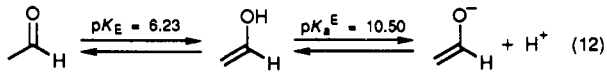
3.72 predicted by a correlation of acidity constants for α-substituted acrylic acids.[37]

The keto–enol equilibrium constant for pyruvic acid determined here, $pK_E = 3.21$, is 3 orders of magnitude greater than that for enolization of the parent unsubstituted carbonyl compound, acetaldehyde (eq 12), for which $pK_E = 6.23$.[38] This difference must



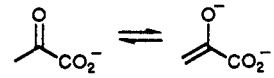
(12)

be due in large part to destabilization of the keto isomer of pyruvic acid by the adjacent carboxylic acid group, as pointed out above. In the case of pyruvate ion with $pK_E = 5.03$, the difference drops considerably to 1 order of magnitude, but it still leaves pyruvate ion with a greater enol content than acetaldehyde. This could be the result of a residual keto isomer destabilizing effect, but stabilization of the enol by carboxylate, for which a sizeable carbon–carbon double-bond-stabilizing effect ($D = 3.9$ kcal mol^{-1}) has been reported,[39] is likely to be a contributing factor.

Pyruvate enol, with $pK_a^E = 11.55$, is a weaker acid than the parent enol, vinyl alcohol (eq 12), for which $pK_a^E = 10.50$.[38] This is undoubtedly due to the presence of the negative charge in pyruvate ion, which will oppose formation of a second negative charge in the enolate ion. The structural relationship between

the substituent and the ionizing hydroxyl group in these two enols is similar to that in the pair formic acid (**3**) and malonate ion (**4**), and it is interesting that the difference in pK_a for the enols, $\Delta K_a = 1.05$, is greater than that between formic acid ($pK_a = 3.75$)[40] and malonate ion ($pK_a = 4.27$),[41] $\Delta pK_a = 0.52$; this is probably because the second negative charge is more delocalized in the malonate ion than it is in pyruvate enol and the unfavorable electrostatic interaction is consequently reduced.

The keto–enol equilibrium constant for pyruvate ion may be combined with the acidity constant of its enol to provide an acidity constant for pyruvate ionizing as a carbon acid, eq 13. The result,



(13)

pK_a^K ($= pK_E + pK_a^E$) $= 16.58 \pm 0.07$,[23] is not very different from that for the parent acetaldehyde system, $pK_a^K = 16.73$; this follows from the fact that the difference in keto–enol equilibrium constants for the two systems, $\Delta pK_E = 1.20$, is very nearly completely offset by the difference in their enol acidity constants, $\Delta pK_a^E = -1.05$.

Pyruvic acid will be converted to pyruvate ion at physiological pH, and it is the keto–enol equilibrium constant for this ion, $pK_E = 5.03$, that is relevant to biological systems. This value gives $\Delta G° = -6.9$ kcal mol^{-1} as the free energy change for the ketonization reaction, which is 47% of the free energy liberated by the hydrolysis of phosphoenolpyruvate (eq 1). This shows that nearly half of the energy content of this high-energy substance resides in its masked enol function.

Acknowledgment. We are grateful to the Natural Sciences and Engineering Research Council of Canada, the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the U. S. National Institutes of Health for financial support of this work.

Supplementary Material Available: Tables S1–S8 containing rate and equilibrium data (8 pages). Ordering information is given on any current masthead page.

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Reduction of Daunomycin in Dimethyl Sulfoxide. Long-Lived Semiquinones and Quinone Methide and Formation of an Enolate at the 14-Position via the Quinone Methide

Giorgio Gaudiano,[†,‡] Massimo Frigerio,[†] Pierfrancesco Bravo,[†] and Tad H. Koch*[,‡]

Contribution from the CNR—Centro di Studio per le Sostanze Organiche Naturali, Dipartimento di Chimica, Politecnico, I-20133 Milano, Italy, and Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309-0215. *Received September 18, 1991*

Abstract: Anaerobic reduction of daunomycin (**1**, daunorubicin) in 5%/95% H_2O/DMSO (DMSO = dimethyl sulfoxide) or in DMSO with sodium dithionite or bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (TM-3 dimer), respectively, yields 7-deoxydaunomycinone (**7**) and a mixture of the diastereomers of bi(7-deoxydaunomycinon-7-yl) (**8**). A precursor to both **7** and **8** is 7-deoxydaunomycinone quinone methide (**4**) formed from glycosidic cleavage of daunomycin hydroquinone (**3**). The hydroquinone **3** is estabished as a precursor to the quinone methide **4** from relative rates. In 5%/95% H_2O/DMSO or DMSO, daunomycin semiquinone (**2**) and quinone methide (**4**) have much longer lifetimes than in 100% protic solvents such as H_2O or methanol. The quinone methide reacts to form the side chain enolate most likely by intramolecular proton transfer from the methyl group at the 14-position to the 7-position.

Introduction

Anthracycline antitumor drugs as represented by daunomycin (**1**, daunorubicin) show complex redox chemistry which is biologically accessible[1,2] and fall in the category of bioreductively activated compounds.[3] The anaerobic reactivity of the transients from reduction of daunomycin in one-electron steps, the semiquinone (**2**), hydroquinone (**3**), and quinone methide (**4**), is medium dependent. In protic solvents such as water and methanol

[†] Politecnico di Milano.
[‡] University of Colorado.

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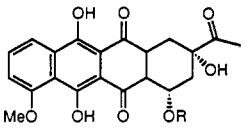
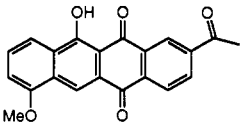
at pH 7–8, the semiquinone disproportionates to the hydroquinone and the starting quinone, and the hydroquinone eliminates the amino sugar daunosamine to form the quinone methide.[4-8] In dimethylformamide (DMF) solvent the semiquinone is long lived and does not seem to disproportionate very rapidly; a second one-electron reduction is required to give the hydroquinone, which then eliminates daunosamine with formation of the quinone methide.[9] The ease of observation of daunomycin semiquinone in dimethyl sulfoxide (DMSO) and DMSO/H_2O by EPR spectroscopy suggests that disproportionation is slow also in this medium.[10] In anhydrous acetonitrile solvent or in protic solvent at acidic pH, the hydroquinone from two-electron reduction of **1** tautomerizes at the B-ring with formation of leucodaunomycin (**5**) in competition with glycosidic cleavage to quinone methide.[11] In THF·H_2O (THF = tetrahydrofuran) solvent at basic pH, the hydroquinone of *N*-acetyldaunomycin generated by saponification of *N*-acetyldaunomycin hydroquinone hexaacetate with lithium hydroxide yields 2-acetyl-11-hydroxy-7-methoxy-5,12-naphthacenedione (**6**) presumably without the intermediacy of a quinone methide.[12] Medium effects are relevant to an understanding of the biological activity of these redox states because both hydrophilic and hydrophobic regions of cells are accessible to anthracyclines. Hydrophobic regions of particular importance in this regard are cellular, mitochondrial, and nuclear membranes.[13-15]



leucodaunomycin **5**



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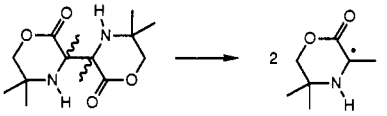
Here we describe in detail the reactivity in DMSO of the transients from reduction of daunomycin in one-electron steps. Glycosidic cleavage occurs at the hydroquinone state to form a relatively long-lived quinone methide which dimerizes in competition with being protonated. Of particular surprise is the observation that the quinone methide is protonated by the methyl group at the 14-position in competition with being protonated by water at the 7-position.

Results and Discussion

Reductions of daunomycin in one-electron steps were accomplished with either sodium dithionite or bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (*dl*-TM-3 dimer). The low solubility of dithionite in DMSO necessitated the use of 5%/95% H_2O/DMSO. Both reducing agents function through spontaneous homolysis of a weak bond to yield one-electron reducing radicals, sulfur dioxide radical anion[16] and 3,5,5-trimethyl-2-oxomorpholin-3-yl (TM-3),[17] respectively. In the region of pH 7–8, both reducing agents have similar reduction potentials in protic solvent, in the range of −0.5 to −0.6 V vs NHE.[18]



TM-3 Dimer TM-3

Daunomycin (**1**) was first reacted with approximately 1 mol equiv of sodium dithionite in 5%/95% H_2O/DMSO. The UV–vis spectral changes which took place are shown in Figures 1 and 2. During the first 60 s of the reaction, the quinone absorption of **1** immediately disappeared to be replaced almost simultaneously by absorption at 440 nm (hydroquinone), 510 nm (semiquinone), and 680 nm (quinone methide). The 440-nm band disappeared in less than 30 s. The 510-nm band almost completely disappeared in ca. 40 s, while the 680-nm band, along with two more quinone methide bands at 382 and 420 nm, reached its maximum intensity. During the following 2 h of reaction, the quinone methide absorbance slowly decreased to be replaced by a new semiquinone band at 504–508 nm with a shoulder at ca. 480 nm indicative of a quinone chromophore (Figure 2). The first half-life for the decay of the absorbance at 680 nm was ca. 2000 s. After 3 days the cell was opened to air; whereby, the UV spectrum quickly and completely changed to that of a typical quinone chromophore, λ_{max} 480 and 494 nm, because of air oxidation of product semiquinones to quinones. The spectral changes are similar to those observed earlier during reduction of daunomycin in methanol with TM-3 dimer, except for the following: in methanol significant absorption by the semiquinone **2** or product semiquinones is not observed, the long wavelength band for the quinone methide appears at 604 nm, and the quinone methide has a half-life of less than 50 s.[5]

The identity of the 680-nm band as arising from the quinone methide was established by reducing daunomycin with TM-3 dimer in methanol and, when the characteristic quinone methide band at 604 nm[5] maximized, diluting with DMSO. The UV–vis spectrum immediately showed a shift of the maximum to 680 nm.

The reduction was also performed with 1 mol equiv of TM-3 dimer in DMSO. The spectral changes were similar to those observed with dithionite as the reducing agent except the semiquinone band maximized after 35 min and the quinone methide band maximized after 54 min. Slower formation of the semiquinone resulted from the slower rate of bond homolysis of TM-3 dimer relative to the rate of bond homolysis of dithionite.[18] The first-order rate constant for bond homolysis of TM-3 dimer in DMSO at 25 °C was determined to be 2.71×10^{-4} s^{-1} by oxidatively trapping the TM-3 radical with Ferriin, a complex of Fe^{3+} with 1,10-phenanthroline. This rate constant gives a half-life for bond homolysis at 25 °C of 43 min. The slow formation of the quinone methide, even after the concentration of the semiquinone maximized, clearly indicates that a second reduction of the semiquinone by TM-3 to the hydroquinone or disproportionation of the semiquinone to quinone and hydroquinone is required prior to glycosidic cleavage, as shown in Scheme I. Also both processes for formation of the hydroquinone must be relatively slow. Reduction of semiquinone **2** to hydroquinone by TM-3 is slow because again homolysis of TM-3 dimer in DMSO is slow.

The band at 510 nm was established as arising from absorption by daunomycin semiquinone through simultaneously monitoring of the UV–vis spectrum and the EPR spectrum of the reaction mixture from reduction with 0.5 mol equiv of TM-3 dimer. The EPR hyperfine pattern established formation of daunomycin semiquinone; coupling constants are reported in Table I. The EPR signal for daunomycin semiquinone maximized and decayed in parallel with the absorption band at 510 nm.

HPLC analysis of the reaction mixture from dithionite reduction showed the formation of 7-deoxydaunomycinone (**7**) and the two diastereomers of bi(7-deoxydaunomycinon-7-yl) (**8**). The products were identified by spectral and chromatographic comparison with

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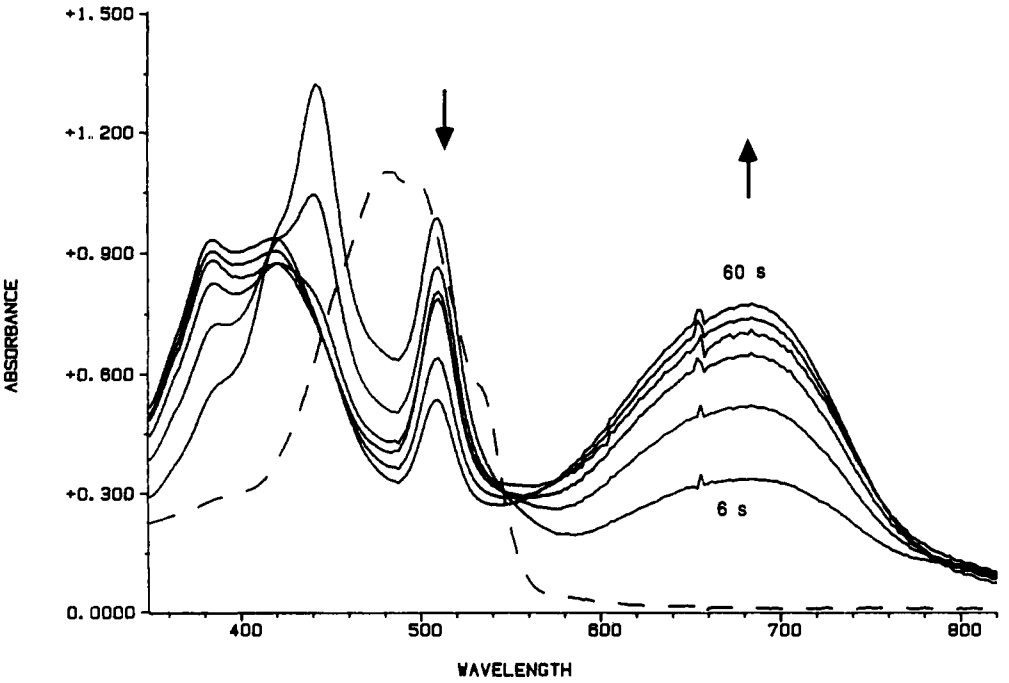

Figure 1. UV–vis spectral changes upon anaerobic reaction of a 9.5×10^{-5} M solution of daunomycin (**1**) with 1 mol equiv of dithionite in 5%/95% H_2O/DMSO at 20 °C as a function of time. Spectra were taken at the following times after mixing of the reagents: 6, 9, 15, 30, 45, and 60 s. The spectrum of **1** prior to mixing is shown with dashed lines.

Table I. EPR Hyperfine Coupling Constants in Gauss for Semiquinones **2** and **11**[a]

entry no.	starting material	semiquinone	medium	reducing agent	a_1	a_2	a_3	$a_{6,11}$	a_{7a}	a_{7b}	a_{10a}	a_{10b}
1	**1**	daunomycin	95%/5% DMSO/D_2O	TM-3 dimer	0.76	0.99	1.42	0.09 0.09	1.52		2.04	2.13
2	**1**	daunomycin	95%/5% DMSO/H_2O	TM-3 dimer	0.77	1.01	1.45	0.56 0.66	1.55		2.09	2.19
3	**1**[b]	daunomycin	50%/50% DMSO/H_2O	xanthine oxidase	0.88	1.01	1.45	0.52 0.63	1.57		2.04	2.22
4	**7**	7-deoxydaunomycinone	DMSO	TM-3 dimer	0.64	1.13	1.39	0.55 0.63	1.66	3.44	2.23	2.46
5	**7**[b]	7-deoxydaunomycinone	50%/50% DMSO/H_2O	xanthine oxidase	0.90	1.01	1.42	0.57 0.61	1.67	3.66	2.31	2.74
6	**1** or **7**	7-deoxydaunomycinone[c]	95%/5% DMSO/D_2O	$S_2O_4^{2-}$	0.68	1.10	1.45	0.07 0.07	1.54	3.37	2.13	2.34

[a] See Scheme I for the numbering system. [b] Taken from ref 10 with reassignment of splitting constants at positions 7 and 10 according to ref 24. [c] Starting with daunomycin, 7-deoxydaunomycinone semiquinone is deuterated at C14.

Table II. Yields of 7-Deoxydaunomycinone (**7**) and Bi(7-deoxydaunomycinon-7-yl) (**8**) from Reduction of Daunomycin as a Function of Reaction Conditions at 20 °C

entry no.	solvent	daunomycin concn (M)	reducing agent	mol equiv	1st half-life for **4** (s)	reacn time (h)	yield of **7** (%)	yield of **8**[a] (%)
1	5%/95% H_2O/DMSO	1.0×10^{-4}	$S_2O_4^{2-}$	1	2000	72	86	12
2	5%/95% H_2O/DMSO	1.0×10^{-4}	$S_2O_4^{2-}$	1	2000	1.5	79	16
3	5%/95% H_2O/DMSO	1.0×10^{-3}	$S_2O_4^{2-}$	1	600	1	75	24
4	5%/95% D_2O/DMSO	1.0×10^{-4}	$S_2O_4^{2-}$	1	5000	24	72	27
5	5%/95% D_2O/DMSO	1.0×10^{-3}	$S_2O_4^{2-}$	1	1000	2	44	52
6	5%/95% D_2O/DMSO	2.0×10^{-3}	$S_2O_4^{2-}$	1		17	61	36
7	DMSO	1.0×10^{-4}	TM-3 dimer	1		20	97	trace
8	5%/95% H_2O/DMSO	2.0×10^{-4}	TM-3 dimer	0.5		20	48	1[b]
9	5%/95% D_2O/DMSO	2.0×10^{-4}	TM-3 dimer	0.5		20	45	6[b]

[a] The yields of aglycon dimer **8** are in chromophoric units. [b] The balance of the reaction mixture was unreacted daunomycin.

authentic samples.[19] The yields as a function of reaction conditions are summarized in Table II. Interpretation of yields is complicated by the instability of the aglycon dimers under the reaction conditions. An independent experiment showed that anaerobic reduction of one of the aglycon dimers with 1 mol equiv of dithionite resulted in 33% cleavage of the dimer to 7-deoxydaunomycinone after 17 h of reaction time. Reductive cleavage of 7-deoxyaglycon dimers was observed earlier in a study of the redox chemistry of aclacinomycin A.[20] Trends apparent in Table II are that shorter reaction times (compare entries 1 and 2), higher concentration of reactants (compare entries 4 and 6), deuterium oxide in place of water (compare entries 3 and 5), and dithionite in place of TM-3 dimer (compare entries 1 and 7) increase the relative yield of dimer. These observations are all consistent with the quinone methide as a precursor to dimer as well as 7-deoxydaunomycinone, as shown in Scheme I. 7-Deoxyaglycon dimer

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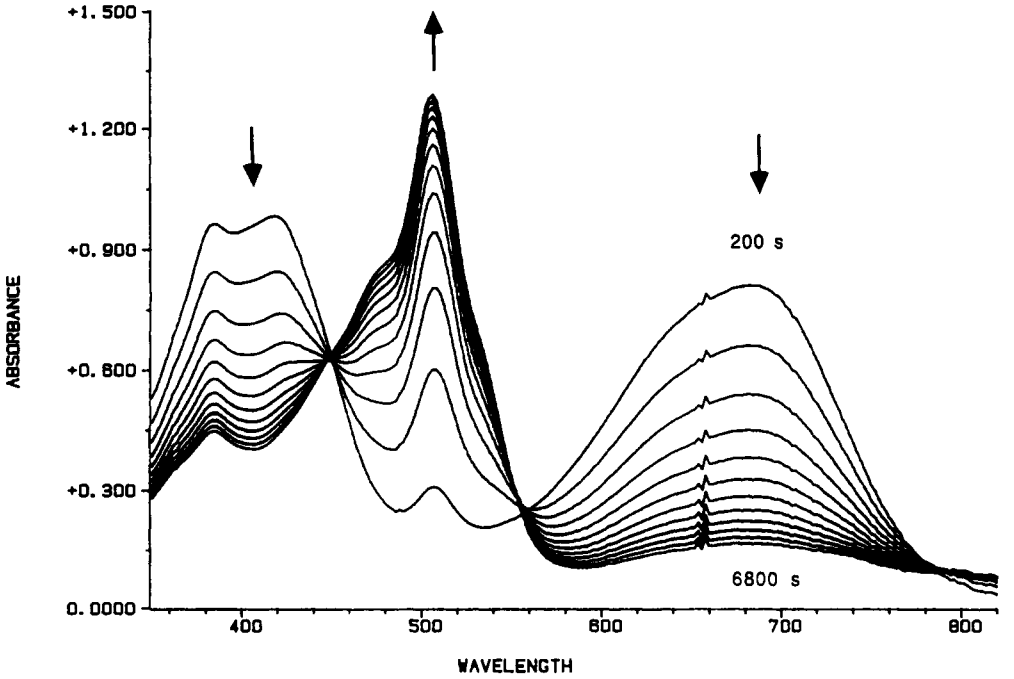
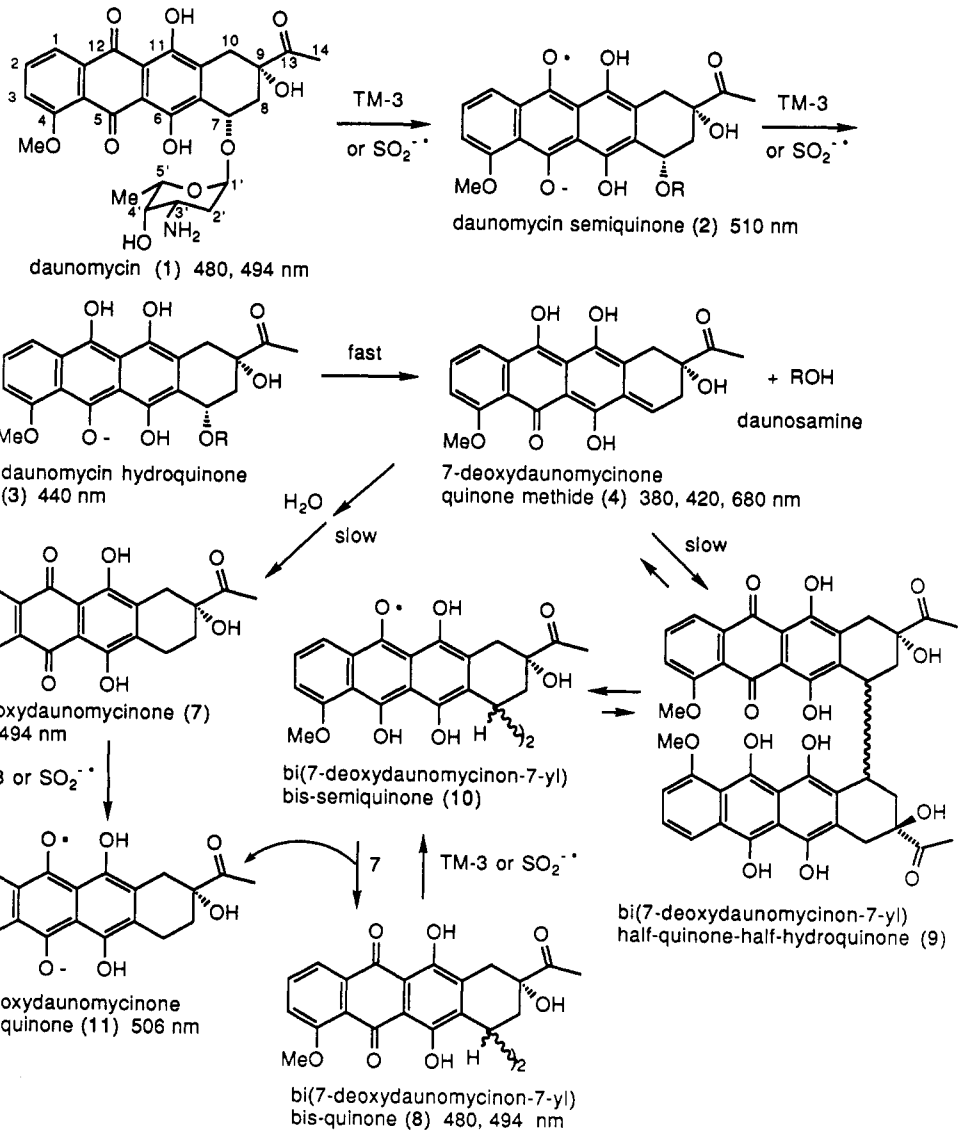


Figure 2. UV–vis spectra for the reaction described in the caption to Figure 1 during the time interval 200–6800 s with spectra taken every 600 s.

Scheme I

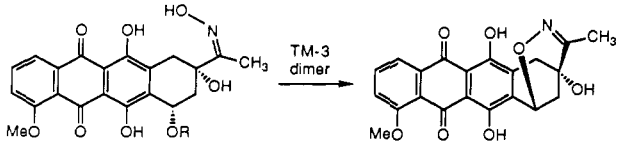


formation is a second-order reaction, and 7-deoxyaglycon formation is a pseudo-first-order reaction. Reaction conditions which increase the concentration of quinone methide should, as observed, increase the yield of dimer. Dithionite yields higher concentrations of quinone methide than does TM-3 dimer because it undergoes bond homolysis faster; bond homolysis is generally the rate controlling step for reduction. A large deuterium kinetic isotope effect was observed earlier for formation of 7-deoxydaunomycinone in methanol versus methanol-*d* solvents.[5] 7-Deoxyaglycon dimer formation is not expected to show a large isotope effect based upon studies of reduction of 11-deoxydaunomycin.[21]

Other features of the mechanism for formation of 7-deoxyaglycon dimers also appear in Scheme I. The initial product from dimerization of quinone methide is most likely the 7-deoxyaglycon dimer in the half-quinone–half-hydroquinone state (**9**). This state of the 7-deoxyaglycon dimer is proposed to exist in equilibrium with the bissemiquinone state (**10**) as suggested by the appearance of a semiquinone band at 504–510 nm upon decay of the quinone methide band. The assignment of this band to a semiquinone is based upon both the position and shape (Figure 2). The bissemiquinone is shown in Scheme I to be oxidized by 7-deoxydaunomycinone (**7**) to 7-deoxyaglycon dimer in the bisquinone state (**8**) with formation of the semiquinone (**11**) of 7-deoxydaunomycinone. Occurrence of this latter process is evident from the EPR spectrum of the reaction mixture from reduction of daunomycin with 1 mol equiv of dithionite after 30 min of reaction time. The spectrum showed the predominant semiquinone species to be **11**. Because of the stoichiometry and the kinetics, **11** could only be formed through reduction of **7** by a semiquinone, which is most likely **10**.

Absorption in the region of 510 nm did not completely disappear during the initial stage of the reaction, as shown in Figure 1, for several reasons. Some product semiquinones were likely formed even during the first 40 s of the reaction period, and product semiquinones can in turn reduce any unreacted daunomycin to daunomycin semiquinone (**2**). In the absence of dithionite, **2** would form hydroquinone **3** slowly. Adding exactly 1 equiv of dithionite was difficult because of its state of purity and its reactivity. This problem coupled with the occurrence of reversible aglycon dimer formation also precluded kinetic analysis of the reaction.

The EPR spectrum of 7-deoxydaunomycinone semiquinone produced from reduction of daunomycin in 5%/95% D_2O/DMSO showed a mixture of **11** and 7-deoxy-7-deuteriodaunomycinone semiquinone (**11**-7-*d*) with **11** being the major semiquinone present. What was anticipated was exclusive formation of **11**-7-*d* resulting from deuteration of the quinone methide at position 7 followed by reduction of the resulting 7-deoxy-7-deuteriodaunomycinone to **11**-7-*d* by the bissemiquinone **10** of 7-deoxyaglycon dimer. The source of the proton for protonation of quinone methide in 5%/95% D_2O/DMSO was shown to be the methyl group of the quinone methide at the 14-position. ^1H NMR analysis of 7-deoxydaunomycinone from reduction of daunomycin in 5%/95% D_2O/DMSO showed more deuterium incorporation at the 14-position than at the 7-position. A control experiment showed no deuterium incorporation at the 14-position from reduction of 7-deoxydaunomycinone with 0.5 mol equiv of dithionite in 5%/95% D_2O/DMSO-d_6. Deuteration at the 14-position via the intramolecular protonation of the quinone methide is proposed in Scheme II. An earlier observation which supports the possibility of an intramolecular proton transfer in terms of the spatial relationship of the groups is the formation of cyclooxime **12** from reductive glycosidic cleavage of daunomycin oxime (**13**) in water or methanol.[22]



daunomycin oxime (13) cyclooxime 12

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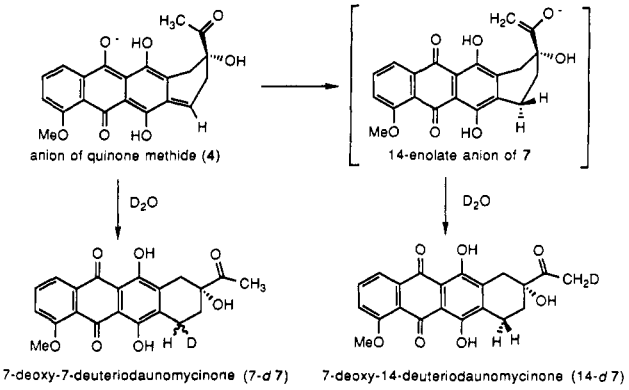
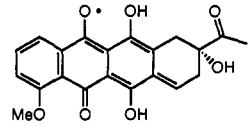
Scheme II. Mechanism Proposed for Deuterium Incorporation in 7-Deoxydaunomycinone from Reduction of Daunomycin in DMSO/D_2O



7-deoxy-7-deuteriodaunomycinone (7-*d* 7) 7-deoxy-14-deuteriodaunomycinone (14-*d* 7)

Table I summarizes EPR hyperfine splitting constants for daunomycin semiquinone (**2**) and 7-deoxydaunomycinone semiquinone (**11**) in DMSO and DMSO/water mixtures. The earlier assignment of the hyperfine coupling constants for protons at positions 7 and 10 in the EPR spectrum of **2** by Mason and co-workers (entry 3) is corrected,[10] based upon a study of **2** in DMF solvent by Stegmann and co-workers. From double resonance and deuterium labeling experiments, Stegmann and coworkers assigned the 1.5–1.6-G splitting constant to the proton at the 7-position.[23] The assignment of the 1.5–1.6-G coupling to a proton at the 7-position of **11** is also consistent with the widths of spectra of **11** and of a mixture of the two diastereomers of **11**–7-*d*, which differ by 1.6 G. Our earlier determination of the hyperfine coupling constants for **11** generated in DMSO with TM-3 dimer[5] is corrected in entry 4.

The mechanism for 7-deoxyaglycon dimer formation differs significantly from that recently proposed in reaction of daunomycin quinone methide with molecular oxygen.[19] 7-Deoxydaunomycinone quinone methide reacts almost instantaneously with molecular oxygen to yield aglycon dimers as well as five other products. A radical mechanism was proposed with the intermediacy of 7-deoxydaunomycinone semiquinone methide from one-electron oxidation of the quinone methide by molecular oxygen.



7-deoxydaunomycinone *semiquinone methide*

We conclude that reduction of daunomycin in DMSO bears some resemblance to the reductions of the 11-deoxyanthracyclines, 11-deoxydaunomycin,[21] aclacinomycin A,[20] and menogaril,[24] in methanol. These anthracyclines yield longer lived quinone methides even in protic solvent because the absence of the 11-hydroxy group makes the quinone methides less basic.

The longer lifetime for the 7-deoxydaunomycinone quinone methide and lower concentration of proton donors in the medium yield a higher probability for reaction with nucleophiles and electrophiles other than protons from solvent. Examples reported here are the dimerization reaction and the intramolecular protonation. A related environment in vivo might be a site for successful coupling of the anthracycline aglycon to a biological macromolecule. A polar aprotic medium also dramatically slows the rate of disproportionation of the daunomycin semiquinone. A consequence in vivo may be more efficient production of reactive

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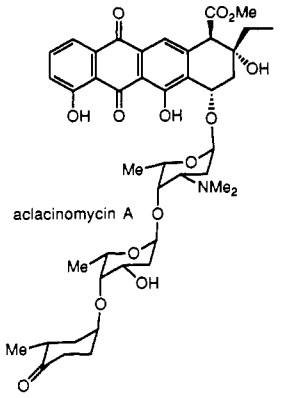

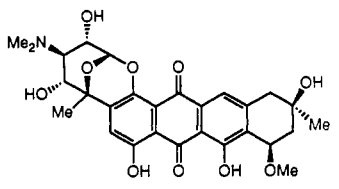

oxygen species through oxidation by molecular oxygen in competition with disproportionation to daunomycin and daunomycin hydroquinone followed by glycosidic cleavage. An important aspect of biological cytotoxicity is thought to be the catalytic oxidation of cellular membranes.[13-15,25,26]

Experimental Section

General Remarks. UV–vis spectra were recorded with a Hewlett-Packard 8452A diode array spectrometer. ^{1}H NMR spectra were obtained from a Varian VXR-300S spectrometer. Chemical shifts are reported in ppm on the δ scale from internal tetramethylsilane or 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt, and coupling constants are in hertz. HPLC analyses were performed with a Hewlett-Packard 1090 liquid chromatograph equipped with a diode array UV–vis detector and work station. Chromatography was performed with a Hewlett-Packard 5-μm C_{18} microbore column, 2.1 mm i.d. × 100 mm, eluting at 0.3 mL/min with a 60/40 mixture of methanol and 0.3% ammonium formate in water, adjusted to pH 4 with 98% formic acid. The percentages of anthracyclines reported from HPLC analyses are the area percentages of the peaks resulting from monitoring at 480 nm. TLC was performed with Merck precoated silica gel 60 F-254 sheets. The experiments involving the use of sodium dithionite or TM-3 dimer as reducing agents were run under anaerobic conditions. The use of nitrogen- or argon-degassed solutions in UV cuvettes provided with a serum stopper proved to be satisfactory only for short-time experiments (<2 h). For long-lasting experiments the freeze–pump–thaw degassing procedure was necessary.[5] Three types of reaction cells were used: (A) a 10-mm Pyrex cuvette fused at a 120° angle to a degassing chamber, 1.6 cm o.d. × 4.5 cm, and a 0.9-cm tube for attachment to a vacuum line with an Ultra-Torr Union; (B) three elements, a 10-mm or 1-mm cuvette, a 2-mL flask, and a 7-mL flask (degassing chamber), all fused perpendicularly to and in the same plane with a 0.9-cm tube to form an "E-shape", and a second 0.9-cm tube for attachment to the vacuum line fused perpendicularly to the first, coaxially with the 2-mL flask, at the center of the E; (C) a type B cell with the addition of a quartz EPR tube fused to the first 0.9-cm tube coaxially to the 7-mL flask. Trizma buffers were prepared from tris(hydroxymethyl)aminomethane (Tris) and Tris hydrochloride (Tris·HCl). Tris and Tris·HCl were purchased from Boehringer Mannheim Gmbh, Germany. Daunomycin hydrochloride was a gift from Farmitalia Carlo-Erba, Milan, Italy. 7-Deoxydaunomycinone and 7-deoxy-7-deuteriodaunomycinone were prepared according to procedures in the literature.[6,27] Sodium dithionite, approximately 80% pure, was obtained from Aldrich Chemical Co., Milwaukee, WI. Its purity was occasionally checked by iodine titration in water containing 2% sodium bicarbonate. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was also obtained from Aldrich.

Reaction of Daunomycin with 1 Mol Equiv of Sodium Dithionite. (a) In 95/5 Dimethyl Sulfoxide–Water, Concentration = 10^{-4} M, Long Reaction Time. A 9.5×10^{-5} M solution of **1** was prepared by dissolving 13.4 mg (0.024 mmol) of daunomycin hydrochloride in 25.0 mL of $(1 + 1) \times 10^{-2}$ M Trizma in dimethyl sulfoxide, followed by 1–10 dilution with dimethyl sulfoxide. The UV–vis spectrum showed λ_{max} = 482 nm ($\epsilon = 1.2 \times 10^4$ M^{-1} cm^{-1}). A 4.0-mL aliquot of this solution (3.8×10^{-4} mmol of **1**) was introduced into the 7-mL arm of a type B cell (10-mm cuvette). Prepurified argon was then bubbled through the solution using Teflon tubing. Sodium dithionite (0.90 mg, 81%, 4.2×10^{-3} mmol) was dissolved in 2.0 mL of argon-bubbled distilled water. Soon 0.20 mL of this solution (4.2×10^{-4} mmol of dithionite) was introduced, via syringe provided with Teflon tubing, into the 2-mL branch of the cell under argon flow. The 2-mL branch had been previously cooled externally at a temperature below −20 °C to provide quick freezing of the solution, in order to minimize air oxidation. Immediately the cell was transferred to the vacuum line, and both the daunomycin and the dithionite solutions were freeze–thaw degassed through three cycles. The cell was flame-sealed under vacuum and brought to 20 °C in a water bath. Very quickly the two solutions were mixed; this mixture was poured into the cuvette portion of the cell, which was immediately introduced into the cell holder of the UV–vis spectrometer thermostated at 20.0 ± 0.1 °C. The spectral changes at 350–820 nm were then monitored at appropriate time intervals and are shown in Figures 1 and 2. HPLC analysis showed 86% 7-deoxydaunomycinone (**7**, R_t = 8 min) and 12% of the two stereoisomeric bi(7-deoxydaunomycinon-7-yl)s (**8**, R_t = 13–14 min). The identities of **7** and **8** were established by HPLC and TLC comparison with authentic samples.[19] NMR and EPR evidence was also provided in analogous separate experiments.

(b) In 95/5 Dimethyl Sulfoxide–Water, Concentration = 10^{-4} M, Short Reaction Time. An experiment was run as described under a, using a serum-stoppered cuvette. The daunomycin solution was deaerated by bubbling argon. The dithionite solution was added from a microsyringe through the serum stopper. After 1.5 h the cell was opened and the solution HPLC analyzed to show 79% **7** and 16% **8**.

(c) In 95/5 Dimethyl Sulfoxide–Water, Concentration = 10^{-3} M, Short Reaction Time. This experiment was run in a sealed type B cell (1-mm cuvette), as described under a, using 10^{-3} M **1**, $(1 + 1) \times 10^{-2}$ M Trizma, and 1 mol equiv of dithionite. The first half-life for the decay of the absorbance at 680 nm was ca. 600 s. The cell was opened 1 h after the beginning of the reaction. HPLC analysis showed 75% **7** and 24% **8**.

(d) In 95/5 Dimethyl Sulfoxide–Deuterium Oxide, Concentration = 10^{-4} M, Long Reaction Time. The reaction was run in a sealed type C cell (10-mm cuvette) as described under a. The first half-life for the decay of the absorbance at 680 nm was ca. 5000 s. After 1 day the cell was opened and HPLC analyzed, showing 72% **7** and 27% **8**. In a similar experiment, when deuterium oxide was only 0.25%, no meaningful change in the rate of decay of the quinone methide was observed.

(e) In 95/5 Dimethyl Sulfoxide–Deuterium Oxide, Concentration = 10^{-3} M, Short Reaction Time. This experiment was run as described under b, using a 10^{-3} M solution of **1**, $(1 + 1) \times 10^{-2}$ M Trizma, and 1 mol equiv of dithionite. The first half-life for the decay of the absorbance at 680 nm was ca. 1000 s. The cuvette was opened after 2 h; HPLC analysis gave 44% **7** and 52% **8**.

Evidence for the Absorbance at 680 nm Being Due to Daunomycin Quinone Methide (4). A 1×10^{-3} M solution of **1** in methanol-*d* containing $(1 + 1) \times 10^{-3}$ M Trizma was deaerated by bubbling nitrogen. A 25-mL aliquot of this solution was introduced into a 1-mm quartz cuvette filled with nitrogen through a serum stopper. Soon 50 μL of air-free methanol containing 10 mol equiv of TM-3 dimer was introduced into the cuvette via a microsyringe. The spectral changes were followed in the 350–820-nm region. The absorbance at 480 nm due to daunomycin dropped to ca. half of its initial intensity in ca. 40 s, while a quinone methide band at 604 nm[5] rose to an optical density (OD) of 0.4. At this point 100 μL of this solution was quickly transferred via syringe into another serum-stoppered cuvette (10 mm), containing 2 mL of deaerated dimethyl sulfoxide. A UV–vis spectrum taken immediately showed a maximum at 680 nm (OD = 0.2, quinone methide) along with another maximum at 508 nm (OD = 0.4, semiquinone) and a shoulder at 440 nm (hydroquinone). During the following hour the bands at 508 and 680 nm decreased to about half of their original intensity, while the absorbance at 440 nm increased to an OD of 0.7.

Rate of Bond Homolysis of *d,l*-TM-3 Dimer in Dimethyl Sulfoxide. A method very similar to that described earlier was employed:[28] A 10^{-4} M solution of Ferriin in dimethyl sulfoxide was made by adding 100 μL of a freshly made 2.5×10^{-2} M aqueous solution of Ferriin to 24.9 mL of dimethyl sulfoxide. The solution gave an absorption max at 357 nm (OD = 0.55). No absorption maximum due to any Ferroin at 516 nm (see below) was seen; only a residual OD of 0.01 was found as the tail of Ferriin absorbance, which slowly increased to 0.04 in 3 h. When a very large excess of solid TM-3 dimer was added, a band at 516 nm, due to Ferroin, soon appeared and reached its maximum ($\epsilon = 1.1 \times 10^4$ M^{-1} cm^{-1}) in ca. 20 min. No appreciable decay was noticed over several hours. A kinetic measurement was run as follows: TM-3 dimer (3.89 mg, 1.37×10^{-2} mmol) was dissolved in 2.0 mL of dimethyl sulfoxide. A 12-μL aliquot of this solution (8.2×10^{-5} mmol) was introduced into a UV cuvette containing 2.0 mL of the 10^{-4} M solution of Ferriin. The

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cuvette was soon introduced into the thermostated cell holder of the spectrometer at 25.0 ± 0.1 °C. The average absorbance at 510–520 nm was recorded as a function of time over a period of 3 h (4.2 half-lives). The increase of the absorbance with time was found to follow a first-order rate law, $k_1 = 2.71 \times 10^{-4}$ s^{-1} ($\sigma = 0.01 \times 10^{-4}$).

Reaction of Daunomycin (1) with 1 Mol Equiv of *d,l*-TM-3 Dimer. *d,l*-TM-3 dimer (1.3 mg, 4.6×10^{-3} mmol) was dissolved in 10 mL of dichloromethane. A 0.5-mL aliquot of this solution (2×10^{-4} mmol of TM-3 dimer) was introduced into the cuvette portion of a type A cell, and the solvent was evaporated with a stream of nitrogen. A 2.0-mL aliquot of a dimethyl sulfoxide solution, 1.0×10^{-4} M in **1** and $(1 + 1) \times 10^{-3}$ M in Trizma, was introduced in the degassing chamber of the cell. After freeze–thaw degassing (3 cycles) the cell was sealed, brought to 25 °C, shaken to dissolve TM-3 dimer, and put in the thermostated cell holder of the spectrometer, at 25.0 ± 0.1 °C. The spectral changes at 350–820 nm were monitored. During the first part of the reaction a slow decrease of the quinone absorbance at 480 nm occurred with a simultaneous rise of a semiquinone band at 510 nm, reaching its maximum (OD = 1.6) after 25 min. A relatively weaker, broad absorption in the 600–820-nm region, where both semiquinone and quinone methide absorb, also rose up. However, the absorbance at 680 nm, where the quinone methide shows its maximum intensity, reached its maximum only after 54 min. During the following 20 h the absorbance due to both semiquinone and quinone methide slowly faded to almost disappear and a quinone-type spectrum was restored. At this time the cell was opened and the reaction mixture HPLC analyzed to show 97% **7** and only traces of **8**.

Reaction of Daunomycin (1) with 0.5 Mol Equiv of *d,l*-TM-3 Dimer; EPR Spectrum of Daunomycin Semiquinone. (a) *dl*-TM-3 dimer (0.071 mg, 2.5×10^{-4} mmol) was introduced, as described in the previous paragraph, in the cuvette portion of a type A cell fused to a quartz EPR tube. A 2.5-mL aliquot of a 95%/5% dimethyl sulfoxide/water solution, 2.0×10^{-4} M in **1** and $2 \times (1 + 1) \times 10^{-3}$ M in Trizma, was introduced into the degassing chamber of the cell. After a four-cycle freeze–thaw degassing, the cell was sealed, brought to 25 °C, shaken to dissolve TM-3 dimer, and put in the cell holder of the UV–vis spectrometer, at 25 ± 0.1 °C. The spectral changes in the 350–820-nm region were monitored with time for ca. 15 min. By this time the rising semiquinone band at 510 nm had reached an OD value of 2.5. The cell was soon transferred to the EPR spectrometer, at 20 °C, and spectra were recorded at different times. The EPR spectrum (Table I) reached a maximum intensity after 1 h, to then slowly decrease to less than 5% of its maximum intensity in 20 h. A set of control UV–vis spectra showed that the band at 510 nm also reached its maximum intensity (OD = 2.9) in ca. 1 h and then slowly disappeared in 20 h. At this time the cell was opened and analyzed by HPLC; **1** (R_t = 6.5 min) and **7** were present in a 1:1 ratio, accounting for 96% of the reaction mixture. Only 1% of **8** was observed.

(b) A similar experiment was run using a 95%/5% dimethyl sulfoxide/deuterium oxide mixture as solvent. A similar result was obtained, except for a larger (6%) amount of **8** and a different hyperfine structure in the EPR spectrum (Table I). The measured *g* value was 2.0039.

EPR Spectrum of 7-Deoxydaunomycinone Semiquinone (11) from the Reduction of Daunomycin (1) with Sodium Dithionite. A reference EPR spectrum of 7-deoxydaunomycinone semiquinone (**11**) was taken from a 95%/5% dimethyl sulfoxide/deuterium oxide solution as follows: 3.0 mL of a 5×10^{-4} M solution of 7-deoxydaunomycinone (**7**, 1.5×10^{-3} mmol) in dimethyl sulfoxide was introduced into the degassing chamber of a type C cell. Nitrogen was bubbled through the solution. A $(1 + 1) \times 10^{-1}$ M solution of Trizma in deuterium oxide (7 mL) was rotary evaporated at 0.5 Torr. The residue was redissolved in 7 mL of deuterium oxide, and the solution was deaerated with a nitrogen stream. Sodium dithionite (81%, 1.26 mg, 6×10^{-3} mmol) was dissolved in 1.2 mL of the buffer. A 0.15-mL aliquot of this solution (7.5×10^{-4} mmol of dithionite) was introduced into the 2-mL branch of the cell, precooled for a quick freezing. After a three-cycle freeze–thaw degassing, the cell was sealed and the reactants mixed before the EPR spectrum was taken. The *g* value was 2.0040, and the spectrum width, 12.6 G. A reference EPR spectrum of the semiquinone of 7-deoxy-7-deuteriodaunomycinone was also taken by reduction of 7-deoxy-7-deuteriodaunomycinone with 0.5 mol equiv of dithionite, as described above for the reduction of **7**. The EPR spectrum was 11.0-G wide. When a 2×10^{-3} M daunomycin solution $(1 + 1) \times 10^{-2}$ M in Trizma in a 95%/5% dimethyl sulfoxide/deuterium oxide mixture (4 mL) was allowed to react with 1 mol equiv of sodium dithionite, as described above, the EPR spectrum recorded after 0.5 h was 12.6 G wide, with the same hyperfine splitting as observed in the spectrum of **11**. Less intense peaks corresponding to the spectrum of 7-deoxy-7-deuteriodaunomycinone semiquinone were also present. Control UV–vis spectra showed the characteristic semiquinone absorption at 508 nm which reached its maximum intensity after ca. 45 min, while the quinone methide absorption at 680 nm had fallen to ca. 10% of its maximum intensity. The cell was opened after 17 h. HPLC analysis showed **7** and **8** in a 1.7 ratio (total yield 97%). The crude reaction mixture was purified by silica gel suction chromatography:[29] 7-deoxydaunomycinone was eluted with 0.2% 2-propanol in chloroform and was used for NMR analysis (see below). In a separate similar experiment the reduction of **1** with sodium dithionite was run using 95%/5% dimethyl sulfoxide/water as solvent. An EPR spectrum was obtained (g = 2.0039, width 14.4 G) virtually identical to that reported in the literature for the reduction of **7** by TM-3 in dimethyl sulfoxide.[5]

A 50%/50% dimethyl sulfoxide/water solution 5×10^{-4} M in 7-deoxydaunomycinone, buffered at pH 8 with phosphate, was reduced with 0.5 mol equiv of sodium dithionite. The EPR spectrum matched that reported by Schreiber and co-workers.[10] The spectrum showed a noteworthy difference with the spectrum obtained in pure dimethyl sulfoxide or 95%/5% dimethyl sulfoxide/water, as reported above.

NMR Spectrum of 7-Deoxydaunomycinone (7) from the Reduction of Daunomycin (1) with Sodium Dithionite. A reference spectrum of **7** was first taken in di(methyl-d_3) sulfoxide. The spectrum showed a signal at 2.88 ppm (A part of an AB pattern, $J_{10a,10b}$ = 19, H10a) and in the 2.64–2.82 ppm region a signal at 2.74 ppm (B part of an AB pattern, $J_{10b,10a}$ = 19, H10b) overlapped with a multiplet (H7a + H7b). The overlapped signals integrated for 3 H, relative to the CH_3O singlet at 3.96 ppm. The MeCO signal appeared at 2.2 ppm and integrated for 2.8 H. A reference spectrum of 7-deoxy-7-deuteriodaunomycinone obtained according to the literature[5,6] was taken in the same solvent. Again in the 2.64–2.82 ppm region the signal from H10b overlapped with a multiplet due to H7a and/or H7b. However, the overlapped signals integrated for 2.2 H. The MeCO signal integrated for 2.8 H. The sample of 7-deoxydaunomycinone obtained from the reduction of **1** in 95%/5% dimethyl sulfoxide/deuterium oxide and purified as described in the previous paragraph, gave the following spectrum: the overlapped signals at 2.64–2.82 ppm (H10b + H7a and/or H7b) integrated for 2.6 H. The MeCO signal integrated for 2.4 H. In a control experiment a 2×10^{-3} M solution of **7** in 95%/5% di(methyl-d_3) sulfoxide/deuterium oxide, $(1 + 1) \times 10^{-2}$ M in Trizma, was reduced to semiquinone with 0.5 mol equiv of sodium dithionite in a type C cell. After 17 h the cell was opened and an NMR spectrum taken: the overlapped signals at 2.64–2.82 ppm integrated for 3.0 H and the MeCO for 2.9 H.

Slow Transformation of Bi(7-deoxydaunomycinon-7-yl) (8) into 7-Deoxydaunomycinone (7) through Reduction. Bi(7-deoxydaunomycinon-7-yl) (isomer **9** of ref 20; 0.31 mg, 4.01×10^{-4} mmol) was dissolved in 0.57 mL of dry dimethyl sulfoxide. A 0.35-mL aliquot of this solution (2.5×10^{-4} mmol of **8**) was introduced into the degassing chamber of a type C cell. Nitrogen was then bubbled through the solution. Sodium dithionite (81%, 1.89 mg, 8.8×10^{-3} mmol) was dissolved in 0.88 mL of deaerated deuterium oxide, $(1 + 1) \times 10^{-1}$ M Trizma. A 0.25-mL aliquot of this solution was mixed with 1.5 mL of deaerated dry dimethyl sulfoxide, and 175 μL of this mixture (2.2×10^{-4} mmol of dithionite) was introduced into the 2-mL branch of the cell previously cooled for a quick freeze. After a three-cycle freeze–thaw degassing, the cell was sealed and brought to 20 °C and the reactants were mixed. Immediately the UV–vis spectrum showed a change from a quinone-type (λ_{max} = 500 nm) to a semiquinone-type (λ_{max} = 510 nm) spectrum. A very broad EPR spectrum was also observed, not enough resolved to be analyzed. After 17 h the UV spectrum showed a shoulder at 480 nm along with the semiquinone band. The cell was opened and analyzed by HPLC. Only starting material (**8**) and 7-deoxydaunomycinone (**7**) were detected in a 2:1 ratio.

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