Evidence for Spontaneous, Low-Temperature Biradical Formation from a Highly Reactive Neocarzinostatin Chromophore–Thiol Conjugate

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Neocarzinostatin chromophore (**1**) and methyl thioglycolate (**2**) combine at −70 °C to form the observable intermediate **3** which, upon warming to −38 °C, decays in a first-order process ($t_{1/2}$ = 2 h). Evidence is presented to support the biradical **4** as the direct product of this unimolecular decomposition.

Reaction of the antitumor antibiotic neocarzinostatin chromophore (**1**) with thiol **2** (0.2 M) in 0.5 M methanolic acetic acid (−78 → 0 °C) has been shown to produce the stable mono- and bisthiol adducts **5** and **6**, respectively, in 1:1 ratio.[1] The sequence **1** → **3** → **4** → **5** (Scheme I) was suggested to account for the formation of **5**.[2] In this pathway, biradical **4** is of particular significance since free-radical intermediates have been implicated in the cleavage of DNA by thiol-activated **1**.[3] In order to gain further insight into the mechanistic details of the transformation **1** + **2** → **5** + **6**, we have studied this reaction by low-temperature ^{1}H NMR spectroscopy.

A solution of **1** (0.01 M) and **2** (0.2 M) in 9:1 tetrahydrofuran-d_8:CD_3CO_2H at −78 °C showed distinct signals for each component in the 400-MHz ^{1}H NMR spectrum. Upon warming to −70 °C, a pseudo-first-order transformation of resonances for **1** to those of a new compound was observed [$t_{1/2}$(−70 °C, 0.2 M **2**) = 1.5 h, $k_1 = (1.2 \pm 0.1) \times 10^{-4}$ s^{-1}, Figure 1]. The chemical shift changes that signalled this conversion were entirely consistent with the proposal **1** → **3**. Thus, signals for H12 (δ 6.80), H11 (δ 6.12), H8 (δ 5.66), and H5 (δ 4.11) of **1** diminished while four new peaks at δ 4.20, 5.72, 6.24, and 5.81 (assigned as H12, H11, H8, and H5 of **3**, respectively) increased. The latter two resonances were observed as a pair of coupled doublets (verified by low-temperature irradiation), J = 5.1 Hz, confirming their assignment as H8 and H5 and providing support for the presence of the cumulene functional group.[4] While stable for days under argon at −70 °C, intermediate **3** rapidly decayed at −38 °C and above to produce as major products (ca. 50% yield) a 1:1 mixture of the mono- and bisthiol adducts **5** and **6**. Careful separation of **5** and **6** and subsequent ^{1}H NMR analysis of each pure compound showed that deuterium had been incorporated at C2 and C6 of **5** to the extent of 35 ± 5% and at C2 of **6** to the same degree, within experimental error. When the above experiment was conducted at 7-fold lower concentration of **2** (0.03 M, ca. 3 equiv), the ratio of **5** to **6** increased to 4:1 and the incorporation of solvent (carbon-bound) deuterium was increased to 80 ± 5% at each of these three positions. These data clearly support the existence of free-radical precursors to **5** and **6** with odd electron density at the labeled carbon atoms. The data also suggest that the bulk of bisthiol adducts **6** does not arise by a cage abstraction–recombination reaction of **4** with **2**, since this pathway would predict incorporation of protium at C2.[5] The data do support the formation of **6** by a process involving initial thiol radical addition into C6 of the cumulene **3** with transannular ring closure (Scheme I). Also consistent with this hypothesis is the fact that the kinetics of decay of **3** under conditions that produced equivalent amounts of **5** and **6** (0.2 M **2**) were complex, approximately second-order in **3**.

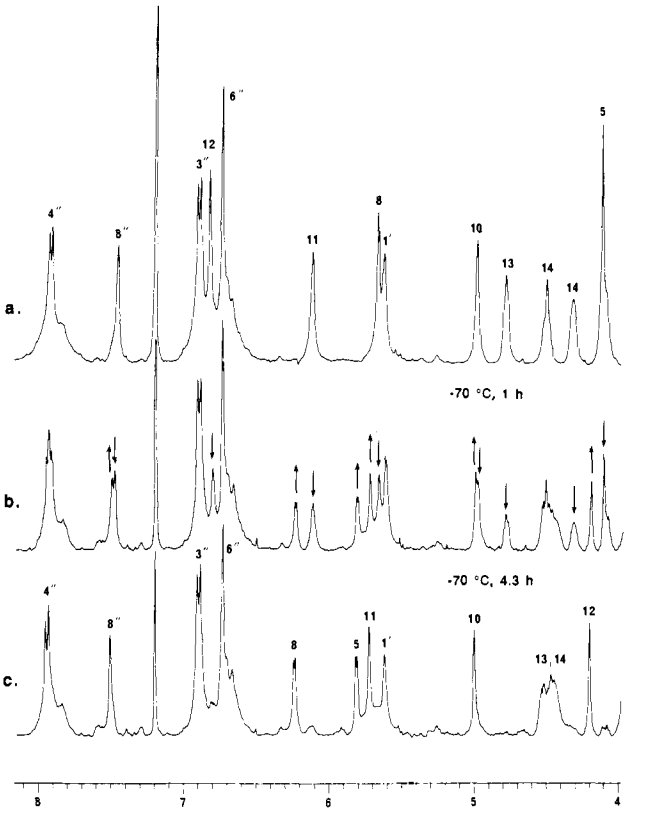

Figure 1. Reaction of **1** (0.01 M) and **2** (0.20 M) at −70 °C (9:1 tetrahydrofuran-d_8:CD_3CO_2H) to produce **3**, as monitored by 400-MHz ^{1}H NMR (δ 4.0–8.1): (a) **1**, −78 °C, prior to addition of **2**; (b) **1** + **2**, −70 °C, 1 h; (c) **1** + **2**, −70 °C, 4.3 h.

By careful optimization of parameters we were able to effectively suppress formation of **6** and thereby obtain first-order kinetics for the decomposition of **3**. Incubation of **1** (0.01 M) with **2** (0.03 M) in 9:1 tetrahydrofuran-d_8:CD_3CO_2H containing 1,4-cyclohexadiene (0.2 M) led to complete conversion of **1** to the cumulene **3** after 74 h at −70 °C. Warming to −38 °C in the probe of a high-field ^{1}H NMR spectrometer (400 MHz, *trans*-1,2-dichloroethylene as internal standard) then led to smooth first-order decay of **3** [$k_{obsd} = (1.0 \pm 0.2) \times 10^{-4}$ s^{-1}] with concomitant production of **5**. The yield of **5** was approximately 68%, the ratio of 5:6 was >10:1, and purified **5** had 40 ± 5% incorporated deuterium at C2 and C6. Following the precedent of Bergman, ΔH for the transformation of **3** to **4** can be estimated to be ≅ +6 kcal/mol, less the strain energy of the nine-membered ring of **3**.[6] Any reasonable estimate of the latter will undoubtedly place **4** below **3** in energy. The reaction **4** → **3** can therefore be excluded

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(2) Myers, A. G. *Tetrahedron Lett.* **1987**, *28*, 4493.

(3) Goldberg, I. H. *Free Radical Biology & Medicine* **1987**, *3*, 41 and references therein.

(4) The theoretical (Karplus, M. *J. Am. Chem. Soc.* **1960**, *82*, 4431) and experimental (Montijn, P. P.; Brandsma, L.; Arens, J. F. *Recl. Trav. Chim. Pays-Bas.* **1967**, *86*, 129) value of $J_{1,4}$ in [3]-cumulenes is about 7.8 Hz. It is anticipated that cyclic cumulenes will exhibit somewhat smaller values: Jackman, L. M.; Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*; Pergamon Press: New York, 1969; p 303 and references therein.

(5) For the same reason, mechanisms involving polar addition of thiol to **3** can be ruled out. We are grateful to Professor Jack Baldwin for stimulating discussions on this topic.

(6) Jones, R. R.; Bergman, R. G. *J. Am. Chem. Soc.* **1972**, *94*, 660. For this calculation, we used the model transformation of 1,2,3,5-cyclononatetraen-7-yne to 3,7-dehydroindene. ΔH_f for the former was determined to be 149 kcal/mol using group activities (Benson, S. W. *Thermochemical Kinetics*, 2nd ed.; Wiley: New York, 1976) and as 155 kcal/mol for the latter by subtraction of the bond dissociation energy of molecular hydrogen (104.2 kcal/mol, Herzberg, G. *J. Mol. Spectrosc.* **1970**, *33*, 147) from the sum of ΔH_f indene (39.08 ± 0.43 kcal/mol; Pedley, J. B.; Naylor, R. D.; Kirby, S. P. *Thermochemical Data of Organic Compounds*, 2nd ed.; Chapman and Hall: New York, 1986) and two benzene CH bond dissociation energies (2 × 110.2 ± 2.0 kcal/mol; Chamberlain, G. A.; Whittle, E. *Trans. Faraday Soc.* **1971**, *67*, 2077).

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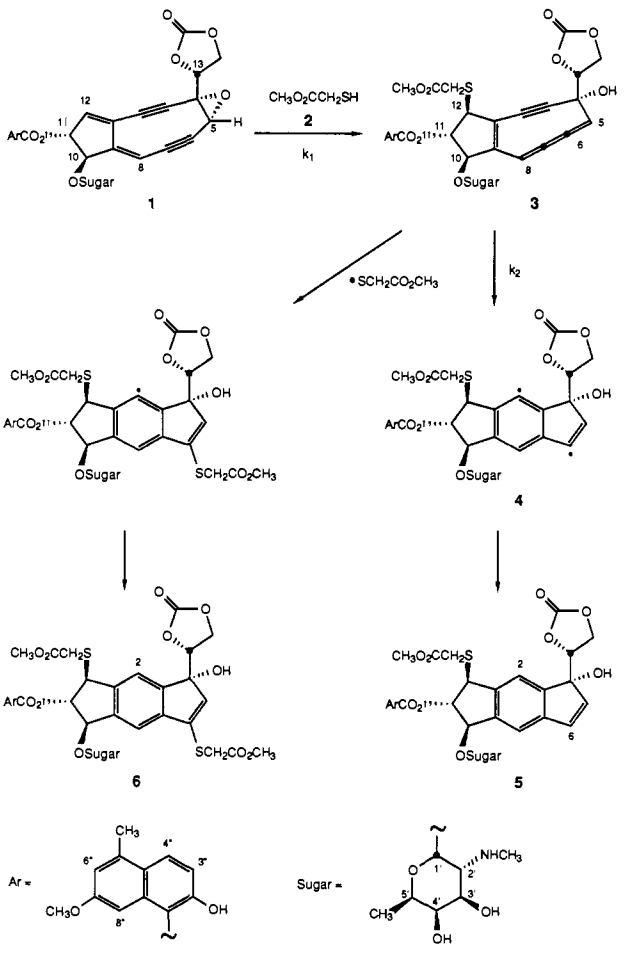
Scheme I



from consideration in light of more rapid trapping of **4** by solvent and we thus conclude that $k_{obsd} = k_2$ (Scheme I) and that $\Delta G^{\ddagger}$ for the process **3** → **4** is 18.0 ± 0.1 kcal/mol.[7]

While the conditions of the experiments outlined above are far from physiological, our results bear on questions regarding the relevance of pathways such as that outlined in Scheme I to the mechanism of action of neocarzinostatin in vivo. In the initial activation event, it is clear that **1** possesses a remarkable affinity for thiols, combining readily with methyl thioglycolate (30 mM, pK_a = 7.9)[8] at −70 °C. Glutathione (pK_a = 8.7), present in mammalian cells at concentrations of 0.5–10 mM,[9] has been strongly implicated as the activating nucleophile in studies of neocarzinostatin toxicity in intact cells.[10] With regard to the second event in activation, aromatization of a thiol–chromophore adduct such as **3** to the corresponding tetrahydroindacenediyl, we calculate a half-life of ~0.5 s for the transformation of **3** to **4** at 37 °C.[11]

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(7) The rate of trapping of **4** can be estimated by the rate of reaction of phenyl radical with diphenylmethane (7.7×10^6 M^{-1} s^{-1} at 60 °C): Lockhart, T. P.; Mallon, C. B.; Bergman, R. G. *J. Am. Chem. Soc.* **1980**, *102*, 5976. Ingold, K. U. In *Free Radicals*; Kochi, J. K., Ed.; Wiley: New York, 1973; Vol. I, Chapter 2.

(8) Jencks, W. P.; Salvesen, K. *J. Am. Chem. Soc.* **1971**, *93*, 4433.

(9) Meister, A.; Anderson, M. E. *Annu. Rev. Biochem.* **1983**, *52*, 711.

(10) DeGraff, W. G.; Russo, A.; Mitchell, J. B. *J. Biol. Chem.* **1985**, *260*, 8312.

(11) This assumes a (likely) weak temperature dependence of $\Delta G^{\ddagger}$.

Nonplanarity in Hückel 2π Aromatic Systems. An NMR-IGLO-ab Initio Proof of the Puckered Structure of Cyclobutadiene Dications

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Although numerous derivatives of the cyclobutadienyl dication (**1** and **2**) have been characterized by NMR spectroscopy in super acid media[1]—the tetramethyl-substituted dication (**1b** and **2b**) was prepared by Olah et al. 20 years ago[1a]—the structures of these species have not been established experimentally.[2]

In contrast to the expectation that 2π electron Hückel aromatics should prefer planar geometries **1**,[1e,3] ab initio calculations predict puckered structures **2** to be more stable.[4] The same preference also was forecast for isoelectronic 1,3-diboracyclobutadiene **3**;[4c] this was verified, subsequently by X-ray crystallography on derivatives[5] and by further ab initio calculations.[6]

We now present evidence, based on comparison of the chemical shifts calculated (IGLO)[7] for **1b** and **2b** with the experimental values, which establishes the nonplanar structure **2b** for the tetramethylcyclobutadienyl dication conclusively.

The fully optimized 6-31G* geometries were employed not only for the parent dication (**1a** and **2a**)[4] but also for the tetramethyl derivatives (**1b** and **2b**). Experience has shown that carbocation structures of at least this quality are desirable for the IGLO calculations, in order to obtain the best comparison with experimental chemical shifts.[7-9]

The symmetries chosen, C_{4h} for **1b** and D_{2d} for **2b**, were based on the methyl group conformational preferences found at 3-21G//3-21G. Frequency analyses at 6-31G* establish **2a** (D_{2d}) to be a minimum and **1a** (D_{4h}) to be the transition structure for ring inversion (one imaginary frequency). We assume the same to hold for **2b** and **1b**. As is shown in Table I, which also summarizes the earlier work, the barrier as well as the puckering angle increase somewhat at higher levels of theory. On the basis of the trends in the **1a–2a** energy differences, we estimate an inversion barrier of about 7 kcal/mol for **1b–2b**.

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(2) Attempts to establish the nonplanarity of cyclobutadiene dications by use of prochiral substituents were unsuccessful, see: Brendel, D. Dissertation, Erlangen, 1985.

(3) See, e.g.: Pittman, C. U., Jr.; Kress, A.; Kispert, L. D. *J. Org. Chem.* **1974**, *39*, 378. Radom, L.; Schaefer, H. F., Jr. *J. Am. Chem. Soc.* **1977**, *99*, 7522.

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