Electrophilic Trapping of the Tautomer of 7-Deoxydaunomycinone. A Possible Mechanism for Covalent Binding of Daunomycin to DNA

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Received April 4, 1983

Bioreductive activation of the antileukemic drug daunomycin (**1**) is proposed to yield a reactive intermediate, the tautomer (**2**) of 7-deoxydaunomycinone (**3**), which reacts with nucleophilic sites in DNA or other macromolecules.[1,2] Reactivity with nucleophiles was proposed because of the quinone methide structure; however, attempts to trap **2** in vitro with nucleophiles have been unsuccessful.[3] The semiquinone (**4**) of daunomycin is also thought to be a reactive intermediate for covalent binding of **1** to DNA.[2,4,5]

We have previously reported that daunomycin reacts in buffered methanol solvent with *dl*-bis(3,5,5-trimethyl-2-oxomorpholin-3-yl) (**5**) to give 7-deoxdaunomycinone (**3**) via the tautomer **2**, characterized by UV-visible absorption at 380 and 608 nm.[6] Under these conditions the half-life of **2** in methanol solvent at 25 °C is 53 s with respect to protonation to form **3**.

We now report the efficient trapping of tautomer **2** with the electrophile benzaldehyde in what is nominally an aldol condensation and propose that reaction of **2** with electrophilic sites is important in covalent binding of duanomycin to DNA.

A rigorously oxygen degassed, methanol-*O-d* solution 7.80×10^{-4} M in daunomycin, 7.80×10^{-4} M in **5**, and 8.0×10^{-3} M in trisma buffer (1:1 Tris/Tris-HCl) at 25.1 °C gave absorption for **2** at 608 nm as a function of time as shown in Figure 1. A similar solution 4.91×10^{-2} M in benzaldehyde showed a 76% decrease in the area under the absorption vs. time plot. HPLC of the product mixture from reaction in the presence of benzaldehyde with a phenyl reverse-phase column eluting with 40% tetrahydrofuran/60% buffered water (0.1% ammonium formate adjusted to pH 4 with formic acid)[7] indicated two major products, the benzaldehyde adduct **6** (47%) and **3** (29%). The benzaldehyde adduct was isolated as a red solid in 61% yield from a similar reaction using 3.72×10^{-4} M **1** and **5** and 7.07×10^{-2} M benzaldehyde by silica gel flash chromatography[8] eluting with 1.7% methanol in methylene chloride. The structure of **6** was assigned as shown in Scheme I from the folowing spectroscopic, chemical, and analytical data: ^{1}H NMR (250 MHz, $DCCl_3$) δ 1.62 (s, CH_3-14), 1.86 (dd, J = 12, 3 Hz, CH-8), 2.40 (broad, OH-9, OH-13, disappears with D_2O), 2.72 (dd, J = 12, 3 Hz, CH-8), 2.74 (d, J = 20 Hz, CH-10), 3.48 (d, J = 20 Hz, CH-10), 3.88 (dt, J = 2, 3 Hz, CH-7), 4.00 (s, OCH_3-4), 5.39 (d, J = 2 Hz, CH-7′), 6.95 (d, J = 6 Hz, *o*-Ph′7′), 7-7.2 (m, *m,p*-Ph′7′), 7.34 (d, J = 9 Hz, CH-3), 7.74 (t, J = 9 Hz, CH-2), 8.04 (d, J = 9 Hz, CH-1), 12.90 (s, OH-6), 13.50 ppm (s, OH-11); the assignment of resonances for the protons in the A ring were verified by decoupling experiments; IR (KBr) 2.90, 3.15, 3.41, 6.19, 6.30 μm; mass spectrum (FAB-glycerin, positive ion), m/z (relative intensity) 489 (56, M + 1), 490 (100, M + 2), 491 (44, M + 3);[9] exact mass calcd for $C_{28}H_{26}O_8$ 490.1627, found 490.1623;

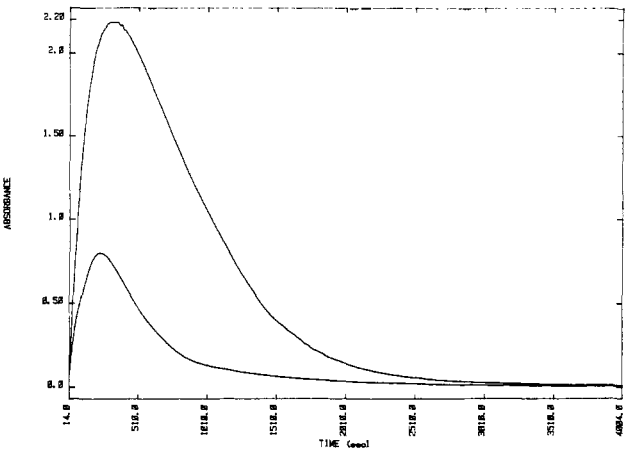
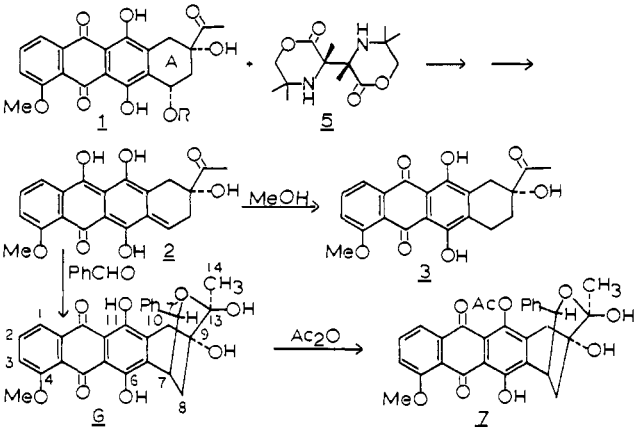


Figure 1. Absorption at 608 nm as a function of time for a freeze-thaw degassed methanol-*O-d* solution 7.80×10^{-4} M in daunomycin, 7.80×10^{-4} M in **5**, and 8.0×10^{-3} M in trisma buffer (1:1 Tris/Tris-HCl) at 25.1 °C: (a) without benzaldehyde, (b) with 4.91×10^{-2} M benzaldehyde.

Scheme I



(FAB-glycerin, negative ion), m/z 488 (87, M), 489 (100, M + 1), 490 (39, M + 2). Anal. Calcd for $C_{28}H_{24}O_8$: C, 68.85; H, 4.95. Found: C, 68.62; H, 5.00. Treatment of **6** with acetic anhydride-pyridine gave the monoacetate **7**, a yellow solid, characterized by an acetate methyl resonance at δ 2.54 and a single phenolic resonance at 12.90 ppm and other spectral data. The stereochemistry for **6** was proposed on the basis of steric effects, the coupling constants for the protons in the A ring,[10] monoacetylation, and formation of the hemiketal.

Methanol-*O-d* solvent was selected for these experiments to facilitate trapping of **2**. The half-life of **2** is 9 times longer in methanol-*O-d* solvent because of the kinetic isotope effect for formation of **3**.[6] In methanol solvent with 7.80×10^{-4} M **1** and **5** and 4.91×10^{-2} M benzaldehyde the HPLC yields of **3** and **6** were 62% and 35%, respectively. The diminished yield of **6** with undeuterated solvent also supports the intermediacy of **2**.

Control experiments showed that benzaldehyde was unreactive with **1**, **3**, and **5**. Benzaldehyde was also shown to be unreactive with the semiquinone (**4**) of daunomycin, which is an intermediate in the reductive cleavage of **1** by **5**.[6] The EPR signal intensity of **4** as a function of time was not altered by 4.91×10^{-2} M benzaldehyde at 0 °C. The experiment was performed at 0 °C to facilitate EPR measurements. The product distribution was not altered by this change in temperature.

The bimolecular rate constant for reaction between **2** and benzaldehyde was calculated to be 9×10^{-2} M^{-1} s^{-1} at 25.1 °C from the ratio of the areas under the curves in Figure 1. This is accomplished by integration of the kinetic expression for the

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concentration of **2** with pseudo-first-order conditions for trapping.

Efficient trapping of the tautomer **2** with benzaldehyde indicates its nucleophilic character and suggests that the enolic functionality dominates the quinone methide functionality. Bioreduction of daunomycin or adriamycin intercalated in DNA might then lead to formation of a covalent bond between the 7-position of **3** or 7-deoxyadriamycinone and the 2- or 4-position of a pyrimidine base or the 2- or 6-position of a purine base on the basis of electron density calculations.[11,12] Trapping experiments with simple pyrimidine and purine bases analogous to trapping with benzaldehyde have been unsuccessful to date because the high concentrations of trapping agent necessary cannot be achieved.

In summary we reported here the first experimental evidence for the nucleophilic reactivity, other than protonation, for the tautomer **2**, proposed as a biologically active form of daunomycin.

Acknowledgment. This investigation was facilitated by the following support: PHS Grant CA-24665, DHHS; University of Colorado, CCRW; Developmental Therapeutics Program, NCI; 3M Co.; University of Colorado Clinical Mass Spectrometry Resource, PHS Grant RR-01152, DHHS. We also thank Robert Murphy and Keith Clay for assistance with the FAB mass spectrometry.

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Photodecomposition of Alkanones in Urea Inclusion Compounds

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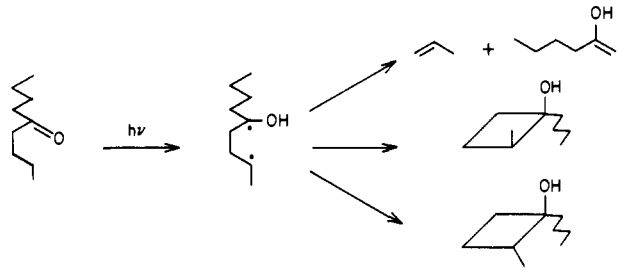
Received February 10, 1983

During recent years, photochemical processes in ordered or constrained systems have been the object of considerable attention. Among such systems have been micelles,[4,5] vesicles,[6] microemulsions,[6,7] monolayers,[5,8] and liquid crystals.[9] Solid-phase studies have been reported on molecules adsorbed on surfaces[10] and in crystalline[11] and polymeric[12] systems. Only crystals and liquid monolayers, so far, offer a well-defined location and environment. In this context the recent reports by Lahav and coworkers of the photoaddition of guest ketones to deoxycholic acid are particularly interesting.[13] In this communication we report that urea inclusion compounds provide an environment wherein photoreactions may be carried out, with consequences different from those observed in homogeneous solution, and that the steric constraints imposed by the host upon the guest in the once-formed inclusion compound are not as severe as the ready steric inhibition of formation might suggest.

Scheme I



The crystal system, unit cell characteristics, and dimensions of urea inclusion compounds are well established.[14,15] Urea in the inclusion compounds crystallizes in an hexagonal lattice (as opposed to tetragonal for free urea) in which there are long channels of ~5 Å internal diameter.[14,15] This is sufficient to accommodate linear paraffin-like molecules in the planar zig-zag conformation. Longitudinal rotation of hydrocarbon segments is relatively free while other motions (e.g., gauche–trans isomerization) are more severely restricted. The steric restraints are such that one methyl substituent in a long chain is enough to make complex formation difficult.[14]

Most of our experiments have been carried out with 5-nonanone. This, in solution undergoes photodecomposition predominantly via the Norrish Type II reaction, leading to 2-hexanone, propylene, and two isomeric cyclobutanols, Scheme I. The inclusion compound is prepared by crystallization from concentrated urea solutions in methanol, following the addition of neat 5-nonanone. The samples were characterized by Raman spectroscopy;[17] this

(1) National Research Council.
(2) University of Western Ontario.
(3) (a) Issued as NRCC-21346. (b) Publication No. 297 from the Photochemistry Unit, University of Western Ontario.
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