Spectroscopic and Kinetic Evidence for the Tautomer of 7-Deoxyalklavinone as an Intermediate in the Reductive Coupling of Aclacinomycin A

Don L. Kleyer, Giorgio Gaudiano,[1] and Tad H. Koch*

Contribution from the Department of Chemistry, University of Colorado, Boulder, Colorado 80309. *Received August 3, 1983*

Abstract: Reduction of aclacinomycin A (**7**) with an excess of *dl*-bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (**11**) in Trizma-buffered methanol solvent in the absence of oxygen gave 7-deoxyalkavinone (**8**), bi(7-deoxyalkavinon-7-yl) (**9**), 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (**12**), and 3,5,5-trimethyl-2-oxomorpholine (**13**). The reducing agent was 3,5,5-trimethyl-2-oxomorpholin-3-yl (**5**) resulting from bond homolysis of **11**. The relative yields of **8** and **9** were a function of the initial concentrations of **7** and **11** and the reaction time. The semiquinone **15** of **7** was observed as an intermediate by EPR spectroscopy. UV–visible spectroscopic monitoring revealed the formation of the tautomer **14** of 7-deoxyalkavinone as a second intermediate in the formation of both **8** and **9**. The tautomer showed absorption at 350 and 548 nm. Reaction of **9** with **11** resulted in reductive cleavage of **9** to **8** and disproportionation of **11** to **12** and **13**. The mechanism proposed for the reduction of **7** is shown in Scheme III and includes protonation of **14** to give **8** and coupling of **14** followed by oxidation with **12** to give **9**. The decay of the tautomer absorption followed mixed kinetics, first and second order in **14**. Nonlinear least-squares analysis of the decay gave a pseudo-first-order rate constant for protonation equal to 3.36×10^{-3} s^{-1} and a second-order rate constant for coupling equal to approximately 180 M^{-1} s^{-1}. The tautomer **14** of 7-deoxyalkavinone is protonated in buffered methanol 15 times slower than the tautomer **6** of 7-deoxydaunomycinone, thus allowing the coupling reaction to take place. The difference in reactivity of the tautomers may be relevant to the tumor response and toxicity of the two anthracyclines.

The anthracycline antibiotics daunomycin (**1**) and adriamycin (**2**) are clinically important antitumor drugs[2] proposed to be bioreductively activated.[3] Anaerobic reduction of **1** and **2** yields 7-deoxydaunomycinone (**3**) and 7-deoxyadriamycinone (**4**), respectively,[4-6] and/or covalent binding of the drugs to biological macromolecules.[7-9] Aerobic reduction leads to the production of reactive oxygen species.[4] These reductive processes are proposed to be responsible at least in part for the biological activity including the acute cardiotoxicity,[10] and consequently, their mechanisms are under intense chemical and biological investigation.

We have demonstrated that anaerobic reduction of **1** with the reducing agent 3,5,5-trimethyl-2-oxomorpholin-3-yl (**5**) yields 7-deoxydaunomycinone (**3**) via two sequential one-electron reductions followed by elimination of daunosamine to give the tautomer **6**, also described as the quinone methide. Protonation of **6** leads to 7-deoxydaunomycinone (Scheme I).[11] The tautomer **6** is characterized by absorption at 380 and 608 nm and expresses nucleophilic reactivity.[12]

A more recently discovered anthracycline antitumor drug is aclacinomycin A (**7**).[13] The aglycon of **7** differs from the aglycon of **1** and **2** in the A-ring substitution and by one less electron-donating substituent in the anthraquinone chromophore. Aclacinomycin A appears to be less cardiotoxic than **1** or **2**.[14] In vivo and in vitro reduction of **7** gives 7-deoxyaklavinone (**8**) and bi-

Scheme I. Mechanism Proposed by Us for Reductive Glycosidic Cleavage of Daunomycin (**1**) and Adriamycin (**2**)

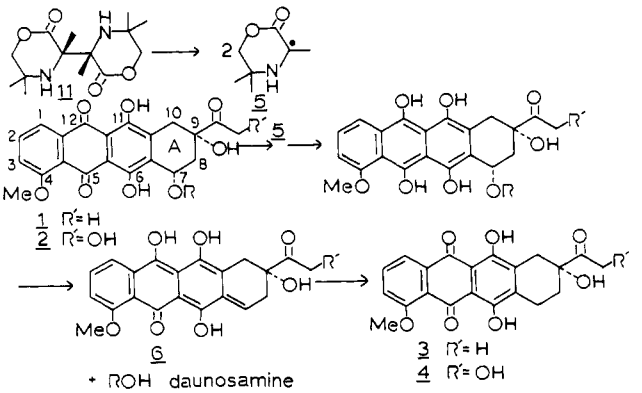

Table I. Yields of 7-Deoxyaklavinone (**8**) and Bi(7-deoxyaklavinon-7-yl) (**9**)

initial concn aclacinomycin A, M	reaction time	ratio **11**:**7**	final concn, M (%) 7-deoxyaklavinone	final concn, M (%) bi(7-deoxyaklavinon-7-yl)
2.9×10^{-3}	24 h	1:1	2.3×10^{-4} (8)	13.1×10^{-4} (92)
2.9×10^{-4}	24 h[a]	1:1	1.4×10^{-4} (46)	0.82×10^{-4} (54)
2.9×10^{-5}	24 h	1:1	2.1×10^{-5} (66)	0.53×10^{-5} (33)
2.9×10^{-4}	24 h	10:1	0.91×10^{-4} (29)	1.1×10^{-4} (71)
2.9×10^{-4}	11 min[b]	10:1	0.40×10^{-4} (13)	1.3×10^{-4} (87)

[a] In this experiment the concentrations of oxazinone **12** and morpholine **13** were found to be $(3.0 \pm 0.1) \times 10^{-3}$ M and $(2.7 \pm 0.1) \times 10^{-3}$ M, respectively. [b] Hydroquinones were still present at the time the reaction solution was terminated by exposure to oxygen.

(1) On leave from the University of L'Aquila, L'Aquila, Italy.
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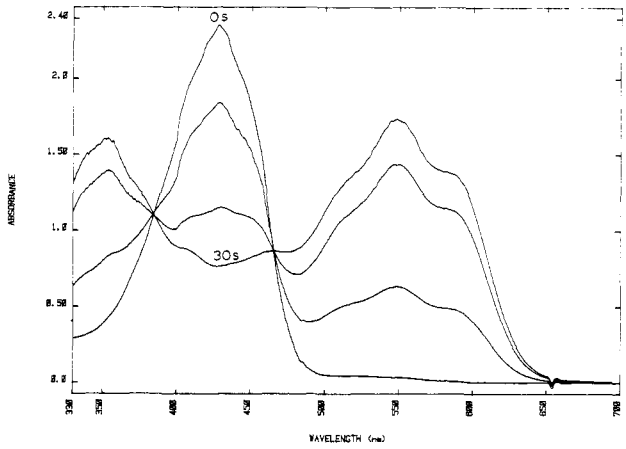


Figure 1. UV–visible absorption of an oxygen-degassed methanol solution 2.02×10^{-4} M in aclacinomycin A, 2.03×10^{-3} M in *dl* dimer **11**, and 4.0×10^{-3} M in Trizma buffer as a function of time at 24.6 ± 0.1 °C. Scans were 1 s in duration and occurred every 10 s in the time period 0–30 s. Absorption at 350 and 548 nm resulted from formation of the tautomer **14** of 7-deoxyalkavinone.

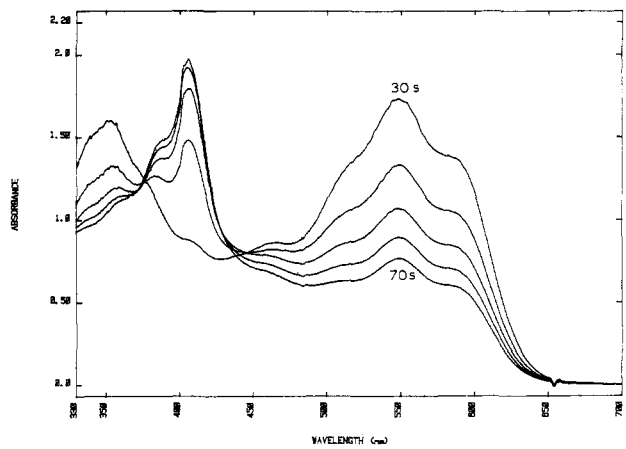
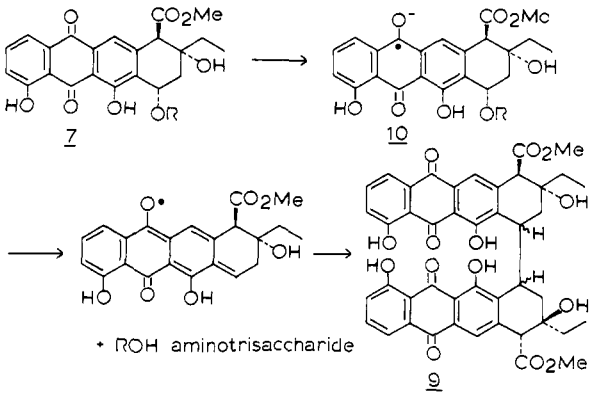
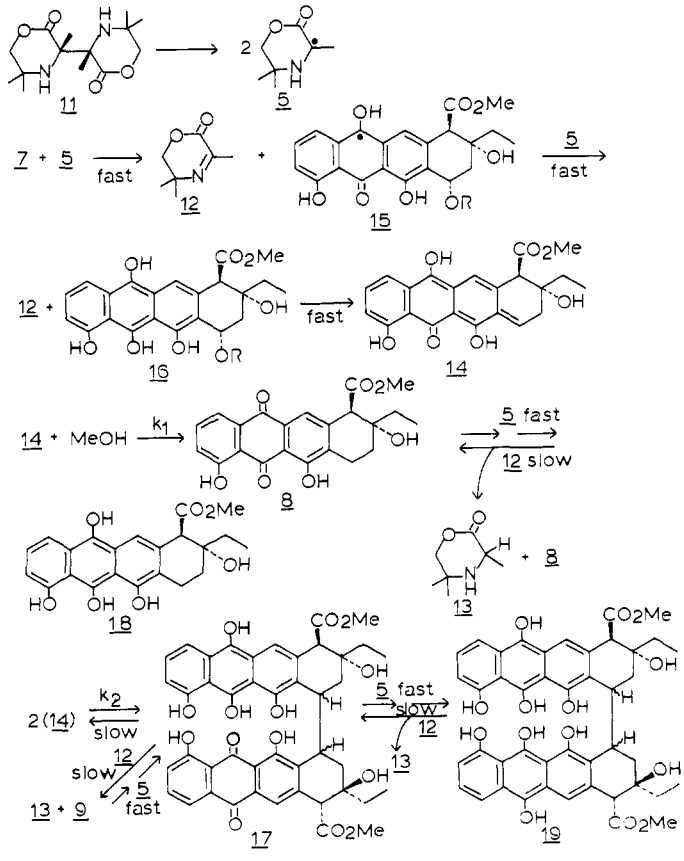


Figure 2. UV–visible absorption of an oxygen-degassed methanol solution 2.02×10^{-4} M in aclacinomycin A, 2.03×10^{-3} M in *dl* dimer **11**, and 4.0×10^{-3} M in Trizma buffer as a function of time at 24.6 ± 0.1 °C. Scans were 1 s in duration and occurred every 10 s in the time period 30–70 s. Absorption at 408 nm resulted from formation of the hydroquinone chromophore.

Scheme II. Mechanism Proposed by Others for Formation of Bi(7-deoxyaklavinon-7-yl) (9) from Reduction of Aclacinomycin A (7)



Scheme III. Mechanism Proposed by Us for Formation of Bi(7-deoxyaklavinon-7-yl) (9) from Reduction of Aclacinomycin A



(7-deoxyaklavinon-7-yl) (**9**).[5,15] The formation of the dimer **9** prompted a proposal of elimination of the sugar moiety at the semiquinone state to give the semiquinone methide, 7-deoxyalkavinon-7-yl (**10**) followed by radical combination (Scheme II).[5,16]

The work described here was performed to establish that the reductive coupling leading to **9** occurs via the quinone methide rather than the semiquinone methide, that daunomycin and aclacinomycin A share similar reactivity with reducing agents, and that the difference in the reductions resides in the reactivity of the quinone methides. The reducing agent employed in this study was again the one-electron reducing agent, 3,5,5-trimethyl-2-oxomorpholin-3-yl (**5**), produced from bond homolysis of bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (**11**).[17] This reducing agent was selected for all of these studies because its kinetic and electronic properties were well established.[17] Furthermore, **11** reduces dramatically the toxicity of adriamycin in mice and might be useful as a rescue agent in high-dose rescue therapy.[18] The mechanism of rescue most likely resides in the reduction reaction.

Results

Reaction of aclacinomycin A with *dl*-bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (**11**) at ambient temperature for 24 h in Trizma-buffered methanol solvent in the absence of oxygen gave 7-deoxyaklavinone (**8**), bi(7-deoxyaklavinon-7-yl) (**9**), 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (**12**), and 3,5,5-trimethyl-2-oxomorpholine (**13**). 7-Deoxyaklavinone and bi(7-deoxyaklavinon-7-yl) were isolated by silica gel flash chromatography[19] and identified by comparison of their spectral properties with those in the literature.[13] Oxazinone **12** and morpholine **13** were identified by GLC and ^{1}H NMR spectroscopy.[17] Yields as a function of initial reactant concentrations are shown in Table

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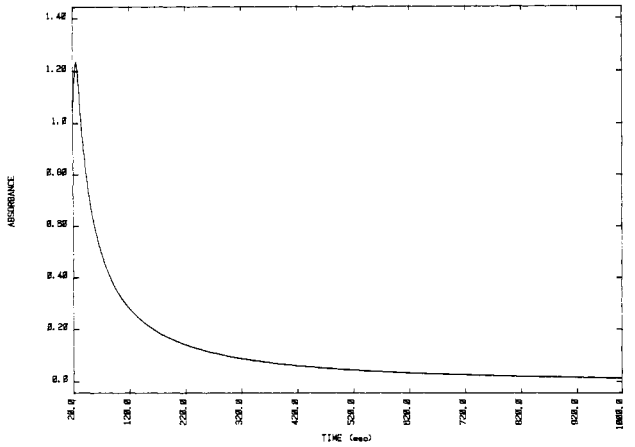

Figure 3. Absorbance at 600 nm of an oxygen-degassed methanol solution 2.02×10^{-4} M in aclacinomycin A, 2.03×10^{-3} M in *dl* dimer **11**, and 4.0×10^{-3} M in Trizma buffer as a function of time at 24.6 ± 0.1 °C.

I. In the experiments, which were run with a molar ratio **11**:**7** ≥ 1, the coupling product **9** was favored at higher concentrations of either aclacinomycin A or **11** or both. When a large excess of **11** was used, terminating the reaction after 11 min by the addition of oxygen also increased the relative yield of **9**. As will be reiterated later with initial concentrations of **7** and **11** determine the concentration of the important reactive intermediate **14** (Scheme III), which then in part determines the relative yields of **8** and **9**.

Monitoring the reaction of aclacinomycin A with 10 mol equiv of **11** at 24.6 ± 0.1 °C by UV–visible spectroscopy gave the spectral changes as a function of time shown in Figure 1 over the time period 0–30 s and in Figure 2 over the time period 30–70 s. Absorption at 430 nm resulted from **7** and absorption at 350 and 548 nm from the tautomer **14** of 7-deoxyaklavinone. The assignment of the 350- and 548-nm bands was based upon the observation of absorption at 380 and 608 nm for the structurally similar tautomer **6**.[11] The absorption in the region of 408 nm in the Figure 2 resulted from formation of product molecules bearing the hydroquinone chromophore. The hydroquinone of 7-deoxydaunomycinone has maximum absorption in methanol solvent at 420 nm.[20] After 24 h the absorption band at 430 nm reappeared and the spectrum was similar to the spectrum at time 0. Figure 3 shows the absorbance change at 600 nm as a function of time. At 600 nm no other species except **14** absorbed, and the absorbance was then proportional to the concentration of this species. As will be discussed later the decay in the absorbance after 80 s followed mixed kinetics, first and second order in **14**. The large excess of **11** was employed in these experiments to simplify the kinetic analysis.

A methanol solution of aclacinomycin A and **11** in the ratio 1:1 at 1 °C gave the EPR signal shown in Figure 4. The spectrum was assigned to the semiquinone **15** of **7** on the basis of a g = 2.0038 and the splitting constants. Determination of the splitting constants reported in the legend of Figure 4 was facilitated by first analyzing the spectrum of **15** taken in methanol-*d* solvent at 5 °C. In deuterated solvent the two phenolic OH proton splittings of 18 G disappeared. The g value of the daunomycin semiquinone similarly generated in methanol is 2.0037.[11] The EPR spectrum of the semiquinone of bi(7-deoxyaklavinon-7-yl) (**9**), generated by reaction of **9** with **5**, was distinct from the EPR spectrum of **15**. The lower temperatures were employed for the EPR experiments in order to slow the reactions to a rate convenient for the measurements. The concentration of **15** was insufficient to produce significant absorption in the visible spectrum.

Reaction of bi(7-deoxyaklavinon-7-yl) (**9**) with 4 mol equiv of **11** in oxygen-degassed methanol for 24 h at ambient temperature

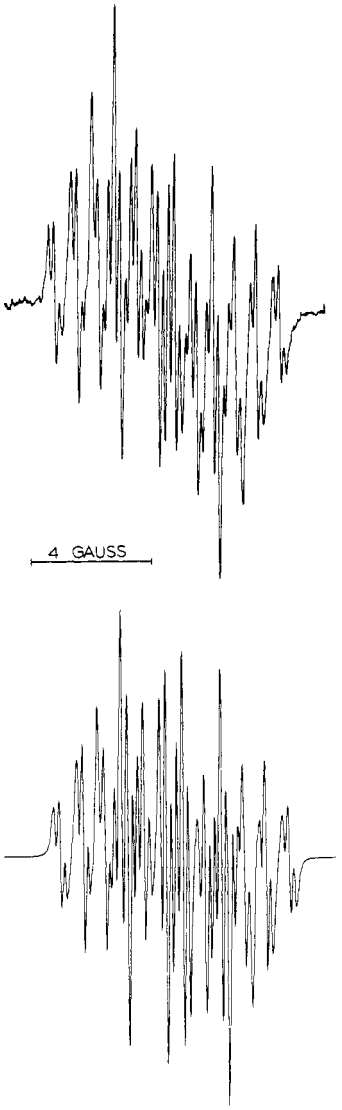

Figure 4. Actual and simulated EPR spectra of aclacinomycin A semiquinone observed at 1 °C in a methanol solution 1.73×10^{-3} M in aclacinomyin A, 4.0×10^{-2} M in Trizma buffer, and 1.74×10^{-3} M in *dl* dimer **11**. The g value is 2.0038 and the hyperfine splittings used in the simulation were 1.81 G (1:1), 1.48 G (1:1), 1.44 G(1:1), 1.23 G (1:1), 0.76 G (1:2:1), 0.18 G (1:2:1).

gave 7-deoxyaklavinone (**8**, 40%), recovered **9** (60%), **12**, and **13**. This reaction occurred very slowly, and the proposed intermediate, tautomer **14** (vide infra), was never present at sufficient concentration to be observed spectroscopically. The spectral changes observed with a solution 5.1×10^{-5} M in **9** and 1.9×10^{-4} M in **11** indicated formation of hydroquinones in the time period 0–180 s followed by regeneration of quinones over a period of hours.

Discussion

These observations coupled with our extensive experience with the mechanism of reductive glycosidic cleavage of daunomycin[11,12,19] suggest that the chemistry described occurred via the mechanism shown in Scheme III. The mechanism involves two rapid sequential one-electron reductions of aclacinomycin A (**7**) by 3,5,5-trimethyl-2-oxomorpholin-3-yl (**5**) to give the hydroquinone **16**, which rapidly eliminates the sugar moiety yielding the tautomer **14**. Tautomer **14** is the important reactive intermediate. Protonation of **14** leads to 7-deoxyaklavinone, while coupling of two molecules of **14**, one serving as an enol and the other as a Michael acceptor, yields reversibly the dimeric quinone–hydroquinone **17**. Bi(7-deoxyalkavinon-7-yl) (**9**) results from slow oxidation of **17** by oxazinone **12**. Oxidations of this type find precedent in the reaction of 7-deoxydaunomycinone hydroquinone with **12** and appear to occur by hydride transfer.[20] Rapid re-

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ductions of **8** to **18** and **17** to **19** also occur with the excess of reducing agent present. The hydroquinones **17**, **18**, and **19** are eventually oxidized by **12**. The data are consistent with this mechanism as follows.

The relative product yields in Table I are explained in terms of **14** as a common intermediate. The initial concentrations of **7** and **11** determined the concentration of **14** as a function of time. At higher concentrations of **14** the bimolecular coupling was the favored reaction. Also in methanol-*d* solvent the yield of **8** dropped essentially to zero consistent with the expected large deuterium kinetic isotope effect on protonation.[11]

The spectral changes in Figures 1 and 2 indicate the time scale for the processes. Figure 1 showed rapid formation of **14** with sharp isosbestic points, indicating that the formation of **14**, in the presence of a large excess of the reducing agent **11**, during the first 30 s was much faster than its destruction. Figure 2, which shows reaction during the 30–70-s period, indicates that as **14** reacted, hydroquinone type products appeared, consistent with rapid reduction of the initially formed quinone products **8** and **17** by the excess reducing agent. Rapid subsequent reduction of **8** and **17** was anticipated because **7**, **8**, and **17** all bear the same electrophore. The broad isosbestic points in Figure 2 primarily reflect simultaneous formation and destruction of **14**. As Figure 3 indicates at 30 s the rate of formation of **14** equaled its rate of destruction, and in the period 30–70 s, formation must still have been occurring at a significant rate.

The increase in the relative yield of **9** upon termination of the reaction with oxygen after 11 min can be explained in terms of a rapid reduction of **17** to **19** by **5** followed by a rapid oxidation of the bis(hydroquinone) **19** to **9** by oxygen. With the back oxidation of **19** to **17** by **12** a slow process, the reverse sequence **19** → **17** → **14**, which would increase the relative yield of **8**, becomes significant only in the late stage of the reaction. The much higher rate of reduction of the quinones by **5** than oxidation of the hydroquinones by **12** as indicated by the accumulation of hydroquinones in the second stage of the reaction (Figure 2). The relative rates of reduction of **8**, **9**, and **17** by **5** and oxidation of **17**, **18**, and **19** by **12** are comparable to analogous processes observed upon reduction of 7-deoxydaunomycinone with **11**.[20]

The intermediacy of **14** in the coupling reaction is established because **14** was formed in amounts exceeding the yield of 7-deoxyaklavinone, and no anthracycline products other than **8** and **9** were obtained. Assuming an excitation coefficient for **14** at 548 nm similar to that determined for the tatuomer **6** of 7-deoxydaunomycinone at 608 nm, the concentration of **14** at maximum was more than half of the initial concentration of **7** for the experiment shown in Figure 1. Hence, a mechanism like that shown in Scheme II or other mechanisms involving, in the coupling step, any intermediate between **7** and **14** is unlikely.

The cleavage of **17** to **14** is indicated by the observation that treatment of **9** with **11** led to **8**. Reversibility and relative rate of the coupling reaction were also indicated by the change in the relative yields of **8** and **9** as a function of reaction time with excess reducing agent as discussed above. Hence, **9** is the kinetic product of reaction of **14** under the conditions employed in this study.

In addition to the previous considerations the slow oxidations of the hydroquinones **17**, **18** and **19** by the oxazinone **12** are indicated by the reappearance of the quinone chromophore after 24 h. The formation of the morpholine **13** in a time period short relative to the time required for disproportionation of **5**[20] is also consistent with the occurrence of these steps.

The decay in the absorption at 600 nm shown in Figure 3 followed clean mixed kinetics, first and second order in **14** concentration. Nonlinear least-squares analysis showed that in the time region 80–700 s, the decay followed the kinetic expression:

$$A_t = (A_0k_1)/\{e^{k_1t}(k_1 + (A_0k_2/\epsilon)) - (A_0k_2/\epsilon)\}$$

where A_t is the absorbance of **14** at time t, A_0 is the absorbance at time 80 s, t is the time from 80 s, k_1 is the pseudounimolecular rate constant for protonation of **14**, k_2 is the bimolecular rate constant for dimerization of **14**, and ϵ is the extinction coefficient for **14** at 600 nm. The initial 80 s was sufficient time for production of enough **5** to reduce all the aclacinomycin A twice since the rate constant for bond homolysis of **11** in methanol at 25 °C is 3.36×10^{-3} s^{-1}.[21] The calculated values of k_1 and k_2/ϵ were $(8.9 \pm 0.1) \times 10^{-4}$ s^{-1} and $(2.6 \pm 0.1) \times 10^{-3}$ s^{-1} cm^{-1}. If the extinction coefficient of **14** at 548 nm equals the extinction coefficient of **6** at its maximum, 608 nm, then the ϵ of **14** at 600 nm equals 7×10^3 and $k_2 = 183$ M^{-1} s^{-1}.

The difference in reactivity of the tautomers **6** and **14** is primarily manifested in the difference in substitution at the 11-position. 7-Deoxydaunomycinone tautomer (**6**) bears an additional electron-donating substituent at the 11-position making it more basic and nucleophilic. The rate constant for protonation of **6** is in fact 15 times larger than the rate constant for protonation of **14**. The absence of the 11-hydroxy group in the tautomer **14** of 7-deoxyaklavinone makes it a better electrophile and a poorer nucleophile; hence, the coupling reaction is enhanced relative to protonation. The coupling reaction is mechanistically related to the base-promoted coupling of tetraisobutrylriboflavin.[22]

Work of others has shown that the relative yields of **8** and **9** can be dramatically altered by changing the pH of the medium[23] or by inhibiting bimolecular interaction of **14**.[24] Reduction of **7** with zinc and acetic acid or with an enzyme system that binds **7** and **14** tightly yielded only **8**.

In summary we have shown that the tautomer **14** of 7-deoxyaklavinone, also described as the quinone methide, is a reactive species formed by reductive cleavage of aclacinomycin A. The difference in product formation relative to reductive glycosidic cleavage of daunomycin results from a difference in the nucleophilicity and/or basicity and electrophilicity of the respective tautomers. This difference in reactivity of the tautomers **6** and **14** may be responsible at least in part for the difference in biological activity.

Experimental Section

General Remarks. EPR spectra were recorded with a Varian Associates 109E spectrometer equipped with field-frequency lock. A Hewlett Packard 8450A rapid-scan spectrometer was used for obtaining UV–visible spectral data. GLC analyses were performed with a Varian Aerograph Model 940 gas chromatograph equipped with a flame ionization detector and a Hewlett Packard 3390A integrator. HPLC analyses were performed with a Tracor liquid chromatograph equipped with a Model 970A variable-wavelength detector and a Hewlett Packard 3390A integrator. ^{1}H NMR spectral data were obtained with a Bruker 250-MHz spectrometer. Solvents were either reagent or spectroscopic grade. Aclacinomycin A was obtained from the National Cancer Institute, Drug Development Branch, and a sample of 7-deoxyaklavinone was obtained from Hoffmann-La Roche, Inc. *dl*-Bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) was prepared by photoreductive dimerization of 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one followed by alumina flash chromatography.[25] Tris, tris (hydroxylmethyl)aminomethane, and Tris·HCl, tris(hydroxymethyl)aminomethane hydrochloride, were obtained from Sigma.

Freeze–Thaw Degassing Procedure. Methanol solutions in multicompartment cells described below were oxygen degassed on a high-vacuum line. Each solution was frozen in liquid nitrogen and evacuated to a pressure of 2×10^{-6} torr. At this time the glass directly above the frozen sample was warmed with a heat gun. The liquid nitrogen was then removed, and the solution was thawed by immersion in an ice bath. The heat-gun warming was necessary to prevent contamination of other compartments with solution from bumping during the first thaw cycle. After thawing, the solution was sonicated for about 5 s before freeze–pump–thawing was repeated. Four cycles were performed; then, the sample was frozen, evacuated, and sealed with a torch. This rigorous degassing technique was necessary to prevent oxidation of *dl* dimer **11** and hydroquinone species by oxygen remaining in solution.

Aclacinomycin A Semiquinone EPR Spectrum. A three-compartment cell was used. Two of the compartments were 1.2-cm o.d., one fused in line with a 0.9-cm tube for attachment to a vacuum line with an Ulatra Torr Union and the second fused perpendicular to the 0.9-cm tube. The third compartment, also fused perpendicular to the 0.9-cm tube, was a Wilmad #705-PQ EPR tube 0.199 ± 0.0013 cm i.d. *dl* dimer **11** (15.1

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mg, 5.32×10^{-5} mol) was diluted to 100 mL in a volumetric flask with dichloromethane, and 0.98 mL (5.21×10^{-7} mol of **11**) of this solution was syringed into the 1.2-cm o.d. perpendicular compartment. The dichloromethane was evaporated with a stream of nitrogen. A 0.30-mL sample of a methanol solution 1.73×10^{-3} M in aclacinomycin A (5.19×10^{-7} mol) and 4.0×10^{-2} M in Trizma buffer (2.0×10^{-2} M in each Tris and Tris·HCl) was syringed into the second 1.2-cm o.d. compartment. The methanol solution was freeze–thaw degassed and sealed. After temperature equilibration of the methanol solution at 0 °C for 5 min, the solution was mixed at 0 °C with the *dl* dimer **11** by shaking in an ice water bath for 2 min. The sample was then placed in the EPR cavity maintained at 1 °C. The spectrum obtained is shown in Figure 4. Under these conditions the signal persisted for at least an hour.

Spectroscopic Monitoring of the Reaction of Aclacinomycin A with *dl* Dimer 11. A two-compartment cell was used consisting of a 1-cm Beckman, Pyrex cuvette fused to a degassing chamber 1.6 cm o.d. × 4.5 cm long and a 0.9-cm o.d. tube for attachment to a vacuum line with an Ultra Torr Union. The angles between the cuvette and the 0.9-cm tube and 1.6-cm chamber were 90° and 120°, respectively. The 120° angle is necessary to prevent concentration changes resulting from solvent distillation in the cell during the course of the reaction. Aclacinomycin A (8.2 mg, 1.01×10^{-5} mol), Tris (12.1 mg, 1.0×10^{-4} mol), and Tris·HCl (15.8 mg, 1.0×10^{-4} mol) were dissolved in 50 mL of methanol in a volumetric flask by magnetic stirring for 2 h. *dl* Dimer **11** (8.8 mg, 3.10×10^{-5} mol) was dissolved in 5.0 mL of methylene chloride. A syringe was used to transfer 0.82 mL (5.08×10^{-6} mol) of the methylene chloride solution to the cuvette compartment of the cell. The methylene chloride was evaporated with a stream of nitrogen. An aliquot of the aclacinomycin A solution (2.5 mL, 5.05×10^{-7} mol) was added to the 1.6-cm chamber. The methanol solution was freeze–thaw degassed and the apparatus sealed. The cell was then transferred to a thermostated cell holder at 24.6 ± 0.1 °C. The cell holder consisted of an aluminum block milled to accomodate the entire apparatus and connected to a refrigerated circulator. The thermostated cell holder replaced the standard cell holder of the Hewlett Packard 8450A spectrometer. The methanol solution was temperature equilibrated at 24.6 ± 0.1 °C for 20 min by placing the cell holder at 90° to its normal position. The *dl* dimer **11** and the aclacinomycin A solution were then rapidly mixed by shaking the cell holder vigorously. The absorbance of the solution from 330 to 700 nm as a function of time was recorded as shown in Figures 1 and 2. After 24 h the spectrum showed only the anthraquinone chromophore and appeared similar to the spectrum at time 0. An identical reaction solution gave the absorbance change at 600 nm vs. time as shown in Figure 3. The reaction was monitored at 600 nm because only the tautomer of 7-deoxyaklavinone absorbed significantly at this wavelength. The decay in the absorbance at 600 nm over the time period 80–700 s fits perfectly to a combined first- and second-order kinetic expression, using a nonlinear least-squares fitting procedure.

Yields of 8 and 9 as a Function of Reactant Concentrations. Two compartment cells were charged and freeze–thaw degassed as described above to give methanol solutions with the concentrations of aclacinomycin A and *dl* dimer **11** indicated in Table I buffered with a 1:1 mixture of Tris and Tris·HCl. Upon mixing, the solutions, except one, were allowed to react at ambient temperature for 24 h. One reaction was terminated after 11 min by opening to oxygen. The solutions were analyzed for **8** and **9** by HPLC with a 0.30 × 0.04 m Alltech RSIL-phenyl column eluting with 3% water–97% methanol at 2.0 mL/min, detecting at 438 nm. The analytical method was calibrated with standard solutions. The yields of **12** and **13** were determined by GLC using a 3.7 m × 0.32 cm SE-30 on 100/120 mesh high-performance Chromosorb W column at 130 °C eluting with helium at 25 mL/min. The concentrations of **12** and **13** from the reaction mixture 2.9×10^{-4} M in **7** and 2.9×10^{-3} M in **11** were $(3.0 \pm 0.1) \times 10^{-3}$ M and $(2.7 \pm 0.1) \times 10^{-3}$ M, respectively, determined relative to standard solutions. Formation of **12** and **13** was also verified by ^{1}H NMR spectroscopy.

7-Deoxyaklavinone (**8**) and bi(7-deoxyaklavinon-7-yl) (**9**) were isolated from two reactions run similarly. The products were separated by flash chromatography[19] on a 1.0-cm o.d. column packed with 15 cm of Merck silica gel 60 (40–63 μm) and eluted at a flow rate of 5 cm/min with 1.8% methanol–98.2% methylene chloride. The products were identified as **8** and **9** by comparison of ^{1}H NMR spectral data with data in the literature.[13]

Reductive Cleavage of Bi(7-deoxyalklavinon-7-yl) (9). A two-compartment cell was charged and freeze–thaw degassed as described above to give a methanol solution 5.1×10^{-5} M in **9**, 1.9×10^{-4} M in *dl* dimer **11**, 4.0×10^{-3} M in Tris, and 4.0×10^{-3} M in Tris·HCl. Upon mixing, the solution was allowed to react at ambient temperature for 24 h. Spectroscopic monitoring showed formation of the hydroquinone chromophore characterized by absorption at 408 nm over the time period 0–180 s. After 24 h the characteristic quinone absorption had reappeared. HPLC analysis as described above indicated that the solution contained 4.1×10^{-5} M 7-deoxyaklavinone (**8**) and 3.1×10^{-5} M bi(7-deoxyaklavinon-7-yl) (**9**). GLC analysis using the condition described above indicated that the concentration of **12** was 2.9×10^{-4} M and the concentration of **13** 0.87×10^{-4} M.

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On the Structure of Micelles

F. M. Menger* and Daniel W. Doll

Contribution from the Department of Chemistry, Emory University, Atlanta, Georgia 30322.
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Abstract: Kinetic studies of micellar olefin oxidation by permanganate ion show that a terminal olefin is oxidized 2 orders of magnitude faster than internal olefins. This is interpreted in terms of coiling and disorder which place chain termini in the water-rich Stern region. The results are not consistent with the Dill–Flory and Fromherz models.

Several years ago Breslow et al.[1] reported that the photolysis of benzophenone-4-carboxylate in SDS or CTAB micelles leads predominantly to oxygen insertion at the terminal methylene of the surfactant tails. As much as 27% of the functionalization occurs at C-11 of SDS. We interpret this observation as evidence for micelle looping and disorder which bring into proximity the chain termini and carbonyls near the micelle surface (where the anionic benzophenone certainly resides[2]). The word "disorder" signifies here a nonradial positioning of the chains as would occur, for example, in a "brush-heap" configuration.[3] Unfortunately, the photolysis experiments required large amounts of benzo-

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