Table III. Extended-Hückel Parameters

| orbital | | $H_{ii}$ (eV) | $\zeta$ |
|---|---|---|---|
| Be | 2s | −10.0 | 1.2 |
| | 2p | −6.0 | 1.2 |
| Si (Ge) | 3s | −17.3 | 1.38 |
| | 3p | −9.2 | 1.38 |
| Ca | 4s | −7.0 | 1.2 |
| | 4p | −4.0 | 1.2 |

Pn = As, Sb, Bi. As Pn goes from As to Bi, the structure changes from the $ThCr_2Si_2$ to the $CaBe_2Ge_2$ type. They argued that this is due to the decreasing Pd–Pd bonding and increasing Pd–pnictogen bonding as the pnictogen size increases. In our main group element case, however, there is no evident Be–Be bonding. Firstly, the Be···Be at site I (**9**) in $CaBe_2Ge_2$ is 2.84 Å, greater than the sum of covalent radii 2.50 Å. Secondly, the occupied states are mostly Ge (>80%) and the empty Be states do not contribute to Be···Be bonding. Were it not for the interlayer interaction, the site occupation would invert in all the layers when Be···Be bonding decreases, as happens in $CaAl_2Zn_2$. We would have the formal oxidation states $Ca^{2+}(Be^{2+})_2(Si^{3-})_2$; the $Be^{2+}$ would be ionic and repulsive to each other and no Be–Be bond would be present.

**Acknowledgment.** This research was supported by the National Science Foundation through Grant CHE 8406119 and Grant DMR 821722702 to the Materials Science Center at Cornell University. C.Z. would like to thank Professor H. Schäfer and Dr. B. Eisenmann of T. H. Darmstadt for stimulating discussions. We are grateful to J. Burdett and the reviewers for helpful comments, to Cora Eckenroth for the typing, and the Jane Jorgensen and Elisabeth Fields for the drawings.

## Appendix

The extended-Hückel method[38] was used in all calculations. Si parameters were used to mimic Ge since no reliable Ge parameters were available. The parameters are assembled in Table III. The geometry was chosen such that Be (Ge) in layer **A** (**D**) is at the center of an ideal $Ge_4$ ($Be_4$) tetrahedron. Be–Be (Ge–Ge) = 2.83 Å, Be–Ge = 2.45 Å. A 28K point set[39] was used for the 2-dimensional layer and a 30K point set for the 3-dimensional structure. The calculations were repeated for the 3-dimensional structure with Ca. We found that the calculations with and without Ca were essentially the same.

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# Tautomeric Instability of 10-Deoxydaunomycinone Hydroquinone[1]

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**Abstract:** 10-Deoxydaunomycinone and daunomycin are reduced by excess sodium dithionite, under anaerobic conditions, to 10-deoxydaunomycinone hydroquinone. This hydroquinone is not stable, having an approximate rate constant (in 48% MeOH, 52% $H_2O$, 10 mM Tris-HCl, 10 mM Tris base) for its disappearance of $2 \times 10^{-4}$ $s^{-1}$. This disappearance results from a number of tautomeric equilibria, which transform this hydroquinone into more stable species. Typically, eight products in addition to 10-deoxydaunomycinone are detected by reverse-phase liquid chromatographic analysis of the product mixture. Thus far seven of these products have been identified and characterized. Three of the products are diastereomers of (2*R*)-2-acetyl-1,2,3,4,4a,12a-hexahydro-2,6,11-trihydroxy-7-methoxy-5,12-naphthacenedione that have differing stereochemistry at the C-4a,C-12a ring juncture. The major diastereomer (50% of the product) has a trans ring juncture, while the other two diastereomers (13% and 5% of the product) both have a cis ring juncture. Two of the products formed, (2*R*)-2-acetyl-1,2,3,4-tetrahydro-2,11-dihydroxy-7-methoxy-5,12-naphthacenedione (4% of the product) and (2*R*)-2-acetyl-1,2,3,4-tetrahydro-2,6-dihydroxy-7-methoxy-5,12-naphthacenedione (2% of the product), involve the loss of an oxygen from the anthracycline's C ring. The last two products, (8*R*)-8-acetyl-7,9,10,12-tetrahydro-6,8,11-trihydroxy-1-methoxy-5(8*H*)-naphthacenone (11% of the product) and (9*R*)-9-acetyl-7,9,10,12-tetrahydro-6,9,11-trihydroxy-4-methoxy-5(8*H*)-naphthacenone (5% of the product), are derived from the reduction of the dihydroxynaphthacenediones by the excess dithionite present in the mixture. The effect of Fe(III) ion chelation of the anthracycline on the anaerobic dithionite reduction of both daunomycin and 10-deoxydaunomycinone is examined. A modest rate increase for the tautomerization of the hydroquinone is observed for the anaerobic dithionite reduction of the 10-deoxydaunomycinone–Fe(III) chelate, whereas little effect is observed for the daunomycin–Fe(III) chelate. This surprising diversity of materials may account for the abundance of aglycon metabolites found in vivo (many as yet uncharacterized), may prove of value in the synthetic elaboration of anthracyclinones, and is likely to be representative of the hydroquinone behavior of the *p*-dihydroxyanthracyclinones of the rhodomycinone, isorhodomycinone, and pyrromycinone families.

Daunomycin (**1a**) and adriamycin (**1b**) are among the most useful of the antitumor antibiotics.[3] Nevertheless the discovery of the chemical attributes of these molecules responsible for their antibiotic efficacy has proven an extraordinarily difficult problem. Several theories of surprising dissimilarity remain at this time, all of which have experimental evidence in their support.[3,4] The focus of one of these theories[5] centers on the ability of these molecules to enter into redox chemistry by virtue of their quinone moiety. In the presence of a suitable redox enzyme (generally

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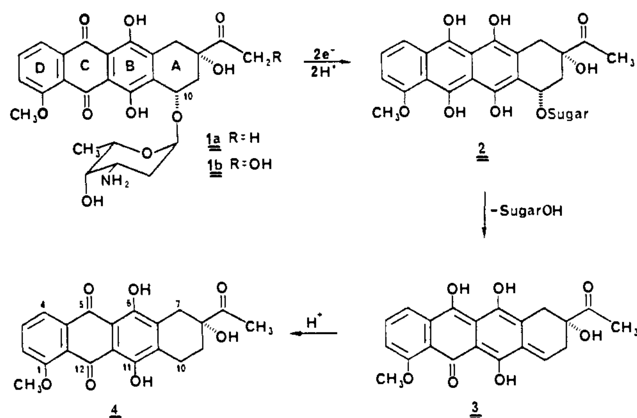
Scheme I



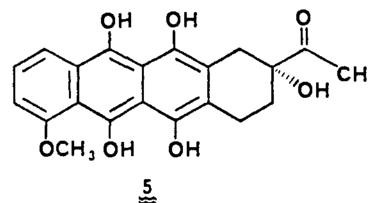
one containing a low-potential flavin or metal center), the quinone may undergo either a one- or two-electron reduction. Should this reduction occur under aerobic circumstances, the electrons are rapidly passed from the reduced quinone to molecular oxygen, generating $O_2^-$ and $H_2O_2$. The first anaerobic reduction, however, is mechanistically unique. It yields an *unstable* hydroquinone, **2**, which is given toward the rapid intramolecular expulsion of the C-10 aminoglycoside, producing the quinone methide **3** (Scheme I). This quinone methide is only somewhat more stable than its hydroquinone precursor. In the case of daunomycin it most frequently undergoes a thermodynamically favorable tautomerization, with acquisition of a solvent proton at C-10, to provide 10-deoxydaunomycinone (**4**).[5,6] These 10-deoxy molecules are well-recognized species which account for an appreciable portion of the metabolites of daunomycin and adriamycin.[3,7] These observations indicate that in vivo reductive metabolism under hypoxic conditions is commonplace. This suggests that the quinone methide is a potentially reactive in vivo species, which by specific alkylation of an as yet conjectural receptor may provide a molecular basis for the therapeutic efficacy of these antibiotics. Although there are no direct experimental data in support of this hypothesis, the chemical competence of this quinone methide **3** as both a nucleophile[8] and an electrophile[9] has been proven.

Regardless of the precise role that the redox chemistry of **1a** and **1b** may have in the expression of their biological activities, their redox conversion in vivo (under essentially anaerobic conditions) must be commonplace. Only the first of the anaerobic turnovers of daunomycin provides the quinone methide, which rapidly tautomerizes to 10-deoxydaunomycinone. Therefore, it is concluded that after the first anaerobic reaction *all ensuing anaerobic redox chemistry involves 10-deoxydaunomycinone* (**4**) as the oxidizing agent. Until this time the redox chemistry of **4** has been considered prosaic. Nevertheless there are two justifications for the belief that this might not be the case. First, as noted above, the evidence requires that redox chemistry involving **4** be thought of as a common in vivo occurrence and hence worthy of examination. Second, anthracycline metabolism is known to give rise to a plethora of metabolites. While three major metabolic transformations which preserve the basic constitution of the quinone nucleus (C-10 reductive deglycosylation, reduction of the C-8 acetyl, and glycoside hydrolysis to the aglycon)[3,7] are known to occur, there is evidence that metabolism may also significantly alter the basic constitution of the aglycon. Schwartz and his colleagues in particular have noted that when chromatographic analysis of daunomycin metabolism is accomplished with electrochemical detection, coupled with fluorometric detection, there are numerous additional species detected.[10] The possibility that some of these might arise during the course of anaerobic redox transformations upon **4** seemed reasonable, particularly in light of our preliminary observations on this issue.[11]

Although the redox chemistry of 10-deoxydaunomycinone in vivo is enzyme-mediated, enzymes cannot be conveniently used in the initial characterization of these redox events. Rather, the reducing agent of choice is sodium dithionite. Dithionite rapidly and cleanly reduces anthracyclines to their hydroquinones, as evidenced by the anerobic conversion of daunomycin to 10-deoxydaunomycinone, first noted by Smith et al.[12] The first indication that the anaerobic reduction of 10-deoxydaunomycinone presents unanticipated chemistry came during the course of an anaerobic dithionite reduction of a daunomycin–aporiboflavin binding protein complex.[11] After addition of excess dithionite and subsequent exposure to $O_2$, the solution's visible spectrum clearly showed the presence of new product(s) in addition to 10-deoxydaunomycinone, all of which remain tightly bound to the protein. Since, upon release from the protein, these new materials could be made to undergo facile conversion to 10-deoxydaunomycinone, it was suggested that they were 10-deoxydaunomycinone tautomers arising as kinetic products of hydroquinone oxidation by oxygen. This assertion is now found to require correction. As is described below, these products are identified as a series of diastereomers, corresponding to more stable tautomers of 10-deoxydaunomycinone *hydroquinone*. Further, the tautomeric instability of this hydroquinone gives rise to additional structures, which are now also described. The full complexity of these tautomerization processes, which may easily account for the aforementioned proliferation of daunomycin metabolites, is now unfolded.

## Results

The intrinsic complexity of the redox transformations of 10-deoxydaunomycinone is clearly illustrated by a simple experiment. When a small excess of dithionite is added to a rigorously anaerobic solution of **4** in buffered methanol, **4** is instantly reduced to its hydroquinone, **5**. This hydroquinone is not stable. Periodically, portions of the reaction mixture are removed and exposed



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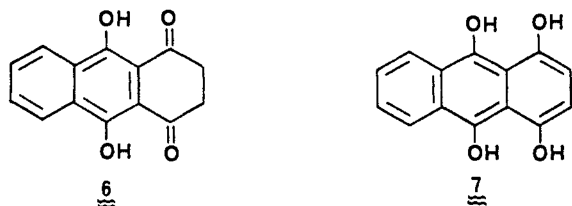
to air by dilution with air-saturated buffer (this rapidly oxidizes any residual hydroquinone), and the oxidized aglycons are recovered by $CHCl_3$ extraction. The visible spectra of these portions (Figure 1) show a progressive loss of 10-deoxydaunomycinone (**4**) and the appearance of new products. Were the hydroquinone **5** stable under anaerobic conditions, this protocol would have provided a quantitative restoration of 10-deoxydaunomycinone at each time point.

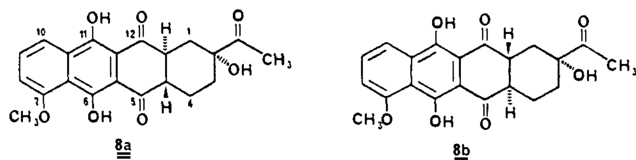
The complexity of the product mixture which ultimately arises from the reaction of **4** with dithionite and subsequent air oxidation is evidenced from chromatographic analysis of the resulting product mixture (Figure 2). A total of eight products, in addition to 10-deoxydaunomycinone, is observed. Upon purification, the examination of the visible spectrum of each delegates it between two categories, those products which are yellow (leuco) and those which are red-orange (chromo). The yellow compounds correspond to peaks 1, 3, 4, 6, and 7 of Figure 2, with peaks 3, 6, and 7 having virtually identical visible spectra. The red-orange compounds correspond to peaks 2, 5, 8, and 9 in Figure 2 and have similar—but not identical—visible spectra. 10-Deoxydaunomycinone (**4**) is found as peak 8. The structural and/or spectroscopic features of each are discussed in turn.

The major set of leuco products comprise peaks 3, 6, and 7 of Figure 2. All are strongly yellow compounds, exhibit an intense blue fluorescence when viewed under long-wavelength UV light, and have virtually identical visible absorption spectra ($\lambda_{max}$ = 398, 418, 442 nm). Hence all have the same chromophore. These characteristics[13] identify the three as classic leuco tautomers of **5**, sharing a diastereomeric relationship by virtue of a difference in the C-4a,C-12a ring-juncture stereochemistry. Leuco tautomers analogous to those arising from **5** are well-known in the 1,4-dihydroxy-9,10-anthracenedione series, appearing as the thermodynamically more stable tautomer **6** of the hydroquinone **7**.[13]



Leuco tautomers in the tetrahydronaphthacenedione series are less common, but have been encountered during the total synthesis of anthracyclinones.[14-16] As is evident by the circumstances of their isolation, these leuco tautomers are quite oxygen stable, in contrast to their hydroquinone tautomers.

A detailed spectral analysis on the major leuco compound (Figure 2, peak 7) substantiates the assignment to this compound of either structure **8a** or **8b**. In particular, the $^{13}C$ NMR spectrum



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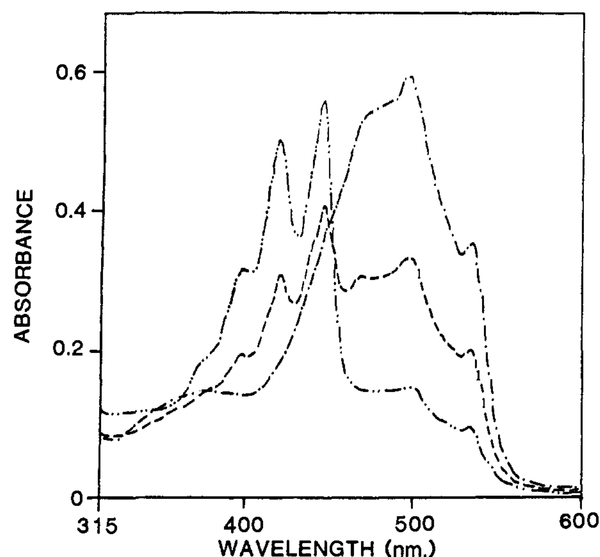


**Figure 1.** A solution of 10-deoxydaunomycinone (0.20 mM) in anaerobic buffered methanol (10 mM Tris-HCl/10mM Tris base) is reduced with excess sodium dithionite, and portions are removed at the indicated times. The anthracyclinones are extracted into $CHCl_3$, and the visible spectrum is obtained; (-·) before addition of dithionite, (--) 20 min, (-··) 150 min.

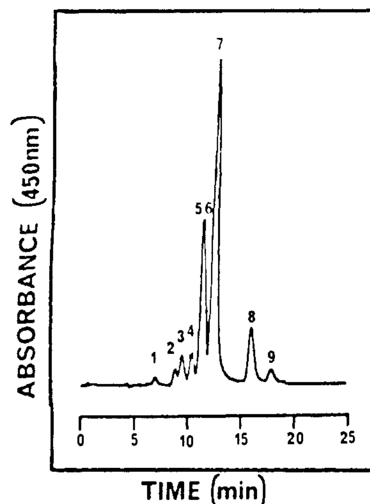


**Figure 2.** Reverse-phase analytical liquid chromatographic analysis, as described in the Experimental Section, of a reaction mixture portion taken 9 h after the addition of excess dithionite to an anaerobic solution of 10-deoxydaunomycinone (**4**) (0.35 mM) in buffered methanol (10 mM Tris-HCl/10 mM Tris base). Peak 1, **14** (retention time relative to **4** = 0.42); peak 2, unknown chromo compound (0.55); peak 3, **9** or **10** (0.59); peak 4, **13** (0.64); peaks 5 and 6, **12**, **9**, or **10** (0.70); peak 7, **8** (0.77); peak 8, **4** (1.00); peak 9, **11** (1.12).

contains three carbonyl resonances ($\delta$ 211.0, 202.2, and 200.6) which are all too deshielded for quinone carbonyls.[17] The least-shielded resonance corresponds to the acetyl carbonyl at C-2, with the remaining two found in the appropriate position for assignment as the C-5 and C-12 of structure **8a** or **8b**. The leuco structure is further substantiated in this spectrum by a pair of methine resonances at $\delta$ 47.2 and 44.1, assigned to C-4a and C-12a.[18] The $^1H$ NMR spectrum establishes the trans diaxial relative stereochemistry at the C-4a,C-12a ring juncture. This follows[14,15] from two large coupling constants for both H-4a ($J_{4a,12a}$ = 13.5 Hz, $J_{4a,4ax}$ = 11.4 Hz) and H-12a ($J_{12a,4a}$ = 13.6 Hz, $J_{12a,1ax}$ = 11.4 Hz). Unfortunately, both **8a** and **8b** have permissible conformations that could account for the observed splitting patterns

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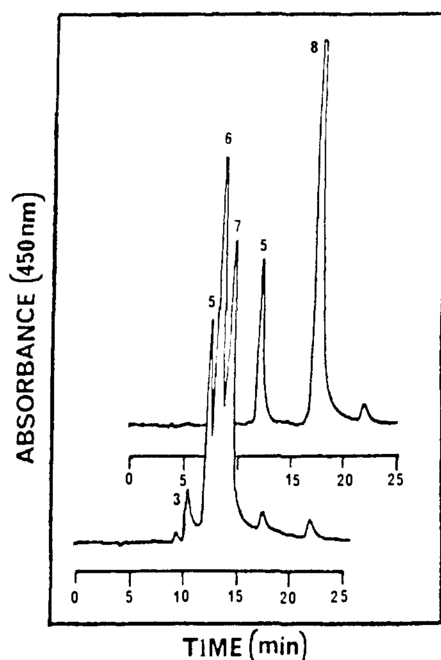
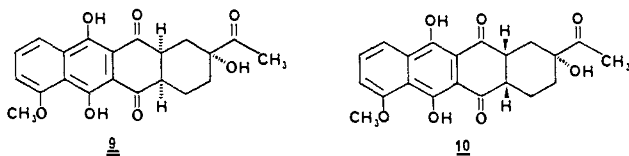



**Figure 3.** (bottom) Reverse-phase analytical liquid chromatographic analysis, as described in the Experimental Section, of a mixture of compounds corresponding to (see Figure 2) peak 3 (5%), peak 5 (11%), peak 6 (45%), peak 7 (37%), peak 8 (1%), and peak 9 (1%), before treatment with NaOMe. (top) Reverse-phase analytical liquid chromatographic analysis of the product mixture arising from the reaction of a methanolic solution of the aforementioned mixture with a slight excess of NaOMe for 2 h followed by neutralization with dilute HCl. The treatment with NaOMe quantitatively converts the leuco diastereomers (peaks 3, 6, and 7) to peak 8 (**4**), without having any affect on peaks 5 (**12**) or 9 (**11**).

in the $^1$H NMR spectrum. Therefore, the spectral data gathered do not differentiate between the two diastereomeric possibilities **8a** and **8b**, and either of these two structures may be assigned to this major leuco compound.

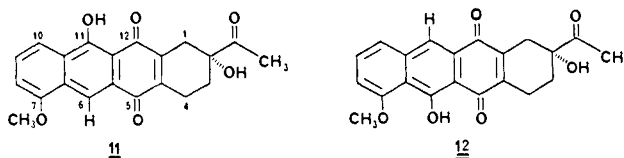
The second most abundant leuco compound produced (Figure 2, peak 6) from the dithionite reduction of **4** is diastereomeric with the major leuco compound **8**. It differs from **8** in that it has a cis relative stereochemistry at the C-4a,C-12a ring juncture. This follows from the size of the bridgehead proton coupling constants. The H-12a proton has one large vicinal coupling constant ($J_{12a,1ax}$ = 12.7 Hz) and two smaller vicinal coupling constants ($J_{12a,4a}$ = 5.4 Hz, $J_{12a,1eq}$ = 4.4 Hz). In addition to its coupling to H-12a, H-4a shows only two, much smaller coupling constants to the H-4 pair. These coupling constants require the cis ring juncture, since a trans diaxial stereochemistry at this ring juncture necessitates *two* large (diaxial) vicinal coupling constants for each bridgehead proton, as is seen for **8**.[14] Likewise, the third and last leuco diastereomer isolated (Figure 2, peak 3) also possesses the cis ring juncture at C-4a,C-12a. Its H-12a proton possesses three small vicinal coupling constants ($J_{12a,4a}$ = 5.8 Hz, $J_{12a,1ax}$ = 5.8 Hz, $J_{12a,1eq}$ = 2.5 Hz), while the H-4a proton has one large vicinal coupling constant ($J_{4a,4ax}$ = 10.8 Hz) and two smaller vicinal coupling constants ($J_{4a,4eq}$ = $J_{4a,12a}$ = 5.9 Hz). There are two possible leuco diastereomers, **9** and **10**, having the cis ring juncture, and the above




data do not differentiate between the two. Both **9** and **10** have permissible conformations that would correspond to the observed coupling constants. Therefore, an absolute stereochemical assignment is not possible.

The leuco compounds **8**, **9**, and **10** are unstable toward base in the presence of oxidants. As evidenced by chromatographic analysis of the product mixture (Figure 3), these compounds quantitatively transform to **4** in an aerobic solution of basic methanol. The isolated product comigrates with **4** and has absorption and fluorescence spectra identical with those of **4**. The fact that these three leuco compounds each produce **4** upon reaction with base lends further credence to their assignment as a set of diastereomers.

The mass spectrum of the most abundant new chromo compound (Figure 2, peak 9) indicates its constitution differs from that of **4** by loss of a single oxygen atom. The lost oxygen must be one of the four from 10-deoxydaunomycinone's B and C rings, as evidenced by the presence of a single phenolic hydroxyl peak ($\delta$ 13.77) in the $^1$H NMR spectrum. Comparison of the spectral data (IR, $^1$H NMR, and $^{13}$C NMR) for **4** and the new chromo compound corroborates this conclusion, as they require that the three oxygens of the A and D rings remain present. Loss of oxygen from C-6 or C-11 of **4**, with retention of a C ring quinone, can be ruled out on the basis of the chromo-like visible spectrum. 6-Deoxydaunomycinone and 11-deoxydaunomycinone derivatives both have $\lambda_{max}$ values 50–70 nm shorter than the observed $\lambda_{max}$ (480.5 nm).[19,20] Since the IR bands[19b] at 1660 and 1632 cm$^{-1}$ and the $^{13}$C NMR resonances at $\delta$ 188.5 and 182.8[17] establish that this chromo product *is* a quinone, it must contain a B ring quinone and correspond to either structure **11** or **12**.




Inspection of the $^1$H NMR spectrum distinguishes between the two. In particular, the chemical shifts of the aryl hydrogen of the C ring, and those of the phenolic hydrogen, are completely diagnostic. Thus, in compounds analogous to **11**, the aryl hydrogen appears near $\delta$ 8.5 and the phenolic hydrogen near $\delta$ 13.8.[21-24] On the other hand, compounds analogous to the isomeric structure **12** have these same hydrogens near $\delta$ 8.0 and 15.0, respectively.[22,23] As the new chromo compound has singlets at $\delta$ 8.50 and 13.77, its assignment as **11** is secure.

The second new chromo product of the dithionite reactions (Figure 2, peak 5) normally appears in a ratio of 1:2 to 1:4 relative to **11**. The mass spectrum indicates that it too has lost a single oxygen atom relative to **4**. Again, the visible spectrum ($\lambda_{max}$ = 504 nm) excludes a 6-deoxy- or 11-deoxydaunomycinone structure. As the $^1$H NMR spectrum shows a single hydrogen-bonded phenol ($\delta$ 15.11) and an aromatic singlet ($\delta$ 8.01), it must correspond to structure **12**.

These two (**11** and **12**) are oxidants and under the circumstances of their formation will therefore be reduced by dithionite to their respective hydroquinones. These hydroquinones are also found to exhibit tautomeric instability and upon standing give rise to

(19) 6-Deoxy (systematic numbering; 11-deoxy traditional numbering): (a) Arcamone, F.; Cassinelli, G.; DiMatteo, F.; Forenza, S.; Ripamonti, M. C.; Rivola, G.; Vigevani, A.; Clardy, J.; McCabe, T. *J. Am. Chem. Soc.* **1980**, *102*, 1462–1463. (b) Krohn, K. *Liebigs Ann. Chem.* **1981**, 2285–2297. (c) Kimball, D. S.; Walt, D. R.; Johnson, F. *J. Am. Chem. Soc.* **1981**, *103*, 1561–1563.

(20) 11-Deoxy (systematic numbering; 6-deoxy traditional): (a) Bauman, J. G.; Barber, R. B.; Gless, R. D.; Rapoport, H. *Tetrahedron Lett.* **1980**, *21*, 4777–4780. (b) Penco, S.; Angelucci, F.; Ballabio, M.; Barchielli, G.; Suarato, A.; Vanotti, E.; Vigevani, A.; Arcamone, F. *Tetrahedron* **1984**, *40*, 4677–4683. (c) Swenton, J. S.; Anderson, D. K.; Coburn, C. E.; Haag, A. P. *Ibid.* **1984**, *40*, 4633–4642.

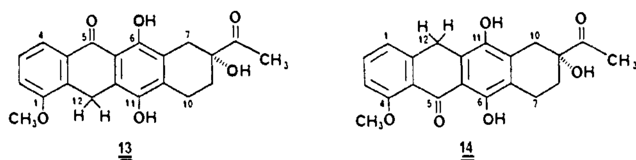
(21) (a) Parker, K. A.; Kallmerten, J. *J. Am. Chem. Soc.* **1980**, *102*, 5881–5886. (b) Russell, R. A.; Vikingur, E. G.; Warrener, R. N. *Aust. J. Chem.* **1981**, *34*, 131–141.

(22) (a) Tamura, Y.; Sasho, M.; Akai, S.; Wada, A.; Kita, Y. *Tetrahedron* **1984**, *40*, 4539–4548. (b) Krohn, K. (Bert Behnke's Ph.D. Thesis, University of Hamburg, 1982; Wahyudi Priyono's Ph.D. Thesis, Technischen Universtat Braunschweig, 1985) to Fisher, J.; private communication.

(23) Laatsch, H.; Anke, H. *Liebigs Ann. Chem.* **1982**, 2189–2215.

(24) Tresselt, D.; Ihn, W.; Horn, G.; Berg, H. *Pharmazie* **1984**, *39*, 417–419.

the respective 5(8*H*)-naphthacenones **13** and **14**. Analogous tautomerizations are known to occur for hydroxyanthracenediones to form dihydroxyanthracenones (anthrones).[23,25]



The reduction of purified **11** proceeds smoothly to give a single product. This material coelutes with material corresponding to peak 4 (Figure 2) and hence is formed during the dithionite reduction of **4**. Its assignment as **13** follows from the $^1$H and $^{13}$C NMR data. The $^1$H NMR spectrum shows only one hydrogen-bonded phenolic hydrogen ($\delta$ 12.84) and a non-first-order AB pattern at $\delta$ 3.87, which is assigned to the H-12 methylene protons. This methylene commonly occurs near $\delta$ 4.0[25,26] in 9(10*H*)-anthracenones. The $^{13}$C NMR spectrum contains two carbonyl resonances ($\delta$ 213.6, 189.7) and four methylene resonances ($\delta$ 31.9, 29.6, 23.5, 21.4) as compared to three carbonyl resonances ($\delta$ 211.0, 188.5, 182.8) and three methylene resonances ($\delta$ 32.1, 29.0, 20.2) in **11**. This disappearance of a quinone carbonyl resonance, coupled with the appearance of an additional methylene resonance, strongly suggests a 5(8*H*)-naphthacenone structure for **13**. Confirmation of this assignment is found in the $^{13}$C NMR spectrum of 1-hydroxy-5-methoxy-9(10*H*)-anthracenone (a mimic of the B, C, and D rings of **13**), which contains these respective resonances at $\delta$ 189.5 and 31.5.[27] Lastly, an aerobic solution of **13** is quantitatively transformed to **11** by sodium methoxide. However, **13** is not indefinitely stable, even in aerobic organic solvent. Over the course of a number of hours it spontaneously tautomerizes, and then oxidizes to **11**. This instability is consistent with the behavior of certain dihydroxyanthracenones.[23]

Likewise, dithionite reduction of **12** to its hydroquinone provides, over a period of several hours, a substantial conversion (ca. 80%) to a single product. This compound has spectral properties consistent with its assignment to structure **14** ($\lambda_{max}$ = 393 nm, $\epsilon \sim 1250$). It also coelutes with the material corresponding to peak 1 (Figure 2). Unfortunately this naphthacenone is much less stable than its isomer **13**. An aerobic $CH_2Cl_2$ solution of **14** undergoes spontaneous tautomerization and oxidation to restore **12** over a several-hour period. This precludes its further characterization.

The final product formed in the dithionite reduction of **4** (peak 2, Figure 2) has a visible absorption spectrum similar to that of the other chromo compounds ($\lambda_{max}$ = 495 nm). This compound typically comprises 0.1–0.3% of the total product mixture. Its retention time in both normal and reverse-phase chromatography indicates that it is more polar than the other chromo compounds found in the product mixture. Further characterization of this material has not been possible thus far because of the small amount of material that has been isolated.

The kinetics of the dithionite reduction of **4** in buffered aqueous MeOH are also examined. The reduction of **4** to the hydroquinone **5** is instantaneous (as evidenced by the color of the reaction mixture turning from orange to yellow). When the anaerobic conditions of the reaction mixture are maintained, **5** is converted to the products mentioned above over a several-hour period. The time course of the conversion of **5** to these products is ascertained by reverse-phase liquid chromatographic analysis of portions withdrawn from the reaction mixture at various time intervals (Figure 4A). The components of the product mixture which show the greatest variance with respect to time are **8**, **4**, and to a lesser extent **13**. All other components are seen to form quickly (within 1 h) and exhibit little variance in their contribution to the product mixture after the 1-h time point. The half-life for both the appearance of **8** and the disappearance of **5** (ascertained by the amount of residual **4**) is approximately 1 h. The final product mixture typically contains 10–15% **4** and 50–55% **8**.

The kinetics of the dithionite reduction of **1a** are examined under conditions similar to those used for the dithionite reduction of **4**. Inspection of these results (Figure 4B) shows a faster conversion of the hydroquinone **5** to products (as evidenced by the more rapid disappearance of **4**) when compared to the results cited above (Figure 4A). There is also a slightly greater variance in product composition with time for material corresponding to peaks 3 and 6 (Figure 2) in this reaction. The final product distributions for the reactions of **1a** and **4** with dithionite are, however, virtually identical. When the aqueous content of the reaction solvent is increased from 55% to 100%, for the dithionite reduction of **1a**, the rate of the tautomerization of **5** is significantly increased (Figure 4C). The reaction is virtually complete after 1 h.[28]

Several observations have suggested that the redox chemistry of anthracyclines in vivo may be modulated by metal ion chelation; in particular, Fe(III) forms a stable chelate with the O-5 and O-6 atoms of daunomycin.[4b,29,30] Since all of the above products may be rationalized in terms of tautomeric equilibria within the central B and C rings, it is reasonable to inquire as to the effect of metal chelation on these processes. Accordingly, the kinetics of the dithionite reduction of the Fe(III) chelates of both **1a** and **4** were investigated. Little, if any, effect is observed upon anaerobic dithionite reduction of the daunomycin–Fe(III) chelate in water (Figure 4C). Both the rate of reaction and the composition of the final product mixture are unchanged by the presence of Fe(III). Yet, anaerobic dithionite reduction of an equimolar solution of **4** and Fe(III)[31] in (1:1) methanol/water shows some effects due to the presence of the metal. Inclusion of Fe(III) in this reaction mixture increases the rate of the disappearance of the 10-deoxydaunomycinone hydroquinone (**5**) approximately 2-fold. The Fe(III) affects the reaction primarily by enhancing the overall conversion of **5** to **11** and its naphthacenone **13** (Figure 4D vs. Figure 4A) but has relatively little effect on the formation of the three leuco diastereomers **8**, **9**, and **10**. In addition, the amount of residual **4** in the final product mixture is reduced when Fe(III) is included in the reaction mixture. Since the relative chelating ability of iron (either Fe(II) or Fe(III)) to the hydroquinone is not established, it is not possible to ascribe a particular explanation of this metal effect under these circumstances. Possibly, the equilibrium constant between the hydroquinone and its anion is modestly increased due to metal-induced stabilization of the anion.

## Discussion

Clearly the most interesting mechanistic question concerns the formation of the structures **8–14** from the aglycon hydroquinone **5**. Although the observations do not directly establish a mechanism, a most reasonable rationalization is that a series of tautomeric equilibria exists involving the monoanion form of this hydroquinone. The involvement of the anion is consistent with the somewhat faster velocities observed in water compared to aqueous methanol or methanol. Sequential keto–enol tautomerization accomplishes the transposition of the carbonyls from the C to B ring for the formation of the leuco diastereomers. As noted previously, this tautomerization is well precedented for the hy-

(25) Cameron, D. W.; Edmonds, J. S.; Raverty, W. D. *Aust. J. Chem.* **1976**, *29*, 1535–1548.

(26) Rao, A. V. Rama; Reddy, K. Bal; Mehendale, A. R. *J. Chem. Soc., Chem. Commun.* **1983**, 564–566.

(27) Tamano, M.; Koketsu, J. *Nippon Kagaku Kaishi* **1983**, 1028–1034.

(28) In buffered methanol, the rates of hydroquinone to leuco tautomerization of both **5** and the hydroquinone derived from 1,4-dihydroxy-2,3-dimethyl-9,10-anthracenedione have been compared; the rate for **5** is approximately 3-fold slower.

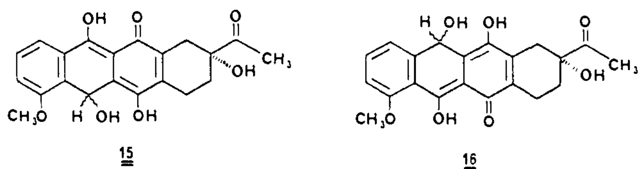
(29) Myers, C. E.; Gianni, L.; Simone, C. B.; Klecker, R.; Greene, R. *Biochemistry* **1982**, *21*, 1707–1713.

(30) (a) May, P. M.; Williams, G. K.; Williams, D. R. *Inorg. Chim. Acta* **1980**, *46*, 221–228. (b) Muinidi, J. R. F.; Sinha, B. K.; Gianni, L.; Myers, C. E. *FEBS Lett.* **1984**, *172*, 226–230. (c) Eliot, H.; Gianni, L.; Myers, C.; *Biochemistry* **1984**, *23*, 928–936. (d) Beraldo, H.; Garnier-Suillerot, A.; Tosi, L.; Lavelle, F. *Ibid.* **1985**, *24*, 284–289.

(31) An equimolar solution of **4** and Fe(III) corresponds to a formal excess of Fe(III), assuming 10-deoxydaunomycinone forms a 3 to 1 chelate with the Fe(III), as is the case with daunomycin.[29,30d]

**Figure 4.** Analytical reverse-phase liquid chromatographic analysis (as described in the Experimental Section) of the anthracyclinones (see Figure 2) recovered from the $CH_2Cl_2$ extraction of portions of the reaction mixture removed at the indicated times from the anaerobic dithionite reduction of either daunomycin (**1a**) or 10-deoxydaunomycinone (**4**): (◇), peak 1 (Figure 2); (△), peak 3; (▲), peak 4; (●), peaks 5 and 6; (□), peak 7; (○), peak 8, (■), peak 9. (A) Dithionite reduction of **4** (0.27 mM) in anaerobic buffered aqueous MeOH (48% v/v) MeOH, 52% $H_2O$, 10 mM Tris-HCl, 10 mM Tris base). (B) Dithionite reduction of **1a** (0.27 mM) in anaerobic buffered aqueous MeOH (55% v/v) MeOH, 45% 50 mM Tris buffer pH 7.4). (C) Dithionite reduction of **1a** (0.27 mM) in anaerobic 25 mM Tris-HCl buffer pH 7.4. The dithionite reduction of daunomycin-Fe(III) chelate under similar conditions gives an analogous product composition vs. time curve. (D) Dithionite reduction of a 10-deoxydaunomycinone-Fe(III) chelate 0.27 mM) in anaerobic buffered aqueous MeOH (48% (v/v) MeOH, 52% $H_2O$, 10 mM Tris-HCl, 10 mM Tris base).

droquinones produced in the reduction of *p*-dihydroxy-anthracenediones.[13] The formation of **11** and **12** is envisioned to involve a similar, yet somewhat more subtle, tautomeric process. Should the monoanion form of the hydroquinone undergo carbon reprotonation to give the respective hydroquinone isomers **15** and **16**, then elimination of the elements of water (analogous to the




quinone methide forming reactions of the hydroquinone **2**) would provide **11** and **12** at the quinone level. As excess dithionite is present, these are reduced forthrightly to their hydroquinones. The tautomeric equilibria of these hydroquinones give rise to appreciable quantities of the naphthacenones **13** and **14**. It is interesting to note that compound **11** has been isolated and characterized ($^1$H NMR, IR, UV-vis, and MS) by Tresselt et al.[24] from the anaerobic electrochemical reduction of 12-imino-daunomycin in buffered methanol. Our spectral data for **11** are in agreement. The appearance of **11** under these circumstances remains consistent with the suggestion of tautomeric equilibria involving carbon protonation of the hydroquinone at C-12. In the case of 12-iminodaunomycin, reductive elimination of the sugar followed by reduction and subsequent protonation at C-12 (of the 12-aminohydroquinone analogous to **5**) and the amino N would allow the formation of **11** by the loss of $NH_3$ as the leaving group.

It is appropriate to contrast our observations with the anaerobic dithionite protocol of Smith et al.,[32] which reports a quantitative production of 10-deoxydaunomycinone (**4**) from daunomycin hydrochloride in a solvent of methanol/tetrahydrofuran/0.6 M $NaHCO_3$ (5:6:6) with a 15-min reaction time. Clearly the longer reaction times in our studies allow for a more complete expression of the tautomeric equilibria of the 10-deoxydaunomycinone hy-

(32) Smith, T. H.; Fujiwara, A. N.; Lee, W. W.; Wu, H. Y.; Henry, D. W. *J. Org. Chem.* **1977**, *42*, 3653–3660.

droquinone (**5**). Furthermore, the omission of $NaHCO_3$ in our reactions may limit the reversion of the three leuco diastereomers to the 10-deoxydaunomycinone hydroquinone. In several experiments run under conditions similar to (but not identical with) those of Smith et al. the formation of modest quantities (approximately 15% of the aglycon mixture) of the leuco diastereomers has been observed. Under conditions of short reaction times, or where the initially formed 10-deoxydaunomycinone precipitates (this may occur in the absence of appropriate quantities of an organic cosolvent), the appearance of the products arising from the tautomeric equilibria of **5** is minimized.

The final discussion addresses the question of whether the tautomerization of the 10-deoxydaunomycinone hydroquinone is likely to occur in vivo. Although the unequivocal answer must await a direct examination, it is our opinion that the circumstances with which these events occur in vitro at least brings forward the reasonable possibility that this is the case. The minimal requirement for this reaction is the anaerobic two-electron reduction of 10-deoxydaunomycinone. The rapidity of 10-deoxydaunomycinone formation from daunomycin in vivo and the comparable oxidizing power of the aglycon to that of the parent glycoside indicate this requirement should be easily met. The validity of this argument is now substantiated.[33] An anaerobic solution of daunomycin, in the presence of excess NADPH, with spinach ferredoxin reductase and ferredoxin serving as catalysts,[8c] yields **4**, **8**, **11**, and **12** over the course of a several-hour reaction. The more difficult decision is then whether the in vivo lifetime of the hydroquinone is sufficient for the tautomerization processes to occur. In the absence of metals, at least several minutes are necessary for the detectable accumulation of new products. Our observations suggest that in the proper environment an appropriate metal ion (which is capable of chelating with the 10-deoxydaunomycinone) may shorten this period severalfold. These events may then occur quickly enough to transpire in vivo. As noted earlier, there are a diversity of aglycons obtained in vivo, many of which are presently unidentified. It is reasonable then to suggest that the tautomeric pathways involving the 10-deoxydaunomycinone hydroquinone may account for some of these, as yet, unaccounted for metabolites.

Last, there may be a generality to these reactions. These tautomeric pathways are predicted not to be limited to the rhodomycinones (as exemplified by **4**), but other anthracyclinones (such as the pyrromycinones and isorhodomycinones) should also be capable of these processes. Krohn and colleagues have already seen several examples of hydroquinone tautomeric instability in the course of dithionite reduction of anthracyclinone precursors.[22b] As interest in the anthracyclinones continues unabated[34] and the dithionite reduction of these quinones remains a reaction of continuing usefulness,[20c,35] the present observations complement the exhaustive efforts to understand the in vitro and in vivo chemistry of these species.

## Experimental Section

**Materials.** Sodium dithionite is obtained from Mallinckrodt and is used as received. Silica gel (Silica Woelm TSC) is from Universal Scientific Inc. Preparative thin-layer chromatography plates (silica gel GF, 1000 μm) are obtained from Analtech Inc. Daunomycin hydrochloride is a generous gift of the Drug Synthesis and Chemistry Branch of the National Cancer Institute.

**General Information.** The $^1H$ NMR spectra are obtained on a Nicolet NT300 spectrometer using the solvent ($CDCl_3$) as the internal standard referenced to $Si(Me)_4$. All coupling constants given are those observed (uncorrected for non-first-order behavior) and are expressed in Hz. The assignment of the $^1H$ resonances is made by comparison with spectra of known compounds and through the use of selectively decoupled spectra. The $^{13}C$ NMR spectra are taken either on a Nicolet NT300 spectrometer (75.46 MHz) or on a Bruker WM 250 spectrometer (62.9 MHz), using the solvent ($CDCl_3$) as the internal standard referenced to $Si(Me)_4$, except where stated otherwise. The assignment of the $^{13}C$ resonances is made by comparison with literature values and is consistent with results obtained by either off-resonance decoupled or psuedoinept techniques. Fast atom bombardment (FAB) mass spectra are obtained on a Kratos MS-50 spectrometer at the Midwest Center for Mass Spectrometry, University of Nebraska. High-resolution electron impact mass spectra are obtained on an AEI MS-30 spectrometer. Emission and excitation fluorescence spectra are obtained on a SPEX Fluorolog 222 equipped with a Datamate data processor. The fluorometric scans are lamp corrected but not photomultiplier corrected. All fluorescence spectra are done on approximately 5 μM solutions in MeOH. Melting points are taken on a hot stage melting point apparatus and are uncorrected.

**Deoxygenation of Solutions.** Buffer solutions are routinely deoxygenated by boiling the solution under nitrogen for a period of 10–15 min. The nitrogen used is deoxygenated by passage through activated BASF catalyst at 90 °C and is fed to the reaction solutions by a glass and stainless steel manifold (Ace Glass). Methanolic solutions are routinely deoxygenated by repeated evacuation and flushing with nitrogen.

**Chromatography.** Analytical liquid chromatographic analyses are performed on a Beckman Model 110A dual pump system utilizing a Beckman 421 controller, a Rainin Microsorb C18 column (4.6 mm × 25 cm, 5-μm particle size), a Hitachi Model 100-10 UV-vis spectrometer with an Altex flow cell, and a Hewlett-Packard Model 3390A integrator. Detection of 10-deoxydaunomycinone reaction products is done at 450 nm, with the integrated yields corrected to take into account variances in the exctinction coefficients among the compounds. Samples (usually 100 μL) are eluted isocratically at room temperature by using a mobile phase of 75% MeOH, 24.9% $H_2O$, and 0.1% $CF_3COOH$ at a flow rate of 0.75 mL $min^{-1}$. Peaks are identified by comparison of elution times (relative to 10-deoxydaunomycinone) to those of authentic samples and by peak collection followed by examination of the compounds' absorption spectrum.

Preparative reverse-phase liquid chromatographic separations are done using the aforementioned Beckman Model 110A dual pump system and a Du Pont Zorbax C18 column (2.1 × 25 cm). Samples (typically 1.7 mL containing 5–10 μmol of an anthracyclinone mixture) are eluted isocratically at room temperature at a flow rate of 4.0 mL $min^{-1}$. The mobile phase for most separations is 80.0% MeOH, 19.9% $H_2O$, and 0.1% $CF_3COOH$. Increasing the aqueous content of the mobile phase to 30.0% enhances the resolution of the separation, but decreased solubility and lengthy retention times become overriding factors.

**(8*R*)-8-Acetyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione (4).** A modification of Smith's procedure[32] is used. Daunomycin hydrochloride (282.6 mg, 0.50 mmol) is dissolved in 5 mL of dimethylformamide and partially deoxygenated by nitrogen bubbling for 20 min. Sodium dithionite (174 mg, ca. 1.0 mmol) is added to a Schlenck tube containing 100 mL of 50 mM tris(hydroxymethyl)-aminomethane hydrochloride (Tris-HCl) pH 7.4 buffer, which has been degassed by boiling under nitrogen. The entire daunomycin solution is transferred by syringe to the Schlenck tube to initiate the reaction. After 20 min the 10-deoxydaunomycinone has substantially precipitated out of solution, and the reaction mixture is opened to the atmosphere. The precipitate is collected by centrifugation and taken up in $CHCl_3$ (100 mL). This solution is washed with $H_2O$ (3 × 100 mL) and then dried ($Na_2SO_4$). The $CHCl_3$ is evaporated off to provide 168 mg (88%) of impure 10-deoxydaunomycinone, containing approximately 10% of the leuco tautomers. This 10-deoxydaunomycinone is of sufficient purity for the dithionite reactions described. Where 10-deoxydaunomycinone of greater purity is desired, it is purified by silica gel chromatography (90.0% $CHCl_3$, 9.9% EtOAc, 0.1% $CF_3COOH$). The spectral data are in agreement with those reported by Broadhurst et al.:[36] mp 224–226 °C (lit. 229–230 °C[37]); $^1H$ NMR (300 MHz) δ 13.82 (s, 1 H, phenolic OH), 13.42 (s, 1 H, phenolic OH), 8.01 (dd, 1 H, $J_{4,3} = 7.7$, $J_{4,2} = 1.1$, H-4), 7.76 (dd, 1 H, $J_{3,4} = 7.8$, $J_{3,2} = 8.4$, H-3), 7.36 (dd, 1 H, $J_{2,3} = 8.5$, $J_{2,4} = 1.0$, H-2), 4.08 (s, 3 H, 1-OMe), 3.81 (s, 1 H, 8-OH), 3.15 (app ddd, 1 H, $J_{10eq,10ax} = 18.9$, $J_{10eq,9ax} = 5.5$, $J_{10eq,9eq} = 2.4$, H-10eq). 3.05 (app ddd, 1 H, $J_{7ax,7eq} = 18.4$, $J_{7ax,10ax} = 2.3$, $J_{7ax,10eq} = 1.1$, H-7ax), 2.93 (m, 1 H, H-10ax; irradiation at δ 1.95 gave a ddd, $J_{10ax,10eq} = 18.9$, $J_{10ax,7ax} = 2.3$, $J_{10ax,7eq} = 1.4$), 2.91 (m, 1 H, H-7eq; irradiation at δ 1.95 gave a dd, $J_{7eq,7ax} = 18.4$, $J_{7eq,10ax} = 1.1$), 2.39 (s, 3 H, COMe), 2.01 (dt, 1 H, $J_{9ax,9eq} = 13.1$, $J_{9ax,10eq} = 5.7$, $J_{9ax,10ax} = 5.7$, H-9ax), 1.94 (d-dt, 1 H, $J_{9eq,9ax} = 13.0$, $J_{9eq,10ax} = 6.6$, $J_{9eq,10eq} = 2.3$, $J_{9eq,7eq} = 2.3$, H-9eq); $^{13}C$ NMR (62.9 MHz) δ 211.1 (C-13), 187.1 and 186.3 (C-5, C-12), 161.0

(33) Brand, D. J., unpublished results.

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(36) Broadhurst, M. J.; Hassall, C. H. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2227–2238.

(37) (a) Arcamone, F.; Franceschi, G.; Orezzi, P.; Cassinelli, G.; Barbieri, W.; Mondelli, R. *J. Am. Chem. Soc.* **1964**, *86*, 5334–5335. (b) Gleim, R. D.; Trenbeath, S.; Mittal, R. S. D.; Sih, C. J. *Tetrahedron Lett.* **1976**, 3385–3388.

(C-1), 156.3 and 156.1 (C-6 C-11), 138.2 and 135.8 (C-6a, C-10a), 135.4 (C-3), 133.2 (C-4a), 122.2 (C-12a), 119.6 (C-4), 118.2 (C-2), 110.6 and 109.7 (C-5a, C-11a), 75.8 (C-8), 56.7 (1-OMe), 32.4 (C-7), 29.2 and 19.9 (C-9, C-10), 23.9 (C-14); IR ($CDCl_3$) inter alia, 1710 (COMe), 1618 (H-bonded quinone), 1589 $cm^{-1}$ (aryl); UV-vis $\lambda_{max}$ (MeOH) 528.0 ($\epsilon$ 7240), 493.0 (12000), 289.0 (7970), 252.5 (29900), 235.0 nm (31700); fluorescence, emission spectrum ($\lambda_{exc}$ 495 nm, excitation slit 0.2 nm, emission slit 5 nm) $\lambda_{max}$ (rel intensity) 626 sh (46), 580 (100), 554 nm (81); fluorescence, excitation spectrum ($\lambda_{em}$ 579 nm, excitation slit 0.2 nm, emission slit 5 nm) $\lambda_{max}$ (rel intensity) 527 (63), 494 (100), 470 sh nm (88).

**Dithionite Reduction of 4.** A typical experiment describing the dithionite reduction of **4** is presented. 10-Deoxydaunomycinone (**4**) (7.7 mg, 20.2 $\mu$mol) is dissolved in 5 mL of $CHCl_3$ and then added to 80 mL of MeOH buffer (10 mM Tris-HCl/10 mM Tris base), which has been made anaerobic by boiling under nitrogen. Sodium dithionite (14.1 mg, ca. 81 $\mu$mol) in 3 mL of anaerobic 50 mM Tris-HCl buffer (pH 7.4) is added to initiate the reaction. The progress of the reaction is followed by removing 1.0-mL portions at various time intervals and extracting the anthracyclinones into $CHCl_3$. The visible spectra of the $CHCl_3$ extracts are obtained and the components of the mixture analyzed by reverse-phase liquid chromatography. Although this analysis showed the reaction to be about 90% complete (on the basis of the percentage of recovered **4**) after 3 h, the reaction was allowed to proceed overnight. After 18.5 h at ambient temperature the remaining reaction mixture is exposed to air by dilution with 300 mL of air-saturated $H_2O$ and is then extracted with $CHCl_3$ (6 × 20 mL). The $CHCl_3$ layer is washed with water (2 × 200 mL) and dried ($Na_2SO_4$), and the $CHCl_3$ is evaporated to provide 7.5 mg of solid (100% corrected weight recovery). The composition of this product mixture (as determined by reverse-phase liquid chromatography) is **8**, 50%; **11**, 14%; **4**, 13%; peak 6 (Figure 2), 11%; **13**, 6%; **12**, 4%; peak 3 (Figure 2), 2%; **14**, 0.1%; and unknown chromo compound, 0.1%.

**Purification of Product Mixture Components from Dithionite Reductions of 4.** Crude fractionation of the product mixtures from the dithionite reduction of **4** is accomplished by performing preparative reverse-phase liquid chromatographic separations using the Du Pont Zorbax column and a mobile phase of 85.0% MeOH, 14.9% $H_2O$, and 0.1% $CF_3COOH$ at a flow rate of 4.0 mL $min^{-1}$. Typically, five fractions (1, 14–20 min; 2, 20–22 min; 3, 22–25 min; 4, 25–28 min; 5, 28–30 min) are collected at this point. These fractions are then subjected to further purification as outlined below to isolate the individual components.

**[2*R*-(4a$\alpha$,12a$\beta$ or 4a$\beta$,12a$\alpha$)]-2-Acetyl-1,2,3,4,4a,12a-hexahydro-2,6,11-trihydroxy-7-methoxy-5,12-naphthacenedione (8).** Fraction 3 from the above crude fractionation is rechromatographed on the Du Pont Zorbax column using a mobile phase of 70.0% MeOH, 29.9% $H_2O$, and 0.1% $CF_3COOH$ at a flow rate of 4.0 mL $min^{-1}$ to afford a 65% yield of **8** (retention time, $R_T$ = 46 min): mp sample darkened at 145–160 °C and melted at 217–219 °C; $^1$H NMR (300 MHz) $\delta$ 14.59 (s, 1 H, 6-OH), 13.32 (s, 1 H, 11-OH), 8.08 (d, 1 H, $J_{10,9}$ = 8.2, H-10), 7.68 (app t, 1 H, $J$ = 8.2, H-9), 7.17 (d, 1 H, $J_{8,9}$ = 7.8, H-8), 4.06 (s, 3 H, 7-OMe), 3.79 (s, 1 H, 2-OH), 3.33 (ddd, 1 H, $J_{12a,4a}$ = 13.6, $J_{12a,1ax}$ = 11.4, $J_{12a,1eq}$ = 3.7, H-12a), 2.81 (ddd, 1 H, $J_{4a,12a}$ = 13.5, $J_{4a,4ax}$ = 11.6, $J_{4a,4eq}$ = 3.9, H-4a), 2.48 (app ddd, analyzed as dddd, 1 H, $J_{4eq,4ax}$ = 13.9, $J_{4eq,3eq}$ = 3.6, $J_{4eq,3ax}$ = 3.6, $J_{4eq,4a}$ = 3.6, H-4eq), 2.33 (s, 3 H, COMe), 2.31 (app dd, 1 H, $J_{1eq,1ax}$ = 13.3, $J_{1eq,12a}$ = 3.6, H-1eq), 2.05 (app ddd, non-first order, 1 H, $J_{4ax,4eq}$ = 13.9, $J_{4ax,3ax}$ = 11.7, $J_{4ax,4a}$ = 11.7, $J_{4ax,3eq}$ = 5.5, H-4ax), 1.92 (dd, 1 H, $J_{1ax,1eq}$ = 13.6, $J_{1ax,12a}$ = 11.5, H-1ax), 1.78 (m, 2 H, $H_2$-3); $^{13}$C NMR (62.9 MHz) $\delta$ 211.0 (C-13), 202.2 and 200.6 (C-5, C-12), 160.0, 158.4, and 153.9 (C-6, C-7, C-11), 132.1 (C-10a), 131.5 (C-9), 119.9 (C-6a), 117.0 (C-10), 111.8 (C-8), 107.8 and 107.1 (C-5a, C-11a), 76.8 (C-2), 56.5 (7-OMe), 47.2 and 44.1 (C-4a, C-12a), 33.6, 32.4, and 20.7 (C-1, C-3, C-4), 23.6 (C-14); IR ($CDCl_3$) inter alia, 1708 (COMe), 1630 and 1605 (H-bonded quinone), 1585 $cm^{-1}$ (aryl); UV-vis $\lambda_{max}$ (MeOH) 442.0 ($\epsilon$ 16000), 417.5 (15200), 397.5 (8740), 376.0 sh (4290), 266.5 (23500), 241.0 nm (27400); MS (FAB, dithiothreitol/dithioerythritol matrix, low-resolution positive ion), $m/z$ (rel intensity) inter alia, 389 (M + 5, 12) 388 (M + 4, 34), 387 (M + 3, 10), 386 (M + 2, 28), 385 (M + 1, 100), 384 (M, 51), 367 (M + H – $H_2O$, 27), 341 (M – COMe, 9), 323 (M – $H_2O$ – COMe, 27), 309 (18); MS (FAB, dithiothreitol/dithioerythritol matrix, low-resolution negative ion), $m/z$ (rel intensity) inter alia, 385 (M + 1, 22), 384 (M, 60), 383 (M – 1, 100), 382 (M – 2, 39), 381 (M – 3, 15), 369 (45); fluorescence, emission spectrum ($\lambda_{exc}$ 420 nm, excitation slit 0.2 nm, emission slit 3 nm) $\lambda_{max}$ (rel intensity) 515 sh (47), 484 (100), 458 nm (99); fluorescence, excitation spectrum ($\lambda_{em}$ 483 nm, excitation slit 0.2 nm, emission slit 3 nm) $\lambda_{max}$ (rel intensity) 443 (100), 420 (93), 400 sh nm (55).

**[2*R*-(4a$\alpha$,12a$\alpha$ or 4a$\beta$,12a$\beta$)]-2-Acetyl-1,2,3,4,4a,12a-hexahydro-2,6,11-trihydroxy-7-methoxy-5,12-naphthacenedione (Peak 6, Figure 2; 9 or 10).** Fraction 3 from the above crude fractionation was rechromatographed on the Du Pont Zorbax column using a mobile phase of 70.0% MeOH, 29.9% $H_2O$, and 0.1% $CF_3COOH$ at a flow rate of 4.0 mL $min^{-1}$ to afford a 25% yield of this compound ($R_T$ = 42 min): $^1$H NMR (300 MHz) $\delta$ 14.90 (s, 1 H, 6-OH), 13.66 (s, 1 H, 11-OH), 8.11 (dd, 1 H, $J_{10,9}$ = 8.2, $J_{10,8}$ = 1.0, H-10), 7.71 (app t, 1 H, $J$ = 8.1, H-9), 7.20 (dd, 1 H, $J_{8,9}$ = 8.3, $J_{8,10}$ = 0.9, H-8), 4.08 (s, 3 H, 7-OMe), 3.82 (s, 1 H, 2-OH), 3.51 (ddd, 1 H, $J_{12a,1ax}$ = 12.7, $J_{12a,4a}$ = 5.4, $J_{12a,1eq}$ = 4.4, H-12a), 3.36 (m, 1 H, H-4a), 2.66 (app ddd, 1 H, $J_{4eq,4ax}$ = 9.6, $J_{4eq,3ax}$ = 2.4, $J_{4eq,3eq}$ = 2.4, H-4eq), 2.13 (s, 3 H, COMe), 1.97–1.90 (m, 2 H, H-4ax, H-1eq), 1.83 (app t, 1 H, $J$ = 12.7, H-1ax), 1.74 (m, 1 H, H-3), 1.55 (m, 1 H, H-3); UV-vis (MeOH) $\lambda_{max}$ (rel absorbance) 443.5 (64), 438.0 sh (55), 419.0 (59), 397.5 (33), 376.5 sh (16), 267.5 (91), 242.0 nm (100).

**[2*R*-(4a$\beta$,12a$\beta$ or 4a$\alpha$,12a$\alpha$)]-2-Acetyl-1,2,3,4,4a,12a-hexahydro-2,6,11-trihydroxy-7-methoxy-5,12-naphthacenedione (Peak 3, Figure 2: 9 or 10).** Fraction 2 from the crude fractionation was chromatographed on silica gel using 99.9% $CHCl_3$ and 0.1% $CF_3COOH$ as the eluent to afford a 10% yield of this compound: $^1$H NMR (300 MHz) $\delta$ 14.99 (s, 1 H, 6-OH), 13.61 (s, 1 H, 11-OH), 8.13 (dd, 1 H, $J_{10,9}$ = 8.3, $J_{10,8}$ = 1.0, H-10), 7.72 (app t, 1 H, $J$ = 8.2, H-9), 7.20 (dd, 1 H, $J_{8,9}$ = 8.0, $J_{8,10}$ = 0.8, H-8), 4.06 (s, 3 H, 7-OMe), 3.42 (ddd, 1 H, $J_{12a,4a}$ = 5.8, $J_{12a,1ax}$ = 5.8, $J_{12a,1eq}$ = 2.5, H-12a) 3.10 (br s, 1 H, 2-OH), 3.02 (app ddd, 1 H, $J_{4a,4ax}$ = 10.8, $J_{4a,4eq}$ = 5.9, $J_{4a,12a}$ = 5.9, H-4a), 2.72 (ddd, 1 H, $J_{1eq,1ax}$ = 14.7, $J_{1eq,12a}$ = 2.2, $J_{1eq,3eq}$ = 2.2, H-1eq), 2.33 (s, 3 H, COMe), 2.00 (dd, 1 H, $J_{1ax,1eq}$ = 14.9, $J_{1ax,12a}$ = 5.8, H-1ax), 1.94 (m, 1 H, H-4ax), 1.76 (m, 2 H, $H_2$-3); UV-vis (MeOH) $\lambda_{max}$ (rel absorbance) 443.5 (59), 437.0 sh (51), 418.5 (55), 398.0 (32), 375.0 sh (16), 267.0 (89), 242.5 nm (100).

**(2*R*)-2-Acetyl-1,2,3,4-tetrahydro-2,11-dihydroxy-7-methoxy-5,12-naphthacenedione (11).** Fraction 5 from the crude fractionation is reacted with a slight excess of NaOMe in MeOH for 2 h (to remove any trace amounts of leuco compounds) and then neutralized with dilute HCl. This is diluted with $H_2O$, extracted into $CHCl_3$, and then dried ($Na_2SO_4$). The $CHCl_3$ is evaporated off and the residue subjected to normal-phase (silica gel) flash column chromatography using 19.9% EtOAc, 80.0% hexanes, and 0.1% $CF_3COOH$ as the eluent. This yielded **11** in >95% purity: mp 200–203 °C; $^1$H NMR $\delta$ 13.77 (s, 1 H, 11-OH), 8.50 (s, 1 H, H-6, non-$D_2O$ exchangeable), 7.97 (d, 1 H, $J_{10,9}$ = 8.4, H-10), 7.57 (app t, 1 H, $J$ = 8.1, H-9), 7.05 (d, 1 H, $J_{8,9}$ = 7.8, H-8), 4.03 (s, 3 H, 7-OMe), 3.86 (s, 1 H, 2-OH, $D_2O$ exchangeable), 3.05 (m, 1 H, H-4eq; irradiation at $\delta$ 1.9 gave a dd, $J_{4eq,4ax}$ = 20.8, $J_{4eq,1ax}$ = 1.7), 2.91 (ddd, 1 H, $J_{1ax,1eq}$ = 19.0, $J_{1ax,4ax}$ = 4.0, $J_{1ax,4eq}$ = 2.0, H-1ax), 2.8 (m, 1 H, H-4ax; irradiation at $\delta$ 1.9 gave a ddd, $J_{4ax,4eq}$ = 20.8, $J_{4ax,1ax}$ = 3.8, $J_{4ax,1eq}$ = 1.9), 2.75 (m, 1 H, H-1eq; irradiation at $\delta$ 1.9 gave a dd, $J_{1eq,1ax}$ = 18.9, $J_{1eq,4ax}$ = 1.9), 2.38 (s, 3 H, COMe), 1.91 (m, 2 H, $H_2$-8); $^{13}$C NMR (75.5 MHz) $\delta$ 211.0 (C-13), 188.5 (C-12), 182.8 (C-5), 161.8 and 157.4 (C-7, C-11), 146.5 and 141.5 (C-4a, C-12a), 129.7 (C-9), 128.6, 127.5, and 126.4 (C-5a, C-6a, C-10a), 116.47 and 116.42 (C-6, C-10), 109.6 (C-8), 109.0 (C-11a), 75.8 (C-2), 55.9 (7-OMe), 32.1, 29.0 and 20.2 (C-1, C-3, C-4), 23.9 (C-14); IR ($CDCl_3$) inter alia, 1713 (COMe), 1660 (non-H-bonded quinone), 1632 (H-bonded quinone), 1605 and 1578 $cm^{-1}$ (aryl); UV-vis $\lambda_{max}$ (MeOH) 480.5 ($\epsilon$ 9070), 342.0 sh (2530), 324.5 (3670), 284.0 sh (14100), 275.0 sh (16200), 242.5 nm (55500); MS (EI, low resolution), $m/z$ (rel intensity) inter alia, 366 (M, 50), 348 (M – $H_2O$, 40), 323 (M – COMe, 100), 305 (M – COMe – $H_2O$, 52), 295 (M – COMe – CO, 34); MS (FAB, low resolution, dithiothreitol/dithioerythritol matrix, positive ion), $m/z$ (rel intensity) inter alia, 369 (M + 3, 45), 368 (M + 2, 100), 367 (M + 1, 99), 366 (M, 25), 349 (M + 1 – $H_2O$, 33), 323 (M – COMe, 30), 309 (M – 2CO, 59); MS (FAB, low resolution, nitrobenzyl alcohol matrix, negative ion), $m/z$ (rel intensity) inter alia, 367 (M + 1, 38), 366 (M, 100), 365 (M – 1, 43), 305 (M – $H_2O$ – COMe, 33); MS (EI, high resolution), $m/z$ 366.1088, calcd for $C_{21}H_{18}O_6$ 366.1103; fluorescence, emission spectrum ($\lambda_{exc}$ 485 nm, excitation slit 0.5 nm, emission slit 5 nm) $\lambda_{max}$ 606 nm; fluorescence, excitation spectrum ($\lambda_{em}$ 606 nm, excitation slit 0.5 nm, emission slit 5 nm) $\lambda_{max}$ 485 nm.

**(2*R*)-2-Acetyl-1,2,3,4-tetrahydro-2,6-dihydroxy-7-methoxy-5,12-naphthacenedione (12).** Material corresponding to peaks 5 and 6 (Figure 2) is reacted with a slight excess of NaOMe in MeOH for 2 h to convert the material corresponding to peak 6 to **4**. This is then neutralized with dilute HCl, diluted with $H_2O$, and then extracted into $CHCl_3$, and these extracts are dried ($Na_2SO_4$). The $CHCl_3$ is evaporated off, and the residue is subjected to reverse-phase chromatography, to remove **4**, on the Du Pont Zorbax column using a mobile phase of 80.0% MeOH, 19.9% $H_2O$, and 0.1% $CF_3COOH$ to afford a 50% yield of **12**: mp 152–153 °C; $^1$H NMR (300 MHz) $\delta$ 15.11 (s, 1 H, 6-OH), 8.01 (s, 1 H, H-11), 7.62 (app t, 1 H, $J$ = 8.1, H-9), 7.51 (d, 1 H, $J_{10,9}$ = 7.9, H-10), 7.06 (d, 1 H, $J_{8,9}$ = 8.0, H-8), 4.07 (s, 3 H, 7-OMe), 3.84 (s, 1 H, 2-OH), 3.07 (m, 1 H, H-4eq; irradiation at $\delta$ 1.9 gave a d, $J_{4eq,4ax}$ = 19.8), 2.93 (m, 1 H, H-1ax; irradiation at $\delta$ 1.9 gave a dd, $J_{1ax,1eq}$ = 20.2, $J_{1ax,4ax}$ = 3.6), 2.82 (m, 1 H, H-4ax, irradiation at $\delta$ 1.9 gave a ddd, $J_{4ax,4eq}$ = 20.4, $J_{4ax,1ax}$ = 3.2, $J_{4ax,1eq}$ = 2.0), 2.79 (m, 1 H, H-1eq, irra-

diation at δ 1.9 gave a dd, $J_{1eq,1ax}$ = 19.8, $J_{1eq,4ax}$ = 2.0), 2.36 (s, 3 H, COMe), 1.92 (m, 2 H, $H_2$-8); IR ($CDCl_3$) inter alia, 1710 (COMe), 1655 (non-H-bonded quinone), 1630 (H-bonded quinone), 1600 and 1575 $cm^{-1}$ (aryl); UV-vis (MeOH) $\lambda_{max}$ (rel absorbance) 504 (18), 344 sh (4), 330 (6), 276.5 (22), 244 nm (100), MS (EI), $m/z$ (rel intensity) inter alia, 366 (M, 65), 348 (M − $H_2O$, 21), 323 (M − COMe, 100), 305 (M − $H_2O$ − COMe, 22), 295 (M − COMe − CO, 29); MS (EI, high resolution), $m/z$ 366.1100, calcd for $C_{21}H_{18}O_6$ 366.1103; fluorescence, emission spectrum ($\lambda_{exc}$ 503 nm, excitation slit 0.5 nm, emission slit 5 nm) $\lambda_{max}$ 605 nm; fluorescence excitation spectrum ($\lambda_{em}$ 605 nm, excitation slit 0.5 nm, emission slit 5 nm) $\lambda_{max}$ 503 nm.

**(8*R*)-8-Acetyl-7,9,10,12-tetrahydro-6,8,11-trihydroxy-1-methoxy-5-(8*H*)-naphthacenone (13).** To 18 mL of a solution of 55% MeOH and 45% 50 mM Tris-HCl buffer pH 7.4 are added **11** (4.4 mg, 12 μmol) and $MnCl_2 \cdot 4H_2O$ (2.56 mg, 13 μmol). The solution is then made anaerobic by repeated evacuation and flushing with nitrogen. Sodium dithionite (5.6 mg, ca. 32 μmol) in 2 mL of anaerobic 50 mM Tris-HCl buffer, pH 7.4, is added to initiate the reaction. After 3 h at ambient temperature the reaction vessel is opened to the atmosphere, and the product mixture is then diluted with 100 mL of 1.0 mM ethylenediaminetetraacetic acid disodium salt (EDTA). This aqueous solution is extracted with $CH_2Cl_2$ (4 × 10 mL), and the organic layer is washed with 100 mL of $H_2O$. After this layer is dried ($Na_2SO_4$), the $CH_2Cl_2$ is evaporated off and the residue immediately subjected to normal-phase (silica gel) flash chromatography using 90% $CHCl_3$ and 10% EtOAc as the eluent. This afforded 3.5 mg (80%) of **13**: $^1H$ NMR (300 MHz) δ 12.84 (s, 1 H, 6-OH, $D_2O$ exchangeable), 7.81 (dd, 1 H, $J_{4,3}$ = 8.0, $J_{4,2}$ = 1.1, H-4), 7.43 (app t, 1 H, $J$ = 8.0, H-3), 7.13 (dd, 1 H, $J_{2,3}$ = 8.1, $J_{2,4}$ = 1.1, H-2), 5.04 (br s, 1 H, 11-OH, $D_2O$ exchangeable), 4.20 (br s, 1 H, 8-OH, $D_2O$ exchangeable), 3.97 (s, 3 H, 1-OMe), 3.871 and 3.867 (app d (non-first-order AB pattern), 2 H, $H_2$-12, non-$D_2O$ exchangeable), 3.03–2.82 (m, 4 H, $H_2$-7 and $H_2$-10), 2.39 (s, 3 H, 8-COMe), 2.10–1.89 (m, 2 H, $H_2$-9); $^{13}C$ NMR (75.5 MHz, $CD_2Cl_2$ + 10% $Me_2SO$-$d_6$, reference Si$(Me)_4$) δ 213.6 (C-13), 189.7 (C-5), 157.1 and 155.4 (C-1, C-6), 143.4 (C-11), 134.5 and 132.2 (C-6a, C-10a), 130.6 (C-12a), 127.4 (C-3), 124.8 (C-4a), 120.2 (C-11a), 118.5 (C-4), 114.0 (C-2), 113.2 (C-5a), 76.3 (C-8), 56.0 (1-OMe), 31.9, 29.6, 23.5, and 21.4 (C-12, C-7, C-9, C-10), 24.7 (C-14); IR ($CDCl_3$) inter alia, 1725 (COMe), 1630 (H-bonded quinone), 1580 $cm^{-1}$ (aryl); UV-vis $\lambda_{max}$ (MeOH) 396.0 (ε 4000), 297.5 (11 300), 269.5 (16 100), 237.0 nm (14 300).

**Reaction of 13 with Sodium Methoxide.** To MeOH are added **13** (0.1 mg, 0.3 μmol) and a slight excess of NaOMe. The reaction mixture is allowed to proceed at ambient temperature for 15 min. The reaction mixture is then neutralized with dilute HCl, diluted with $H_2O$, and extracted into $CH_2Cl_2$. Analytical reverse-phase liquid chromatographic analysis of the $CH_2Cl_2$ extracts of this product mixture showed it to be 96% **11** and 4% **13**.

**(9*R*)-9-Acetyl-7,9,10,12-tetrahydro-6,9,11-trihydroxy-4-methoxy-5-(8*H*)-naphthacenone (14).** To 9 mL of a solution of 55% MeOH and 45% 50 mM Tris-HCl buffer pH 7.4 are added **12** (0.1 mg, 0.27 μmol) and $MnCl_2 \cdot 4H_2O$ (0.05 mg, 0.27 μmol). The solution is then made anaerobic by repeated evacuation and flushing with nitrogen. Sodium dithionite (0.21 mg, ca. 1.2 μmol) in 1 mL of anaerobic 50 mM Tris-HCl buffer pH 7.4 is added to initiate the reaction. Periodically, 1.0-mL portions are removed from the reaction mixture and exposed to air by dilution with air-saturated 1.0 mM EDTA, and the aglycons are extracted into $CH_2Cl_2$. Reverse-phase liquid chromatographic analysis shows that the conversion is approximately 75–80% complete after 15 min. Letting the reaction proceed for an additional 4 h shows no further conversion of **12** to **14**. After 4 h, the remainder of the reaction mixture is worked up by diluting the mixture with 20 mL of 1.0 mM EDTA and extracting it with 5 mL of $CH_2Cl_2$. Reverse-phase liquid chromatographic analysis shows it is approximately 55–60% **14** and 35–40% **12**. The visible absorption spectrum of **14**, corrected for residual **12**, showed $\lambda_{max}$ ($CH_2Cl_2$) at 393 (rel absorbance, 40) and 331 nm (100). Further characterization of **14** is not possible because of its rapid tautomerization to **12** under aerobic conditions.

**Unknown Chromo Compound (Peak 2, Figure 2).** Fraction 2 from the crude fractionation of the dithionite reduction of **4** is rechromatographed on silica gel using 99.9% $CHCl_3$ and 0.1% $CF_3COOH$ as the eluent. Fractions from this column are analyzed by reverse-phase liquid chromatography, and those containing material corresponding to the unknown chromo compound are subjected to preparative thin-layer chromatography using 70.0% hexane, 29.9% EtOAc, and 0.1% $CF_3COOH$ as the eluting solvent. The absorption spectrum of the unknown chromo compound shows $\lambda_{max}$ (MeOH) at 516 sh (rel absorbance, 32), 495 (35), 277 sh (39), 251 sh (85), and 238.5 nm (100).

**Reaction of Daunomycin–Fe(III) Chelate with Dithionite.** A modification of Myer's procedure[29] is used to form the ferric ion chelate of daunomycin. Daunomycin-HCl (3.1 mg, 5.5 μmol) is transferred to a Schlenck tube and dissolved in 10 mL of pH 3.0 $H_2O$. $FeCl_3$ (0.89 mg, 5.5 μmol) is added to the reaction mixture and the pH adjusted to 7.4 using 50 mM Tris base. The volume of the solution is then adjusted to 18 mL using 50 mM Tris-HCl buffer pH 7.4. The solution is made anaerobic by repeated evacuation and flushing with nitrogen. Sodium dithionite (5.1 mg, ca. 29 μmol) in 2 mL of anaerobic 50 mM Tris-HCl buffer pH 7.4 is added to initiate the reaction. The progress of the reaction is followed by removing 0.5-mL portions at various time intervals, diluting these with 8 mL of 1.0 mM EDTA, and extracting with 3 mL of $CH_2Cl_2$. The $CH_2Cl_2$ extracts are then analyzed by reverse-phase liquid chromatography (Figure 4C). This analysis showed that the reaction was over 90% complete within 1 h. After 7 h at ambient temperature the remaining reaction mixture is exposed to oxygen and diluted with 100 mL of 1.0 mM EDTA. This solution is extracted with $CH_2Cl_2$ (4 × 20 mL) and the $CH_2Cl_2$ layer washed with 100 mL of $H_2O$. After this layer is dried ($Na_2SO_4$), the $CH_2Cl_2$ is evaporated to provide 2.1 mg of solid (100% weight recovery). Reverse-phase liquid chromatographic analysis of the product mixture shows its composition to be **8**, 66.8%; peak 6 (Figure 2), 13%; **13**, 8.7%; peak 3 (Figure 2), 4.0%; **14**, 2.5%; **12**, 2%; **11**, 1.6%; **4**, 1.4%; and unknown chromo compound, 0.1%.

**Dithionite Reduction of 1a.** Daunomycin hydrochloride (3.1 mg, 5.5 μmol) is reduced by sodium dithionite (5.1 mg, ca. 29 μmol) by using a procedure analogous to that outlined previously for the daunomycin–Fe(III) chelate (except that $FeCl_3$ is not included in the reaction mixture). Periodically, 0.5-mL portions of the reaction mixture are removed and analyzed as above (Figure 4C). The reaction is worked up, as above, after 7 h to yield 2.1 mg of products (100% weight recovery). Reverse-phase liquid chromatographic analysis of the final product mixture shows its composition to be **8**, 67.2%; peak 6 (Figure 2), 13%; **13**, 8.0%; peak 3 (Figure 2), 4.3%; **14**, 3.1%; **12**, 2%; **11**, 1.5%; **4**, 1.4%; and unknown chromo compound, 0.1%.

**Reaction of 10-Deoxydaunomycinone–Fe(III) Chelate with Dithionite.** A modification of Myer's procedure[29] is used to form the ferric ion chelate of 10-deoxydaunomycinone. 10-Deoxydaunomycinone (**4**) (2.2 mg, 5.8 μmol) is placed in a Schlenck tube and then is dissolved in 18 mL of a solution which contains 10 mM Tris-HCl and is 55% MeOH, 45% $H_2O$. $FeCl_3$ (0.97 mg, 6.0 μmol) is added to the reaction mixture to form the ferric ion chelate of **4**. Immediately after the addition of $FeCl_3$, Tris base (21.8 mg, 180 μmol) is added so that the reaction mixture is 10 mM in both Tris-HCl and Tris base. The solution is made anaerobic by repeated evacuation and flushing with nitrogen. Sodium dithionite (4.0 mg, ca. 23 μmol) in 3.0 mL of anaerobic 50 mM Tris-HCl buffer, pH 7.4, is added to initiate the reaction. The progress of the reaction is followed by using the same procedure cited above for the dithionite reaction of the ferric ion chelate of **1a**. This reaction is approximately 90% complete after 3 h (Figure 4D). After 7 h at ambient temperature the remaining reaction mixture is exposed to oxygen and then diluted with 100 mL of 1.0 mM EDTA. This solution is extracted with $CH_2Cl_2$ (4 × 20 mL), and the $CH_2Cl_2$ layer is then washed with 100 mL of $H_2O$. After the solution is dried ($Na_2SO_4$), the $CH_2Cl_2$ is evaporated to provide 1.9 mg of solid (100% corrected weight recovery). Reverse-phase liquid chromatographic analysis of the final product mixture shows its composition to be **8**, 52.8%; **13**, 15.3%; peak 6 (Figure 2), 15%; **11**, 4.9% peak 3 (Figure 2), 4.8%; **14**, 3.0%; **4**, 2.2%; and **12**, 2%.

**Reaction of 4 with Dithionite in Aqueous Methanol.** 10-Deoxydaunomycinone (**4**) (2.2 mg, 5.8 μmol) is reduced by sodium dithionite (4.0 mg, ca. 23 μmol) by using a procedure analogous to that outlined above for the dithionite reduction of the ferric ion chelate of **4** (except that $FeCl_3$ is not present in the reaction mixture). This reaction is approximately 90% complete after 5 h (Figure 4A). After 7 h the reaction is worked up, as above, to yield 1.9 mg of products (100% corrected weight recovery). Reverse-phase liquid chromatographic analysis of the final product mixture showed its composition to be **8**, 51.5%; peak 6 (Figure 2), 11%; **13**, 10.7%; **4**, 10.2%; peak 3 (Figure 2), 5.3%; **14**, 5.0%; **11**, 4.4%; and **12**, 2%.

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