Acknowledgement is made to the National Science Foundation (CHE-8705534) for support and to Johnson Matthey for a loan of $RhCl_3$. S.S.-E. acknowledges partial support from a CNRS-NSF grant.

Supplementary Material Available: A listing of the synthetic procedures used to prepare **4**, **5**, **8**, and **9**, ^1H and ^{13}C NMR spectral data for **4**, **5**, **8**, and **9**, and microanalytical data for **5** and **9** (5 pages). Ordering information is given on any current masthead page.

(+)-CC-1065 DNA Alkylation: Observation of an Unexpected Relationship between Cyclopropane Electrophile Reactivity and the Intensity of DNA Alkylation

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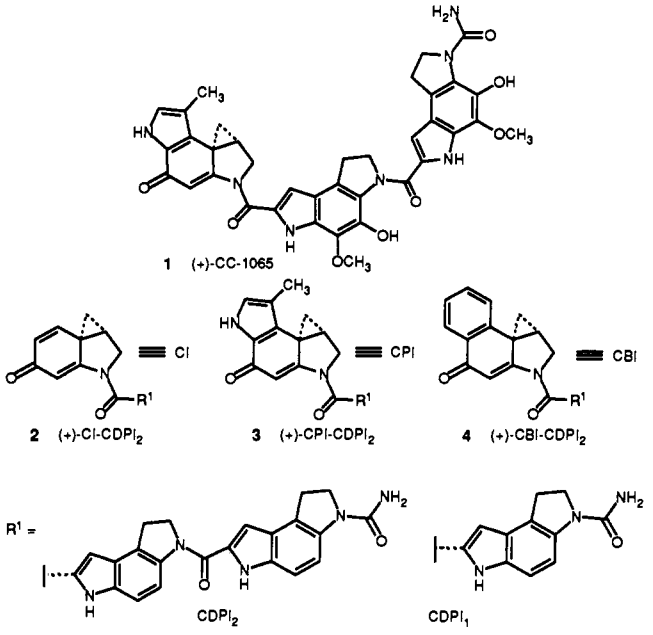
Received October 1, 1990

(+)-CC-1065 (**1**), a potent antitumor antibiotic and the subject of extensive investigations,[2-4] has been shown to exert its effects through the selective alkylation of DNA within the minor groove.[2-5] For (+)-CC-1065, the alkylation has been demonstrated to proceed by 3′-adenine N-3 alkylation of the electrophilic cyclopropane present in the left-hand (CPI) subunit within A-T-rich minor groove regions of DNA.[5,6] In an extensive evaluation of a series of agents possessing the natural enantiomer of the authentic alkylation subunit of (+)-CC-1065, the relative in vitro cytotoxic potency of the agents has been correlated with the relative intensity of the adenine N-3 alkylation with cell-free double-stranded DNA.[2,4,5] It has been further suggested that the cytotoxic potency of the agents may be a direct expression of their rate of DNA covalent alkylation[2] and directly related to the agent relative rate of acid-catalyzed solvolysis[4] by virtue of a sequence-dependent autocatalytic activation of the alkylation event by a strategically located phosphate (carbonyl protonation/complexation).[5] Thus, in the course of our investigations which have resulted in the preparation and evaluation of (+)-CPI-CDPI$_n$,[7-10] (+)-CI-CPDI$_n$,[6,11-13] and (+)-CBI-CDPI$_n$,[14-16] the observation that an inverse versus direct relationship between the solvolytic reactivity and cytotoxic potency of the agent may constitute a relevant relationship in the design of functional analogues has proven unexpected.[15,16] Herein, we detail the results of a comparative study of the DNA alkylation properties of (+)-CPI-CDPI$_2$ (**3**), (+)-CI-CDPI$_2$ (**2**), and (+)-CBI-CDPI$_2$ (**4**) representative of comparisons made with a full series of agents[17] which additionally demonstrate that the intensity of DNA alkylation follows *the same inverse relationship*: (+)-*CBI-CDPI*$_2$ > (+)-*CPI-CDPI*$_2$ > (+)-*CI-CDPI*$_2$.

Table I

	(+)-CBI-CDPI$_2$ (**4**)	(+)-CPI-CDPI$_2$ (**3**)	(+)-CI-CDPI$_2$ (**2**)
rel int (37 °C, 24 h) of DNA alkylation[a] (%)	1.1 (100%)	1.0 (100%)	0.6 (100%)
rel int (4 °C, 24 h) of DNA alkylation[a] (%)	1.0 (90%)	0.2 (20%)	0.02 (3%)
k (rel), 4 °C[b]	1.0	0.05	nd[e]
rel stability[c]	1.0	0.27	0.0001
rel in vitro cytotoxic act. (L1210)[d]	1.0	0.24	0.0005

[a] Relative intensity of alkylation (thermally induced strand cleavage) at the high-affinity alkylation site [5′-d(AATTA)-3′] within w794 DNA determined by using a scanning densitometer. Percent reaction (%) is expressed as the percentage of the total alkylation at this site observed when the alkylation is taken to >90% completion (37 °C, 24 h); the relative intensities of alkylation detectable at 10^{-8} M at this point are compared to (+)-**4** (4 °C, 24 h) = 1.0. [b] Relative first-order rate constants for DNA alkylation at the high-affinity site taken from plots of the intensity of DNA cleavage versus time (4 °C, 10^{-7} M agent; 12, 24, 48, 96, and 192 h). [c] Taken from refs 13, 15 and 16. Solvolysis studies conducted spectrophotometrically (UV) at pH = 3 (50% buffer–CH_3OH, buffer = 4:1:20 (v/v/v) 0.1 M citric acid, 0.2 M Na_2HPO_4, and H_2O). [d] Relative IC_{50} for in vitro cytotoxic activity against L1210 mouse lymphocytic leukemia, (+)-**2**, 10000 pM; (+)-**3**, 20 pM; (+)-**4**, 4.8 pM. [e] Not determined.



Singly 5′ ^{32}P end labeled double-stranded DNA constituting SV40 DNA nucleotides no. 5238-138 (144 base pairs) cloned into the *Sma*I site of M13mp10 was prepared by treatment of single-stranded templates (clone w794)[18] with 5′ ^{32}P end labeled universal primer [5′-d(GTAAAACGACGGCCAGT)-3′], extension of the primer–template duplex with the Klenow fragment of DNA polymerase I, and subsequent *Eco*RI cleavage of the double-stranded DNA immediately following the inserted DNA.

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(17) Similarly, the following trends in the intensity of DNA alkylation (4 °C, 24 h) and cytotoxic activity (L1210) have been observed: (+)-CBI-CDPI$_1$ (1, 5 pM) > (+)-CPI-CDPI$_1$ (0.2, 40 pM) > (+)-CI-CDPI$_1$ (24000 pM); (+)-CBI-(indole)$_2$ (1, 5 pM) > (+)-CPI-(indole)$_2$ (0.1, 20 pM).
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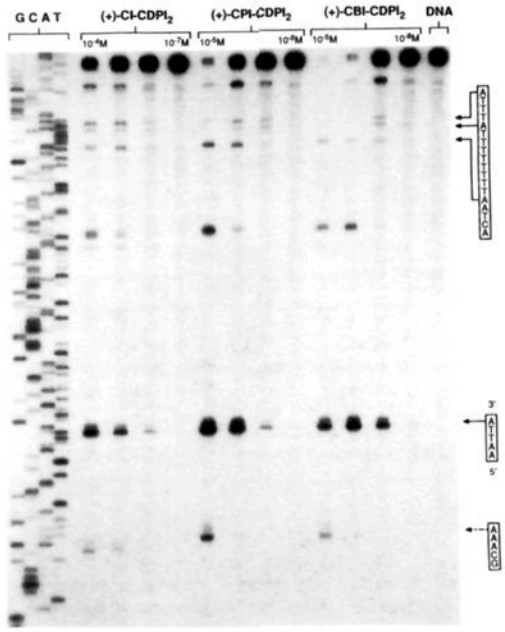

Figure 1. Thermally induced strand cleavage of double-stranded DNA (SV40 DNA fragment, nucleotide no. 138-5238, clone w794) after 24-h incubation of agent–DNA at 4 °C followed by removal of unbound agent and 30-min warming at 100 °C, gel electrophoresis, and autoradiography. Lanes 1–4, Sanger G, C, A, and T sequencing reactions; lanes 5–8, (+)-CI-CDPI$_2$ [$(5.6 \times 10^{-4}) - (5.6 \times 10^{-7}$ M)]; lanes 9–12, (+)-CPI-CDPI$_2$ [$(5.6 \times 10^{-5}) - (5.6 \times 10^{-8}$ M)]; lanes 13–16, (+)-CBI-CDPI$_2$ [$(5.6 \times 10^{-5}) - (5.6 \times 10^{-8}$ M)]; lane 17, control DNA. The origin of the double bands observed for a single adenine N-3 alkylation with 5′-end-labeled double-stranded DNA has been detailed elsewhere (ref 5 and 6).

The resultant DNA was treated with the agents at 4 or 37 °C (24 h) at a range of concentrations. Removal of the unreacted agent through ethanol precipitation of the DNA, redissolution of the alkylated DNA in aqueous buffer, thermally induced cleavage of the DNA at the sites of alkylation (100 °C, 30 min),[2,5] gel electrophoresis of the resultant DNA alongside Sanger dideoxynucleotide sequencing reactions, and subsequent autoradiography revealed the agent sites of alkylation and their relative intensities, Figure 1.[6] As illustrated in Figure 1, the most stable and least reactive agent [(+)-**4**] exhibits the most intense DNA alkylation at 4 °C, and the exceptionally reactive agent [(+)-**2**] exhibits the least intense DNA alkylation. Thus, an inverse (versus direct) relationship between the intensity of DNA alkylation and the chemical solvolytic reactivity of the agents was observed. In addition, the most reactive agent [(+)-**2**] exhibits slightly less selectivity among the available alkylation sites than (+)-**4** or (+)-**3**. As detailed elsewhere, this more selective and productive covalent modification of DNA by the more stable agent presumably arises in part from the enhanced agent availability (stability, selectivity).[16] However, when the DNA alkylation reactions were taken to >90% completion (37 °C, 24 h) under conditions where excess DNA was present and the alkylation would be expected to follow first-order kinetics with respect to the agent concentration (10^{-7}–10^{-8} M agent), each of the three agents exhibited a comparably intense DNA alkylation, Table I. Under such conditions where the agents are effectively sequestered by double-stranded DNA,[19] the results suggest that the nonproductive solvolysis[20] and alkylation selectivity differences account for only part of the distinctions in the observed DNA alkylation intensity at 4 °C. In particular, the comparisons of (+)-**4** and (+)-**3** made at 4 °C (24 h)[20] additionally reflect the relative rates of DNA alkylation by the two agents, which follow the unexpected order[19] of (+)-CBI-CDPI$_2$ > (+)-CPI-CDPI$_2$, Table I. The precise origin of this relationship and its generality are under investigation.[21]

Although limited to the three classes of agents presently available for comparison,[17] the inverse correlation between the cytotoxic potency of the agents and the solvolytic reactivity of the cyclopropane suggests that the direct relationship between the agent stability and cytotoxic activity may constitute a relevant feature in the further design of functional analogues, Table I. Potentially contributing to the distinctions in the properties of the agents examined herein[17] may be the unexpected observation of the more rapid (rate), more discriminate (selectivity), and more productive (intensity) DNA alkylation by the more stable agent.

Acknowledgment. This work was supported financially by the National Institutes of Health (CA 41986) and the American Cancer Society (PF-3311, S.A.M.). We gratefully acknowledge Professor M. Bina and her group for assistance in developing the DNA alkylation/cleavage protocols (ref 6) and for the gift of M13mp10 clones (w794).

Supplementary Material Available: Table of IC_{50}, k, $t_{1/2}$, and DNA cleavage data and graph of $-\log k$ vs $\log IC_{50}$ for various agents and listing of agents and their IC_{50} values (2 pages). Ordering information is given on any current masthead page.

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(21) Other agent properties may correlate with the DNA alkylation intensity. Such correlations are under examination.

The Unusual Electron Spin Resonance of Fullerene $C_{60}^{\bullet-}$

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Received January 18, 1991

The success in the preparation of the fullerenes[1-3] and the confirmation[4,5] of the theoretical prediction[6] that C_{60} is a relatively electronegative molecule prompted us to prepare the first fulleride salt and evaluate its condensed matter properties. Here we report on electron spin resonance and transport properties.

Samples of the pure fullerene C_{60} were obtained as described previously.[3] We discovered five interesting features:

1. Upon bulk electrolysis, fullerene C_{60} deposits the $C_{60}^{\bullet-}$ $Ph_4P^+ \cdot (Ph_4PCl)_2$ salt as a microcrystalline powder[7] at a platinum

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(7) X-ray powder pattern determination indicates a pure, crystalline phase, with a pattern that is unrelated to, and much simpler than, the powder pattern of $Ph_4P^+Cl^-$. Attempts to index and obtain unit cell parameters are in progress.

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