Synthesis and Redox Chemistry of 5-Deoxydaunomycin. A Long-Lived Hydroquinone Tautomer

Barbara Ann Schweitzer and Tad H. Koch*

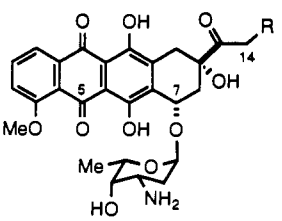
Contribution from the Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309-0215

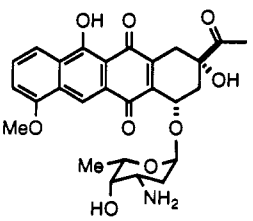
Received January 25, 1993

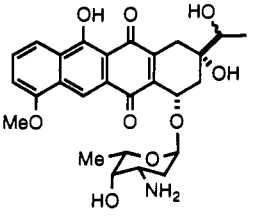
Abstract: Reduction of 5-iminodaunomycin with dithionite in anaerobic methanol followed by lowering the pH to 3 and saturating with air led to deamination without glycosidic cleavage to yield 89% 5-deoxydaunomycin. An intermediate observed during the reaction is proposed to be the hydroquinone tautomer, 8-acetyl-12-amino-10-[(3-amino-2,3,6-trideoxy-α-lyxo-hexopyranosyl)oxy]-1-methoxy-7,9,10,12-tetrahydro-6,8,11-trihydroxy-5(8*H*)-naphthacenone hydrochloride (**1**), which loses ammonia with a half-life of 49 min. Anaerobic reduction of 5-deoxydaunomycin with bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (TM-3 dimer) in methanol buffered to an apparent pH of 8 yielded 26% recovered 5-deoxydaunomycin, 56% 5,7-dideoxydaunomycinone, and 18% 2-acetyl-11-hydroxy-7-methoxy-5,12-naphthacenedione (**5**) after 42 h and subsequent exposure to molecular oxygen. The reduction leads to relatively rapid formation of a long-lived transient proposed to be, 8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-lyxo-hexopyranosyl)oxy]-1-methoxy-7,9,10,12-tetrahydro-6,8,11-trihydroxy-5(8*H*)-naphthacenone (**4**). Exposure of **4** at its maximum concentration to molecular oxygen yielded 88% recovered 5-deoxydaunomycin and 12% **5**. Tetrahydronaphthacenone **4** disappeared with a half-life of 2283 min in the absence of oxygen and 16 min in air-saturated methanol. Mechanistic pathways to the products are proposed in Scheme II. Analysis of the apparent rate constants for disappearance of **4** indicates that 5-deoxydaunomycin undergoes glycosidic cleavage to its 7-deoxyaglycon 8000 times slower than daunomycin upon reduction to the hydroquinone state.

Introduction

Anaerobic reduction of the quinone functionality of the anthracycline antitumor drugs, daunomycin (daunorubicin), adriamycin (doxorubicin), aclacinomycin A, and menogaril, in protic solvent with bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (TM-3 dimer),[1] carbon dioxide radical anion,[2] dithionite,[1,3] or reducing enzymes[3] leads to deoxygenation at the 7-position via, sequentially, semiquinone, hydroquinone, and quinone methide intermediates.[4-6] The quinone methides tautomerize to 7-deoxydaunomycinone, 7-deoxyadriamycinone, 7-deoxyaklavinone, and 7-deoxynogarol, respectively. 7-Deoxyaklavinone and 7-deoxynogarol quinone methides also dimerize.[7,8] Quinone methides are of general interest as potential biological alkylating agents.[9-12] Subsequent reduction of 7-deoxydaunomycinone yields 5,7-dideoxydaunomycinone amongst other products.[13-15] Cameron and co-workers[16] have reported that catalytic hydrogenation of daunomycin on platinum in methanol containing chloroacetic acid yielded 57% of 5-deoxydaunomycin and 25% of a mixture of 5-deoxydaunomycinol and 5-deoxyepidaunomycinol upon reoxidation of the crude product mixture with molecular oxygen. Similar results were obtained upon reduction of adriamycin. The authors further reported that the 5-deoxyanthracyclines showed significant anticancer activity, comparable with that of the parent anthracyclines.







We now report a high-yield synthesis of 5-deoxydaunomycin without side chain reduction at the 14-position from reaction of 5-iminodaunomycin with dithionite at reduced pH. The redox chemistry of 5-iminodaunomycin continues to be of interest

(1) Gaudiano, G.; Koch, T. H. *Chem. Res. Toxicology* **1991**, *4*, 2.
(2) Land, E. J.; Mukherjee, T.; Swallow, A. J.; Bruce, J. M. *Br. J. Cancer* **1985**, *51*, 515. Mukherjee, T.; Land, E. J.; Swallow, A. J.; Bruce, J. M. *Arch. Biochem. Biophys.* **1989**, *272*, 450. Lea, J. S.; Rushton, F. A. P.; Land, E. J.; Swallow, A. J. *Free Radical Res. Commun.* **1990**, *8*, 241.
(3) Fisher, J.; Abdella, B. R. J.; McLane, K. E. *Biochemistry* **1985**, *24*, 3562.
(4) Abdella, B. R. J.; Fisher, J. *Environ. Health Perspect.* **1985**, *64*, 3. Fisher, J. F.; Aristoff, P. A. *Prog. Drug Res.* **1988**, *32*, 411.
(5) Powis, G. *Free Radicals Biol. Med.* **1989**, *6*, 63.
(6) *Anthracyclines and Anthracenedione-Based Anti-Cancer Agents*; Lown, J. W., Ed.; Elsevier: Amsterdam, 1988.
(7) Kleyer, D. L.; Gaudiano, G.; Koch, T. H. *J. Am. Chem. Soc.* **1984**, *106*, 1105.
(8) Boldt, M.; Gaudiano, G.; Haddadin, M. J.; Koch, T. H. *J. Am. Chem. Soc.* **1989**, *111*, 2283.
(9) Lin, T.-S.; Antonini, L.; Cosby, L. A.; Sartorelli, A. C. *J. Med. Chem.* **1984**, *27*, 813.
(10) Moore, H. W.; Czerniak, R.; Hamdan, A. *Drugs Exptl. Clin. Res.* **1986**, *12*, 475.
(11) Angle, S. R.; Yang, W. *J. Org. Chem.* **1992**, *57*, 1092.
(12) Lemus, R. H.; Skibo, E. B. *J. Org. Chem.* **1992**, *57*, 5649. Skibo, E. B. *J. Org. Chem.* **1992**, *57*, 5874.
(13) Brand, D. J.; Fisher, J. *J. Am. Chem. Soc.* **1986**, *108*, 3088. Brand, D. J.; Fisher, *J. Org. Chem.* **1990**, *55*, 2518.
(14) Tresselt, D.; Ihn, W.; Horn, G.; Berg, H. *Pharmazie* **1984**, *39*, 417. Berg, H. *Studia Biophysica* **1984**, *104*, 13.
(15) Schweitzer, B. A.; Egholm, M.; Koch, T. H. *J. Am. Chem. Soc.* **1992**, *114*, 242.
(16) Cameron, D. W.; Feutrill, G. I.; Griffiths, P. G. *Tetrahedron Lett.* **1988**, *29*, 4629.

0002-7863/93/1515-5440$04.00/0

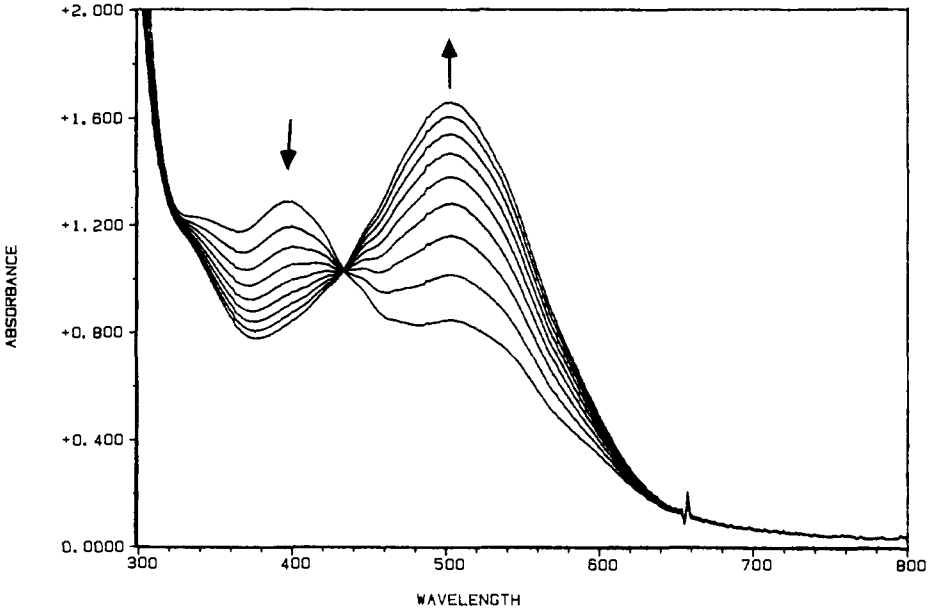


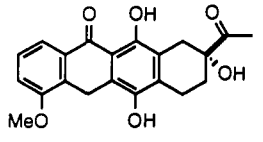
Figure 1. UV–vis spectra versus time showing formation of 5-deoxydaunomycin from aminotetrahydronaphthacenone **1** in the time period 1200–6000 s at 25 °C. Scans were every 600 s and were 2 s in duration. Intermediate **1** was created by reacting 5-iminodaunomycin with 1.3 mol equiv of dithionite in an anaerobic environment and then lowering the pH to 3.

because 5-iminodaunomycin shows less cardiotoxicity than daunomycin while retaining significant anti-tumor activity.[17] We then compare the redox chemistry of the potential anti-tumor drug, 5-deoxydaunomycin, with that of daunomycin.

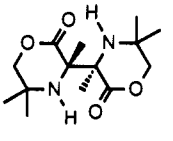
Results and Discussion

Synthesis of 5-Deoxydaunomycin. 5-Iminodaunomycin was reduced with 1.3 equiv of sodium dithionite in nitrogen degassed methanol to yield 5-iminodaunomycin hydroquinone. Approximately 25 s after the addition of the dithionite, the pH was lowered to 3 with hydrochloric acid, and the solution was saturated with molecular oxygen. Monitoring of the reaction by UV–vis spectroscopy showed immediate formation of a transient with maximum absorption at 396 nm upon addition of the hydrochloric acid. Over a period of 5 h the 396-nm band decreased with first-order kinetics and with appearance of a band at 500 nm corresponding to absorption by 5-deoxydaunomycin. Spectral changes as a function of time are shown in Figure 1. HPLC analysis showed 89% yield of 5-deoxydaunomycin.

A mechanism for deamination of 5-iminodaunomycin is proposed in Scheme I. The transient with absorption at 396 nm is assigned the structure 8-acetyl-12-amino-10-[(3-amino-2,3,6-trideoxy-α-lyxo-hexopyranosyl)oxy]-1-methoxy-7,9,10,12-tetrahydro-6,8,11-trihydroxy-5(8*H*)-naphthacenone hydrochloride (**1**) based upon absorption by 8-acetyl-1-methoxy-7,9,10,12-tetrahydro-6,8,11-trihydroxy-5(8*H*)-naphthacenone (**2**) at 397 nm.[18] Protonation of the amino substituent of 5-iminodaunomycin



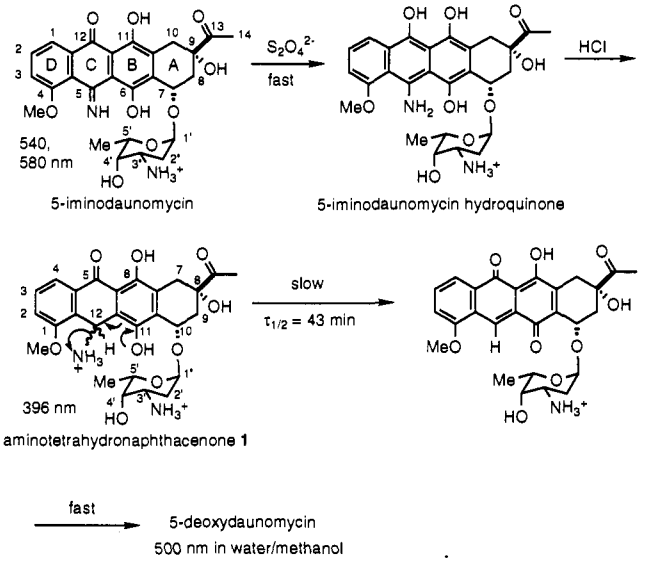
tetrahydronaphthacenone **2**



dl-TM-3 dimer

hydroquinone results in isomerization of the C-ring to the naphthacenone tautomer. In this form glycosidic cleavage to

Scheme I



yield 7-deoxydaunomycinone quinone methide is not competitive with deamination to yield 5-deoxydaunomycin. In Tris buffered methanol at an apparent pH of 8, 5-iminodaunomycin hydroquinone undergoes glycosidic cleavage without significant deamination with a half-life of 32 s at 25 °C.[18] At apparent pH 3 the half-life for deamination of **1** is 49 min. Reduction of 7-deoxy-5-iminodaunomycin at an apparent pH of 8 in methanol at 25 °C results in deamination to form 5,7-dideoxydaunomycinone via aminotetrahydronaphthacenone **3** with a half-life of only 17 s. The significant difference in the rates of deamination of 5-iminodaunomycin at pH 3 and 7-deoxy-5-iminodaunomycin at pH 8 probably result from differences in protonation of the phenolic oxygen at the 6-position. These rates together with others to be discussed are summarized in Table I.

Redox Chemistry of 5-Deoxydaunomycin. 5-Deoxydaunomycin in methanol at apparent pH 8 was reduced anaerobically with 1.2 mol equiv of *dl*-TM-3 dimer. UV–vis spectroscopic monitoring

(17) Factors in the Selection of New Anthracyclines. Acton, E. M.; Jensen, R. A.; Peters, J. H. In *Anthracycline Antibiotics in Cancer Therapy;* Muggia, F. M., Young, C. W., Carter, S. K., Eds.; Martinus Nijhoff: Boston, 1982; pp 205–219. Glazer, R. I.; Hartman, K. D.; Richardson, C. L. *Cancer Res.* **1982**, *42*, 117. Jensen, R. A.; Acton, E. M.; Peters, J. H. *Cancer Res.* **1984**, *44*, 4030.

(18) Bird, D. M.; Boldt, M.; Koch, T. H. *J. Am. Chem. Soc.* **1987**, *109*, 4046.

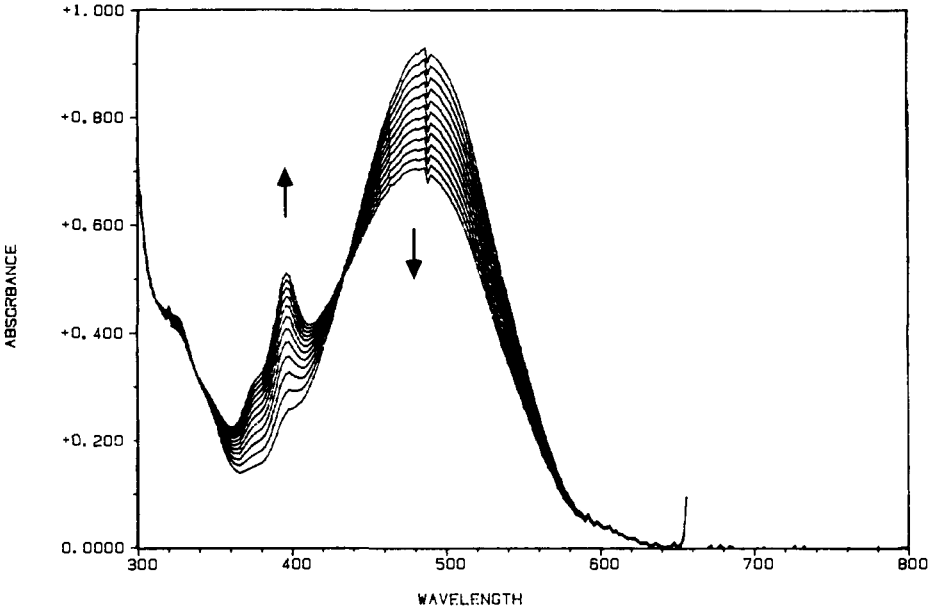


Figure 2. UV–vis spectra versus time of a reaction of 1.25×10^{-4} M 5-deoxydaunomycin and 1.35×10^{-4} M *dl*-TM-3 dimer in Tris buffered methanol at an apparent pH of 8 and 25 °C. Scans were every 4 s and were 0.5 s in duration during the time period 0–44 s.

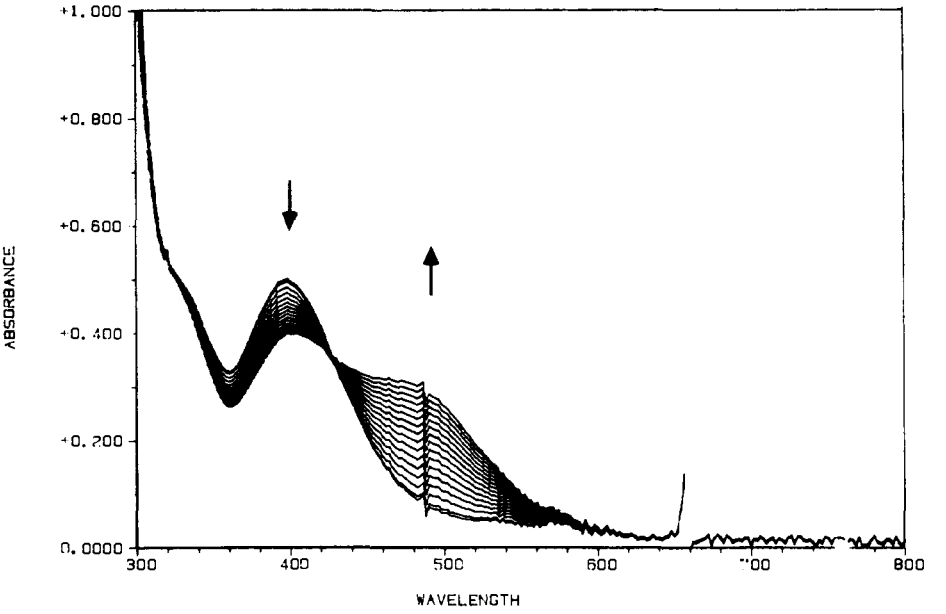
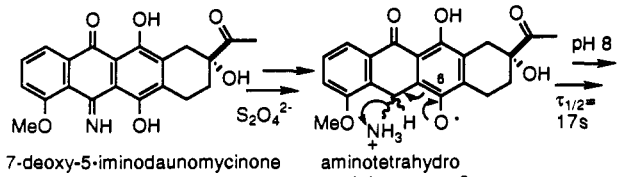
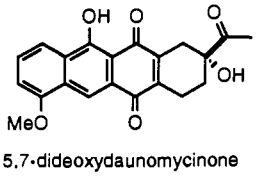


Figure 3. UV–vis spectra for the reaction described in the caption to Figure 2 as a function of time during the period 1.1–16.1 h. Scans were every 3600 s and were 2 s in duration.

of the reaction showed a decrease in the absorption by 5-deoxydaunomycin at 490 nm with an increase in absorption at 396 nm during the first 44 s corresponding to formation 5-deoxydaunomycin hydroquinone as shown in Figure 2. During the next 120 s the 396-nm band decreased with a slight shift to 398 nm and band broadening. The 398-nm band then slowly decreased with first-order kinetics and an increase in absorption at 480 nm during the next 42 h. A portion of the spectral changes are shown in Figures 2 and 3. The reaction mixture was exposed to molecular oxygen before complete reaction; HPLC analysis showed 26% recovery of 5-deoxydaunomycin, 56% 5,7-dideoxydaunomycinone, and 18% 2-acetyl-11-hydroxy-7-methoxy-5,12-naphthacenedione (**5**). Product structures were assigned from chromatographic and UV–vis spectral comparison with authentic samples. A second, analogous, anaerobic reduction was performed except the reaction mixture was saturated with air when the 398-nm band appeared to be at its maximum. The 398-nm band disappeared much faster, still with first-order kinetics and increased absorption at 480 nm. The observed first-order rate constants are reported in Table I. HPLC analysis of this reaction showed 88% recovered 5-deoxydaunomycin and 12% naphthacenedione **5**. A control experiment showed no reaction of 5-deoxydaunomycin in the absence of a reducing agent.



7-deoxy-5-iminodaunomycinone aminotetrahydro naphthacenone **3**



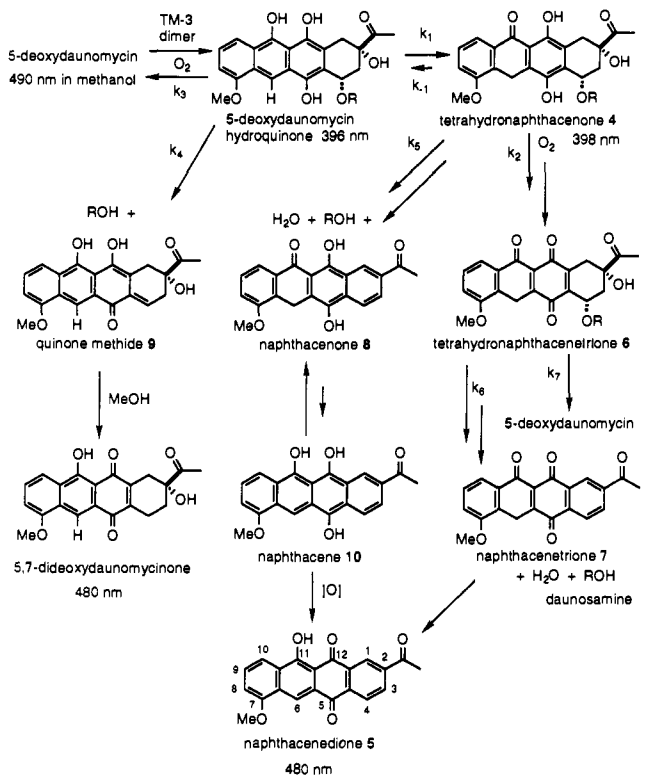
5,7-dideoxydaunomycinone (I)

Table I. Rate Constants and Product Ratios for the Formation and Reduction of 5-Iminodaunomycin and 5-Deoxydaunomycin at 25 °C in Methanol

reactants	apparent pH	rate constant k_{obs} (s^{-1})	half-life (min)	products
aminotetrahydronaphthacenone **1**	3	$(2.35 \pm 0.03) \times 10^{-4}$	49	5-deoxydaunomycin (89%)
aminotetrahydronaphthacenone **3**[a]	8	4.0×10^{-2}	0.28	5,7-dideoxydaunomycinone
tetrahydronaphthacenone **4** + O_2	8	$(7.18 \pm 0.08) \times 10^{-4}$	16	5-deoxydaunomycin (88%) + naphthacenedione **5** (12%)
tetrahydronaphthacenone **2** + O_2[a]	8	3.3×10^{-4}	35	5,7-dideoxydaunomycinone
tetrahydronaphthacenone **4** (anaerobic)	8	$(1.46 \pm 0.05) \times 10^{-5}$	2283	5-deoxydaunomycin (26%) + naphthacenedione **5** (18%) + 5,7-dideoxydaunomycinone (56%)

[a] From ref 18.

Scheme II



A mechanism for the redox chemistry of 5-deoxydaunomyin consistent with the spectral changes, kinetics, and products is proposed in Scheme II. The transient with absorption at 398 nm is proposed to be 8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-*lyxo*-hexopyranosyl)oxy]-1-methoxy-7,9,10,12-tetrahydro-6,8,11-trihydroxy-5(8*H*)-naphthacenone (**4**). Under aerobic conditions **4** is oxidized to 5-deoxydaunomycin via 5-deoxydaunomycin hydroquinone and also via tetrahydronaphthacenetrione **6** which is also proposed to undergo elimination of water and daunosamine to form naphthacenetrione **7**. Tautomerization of **7** yields naphthacenedione **5**. Under anaerobic conditions tetrahydronaphthacenone **4** undergoes glycosidic cleavage to form quinone methide **9** via reformation of 5-deoxydaunomycin hydroquinone. In competition with the glycosidic cleavage pathway is elimination of water and daunosamine to form naphthacenone **8**. Tautomerization of **8** to naphthacene **10** followed by oxidation, either with molecular oxygen under aerobic conditions or with 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (**11**) under anaerobic conditions, yields naphthacenedione **5**. Oxazinone **11** is the byproduct of reduction with TM-3 dimer and functions as a slow oxidizing agent of hydroquinones with reduction to 3,5,5-trimethyl-2-oxomorpholine (**12**).[19] Two pathways to naphthacenedione **5** are required because the ratio of 5-deoxydaunomycin to naphthacenedione differs as a function of the anaerobic time period.

(19) Kleyer, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1984**, *106*, 2380.

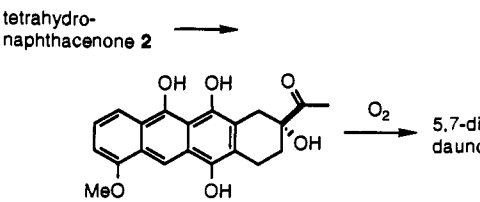
(II)

The observed rate constant for disappearance of tetrahydronaphthacenone **4** in the presence of a large excess of oxygen in terms of the rate constants defined in Scheme II is given in eq 1. The product ratio (88/12) is defined in terms of the rate constants as shown in eq 2. If the path defined by k_7 is a minor

$$k_{obs}(\text{aerobic}) = k_2[O_2] + (k_{-1}k_3[O_2])/(k_1 + k_3[O_2]) \quad (1)$$

$$88/12 = \{(k_{-1}k_3[O_2])/(k_1 + k_3[O_2]) + k_2[O_2]k_7/(k_6 + k_7)\}/\{k_2[O_2]k_6/(k_6 + k_7)\} \quad (2)$$

one, the equations can be solved for $k_2[O_2]$ and $k_{-1}k_3[O_2]/(k_1 + k_3[O_2])$. With this assumption, the apparent rate constant for air oxidation of **4** via 5-deoxydaunomycin hydroquinone, $k_{-1}k_3[O_2]/(k_1 + k_3[O_2])$, is 6.3×10^{-4} s^{-1}; this rate constant is for reaction in an air saturated solution at approximately 630 mm atmospheric pressure. The half-life then is about 18 min. The rate is comparable with that reported for similar air oxidation of tetrahydronaphthacenone **2** to 5,7-dideoxydaunomycinone which occurs with a half-life of 35 min[18] (Table I). In contrast,



(III)

a solution of daunomycin hydroquinone reacts almost instantaneously when shaken with air. Similarly, the observed rate constant for disappearance of tetrahydronaphthacenone **4** in the absence of oxygen is given by eq 3. The ratio of 5,7-dideoxydaunomycinone to naphthacenedione **5**, correcting for the amount of **5** produced during the aerobic portion of the reaction (56/15), is given by eq 4. Solution of these equations gives k_5, the pseudo-

$$k_{obs}(\text{anaerobic}) = \{k_{-1}k_4/(k_1 + k_4)\} + k_5 \quad (3)$$

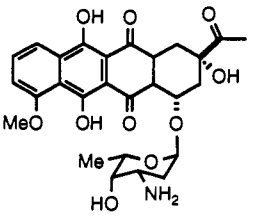
$$56/15 = \{k_{-1}k_4/(k_1 + k_4)\}/k_5 \quad (4)$$

first-order rate constant for the slow step in the elimination of water and daunosamine from tetrahydronaphthacenone **4**, equal to 3.08×10^{-6} s^{-1} and $k_{-1}k_4/(k_1 + k_4)$, the apparent rate constant for glycosidic cleavage of **4**, equal to 1.15×10^{-5} s^{-1}. Daunomycin undergoes glycosidic cleavage in buffered methanol directly from

the hydroquinone redox state with a rate constant of 0.095 s^{-1} (see Experimental Section). At least in part, because of the rapid tautomerization of 5-deoxydaunomycin hydroquinone to tetrahydronaphthacenone **4**, the apparent rate constant for glycosidic cleavage of reduced 5-deoxydaunomycin to its 7-deoxyaglycon is 8000 times smaller than the actual rate constant for glycosidic cleavage of reduced daunomycin. The actual rate constant for glycosidic cleavage of 5-deoxydaunomycin hydroquinone, k_4, may also be smaller because the hydroquinone, bearing one less electron donating hydroxyl group, is less electron rich than the hydroquinone of daunomycin. Lemus and Skibo have recently demonstrated a linear free energy relationship between the rate of quinone methide formation and the quinone reduction potential for a series of anthraquinone mimics.[12] The more positive or less negative the reduction potential the smaller is the rate constant for elimination to form the quinone methide. Although the reduction potential of 5-deoxydaunomycin is unknown, it is likely to be less negative than that of daunomycin. The apparent rate constants for air oxidation and glycosidic cleavage of **4** are consistent with the observation that oxidation in air saturated methanol occurs virtually to the exclusion of glycosidic cleavage.

Other deoxydaunomycins studied with respect to redox chemistry are 11-deoxydaunomycin, 4-demethoxydaunomycin, 4-demethoxy-11-deoxydaunomycin, and 4-demethoxy-6-deoxydaunomycin. All but 4-demethoxy-6-deoxydaunomycin undergo rapid glycosidic cleavage upon anaerobic reduction to their respective hydroquinone states.[3,20,21] The absence of the hydroxy group in the 6-position presumably leads to an anthracycline A-ring conformation with the sugar substituent in a pseudoequatorial position, less favorable for elimination.[20]

In summary, the B-ring quinone derivative of daunomycin, 5-deoxydaunomycin, shows some differences in its chemistry when reduced to the hydroquinone state from that of daunomycin and substantial differences in the apparent rates of molecular oxygen oxidation and glycosidic cleavage. Of particular note are the tautomerization to tetrahydronaphthacenone **4** and the elimination of water and daunosamine to form ultimately naphthacenedione **5**. The lower rate of air oxidation predicts less *in vivo* redox cycling to produce reactive oxygen species and possibly lower cardiotoxicity.[22] The similarity in tumor response of daunomycin and 5-deoxydaunomycin brings into question the role of glycosidic cleavage and the quinone methide state. Possibly, the tetrahydronaphthacenone **4** protects the hydroquinone until it finds a suitable target. An analogous protected form of the hydroquinone of daunomycin is leucodaunomycin which forms upon reduction of daunomycin in aprotic solvent or in protic solvent at lower pH.[23] Upon raising the pH, leucodaunomycin tautomerizes back to daunomycin hydroquinone which subsequently either air oxidizes to daunomycin or undergoes glycosidic cleavage to 7-deoxydaunomycinone quinone methide. Derivatives of adriamycin and daunomycin with improved therapeutic response and/or pharmacological properties are of continued interest. Important examples are 4′-epidoxorubicin and 4-demethoxydaunorubicin (idarubicin).[24] We have also demonstrated a change in the chemistry of 5-iminodaunomycin hydroquinone upon lowering the pH. Deamination significantly predominates over glycosidic cleavage and molecular oxygen oxidation because of favorable tautomerization to the transient aminotetrahydronaphthacenone **1**. Hence, both the absence of the hydroxy group and the presence of a positively charged amminium substituent at the 5-position foster tautomerization of the anthracycline hydroquinone functionality to the naphthacenone functionality in competition with glycosidic cleavage.



leucodaunomycin

Experimental Section

General Remarks. UV–vis spectra were recorded with a Hewlett-Packard 8452 diode array spectrometer. ^1H NMR spectra were obtained with a Varian VXR 300-MHz instrument, and chemical shifts are reported in ppm on the δ scale with the solvent as an internal reference. HPLC was performed with a Hewlett Packard Model 1090 chromatograph equipped with a diode array UV–vis detector and data processing work station. Chromatography was performed with a 5-μm ODS Hypersil reverse phase microbore column, 2.1 mm i.d. × 100 mm from Hewlett-Packard. The column was eluted with a mixture of methanol (A) and 0.3% ammonium formate buffer adjusted to pH 4 with formic acid (B) with an A:B gradient from 30:70 to 60:40 at 12 min and to 80:20 at 20 min. Materials eluting from the column were detected by absorptions at 400, 480, and 500 nm. HPLC yields were determined by integration of peaks from monitoring at 455 nm, the point of equal absorptivity for most of the chromophores. Preparative HPLC was performed with a Rainin reverse phase column, 10.0 mm i.d. × 5 cm, 3 μm C-18. The column was eluted at 3.0 mL/min with a mixture of methanol (A) and 0.3% ammonium formate buffer adjusted to pH 4 with formic acid (B) with an initial A:B gradient of 30:70 to 80:20 at 15 min.

Tris(hydroxymethyl)aminomethane (Tris) and Tris·HCl were obtained from Sigma. Sodium dithionite was purchased from Aldrich and its purity determined by iodine titration in water containing 2% sodium bicarbonate. Daunomycin was a gift of Famitalia Carlo-Erba, Milan, Italy. *dl*-Bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (*dl*-TM-3 dimer),[25] 5,7-dideoxydaunomycinone,[18] and 2-acetyl-11-hydroxy-7-methoxy-5,12-naphthacenedione (**5**)[26] were prepared as previously described. All other solvents and chemicals were obtained form Aldrich, Sigma, J. T. Baker (Phillipsburg, NJ), or Fisher (Fairlawn, NJ).

A two compartment cell was employed for the reductions. The first compartment could hold a volume of 3 mL, and the second compartment, a 10-mm pathlength Pyrex cuvette, was attached at a 90° angle. The cell was equipped with a glass tube that allowed connection to a high vacuum line for the freeze–thaw–degassing.

Solutions placed in the multicompartment cell were oxygen-degassed on a high vacuum line. Each cell was frozen in liquid nitrogen and evacuated to 3 × 10^{-6} Torr or less. The liquid nitrogen was removed, and the solutions were thawed gently with a heat gun. This was repeated four times. On cycles 3 and 4 the solutions were sonicated 5–10 s before refreezing. After sonication on the fourth cycle, the solutions were frozen, evacuated for the final time, and sealed off with a torch. Unless otherwise indicated, reactions were run at ambient temperature.

Synthesis of 5-Deoxydaunomycin (2-Acetyl-4-[(3-amino-2,3,6-trideoxy-α-*lyxo*-hexopyranosyl)oxy]-2,11-dihydroxy-7-methoxy-1,2,3,4-tetrahydro-5,12-naphthacenedione). 5-Iminodaunomycin, prepared as previously described[27] (28.1 mg, 5.30 × 10^{-5} mol), was dissolved in 30 mL of methanol. The solution was degassed with prepurified nitrogen. To this solution was added 13.9 mg (1.3 equiv) of sodium dithionite (80% pure). Dilute hydrochloric acid was added 20–30 s after addition of sodium dithionite to bring the solution to pH 3. The solution was saturated with molecular oxygen and stirred overnight. The formation of 5-deoxydaunomycin was monitored by UV–vis spectroscopy over a period of 5 h and the changes are reported in Results and Discussion and shown in part in Figure 1. Kinetic analysis at an average absorption of 406–410 nm gave a first order rate constant for the formation of 5-deoxydaunomycin from the intermediate as reported in Table I. The reaction solution was

(20) Malatesta, V.; Penco, S.; Sacchi, N.; Valentini, L.; Vigevani, A.; Arcamone, F. *Can. J. Chem.* **1984**, *62*, 2845.
(21) Boldt, M.; Gaudiano, G.; Koch, T. H. *J. Org. Chem.* **1987**, *52*, 2146.
(22) Lown, J. W.; Chen, H.-H.;, Plambeck, J. A.; Acton, E. M. *Biochem. Pharmacol.* **1982**, *31*, 575. Gianni, L.; Corden, B. J.; Myers, C. E. *Rev. Biochem. Toxicol.* **1983**, *5*, 1. Doroshow, J. H.; *Cancer Res.* **1983**, *43*, 460. Doroshow, J. H.; Davies, K. J. A. *J. Biol. Chem.* **1986**, *261*, 3068.
(23) Bird, D. M.; Gaudiano, G.; Koch, T. H. *J. Am. Chem. Soc.* **1991**, *113*, 308.
(24) Arcamone, F. *Cancer Res.* **1985**, *45*, 5995.
(25) Bennett, R. W.; Wharry, D. L.; Koch T. H. *J. Am. Chem. Soc.* **1980**, *102*, 2345.
(26) Schweitzer, B. A.; Koch, T. H. *J. Am. Chem. Soc.*, following paper in this issue.

extracted with CH_2Cl_2 (4 × 20 mL) and the aqueous layer lyophilized to yield 30.1 mg of a purple solid. HPLC analysis of the lyophilized material showed the product to be 89% pure. The other 11% consisted of the following: starting material (5%), 5-imino-7-deoxydaunomycin (3%), and unidentified products (3%). An aliquot (10 mg) of this solid was purified by HPLC to obtain 3.0 mg of pure product which showed the following spectral properties: ^{1}H NMR (CD_3OD) δ 8.39 (s, 1 H, H-5), 7.95 (d, 1H, J = 8, H-1), 7.64 (t, 1H, J = 8, H-2), 7.22 (d, 1H, J = 8, H-3), 5.47 (br s, 1H, H-1′), 4.95 (br s, 1H, H-7), 4.24 (q, 1H, J = 6, H-5′), 4.04 (s, 3H, 4-OCH_3), 3.51 (s, 1H, H-4′), 3.27–3.29 (m, 1H, H-3′), 2.91 (br s, 2H, H-10), 2.32 (s, 3H, H-14), 2.11–2.23 (m, 2H, H-8), 2.01 (td, 1H, J = 4, 12 ,H-2′), 1.77 (dd, 1H, J = 5, 12, H-2′), 1.25 (d, 3H, J = 6, H-5′); FAB^+ (3-nitrobenzyl alcohol matrix) mass spectrum, m/z 512 (M^+). Several ^{1}H NMR signals for 5-deoxydaunomycin are reported in the literature,[16] and these compared favorably with the corresponding signals reported here.

Synthesis of *N*-Acetyl-5-deoxydaunomycin (2-Acetyl-4-[3-acetamido-2,3,6-trideoxy-α-*lyxo*-hexopyranosyl)oxy]-2,11-dihydroxy-7-methoxy-1,2,3,4-tetrahydro-5,12-naphthacenedione). 5-Deoxydaunomycin (15.4 mg, 2.79 × 10–5 mol) was dissolved in 5 mL of methanol, and 50 μL of acetic anhydride and 2 μL of pyridine were added. The solution was stirred for 4 h, and then volatile materials were removed with a high vacuum rotary evaporator. Normal phase suction chromatography[28] eluting with 5:0.5 chloroform to methanol (v/v) yielded 3.5 mg (23%) of pure *N*-acetyl-5-deoxydaunomycin which was characterized from the following spectral data: ^{1}H NMR (CD_3OD) δ 8.26 (s, 1H, H-5), 7.86 (d, 1H, J = 8 Hz, H-1), 7.58 (t, 1H, J = 8 Hz, H-2), 7.17 (d, 1H, J = 8 Hz, H-3), 5.41 (d, 1H, J = 3 Hz, H-1′), 4.94 (br s, 1H, H-7), 4.25 (q, 1H, J = 7 Hz, H-5′), 4.10 (m, 1H, H-3′), 4.02 (s, 3H, 4-OCH_3), 3.57 (br s, 1H, H-4′), 2.86 (br s, 2H, H-10), 2.34 (s, 3H, 14-CH_3), 2.22 (dd, 1H, J = 3, 15 Hz, H-8), 2.08 (dd, 1H, J = 5, 15 Hz, H-8), 1.96 (td, 1H, J = 4, 13 Hz, H-2′), 1.89 (s, 3H, 3′-NAc), 1.58 (dd, 1H, J = 5, 13 Hz, H-2′), 1.23 (d, 1H, J = 7 Hz, 5′-CH_3); FAB (glycerine matrix) mass spectrum, m/z 554.2044 ($M + H^+$, calculated for 554.2026) and 556.2158 ($M + H_3^+$, calculated for 556.2183). The $M + H_3^+$ signal is protonated *N*-acetyl-5-deoxydaunomycin hydroquinone from reduction and protonation of *N*-acetyl-5-deoxydaunomycin in the mass spectrometer during fast atom bombardment. Similar reduction in FAB MS of anthracycline derivatives has been noted earlier.[29,30]

Aerobic Chemistry of 8-Acetyl-10-[(3-amino-2,3,6-trideoxy-α-*lyxo*-hexopyranosyl)oxy]-1-methoxy-7,9,10,12-tetrahydro-6,8,11-trihydroxy-5(8*H*)-naphthacenone (Tetrahydronaphthacenone 4). 5-Deoxydaunomycin (0.91 mg, 1.66 × 10–6 mol, 87% pure, containing 13% ammonium formate from preparative HPLC) was dissolved in 5.0 mL of Tris/Tris·HCl buffered methanol with an apparent pH of 8. The concentration of 5-deoxydaunomycin was determined from the extinction coefficient for its chromophore and the optical density of the solution. The extinction coefficient for the chromophore was determined to be 7570 by measuring the optical density of a known concentration of *N*-acetyl-5-deoxydaunomycin. *N*-acetyl-5-deoxydaunomycin was prepared as described above. A solution of *dl*-TM-3 dimer was prepared by dissolving 7.09 mg of the solid in 25.0 mL of CH_2Cl_2. An aliquot of the TM-3 dimer solution (0.70 mL, 7.0 × 10–7 mol) was placed in one compartment of a two compartment cell, and the solvent was evaporated with a stream of nitrogen. A 2.0 mL aliquot (5.8 × 10–7 mol) of 5-deoxydaunomycin was placed in the other compartment. The cell was freeze–thaw–degassed (4 cycles) and sealed under vacuum. The solution was thawed and the contents of the two compartments were mixed. Spectroscopic monitoring at 25 °C showed a decrease in absorption at 490 nm with an increase at 396 nm. The band at 396 nm then decreased slightly with a shift to 398 nm with broadening. Exposure of the solution to oxygen regenerated the band at 490 nm. Kinetic analysis of the spectral data for the reaction with oxygen at an average absorption of 478–482 nm gave the first-order rate constant shown in Table I. HPLC analysis showed recovery of the starting material and formation of a minor product at 21.8 min (12%) which had a chromophore similar to that of the starting material. Coinjection with an authentic sample confirmed this product to be **5**.

Anaerobic Chemistry of Tetrahydronaphthacenone (4). 5-Deoxydaunomycin was dissolved in Tris/Tris-HCl buffered methanol with an apparent pH of 8 to give a concentration of 1.25 × 10–4 M. An aliquot of this solution (2.0 mL, 2.5 × 10–7 mol) was placed in one compartment of a two compartment cell. A solution of *dl*-TM-3 dimer was prepared by dissolving 7.10 mg of the solid in 25 mL of dichloromethane. An aliquot (0.27 mL, 2.7 × 10–7 mol) of this solution was placed in the second compartment of the two compartment cell, and the solvent was evaporated with a steady stream of nitrogen. The cell was freeze–thaw–degassed for 5 cycles and sealed under vacuum. The solution of 5-deoxydaunomycin was thawed and the contents of the two compartments were mixed. The reaction was monitored by UV–vis spectroscopy, and the spectral changes are described in Results and Discussion and in part shown in Figures 2 and 3. Kinetic analysis of the spectral changes at 394–398 nm as a function of time gave the first-order rate constant reported in Table I. After 42 h the cell was opened, and residual **4** was allowed to react with molecular oxygen. HPLC analysis showed 26% recovery of the starting material, 56% 5,7-dideoxydaunomycinone, and 18% 2-acetyl-11-hydroxy-7-methoxy-5,12-naphthacenedione (**5**).

Kinetic Analysis of the Reduction of Daunomycin with Sodium Dithionite. Daunomycin (1.1 mg, 1.95 × 10–6 mol) was dissolved in 25.0 mL of pH 8, Tris buffered methanol. A 2.0 mL aliquot (1.56 × 10–7 mol) of daunomycin was placed in a quartz cuvette equipped with a septum cap and degassed with prepurified nitrogen. Meanwhile, sodium dithionite (79%, 6.1 × 10–5 mol) was dissolved in 10.0 mL of degassed water. A 34 μL (2.07 × 10–7 mol) aliquot of the sodium dithionite was injected into the daunomycin solution. Spectroscopic monitoring was at an average absorption of 628–632 nm where 7-deoxydaunomycinone quinone methide absorbs.[31] HPLC analysis showed an 81% conversion of the starting material to 7-deoxydaunomycinone. Consecutive first-order kinetic analysis of the spectral data as a function of time gave a rate constant for formation of quinone methide equal to (9.5 ± 0.2) × 10–2 s^{-1} and a rate constant for decay of the quinone methide equal to (7.36 ± 0.03) × 10–2 s^{-1}.

Acknowledgment. Helpful suggestions from Giorgio Gaudiano are gratefully acknowledged as well as a gift of daunomycin from Sergio Penco of Farmitalia Carlo-Erba, Milan, Italy. The authors thank the U.S. PHS for financial support in the form of Grant CA-24665 from the National Cancer Institute.

(27) Tong, G. L.; Henry, D. W.; Acton, E. M. *J. Med. Chem.* **1979**, *22*, 36–39.
(28) Harwood, L. M. *Aldrichimica Acta* **1985**, *18*, 25.
(29) Kleyer, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1983**, *105*, 5145.
(30) Dass, C.; Seshadri, R.; Israel, M.; Desiderio, D. *Biomed. Environ. Mass Spectrom.* **1988**, *17*, 37.
(31) Kleyer, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1983**, *105*, 2504.