chanically stirred solution of **12** (70 mg, 0.166 mmol) in 3 mL of methanol at 20 °C was added 1.0 mg of potassium hydroxide. The solution was stirred at 20 °C for 12 h. To the reaction mixture was added small pieces of dry ice. The solution was diluted with chloroform and washed with brine. The organic layer was dried and concentrated. The crude product was purified by silica gel chromatography using 5% methanol in chloroform to afford 37 mg (64% yield from **12**) of **4**.

4: 400-MHz ^{1}H NMR ($CDCl_3$) δ 0.7 (1 H, br s, 1a-NH), 1.82 (1.4 H, t, J = 2.2 Hz, 6-CH_2D), 1.85* (0.9 H, s, 6-CH_3), 2.83 (1 H, dd, J = 4.2, 1.7 Hz, 2-H), 2.92 (1 H, d, J = 4.2 Hz, 1-H), 3.22 (3 H, s, 9a-OCH_3), 3.48 (1 H, dd, J = 12.8, 1.7 Hz, 3α-H), 3.62 (1 H, dd, J = 10.6, 4.4 Hz, 9-H), 4.04 (3 H, s, 7-OCH_3), 4.05 (1 H, d, J = 12.8 Hz, 3β-H), 4.56 (1 H, t, J = 10.6 Hz, 10-Ha), 4.74 (1 H, dd, J = 10.6, 4.4 Hz, 10-Hb), 4.89 (2 H, br s, $OCONH_2$) (*, peak due to natural mitomycin A (**2**)); 61-MHz ^{2}H NMR ($CHCl_3$) δ 1.85 (t, J = 2.2 Hz); EIMS m/z 350 (M^+); HRMS m/z for $C_{16}H_{18}DN_3O_6$ calcd 350.1337, found 350.1347; IR (KBr) 3380, 1706, 1633, 1551, 1434, 1323, 1219, 1061 cm^{-1}.

Acknowledgment. We are extremely grateful to Hitoshi Arai for carrying out HPLC analysis and for his valuable discussion.

Registry No. **3**, 125685-49-6; **4**, 125685-50-9; **7**, 4901-84-2; **8** (isomer 1), 125761-41-3; **8** (isomer 2), 125761-42-4; **9**, 125685-48-5; **10** (isomer 1), 122675-60-9; **10** (isomer 2), 122644-74-0; **11**, 122644-75-1; **12**, 122644-76-2.

Reductive Transformations of 10-Deoxydaunomycinone[†,1,2]

David J. Brand*[,‡] and Jed F. Fisher*[,§]

Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455, and Cardiovascular Diseases Research, The Upjohn Company, Kalamazoo, Michigan 49001

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An enzyme system consisting of spinach ferredoxin-$NADP^+$ reductase (ferredoxin reductase; EC 1.18.1.2) and pig heart isocitric dehydrogenase($NADP^+$) (isocitric dehydrogenase; EC 1.1.1.42), along with spinach ferredoxin and either of the coenzymes NAD(P)H, was utilized in the reduction of aqueous anaerobic solutions of daunomycin and related aglycons. Typically, reductive transformations of daunomycin yielded three major and several minor aglycon products, as detected by reverse-phase liquid chromatography analysis. The ferredoxin reductase component of the enzymatic system elicited the reductive deglycosylation of the daunomycin. Subsequent to the loss of the daunosamine sugar were several keto-enol tautomeric equilibria of the hydroquinone intermediates that result in the transformation of the anthracycline ring system in a fashion identical with that observed during the sodium dithionite reduction of daunomycin (Brand, D. J.; Fisher, J. *J. Am. Chem. Soc.* **1986**, *108*, 3088-3096). Additionally, an aldo-keto reductase contaminant in the isocitric dehydrogenase enzyme preparation caused the stereoselective reduction of the acetyl side chain found in daunomycin and related aglycons, resulting in all the product aglycons having a 1-hydroxyethyl side chain bearing an *S* configuration at the C-1′ stereogenic center. The three major products of the ferredoxin reductase and isocitric dehydrogenase enzyme-catalyzed reduction of daunomycin have been characterized. Two of the major products of the reaction, [*S*-(*R**,*S**)]-1,2,3,4-tetrahydro-2,11-dihydroxy-2-(1-hydroxyethyl)-7-methoxy-5,12-naphthacenedione (**16**; 50–55% of the product) and [*S*-(*R**,*S**)]-1,2,3,4-tetrahydro-2,6-dihydroxy-2-(1-hydroxyethyl)-7-methoxy-5,12-naphthacenedione (**17**; 15–20% of the product), involve both the loss of an oxygen from the anthracycline's C ring and the reduction of the side-chain acetyl group. The third major product and two of the minor products were a diastereomeric set of "leuco" tautomers of the hydroquinone produced upon reduction of the anthracycline ring system; reduction of the acetyl side chain also occurred. These three diastereomers of [2*R*-[2α,2(*S**)]]-1,2,3,4,4a,12a-hexahydro-2,6,11-trihydroxy-2-(1-hydroxyethyl)-7-methoxy-5,12-naphthacenedione differ in the stereochemistry at the C-4a, C-12a ring juncture. The major diastereomer (**19**; 20–25% of the product) has a trans ring juncture, while the two minor diastereomers (**20** and **21**; 0–3% and 5–8% of the product) have a cis ring juncture. Virtually identical results were observed when either daunomycinone or (1′*S*)-1′-dihydrodaunomycinone (**11**), instead of daunomycin, were used as a substrate for the enzyme system. However, a chromatographically different set of products, epimeric to those formed when (1′*S*)-1′-dihydrodaunomycinone was the substrate, were produced when (1′*R*)-1′-dihydrodaunomycinone (**12**) was used. All of these products had the *R* configuration at the C-1′ stereogenic center. This series of results may prove of value in the synthetic preparation of 1′-anthracyclinols and in the study of the possible role of 1′-anthracyclinol aglycons and glycosides in the expression of the anthracycline's dose-limiting cardiotoxicity.

A viable antineoplastic agent is one that is selectively toxic to the neoplasm rather than toward the host organism. Quinones account for a disproportionate number of these cytotoxic substances. This functional group is found in two of the most useful antitumor antibiotics classes (mitomycins and anthracyclines), as well as in several of the newer antitumor structures (e.g., mitoxantrone and fredericamycin). The cytotoxicity of the quinones has led to the speculation that there is an intrinsic chemical property of the quinone moiety that expresses antitumor activity.[3] An obvious possibility is the quinone's capacity

[†] This paper is dedicated to the memory of Paul F. Wiley, in recognition of his achievements in natural product chemistry, in general, and in the anthracyclines in particular.

[‡] Present address: 14 D Oak Leaf Lane, Circle Pines, MN 55014.

[§] Present address: The Upjohn Company.

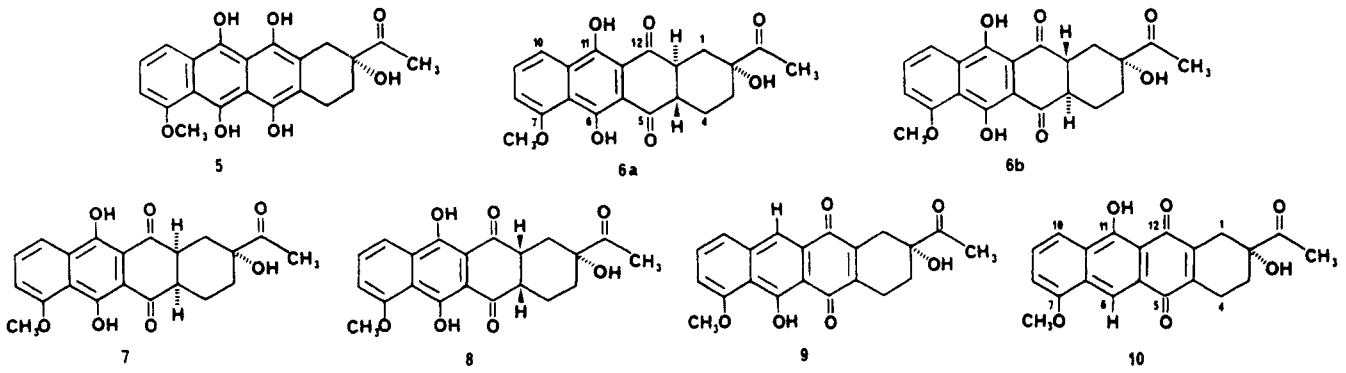
(1) For indexing purposes the anthracyclinone ring system is numbered in this paper in accordance with the numbering system of *Chemical Abstracts*, which is at variance with the numbering system introduced by Brockmann[2] that is used by most workers in the anthracycline field. However, for reasons of convenience to the reader, the 8-(1-hydroxyethyl) aglycons will be referred to as 1′-dihydro aglycons.

(2) Brockmann, H. *Prog. Chem. Org. Nat. Prod.* **1963**, *21*, 121-182.

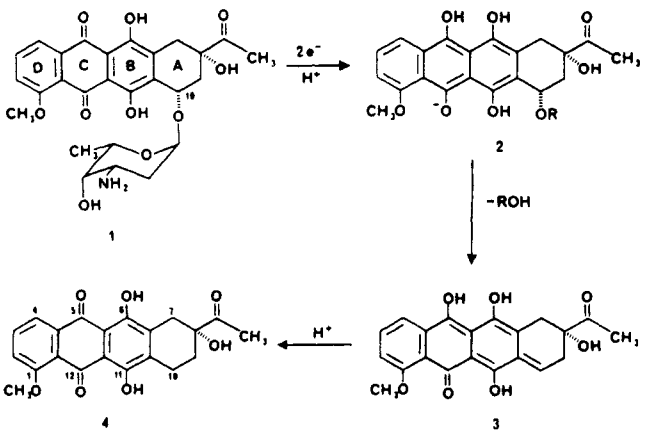
(3) For recent, general discussions of this phenomenon, see: (a) Pullman, B. *Int. J. Quantum Chem., Quantum Biol. Symp.* **1986**, *13*, 95-105. (b) Cohen, G. M.; d'Arcy Doherty, M. *Br. J. Cancer, Suppl.* **1987**, *55*, 46-52. (c) Butler, J.; Hoey, B. M. *Br. J. Cancer, Suppl.* **1987**, *55*, 53-59.

0022-3263/90/1955-2518$02.50/0

Chart I



Scheme I



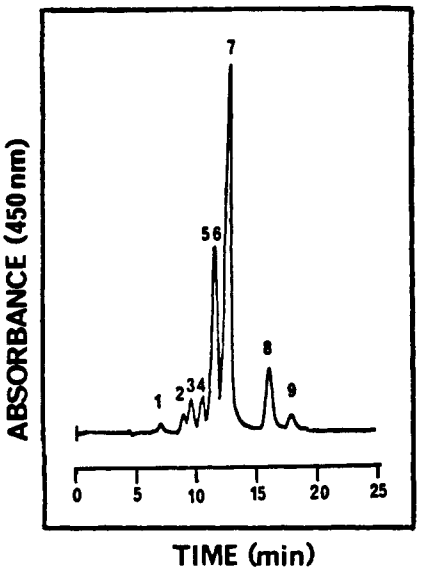


Figure 1. Reverse-phase analytical high performance liquid chromatographic analysis, as described in the Experimental Section, of a reaction mixture portion taken 9 h after the addition of excess dithionite to an anaerobic solution of 10-deoxydaunomycinone (4, 0.35 mM) in buffered methanol (10 mM Tris-HCl/10 mM Tris base).[20] Peak 1, (*R*)-9-acetyl-7,9,10,12-tetrahydro-6,9,11-trihydroxy-4-methoxy-5(8*H*)-naphthacenone; peak 2, unknown chromo compound; peak 3, cis-leuco A **7** or **8**; peak 4, (*R*)-8-acetyl-7,9,10,12-tetrahydro-6,8,11-trihydroxy-1-methoxy-5(8*H*)-naphthacenone; peak 5, **9**; peak 6, cis-leuco B **7** or **8**; peak 7, trans-leuco **6a** or **6b**; peak 8, **4**; peak 9, **10**.

to participate in redox chemistry. Moore[4] has made note of the fact that many cytotoxic quinones contain appropriately placed leaving groups that may be activated to alkylating intermediates by quinone reduction. Likewise, Sartorelli, Lin, and colleagues[5] have focused on this concept of "bioreductive alkylation" as the theme to their efforts to develop a solid-tumor active antibiotic. It should be noted that to date only in the case of mitomycin C[6] has substantial evidence accrued in support of bioreductive alkylation playing a role in a compound's antineoplastic properties. Bioreductive alkylation, however, still remains a viable hypothesis both to account for the activity of other known antitumor antibiotics and as a basis for designing new structures.

The relevance of bioreductive alkylation to the antitumor efficacy[7] of the anthracyclines remains unproven.[8a,b] Nevertheless it is certain that a portion of the anthracyclines are metabolized via reduction and with subsequent loss of their benzylic glycoside to provide a quinone methide intermediate.[8c] This sequence is illustrated for daunomycin (1) in Scheme I. Daunomycin can be reduced, with participation of a suitable enzyme catalyst, to its hydroquinone. In the absence of oxygen,[9] glycoside loss proceeds from this hydroquinone anion **2** (or tautomer thereof)[10] to yield[11] the quinone methide **3**. In the course of anthracycline metabolism, this intermediate functions as a nucleophile and is intercepted by a proton to provide[12] the 10-deoxyaglycon **4**. Koch and Kleyer have demonstrated that this quinone methide is also trapped by other

(4) (a) Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249–280. (b) Moore, H. W.; Czerniak, R.; Hamdan, A. *Drugs Exp. Clin. Res.* **1986**, *12*, 475–494.

(5) (a) Kirkpatrick, D. L.; Johnson, K. E.; Sartorelli, A. C. *J. Med. Chem.* **1986**, *29*, 2048–2052. (b) Wilson, I.; Wardman, P.; Lin, T. S.; Sartorelli, A. C. *Chem.-Biol. Interact.* **1987**, *61*, 229–240.

(6) (a) Pan, S.-S.; Iracki, T.; Bachur, N. R. *Mol. Pharmacol.* **1986**, *29*, 622–628. (b) Zein, N.; Kohn, H. *J. Am. Chem. Soc.* **1987**, *109*, 1576–1577. (c) Tomasz, M.; Lipman, R.; Lee, M. S.; Verdine, G. L.; Nakanishi, K. *Biochemistry* **1987**, *26*, 2010–2027. (d) Tomasz, M.; Lipman, R.; Chowdary, D.; Pawlik, J.; Verdine, G. L.; Nakanishi, K. *Science (Washington, D.C.)* **1987**, *235*, 1204–1208. (e) Sartorelli, A. C. *Biochem. Pharmacol.* **1986**, *35*, 67–69. (f) Franck, R. W.; Tomasz, M. In *Chemistry of Antitumor Agents*; Wilman, D. F. V., Ed.; Blackie and Sons Ltd.: Scotland (in press), 1989.

(7) Arcamone, F. *Cancer Res.* **1985**, *45*, 5995–5999.

(8) (a) Abdella, B. R. J.; Fisher, J. *EHP, Environ. Health Perspect.* **1985**, *64*, 3–18. (b) Fisher, J. F.; Aristoff, P. A. *Prog. Drug Res.* **1988**, *32*, 411–498. (c) Powis, G. *Pharmacol. Ther.* **1987**, *35*, 57–162.

(9) Bachur, N. R.; Gordon, S. L.; Gee, M. V.; Kon, H. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 954–957.

(10) (a) Blankespoor, R. L.; Schutt, D. L.; Tubergen, M. B.; De Jong, R. L. *J. Org. Chem.* **1987**, *52*, 2059–2064. (b) Blankespoor, R. L.; Hsung, R.; Schutt, D. L. *J. Org. Chem.* **1988**, *53*, 3032–3035.

(11) (a) Kleyer, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1983**, *105*, 2504–2505. (b) Kleyer, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1984**, *106*, 2380–2387.

(12) (a) Fisher, J.; Ramakrishnan, K.; Becvar, J. E. *Biochemistry* **1983**, *22*, 1347–1355. (b) Boldt, M.; Gaudiano, G.; Koch, T. H. *J. Org. Chem.* **1987**, *52*, 2146–2153.

electrophiles (e.g., benzaldehyde).[13] Alternatively, the methide can act as an electrophile and react with thiol nucleophiles to yield, after oxidation, 10-alkylated thiol ethers.[14] In the course of examining these events we became intrigued by the possibility that interesting redox chemistry might be found even after the quinone methide generation and subsequent trapping has occurred. In particular, focus was placed on the consequences of 10-deoxydaunomycinone (4) reduction. This is the major product of the *first* anaerobic reduction of daunomycin and possesses virtually the same quinone oxidant as 1. Should this quinone now encounter the same enzyme that accomplished the daunomycin reduction, it will be reduced to the hydroquinone 5. In the near anaerobic environment of the anthracycline metabolizing cell,[15] this hydroquinone may persist, thus potentially allowing for further transformation. But what chemical opportunities remain to this hydroquinone? It cannot participate in quinone methide chemistry. It can, however, act as a reductant to oxygen, other quinones, and metals.[16,18] And, lastly, if left to itself it will metamorphose by way of several competing tautomeric equilibria to produce a total of nine products[20] (as indicated by the RP-HPLC chromatogram shown in Figure 1). These tautomeric equilibria are our present focus.

The competing equilibria are of two types. In the first, a bis enol to ketone transposition of the B ring of 5 yields three (one trans and two cis) "leuco" diastereomers (6, 7, and 8) (Chart I). The most abundant is trans at the A–B ring juncture (**6a**, 4aβ,12aα, or **6b**, 4aα,12aβ). The second equilibrium also is an enol to ketone tautomerization of the B ring of 5, but with carbon protonation occurring at one of the two available sites in the C ring. This permits rearomatization by an irreversible loss of water, and both possible isomers (9 and 10) are obtained. Precedence exists for both types of tautomeric equilibration. The formation of leuco structures is occasionally encountered during synthetic efforts,[21] and it has been suggested that fusarubin is biosynthesized as the leuco tautomer.[22] The second tautomerization process has been observed by several others,[23,24a] and a completely analogous process accounts for the kinetic peculiarities of 12-iminodaunorubicin reduction.[24] Although no biological significance has yet to be associated to 10-deoxydaunomycinone hydroquinone tautomerization, our focus was to establish chemical competence for these processes under more biomimetic conditions than in our first study.[20] In that case rather high hydroquinone concentrations were sustained by chemical reduction of 4 (using sodium dithionite) in an aqueous methanol solution. Since efficient enzymatic catalysis of these events is easily arranged,[17a] and authentic materials were in hand, a comparison of the enzymatic versus chemical reduction of 10-deoxydaunomycinone was in order.

Results

The key experiment was no more difficult than to provide a suitable reductant and enzyme catalyst to an anaerobic aqueous solution of daunomycin and to observe the spectrum of products as it evolved. The reductant used was NADPH, and the catalyst used was spinach ferredoxin–$NADP^+$ reductase (ferredoxin reductase; EC 1.18.1.2). Since inhibition of the ferredoxin reductase by the oxidation product, $NADP^+$, limits the practical efficiency of this system for anthracycline reduction,[17a,25] an enzymatic $NADP^+$ to NADPH recycling system was also provided. While there are several available recycling systems, the most convenient [oxidation of isocitrate by $NADP^+$ using the Mn^{2+}-dependent pig heart isocitric dehydrogenase($NADP^+$) (isocitric dehydrogenase; EC 1.1.1.42)] was chosen. Lastly, the iron–sulfur protein ferredoxin was included to increase the forward reduction velocity and mitigate $NADP^+$ inhibition.[17a] The complete reaction mixture thus consists of an anaerobic solution of NADPH, ferredoxin reductase, ferredoxin, Mn^{2+}, DL-isocitrate, isocitric dehydrogenase, and the anthracycline. At anthracycline concentrations of ca. 50 μM a catalytic velocity (relative to ferredoxin reductase) for hydroquinone formation of ca. 1 s^{-1} was obtained.

Under these conditions daunomycin was rapidly transformed through the sequence: daunomycin hydroquinone ... quinone methide ... 10-deoxydaunomycinone ... 10-deoxydaunomycinone hydroquinone (5). During the course of these events portions were removed from the reaction, exposed to oxygen (to oxidize all remaining hydroquinones), and then chromatographically analyzed to determine the product distribution. From previous experience (e.g., Figure 1) a complex chromatogram was anticipated as 5 dissipates by the tautomeric equilibria. In point of fact, the chromatogram after a 0.5-h reaction period was complex beyond our expectations, showing no less than nine products arising from 5 (Figure 2a). In particular, several of these products had longer retention times than those produced from the sodium dithionite reduction[20] of 1. Figure 2a also indicates that, under these conditions, no 5 remains after the 0.5 h point, as evidenced by the absence of 4 in the chromatogram.[26] The compounds that were present in the product mixture shown in Figure 2a include a trans-leuco[27] (**6a** or **6b**, peak 7 of Figure 1), a cis-leuco (7 or 8, peak 6 of Figure 1), 9, and 10. The other cis-leuco (7 or 8, peak 3 of Figure 1) was probably present in the chromatogram, but was obscured by the other peaks present. Subsequent chromatograms suggested that the remaining peaks found in Figure 2a were derived from these initially formed compounds.[28] Further analysis at intermediate time points showed that the major initial

(13) Kleyer, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1983**, *105*, 5154–5155.
(14) (a) Ramakrishnan, K.; Fisher, J. *J. Am. Chem. Soc.* **1983**, *105*, 7187–7188. (b) Ramakrishnan, K.; Fisher, J. *J. Med. Chem.* **1986**, *29*, 1215–1221.
(15) (a) Nastainczyk, W.; Ullrich, V.; Sies, H. *Biochem. Pharmacol.* **1978**, *27*, 387–392. (b) Gewirtz, D. A.; Yanovich, S. *Biochem. Pharmacol.* **1986**, *35*, 4059–4064. (c) Gewirtz, D. A.; Yanovich, S. *Biochem. Pharmacol.* **1987**, *36*, 1793–1798.
(16) The oxygen lability of the hydroquinone and its ability to act as a hydride donor are well-known.[17]
(17) (a) Fisher, J.; Abdella, B. R. J.; McLane, K. E. *Biochemistry* **1985**, *24*, 3562–3571. (b) Kleyer, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1983**, *105*, 5911–5912.
(18) In the reactions with metals it is not generally possible to distinguish between semiquinone and hydroquinone reduction.[19]
(19) (a) Demant, E. J. F. *FEBS Lett.* **1984**, *176*, 97–100. (b) Thomas, C. E.; Aust, S. D. *Arch. Biochem. Biophys.* **1986**, *248*, 684–689. (c) Gianni, L.; Zweier, J. L.; Levy, A.; Myers, C. E. *J. Biol. Chem.* **1985**, *260*, 6820–6826.
(20) Brand, D. J.; Fisher, J. *J. Am. Chem. Soc.* **1986**, *108*, 3088–3096.
(21) Gupta, R. C.; Jackson, D. A.; Stoodley, R. J.; Williams, D. J. *J. Chem. Soc., Perkin Trans. 1* **1985**, 525–533.
(22) Kurobane, I.; Zaita, N.; Fukuda, A. *J. Antibiot.* **1986**, *39*, 205–214.
(23) (a) Krohn, K.; Priyono, W. *Liebigs Ann. Chem.* **1986**, 1506–1527. (b) Laatsch, H.; Anke, H. *Liebigs Ann. Chem.* **1982**, 2189–2215.
(24) (a) Tresselt, D.; Ihn, W.; Horn, G.; Berg, H. *Pharm.* **1984**, *39*, 417–419. (b) Bird, D. M.; Boldt, M.; Koch, T. H. *J. Am. Chem. Soc.* **1987**, *109*, 4046–4053.
(25) Dykes, J.; Davis, D. J. *Arch. Biochem. Biophys.* **1982**, *218*, 46–50.
(26) Any untautomerized hydroquinone 5 should be converted to 10-deoxydaunomycinone (4) upon exposure to oxygen.
(27) We have utilized the term leuco to describe yellow-colored aglycons that have two λ_{max} at ca. 442 and 417 nm.[20] Trans-leuco compounds have a trans A,B ring juncture (e.g. 6), while cis-leuco compounds (e.g. 7) have a cis A,B ring juncture.
(28) Brand, D. J. Ph.D. Thesis, University of Minnesota, Feb. 1987 (*Chem. Abstr.* 1988, *107*, 216084f).

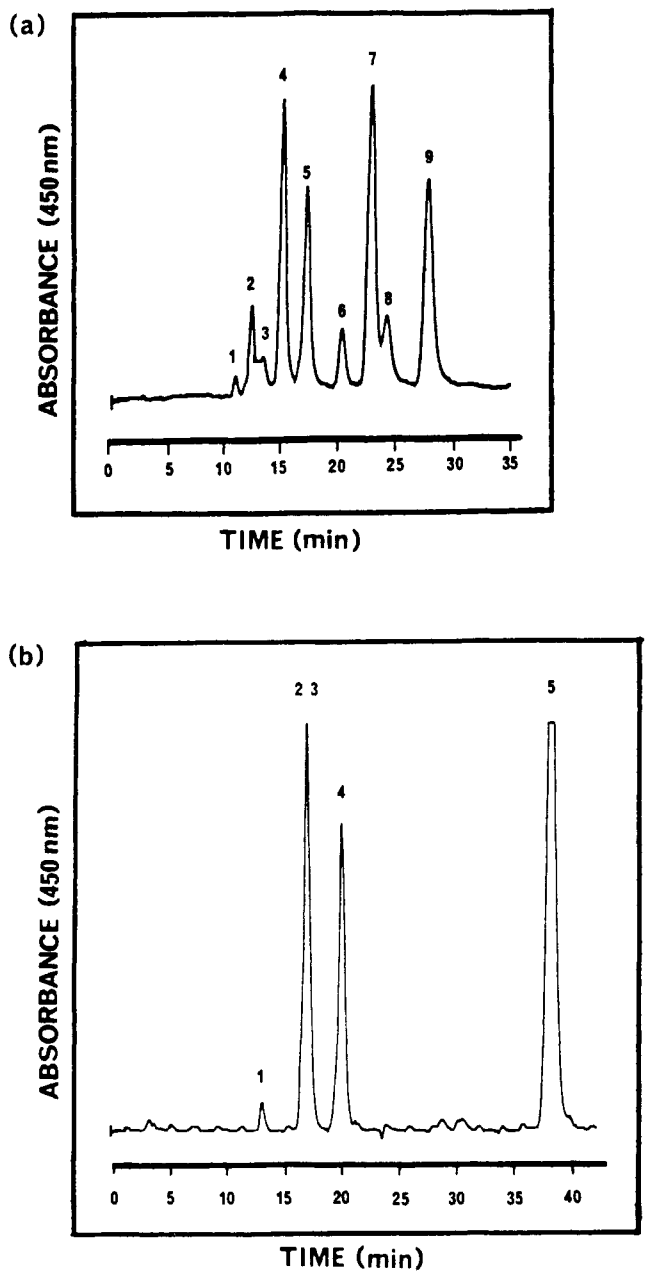


Figure 2. Reverse-phase analytical high performance liquid chromatographic (RP-HPLC) analyses, as described in the Experimental Section, of reaction mixture portions taken after the addition of the ferredoxin reductase/isocitric dehydrogenase enzyme system to solutions of daunomycin (1) in anaerobic 50 mM Hepes pH 7.0 buffer. (a) RP-HPLC analysis of reaction portion taken 0.5 h after initiation of reaction [daunomycin, 78 μM; ferredoxin reductase, 0.20 μM; ferredoxin, 0.67 μM; isocitric dehydrogenase, 1.3 U/mL; $NADP^+$, 0.47 mM; Mn(II), 4.0 mM; trisodium isocitrate, 25 mM]. Peak 1, 1′*S*-cis-leuco-ol A **20** or **21**, 1%; peak 2, cis-leuco B **7** or **8**, 5%; peak 3, **17**, 4%; peak 4, 1′*S*-trans-leuco-ol **19a** or **19b**, 21%; peak 5, trans-leuco **6a** or **6b**, 16%; peak 6, **18**, 3%; peak 7, **4**, 21%; peak 8, **16**, 7%; peak 9, **10**, 22%. (b) RP-HPLC analysis of the final product mixture derived from an overnight reaction [daunomycin, 25 μM; ferredoxin reductase, 0.62 μM; ferredoxin, 2.2 μM; isocitric dehydrogenase, 0.33 mg/mL; $NADP^+$, 1.2 mM; Mn(II), 4.8 mM; trisodium isocitrate, 15 mM]. Peak 1, 1′*S*-cis-leuco-ol A **20** or **21**, 1%; peak 2, 1′*S*-cis-leuco-ol B **20** or **21**, 9%; peak 3, **17**, 20%; peak 4, 1′*S*-trans-leuco-ol **19a** or **19b**, 17%; peak 5, **16**, 52%. Note: Compounds corresponding to peaks 2 and 3 normally coeluted under the standard chromatographic conditions. The relative percentages of these two compounds, in any given sample, were determined by comparing the area of their combined peak (relative to the area of peak 5 of Figure 2b) at 450 nm vs 485 nm.

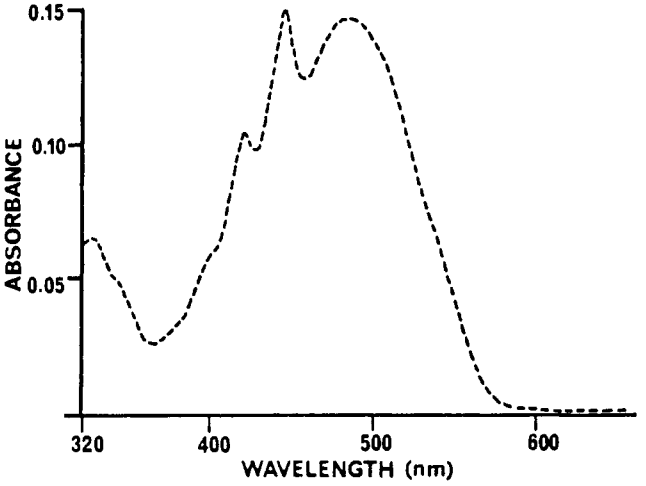


Figure 3. Visible spectrum of a chloroform solution of the final product mixture derived from an overnight reaction of daunomycin (1, 25 μM) with the ferredoxin reductase/isocitric dehydrogenase enzyme system [ferredoxin reductase, 0.62 μM; ferredoxin, 2.2 μM; isocitric dehydrogenase, 0.33 mg/mL; $NADP^+$, 1.2 mM; Mn(II), 4.8 mM; trisodium isocitrate, 15 mM] in anaerobic 50 mM Hepes pH 7.0 buffer.

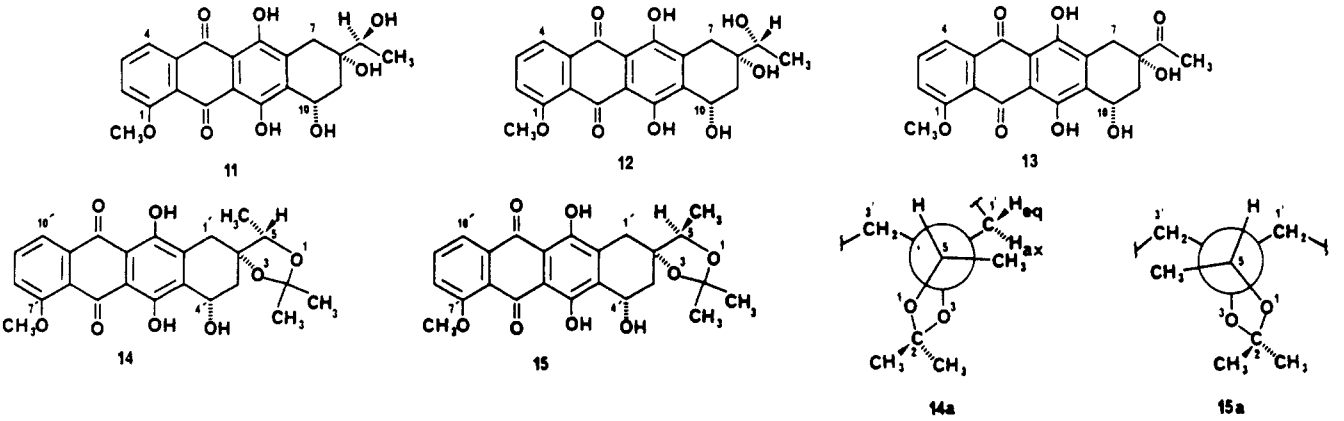
products [including trans-leuco (**6a** or **6b**), **9**, and **10**] progressively diminished, while a total of five ultimate products intensified (Figure 2b). None of these five end products was identical with **6–10**. Yet, examination of an absorption spectrum (Figure 3) of the crude, oxidized final product mixture showed that it was nearly identical with that from chemical reduction.[20] Hence the chromophores of **6–10** were present, but the compounds themselves were not.

Only one explanation reconciles these observations. The expected products of 10-deoxydaunomycinone hydroquinone tautomerization, i.e., **6–10**, were indeed initial products of ferredoxin reductase catalysis. However, these compounds serve as substrates for a second reaction process and go on to produce the final set of products. This ultimate set of products must however maintain the basic constitution of the respective naphthacenediones from which they were derived. The nature of this second reaction was revealed by the isolation and characterization of the three major end products (peaks 3, 4, and 5 of Figure 2b). Each of these three compounds was found to possess a mass of 2 greater than its parent and to have in its ^1H NMR spectrum a one-hydrogen δ 3.6 quartet coupled to a three-hydrogen δ 1.4 doublet. In addition, the acetyl methyl singlet (δ 2.3–2.4) was not present. This indicated that a $CH(OH)CH_3$ moiety, rather than an acetyl group, was found in each final product. Peak 3 (Figure 2b) was the reduction product of **9**, while peak 4 was derived from trans-leuco compound **6** and peak 5 was derived from **10**. The ultimate transformation was then acetyl side-chain reduction of the expected products of 10-deoxydaunomycinone tautomerization.

Aldo–Keto Reductase. Two issues remained unresolved at this point. First, what was the component of the enzymatic reaction system responsible for the acetyl reduction? Second, what was the absolute configuration of the newly produced stereogenic center?[29] The answer to the first question was clearly to be found with the NADPH recycling system. All other components were immediately excluded since each had been used previously in enzyme-catalyzed reductions of anthracyclines, without acetyl

(29) (a) Mislow, K.; Siegel, J. *J. Am. Chem. Soc.* **1984**, *106*, 3319–3328. (b) Brand, D. J.; Fisher, J. *J. Chem. Educ.* **1987**, *64*, 1035–1038.

Chart II



reduction having taken place. This conclusion was confirmed by the following series of parallel experiments. A single pure 10-deoxyaglycon, trans-leuco **6**, was combined with the isocitric dehydrogenase, isocitrate, Mn^{2+}, and $NADP^+$ under anaerobic conditions.[30] A clean conversion to a single product, peak 4 of Figure 2b, ensued. In a like fashion, pure **9** was reduced to a material coinciding with peak 3, and **10** was reduced to the material of peak 5. In each instance, there was no evidence for the presence of more than one product diastereomer.

The stereospecificity of this side-chain reduction process implicated an enzyme catalyst (an aldo–keto reductase) that consumed NADPH to effect acetyl reduction. Since there was only one protein source used in these studies of the side-chain reduction of the 10-deoxyaglycons, this reductase must be a component of the commercial isocitric dehydrogenase. Indeed, this is not a homogeneous enzyme.[31] Using commercial isocitric dehydrogenase of increasing purity, it was observed that the aldo–keto reductase was most abundant in the crudest grade of the enzyme and progressively decreased in content (but was not lost completely) as purer commercial grades of the enzyme were used.[28] Therefore, although the isocitric dehydrogenase replaced the NADPH, the aldo–keto reductase was simultaneously consuming it by acetyl reduction. Most importantly, in the context of the complete reaction system (containing ferredoxin reductase and the isocitric dehydrogenase enzyme preparation), Figure 2 indicates that acetyl side-chain reduction occurs concurrently with the quinone reduction and tautomerization.

Absolute Configuration of the $CH(OH)CH_3$ Segment. Reduction of the acetyl side chain is the major metabolic transformation of this anthracycline family in mammalian tissues.[8c,32] Human liver tissue, for example, has been shown to contain two separate reductase enzymes,[33] with each enzyme capable of producing the same 1′-dihydro isomers. After initial uncertainty,[34] the absolute configuration of the stereogenic center formed upon the enzyme-catalyzed acetyl reduction of a rhodomycinone was established as *S* by Broadhurst et al.[35] and has since been independently confirmed.[36] The standards commonly used to evaluate the stereochemistry of this side-chain reduction are the two epimers of 1′-dihydrodaunomycinone, **11** and **12**. The two epimers are distinguished by their relative polarities (the enzymatic product being the less polar) and by their ^1H NMR spectra. Both epimers (**11** and **12**) were obtained by sequential reduction ($NaBH_4$, pH 10)[37] and acid hydrolysis of daunomycin (**1**), followed by chromatographic separation (Chart II). Independently, a single diastereomer was obtained by treating daunomycinone (**13**) with NADPH and the pig heart aldo–keto reductase (found in the isocitric dehydrogenase). The two diastereomers, **11** and **12**, were easily distinguished by reverse phase chromatography; the enzymatic product coelutes with the longer retention time (less polar) epimer. Additionally, the ^1H NMR spectrum of the enzymatic diastereomer was identical with that reported[36b,c] for the 1′*S* isomer **11**. Final confirmation was obtained by the chemical conversion of the enzymatic isomer to its 1′,8-isopropylidene derivative.[36a,38] For the (1′*S*)-1′-dihydrodaunomycinone (**11**), the corresponding derivative would be **14**, whereas for the 1′*R* epimer the isopropylidene derivative would be **15**. As noted by Dornberger et al.,[36a] the two diastereomers have quite different spatial relationships (as represented by their respective Newman projections, **14a** and **15a**) that are reflected in the spatial transfer of spin polarization (NOE effect) in the ^1H NMR spectra. Irradiation of the enzymatically derived 1′-dihydrodaunomycinone isopropylidene derivative at its δ 1.36 C-5 methyl[38] resulted in a positive NOE effect at H-1′ax and H-1′eq. Irradiation at the δ 4.14 C-5 methine resulted in a positive NOE at H-3′$_{eq}$. Structure **14a** alone is consistent with these results. The product of pig heart aldo–keto reductase reduction of **13** was therefore (1′*S*)-1′-dihydrodaunomycinone (**11**).

Homochiral $CH(OH)CH_3$ Moieties. Although all observations were consistent with a homochiral $CH(OH)CH_3$ segment present across the spectrum of final

(30) The anaerobicity is not required for the ketone reduction, but rather to prevent 10-deoxydaunomycinone formation by oxidation of its hydroquinone (obtained via tautomeric equilibria).

(31) The ferredoxin reductase and ferredoxin utilized were homogeneous.

(32) (a) Felsted, R. L.; Bachur, N. R. *Enzym. Basis Detoxicat.* **1980**, *1*, 281–293. (b) Felsted, R. L.; Bachur, N. R. *Drug Metab. Rev.* **1980**, *11*, 1–60.

(33) (a) Felsted, R. L.; Bachur, N. R. *Prog. Clin. Biol. Res.* **1982**, *114*, 291–305. (b) Vasanthakumar, G.; Ahmed, N. K. *Cancer Chemother. Pharmacol.* **1985**, *15*, 35–39. (c) Ahmed, N. K.; Felsted, R. L.; Bachur, N. R. *J. Pharmacol. Exp. Ther.* **1979**, *209*, 12–19.

(34) Cassinelli, G.; Grein, A.; Merli, S.; Penco, S.; Rivola, G.; Vigevani, A.; Zini, P.; Arcamone, F. *Gazz. Chim. Ital.* **1984**, *114*, 185–188.

(35) Broadhurst, M. J.; Hassall, C. H.; Thomas, G. J. *Tetrahedron Lett.* **1984**, *25*, 6059–6062.

(36) (a) Dornberger, K.; Hubener, R.; Ihn, W.; Thrum, H.; Radics, L. *J. Antibiot.* **1985**, *38*, 1219–1225. (b) Penco, S.; Cassinelli, G.; Vigevani, A.; Zini, P.; Rivola, G.; Arcamone, F. *Gazz. Chim. Ital.* **1985**, *115*, 195–197. (c) Russell, R. A.; Irvine, R. W.; Warrener, R. N. *J. Org. Chem.* **1986**, *51*, 1595–1599.

(37) A slightly modified procedure of Cassinelli et al.,[34] utilizing pH 10 buffer, was used. Running the synthesis in unbuffered aqueous solution gave an entirely different reaction outcome (vide infra).

(38) The numbering of the isopropylidene derivative is that of a spiro compound having a 1,3-dioxolane parent ring system.

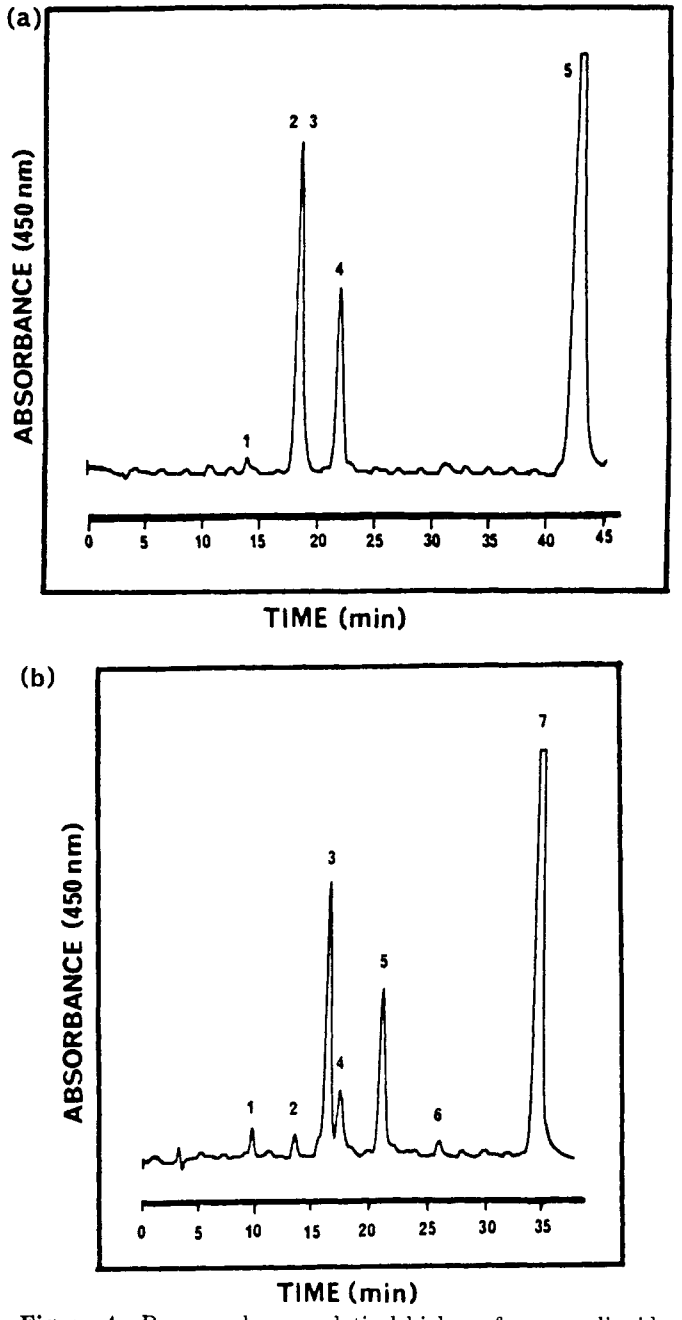


Figure 4. Reverse-phase analytical high performance liquid chromatographic analyses, as described in the Experimental Section, of the final product mixture derived from an overnight reaction of each 1′-dihydrodaunomycinone (**11** or **12**) with the ferredoxin reductase/isocitric dehydrogenase enzyme system [ferredoxin reductase, 1.0 μM; ferredoxin, 1.9 μM; isocitric dehydrogenase, 0.16 mg/mL; $NADP^+$, 0.6 mM; Mn(II), 5.0 mM; trisodium isocitrate, 25 mM] in anaerobic 50 mM Hepes pH 7.0 buffer. (a) Reaction with (1′*S*)-1′-dihydrodaunomycinone (**11**, 25 μM): peak 1, 1′*S*-cis-leuco-ol A **20** or **21**, 1%; peak 2, 1′*S*-cis-leuco-ol B **20** or **21**, 4%; peak 3, **17**, 23%; peak 4, 1′*S*-trans-leuco-ol **19a** or **19b**, 13%; peak 5, **16**, 59%. (b) Reaction with (1′*R*)-1′-dihydrodaunomycinone (**12**, 25 μM): peak 1, unknown, 2%; peak 2, 1′*R*-cis-leuco-ol A **26** or **27**, 2%; peak 3, **22**, 26%; peak 4, 1′*R*-cis-leuco-ol B **26** or **27**, 7%; peak 5, 1′*R*-trans-leuco-ol **25a** or **25b**, 13%; peak 6, **23**, 2%; peak 7, **24**, 50%.

products (Figure 2b), direct experimental proof was easily obtained. Inasmuch as the 10-hydroxyl of (1′*S*)-1′-dihydrodaunomycinone can act as a leaving group, albeit poorer than the daunosamine of daunomycin,[39-41] the (1′*S*)-1′-dihydrodaunomycinone has the potential for quinone methide formation. The quinone methide can then be protonated to give the corresponding 10-deoxyaglycon, which subsequently undergoes quinone reduction. Enzymatic reduction of this aglycon (**11**) gratifyingly produced the same product mixture as daunomycin (Figure 4a). In contrast, the reduction of (1′*R*)-1′-dihydrodaunomycinone (**12**) produced a spectrum of products chromatographically distinguishable from those of (1′*S*)-1′-dihydrodaunomycinone (Figure 4b). This established not only that chromatographic separation of all diastereomers could be achieved but also excluded the possibility of epimerization at the C-1′ stereogenic center. The stereochemical outcome at C-1′ was independent of reaction aerobicity or anaerobicity, which implied that both the quinone and hydroquinone species serve as substrates of the aldo–keto reductase. In addition, all tautomeric species serve as substrates for this enzyme. Thus, it was concluded that the pig heart enzyme accepts a wide diversity of aglycon structures and yet maintains stereochemical fidelity for acetyl reduction.

Products of Combined Ferredoxin Reductase/Aldo–Keto Reductase Reduction of Daunomycinone. The components of the final product mixture (Figure 2b) have now been deduced inasmuch as both the constitutional and stereochemical problems have been solved. The most abundant component (peak 5, Figure 2b; relative yield of 52%)[42] was **16** (Chart III). The basis for this assignment was the close semblance of its ^1H NMR and UV–vis spectra to that of **10**. In a like fashion, structure **17** was assigned to the next most abundant product (peak 3, Figure 2b) on the basis of its similarity to **9**. The third major component (peak 4, Figure 2b) was a yellow (λ_{max} at 442, 417, 397, 264.5, and 238.5 nm), intensely blue fluorescent material. These two features established it as a "classic" leuco[43] hydroquinone tautomer. An *S* absolute configuration at C-1′ followed from the identity with the material obtained from (1′*S*)-1′-dihydrodaunomycinone reduction, and the conversion of the reduction product (in basic, aerobic solution)[44] to (1′*S*)-10-deoxy-1′-dihydrodaunomycinone (**18**). The compound's (peak 4, Figure 2b) ^1H NMR spectrum was similar to that of trans-leuco **6**, showing two large vicinal coupling constants for both C-4a ($J_{4a,12a}$ = 13.3 Hz, $J_{4a,4ax}$ = 11.2 Hz) and C-12a ($J_{12a,4a}$ = 13.2 Hz, $J_{12a,1ax}$ = 11.5 Hz).[21,45] Hence this isomer, 1′*S*-trans-leuco-ol,[46] was assigned to structure **19**; there was no spectral basis to distinguish between the two possibilities **19a** or **19b**. A complete proof of structure was not un-

(39) Malatesta, V.; Penco, S.; Sacchi, N.; Valentini, L.; Vigevani, A.; Arcamone, F. *Can. J. Chem.* **1984**, *62*, 2845–2850.

(40) Our earlier suggestion that the reductive elimination of the leaving group from daunomycin hydroquinone and daunomycinone hydroquinone are comparable[17a] is incorrect.

(41) Abdella, B. R. J. Ph.D. Thesis, University of Minnesota, 1986 (*Chem. Abstr.* **1988**, *107*, 397n).

(42) The relative yields of the five end products from the combined ferredoxin reductase/aldo–keto reductase catalyzed reduction of daunomycin were quite reproducible. Yields typically did not vary more than one-tenth from the reported values [e.g. the relative yield for **16** (peak 5) among several reactions was 52 ± 5%].

(43) (a) Bloom, S. M.; Hutton, R. F. *Tetrahedron Lett.* **1963**, 1993–1997. (b) Kikuchi, M.; Yamagishi, T.; Hida, M. *Dyes Pigm.* **1981**, *2*, 143–151.

(44) This reaction is analogous to the conversion of trans-leuco **6** to 10-deoxydaunomycinone (**4**) in aerobic alkaline solution.[20]

(45) (a) Chandler, M.; Stoodley, R. J. *J. Chem. Soc., Chem. Commun.* **1978**, 997–998. (b) Gupta, R. C.; Harland, P. A.; Stoodley, R. J. *Tetrahedron* **1984**, *40*, 4657–4667.

(46) The terms 1′*S*-trans-leuco-ol, 1′*S*-cis-leuco-ol A, and 1′*S*-cis-leuco-ol B are used to refer to the 10-deoxy-1′-dihydroaglycons that possess a leuco structure and a *S* configuration at the C-1′ stereogenic center. An analogous set of terms, 1′*R*-trans-leuco-ol, 1′*R*-cis-leuco-ol A, and 1′*R*-cis-leuco-ol B, is used to indicate the 10-deoxy-1′-dihydroaglycons that possess a leuco structure and a *R* configuration at the C-1′ stereogenic center.

Chart III

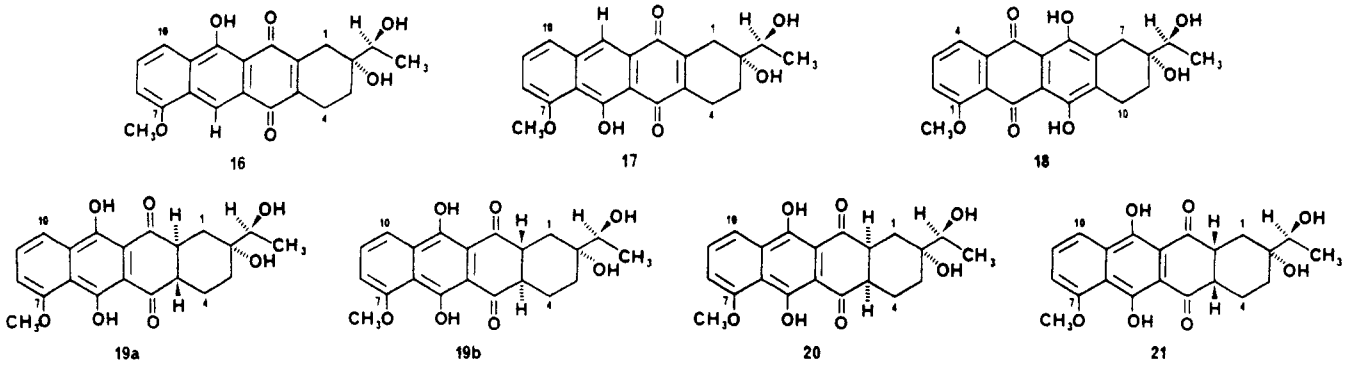
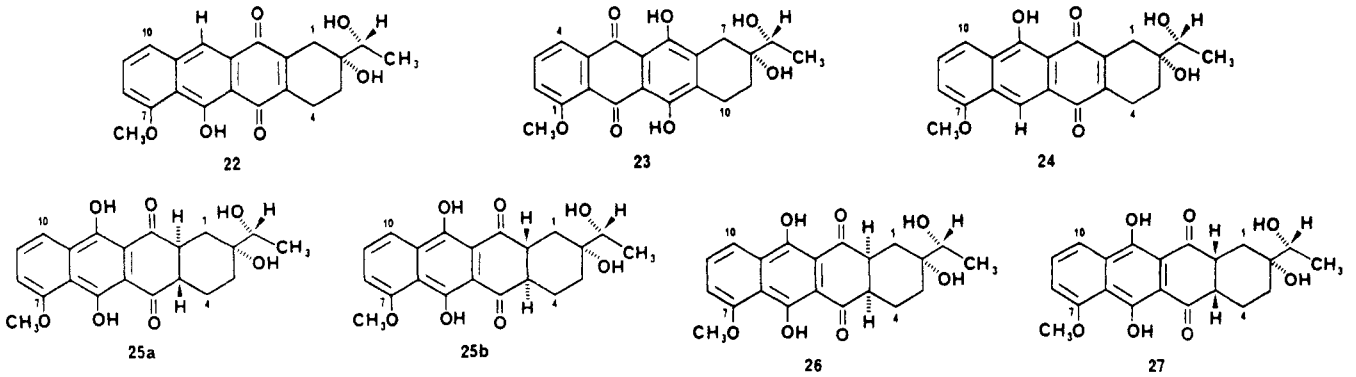


Chart IV



dertaken for the remaining minor components; nevertheless, a tentative assignment can be made. The first minor product (peak 1, Figure 2b) may be reasonably assigned to either structure **20** or **21**. This was based upon the reduction of the more polar cis-leuco isomer (peak 6, Figure 1; **7** or **8**) with the aldo–keto reductase. In this reaction the first product (15% yield) coelutes with peak 1 (Figure 2b) and does not absorb at 485 nm (consistent with a leuco structure). The remaining product (85% yield) was **18**. Thus, the C-4a,C-12a ring juncture of this minor product (peak 1, Figure 2b; 1′*S*-cis-leuco-ol A) was cis and the stereochemistry at C-1′ is *S*. The second minor component (peak 2, Figure 2b) was the second dihydro cis-leuco diastereomer. Reaction of the less polar cis-leuco isomer (peak 3, Figure 1; **7** or **8**) with the aldo–keto reductase found in the NADPH recycling systems gives a 19% yield of material (1′*S*-cis-leuco-ol B) corresponding to peak 2 (Figure 2b), which upon reverse liquid chromatographic analysis (monitoring at different wavelengths) gave results consistent with the leuco structure having been retained. The remaining product from the reaction is once again **18**. Aside from the issue of acetyl reduction, the tautomeric behavior of 10-deoxydaunomycinone hydroquinone was therefore similar when either enzymatic reduction or chemical reduction[20] was carried out.

Products of Combined Ferredoxin Reductase/Aldo-Keto Reductase Reduction of (1′*R*)-1′-Dihydrodaunomycinone. The $NaBH_4$ reduction of daunomycin (**1**) under basic conditions (see above), when followed by acid hydrolysis, gives a near equimolar mixture of (1′*R*)-1′-dihydrodaunomycinone and (1′*S*)-1′-dihydrodaunomycinone. Separation of the two diastereomers was achieved by normal phase chromatography. The use of the combined enzyme system to reduce the purified 1′*R* isomer **12** led to four major and three minor products (Figure 4b). The absorption spectrum of the crude oxidized reaction showed the presence of both leuco and "chromo"[47] aglycons. The characterization of the major products was simplified greatly by an observation made in optimizing the above $NaBH_4$ reduction. When 10-deoxydaunomycinone (**4**) was reduced with $NaBH_4$ in EtOH, diasteromeric sets of three chromo products were obtained (Figure 5a). Peaks 2, 4, and 6 were identified, by comparison with authentic materials, as the 1′*S* species **17**, **18**, and **16**, respectively. Isolation of the three remaining peaks indicated that peak 1 was the 1′*R* diastereomer (**22**) of peak 2, peak 3 was the 1′*R* diastereomer (**23**) of peak 4, and peak 5 was the 1′*R* diastereomer (**24**) of peak 6 (Chart IV). Furthermore, the conditions of the $NaBH_4$ reduction could be adjusted so as to produce **18** and **23** as the main products. If sufficient NaOMe was added to convert **4** to its phenolate anion (as evinced by the reaction becoming a translucent violet), then $NaBH_4$ reduction yielded predominantly **18** and **23** (Figure 5b). This indicated that the C ring quinone of **4** was less likely to be reduced by the $NaBH_4$ when the B ring was deprotonated, but this deprotonation did not affect the ease of side-chain reduction. The reverse phase liquid chromatographic analyses depicted in Figure 5 illustrate a common feature of the 1′-dihydro aglycons. Those aglycons possessing a 1′*S* configuration have longer retention times than their 1′*R* epimers. This observation agrees with previous analyses.[34,35] The only observed exceptions to the generality that the 1′*S* stereoisomer was the less polar epimer were the leuco compounds, as exemplified by the 1′*R* and 1′*S* trans-leuco diastereomers, 1′*R*-trans-leuco-ol and 1′*S*-trans-leuco-ol. As noted below these compounds coeluted under our reverse phase conditions.

With the $NaBH_4$ reduction of **4** providing a generous supply of the 1′*R* chromo diastereomers, an analysis of the enzymatic reduction products of (1′*R*)-1′-dihydrodaunomycinone was simplified. Peaks 1, 3, 6, and 7 of Figure 4b absorbed at 485 nm and hence were chromo diaste-

(47) We have utilized the term chromo to characterize red-orange aglycons that have a λ_{max} of ca. 480–500 nm in solution.[20]

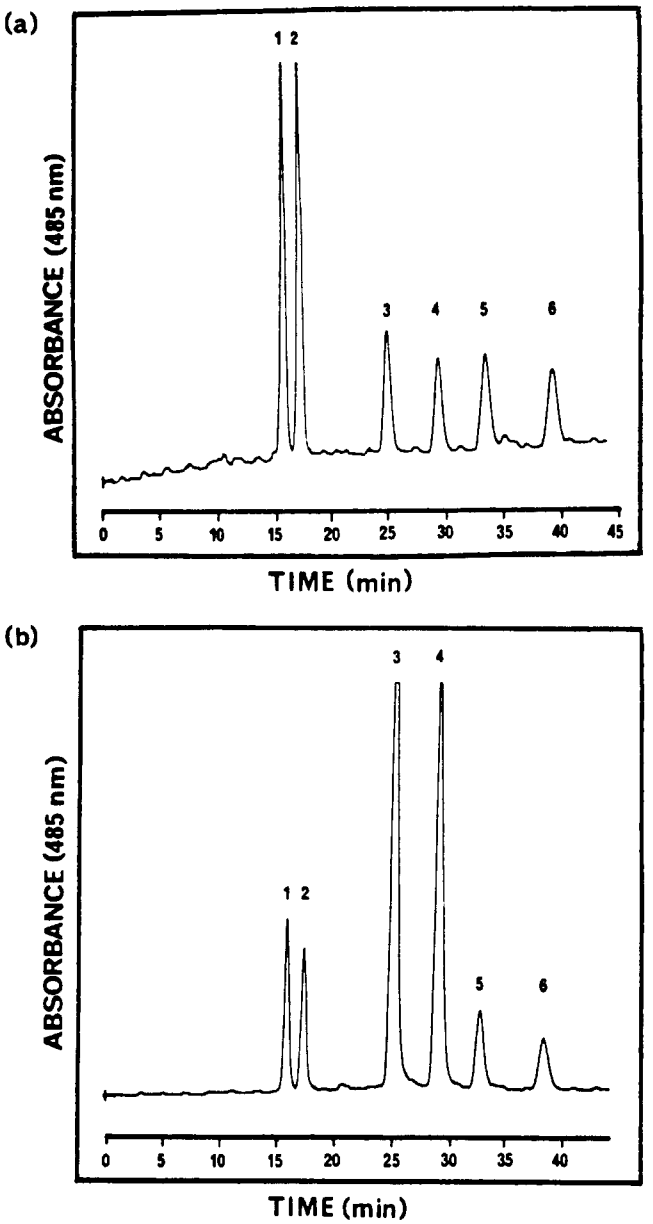
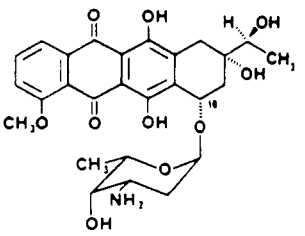


Figure 5. Reverse-phase analytical high performance liquid chromatographic analysis, as described in the Experimental Section, of product mixtures derived from a 0.5-h sodium borohydride reduction of 10-deoxydaunomycinone (**4**) in EtOH. (a) In the absence of excess NaOMe: peak 1, **22**, 30.1%; peak 2, **17**, 29.3%; peak 3, **23**, 9.5%; peak 4, **18**, 7.8%; peak 5, **24**, 12.9%; peak 6, **16**, 10.5%. (b) In the presence of excess NaOMe: peak 1, **22**, 9.9%; peak 2, **17**, 8.5%; peak 3, **23**, 39.0%; peak 4, **18**, 30.4%; peak 5, **24**, 6.5%; peak 6, **16**, 5.5%.

reomers.[20] Comparison with authentic materials allowed peaks 3, 6, and 7 to be readily assigned as **22**, **23**, and **24**, respectively. The identity of peak 1 remains unknown. The remaining three peaks (which did not absorb at 485 nm) have not been rigorously assigned, but most likely correspond to the three members of the leuco diastereomers (one trans-leuco stereoisomer, **25**, and two cis-leuco stereoisomers, **26** and **27**). Since the trans-leuco diastereomers generally have longer reverse phase retention times than the two cis-leuco diastereomers (Figure 1, Figure 2b) and were generally the dominant leuco diastereomer, peak 5 of Figure 4b probably corresponds to either **25a** or **25b** (1′*R*-trans-leuco-ol). Curiously, it was observed that admixture of 1′*S*-trans-leuco-ol (**19a** or **19b**) with the material of peak 5 of Figure 5b (1′*R*-trans-leuco-ol) resulted in a single chromatographic peak. Since the side-chain stereochemistry of material corresponding to peak 5 must be 1′*R*, these two trans-leuco 1′-dihydro diastereomers must coelute under the reverse phase chromatographic conditions used. In conclusion, overall comparison of the general constitution of the products from the respective hydroquinone tautomerizations of (1′*S*)- and (1′*R*)-1′-dihydrodaunomycinones indicated that the nature of the product outcome was nearly identical.

Pig Heart Aldo–Keto Reductase. A detailed study of this enzyme has not yet been undertaken. It was however of more than idle interest to address two unrelated issues. The first is the competence of NADH as a coenzyme for this enzyme. From the standpoint of the synthetic use of this enzyme, NADH has the advantage over NADPH in that it is more stable[48] and less expensive. The experiment to answer this question was simply the substitution of NADH for NADPH under the same reaction conditions as those utilized to produce the results depicted in Figure 2. Reverse phase analysis showed the reaction velocity was very similar to that observed when NADPH was used, and the final product composition was comparable regardless of the coenzyme used.[28] The observed differences were a smaller quantity of leuco diastereomers and a greater quantity[49] of **18** when NADH was utilized. Clearly, the NADH was a substrate for both the ferredoxin reductase (as seen previously[41]) and the aldo–keto reductase. This aldo–keto reductase differs from the better studied liver enzymes in this respect, as these are highly specific for NADPH.[32,33a,c,50] The second issue is the obvious one of the relative efficacy of acetyl reduction for daunomycin relative to the aglycons. Preliminary experiments indicated that conversion of daunomycin to 1′-dihydrodaunomycin (**28**) was substantially slower than the transformation of any aglycon to its 1′-dihydro derivative; indeed under conditions where quantitative conversion of the aglycons has occurred, there was only partial formation of 1′-dihydrodaunomycin (**28**) from daunomycin. It is apparent therefore that the aglycons are generally the better substrates for this aldo–keto reductase.



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Discussion

The primary objective of this study was to establish that the tautomeric chemistry of 10-deoxydaunomycinone hydroquinone (**5**) was an intrinsic attribute and would be expressed regardless of whether it was produced by enzymatic or chemical reduction. This has now been established. This type of tautomeric behavior is not limited to the 10-deoxydaunomycinone hydroquinone. Recently, conditions that allow for tautomerization of the daunomycin hydroquinone (**2**) prior to glycoside loss have been demonstrated. Cameron et al. have obtained,[51] via selective

(48) Wong, C.-H.; Whitesides, G. M. *J. Am. Chem. Soc.* **1981**, *103*, 4890–4899.

(49) Curiously, we have observed that omission of the DL-isocitrate results in a significant increase in the amount of **18** in the product mixture. Control experiments suggest that this is not a consequence of its acting as a reducing agent toward NAD^+, catalyzed by isocitric dehydrogenase.[28] Yet no other plausible explanation is apparent.

(50) Bachur, N. R. *Science (Washington, D.C.)* **1976**, *193*, 595–597.

catalytic reduction of daunomycin, glycosidic compounds having a 1,2,3,4-tetrahydro-2,11-dihydroxy-7-methoxy-5,12-naphthacenedione anthracycline ring system (analogous to **10**, **16**, and **24**) that have retained their glycoside moiety. In addition, Bird et al. have prepared[52] leuco daunomycin glycosidic species via the sodium dithionite reduction of daunomycin, followed by an immediate acid quench, resulting in the isolation of four leuco tautomers of the daunomycin hydroquinone. These results point to the possibility that hydroquinone tautomerism may impact on an overall understanding of anthracycline behavior.[53] A second aspect of this study—the fortuitous discovery of a pig heart carbonyl reductase mediated reduction of the aglycon (and to a lesser extent the glycon) carbonyl—may also prove serendipitous in the identical fashion, as discussed below.

No doubt remains that the four major metabolic transformations for the anthracyclines—ketone reduction, anaerobic reductive deglycosylation, aerobic redox cycling, and glucuronide conjugate formation—profoundly determine the biological activities of the anthracyclines. Much more problematic is the assessment of each pathway's role in the manifestation of the anthracycline's toxicity toward the neoplasm relative to the dose-limiting cardiotoxicity exhibited by these therapeutic agents. The present study bears tangentially on these aspects. It confirms once again the ease of anthracycline reduction to the hydroquinones. In the instance of daunomycin hydroquinone, the hydroquinone is converted (under anaerobic conditions) to the quinone methide via reductive deglycosylation, whereas in the case of the 10-deoxydaunomycinone hydroquinone the various leuco and chromo entities enumerated above are derived from the hydroquinone. The role these compounds play in the therapeutic action of the anthracyclines is not well understood. At present, for example, the issue of whether reductive deglycosylation is a significant metabolic pathway remains unresolved.[8,15b,c,54,55a] Likewise, the question as to whether the quinone methide expresses antitumor activity by means such as DNA alkylation is as yet without direct evidence.[8b] Nevertheless, the relative ease of these reductive pathways in vitro coupled with the substantial circumstantial evidence that the anthracyclines participate in redox chemistry in vivo[55,56] makes it reasonable to believe that some manifestation of these reductions and tautomerisms will be discerned in vivo. At the very least the existence of these pathways may suggest answers to existing structural conundrums in anthracycline chemistry; an approach that has been elegantly demonstrated by Koch et al. to account for the inability of 12-iminodaunomycin to effectively catalyze the in vivo reduction of molecular oxygen.[24b]

The discovery of an aldo–keto reductase activity in the commercial pig heart isocitric dehydrogenase, capable of anthracycline ketone reduction, is likely to receive continuing scrutiny. A resurgence of interest in anthracycline ketone reductions[57,58] and aglycon formation[59] has occurred, due in part to their use as a means of explaining the cardiac toxicity of anthracyclines. The postulate that anthracyclinols may play a role in explaining anthracycline's cardiac toxicity is supported by evidence that anthracyclinols accumulate in the heart by in situ reduction.[58,60] At a minimum the aldo–keto reductase activity in the commercial pig heart isocitric dehydrogenase provides a convenient access to enantiomerically pure 1′-anthracyclinol reference materials for the continuing studies on anthracycline metabolism and enzyme inhibition. In addition, the ready availability of this activity will permit an assignment of its place among the rather heterogeneous carbonyl reductase family.[61,62] It is already clearly separated from the known carbonyl reductases that participate in anthracycline metabolism[32] by its acceptance of NADH as a coenzyme.[28] The preference of this pig heart enzyme for the aglycons and the (albeit at times conflicting) evidence for substantial aglycon formation within the heart permit the speculation that the enzyme inhibitory activity of the 1′-anthracyclinol aglycons may be as significant as that of the 1′-anthracyclinol glycosides.

Experimental Section

Materials. Porcine heart isocitric dehydrogenase(NADP$^+$) (EC 1.1.1.42; isocitric dehydrogenase) [type I (crude), Sigma I 1877 (similar outcomes were obtained by using samples from different lot numbers); type IV (partially purified), Sigma I 2002; and type VI (highly purified), Sigma I 5882], glucose oxidase (type V, EC 1.1.3.4), catalase (bovine liver, EC 1.11.1.6), DL-trisodium isocitrate, NADP$^+$ (sodium salt, Sigma grade), NADPH (type III), and the buffering agents *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (Hepes), bis[(2-hydroxyethyl)imino]tris(hydroxymethyl)-methane (Bis-Tris), and tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) were obtained from the Sigma Chemical Company. The concentrations of the enzymes (in units, U) given in the experimental procedures indicate the activity, as defined and measured by the supplier, for the portions used. Spinach ferredoxin–NADP$^+$ reductase (EC 1.18.1.2); ferredoxin reductase) and spinach ferredoxin were purified from spinach according to established procedures.[17a,63] Daunomycin hydrochloride was a generous gift of the Drug Synthesis and Chemistry Branch of the National Cancer Institute. Daunomycinone was a generous gift from Dr. F. Arcamone, Farmitalia Carlo Erba. All other chemicals were of the highest quality available and were used as received.

Reverse-phase phenyl resin was prepared by using phenyltrichlorosilane, trimethylchlorosilane, and Merck silica gel (grade 60, 230–400 mesh) from Aldrich Chemical Company according to a slightly modified literature procedure.[64] Preparative thin

(51) Cameron, D. W.; Feutrill, G. I.; Griffiths, P. G. *Tetrahedron Lett.* **1988**, *29*, 4629–4630.
(52) Bird, D. M.; Boldt, M.; Koch, T. H. *J. Am. Chem. Soc.* **1989**, *111*, 1148–1150.
(53) Myers, C. *Cancer Chemother. Biol. Response Modifiers* **1987**, *9*, 36–49.
(54) (a) Cummings, J.; Willmott, N.; More, I.; Kerr, D. J.; Morrison, J. G.; Kaye, S. B. *Biochem. Pharmacol.* **1987**, *36*, 1521–1526. (b) Loveless, H.; Arena, E.; Felsted, R. L.; Bachur, N. R. *Cancer Res.* **1978**, *38*, 593–598. (c) Vrignaud, P.; Londos-Gagliardi, D.; Robert, J. *Eur. J. Drug Metab. Pharmacokineti.* **1986**, *11*, 101–105. (d) Le Bot, M. A.; Begue, J. M.; Kernaleguen, D.; Robert, J.; Ratanasavanh, D.; Airiau, J.; Richie, C.; Guillouzo, A. *Biochem. Pharmacol.* **1988**, *37*, 3877–3887.
(55) (a) Powis, G. *Free Radical Biol. Med.* **1989**, *6*, 63–101. (b) Myers, C. *Cancer Chemother. Biol. Response Modifiers* **1988**, *10*, 33–39.
(56) (a) Davies, K. J. A.; Doroshow, J. H. *J. Biol. Chem.* **1986**, *261*, 3060–3067. (b) Doroshow, J. H.; Davies, K. J. A. *J. Biol. Chem.* **1986**, *261*, 3068–3074. (c) Wallace, K. B.; Johnson, J. A. *Mol. Pharmacol.* **1987**, *31*, 307–311. (d) Nohl, H. *Biochem. Pharmacol.* **1988**, *37*, 2633–2637.
(57) Del Tacca, M.; Danesi, R.; Ducci, M.; Bernardini, C.; Romanini, A. *Pharmacol. Res. Commun.* **1985**, *17*, 1073–1084.
(58) (a) Boucedk, R. J., Jr.; Olson, R. D.; Brenner, D. E.; Ogunbunmi, E. M.; Inui, M.; Fleischer, S. *J. Biol. Chem.* **1987**, *262*, 15851–15856. (b) Olson, R. D.; Mushlin, P. S.; Brenner, D. E.; Fleischer, S.; Cusack, B. J.; Chang, B. K.; Boucek, R. J., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 3585–3589. (c) Cusack, B. J.; Tesnohlidek, D. A.; Loseke, V. L.; Vestal, R. E.; Brenner, D. E.; Olson, R. D. *Cancer Chemother. Pharmacol.* **1988**, *22*, 294–298.
(59) Sokolove, P. M.; Shinaberry, R. G. *Biochem. Pharmacol.* **1988**, *37*, 803–812.
(60) (a) Peters, J. H.; Gordon, G. R.; Kashiwase, D.; Acton, E. M. *Cancer Chemother. Pharmacol.* **1981**, *7*, 65–69. (b) Rossini, L.; Monti, E.; Cova, D.; Piccinini, F. *Arch. Toxicol., Suppl.* **1986**, *9*, 474–478.
(61) (a) Wermuth, B.; Omar, A.; Forster, A.; di Francesco, C.; Wolf, M.; van Wartburg, J.-P.; Bullock, B.; Gabbay, K. H. *Prog. Clin. Biol. Res.* **1987**, *232*, 297–307. (b) Carper, D.; Nishimura, C.; Shinohara, T.; Dietzchold, B.; Wistow, G.; Craft, C.; Kador, P.; Kinoshita, J. H. *FEBS Lett.* **1987**, *220*, 209–213. (c) Wermuth, B.; Bohren, K. M.; Heinemann, G.; von Wartburg, J.-P.; Gabbay, K. H. *J. Biol. Chem.* **1988**, *263*, 16185–16188.
(62) (a) Wermuth, B. *J. Biol. Chem.* **1981**, *256*, 1206–1213. (b) Wermuth, B. *Prog. Clin. Biol. Res.* **1985**, *174*, 209–230.
(63) (a) Zanetti, G.; Curti, B. *Methods Enzymol.* **1980**, *69*, 250–255. (b) Batie, C. J.; Kamin, H. *J. Biol. Chem.* **1981**, *256*, 7756–7763.

layer chromatography plates (silica gel GF, 500 μm, and silica gel GF uniplate-T tapered plates) were from Analtech Inc. and were predeveloped in EtOAc (0.1% CF_3COOH) before use.

General procedures [^1H NMR, ^{13}C NMR, electron impact mass spectra (MS-EI), and fast atom bombardment mass spectra (MS-FAB)] utilized were identical with those formerly described.[20] Analytical and preparative reverse phase high performance liquid chromatographic (RP-HPLC) analyses were performed isocratically by using conditions similar to those cited previously.[20] Analytical RP-HPLC utilized a Rainin Microsorb column (4.6 mm × 25 cm, 5-μm particle size) and a mobile phase of $MeOH/H_2O/CF_3COOH$ (62.0:37.9:0.1) at a flow rate of 1.0 mL min^{-1}. Preparative RP-HPLC separations were done on a Du Pont Zorbax C18 column (2.1 × 25 cm) and a mobile phase of $MeOH/H_2O/CF_3COOH$ (65.0:34.9:0.1) at a flow rate of 4.0 mL min^{-1}. Detection of anthracyclinone reaction products was done at 450 nm (unless specified otherwise).

Anaerobic Anthracycline Reductions. Reductions were performed by using standard anaerobic techniques. Buffers were made oxygen free by boiling under N_2, followed by cooling under a positive N_2 flow. Enzyme solutions were made anaerobic by equilibration with an N_2 atmosphere for at least 30 min. All anaerobic transfers were made with gas-tight syringes. Glucose (5 mM), glucose oxidase (40 U), and catalase (10000 U) were added to the reaction mixtures to help maintain anaerobicity throughout the course of the reaction. Reaction mixtures, unless otherwise stated, routinely contained Mn^{2+}, trisodium isocitrate, and isocitric dehydrogenase so as to generate NADPH from $NADP^+$.

Enzyme Assays. The specific activity of the ferredoxin reductase was measured at 30 °C in 50 mM Tris-HCl pH 8.0 buffer, with NADPH and $K_3Fe(CN)_6$ serving as electron acceptors as detailed elsewhere.[17a] Isocitric dehydrogenase was assayed by using trisodium DL-isocitrate, $NADP^+$, and $MnCl_2$, utilizing a modified version of a protocol described by the Sigma Chemical Company.[65] The specific activity of the isocitric dehydrogenase was measured at 30 °C in 50 mM Hepes pH 7.0 buffer by monitoring the increase in absorbance at 340 nm that occurs when $NADP^+$ is reduced to NADPH.

[8*S*-(8α,8*R,10α)]-7,8,9,10-Tetrahydro-6,8,10,11-tetrahydroxy-8-(1-hydroxyethyl)-1-methoxy-5,12-naphthacenedione [(1′*S*)-1′-Dihydrodaunomycinone, 11].** Daunomycinone (13) (10.2 mg, 25.6 μmol) was dissolved in 20 mL of dimethylformamide (DMF) and added to 350 mL of 50 mM Hepes pH 7.0 buffer containing $MnCl_2 \cdot 4H_2O$ (400 mg, 2.0 mmol), $NADP^+$ (68.4 mg, 80 μmol), and trisodium isocitrate (1.89 g, 6.0 mmol). Isocitric dehydrogenase [255 mg (type I) in 50 mL of 50 mM Hepes pH 7.0 buffer] was added to initiate the reaction. Final reagent concentrations were daunomycinone, 61 μM; $NADP^+$, 0.19 mM; isocitrate, 14.4 mM; Mn^{2+}, 4.8 mM; and isocitric dehydrogenase, 0.61 mg mL^{-1} (51 U). After 26 h at ambient temperature, the reaction mixture was filtered through glass wool and the filtrate was diluted with 400 mL of H_2O. This solution was then extracted with $CHCl_3$ (5 × 25 mL); any emulsions that formed were clarified by centrifugation. The combined $CHCl_3$ extracts were washed with H_2O (4 × 500 mL) and dried (Na_2SO_4). The $CHCl_3$ was evaporated off to yield 8.7 mg (21.7 μmol, 84% yield) of 11 (97% pure by RP-HPLC): mp 184–186 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 13.97 (s, 1 H, 11-OH), 13.30 (s, 1 H, 6-OH), 8.03 (d, 1 H, $J_{4,3}$ = 7.5, H-4), 7.78 (app t, 1 H, J = 8.1, H-3), 7.39 (d, 1 H, $J_{2,3}$ = 8.4, H-2), 5.34 (dd, 1 H, $J_{10eq,9ax}$ = 4.9, $J_{10eq,9eq}$ = 1.3, H-10eq), 5.30 (s, 1 H, 1′-OH), 4.09 (s, 3 H, 1-OMe), 3.73 (q, 1 H, $J_{1',2'}$ = 6.4, H-1′), 3.64 (s, 1 H, 8-OH), 3.20 (dd, 1 H, $J_{7eq,7ax}$ = 18.6, $J_{7eq,9eq}$ = 2.0, H-7eq), 2.55 (br d, 1 H, $J_{9eq,9ax}$ = 14.7, H-9eq), 2.52 (d, 1 H, $J_{7ax,7eq}$ = 18.8, H-7ax), 1.80 (dd, 1 H, $J_{9ax,9eq}$ = 14.8, $J_{9ax,10eq}$ = 4.9, H-9ax), 1.33 (d, 3 H, $J_{2',1'}$ = 6.4, H-2′); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 185.0 and 184.9 (C-5, C-12), 159.4 (C-1), 155.1 and 154.7 (C-6, C-11), 136.7, 134.1, and 133.7 (C-4a, C-6a, C-10a), 134.3 (C-3), 119.2 (C-12a), 117.9 (C-4), 117.4 (C-2), 109.3 and 109.0 (C-5a, C-11a), 71.4 (C-8), 71.2 (C-1′), 60.1 (C-10), 55.3 (1-OMe), 32.6 and 31.1 (C-7, C-9), 15.9 (C-2′); IR ($CDCl_3$) 3270, 3155, 1660, 1640, 1620, 1602, 1585, 1540, 1465, 1380, 1340, 1285, 1242, 1210, 1165, 1090, 1055, 990 cm^{-1}; UV–vis (MeOH) λ_{max} (rel absorbance) 530.0 (19), 495.0 (34), 289.5 (24), 251.0 (76), 233.5 nm (100); MS (EI, low resolution),[66] m/z (rel intensity) inter alia, 401 (M + 1, 1), 400 (M, 6), 384 (M − O, 11), 382 (M − H_2O, 6), 364 (M − $2H_2O$, 100), 346 (M − $3H_2O$, 31), 321 (13), 303 (13); MS (EI, high resolution), m/z 400.1158, calcd for $C_{21}H_{20}O_8$ 400.1159.

[8*S*-(8α,8*S,10α)]-7,8,9,10-Tetrahydro-6,8,10,11-tetrahydroxy-8-(1-hydroxyethyl)-1-methoxy-5,12-naphthacenedione [(1′*R*)-1′-Dihydrodaunomycinone, 12].** A slightly modified version of the procedure developed by Cassinelli et al. was used.[34] $NaBH_4$ (1.1 mg, 29 μmol) was added to 10 mL of a 1.7 mM solution of daunomycin hydrochloride (9.7 mg, 17 μmol) in pH 10 buffer. After 10 min at ambient temperature, the reaction was quenched by the addition of 15 mL of 0.2 N HCl and stirred for 30 min. This mixture was then subjected to acid hydrolysis (30 min at 100 °C). A red-orange precipitate appeared after 10 min. This precipitate was isolated by centrifugation (5000 rpm for 5 min at 4 °C). The weight of isolated solid was 5.9 mg (15 μmol, 88% yield). RP-HPLC analysis of this residue indicated a composition of 50.8% 11 and 43.2% 12. This mixture was then chromatographed on a Uniplate-T tapered preparative thin-layer plate using hexane/EtOAc/CF_3COOH (15:85:0.2) as the developing solvent. A partial separation was achieved, with this purification step yielding 0.6 mg of a mixture of 90% 12 and 10% 11. A second preparative thin-layer plate was utilized to purify 12 from the first plate by using identical conditions. This afforded 0.3 mg (0.7 μmol) of 12 (99% pure by RP-HPLC): mp 178–180 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 14.01 (s, 1 H, 11-OH), 13.31 (s, 1 H, 6-OH), 8.05 (dd, 1 H, $J_{4,3}$ = 7.7, $J_{4,2}$ = 0.8, H-4), 7.79 (app t, 1 H, J = 8.1, H-3), 7.40 (d, 1 H, $J_{2,3}$ = 8.4, H-2), 5.38 (dd, 1 H, $J_{10eq,9ax}$ = 4.9, $J_{10eq,9eq}$ = 1.2, H-10eq), 4.10 (s, 3 H, 1-OMe), 3.86 (q, 1 H, $J_{1',2'}$ = 6.5, H-1′), 3.18 (dd, 1 H, $J_{7eq,7ax}$ = 18.7, $J_{7eq,9eq}$ = 2.0, H-7eq), 2.64 (d, 1 H, $J_{7ax,7eq}$ = 18.7, H-7ax), 2.42 (app. ddd, 1 H, $J_{9eq,9ax}$ = 14.7, $J_{9eq,10eq}$ = $J_{9eq,7eq}$ = 2.0, H-9eq), 1.86 (dd, 1 H, $J_{9ax,9eq}$ = 14.7, $J_{9ax,10eq}$ = 4.9, H-9ax), 1.29 (d, 3 H, $J_{2',1'}$ = 6.5, H-2′); UV–vis (MeOH) λ_{max} (rel absorbance) 535.0 (19), 495.0 (33), 289.5 (23), 251.0 (76), 233.5 nm (100); MS (EI, low resolution), m/z (rel intensity) inter alia, 400 (M, 15), 382 (M − H_2O, 9), 364 (M − $2H_2O$, 100), 346 (M − $3H_2O$, 38), 321 (20), 303 (32), 275 (23); MS (EI, high resolution), m/z 400.1159, calcd for $C_{21}H_{20}O_8$ 400.1159.

[4*S*-(4α,4*R,5β)]-3′,4′-Dihydro-4′,5′,12′-trihydroxy-7′-methoxy-2,2,5′-trimethylspiro[1,3-dioxolane-4,2′(1′*H*)-naphthacene]-6′,11′-dione (14).** A slightly modified version of the procedure developed by Dornberger et al. was used.[36a] Compound 11 (17.1 mg, 43 μmol) and a catalytic amount of *p*-toluenesulfonic acid monohydrate (0.8 mg, 4.2 μmol) were dissolved, with sonication, in 10 mL of 2,2-dimethoxypropane. After 7.5 h at ambient temperature, the reaction mixture was diluted with 75 mL of H_2O and extracted with $CHCl_3$ (4 × 10 mL). These extracts were washed with H_2O (2 × 75 mL) and then dried (Na_2SO_4). The solvent was evaporated off and the residue placed under vacuum to remove residual volatiles. Purification by normal-phase flash chromatography [2 × 21 cm silica gel column, $CHCl_3$/MeOH (93:7)] provided 14.8 mg (33.6 μmol, 78% yield) of 14: ^1H NMR (300 MHz, $CDCl_3$) δ 13.95 (s, 1 H, 5′-OH), 13.31 (s, 1 H, 12′-OH), 7.96 (d, 1 H, $J_{10',9'}$ = 7.5, H-10′), 7.73 (app t, 1 H, J = 8.0, H-9′), 7.36 (d, 1 H, $J_{8',9'}$ = 8.4, H-8′), 5.17 (dd, 1 H, $J_{4'eq,3'ax}$ = 4.8, $J_{4'eq,3'eq}$ = 1.0, H-4′eq), 4.14 (q, 1 H, $J_{5,6}$ = 6.2, H-5), 4.07 (s, 3 H, 7′-OMe), 3.15 (dd, 1 H, $J_{1'eq,1'ax}$ = 18.3, $J_{1'eq,3'eq}$ = 2.0, H-1′eq), 2.53 (d, 1 H, $J_{1'ax,1'eq}$ = 18.3, H-1′eq), 2.21 (app ddd, $J_{3'eq,3'ax}$ = 14.0, $J_{3'eq,4'eq}$ = $J_{3'eq,1'eq}$ = 2.0, H-3′eq), 2.01 (dd, 1 H, $J_{3'ax,3'eq}$ = 14.0, $J_{3'ax,4'eq}$ = 4.8, H-3′ax), 1.423 (s, 3 H, dimethyl ketal), 1.416 (s, 3 H, dimethyl ketal), 1.35 (d, 3 H, $J_{6,5}$ = 6.2, H-6); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 186.8 and 186.6 (C-6′, C-11′), 161.0 (C-7′), 156.7 and 156.0 (C-5′, C-12′), 138.0, 135.52, and 134.3 (C-4a′, C-10a′, C-12a′), 135.47 (C-9′), 121.0 (C-6a′), 119.6 (C-10′), 118.3 (C-8′), 111.3 and 110.9 (C-5a′, C-11a′), 108.0 (C-2), 80.4 (C-4), 79.3 (C-5), 62.7 (C-4′), 56.8 (7′-OMe), 37.8 (C-3′), 30.0 (C-1′), 28.5 and 26.9 (2 C-2 Me), 14.9 (C-5 Me); MS (EI, low resolution), m/z (rel intensity) inter alia, 441 (M + 1, 5), 440 (M, 22), 422 (M − H_2O, 10), 378 (12), 364 (100), 346 (53), 321 (20), 312 (23), 284 (18); MS

(64) (a) Kuhler, T. C.; Lindsten, G. R. *J. Org. Chem.* **1983**, *48*, 3589–3591. (b) Evans, M. B.; Dale, A. D.; Little, C. J. *Chromatographia* **1980**, *13*, 5–10.

(65) Sigma Chemical Company Technical Bulletin No. 153-UV.

(66) Low resolution MS-EI utilized an ionizing voltage of 20 eV. All samples used for high resolution mass spectral analysis were greater than 95% pure (based on analytical RP-HPLC analyses).

(EI, high resolution), m/z 440.1469, calcd for $C_{24}H_{24}O_8$ 440.1471.

Preparative Scale Reduction of Daunomycin, Catalyzed by the Ferredoxin Reductase/Isocitric Dehydrogenase Enzyme System. Daunomycin hydrochloride (12.6 mg, 22.4 μmol) was dissolved in 1.0 mL of DMF and then added to 200 mL of anaerobic 50 mM Hepes pH 7.0 buffer, which contained $NADP^+$ (83.0 mg, 97.3 μmol), $MnCl_2 \cdot 4H_2O$ (200 mg, 1.0 mmol), trisodium isocitrate (1.57 g, 5.0 mmol), glucose (1.0 g, 5.6 mmol), glucose oxidase (600 U), and catalase (84 000 U). Isocitric dehydrogenase (type IV, 250 U) was transferred to the reaction mixture and allowed to react for 15 min before spinach ferredoxin (89 nmol) and ferredoxin reductase (24 nmol) were added. Final reagent concentrations were as follows: daunomycin, 0.11 mM; $NADP^+$, 0.46 mM; isocitrate, 23.7 mM; Mn^{2+}, 4.8 mM; glucose, 26.4 mM; spinach ferredoxin, 0.42 μM, and ferredoxin reductase, 0.11 μM. The reaction was then slowly stirred at ambient temperature for 28 h. After this time, the reaction was opened to the atmosphere and diluted with 300 mL of 3.0 mM ethylenediaminetetraacetic acid disodium salt (Na_2EDTA). This aqueous solution was then extracted with $CHCl_3$ (8 × 25 mL). The emulsion that formed during each extraction was clarified by centrifugation for 5 min in a clinical centrifuge. The combined extracts were washed with H_2O (3 × 500 mL) and dried (Na_2SO_4), and the $CHCl_3$ was evaporated off. The dark red solid was then placed under a vacuum for several hours to remove any residual DMF. This yielded 8.5 mg (100% weight recovery) of material. The composition of this product mixture [as determined by reverse-phase liquid chromatography, (RP-HPLC)] was as follows: **16**, 49.2%; 1′*S*-trans-leuco-ol **19a** or **19b**, 24.2%; **17**, 17%; 1′*S*-cis-leuco-ol B **20** or **21**, 8%; 1′*S*-cis-leuco-ol A **20** or **21**, 1.2%; and **18**, 0.6%. Crude fractionation of a portion of the aglycon mixture was accomplished by preparative reverse-phase chromatography on a (1.5 × 13 cm) phenyl silica gel column. The product mixture (ca. 15 μmol) was dissolved in tetrahydrofuran (THF) and then diluted with 20 volumes of H_2O (containing 0.1% CF_3COOH). The sample was applied to the phenyl column and eluted by using a gradient of $THF/H_2O/CF_3COOH$ (10:90:0.1 to 50:50:0.1). Compound **17** eluted at ca. 30% THF, whereas 1′*S*-trans-leuco-ol **19** eluted at ca. 35% THF and **16** eluted at ca. 50% THF. Compound **16** (2.4 mg, 6.5 μmol) was 95% pure after the phenyl column, whereas compounds 1′*S*-trans-leuco-ol **19** and **17** were ca. 75–80% pure and were subjected to further purification as outlined below.

[*S*-(*R,*S**)]-1,2,3,4-Tetrahydro-2,11-dihydroxy-2-(1-hydroxyethyl)-7-methoxy-5,12-naphthacenedione (16)**: mp 195–197 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 13.88 (s, 1 H, 11-OH), 8.54 (s, 1 H, H-6), 8.00 (d, 1 H, $J_{10,9}$ = 8.1, H-10), 7.58 (app t, 1 H, J = 7.9, H-9), 7.05 (d, 1 H, $J_{8,9}$ = 8.0, H-8), 4.03 (s, 3 H, 7-OMe), 3.77 (q, 1 H, $J_{1',2'}$ = 6.2, H-1′), 2.97 (app dddd, 1 H, $J_{4eq,4ax}$ = 19.8, $J_{4eq,3ax}$ = 6.0, $J_{4eq,3eq}$ = 2.0, $J_{4eq,1ax}$ = 1.7, H-4eq), 2.83 (dd, 1 H, $J_{1eq,1ax}$ = 19.4, $J_{1eq,4ax}$ = 2.2, H-1eq), 2.7 (m, 1 H, H-4ax; irradiation at δ 2.0 gave an app dddd, $J_{4ax,4eq}$ = 19.8, $J_{4ax,3ax}$ = 10.2, $J_{4ax,3eq}$ = 2.8, $J_{4ax,1eq}$ = 2.8), 2.48 (ddd, 1 H, $J_{1ax,1eq}$ = 19.3, $J_{1ax,4ax}$ = 3.8, $J_{1ax,4eq}$ = 2.0, H-1ax), 2.1 (m, 1 H, H-3eq), 1.52 (ddd, 1 H, $J_{3ax,3eq}$ = 13.4, $J_{3ax,4ax}$ = 11.8, $J_{3ax,4eq}$ = 6.0, H-3ax), 1.34 (d, 3 H, $J_{2',1'}$ = 6.4, H-2′); IR ($CDCl_3$) 3270, 3155, 2930, 1660, 1638, 1603, 1570, 1540, 1500, 1455, 1420, 1380, 1320, 1290, 1265, 1230, 1210, 1185, 1170, 1140, 1090, 1070, 1002 cm^{-1}; UV–vis (MeOH) λ_{max} (rel absorbance) 479.0 (17), 342.5 sh (4), 323.5 (6), 275.0 (27), 242.0 nm (100); MS (EI, low resolution), m/z (rel intensity) inter alia, 370 (M + 2, 10), 369 (M + 1, 25), 368 (M, 100), 350 (M − H_2O, 10), 323 (M − $CH(OH)CH_3$, 84), 305 (M − $CH(OH)CH_3$ − H_2O, 33), 295 (M − $CH(OH)CH_3$ − CO, 33); MS (EI, high resolution), m/z 368.1277, calcd for $C_{21}H_{20}O_6$ 368.1260; MS (FAB, dithiothreitol/dithioerythritol matrix, low resolution positive ion), m/z (rel intensity) inter alia, 371 (M + 3, 17), 370 (M + 2, 53), 369 (M + 1, 100), 368 (M, 26), 351 (M + 1 − H_2O, 16), 323 (M − $CH(OH)CH_3$, 10), 309 (67), 279 (12); MS (FAB, dithiothreitol/dithioerythritol matrix, low resolution negative ion), m/z (rel intensity) inter alia, 369 (M + 1, 47), 368 (M, 100), 367 (M − 1, 52), 353 (19), 333 (68), 307 (93), 305 (M − $CH(OH)CH_3$ − CO, 78), 273 (43); MS (FAB, mononitrobenzyl alcohol matrix, high resolution, positive ion), m/z (M + 1) 369.1362, calcd for $C_{21}H_{21}O_6$ 369.1338.

[*S*-(*R,*S**)]-1,2,3,4-Tetrahydro-2,6-dihydroxy-2-(1-hydroxyethyl)-7-methoxy-5,12-naphthacenedione (17).** The fractions (total weight ca. 2 mg), from the above phenyl column, which contained **17**, were subjected to a normal-phase separation on a 500-μm thin layer plate, using hexane/EtOAc/CF_3COOH (45:55:0.2) as the eluting solvent. This yielded 0.4 mg (1.1 μmol) of **17**: mp sample darkened at 100–110 °C and melted at 118–121 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 15.14 (s, 1 H, 6-OH), 8.00 (s, 1 H, H-11), 7.61 (app t, 1 H, J = 7.9, H-9), 7.50 (d, 1 H, $J_{10,9}$ = 7.9, H-10), 7.05 (d, 1 H, $J_{8,9}$ = 7.9, H-8), 4.07 (s, 3 H, 7-OMe), 3.75 (q, 1 H, $J_{1',2'}$ = 6.3, H-1′), 2.97 (app dddd, 1 H, $J_{4eq,4ax}$ = 19.7, $J_{4eq,3ax}$ = 5.9, $J_{4eq,3eq}$ = $J_{4eq,1ax}$ = 1.7, H-4eq), 2.80 (dd, 1 H, $J_{1eq,1ax}$ = 19.7, $J_{1eq,4ax}$ = 1.8, H-1eq), 2.7 (m, 1 H, H-4ax), 2.47 (ddd, 1 H, $J_{1ax,1eq}$ = 19.7, $J_{1ax,4ax}$ = 3.9, $J_{1ax,4eq}$ = 1.7, H-1ax), 2.10 (app dddd, 1 H, $J_{3eq,3ax}$ = 13.3, $J_{3eq,4ax}$ = 5.9, $J_{3eq,4eq}$ = $J_{3eq,1eq}$ = 2.2, H-3eq), 1.52 (ddd, 1 H, $J_{3ax,3eq}$ = $J_{3ax,4ax}$ = 12.7, $J_{3ax,4eq}$ = 6.1, H-3ax), 1.33 (d, 3 H, $J_{2',1'}$ = 6.4, H-2′); IR ($CDCl_3$) 3270, 3155, 1662, 1640, 1603, 1575, 1547, 1493, 1460, 1378, 1342, 1328, 1282, 1262, 1218, 1187, 1165, 1137, 1095, 1027 cm^{-1}; UV–vis (MeOH) λ_{max} (rel absorbance) 502.0 (18), 484.0 sh (18), 349.5 sh (4), 329.5 (6), 277.5 (22), 244.0 nm (100); MS (EI, low resolution), m/z (rel intensity) inter alia, 369 (M + 1, 22), 368 (M, 100), 350 (M − H_2O, 7), 323 (M − $CH(OH)CH_3$, 38), 305 (M − $CH(OH)CH_3$ − H_2O, 11), 295 (M − $CH(OH)CH_3$ − CO, 19); MS (EI, high resolution), m/z 368.1261, calcd for $C_{21}H_{20}O_6$ 368.1260.

[2*R*-[(2α,2*S,4aβ,12aα)- or -(2α,2*S**,4aα,12aβ)]]-1,2,3,4,4a,12a-Hexahydro-2,6,11-trihydroxy-2-(1-hydroxyethyl)-7-methoxy-5,12-naphthacenedione (1′*S*-trans-leuco-ol 19a or 19b).** Those fractions (total weight ca. 1.0 mg), from the above phenyl column, containing 1′*S*-trans-leuco-ol were further purified on the Du Pont Zorbax C18 column (followed by purifying the sample utilizing a silica gel thin layer plate to remove any residual reverse phase resin) under the previously cited conditions. This afforded 0.3 mg (0.8 μmol) of 1′*S*-trans-leuco-ol **19a** or **19b**: mp 121–123 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 14.65 (s, 1 H, 6-OH), 13.45 (s, 1 H, 11-OH), 8.10 (d, 1 H, $J_{10,9}$ = 8.3, H-10), 7.69 (app t, 1 H, J = 8.1, H-9), 7.17 (d, 1 H, $J_{8,9}$ = 8.1, H-8), 4.06 (s, 3 H, 7-OMe), 3.68 (q, 1 H, $J_{1',2'}$ = 6.5, H-1′), 3.27 (ddd, 1 H, $J_{12a,4a}$ = 13.2, $J_{12a,1ax}$ = 11.5, $J_{12a,1eq}$ = 3.7, H-12a), 2.70 (ddd, 1 H, $J_{4a,12a}$ = 13.3, $J_{4a,4ax}$ = 11.2, $J_{4a,4eq}$ = 3.7, H-4a), 2.5–2.3 (m, 2 H, H-1eq, H-4eq), 1.9 (m, 1 H, H-4ax), 1.7–1.5 (m, 2 H, H-3ax, H-3eq), 1.48 (dd, 1 H, $J_{1ax,1eq}$ = 13.9, $J_{1ax,12a}$ = 11.7, H-1ax), 1.29 (d, 3 H, $J_{2',1'}$ = 6.5, H-2′); IR ($CDCl_3$) 3270, 3155, 2955, 2930, 2855, 1660, 1640, 1585, 1540, 1465, 1390, 1380, 1340, 1325, 1290, 1265, 1180, 1165, 1100, 1010 cm^{-1}; UV–vis (MeOH) λ_{max} (rel absorbance) 442.0 (44), 436.0 sh (39), 417.0 (43), 397.0 (27), 264.5 (80), 238.5 nm (100); MS (FAB, dithiothreitol/dithioerythritol matrix, low resolution positive ion), m/z (rel intensity) inter alia, 388 (M + 2, 31), 387 (M + 1, 100), 386 (M, 51), 369 (M + 1 − H_2O, 31), 368 (M − H_2O, 23), 351 (M + 1 − $2H_2O$, 31), 341 (M − $CH(OH)CH_3$, 23), 324 (M + 1 − H_2O − $CH(OH)CH_3$, 35), 323 (M − H_2O − $CH(OH)CH_3$, 56), 295 (M + 1 − H_2O − $CH(OH)CH_3$ − CO, 33); MS (FAB, dithiothreitol/dithioerythritol matrix, low resolution negative ion), m/z (rel intensity) inter alia, 387 (M + 1, 30), 386 (M, 78), 385 (M − 1, 100), 384 (M − 2, 48), 383 (M − 3, 22), 371 (84); MS (FAB, dithiothreitol/dithioerythritol matrix, high resolution positive ion), m/z 387.1461, calcd for $C_{21}H_{23}O_7$ (M + 1) 387.1444.[67]

Small-Scale Reductions of the Acetyl Group in Anthracyclinones Catalyzed by the Porcine Heart Aldo–Keto Reductase Present in the Commercial Preparations of Isocitric Dehydrogenase (General Procedure Utilizing Aerobic Conditions). $NADP^+$ (1.3 mg, 1.5 μmol), trisodium isocitrate (23.5 mg, 75.3 μmol), and $MnCl_2 \cdot 4H_2O$ (2.7 mg, 13.7 μmol) were dissolved in 2.7 mL of 50 mM Hepes pH 7.0 buffer. The appropriate anthracyclinone (150 μL of a 0.50 mM solution in DMF) was added, followed by the addition of isocitric dehydrogenase (type IV, 4 U), which initiated the reaction. The reaction proceeded for 18–22 h at ambient temperature. It was then diluted with 15 mL of 1.0 mM Na_2EDTA and extracted with CH_2Cl_2 (4 × 3 mL). The CH_2Cl_2 extracts were washed with H_2O (4 × 10 mL) to remove the DMF and then dried (Na_2SO_4). The CH_2Cl_2 was evaporated off and the product composition analyzed by RP-HPLC. Reduction of **4** gave **18** (48.6%) and **4**, (44.7%). (The

(67) Compound 1′*S*-trans-leuco-ol **19a** or **19b** reacted with NaOMe/MeOH under aerobic conditions to quantitatively form **18**. This is similar to the reaction observed when trans-leuco **6a** or **6b** is treated under analogous conditions[20] to form **4**.

sum of the relative yields of the products does not equal 100% due to the standard error associated with the RP-HPLC analysis.) Reduction of **10** gave **16** (55.1%) and **10** (41.8%). Reduction of **9** gave **17** (95.6%). Reduction of trans-leuco **6a** or **6b** gave 1′*S*-trans-leuco-ol **19a** or **19b** (55.4%) and **18** (36.1%). Reduction of a mixture of cis-leuco B **7** or **8** (64%) and trans-leuco **6a** or **6b** (35%) gave 1′*S*-cis-leuco-ol B **20** or **21** (17.2%), 1′*S*-trans-leuco-ol **19a** or **19b** (38.0%), and **18** (45.0%). Reduction of cis-leuco A **7** or **8** was carried out by using the same reagent concentrations as cited above, but the reaction was run under anaerobic conditions and worked up after 1 h. The product composition for this reaction was 1′*S*-cis-leuco-ol A **20** or **21** (17%) and **18** (83%). If this reaction was allowed to proceed for 4 h under anaerobic conditions, only **18** was observed in the product mixture.

The appropriate control reactions (NADPH without isocitric dehydrogenase and isocitric dehydrogenase without $NADP^+$) were run with **4** as the substrate. There was no observable formation of the 1′-dihydro compound **18** in either instance. Typically, yields of greater than 90% for the corresponding 1′-dihydro compounds were observed when the type I grade of isocitric dehydrogenase (0.5–1.0 mg mL^{-1}, 0.3–0.6 U) was used under the above conditions. Finally, similar outcomes were observed regardless of whether the reactions were done under anaerobic or aerobic conditions.

[*S*-(*R,*S**)]-7,8,9,10-Tetrahydro-6,8,11-trihydroxy-8-(1-hydroxyethyl)-1-methoxy-5,12-naphthacenedione (18).** 10-Deoxydaunomycinone (1.9 mg, 5.0 μmol) was dissolved in 10 mL of DMF, and this solution was then added to 200 mL of 50 mM Bis-Tris pH 6.5 buffer that contained $NADP^+$ (52.0 mg, 61 μmol), $MnCl_2 \cdot 4H_2O$ (197 mg, 1.0 mmol), and trisodium isocitrate (0.936 g, 3.0 mmol). The isocitric dehydrogenase (type I, 189 mg, 38 U) was dissolved in 50 mL of 50 mM Bis-Tris pH 6.5 buffer and then added to the reaction mixture to initiate the reaction. Final reagent concentrations were 10-deoxydaunomycinone, 25 μM; $NADP^+$, 0.31 mM; Mn^{2+}, 5.0 mM; isocitrate, 15.0 mM; and isocitric dehydrogenase, 0.95 mg mL^{-1}. The reaction mixture was slowly stirred for 24 h at ambient temperature. The product mixture was then diluted with 350 mL of 3.0 mM Na_2EDTA and extracted with $CHCl_3$ (4 × 25 mL). Any emulsions that formed during the extraction process were clarified by centrifugation. The $CHCl_3$ extracts were washed with H_2O (3 × 500 mL) to remove any residual DMF and then dried over Na_2SO_4. The solvent was evaporated off and the product mixture was subjected to a normal-phase separation on a 500-μm thin layer plate and hexane/EtOAc/CF_3COOH (50:50:0.2) as the developing solvent. This yielded 1.1 mg (2.9 μmol; 58% yield) of **18**. The spectral properties of **18** are in agreement with those reported by Russell et al.:[36c] mp 200–202 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 13.87 (s, 1 H, 11-OH), 13.52 (s, 1 H, 6-OH), 8.03 (d, 1 H, $J_{4,3}$ = 7.8, H-4), 7.75 (app t, J = 8.1, H-3), 7.36 (d, 1 H, $J_{2,3}$ = 8.5, H-2), 4.08 (s, 3 H, 1-OMe), 3.78 (q, 1 H, $J_{1',2'}$ = 6.3, H-1′), 3.11 (ddd, 1 H, $J_{10eq,10ax}$ = 19.6, $J_{10eq,9ax}$ = 4.9, $J_{10eq,9eq}$ = 2.2, H-10eq), 2.95 (dd, 1 H, $J_{7eq,7ax}$ = 18.4, $J_{7eq,9eq}$ = 1.5, H-7eq), 2.9 (m, 1 H, H-10ax), 2.63 (d, 1 H, $J_{7ax,7eq}$ = 18.5, H-7ax), 2.10 (app dddd, 1 H, $J_{9eq,9ax}$ = 13.5, $J_{9eq,10ax}$ = 5.5, $J_{9eq,10eq}$ = 2.8, $J_{9eq,7eq}$ = 1.5, H-9eq), 1.6 (m, 1 H, H-9ax), 1.33 (d, 3 H, $J_{2',1'}$ = 6.4, H-2′); IR ($CDCl_3$) 3600, 3285, 3150, 2925, 2850, 1660, 1640, 1620, 1580, 1545, 1450, 1380, 1345, 1280, 1255, 1210, 1130, 1100, 1070 cm^{-1}; UV–vis (MeOH) λ_{max} (rel absorbance) 529.0 (25), 494.0 (40), 480.0 sh (35), 289.5 (25), 253.0 (100), 235.5 nm (90); MS (EI, low resolution), m/z (rel intensity) inter alia, 386 (M + 2, 4), 385 (M + 1, 19), 384 (M, 61), 366 (M − H_2O, 6), 339 (M − $CH(OH)CH_3$, 100), 321 (M − $CH(OH)CH_3$ − H_2O, 20), 311 (M − $CH(OH)CH_3$ − CO, 14), 296 (16); MS (EI, high resolution), m/z 384.1210, calcd for $C_{21}H_{20}O_7$ 384.1209.

Reaction of Daunomycin (or Daunomycinone) with the NADPH Recycling System. Daunomycin hydrochloride (0.56 mg, 1.0 μmol), $NADP^+$ (17.3 mg, 20.3 μmol), $MnCl_2 \cdot 4H_2O$ (20.2 mg, 0.10 mmol), trisodium isocitrate (93.6 mg, 0.30 mmol), and glucose (100 mg, 0.56 mmol) were placed in a Schlenck tube and dissolved in 15.0 mL of anaerobic 50 mM Hepes pH 7.0 buffer. The vessel was then evacuated and flushed with N_2 repeatedly. Isocitric dehydrogenase (type I, 20.0 mg, 4 U), glucose oxidase (280 U), and catalase (70 000 U) were dissolved in 5.0 mL of anaerobic 50 mM Hepes pH 7.0 buffer and then transferred to the Schlenck tube via syringe. Final reagent concentrations were daunomycin, 50 μM; $NADP^+$, 1.0 mM; isocitrate, 15.0 mM; Mn^{2+}, 5.0 mM; and isocitric dehydrogenase, 1.0 mg mL^{-1}. The progress of this reaction was followed by periodically removing 2.0-mL portions from the reaction mixture, diluting each with 25 mL of 1.0 mM Na_2EDTA, and adjusting the pH of this solution to 8.2 with saturated $NaHCO_3$. This solution was extracted with CH_2Cl_2/MeOH (9:1) (4 × 5 mL), and these extracts were washed once with 20 mL of pH 8.2 buffer. These extracts were then analyzed by RP-HPLC. The percentage of 1′-dihydrodaunomycin (daunomycinol, **28**) observed in these reaction aliquots was as follows [% **28** (reaction time)]: 2.2% (1.3 h); 2.9% (4.3 h); 5.3% (6.8 h); and 16.0% (21.7 h). Twenty-four hours after the reaction was initially started, additional isocitric dehydrogenase (type I, 11.6 mg, 2.3 U), $NADP^+$ (9.4 mg, 11 μmol), and trisodium isocitrate (41.7 mg, 0.14 mmol) dissolved in 3.0 mL of anaerobic 50 mM Hepes pH 7.0 buffer was added to the remaining 12 mL of the reaction mixture. The reaction was allowed to proceed for an additional 25 h (total reaction time of 49 h) and then worked up and analyzed as above. The final product composition was 42% **28** and 58% **1**. The identity of the product (**28**) was established by subjecting this material to acid hydrolysis (0.2 N HCl, 100 °C, 0.5 h) and confirming (by MS-EI and RP-HPLC) that the aglycon product was **11**.

Daunomycinone (**13**) was examined as a substrate for the aldo–keto reductase (present in the commercial preparations of isocitric dehydrogenase) by using conditions analogous to those cited above. The only variation from the above procedure was that sufficient DMF (5% of the total volume) was included to aid in the solubilization of **13** in the aqueous reaction mixture. Final reagent conditions were daunomycinone, 50 μM; $NADP^+$, 1.0 mM; isocitrate, 15.0 mM; Mn^{2+}, 5.0 mM; and isocitric dehydrogenase, 1.0 mg mL^{-1}. The reaction aliquots were worked up as above, except that the portions were diluted with the Na_2EDTA solution without subsequently adjusting the pH to 8.2. The percentage of **11** observed in these reactions aliquots was as follows [% **11** (reaction time)]: 40.3% (1.3 h); 96.3% (6.8 h); and 100% (21.7 h).

Small-Scale $NaBH_4$ Reductions (without NaOMe) of Anthracyclinones. The anthracyclinone (0.2–0.4 μmol) was dissolved in 3 mL of absolute EtOH and excess $NaBH_4$ (1–3 μmol) was added. After 0.5 h at ambient temperature the reaction was quenched by the addition of 1.0 mL of 0.4 N HCl, stirred 20 min, and then diluted with 20 mL of H_2O. This solution was extracted with CH_2Cl_2 (4 × 3 mL), followed by washing these extracts with H_2O (3 × 5 mL). After drying (Na_2SO_4), the solvent was evaporated off and the composition of the product mixture ascertained by RP-HPLC (detection at 485 nm). The product compositions for the $NaBH_4$ reduction of specified anthracyclinones are listed below. Reaction of **4** with $NaBH_4$ gave **22** (30.1%), **17** (29.3%), **24** (12.9%), **16** (10.5%), **23** (9.5%), and **18** (7.8%). (If the reaction period was increased from 0.5 h to 2 h, the product composition was **22** (41.1%), **17** (40.5%), **24** (6.7%), **16** (6.9%), **23** (2.8%), and **18** (1.8%).) Reaction of **10** with $NaBH_4$ gave **24** (46.0%), **16** (44.3%), and unknown compounds (9.6%). Reaction of **9** with $NaBH_4$ gave **22** (53.0%) and **17** (47.0%).

Small-Scale $NaBH_4$ Reductions (with NaOMe) of Anthracyclinones. The reaction conditions used were similar to those described above except that excess NaOMe was present in the initial reaction mixture. NaOMe was added to the reaction mixture (before the addition of the $NaBH_4$) until the solution became violet in color. The reaction was then run for 0.5 h in the dark at ambient temperature. The product composition from the $NaBH_4$ reduction (in the presence of NaOMe) of a specified anthracyclinone was determined by RP-HPLC analysis. Reaction of **4** with $NaBH_4$ and NaOMe gave **23** (44%), **18** (36%), **22** (6%), **17** (5%), **24** (3%), **16** (3%), and **4** (3%). (If the reaction period was increased from 0.5 h to 2 h, the product composition was **23** (55%), **18** (36%), **22** (1%), **17** (1%), **24** (2%), **16** (2%), and **4** (3%).) Reaction of **10** with $NaBH_4$ and NaOMe gave **24** (45.9%), **16** (45.1%), and **10** (7.7%).

[*R*-(*R,*R**)]-1,2,3,4-Tetrahydro-2,6-dihydroxy-2-(1-hydroxyethyl)-7-methoxy-5,12-naphthacenedione (22).** 10-Deoxydaunomycinone (**4**) (6.9 mg, 18.0 μmol) and $NaBH_4$ (5.1 mg, 0.14 mmol) were dissolved in 80 mL of absolute EtOH. After 3 h, the reaction was quenched by the addition of 1.0 mL of 0.4 N HCl, stirred for 15 min, and then diluted with 150 mL of H_2O. This solution was extracted with CH_2Cl_2 (4 × 25 mL), and these extracts were washed with H_2O (4 × 25 mL). After drying

(Na_2SO_4), the CH_2Cl_2 was evaporated off to yield 6.9 mg of solid. RP-HPLC analysis (detection at 485 nm) showed the composition of this product mixture to be **22** (45.2%), **17** (43.3%), **24** (4.8%), **16** (4.9%), **23** (1.0%), and **18** (1.1%). Compounds **17** and **22** were purified from this mixture by preparative RP-HPLC on the Du Pont Zorbax C18 column under the previously stipulated conditions (t_R(**22**) = 45 min, t_R(**17**) = 48 min). This purification process yielded 2.1 mg of **22** (5.7 μmol, 32% overall yield, 99% pure): mp sample darkened at 105–113 °C and melted at 111–113 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.11 (s, 1 H, 6-OH), 7.98 (s, 1 H, H-11), 7.61 (app. t, 1 H, J = 8.0, H-9), 7.49 (d, 1 H, $J_{10,9}$ = 7.9, H-10), 7.05 (d, 1 H, $J_{8,9}$ = 7.9, H-8), 4.07 (s, 3 H, 7-OMe), 3.84 (q, 1 H, $J_{1',2'}$ = 6.4, H-1′), 2.95 (ddd, 1 H, $J_{4eq,4ax}$ = 19.4, $J_{4eq,3ax}$ = 5.6, $J_{4eq,3eq}$ = 2.6, H-4eq), 2.84 (d, 1 H, $J_{1eq,1ax}$ = 18.7, H-1eq), 2.71 (m, 1 H, H-4ax; irradiation at δ 1.6 gave an app ddd, $J_{4ax,4eq}$ = 21.8, $J_{4ax,3eq}$ = 6.3, $J_{4ax,1ax}$ = 3.1), 2.63 (dd, 1 H, $J_{1ax,1eq}$ = 19.4, $J_{1ax,4ax}$ = 2.3, H-1ax), 1.96 (app dddd, 1 H, $J_{3eq,3ax}$ = 13.3, $J_{3eq,4ax}$ = 5.6, $J_{3eq,4eq}$ = $J_{3eq,1eq}$ = 2.0, H-3eq), 1.57 (ddd, 1 H, $J_{3ax,3eq}$ = 13.5, $J_{3ax,4ax}$ = 10.9, $J_{3ax,4eq}$ = 6.3, H-3ax), 1.30 (d, 3 H, $J_{2',1'}$ = 6.5, H-2′); UV–vis (MeOH) λ_{max} (rel absorbance) 501.0 (19), 485.0 sh (19), 353.0 sh (4), 331.0 (6), 278.0 (21), 244.0 nm (100); MS (EI, low resolution, 190 °C),[68] m/z (rel intensity) inter alia, 370 (M + 2, 19), 369 (M + 1, 24), 368 (M, 65), 350 (M – H_2O, 38), 348 (100), 334 (58), 323 (M – $CH(OH)CH_3$, 25), 305 (M – $CH(OH)CH_3$ – H_2O, 52), 287 (M – $CH(OH)CH_3$ – $2H_2O$, 59) [if the temperature was increased to 230 °C the spectrum changed to 370 (M + 2, 53), 368 (M, 44), 350 (M – H_2O, 9), 348 (100), 334 (10), 323 (M – $CH(OH)CH_3$, 19), 315 (43), 307 (41), 287 (M – $CH(OH)CH_3$ – $2H_2O$, 28), indicating that the m/z 370 peak could be due to some thermal decomposition process]; MS (EI, high resolution), m/z 368.1257, calcd for $C_{21}H_{20}O_6$ 368.1260; MS (FAB, dithiothreitol/dithioerythritol matrix, low resolution positive ion), m/z (rel intensity) inter alia, 371 (M + 3, 34), 370 (M + 2, 100), 369 (M + 1, 83), 368 (M, 36), 351 (M + 1 – H_2O, 39), 324 (M + 1 – $CH(OH)CH_3$, 11), 323 (M – $CH(OH)CH_3$, 15), 307 (37), 291 (18), 281 (12); MS (FAB, mononitrobenzyl alcohol matrix, low resolution positive ion), m/z (rel intensity) inter alia, 370 (M + 2, 11), 369 (M + 1, 22), 368 (M, 12), 351 (M + 1 – H_2O, 7), 323 (M – $CH(OH)CH_3$, 5), 307 (100), 289 (59), 281 (23); MS (FAB, dithiothreitol/dithioerythritol matrix, low resolution negative ion), m/z (rel intensity) inter alia, 369 (M + 1, 43), 368 (M, 100), 367 (M – 1, 58), 353 (16), 333 (32), 307 (25), 305 (M – $CH(OH)CH_3$ – CO, 25), 273 (17); MS (FAB, mononitrobenzyl alcohol matrix, low resolution negative ion), m/z (rel intensity) inter alia, 369 (M + 1, 7), 368 (M, 19), 367 (M – 1, 17), 352 (9), 306 (98), 305 (M – $CH(OH)CH_3$ – CO, 100); MS (FAB, mononitrobenzyl alcohol matrix, high resolution, positive ion), m/z (M + 1) 369.1330, calcd for $C_{21}H_{21}O_6$ 369.1338.

[*R*-(*R,*R**)]-7,8,9,10-Tetrahydro-6,8,11-trihydroxy-8-(1-hydroxyethyl)-1-methoxy-5,12-naphthacenedione (23).** 10-Deoxydaunomycinone (**4**) (5.3 mg, 14 μmol) was dissolved in 80 mL of absolute EtOH. NaOMe (11.0 mg, 0.20 mmol) was added to the reaction mixture until the color of the solution changed from reddish orange to a translucent purple. $NaBH_4$ (2.0 mg, 52 μmol) was then added and the reaction proceeded for 1.5 h in the dark. After this time, the reaction was quenched by the addition of 2.0 mL of 0.4 N HCl, stirred 10 min, and then diluted with 300 mL of H_2O. The anthracyclinones were extracted into CH_2Cl_2 (5 × 25 mL), and these extracts were then washed with H_2O (2 × 200 mL) and dried (Na_2SO_4). Evaporation of the solvent provided 5.3 mg of solid. RP-HPLC analysis (detection at 485 nm) indicated that the product composition from this reaction was **23** (39.2%), **18** (30.4%), **22** (9.8%), **17** (8.5%), **24** (6.5%), and **16** (5.5%). Compound **23** was purified from this mixture by preparative RP-HPLC on the Du Pont C18 Zorbax column with the aforementioned chromatography conditions (t_R(**23**) = 55 min; t_R(**18**) = t_R(**24**) = 63 min, thus **18** could not be purified to homogeneity under these conditions). This purification yielded 1.6 mg (4.2 μmol) of **23** (30% overall yield, 99% pure). The spectral properties of **23** are in agreement with those reported by Russell et al.[36c]: mp 236–238 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.86 (s, 1 H, 11-OH), 13.48 (s, 1 H, 6-OH), 8.02 (d, 1 H, $J_{4,3}$ = 7.5, H-4), 7.76 (app t, J = 8.1, H-3), 7.37 (d, 1 H, $J_{2,3}$ = 8.4, H-2), 4.09 (s, 3 H, 1-OMe), 3.85 (q, 1 H, $J_{1',2'}$ = 6.4, H-1′), 3.05 (ddd, 1 H, $J_{10eq,10ax}$ = 18.6, $J_{10eq,9ax}$ = 5.3, $J_{10eq,9eq}$ = 3.2, H-10eq), 2.96 (d, 1 H, $J_{7eq,7ax}$ = 19.8 H-7eq), 2.9 (m, 1 H, H-10ax), 2.80 (d, 1 H, $J_{7ax,7eq}$ = 18.8 H-7ax), 2.01 (ddd, 1 H, $J_{9eq,9ax}$ = 13.1, $J_{9eq,10ax}$ = 5.3, $J_{9eq,10eq}$ = 2.9, H-9eq), 1.66 (ddd, 1 H, $J_{9ax,9eq}$ = 13.3, $J_{9ax,10ax}$ = 10.9, $J_{9ax,10eq}$ = 6.3, H-9ax), 1.32 (d, 3 H, $J_{2',1'}$ = 6.5, H-2′); UV–vis (MeOH) λ_{max} (rel absorbance) 529.0 (25), 493.5 (40), 290.5 (25), 253.0 (100), 235.5 nm (88); MS (EI, low resolution), m/z (rel intensity) inter alia, 386 (M + 2, 6), 385 (M + 1, 22), 384 (M, 62), 366 (10), 339 (M – $CH(OH)CH_3$, 100), 321 (M – $CH(OH)CH_3$ – H_2O, 22), 311 (M – $CH(OH)CH_3$ – CO, 18), 298 (24); MS (EI, high resolution), m/z 384.1207, calcd for $C_{21}H_{20}O_7$ 384.1209.

[*R*-(*R,*R**)]-1,2,3,4-Tetrahydro-2,11-dihydroxy-2-(1-hydroxyethyl)-7-methoxy-5,12-naphthacenedione (24).** NaOMe (11.5 mg, 0.21 mmol) was added to 80 mL of a 0.27 mM solution of **10** (8.0 mg, 22 μmol) in absolute EtOH until the mixture became purple. $NaBH_4$ (3.4 mg, 90 μmol) was added and the reaction mixture was placed in the dark to minimize photodecomposition. After 2 h, the reaction was quenched with 2.0 mL of 0.4 N HCl, stirred for 20 min, and diluted with 300 mL of H_2O. This solution was extracted with CH_2Cl_2 (5 × 25 mL), and these extracts were washed with H_2O (3 × 200 mL). After drying (Na_2SO_4), the CH_2Cl_2 was evaporated off to yield 8.0 mg of solid. RP-HPLC analysis indicated that the composition of this product mixture was 56.4% **24** and 40.9% **16**. A portion of this mixture (16 μmol) was subjected to preparative RP-HPLC on the Du Pont Zorbax column and with the aforementioned chromatographic conditions (t_R(**24**) = 61 min, t_R(**16**) = 69 min). This yielded 2.5 mg of **24** (6.8 μmol, 42% yield, 99% pure): mp 194–196 °C; ^{1}H NMR (300 MHz, $CDCl_3$ + 0.5% CF_3COOH)[69] δ 13.79 (s, 1 H, 11-OH), 8.55 (s, 1 H, H-6), 8.00 (d, 1 H, $J_{10,9}$ = 8.2, H-10), 7.62 (app. t, 1 H, J = 8.1, H-9), 7.08 (d, 1 H, $J_{8,9}$ = 7.9, H-8), 4.04 (s, 3 H, 7-OMe), 4.02 (q, 1 H, $J_{1',2'}$ = 6.5, H-1′), 2.95 (app ddd, 1 H, $J_{4eq,4ax}$ = 18.8, $J_{4eq,3eq}$ = 6.7, $J_{4eq,3eq}$ = 2.0, H-4eq), 2.94 (d, 1 H, $J_{1eq,1ax}$ = 18.0, H-1eq), 2.74 (app ddd, 1 H, $J_{4ax,4eq}$ = 19.8, $J_{4ax,3ax}$ = 10.3, $J_{4ax,3eq}$ = 6.2, H-4ax), 2.73 (d, 1 H, $J_{1ax,1eq}$ = 18.3, H-1ax), 2.04 (app ddd, 1 H, $J_{3eq,3ax}$ = 13.8, $J_{3eq,4ax}$ = 6.0, $J_{3eq,4eq}$ = 2.6, H-3eq), 1.66 (app ddd, 1 H, $J_{3ax,3eq}$ = 13.7, $J_{3ax,4ax}$ = 10.6, $J_{3ax,4eq}$ = 5.8, H-3ax), 1.37 (d, 3 H, $J_{2',1'}$ = 6.5, H-2′); UV–vis (MeOH) λ_{max} (rel absorbance) 479.5 (17), 342.0 sh (4), 324.0 (6), 278.0 (25), 242.5 nm (100); MS (EI, low resolution), m/z (rel intensity) inter alia, 370 (M + 2, 4), 369 (M + 1, 33), 368 (M, 100), 350 (M – H_2O, 10), 323 (M – $CH(OH)CH_3$, 52), 305 (M – H_2O – $CH(OH)CH_3$, 31), 295 (M – $CH(OH)CH_3$ – CO, 29), 277 (M – $CH(OH)CH_3$ – H_2O – CO, 12); MS (EI, high resolution), m/z 368.1259, calcd for $C_{21}H_{20}O_6$ 368.1260.

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Registry No. 1·HCl, 23541-50-6; **4**, 32384-98-8; **6a**, 101810-83-7; **6b**, 101810-84-8; **7**, 101834-86-0; **8**, 101914-74-3; **9**, 101810-85-9; **10**, 98151-30-5; **11**, 28008-51-7; **12**, 125515-17-5; **13**, 21794-55-8; **14**, 97068-04-7; **16**, 125438-66-6; **17**, 125438-67-7; **18**, 40940-87-2; **19a**, 125438-68-8; **19b**, 125438-75-7; **20**, 125438-69-9; **21**, 125438-70-2; **22**, 125438-71-3; **23**, 100992-86-7; **24**, 120001-57-2; **25a**, 125438-72-4; **25b**, 125438-76-8; **26**, 125438-73-5; **27**, 125438-74-6; **28**, 28008-55-1; isocitric dehydrogenase, 9001-58-5; ferredoxin reductase, 39369-37-4; aldo-keto reductase, 106640-75-9.

(68) The mass spectral behavior of **22** was complex. During MS-EI analyses of **22** a fragment at m/z = 370 (M + 2) was observed, which became more intense as the solid probe's temperature was increased beyond 200 °C. The MS-FAB analyses of **22** were also complex with the base peak, changing from m/z = 370 to m/z = 307 depending on the matrix used. This was the only 1′-dihydro aglycon compound that showed such behavior.

(69) Compound **24** appeared to readily deprotonate and precipitate out from $CDCl_3$ during ^{1}H NMR analyses. This problem was alleviated by the inclusion of trifluoroacetic acid (0.5%) in the NMR sample. This was the only 1′-dihydro aglycon that exhibited such behavior.