Electronic Spectra of Crystalline 9-Ethylguanine and Guanine Hydrochloride

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Abstract: Polarized reflection spectra from crystals of 9-ethylguanine and guanine hydrochloride dihydrate have been measured. Correlation of the absorption spectra obtained by Kramers–Krönig analyses of the reflection data permits assignment of the transition moment directions of the first four strong, in-plane polarized transitions. Two or three additional bands in the vacuum ultraviolet region are observed for 9-ethylguanine. Evidence is presented for the possible existence of an n → π* band in 9-ethylguanine crystals at 3003 Å and another weak, in-plane transition at about 2300 Å in both systems.

The theories which deal with the optical properties of molecular aggregates involve matrix elements which can be empirically evaluated from a knowledge of the electric dipole transition moments of the constituent molecules. A knowledge of the transition moment magnitudes and directions of the low lying excited states of the purine and pyrimidine bases are thus important in dealing with effects of intermolecular coupling in DNA and other polymers.

Information is now available for the lowest one or two transitions of adenine,[1] thymine,[2] and cytosine[3] (or their methyl derivatives). A report concerning the transition moment directions of the one remaining DNA base, guanine, has recently been published by Callis, Fanconi, and Simpson.[4] These workers coupled the results of polarized specular reflection from single crystals of 9-ethylguanine with the constraints obtained from a fluorescence polarization study to restrict the polarization directions of the lowest two transitions in 9-ethylguanine to two possible definite combinations. The purpose of the present paper is to present additional data which resolve the above mentioned ambiguity as well as to provide information about the higher energy transitions.

The ambiguity can never be resolved from the examination of single crystals of 9-ethylguanine alone due to the tetragonal crystal symmetry. However a clear choice can be made through the comparison of polarized spectra of single crystals of 9-ethylguanine and guanine·HCl·2H_2O. Here it is necessary to make the assumption that the transition moment directions of 9-ethylguanine and protonated guanine are not dissimilar. Two lines of reasoning appear to support this assumption. On the one hand the absorption spectrum of a neutral solution of guanine (or 9-alkyl derivatives) does not change in any large way upon acidification (see Figure 1). On the other hand the crystal spectra of the two systems are neatly interpreted with this assumption (vide infra).

Experimental Section

Polarized reflection spectra were obtained with instruments which have been described earlier.[5,6] Crystals were grown from saturated solutions; all showed uniform extinction. The crystal faces were identified by morphologic examination, while the indices of refraction at the Na D line were obtained by standard immersion techniques. The solution spectra were obtained with a Cary 15 spectrophotometer. 9-Ethylguanine and guanine were purchased from Cyclo Chemical Co. and Calbiochem, respectively, and were used directly from the bottles. Equations and procedures used for the Kramers–Krönig transformation have been given before.[5,7]

Results

9-Ethylguanine. 9-Ethylguanine crystallizes in the tetragonal, $P4_12_12$ space group.[8] There are 16 molecules per unit cell assembled in pairs (the asymmetric unit). The long axis of every molecule is approximately perpendicular to the unique *c* crystal axis and almost parallel to the *a* or *b* axis depending upon the particular pair (see inset Figure 2). Crystals were grown from dimethyl sulfoxide as bipyramids bounded by the forms {111} and {113}. The spectra obtained from the (111) face are reported here.

The reflection spectra are shown in Figure 2. The quality of the surface was judged satisfactory by the agreement of the measured reflection at 589 mμ with the reflectivity calculated (small circles in Figure 2) from refractive index data.[9]

Corresponding absorption spectra obtained by Kramers–Krönig analyses of the reflection data are shown in Figure 3. The four bands which are observed in the solution spectrum are clearly identifiable at 36 000, 39 300, 49 000, and 53 000 cm^{-1} (labeled I–IV). The higher energy region beyond 58 000 cm^{-1} is badly overlapped with an apparent underlying continuum. However, there appear to be three additional bands in this region at 63 000, 65 000, and 69 000 cm^{-1} (labeled V–VII).[10] The possibility that these higher energy bands are composite in nature of course cannot be ruled out.

It is to be expected that the strong transitions are polarized in the molecular plane. We also assume the oriented gas model in which the observed oscillator strength of a given transition along any particular crystal axis is simply the sum of the oscillator strengths obtained from the projections onto that axis of the transition moment of all the molecules. In general a given dichroic ratio will be consistent with two possible in-plane transition moment directions θ.[11]

The spectra are resolved into components which are also shown in Figure 3. Of course the ambiguity in resolving the overlapped bands leads to an uncertainty in establishing the dichroic ratios, and in this regard we will attach uncertainty limits. Thus we maximize and minimize the observed dichroic ratio within the bounds of plausible band shapes. For example, the dichroic ratio $f_{\parallel}:f_{\perp}$ of the resolution of transition I in Figure 3 is 9.3, but it could be as high as 14 or as low as 6. The ranges of the two possible transition moment directions, θ, work out to be −1 to −7° or 31–39°. The average values become either −4 ± 3° or 35 ± 4°. The corresponding free molecule oscillator strengths are 0.16 ± 0.00 or 0.15 ± 0.01, respectively.

The analysis of transition II is slightly different. Here a distinct component is observed only in the ⊥ direction. An uncertainty, however, still obtains due to the possible small component of $II_{\parallel}$ obscured by $I_{\parallel}$. The results of this sort of analysis for transitions I through IV are given in Table I. No conclusive results for the higher energy bands (V–VII) can be obtained due to their diffuseness. It appears that V and VII are possibly similar to I and IV. We will discuss the M band later.

Guanine·HCl·2H_2O. Guanine hydrochloride dihydrate was grown from dilute hydrochloric acid solutions by slow evaporation. The crystals were needles which were elongated along *c*. The common bounding form were {110}, {210}, and {011}.

Table I. Solution Data and Crystal Results for 9-Ethylguanine Transitions

Transition	ν_{solution},[a] cm^{-1}	ν_{crystal}, cm^{-1}	θ[b]	f_{crystal}	f_{solution}[a]
I	36 700	36 000	−4 ± 3°	0.16 ± 0.00	
			35 ± 4°	0.15 ± 0.01	0.14 ± 0.01
II	40 200	39 400	−75 ± 3°	0.25 ± 0.00	0.21 ± 0.01
III	48 600	49 000	−71 ± 4°	0.41 ± 0.01	
			−79 ± 4°	0.42 ± 0.01	0.38 ± 0.03
IV	53 500	53 000	−9 ± 4°	0.48 ± 0.04	
			41°	0.52 ± 0.04	0.42 ± 0.03
V		63 000	~0°		
			~40°		
VI		65 000			
VII		69 000	~0°		
			~40°		

[a] Aqueous solution, pH 6.8. [b] θ measured from C(4)-C(5) axis toward C(6). Two values correspond to the two possible transition moment directions consistent with the observed dichroic ratios. The uncertainties pertain to the ambiguity in resolving the overlapped spectra.

Table II. Solution Data and Crystal Results for Guanine Hydrochloride Dihydrate

Transition	Solution,[a] cm^{-1}	Crystal, cm^{-1}	θ[b]	f_{crystal}	f_{solution}[a]
I	36 300	34 500	2°	0.15	0.13 ± 0.01
	(35 800)		77°	0.10	(0.12)
II	40 600	41 400	−82°	0.20	0.22 ± 0.01
			−14°	0.31	(0.23)
III	51 800	51 000	−75°	0.63	0.7
	(49 700)		−17°	0.86	(0.5)

[a] Aqueous hydrochloric acid, pH 2.1. Values in parentheses refer to aqueous 9-ethylguanine, pH 2.1. [b] θ measured from C(4)-C(5) axis toward C(6). Two values correspond to the two possible transition moments consistent with the observed dichroic ratios. The experimental uncertainty for these angles is estimated to be no larger than ±5° even for transition III.

Occasionally the (001) face developed on the ends of the needles, and this face was used. The projection onto the (001) plane of the four spectroscopically equivalent molecules of the unit cell (monoclinic, space group $P2_1/c$)[12] is shown in Figure 4.

The reflection spectra for radiation polarized along the *a* and *b* crystal axes are given in Figure 5. The small circles at 589 mμ correspond to reflectivity calculated from published data on the Na D line indicatrix.[13] Reflection measurements below about 190 mμ failed, since the crystals apparently lose the water of hydration and become opaque in a dry nitrogen atmosphere or in a vacuum. The first three bands, however, are distinct and the fourth band is indicated by the turning up of the reflection at about 54 000 cm^{-1} in the *a* axis spectrum.

The absorption spectra derived from Kramers-Krönig analyses are shown in Figure 6. The resolution of the spectra into band components is reasonably straightforward. In general a given dichroic ratio will correspond to two possible in-plane polarization directions. However, different free molecule oscillator strengths will be required for the two directions in order to yield the observed crystal intensities. For example, the observed dichroic ratio (f_a/f_b) for transition I is 2.9. The two possible in-plane transition moment directions consistent with this dichroic ratio are 2 or 77°. The free molecule oscillator strength for transition I with these two possible polarization directions must be either 0.15 or 0.10, respectively, in order to produce the observed crystal oscillator strengths. The results for the hydrochloride are tabulated in Table II.

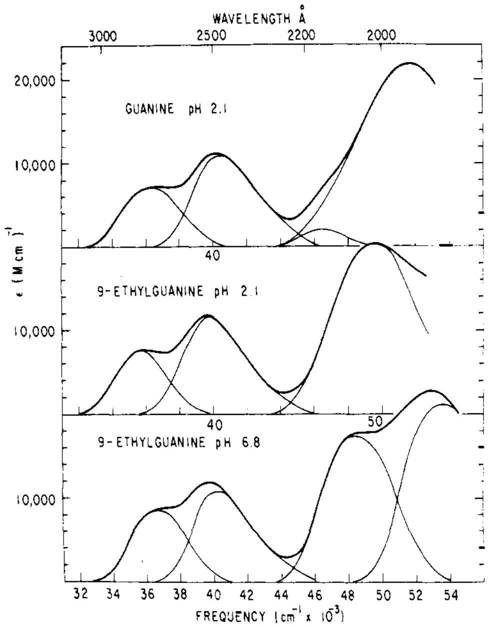

Figure 1. Aqueous solution spectra of guanine and 9-ethylguanine. Oscillator strengths were evaluated from the resolutions shown.

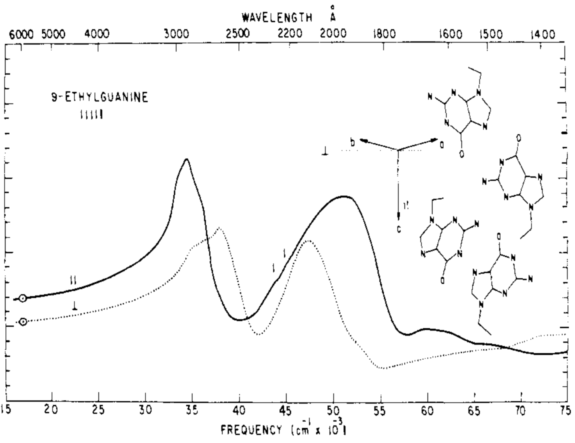

Figure 2. Polarized reflection spectra of the (111) plane of 9-ethylguanine single crystals. A projection of two representative asymmetric units onto (111) is shown. The small circles at 5890 Å correspond to reflectivities calculated from refractive index data.

Polarization Assignments. The possible transition moments consistent with the data for transitions I and II are compared in Figure 7. The directions in guanine·HCl are either essentially short axis or long axis. The −4° direction in 9-ethylguanine easily correlates with the 2° possibility in the hydrochloride. Any other choice would require large changes upon protonation. We therefore choose the short axis assignment for I. There is no ambiguity with transition II in the 9-ethylguanine analysis, and the long-axis direction of II in the hydrochloride is selected over the short-axis possibility. The analysis of transition III is much like that for II. The fact that

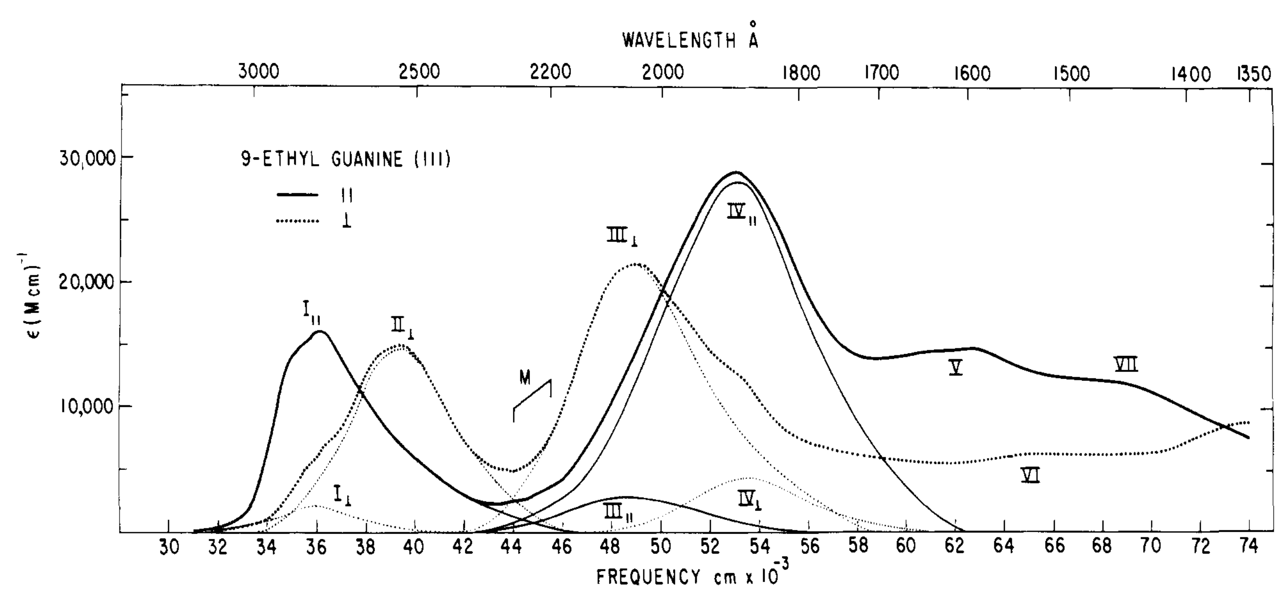

Figure 3. Polarized absorption spectra of the (111) plane of 9-ethylguanine obtained by Kramers-Krönig analyses of the reflection spectra shown in Figure 2.

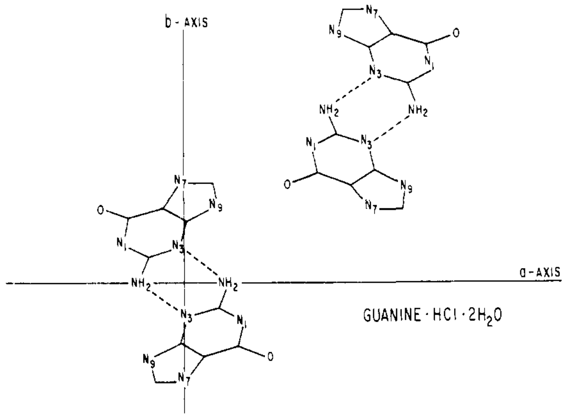

Figure 4. Projection of the four guanine moieties in the unit cell of guanine·HCl·2H$_2$O onto the (001) plane.

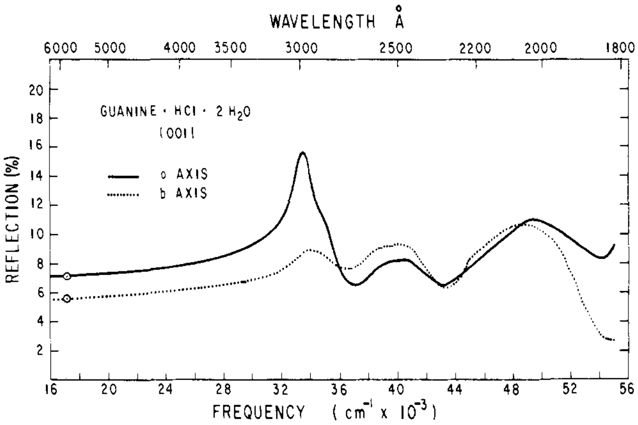

Figure 5. Polarized reflection spectra of the (001) face of guanine·HCl·2H$_2$O. The circled points correspond to reflectivities calculated from refractive index data.

IV seems to be appearing more strongly along *a* than *b* in the hydrochloride suggests that the short-axis direction should be selected for this transition. The final results are tabulated in Table III. Our results are reasonably close to the case 2 possibility in ref 4.

An alternative interpretation of the 50 000 cm^{-1} absorption region (bands III and IV) of the hydrochloride is that protonation causes bands III and IV to become superimposed. Such a situation cannot be ruled out by oscillator strength arguments. For example, the solution spectrum of 9-EG shows $f_{III} = 0.38$ and $f_{IV} = 0.42$ for a combined total of 0.80. The third strong band of protonated 9-EG in solution has an f of about 0.5-0.6. If this third band correlated solely with band III, then a substantial intensification (>30%) must have occurred. If on the other hand both III and IV are superimposed, then a substantial loss of intensity (>25%) is observed. Clearly no choice can be made with the data available.

However, if it be maintained that the transition moment directions are not changed in any *large* way upon protonation, then we believe a resolution of the 9-EG band IV ambiguity can be obtained even if III and IV are superimposed in the protonated form. Here we simply insert the 9-EG results in the hydrochloride structure and calculate the intensities to be expected along the *a* and *b* axes. Thus combining the intensities from III and IV with the −9° choice of IV yields $f_a = 0.76$ and $f_b = 0.87$, whereas with the 41° choice of IV yields 1.82 and 0.58, respectively. The experimental values of 0.6 and 0.8 are consistent only with the −9° choice. Thus the same choice for the polarization of IV is obtained whether III and IV are superimposed or not upon protonation.

Oscillator Strengths. Oscillator strengths obtained from the solution spectra have been given in Tables I and II. The agreement between the solution values and the numbers obtained from the crystal data support the polarization choices made and, in fact, can sometimes be used as an independent criterion for selection. For example, the discarded polarization of II in the hydrochloride would require an oscillator strength of 0.31. This number does not correlate with either the solution value or the 9-ethylguanine result.

Crystal Interactions and Uncertainties. The oriented gas model ignores intermolecular interactions and the concomitant state mixing in crystals. Such mixing alters the observed band intensities and, therefore, the dichroic ratios over those predicted from the simple projection of free molecule transition moment vectors. In view of the complicated nature of the crystal structure of 9-ethylguanine and the fact that we have data on but a single $\hat{k}$ direction for each system, we choose to let the matter rest rather than attempt any type of detailed analysis of the effects of intermolecular coupling. Gauging from the coupling effects noted in adenine·HCl crystals[7] we assign an uncertainty for the oriented gas model of ±10° in the

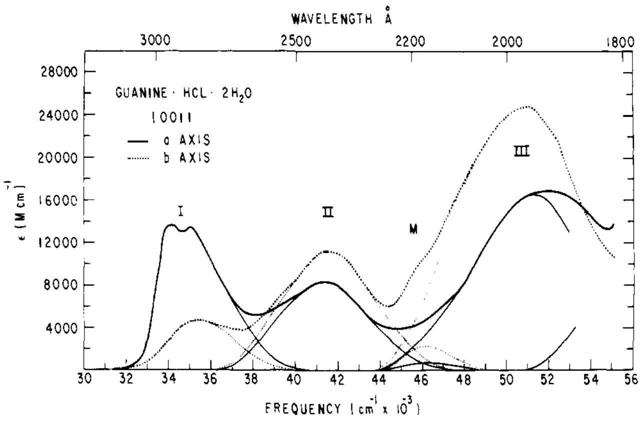

Figure 6. Absorption spectra of the (001) face of guanine•HCl•$2H_2O$ obtained by Kramers–Krönig analyses of the reflection spectra shown in Figure 5.

Table III. Final Transition Moment Directions

	9-Ethylguanine			Guanine•HCl•$2H_2O$		
Transition	ν	f	θ^a	ν	f	θ^a
n → π^* (?)	33300	Weak	⊥			
I	36000	0.16	−4 ± 3°	34500	0.15	2°
II	39400	0.25	−75 ± 3°	41400	0.20	−82°
M	44000			46500		
III	49000	0.41	−75 ± 5°	51000	0.63	−75°
IV	53000	0.48	−9 ± 4°			

[a] θ measured from C(4)–C(5) axis toward C(6).

transition moment directions. Although the angular differences between 9-ethylguanine and protonated guanine may be real and due to ethyl substitution and/or protonation, such a conjecture cannot be validated.

n → π^* Transition. No n → π^* transitions have ever been observed in any guanine moiety. These weak transitions which are polarized perpendicular to the molecular plane must be obscured by the strong, in-plane, $\pi \rightarrow \pi^*$ bands. The only chance of observing such weak bands in absorption spectra would be if the transition energy was below that of the first strong band. Hydrogen bonding of lone pair nitrogen electrons commonly produces a substantial blue shift of n → π^* bands. The observation of such a long wavelength band is therefore precluded in aqueous solution spectra or crystal spectra like guanine•HCl•$2H_2O$. This situation, however, does not occur in crystals of 9-ethylguanine.

In the search for such a band system we have measured the reflection spectra of the (111) face of 9-ethylguanine at 77 K (Figure 8). Perpendicularly polarized transitions should appear only in the ⊥ crystal spectrum. Reference to Figure 8 shows a weak bump in the ⊥ spectrum at 33 300 cm^{-1} (3003 Å). No corresponding feature is observed in the ∥ spectrum, and we tentatively assign the weak band as an n → π^* transition. However, it cannot be ruled out that this band is the shifted ∥ component of 0–0 of transition I. The lowest n → π^* transition in guanine has been predicted by Hug and Tinoco to occur at substantially higher energy.[14]

The M Band. The solution spectrum of protonated guanine shows a weak inflection at 46 500 cm^{-1}. The oscillator strength appears to be in the range 0.01–0.03. Corresponding features are observed in the crystal spectra of both systems examined here. This band may correspond to the third $\pi \rightarrow \pi^*$ transition predicted by Hug and Tinoco to be in this region.[15]

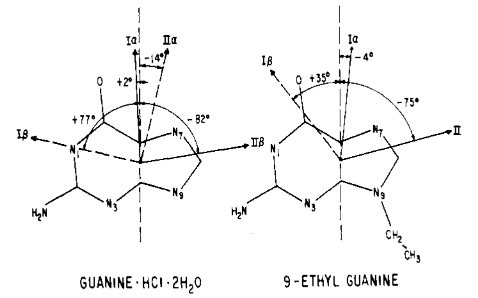

Figure 7. Comparison of possible transition moment directions for the first two in-plane transitions (I and II) observed in guanine•HCl•$2H_2O$ and 9-ethylguanine. The two different directions consistent with a given dichroic ratio are labeled α and β.

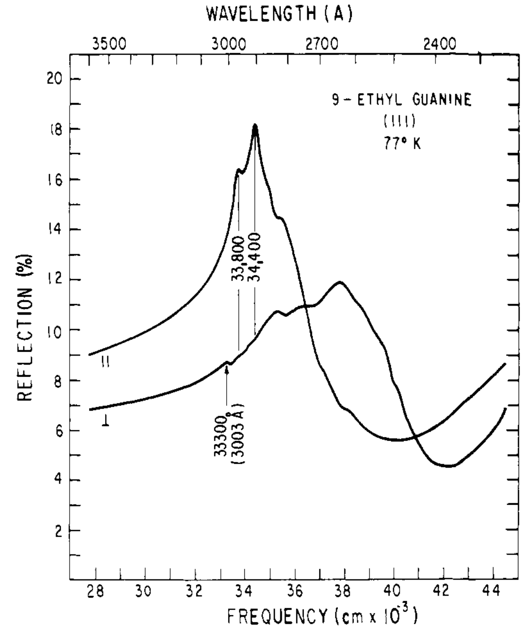

Figure 8. Polarized reflection spectra of 9-ethylguanine at 77 K. The feature at 33 300 cm^{-1} may result from an out-of-plane polarized transition.

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References and Notes

(1) (a) 9-Methyladenine: R. F. Stewart and N. Davidson, *J. Chem. Phys.*, **39**, 255 (1963); (b) protonated adenine: H. H. Chen and L. B. Clark, *J. Chem. Phys.*, **58**, 2593 (1973); (c) see also A. F. Fucaloro and L. S. Forster, *J. Am. Chem. Soc.*, **93**, 6443 (1971) (stretched film spectra of all the bases).
(2) (a) 1-Methylthymine: see ref 1a; (b) 1-methyluracil; W. A. Eaton and T. P. Lewis, *J. Chem. Phys.*, **53**, 2164 (1970).
(3) (a) 1-Methylcytosine: P. R. Callis and W. T. Simpson, *J. Am. Chem. Soc.*, **92**, 3593 (1970); (b) T. O. Lewis and W. A. Eaton, *ibid.*, **93**, 2054 (1971).
(4) P. R. Callis, B. Fanconi, and W. T. Simpson, *J. Am. Chem. Soc.*, **93**, 6679 (1971).
(5) H. H. Chen and L. B. Clark, *J. Chem. Phys.*, **51**, 1862 (1969).
(6) L. B. Clark, *J. Chem. Phys.*, **53**, 4092 (1970).
(7) See ref 5 and L. B. Clark and M. R. Philpott, *J. Chem. Phys.*, **53**, 3790 (1970).
(8) R. Destro and R. E. Marsh, private communication.
(9) Principal indices of refraction at Na D for radiation propagating normal to (111) were found to be $n_{\parallel}$ = 1.71 ± 0.005 and $n_{\perp}$ = 1.60 ± 0.005.
(10) A sublimed film of guanine shows peaks at 35 000, 40 300, 50 300, 54 100,

64 500, and 69 900 cm^{-1}; T. Yamada and H. Fukutome, *Biopolymers*, **6**, 43 (1968).
(11) For the angle convention, see H. DeVoe and I. Tinoco, *J. Mol. Biol.*, **4**, 518 (1962).
(12) I. Iball and H. R. Wilson, *Proc. R. Soc. London, Ser. A*, **288**, 418 (1965).
(13) J. A. Biles, N. F. Witt, and C. F. Poe, *Mikrochem.*, **38**, 593 (1951).
(14) W. Hug and I. Tinoco, *J. Am. Chem. Soc.*, **96**, 665 (1974).
(15) W. Hug and I. Tinoco, *J. Am. Chem. Soc.*, **95**, 2803 (1973).

The Rotation–Inversion Dichotomy in Trialkylamines. Direct ^{1}H DNMR Observation of Distinctly Different Rates of Nitrogen Inversion and Carbon–Nitrogen Bond Rotation in Isopropylmethylethylamine

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Abstract: Examination of the ^{1}H[^{2}H] DNMR spectrum of (isopropyl-*2-d*)(methyl-*d*$_3$)(ethyl-*2,2,2-d*$_3$)amine in CBrF$_3$ reveals for the first time in an acyclic trialkylamine two distinctly different coalescence phenomena one of which can be assigned to nitrogen *inversion* ($\Delta H^{\ddagger} = 8.3 \pm 0.5$ kcal/mol; $\Delta S^{\ddagger} = 5.5 \pm 4.0$ gibbs; $\Delta G^{\ddagger} = 7.5 \pm 0.2$ kcal/mol at -121.3 °C) and the other to C–N bond *rotation* ($\Delta H^{\ddagger} = 5.3 \pm 0.3$ kcal/mol; $\Delta S^{\ddagger} = -3 \pm 2$ gibbs; $\Delta G^{\ddagger} = 5.6 \pm 0.2$ kcal/mol at -157.8 °C).

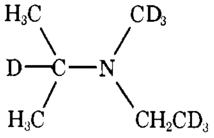
In a recent paper concerning *hindered N-tert*-butyl-*N,N*-dialkylamines, we presented evidence for a stereodynamical itinerary which *couples tert*-butyl rotation and nitrogen inversion.[3] Indeed, it is this pathway that provides the lowest barrier route for equilibrating the *tert*-butyl methyl groups as well as inverting the nitrogen atom. The implication in this dynamical model is that the barrier to isolated *tert*-butyl rotation with *no* concomitant inversion at nitrogen is *higher* than that for the coupled inversion–rotation process.[3] Since the DNMR method is sensitive only to the lowest barrier itinerary of several pathways by which the same net conformational exchange may occur, isolated *tert*-butyl rotation in the hindered *N-tert*-butyl-*N,N*-dialkylamines is invisible to the DNMR technique. This situation precludes the observation of two separate DNMR coalescence phenomena over different respective temperature ranges due to nitrogen inversion in one case and isolated *tert*-butyl rotation in the other. In *less hindered* trialkylamines such as methylamine, dimethylamine, and trimethylamine, theoretical calculations[3] and experimental measurements[4] indicate that the barrier to isolated C–N rotation is consistently *lower* than that for inversion. However, in all of the reported DNMR investigations of less hindered acyclic trialkylamines such as dibenzylmethylamine,[3,5] examination of the ^{1}H DNMR spectra of pertinent diastereotopic groups revealed changes consistent with slowing only nitrogen inversion. Separate DNMR coalescence phenomena attributable to nitrogen inversion in one instance and isolated C–N rotation in the other have not been observed. In these *less hindered* trialkylamines, this situation is due most likely to barriers for C–N rotation which are below the lower limit of DNMR detection (~4 kcal/mol).

We also presented data for a series of *N-tert*-butyl-*N*-chloramines indicating that an electronegative N-substituent such as chlorine effectively *decouples tert*-butyl rotation from nitrogen inversion by *raising* the barrier to inversion relative to isolated *tert*-butyl rotation.[6] In this instance, two separate DNMR coalescence phenomena due to inversion and *tert*-butyl rotation are observed over two different temperature ranges.

This report concerns what we believe to be the first direct observation for an *acyclic trialkylamine* of two separate and distinct ^{1}H DNMR coalescence phenomena one of which can be ascribed to nitrogen *inversion* and the other to C–N bond *rotation.*

Results and Discussion

Examination of the ^{1}H[^{2}H] DNMR spectrum (60 MHz) of **1** (5% v/v in CBrF$_3$; Figure 1) at -65.4 °C shows two singlet resonances at δ 0.98 [6 H, (CH$_3$)$_2$CD] and δ 2.37 [2 H, CH$_2$].



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At lower temperatures (Figure 1), each of these two resonances undergoes two separate and clearly defined coalescence phenomena. At -140.3 °C (Figure 1), the CH$_2$ resonance consists of *one* AB spectrum albeit distorted while the (CH$_3$)$_2$CD resonance is separated into two singlets. The spectral changes for the CH$_2$ group can be rationalized with the aid of eq 1 (projection down H$_2$C–N bond) and the results of theoretical calculations performed in our laboratory.

Using standard bond lengths (C–N, 1.47 Å; C–C, 1.54 Å; C–H, 1.09 Å) and all methyl groups essentially perfectly staggered, a geometry optimization approach using the INDO-A method[7] led to the prediction of four stable conformations, i.e., two pairs of enantiomers. One enantiomer from each pair is illustrated respectively in structures **8** and **9** for nondeuterated **1.** Conformer **8** is calculated to be 0.18 kcal/mol more stable than **9** with both **8** and **9** having optimized CNC bond angles of 113.1°. In **8,** the lone pair is gauche to the two isopropyl methyl groups with a dihedral angle of 159° between the central isopropyl C–H bond and the lone pair (methine hydrogen leaning toward the ethyl group). In **9,** the dihedral angle between the CH$_2$CH$_3$ carbon–carbon bond and the lone pair is 10° with an essentially flat potential minimum for di-