2D HOHAHA spectrum, obtained with a 33-ms MLEV17 mixing and a 7.9-kHz rf field strength for all pulses (32-μs 90° pulse); $\tau_1 = 94$ μs, $\tau_2 = 220$ μs. (B) 2D ROESY spectrum, recorded with a 60-ms spin lock duration, using a 6.25-kHz rf field for all pulses (40-μs 90° pulse); $\tau_1 = 90$ μs, $\tau_2 = 212$ μs. A 4-ms saturation pulse was used in both experiments. Both spectra result from $2 \times 320 \times 1024$ data matrices with 128 scans per t_1 value, a recycle delay of 1.3 s, and a total measuring time of 16 h per spectrum. Along the top and side of spectrum A the corresponding regions are shown of the 1D spectrum, recorded with presaturation of H_2O. Along the side of spectrum B a section at the F_1 H_2O frequency through the 2D spectrum is shown, displaying the protons that show rapid exchange with H_2O. Some NH(i)–$C^\alpha(i)$H connectivities are labeled. Assignments are taken from ref 20.

2D HOHAHA spectrum, recorded with a 33-ms MLEV17 mixing scheme[6] where the flip angle of the 17th pulse has been reduced from 180° to 60°.[14] The MLEV17 cycle was preceded and followed by 2-ms trim pulses[6] to eliminate longitudinal H_2O magnetization that builds up during the evolution period. The spectrum shows very clear connectivities between amide protons on the one hand and the C^α and side-chain protons on the other. Cross peaks between NH and H_2O protons (at $F_1 = 4.6$ ppm) are caused by hydrogen exchange during the mixing period.

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Figure 2B shows part of the 2D ROESY spectrum, recorded with a 60-ms mixing time. A large number of intense NOE interactions can be seen between the amide/aromatic resonances and the aliphatic resonances. In the spin-locked NOE method, originally proposed by Bothner-By et al.,[12] the NOE is always positive, independent of the molecular correlation time. Spin diffusion effects are therefore generally not observed[17] and the interpretation of NOE intensities may be more straightforward. Relay of NOE intensity via the HOHAHA effect[17,18] could in principle give rise to false NOE cross peaks. In practice, these types of relay peaks in proteins are limited almost exclusively to NOE interactions with a methylene proton, where Hartmann-Hahn relay to the second methylene proton can occur. Because methylene protons are commonly treated with the pseudoatom approach,[19] this does not affect the structure refinement process. Another interesting feature of the spin-locked NOE spectrum is that NOE and exchange cross peaks are of opposite sign. Exchange cross peaks between NH and H_2O resonances in Figure 2B are not visible (apart from small truncation artifacts from these intense resonances) because only resonances with opposite sign to the diagonal (NOE cross peaks) are displayed in this spectrum. A cross section taken at the H_2O F_1 frequency is shown along the left-hand side of the 2D spectrum and displays the amide protons that show a significant amount of hydrogen exchange during the 60-ms mixing period.

The approach described above makes it possible to record HOHAHA and ROESY spectra in H_2O solution in a routine fashion. Neither of the two experiments is particularly critical to fine tuning of parameters and the final spectra do not require any base-line correction procedure. To the best of our knowledge, Figure 2B also represents the first application of spin-locked NOE to a protein. The spectrum indicates that the quality is comparable to that of a NOESY spectrum; major advantages are that spin diffusion is eliminated and that chemical exchange peaks are identified by their opposite sign.

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Formation and Dehydration of an (α,β-Dihydroxyethyl)rhodium Porphyrin Complex: Potential Relevance to Coenzyme B_{12}-Substrate Complexes

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Diol dehydratase functions in concert with coenzyme B_{12}, (5'-deoxyadenosyl)cobalamin ($Co-CH_2R$), to catalyze the dehydration of vicinal diols to aldehydes through the intermediacy of geminal diol species (eq 1),[1,2] One of the proposed pathways

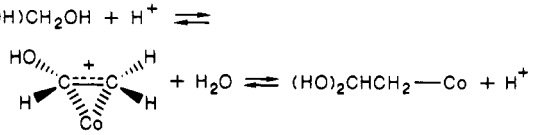
$$RCH(OH)CH_2(OH) \rightarrow RCH_2CH(OH)_2 \rightarrow RCH_2CHO + H_2O \quad (1)$$

for diol dehydratase catalyzed dehydration of ethylene glycol involves the formation and interconversion of organometallic intermediates derived from B_{12r}, ($Co^{II\bullet}$), and the substrate radical ($^\bullet CH(OH)CH_2OH$) (eq 2 and 3).[1-4] Dehydroxylation of the

$$Co^{II\bullet} + {}^\bullet CH(OH)CH_2(OH) \rightleftharpoons Co-CH(OH)CH_2(OH) \quad (2)$$

$$Co-CH(OH)CH_2(OH) \rightleftharpoons Co-CH_2CH(OH)_2 \quad (3)$$

β-OH group of $Co-CH(OH)CH_2(OH)$ by an acid site, assisted by formation of a vinyl alcohol π-complex and subsequent readdition of hydroxide to the π-complex, has been suggested as a convenient route to the required geminal diol species.[2] Model



studies focused on emulating the pathway utilizing organometallic intermediates have had considerable success,[5-10] but investigations using cobalt macrocycles are limited by the thermodynamic and kinetic instability of α-hydroxyalkyl complexes relative to dissociation into aldehyde and metal hydride.[11,12] Mulac and Meyerstein have succeeded in forming the proposed cobalamin-substrate complex $Co-CH(OH)CH_2(OH)$ by the reaction of $Co^{II\bullet}$ (B_{12r}) with the substrate radical $^\bullet CH(OH)CH_2(OH)$,[6a] which mimics the manner by which the cobalamin-substrate complex would form in the enzymatic reaction. $Co-CH(OH)CH_2(OH)$ is a transient species in neutral aqueous media and dissociates into $Co(I)^-$, H^+, and $CH_2(OH)CHO$ without observation of the dehydration reaction,[6] and this result has been used as evidence against invoking $Co-CH(OH)CH_2(OH)$ as an intermediate in the enzymatic dehydration of vicinal diols.[13] However, $Co-CH(OH)CH_2(OH)$ would be kinetically stabilized toward formation of glycoaldehyde in the enzyme environment by use of the α-OH group in substrate-protein binding. A thermodynamically stable complex that models $Co-CH(OH)CH_2(OH)$ outside of the enzyme environment is needed in order to evaluate whether or not this type of complex could be useful in the enzymatic substrate reactions.

Rhodium macrocycles are potentially useful model complexes for the cobalt macrocycle in B_{12} due to their related electronic structures (Co(III), $3d^6$; Rh(III), $4d^6$) and because the substantially stronger Rh-C bond[14,15] provides thermodynamic stabilization for organometallic species that may have only transient existence in the analogous cobalt species. One of the consequences of the strong Rh-C bond is the observation of α-hydroxyalkyl complexes of rhodium porphyrins[12,16] in equilibrium with rhodium porphyrin hydrides and aldehydes, where the corresponding cobalt porphyrin derivatives have not been detected. This paper reports on the formation and dehydration of the α,β-dihydroxyethyl derivative of rhodium octaethylporphyrin, (OEP)Rh-CH(OH)-CH_2OH (**1**), which may be relevant to the structure and reactivity

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