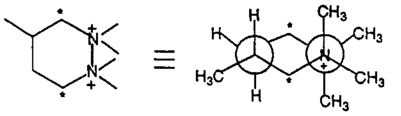
I prefers an anti configuration when bound to several nucleic acids.

Double-^{13}C-labeled spermidine (Scheme I) at pD = 2–7, where it is fully protonated, gave $^3J_{cc} = 5.9$ Hz.[9] We assume, partly on the basis of MACROMODEL calculations,[10] that 5.9 Hz reflects an average value heavily weighted toward the anti conformation. In order to obtain $^3J_{cc}$ for a pure gauche relationship, we synthesized the double-^{13}C-labeled heterocycle II (Scheme II) in which a ring structure enforces a gauche disposition upon the isotopic carbons. Compound II provided $^3J_{cc}$ (gauche) = 4.3 Hz,



II

a value that probably represents an upper limit for gauche interactions in acyclic compounds where there exists only a single "coupling route" between carbons.[11] The minimum 1.6-Hz difference between anti and gauche allowed for easy NMR discrimination between the two states.

In a typical experiment, 0.2 mM 98% isotopically labeled spermidine was mixed with a 25-fold base-pair excess of DNA (0.1 M phosphate buffer in D_2O, pD = 7.0, 0.1 M NaCl, 25 °C).[12] A low concentration of I was required to avoid precipitate formation, while large amounts of DNA were needed to bind all the spermidine and assure that free spermidine contributed nothing to the couplings. Total binding of I to DNA was demonstrated by observing a 0.7-ppm upfield shift of the labeled carbons in the presence of excess DNA. A plot of chemical shift vs [DNA] reached "saturation" (i.e. no further change) at only a 5-fold base-pair excess of calf thymus DNA.[13] NMR spectra were recorded on a 500-MHz instrument (3600-Hz spectral width and 15 000 aquisitions). Use of a 32-phase INADEQUATE pulse sequence[14] suppressed all signals except the two doublets from the labeled spermidine.

Six forms of DNA, given here with their classifications and excess over I, were investigated:[15] calf thymus (random, 53×); poly(dA)·poly(dT) (duplex, 30×); poly(dA)·poly(dT)$_{12-18}$ and poly(dC)·poly(dG)$_{12-18}$ (template primers, 12×); poly(dA-dT)·poly(dA-dT), and poly(dG-dC)·poly(dG-dC) (alternating copolymers, 20×). Calf thymus (5–45 °C) and other DNA's (25 °C) all gave observed coupling constants of 6.0 ± 0.1 Hz. This establishes conclusively that, as in free solution, the 4-carbon segment of spermidine prefers to be predominantly anti when associated with the nucleic acids. Note that our experiments cannot, of course, differentiate between (a) an anti spermidine fixed rigidly to a discrete DNA binding site and (b) a mobile and flexible spermidine whose configuration averaged over many binding sites is heavily weighted toward anti.[16] Although spermidine may dance and cartwheel across the DNA surface, it certainly does *not* do so while contorted in a gauche conformation. In this regard, the behavior differs from the spermine/B-DNA complex where multiple negative charges stabilize the gauche form.

Docking calculations[17] were carried out on spermidine in the major and minor grooves of the oligonucleotide, poly(dG-dC)$_{10}$. It was found that spermidine prefers the minor groove by 6 kcal/mol and that, in agreement with the NMR data, the triamine adopts therein an anti configuration. Our computations have a measure of uncertainty owing, in part, to their neglect of solvent. Double-^{13}C-labeling, on the other hand, pertains to aqueous media where biomolecules normally reside. Only the need to synthesize the dilabeled compounds, admittedly an arduous task, tarnishes an otherwise simple and informative method applicable to a variety of biochemical problems.

Acknowledgment. This work was supported by the National Institutes of Health.

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(8) The tentative conclusion of Bunce and Knong[7] disagrees with an anti conformation evident from our coupling data on mixtures of I and a 55-fold excess of 5′-AMP.

(9) In organic solvents ($CDCl_3$) and aqueous base (pD = 13), $^3J_{CC}$ for the unprotonated triamine falls between 4.0 and 4.4 Hz. Molecular mechanics calculations show that this corresponds to about 31% gauche and 69% anti.

(10) W. Clark Still, Columbia University.

(11) Both $^3J_{cc}$ (anti) = 5.9 Hz and $^3J_{cc}$ (gauche) = 4.3 Hz are about 2.3 Hz larger than those observed and calculated[1,2] for simple hydrocarbons; we presume the difference arises from an inductive effect of the cationic nitrogens.

(12) Spermidine is tricationic under these conditions. See pK_a values in Kimberly, M. M.; Goldstein, J. H. *Anal. Chem.* 1981, *53*, 789.

(13) The DNA/spermidine association constant is reported to be 10^6 M^{-1}. Rubin, R. L. *J. Bacteriol.* 1977, *129*, 916. Assuming this value, it can be calculated that under our conditions the spermidine is >99% bound to DNA.

(14) Bax, A.; Freeman, R.; Kempsell, S. P. *J. Am. Chem. Soc.* 1980, *102*, 4849.

(15) Nucleic acids were purchased from Pharmacia.

(16) Wemmer, D. E.; Srivenugopal, K. S.; Reid, B. R.; Morris, D. R. *J. Mol. Biol.* 1985, *185*, 457. These authors discuss the uncertainty with regard to spermidine/DNA complexes. Relatively sharp spermidine line widths in our case suggest substantial mobility.

(17) The united-atom AMBER force field as implemented in the MACROMODEL program[10] was used in the calculations (rms set to 0.2 kcal mol^{-1} Å^{-1}). Calculations were performed with the aid of an Evans and Sutherland PS 390 workstation interfaced to a Micro-Vax II.

A Tritiated Anion Radical

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Summary: The first tritiated anion radical (that of monotritiated [8]annulene) has been prepared and its EPR spectrum recorded.

Since its original observation, ^{3}H NMR is routinely studied without ^{1}H interference by selective tuning of the probe to the Larmor frequency of ^{3}H.[1-2] However, EPR spectroscopists have not been able to make use of tritiated

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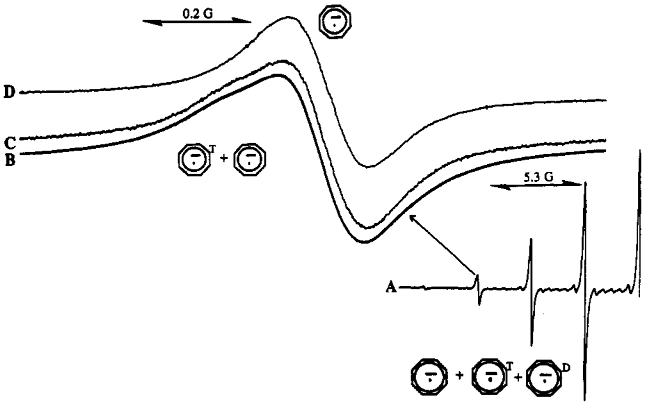
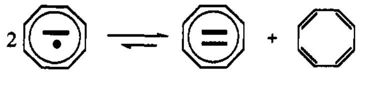


Figure 1. (A) The low-field half of an EPR spectrum (sweep width = 40 G, recorded at 220 K) of an HMPA/THF solution of 0.1 mmol of COT partially reduced with 0.04 mmol of potassium 15 h after the addition of 1 Ci of T_2O. The EPR spectrum clearly exhibits the presence of three different species: $C_8H_8^{\bullet-}$ (A_H = 3.242 G); $C_8H_7D^{\bullet-}$ (A_H = 3.242 G, A_D = 0.497 G); and $C_8H_7T^{\bullet-}$ (A_H = 3.242 G, A_T = 3.470 G) in a ratio of $[C_8H_7T^{\bullet-}]/[C_8H_7D^{\bullet-}]/[C_8H_8^{\bullet-}]$ = 0.167/0.042/1.0. The small difference between the proton and tritium coupling constants reduces the ostensible effects of the tritium to an anisotropy in the hyperfine lines and renders it difficult to obtain accurate coupling constants at this scan range. (B) The computer simulation generated using a ratio of A_T/A_H = 1.070. (C) An EPR spectrum (sweep width = 1.0 G, recorded at 220 K) and simulation showing only the second hyperfine component. The effects of $C_8H_7D^{\bullet-}$ are not seen at this scan range and position. The tritium hyperfine component is clearly seen as a partially resolved line on the low field side of the proton line. (D) An EPR spectrum of the same solution prior to the addition of the T_2O. Computer simulation shows this line to be Lorentzian in shape. Since the gyromagnetic ratio of T is just slightly larger than that of H, the differences in the peak positions of $C_8H_8^{\bullet-}$ and $C_8H_7T^{\bullet-}$ grows larger as we approach the out edges of the spectrum. Thus, the best resolution of the two species is seen on the outermost lines. However, due to signal to noise consideration, we choose to exhibit the second outermost lines.

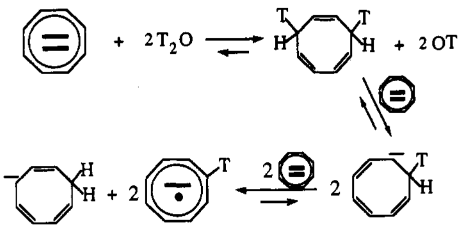
anion radicals despite great potential for profitable applications (i.e. the study of vibronic interactions,[3] Jahn–Teller and pseudo-Jahn–Teller distortions,[4] electronic effect of isotopic substitution upon molecular orbitals,[5] etc.). Similarity of gyromagnetic ratios of ^1H and ^3H ($\gamma_{^3H}/\gamma_{^1H}$ = 1.06664), coupled with the necessarily very small amounts of tritium that can be substituted into a sample, and the inability of the EPR spectroscopists to "tune away" from the proton effects have precluded EPR studies of tritiated anion radicals.

Potassium reduction of a solution of [8]annulene in a 2/1 HMPA/THF mixture results in the formation of $C_8H_8^{\bullet-}$ and $C_8H_8^{2-}$ whose concentrations are controlled by reaction 1.[6] About 15 h after the addition of 17 μmol[7] of D_2O to 1.0 mL of a solution 0.05 M in both C_8H_8 and $C_8H_8^{2-}$, the EPR signal for $C_8H_8^{\bullet-}$ began to be replaced by that of $C_8H_7D^{\bullet-}$, and the $[C_8H_7D^{\bullet-}]/[C_8H_8^{\bullet-}]$ ratio increased for several days.



(1)

Scheme I



We were motivated to investigate an analogous reaction with T_2O in an effort to test for an isotopic spin density distortion and to carry out an EPR measurement involving a tritium-substituted anion radical in a manner that could become of general use. The high specific activity of tritiated materials (58000 Ci/mol) causes self radiolytic decomposition of pure condensed phase tritiated compounds.[8] Thus, in our case, it was convenient to store tritium as elemental gas and convert it to T_2O just prior to substitution into the anion radical. This was done via transport of 1-Ci or 17-μmol portions of T_2 gas along with 1 μmol of D_2 (to facilitate monitoring of the progress of the substitution reaction) through a vacuum system equipped with a Cu/CuO furnace ($T_2 + CuO \rightarrow T_2O + Cu$) into previously prepared EPR samples of $C_8H_8^{\bullet-}$. Conversion of tritium gas to ca. 0.4 μL of liquid T_2O was monitored via pressure decrease, and the T_2O was introduced into the $C_8H_8^{\bullet-}$ solutions immediately after its formation. The sample tube was then sealed from the line and submitted to EPR analysis. Fifteen hours later, EPR analysis showed the presence of all three isotopically different anion radicals ($C_8H_8^{\bullet-}$, $C_8H_7D^{\bullet-}$, and $C_8H_7T^{\bullet-}$), Figure 1. After the experiment, the tritium and tube could be safely disposed of as a sealed unit.

There are only two stable annulene anion radicals capable of maintaining the shape of a regular polygon on the EPR time scale. In both systems the odd electron is equally distributed between degenerate MO's, which are nonbonding in [8]annulene and antibonding in [6]-annulene. Note that a single deuterium substituent on $C_6H_6^{\bullet-}$ is sufficient to result in a relative decrease in the spin density in the symmetric wave function (Ψ_s), which is increased in energy ($A_{H(meta)}/A_D$ = 7.28).[5,9] In contrast, the analogous isotope effect in $C_8H_7D^{\bullet-}$ is too small for observation.[4] A_H/A_D is within experimental uncertainty of $\gamma_{^1H}/\gamma_{^2H}$ = 6.51. This may simply be a manifestation of the larger ring system (relative to that of benzene) coupled with the large EPR line widths for $C_8H_7D^{\bullet-}$ "diluting" the effect to the point of obscurity. However, Carrington and co-workers[4] offered an explanation based upon differences in the way the Jahn–Teller effect operates in the $C_6H_5D^{\bullet-}$ systems. If their explanation is correct, orbital degeneracy should not be lifted even upon substitution with a heavier isotope of hydrogen. Our results (A_T/A_H = 1.070) support this latter explanation.

This procedure represents a simple method for incorporation of tritium into anion radical systems, which can then be used in a wide variety of studies for which the EPR active and radioactive label might be useful. After the fast Birch reduction of the [8]annulene dianion,[10] the ditriti-

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[7] D_2 gas was released into the vacuum line, for which the volume was known. The amount of D_2 was determined form its pressure, temperature, and volume. It was then completely converted to D_2O with a Cu/CuO furnace. The D_2O was then distilled directly into the anion radical solution.

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ated cyclooctatriene acts as a slow donor of tritium resulting in the formation of $C_8H_7T^{\bullet-}$, Scheme I. This mechanism is supported by the fact that addition of independently synthesized dideuteriocyclooctatriene to a $C_8H_8^{\bullet-}$ solution results in formation of $C_8H_7D^{\bullet-}$.

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Synthesis and Characterization of the First Water-Soluble Closely Interspaced Cofacial Porphyrin Dimer

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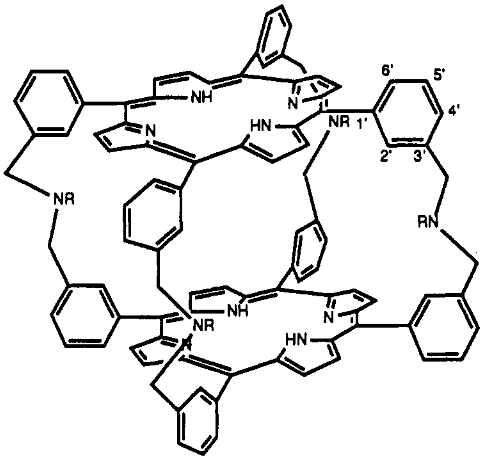
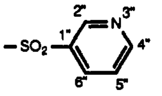
Received February 6, 1991

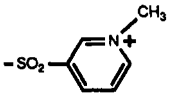
Summary: The first closely spaced quadruply bridged water-soluble cofacial dimer (**5**) was successfully synthesized by the reaction of porphyrin **2** with sulfonamide **3** followed by N-methylation with methyl iodide. The conformations of **5** and its precursor **4** are largely dependent on the solvent, and the solvent effect is inhibited by protonation or metalation of the porphyrin cores.

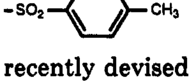
Metal-centered cofacial dimeric prophyrins have been recognized as intriguing compounds since they were first prepared and reported.[1] They have been offered as putative models for the study of cytochrome c_3,[2] cytochrome-c-oxidase,[3] and other[4,5] metallo enzymes. Binding affinities of dioxygen and carbon monoxide[6] by metal-centered cofacial dimeric prophyrins have been studied, and it has been shown that they may serve as effective catalysts for the four-electron reduction of dioxygen to water.[3] These agents have been employed in the study of antiferromagnetic coupling as seen with superoxide dismutase and cytochrome-c-oxidase.[7] To this date, studies have been restricted to organic solvents. Cofacial dimeric tetraphenylporphyrins may be singly, doubly, triply, or quadruply bridged. The number of bridges determine the rigidity of the structure, and the length of the bridges determine the closeness of interspacing of the porphyrin planes.

With the exception of the single compound prepared by Kagan in 1977,[8] quadruply bridged *closely interspaced* dimeric tetraphenylporphyrins were unknown. We report now the synthesis of and preliminary observations on the first *closely spaced* water-soluble cofacial dimeric tetraphenylporphyrin **5** and its biszinc(II) complex (Zn_2–**5**).



4 R = 

, **5** R = 

6 R = 

The synthesis of **5** is based on recently devised technology for the synthesis of quadruply two- and three-atom, aza-bridged cofacial tetraphenylporphyrins.[9] Porphyrin

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