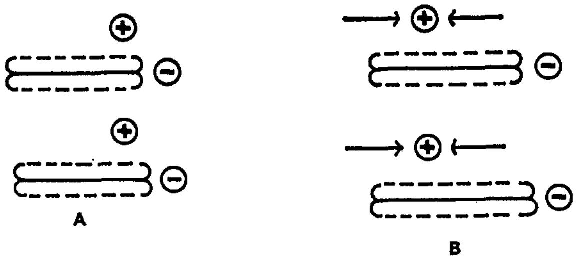

Figure 1. A represents an aggregate of contact ion pairs $(F^-,M^+)_n$ and B that of solvent-separated ion pairs. The arrow denotes solvating molecules inducing a shift of the counterion to a new average position away from the center of negative charge.

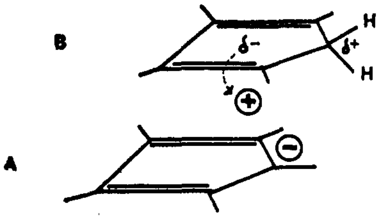

Figure 2. Activating role of the positive ion in proton-abstraction reactions. A and B represent the fluorenyl carbanion and the benzofluorene molecule, respectively.

the counterion to the new carbanion. For the tetrabutylammonium salt, the reactivity is found to be the same in THF as in DME. No solvent-separated ion pairs are observed for this salt in either solvent! However, the Coulomb interaction between carbanion and counterion is not the only rate-determining factor, since fluorenylcesium would then be expected to have a higher reactivity than fluorenylsodium in both dioxane and THF. The reverse is true, which suggests that the counterion activates the benzofluorene molecule and facilitates the proton-abstraction process (see Figure 2). Such an activation has been suggested in other reactions involving organometallic reagents.[4] The effect will rapidly decrease with increasing size of the counterion and may be essentially absent for cesium and tetrabutylammonium (the association of the lithium salts prevented us from obtaining the rate constant for the lithium ion pair).

It is significant that addition of the strongly dissociable $NaB(C_6H_5)_4$ does not decrease the rate of the reaction but seems to even increase the rate slightly. Apparently, the free carbanion is not more, and presumably even less, reactive than the contact ion pair. This would also explain the concentration independence of the rate constants for the sodium and cesium salts in THF in spite of the increased free ion formation at low concentration.[5] These results are in agreement with the catalyzing role assigned to the counterion.

The solvent-separated ion pair, where Coulomb interaction has greatly weakened, would be highly reactive as long as the counterion can effectively activate the benzofluorene molecules. This appears to be the case in 1,2-dimethoxyethane. However, when the solvating entity prevents a close approach between the benzofluorene molecule and the counterion, the solvent-separated ion pair reactivity is expected to be less than that of the contact ion pair and may approach that of the free ion. Such appears to be the case in the system fluorenylsodium–tetrahydrofuran to which small quantities of tetraethylene glycol dimethyl ether were added. In the presence of this powerful solvating agent[6] the spectrum shows complete solvent-separated ion pair formation, yet the rate of proton abstraction is slower than in the absence of this reagent. Its high solvating power arises from the fact that probably all five oxygens are utilized in the solvation of the sodium ion and therefore effectively shield this ion from interaction with the benzofluorene molecule.

The behavior of these carbanion salts in proton-abstraction reactions is the more interesting when the results are compared with those of addition reactions involving carbanions. In the anionic polymerization of styrene in THF[2] the free polystyryl ion was found to be about 800 times more reactive than the sodium contact ion pair, while the solvent-separated ion pair reactivity approaches that of the free ion.[7] In dioxane the ion pair reactivity increases substantially in the order Li < Na < Cs.[8] Both results indicate that in these reactions the Coulombic interaction between the carbanion and the counterion is of far greater importance than any possible interaction between the counterion and the reacting monomer, at least in ethereal solvents. Other types of carbanions and substrates will be investigated in order to establish whether the behavior of fluorene and its derivatives in proton abstraction reactions is unique in this respect.

Acknowledgment. The support of this research through a grant from the Petroleum Research Fund, administered by the American Chemical Society, is gratefully acknowledged.

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[4] A. A. Morton, "Solid Organoalkalimetal Reagents," Gordon and Breach Science Publishers Inc., New York, N. Y., 1964.

[5] T. E. Hogen-Esch and J. Smid, *J. Am. Chem. Soc.*, **88**, 318 (1966).

[6] L. L. Chan and J. Smid, to be published.

[7] T. Shimomura, K. J. Tölle, J. Smid, and M. Szwarc, *J. Am. Chem. Soc.*, **89**, 796 (1967).

[8] D. N. Bhattacharyya, J. Smid, and M. Szwarc, *J. Phys. Chem.*, **69**, 624 (1965).

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Indirect ^{15}N–^{1}H Spin–Spin Couplings in Quinoline-^{15}N, Its Ethiodide, and Its N-Oxide. Relative Signs and Solvent Dependence

Sir:

Previous observations,[1,2] of some interest, on the spin couplings between a nitrogen-15 nucleus in an aromatic ring and ring protons have prompted us to report here the correlation of indirect ^{15}N–^{1}H couplings with the electronic nature of the ^{15}N atom as studied by using quinoline-^{15}N (I), its ethiodide (II), and its N-oxide (III).[3] Coupling constants, *J*, in these compounds were obtained by comparing the pmr spectra of the ^{15}N compounds with those of the corresponding ^{14}N compounds in a variety of solvents (see Table I).[4] A pair of the pmr spectra of quinoline-^{14}N and -^{15}N in acetone-d_6 is shown in Figure 1 as an example.

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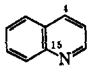
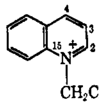
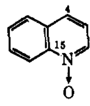
[1] Y. Kawazoe, M. Ohnishi, and N. Kataoka, *Chem. Pharm. Bull.* (Tokyo), **13**, 396 (1965).

[2] B. W. Roberts, J. B. Lambert, and J. D. Roberts, *J. Am. Chem. Soc.*, **87**, 5439 (1965).

[3] For the synthesis of III (97 atom %), see ref 1. I and II were successively prepared from III in the usual way.

[4] Pmr spectra were taken with a Varian HA-100 spectrometer operating at 100 MHz in the frequency-swept and TMS-locked mode,

Table I. Summary of ^{15}N-^{1}H Spin-Coupling Constants (in Hz)

Compound		Solvent		
		Aprotic solvents[a]	CH_3COOD	D_2SO_4 and CF_3COOD
I 	$J_{^{15}N,H(2)}$	−11.1	−4.5	−2.0
	$J_{^{15}N,H(3)}$	−1.4	−3.1	−4.5
II 	$J_{^{15}N,H(2)}$	−1.6[b]	−1.6	. . .[c]
	$J_{^{15}N,H(3)}$	−4.3[b]	−4.3	−4.4
III 	$J_{^{15}N,H(2)}$	~0	~+1	+1.2
	$J_{^{15}N,H(3)}$	−5.0	−5.5	−6.0

[a] Aprotic solvents used were cyclohexane, CCl_4, C_6D_6, C_5D_5N, CH_3CN, dioxane, acetone-d_6, and/or DMSO-d_6. Other solvents used as protic ones were $CDCl_3$, D_2O, and/or D_2O–dioxane (1:1)–DCl system. [b] Values obtained in D_2O. [c] Obscured by overlapping of other signals.

Since coupling constants between ring protons in quinoline were already found to have a positive sign,[5] the relative sign of $J_{^{15}N,H(2)}$ and $J_{^{15}N,H(3)}$ was determined in an acetone-d_6 solution by using partial spin-decoupling technique. The ^{15}N, H(2), H(3), and H(4) nuclei can be treated as an isolated first-order, four-spin system by neglecting $J_{H(4),H(8)}$. On double irradiation on the lines q, r, s, and t, the lines a and c and the lines e and g collapse to two single peaks, whereas irradiation on the lines u, v, w, and x causes collapsing of the lines b and d, and the lines f and h (see Figure 1). These results can be well explained by the spin states shown in Figure 1 (botton); this indicates that $J_{^{15}N,H(2)}$ and $J_{^{15}N,H(3)}$ are of the same sign although the sign of these J's relative to $J_{^{15}N,H(4)}$ could not be determined because of the small magnitude of the latter, $J_{H(2),H(4)}$, and $J_{H(4),H(8)}$.[6] Since the magnetogyric ratio of the ^{15}N nucleus has a negative sign, the absolute sign of these J values can reasonably be assumed to be negative by analogy with the fact that the $J_{^{14}N^+\text{-}C\text{-}C\text{-}H}$ values in trimethylvinylammonium bromide[7] and alkyl isonitriles[8] have a positive sign.[9]

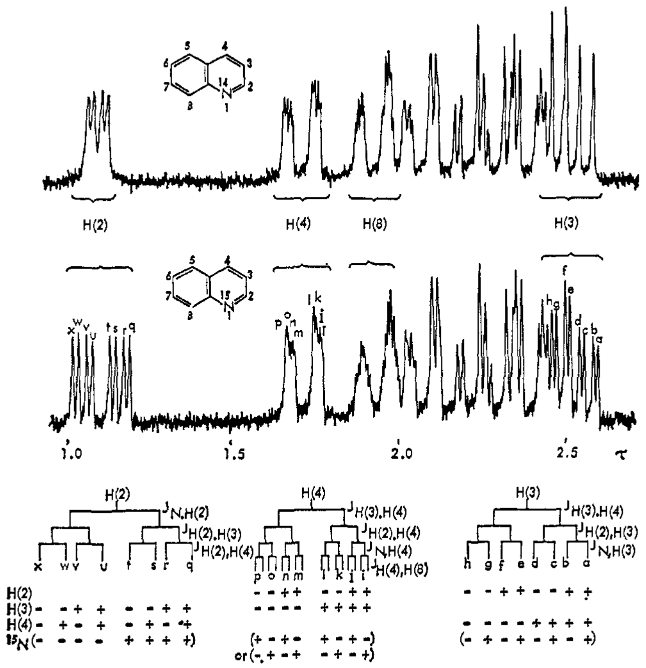


Figure 1. Pmr spectra of quinoline-^{14}N and -^{15}N in acetone-d_6 at 100 MHz and the spin-state diagram.

The large negative value for $J_{^{15}N,H(2)}$ was obtained for I as for pyrimidine derivatives in aprotic solvents.[2] However, this value increases algebraically to a great extent with an increase in the acidity of the solutions, whereas the value for $J_{^{15}N,H(3)}$ decreases reversely, as summarized in Table I. These J values were plotted against pH values of the aqueous solutions examined (D_2O-dioxane–DCl system) to obtain the proportional correlation of the J values with the degree of protonation toward the ^{15}N atom in question. The J values in II and III, where the ^{15}N atom is cationic even in aprotic solvents, depend a little upon the solvent acidity. The variation in the J values in going from I through III is such that the J values in II and III are brought to values which I would show in far more strongly acid media than have been examined here. On the basis of such presumption, the $J_{^{15}N,H(2)}$ in III, which is about 0 Hz in aprotic solvents, may reasonably be inferred to increase finally to +1.2 Hz in strongly acid media, while the $J_{^{15}N,H(3)}$ value decreases to −6.0 Hz. Thus it was revealed that either N oxygenation, quaternization, protonation, or hydrogen bonding, which changes the s character of the ^{15}N-atom bonding orbitals to carbon, results in a remarkable increase in the $J_{^{15}N,H(2)}$ value and a decrease in the $J_{^{15}N,H(3)}$ value simultaneously. The s character of the ^{15}N-atom bonding orbitals to carbon, which is supposed to increase in the order of $\geqslant N < \geqslant N^+\text{-}H < \geqslant N^+\text{-}C < \geqslant N^+ \rightarrow O^- < \geqslant N^+\text{-}OH$,

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by using about 5% (w/v) solutions of the samples. Errors in coupling constants are less than ±0.1 Hz. Spin-decoupling experiments were performed by using a Hewlett-Packard HP-200ABR audiooscillator and an HP-5212A electronic counter. For the spectral assignments of quinoline and its N-oxide, the reader is referred to P. J. Black and M. L. Heffernan, *Australian J. Chem.*, **17**, 558 (1964), and K. Tori, M. Ogata, and H. Kano, *Chem. Pharm. Bull.* (Tokyo), **11**, 681 (1963), as well as ref 1, respectively.

(5) W. G. Paterson and G. Bigam, *Can. J. Chem.*, **41**, 1841 (1963).

(6) The determination of relative signs of $J_{^{15}N,H}$ to $J_{H,H}$ is impossible solely from pmr spectra; see E. W. Randall and J. D. Baldeschwieler, *J. Mol. Spectry.*, **8**, 365 (1962).

(7) (a) M. Ohtsuru and K. Tori, *Chem. Commun.*, 750 (1966); (b) J. M. Lehn and R. Seher, *ibid.*, 847 (1966).

(8) (a) See footnote 12 in ref 10a; (b) J. P. Maher, *J. Chem. Soc., Sect. A*, 1855 (1966).

(9) Refer to A. J. R. Bourn, D. G. Gillies, and E. W. Randall, "Nuclear Magnetic Resonance in Chemistry," B. Pesce, Ed., Academic Press Inc., New York, N. Y., 1965, p 277.

might play a significant role in these facts, although the contribution due to changes in π-electron structures cannot completely be excluded. Further, this conclusion is supported by the similar behavior reported on $J_{^{31}P\text{-}C\text{-}H}$ and $J_{^{31}P\text{-}C\text{-}C\text{-}H}$,[10] when the reduced coupling constants are compared. The former J value decreases algebraically whereas the latter, which is always positive, increases as the ^{31}P atom becomes more cationic ($P \rightarrow P^+$).

It should also be noted that whenever the signals concerned were separately observed, the presence of weak couplings (about 1 Hz or less), although not exactly measured, were clearly discernible for $|J_{^{15}N,H(4)}|$ and $|J_{^{15}N,H(8)}|$, which are considerably smaller than $|J_{^{15}N,H(3)}|$ in all the cases examined (see Figure 1, for example).[11]

(10) (a) S. L. Manatt, G. L. Juvinall, and D. D. Elleman, *J. Am. Chem. Soc.*, **85**, 2664 (1963); (b) A. R. Cullingworth, A. Pidcock, and J. D. Smith, *Chem. Commun.*, 89 (1966); (c) S. L. Manatt, G. L. Juvinall, R. I. Wagner, and D. D. Elleman, *J. Am. Chem. Soc.*, **88**, 2689 (1966); (d) W. McFarlane, *Chem. Commun.*, 58 (1967).

(11) We thank the referee for his valuable comments on the sign of coupling constants.

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The Synthesis of N⁴-Acetyl-3,4,5,6-tetrahydrocytidine and Copolymers of Cytidylic Acid and N⁴-Acetyl-3,4,5,6-tetrahydrocytidylic Acid

Sir:

Recent studies of the optical rotatory dispersion curves of poly C[1,2] and poly U[3] at neutral pH, and of the reaction of poly C[2] and poly A[4] with formaldehyde, led to the conclusion that intra- or interstrand hydrogen bonding plays a negligible role in the stabilization of the secondary structure of these polymers. It has been postulated for these polymers that a single-stranded helical structure is stabilized by interactions of the stacked bases. Polynucleotides containing residues with a saturated and therefore nonplanar heterocyclic portion are suitable model compounds for studying this hypothesis. Saturated nucleotides are unlikely to participate in base stacking due to their stereochemistry and their shortened π-electron system.[5] Copolymers containing saturated nucleotide residues are also interesting model compounds for yeast transfer RNA, where 5,6-dihydrouridine has been detected as a minor constituent.[6] We wish to report the synthesis of N^4-acetyl-3,4,5,6-tetrahydrocytidine and copolymers of cytidylic acid containing varying amounts of N^4-acetyl-3,4,5,6-tetrahydrocytidylic acid as nonplanar constituents.

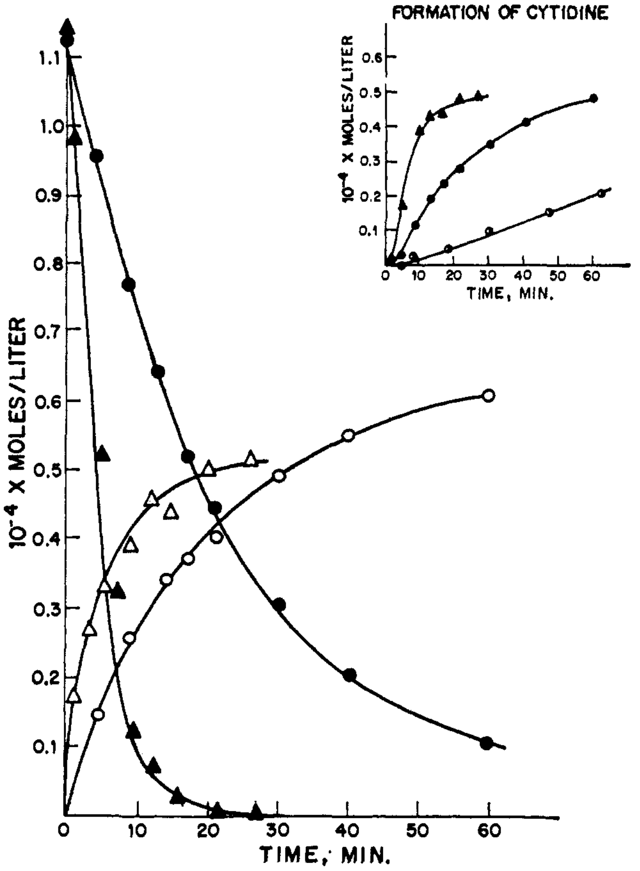

Figure 1. The reduction and deacetylation of N^4-acetylcytidine in the presence of sodium borohydride in the dark. Disappearance of N^4-acetylcytidine calculated from the absorption at 310 mμ in 1 *N* HCl (●, 14 *M* excess NaBH$_4$; ▲, 140 *M* excess NaBH$_4$; ϵ_{310} N^4-acetylcytidine 18 × 10^3); formation of N^4-acetyl-3,4,5,6-tetrahydrocytidine, orcinol assay (○, 14 *M* excess NaBH$_4$; △, 140 *M* excess NaBH$_4$). Inset: formation of cytidine calculated from the absorption at 310 mμ and 270 mμ in 1 *N* HCl (●, 14 *M* excess NaBH$_4$; ▲, 140 *M* excess NaBH$_4$; ϵ_{310} N^4-acetylcytidine 18 × 10^3, ϵ_{270} N^4-acetylcytidine 23 × 10^2, ϵ_{310} cytidine 0, ϵ_{270} cytidine 10 × 10^4); deacetylation of N^4-acetylcytidine in the absence of NaBH$_4$ at pH 10 (◑, from the disappearance of the absorption at 310 mμ measured in 1 *N* HCl; ϵ_{310} N^4-acetylcytidine 18 × 10^3).

While cytidine is completely resistant toward sodium borohydride in the dark,[7] N^4-acetylcytidine is reduced to N^4-acetyl-3,4,5,6-tetrahydrocytidine and partially deacetylated to cytidine in the presence of sodium borohydride at pH 10. The rate of the reduction and deacetylation[8] are both dependent on the concentration of sodium borohydride. N^4-Acetylcytidine is also deacetylated at a slower rate at pH 10 in the absence of the reducing agent. The extent of the reduction can be determined with the orcinol assay for ribose due to the labilization of the N-glycoside bond upon saturation of the 5,6 double bond in N^4-acetylcytidine. The disappearance of N^4-acetylcytidine and the formation of cytidine can be followed spectrophotometrically and the composition of the reaction mixture calculated at each point of the reaction from the absorbance at 310 and 270 mμ in 1 *N* HCl[9] (see Figure 1). The final yield of N^4-acetyl-3,4,5,6-tetrahydrocytidine was 54% if a 14 *M* excess of sodium borohydride was used and 45% at a 140 *M* excess of the reducing agent. In both cases

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(1) Abbreviations: polyuridylic acid, poly U; polycytidylic acid, poly C; polyadenylic acid, poly A.

(2) G. D. Fasman, C. Lindblow, and L. Grossman, *Biochemistry*, **3**, 1015 (1964).

(3) A. M. Michelson and C. Monny, *Proc. Natl. Acad. Sci. U. S.*, **56**, 1528 (1966).

(4) C. L. Stevens and A. Rosenfeld, *Biochemistry*, **5**, 2714 (1966).

(5) P. Cerutti, H. Miles, and J. Frazier, *Biochem. Biophys. Res. Commun.*, **22**, 466 (1966).

(6) J. T. Madison and R. W. Holley, *ibid.*, **18**, 153 (1965).

(7) P. Cerutti and N. Miller, *J. Mol. Biol.*, in press.

(8) The deacetylation reaction is reminiscent of the reductive deacylation of N-acylindoles and carbazoles with NaBH$_4$ (K. Banholzer, T. W. Campbell, and H. Schmid, *Helv. Chim. Acta*, **35**, 1577 (1952)).

(9) N^4-Acetyl-3,4,5,6-tetrahydrocytidine has no appreciable absorption at wavelengths longer than 250 mμ.