

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 electropositive¹⁴ C(6) by the electro-negative^{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-azauridine⁶ and in formycin.¹³ This may explain why 6-azacytidine 5'-diphosphate acts as a substrate¹⁸ for polynucleotide phosphorylase whereas 6-azauridine 5'-diphosphate acts as an inhibitor¹⁹ 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, there-

fore, that the suggestion¹³ 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 out⁶ 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 activity¹) 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

(21) W. Saenger and D. Suck, *Nature (London)*, **242**, 610 (1973).

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Experimental and Theoretical Studies of Vicinal ¹³C-¹³C 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 ¹³C-¹³C coupling constants often will not conform to those features found in vicinal H-H coupling. The experimental and theoretical results for vicinal ¹³C-¹³C coupling presented here substantiate this.

Experimental values of vicinal ¹³C-¹³C coupling constants for a series of aliphatic and alicyclic alcohols having ¹³C enriched methyl groups are entered in Table I. These compounds were prepared *via* Grignard reactions between the appropriate ketone and 67% ¹³C 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

(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).

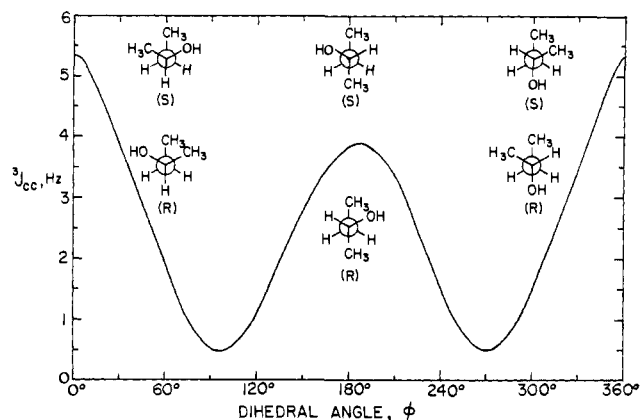


Figure 1. A plot of the calculated vicinal ^{13}C - ^{13}C coupling constant in 2-butanol as a function of the dihedral angle ϕ . The curve was based on calculations at 15° intervals of the dihedral angles. The (R) and (S) optical isomers are depicted at 60, 180, and 300° .

Table I. Experimental ^{13}C - ^{13}C Coupling Constants in a Series of Alcohols Having ^{13}C -Enriched Methyl Groups and Calculated Values for the Coupling in 2-Butanol for the Estimated Dihedral Angles

	Carbon no., C_i	Dihedral angle ϕ , deg	$^3J(^*\text{CH}_3-\text{C}_i)$	
			Exptl Hz	Calcd Hz
1.	4	a	1.9	2.4 ^a
2.	3	$\sim 180^b$	3.2	3.8
3.	3	$< 300^b$	c	1.5
4.	4	120	0.9	1.4
	6	170	1.8	3.6
	7	275	< 0.35	0.5
5.	4	~ 125	0.8	1.4
	6	d	0.9	d
	7	~ 270	< 0.4	0.5
	8	~ 0	5.4	5.3
	9	~ 90	< 0.4	0.5
	10	~ 40	2.6	3.1

^a The calculated number was obtained as the average of the 60, 180, and 300° values for 2-butanol from Table II. This assumes that the three rotamer conformations would have equal statistical weights. ^b Estimates for these dihedral angles were based on the assumption that the *t*-butyl group effectively "locks" the ring in the conformation depicted. For item 3 introduction of an axial methyl may distort the dihedral angle to a value less than 300° . ^c This splitting could not be resolved because of interference from other spectral lines. ^d This angle is difficult to estimate because of strong interactions between the labeled methyl group and the C8 methyl group. As a consequence, it would certainly be less than 170° .

limit on the smallest coupling constant which can be readily measured. The vicinal coupling constant for item 1 in Table I was based on spectral widths of 300–1200 Hz on a Bruker Scientific WH-90 Fourier trans-

Table II. Calculated Vicinal ^{13}C - ^{13}C Coupling Constants for Butane, 2-Butanol, and Butanoic Acid at 30° Intervals of the Dihedral Angle ϕ

Dihedral angle ϕ , deg	$J(^{13}\text{C}-^{13}\text{C})$, Hz		
	Butane	2-Butanol ^a	Butanoic acid ^b
0	5.79	5.34	1.76
30	3.96	3.79	0.90
60	1.94	1.97	c
90	0.56	0.50	0.13
120	1.45	1.10	c
150	3.34	2.77	4.24
180	4.27	3.82	5.87
210	3.34	3.31	4.24
240	1.45	1.57	c
270	0.56	0.51	0.13
300	1.94	1.56	c
330	3.96	3.40	0.90

^a The orientation of the OH is trans to the C_2 -H bond. See Figure 1 for the sense of measurement of ϕ . ^b The carboxyl was oriented in such a way that the carbonyl eclipses the C_3 carbon atom. ^c Calculated values for these conformations were considered unreliable because of the very high energies associated with steric interactions between the C_4 methyl group and the carboxyl.

form nmr spectrometer operating at a frequency of 22.64 MHz. Coupling constant data for items 2–5 in the table were obtained in the CW mode on a Varian Associates XL-100 nmr spectrometer operating at a frequency of 24.14 MHz. Errors in the reported coupling constants are estimated to be less than 0.2 Hz. Also included in Table I are estimates of the dihedral angles from molecular models. Theoretical results are given in the last column of Table I.

The theoretical results for the model compounds butane, 2-butanol, and butanoic acid, which are listed in Table II at 30° intervals of the dihedral angle ϕ , were obtained in the finite perturbation theory¹ formulation for nuclear spin-spin coupling in the approximation of intermediate neglect of differential overlap.

The spread in values of more than 5 Hz in the coupling constants in Tables I and II indicates that the vicinal coupling constants have potential in structural studies provided that the other relevant factors can be identified. For example, there are substantial differences between the results for 2-butanol and butanoic acid, especially for small values of the dihedral angles in Table II. Although the predicted minima near 90 and 270° are in conformity with the usual Karplus-type dependency² for vicinal H-H coupling, cis/trans ratios greater than one for butane and 2-butanol and much less than one for butanoic acid, in Table II, were unexpected. The experimental results in Table II suggest that the predicted behavior is correct. For dihedral angles near 90 and 270° (items 4 and 5 in Table I) vicinal ^{13}C - ^{13}C coupling constants could not be detected. Furthermore, coupling in the cis orientation (a coupling constant of 5.4 Hz to the C8 carbon atom for item 5 in Table I) is substantially larger than that observed for the trans arrangement (3.2 Hz for item 2 in Table I). These values are in reasonable agreement with the calculated values of 5.3 and 3.8 Hz, respectively, for 2-butanol.

(1) J. A. Pople, J. W. McIver, Jr., and N. S. Ostlund, *J. Chem. Phys.*, **49**, 2960, 2965 (1968).

(2) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

In a recent study³ of vicinal ¹³C-¹³C coupling in aliphatic and alicyclic ¹³C-labeled acids, the experimental magnitudes for dihedral angles near 0° ranged from 1.9 to 2.4 Hz and for angles near 180° the values ranged from 3.6 to 5.2 Hz, in reasonable correspondence with calculated results for butanoic acid in Table II. The shifts in the maxima from the usual 0 and 180° values³ may be attributable to the dependence of this type of coupling on two dihedral angles.

The large differences in the ¹³C-¹³C coupling constants for the *cis* conformations of 2-butanol and butanoic acid follow the experimental trends but have no counterpart in vicinal H-H coupling. For the *cis* conformation of butanoic acid the oxygen atom is in close proximity (1.70 Å) to the hydrogen atoms on the C₄ carbon atom. It is possible that the oxygen and hydrogen atoms constitute the electronic path for transfer of spin information between the two carbon atoms.

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(3) J. L. Marshall and D. E. Miiller, *J. Amer. Chem. Soc.*, **95**, 8305 (1973).

(4) Queen Elizabeth II Research Fellow, 1973-1974.

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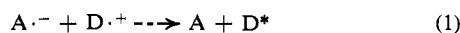
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A Comparison of Optical Excitation and Chemiluminescence. Variation of Fluorescence Quantum Efficiency with Selective Population of Vibrational Levels

Sir:

This paper reports the first example of systematically increasing the fluorescence efficiency of a compound by selectively populating various vibrational levels of the excited state, based on the specific difference between optical excitation and chemiluminescence.^{1,2} In chemiluminescence, energy from a chemical reaction is converted into light; the type of chemiluminescence involved in this report is due to homogeneous charge transfer in a cation-anion annihilation reaction, which can be represented as



followed by



When the ions A^{·-} and D^{·+} are prepared *in situ* by electrochemical means, the term "electrogenerated

chemiluminescence," often abbreviated as "ECL," applies.³⁻⁹ The conversion of one form of energy into another is among the most fundamental problems in science, and recent advances in ECL have been related to improving that conversion efficiency (Φ_{ECL}). Advantageous experimental conditions were found to be the use of low polarity solvents including mixed "heteropolar" solvents such as acetonitrile-benzene⁷⁻⁹ and lowering the supporting electrolyte concentration.⁹ These improvements⁷⁻⁹ did not directly involve the fluorescence efficiency, Φ_f , and the relevant literature^{3,4} including the recent paper by Schwartz, Blakeley, and Robinson¹⁰ assumed that the optical quantum yield has a limit set by the fluorescence quantum efficiency measured from optical excitation ($\Phi_{f, optic}$); *i.e.*, even if eq 1 were 100% efficient, Φ_{ECL} , representing the combined process of eq 1 and eq 2, was regarded as having the upper limit $\Phi_{f, optic}$.

The results presented here demonstrate that such need not be the case and indeed that an upper limit on Φ_{ECL} set by $\Phi_{f, optic}$ does not exist in the general sense, the reason being that fluorescence efficiency may strongly depend for some compounds on the mode of excitation. Specifically, the fluorescence quantum efficiency of a luminescor may be much lower when optical excitation is used than when chemical energy is converted to light.

To readily demonstrate such a difference in fluorescence quantum efficiency, a compound having a low $\Phi_{f, optic}$ is best suited. In addition, the chemiluminescence route to excited-state production should clearly avoid some established causes of the low $\Phi_{f, optic}$. A suitable compound is thianthrene (TH) having $\Phi_{f, optic} = 3.6\%$, $\Phi_{f, optic} = 94\%$.¹¹ In addition, for thianthrene the optical excitation maximum (4.77 eV) differs significantly from the emission maximum (2.84 eV), and this leads to a situation where intersystem crossing from the vibrationally "hot" Franck-Condon state (R*†) to the triplet becomes a prominent route in the time evolution of TH*.

The chemical energy available from eq 1 is found from the standard oxidation and reduction potential of D and A, respectively, according to

$$\Delta H_r^\circ = E^\circ_{(A/A^{\cdot-})} - E^\circ_{(D/D^{\cdot+})} + 0.10 \text{ eV} \quad (3)$$

where the last term is the currently used estimate of $T\Delta S^\circ$, needed when direct measurement of $[\partial\Delta G^\circ/\partial T]_p$ is lacking for the system. The reduction potentials of the various A species are given in Table I, together with the calculated ΔH_r° values. We note that the lesser $|\Delta H_r^\circ|$ values are still sufficient to populate the ¹TH* state (2.84 eV) but progressively lower

(3) D. M. Hercules, *Accounts Chem. Res.*, **2**, 301 (1969).

(4) R. Bezman and L. R. Faulkner, *J. Amer. Chem. Soc.*, **94**, 6331 (1972).

(5) C. P. Keszthelyi, H. Tachikawa, and A. J. Bard, *J. Amer. Chem. Soc.*, **94**, 1522 (1972).

(6) N. E. Tokel, C. P. Keszthelyi, and A. J. Bard, *J. Amer. Chem. Soc.*, **94**, 4872 (1972).

(7) C. P. Keszthelyi and A. J. Bard, *J. Electrochem. Soc.*, **120**, 241 (1973).

(8) C. P. Keszthelyi, *J. Electrochem. Soc.*, **120**, 39C (1973).

(9) A. J. Bard, C. P. Keszthelyi, H. Tachikawa, and N. E. Tokel, "Chemiluminescence and Bioluminescence, Proceedings of the 1972 International Conference," M. J. Cormier, D. M. Hercules, and J. Lee, Ed., Plenum Press, New York, N. Y., 1973, pp 193-208.

(10) P. M. Schwartz, R. A. Blakeley, and B. B. Robinson, *J. Phys. Chem.*, **76**, 1868 (1972).

(11) J. M. Bonnier and R. Jardon, *J. Chim. Phys. Physicochim. Biol.*, **68**, 428 (1971).

(1) F. McCapra, *Essays Chem.*, **3**, 101 (1972).

(2) C. P. Keszthelyi, *Extended Abstr.*, **72-1**, 234 (1972).