one another in the polypeptide chain, its intactness indicates that no major distortion of the protein's native, folded structure occurs when the dry enzyme is suspended in anhydrous acetone or octane. Furthermore, there is no evidence of appreciable structural heterogeneity in the population of enzyme active centers in these solvents. Thus, the vast difference in the catalytic efficiency of α-LP in water, acetone, and octane (k_{cat}/K_m = 5800, 3.3, and 5400 M^{-1} min^{-1}, respectively)[14] is not due to disruption of conformation but rather to another effect of the solvent, e.g., a change in the binding affinities of substrates or in protein dynamics (perhaps by stripping bound water[1,15]).

In contrast to the above results, dimethyl sulfoxide (DMSO, in which proteins are soluble[16]) was found to disrupt the catalytic triad. The MAS spectrum of $^{15}N^{\delta 1}$-His α-LP ("pH 8.6") reclaimed from DMSO[17] displayed (Figure 1C), in addition to the peptide backbone peak, a single resonance at 120 ppm, indicating the exclusive existence of the $N^{\epsilon 2}$–H tautomer.[9] This is consistent with the observations that (i) DMSO was the only solvent of seven tested[14] in which α-LP was inactive and (ii) α-LP lyophilized from DMSO (as opposed to water) was inactive when suspended in octane.

In summary, solid-state MAS NMR spectroscopy has been used to show that the catalytic triad of α-LP is intact in acetone and octane, thus providing the first direct physical evidence that enzymes do not denature when suspended in neat organic solvents.

Registry No. His, 71-00-1; α-lytic proteinase, 37288-76-9; acetone, 67-64-1; octane, 111-65-9; dimethyl sulfoxide, 67-68-5.

(14) α-LP was assayed in organic solvents by following transesterification of N-Ac-L-Ala-OCH_2CH_2Cl with ethanol as described for subtilisin[15] and in water by following hydrolysis of the same substrate using a pH-stat. α-LP preinhibited with phenylmethanesulfonyl fluoride was inactive in organic solvents.

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(18) This work was financially supported by NSF Grant CBT-8710106 to A.M.K.

Coupling of the Anthracycline Antitumor Drug Menogaril to 2′-Deoxyguanosine through Reductive Activation[1]

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The concept of bioreductive activation as applied by Moore and Czerniak to the anthracycline antitumor drugs, most notably daunomycin and adriamycin, proposes formation of a reactive quinone methide from a hydroquinone state.[2,3] The hydroquinone state is formed through bioreduction, and the quinone methide reacts with a nucleophilic site in a biological macromolecule. The tumor cell toxicity is proposed to result from the reaction of the quinone methide with a nucleophilic site in DNA such as the 2-amino group of 2′-deoxyguanosine. Although such a coupling in itself may not be cytotoxic, it attaches an effective catalyst for the production of reactive oxygen species to DNA. Subsequent catalytic production and reaction of reactive oxygen species may damage the DNA beyond repair.[4] Reductive activation of the quinone antitumor drug mitomycin C leads to coupling to guanine moieties,[5] and the elegant work of Tomasz and Nakanishi has further established that reductively activated mitomycin C cross-links duplex DNA through reaction with two guanine bases.[6] However, Fisher and Aristoff have recently stated in their extensive review of the chemistry of DNA modification by antitumor antibiotics with regard to the quinone methide from reductive activation of anthracyclines: "in principle this quinone methide may be thought of as a DNA-reactive entity, but its behavior with DNA on this score in vitro is ambiguous and numerous efforts to obtain mononucleotide adducts have been unsuccessful" and further "there is no in vitro experiment to provide even the suggestion of the chemistry leading to adduct formation".[7]

We have recently reported that reduction of the anthracycline antitumor drug menogaril (**1**) with substoichiometric quantities of the one-electron-reducing agent bi(3,5-dimethyl-5-(hydroxymethyl)-2-oxomorpholin-3-yl) (**2**, DHM-3 dimer) in an aqueous medium yields a long-lived quinone methide transient, which can be trapped with the sulfur and nitrogen nucleophiles *N*-acetylcysteine[8] and imidazole,[9] respectively. These results suggest that the quinone methide can react with cysteine and histidine residues in peptides and proteins. We now describe the successful reductive coupling of menogaril at the 7-position to 2′-deoxyguanosine at the 2-amino position.

Menogaril (18 mg, 2.0×10^{-4} M) was reacted anaerobically with 25 mol % of DHM-3 dimer (**2**) in 10% dimethylformamide–90% pH 8.2, aqueous, Tris buffer (v/v) in the presence of a 100-fold excess of 2′-deoxyguanosine for 6 days at ambient temperature. Reverse-phase HPLC analysis of the reaction mixture eluting with a gradient consisting of a formate buffer and methanol and detecting at 480 nm showed peaks with retention times of 1.7 min with a shoulder at 2.0 min (17%, 7-(2″-*N*-(2‴-deoxyguanosyl))-7-deoxynogarol stereoisomers,[10] **3**), 5.3 min (17%, menogaril, **1**), 5.6 min (30%, 7-deoxynogarol, **4**), 6.8 min (23%, bi(7-deoxynogarol-7-yl), **5**), and 9.7 min (10%, probably a stereoisomer of **5**). Minor unidentified product peaks appeared at 2.5 min (2%) and 2.8 min (1%). Menogaril, 7-deoxynogarol, and the major stereoisomer of bi(7-deoxynogarol-7-yl) were identified by coinjection onto the HPLC column with authentic samples[8] and purity evaluation of the peaks with a Hewlett-Packard diode array detector. The deoxynogarol dimer **5** was further characterized by isolation from a chloroform extract of the reaction mixture and FAB mass spectral analysis, which showed a characteristic molecular ion at m/z 1021.[8] The major stereoisomer of the deoxyguanosyl–deoxynogarol adduct **3** was isolated from the aqueous phase after the chloroform extraction by evaporation of the water followed by a series of ethanol extractions of the solid material to remove excess deoxyguanosine and chloroform extractions of aqueous solutions to remove byproducts. Extraction was followed by reverse-phase flash chro-

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(10) The minor product was not isolated and is presumed to be a diastereomer of **3** on the basis of its similar chromatographic behavior.

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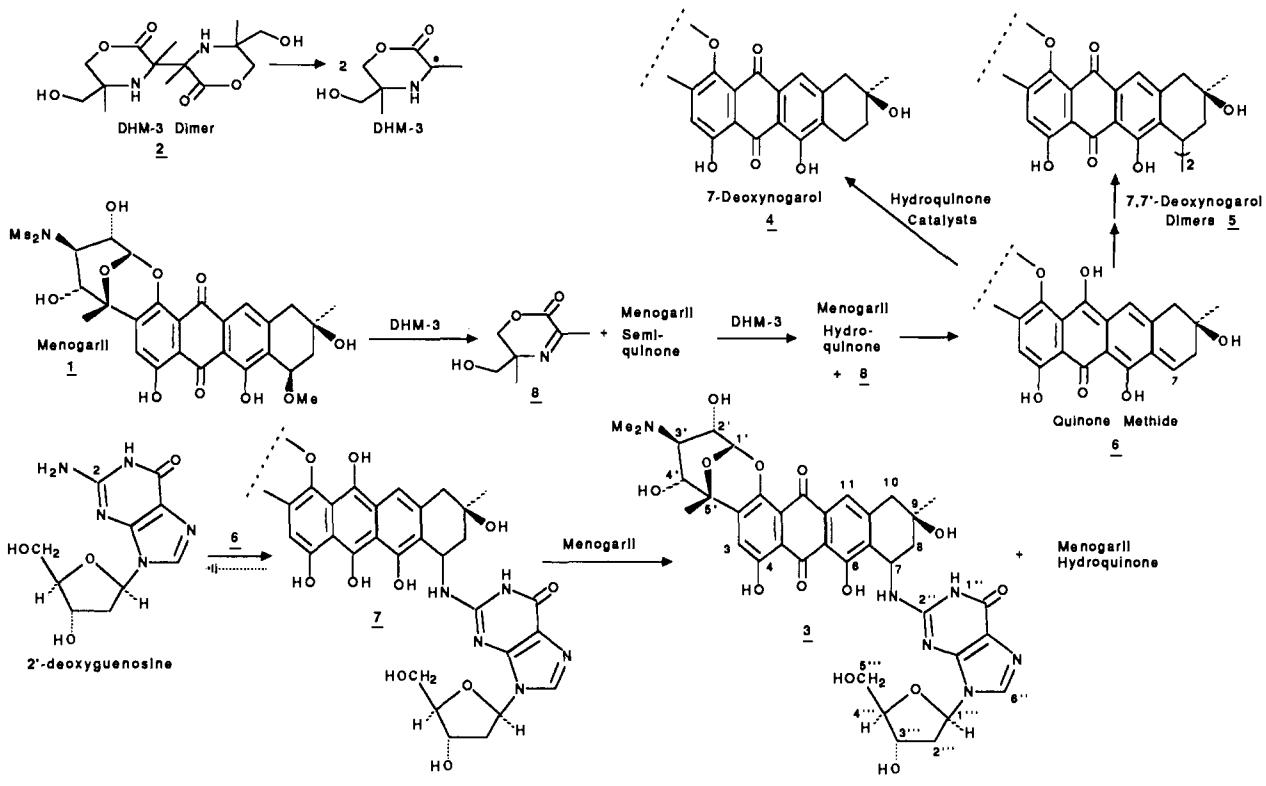
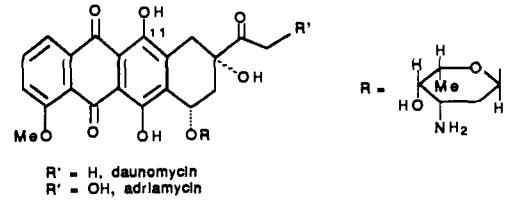
Scheme I



matography eluting with 40% of 0.1% trifluoroacetic acid–60% methanol (v/v). A major fraction from the column contained one stereoisomer of **3** contaminated with about 10% of the other isomer and only 4% guanosine (peak area percent) as indicated by HPLC analysis detecting at 260 nm. The structure of the major isomer isolated was established by a characteristic menogaril quinone UV–vis spectrum, by a strong FAB M + 1 mass spectral peak at m/z 777 with a fragment ion at m/z 645 corresponding to loss of the deoxyribose group, and by one- and two-dimensional ^{1}H NMR spectroscopy. The one-dimensional 500-MHz ^{1}H NMR spectrum of **3** in DMSO-d_6 solvent was assigned as follows: δ 12.35 and 12.20 (s, 4- and 6-OH), 10.66 (s, 1″-NH), 9.01 (br s, 3′-N$^+$HMe$_2$), 7.95 (s, 8″), 7.42 (s, 11), 7.16 (s, 3), 6.67 (d, J = 8 Hz, 2″-NH), 6.21 (t, J = 7 Hz, 1‴), 5.74 (d, J = 4 Hz, 1′), 5.56 (m, 7), 5.2 (br, aliphatic OH's), 4.4 (m, 3‴), 4.19 (dd, J = 4, 10 Hz, 2′), 3.99 (d, J = 10 Hz, 4′), 3.6–3.85 (m, 5‴), 3.04 (d, J = 18 Hz, 10 (a or b)), 2.88 (s, 3′-NMe$_2$), 2.83 and 2.85 (s, 3′-N$^+$HMe$_2$),[11] 2.7–2.9 (m, 10 (a or b), 3′, 2‴ (a or b)), 2.23 (m, 2‴ (a or b)), 1.9–2.1 (m, 8 (a and b)), 1.64, (s, 5′-CH$_3$), and 1.32 (s, 9-CH$_3$). A double quantum filtered 500-MHz homonuclear COSY spectrum established connectivity between the 7-position and the 2″-NH position. The assignment of the 2″-NH was further established by D$_2$O exchange. The COSY spectrum and the spectrum with deuterium exchange also facilitated the chemical shift assignments as reported above, particularly when patterns overlapped such as those for protons at positions 10, 3′, and 2‴.

Formation of 7-(2″-*N*-(2‴-deoxyguanosyl))-7-deoxynogarol (**3**), 7-deoxynogarol (**4**), and bi(7-deoxynogarol-7-yl) (**5**) is proposed to occur via the quinone methide intermediate **6** as shown in Scheme I. Tautomerization of **6**, catalyzed by any hydroquinone present such as from reduction of starting material or product, gives 7-deoxynogarol.[8] Dimerization of **6** gives initially **5** in the half quinone–half hydroquinone state. Nucleophilic addition of the 2′-NH$_2$ of guanosine to the 7-position of **6** followed by proton shifts gives adduct **3** in its hydroquinone state **7**. The nucleophilic addition and dimerization steps are possibly reversible.[12,13] Oxidation of products formed in hydroquinone states is proposed to occur via reaction with starting menogaril to produce menogaril hydroquinone. This latter step is the propagation step in the Fisher mechanism[13] for nucleophilic trapping of quinone methides produced by reductive activation. The quinone methide **6** results from elimination of methanol from the menogaril hydroquinone state produced by reduction of menogaril by DHM-3 radical in one-electron steps[8,14] or from the chain propagation step described above. The byproduct of reduction by DHM-3 is 5,6-dihydro-3,5-dimethyl-5-(hydroxymethyl)-1,4-oxazin-2-one (**8**).[14] The chain mechanism[13] and the catalytic effect of hydroquinones on the undesired tautomerization of **6**[8] explain the use of substoichiometric amounts of the reducing agent, DHM-3 dimer, to maximize the yield of **3**. The competing process of dimerization is slow because of the low concentration of quinone methide during the course of the reaction. The successful coupling is also consistent with the higher reactivity with nucleophiles and the lower reactivity with electrophiles, such as the proton, at the 7-position anticipated for anthracyclines deprived of the OH group at the 11-position (menogaril,[8] aclacinomycin A,[12,13] and 11-deoxydaunomycin[13,15]).



Similar reduction of menogaril in the presence of a large excess of 2′-deoxyadenosine did not result in adduct formation. The predominant species present after reaction were menogaril (**1**,

(11) The material obtained from the reverse-phase column was partially protonated by the trifluoroacetic acid eluent. The N–Me$_2$ signal appears as one large singlet and two small singlets; the two small singlets result from the fact that in the protonated form the methyls are diastereotopic and proton exchange in DMSO solvent is slow. Weak coupling with the NH is apparent in the COSY spectrum.

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34%), 7-deoxynogarol (**4**, 43%), and bi(7-deoxynogarol-7-yl) (**5**, 9%). The unidentified minor product peaks with the exception of a peak at 7.2 min (5%) had the same retention times as the minor products from reduction in the presence of deoxyguanosine. The 7.2-min peak could not have been an adduct since an adduct would be considerably more polar than menogaril and would have eluted before menogaril. Its UV-vis spectrum also precluded it being an adduct.

The result described here indicates for the first time that an anthracycline quinone methide transient can covalently bind to a nucleophilic site in a nucleic acid. Studies of menogaril metabolism show low levels of recovery of drug and its known metabolites.[16] Possibly, covalent binding to nucleophilic sites in proteins and/or nucleic acids through reductive activation is an explanation. The quinone methide from reductive activation of adriamycin or daunomycin bears an 11-hydroxy substituent and consequently is short lived and less likely to bind covalently to sites of lower nucleophilicity.[13,15,17] In vivo modification of the 11-hydroxy group of these drugs prior to reductive activation might change the reactivity of their quinone methide states with nucleic acids.

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Chemical Reaction Mechanisms at Unusually Low Temperatures: The Gas-Phase Reaction of $C_2H_2^+ + H_2$

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We have recently developed a supersonic flow kinetic technique that allows reaction rate studies at extremely low collision energies.[1-5] We report the observation of gas-phase reactivity between the acetylene radical cation, $C_2H_2^+$, and molecular hydrogen at collision energies of less than 0.5 meV (translational temperatures near 2 K).

$$C_2H_2^+ + H_2 \rightarrow C_2H_3^+ + H \qquad (1)$$

Reaction 1 has been well studied at temperatures above 80 K and is found to increase in rate with increasing temperature.[6-10] The observed rate coefficient at 300 K is 1.0×10^{-11} cm^3 s^{-1}, and the reaction is not observed to occur at 80 K.[6,7] The reaction cross section is observed to increase with collision energy between 0.05 and 0.8 eV.[8] Vibrational excitation in the $C_2H_2^+$ also accelerates the rate at low energies.[9,10] Appearance potential measurements for $C_2H_2^+$ and $C_2H_3^+$, from the photoionization of C_2H_4, have suggested that the heat of formation of $C_2H_3^+$ lies in the range

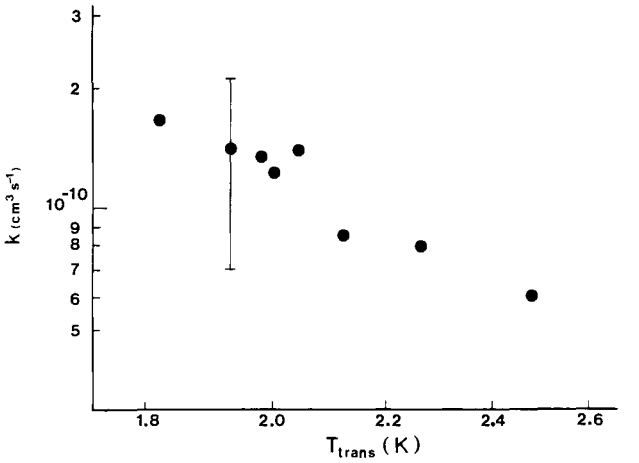

Figure 1. The observed translational temperature dependence of the experimentally measured rate coefficients for reaction 1. Temperatures are a neutral density weighted average of the perpendicular and parallel temperatures present in the jet over the range of an experiment.

$\Delta H°_{f_0}$ = 267-269 kcal mol^{-1}.[11,12] All of these results have led to an acceptance of an endothermicity for reaction 1 of 2.0 kcal mol^{-1} at 0 K.

Our experiment is outlined below. Briefly, a 1-3% mixture of C_2H_2 in H_2 at 0.5-1.4 atm is formed into a free jet by expansion through a 0.03- or 0.05-cm orifice into a vacuum chamber. Downstream, a pulsed dye laser is used to selectively ionize C_2H_2 via resonant multiphoton ionization through the G $^1\Pi_u$ $v = 0$ state of C_2H_2. This ionization technique allows selective production of $C_2H_2^+$ (or $C_2D_2^+$ in separate experiments using C_2D_2) in the ground vibrational state with 100% state purity.[13] The photoions remain in translational equilibrium with the surrounding neutrals. Velocity and temperature slip under our expansion conditions are negligible.[5] The properties of both the reactor and the expansion assure that all collisions occur at the extremely low energies characteristic of a free jet flow. Temporal monitoring of the chemical composition of the cold ion packet via mass spectrometry allows determination of an absolute reaction rate coefficient.

The experimentally observed rate coefficient is actually an average over two distribution functions, the relative ion-neutral collision energy and the neutral rotational state distribution function.[14] These degrees of freedom are not in equilibrium in the jet, therefore,

$$k_{expt} = \sum_{j=0}^{\infty} f_{rot}(j) \int_0^{\infty} k(\epsilon,j) f_{trans}(\epsilon)\, d\epsilon \qquad (2)$$

The f_i are the appropriate distribution functions, $k(\epsilon,j)$ are the microcanonical rate coefficients, and ϵ is the relative collision energy, while j is the rotational quantum number for the diatomic reactant. It is well-known that H_2 does not efficiently rotationally relax within a free jet, and the resulting rotational temperature is between 150-200 K under our expansion conditions.[15] Even at these temperatures, 98% of the H_2 population resides in rotational levels with j less than or equal to 2. At the same time, the translational distribution can be well represented by an average temperature in the range 1.8-2.5 K. These translational temperatures are averages over the parallel and perpendicular degrees of freedom in the jet. They are calculated by using the thermal conduction model of Klots[16] and the observed H_2 rotational tem-

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