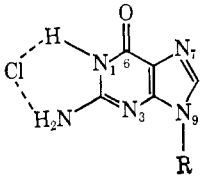
Cl^- interacting with uncoordinated guanosine, (2) Cl^- interacting with coordinated guanosine, and (3) the coordination of guanosine to the metal. This complexity was obviously not recognized when the stability constants were calculated,[2,3] and, thus, such constants are inaccurate.

We believe that the charge reversed chelate complex (I), in which two positive centers chelate a negative ion,



I

is formed. Our reasons for suggesting this particular model are as follows. (1) Downfield shifts are expected from hydrogen bonding.[10] (2) Only when a chelate is possible are large shifts seen. (No large shifts were observed for adenosine.)[2,3] (3) Almost identical interactions have been observed in the solid.[11] (4) Both N_1H and C_2NH_2 pmr signals shift substantially and simultaneously. The shifts level off simultaneously,[3] and this is inconsistent with one-point attachment. (5) The interaction is observed in solvents (DMSO, M-pyrol) which do not solvate anions, whereas water decreases the interaction. (6) Anions which do not accept hydrogen bonds readily do not show this interaction. (7) Modified guanosine derivatives which leave the six-membered ring unchanged (the triacetyl[2] and platinum derivatives) exhibit the same effect. (8) The Cl^- ion is remote from the C_8H group, and the pmr signal of this group is not expected to shift.

If the effect is due entirely to chloride ion, then we must explain the different stability constants found for the four alkaline earth salts.[3] We attribute these differences to (1) failure to maintain constant ionic strength, (2) variation in moisture content of the solution,[12] (3) data analysis based on an incorrect model, and (4) ion pairing. If we assume a one-to-one chloride ion–guanosine complex, we calculate[13] $K = 2.8\ M^{-1}$. The observation[2] of increased acidity of guanosine induced by $CaCl_2$ merely requires that deprotonated guanosine form a calcium complex.

We believe that the amino group is rotating rapidly on the pmr time scale because its resonance remains relatively sharp. If dissociation of guanosine from I is rapid, the magnitude of the effect of Cl^- on the shift of the amino proton resonance represents an average value for the two nonequivalent positions. Thus, the equivalent shifts of both the N_1H and the NH_2 pmr resonances of guanosine upon formation of I are coincidental. This reasoning is supported by the three times greater shift of the pmr signal of the NH_2 group than of the N_1H group in guanosine upon formation of the bromide analog of I.

Salt effects on biopolymers were recently reviewed[14] and are not completely understood. Geiduschek[15] has found that the melting temperature of DNA's can vary as much as 60° by varying salt concentrations and the phenomenon is due mainly to the anions. The magnitude of the effect is dependent on the base composition of DNA. It is interesting to note that the influence of anions on stabilizing DNA follows the order Cl^-, $Br^- > I^- > ClO_4^- > SCN^-$, and we find that effectiveness of these anions in causing shifts follows this same order. Our results provide evidence that specific interactions between anions and bipolymers are possible.

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(10) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High-Resolution Nuclear Magnetic Resonance," McGraw-Hill, New York, N. Y., 1959, p 400.

(11) T. J. Kistenmacher and T. Shigematsu, observed for adenine dihydrochloride, *Acta Crystallogr.*, in press. We note that interactions between halide ions and purine bases normally involve in plane hydrogen bonds. However, guanosine hydrobromide involves a charge-transfer type interaction (crystallographic evidence) between bromide and the six-membered ring: P. Tougard in "The Purines. Theory and Experiment," E. D. Bergmann and B. Pullman, Ed., Academic Press, New York, N. Y., 1972, p 217. We cannot exclude such an interaction in our studies, but we feel the hydrogen bond interaction is more consistent with our results.

(12) We find that the effect is not very sensitive to water in the concentration ranges 2–25% water but diminishes gradually; however, all the salts, guanosine, and the solvent are hygroscopic. It seems advisable that future studies involving metal ion interactions with nucleosides employ partially aqueous systems, rather than deal with the uncertainties of a small but unknown amount of water. Addition of water to a 0.2 *M* guanosine solution (DMSO) had the following effects: (a) the N_1H proton resonance shifted downfield (~2–3 Hz) and broadened and became lost in the noise as the water content reached 10% by volume and (b) the NH_2 and C_8H proton resonances did not shift up to 40% by volume water. This last solution was supersaturated. Guanosine precipitated before more water was added but after the spectrum was recorded.

(13) The calculation of the equilibrium constants in ref 2 requires several assumptions. These are that the chemical shifts of both the free guanosine and the complex guanosine pmr signals are not dependent on (1) the concentration of these species and (2) the concentration of the $CaCl_2$ salt. Most of these assumptions cannot be checked. However, in the absence of $CaCl_2$, the guanosine resonances *are* concentration dependent.[2,4] Strictly speaking, the calculations[2] have assumed Δ_t should be a constant (Δ_t = total shift difference between complexed and free guanosine). This may not be true unless the effect of $CaCl_2$ concentration exactly balances the change in chemical shifts attending the decrease in free guanosine concentration. Alternatively, the concentration dependence of the shift of the amino group of the complex must accomplish this result. Neither of these possibilities is likely. If we use the data in ref 2 and assume that $\Delta_t = 46$ Hz and the $[ClG^-] = (\Delta_0/46\ \text{Hz}) \times 0.282\ M$, the equilibrium constant for $Cl^- + G \rightleftharpoons ClG^-$ can be calculated to be $3.9 \pm 0.5\ M^{-1}$. If we calculate this equilibrium constant using the data in ref 2 and a method of varying Δ_{max} for NH_2 similar to that in ref 2, we calculate $K = 2.82 \pm 0.09\ M^{-1}$ ($\Delta_{max} = 52.5$ Hz). The calculation performed as in ref 3 gives $K = 2.83$, $\Delta_{max} = 52.4$ Hz.

(14) P. H. von Hippel and T. Schleich, *Accounts Chem. Res.*, 2, 257 (1969).

(15) K. Homaguchi and E. P. Geiduschek, *J. Amer. Chem. Soc.*, **84**, 1329 (1962).

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Long-Range ^{13}C–^1H Coupling Constants. I. Cyanopyridines

Sir:

Until now, ^{13}C–^1H coupling constants have been measured either from the satellite peaks of proton nmr spectra[1] or directly from ^{13}C nmr spectra with the aid of computers of average transients.[2] Hence

(1) K. Tori and T. Nakagawa, *J. Phys. Chem.*, **68**, 3163 (1964).
(2) K. Takahashi, T. Sone, and K. Fujieda, *J. Phys. Chem.*, **74**, 2765 (1970).

Table I. ^{13}C Chemical Shifts (δ) and One-Bond ^{13}C–^{1}H Coupling Constants (^{1}J) of Cyanopyridines **1–3** in Dimethyl-d_6 Sulfoxide[a,b]

Compound	δ[a,b] C_2	C_3	C_4	C_5	C_6	C_c	^{1}J[c] C_2	C_3	C_4	C_5	C_c
2-Cyano(**1**)	133.6	129.3	137.9	127.9	151.5	117.1		170	169	168	182
	(134.3)	(129.2)	(138.4)	(127.8)	(151.8)	(118.4)					
3-Cyano(**2**)	153.3	110.0	140.8	124.9	154.1	117.8	188		174	170	187
	(153.7)	(111.2)	(140.5)	(124.7)	(153.7)	(117.5)					
4-Cyano(**3**)	151.4	126.7	120.9	126.7	151.4	117.4	183	168		168	183
	(151.5)	(126.7)	(120.9)	(126.7)	(151.5)	(117.5)					

[a] In ppm relative to ^{13}C resonance of Me_4Si. [b] Values in parentheses are taken from ref 5. δ values relative to CS_2 are converted to δ relative to Me_4Si. [c] In Hz.

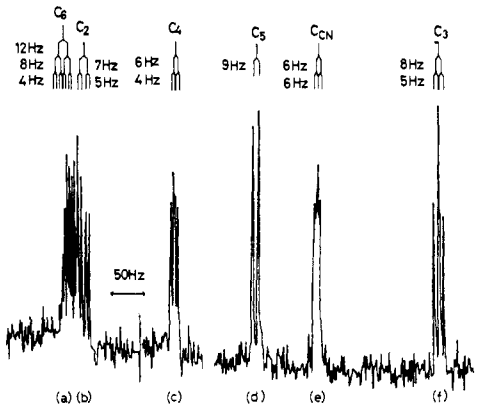

Figure 1. A part of the proton-noise undecoupled ^{13}C FT nmr spectrum of 3-cyanopyridine (**2**). Except for the C_3 and C_{CN} signals, only one of the two multiplets is reproduced: pulse width, 10 μsec; sweep width, 2500 Hz with 4096 memory points; number of pulses accumulated, 3500; resolution, 2 Hz.

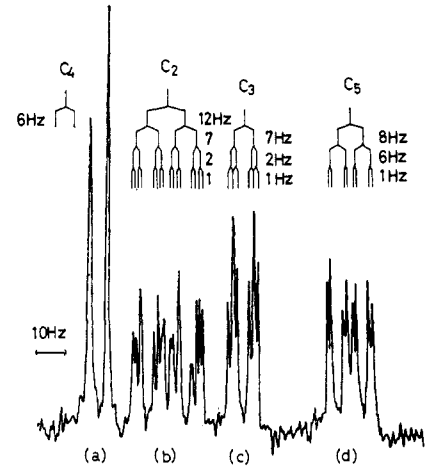

Figure 2. A part of the proton-noise undecoupled ^{13}C FT nmr spectrum of 2-cyanopyridine (**1**). Except for the C_2 signal, only one of the two multiplets is reproduced: pulse width, 10 μsec; sweep width, 1000 Hz with 4096 memory points; number of pulses accumulated, 2500; resolution, 1 Hz.

determination of long-range (*i.e.*, through more than two bonds) coupling constants was difficult and the available long-range coupling constants are necessarily scarce.[3] We report here direct determination of long-range ^{13}C–^{1}H coupling constants of three cyanopyridines by ^{13}C FT nmr spectra and describe their diagnostic value.

^{13}C nmr spectra of 2-cyano-, 3-cyano-, and 4-cyanopyridines **1–3** were measured with proton-noise decoupling (to assess chemical shifts) and without (to assess coupling constants).[4] In Table I, the chemical shifts (δ) and one-bond ^{13}C–^{1}H coupling constants (^{1}J) are listed. Values in parentheses are those of Retcofsky and Friedel.[5] In Table II, two-bond coupling constants (^{2}J) and three-bond coupling constants (^{3}J) are listed. The assignments (both δ and J) were made as described below, based on the undecoupled spectra.

In Figure 1, a part of the undecoupled spectrum of **2** is given. Peaks e and f are associated with C_{CN} and C_3 because of the lack of one-bond coupling. A poorly resolved triplet is characteristic of the C_{CN} peak for three compounds investigated. Peaks a and b, with larger one-bond couplings, are associated with carbons adjacent to nitrogen.[1] The identification of C_2 and C_6 peaks can be made from the analysis of the splittings of (a) and (b) multiplets. The reported J values for

Table II. Two-Bond ^{13}C–^{1}H Coupling Constants (^{2}J) and Three-Bond Coupling Constants (^{3}J) of Cyanopyridines **1–3**[a]

Compound	^{2}J C_2–H_3	C_3–H_2	C_3–H_4	C_4–H_3	C_4–H_5	C_5–H_4	C_5–H_6	C_6–H_5
2-Cyano(**1**)	2		2	<1	<1	1	8	4
3-Cyano(**2**)		8	<1		<1	<1	9	4
4-Cyano(**3**)	2	7		<1	<1		7	2

Compound	^{3}J C_2–H_4	C_2–H_6	C_3–H_5	C_4–H_2	C_4–H_6	C_5–H_3	C_6–H_2	C_6–H_4
2-Cyano(**1**)	7	12	7		6	6		8
3-Cyano(**2**)	5	13	5	4	6		12	8
4-Cyano(**3**)		12	3	7	7	3	12	

[a] In Hz.

benzene[6] and a few substituted benzenes[7] can be used as the basis for comparison in the present analysis. Thus, ^{3}J is in the range of 5–8 Hz and is larger than ^{2}J which is in the range of 1–4 Hz. ^{4}J is in most cases 1 Hz or less and often unresolved.

Both C_2 and C_6 of **2** enjoy two three-bond couplings, but, for C_6, an additional splitting due to ^{2}J (C_6–H_5) is expected. Thus, peak a, an octet, is associated with C_6 while peak b, a quartet, is associated with C_2. A large ^{3}J (12–13 Hz), common to both C_2 and C_6, should be

(3) J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, N. Y., 1971.
(4) The ^{13}C spectra were recorded with Varian XL-100-FT spectrometer operating at 25.2 MHz. The solutions were prepared by adding 1 ml of dimethyl-d_6 sulfoxide to 2.5 g of the sample.
(5) H. L. Retcofsky and R. A. Friedel, *J. Phys. Chem.*, 71, 3592 (1967); 72, 290, 2619 (1968).
(6) F. J. Weigert and J. D. Roberts, *J. Amer. Chem. Soc.*, **89**, 2967 (1967).
(7) G. Govil, *J. Chem. Soc. A*, 1420 (1967).

due to the coupling through nitrogen. This assignment is further confirmed by the large splitting of the $C_{2,6}$ peak of **3** (12 Hz) where ^3J (C_2–H_6) is the only three-bond coupling involved. This enhancement by nitrogen is interesting because it makes a sharp contrast with ^1H–^1H coupling through nitrogen in pyridine. Thus, ^4J (H_2–H_6) is less than one-tenth of ^4J (H_2–H_4) or ^4J (H_3–H_5).[8,9] ^3J (C_2–H_4) of **2** is small as a three-bond coupling (4 Hz). Any ^3J through the substituted carbon (the carbon carrying the cyano group) is generally small. Thus, C_4, peak c, is a quartet with one normal and one small ^3J, *i.e.*, ^3J (C_4–H_6) (6 Hz) and ^3J (C_4–H_2) (4 Hz). Although no three-bond coupling is involved, the splitting of the C_5 peak is very large (9 Hz). This should either be ^2J (C_5–H_6) or ^2J (C_5–H_4). The last interpretation is incompatible with a very small ^2J such as ^2J (C_4–H_5) or ^2J (C_4–H_3). If the analogy between ^2J (C–H) and ^2J (H–H) is taken for granted,[10] ^2J (C_5–H_6), a two-bond coupling constant between ^{13}C and ^1H adjacent to sp^2-hybridized nitrogen, can be very large as is ^{gem}J (H–H) for the $H_2C{=}NR$ system.[11] This large two-bond coupling is characteristic for all pyridine derivatives and has some diagnostic value.[12]

These observations can be summarized as below. (i) ^3J is normally in the range of 5–8 Hz; the coupling through nitrogen is enhanced to 12–13 Hz, and the one through the substituted carbon is reduced to 3–5 Hz. (ii) ^2J is normally in the range of 1–4 Hz; the coupling between ^{13}C and the proton adjacent to nitrogen is remarkably enhanced to 8–9 Hz. (iii) ^4J is not larger than 2 Hz.

The multiplets observed for **1** and **3** are interpretable with the aid of these criteria. In Figure 2, a part of the undecoupled spectrum of **1** is reproduced. In this spectrum, the resolution is so good that a number of the small four-bond couplings (^4J = 1 Hz) are easily resolved. Here again, ^2J with the proton adjacent to nitrogen and ^3J through nitrogen are enhanced while other ^2J and ^3J values are within the range of normal values. Much the same is true for **3**. Thus, both $C_{2,6}$ and $C_{3,5}$ signals consist of a pair (^1J) of a doublet of doublet (^2J and ^3J), while both C_4 and C_{CN} signals are a a triplet (two ^3J).

Previously the assignment of signals of ^{13}C spectra of substituted pyridines[5] (and any other aromatic and heteroaromatic compounds) was made by the additivity principle, *i.e.*, by the comparison of observed shifts with those estimated from the appropriate substituent parameters (obtained from ^{13}C spectra of monosubstituted benzenes). With this, however, there often remains some uncertainty in the assignment when the estimated (hence observed) chemical shifts are close. In fact such is the case with C_3 and C_5 for **1**, and C_2 and C_6 for **2**. Our assignment based on the coupling constants is unequivocal and completely free from such an ambiguity. We believe that the long-range ^{13}C–^1H coupling constants are very valuable as a means for the structure determination of organic compounds and as the touchstone of the theory of coupling constants.[13]

Acknowledgment. We thank Professor A. R. Katritzky and the University of East Anglia for the use of the instrument.

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(11) R. C. Cookson, T. A. Crabb, J. J. Frankel, and J. Hudec, *Tetrahedron Suppl.*, No. 7, 355 (1966).
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(13) NOTE ADDED IN PROOF. M. Hansen and H. J. Jacobsen (*J. Magn. Resonance*, 10, 74 (1973)) measured narrow-range proton-noise undecoupled spectra of pyridine and 2-bromopyridine, and determined long-range coupling constants from complete iterative computer analysis. Our values are in good agreement with theirs.

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Stereochemistry of the Solvolysis of Menthyl Tosylate. An Example of Retained Chair Conformation in the Transition State[1]

Sir:

We wish to report that the solvolysis of menthyl tosylate (**1**-OTs) in both aqueous ethanol (E) and aqueous trifluoroethanol (T) gives hydroxylic substitution products of predominantly retained configuration (58% in E, 83% in T). This result and the observed significantly reduced β,β'-d_3 isotope effect (k_H/k_D = 1.30), which contradict the expected behavior of most cyclohexyl derivatives,[2–6] suggest a retained chair conformation in the rate-determining transition state and the intermediate ion pair(s).

Conformational rigidity has been proposed by Winstein and Holness[7] for transition states in solvolyses of both *cis*- and *trans*-4-*tert*-butylcyclohexyl tosylates. However, in a recent study, Lambert, *et al.*,[8] found that the product distribution in the solvolysis of the unsubstituted conformationally flexible cyclohexyl tosylate is essentially identical with that from the supposedly rigid *trans*-4-*tert*-butylcyclohexyl brosylate. Doubts about the generality of the Winstein and Holness[6] assumption have been repeatedly expressed in the past, but satisfactory explanations were not given.[9,10] Half-chair transition states in solvolyses of uncomplicated

(1) This work was supported by the Research Council of Croatia and Grant No. 02-011-1 (PL-480) administrated by the National Institutes of Health.
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