Conformation and Dynamics of Carcinogenic N-Substituted 2-Aminofluorene Compounds Studied by Nuclear Magnetic Resonance Spectroscopy[1]

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Abstract: The conformation and dynamics associated with the aryl–nitrogen bond and the amide bond of 2-(acetylamino)fluorene (AAF) as well as the *N*-hydroxy, *N*-acetoxy, and *N*-sulfate derivatives were investigated as an initial step toward analysis of the orientation of AAF in modified nucleic acids. ^{1}H, ^{13}C, and ^{15}N nuclear magnetic resonance spectroscopic data at low temperatures were obtained for each compound. The equilibrium distribution of conformers about the amide bond in *N*-hydroxy-AAF and *N*-acetoxy-AAF, which was unusually favorable for aryl amides, enabled a detailed structural study. A barrier to internal rotation about the amide bond of 14.4 and 13.7 kcal/mol was computed for *N*-hydroxy-AAF and *N*-acetoxy-AAF, respectively. There was rapid rotation about the aryl–nitrogen bond, with the preferred torsion angle about the aryl–nitrogen bond being interrelated to the conformation about the amide bond and the nature of the N substituent. This resulted in interesting chemical shift differences between subspectra obtained at low temperatures, which were interpreted in terms of conformation.

The potent carcinogen 2-(acetylamino)fluorene (AAF)[2] and its derivatives are some of the most widely studied chemical carcinogens.[3,4] It is generally considered that AAF in vivo is activated by N-esterification into compounds that covalently bind to macromolecules, and in particular to nucleic acids.[3-6] The nucleic acid–carcinogen adducts that are formed by reaction with a carcinogenic aryl amide characteristically possess a nitrogen atom as the link between the carcinogen and the nucleic acid.[3,4,6-9] We have proposed that an understanding of the orientation of an aryl amide carcinogen in modified nucleic acids in solution can be approached from an investigation of the conformation and dynamics associated with the aryl nitrogen atom at the site of attachment.[10] A detailed structural investigation of free aryl amide carcinogens in solution is a logical step in this direction.

Structural studies on AAF and several hydroxylated derivatives have been conducted utilizing X-ray crystallography.[11,12] Each of these compounds possessed a near coplanar orientation between the fluorene ring and the acetamido moiety, with the fluorene ring being cis to the carbonyl oxygen atom.[13] However, information on dynamics cannot be obtained from these data. In this manuscript, we have utilized high-field multinuclear NMR spectroscopy to investigate the conformation and dynamics associated with the nitrogen atom of AAF and its *N*-hydroxy, *N*-acetoxy, and *N*-sulfate derivatives in solution at low temperatures. To our knowledge, this is the first such investigation on any carcinogenic aryl amide as well as any aryl amide with an oxygen attached to the nitrogen atom.

Experimental Section

Nuclear magnetic resonance spectra were recorded in the ^{1}H configuration on a Bruker WM 500 spectrometer and in the ^{13}C configuration on a Bruker WH 270 spectrometer. The ^{15}N measurements were carried out on a Bruker WH 400 located at the NSF facility in NMR spectroscopy at the University of South Carolina. Samples were dissolved in deuterated methanol. Me_4Si was added as an internal standard for the ^{1}H and ^{13}C measurements and chemical shifts were reported on the δ scale. Typical ^{1}H and ^{13}C data acquisition and processing conditions are listed in the legends of the first two figures. The ^{15}N chemical shifts were reported in ppm downfield from anhydrous liquid ammonia by assigning the resonance from external dimethylformamide to 103.8 ppm. The ^{15}N measurements at 40.55 MHz were carried out with gated proton decoupling, using a relaxation delay of 10 s and a flip angle of 33°.

AAF and 2-nitrofluorene were obtained commercially (Aldrich). The ^{15}N-enriched (99%) N-HO-AAF and N-AcO-AAF were obtained on contract from The Midwest Research Institute. The N-substituted compounds were synthesized according to established methods.[14-16] Synthesis of *N*-hydroxy-AAF (N-HO-AAF) can be carried out by partial reductive acetylation of 2-nitrofluorene with zinc, ammonium sulfate, and acetic anhydride in tetrahydrofuran (THF) under argon.[14] Some batches of THF contained copious amounts of peroxide which hampered the progress of the reduction. This problem could be circumvented by modification of the procedure to use of glyme as the solvent with equally good yields of N-HO-AAF. The *N*-acetoxy-AAF (N-AcO-AAF) was prepared by stirring N-HO-AAF under argon in acetic anhydride and pyridine.[15] The sulfonation of N-HO-AAF to form the N-sulfate of AAF (N-O_3SO-AAF) was accomplished by using dicyclohexylcarbodiimide and sulfuric acid in dimethylformamide.[16]

Rate constants were measured by complete band-shape analysis utilizing the DNMR program[17] obtained from The Quantum Chemistry Program Exchange, Indiana University. The sample temperature was determined from the chemical shifts of methanol as measured both before and after the spectral acquisition. The barrier to rotation was determined

(1) A preliminary account of this work has appeared in the program of the "International Symposium on Structure and Dynamics of Nucleic Acids and Proteins", La Jolla, California, September, 1982, p 40.

(2) Abbreviations used: AAF, 2-(acetylamino)fluorene; N-HO-AAF, *N*-hydroxy-2-(acetylamino)fluorene; N-AcO-AAF, *N*-acetoxy-2-(acetylamino)fluorene; N-O_3SO-AAF, 2-(*N*-sulfate acetylamino)fluorene; Me_4Si tetramethylsilane; NOE, nuclear Overhauser effect; NMR, nuclear magnetic resonance.

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Table I. ^{1}H NMR Chemical Shifts in ppm of N-Substituted 2-Aminofluorene Compounds

compd[a]	temp, °C	conf[b]	assignments 1	3	4	5	6	7	8	9	$NCOCH_3$
AAF	52	av	7.81	7.46	7.71	7.72	7.32	7.23	7.50	3.85	2.14
	−50	cis	7.89	7.48	7.75	7.76	7.34	7.26	7.54	3.87	2.15
N-HO-AAF	52	av	7.76	7.57	7.82	7.79	7.36	7.29	7.54	3.91	2.32
	−50	cis	7.80	7.60	7.82	7.80	7.36	7.29	7.55	3.90	2.33
	−50	trans	7.61	7.42	7.92	7.87	7.40	7.35	7.60	3.95	2.00
N-AcO-AAF[c]	52	av	7.62	7.45	7.84	7.80	7.35	7.30	7.53	3.88	2.06
	−50	cis	7.64	7.44	7.80	7.79	7.36	7.31	7.54	3.84	2.21
	−50	trans	7.69	7.51	7.91	7.86	7.40	7.36	7.59	3.91	2.03
$N\text{-}O_3SO\text{-}AAF$	52	av	7.67	7.49	7.79	7.79	7.35	7.28	7.54	3.91	2.42
	−50	cis	7.64	7.47	7.81	7.81	7.36	7.29	7.55	3.89	2.45
	−50	trans	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.97

[a] Concentrations were approximately 25 mg/mL in methanol-d_4. [b] Conformation about the amide bond was determined from the chemical shift of methyl protons and from the ^{13}C NMR spectral data (see text). [c] The chemical shifts of the *O*-acetyl protons were 2.17 at 52 °C and 2.28 (cis) and 2.17 (trans) at −50 °C.

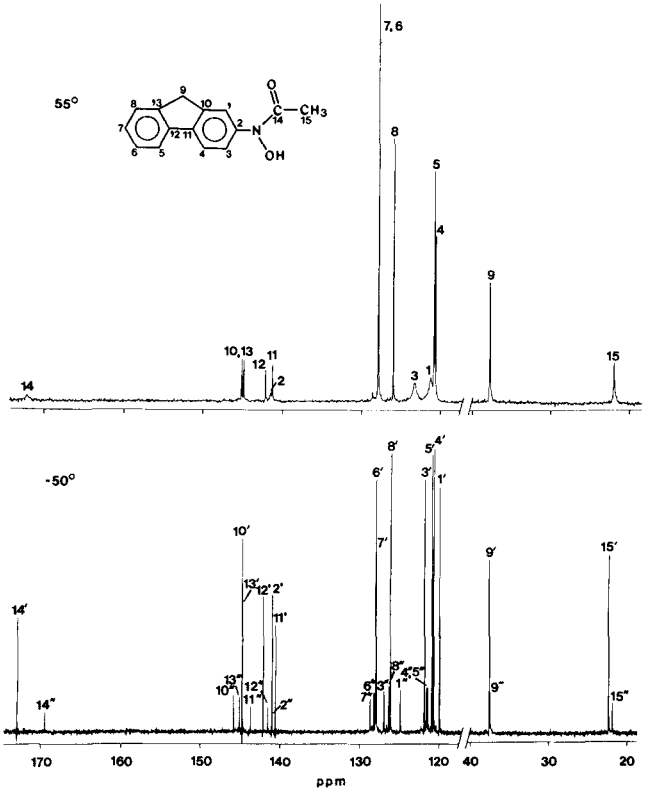

Figure 1. (top) The 67.89-MHz ^{13}C NMR spectrum of N-HO-AAF (25 mg/mL) recorded at 52 °C in methanol-d_4 with resonance assignments. The molecular structure is in the cis form to correspond with the preferred solution conformation. Data acquisition and processing conditions were as follows: sweep width, 15 151 Hz; data size, 16K; flip angle, 45°; number of scans, 18 000; relaxation delay, 0; free induction decay processed with exponential line broadening of 1 Hz. (bottom) The spectrum of N-HO-AAF recorded at −50 °C. Data acquisition conditions were the same as above except that the relaxation delay was 1 s and the number of scans 5000. The free induction decay was processed using a Lorenzian to Gaussian resolution enhancement function with a line broadening of −0.8 Hz and Gaussian broadening of 0.2 of the acquisition time and with zero filling to 32K. The resonances marked with a single prime are those of the cis form, and those double primed belong to the trans form.

from the standard Eyring equation. The propagated error in the free energies was computed from the inherent uncertainties in the measured temperatures and rate constants.[18]

Results

Resonance Assignments in High-Temperature Spectra. The ^{1}H resonance assignments of AAF, N-HO-AAF, N-AcO-AAF, and

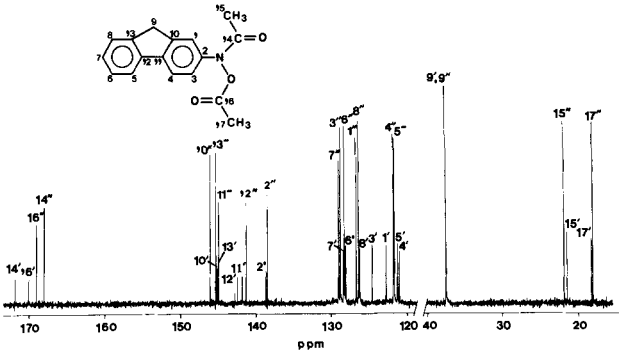

Figure 2. The 67.89-MHz ^{13}C spectrum of N-AcO-AAF (25 mg/mL) recorded at −50 °C in methanol-d_4. The structure is in the trans form to correspond with the preferred solution conformation. The assigned resonances labeled with a single prime correspond to the cis form and those with a double prime correspond to the trans form. Data acquisition conditions were as follows: sweep width, 15 151 Hz; data size, 16K; flip angle, 75°; number of scans, 750; relaxation delay 1 second. The free induction decay was processed using a Lorentzian to Gaussian resolution enhancement function with a line broadening of −1.1 Hz and Gaussian broadening of 0.22 of the acquisition time and with zero filling to 32K.

$N\text{-}O_3SO\text{-}AAF$ were determined from 500-MHz spectra (Table I). All aromatic protons were assigned by the combination of standard homonuclear decoupling studies on the aromatic protons and by decoupling of the benzylic protons in resolution-enhanced spectra. The latter step[19] enabled confirmation of the relatively large long-range couplings which exist between protons ortho and protons para to a benzylic group. Assignment of H-1 and H-8 was further substantiated by the nuclear Overhauser enhancement (NOE) observed for both resonances following saturation of the adjacent benzylic protons.[6] The two acetyl proton resonances of N-AcO-AAF were assigned on the basis of the ^{13}C assignments of the methyl groups and selective heteronuclear decoupling experiments on each acetyl proton resonance.

The ^{13}C assignments in the 67.89-MHz spectra of AAF, N-HO-AAF, N-AcO-AAF, and $N\text{-}O_3SO\text{-}AAF$ recorded at elevated temperature (Table II) were derived mainly from the hydrogen assignments and selective heteronuclear decoupling experiments. Assignments were determined by selective decoupling studies involving each of the one-bond $^1J_{C\text{-}H}$ couplings (155–165 Hz). Spectra were also recorded without decoupling and with very low power selective decoupling of aromatic and benzylic protons so that the fine structure from small couplings could be used in making assignments. The two-bond benzylic coupling constants and the three-bond coupling constants between aromatic carbons and aromatic hydrogens (5–9 Hz) were used to assign C-2, C-10, C-11, C-12, and C-13. The methyl-carbon resonances of N-AcO-AAF were assigned by comparison to that of AAF, N-

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Table II. ^{13}C NMR Chemical Shifts in ppm of N-Substituted 2-Aminofluorene Compounds[a]

compd	temp, °C	conf	assignments												
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
AAF	52	av	118.4	138.9	120.4	120.9	120.5	127.9	127.5	126.0	37.8	145.5	139.3	142.8	144.6	171.7	23.9
	−50	cis	117.7	138.9	119.6	121.0	120.5	127.9	127.5	126.1	37.7	145.3	138.9	142.6	144.4	171.7	23.8
N-HO-AAF	52	av	121.3	141.4	123.8	120.8	121.0	128.0	128.0	126.1	37.8	145.2	141.6	142.3	145.0	172.4	22.0
	−50	cis	119.9	141.0	121.8	120.6	120.9	128.0	127.9	126.1	37.6	144.8	140.6	142.1	144.7	173.0	22.3
	−50	trans	124.8	141.0	126.9	121.5	121.4	128.1	128.6	126.2	37.5	145.8	143.6	141.5	145.0	169.5	21.8
N-AcO-AAF[b]	52	av	125.4	139.0	127.5	121.5	121.5	128.2	128.8	126.3	37.8	146.0	144.3	141.7	145.4	N.D.	21.6
	−50	cis	122.8	138.7	124.6	121.0	121.3	128.0	128.3	126.2	37.5	145.2	141.8	142.5	145.0	171.9	21.4
	−50	trans	126.7	138.5	128.7	121.8	121.7	128.2	129.0	126.4	37.5	146.1	144.9	141.3	145.3	168.0	21.8
N-OSO_3-AAF	−50	cis	122.6	140.3	124.4	120.5	121.1	128.0	128.0	126.2	37.5	144.5	141.1	142.1	144.9	175.1	22.4

[a] See legend in Table I for sample concentrations. The determination of the conformation about the amide bond from the spectral data is described in the text. [b] The chemical shifts of C-16 were 169.7 at 52 °C and 170.1 (cis) and 169.1 (trans) at −50 °C. The chemical shifts of C-17 were 18.1 at 52 °C and 18.3 (cis) and 18.1 (trans) at −50 °C.

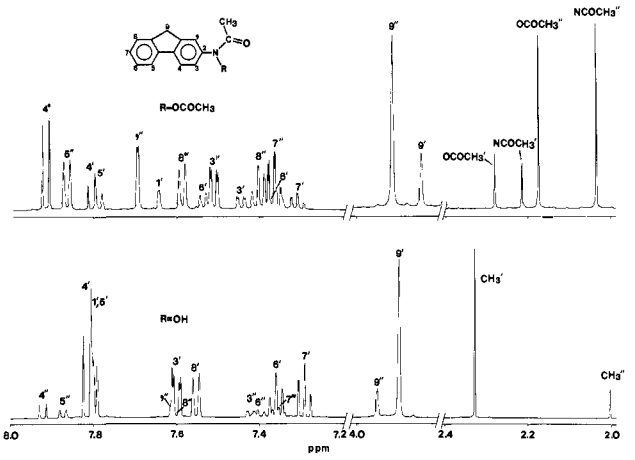

Figure 3. The 500-MHz ^1H NMR spectra recorded at −50 °C in methanol-d_4 of (top) N-AcO-AAF (25 mg/mL) and (bottom) N-HO-AAF (25 mg/mL) The resonance numbers with a single prime correspond to the cis form and the double prime correspond to the trans form. Data acquisition and processing conditions for both samples were as follows: sweep width, 6024 Hz; data size, 32K; flip angle, 75°; relaxation delay, 0; number of scans, 64; free induction decay was multiplied by a Lorentzian to Gaussian resolution enhancement function with a line broadening of −1.0 Hz and Gaussian broadening of 0.22 of the acquisition time.

HO-AAF, and N-O_3SO-AAF. The carbonyl resonances of N-AcO-AAF were assigned by selective decoupling of the *N*-acetyl and *O*-acetyl protons. All assignments were in accord with results expected from empirical substituent effects.

Temperature Dependence of Spectra and Resonance Assignments in Low-Temperature Spectra. The spectral patterns of N-HO-AAF and N-AcO-AAF exhibited a strong dependence on temperature. At 52 °C, the C-1, C-3, C-11, and C-14 resonances in the ^{13}C spectra of N-HO-AAF (Figure 1) and N-AcO-AAF were unusually broad. Lower temperatures resulted in a broadening of the other resonances. At −50 °C each resonance was narrow and had been split into two resonances of unequal intensity (Figures 1 and 2). A temperature dependence was also observed in the ^1H spectra, as illustrated in the low-temperature spectrum of N-HO-AAF and N-AcO-AAF (Figure 3). The temperature dependence was characteristic of an exchange between two conformations having differences in chemical shifts and in population.[17,18,20] At low temperature, the residence time in each conformation was long enough to directly measure separate spectral parameters, whereas at the elevated temperature a weighted average was observed.

A small temperature dependence was observed in the ^{13}C spectra for AAF in methanol. The ^{13}C resonances were sharp at all temperatures except in the 20–40 °C range. Here a small amount of line broadening was detected for C-1, C-3, and C-11. It was not possible to confirm the presence of a second set of resonances at low temperature in either the ^{13}C or ^1H spectra. A temperature dependence was detected for the *N*-acetyl proton resonance of N-O_3SO-AAF, including the presence of two separate resonances at low temperatures which were confirmed by saturation transfer experiments. A ^{13}C temperature dependence study was not conducted because of the instability of N-O_3SO-AAF at high temperature and the long signal averaging times required for the ^{13}C data acquisition.

It is noteworthy that the ^1H and ^{13}C spectral parameters of the major and minor components of N-HO-AAF and N-AcO-AAF could in most cases be resolved in the high-field spectra (Figure 1–3). Accordingly, complete spectral analysis of these fairly large bio-organic molecules was possible, with assignments being made by the same types of decoupling studies already described. In several cases saturation transfer difference spectra[21] were recorded

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Table III. ^{15}N NMR Chemical Shifts of *N*-Hydroxy- and *N*-Acetoxy-2-(acetylamino)fluorene[a]

compd	temp, °C	conf	shift
N-HO-AAF	32	av	181.3
	−50	cis	181.4
	−50	trans	179.6
N-AcO-AAF	32	av	188.9
	−50	cis	190.5
	−50	trans	186.8

[a] Compounds dissolved in methanol-d_4 at a concentration of 25 mg/mL. Chemical shifts (δ) are reported in ppm downfield from external ammonia. The determination of the conformation about the amide bond is described in the text.

Table IV. Nuclear Overhauser Enhancements (NOE) for N-Substituted 2-(Acetylamino)fluorene Compounds[a]

compd	protons saturated	NOE, % H-1	H-3	H-8
N-HO-AAF	CH_2	3	0	3
	N-CH_3	0	0	0
N-AcO-AAF	CH_2	2	0	2
	N-CH_3	0.4	0.4	0
N-O_3SO-AAF	CH_2	3	0	3
	N-CH_3	0	0	0

[a] Samples dissolved in methanol-d_4 (25 mg/mL) without degassing. Spectra were recorded at 50 °C.

to assign corresponding resonances of the two subspectra. For example, in the case of N-AcO-AAF in methanol at −50 °C, saturation of the *N*-acetyl proton resonance at δ 2.21 resulted in a transfer of saturation to the *N*-acetyl proton resonance at δ 2.03. Subtraction of the spectrum so obtained from a spectrum recorded without saturation, but otherwise under identical conditions, yielded a difference spectrum consisting only of the two resonances due to saturation transfer.

Chemical shift differences were the primary basis for assigning subspectra to a specific cis or trans conformation about the amide bond. The upfield shifts of the C-1, C-3, and C-11 carbon resonances as well as the downfield shift of the *N*-acetyl proton resonance correlated to the cis orientation between the fluorene ring and the carbonyl oxygen. The reverse was true for the trans form. A detailed discussion of the use of the spectral parameters to identify the cis and trans forms will be discussed in another part of the manuscript. The relative intensities of the specific subspectra, which were consistent between ^1H, ^{13}C, and ^{15}N spectral data, were the basis for assignment of the ^{15}N subspectra (Table III).

Chemical Shift Differences between Cis and Trans Conformations. The ^{13}C, ^1H, and ^{15}N spectra of N-HO-AAF and N-AcO-AAF (Figures 1–3, Tables I–III) at low temperature closely resembled each other except the chemical shift data for the major and minor components were reversed. This interesting result was a consequence of a similarity in the spectral parameters associated with both cis forms and both trans forms, but a reversal in relative populations between the two compounds. The ^{13}C chemical shift differences between subspectra for corresponding resonances were by far the largest for C-1, C-3, C-11, and C-14. The chemical shift differences were 3–5 ppm for these resonances, and only 1.2 ppm or less for all other carbon resonances (Table II). Most ^1H chemical shift differences of aromatic protons, except for H-1 and H-3, were less than 0.06 ppm. The largest ^1H chemical shift difference between subspectra was in the *N*-acetyl protons which ranged from 0.18 to 0.48 ppm for N-HO-AAF, N-AcO-AAF, and N-O_3SO-AAF.

The ^{13}C and ^1H spectral data for N-HO-AAF and N-AcO-AAF exhibited similarities in the direction of the chemical shift changes between subspectra of the cis and trans forms. All aromatic ^{13}C resonances except C-12 and C-2 of the cis form were upfield of the corresponding resonances of the trans form (Table II). Likewise, all aromatic proton resonances of the cis form were upfield of those of the trans form with the exception of H-1 and H-3 of N-HO-AAF (Table I). The low population of the trans form prevented similar measurements on AAF and N-O_3SO-AAF.

The chemical shift differences between selected pairs of resonances could imply that the methylene bridge had little effect on the orientation of the acetamido moiety. The chemical shift difference of H-1 between subspectra was approximately equal to that of H-3, and H-6 was equal to that of H-8 (Table I). Similarly, the chemical shift difference of C-1 equalled C-3, C-4 equalled C-10, C-5 equalled C-13, and C-6 equalled C-8 (Table II).

Population of Cis and Trans Forms. The populations, which were measured from the methyl resonances in deuterated solvents at −50 °C, were highly dependent on the nature of the N substituent. The percentage of cis form was in excess of 99% in AAF. For N-O_3SO-AAF, N-HO-AAF, and N-AcO-AAF the populations of the cis form were 92%, 83%, and 23%, respectively, with the remainder being in the alternate trans form.[22]

Barrier to Internal Rotation about the Amide Bond. The equilibrium distribution of conformers of N-HO-AAF (10 mg/mL) and N-AcO-AAF (10 mg/mL) in methanol-d_4 solution was suitable for calculation of the barrier to rotation about the amide bond. The *N*-methyl proton resonance of N-HO-AAF and both the *N*-methyl and *O*-methyl proton resonances of N-AcO-AAF were utilized in a complete band-shape analysis. A barrier of 14.4 ± 0.1 kcal/mol and a rate constant of 15.25 ± 0.25 s^{-1} for N-HO-AAF at −1.3 ± 1.0 °C were computed from the spectral data. For N-AcO-AAF at −0.8 ± 1 °C, the barrier and rate were 13.7 ± 0.1 kcal/mol and 59.5 ± 0.5 s^{-1}. The average deviate over a temperature range of −26 to +3 °C was 0.07 kcal/mol for N-HO-AAF. The average deviate for N-AcO-AAF was 0.05 kcal/mol in the temperature range of −26 to +12 °C.

Nuclear Overhauser Enhancement. Nuclear Overhauser enhancements (NOE) were measured for N-HO-AAF, N-AcO-AAF, and N-O_3SO-AAF (Table IV). Saturation of the benzylic protons induced a 2–3% NOE at the adjacent H-1 and H-8 protons. This result is similar to that previously reported in the structure elucidation of *N*-(deoxyguanosin-8-yl)-2-aminofluorene.[6] A much smaller NOE was observed for H-1 and H-3 in N-AcO-AAF when the methyl protons of the N-acetyl moiety were saturated. No such NOE was detected for H-1 and H-3 in N-HO-AAF or N-O_3SO-AAF.

Discussion

Though the general phenomenum of restricted rotation about amide bonds is well known,[20,23,24] the case for aryl amides has been difficult to investigate because the equilibrium is almost always shifted far in favor of one conformation or else the barrier may in some instances be too small.[20,23-25] This has greatly limited the amount of information available about conformation and dynamics, as well as the associated spectral parameters. Acetanilide is generally considered to be in the cis form at a level in excess of 99.9%.[23] In *N*-methylacetanilide, the alternate trans form is at a level of 99.5%.[23] However, because of the low populations of the minor component, information on barriers to rotation as well as ^{13}C and ^{15}N parameters of subspectra have not to our knowledge been reported. The present data on N-HO-AAF and N-AcO-AAF are unusually favorable cases for aryl amides because the relative populations enabled the measurements required for computation of energy barriers and for interpretation of conformation from the subspectra.

(22) The error in measurement was ±2%, except for N-O_3SO-AAF which was ±5% due to impurities in the methyl region of the spectrum. The populations were also highly dependent on solvent, especially in the case of N-OH-AAF (unpublished data).

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(24) Y. Shvo, E. C. Taylor, K. Mislow, and M. Raban, *J. Am. Chem. Soc.*, **89**, 4910 (1967).

(25) An exception is the class of aryl amides with an ortho substituent,[23] which can be considered as a special case not applicable to the present study because of the interaction between the ortho substituent and the acetamido moiety.

The barrier to rotation about the amide bond of 14.4 kcal/mol in N-HO-AAF and 13.7 kcal/mol in N-AcO-AAF indicated little difference between the two N substituents. This is considerably lower than the 20-kcal/mol barrier found in common amides such as dimethylamide.[23] Conjugatative and electron-withdrawing effects of the substituents cannot be distinguished as the primary cause of lowering of the barrier without additional data. The N substituent had a significant effect on the population of conformers. Each of the N substituents caused an increase in the population of the trans form at the expense of the cis form as compared to the parent compound AAF, but the latter remained the preferred conformation in N-HO-AAF and N-O_3SO-AAF. Only in N-AcO-AAF was the trans form the major conformer.

The presence of major conformational differences other than simple cis–trans isomerism is implicated by the large chemical shift differences in carbon chemical shifts of the resonances ortho and para to the acetamido moiety. Since this has not previously been reported for any aryl amides, it may deserve special attention in order to interpret the data. The C-1, C-3, and C-11 chemical shifts were highly sensitive to whether the compounds were in the cis or trans form about the amide bond because the conformation about the amide bond was interrelated to the orientation about the fluorenyl–nitrogen bond. In both N-HO-AAF and N-AcO-AAF the carbons ortho and para to the acetamido moiety (C-1, C-3, and C-11) were 3–5-ppm upfield in the cis form as compared to the trans form primarily because of delocalization of π-electron density from the nitrogen to the fluorene ring. Delocalization should be greatest in an exact coplanar orientation of the acetamido and fluorene planes. Theoretically, the resonance energy due to conjugation decreases by a $\cos^2 \phi$ function,[26-28] where in this case ϕ is the torsion angle between the fluorene and acetamido planes. Thus the upfield shifts can be accounted for from the increase in electron density. However, an alternative nonconjugative mechanism by which the ^{13}C chemical shifts should depend on angle arises from anisotropy of the acetamido bond network and steric crowding between the acetamido moiety and the ortho hydrogens. Though no theoretical dependence between these factors is available, it is reasonable to assume that the magnitude of the chemical shift change would be greatest in a coplanar orientation. We propose that an empirically based estimate for the nonconjugative effects can be obtained from analysis of the low-temperature ^{13}C data on *N*-acetylcarbazole[29] because the *N*-acetyl moiety is almost coplanar with the ring system in *N*-acetylcarbazole. A downfield shift of 3.0 ppm was reported for the ortho carbon closest to the carbonyl oxygen. Chemical shift differences at other positions of *N*-acetylcarbazole were approximately 1 ppm, with the magnitude for the chemical shift difference of the para carbon being 0.7 ppm. By analogy an estimated 1.5-ppm deshielding of C-1 and C-3 would be expected from nonconjugative effects in AAF and the N-substituted compounds due to rapid rotation about the fluorenyl–nitrogen bond. The large *upfield* shifts actually observed (Table II) indicated that the conjugative effects predominated over nonconjugative effects in determining the chemical shift differences for C-1, C-3, and C-11 and that the cis form had more coplanar character than the trans form. Thus the large upfield shifts of the carbons ortho and para to the acetamido moiety were characteristic of the cis form because the cis form was closer to coplanarity than the trans form. Apparently a small fraction of the electron density was delocalized from the nitrogen to the second aromatic ring as evidenced by the upfield shift of C-5, C-7, and C-13.

In contrast to the ^{13}C spectral results discussed above, it is well known from high-temperature ^1H NMR studies of ortho-substituted acetanilide compounds that the anisotropy of the acetamido bond system dominates over the conjugative effect yielding a downfield shift for ortho protons as compared to the parent compound acetanilide.[23] On the basis of these results, H-1 and H-3 of AAF compounds in a coplanar cis form may be expected to be significantly downfield from those of the trans form because the ortho protons are moved away from the deshielding zone of the carbonyl in the trans form. However, for N-HO-AAF, the ortho protons were deshielded by only 0.2 ppm, while in N-AcO-AF the ortho protons were slightly shielded in the cis form (Table I). This is apparently the first case in which shielding was observed for ortho protons in a trans conformation. This may be attributed to a larger than usual average torsion angle in the N-substituted AAF compounds and a subsequent placement of the ortho protons in a less deshielded region of the anisotropic field of the acetamido bond system. Therefore, caution should be used in assigning cis and trans subspectra on the basis of chemical shifts of H-1 and H-3. The chemical shifts of H-4, H-5, H-6, H-7, and H-8 appear to provide an alternative method for assigning cis and trans subspectra, since the chemical shifts of the cis form were consistently upfield of those of the trans form.

The ^{15}N resonances were downfield in the cis form for both N-HO-AAF and N-AcO-AAF. The chemical shift difference of 1.8 and 3.7 ppm respectively indicated that the orientation about the adjacent aryl–nitrogen bond did not have the large effect known to occur for aryl amines in general. Thus the ^{15}N chemical shift of aryl amides does not appear to be as sensitive as indicator of conformation as may be hoped for from delocalization considerations.

The lack of detection of subspectra from possible hindered rotation about the aryl–nitrogen bond[30] may in principle be due to a small barrier to internal rotation or to an equilibrium shifted far in favor of one conformer.[20] However, the near identity between the chemical shift differences of H-1 and H-3 between cis and trans forms was indicative of the preferred orientations about the fluorenyl–nitrogen bond. Since the ^1H chemical shift differences for the ortho protons were strongly dependent on the anisotropic acetamido bond system, the chemical shift data for H-1 and H-3 strongly suggested that there was rapid rotation about the fluorene–nitrogen bond and that the methylene bridge had little effect on whether the oxygen was directed toward H-1 or toward H-3. Therefore, the small barrier to rotation about the aryl–nitrogen band is the reason that additional subspectra were not detected.

It should be possible to obtain information about the average aryl–nitrogen torsion angle from NOE measurements because of the proximity of the methyl protons to the protons ortho to the acetamido moiety. Examination of molecular models revealed that in a hypothetical coplanar-trans conformation, close approach and severe steric crowding between the methyl group and the ortho protons would result in a relatively large NOE for H-1 and H-3 when the methyl protons were saturated. No significant NOE would be expected for the cis form regardless of the torsion angle. Thus deviation from coplanarity for the trans form should result in progressively smaller NOEs. The magnitude of NOEs is an indication of the relative distances and geometry of the methyl group to H-1, H-3, and the methylene protons. The observation that the NOE induced as a result of saturation of the methylene protons was larger than that induced by the *N*-methyl protons indicated that the orientation of the fluorene–nitrogen bond had positioned the methyl protons further from H-1 and H-3 than from the adjacent methylene protons. This restriction placed the acetamido moiety orthogonal or nearly orthogonal to the plane of the fluorene ring. The slightly larger NOE for H-1 and H-3 of N-AcO-AAF also indicated a near orthogonal-trans conformation, but with less orthogonal character than in N-HO-AAF and N-O_3SO-AAF. The upfield shift of the *N*-acetyl proton resonance in the trans form of N-HO-AAF and N-O_3SO-AAF was also consistent with an orthogonal orientation because of ring current deshielding of the *N*-acetyl protons by the fluorene ring. This result was consistent with upfield shifts previously reported

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in ortho-substituted acetanilide compounds.[23]

The predominant cis and nearly coplanar conformation found for AAF in solution resembled results previously reported for acetanilide in solution.[23] Though no attempt was made to estimate the average torsion angle about the aryl–nitrogen level of the cis form of AAF in solution, it may be thought of as being a compromise between the unfavorable steric interactions between the oxygen and the ortho protons and the resonance energy stabilization from π-electron delocalization from the nitrogen to the fluorene ring system. In the trans form, the average torsion angle for the aryl–nitrogen bond is predicted to be approximately 90° in solution, like that of N-substituted AAF compounds. Unfavorable steric interactions between the methyl group and the ortho protons apparently contribute to the destabilization and a coplanar conformation.

X-ray crystallography results have been reported for AAF[11] and N-HO-AAF,[12] each of which was in the cis form. The torsion angle between the planes of the fluorene ring and the acetamido moiety for AAF and N-HO-AAF was 42.2° and 16.6°, respectively. The reason for the apparent deviation from near coplanarity for AAF in the solid state is unclear. In solution, the upfield shifts of the carbons ortho and para to the acetamido moiety in the cis form of AAF and N-HO-AAF indicated that the averge torsion angle of the cis form of AAF was similar to and possibly less than that of N-HO-AAF.

Conclusion

The conformation and dynamics about the aryl–nitrogen bond and the amide bond of AAF and several aryl amides with oxygen on the nitrogen atom have been investigated for the first time. An unusually favorable equilibrium distribution in N-HO-AAF and N-AcO-AAF enabled calculation of the barrier to rotation about the amide bond. The barrier for the amide bond was 14.4 ± 0.1 kcal/mol for N-HO-AAF and 13.7 ± 0.1 kcal/mol for N-AcO-AAF. Rotation about the aryl–nitrogen bond was rapid at the low temperatures that were utilized, but pronounced conformational preferences were observed. In the cis conformation about the amide bond, the acetamido moiety was approximately coplanar to the fluorene ring while in the trans conformation the acetamido and fluorene moieties were nearly orthogonal. This was manifested in large chemical shift differences between subspectra of the resonances from carbons ortho and para to the acetamido moiety and smaller differences in the second ring due mainly to delocalization of π-electron density from the nitrogen to both aromatic rings of fluorene. Resonances of the ortho protons could be shifted either upfield or downfield depending on the average torsion angle about the aryl–nitrogen band. The NMR data suggest a greater access to the conformational domains than is suggested from X-ray crystallography data. The results should be of value for interpretations concerning the orientation of the AAF moiety in modified oligonucleotides.

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On the Structure of Anions Derived from Cycloheptatriene in the Gas Phase

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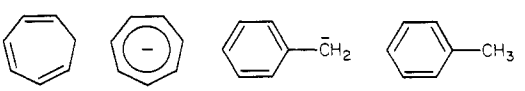
Abstract: Fourier transform mass spectrometric studies of hydrogen–deuterium exchange in gas-phase anion–molecule reactions at 6×10^{-6} torr have led to the conclusion that a species with the time-averaged symmetry of the cycloheptatrienyl anion (**2**) is formed by proton abstraction from cycloheptatriene (**1**) and then rapidly rearranges to the benzyl anion (**3**) when OD^- (and possibly ND_2^-) is employed as the abstracting base. These conclusions are supported by the results for **1**-*7,7-d*$_2$ or **1**-*1,2,3,4,5,6-d*$_6$ and OH^-, which show the required scrambling of the label in **3**, and by results for $C_7H_7^-$ from toluene, which parallel those for $C_7H_7^-$ from **1** with ND_3 or D_2O but not with CD_3OD. Anion **2** undergoes exchange but does not rearrange to benzyl in the presence of CD_3OD. Evidence was not obtained for electron autodetachment from **3** formed from **1**. Since such behavior is expected for unimolecular isomerization, it is concluded that rearrangement occurs predominantly in a complex between **2** and water.

Previously reported thermochemical,[3] hydrogen–deuterium (H–D) exchange,[4] and infrared multiphoton electron detachment[5] data have indicated that a stable cycloheptatrienyl anion (**2**) can be formed by deprotonation of cycloheptatriene (**1**) in the gas phase. Isomerization of **2** ($\Delta H_f°$ = 51.2 ± 3 kcal/mol)[3] to the more stable benzyl anion (**3**) ($\Delta H_f°$ = 23.8 ± 3 kcal/mol)[3] would



1 2 3 4

be expected if the energy barrier(s) for this reaction were relatively small. However, this isomerization has not been observed either in the gas phase[3-5] or in solution.[6-8] We now report H–D exchange and isotopic labeling studies (by Fourier transform mass spectrometry (FTMS))[9] which demonstrate that this thermody-

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