plated in triplicate. Survivors were counted as previously described.

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Reduction of Daunomycin and 11-Deoxydaunomycin with Sodium Dithionite in DMSO. Formation of Quinone Methide Sulfite Adducts and the First NMR Characterization of an Anthracycline Quinone Methide

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Abstract: Daunomycin (**1**) yields 7-deoxydaunomycinon-7-yl sulfonates (**6** and **7**) upon anaerobic reaction with 6 mol equiv of dithionite in 5% H_2O–95% DMSO followed by oxidation with molecular oxygen. The reaction is proposed to occur via reductive glycosidic cleavage to 7-deoxydaunomycinone quinone methide (**4**) followed by reversible dithionite addition to form adduct hydroquinone **17** and subsequent molecular oxygen oxidation at the hydroquinone and at the sulfur functional groups to yield **6** and **7**. Byproducts 7-deoxydaunomycinone (**2**) and bi(7-deoxydaunomycinon-7-yl) (**3**), epidaunomycinone (**8**), 7-deoxy-7-ketodaunomycinone (**9**), 7-deoxy-7,13-epidioxydaunomycinol (**10**), and daunomycinone (**11**) result from protonation of **4** and molecular oxygen oxidation of **4**, respectively. Sulfonate adducts **6** and **7** are relatively stable even in semiquinone and hydroquinone redox states. 11-Deoxydaunomycin (**12**) yields 7,11-dideoxydaunomycinon-7-yl sulfonates (**14** and **15**) upon similar reduction with even 1 mol equiv of dithionite. Sulfonates **14** and **15** are proposed to form by both dithionite and hydrogen sulfite addition to intermediate 7,11-dideoxydaunomycinone quinone methide (**16**), with hydrogen sulfite being a byproduct of quinone reduction by dithionite. With these reaction conditions, quinone methide **16** is long-lived and is characterized by ^1H NMR spectroscopy; the spectrum suggests a B-ring quinone methide structure.

Introduction

Anthracyclines such as daunomycin (**1**) and 11-deoxydaunomycin (**12**) are potentially bioreductively activated to quinone methide transients[1,2] via sequential semiquinone and hydroquinone redox states.[3,4] In vitro reductive activation with chemical,[5,6] electrochemical,[7] and enzymatic[8-10] reducing agents has been observed. Reaction of quinone methides with nucleophilic sites in critical biological molecules to yield adducts is possibly a source of cytotoxicity, and reaction with a proton source such as water to yield the 7-deoxyaglycons is possibly a detoxification pathway. Reactive free radical transients from reaction of the quinone methide with molecular oxygen have also been reported.[11] Consequently, the chemistry of quinone methides, generally,[12] and quinone methides from anthracyclines, specifically, is being actively studied.

Anaerobic reactivity is substituent-dependent especially with regard to the 11-position. The 11-deoxyanthracycline quinone methides, such as 7,11-dideoxydaunomycinone quinone methide (**16**), are less reactive with protic solvents to form 7-deoxyaglycons than 7-deoxydaunomycinone quinone methide (**4**). Consequently, they have longer lifetimes. Formation of aglycon dimers is sometimes the primary reaction pathway.[13-16] Dimerization occurs with one quinone methide serving as a nucleophile and the other as an electrophile with formation of the aglycon dimer initially in a half quinone, half hydroquinone state.[14] In aqueous media, both **16** and **4** also react with divalent sulfur nucleophiles such as *N*-acetylcysteine to give adducts[10,17] initially in hydroquinone states; subsequent oxidation yields stable cysteinyl adducts in quinone states. The adduct isolated yield is higher from 11-

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(1) Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249. Moore, H. W.; Czerniak, R.; Hamdan, A. *Drugs Exp. Clin. Res.* **1986**, *12*, 475.
(2) Lin, A. J.; Crosby, L. A.; Shansky, C. W.; Sartorelli, A. C. *J. Med. Chem.* **1972**, *15*, 1247. Lin, A. J.; Sartorelli, A. C. *J. Med. Chem.* **1976**, *19*, 1336.
(3) Gaudiano, G.; Koch, T. H. *Chem. Res. Toxicol.* **1991**, *4*, 2.
(4) Abdella, B. R. J.; Fisher, J. *Environ. Health Perspect.* **1985**, *64*, 3.
(5) Kleyer, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1984**, *106*, 2380.
(6) Lown, J. W.; Chen, H.-H. *Can. J. Chem.* **1981**, *59*, 390.
(7) Lown, J. W.; Chen, H.-H.; Plambeck, J. A.; Acton, E. M. *Biochem. Pharmacol.* **1982**, *31*, 575.
(8) Bachur, N. R.; Gordon, S. L.; Gee, M. V.; Kon, H. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 954.
(9) Fisher, J.; Ramakrishnan, K.; Becvar, J. E. *Biochemistry* **1983**, *22*, 1347.
(10) Fisher, J.; Abdella, B. R. J.; McLane, K. E. *Biochemistry* **1985**, *24*, 3562.
(11) Gaudiano, G.; Koch, T. H. *J. Am. Chem. Soc.* **1990**, *112*, 9424.
(12) Angle, S. R.; Yang, W. *J. Am. Chem. Soc.* **1990**, *112*, 4524. Angle, S. R.; Turnbull, K. D. *J. Am. Chem. Soc.* **1990**, *112*, 3698.
(13) Komiyama, T.; Oki, T.; Inui, T.; Takeuchi, T.; Umezawa, H. *Gann* **1979**, *70*, 403.
(14) Kleyer, D. L.; Gaudiano, G.; Koch, T. H. *J. Am. Chem. Soc.* **1984**, *106*, 1105.
(15) Boldt, M.; Gaudiano, G.; Koch, T. H. *J. Org. Chem.* **1987**, *52*, 2146.
(16) Boldt, M.; Gaudiano, G.; Haddadin, M. J.; Koch, T. H. *J. Am. Chem. Soc.* **1989**, *111*, 2283.
(17) Ramakrishnan, K.; Fisher, J. *J. Med. Chem.* **1986**, *29*, 1215.

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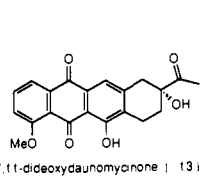
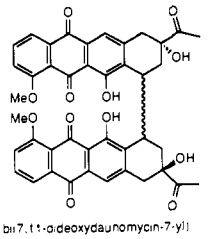
deoxydaunomycin than from daunomycin.

Medium is also important in directing the anaerobic chemistry. In protic media, 7,11-dideoxydaunomycinone quinone methide (**16**), but not 7-deoxydaunomycinone quinone methide (**4**), dimerizes to form, after oxidation, bi(7,11-dideoxydaunomycin-7-yl) in competition with protonation to form 7,11-dideoxydaunomycinone (**13**).[15] However, in 5% water–95% DMSO, **4** also dimerizes to form bi(7-deoxydaunomycinon-7-yl) (**3**) in competition with protonation.[18]





Quinone methide **4** reacts almost instantly with molecular oxygen to yield bi(7-deoxydaunomycinon-7-yl) (**3**), epidaunomycinone (**8**), 7-deoxy-7-ketodaunomycinone (**9**), 7-deoxy-7,13-epidioxydaunomycinol (**10**), and daunomycinone (**11**).[11] In this case, **3** has been proposed to result from initial one-electron oxidation of **4** to 7-deoxydaunomycinone semiquinone methide by molecular oxygen.

7-Deoxydaunomycinone quinone methide (**4**) and 7,11-dideoxydaunomycinone quinone methide (**16**) have been characterized spectroscopically only from visible absorption bands at 380 + 610[5] and 340 + 530 nm,[15] respectively, in protic medium. The 380 + 610 nm bands of **4** shift to 420 + 680 nm in DMSO solvent.[18]

We now report the anaerobic formation and reaction of **4** and **16** with excess dithionite in a predominantly DMSO solvent to yield the sulfite adducts 7-deoxydaunomycinon-7-yl sulfonates (**6** and **7**) and 7,11-dideoxydaunomycinon-7-yl sulfonates (**14** and **15**), respectively. The actual nucleophile in the formation of **6** and **7** appears to be dithionite, and in the formation of **14** and **15** either dithionite or bisulfite. Further, we present the first ^{1}H NMR characterization of a transient, anthracycline quinone methide, that of **16**.

Results and Discussion

Daunomycin was reacted with 6 mol equiv of dithionite in 5% water–95% DMSO under anaerobic conditions. UV–vis spectroscopy showed the following spectral changes as a function of time. The first spectrum, taken within 10 s from the beginning of the reaction, showed a typical hydroquinone absorption, λ_{max} = 445 nm (A = 1.6), with shoulders at 422 (A = 1.3) and 530 nm (A = 0.3). The spectrum was featureless ($A \leq 0.01$) at $\lambda >$ 580 nm. No significant spectral changes were observed during the following 4 h except a hypsochromic shift of the λ_{max} value to 440 nm. During the next 2 h, the hydroquinone band was replaced by a semiquinone band at 506 nm, which in turn changed to a quinone band (λ_{max} = 500 nm) in a few hours. In a second experiment, aliquots of the reaction were quenched by exposure to molecular oxygen at sequential time intervals. Reverse-phase HPLC analysis showed the following aglycon products in order of retention time: diastereomeric 7-deoxydaunomycinon-7-yl sulfonates (**6** and **7**), epidaunomycinone (**8**), 7-deoxy-7-ketodaunomycinone (**9**), 7-deoxy-7,13-epidioxydaunomycinol (**10**), daunomycinone (**11**), 7-deoxydaunomycinone (**2**), and diastereomeric bi(7-deoxydaunomycinon-7-yl)s (**3**) (Scheme I). Yields as a function of time interval are shown in Table I. Quenching with acid at similar time intervals yielded predominantly 7-deoxydaunomycinone (**2**). Products **8–11**, **2**, and **3** were characterized by chromatographic and spectroscopic comparison with authentic samples.[11] The sulfonates **6** and **7** were isolated by reverse-phase chromatography in 8% and 19% yields, respectively. The highest state of purity achieved was about 95%. The structures were evident from spectroscopic and analytical data. Of particular significance were the characteristic ^{1}H NMR spectra and the negative ion FAB molecular ions. The proton at the 7-position characteristically appeared in DMSO-d_6 in the region of δ 4.5–5 ppm with significant coupling to only one (isomer **6**) or both protons (isomer **7**) at the 8-position (see Scheme I for the anthracycline numbering system).

Scheme I

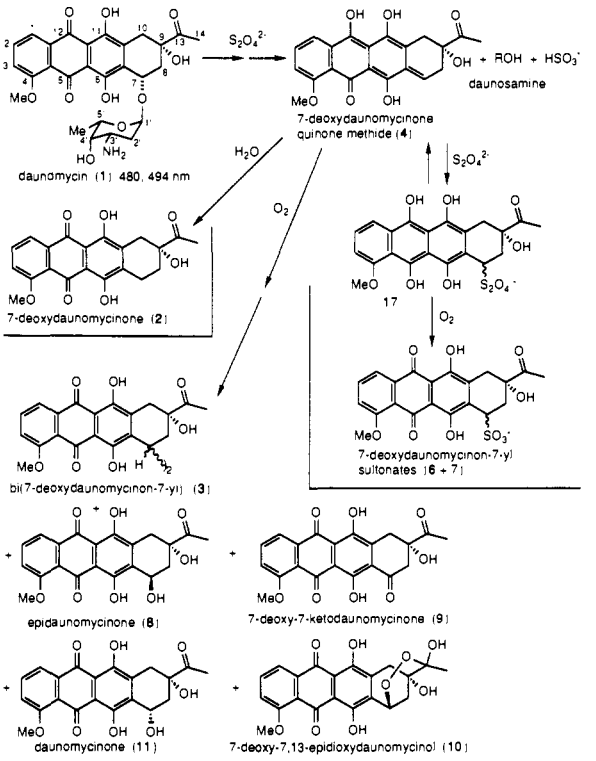


Table I. Product Yields from Quenching with Molecular Oxygen the Reaction of Daunomycin with Excess Dithionite as a Function of Quenching Time[a]

	yield, %		
quenching time	sulfonates **6** + **7**	oxidation products **3** + (**8–11**)	7-deoxydaunomycinone **2**
2 min	50	35	15
15 min	30	31	38
140 min	5	13	81
24 h		traces	90

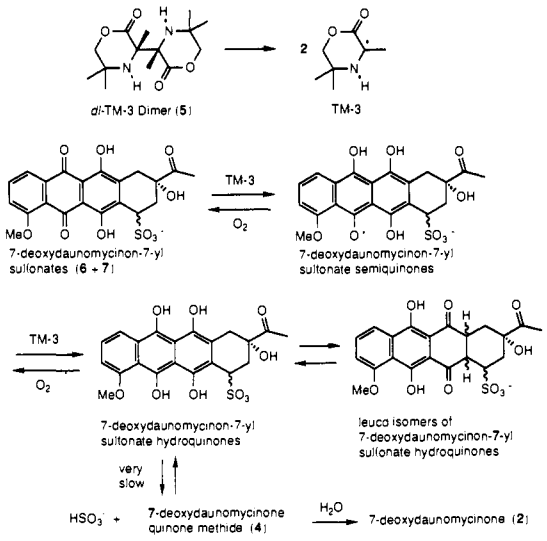
[a] Yields were simply determined from peak areas; the yield of product **3** was not corrected for its two chromophoric units. No correction was made for the anticipated difference in detector response to product **9**.

The reduction was also performed with 1 mol equiv of dithionite in the presence of 11 mol equiv of sodium hydrogen sulfite with monitoring of the UV–vis spectral changes. Significant absorption by quinone methide **4** immediately appeared at 680 nm upon addition of 1 mol equiv of dithionite. The disappearance of this band required approximately 2 h in both the presence and absence of 11 mol equiv of sodium hydrogen sulfite. Consequently, sodium hydrogen sulfite, a byproduct of reduction with dithionite, does not appear to be the nucleophile responsible for the formation of **6** and **7**. The nucleophile was shown to be dithionite by generating quinone methide **4** with 1 mol equiv of dithionite[18] and subsequently adding 1 mol equiv of dithionite in 0.5 mol equiv steps. The first addition instantly halved the quinone methide absorbance, and the second addition reduced it to zero. Concomitant with the reduction in the quinone methide absorbance at 680 nm was an increase in the hydroquinone absorbance at 440 nm.

These results are rationalized by the mechanism shown in Scheme I. Reduction with excess dithionite initially yields quinone methide **4** via daunomycin semiquinone and hydroquinone redox

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

Scheme II



states. The actual reducing species is most likely $SO_2^{\cdot-}$ from homolysis of the sulfur–sulfur bond,[18] although $S_2O_4^{2-}$ can also react directly as a reducing agent.[19] The quinone methide then reacts reversibly with dithionite to form predominantly diastereomeric adduct hydroquinones **17**. In competition is protonation of **4** by water to yield 7-deoxydaunomycinone (**2**). Because **17** is formed reversibly and **2** is formed irreversibly, long reaction times favor **2** (Table I). Quenching with molecular oxygen first consumes excess dithionite, which shifts the equilibrium between **4** and **17** toward **4**. Subsequently, and no doubt to some extent simultaneously, oxygen oxidizes **17** to 7-deoxydaunomycinon-7-yl sulfonates (**6** and **7**) and reacts with **4** to yield **3** and **8–11**. Some **3** may also arise via the anaerobic dimerization of **4**, which was observed earlier in 5% H_2O–95% DMSO.[18] Predominant formation of 7-deoxyaglycon from quenching with acid similarly supports the equilibrium between **17** and **4** with the acid protonating **4** and driving the reaction toward 7-deoxydaunomycinone (**2**).

The sulfur species initially adding to **4** might also be $SO_2^{\cdot-}$; dithionite is proposed here because the equilibrium constant for formation of $SO_2^{\cdot-}$ is only 1.4×10^{-9} M in water at 25 °C.[20] If the equilibrium constant in 95% DMSO–5% water were the same, 99.9% of the dithionite would be undissociated. Addition of dithionite to **4** caused instant conversion to a hydroquinone.

A curious result was the observation of a quinone band preceded sequentially by semiquinone and hydroquinone bands from anaerobic reduction of daunomycin with 6 mol equiv of dithionite after about 7 h of reaction time. What was expected on the basis of the above discussion was the ultimate formation of 7-deoxydaunomycinone hydroquinone (λ_{max} = 440 nm) with residual dithionite. A control experiment provides an explanation. An anaerobic solution of dithionite at the same concentration in 5% H_2O–95% DMSO was monitored by UV–vis spectroscopy at the absorbance of dithionite (λ_{max} = 298 nm). Even with no daunomycin present, dithionite was unstable and completely disappeared in 4.5 h, possibly through slow reaction with DMSO. A similar control experiment in water showed no destruction of dithionite. Consequently, the final quinone band, namely, that arising from 7-deoxydaunomycinone, preceded by semiquinone and hydroquinone bands results from the combination of reversible dithionite addition to the quinone methide, irreversible protonation of the quinone methide, destruction of the dithionite, and equilibrium of semiquinones with quinones and hydroquinones.

Reaction of **6** in 5% H_2O–95% DMSO with 2 mol equiv of the one-electron reducing agent 3,5,5-trimethyl-2-oxomorpholin-3-yl (TM-3) from bond homolysis of bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (TM-3 dimer, **5**)[5,21] yielded a free radical transient with a semiquinone type visible absorption at 514 nm[18] and a semiquinone type EPR spectrum. The semiquinone absorption maximized in about 30 min to slowly decay during the next 18 h with the formation of bands at 420 and 440 nm possibly due to hydroquinone and leuco[22] chromophores (Scheme II). Leuco isomers of hydroquinones such as that of 7-deoxydaunomycinon-7-yl sulfonate form reversibly when elimination of a group at the 7-position is less favorable.[22,23] After a total reaction time of 24 h and subsequent exposure to molecular oxygen, HPLC analysis showed only the presence of **6**. Similar UV–vis spectroscopic changes occurred upon reduction of **7** with TM-3 dimer, although the changes occurred more rapidly. HPLC analysis of the reaction mixture at termination showed 90% recovery of **7** with 4% formation of 7-deoxydaunomycinone (**2**). These observations indicate that **6** and **7** undergo cleavage of their sulfonyl groups in reduced states in 5% H_2O–95% DMSO very slowly (Scheme II).

The EPR spectrum of the semiquinone of **6** showed a g value of 2.0030 and was simulated with the following hyperfine coupling constants: a_H = 1.46, 1.36, 1.06, 0.99, 0.78, 0.72, 0.66, 0.62, and 0.19 G. The EPR spectrum was also obtained in 5% D_2O–95% DMSO and simulated with the following splitting constants: a_H = 1.48, 1.37, 1.00, 0.96, 0.81, and 0.75 G and a_D = 0.11, 0.10, and 0.03. This indicates that the three smallest splittings observed in 5% H_2O–95% DMSO represent exchangeable protons, most likely the phenolic protons. The EPR spectra of semiquinones of daunomycin and 7-deoxydaunomycinone in DMSO and DMSO–H_2O show only two exchangeable protons.[18,24] A reasonable explanation is that the semiquinone of **6** is anionic on the sulfonyl group and the semiquinones of daunomycin and 7-deoxydaunomycinone are anionic on the semiquinone functionality. A similar difference in the location of negative charge in the respective hydroquinone states may be an explanation for the lack of glycosidic cleavage upon reduction of **6** and **7** as mentioned above.

UV–vis monitoring of an anaerobic reaction of 11-deoxydaunomycin (**12**) with 1 mol equiv of dithionite in 2% D_2O–30% acetone-d_6–70% DMSO-d_6 showed rapid formation of 7,11-dideoxydaunomycinone quinone methide (**16**) followed by slow formation of a hydroquinone over a period of 16 h. Addition of molecular oxygen led to the formation of quinones. The solvent system was chosen for this and subsequent experiments to permit ultimate NMR characterization of **16** (vide infra). HPLC analysis of the reaction mixture showed a 71% yield of a mixture of the two diastereomeric 7,11-dideoxydaunomycinon-7-yl sulfonates (**14** and **15**). Reduction of daunomycin under similar conditions would have yielded little if any sulfonate adducts. Reduction of **12** with 2 mol equiv of dithionite showed only immediate formation of hydroquinones. Quenching with molecular oxygen after 10 min of reaction yielded 51% of a 1:2 mixture of **14** and **15**. The balance of reaction products was a complicated mixture of unknown products which were probably equivalent to those observed from the molecular oxygen oxidation of 7-deoxydaunomycinone quinone methide (**4**) shown in Scheme I. The presence of semiquinones was not observed by UV–vis or EPR spectroscopy during reduction of **12**, suggesting that disproportionation was relatively rapid. The sulfonate adducts were isolated by extraction followed by chromatography and were characterized from spectral properties. Again, molecular ions were observed by negative ion FAB mass spectrometry. The protons at the 7-position appeared in the region of δ 4.5–5 ppm, this time as doublet of doublet patterns with 2- and 8-Hz splittings by the protons at the 8-position.

(19) McKenna, C. E.; Gutheil, W. G.; Song, W. *Biochim. Biophys. Acta* **1991**, *1075*, 109. Tsukahara, K.; Wilkins, R. G. *J. Am. Chem. Soc.* **1985**, *107*, 2632.
(20) Lambeth, D. O.; Palmer, G. *J. Biol. Chem.* **1973**, *248*, 6095.
(21) Burns, J. M.; Wharry, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1981**, *103*, 2345.
(22) Brand, D. J.; Fisher, J. *J. Am. Chem. Soc.* **1986**, *108*, 3088. Brand, D. J.; Fisher, J. *J. Org. Chem.* **1990**, *55*, 2518.
(23) Bird, D. M.; Gaudiano, G.; Koch, T. H. *J. Am. Chem. Soc.* **1991**, *113*, 308.
(24) Schreiber, J.; Mottley, C.; Sinha, B. K.; Kalyanaraman, B.; Mason, R. P. *J. Am. Chem. Soc.* **1987**, *109*, 348.

Table II. ^{1}H NMR Spectral Data at 300 MHz for 11-Deoxydaunomycin (**12**) and Its Quinone Methide in 70:30:5 DMSO-d_6–Acetone-d_6–D_2O

compound	H3(1)	H2	H1(3)	H11	H7	CH_3O	H10a	H10b	H8a	H8b	CH_3CO
12[a]	7.90	7.84	7.64	7.46	5.31	3.99	3.15	3.01	1.6–2.3	1.6–2.3	2.33
	d, J = 8	t, J = 8	d, J = 8	s	br s	s	d, J = 17	d, J = 17	m[b]	m[b]	s
quinone methide **16**[c]	7.74	7.62	7.02	6.95	7.27	3.90	2.79	2.70	2.86	2.58	2.26
	d, J = 8	t, J = 8	d, J = 8	s	m[d]	s	d, J = 16	d, J = 16	dd,[e] J = 4, 20	dd,[f] J = 5, 20	s

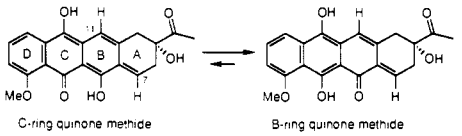
[a] The proton signals from the daunosaminyl group are not reported. [b] Overlapped with the proton signals from the daunosaminyl group. [c] Approximately 80% of **16** from inspection of the aromatic region. [d] X part of an AMX pattern; appears as a broadened triplet. [e] A part of an AMX pattern. [f] M part of an AMX pattern.

The effect of sodium hydrogen sulfite on the disappearance of the quinone methide band was similarly determined by creating quinone methide **16** with TM-3 dimer and then adding a 5 mol excess of sodium hydrogen sulfite. The addition caused the quinone methide band to disappear in 1 h to be replaced by a hydroquinone band. In comparison, the quinone methide from reduction with 1 mol equiv of dithionite required 16 h. These observations are consistent with sulfonate adducts **14** and **15** forming as shown in Scheme III (note: **16** is shown as a B-ring quinone methide on the basis of discussion presented below). Even hydrogen sulfite is sufficiently nucleophilic to react with the more electrophilic quinone methide **16**. Dithionite is still the more reactive nucleophile. In the reduction with 2 mol equiv of dithionite, **16** disappeared as soon as it was formed.

An analogous sulfonate derivative of the quinone antitumor drug mitomycin C has recently been characterized from reductive activation with dithionite.[25] The derivative was proposed to result from bisulfite addition to a transient from the reductive activation.

The relative rates of formation and destruction of the 7,11-dideoxydaunomycinone quinone methide (**16**) permitted determination of the molar extinction coefficients for the UV–vis bands, 354 (8000 M^{-1} cm^{-1}) and 530 nm (12 000 M^{-1} cm^{-1}), and the ^{1}H NMR resonances of **16**. The NMR spectrum of **16** compared with that of the aglycon portion of **12** is reported in Table II. The assignment of the resonances for the C-7 and C-8 protons was consistent with spin decoupling experiments (Experimental Section). As expected, the most significant differences in chemical shift occur for protons at positions 7 and 8, which are olefinic and allylic in **16** and benzylic and saturated in **12**, respectively. Similar attempts to observe the ^{1}H NMR spectrum of 7-deoxydaunomycinone quinone methide (**4**) were much less successful because of paramagnetic broadening from semiquinones whose presence could not be eliminated.

The proton at the 7-position of **16** is significantly deshielded, consistent with it being β to a carbonyl group. An internal chemical shift reference is the proton at the 11-position which is peri to the hydroxy substituent at the 12-position and resonates at a chemical shift 0.32 ppm higher than the proton at the 7-position. The structure, then, is more like a B-ring quinone methide than a C-ring quinone methide, which has traditionally been assigned. Interconversion between the B-ring and C-ring quinone methides involves simply the tautomeric shift of a hydrogen-bonded proton. Thermodynamically, the B-ring structure also seems reasonable since it contains two aromatic rings; group equivalent calculations[26] estimate the B-ring structure to be 19 kcal/mol more stable than the C-ring structure. Also from a kinetic perspective, formation of a B-ring quinone methide might allow intramolecular proton transfer concomitant with cleavage of the glycosidic bond. As reported in the Experimental Section, paramagnetic transients precluded the NMR characterization of **4**. The results of pulse radiolysis experiments on daunomycin in protic media have been interpreted in terms of the formation of a B-ring quinone methide preceding a C-ring quinone methide;[27] the investigators also pointed out that the B-ring structure is consistent with a quantum semiempirical calculation.[28] Formation of the B-ring structure may be relevant to the lack of glycosidic cleavage observed upon reduction of 4-demethoxy-6-deoxydaunomycin[29] and the pentaacetate of daunomycin.[30]



Several important ideas about anthracycline aglycon quinone methide structure and reactivity result from the observations reported here. A transient anthracycline-derived quinone methide is now characterized by an ^{1}H NMR spectrum which suggests a B-ring quinone methide tautomer structure. The aprotic medium DMSO promotes nucleophilic addition in competition with electrophilic protonation by 5% H_2O. Dithionite or $SO_2^{\cdot-}$ appears to be a better nucleophile than hydrogen sulfite for additions to quinone methides. Dithionite or $SO_2^{\cdot-}$ adduct hydroquinones also eliminate dithionite or $SO_2^{\cdot-}$, respectively, and irreversible adduct formation is achieved by molecular oxygen oxidation to sulfonate adduct quinones. Sulfonate adducts even in hydroquinone redox states do not eliminate sulfite or hydrogen sulfite to reform quinone methides very rapidly.

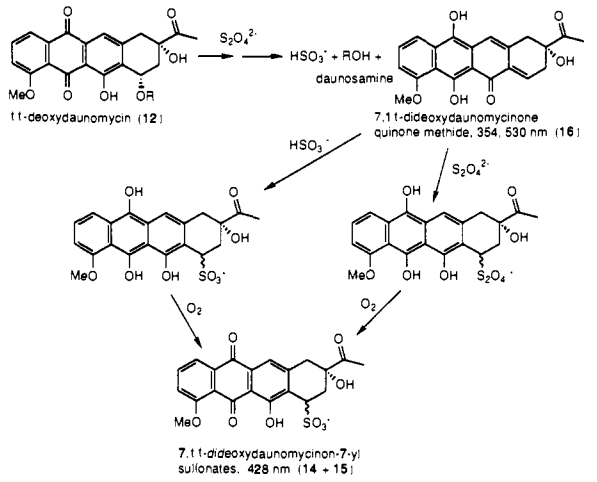
The biological implications include the fact that covalent binding of anthracycline-derived quinone methides to biological molecules via nucleophilic addition at the 7-position would be favored in aprotic regions of cells. The observation that a nucleophile can be a poor leaving group for an anthracycline aglycon in its hydroquinone state suggests that nucleophilic addition to a quinone methide might yield a stable lesion to a biological molecule. Another example of irreversible nucleophilic addition to an anthracycline-derived quinone methide was the intramolecular formation of a cyclo-semicarbazone from the reduction of daunomycin semicarbazone.[31] Recent experiments by Cummings and co-workers suggest that adriamycin (14-hydroxydaunomycin) becomes covalently bound to the DNA of MCF-7 human breast cancer cells in culture.[32]

Experimental Section

General Remarks. UV–visible spectra were recorded with a Hewlett-Packard 8452 A diode array spectrometer. ^{1}H NMR spectra were obtained with a Varian VXR-300S or a Bruker Pulse CXP BR 30/60K spectrometer. Chemical shifts are reported (in ppm) on the δ scale from internal tetramethylsilane or 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt. Coupling constants are in hertz. Mass spectra were obtained with a VG 7070 EQ-HF high-resolution mass spectrometer. FAB spectra were obtained by bombarding samples in dithiothreitol–dithioerythritol with argon atoms unless otherwise specified. HPLC analyses were performed with a Hewlett-Packard 1090 liquid chromatograph equipped with a diode array detector and work station. Chromatography was performed with a Hewlett-Packard 5-μm C_{18} microbore column, 2.1 mm i.d. × 100 mm, eluting with a 60:40 (method A) or 45:55 (method B) mixture of methanol and 0.3% ammonium formate in water, adjusted

(25) McGuinness, B. F.; Lipman, R.; Nakanishi, K.; Tomasz, M. *J. Org. Chem.* **1991**, *56*, 4826.
(26) Benson, S. W. *Thermochemical Kinetics*, 2nd ed.; Wiley: New York, 1976.
(27) Houée-Levin, C.; Gardès-Albert, M.; Ferradini, C. *J. Free Radicals Biol. Med.* **1986**, *2*, 89.
(28) Houée-Levin, C.; Blain, M.; Odiot, S. *THEOCHEM* **1986**, *136*, 229.
(29) Malatesta, V.; Penco, S.; Sacchi, N.; Valentini, L.; Vigevani, A.; Arcamone, F. *Can. J. Chem.* **1984**, *62*, 2845.
(30) Sulikowski, G. A.; Turos, E.; Danishefsky, S. J.; Shulte, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 1373.
(31) Gaudiano, G.; Frigerio, M.; Bravo, P.; Koch, T. H. *J. Am. Chem. Soc.* **1990**, *112*, 6704.
(32) Cummings, J.; Bartoxzek, A.; Smyth, J. F. *Anal. Biochem.* **1991**, *194*, 146.

Scheme III



to pH 4 with 98% formic acid at a 0.3 mL/min flow rate. Method C used gradient elution from 45:55 to 60:40 of the same solvent mixture over 7 min. The percentages of anthracyclines reported from HPLC analyses are the area percentages of the peaks resulting from monitoring at 480 (for daunomycin derivatives) or 420 nm (for 11-deoxydaunomycin derivatives). Preparative HPLC was performed with an 8-μm C_{18} reverse-phase Dynamax column, 4.6 mm i.d. × 250 mm from Rainin. TLC was performed with Merck precoated silica gel 60 F-254 sheets. Elemental analyses were performed by Atlantic Microlab, Norcross, GA. The experiments involving the use of sodium dithionite or TM-3 dimer as reducing agents were run under anaerobic conditions. The use of nitrogen- or argon-degassed solutions in UV cuvettes provided with serum stoppers proved to be satisfactory only for short-time experiments (less than 2 h). For long-lasting experiments, the freeze–pump–thaw degassing procedure was necessary.[5] Three types of reaction cells were used: (A) a 10-mm Pyrex cuvette fused at a 120° angle to a degassing chamber 1.6 cm o.d. × 4.5 cm and a 0.9-cm tube for attachment to a vacuum line with an Ultra-Torr Union; (B) a 10-mm or 1-mm cuvette, a 2-mL flask, and a 7-mL flask (degassing chamber) all fused perpendicularly to and in the same plane with a 0.9-cm tube to make an "E" shape, and a second 0.9-cm tube for attachment to the vacuum line fused perpendicularly to the first and coaxially with the 2-mL flask; (C) a type B cell with the addition of a quartz EPR tube fused to the first 0.9-cm tube coaxial to the 7-mL flask.[18] Trizma buffers were prepared from tris(hydroxymethyl)aminomethane (Tris) and Tris hydrochloride (Tris·HCl). Tris and Tris·HCl were purchased from Boehringer Mannheim GmbH, Germany. Daunomycin, 11-deoxydaunomycin, and daunosamine hydrochorides were a gift from Farmitalia Carlo-Erba, Milan, Italy. 7-Deoxydaunomycinone[33] and *dl*-bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (*dl*-TM-3 dimer)[34] were prepared as described in the literature. Sodium dithionite, approximately 80% pure, was obtained from Aldrich Chemical Co., Milwaukee, WI, or E. Merck, Darmstadt, Germany. Its purity was occasionally checked by iodine titration in water containing 2% sodium bicarbonate. Sodium hydrogen sulfite and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were also obtained from Aldrich.

Reaction of Daunomycin (1) with Sodium Dithionite (6 mol equiv). (a) A 3-mL aliquot of a dimethyl sulfoxide solution 9.3×10^{-5} M in **1** (2.8×10^{-4} mmol) and $(1 + 1) \times 10^{-3}$ M in Trizma were introduced into the 7-mL arm of a type B cell. Nitrogen was bubbled through the solution via Teflon tubing. Sodium dithionite (81%, 2.82 mg) was dissolved in 1.2 mL of deaerated water. In less than 1 min, 0.15 mL of this solution (1.6×10^{-3} mmol of dithionite) was introduced via a syringe provided with Teflon tubing into the 2-mL arm of the cell under nitrogen flow. The arm had been previously externally cooled at a temperature below −20 °C to freeze the solution in order to minimize air oxidation. Immediately, the cell was transferred to the vacuum line, and both the daunomycin and the dithionite solutions were freeze–thaw degassed through three cycles. The cell was flame-sealed under vacuum and brought to ambient temperature. Very quickly the two solutions were mixed and poured into the cuvette portion of the cell, which was immediately transferred to the UV–vis spectrometer for spectral monitoring over a period of several hours. The cell was opened after a total of 21 h. The solvent was rotary-evaporated (40 °C, 0.1 Torr), and the residue was dissolved in methanol and analyzed by HPLC (method A) to show 87% of 7-deoxydaunomycinone (**2**), t_R 8.1 min, along with at least five minor side products.

(b) **Quenching with Oxygen.** A dimethyl sulfoxide solution (20 mL) 9.2×10^{-5} M in daunomycin (**1**, 1.8×10^{-3} mmol) and $(1 + 1) \times 10^{-3}$ M in Trizma was introduced into a 25-mL flask. The flask was serum-stoppered, and nitrogen was bubbled through the solution via two needles. A deaerated solution of dithionite (2.3 mg of an 81% sample in 1.0 mL of water, 1.1×10^{-2} mmol) was injected into the daunomycin solution, which immediately turned from orange to yellow. Four 2-mL portions of the reaction mixture were then withdrawn at different times (2 min, 15 min, 140 min, 24 h) via syringe, and each was transferred to a 20-mL flask and quickly shaken with air. The solvent from each portion was then rotary-evaporated, and the residue was dissolved in 1 mL of methanol and analyzed by HPLC (method A). The reaction products appeared in the following order: 7-deoxydaunomycinon-7-yl sulfonates (**6** + **7**, t_R 1–2 min, partially overlapped), 7-epidaunomycinone (**8**, t_R 3 min), 7-deoxy-7-ketodaunomycinone (**9**, t_R 3.5 min, overlapped with **8**), 7-deoxy-7,13-epidioxydaunomycinol (**10**, t_R 4.2 min), daunomycinone (**11**, t_R 11.2 min), 7-deoxydaunomycinone (**2**, t_R 8.2 min), bi(7-deoxydaunomycinon-7-yl) (**3**, two stereoisomers, t_R 12–13 min). They were identified both by HPLC and TLC comparisons with authentic samples.[11] The results are summarized in Table I. When 2 mL of the 21-mL original solution was added to 2 mL of dimethyl sulfoxide in a UV cuvette at time 3 min in the presence of air, a short-lived (few seconds) green color, λ_{max} = 680 nm, appeared, typical of **4**, which was then replaced by the orange color, λ_{max} = 496 nm, of the quinone chromophore.

(c) **Quenching with Acid.** Using the same 21-mL solution obtained as described above from **1** and sodium dithionite, quenching experiments with acid were performed at different times (2 min, 15 min, 140 min) as follows: 2-mL portions were transferred via syringe from the reaction flask, each into a 5-mL vial containing 0.3 mL of a 0.1 M aqueous hydrochloric acid solution. In all cases, HPLC analysis showed 82–95% of **2** along with a mixture of small amounts of at least five side products.

Isolation and Characterization of the Stereoisomeric 7-Deoxydaunomycinon-7-yl Sulfonates (6 and 7). Sodium dithionite (246 mg of an 85% sample, 1.2 mmol) dissolved in 5 mL of deaerated water was added to a 100-mL dimethyl sulfoxide solution of daunomycin hydrochloride (**1**, 113 mg, 0.20 mmol), $2 \times (1 + 1) \times 10^{-2}$ M in Trizma, under nitrogen bubbling. After 1 min, the solution was poured into 100 mL of dimethyl sulfoxide saturated with pure oxygen. The solvent was rotary-evaporated at 40 °C, 0.1 Torr, down to a volume of a few milliliters. Water (15 mL) was added to the residue, and the mixture was extracted three times with 30 mL of chloroform each time. HPLC analysis (method B) of the filtered aqueous solution showed 90% of a mixture of **6**, t_R 1.8 min, and **7**, t_R 4.6 min, in a 0.6:1 ratio. The aqueous solution was again rotary-evaporated and kept at 0.1 Torr, 40 °C, for 0.5 h. Hydrochloric acid (0.1 M, 20 mL) was added, and **6** and **7** were separated by suction chromatography[35] on Baker 40-μm reverse-phase Octylsilane (C_8) bonded to silica gel. Deoxy sulfonate **6** was first eluted with water; **7** was then eluted with a 95:5 water–methanol mixture. Deoxy sulfonate **7**, 20 mg of material 89% pure by HPLC (19% yield), from the chromatography was further purified to approximately 95% purity by crystallization from acetonitrile (30 mL): UV [$(1 + 1) \times 10^{-3}$ M Trizma in dimethyl sulfoxide] λ_{max}, nm (ϵ, M^{-1} cm^{-1}) 372 (2.8×10^3), 486 (sh, 8.7×10^3), 504 (9.3×10^3), 540 (sh, 5.7×10^3); ^{1}H NMR (DMSO-d_6) δ 7.85–7.95 (m, H2 + H3), 7.64 (dd, $J_{1,2}$ = 7, $J_{1,3}$ = 3, H1), 6.68 (s, C9-OH, disappeared with D_2O), 4.54 (dd, $J_{7,8a}$ = 2, $J_{7,8b}$ = 8, H7), 3.99 (s, CH_3O), 3.35 (d, $J_{10a,10b}$ = 17, H10a), 2.92 (d, $J_{10b,10a}$ = 17, H10b), 2.53 (d, $J_{8a,7}$ = 2, the downfield part of a dd, $^1/_2$ H8a), 2.48 (part of a dd, partially overlapped with the solvent peak, $^1/_2$ H8a), 2.19 (dd, $J_{8b,7}$ = 8, $J_{8b,8a}$ = 14, H8b), 2.14 (s, CH_3CO); ^{1}H NMR (D_2O) δ 7.3–7.5 (m, H2 + H3), 7.19 (d, $J_{1,2}$ = 8, H1), 4.97 (d, $J_{7,8a}$ = 8, H7), 3.83 (s, CH_3O), 3.56 (d, $J_{10a,10b}$ = 16.5, H10a), 3.03 (d, $J_{10b,10a}$ = 16.5, H10b), 2.74 (dd, $J_{8a,7}$ = 8, $J_{8a,8b}$ = 15, H8a), 2.62 (d, $J_{8b,8a}$ = 15, H8b), 2.37 (s, CH_3CO); FAB mass spectrum, negative ion m/z 461 (M − 1). Anal. Calcd for $C_{21}H_{18}SO_{10}$: S, 6.93. Found: S, 6.67. Isomer **6** [10 mg, 78% pure (by HPLC), 8% yield], as obtained from suction chromatography, was further purified by preparative HPLC, eluting with 43:57 water–methanol at a 0.55 mL/min flow rate. The solvent from the combined chromatography fractions was rotary-evaporated, and the residue was washed with chloroform and a little 2-propanol to give approximately 95% pure **6**: UV [$(1 + 1) \times 10^{-3}$ M Trizma in dimethyl sulfoxide] λ_{max}, nm (ϵ, M^{-1} cm^{-1}) 292 (6.2×10^3), 375 (3.6×10^3), 485 (sh, 8.5×10^3), 506 (9.8×10^3), 542 (sh, 6.2×10^3); ^{1}H NMR (DMSO-d_6) δ 7.85–8.0 (m, H2 + H3), 7.64 (d, $J_{1,2}$ = 8, H1), 5.11 (s, C9-OH, disappeared with D_2O), 4.51 (d, $J_{7,8b}$ = 10, H7), 3.99 (s, CH_3O), 3.03 (s (sic!), 2H10), 2.71 (d, $J_{8a,8b}$ = 15, H8a), 2.26 (s,

(33) Barone, A. D.; Atkinson, R. F.; Wharry, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1981**, *103*, 1606.

(34) Bennett, R. W.; Wharry, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1980**, *102*, 2345.

(35) Harwood, L. M. *Aldrichimica Acta* **1985**, *18*, 25.

CH_3CO), 2.02 (dd, $J_{8b,7}$ = 10, $J_{8b,8a}$ = 15, H8b); ^1H NMR (D_2O) δ 7.1–7.4 (m, H2 + H3), 7.0 (d, $J_{1,2}$ = 8, H1), 5.00 (d, $J_{7,8b}$ = 9, H7), 3.72 (s, CH_3O), 3.38 (d, $J_{10a,10b}$ = 16, H10a), 3.09 (d, $J_{10b,10a}$ = 16, H10b), 2.95 (d, $J_{8a,8b}$ = 16, H8a), 2.51 (s, CH_3CO), 2.38–2.5 (m, H8b); FAB mass spectrum, negative ion m/z 461 (M − 1); FAB exact mass (polyethylene glycol PEG 600 as reference) 461.0523, calcd for $C_{21}H_{17}SO_{10}$ 461.0542.

Reaction of Daunomycinon-7-yl Sulfonates (6 and 7) with *d,l*-TM-3 Dimer (5) (1 mol equiv). EPR Spectrum of the Semiquinone of 6. (a) *d,l*-TM-3 dimer (**5**, 0.95 mg, 3.35×10^{-3} mmol) was dissolved in 10 mL of dichloromethane. A 0.67-mL aliquot of this solution (2.2×10^{-4} mmol of **5**) was introduced into the cuvette portion of a type C cell, and the solvent was evaporated with a nitrogen stream. A 2.0-mL volume of a 1.1×10^{-4} M solution of **6** (2.2×10^{-4} mmol) and $(1 + 1) \times 10^{-3}$ M in Trizma in 95:5 dimethyl sulfoxide–deuterium oxide was introduced into the degassing chamber of the cell. The solution was freeze–thaw degassed through three cycles, and the cell was sealed under vacuum and brought to ambient temperature. The reactants were mixed by shaking. The following spectral changes at 25.0 ± 0.1 °C were observed: during the first 30 min the quinone band at 504 nm slowly decreased to half of its original intensity, while a semiquinone band at 514 nm rose and reached its maximum intensity in ca. 30 min. During this time, EPR spectra were also recorded, showing a multiline signal with g = 2.0030 and the following hyperfine splitting constants: a_H = 1.48, 1.37, 1.00, 0.96, 0.81, and 0.75 G and a_D = 0.11, 0.10, and 0.03 G. During the next 18 h, the 514-nm band slowly decreased to almost disappear. Two bands at 420 and 440 nm appeared instead, due to hydroquinone and possibly "leuco" chromophores,[22,23] with their relative intensity changing from 0.9 to 1.1. After a total of 24 h the cell was opened. HPLC analysis showed the presence of only **6**.

(b) When a similar experiment was run using 95:5 dimethyl sulfoxide–water as solvent, the EPR spectrum showed the following hyperfine couplings: a_H = 1.46, 1.36, 1.06, 0.99, 0.78, 0.72, 0.66, 0.62, and 0.19 G.

(c) When a similar experiment was run using a 10^{-4} M solution of **7** in 100% dimethyl sulfoxide and $(1 + 1) \times 10^{-3}$ M in Trizma in a type A cell, UV–vis spectroscopic changes were observed similar to those described in paragraph a, but at a faster pace. The 514-nm semiquinone band reached its maximum intensity in 20 min and faded down in ca. 2 h. The cell was opened after a total of 4 h. HPLC analysis showed 90% of **7** and 4% of **2**.

Attempted Trapping of Daunomycin Quinone Methide (4) by Sodium Hydrogen Sulfite. A 3.0-mL volume of dimethyl sulfoxide solution 1.1×10^{-4} M in daunomycin and $(1 + 1) \times 10^{-2}$ M in Trizma was introduced into the degassing chamber of a type A cell. Nitrogen was bubbled through the solution. A mixture of 5.6 mg of sodium hydrogen sulfite and 1.06 mg of 81% sodium dithionite was dissolved in 2.2 mL of deaerated $(1 + 1) \times 10^{-1}$ M Trizma solution in deuterium oxide. A 150-μL aliquot of this solution, corresponding to 11 mol equiv of sodium hydrogen sulfite and 1.0 mol equiv of sodium dithionite relative to **1**, was introduced via a syringe provided with Teflon tubing into the cuvette compartment of the cell, externally cooled at a temperature below −20 °C for a quick freezing. Immediately, the cell was transferred to the vacuum line, and both solutions in the cell were freeze–thaw degassed through three cycles. The cell was flame-sealed under vacuum and brought to 20 °C. The solutions were mixed, and the cuvette was immediately introduced into the cell holder of the UV–vis spectrometer kept at 20.0 ± 0.1 °C. The following spectral changes were observed: the quinone band immediately disappeared to be replaced by a semiquinone band at 510 nm (A = 1.6) and a quinone methide band at 680 nm (A = 0.59). The semiquinone band slowly decreased to A = 1.3 in 5 min and then slowly changed into a quinone type band, λ_{max} = 500 nm, in 2 h. The quinone methide band, which had reached its maximum intensity (A = 0.61) in 1 min, slowly disappeared over a 2-h period. At this time the cell was opened and the solution HPLC analyzed: 80% of **2**, 3% of **3**, and five more unidentified products were found in the reaction mixture. Little if any 7-deoxydaunomycinon-7-yl sulfonates (**6** and **7**) were observed.

Reaction of Daunomycin Quinone Methide (4) with Sodium Dithionite. A 0.6-mL aliquot of a dimethyl sulfoxide solution 9.5×10^{-4} M in daunomycin (**1**) and $(1 + 1) \times 10^{-2}$ M in Trizma was introduced into a quartz 1-mm path UV cuvette provided with a serum stopper. Argon was bubbled through the solution using two syringe needles through the stopper. The needles were withdrawn, and 15 μL of an argon-deaerated deuterium oxide solution of dithionite from a 2.0-mL solution containing 16.0 mg of 81% sodium dithionite, corresponding to 1 mol equiv with respect to **1**, was injectd into the cell through the septum. Spectral changes were monitored with time at 20 °C. In less than 60 s, the spectrum of **1** was replaced by a quinone methide spectrum (A_{680} = 0.7).[18] A certain amount of semiquinone species was also present (A_{506} ca. 0.5). After an additional 60 s, 7.5 μL of the dithionite solution was added, whereby an instantaneous drop of the quinone methide absorbance (A_{689} from 0.7 down to 0.35) occurred with the simultaneous appearance of a hydroquinone band, λ_{max} = 440 nm (A = 1.22). No change in the semiquinone region occurred. After an additional 100 s, a second 7.5-μL portion of dithionite caused the instantaneous complete disappearance of the quinone methide band and a further increase of the hydroquinone band at 440 nm up to A = 1.75, with a shoulder at 505 nm. During the next 10 min, no significant spectral changes were observed. At this time another addition of dithionite (30 μL, 2 mol equiv) only caused the disappearance of the semiquinone shoulder at 505 nm and a small increase of the hydroquinone band. The cell was opened after a total of 135 min. HPLC analysis showed 74% of **2** and 21% of **3**, with very little, if any, **6** or **7**.

Reaction of 11-Deoxydaunomycin (12) with Sodium Dithionite (1 mol equiv). A 1.0×10^{-3} M solution of 11-deoxydaunomycin was made by dissolving the hydrochloride of **12** (1.62 mg, 3×10^{-3} mmol) in 3.0 mL of a 70:30:2 dimethyl sulfoxide-d_6–acetone-d_6–deuterium oxide mixture. The UV spectrum showed λ_{max} = 418 nm ($\epsilon = 1.0 \times 10^4$ M^{-1} cm^{-1}). The solution was transferred into the degassing chamber of a type C cell (1.0-mm cuvette) and deaerated with a flow of nitrogen. Sodium dithionite (3.50 mg of a 81% sample, 1.63×10^{-2} mmol) was dissolved in 1.1 mL of deaerated deuterium oxide. A 0.20-mL aliquot of this solution (3.0×10^{-3} mmol of dithionite) was immediately introduced into the 2-mL arm of the cell, precooled at a temperature below −20 °C. The solution was freeze–thaw degassed through three cycles. When the solutions were mixed at 25 °C, a purple color developed. The cell was put in the thermostated cell holder of the UV–vis spectrometer at 24.9 ± 0.1 °C, and the following spectral changes were recorded. The original quinone chromophore was not present, even in the first spectrum (time 45 s). An absorption with maxima at 354 ($\epsilon = 7.8 \times 10^3$ M^{-1} cm^{-1}) and 530 nm ($\epsilon = 1.2 \times 10^4$ M^{-1} cm^{-1}), where a quinone methide was expected to absorb, was observed instead. During the following 40 min, the 530-nm band decreased to ca. half of its original intensity while a hydroquinone band at 400 nm rose, with an isosbestic point at 462 nm. During the following 16 h, the quinone methide absorption kept decreasing to less than 10% of its original intensity, while the 400-nm hydroquinone band reached its maximum intensity (A = 1.4). No semiquinone bands were observed. The lack of semiquinone species was also proven by EPR spectroscopy, checked at time 1.8 h. After 19 h the cell was opened, whereby the spectrum changed to a quinone type chromophore, λ_{max} = 428 nm, in less than 1 min. The solvent was rotary-evaporated (0.5 Torr, 40 °C), and the residue was dissolved in methanol and analyzed by HPLC (method A, monitoring at 420 nm) to show 7,11-dideoxydaunomycinon-7-yl sulfonates (**14** and **15**, two partially overlapped peaks, t_R ca. 1 min, 71%) and at least seven more minor unidentified products, t_R from 2 up to 12 min. Adducts **14** and **15** were identified as shown below.

Reduction of 11-Deoxydaunomycin (12) with Sodium Dithionite (2 mol equiv). A 0.6-mL sample of a 1.0×10^{-3} M solution of 11-deoxydaunomycin hydrochloride (**12**·HCl) in 70:30 dimethyl sulfoxide–acetone was introduced into a quartz 1-mm pathlength UV cuvette provided with a serum stopper. Argon was bubbled through the solution using two syringe needles through the stopper. The needles were withdrawn, and 30 μL of an argon-deaerated deuterium oxide solution of dithionite from a 0.87-mL solution containing 7.49 mg of 81% sodium dithionite (corresponding to 2 mol equiv relative to **12**) was injected into the cell through the stopper. Immediately, the spectrum changed from a quinone type (λ_{max} = 418 nm) to a hydroquinone type (λ_{max} = 406 nm) absorption. No further changes were observed during the next 10 min. At this time the cell was opened to the air and shaken. A pink color appeared in less than 1 min. The spectrum showed a maximum at 530 nm, A = 0.25 (corresponding to ca. 20% of quinone methide), and still the hydroquinone absorption at 406 nm, A = 1.5. Only after bubbling air for 15 min did the quinone methide band disappear; at this time the spectrum only showed a quinone chromophore. The solvent was rotary-evaporated, and the residue was dissolved in a little methanol. HPLC analysis (method C) showed 51% of **14** and **15** in a 1:2 ratio, along with other unidentified products with retention times of 3.5, 4.1, 5.1 (25%), 7.3, 7.6, 8.4, 9.5, and 10.2 min. Products **14** and **15** were identified by coinjection with authentic samples prepared as described below and comparison of spectral properties.

Isolation and Characterization of the Stereoisomeric 7,11-Dideoxydaunomycinon-7-yl Sulfonates (14 and 15). 11-Deoxydaunomycin hydrochloride (**12**·HCl, 11 mg, 2×10^{-2} mmol) was dissolved in 7.0 mL of a 70:30 dimethyl sulfoxide–acetone mixture. The solution was introduced into one of the chambers of a cell consisting of two 15-mL Pyrex flasks sealed to 9-mm Pyrex tubing for connection to the vacuum line. Nitrogen was then bubbled through the solution. In the meantime, sodium dithionite (14.7 mg of an 81% sample, 6.8×10^{-2} mmol) was dissolved in 1.7 mL of deaerated deuterium oxide. Immediately, 0.50 mL of this

solution (2×10^{-2} mmol of dithionite) was introduced into the empty flask of the cell, precooled at a temperature below −20 °C. The solution was freeze–thaw degassed through three cycles, sealed under vacuum, and brought to ambient temperature. The two solutions were mixed, whereby a crimson color appeared. The cell was kept at 30 °C for 3 h. During this time the crimson color changed to yellow-orange. The cell was opened, the solvent was rotary-evaporated (5 Torr, 40 °C), and the residue was dissolved in methanol. HPLC analysis (method C) showed 67% of **14** + **15**, t_R 1.2 and 2.0 min, in a 1:1.2 ratio. The solvent was rotary-evaporated, and the solid residue was treated with a few milliliters of chloroform. The undissolved yellow solid contained only **14** and **15** (by HPLC), in the form of the sodium salt, and daunosamine (by NMR). The FAB mass spectrum (3:1 dithiothreitol–dithioerythritol matrix) showed the following negative ions: m/z (relative intensity) 468 (50, $C_{21}H_{17}SO_9Na$), 467 (75, $C_{21}H_{16}SO_9Na$), 446 (45, $C_{21}H_{18}SO_9$), 445 (100, $C_{21}H_{17}SO_9$); the intensity of the isotope peaks at 469 and 447 was consistent with the presence of a sulfur atom. The occurrence of sodium in negative ions is precedented in the negative ion mass spectra of glycosphingolipids.[36] The solid dissolved readily in 0.5 mL of a 1:1 water–methanol mixture. The solution was loaded onto a silica gel column and eluted with a 93:7 chloroform–methanol mixture. After rotary evaporation of the solvent from the combined yellow fractions, a mixture of pure **14** and **15** was obtained. The two isomers were separated by preparative HPLC. A gradient from 45:55 to 60:40 methanol–water over 10 min at a 0.60 mL/min flow rate was used. Adduct **14** was eluted after 4–5 min and **15** after 7–8 min. The combined fractions containing **14** were rotary-evaporated down to 5 mL, washed three times with 3 mL of chloroform each time, and rotary-evaporated to dryness, and the residue was washed thoroughly with 2-propanol with sonication. The resulting residue was >95% pure **14**: UV (H_2O) λ_{max} (relative intensity) 229 (3.2), 260 (2.4), 286 (sh, 0.95), 426 nm (1.0); ^1H NMR (DMSO-d_6) δ 13.38 (s, C6-OH, disappeared with D_2O), 7.87 (d, $J_{3,2}$ = 8, H3), 7.81 (t, $J_{2,1} = J_{2,3}$ = 8, H2), 7.62 (d, $J_{1,2}$ = 8, H1), 7.38 (s, H11), 4.99 (s, C9-OH, disappeared with D_2O), 4.46 (d,d, $J_{7,8a}$ = 2, $J_{7,8b}$ = 10, H7), 3.98 (s, CH_3O), 3.53 (d, $J_{10a,10b}$ = 15, H10a), 2.73 (d,d, $J_{8a,8b}$ = 15, $J_{8a,7}$ = 2, H8a), 2.62 (d, $J_{10b,10a}$ = 15, H10b), 2.24 (s, CH_3CO), 1.90–2.05 (m, H8b). Sufficient material was not available for elemental analysis. Isomer **15**, purified as described for **14**, gave a virtually identical UV–vis spectrum: ^1H NMR (DMSO-d_6) δ 8.3 (br s, C6-OH, slowly disappeared with D_2O), 7.87 (d, $J_{3,2}$ = 8, H3), 7.80 (t, J = 8, H2), 7.61 (d, $J_{1,2}$ = 8, H1), 7.38 (s, H11), 6.42 (s, C9-OH, disappeared with D_2O), 4.50 (dd, $J_{7,8a}$ = 2, $J_{7,8b}$ = 8, H7), 3.98 (s, CH_3O), 3.12 (d, $J_{10b,10a}$ = 16, H10b, the signal for H10b was probably buried under a large water peak at δ 3.2–3.6), 2.45 (d, the upfield portion of a dd, $J_{8a,7}$ = 2, $^1/_2$ H8a, the downfield portion was probably buried under the solvent peak), 2.23 (dd, $J_{8b,7}$ = 8, $J_{8b,8a}$ = 15, H8b), 2.11 (s, CH_3CO); ^1H NMR (D_2O) δ 7.19 (s, H11), 6.98–7.15 (m, H2 + H3), 6.87 (d, $J_{1,2}$ = 7, H1), 4.81 (d, $J_{7,8a}$ = 9, H7), 3.57 (s, CH_3O), 3.32 (A part of AB pattern, $J_{10a,10b}$ = 15, H10a), 3.13 (B part of AB pattern, $J_{10b,10a}$ = 15, H10b), 2.61 (dd, $J_{8a,7}$ = 9, $J_{8a,8b}$ = 15, H8a), 2.41 (d, $J_{8b,8a}$ = 15, H8b), 2.17 (s, CH_3CO); FAB mass spectrum (polyethylene glycol matrix), weak negative ion m/z 445.0709, calcd for $C_{21}H_{17}SO_9$ 445.0593.

Generation of the Quinone Methide from 11-Deoxydaunomycin by Reduction with *d,l*-TM-3 Dimer and Its Reaction with Sodium Hydrogen Sulfite. A 0.5-mL sample of a 1.0×10^{-3} M solution of 11-deoxydaunomycin hydrochloride (**12**·HCl) in 70:30 dimethyl sulfoxide–acetone was introduced into a quartz 1-mm pathlength UV cuvette and degassed as described in the previous paragraph. A 50-μL sample of an argon-bubbled dimethyl sulfoxide solution containing 1.42 mg (10 mol equiv) of *d,l*-TM-3 dimer was injected into the cuvette, and the spectral changes were monitored with time at 25 °C. The quinone band at 418 nm gradually decreased to be replaced by a quinone methide band at 532 nm, whose intensity reached its maximum (A = 1.2) in 5 min. Isosbestic points were observed at 380 and 460 nm. After an additional 5 min (A_{532} was still 1.2), 25 μL of an argon-bubbled deuterium oxide solution containing 0.26 mg of sodium bisulfite was injected into the cuvette. The quinone methide band decreased to 20% of its initial intensity in 7 min, being replaced by a hydroquinone band at 404 nm. After 1 h, when no more quinone methide was present, the cuvette was opened, the solvent was rotary-evaporated, and the residue was dissolved in a little methanol. HPLC analysis (method C) showed **14** and **15** in a 3:4 ratio (total 71%), along with other products at 4.0, 5.0 (4%), 8.2, 9.4 (12%), 10.6, and 11.8 min. Adducts **14** and **15** were identified by coinjection with authentic samples.

NMR Spectra of Quinone Methides from Daunomycin (1) and 11-Deoxydaunomycin (12). (A) Preliminary Experiments for the Reduction of Daunomycin (1) with Sodium Dithionite in Dimethyl Sulfoxide–Acetone. (a) Using a 10^{-4} M Solution of 1 and 1.2 mol equiv of Dithionite at 25 °C. A 2.0-mL volume of a 1.3×10^{-4} M solution of **1** in 70:30 anhydrous dimethyl sulfoxide–acetone was introduced into a UV cuvette provided with a serum stopper. The spectrum showed two maxima at 480 ($\epsilon = 1.2 \times 10^4$ M^{-1} cm^{-1}) and 500 nm ($\epsilon = 1.2 \times 10^4$ M^{-1} cm^{-1}). Nitrogen was bubbled through the solution using two syringe needles through the stopper. The needles were withdrawn, and 100 μL of a deaerated deuterium oxide solution containing 1.2 mol equiv of sodium dithionite (0.072 mg of a 73% pure sample) was injected into the cell through the septum. The following spectral changes were observed at 25 °C: the spectrum of the semiquinone of **1** appeared[18] immediately, which very rapidly faded during the first minute to be replaced by the quinone methide spectrum, λ_{max} = 380, 420, 678 nm. After reaching its maximum intensity (A_{678} = 0.88), the quinone methide absorbance began to decrease (to 20% of its maximum intensity in ca. 2 h), being replaced by a broad absorption band, λ_{max} = 480–490 nm, suggesting the presence of mostly quinone and possibly a little semiquinone species. The first half-life of the quinone methide was ca. 45 min.

(b) Using a 10^{-3} M Solution of 1 and 1.2 mol equiv of Dithionite at 25 °C. The experiment was similar to that described in the previous paragraph. However, the absorbance of the species generated during the decay of the quinone methide was mostly of a semiquinone type. The first half-life of the quinone methide was ca. 10 min.

(c) Using a 10^{-3} M Solution of 1 and 1.2 mol equiv of Dithionite at 0 °C. The experiment was run as above, but at 0 °C with a stream of dry air surrounding the cuvette to prevent water condensation. The first half-life of quinone methide was ca. 40 min.

(B) ^1H NMR Spectra. (a) General Remarks. The NMR spectra were taken using a 70:30:5 DMSO-d_6–acetone-d_6–D_2O mixture as solvent at 0 °C. First reference spectra were taken of daunomycin (**1**), 7-deoxydaunomycinone (**2**), daunosamine, and 11-deoxydaunomycin (**12**) (see Table II). The spectra of the quinone methides were taken according to the following procedure: a 10^{-3} M solution of the anthracycline (**1** or **12**) hydrochloride in 70:30 DMSO-d_6–acetone-d_6 (1 mL) was introduced into a 5-mm NMR tube that had been narrowed, for ca. 5 mm length, 1 cm below the top. Using a glass capillary, nitrogen was bubbled through the solution for a few minutes with external ice cooling to minimize solvent evaporation (acetone-d_6 was added, if necessary, to restore the original volume). The capillary was withdrawn up to the mouth of the tube, just below the rim, keeping a strong flow of nitrogen, and the tube was cooled at a temperature below −15 °C by immersion in an ice–salt bath. In the meantime, 1–2 mL of a nitrogen-bubbled solution 2×10^{-2} M in sodium dithionite in D_2O had been prepared. A 50-μL aliquot of this solution was introduced into the tube via a microsyringe and deposited on the wall of the tube, between the constriction and the anthracycline solution, to make the dithionite solution freeze to prevent premature contact with the anthracycline. While still in the cooling bath under nitrogen flow, the tube was immediately flame-sealed at the constriction. In the meantime, the spectrometer (Bruker) had been set for operation at 0 °C, and a preliminary tuning had been done with a similar NMR tube, with the same solvent mixture, to minimize the tuning time required for the experiment. Immediately before the spectra were taken, the NMR tube containing the reactants was removed from the cooling bath, plunged into an ice–water bath to thaw and mix the solutions by shaking, and dropped into the probe. After a quick tuning (2–5 min), data collection was started. The spectrometer was set to allow for interruption and saving of data from partial accumulation in such a way that any successive accumulation would include all of the previous data, from time zero. Typically spectra were saved after 15 min, 30 min, 60 min, and 2 h.

(b) NMR Spectrum of the Quinone Methide 16. After 10–15 min of accumulation, in addition to the peaks corresponding to daunosamine (mostly one epimer) the spectrum showed fairly sharp peaks, as reported in Table II. In a decoupling experiment, irradiation at δ 7.27 (H7) made the 4-Hz $J_{8a,7}$ and the 5-Hz $J_{8b,7}$ disappear. Irradiation at δ 2.85 (H8a) made the 7.27 peak sharpen.

(c) NMR Spectrum of the Quinone Methide 4. Due to the presence of semiquinone radicals, the quality of the spectra was very poor, even when slightly less or slightly more than the stoichiometric amount of dithionite was used. Broadening of the peaks, especially in the aromatic region, prevented any accurate analysis of the spectra. In an experiment run using only 0.8 mol equiv of dithionite, the spectrum obtained during the first 15 min of accumulation showed in the aromatic region broad multiplets at δ 7.6–8.7, a very broad multiplet at 6.9–7.2, and a broad multiplet at 6.5–6.7, the latter probably due to H7. In the CH_3CO region, a singlet was present at δ 2.27, well separated from another singlet at δ 2.21. The latter was assigned to the quinone methide because the 2.21:2.27 peak intensity ratio decreased with the accumulation time from 1.5 (15 min) to 1.2 (30 min) and finally to 0.8 in 160 min.

(36) Arita, M.; Iwamori, M.; Higuchi, T.; Nagai, Y. *J. Biochem.* **1984**, *95*, 971.

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The Remarkable Nucleophilic Reactivity of Molybdate†

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Abstract: Dianionic molybdate (pH 7.5, 27 °C, μ = 1.6 M) is a good nucleophilic catalyst for the hydrolysis of *p*-nitrophenyl acetate (k_2 = 0.26 M^{-1} min^{-1}) and *p*-nitrophenyl thioacetate (k_2 = 0.34 M^{-1} min^{-1}). The solvent kinetic isotope effect for the molybdate-catalyzed hydrolysis of each ester is 1.0 (±0.1). In the presence of 0.1 M molybdate, the hydrolysis rate at pH 7.5 is increased by a factor of 50 for *p*-nitrophenyl acetate and by a factor of 80 for the thioester. The second-order rate constant for the reaction of the molybdate dianion with *p*-nitrophenyl acetate is 35-times larger than that for the reaction of the phosphate dianion in spite of the 1000-fold greater basicity of the latter species. This may reflect a greater degree of solvation of the nucleophilic site on phosphate than on the larger molybdate ion.

Acyl transfer reactions are widespread in nature, and the mechanism has been extensively studied with a vast range of nucleophilic acceptors. In a classic paper Jencks and Carriuolo[1] examined the nucleophilic reactivity of over 45 nucleophilies with *p*-nitrophenyl acetate. They showed a reasonable correlation of nucleophilic reactivity with the pK_a of the attacking group ($\beta \sim 0.8$) with the noteworthy negative deviations (by over two orders of magnitude) for the dianions phosphate and carbonate. In order to explore some of the factors responsible for this low reactivity, the reaction of a series of phosphate analogs (i.e., phosphonates) with various *p*-nitrophenyl esters was examined.[2] This study revealed an unusually small Bronsted coefficient (β = 0.3). The low reactivity and the small sensitivity of the rate constant to basicity, as well as the unusually small magnitude of the activation entropy for the bimolecular reaction,[3] have been attributed, at least in part, to the requirement for desolvation from a nucleophilic site on the oxydianion.[2] In view of this, another interesting feature appears in the data of Jencks and Carriuolo.[1] They found that although the dianions of phosphate and arsenate have nearly identical pK_as, arsenate is 5.5-times more reactive with *p*-nitrophenyl acetate than is phosphate. In order to further investigate this phenomenon, we examined the reactivity of the less basic molybdate dianion. Since arsenate and molybdate[4] can substitute for phosphate in a variety of enzymic reactions, it was of interest to examine the intrinsic reactivity of these species. Surprisingly, we found molybdate to be *more reactive* than either phosphate or arsenate with *p*-nitrophenyl acetate and its thiol ester analog.

Experimental Section

Materials. *p*-Nitrophenyl acetate was obtained from Aldrich Chemical Co. and *p*-nitrophenyl thioacetate from U.S. Biochemical Corp. The HEPES† buffer was obtained from Sigma Chemical Co. as a 1:1 mixture of the free acid and the sodium salt. Sodium molybdate (dihydrate) was obtained from Matheson, Coleman and Bell and stock solutions (0.5 M) were prepared by dissolving Na_2MoO_4 in buffer solutions (0.01 M HEPES, 0.1 M NaCl) and adjusting the pH to 7.5 with HCl (or DCl in D_2O). At this pH there is negligible polymerization of molybdate.[5] D_2O (99.8%) was from Sigma Chemical Co.

Methods. pH measurements were made with a Metrohm (Brinkmann) combititrator. pD values were estimated by adding 0.41 to the pH meter reading.[6] Kinetics were followed by measuring the absorbance (λ = 400 nm for *p*-nitrophenoxide or 412 nm for *p*-nitrobenzenethiolate) on either a Hewlett-Packard Model 8452A diode array spectrophotometer or a Beckman 3600 spectrophotometer. The reactions were initiated by adding 10 μL of the ester (10 mM in CH_3CN) to 1 mL of a thermally equilibrated (27 °C) buffer solution (ionic strength maintained at 1.6 M with Na_2SO_4). At the higher molybdate concentrations (0.3–0.5 M) the release of *p*-nitrophenoxide or *p*-nitrobenzenethiolate was demonstrated to follow first-order kinetics by monitoring the reaction for 9 half-lives. In general the increase in the absorbance was typically followed for at least 4 half-lives and the data analyzed by nonlinear regression to a first-order curve.[7] The internal standard deviations of the pseudo-first-order rate constants were consistently less than 1%. Rate constants were determined at least in triplicate for each reaction.

Table I. Effectors of *p*-Nitrophenyl Acetate Hydrolysis

$[Na_2SO_4]$	$[Na_2MoO_4]$	[NaCl]	μ, M	k,[a] min^{-1}
0	0	0.1	0.1	3.8 (±0.1) × 10^{-4}
0.5	0	0.1	1.6	4.2 (±0.1) × 10^{-4}
0	0.1	0.1	0.4	2.0 (±0.1) × 10^{-2}
0	0.1	1.3	1.6	1.7 (±0.1) × 10^{-2}
0.4	0.1	0.1	1.6	2.24 (±0.04) × 10^{-2}

[a] 1% v/v CH_3CN, 0.1 mM ester, 0.01 M HEPES, pH 7.5, 27 °C.

Results

In order to evaluate the influence of the reaction medium (i.e., $[Na_2SO_4]$ and ionic strength) on the rate of hydrolysis of pNPA,[1] the concentration of Na_2SO_4 and NaCl was varied in the presence and absence of Na_2MoO_4 (0.1 M). The results (Table I) indicate negligible influence of sodium sulfate on the hydrolysis rate. The pseudo-first-order rate constants for release of *p*-nitrophenoxide or *p*-nitrobenzenethiolate from the corresponding acetate esters shows a linear dependence on the molybdate concentration (up to at least 0.5 M) at pH 7.5, 27 °C. The second-order rate constant for the molybdate-catalyzed hydrolysis is 0.26 (±0.03) M^{-1} min^{-1} with *p*-nitrophenyl acetate (pNPA) and 0.337 (±0.009) M^{-1} min^{-1} with *p*-nitrophenyl thioacetate (pNPTA). Thus, there is a significant enhanced hydrolysis rate in the presence of

† Abbreviations: GPD, glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12); HEPES, *N*-2-hydroxyethylpiperizine-*N*'-2-ethanesulfonic acid; pNPA, *p*-nitrophenyl acetate; pNPTA, *p*-nitrophenyl thioacetate.

(1) Jencks, W. P.; Carriuolo *J. Am. Chem. Soc.* **1960**, *82*, 1778–1786.
(2) Shames, S. L.; Byers L. D. *J. Am. Chem. Soc.* **1981**, *103*, 6170–6177.
(3) Shames, S. L.; Byers, L. D. *J. Am. Chem. Soc.* **1981**, *103*, 6177–6184.
(4) Byers, L. D.; She, H. S.; Alayoff, A. *Biochemistry* **1979**, *18*, 2471–2480.
(5) Aveston, J.; Anacker, E. W.; Johnson, J. S. *Inorg. Chem.* **1964**, *3*, 735–746.
(6) Covington, A. K.; Paabo, M.; Robinson, R. A.; Bates, R. G. *Anal. Chem.* **1968**, *40*, 700–706.
(7) Leatherbarrow, R. J. *Enzfitter: A Non-linear Regression Data Analysis Program for the IBM PC*; Elsevier Science Publishers: New York, 1987.

0002-7863/92/1514-5553$03.00/0