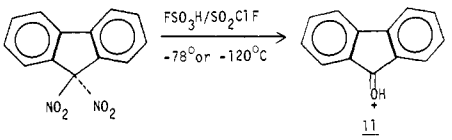
at δ ^{13}C 186.0 and the para carbon at δ ^{13}C 170.4 indicates extensive charge delocalization into the aromatic ring. The complete assignment is shown in Table I. Upon warming of the solution to −40 °C for several hours the cation **2** completely, and irreversibly fragments to the O-protonated 4,4′-dimethylbenzophenone (**9**).[18b] The intermediate O-nitrosated 4,4′-dimethylbenzophenone (or its protonated form) was not observed in this case.

Attempts to prepare 9-nitro-9-fluorenyl cation (**10**) by the ionization of 9,9-dinitrofluorene in FSO_3H/SO_2ClF either at −78 or −120 °C was not successful. Only protonated fluorenone **11**[18a]



11

was observed in the media, indicating the instability of 9-nitro-9-fluorenyl cation (**10**). This can be readily rationalized by the similar failure to observe the parent 9-fluorenyl cation under stable ion conditions.[18a] Only 9-fluorenyl cations with strongly stabilizing substituents were observed.

We were also unsuccessful in preparing α-nitrodialkylmethyl cations under stable ion conditions. Therefore, it appears that to counteract the electron-withdrawing effect of the nitro group strongly positive charge stabilizing substituents are needed to obtain long-lived α-nitromethyl cations. We are continuing our study of nitro carbocations.

Experimental Section

The starting α,α-dinitro compounds were prepared by the oxidation of the corresponding ketoximes with N_2O_4 following the procedure of Frojmovic and Just.[12]

General Procedure. To a N_2O_4-saturated stirred solution of dry dichloromethane (50 mL) in a 250-mL round bottom flask at 0 °C is rapidly added the corresponding ketoxime (20 mmol) in 15 mL of dichloromethane. The resulting mixture is brought to room temperature and stirred for a period of 10 min. Subsequently the mixture is evaporated to dryness in a Rotavap at ambient temperature in a well-ventilated hood. The resulting residue is chromatographed on silica gel (50 g) with hexane–benzene (7:3) as eluant. The collected eluant is evaporated to obtain the gem dinitro compound.

α,α-Dinitrodiphenylmethane: mp 80.4 °C (lit.[12] 79–79.5 °C); ^{13}C NMR ($CDCl_3$, ambient), δ 131.6 (d), 131.1 (s), 129.8 (d), 128.6 (d), 126.6 (s).

α,α-Dinitroditolylmethane: Viscous yellow oil; ^{13}C NMR ($CDCl_3$, ambient), δ 142.1 (s), 129.6 (d), 129.1 (d), 128.9 (s), 128.2 (s).

9,9-Dinitrofluorene: mp 134.2 °C (lit.[12] 131–133 °C); ^{13}C NMR ($CDCl_3$, ambient), δ 142.1 (s), 133.7 (d), 132.8 (s), 129.3 (d), 127.2 (d), 120.9 (d), 119.5 (s).

The ~5% ^{15}N-enriched α,α-dinitrodiphenylmethane was prepared by using 15% ^{15}N-enriched benzophenone oxime and N_2O_4. There was loss of enrichment during the oxidation. This can be rationalized by a radical-type reaction. ^{15}N-enriched benzophenone oxime (15%) was prepared from 15% ^{15}N-enriched hydroxylamine hydrochloride and benzophenone; 99% ^{15}N-enriched hydroxylamine hydrochloride was purchased from MSD Isotopes, Canada.

Preparation of Carbocations. To a cooled solution of FSO_3H in SO_2ClF at −78 °C (dry ice/acetone bath) or −120 °C (ethanol/liquid N_2 slush) is added a SO_2ClF slurry of dinitro compound in small portions with stirring so as to obtain ~15% of the ionic solution.

The ^1H NMR spectra were recorded by using a Varian Associates XL-200 NMR spectrometer equipped with a variable temperature probe. ^{15}N and ^{13}C NMR spectra were obtained on a Varian Associates Model FT-80 NMR spectrometer equipped with a variable temperature probe. The ^1H and ^{13}C NMR chemical shifts are referenced to those of external capillary Me_4Si. The ^{15}N shifts are with respect to that of anhydrous NH_3.

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Registry No. 1, 89196-81-6; **2**, 89196-82-7; **3**, 89196-84-9; **5**, 87963-52-8; **6**, 89196-86-1; **6** (deprotonated), 89196-85-0; **7**, 89196-87-2; **8**, 25721-22-6; **10**, 89196-80-5; **11**, 25721-28-2; ^{15}N, 14390-96-6; N_2O_4, 10544-72-6; α,α-dinitrodiphenylmethane, 21160-03-2; α,α-dinitrodi-*p*-tolylmethane, 89196-83-8; 9,9-dinitrofluorene, 21159-97-7; ^{15}N-enriched α,α-dinitrodiphenylmethane, 89196-88-3; ^{15}N-enriched benzophenone oxime, 89196-89-4; ^{15}N-enriched hydroxylamine hydrochloride, 40711-48-6; benzophenone, 119-61-9.

Mechanistic Investigation of Reduction of Daunomycin and 7-Deoxydaunomycinone with Bi(3,5,5-trimethyl-2-oxomorpholin-3-yl)

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Contribution from the Department of Chemistry, University of Colorado, Boulder, Colorado 80309. *Received August 15, 1983*

Abstract: The mechanisms of reduction of 7-deoxydaunomycinone (**3**) and daunomycin (**1**) by 3,5,5-trimethyl-2-oxomorpholin-3-yl (**5**), the one-electron reducing agent formed from bond homolysis of *dl*-bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (**4**), are described. Reaction of **3** with **5** in buffered methanol gave the semiquinone **9** of 7-deoxydaunomycinone characterized by EPR spectroscopy. The semiquinone was further reduced to the hydroquinone **8** characterized by a transient absorption maximum at 420 nm (ϵ 12 000) in the visible spectrum. The hydroquinone **8** subsequently reacted in a single step with 1 equiv of 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (**6**), the product of oxidation of **5**, to give 3,5,5-trimethyl-2-oxomorpholine (**7**) with a rate constant of 2 M^{-1} s^{-1} at 25 °C and a deuterium kinetic isotope effect of 3. The reduction of **6** is proposed to occur by hydride transfer. The overall reaction, disproportionation of **5**, was facilitated by the creation of a two-electron reducing agent from a one-electron reducing agent. Reduction of **1** with **5** in buffered methanol gave the semiquinone **12**, which was further reduced to the hydroquinone **10** also characterized by a very transient absorption at 420 nm. The hydroquinone rapidly eliminated daunosamine to give the tautomer **11** of 7-deoxydaunomycinone. Tautomer **11** showed transient absorption at 380 and 608 nm (ϵ 9400). Protonation of **11** by methanol gave 7-deoxydaunomycinone (**3**) and occurred with a pseudounimolecular rate constant of 0.013 s^{-1} and a deuterium kinetic isotope effect of 9. Tautomer **11** reacted as a nucleophile with benzaldehyde to give the aldol adduct **19** with a rate constant of 0.09 M^{-1} s^{-1}. This work represents the first spectroscopic observation and chemical trapping of **11**, proposed as a biologically active form of daunomycin.

Daunomycin (**1**) is an anthracycline antitumor drug produced by a mutant strain of *Streptomyces peucetius*.[1] Because of its value in the treatment of acute leukemia and the value of the structurally related anthracycline, adriamycin (**2**), as a broad

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spectrum antitumor drug, the metabolism and mode of action of both **1** and **2** have been extensively investigated. Current thought concerning the mode of action focuses on the redox chemistry. Reduction of daunomycin and related antitumor drugs in the presence of oxygen leads to catalytic production of reactive oxygen species which may be responsible for the antitumor activity[2-11] as well as the serious side effects of acute cardiotoxicity.[12-14] Anerobic reduction of daunomycin leads to glycosidic cleavage and formation of 7-deoxydaunomycinone (**3**).[2-4,15] The mechanism of glycosidic cleavage has been intensely investigated because of its possible role in the covalent binding of the anthracyclines to biological macromolecules including DNA.[16-23] Glycosidic cleavage after a one or a two electron reduction of the anthraquinone electrophore has been debated[2,24] as well as the possible role of reactive intermediates in the covalent binding of **1** and **2** to biological macromolecules.[16-18,22,23]

We have observed that the radical dimer, bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (**4**) dramatically reduces the toxicity of adriamycin in mice and have proposed that **4** might be useful in high-dose anthracycline-rescue therapy.[25] This observation prompted our investigation of the in vitro reactivity between **1** and **4** which might be the basis of rescue.

Earlier we demonstrated that **4** efficiently caused the glycosidic cleavage reaction.[26] We now have employed **4** as a tool for studying the mechanism of glycosidic cleavage. Reductions with **4** occur in one-electron steps, and the actual reducing agent is the 3,5,5-trimethyl-2-oxomorpholin-3-yl radical (**5**) resulting from bond homolysis of **4**.[27] Commonly reduction occurs much faster than radical recombination.[28] In retrospect, the radical dimer

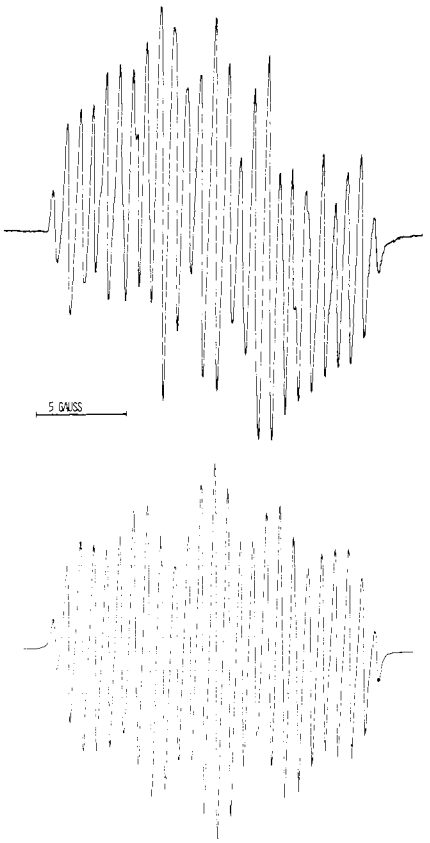

Figure 1. EPR spectrum of the semiquinone **9** of 7-deoxydaunomycinone observed at 40 °C with an oxygen-degassed dimethyl sulfoxide solution 2.09×10^{-3} M in **3**, 2.09×10^{-3} M in *dl*-dimer **4**, 4.0×10^{-2} M in Tris, and 4.0×10^{-2} M in Tris·HCl: top, experimentally observed spectrum; bottom, computer simulation generated with the following parameters, 0.59 (1:3:3:1), 1.18, (1:1), 5.80 (1:1), 2.32 (1:1), 3.06 G (1:1). The measured *g* value was 2.0037.

4 was particularly useful for these studies because it produced all of the important redox states of the anthraquinone electrophore and because the kinetics of reductions with **4** were reasonably well understood. The *dl*-stereoisomer of **4** was employed because it cleaves more slowly than the *meso*-stereoisomer, which made kinetic measurements easier.

Results and Discussion

Redox Chemistry of 7-Deoxydaunomycinone. Because 7-deoxydaunomycinone (**3**), the product of reductive glycosidic cleavage of daunomycin, bears the same electrophore as daunomycin, the redox chemistry of **3** with **4** was studied initially. This was done in order to understand possible competitive processes which might occur during the reduction of daunomycin.

An oxygen-degassed, Trizma-buffered, methanol solution of **3** and **4** in the mole ratio 1:2 after reaction for 24 h at ambient temperature gave a quantitative yield of 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (**6**) and 3,5,5-trimethyl-2-oxomorpholine (**7**), the products of disproportionation of **5**, without any destruction of **3**. In the absence of **3**, no disproportionation of **5** occurred even under harsher conditions. Participation of **3** was revealed by monitoring the reaction by rapid-scan UV–visible spectroscopy. The spectral changes as a function of time included a drop in the absorption of 7-deoxydaunomycinone at 480 nm with a rise in the absorption at 420 nm with a reasonably sharp isosbestic point at 445 nm in the time period 0–18 min. These spectral changes are shown in Figure 1 of ref 19. The 420-nm band was assigned to the hydroquinone (**8**) of 7-deoxydaunomycinone consistent with absorption at 417 nm by the pentaacetate of 7-deoxydaunomycinone hydroquinone in methanol solvent.[26] Over the subsequent time period of 18–240 min the band at 420

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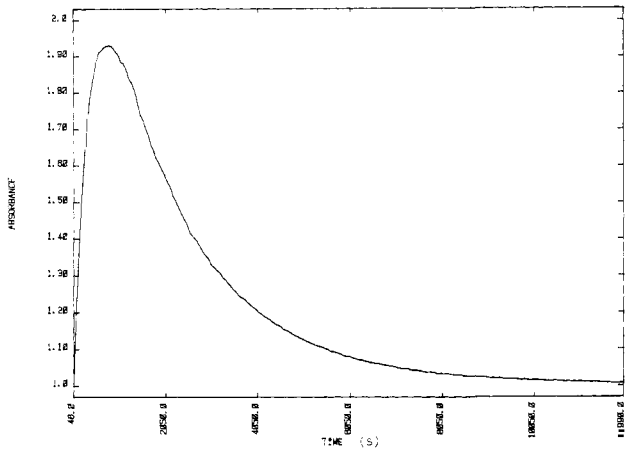


Figure 2. Average absorbance at 418 and 420 nm vs. time of an oxygen-degassed methanol solution 2.04×10^{-4} M in 7-deoxydaunomycinone (3), 2.04×10^{-4} M in *dl*-dimer 4, 4.0×10^{-3} M in Tris, and 4.0×10^{-3} M in Tris·HCl at 25.3 ± 0.1 °C.

nm dropped and the band at 480 nm rose with a sharp isosbestic point at 445 nm. Subtraction of the absorbance at 420 nm predicted to result from residual **3**, on the basis of absorption at 530 nm at 18 min, from the absorbance at 420 nm at 18 min gave an extinction coefficient for **8** of 12000. This procedure assumed no absorption from **8** at 530 nm. This is the second reported observation of hydroquinone **8**. The first report by Fisher and co-workers assigned a band at 407 nm to enzyme-bound **8** at pH 7.0 produced by sodium dithionite reduction of enzyme-bound 7-deoxydaunomycinone in sodium phosphate buffer.[30]

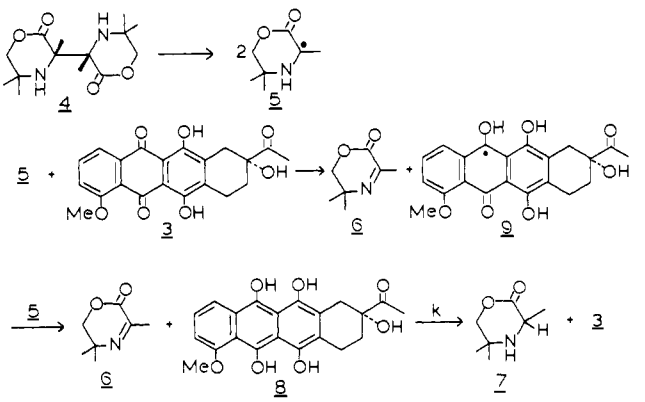
Examination of a degassed methanol solution of **3** and **4** by EPR spectroscopy at −20 °C gave a spectrum characteristic of the anthracycline semiquinone with a *g* value of 2.0037. Signal intensity and anisotropic effects precluded an assignment of the splittings. In dimethyl sulfoxide solvent at 40 °C the signal from a mixture of **3** and **4** was stronger because a higher concentration of **4** could be achieved. The spectrum was assigned to the semiquinone (**9**) of **3** and appears together with a reasonable computer simulation in Figure 1. The weak EPR signal intensity even in Me_2SO solvent at 40 °C suggested that **9** is never present in sufficient concentration to appear significantly in the UV–visible spectrum. The semiquinone **9** was efficiently reduced by **5** to the hydroquinone **8** or efficiently disproportionated to **8** and **3**.

The destruction of the hydroquinone **8** possibly resulted from reaction with the oxazinone **6** produced during the reduction of **3**. This possibility was indicated by the observation of a complete suppression of spectral changes at 420 nm by inclusion of 220 mol equiv of **6** in the mixture of **3** and **4**. The kinetics of decay in the absorption at 420 nm resulting from a 1:1 mixture of **3** and **4** further established this step. The absorption at 420 nm as a function of time is shown in Figure 2. The decay after 10 half-lives of **4** (2040 s, vide infra) followed clean second-order kinetics, first order in both **6** and **8**. A nonlinear least-squares fitting of the data to the integrated rate law, correcting for absorption by **3** (eq 1), gave a rate constant of 2.06 ± 0.02 M^{-1} s^{-1} for reduction of **6** by **8** and an extinction coefficient for **8** at 420 nm of 12700 ± 400.

$$A_t = \epsilon_3[\mathbf{3}]_0 + (\epsilon_8 - \epsilon_3)\left[\frac{e^{-kt[\mathbf{3}]_0}[\mathbf{3}]_0\left\{\frac{A_0 - \epsilon_3[\mathbf{3}]_0}{\epsilon_8 - \epsilon_3}\right\}}{[\mathbf{3}]_0 + \left\{\frac{A_0 - \epsilon_3[\mathbf{3}]_0}{\epsilon_8 - \epsilon_3}\right\}\{1 - e^{-kt[\mathbf{3}]_0}\}}\right] \quad (1)$$

where A_t = absorbance at 420 nm at time *t* from 2040 s, ϵ_8 and ϵ_3 are the extinction coefficients for **8** and **3** at 420 nm, *k* is the rate constant for reaction of **8** with **6**, $[\mathbf{3}]_0$ is the initial concentration of **3**, A_0 is the absorbance at 420 nm at 2040 s.[31] The calculated molar extinction coefficient of 12700 is in close agreement with the measured value of 12000 as described above. A similar experiment in methanol-*O*-*d* solvent, assuming a molar extinction coefficient of 12700 for **8**, gave a second-order rate constant *k* of 0.69 ± 0.01 M^{-1} s^{-1}. The deuterium kinetic isotope effect is thus 3.0, consistent with the breakage of a bond to hydrogen in the transition state.

Scheme I



The evidence is consistent with 7-deoxydaunomycinone-catalyzed disproportionation of **4** occurring via reduction of **3** in single-electron steps to give hydroquinone **8** followed by transfer of a hydride from **8** to oxazinone **6** as shown in Scheme I. Thus, catalysis occurs because a one-electron reducing agent creates a two-electron reducing agent.

Redox Chemistry of Daunomycin. Earlier we presented kinetic evidence that the reduction of daunomycin (**1**) to its hydroquinone state by *dl*-dimer **4** occurred in a single step possibly by hydride transfer.[26] The kinetic argument was based upon spectral changes at 480 nm, the wavelength of maximum absorption in the visible spectrum of daunomycin, assuming no long-lived intermediates. We have now reinvestigated the reaction mechanism by using the technique of rapid scan UV–visible spectroscopy and have observed a reasonably long-lived intermediate which absorbs in the visible region. Absorption by this intermediate negates the previous kinetic analysis.

An oxygen-degassed, Trizma buffered, methanol-*Od* solution of daunomycin and *dl*-dimer **4** in the mole ratio 1:11 at 25.0 ± 0.1 °C with daunomycin concentration 1.79×10^{-4} M showed UV–visible spectral changes in the time regime 10–130 s as follows: a fall in the absorption at 480 nm coupled with a short rise at 420 nm followed by a substantial rise at 380 and 608 nm. During the 380- and 608-nm band rise, the 420-nm band disappeared. Broad isosbestic points appeared at 425 and 530 nm. The isosbestic point at 425 nm was especially perturbed by the absorption at 420 nm during the early stages of the reaction. Scans beyond 130 s revealed a fall in the 380- and 608-nm bands with a rise in the 480-nm band. The final absorption at 480 nm resulted from the product 7-deoxydaunomycinone. The 420-nm band was assigned to the hydroquinone **10** of daunomycin consistent with absorption at this wavelength by the hydroquinone of 7-deoxydaunomycinone. The bands at 380 and 608 nm were assigned to the tautomer **11** of 7-deoxydaunomycinone on the basis of experiments to be described. The spectral changes for the time period 10–130 s are shown in Figure 1 of ref 32.

A degassed, buffered, methanol solution of **1** and **4** in the ratio 1:1 was observed by EPR spectroscopy at 0.6 °C. The spectrum obtained is shown in Figure 3 together with a computer simulation. The signal was assigned to the semiquinone **12** of daunomycin on the basis of the *g* value and splitting constants which are similar to those determined by Lown and co-workers for **12** in aqueous medium.[33] The signal is significantly different from that observed

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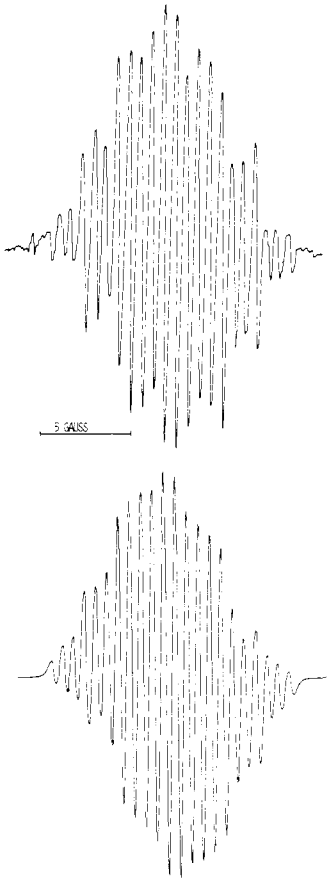


Figure 3. EPR spectrum of the semiquinone **12** of daunomycin observed at 0.6 °C with an oxygen-degassed methanol solution 1.56×10^{-4} M in daunomycin, 1.56×10^{-4} M in *dl*-dimer **4**, 1.0×10^{-3} M in Tris, and 1.0×10^{-3} M in Tris·HCl: top, experimentally observed spectrum; bottom, computer simulation generated with the following parameters, 0.50 (1:2:1), 0.92 (1:2:1), 1.44 (1:1), 1.57 (1:1), 1.98 (1:1), 2.53 G (1:1). The measured *g* value was 2.0037.

for semiquinone **9** in methanol. Determination of the splitting constants reported in the legend of Figure 3 was faciliated by first analyzing the spectrum of **12** in methanol-*Od* solvent. In deuterated solvent the two phenolic-OH proton splittings of 0.50 G disappeared. Our experience in measuring the concentrations of paramagnetic materials by EPR suggests that **12** is never present in sufficient amount to produce a significant UV–visible absorption. No additional paramagnetic species were observed including morpholinyl radical **5**. Under these reaction conditions **5** was present at very low concentration because it was being efficiently scavenged by daunomycin.

A degassed, buffered, methanol-*Od* solution of **1** and **4** in the ratio 2:1 with daunomycin concentration 1.79×10^{-4} M at 25.1 ± 0.1 °C showed the spectral changes described above except the 420-nm band did not appear, the isosbestic points at 425 and 530 nm were sharp, and the spectral changes occurred more slowly. The decay in the 380- and 608-nm bands also proceeded with sharp isosbestic points. The 420-nm band resulting from the hydroquinone **10** did not appear with the condition of lower reducing agent concentration because its rate of destruction exceeded its rate of formation. A similar reaction in methanol solvent also showed sharp isosbestic points. The sharp isosbestic points appeared in spectral changes for these reactions because **11** was the only long-lived intermediate, and **1** and **3** have almost identical absorption in the region of 480 nm.

The change in the concentration of **11** as represented by absorption in the region 618–620 nm as a function of time for the reaction in methanol solvent with **1** and **4** in the ratio 2:1 is shown in Figure 4. The formation and destruction of **11** followed

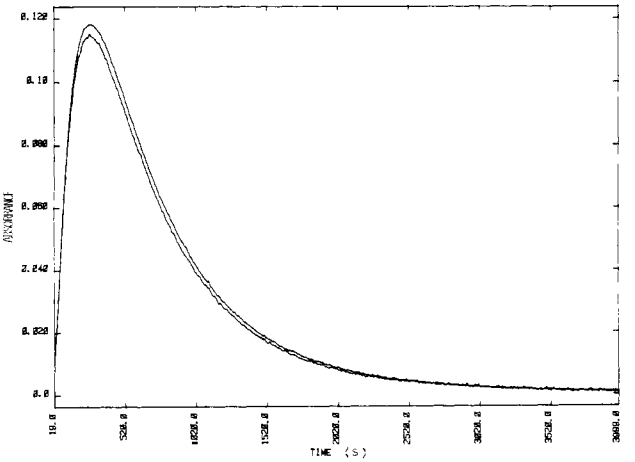


Figure 4. Average absorbance at 618 and 620 nm vs. time for two independent observations at 19.8 ± 0.1 °C of an oxygen-degassed methanol solution 1.71×10^{-4} M in daunomycin, 0.86×10^{-4} M in *dl*-dimer **4**, 1.0×10^{-3} M in Tris, and 1.0×10^{-3} M in Tris·HCl.

Table I. Rate Constants for Bond Homolysis of *dl*-Dimer 4

temp, °C	rate constant k_1, s^{-1}	solvent
25.1 ± 0.1	$(2.49 \pm 0.01) \times 10^{-3}$ [a]	CH_3OD
25.1 ± 0.1	$(3.36 \pm 0.06) \times 10^{-3}$ [b]	CH_3OH
19.8 ± 0.1	$(1.91 \pm 0.01) \times 10^{-3}$ [a]	CH_3OH
14.8 ± 0.1	$(0.90 \pm 0.01) \times 10^{-3}$ [a]	CH_3OH

[a] The error represents the standard deviation in the slope of the kinetics plot. [b] The error represents the average deviation from the mean of three independent measurements.

Table II. Average Extinction Coefficient for Tautomer 11 at 618 and 620 nm and Rate Constants for Tautomerization[a]

temp, °C	solvent	rate constant k_2 for tautomerization, s^{-1}	extinction coefficient
25.1 ± 0.1	CH_3OH	$(13.0 \pm 0.1) \times 10^{-3}$	9800 ± 200
25.1 ± 0.1	CH_3OD	$(1.42 \pm 0.04) \times 10^{-3}$	9500 ± 400
19.8 ± 0.1	CH_3OH	$(7.26 \pm 0.06) \times 10^{-3}$	8800 ± 100
14.8 ± 0.1	CH_3OH	$(4.20 \pm 0.06) \times 10^{-3}$	9400 ± 500

[a] The errors represent the average deviation from the mean of two independent measurements. The standard deviation in k_2 from the nonlinear least-squares analysis was in the range of 0.06×10^{-3}.

consecutive first-order kinetics, first order in **4** and **11**. The kinetics were established by regressional, nonlinear least-squares fitting of the data in Figure 4 to eq 2[31]

$$A_t = \{\epsilon_{11} k_1 [\mathbf{4}]_0 / (k_2 - k_1)\}(e^{-k_1 t} - e^{-k_2 t}) \qquad (2)$$

where A_t is the absorbance of **11** at time t, ϵ_{11} is the average extinction coefficient for **11** at 618 and 620 nm, k_1 is the rate constant for bond homolysis of **4**, and k_2 is the rate constant for tautomerization of **11**. The rate constants k_1 for bond homolysis of **4** as a function of temperature were measured independently as described in the Experimental Section and are reported in Table I. The rate constants k_2 for tautomerization of **11** and the average extinction coefficient for **11** at 618 and 620 nm as a function of temperature and solvent deuteration are shown in Table II. The kinetic analysis was further substantiated by allowing the regressional, nonlinear least-squares calculation to determine k_1 as well as k_2 and ϵ_{11} for the data obtained at 25.1 °C in methanol solvent. The calculated value of k_1 was 3.4×10^{-3} s^{-1}, identical within experimental error to the measured value shown in Table I. A plot of ln k_2 vs. reciprocal of temperature was linear and gave an enthalpy of activation of 18.0 ± 0.5 kcal/mol and an entropy of activation of −6 ± 1 cal/deg·mol for the tautomerization. The deuterium kinetic isotope effect at 25.1 °C was 9.1 ± 0.5, consistent with a protonation–deprotonation mechanism.

Further evidence that the slow step in formation of tautomer **11** was bond homolysis of *dl*-dimer **4** was the independence of the

(33) Lown, J. W.; Chen. H.-H. *Can. J Chem.* **1981**, *59*, 3212.

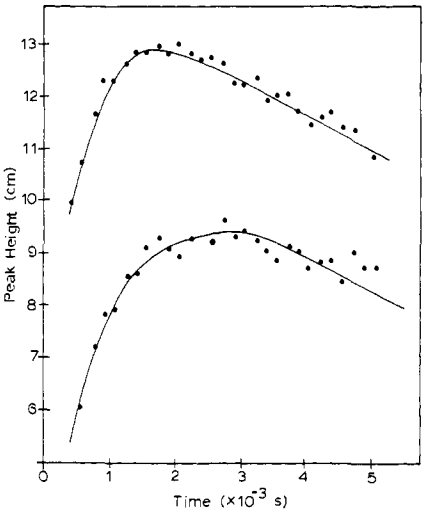
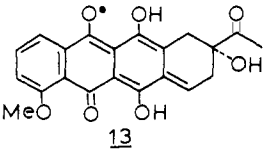


Figure 5. Average EPR signal intensity: top, a methanol solution 1.57 × 10^{-4} M in daunomycin, 1.57 × 10^{-4} M in *dl*-dimer **4**, 1.0 × 10^{-3} M in Tris, and 1.0 × 10^{-3} M in Tris·HCl; bottom, a methanol solution 1.57 × 10^{-4} M in daunomycin, 1.18 × 10^{-4} M in *dl*-dimer **4**, 1.0 × 10^{-3} M in Tris, and 1.0 × 10^{-3} M in Tris·HCl. Both experiments were performed at 0.6 °C under identical instrumental conditions.

calculated ϵ_{11} and k_2 to a change in the daunomycin concentration. Doubling the daunomycin concentration did not result in a change in the calculated values of ϵ_{11} and k_2 from the corresponding tautomer absorption vs. time curve. This experiment also showed that there was no competing reduction of 7-deoxydaunomycinone by **4** as described above when the initial concentration of daunomycin was at least twice that of **4**.

The data presented so far suggest that the tautomer **11** was produced by elimination of daunosamine from the hydroquinone **10** because the hydroquinone was observed as a transient when a high concentration of reducing agent was employed. An alternative mechanism debated in the literature proposes elimination of daunosamine at the semiquinone state to give the semiquinone methide **13**, which is then reduced to **11**.[4,18,19,34] Some advocates of elimination at the semiquinone state propose that any hydroquinone formed undergoes comproportionation with quinone to give semiquinone.



13

If we assume for the moment that elimination occurs at the hydroquinone state, there are also two alternatives for production of the hydroquinone, reduction of **12** by **5** or disproportionation of **12** to **10** and **1**. These two pathways may be in competition depending upon the relative concentrations of **5** and **12**.

All of these mechanistic details are difficult to distinguish. To find a distinction, we have closely examined the reaction mixture by EPR spectroscopy. Two buffered, degassed, methanol solutions 1.57 × 10^{-4} M in daunomycin, one 1.57 × 10^{-4} M and the other 1.18 × 10^{-4} M in *dl*-dimer **4**, were continuously monitored by EPR spectroscopy at 0.6 °C as a function of time. Plots of the signal intensity for **12**, calculated by averaging the 10 most intense peaks of the spectrum, vs. time are shown in Figure 5. To calibrate production of **12** with production of **11** a similar solution 1.56 × 10^{-4} M in both **1** and **4** was observed at 0.6 °C by visible spectroscopy at 618–620 nm. The tautomer **11** reached its maximum concentration at 3000 s compared with 1600 s for the semiquinone **12**. Monitoring the EPR signal revealed no additional paramagnetic species. If **13** had ever been present at concentration levels even a factor of 5 less than those of **12**, its presence should have perturbed the EPR spectrum. The EPR signal from **13** is predicted to be significantly wider than that of **12** because **13** bears two additional protons involved in hyperfine coupling.

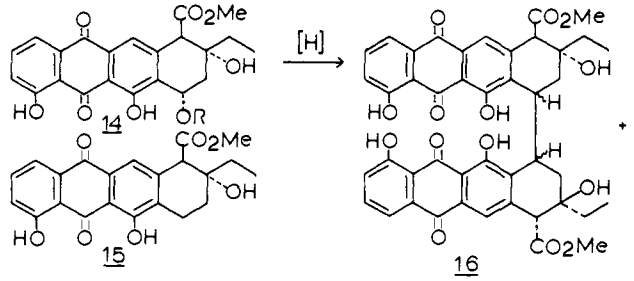
The two alternatives for production of the hydroquinone state of **1** were distinguished, at least in concentrations of **4** employed in the EPR experiments, from the maxima of the plots in Figure 5 and the times at which they were reached. Equation 3 gives the ratio of the signal intensities at maximum for the mechanism in which **12** is reduced by **5** and eq 4 gives the ratio for the mechanism in which **12** disproportionates.

$$\frac{[12]_{m_1}}{[12]_{m_2}} = \frac{h_{m_1}}{h_{m_2}} = \frac{[4]_{o_1}e^{-k_1 t_{m1}}}{[4]_{o_2}e^{-k_1 t_{m2}} + [1]_{o_2} - [4]_{o_2}} \quad (3)$$

$$\frac{[12]_{m_1}}{12]_{m_2}} = \frac{h_{m_1}}{h_{m_2}} = \left[\frac{[4]_{o_1}}{[4]_{o_2}} e^{k_1(t_{m2}-t_{m1})}\right]^{1/2} \quad (4)$$

where h_{m_1} is the average height of the EPR signal at maximum and t_{m_1}, the time the maximum was reached for the experiment at higher initial dimer concentration, and h_{m_2} and t_{m_2} are similarly defined for the experiment at the lower initial dimer concentration.[31,35] With $h_{m_1}/h_{m_2} = 1.34$, $k_1 = 1.3 \times 10^{-4}$ s^{-1}, $t = 1600$ s, the calculated value of t_{m_2} by using eq 3 is 5900 and using eq 4, 3900 s. The observed t_{m_2} is in the range of 3000 s, suggesting that hydroquinone **10** arises from disproportionation of semiquinone **12** when the reagents at time 0 are in the range of (1–2) × 10^{-4} M. A mechanism involving elimination of daunosamine at the semiquinone state is kinetically too complex for this type of analysis.

Two observations in the literature have led to the postulate of elimination at the semiquinone state. Reductive glycosidic cleavage of aclacinomycin A (**14**) gave 7-deoxyalkavinone (**15**) and bi(7-



14 **15** **16**

deoxyaklavinon-7-yl) (**16**).[24,34] Production of **16** was proposed to occur by combination of two semiquinone methides resulting from elimination of the sugar moiety from the semiquinone of aclacinomycin A.[24,36] Our recent work with aclacinomycin A indicates that **16** results from coupling of two tautomers, analogous in structure to the tautomer **11** of daunomycin, followed by oxidation.[37] The other observation is reduction of **1** with potassium borohydride at position-13 without glycosidic cleavage.[38] During reduction at the side chain the quinone must also have been reduced. The role of molecular oxygen[1] and the inermediacy of borate esters may be important. Oxygen rapidly reoxidizes the hydroquinone functionality in competition with glycosidic cleavage.

The results of the UV–visible spectroscopic and EPR experiments can be rationalized in terms of the mechanism in Scheme II. When **5** is in short supply in reactions with near stoichiometric concentrations, **12** is the major radical in solution and it reacts predominantly with itself to give **10** and **1**. With a large excess of **4**, **5** is also available to react with **12**, and the hydroquinone is produced by an additional pathway. The observation of ab-

(34) Komiyama, T.; Oki, T.; Inui, T.; Takeuchi, T.; Umezawa, H. *Gann* **1979**, *70*, 403.

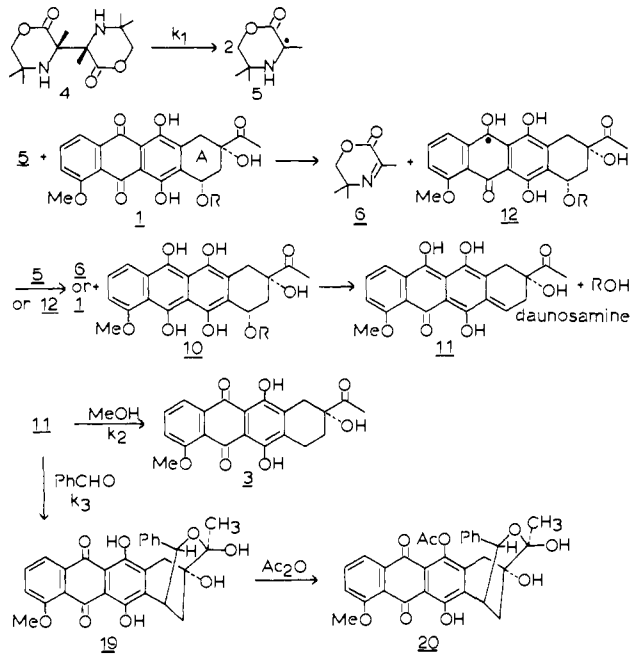
(35) An error was made in the previous derivation of eq 3 presented in ref 32.

(36) Komiyama, T.; Oki, T.; Inui, T. *J. Antibiot.* **1979**, *32*, 1219.

(37) Kleyer, D. L.; Gaudiano, G.; Koch, T. H. *J. Am. Chem. Soc.* **1984**, *106*, 1105.

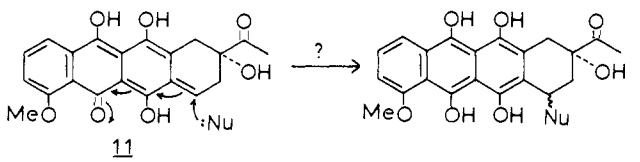
(38) Jolles, J.; Ponsinet, G. Ger. Patent 2 202 690 July 27, 1972; *Chem. Abstr.* **1972**, *77*, 164320.

Scheme II

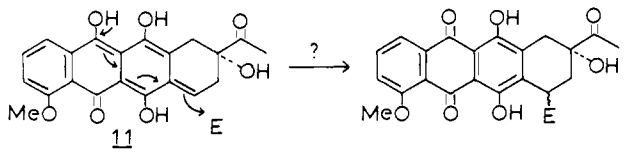


sorption at 420 nm in the reaction of **1** with 10-fold excess of **4** is consistent with this proposal.

Electrophilic Trapping of Tautomer 11. An examination of the structure of tautomer **11** as a possible intermediate that could lead to covalent binding of 7-deoxydaunomycinone to biological macromolecules reveals two possible pathways. In the first pathway the 7-position of **11** serves as an electrophilic site for reaction with nucleophilic sites in the macromolecules. The initial product would be a hydroquinone. This mode of reactivity is the one generally considered in terms of bioreductive activation, extensively discussed by Moore and co-workers.[22,23] To date nucleophilic trapping of intermediates in the reductive cleavage of daunomycin has been unsuccessful.[24] The lack of fruitful reactivity with nucleophiles is reasonable since the hydroquinone formed, **17**, can readily eliminate the nucleophile as the hydroquinone of daunomycin eliminates daunosamine. In the second pathway the



7-position of **11** serves as a nucleophilic site for reaction with electrophilic sites in the macromolecules. Reaction of **11** with an electrophile gives the quinone **18**. Subsequent reduction of **18** would not be expected to lead to cleavage of the new bond at the 7-position. An example of reaction of **11** with an electrophile of course is protonation to give 7-deoxydaunomycinone (**3**).



The reactivity of **11** was investigated with benzaldehyde as the electrophile. Monitoring of a degassed, buffered methanol-*Od* solution 7.8×10^{-4} M in both daunomycin and *dl*-dimer **4** showed the formation and destruction of **11** analogous to that shown in Figure 3. Including 63 mol equiv of benzaldehyde in the reaction mixture significantly attenuated the absorbance at 618–620 nm as a function of time without disturbing significantly the shape of the plot as shown in Figure 1 of ref 39. The percent of **11** which disappeared on a time average basis with inclusion of benzaldehyde was 76%, determined from the ratio of the areas under the two plots. Methanol-*Od* was selected for these experiments to exploit the 9.1-fold increase in the half-life of **11** in deuterated solvent. Identical experiments in methanol solvent showed a 53% decrease in the time average concentration of **11** with inclusion of 63 mol equiv of benzaldehyde.

HPLC of the reaction solution showed the formation of 7-deoxydaunomycinone and a new product ultimately assigned the adduct structure **19**. The HPLC yields for reactions in methanol-*O-d* and methanol solvents were 29% **3**, 47% **19** and 62% **3**, 35% **19**, respectively. Adduct **19** was isolated in 61% yield from a reaction mixture initially 3.72×10^{-4} M in both **1** and **4** by silica gel flash chromatography. The adduct was assigned structure **19** from the spectroscopic and analytical data reported in the Experimental Section. The stereochemistry proposed is consistent with the coupling constants for protons in the A-ring, formation of the hemiketal functionality, and monoacetylation with acetic anhydride–pyridine to give **20**. The monoacetylation was rationalized in terms of steric shielding of the 11-OH by the phenyl substituent. 7-Deoxydaunomycinone is readily acetylated at both the 6- and 11-hydroxy substituents.[26] Control experiments showed that benzaldehyde was unreactive with **1**, **3**, **4**, and **12**.

Integration of eq 2 gives eq 5 which represents the area under

$$\text{area} = \frac{\epsilon_{11} k_1 [\mathbf{4}]_0}{k_2 - k_1}\left\{\frac{1}{k_2} e^{-k_2 t} - \frac{1}{k_1} e^{-k_1 t}\right\} + \frac{\epsilon_{11}[\mathbf{4}]_0}{k_2} \qquad (5)$$

the absorption vs. time plot in Figure 1 of ref 39 without benzaldehyde. In the presence of a large excess of benzaldehyde the correct equation was obtained by replacing the first-order rate constant for destruction of **11**, k_2, by $k_2 + k_3$[benzaldehyde]. In the time period of the measurements, 0–4000 s, the exponential term of eq 5 contributes negligibly to the areas. Consequently, the ratio of the areas under the curves in Figure 1 of ref 39 equals $k_2/(k_2 + k_3)$[benzaldehyde]), which then gives a value for k_3 of 9×10^{-2} M^{-1} s^{-1} at 25.1 °C.

The reaction of tautomer **11** with benzaldehyde is a simple aldol condensation. The structure established for **19** confirms the structural assignment for **11** and indicates the nucleophilic character of **11**. Bioreduction of daunomycin or adriamycin associated with DNA might lead to formation of a covalent bond between the 7-position of **3** or 7-deoxyadriamycinone and an electrophilic site in DNA such as the 2- or 4-position of a pyrimidine base or the 2- or 6-position of a purine base. Electron density calculations indicate that these are the more electron deficient centers of the bases and suggest that guanine ought to be more reactive than adenine and cytosine more reactive than thymine.[40,41] Trapping experiments with simple pyrimidine or purine bases have been unsuccessful to date because the high concentrations of trapping agent cannot be achieved due to solubility. In the absence of association a high concentration of the electrophile is necessary because the half-life of **11** with respect to protonation is short, 53 s at 25 °C in buffered methanol solvent.

In summary we report here the first experimental evidence for formation of the tautomer of 7-deoxydaunomycinone as an intermediate in reductive glycosidic cleavage of daunomycin and have demonstrated that it expresses nucleophilic reactivity. Tautomer **11** is a viable intermediate for covalent binding of anthracycline antibiotics such as daunomycin or adriamycin to biological macromolecules. Once **11** is covalently bound at the 7-position by reaction with an electrophilic site, the quinone electrophore is intact allowing it to function catalytically in the production of reactive, reduced oxygen species or reduction of other cellular constituents in one- or two-electron steps. The reactivity of reduced daunomycin described here probably accounts for at least a portion of the cytotoxicity of the anthracycline antitumor drugs.

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(40) Pullman, B.; Pullman, A. *Adv. Heterocycl. Chem.* **1971**, *13*, 77.

(41) Kwiatkowski, J. S.; Pullman, B. *Adv. Heterocycl. Chem.* **1975**, *18*, 199.

Experimental Section

General Remarks. Infrared spectra were recorded with a Perkin-Elmer Model 337 infrared spectrophotometer and ^1H NMR spectra with a Bruker Model 250-MHz spectrometer. Chemical shifts are reported in parts per million on the δ scale from internal Me_4Si. EPR spectra were recorded with a Varian Associates E109 spectrometer equipped with field/frequency lock. Fast-atom-bombardment mass spectral data were obtained with a modified V.G. Micromass 7070H mass spectrometer. A Varian Aerograph Model 940 gas chromatograph with flame ionization detector and Hewlett Packard Model 3390A integrator were used for analytical GLC. HPLC was performed with a Tracor liquid chromatograph with Model 970A variable-wavelength detector. A Hewlett-Packard 8450A rapid-scan spectrometer was used for obtained ultraviolet and visible spectral data. Microanalyses were performed by Atlantic Microlab, Atlanta, GA.

All solvents were reagent grade or spectroanalyzed. Daunomycin was obtained from the Drug Development Branch of the National Cancer Institute. 7-Deoxydaunomycinone (**3**) was prepared by dithionite reduction of daunomycin.[15] *dl*-**Bi**(3,5,5-trimethyl-2-oxomorpholin-3-yl) (**4**) was prepared by photoreduction of 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (**6**)[42] and 3,5,5-trimethyl-2-oxomorpholine (**7**) by catalytic hydrogenation of **6**.[27,43]

The flash chromatography used was essentially that described by Still and co-workers.[44] A 1.0-cm o.d. column was packed with 15 cm of Merck Silica Gel 60 (40–63 μm) and eluted at a flow rate of 5 cm/min. A volume of 5 mL was collected for each fraction. Fractions were analyzed by silica gel TLC.

All reaction mixtures were freeze(−196 °C)–pump(2×10^{-6} torr)–thaw(0 °C)–sonnicate degassed through four cycles as described earlier. The cells and apparatus used for the kinetics and EPR experiments have also been described elsewhere.[37]

Kinetics of Reaction of the Hydroquinone (8) of 7-Deoxydaunomycinone with 5,6-Dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (6). To 7.8 mg (2.04×10^{-5} mol) of 7-deoxydaunomycinone, 48.8 mg (4.03×10^{-4} mol) of tris(hydroxymethyl)aminomethane (Tris), and 63.1 mg (4.00×10^{-4} mol) of tris(hydroxymethyl)aminomethane hydrochloride (Tris·HCl) in a 100-mL volumetric flask were added methanol and a stirbar. After 8 h of stirring all of the 7-deoxydaunomycinone had dissolved. To a 100-mL volumetric flask was added 15.8 mg (5.56×10^{-5} mol) of *dl*-dimer **4**. The flask was then filled with dichloromethane, and 0.92 mL (5.12×10^{-7} mol) of the solution was transferred via syringe to the chilled (0 °C) spectrophotometric compartment of a two-compartment cell (vide supra) with a 0.9-cm attachment for connection to the vacuum line with an Ultra Torr Union. The dichloromethane was then evaporated with a steady stream of nitrogen. A syringe was used to transfer 2.5 mL of the 7-deoxydaunomycinone solution (5.12×10^{-7} mol) to the second compartment. The sample was freeze–thaw degassed and placed in a thermostatted cell holder at 25.3 ± 0.1 °C for 25 min prior to mixing the solution with the dimer **4**. The average absorbance at 420 and 422 nm was then recorded vs. time as shown in Figure 2. A nonlinear least-squares treatment of the data as discussed (vide supra), starting after 10 half-lives of the dimer **4** with respect to bond homolysis had occurred, gave a molar extinction of 12 700 ± 400 for hydroquinone **8** and a second-order rate constant of 2.06 ± 0.02 M^{-1} s^{-1} for reaction of **8** with oxazinone **6**. Analysis of a similar experiment in methanol-*Od* solvent with 1.57×10^{-4} M 7-deoxydaunomycinone and dimer **4**, using the molar extinction coefficient of 12 700 for *8*, gave the second-order rate constant equal to 0.69 ± 0.01 M^{-1} s^{-1}. Hence the deuterium kinetic isotope effect was 3.0. All data were base-line corrected by subtracting the residual absorbance at an infinity point, 24 000 s for the experiment in methanol and 48 000 s for the experiment in methanol-*Od*, from each measurement.

An experimental nearly identical with that in which the second-order kinetics were determined in methanol solvent was repeated with 220 mol equiv of oxazinone **6** added. The hydroquinone **8** of 7-deoxydaunomycinone was not observed as evident from no change in the absorbance at 420 nm during the course of the experiment.

Spectral changes over the region 402–600 nm as a function of time were observed in methanol solvent by using the same procedure. The reaction solution at time 0 was 1.39×10^{-4} M in 7-deoxydaunomycinone, 1.39×10^{-3} M in *dl*-dimer **4**, 1.0×10^{-3} M in Tris, and 1.0×10^{-3} M in Tris·HCl. The results in the time period 0–18 min appear in Figure 1 of ref 29.

Products from the Reaction of 7-Deoxydaunomycinone (3) with *dl*-Bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (4). Two 2.5-mL methanol reaction solutions each containing 1.39×10^{-4} M 7-deoxydaunomycinone, 2.68×10^{-4} M *dl*-dimer **4**, 1.0×10^{-3} M Tris, and 1.0×10^{-3} M Tris·HCl were prepared and degassed as described above. The reagents were mixed, and the reaction mixtures were allowed to stand 24 h at ambient temperature. The solutions were then cooled to −78 °C and opened. TLC analysis on silica gel by eluting with 5% methanol in dichloromethane at 0 °C and visualizing with 10% phosphomolybdic acid in ethanol showed the absence of any **4**. The solutions were then allowed to warm to room temperature and analyzed by GLC on a 4 × 0.0032 m column packed with 10% SE-30 on 100/120 mesh Chromosorb W with a column temperature of 150 °C and a helium flow rate of 25 mL/min.

Analysis of the first sample showed that 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (**6**) and 3,5,5-trimethyl-2-oxomorpholine (**7**) were formed in a mole ratio of 1.09 ± 0.045 with $(6.6 \pm 0.1) \times 10^{-7}$ mol of morpholine **7** present in the sample. Analysis of the second sample showed a mole ratio of 1.00 ± 0.08 with $(6.8 \pm 0.1) \times 10^{-7}$ mol of morpholine (**7**) present. GLC results were detector-response corrected. The reaction solution at time 0 contained 6.7×10^{-7} mol of **4**. HPLC analysis on a 0.3 × 0.0046 m Alltech RSIL–phenyl column by eluting with 40% THF–60% buffered water (buffer consisted of 0.1% ammonium formate adjusted to pH 4.0 with formic acid)[45] at 2.0 mL/min and observing at 480 nm showed the presence of $(3.55 \pm 0.06) \times 10^{-7}$ mol of 7-deoxydaunomycinone in the first sample and $(3.50 \pm 0.08) \times 10^{-7}$ mol in the second sample. HPLC yields were relative to a standardized solution. The reaction solution at time 0 contained 3.48×10^{-7} mol of **3**. The identities of all the products were confirmed by ^1H NMR spectroscopy in deuteriochloroform solvent.

Attempted Disproportionation of Bi(3,5,5-trimethyl-2-oxomorpholin-3-yl). To an NMR tube equipped with a connection for attachment to a vacuum line were added 7.9 mg (2.78×10^{-5} mol) of *dl*-dimer **4** and 0.5 mL of tetradeuteriomethanol. The sample was freeze–thaw degassed, sealed, and placed in a constant temperature bath at 35.0 ± 0.1 °C. An ^1H NMR spectrum taken after 135 h showed that no disproportionation had occurred.

Kinetics of Reductive Elimination of Daunosamine from Daunomycin. A two-compartment cell was charged with 2.15×10^{-7} mol of *dl*-dimer **4** and 2.5 mL of a methanol solution 1.71×10^{-4} M in daunomycin hydrochloride, 1.0×10^{-3} M in Tris, and 1.0×10^{-3} M in Tris·HCl as described above. The methanol solution was freeze–thaw degassed, sealed, and temperature equilibrated in the thermostatted cell holder for 25 min. After mixing the average absorbance from 618 to 620 nm was measured as a function of time. The data obtained from two independent measurements of this type are shown in Figure 4. The wavelength range chosen had no absorption due to either 7-deoxydaunomycinone or daunomycin. The absorbance vs. time data were analyzed according to eq 2 by a nonlinear least-squares regression method. The entire procedure was performed at a total of three different temperatures. The deuterium kinetic isotope effect was determined by this procedure with a buffered 1.79×10^{-4} M daunomycin deuteriomethanol solution. The kinetic data obtained are summarized in Table II.

Spectral changes over the region 320–600 nm as a function of time at 25.3 ± 0.1 °C were observed in methanol-*Od* solvent by the same procedure. The reaction solution at time 0 was 1.79×10^{-4} M in daunomycin, 1.79×10^{-3} M in *dl*-dimer **4**, 1.0×10^{-3} M in Tris, and 1.0×10^{-3} M in Tris·HCl. The results in the time period 10–130 s appear in Figure 1 of ref 32.

The Lack of Effect of Daunomycin Concentration on the Kinetics of Reductive Cleavage. A 3.76×10^{-4} M daunomycin solution buffered with 2.0×10^{-3} M Tris and 2.0×10^{-3} M Tris·HCl was used to study the effect. The same procedure was used (vide supra) except the concentration of *dl*-dimer **4** was 0.90×10^{-4} M, giving a mole ratio of daunomycin:**4** of 1.0:0.24. Nonlinear least-squares regressional treatment of the data gave a rate constant for tautomerization of **11** equal to 1.3×10^{-2} s^{-1} and a molar extinction coefficient of 10 200 for the tautomer **11** at 618–620 nm.

Kinetics of Bond Homolysis of *dl*-Bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (4) in Methanol. A two-compartment cell was charged with 2.59×10^{-6} mol of *dl*-dimer **4** and 2.75 mL of a methanol solution 2.08×10^{-3} M in *N*-methylisatin, degassed, and sealed (vide supra). After temperature equilibration and mixing, the average absorbance from 418 to 420 nm was measured as a function of time. A plot of the natural logarithm of the difference between the absorbance at time t and absorbance at time infinity was linear, and the slope was equal to the rate of bond homolysis. This procedure was repeated at each of three temperatures used in the kinetic study of the reductive elimination reaction of daunomycin. The deuterium kinetic isotope effect was determined by using a 1.96×10^{-3} M *N*-methylisatin deuteriomethanol solution and this procedure. The

(42) Koch, T. H.; Olesen, J. A.; DeNiro, J. *J. Org. Chem.* **1975**, *40*, 14.
(43) Koch, T. H.; Olesen, J. A.; DeNiro, J. *J. Am. Chem. Soc.* **1975**, *97*, 7285.
(44) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
(45) Andrews, P. A.; Brenner, D. E.; Chou, F. E.; Kubo, H.; Bachur, N. *Drug Metab. Dispos.* **1980**, *3*, 152.

kinetic data obtained are summarized in Table I.

Daunomycin Semiquinone EPR Signal Intensity as a Function of Time and *dl*-Dimer 4 Concentration. Two three-compartment cells as described earlier[37] were employed. One compartment of each cell was a Wilmad 705-PQ quartz EPR tube 0.199 ± 0.0013 cm i.d. and the other two compartments were used for the temporary isolation of **1** and **4**. A dichloromethane solution 5.92×10^{-5} M in *dl*-dimer **4** and a methanol solution 1.56×10^{-4} M in daunomycin with 1.0×10^{-3} M Tris and 1.0×10^{-3} M Tris·HCl were used as stock solutions. A syringe was used to transfer 0.79 mL (4.67×10^{-8} mol) of the dichloromethane solution to one compartment of the apparatus; and the dichloromethane was evaporated with a stream of nitrogen. For the second concentration of **4** of interest 0.59 mL (3.49×10^{-8} mol) of the dichloromethane solution was transferred to one compartment of a second cell, and the dichloromethane evaporated. A syringe was used to transfer 0.30 mL (4.70×10^{-8} mol) of the daunomycin solution to the second compartment of each cell. The methanol solutions were freeze–thaw degassed and the cells sealed. Each solution was then reacted and analyzed as follows. The whole cell was immersed to an ice bath (0 °C) for 5 min for temperature equilibration purposes. The solution was then mixed with the *dl*-dimer **4** for 2 min while still immersed in the ice bath. The resulting solution was poured into the EPR Tube compartment, and the tube was quickly dried and placed in the cavity of the EPR spectrometer, which had been previously thermostatted at 0.6 °C and tuned. A 40-G scan of the EPR signal was recorded every 151 s. A plot of the signal intensity, determined by taking the average height of the ten most intense peaks vs. time is shown in Figure 5.

Trapping of the Tautomer 11 of 7-Deoxydaunomycinone with Benzaldehyde. A deuteriomethanol solution 7.80×10^{-4} M in daunomycin and 8.0×10^{-3} M in both Tris and Tris·HCl was used as a stock solution for two reactions that follow. One reaction solution was carried out by placing 0.075 mL of benzaldehyde into a 3-mL volumetric flask and diluting to the mark with the daunomycin stock solution. A syringe was then used to transfer 2.5 mL of the solution containing benzaldehyde to the second compartment of one two-compartment cell and 2.5 mL of the original daunomycin solution to the second compartment of the second two-compartment cell. The first compartment, or spectrophotometric compartment, of each cell contained 1 equiv (1.95×10^{-6} mol) of *dl*-dimer **4**. The solutions were freeze–thaw degassed and sealed. The progress of the reactions was monitored by following the average absorbance from 618 to 620 nm vs. time after temperature equilibration at 25.0 ± 0.1 °C as previously described. The resulting data are shown in Figure 1 of ref 39. After 24 h the cells were opened and analyzed by HPLC (vide supra). The yield of the benzaldehyde adduct (**19**) was 47% and the yield of 7-deoxydaunomycinone (**3**) was 29%. The same reactions were also performed with 7.80×10^{-4} M daunomycin in methanol solvent, and the yields of **19** and **3** were 35% and 62%, respectively. The HPLC yields were obtained relative to standard solutions.

Isolation of Adduct 19 from the Reaction of Benzaldehyde with the Tautomer 11 of 7-Deoxydaunomycinone. To a 50-mL volumetric flask were added 10.5 mg (1.86×10^{-5} mol) of daunomycin hydrochloride, 12.1 mg (1.0×10^{-4} mol) of Tris, 15.8 mg (1.0×10^{-4} mol) of Tris·HCl, and 50 mg of deuteriomethanol. To a 5-mL volumetric flask were added 10.1 mg (3.56×10^{-5} mol) of *dl*-dimer **4** and 5 mL of dichloromethane. A syringe was used to transfer to each of three two-compartment cells 0.73 mL (2.58×10^{-8} mol) of the dichloromethane solution. A stream of nitrogen was used to evaporate the dichloromethane. Syringes were also used to transfer 13 mL of the daunomycin solution and 0.093 mL of benzaldehyde to each of three larger compartments. The samples were freeze–thaw degassed as described except that seven cycles were employed. After mixing and a 24 h reaction period at ambient temperature, cells were opened and the solutions transferred to a 100-mL flask. The methanol was removed by rotary evaporation at aspirator vacuum and the excess benzaldehyde by rotary evaporation at 0.25 torr. The resulting red material was transferred to another 100-mL flask with 10, 5-mL portions of dichloromethane with filtering of each portion to remove suspended particles of buffer. After removal of the solvent, the material was flash chromatographed on silica gel with 1.7% methanol in dichloromethane eluent to give 4.3 mg (61%) of a red solid assigned structure **19** from the following analytical and spectroscopic properties: IR (KBr) 2.90, 3.15, 3.41, 6.19, and 6.30 μm; fast-atom-bombardment mass spectrum (in glycerin; positive ion) *m/z* (relative intensity) 489 (56, M + 1); 490 (100, M + 2), 491 (44, M + 3); exact mass calcd for $C_{28}H_{24}O_8$, 490.1627; found, 490.1623; (FAB–glycerin; negative ion) *m/z* 488 (87, M), 489 (100, M + 1), 490 (39, M + 2). Anal. Calcd for $C_{28}H_{24}O_8$: C, 68.85; H, 4.95; Found: C, 68.62; H, 5.00; ^{1}H NMR (250 mHz; $DCCl_3$) δ 1.62 (s, CH_3-14), 1.86 (dd, *J* = 12, 3 Hz, CH-8), 2.40 (br, OH-9, OH-13, disappeared with D_2O), 2.74 (d, *J* = 20 Hz, CH-10), 2.72 (dd, *J* = 12, 3 Hz, CH-8), 3.48 (d, *J* = 20 Hz, CH-10), 3.88 (dt, *J* = 2, 3 Hz, CH-7), 4.00 (s, OCH_3-4), 5.39 (d, *J* = 2 Hz, CH-7′), 6.95 (d, *J* = 6 Hz, *o*-Ph-7′), 7–7.2 (m, *m,p*-Ph-7′), 7.34 (d, *J* = 9 Hz, CH-3), 7.74 (t, *J* = 9 Hz, CH-2), 8.04 (d, *J* = 9 Hz, CH-1), 12.90 (s, OH-6), 13.50 (s, OH-11); the assignment of resonances for the protons in the A-ring were verified by decoupling experiments. The adduct **19** was isolated in 20% yield (2.8 mg) from a 37.5-mL methanol solution 7.87×10^{-4} M in daunomycin and *dl*-dimer **4**, buffered as before. The procedure used was similar to that just described with 0.40 mL of benzaldehyde added before splitting the methanol solution into three portions for degassing. Three separate control experiments showed that with the reaction conditions described *dl*-dimer **4**, 7-deoxydaunomycinone, and daunomycin were unreactive with benzaldehyde individually.

Acetylation of Adduct 19. To a 25-mL round-bottom flask were added 2.0 mg (4.1×10^{-6} mol) of **19**, 2 mL of pyridine, 2 mL of acetic anhydride, and a stirbar. After stirring for 18 h at ambient temperature, the pyridine and acetic anhydride were removed by rotary evaporation at 0.2 torr. Silica gel flash chromatography by eluting with 1.5% methanol in dichloromethane followed rotary evaporation of the solvent afforded 1.1 mg (51%) of a yellow solid assigned structure **20** from the following spectroscopic properties: IR (KBr) 2.90, 3.41, 5.66, *o*-Ph-7′), 6.13, 6.30 μm; mass spectrum (fast-atom bombardment in glycerin; positive ion) *m/z* (relative intensity) 531 (42, M + 1), 532 (100, M + 2), 533 (79, M + 3);[46] ^{1}H NMR (250 MHz; $DCCl_3$) δ 1.63 (s, CH_3-14), 1.91 (dd, *J* = 12, 3 Hz, CH-8), 2.43 (br, OH-9, OH-13), 2.56 (s, OAc-11), 2.65 (d, *J* = 17 Hz, CH-10), 2.76 (dd, *J* = 12, 3 Hz, CH-8), 3.50 (d, J = 17 Hz, CH-10), 3.93 (dt, *J* = 2, 3 Hz, CH-7), 4.03 (s, OCH_3-4), 5.40 (d, *J* = 2 Hz, CH-7′), 6.92 (d, *J* = 7 Hz, *o*-Ph-7′), 7–7.2 (m, *m,p*-Ph-7′), 7.29 (d, *J* = 8 Hz, CH-3), 7.71 (t, *J* = 8 Hz, CH-2), 7.89 (d, *J* = 8, CH-1), 12.83 (s, OH-6).

Daunomycin Semiquinone (12) EPR Signal Intensity as a Function of Time and Benzaldehyde Concentration. The same three-compartment EPR cell and procedure as previously described were used for two experiments. A 7.80×10^{-4} M daunomycin deuteriomethanol solution with 2.0×10^{-3} M Tris and 2.0×10^{-3} M Tris·HCl was prepared in a 25-mL volumetric flask. To a 3-mL volumetric flask was added 0.030 mL of either toluene or benzaldehyde and 2.97 mL of the daunomycin solution. A syringe was used to transfer 0.25 mL of the solution to the cell where 1 mol equiv of *dl*-dimer **4** had previously been deposited in a separate compartment. After rigorously degassing the samples, they were treated and observed by EPR spectroscopy as previously described. After completion of the reactions, the mixtures were analyzed by HPLC (vide supra). The yields of **3** and **19** were 23% and 45%, respectively. Plots of EPR signal intensity vs. time were identical for the two experiments.

The Semiquinone of Daunomycin and 7-Deoxydaunomycinone. The same three-compartment apparatus and procedure as previously described were used for obtaining EPR spectra of the semiquinones of both daunomycin and 7-deoxydaunomycinone. For daunomycin the following solutions were used: 1.56×10^{-4} M **1** with 1.0×10^{-4} M Tris and 1.0×10^{-4} M Tris·HCl in methanol and 1.79×10^{-4} M **1** with 1.0×10^{-4} M Tris and 1.0×10^{-4} M Tris·HCl in deuteriomethanol solvent. For 7-deoxydaunomycinone the concentrations were 2.09×10^{-3} M **3**, 4.0×10^{-2} M Tris, and 4.0×10^{-2} M Tris·HCl in dimethyl sulfoxide solvent. The simulated and observed EPR spectra appear in Figures 1 and 3.

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Registry No. 1, 20830-81-3; **3**, 32384-98-8; **4**, 53153-53-0; **5**, 57765-64-7; **6**, 53153-46-1; **7**, 57765-62-5; **8**, 86632-73-7; **9**, 89164-68-1; **10**, 85404-45-1; **11**, 85337-17-3; **12**, 89164-69-2; **19**, 86470-90-8; **20**, 89164-70-5; benzaldehyde, 100-52-7.

(46) Fast-atom-bombardment mass spectrometry of quinones of this type commonly gives large M + 2 and M + 3 peaks. Similar behavior was observed for **3** and 7-deoxyaklavinone.