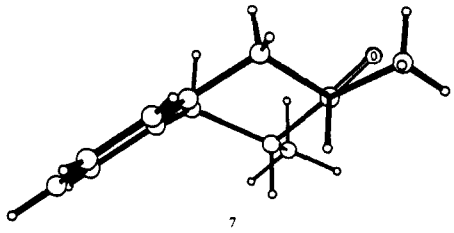
only prove the structure but show the similarity of conformation to those of the six-membered analogues. In the case of **3c** and the acetate derived from the major reduction product, X-ray crystal structures were also determined. The products resulting from equatorial attack are favored upon reduction of both **3b** and **3c**. From **3b**, the ratio of equatorial/axial attack is 60:40, just as predicted, while only the product of equatorial attack can be detected by 500 MHz NMR spectra of the alcohol or derived acetate formed from **3c**. As shown by the Newman projection, **7**, of the crystal structure of **3c**, equatorial attack can clearly occur with less eclipsing than axial attack.



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In general, flattened cyclic ketones such as cyclohexanone itself give axial attack, while puckered ones such as **3**, or dithia analogues of **1** give equatorial attack. This has been summarized as Anh's "flattening rule".[14b,22] Equatorial substituents α to the carbonyl group of **1** or **3** flatten the ring and promote equatorial attack, even though this attack occurs near the substituents. Electronic effects are of minor significance in LAH reductions of cyclic ketones.[23]

In summary, we have demonstrated that the stereoselectivity of LAH reductions of benzocycloheptenones can be correctly predicted by our quantitative calculational model and by Felkin's torsional strain model but not by other models.

Acknowledgment. We are grateful to the National Institutes of Health for financial support of this research.

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Formation and Autocatalytic Destruction of the Quinone Methide from Reductive Cleavage of Menogaril[1]

Marybeth Boldt, Giorgio Gaudiano, Makhluf J. Haddadin,[2] and Tad H. Koch*

Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309-0215

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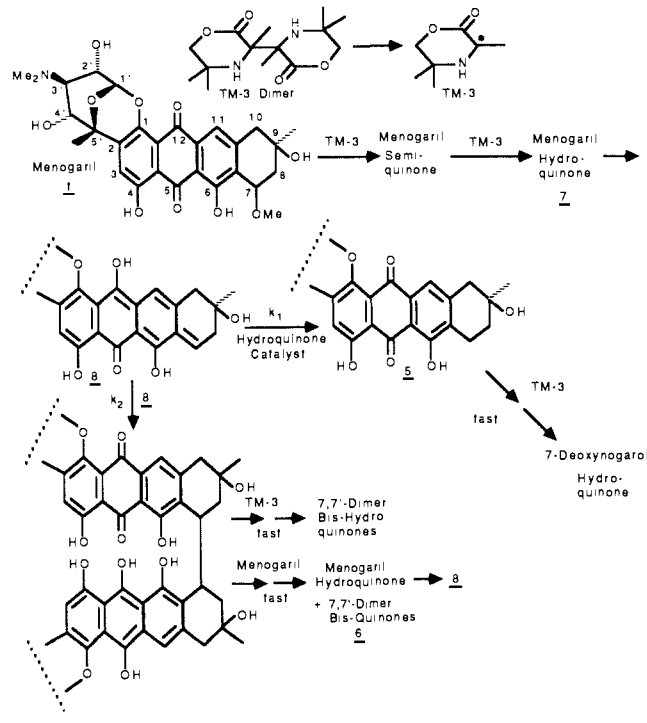
Menogaril, 7-*con*-*O*-methylnogarol (**1**), is a semisynthetic antitumor drug of the anthracycline class presently in clinical trials.[3] It is formed from nogalamycin, a product of the organism *Streptomyces nogalater*.[4] Because of the oxygen substitution

Scheme I



pattern, in particular the presence of an alkoxy substituent at the 7-position and the absence of a hydroxyl substituent at the 11-position, menogaril has the potential for bioreductive activation[5] analogous to that of aclacinomycin A (**2**)[6] and 11-deoxydaunomycin (**3**).[7] We[6,7] and others[8,9] have demonstrated that reduction of **2** and **3** yields the respective 7-deoxyaglycons, 7-substituted aglycons, and 7,7'-dimers of the 7-deoxyaglycons via glycosidic cleavage at the hydroquinone states to form transient quinone methides. 7-Deoxyaglycons result from tautomerization, 7-substituted aglycons from nucleophilic addition, and 7,7'-deoxydimers from combination, one quinone methide serving as a nucleophile and one as an electrophile.[6-9]

Fisher and co-workers have reported that reduction of menogaril with spinach ferrodoxin-$NADP^+$ reductase gives 7-deoxynogarol with only the hydroquinone as an observable transient.[8] We report here that reduction of menogaril with *dl*-bi(3,5,5-trimethyl-2-oxomorpholin-3-yl)(TM-3 dimer)[10] gives 7-deoxynogarol (**5**) and stereoisomers of bi(7-deoxynogarol-7-yl) (**6**) via the observable quinone methide state. Surprisingly, formation of **5** was catalyzed by the presence of hydroquinones including its own hydroquinone and did not occur to any significant extent in the absence of hydroquinones at an apparent pH of 8.

Anaerobic reduction of 1.0×10^{-4} M **1** with 2 or more equiv of TM-3 dimer in apparent pH 8 methanol buffered with tris(hydroxymethyl)aminomethane/tris(hydroxymethyl)aminomethane hydrochloride at ambient temperature gave 85% **5** and 15% **6**. Similar reduction with 0.5 equiv of TM-3 dimer gave 30% **5**, 40% **6**, and 30% recovered **1**. The products, **5** and **6**, were isolated by reverse phase, flash chromatography and characterized from spectroscopic data[11] and in the case of **5** by comparison with

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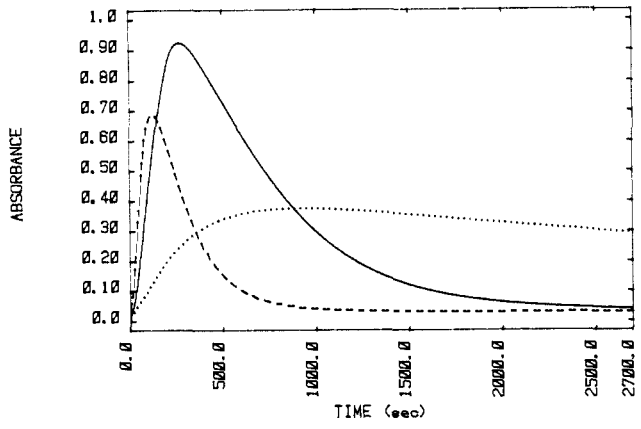

Figure 1. The change in the absorbance at 648 nm as a function of time at 25 ± 0.1 °C of anaerobic, buffered, methanol solutions: (—) 1.0×10^{-4} M **1** and 2.0×10^{-4} M TM-3 dimer; (– – –) 1.0×10^{-4} M **1**, 1.0×10^{-4} M **5**, 5.0×10^{-4} M TM-3 dimer; (···) 1.0×10^{-4} M **1** and 0.50×10^{-4} M TM-3 dimer. The rise and fall resulted from formation and destruction of the quinone methide (**8**) from reductive cleavage.

an authentic sample. The reactions and a kinetic mechanism for formation of **5** and **6** as discussed below are shown in Scheme I.

UV–vis spectroscopic monitoring of the reduction of **1** by TM-3 dimer showed initial formation of the hydroquinone state **7** absorbing at λ_{max} 420 nm followed by formation of the quinone methide state **8** absorbing at λ_{max} 375 and 602 nm with a shoulder at 648 nm. With the disappearance of the quinone methide was appearance of the quinone band of **5** and **6** at λ_{max} 475 nm when substoichiometric quantities of reducing agent were employed and of the hydroquinone band of **5** and **6** at λ_{max} 420 nm when excess reducing agent was employed. The decay of the 602-nm band of **8** was significantly dependent upon whether final products were formed in quinone or hydroquinone states as shown in Figure 1. When final products were formed in hydroquinone states, the decay of the 602-nm band, significantly past its maximum absorbance, appeared first order; however, the apparent first-order rate constant, which was independent of the initial TM-3 dimer concentration, was proportional to the initial menogaril concentration. When final products were formed in quinone states, the decay of the 602-nm band was much slower, second order, and independent of initial menogaril and TM-3 dimer concentrations.

Although the spectroscopic changes occurring during reduction of menogaril are completely analogous to those observed during reduction of the other anthracyclines, the product ratio and the rate of disappearance of the quinone methide state, both as a function of the initial reactant concentrations, were unique. A rationale was evident from the observation that the decay of the 602-nm band in the presence of excess reducing agent was faster when the reaction was run with either product, **5** or **6**, present at time zero as also shown in Figure 1. The product present at time zero was rapidly reduced to its hydroquinone state and served as a catalyst for the tautomerization of the quinone methide to **5**. In the absence of initial product the reaction was autocatalyzed since the hydroquinone states of **5** and **6** were formed as the reaction proceeded. The decay of the 602-nm band in the presence of excess reducing agent was fit by nonlinear least-squares analysis to the following integrated rate law for catalyzed decay of **8** to the hydroquinone of **5** and uncatalyzed dimerization of **8** to the bis-hydroquinone of **6**

$$A_t = \{A_0 k_1([\mathbf{1}]_0 + [\mathbf{5}]_0)\}/[e^{k_1([\mathbf{1}]_0+[\mathbf{5}]_0)t}\{k_1([\mathbf{1}]_0 + [\mathbf{5}]_0) - A_0(k_1 - k_2)/\epsilon\} + A_0(k_1 - k_2)/\epsilon]$$

where A_t is the absorbance at 648 nm at time t from a time zero selected such that all menogaril hydroquinone had eliminated methanol to form quinone methide **8**, A_0 is the absorbance at the selected time zero, $[\mathbf{1}]_0$ is the initial concentration of menogaril, $[\mathbf{5}]_0$ is the initial concentration of the catalyst 7-deoxynogarol (**5**), k_1 is the second-order rate constant for formation of **5** catalyzed by hydroquinones, k_2 is the second-order rate constant for dimerization of **8**, and ϵ is the extinction coefficient for **8** at 648 nm estimated to be 10000. The rate law was derived assuming that the only species present during reaction were **8**, the hydroquinone of **5**, and the bis-hydroquinone of **6** and that all hydroquinones units catalyzed the reaction equally. This assumption was possible because under these conditions, the predominant hydroquinone unit was the hydroquinone of **5**, and an experiment with only **6** added gave a similar value for k_1. The rate constants k_1 and k_2 were 27 ± 2 and 11 ± 1 M^{-1} s^{-1}, respectively, with initial concentrations of menogaril varying from 0.5 to 2.0×10^{-4} M and with initial concentrations of **5** varying from 0 to 1.5×10^{-4} M. With 0.5 equiv of TM-3 dimer as the amount of reducing agent, the integrated rate law for decay of the 602-nm band was simply the integrated second-order rate law, and the rate constant was 5 ± 1 M^{-1} s^{-1}, half the constant observed in the presence of excess reducing agent. The rate constant was half as large because the overall process destroyed two quinone methides and formed one quinone methide, whereas in the presence of excess TM-3 the overall process destroyed two quinone methides (see Scheme I).

The kinetic analysis described above explains all the unusual observations about the rate of disappearance of quinone methide and the product ratio as a function of reaction conditions. With excess reducing agent the decay of the 602-nm band appeared first-order because the exponential term dominates under these conditions. The apparent first-order rate constant was proportional to the initial menogaril concentration because the initial menogaril concentration appears in the exponential term of the integrated, autocatalyzed rate law as shown above. When the decay of the 602-nm band under autocatalyzed reaction conditions was fit by nonlinear least-squares to an integrated rate law which also included an uncatalyzed unimolecular tautomerization of the quinone methide, the fitting procedure made the first-order rate constant very small, less than 5×10^{-5} s^{-1}. Furthermore, when the decay in the absence of hydroquinones was fit to a combined first- and second-order integrated rate law as was appropriate for the decay of the quinone methides from reduction of aclacinomycin A and 11-deoxydaunomycin,[6,7] the fitting procedure again made the first-order rate constant very small, less than 5×10^{-6} s^{-1}. Consequently, at the pH employed here, which was the same as that employed in all of our preceding studies of anthracycline redox chemistry, the quinone methide from reduction of menogaril does not undergo unimolecular tautomerization to 7-deoxynogarol. Formation of the 7-deoxynogarol, observed with 0.5 equiv of TM-3 dimer as the initial amount of reducing agent, must have occurred via menogaril hydroquinone serving as a catalyst for the tautomerization of the quinone methide in the early stages of the reaction. UV–vis monitoring of the reduction under these conditions showed the presence of menogaril hydroquinone as the concentration of quinone methide was rising. Significant quantities of **5** were formed because the catalyzed process is relatively rapid. A possible explanation for the catalysis of tautomerization is that a head-to-head encounter places the acidic 6-hydroxyl of the hydroquinone in the vicinity of the basic 7-position of the quinone methide. Similar, though less effective catalysis by 7-deoxydaunomycinone or alizarin hydroquinone was also observed.

A consequence of the long lifetime of the quinone methide from reduction of menogaril in the absence of hydroquinone species is the potential for nucleophilic trapping.[12] The concept of bioreductive activation of the anthracyclines[5] calls for addition of a nucleophilic site in a critical biological macromolecule such as DNA to the quinone methide. This now seems more likely for the quinone methide from menogaril than the quinone methide

(11) Major diastereoisomeric 7,7′-dimer **6**: ^{1}H NMR (DMSO-d_6) δ 12.78 (s, 1 H, phenolic OH), 12.18 (s, phenolic OH), 7.40 (s, C_{11}-H), 7.17 (s, C_3-H), 6.68 and 6.16 (two d, $C_{2'}$ and $C_{4'}$-OH), 5.75 (d, J = 3.4 Hz, C_1-H), 4.80 (s, C_9-OH), 4.56 (t, J = 6.6 Hz, C_7-H), 4.19 (dd, J = 3.4, 11 Hz, $C_{2'}$-H), 3.99 (d, J = 10.8 Hz, $C_{4'}$-H), 2.70–2.98 (m, C_{10}-H and C_3-H), 2.81 (s, $N(CH_3)_2$), 1.66 (s, 3 H, C_5-CH_3), 1.33 (d, J = 6.6 Hz, C_8-H), 0.85 (s, C_9-CH_3); MS (FAB, positive ion, racemic mixture of $HSCH_2CHOHCHOHCH_2SH$ matrix), m/z (rel intensity) 1022 (M + 2, 75, protonated semiquinone), 511 (100, protonated monomer).

(12) Efficient nucleophilic trapping of **8** with *N*-acetylcysteine has been observed and will be reported in a full paper.

from daunomycin for which the half-life under similar conditions is 53 s.[13] Furthermore, the availability of reducing enzymes and the concentration of hydroquinones produced by them may have an important effect on the binding of the quinone methide from menogaril to biological macromolecules. The slow uncatalyzed tautomerization of the quinone methide and its reactivity with nucleophiles may explain the low recovery of menogaril and its metabolites in animal[14] and human[15] metabolic studies.

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High-Resolution Electrophoretic NMR

Timothy R. Saarinen and Charles S. Johnson, Jr.*

Department of Chemistry, University of North Carolina Chapel Hill, North Carolina 27599-3290

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Here we report a high-resolution electrophoretic NMR experiment which is based on pulsed field gradient FT-NMR. The NMR system is described, and spectra in the presence of electric fields are shown for alkylammonium ions separately and in a mixture. The combination of electrophoresis with high-resolution NMR offers relatively painless determination of mobilities μ for ions in mixtures with internal calibration and with direct indication of complicating effects such as convection and electro-osmosis. It also opens new possibilities for spectral editing in NMR and for the measurement of rates of ionic reactions.

Pulsed field gradient NMR (PFGNMR) was developed by Tanner and Stejskal for the measurement of diffusion and flow.[1,2] The application of this technique to the study of ionic drift in electric fields was apparently first suggested by Packer.[3] Holz and Muller investigated magnetic fields resulting from current flow and found that undesirable gradients are minimized when the electric and magnetic fields are parallel.[4] They then used the parallel field geometry for a demonstration of electrophoretic NMR in a low field proton spin-echo NMR system which permitted no resolution of chemical shifts. In their experiment large concentrations of ions were apparently required to permit detection. The ionic strengths were large, and currents up to 250 mA were used to obtain the necessary electric fields. Accordingly, heating was a severe problem, and the samples required stabilization by a gelling agent.[5]

The experiment described here involves electrophoresis in free solution in a capillary tube. The total ionic concentrations are in the 10-mM range, and current densities and durations are kept below 0.5 A/cm^2 and 1 s, respectively, so that heating effects and convection can be avoided.[6] In the standard PFGNMR sequence, two magnetic field gradient pulses, of amplitude G and duration δ, are inserted into the spin echo sequence (90°-τ-180°). The first is applied between the 90 and 180° pulses, and the second is between the 180° pulse and the echo as shown in Figure 1. In our version of the electrophoretic experiment, an electric field pulse of amplitude E_{dc} is applied during the interval t_f between the two gradient pulses, and the polarity of this field is alternated in successive pulses. For convenience in dealing with the electrodes,

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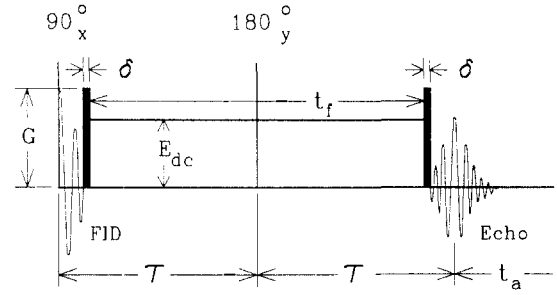

Figure 1. The pulse sequence for high resolution electrophoretic NMR. The acquisition time variable is t_a (see text).

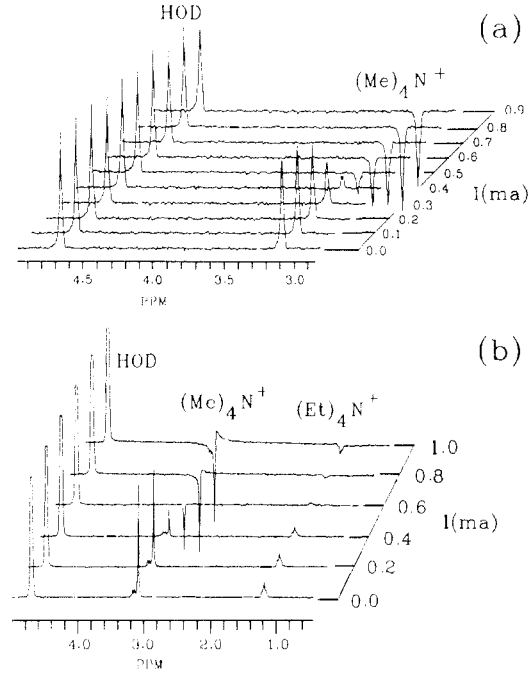

Figure 2. NMR spectra obtained with K = 310 cm^{-1}, δ = 0.75 ms, t_f = 0.75 s, and T = 298 K: (a) 10 mM Me_4NCl in D_2O and (b) 5 mM Me_4NCl and 5 mM Et_4NCl in D_2O. For an ion with mobility μ, the signal amplitudes are proportional to $\cos[K\mu t_f I/(\sigma\kappa)]$ (see text).

a vertical U-tube cell was adopted which gives a counterflow of ions.[4] With this arrangement the echo amplitude at 2τ is given by[2,4,7]

$$M(2\tau) = M(0) \cos(Kvt_f) \exp[-DK^2(t_f + 2\delta/3) - 2\tau/T_2] \quad (1)$$

where $K = \gamma G\delta$, $v = \mu E_{dc}$, D is the tracer diffusion coefficient, and T_2 is the nuclear spin–spin relaxation time. Also, $E_{dc} = I/(\sigma\kappa)$ where I is the current, σ is the cross-sectional area of the tube, and κ is the conductivity. In the FT experiment, data collection commences at the peak of the echo.[8] Fourier transformation then produces an NMR spectrum in which the peaks of the various species show attenuations as described by eq 1.

The experiments were performed on a Bruker WM 250 NMR spectrometer with a custom 10-mm ^{1}H probe built by Cryomagnet Systems. A computer-controlled gradient generator, built in-house for this experiment, produced 0–10 A current pulses for periods up to 12 ms with areas (A·s) reproducible to 1 ppm. Such accurate control is achieved by means of active regulation of pulse amplitudes.[9] This unit drives a pair of opposed Helmholz coils with a separation of 13 mm which produce a gradient of 23.3 G cm^{-1} A^{-1}.[6] A 550 V electric field generator capable of providing gateable currents from 0 to 100 mA was also constructed in-house.[9] The cell consisted of Pt electrodes in a glass U-tube with a cross-sectional area of σ = 0.0268 cm^2.

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