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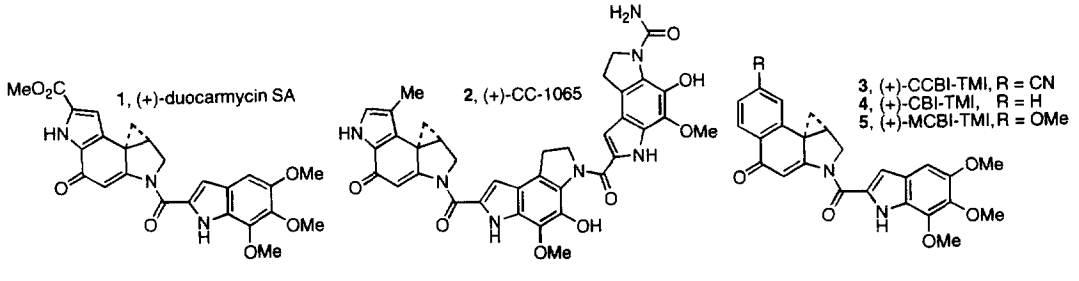
pH DEPENDENCE OF THE RATE OF DNA ALKYLATION FOR (+)-DUOCARMYCIN SA AND (+)-CCBI-TMI

Dale L. Boger,* Christopher W. Boyce, and Douglas S. Johnson
Department of Chemistry, The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, California 92037, U.S.A.

Abstract. A study of the DNA alkylation rate pH dependence with the establishment of pseudo first-order rate constants for **1** and **3** at a single high affinity site in w794 DNA is detailed. This dependence proved to be remarkably small with the rates increasing only 1.89× and 2.5×, respectively, over 2 pH units (pH 6–8).

Duocarmycin SA (**1**)[1] and duocarmycin A[2,3] along with CC-1065 (**2**)[4] constitute the parent members of a class of potent antitumor antibiotics that derive their biological effects through reversible, sequence-selective alkylation of DNA.[5,6] Since their disclosure, extensive efforts have been devoted to defining the fundamental principles underlying the relationships between structure, chemical reactivity, and biological properties. In these studies, the use of synthetic samples of the natural and unnatural enantiomers of the natural products and synthetic analogs bearing deep-seated structural changes have provided important insights into the structural origin of their properties. Despite the efforts that have defined the details of the DNA alkylation reaction, the origin of catalysis for the reaction has remained largely unaddressed.[6,7] The remarkable chemical stability of **1–3** and the requirement of acid catalysis for addition of typical nucleophiles to the activated cyclopropane has led to the assumption that the DNA alkylation reaction similarly must be an acid-catalyzed reaction and significant efforts have been made to support the extent and role of this acid catalysis.[6–8] In past studies, we have qualitatively observed only a small pH dependence on the rate of DNA alkylation and that it was of an insufficient magnitude to account for the extent of catalysis.[8] Moreover, in recent studies we have demonstrated that the rate of DNA alkylation for a series of closely



related agents **3–5** does not parallel the relative rates of acid-catalyzed nucleophilic addition to the activated cyclopropane[9,10] and these studies accurately reflected a growing set of observations.[11–16] Since these observations sharply contrast the proposed important role of acid catalysis for activation of the agents toward DNA alkylation which has served as the basis for one prominent proposal for the origin of the DNA alkylation selectivity,[6,7,17,18] we have examined in detail the pH dependence of the rate of DNA alkylation for duocarmycin SA (**1**) and CCBI-TMI (**3**)[9] over a physiologically relevant range.

The study was conducted by quantitating the relative (k_{rel}) and pseudo first-order rate constants (k_{obs}) for alkylation of the single, w794 high affinity alkylation site (5' -AATTA)[9–16] at a pH of 6.0, 6.6, 7.1, 7.6, and 8.1 for (+)-duocarmycin SA (**1**) and at a pH of 6.1, 6.8, 7.6, and 8.0 for (+)-CCBI-TMI (**3**, 10^{-6} M, 25 °C, 10 mM phosphate buffer, 0–4 h). Although the rate of DNA alkylation was found to increase with decreasing pH in both cases, the rate change was remarkably small (<3× over 2 pH units) and inconsistent with a first-order dependence

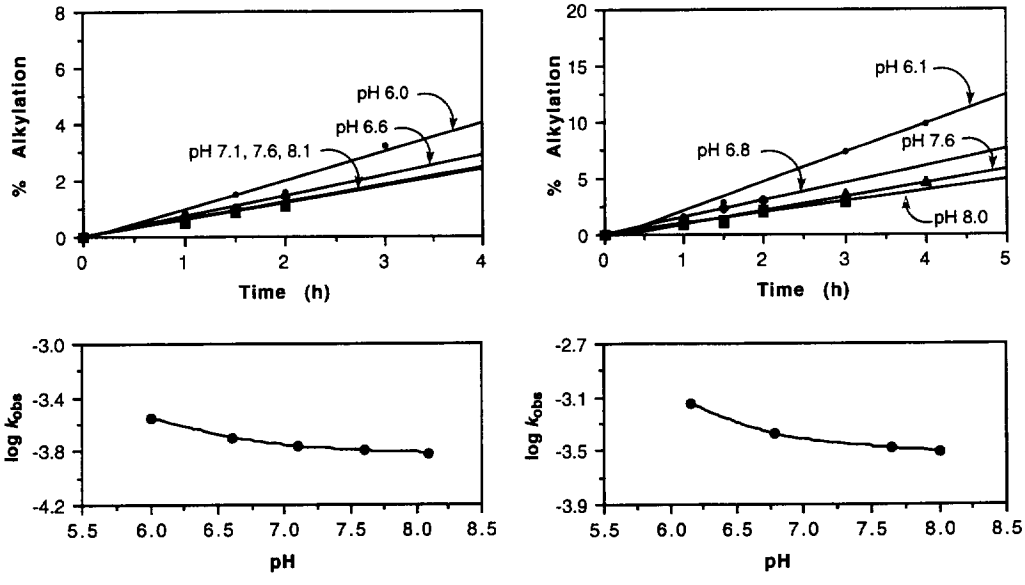
Figure 1

(+)-duocarmycin SA

pH	$k_{obs}(s^{-1})$	k_{rel}
6.0	2.83×10^{-4}	1.89
6.6	1.99×10^{-4}	1.32
7.1	1.69×10^{-4}	1.12
7.6	1.58×10^{-4}	1.05
8.1	1.50×10^{-4}	1.0

(+)-CCBI-TMI

pH	$k_{obs}(s^{-1})$	k_{rel}
6.1	7.06×10^{-4}	2.55
6.8	4.17×10^{-4}	1.50
7.6	3.36×10^{-4}	1.20
8.0	2.78×10^{-4}	1.00



of acid concentration (Figure 1). Between pH 6–7, the slope of the plot of pH versus log k_{obs} was 0.2–0.3, far lower than that of 1.0 required of a first-order dependence on acid concentration. Moreover, between pH 7 and 8, which may be considered the physiologically relevant range, the rate dependence on pH essentially disappeared. A qualitative comparison of the pseudo first-order rate constant for DNA alkylation at this site, $k = 1.58 \times 10^{-4}$ s^{-1} (pH 7.6) for **1** and $k = 3.36 \times 10^{-4}$ (pH 7.6) for **3** with the calculated first-order rate constants for acid-catalyzed solvolysis ($k = 2.7 \times 10^{-11}$ s^{-1} and $k = 2.5 \times 10^{-11}$ s^{-1}, respectively) at pH 7.6 suggest that the bulk of catalysis for the DNA alkylation reaction cannot be accounted for by this source. Perhaps the magnitude of this difference is best recognized by simply noting that at pH 7.6, the calculated $t_{1/2}$ for solvolysis of **1** is 820 years (3×10^5 d) while that of the DNA alkylation is 1.2 h.

Moreover, this rate of DNA alkylation was relatively independent of the buffer exhibiting only small differences in k_{obs} and the buffer had little impact on the pH dependence (Table 1). The presence or absence of EDTA had or no effect on the rate of DNA alkylation for CCBI-TMI in either Tris or phosphate buffer and the alkylation was somewhat slower in phosphate versus Tris buffer (1.7-1.5×).

Table 1

k_{rel} of (+)-CCBI-TMI in various buffers, pH 7.5	
10 mM Tris	1.74
10 mM Tris, 1 mM EDTA	1.51
10 mM NaH_2PO_4-Na_2HPO_4	1.05
10 mM NaH_2PO_4-Na_2HPO_4, 1 mM EDTA	1.0

Throughout the pH range, (+)-CCBI-TMI (**3**) was shown to alkylate the w794 high affinity alkylation site 1.9–2.5× faster than (+)-**1** (Figure 1), corresponding nicely with earlier studies[9] in which CCBI-TMI proved to exhibit the fastest relative rate at this same site: CCBI-TMI (2.5×) > MCBI-TMI (1.9×) > CBI-TMI (1.0×) > DSA (0.9×) (pH 7.5, Table 2). In these studies, the rate of DNA alkylation did not correlate with the relative reactivity of the agents toward acid-catalyzed solvolysis (MCBI > CBI > CCBI, Figure 2) suggesting that other factors are responsible for the catalysis of the DNA alkylation reaction.[8]

Table 2. Relative Rates of DNA Alkylation

Agent	k_{rel}	Agent	k_{rel}	vs. k_{rel} (solv)
3 (+)-CCBI-TMI	2.5	**6** (+)-CCBI-indole$_2$	2.5	0.7
5 (+)-MCBI-TMI	1.9	**7** (+)-MCBI-indole$_2$	1.4	1.2
4 (+)-CBI-TMI	1.0	**8** (+)-CBI-indole$_2$	1.0	1.0
1 (+)-DSA	0.9			

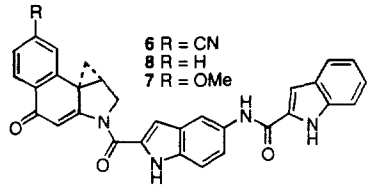
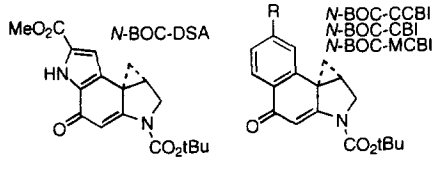


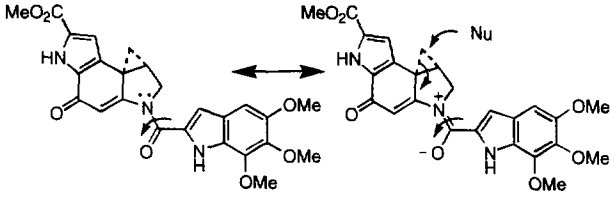
Figure 2. Solvolysis Rates

Agent	k (sec^{-1})	$t_{1/2}$ (h, pH 3)	k_{rel} (solv)
N-BOC-DSA	1.08 x 10^{-6}	177	0.75
N-BOC-CCBI	0.99 x 10^{-6}	213	0.68
N-BOC-CBI	1.45 x 10^{-6}	133	1.00
N-BOC-MCBI	1.75 x 10^{-6}	110	1.21



The relative lack of dependence on acid-concentration (pH) especially in the relevant pH range of 7–8, in conjunction with other studies[8,19] have suggested an alternative source of catalysis responsible for the rapid rate of DNA alkylation by **1–3**. We have proposed that catalysis for the DNA alkylation reaction is derived from a DNA binding induced conformational change in the agent that disrupts the N^2 vinylogous amide stabilization of the alkylation subunit and activates the agent for nucleophilic addition.[8,19] This conformational change results from adoption of a helical bound conformation that follows the curvature and pitch of the DNA minor groove. The helical rise in the bound conformation of the rigid agents is adjusted by twisting the linking N^2 amide which is the only available flexible site. The twisting of the linking amide diminishes the N^2 lone pair conjugation with the cyclohexadienone, disrupts the vinylogous amide stabilization of the alkylation subunit, and increases its inherent reactivity (Figure 3). For the substituted CBI series of agents **3–5** and **6–8**, the impact of the C7 substituent (R = CN≥ OCH_3 > H) is unrelated to its effect on the rate of acid-catalyzed nucleophilic addition (R = OCH_3 > H > CN) and seems to be related simply to its presence rather than electronic nature. We suggest this is due to the resulting extended length of the alkylation subunit with substituent placement in the minor groove and the corresponding increase in the inherent twist of the linking N^2 amide that accompanies minor groove binding.[20] The documentation of remarkably large and appropriate reactivity increases that accompany the decoupling of the vinylogous amide stabilization within the alkylation subunits support this as the source of catalysis.[19]

Figure 3



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