Glycosidic Cleavage from Anaerobic Saponification of the Heptaacetate of Daunomycin Hydroquinone

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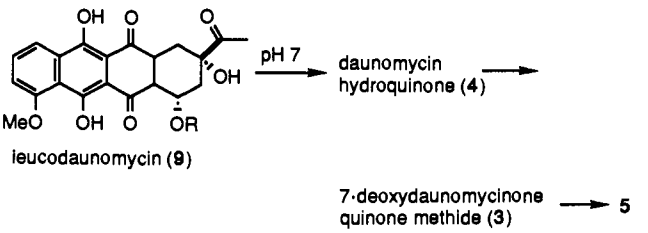
Abstract: Saponification of the heptaacetate of daunomycin hydroquinone (**6**) with 7 equiv of lithium hydroxide to *N*-acetyldaunomycin hydroquinone (**7**) in argon-degassed tetrahydrofuran (THF)/water medium was reported earlier not to give rise to glycosidic cleavage to yield 7-deoxydaunomycinone (**5**) (*J. Am. Chem. Soc.* **1991**, *113*, 1373). The only product characterized after exposure to air was *N*-acetyldaunomycin (**8**). The result was interpreted as possible evidence against glycosidic cleavage of daunomycin at the hydroquinone redox state. We now report that performing the saponification in freeze–thaw–degassed THF/water *in vacuo* yielded 10% (**5**), 17% **8**, 28% *N*-acetyl-5-deoxydaunomycin (**12**), 7% 7-epidaunomycinone (**13**), 2% daunomycinone (**14**), 9% 2-acetyl-11-hydroxy-7-methoxy-5,12-naphthacenedione (**15**), and 14% distributed between three unidentified products. In contrast, reduction of daunomycin at pH 13 in THF/water with bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (TM-3 dimer) *in vacuo* yielded 71% **5** and 23% **14** via spectroscopically observed 7-deoxydaunomycinone quinone methide (**3**). UV–vis spectral changes during the saponification and product structures are rationalized in terms of the mechanism proposed in Scheme II with the intermediacy of a long-lived, daunomycin hydroquinone still acetylated at the 5- and 6-positions (**16**) in equilibrium with a naphthacenone tautomer (**17**). Additional saponification at the 6-position leads to loss of acetate at the 5-position with subsequent formation of **12**, and additional saponification at the 5-position leads to glycosidic cleavage to a quinone methide with subsequent formation of 7-deoxydaunomycinone, daunomycinone, and 7-epidaunomycinone. The anaerobic saponification of **6** is no longer inconsistent with glycosidic cleavage at the hydroquinone redox state.

Introduction

Anthracycline antitumor drugs including daunomycin, adriamycin, aclacinomycin A, and menogaril are proposed to be bioreductively activated to semiquinone methide and/or quinone methide states.[1-3] As illustrated with daunomycin in Scheme I, 7-deoxydaunomycinone semiquinone methide (**1**) might logically be formed via glycosidic cleavage after one-electron reduction of daunomycin to daunomycin semiquinone (**2**) and 7-deoxydaunomycinone quinone methide (**3**), via glycosidic cleavage after two-electron reduction to daunomycin hydroquinone (**4**). Quinone methide **3** could also result from one-electron reduction of **2**; as well, semiquinone methide **2** could result from one-electron oxidation of **3**. In protic medium the product isolated is 7-deoxydaunomycinone (**5**), commonly thought to arise from tautomerization of **3**. The state or states at which glycosidic cleavage occurs continues to be debated. The point is more than academic because the state determines the transient formed and consequently, the subsequent *in vivo* chemistry.

Design of unambiguous experiments to establish the reactive state at least in protic solvent is complicated by the rapid equilibration of anthracycline semiquinones with quinones and hydroquinones.[4] In the aprotic solvents dimethylformamide[5] and dimethyl sulfoxide,[6] differences in the one-electron redox potentials and relative rates of the one-electron reductions, respectively, have led to assignment of the hydroquinone state as the state responsible for glycosidic cleavage. UV–vis monitoring of the reduction of adriamycin and daunomycin by carbon dioxide radical anion generated by pulse radiolysis in aqueous medium also showed sequential formation of semiquinone, hydroquinone, and quinone methide states.[4,7]

Recently Danishefsky and co-workers[8] reported the results of an interesting experiment with the heptaacetate of daunomycin hydroquinone (**6**). Saponification of **6** with 7 equiv of lithium hydroxide in argon-degassed tetrahydrofuran/water medium yielded "no 7-deoxydaunomycinone". Upon neutralization and air oxidation, 43% of *N*-acetyldaunomycin (**8**) was isolated. They favored a reaction pathway in which *N*-acetyldaunomycin hydroquinone (**7**) was formed during the saponification and yet did not undergo glycosidic cleavage. Careful reading of the Experimental Section of the paper revealed a potential problem. Prior to neutralization, the reaction mixture was described as "a purple-blue solution". This is not the color of a hydroquinone or hydroquinone anion but the color of a quinone anion. Possibly oxygen leaked into the reaction vessel during the 14-h reaction period and oxidized hydroquinones to quinones! The authors' summary of the saponification experiment, unfortunately, now appears in the secondary literature as evidence against cleavage at the hydroquinone redox state.[9]



The saponification experiment was inconsistent with an earlier parallel experiment which we reported. A tautomer of dauno-

(1) Lin, A. J.; Cosby, L. A.; Shansky, C. W.; Sartorelli, A. C. *J. Med. Chem.* **1972**, *15*, 1247. Lin, A. J.; Sartorelli, A. C. *J. Med. Chem.* **1976**, *19*, 1336. Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249. Moore, H. W.; Czerniak, R.; Hamdan, A. *Drugs Exptl. Clin. Res.* **1986**, *12*, 475.

(2) Abdella, B. R. J.; Fisher, J. *Environ. Health Perspect.* **1985**, *64*, 3. Powis, G. *Pharmacol. Ther.* **1987**, *35*, 57.

(3) Gaudiano, G.; Koch, T. H. *Chem. Res. Toxicology* **1991**, *4*, 2.

(4) Mukherjee, T.; Land, E. J.; Swallow, A. J.; Bruce, J. M. *Arch. Biochem. Biophys.* **1989**, *272*, 450.

(5) Anne, A.; Moiroux, J. *Nouv. J. Chim.* **1985**, *9*, 83.

(6) Gaudiano, G.; Frigerio, M.; Bravo, P.; Koch, T. H. *J. Am. Chem. Soc.* **1992**, *114*, 3107.

(7) Lea, J. S.; Rushton, F. A. P.; Land, E. J.; Swallow, A. J. *Free Rad. Res. Comms.* **1990**, *8*, 241. Houée-Levin, C.; Gardès-Albert, M.; Rouscilles, A.; Ferradini, C.; Hickel, B. *Biochemistry* **1991**, *30*, 8216.

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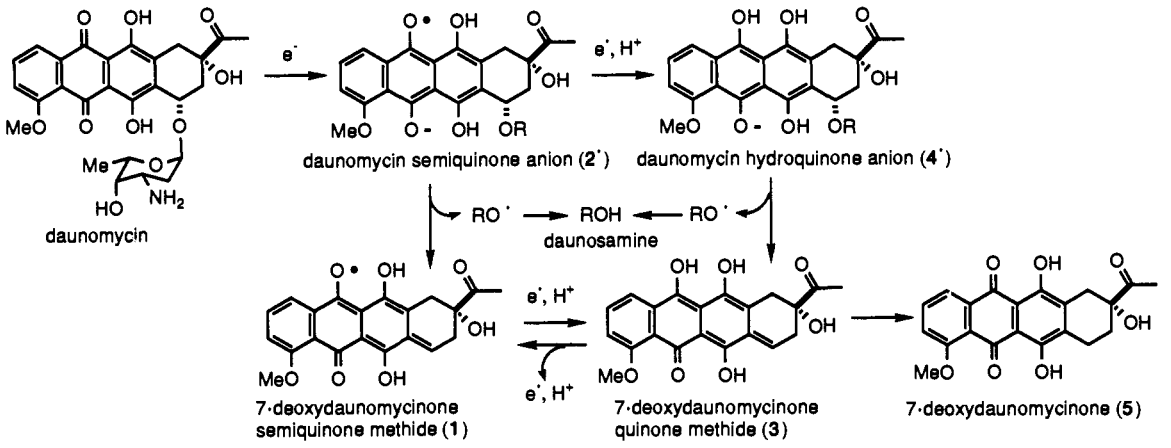
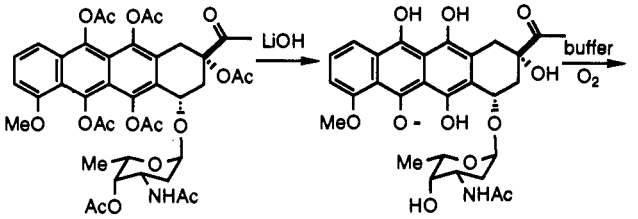
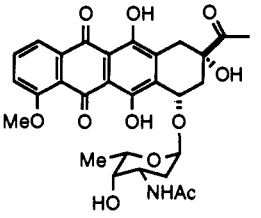
Scheme I



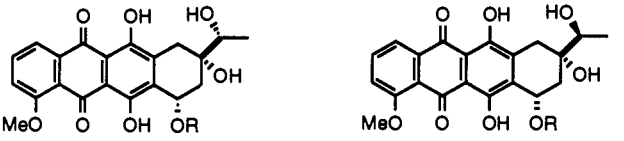
mycin hydroquinone, leucodaunomycin (**9**), was isolated from reduction of daunomycin at reduced pH. Dissolving **9** in aqueous, anaerobic medium at pH 7 yielded 98% 7-deoxydaunomycinone via quinone methide **3**.[10] Presumably, **3** was formed via daunomycin hydroquinone (**4**) from tautomerization of **9**.





Until recently, another inconsistency with glycosidic cleavage at the hydroquinone state of anthracyclines was the report that reduction of daunomycin with sodium borohydride in nitrogen degassed aqueous medium yielded daunomycinol (**10**) and epidaunomycinol (**11**). The color change during the reaction implicated formation of a hydroquinone; yet, glycosidic cleavage did not occur. The result was interpreted in terms of the hydroquinone not being the state responsible for glycosidic cleavage.[11] We have now established that borate esters are intermediates and that given sufficient time and a strictly anaerobic medium the borate esters of daunomycinol and epidaunomycinol hydroquinones do undergo glycosidic cleavage.[12]

On the basis of the inconsistency of the saponification reaction with other experimental results in the literature as described above and our success in explaining the sodium borohydride mystery,



we have investigated saponification of daunomycin hydroquinone heptaacetate (**6**) in aqueous THF under strictly anaerobic conditions. We now report evidence for slow glycosidic cleavage to form a quinone methide as well as slow elimination of acetate and offer a rationale for the differences in the kinetics vs the kinetics for formation of quinone methide **3** via two-electron reduction of daunomycin.

Results and Discussion

Synthesis and Characterization. The heptaacetate of daunomycin hydroquinone (**6**) was synthesized as described by Danishefsky and co-workers by first peracetylating daunomycin with acetic anhydride to give daunomycin pentaacetate, reducing the quinone functionality with hydrogen over palladium on carbon to the pentaacetate hydroquinone and then acetylating the remaining phenolic hydroxyl groups.[8] The procedure is analogous to that reported by us earlier for the synthesis of 7-deoxydaunomycinone hydroquinone pentaacetate except for the reducing agent.[13] Since this report, we have noticed an omission in the NMR spectral data for 7-deoxydaunomycinone hydroquinone pentaacetate, and the omission is now corrected in the Experimental Section.

For the purpose of product identification, *N*-acetyldaunomycin (**8**) was prepared by monoacetylation of daunomycin, and *N*-acetyl-5-deoxydaunomycin (**12**) was prepared by monoacetylation of 5-deoxydaunomycin. A sample of 5-deoxydaunomycin was also transformed to 2-acetyl-11-hydroxy-7-methoxy-5,12-naphthacenedione (**15**) by heating with acetic acid and hydrobromic acid.

Saponification of Daunomycin Hydroquinone Heptaacetate. A tetrahydrofuran (THF) solution of heptaacetate **6** was reacted with 7 mol equiv of aqueous lithium hydroxide in the absence of molecular oxygen. The volume ratio of THF to water was 3:1. Oxygen was removed by a high-vacuum freeze–thaw procedure and the cell was sealed with a torch. After 19 h at 18 °C, the color had changed from yellow to purple-blue. The cell was opened, and the pH was lowered from 10 to 7 with pH 7 phosphate buffer. HPLC analysis of material in solution and material which had precipitated showed 10% 7-deoxydaunomycinone (**5**), 17% *N*-acetyldaunomycin, 28% *N*-acetyl-5-deoxydaunomycin (**12**),

(8) Sulikowski, G. A.; Turos, E.; Danishefsky, S. J.; Shulte, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 1373.
(9) Silverman, R. B. *The Organic Chemistry of Drug Design and Drug Action*; Academic Press: New York, 1992; p 258.
(10) Bird, D. M.; Gaudiano, G.; Koch, T. H. *J. Am. Chem. Soc.* **1991**, *113*, 308.
(11) Pan, S.-S.; Pedersen, L.; Bachur, N. R. *Mol. Pharmacol.* **1981**, *19*, 184.
(12) Schweitzer, B. A.; Egholm, M.; Koch, T. H. *J. Am. Chem. Soc.* **1992**, *114*, 242.
(13) Barone, A. D.; Atkinson, R. F.; Wharry, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1981**, *103*, 1606.

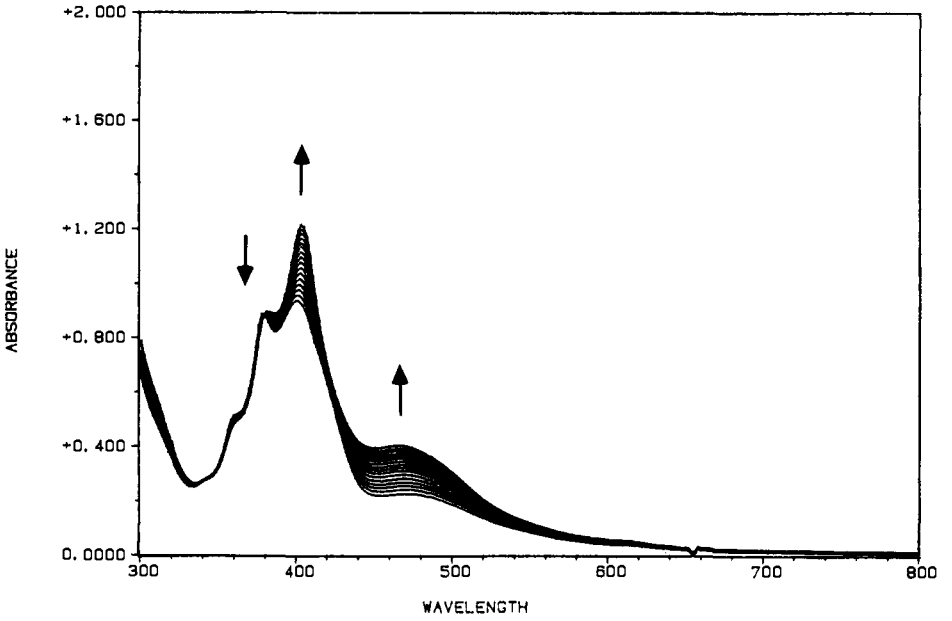


Figure 1. UV–vis spectra of the reaction of 1.21×10^{-4} M daunomycin hydroquinone heptaacetate with 0.012 M lithium hydroxide in aqueous THF as a function of time during the period 6–16 min at 25 °C.

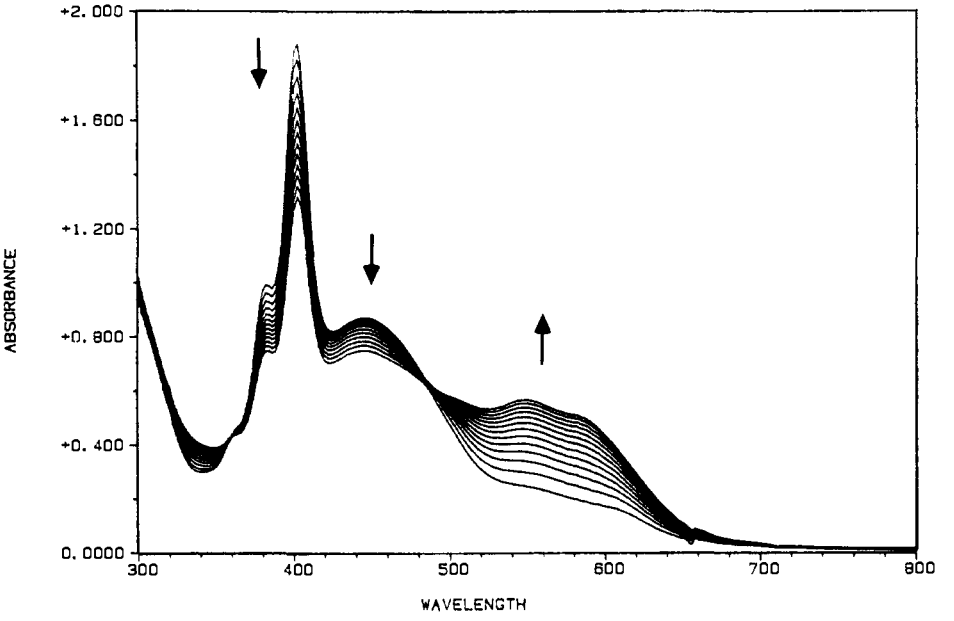


Figure 2. UV–vis spectra for the reaction described in Figure 1 during the time period 2 h to 26 h.

7% 7-epidaunomycinone (**13**), 2% daunomycinone (**14**), 9% **15**, and 14% distributed between three unidentified products. Product identifications were established by chromatographic comparison with standard samples; of particular note was HPLC analysis with co-injection of standards and purity determination with a diode array UV–vis detector. The remainder of the material balance (13%) is likely a result of experimental error and/or additional unknown products. Clearly, the reaction is not simple or clean.

A separate anaerobic solution at lower heptaacetate **6** concentration was monitored by UV–vis spectroscopy for a period of 5 days. The spectral changes as a function of time for portions of the first 26 h are shown in Figures 1 and 2. Figure 1 shows disappearance of heptaacetate bands at 362, 378, 394, and 414 nm with appearance of a sharp band at 406 nm and a broad band extending from 440–480 nm during the time period 6–16 min. Figure 2 shows disappearance of the 406- and 440–480-nm bands with appearance of bands at 545 and 585 nm during the time period 2–26 h. This trend continued during the 5-day reaction period. Again the color change was from yellow to purple-blue. The reaction solution was neutralized *in vacuo* and then opened, and the material in solution was analyzed by HPLC to show 15% 7-deoxydaunomycinone (**5**) and no *N*-acetyldaunomycin (**8**). Products which had precipitated were not analyzed.

The visible bands at 545 and 585 nm are characteristic of the quinone anion chromophore (vide infra) and the ones which give rise to the purple-blue color. The bands at 406 and 440–480 nm move in unison and are proposed to result from an equilibrium mixture of a partially acetylated *N*-acetyldaunomycin hydroquinone **16** and its naphthacenone tautomer **17**. This proposal stems from the observation that reduction of 5,7-dideoxydaunomycinone with dithionite gives a transient hydroquinone **18** with sharp absorption at λ_{max} 392 nm and that 7-deoxydaunomycinone hydroquinone absorbs at λ_{max} 420 nm.[14] Furthermore, the reduction of daunomycin with sodium borohydride gives transient **19** which shows broad absorption in the region of 450 nm.[12] For

(14) Kleyer, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1983**, *105*, 5911.

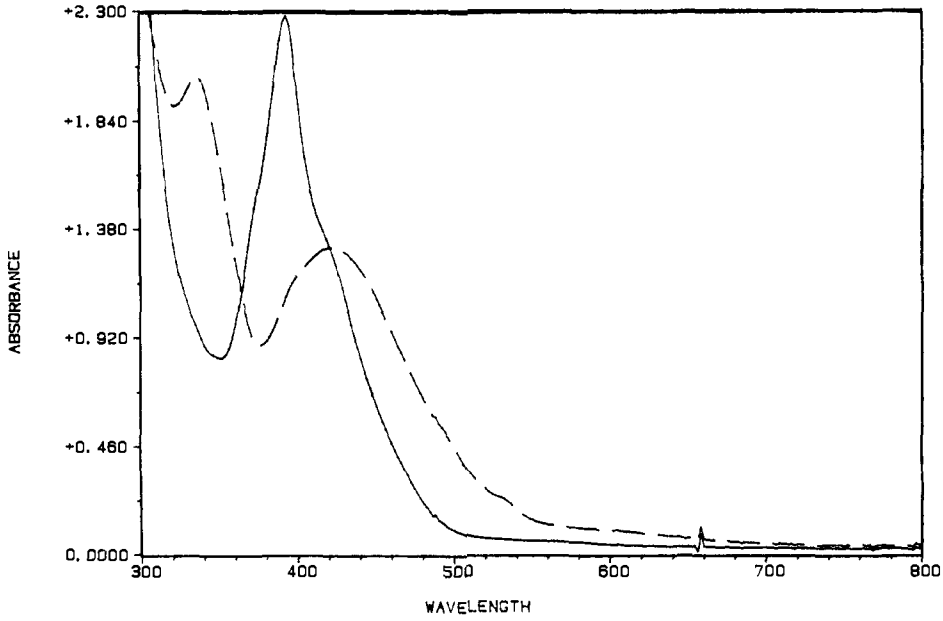
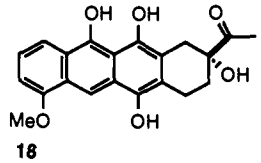


Figure 3. UV–vis spectra of 3.4×10^{-4} M **18** (—) and 3.0×10^{-4} M **19** in methanol (– –).

comparison, the absorption spectra of transients **18** and **19** are shown at approximately equal concentrations in Figure 3.



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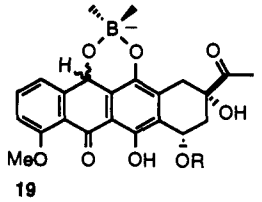

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A mechanism consistent with the spectral changes and the formation of the various products is proposed in Scheme II. Saponification of the acetates at positions 11 and 12 occurs first. This permits equilibration of **16** with tetrahydronaphthacenone **17**. Product formation is determined by competition between saponification of the acetates at positions 5 and 6. Saponification of the acetate at position 6 in structure **17** leads to elimination of acetate at position 5 to form *N*-acetyl-5-deoxydaunomycin (**12**). Competitive with saponification at the 6-position is elimination of *N*-acetyldaunosamine and acetic acid from the A-ring to yield ultimately naphthacenedione **15**. Saponification of the acetate at position 5 followed by glycosidic cleavage yields a quinone methide. Tautomerization of the quinone methide to 7-deoxydaunomycinone (**5**) competes with nucleophilic addition of hydroxide which gives daunomycinone (**14**) and 7-epidaunomycinone (**13**) after aerobic workup. A few percent nucleophilic addition of water to 7-deoxydaunomycinone quinone methide was observed earlier when quinone methide was generated from tautomerization of leucodaunomycin at pH 7.[10] Hydroquinone **20** is likely to be in equilibrium with tetrahydronaphthacenone **21** which can also form *N*-acetyl-5-deoxydaunomycin (**12**) and naphthacenedione **15**. Slow saponification of the acetate at the 6-position is proposed because the 6-position is sterically hindered and because Danishefsky and co-workers demonstrated that reduction of daunomycin pentaacetate to the hydroquinone state did not lead to rapid glycosidic cleavage.[8]

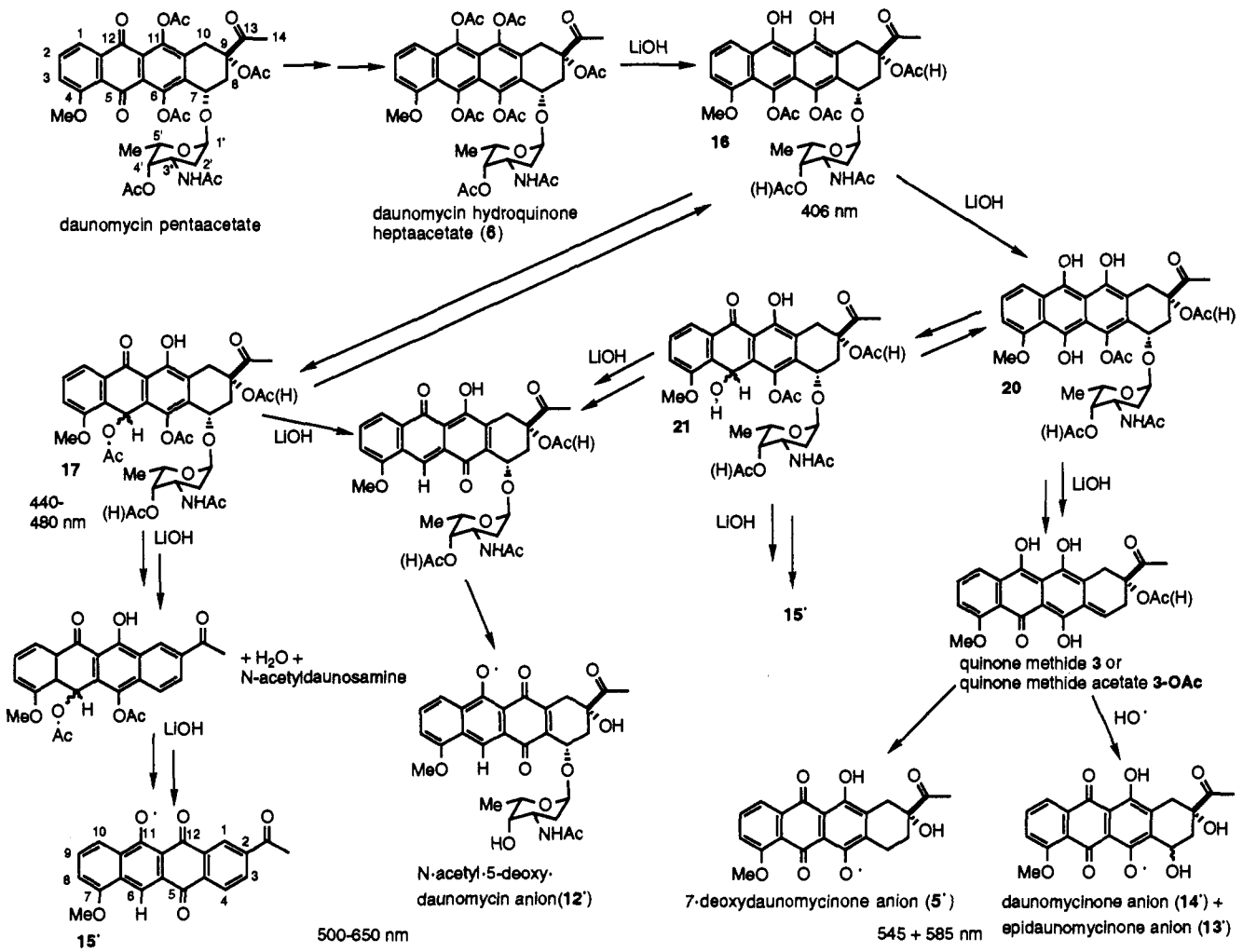
The formation of 17% *N*-acetyldaunomycin after 19 h of reaction likely occurred by molecular oxygen oxidation of hydroquinone **20** during workup as proposed in Scheme III. Spectroscopic monitoring showed that complete consumption of intermediates **16** and **17** required 5 days. Similarly, air oxidation of **20** during the reaction and after opening the reaction vessel could well account for the 43% *N*-acetyldaunomycin observed by Danishefsky and co-workers.[8] Oxidation during the reaction would have led to the purple-blue color observed as well as the lack of glycosidic cleavage. Our experience is that without freeze–thaw–degassing and sealing with a torch, molecular oxygen will slowly leak into a reaction vessel purged of oxygen with nitrogen or argon. The balance of their reaction mixture, although not reported,[8] probably contained **12** and **15**.

The effect of high pH and THF/water medium on glycosidic cleavage of daunomycin hydroquinone was also investigated by reducing daunomycin in the presence of lithium hydroxide, pH 13, with an excess of *dl*·bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (*dl*·TM·3 dimer) in THF/water. We have used TM-3 dimer extensively to generate the redox states of quinone antitumor drugs.[3] Formation and destruction of quinone methide was observed by UV–vis spectroscopy and occurred within a period of 780 s. Bands at 545 and 585 nm, assigned to daunomycin anion, decreased as bands at 390 and 608 nm, characteristic of the quinone methide,[15] increased, maximizing at 360 s. With disappearance of the quinone methide bands was appearance of a band at 498 nm characteristic of semiquinone. The band at 498 nm then disappeared over a period of 8 h with appearance of bands at 545 and 585 nm, again characteristic of quinone anion. HPLC analysis showed 71% 7-deoxydaunomycinone and 23% daunomycinone. The 498-nm band is assigned to a mixture of semiquinones and the 545- and 585-nm bands to a mixture of quinone anions of 7-deoxydaunomycinone and daunomycinone. In aprotic solvent the equilibrium between quinone plus hydroquinone and semiquinone favors semiquinone; the semiquinone predominates even in mixtures of dimethyl sulfoxide and water.[6] Under anaerobic conditions with an excess of reducing agent, the final products were observed in the quinone state most likely because the product of reduction with TM-3 is 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (**22**) which oxidizes hydroquinones to quinones with formation of 3,5,5-trimethyl·2-oxomorpholine (**23**).[14,16] These transformations are summarized in Scheme IV.

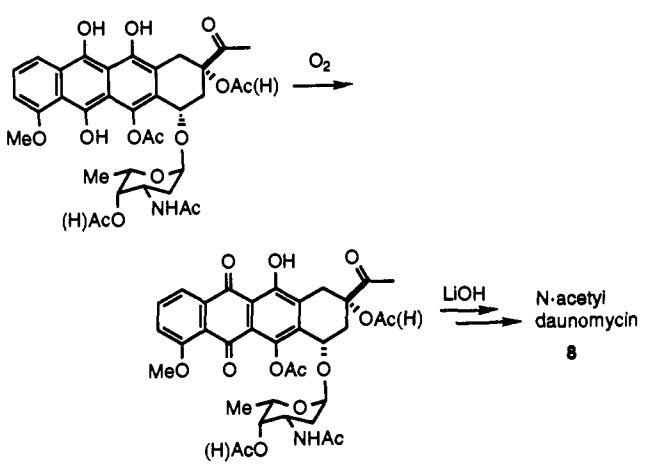
We note that Bachur and co-workers reported the UV–vis absorption spectra for anions of daunomycin, daunomycinone, and 7·deoxydaunomycinone generated with potassium superoxide dicyclohexano-18-crown-6 in dimethyl sulfoxide.[17] The long wavelength bands appear at 604 and 652 nm. Anne and Morioux reported a long wavelength band at 610 nm for daunomycin anion in dimethylformamide created by deprotonation with tetrabu-

(15) Kleyer, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1983**, *105*, 2504.
(16) Kleyer, D. L.; Koch, T. H. *J. Am Chem. Soc.* **1984**, *106*, 2380.
(17) Nakazawa, H.; Andrews, P. A.; Callery, P. S.; Bachur, N. R. *Biochem. Pharmacol.* **1985**, *34*, 481.

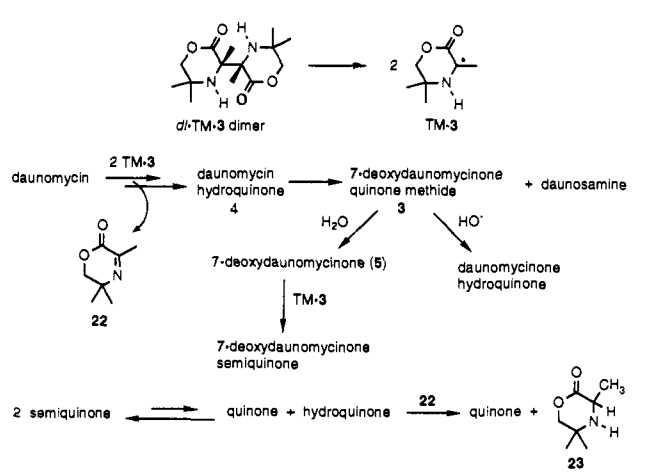
Scheme II



Scheme III



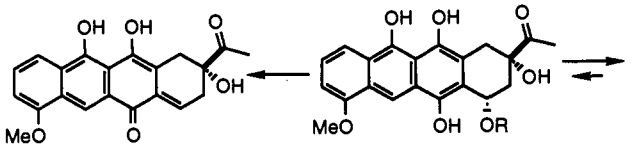
Scheme IV



tylammonium hydroxide.[5] The difference between these band positions and what we observe for lithium salts, 545 and 585 nm, most likely resides in the counter ion. The O–Li^+ bond is more covalent in character than the O–K^+ bond, especially in the presence of a crown ether, and the $O^-(Bu)_4N^+$ bond.

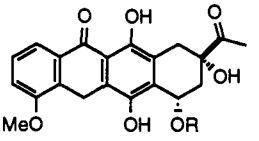
The apparent rate of glycosidic cleavage upon saponification of daunomycin hydroquinone heptaacetate is very slow relative to the apparent rate of glycosidic cleavage upon reduction of daunomycin. The rate controlling step in formation of **5**, **13**, and **14** during the multiple saponification is proposed in Scheme II to be saponification of the acetate at position 5 because *N*-acetyldaunomycin hydroquinone and quinone methide **3** are not observed spectroscopically during the course of the reaction. At the very least, glycosidic cleavage and reactions of the quinone methide occur faster than saponification and probably occur much faster. The rate-controlling steps in formation of **5** when daunomycin is reduced with TM-3 dimer are bond homolysis of the TM-3 dimer to produce TM-3 radical and tautomerization of **3**. With a faster reducing agent such as dithionite, the rate controlling steps are glycosidic cleavage and tautomerization of **3**.

Reduction of 5-deoxydaunomycin bears some resemblance to the saponification of daunomycin hydroquinone heptaacetate. 5–Deoxydaunomycin hydroquinone exists in equilibrium with tetrahydronaphthacenone **24**.[18] The equilibrium mixture of the hydroquinone and **24** undergoes glycosidic cleavage to a quinone methide very slowly, half-life approximately 17 h at 25 °C in Tris buffered methanol. In competition with glycosidic cleavage is elimination of water and daunosamine to form naphthacenedione **15**.



5,7-dideoxydaunomycinone quinone methide

5-deoxydaunomycinone hydroquinone



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In summary, we conclude that saponification of daunomycin hydroquinone heptaacetate (**6**) does lead slowly to formation of a daunomycin hydroquinone which undergoes glycosidic cleavage to form 7-deoxydaunomycinone, daunomycinone, and epidaunomycinone most likely via a quinone methide. The reaction is complex because the acetates are not all saponified simultaneously. An important side reaction pathway involves tautomerization of **16** to tetrahydronaphthacenone **17** which yields *N*-acetyl-5-deoxydaunomycin (**12**) and naphthacenedione **15**.

Experimental Section

General Remarks. UV–vis spectra were recorded with a Hewlett-Packard 8452 diode array spectrometer. ^{1}H 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. Analytical HPLC was performed with a Hewlett-Packard Model 1090 chromatograph equipped with a diode array UV–vis detector and data processing workstation. 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 unless otherwise stated. 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 480 nm, λ_{max} for most of the chromophores, and are uncorrected for small differences in molar absorptivity at this wavelength. Suction chromatographic[19] separations were performed by reverse phase using Bakerbond octadecyl (C_{18}) packing obtained from J. T. Baker or normal phase using silica gel, Kiesgel 60, from EM Science in a 15-mL sintered glass filter. Eluting conditions for reverse phase chromatography were initially 40:60 methanol/water and then a gradual increase to 60:40 methanol/water. Preparative HPLC was performed on a Rainin 10 mm i.d. × 5 cm column of 3 μm C-18 reverse phase silica gel with a Rainin HPLC instrument equipped with a Model UV-1 preparative UV–vis detector. 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 A:B gradient from 30:70 to 80:20 at 15 min.

Tetrahydrofuran (THF) was distilled from sodium/benzophenone and stored over molecular sieves and water was doubly distilled and deionized. Lithium hydroxide was obtained from Aldrich, Milwaukee, WI, and tris(hydroxymethyl)aminomethane (Tris) and Tris·HCl from Sigma, St. Louis, MO. Daunomycin was a gift of Farmitalia Carlo-Erba, Milan, Italy. 7-Deoxydaunomycinone (**5**),[13,20] *N*-acetyl-5-deoxydaunomycin (**12**),[18] and *dl*-bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (*dl*-TM-3 dimer)[21] were prepared as described elsewhere. All other solvents and chemicals were obtained from Aldrich, Sigma, J. T. Baker (Phillipsburg, NJ), or Fisher (Fairlawn, NJ).

Three types of multicompartment cells were employed. The first was a two-pronged, fork-like cell having a 5-mL, medium-walled degassing chamber at the end of one prong and a 1 mm-path, Pyrex cuvette at the end of the other prong. The second consisted of two compartments, a 3-mL medium-walled degassing chamber and a 10-mm path, Pyrex cuvette attached at a 90° angle. The third consisted of four compartments, a medium-walled glass tube which could hold a volume of 3 mL, an identical compartment attached at a 45° angle, and a 10-mm path, Pyrex cuvette attached at a 90° angle. The fourth compartment, also constructed of medium-walled glass, was separated from the others by a break seal. The cells were equipped with 9-mm glass tubes for connection to a high-vacuum line with an Ultra-torr union. The third cell had a second 9-mm glass tube for connection of its fourth compartment to the high vacuum.

Solutions placed in the cells 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 three to 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.

Heptaacetate of Daunomycin Hydroquinone (6). The heptaacetate was prepared as previously described.[8] The crude material was purified by reverse phase suction chromatography rather than by the literature method using 2% KH_2PO_4–silica gel. The ^{1}H NMR spectrum in pyridine-d_6 at 60 °C matched the published spectral data.[8] The UV–vis bands were the same as the literature values;[8] however, the molar extinction coefficients for our heptaacetate in methanol were significantly higher: λ_{max} (ϵ, L M^{-1} cm^{-1}), 228 (1.9×10^4), 252 (sh, 4.3×10^4), 268 (1.1×10^5), 362 (sh, 5.4×10^3), 378 (9.0×10^3), 394 (7.9×10^3), and 414 nm (sh, 6.0×10^3).

2-Acetyl-11-hydroxy-7-methoxy-5,12-naphthacenedione (15). 5-Deoxydaunomycin[18] (10.0 mg, 1.83×10^{-5} mol) was dissolved in 0.65 mL of glacial acetic acid and 0.26 mL of 40% aqueous hydrobromic acid and heated on a steam bath for 30 min. Upon cooling, a precipitate formed which was collected by filtration and washed with acetic acid. After drying in vacuo, pure **15** was obtained in 35% yield. Mass spectral and ^{1}H NMR data on **15** agreed with literature values.[8]

***N*-Acetyldaunomycin (8).** Daunomycin (21.4 mg, 3.80×10^{-5} mol) was dissolved in 5 mL of methanol. To this solution was added 30 mol equiv of acetic anhydride, 2 mg of dimethylaminopyridine (DMAP), and 1 equiv of pyridine. The solution was stirred in the dark for 10–12 h. Analysis of the crude reaction mixture by HPLC showed 63% **8** and 34% recovered daunomycin. The reaction mixture was concentrated on a high vacuum rotary evaporator and purified by normal phase suction chromatography eluting with chloroform/methanol/water (80:10:1, v/v). *N*-Acetyldaunomycin fractions were combined, the solvent evaporated, and the solid dried in vacuo to yield 11.0 mg (47%) of **8**. The ^{1}H NMR spectrum of the purified material in $CDCl_3$ matched the published spectrum.[8]

Pentaacetate of 7-Deoxydaunomycinone Hydroquinone. The signal for the protons at position 8 was omitted in an earlier report[13] and assignments were not made. The complete 90 MHz ^{1}H NMR spectrum was as follows: ($CDCl_3$) δ 1.6–1.8 (m, 2H, H-8), 2.00 (s, 3H, 14-CH_3), 2.16 (s, 3H, 9-OAc), 2.36–2.46 (overlapping s, 12H, aromatic OAc), 6.7 (dd, 1H, J = 2, 8 Hz, H-3), 7.1–7.53 (m, 2H, H-1 and H-2); the aromatic pattern was simulated with chemical shifts 7.27 and 7.40 for H-2 and H-1, respectively, and $J_{1,2} = J_{2,3} = 8$ Hz.

Saponification of the Heptaacetate of Daunomycin Hydroquinone under Anaerobic Conditions. A 6.3×10^{-3} M solution of heptaacetate **6** was made by dissolving 10.6 mg in 2.0 mL of tetrahydrofuran. A 1.5-mL aliquot of this solution was syringed into the degassing chamber of the fork-like cell using Teflon tubing. Argon was then bubbled through the solution while cooling with dry-ice. A 0.10 M solution of lithium hydroxide was prepared by dissolving 13 mg of $LiOH \cdot H_2O$ in 3.0 mL of argon-bubbled doubly distilled water. A 0.65 mL aliquot of this solution (7.0 mol equiv relative to heptaacetate) was introduced via syringe and Teflon tubing into the dry-ice cold degassing chamber still under argon. The cell with the frozen mixture was quickly transferred to the vacuum line. After freeze–thaw–degassing (three cycles) the cell was sealed under vacuum with a torch and brought to ambient temperature. At this point the solution was brown yellow. The cell was kept in a bath at 18 °C for 19 h. UV–vis spectra were taken occasionally by pouring a small amount

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of the solution into the cuvette and diluting it through distillation of some solvent from the degassing chamber by cooling the cuvette with dry-ice. The spectra showed the change of the original 378-nm band into a new band at 406 nm, the strongest band in the visible region of the spectrum. A broad band at 440–480 nm was also present. In the meantime broad absorption in the region 500–650 nm, indicative of quinone anion, rose up to ca. 25% of the intensity of the 406-nm band. After 19 h the cell was opened and the pH was measured to be 10. The dark solution was immediately poured into an argon-bubbled pH 7, 0.25 M phosphate buffer (4 mL). Some dark solid separated out. After 5 min the precipitate (3 mg) was collected by suction filtration and washed with a little water. The precipitate and the filtrate were analyzed separately.

HPLC analysis of the precipitate dissolved in DMSO, using the microbore ODS column eluting at 0.30 mL/min with 60/40 methanol/0.3% pH 4 formate buffer, isocratic from 0 to 10 min, then a gradient to 80/20 from 10 to 20 min and monitoring at 480 nm, showed 7-epidaunomycinone (**13**) (3.2 min, 9%), daunomycinone (**14**) (5.6 min, 3%), *N*-acetyldaunomycin (**8**) (7.7 min, 15%), 7-deoxydaunomycinone (**5**) (8.9 min, 13%), *N*-acetyl-5-deoxydaunomycin (**12**) (12.7 min, 29%), 2-acetyl-11-hydroxy-7-methoxy-5,12-naphthacenedione (**15**) (23.3 min, 9%) plus three more unidentified products at 18 min (5%), 20 min (3%), and 22.7 min (6%). No 5,7-dideoxydaunomycinone was detected as indicated by comparison with an authentic sample.[20,22,23] The identity of **5**, **8**, **12**, **13**, **14**, and **15** was established by coinjection on the HPLC with peak purity analysis via the diode array detector and TLC comparison with authentic samples (silica gel plates, 5% v/v isopropyl alcohol in chloroform for **5**, **13**, **14** and **15**; 10% v/v methanol in chloroform for **8** and **12**).

The filtrate, including washings (total volume 6.2 mL, $A_{370} = 1$, $A_{480} = 0.4$ after a 1:3 dilution), when analyzed by HPLC (60/40 methanol/pH 4 buffer, isocratic) showed a ca 1:1:1 mixture of an unidentified compound (retention time, 3.2 min, $\lambda_{max} = 380$ nm; possibly a decomposition product of **13**, as suggested by comparison with an old partially decomposed sample of **13**,[10,24]) *N*-acetyldaunomycin (**8**) and *N*-acetyl-5-deoxydaunomycin (**12**).

Assuming an average molecular weight of 490 amu for the material in the precipitate and $\epsilon = 10^4$ L mol^{-1} cm^{-1} for the 370 and 480 nm partially overlapped bands for the material in the filtrate, and assuming a 10% loss during the workup and analyses, the following yields were calculated: **5** (10%), **8** (17%), **12** (28%), **13** (7%), **14** (2%), **15** (9%), and the three unidentified products which gave rise to significant HPLC peaks (14%).

Spectroscopic Monitoring of the Saponification of the Heptaacetate of Daunomycin Hydroquinone. The heptaacetate **6** (1.30 mg, 1.58×10^{-6} mol) was dissolved in 10.0 mL of THF. Lithium hydroxide (50.4 mg, 1.20×10^{-3} mol) was dissolved in 10.0 mL of nitrogen degassed water. An aliquot of the heptaacetate (2.0 mL, 3.2×10^{-7} mol) was placed in one compartment of the multicompartment cell described above. An aliquot of the lithium hydroxide was placed in a second compartment. The cell was freeze–thaw–degassed for 5 cycles and sealed under vacuum. The solutions were thawed and then mixed, and the cell was placed in a thermostatted (25.0 ± 0.1 °C) cell holder. The other empty compartments of the cell were wrapped with heating tape to prevent distillation of the solvent into these compartments. The reaction was followed for 5 days by UV–vis spectrophotometry; spectra covering the time periods 6–16 min and 2–26 h appear in Figures 1 and 2, respectively. After five days a Tris/Tris·HCl buffer solution of pH 8 (0.10 M, 0.60 mL) was added to the compartment of the cell separated by the break seal. The solution was freeze–thaw–degassed and sealed under vacuum. The buffer solution was thawed and added to the heptaacetate–lithium hydroxide reaction mixture. After 5 min the cell was opened, the solvent rotary evaporated, and the solid redissolved in methanol. HPLC analysis showed 15% 7-deoxydaunomycinone (**5**) and no *N*-acetyldaunomycin (**8**). Materials not soluble in methanol were not analyzed.

Reduction of Daunomycin with *dl*-TM-3 Dimer at pH 13. Daunomycin (1.24 mg, 2.20×10^{-6} mol) was dissolved in 10.0 mL of THF. Lithium hydroxide (9.0 mg, 2.1×10^{-4} mol) was dissolved in 25.0 mL of nitrogen degassed water. *dl*-TM-3 dimer (12.9 mg, 4.5×10^{-5} mol) was dissolved in 25.0 mL of CH_2Cl_2. To an aliquot of daunomycin solution (2.0 mL, 4.4×10^{-7} mol) was added 0.85 mL of the lithium hydroxide solution. An aliquot of TM-3 dimer solution (2.0 mL, 3.6×10^{-6} mol) was placed in the UV–vis compartment of a two-compartment cell and the CH_2Cl_2 was evaporated with a stream of nitrogen. The pH adjusted daunomycin was placed in the other compartment. The solutions were freeze–thaw–degassed for 5 cycles and sealed under vacuum. The solutions were thawed and mixed. The progress of the reaction was monitored by UV–vis spectroscopy. After 15 h the cell was opened, the solvent evaporated, and the solid redissolved in methanol. HPLC analysis showed 71% 7-deoxydaunomycinone (**5**), 23% daunomycinone (**14**), and 6% of unidentified products.

Reduction of 5,7-Dideoxydaunomycinone (2-Acetyl-2,11-dihydroxy-7-methoxy-1,2,3,4-tetrahydro-5,12-naphthacenedione) with Sodium Dithionite. 5,7-Dideoxydaunomycinone[23] (0.67 mg, 1.8×10^{-6} mol) was dissolved in 5.0 mL of unbuffered methanol. An aliquot of the solution (2.0 mL, 7.4×10^{-7} mol) was placed in a quartz cuvette and degassed with prepurified nitrogen. Meanwhile, sodium dithionite (79%, 8.7×10^{-5} mol) was dissolved in 10.0 mL of degassed water. A 0.11-mL aliquot (9.6×10^{-7} mol) was added to the 5,7-dideoxydaunomycinone solution. The reaction was monitored by UV–vis spectroscopy over the range 300–800 nm which showed disappearance of the band at 500 nm with appearance of a band at 392 nm resembling an anthracycline hydroquinone band and assigned to structure **18**. The band at 392 nm maximized almost immediately to decrease with formation of a broader band at 400 nm during the next 40 s. The 400-nm band was assigned to 8-acetyl-1-methoxy-7,9,10,12-tetrahydro-6,8,11-trihydroxy-5(8*H*)-naphthacenone.[22,23]

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