

Interaction of the Radical Ion of Chlorpromazine with Deoxyribonucleic Acid

Sir:

Piette, Bulow, and Yamazaki¹ have recently proposed that the positive ion radical of chlorpromazine (cpz^+) may be responsible for the psychotropic activity of this tranquilizer. It occurred to us that because of the chemical and structural similarity of cpz^+ to the mutagenic acridine dye ions (e.g., acridine, proflavine, and acridine orange), cpz^+ might *intercalate* in DNA in the same manner as that described by Lerman² for the acridine dyes. In this case, the aromatic molecular plane of cpz^+ would be perpendicular to the DNA helix axis. In this communication, we present strong evidence for this perpendicularity based on paramagnetic resonance. A direct casual relationship between intercalation in DNA (or RNA) and the psychotropic activity of cpz^+ is thus an interesting possibility.

The radical cpz^+ was prepared by persulfate oxidation³ and added to solutions of calf thymus DNA so that the resultant concentration of nucleotide base pairs was $3 \times 10^{-3} M$; there was approximately one cpz^+ ion for five base pairs. The solution pH was 5.0. The radical was found to be markedly stabilized by the presence of DNA. Figure 1 gives three spectra obtained with a Varian 35-kMc. spectrometer. Figure 1a gives the "no flow" magnetic resonance of cpz^+ bound to DNA; the line shape is typical of a "polycrystalline" sample and of course bears no resemblance to the known spectrum of cpz^+ in aqueous solution.^{3,4,4a} Figure 1b shows the "perpendicular flow" resonance of DNA-bound cpz^+ when the DNA helix axes are oriented perpendicularly to the applied field by flowing the solution through a capillary tube in the resonance cavity (shear rate $\sim 3000 \text{ sec.}^{-1}$). Figure 1c gives the corresponding "parallel" resonance spectrum.

The observed spectra are readily interpreted in terms of Lerman's intercalation model² where the helix axis is perpendicular to the aromatic molecular plane of cpz^+ . Consider first the N^{14} hyperfine splitting. The isotropic N^{14} hyperfine splitting, a , measured from the solution spectrum is *ca.* 6 gauss.^{3,4} By analogy with known C^{13} anisotropic π -electron hyperfine interactions,⁵ one expects a hyperfine splitting A parallel to the π -orbital axis of the aromatic nitrogen atom to be approximately twice the isotropic splitting, and the hyperfine splitting B perpendicular to the π -orbital axis to be much less than even the isotropic splitting. These expectations are borne out, for example, by the anisotropy of the N^{14} hyperfine interaction in di-*t*-butyl nitroxide⁶ where $A/a = 2.35$ and $B/a = 0.33$. We therefore expect an N^{14} hyperfine splitting of $\sim 2.35 \times 6 = 14$ gauss when the plane of cpz^+ is perpendicular to the applied field. The triplet splittings seen in the

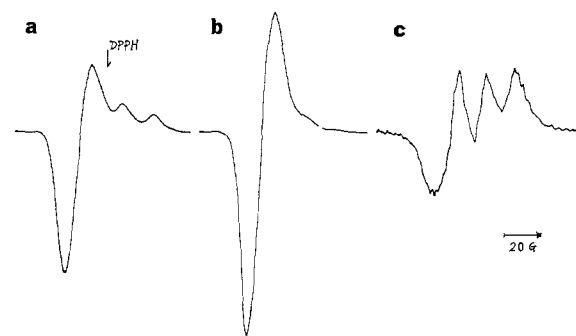


Figure 1. Paramagnetic resonance of the chlorpromazine cation bound to DNA: (a) "no flow" (see text), (b) perpendicular flow, (c) parallel flow.

parallel flow spectrum (Figure 1c) are *ca.* 17 gauss, whereas no splittings are seen in the perpendicular flow experiments. These are just the results expected if the plane of cpz^+ is perpendicular to the helix axis. The asymmetry in the spectra in Figures 1b and 1c is attributed to incomplete orientation of the DNA helices.

Our argument favoring perpendicularity of cpz^+ to the helix axis is actually more general than that given above. When $|A| \gg |B|$, the hyperfine splittings just detectable in the no flow spectrum of Figure 1a must be equal to $|A|$, irrespective of the relative orientation of cpz^+ to the helix axis. Thus, the observed equality of the splittings in Figures 1a and 1c is a necessary condition for perpendicularity of the cpz^+ plane to the helix axis. A sufficient condition for perpendicularity is that this equality must persist for all laminar shear rates, no matter how high; this is certainly true up to the maximum shear we have used (9000 sec.^{-1}).

The observed g -factor anisotropies are also in complete agreement with the intercalation geometry and current knowledge of g -factors in π -electron radicals.⁷ From Figure 1b, g_{\perp} is estimated to be 2.006, and from Figure 1c, g_{\parallel} is estimated to be 2.003.

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(7) H. M. McConnell and R. E. Robertson, *J. Phys. Chem.*, **61**, 1018 (1957).

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The Intramolecular Insertion Mechanism of α -Haloneopentyllithium

Sir:

Transformation of neopentylidene iodide to 1,1-dimethylcyclopropane (**2**) by methyllithium¹ is typical of many in which α -elimination has hitherto been presumed to require a divalent carbon intermediate.² We

(1) W. Kirmse and B. G. v. Wedel, *Ann.*, **666**, 1 (1963).

(2) W. Kirmse, "Carbene Chemistry," Academic Press Inc., New York, N. Y., 1964; J. Hine, "Divalent Carbon," Ronald Press Co., New York, N. Y., 1964.

(1) L. H. Piette, G. Bulow, and I. Yamazaki, *Biochim. Biophys. Acta*, **88**, 120 (1964).

(2) L. S. Lerman, *J. Mol. Biol.*, **3**, 18 (1961); *Proc. Natl. Acad. Sci. U. S. A.*, **49**, 94 (1963); *J. Mol. Biol.*, **10**, 367 (1964).

(3) D. C. Borg and G. C. Cotzias, *Proc. Natl. Acad. Sci. U. S. A.*, **48**, 617, 623 (1962).

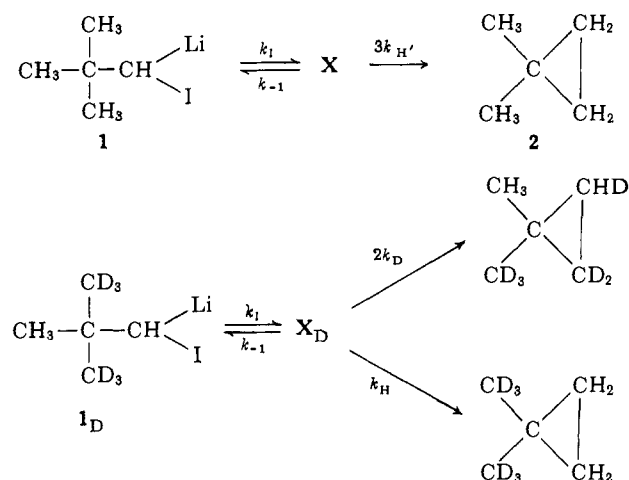
(4) L. H. Piette and I. S. Forrest, *Biochim. Biophys. Acta*, **57**, 419 (1962).

(4a) NOTE ADDED IN PROOF. The resonance of cpz^+ in the presence of *E. coli* transfer RNA is similar to that in Figure 1a, showing that cpz^+ also binds to RNA.

(5) H. M. McConnell and R. W. Fessenden, *J. Chem. Phys.*, **31**, 1688 (1959).

(6) N. Edelstein, A. Kwok, and A. H. Maki, *ibid.*, **41**, 179 (1964).

here (a) confirm that α -iodoneopentyllithium (**1**) is an initial intermediate, (b) provide kinetic evidence consistent with the *absence* of a subsequent intermediate (X, purportedly a carbene), and (c) further demonstrate that if X were in fact to intrude, it could not be a carbene.



The first conclusion was the most simply achieved by preparation of **1** and its protolytic characterization³ (ether, -116°) to provide $>91\%$ neopentyl iodide.^{4,5} The second derives from a comparison of *intramolecular* ($k_{\text{H}}/k_{\text{D}}$) and *intermolecular* (k_{d_0}/k_{d_6} , *vide infra*) deuterium isotope effects obtained at $-90 \pm 1^\circ$ in the subsequent transformation of **1** to **2** ($97 \pm 3\%$ yield).

$k_{\text{H}}/k_{\text{D}}$ (Table I), evaluated⁷ from n.m.r. spectra of

Table I. Intramolecular Kinetic Isotope Effects of α -Haloneopentyl- d_6 -lithium

Halide	Temp., $^\circ\text{C}$.	$k_{\text{H}}/k_{\text{D}}$
I	-90 ± 1	2.76 ± 0.09
I	-44 ± 1	2.43 ± 0.02
Br	-44 ± 1	2.06 ± 0.10
Cl ^a	-68 ± 3	1.71 ± 0.06

^a In Tetrahydrofuran, ref. 6, 7; all others in ether.

gas chromatographically purified product of 1-iodo-2,2-di(methyl- d_3)propyllithium⁸ (**1_D**), measures differences between the force field about the hydrogen transferred and that surrounding those of intact methyl groups in the *transition state for hydrogen transfer*. k_{d_0}/k_{d_6} , the relative rate constants of light (d_0) and heavy (d_6) reactants, evaluated by low voltage mass spectrometric analysis of identically obtained product from **1**, **1_D** mixtures of known composition after 3.6–16% reaction,¹⁰

(3) W. T. Miller, Jr., and D. M. Whalen, *J. Am. Chem. Soc.*, **86**, 2089 (1964).

(4) α -Chloroneopentyllithium (from *t*-butyllithium and methylene chloride) was similarly characterized.⁶ For other recent reports of α -haloalkyllithium scavenging, *cf.* W. Kirmse and B. G. v. Wedel, *Ann.*, **676**, 1 (1964); G. Köbrich, K. Flory, and W. Drischell, *Angew. Chem.*, **76**, 536 (1964); ref. 3.

(5) Lacking the further data needed to formulate associated, complexed, and/or polymeric structures, we employ a monomeric description throughout but without prejudice to other, more probable, possibilities.

(6) M. J. Goldstein and W. R. Dolbier, Jr., to be published.

(7) M. J. Goldstein and S. J. Baum, *J. Am. Chem. Soc.*, **85**, 1885 (1963).

(8) Neopentylidene- d_6 iodide was prepared from *t*-butyl- d_6 -magnesium chloride *via* deuterated pivalaldehyde and its hydrazone.⁹

(9) D. H. R. Barton, R. E. O'Brien, and S. Sternhell, *J. Chem. Soc.*, 470 (1962).

measures a similar quantity for the *rate-limiting transition state*. That both transition states are the same follows from the minimally elaborated kinetic scheme (above), eq. 1 which derives from it, and the experimental values $k_{\text{H}}/k_{\text{D}} = 2.76 \pm 0.09$ and $k_{d_0}/k_{d_6} = 1.50 \pm 0.12$.

$$k_{d_0}/k_{d_6} = \frac{3(k_{\text{H}'}/k_{\text{H}})(k_{\text{H}}/k_{\text{D}}) + \beta(2 + k_{\text{H}}/k_{\text{D}})}{(1 + \beta)(2 + k_{\text{H}}/k_{\text{D}})} \quad (1)$$

where $\beta = 3k_{\text{H}'}/k_{-1}$. If X be absent (or the pre-equilibrium fully established and hence kinetically irrelevant), $\beta = 0$, and $k_{\text{H}'}/k_{\text{H}} = 0.9 \pm 0.3$, effectively unity as expected.^{11,12}

The last, most significant conclusion derives from comparison of intramolecular isotope effects in the insertion of I_{D} and of its bromo analog¹³ (Table I). The unambiguous halogen dependence of $k_{\text{H}}/k_{\text{D}}$ demands the presence of this atom in the transition state for hydrogen transfer,¹⁴ most probably still (at least partly) bonded to its original carbon atom.^{15,16} The plausible, more detailed hypothesis, that hydrogen transfer be concerted with carbon-halogen cleavage, though *not required* by the data finds comforting analogy in the Fritsch-Buttenberg-Wiechell rearrangement for which a carbene mechanism had been considered and then clearly excluded some time ago.¹⁷

Others have demonstrated that olefin formation, from reaction of alkylolithium with α, α -dichloroalkyllithium compounds, by-passes the carbene¹⁸ and that the olefin addition of α -haloalkylzinc,¹⁹ -aluminum,²⁰

(10) *Cf.* eq. V.21, J. Bigeleisen and M. Wolfsberg, *Advan. Chem. Phys.* **1**, 68 (1958). The necessary assumption of first-order kinetics derives from the insensitivity of k_{d_0}/k_{d_6} to a 4.2-fold variation in R_{aq} . Duplicate n.m.r. analyses agreed with the mass spectrometric within experimental uncertainty.

(11) Alternatively, if $k_{\text{H}'}/k_{\text{H}}$ be set equal to unity, $\beta = 0.3 \pm 1.1$, effectively zero.

(12) We are unaware of previous exploitation of a comparison of intra- and intermolecular kinetic isotope effects for such a purpose. It is hoped that related studies will more fully illustrate the utility of this technique.

(13) Neopentylidene- d_6 bromide was prepared in much the same manner as the iodide.⁸ Both the hitherto unreported undeuterated and deuterated bromides were characterized by elemental analyses (*Anal.* Calcd.: C, 26.3; H, 4.4; Br, 69.5. Found: C, 26.4; H, 4.4; Br, 69.2. Calcd.: C, 25.5; "H," 4.6; Br, 67.7. Found: C, 25.5; "H," 4.5; Br, 68.6) and n.m.r. spectra (τ 4.25, 8.80 for both; area ratio 1:9.4 for undeuterated material). 1,1-Dimethylcyclopropane- d_5 obtained from both neopentylidene halides was found only to be contaminated with 6.2% d_6 compound.

(14) A superficially acceptable alternative would require carbenes of varying excess energy to have been generated. Unprecedented in such an environment and unlikely for other reasons, this hypothesis will be more fully considered at another time.

(15) Changes in a remote mass (or in its geometry) should, *alone*, be ineffectual in the absence of force field changes at the hydrogen transferred; *cf.* M. Wolfsberg and M. J. Stern, *Pure Appl. Chem.*, **8**, 225, 325 (1964).

(16) G. L. Closs and R. A. Moss, *J. Am. Chem. Soc.*, **86**, 4042 (1964), have defined the noun "carbenoid" to include intermediates which resemble carbenes in reactivity though not "necessarily" in structure. Traditionally, however, reactivity patterns have assumed the names of previously well-defined structural concepts (e.g., "radical chain reaction"). Since the carbene is structurally well defined, "carbenoid" necessarily carries with it an unintended (and here incorrect) structural implication and so too an unfortunate mechanistic bias.

(17) A. A. Bothner-By, *J. Am. Chem. Soc.*, **77**, 3293 (1955); J. G. Pritchard and A. A. Bothner-By, *J. Phys. Chem.*, **64**, 1271 (1960); D. Y. Curtin, E. W. Flynn, and R. F. Nystrom, *J. Am. Chem. Soc.*, **80**, 4599 (1958).

(18) W. Kirmse and B. G. v. Bulow, *Chem. Ber.*, **96**, 3323 (1963).

(19) E. P. Blanchard and H. E. Simmons, *J. Am. Chem. Soc.*, **86**, 1337 (1964); H. E. Simmons, E. P. Blanchard, and R. D. Smith, *ibid.*, **86**, 1347 (1964); G. Wittig and F. Wingler, *Chem. Ber.*, **97**, 2139 (1964).

(20) H. Hoberg, *Ann.*, **656**, 1, 15 (1962).

and -lithium^{3,16,21} compounds is difficultly reconcilable with such an intermediate. Intramolecular insertion thus becomes the third of three "diagnostic carbene reactions" now shown to be characteristic, instead, of a precursor.

It is clear that the carbene need arise no more often in reactions of an organometallic carrying a labile alkoxide,²² halide, or other Lewis base in the α -position than does its conjugate acid in reactions of a similar substrate which lacks the metal atom. By analogy with carbonium ion chemistry then, one might hope to achieve both deeper understanding and more reliable predictions if the carbene were no longer routinely invoked in α -eliminations but, rather, only after its many precursors had first been excluded from their simpler role: immediate precursors of the product.

(21) G. L. Closs and L. E. Closs, *Angew. Chem.*, **74**, 431 (1962); C. L. Closs and J. J. Coyle, *J. Am. Chem. Soc.*, **84**, 4350 (1962); T. J. Katz and P. J. Garratt, *ibid.*, **86**, 4876 (1964).

(22) K. Ziegler and H. G. Gellert, *Ann.*, **567**, 185 (1950).

(23) Proctor and Gamble Company Fellow, 1963-1964. Partial support was also provided by a grant (GP-1171) from the National Science Foundation.

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Concerning the Acid-Catalyzed Hydration of Acetylenes

Sir:

We wish to report evidence indicating the rate-determining formation of a vinylic cation in the acid-catalyzed hydration of phenylpropionic acids and phenylacetylenes.

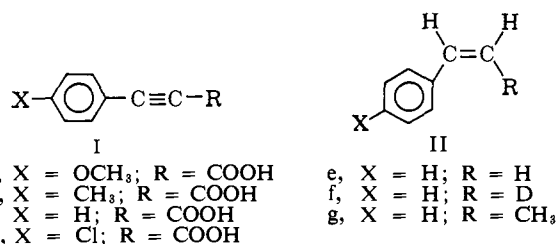
Table I. Hydration of Phenylpropionic Acids in H₂SO₄ at 25°

Compound	10 ⁶ k, sec. ⁻¹ ^a	Acidity range, % H ₂ SO ₄	-d log k/dH ₀	k _{H₂SO₄}/k_{D₂SO₄} ^b}}
Ia	2820	23.6-43.8	1.13	3.93
Ib	13.5	48.4-61.3	0.98	3.70
Ic	0.444	62.3-70.4	0.96	4.07
Id	0.165	64.8-73.4	0.97	3.75

^a At 50% H₂SO₄ (H₀ = -3.38), extrapolated where necessary.

^b Compared at that mole fraction SO₄²⁻ at which the hydration rate in H₂SO₄ is 1 × 10⁻⁸ sec.⁻¹.

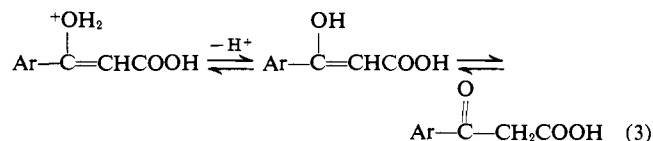
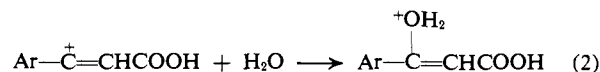
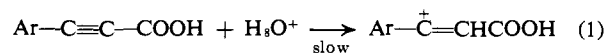
Pseudo-first-order rate constants (measured spectrophotometrically) for the hydration of four phenylpropionic acids (Ia-d) in H₂SO₄ and D₂SO₄ are summarized in Table I, with derived information.



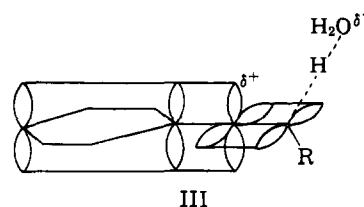
The rate data for the phenylpropionic acids correlate with σ^+ , $\rho = -4.79 \pm 0.02$, indicating a high degree of positive charge on the benzylic carbon at the transition state. The solvent kinetic isotope effects indicate rate-

determining proton transfer to the α -carbon atom; they are similar to those occurring in olefin hydration¹ and aromatic exchange.²

The experimental data thus rule out a mechanism involving 1,4-addition of water to phenylpropionic acid and support the following mechanism.



The large negative ρ shows that C-OH₂ bond formation (2) lags appreciably behind proton transfer. The addition of the two fragments to the triple bond is thus not simultaneous, and the transition state can be described best as a solvated vinylic cation of the following geometry.



Additional evidence (Table II) supporting this interpretation is provided by rate data on hydration of phenylacetylene, phenylacetylene-*d*₁, and methylphenylacetylene.

Table II. Phenylacetylene Hydration in H₂SO₄ at 25°

Compound	10 ⁶ k, sec. ⁻¹ ^a	Acidity range, % H ₂ SO ₄	k _{H₂SO₄}/k_{D₂SO₄} ^b}}
Ie	83.0	32.4-48.4	2.98
If	75.0	31.8-46.5	...
Ig	2.82	48.7-56.7	2.25

^a At 44.0% H₂SO₄, H₀ = -2.78 (solvent 5% ethanol). ^b Compared at that mole fraction SO₄²⁻ at which the hydration rate in H₂SO₄ is 1 × 10⁻⁸ sec.⁻¹.

The enhanced reactivity of phenylacetylene relative to methylphenylacetylene parallels the difference in heats of hydrogenation of 1-propyne and 2-butyne.³ The major factor responsible for this effect would seem to be the difference in rehybridization energy involved in going from a C_{sp}-H bond to a C_{sp²}-H bond in the transition

(1) (a) W. M. Schubert, B. Lamm, and J. R. Keeffe, *J. Am. Chem. Soc.*, **86**, 4727 (1964); (b) D. S. Noyce, H. S. Avarbock, and W. L. Reed, *ibid.*, **84**, 1647 (1962); (c) D. S. Noyce, D. R. Hartter, and F. B. Miles, *ibid.*, **86**, 3583 (1964).

(2) (a) V. Gold, R. W. Lambert, and D. P. N. Satchell, *J. Chem. Soc.*, 2461 (1960); (b) A. J. Kresge and Y. Chiang, *J. Am. Chem. Soc.*, **84**, 3976 (1962); (c) J. Schulze and F. A. Long, *ibid.*, **86**, 331 (1964).

(3) J. B. Conn, G. B. Kistiakowsky, and E. A. Smith, *ibid.*, **61**, 1868 (1939).