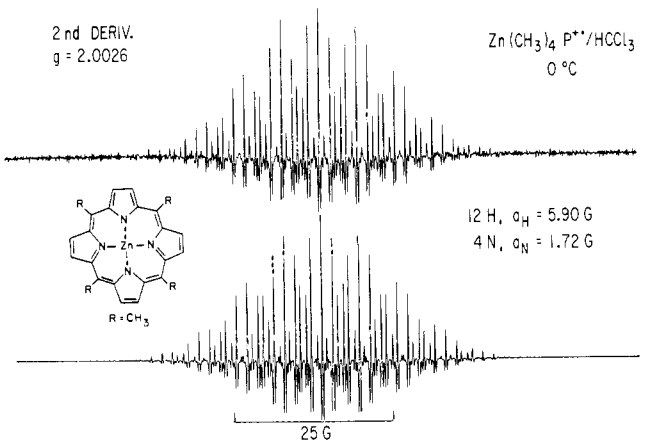

Figure 1. Second derivative esr spectrum of $Zn(CH_3)_4P\cdot^+$ in $CHCl_3$ at 0° (top) and a computer simulation assuming four nitrogens and four methyl groups (bottom).

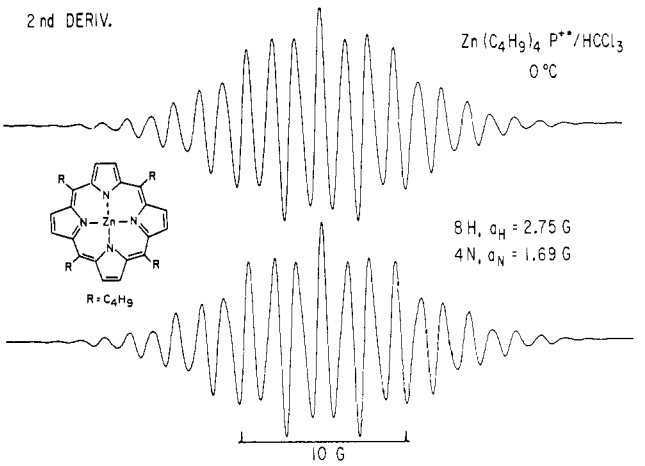

Figure 2. Second derivative esr spectrum of $Zn(C_4H_9)_4P\cdot^+$ in $CHCl_3$ at 0° (top) and a computer simulation assuming four nitrogens and four methylene groups (bottom).

is therefore assigned to restricted rotation of the alkyl groups larger than methyl caused by the adjacent pyrroles. This model is also supported by X-ray data on the structure of $H_2(C_3H_7)_4P$ which indicate short distances between the middle group of the alkyl substituent and the adjacent β-carbon atom of the pyrrole ring.[17]

For a freely rotating methyl group on a cation radical,[15,18] eq 1 is simply $a_{CH_3} \cong 39\rho_C$. In $Zn(CH_3)_4P\cdot^+$, a_{CH_3} = 5.90 G, and the spin density at the meso positions is therefore 0.15. The MO values calculated[4] for a $^2A_{2u}$ state are 0.158 before and 0.193 after configuration interaction. The narrow line widths observed in $Zn(CH_3)_4P\cdot^+$ and the close agreement between the observed and simulated esr spectra exclude any significant unresolved interactions with nuclei other than the nitrogens and the methyl groups. Only negligible unpaired spin densities can therefore exist on the β carbons of the pyrrole rings, in accord with observations made on $^2A_{2u}$ *meso*-tetraphenylporphyrins.[7] The predicted spin densities[4] are 0.019 before and 0.013 after configuration interaction.

Delocalization of spin onto the metal is observed in the radical of cobaltic tetrapropylporphyrin $[Co^{III}(C_3H_7)_4P]\cdot^{2+}$ with $a_{^{59}Co}$ = 6 G in CH_2Cl_2, a value comparable to that observed in the radical of cobaltic tetraphenylporphyrin[3,4] (nuclear spin, I, of ^{59}Co = $^7/_2$). Bromine oxidations of $Zn(C_3H_7)_4P$, $Zn(C_4H_9)_4P$, and $Zn(C_8H_{17})_4P$ yield the bromide-complexed radicals $Zn(alkyl)_4P\cdot^+Br^-$ with bromine splittings (I = $^3/_2$) $a_{^{79}Br}$ = 8.8 G and g values of 2.0045. The tetraalkylporphyrin radicals described here clearly fit the theoretical and experimental criteria for $^2A_{2u}$ states, high spin density at the meso positions coupled with nitrogen, metal, and anion interactions. In addition, negligible spin density is found at the β-pyrrole positions.

The two radical ground states discussed above are of special interest because the oxidized forms (compounds I) of the enzymes catalase (Cat I) and horseradish peroxidase (Hrp I) exhibit optical spectra which suggest that the enzymes function *via* these radicals.[5-7] On the basis of its optical absorption spectrum, Hrp I is believed to contain the $^2A_{2u}$ radical while the electronic absorption of Cat I resembles that of a $^2A_{1u}$ porphyrin radical,[5-7] with lower spin density at the meso carbons (0.05) and most of the unpaired electron delocalized onto the α carbons adjacent to the pyrrole nitrogens.[4,7,9] The different ground states of Hrp I and Cat I may reflect the different apoproteins[19] of the two enzymes since changes in anionic ligands[5-7,10] can switch the ground state of porphyrin radicals between $^2A_{1u}$ and $^2A_{2u}$. In other words, the ground states of Hrp I and Cat I reflect their specific protein environments, but the resulting differences in spin delocalization of the ground states actually control the reactions with substrates.

Furthermore, since the sequestration of the porphyrins of cytochromes b_5 and c within hydrophobic crevices[20,21] appears to preclude direct electron transfer between substrates and heme iron, whereas the peripheries of the porphyrins remain exposed, it is attractive to speculate that electron transfer to and from the iron[5,6,22] occurs *via* porphyrin radical transients with the π-electron configurations described here.

(17) P. W. Codding and A. Tulinsky, *J. Amer. Chem. Soc.*, **94**, 4151 (1972).
(18) R. M. Dessau, S. Shih, and E. I. Heiba, *J. Amer. Chem. Soc.*, **92**, 412 (1970); I. H. Elson and J. K. Kochi, *ibid.*, **95**, 5061 (1973); R. Hulme and C. R. Symons, *J. Chem. Soc.*, 1120 (1965).
(19) A. Deisseroth and A. L. Dounce, *Physiol. Rev.*, **50**, 319 (1970).
(20) F. S. Mathews, P. Argos, and M. Levine, *Cold Spring Harbor Symp. Quant. Biol.*, **36**, 387 (1971).
(21) T. Takano, R. Swanson, O. B. Kallai, and R. E. Dickerson, *Cold Spring Harbor Symp. Quant. Biol.*, **36**, 397 (1971).
(22) C. E. Castro, *J. Theor. Biol.*, **33**, 375 (1971).
(23) The work at Brookhaven National Laboratory was performed under the auspices of the U. S. Atomic Energy Commission.

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Aza Analogs of Nucleic Acid Constituents. III. The Molecular Structure of 6-Azacytidine

Sir:

6-Azacytidine is a powerful carcinostatic agent whose mode of action is postulated to be similar to

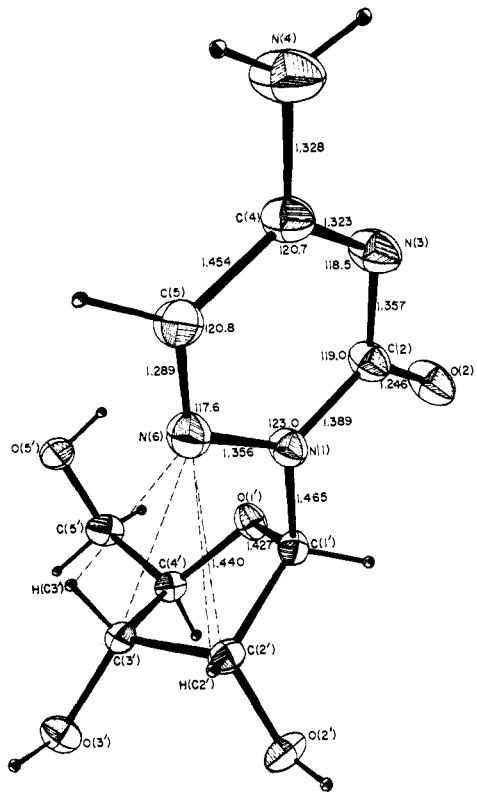

Figure 1. View of the 6-azacytidine molecule. The thermal ellipsoids of the hydrogen atoms have been reduced artificially for clarity. Dashed lines indicate short intramolecular contacts between N(6) and ribose atoms C(2') 2.76, H(C2') 2.46, C(3') 3.30, and H(C3') 2.69 Å.

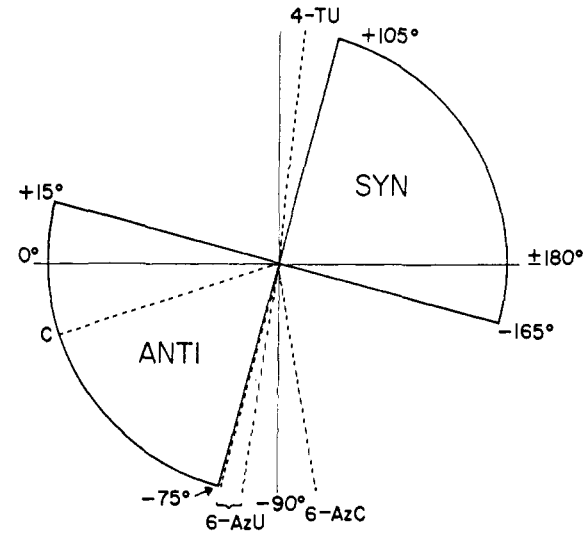

Figure 2. Schematic drawing of the syn and anti ranges of the glycosidic torsional angle ϕ_{CN} for cytidine (C) (S. Furberg, C. S. Peterson, and Chr. Romming, *Acta Crystallogr.*, **18**, 313 (1965)), 4-thiouridine (4-TU), 6-azauridine (6-AzU), and 6-azacytidine (6-AzC).

that of 6-azauridine.[1] After phosphorylation these nucleosides act as inhibitors of orotidylic acid decarboxylase, thereby reducing the rate of pyrimidine synthesis since the decarboxylation of orotidylic acid is a principal pathway for pyrimidine production. Moreover, it has been shown[2] that the replacement of cytidine or uridine by 6-azacytidine or 6-azauridine in a trinucleoside diphosphate, *e.g.*, in GpUpU or GpUpC, both of which code for valine, results in a codon triplet which is nonfunctional in both coding and ribosome binding.

6-Azapyrimidine nucleosides are also of great interest from a stereochemical viewpoint, since the replacement of CH by N adjacent to the glycosidic bond removes a significant barrier to rotation around the glycosidic bond C(1')–N(1).[3] We have, therefore, undertaken a systematic structural investigation of the 6-azapyrimidines[4] and their nucleosides; we report here the structure of 6-azacytidine, and compare it with the known structure of 6-azauridine.[5,6] Details of the structural analysis will be published elsewhere.

Colorless crystals of 6-azacytidine were grown from aqueous solution. The material crystallizes in the orthorhombic space group $P2_12_12_1$ with $a = 7.623$ (6), $b = 6.993$ (7), and $c = 19.622$ (14) Å. Data (1557) whose intensities were greater than 3σ were obtained using a Picker automatic diffractometer equipped with Mo Kα radiation and a graphite monochromator. The structure was solved by direct methods[7] using the multiple solution program MULTAN,[8] and the positional and thermal parameters of all atoms (including hydrogen atoms) were refined by full-matrix least-squares procedures to an R factor of 0.031.[9]

A view of the molecule, including the usual numbering scheme and the principal bond distances and angles, is shown in Figure 1; the distances and angles associated with the ribose moiety are listed in Table I.

A parameter of considerable interest in nucleoside conformation studies is the torsional angle between the pyrimidine plane and the sugar. Sundaralingam and Jensen[10a] define the angle ϕ_{CN}, the dihedral angle O(1')–C(1')–N(1)–C(6), as zero if O(1')–C(1') and N(1)–C(6) are coplanar, with ϕ_{CN} measured positive if the N(1)–C(6) bond is rotated counterclockwise relative to O(1')–C(1') when viewed along C(1')–N(1). From a study of molecular models, Donohue and Trueblood[10b] concluded that the probable ranges for ϕ_{CN} are -75 to $+15°$ (anti) and 105 to 195° (or $-165°$) (syn). The value of ϕ_{CN} observed for 6-azacytidine is $-99.1°$. Hence, as is depicted in Figure 2, the conformation of 6-azacytidine lies 24.1° outside of the anti and 65.9° outside of the syn range. As is also shown in Figure 2, 4-thiouridine[11,12] and both crystallographically independent molecules of 6-azauridine[6] also lie outside of the conventional ranges, as does

(1) J. Skoda, *Progr. Nucl. Acid Res. Mol. Biol.*, **2**, 197 (1963).
(2) J. Skoda in "Biochemical Aspects of Antimetabolites and Drug Hydroxylation," Vol. 16, D. Shugar, Ed., Academic Press, New York, N. Y., 1969, p 23.
(3) A. E. V. Haschemeyer and A. Rich, *J. Mol. Biol.*, **27**, 369 (1967); B. Pullman and H. Berthod, *Fed. Eur. Biochem. Soc. Lett.*, **20**, 341 (1972).
(4) P. Singh and D. J. Hodgson, *J. Chem. Soc., Chem. Commun.*, 439 (1973).
(5) C. H. Schwalbe, W. Saenger, and J. Gassmann, *Biochem. Biophys. Res. Commun.*, **44**, 57 (1971).
(6) C. H. Schwalbe and W. Saenger, *J. Mol. Biol.*, **75**, 129 (1973).
(7) J. Karle and I. L. Karle, *Acta Crystallogr.*, **21**, 849 (1966).
(8) P. Main, M. M. Woolfson, and G. Germain, University of York, York, United Kingdom.
(9) W. R. Busing and H. Levy, Oak Ridge National Laboratory Report. The program used was a local modification of J. A. Ibers' version of this program.
(10) (a) M. Sundaralingam and L. H. Jensen, *J. Mol. Biol.*, **13**, 914 (1965); (b) J. Donohue and K. N. Trueblood, *J. Mol. Biol.*, **2**, 363 (1960).
(11) Calculated from the positional parameters given in ref 12.
(12) W. Saenger and K. H. Scheit, *J. Mol. Biol.*, **50**, 153 (1970).

Table I. Bond Lengths and Angles in the Ribose Moiety

Bond	Length, Å[a]	Angle	Deg[a]
C(1′)–O(1′)	1.427	C(1′)–N(1′–C(2)	119.3
C(1′)–C(2′)	1.537	C(1′)–N(1)–N(6)	117.6
C(2′)–C(3′)	1.530	N(1)–C(1′)–O(1′)	109.9
C(2′)–O(2′)	1.421	N(1)–C(1′)–C(2′)	113.5
C(3′) O(3′)	1.415	O(1′)–C(1′)–C(2′)	107.3
C(3′)–C(4′)	1.522	C(1′)–C(2′)–C(3′)	101.2
C(4′)–O(1′)	1.440	C(1′)–C(2′)–O(2′)	109.1
C(4′)–C(5′)	1.511	C(3′)–C(2′)–O(2′)	109.0
C(5′)–O(5′)	1.428	C(2′)–C(3′)–C(4′)	102.4
		C(2′)–C(3′)–O(3′)	115.6
		C(4′)–C(3′)–O(3′)	110.3
		C(3′)–C(4′)–C(5′)	115.0
		C(3′)–C(4′)–O(1′)	104.5
		C(5′)–C(4′)–O(1′)	108.5
		C(4′)–O(1′)–C(1′)	110.0
		C(4′)–C(5′)–O(5′)	112.6

[a] The esd for the bond lengths is approximately 0.003 Å, and that for the angles is 0.2°.

formycin.[13] It is not altogether surprising that the 6-azanucleosides lie outside of the expected ranges since the substitution of N(6) for C(6)–H(6) removes the rotational barrier caused by interactions between H(6) and both C(2′) and H(C2′); similarly, the substitution of N(8) for C(8)–H(8) in the pseudopurine nucleoside formycin removes the H(8)–C(2′) and H(8)–H(C2′) barriers. It has been suggested,[6,13] however, that the replacement of the electropositive[14] C(6) by the electronegative[6,13] N(6) (or N(8) in azapurines) also gives rise to electrostatic repulsions between N(6) and both O(1′) and O(5′) and that these interactions may also have profound effects on both the glycosidic torsional angle and the conformation around the C(5′)–C(4′) bond (*vide infra*). It is evident, however, that in 6-azacytidine the approach of C(2′) and its hydrogen atom to N(6) is much closer than that in 6-azauridine. Several of the shorter separations between N(6) and the sugar moiety are indicated in Figure 1, and it is apparent that all of them are considerably shorter than the sums of the appropriate van der Waals radii.[15]

The ribose is in the C(3′)-endo envelope conformation, ^3E.[16] The conformation around the exocyclic bond C(5′)–C(4′) is the commonly occurring gauche–gauche,[17] in which O(5′) lies above the sugar ring; this is in contrast to the gauche–trans conformation found in 6-azauridine[6] and in formycin.[13] This may explain why 6-azacytidine 5′-diphosphate acts as a substrate[18] for polynucleotide phosphorylase whereas 6-azauridine 5′-diphosphate acts as an inhibitor[19] of this enzyme; it should be noted, however, that the conformational energetics of nucleotides are not always similar to those of the corresponding nucleosides.[20] We infer, therefore, that the suggestion[13] that base modification leads to a change in the favored conformation of the sugar–phosphate backbone is not general. The interplanar angle between the pyrimidine plane and the best least-squares plane through the ribose atoms O(1′), C(1′), C(2′), and C(4′) (*vide supra*) is 113.6°.

All hydrogen atoms attached to an oxygen or a nitrogen atom in the 6-azacytidine crystal take part in hydrogen bonding. There are no hydrogen bonds to any of the ring atoms N(3), N(6), or O(1′), all of which would have to form acceptor hydrogen bonds. In 6-azauridine, O(1′) is involved in an intermolecular hydrogen bond with N(3);[6] such a hydrogen bond is not possible in 6-azacytidine since neither atom is protonated. A further distinction between these two nucleosides is that the regular alternation between angles smaller than 120° and greater than 120° found in the base in 6-azauridine is not observed in 6-azacytidine (see Figure 1). It has been pointed out[6] that this regular alternation and orientation of the C(5′)–O(5′) bond in 6-azauridine are similar to those in orotidine, and hence 6-azauridine is conformationally acceptable to orotidylic acid decarboxylase.[21] Evidently, unless 6-azacytidine is converted to 6-azauridine (which is known to account for only part of its activity[1]) the inhibitory action of 6-azacytidine must follow some other pathway, which is probably the result of the established "high-anti"[13] conformation about the glycosidic bond.[22]

(13) P. Pruisner, T. Brennan, and M. Sundaralingam, *Biochemistry*, **12**, 1196 (1973).
(14) F. Jordan and B. Pullman, *Theor. Chim. Acta*, **9**, 242 (1968).
(15) L. Pauling, "Nature of the Chemical Bond," 3rd ed, Cornell University Press, Ithaca, N. Y., 1960.
(16) M. Sundaralingam, *J. Amer. Chem. Soc.*, **93**, 6644 (1971).
(17) E. Shefter and K. N. Trueblood, *Acta Crystallogr.*, **18**, 1065 (1965); M. Sundaralingam, *J. Amer. Chem. Soc.*, **87**, 599 (1965).
(18) J. Skoda and F. Sorm, *Biochim. Biophys. Acta*, **91**, 352 (1964).
(19) J. Skoda, J. Kara, Z. Sormova, and F. Sorm, *Biochim. Biophys. Acta*, **33**, 579 (1959).
(20) M. Sundaralingam, "Conformations of Biological Molecules and Polymers," The Jerusalem Symposia on Quantum Chemistry and Biochemistry, Israel Academy of Sciences and Humanities, Jerusalem, **5**, 417 (1973).
(21) W. Saenger and D. Suck, *Nature (London)*, **242**, 610 (1973).
(22) This research was supported by the National Institutes of Health through Grant No. CA 15171-01 from the National Cancer Institute.

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Experimental and Theoretical Studies of Vicinal ^{13}C–^{13}C Coupling Constants

Sir:

The physical situation presented by nuclear spin–spin coupling between two carbon atoms is substantially more complicated than the analogous H–H coupling constants. This complexity arises because each carbon atom has four valence electrons instead of one for a hydrogen atom. For this reason the number of possibilities for substitution and hybridization effects at each of the coupled carbon atoms becomes enormous, and it may reasonably be assumed that conformational and substituent effects on vicinal ^{13}C–^{13}C coupling constants often will not conform to those features found in vicinal H–H coupling. The experimental and theoretical results for vicinal ^{13}C–^{13}C coupling presented here substantiate this.

Experimental values of vicinal ^{13}C–^{13}C coupling constants for a series of aliphatic and alicyclic alcohols having ^{13}C enriched methyl groups are entered in Table I. These compounds were prepared *via* Grignard reactions between the appropriate ketone and 67% ^{13}C enriched methylmagnesium iodide. At this level of enrichment the coupled peaks appear on either side of the peak for the unlabeled molecules and with about the same intensity. This puts a lower