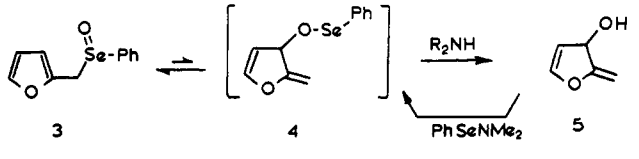
tectable change. It can be shown that the observed and [2,3] sigmatropic rate constants are related as follows, provided that $k_N \gg k_X$:[6] $k_{2c2t}^{Se} \approx 2k_{2X}^{Se}$ and $k_{12}^{Se} \approx k_{1N}^{Se}$. Using the directly measured value for k_{12}^{Se} and the value for k_{2X}^{Se} obtained by extrapolating k_{2c2t}^{Se} to −80 °C ($\Delta H^{\ddagger}$ = 24.9 kcal/mol, $\Delta S^{\ddagger}$ = −7.0 eu), it is possible to construct a partial free-energy diagram for the **1**-Se/**2**-Se equilibration (Figure 1a). The free-energy difference of interest separating selenoxide and selenenate is 12.5 − $\Delta\Delta G^{\ddagger}_{N/X}$ kcal/mol, where $\Delta\Delta G^{\ddagger}_{N/X}$ is the separation between the endo and exo transition states (i.e. k_N/k_X).

Since k_N/k_X cannot be directly measured for Y = Se, we decided to provide a partial answer by studying the sulfur analogue for which K_{eq} is directly measureable. The sulfenate ester **2**-S was prepared from 2-methyl-3-buten-2-ol and *o*-nitrobenzenesulfenyl chloride at −50 °C, and the rate of equilibration ($k_{12}^S + k_{21}^S$) with sulfoxide **1**-S was measured at −29.7 °C ($k_{12}^S + k_{21}^S$ = 0.000 216 s^{-1}, K_{eq} = 23.9). When the deuterium-labeled compound **2c**-S[7,9] was used, only a single diastereomer[6] of **1**-S was formed (>98%). Equilibration (k_{1a1b}^S) occurred at higher temperatures, and the rate was extrapolated to −29.7 °C ($\Delta H^{\ddagger}$ = 21.7 kcal/mol, $\Delta S^{\ddagger}$ = −1.6 eu). From the three experimentally determined numbers k_{21}^S, k_{1a1b}^S, and K_{eq}^S, it was possible to calculate the [2,3] sigmatropic rate constants and construct the free-energy diagram (Figure 1b).

The most striking finding is the high value (275) of k_N^S/k_X^S,[6] corresponding to a $\Delta\Delta G_{N/X}^{\ddagger}$ of 2.7 kcal/mol. The k_N/k_X value represents the maximum possible asymmetry transfer from chiral sulfur to chiral carbon (if there is one) of the sulfenate. That such high values have been rarely achieved by using optically active sulfoxides for the synthesis of chiral allyl alcohols[5] could be due in part to the inefficient cleavage of allyl sulfenates, but more likely reflect some peculiarityof the present system.[5b,10]

Returning now to the original question of the selenenate–selenoxide equilibration we can esimate $\Delta\Delta G_{N/X}^{\ddagger} \approx 2$ kcal/mol, and thus $\Delta G^{\circ}_{1Se/2Se} \approx 11$ kcal/mol. Because of the long temperature extrapolation involved, we estimate a possible error of ±2.5 kcal/mol. Since $\Delta G^{\circ}_{1S/2S}$ = −1.5 kcal/mol, the equilibrium of eq 2 shifts by 12 kcal/mol on going from S to Se. The two principal contributors are the weaker C–Se bond strength compared to C–S and the smaller degree of multiple bonding in the dipolar Se–O vs. S–O bond. Some of the more dramatic differences between S and Se chemistry can be traced to the effect discussed here, (e.g., the fact that selenoxide syn eliminations are irreversible and much more rapid than those of sulfoxides[11]).

The isomerization of selenoxide to selenenate can be facile even in situations where the double bond is part of an aromatic ring such as furan or phenanthrene. Even though the selenoxide **3** is the only species detected by NMR,[12] it is in rapid equilibrium with its selenenate isomer **4** since treatment with pyrrolidine gives alcohol **5** in a crude (NMR) yield of 87%.[13] Purification by distillation is usually accompanied by some isomerization to furfuryl alcohol, as well as reversal to selenoxide **3**. 9-Phenanthrenylmethyl phenyl selenoxide can similarly be converted to 9-methylene-10-hydroxy-9,10-dihydrophenanthrene.



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Supplementary Material Available: Tables of observed and calculated rate constants used to construct Figure 1 (2 pages). Ordering information is given on any current masthead page.

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Spectroscopic Observation of the Tautomer of 7-Deoxydaunomycinone from Elimination of Daunosamine from Daunomycin Hydroquinone[1]

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Anaerobic reduction of daunomycin (**1**) in microsomes by NADPH[2,3] and in solution by dithionite gives 7-deoxydaunomycinone (**2**).[4] In vivo reductive elimination has been proposed to occur from the semiquinone (**3**) by some[5-7] and from the hydroquinone (**4**) by others[7,8] and to be at least in part responsible for covalent binding of the drug to DNA.[6-8]

Earlier we reported the efficient reduction of daunomycin to 7-deoxydaunomycinone by **6** and kinetic evidence that the reduction occurred possibly via hydride transfer.[9] The kinetic measurements presumed no long-lived intermediates as suggested by prior electrochemical studies.[10] This presumption has now been found to be inaccurate. Kinetics and spectroscopy establish that the reducing agent is **7**[11] and reveal the elusive tautomer **5** of 7-deoxydaunomycinone.

A rigorously oxygen-degassed, methanol-*d* solution containing 1.79×10^{-4} M **1**, 1.79×10^{-3} M **6**, and 2.0×10^{-3} M trisma buffer (1:1 Tris/Tris·HCl) at 25.0 ± 0.1 °C gave the spectral changes shown in Figure 1 during the time regime 10–130 s with scans every 10 s. The sequence of events was 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. Scans beyond 130 s

(1) Support: NCI, CA-24665, Developmental Therapeutics Program; University of Colorado, CCRW; 3M Co.

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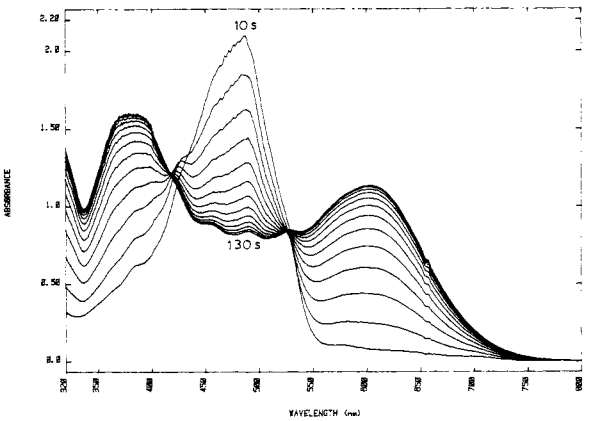


Figure 1. UV–visible absorption of a rigorously oxygen-degassed solution of 1.79×10^{-4} M daunomycin, 1.79×10^{-3} M **6**, and 2.0×10^{-3} M trisma in methanol-*d* solvent at 25.0 ± 0.1 °C as a function of time. Scans were 1 s in duration and occurred every 10 s in the time period 10–130 s.

revealed a fall in the 380- and 608-nm bands with a rise in the 480-nm band. The 420-nm band was assigned to the hydroquinone **4** consistent with absorption in this region for the hydroquinone of 7-deoxydaunomycinone.[12] The bands at 380 and 608 nm were assigned to the tautomer **5**. Daunomycin and 7-deoxydaunomycinone both absorb at 480 nm. The semiquinone **3** is observable by EPR spectroscopy, but its concentration even at maximum is insufficient to produce a significant UV–visible absorption.[13] No additional paramagnetic species were observed. The appearance and breadth of the isosbestic points at 417 and 525 nm are consistent with initial formation of the hydroquinone **4** followed by elimination to the tautomer **5** possibly with some isomerization of tautomer to **2** during the 130-s time period.

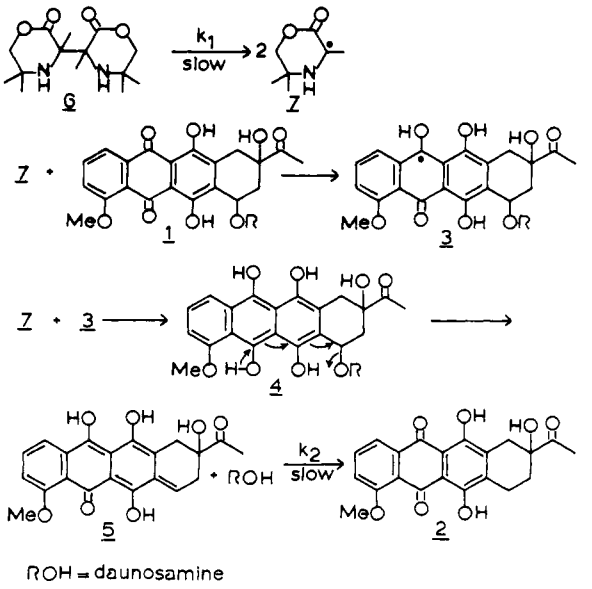
The kinetics of formation and destruction of **3** and **5** were observed with 1.56×10^{-4} M **6** and daunomycin and 2.0×10^{-3} M trisma buffer in methanol. At 0.6 °C the EPR signal rose and fell as a function of time with a maximum at 1800 s. The visible absorption signal at 608 nm similarly rose and fell, however, with a maximum at 3000 s clearly distinguishing these two intermediates.

At 0.86×10^{-4} M **6**, 1.71×10^{-4} M **1**, and 2.0×10^{-3} M trisma buffer in methanol in the temperature range 15–25 °C, tautomer concentration as a function of time followed clean consecutive first-order kinetics with the slow steps being bond homolysis of **6** (k_1) and tautomerization (k_2) as shown in Scheme I. At this concentration of **6**, hydroquinone **4** was not observed because it is produced more slowly, and the isosbestic points at 417 and 525 nm are sharp for both formation and destruction of **5**. The first-order rate constant k_2 (0.013 ± 0.001 s^{-1}) and the molar extinction coefficient for **5** at 620 nm (9400 ± 400) were determined at 25.0 ± 0.1 °C by a nonlinear least-squares fitting of the absorption as a function of time using an independently measured value for k_1.[9] These experiments and a similar experiment using methanol-*d* solvent gave a $k_H/k_D = 9.1 \pm 0.5$ and $\Delta H^{\ddagger} = 18 \pm 0.5$ kcal/mol and $\Delta S^{\ddagger} = -6 \pm 1$ cal/(deg·mol) for the tautomerism.

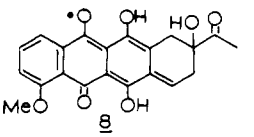
Kinetic evidence that the semiquinone **3** reacted via reduction to hydroquinone **4** as shown in Scheme I rather than elimination to radical **8** as proposed by others for reaction under somewhat different conditions[5-7] was obtained by EPR spectroscopy. At maximum semiquinone concentration, the rate of formation of **3** equals its rate of destruction. Consistent with Scheme I, the height of the EPR signal for **3** at maximum (h_m) is related to the time (t_m) at which the maximum is achieved at two different initial concentrations of **6** according to

$$h_{m_1}/h_{m_2} = ([\mathbf{6}]_{0_1}/[\mathbf{6}]_{0_2})e^{k_1(t_{m_2}-t_{m_1})}$$

Scheme I



With 1.57×10^{-4} and 1.17×10^{-4} M initial concentrations of **6**, the observed and calculated values of h_{m_1}/h_{m_2} were both 1.4 ± 0.1. Elimination via **3** to radical **8** does not fit this simple relationship unless reduction of **8** to **5** is slow. If reduction of **8** were slow, **8** would have been observed in the EPR.



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In summary we reported here the first experimental evidence for the tautomer **5**, an intermediate resulting from a sequential two-electron reductive glycosidic cleavage of daunomycin. Evidence is thus presented for an intermediate that has been previously proposed as a biologically active form of daunomycin.

(12) The hydroquinone of **2** was generated by anaerobic reaction of **2** with **6**.

(13) Semiquinone: $g = 2.0037$, 2.53 (1:1), 1.98 (1:1), 1.57 (1:1), 1.44 (1:1), 0.92 (1:2:1), 0.50 G (1:2:1).[14]

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Biosynthesis of Riboflavin. Analysis of Biosynthetically ^{13}C-Labeled Riboflavin by Double-Quantum and Two-Dimensional NMR

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The biosynthesis of riboflavin (**3a**, Scheme I) has been the subject of many recent reports.[1] The terminal step in this pathway is the transfer of a four-carbon unit consisting of carbons 6, 6a, 7, and 7a from one molecule of 6,7-dimethyl-8-ribityllumazine (**2**) to a second molecule of **2**. Diacetyl, acetoin, tetroses, and

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