Nucleophilic Substitution on the Ultimate Hepatacarcinogen *N*-(Sulfonatooxy)-2-(acetylamino)fluorene by Aromatic Amines

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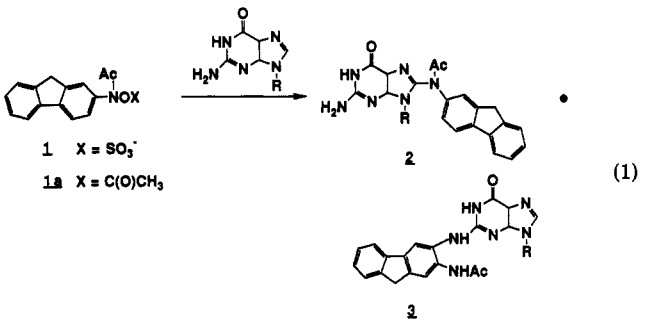
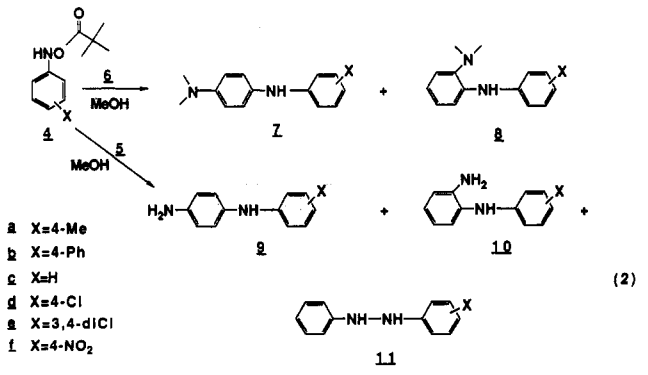
The kinetics and products of the reactions of the title compound 1 with aniline (5) and *N,N*-dimethylaniline (6) were investigated in MeOH. Addition of 5 (0.1–0.4 M) to a solution of 1 in MeOD-d_4 has no effect on the overall rate of decomposition of 1 but generates a number of adducts (20–24) in moderate to high yield. The yields of all solvolysis products, except the rearranged *O*-sulfates 18 and 19, are suppressed by the addition of 5. The kinetic and product data are consistent with an S_N1 mechanism (Scheme IV) in which 18 and 19 are generated by internal return from a tight ion pair, but all other products are generated by nucleophilic attack on a free nitrenium ion or solvent separated ion pair. The reaction of 6 with 1 shows similar characteristics to that of 5 with the exception that 6 reduces 1 in moderate yield to generate 2-(acetylamino)fluorene (25). This reduction occurs in competition with reaction to generate adducts (26, 27) similar to those obtained with 5. Kinetic and product data indicate that 25 is generated by reaction of 6 with a nitrenium ion intermediate. The differences in the behavior of 5 and 6 may be explained by cyclic voltammetry results which show that 6 is oxidized in MeOH more readily than 5 by about 2.5 kcal/mol. The reaction of 1 with 5 and 6 is considerably different from the reactions of the same amines with the *N*-aryl-*O*-pivaloylhydroxylamines, which were previously shown to proceed via an S_N2 mechanism. This change in mechanism may be attributed, in part, to increased steric hindrance at the nitrogen of 1 due to the *N*-acetyl group.

N-(Sulfonatooxy)-2-(acetylamino)fluorene (1) is a putative ultimate carcinogenic metabolite of 2-(acetylamino)fluorene.[1] It and its acetic acid ester analogue 1a have been shown to react with deoxyguanosine residues of DNA in vivo and in vitro and with deoxyguanosine itself, in low yield, to generate the adducts shown in eq 1.[2] These



(1)

adducts are thought to be responsible for the carcinogenic properties of 1.[2e,f] Previously, we have shown that the *N*-aryl-*O*-pivaloylhydroxylamines 4 react with aniline (5) and *N,N*-dimethylaniline (6) to produce the adducts 7–11 (eq 2) via an S_N2 mechanism.[3] It is not clear what the



(2)

effect of the *N*-acyl group of 1 would be on the mechanism of nucleophilic substitution by arylamines since the acyl group adds to the steric bulk around nitrogen but is a well-known activator of S_N2 substitutions at an α-carbon. The effect of the change in leaving group from carboxylate to sulfate is also not known. Therefore, we commenced a study of the reactions of 1 with 5 and 6 in dry MeOH. The results of that study, which show that a large number of adducts are generated by an apparent S_N1 mechanism, are presented herein.

Experimental Section

The synthesis of 1 as its K^+ salt and the purification of 5, 6 and MeOH have been previously described.[2d,3,4] The 3- and 1-hydroxy-2-(acetylamino)fluorenes (14 and 15) were available from a previous study,[5] and the K^+ salts of the corresponding *O*-sulfates (18 and 19) were synthesized from 14 and 15 by published procedures.[6] *N*-Hydroxy-2-(acetylamino)fluorene (17) and 2-(acetylamino)fluorene (25) were also prepared as described in the literature.[7]

Kinetics. The kinetics of the decomposition of 1 in MeOD-d_4 (99.8% deuterated) at 35 ± 1 °C in the presence or absence of 5 or 6 (0.1–0.4 M) were monitored by ^1H NMR spectroscopy at 300 MHz. The acyl methyl peaks of 1 and its various decomposition products, which appear in the range from ca. δ 2.7 to 1.9, were used to monitor concentrations. Initial concentrations of 1 of ca. 4 mM were obtained by injection of 20 μL of a 0.1 M stock solution of 1 in DMF-d_7 (99.5% deuterated) into 0.5 mL of the MeOD-d_4 solution previously incubated in the probe of the NMR spectrometer for at least 20 min. Automatic data collection was initiated as soon as temperature equilibrium was reestablished (ca. 4–5 min). Methods used to fit normalized peak area vs time data to first-order rate equations have been described.[3,8] Data were routinely taken for at least 5 half-lives of the decomposition of 1.

† On leave from the University of Mysore, India.

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Products. Reaction products were isolated from larger scale reactions (5 mM in 1, 50-mL volume) done in the presence or absence of 1.0 M **5** or **6** in dry MeOH under a N_2 atmosphere. These reactions were done at 25 °C, and the course of the reactions was monitored by HPLC (see below). Reactions appeared to be complete within 20–24 h, which is in accord with a rate constant of 9.9×10^{-5} s^{-1} for the decomposition of **1** in MeOD-d_4 determined at 25 °C by NMR. The MeOH was then removed by rotary evaporation, and **5** or **6** was removed, if present, under reduced pressure (~0.1 Torr) to yield a brown to black solid residue. This material was taken up into methylene chloride and filtered to remove insoluble materials that proved to be inorganic salts by ^1H and ^{13}C NMR in DMSO or D_2O, and the components of the mixture were separated by preparative layer chromatography on silica gel (CH_2Cl_2/EtOAc eluent (5/1, 3/1, or 3/2). Products were subjected to repeated chromatography until HPLC analysis (μ-Bondapak C-18 or Altex C-8 column, MeOH/H_2O (60/40, 70/30, or 80/20) containing 0.05 M 1/1 HOAc/KOAc as eluent, 1 mL/min) with UV detection at 250 nm showed only one peak. It was not possible to separate two of the minor isomeric products (**23** and **24**) by chromatographic means. These were analyzed as a mixture. Some materials were recrystallized prior to characterization. Some solvolysis products (**14**, **15**, **17–19**) were identified by comparison to authentic samples. The redox products **25** and **28** were identified by comparison to an authentic sample (**25**) and to physical and spectral data in the literature (**28**).[9] All other materials were previously unreported. IR and ^1H NMR data for all new compounds are included in the supplementary material, with ^{13}C NMR spectra and selected H,H-COSY spectra.

2-Methylfluoreno[2,1-*d*]oxazole (12): recrystallized from MeOH, mp 166–167 °C; ^{13}C NMR (75.5 MHz, CD_2Cl_2) δ 14.7 (q), 34.1 (t), 116.3 (d), 118.4 (d), 120.2 (d), 124.9 (s), 125.5 (d), 127.0 (d), 127.3 (d), 140.1 (s), 141.6 (s), 142.0 (s), 143.1 (s), 148.3 (s), 164.1 (s); high-resolution MS *m/e* 221.0837, $C_{15}H_{11}NO$ requires 221.0841.

2-Methylfluoreno[2,3-*d*]oxazole (13): recrystallized from MeOH, mp 143–144 °C; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.7 (q), 36.6 (t), 101.4 (d), 115.5 (d), 119.7 (d), 125.1 (d), 126.7 (d), 126.9 (d), 139.0 (s), 139.5 (s), 141.1 (s), 141.3 (s), 143.8 (s), 150.8 (s), 164.0 (s); high-resolution MS *m/e* 221.0838, $C_{15}H_{11}NO$ requires 221.0841.

4-Methoxy-2-(acetylamino)fluorene (16): mp 186–188 °C; ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 24.2, 37.0, 55.2, 100.5, 107.9, 122.3, 123.9, 124.4, 125.1, 126.6, 139.7, 140.3, 142.1, 145.2, 155.2, 168.3; high-resolution MS *m/e* 253.1125, $C_{16}H_{15}NO_2$ requires 253.1103. Treatment of 4-hydroxy-2-(acetylamino)fluorene[5] (**31**) with diazomethane yielded identical material.

***N*-(4′-Aminophenyl)-2-(acetylamino)fluorene (20)**: recrystallized from MeOH, mp 194–198 °C; ^{13}C NMR (75.5 MHz, DMSO-d_6, 70 °C) δ 22.8 (q), 36.1 (t), 114.0 (d), 119.5 (d), 119.6 (d), 123.2 (d), 124.6 (d), 125.2 (d), 126.2 (d), 126.4 (d), 128.2 (d), 131.8 (s), 138.6 (s), 140.2 (s), 142.4 (s), 142.9 (s), 143.3 (s), 147.4 (s), 169.3 (s); high-resolution Ms *m/e* 314.1445, $C_{21}H_{18}N_2O$ requires 314.1419.

3-(Phenylamino)-2-(acetylamino)fluorene (21): recrystallized from MeOH/CH_2Cl_2, mp 209–212 °C; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 24.7 (q), 36.9 (t), 115.7 (d), 116.6 (d), 118.2 (d), 119.6 (d), 120.2 (d), 125.0 (d), 126.5 (d), 126.7 (d), 129.5 (d), 131.6 (s), 132.5 (s), 138.7 (s), 141.0 (s), 141.1 (s), 143.6 (s), 145.7 (s), 168.7 (s); high-resolution MS *m/e* 314.1393, $C_{21}H_{18}N_2O$ requires 314.1419.

1-(Phenylamino)-2-(acetylamino)fluorene (22): recrystallized from MeOH, mp 231–232 °C; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 24.7 (q), 35.5 (t), 114.7 (d), 118.3 (d), 119.8 (d), 120.7 (d), 124.9 (d), 125.0 (d), 126.5 (d), 126.9 (d), 128.3 (s), 129.5 (d), 133.8 (s), 139.1 (s), 140.5 (s), 141.4 (s), 142.7 (s), 145.2 (s), 168.7 (s); high-resolution MS *m/e* 314.1397; $C_{21}H_{18}N_2O$ requires 314.1419.

3-(4′-Aminophenyl)-2-(acetylamino)fluorene (23) and 1-(4′-Aminophenyl)-2-(acetylamino)fluorene (24). These materials were obtained as a chromatographically inseparable yellow wax: IR (KBr) 1650, 1507 cm^{-1}. ^1H NMR showed that the materials were obtained in a ratio (**23**/**24**) of ca. 7/1. The ^1H NMR of the major isomer **23** could be obtained without interference from **24**: ^1H NMR (500 MHz, CD_2Cl_2) δ 2.02 (3 H, s), 3.89 (2 H, s, broad), 3.95 (2 H, s), 6.81 (2 H, AA′BB′pattern (upfield part), *J* = 8.4 Hz), 7.22 (2 H, AA′BB′pattern (downfield part), *J* = 8.4 Hz), 7.27 (1 H, t, *J* = 7.4 Hz), 7.30 (1 H, s), 7.35 (1 H, t, *J* = 7.5 Hz), 7.55 (1 H, d, *J* = 7.4 Hz), 7.62 (1 H, s), 7.71 (1 H, d, *J* = 7.5 Hz), 8.45 (1 H, s). The NMR of **24** could be obtained in part: ^1H NMR (500 MHz, CD_2Cl_2) δ 1.97 (3 H, s), 3.65 (2 H, s), 6.84 (2 H, AA′BB′pattern (upfield part), *J* = 8.4 Hz), 7.14 (2 H, AA′BB′pattern (downfield part), *J* = 8.4 Hz), 7.44 (1 H, d, *J* = 7.6 Hz), 7.73 (1 H, d, *J* = 7.5 Hz), 7.77 (1 H, d, *J* = 7.6 Hz), 8.30 (1 H, d, *J* = 7.5 Hz). A COSY-90 spectrum, included in the supplementary material, shows that the doublets at δ 7.73 and δ 8.30 are coupled to each other and that the doublets at δ 7.44 and 7.77 are coupled to signals at δ 7.25 and 7.35, respectively. These are buried under much larger signals for **23**. The ^{13}C NMR of **23** was also obtainable: ^{13}C NMR (75.5 MHz, CD_2Cl_2) δ 24.8 (q), 37.4 (t), 115.7 (d), 118.1 (d), 119.8 (d), 121.7 (d), 125.4 (d), 126.7 (d), 127.1 (d), 128.3 (s), 130.8 (d), 131.8 (s), 134.6 (s), 138.0 (s), 141.7 (s), 143.3 (s), 144.0 (s), 147.1 (s), 168.3 (s); high-resolution MS *m/e* 314.1417, $C_{21}H_{18}N_2O$ requires 314.1419.

***N*-(4′-(Dimethylamino)phenyl)-2-(acetylamino)fluorene (26)**: recrystallized from MeOH, mp 151–152 °C; ^{13}C NMR (75.5 MHz, DMSO-d_6, 70 °C) δ 22.9 (q), 36.1 (t), 112.3 (d), 119.5 (d), 119.6 (d), 123.4 (d), 124.7 (d), 125.4 (d), 126.3 (d), 126.4 (d), 128.1 (d), 132.1 (s), 138.7 (s), 140.2 (s), 142.3 (s), 142.9 (s), 143.4 (s), 149.0 (s), 169.3 (s); one ^{13}C resonance obscured by the solvent is observed in $CDCl_3$, δ 40.5 (q); high-resolution MS *m/e* 342.1731; $C_{23}H_{22}N_2O$ requires 342.1731.

3-(4′-(Dimethylamino)phenyl)-2-(acetylamino)fluorene (27): mp 197–201 °C; high-resolution MS *m/e* 342.1738; $C_{23}H_{22}N_2O$ requires 342.1731.

Quantification of reaction products was performed by ^1H NMR of the kinetic reaction mixtures after 10 half-lives for the decomposition of **1**. ^1H NMR spectra of each of the reaction products were taken in MeOD-d_4 under the kinetic conditions to provide reliable chemical shift standards. Identification of products in the kinetic mixtures was based on chemical shift coincidence of the acyl methyl and 9-methylene resonances. In all cases agreement between the standard and reaction mixture resonances was ±0.005 ppm. HPLC methods previously described[3] and the column conditions outlined above were also used to quantify some products.

Cyclic Voltammetry. Cyclic voltammetry was performed with a BAS-100 electrochemical analyzer. The cell was equipped with a Pt disc working electrode, a Pt wire counter electrode, and a saturated NaCl–SCE reference electrode. The reference compartment was isolated from the working compartment by a cracked-tip glass junction to minimize introduction of H_2O into the working solution. The oxidation of **5** and **6** (ca. 1 mM), in MeOH containing 0.1 M tetraethylammonium perchlorate, was monitored at scan rates ranging from 50 to 200 mV/s. Solutions were maintained under a N_2 atmosphere and were actively outgassed prior to measurement. Ferrocene was used as an internal standard to calibrate the voltage axis. This eliminated errors from varying junction potentials.

Table I. Kinetics of Decomposition of 1 in MeOD-d_4 at 35 °C[a]

condns	k_{obs} (10^4 s^{-1})[b]	condns	k_{obs} (10^4 s^{-1})[b]
MeOD-d_4	1.63 ± 0.05	0.3 M **5**	1.57 ± 0.03
0.1 M **5**	1.50 ± 0.05	0.4 M **5**	1.59 ± 0.05
0.2 M **5**	1.55 ± 0.05	0.1 M **6**	1.65 ± 0.03

[a] Initial concentration of **1** is 4.0 mM. [b] Determined by a linear fit of ln (normalized peak area) for the ^1H NMR acyl methyl peak of **1** vs time. Plots were linear for at least 5 half-lives. Error limits are 2.5 standard deviations of the slope.

Results

Kinetics were monitored in MeOD-d_4 at 35 ± 1 °C by ^1H NMR. Plots of the ln of the normalized acyl methyl peak area of **1** vs time were linear for at least 5 half lives (Figures 1 and 2). The rate constants determined from the slopes of these plots under various conditions are shown in Table I. Although there is some scatter in k_{obs},

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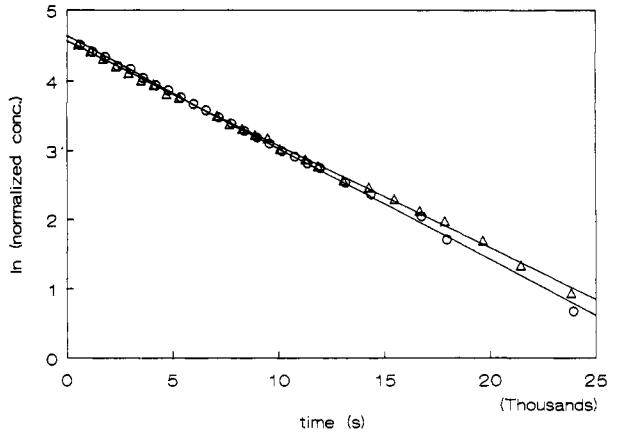


Figure 1. Plots of the ln of the normalized peak area for the acyl methyl ^{1}H NMR peak of 1 vs time at 35 °C in MeOD-d_4 (circles) and in MeOD-d_4 containing 0.1 M **5** (triangles). Initial concentration of 1 is 4 mM.

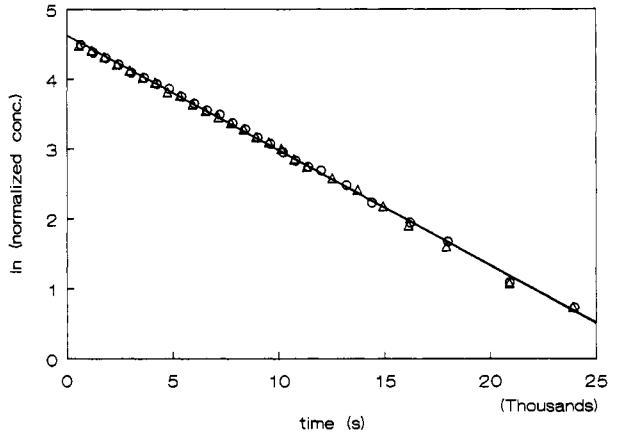


Figure 2. Plots of the ln of the normalized peak area for the acyl methyl ^{1}H NMR peak of 1 vs time at 35 °C in MeOD-d_4 (circles) and in MeOD-d_4 containing 0.1 M **6** (triangles). Initial concentration of 1 is 4 mM.

Table II. Yields of Solvolysis Products of 1 in MeOD-d_4 at 35 °C

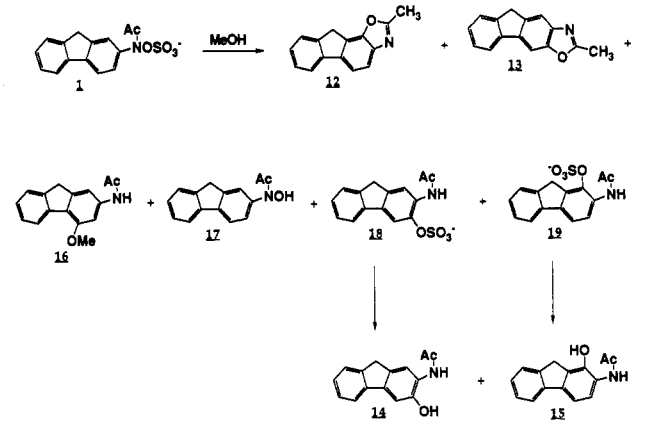
product	% yield[a]	product	% yield[a]
12	22 ± 2	15[b]	12 ± 1
13	20 ± 2	16	13 ± 1
14[b]	14 ± 2	17	3.5 ± 0.5

[a] Determined by NMR peak integration at the completion of the kinetic run. Triplicate runs showed that the reproducibility of the yields is ca. ±10%. Initial concentration of 1 was 4 mM. [b] These are decomposition products of 18 and 19. See Results and Discussion.

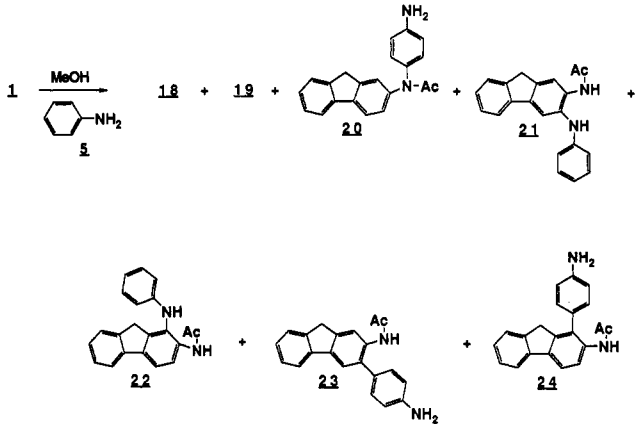
all values are within 5% of an average k_{obs} of 1.58×10^{-4} s^{-1}. It is clear that the aromatic amines **5** and **6** have no significant effect on the rate of decomposition of 1 in MeOD-d_4. Normalized peak area vs time data for solvolysis products and adducts of **5** or **6** with 1 also fit the first-order rate equation with two exceptions discussed below. The rate constants determined from these fits are comparable to those listed in Table I.

Solvolysis products of 1 in MeOH are shown in Scheme I, and their yields determined by ^{1}H NMR in MeOD-d_4 at 35 °C are shown in Table II. The six materials shown in Table II constitute ca. 85% of the yield of the solvolysis products of 1. Several minor (≤3% yield) unidentified products were observed in the NMR spectrum of the kinetic reaction mixture. The hydroxy compounds **14** and **15** are not the immediate solvolysis products of 1, but are formed by decomposition of the *O*-sulfates **18** and **19**. The

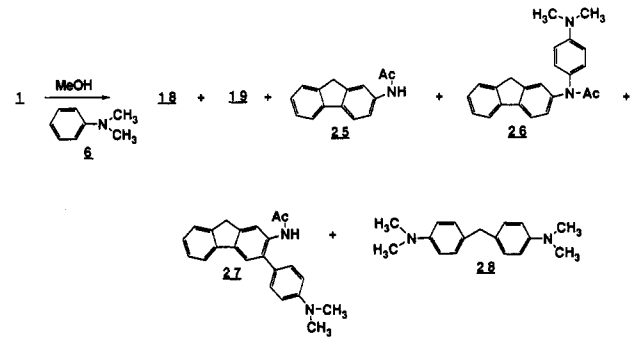
Scheme I



Scheme II



Scheme III



NMR data show that **18** and **19** are formed as 1 decomposes, but these materials in turn decompose slowly into **14** and **15**. All four compounds were identified by comparison to authentic materials, and control experiments showed that the authentic *O*-sulfates did decompose into the corresponding hydroxy compounds under the reaction conditions. Both **18** and **19** were observed as products of the hydrolysis of 1 in H_2O, as was the 4-hydroxy compound analogous to **16**.[5,6] Less reactive analogues of 1 yield the *N*-hydroxy compound formed by S–O bond cleavage as the major solvolysis product in MeOH,[10] but **17** is only a minor solvolysis product of 1. No compounds analogous to the oxazoles **12** and **13** were reported among the hydrolysis products of 1.[5,6]

Decomposition of 1 in the presence of the aromatic amines **5** or **6** led to a very large change in the reaction

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Table III. Yields of Decomposition Products of 1 in the Presence of 0.1 M 5 in MeOD-d_4 at 35 °C

	% yield[a]			
product	0.1 M 5	0.2 M 5	0.3 M 5	0.4 M 5
18	13	14	13	13
19	13	12	11	11
20	22	22	21	22
21	17	17	17	19
22	5	6	7	7
23	9	9	7	8
24	1.1	0.8	1.8	1.1

[a] Determined by NMR peak integration at the completion of the kinetic run. Initial concentration of 1 was 4 mM. Duplicate runs showed that reproducibility of individual yields is ca. ±10%.

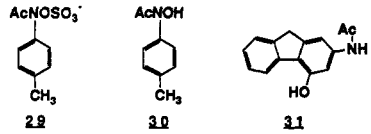
Table IV. Yields of Decomposition Products of 1 in the Presence of 0.1 M 6 in MeOD-d_4 at 35 °C

products	% yield[a]	products	% yield[a]
18	14 ± 1	26	29 ± 3 (30 ● 3)[c]
19	12 ± 1	27	12 ± 1 (15 ± 1)[c]
25	30 ± 3 (28 ● 3)[c]	28	*b* (35 ± 3)[c]

[a] Determined by NMR peak integration at the completion of the kinetic run. Initial concentration of 1 was 4 mM. [b] Not determined by NMR method. [c] Determined by HPLC peak integration of a reaction run in 1.0 M 6 in MeOH. The % yield of 28 is based on 1 initially present. Initial concentration of 1 was 5 mM.

product distribution. The products that have been isolated and characterized in these reactions are shown in Schemes II and III, and the yields of these products determined under the kinetic conditions are shown in Tables III and IV. In the case of the reaction done in the presence of 5 the identified products account for ca. 80% of the overall product yield. A number of unidentified minor products can be seen in the ^{1}H NMR of the kinetic mixtures. For the reaction performed in the presence of 6, no significant minor products remain unidentified and the observed products quantitatively account for 1 within experimental error.

The only solvolysis products still observed in these reactions are 18 and 19 which do not decompose into 14 and 15 in the presence of 5 or 6. Control experiments with the authentic samples confirm this, and the decomposition of 1 to yield the *N*-hydroxy compound 17 is also apparently suppressed by these amines. Preliminary experiments with the less reactive compound 29 show that the *N*-hydroxy compound 30 accounts for ca. 85% of the solvolysis products of 29 in MeOD-d_4, but in the presence of 0.1 M 5 this product can no longer be detected.[11] The suppression of S–O bond cleavage by 5 and 6 was not investigated further.



29 30 31

The five isomeric adducts 20–24 isolated in the reaction done in the presence of 5 were identified after separation primarily by ^{1}H and ^{13}C NMR. H,H-COSY[12] spectra of the aromatic region, such as those shown for 21 and 22 in Figure 3, were particularly useful in assigning structure by establishing the substitution pattern on all aromatic rings. The number of hydrogens bound to each carbon, determined by off-resonance decoupled ^{13}C NMR or DEPT[13] experiments, was also a useful tool in distinguishing isomers. The adducts with 6, 26, and 27, were similarly identified.

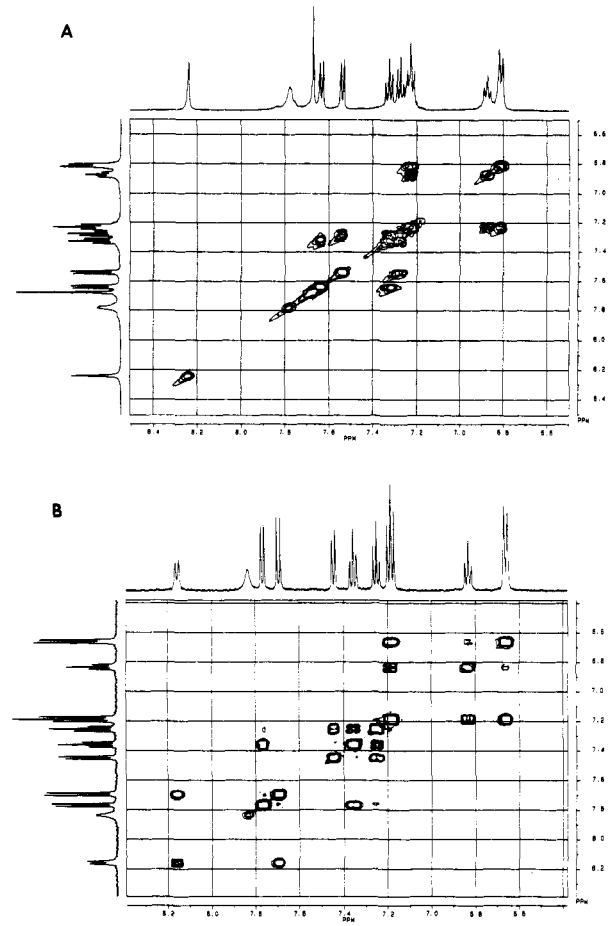


Figure 3. 500-MHz COSY-90 contour spectra of the aromatic regions of 21 (A) and 22 (B). Both spectra are of ca. 5 mg of material dissolved in 0.5 mL of CD_2Cl_2; 400 measurements of 32 FIDs each were made; spectrum size is 2K × 1K.

Table III shows that product yields are essentially invariant in the range from 0.1 to 0.4 M in 5 and that the yields of 18 and 19 are equivalent, within experimental error, to their yields (as 14 and 15) in the absence of 5. These observations are also duplicated in the presence of 6 (Table IV). One significant difference between the reactions performed in the presence of 5 and 6 is the large yield of the reduction product 25 observed in the reaction done in the presence of 6. Reduction of 1, and compounds similar to it, has been observed previously under solvolysis conditions, in the presence of reducing agents such as I^- or Fe^{2+},[6,10,14] but reduction in the presence of simple tertiary amines has not been previously reported. The apparent oxidation product generated in this reaction is 28 which has been detected in yields approximately equivalent to 25 (Table IV). No similar reaction occurs at a detectable level in the presence of 5.

The oxidations of 5 and 6 at a platinum disc electrode were examined in MeOH containing 0.1 M tetraethylammonium perchlorate by cyclic voltammetry. Both ox-

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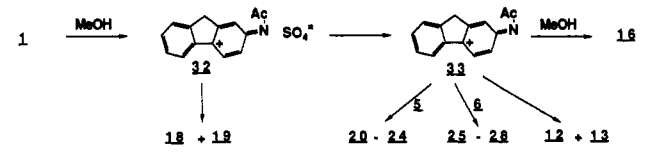
Scheme IV



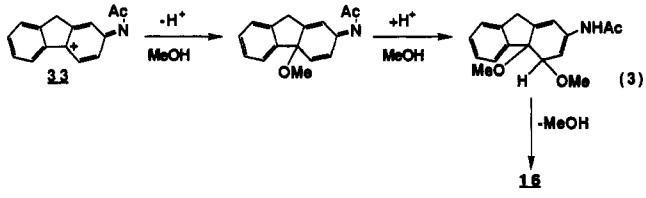
idations were irreversible, but peak potentials were essentially invariant to scan rates in the range of 50–200 mV/s. At 100 mV/s the peak potentials were 820 mV for the oxidation of **5** and 713 mV for the oxidation of **6**. This corresponds to a difference in ΔG for the one-electron oxidation of **5** and **6** of 2.5 kcal/mol.

Discussion

The methanolysis products obtained from **1** (Table II) are similar to its previously reported hydrolysis products.[5,6] Under neutral pH conditions (pH 5–9, μ = 0.5 M (KCl)) the hydroxy analogue of **16**, **31**, is the major hydrolysis product of **1** (40–70% yield), and low yields (ca. 2%) of the *O*-sulfates **18** and **19** can also be detected.[5] The oxazoles **12** and **13** were not reported in these studies, but low yields of these unexpected products may have escaped detection. We will examine this possibility shortly. The hydrolysis products were previously explained in terms of a nitrenium ion mechanism[5] which can also be used to explain the methanolysis products, with the exception of **17**, and the products of the reaction of **1** with **5** and **6**. The mechanism of Scheme IV will fit the available data provided that N–O bond cleavage is rate limiting.

The *O*-sulfates **18** and **19** must be produced by a pathway different from the other major solvolysis products because **5** and **6** do not affect the yields of these materials but do suppress the formation of **12**, **13**, and **16** (Tables III and IV) without affecting the overall rate of the reaction. According to Scheme IV, **18** and **19** are obtained by internal return from a tight ion pair **32**, which can also decompose to the free ion **33**, or a solvent-separated ion pair, but are not susceptible to attack by other reagents. All other products (except **17**) are apparently obtained from intra- or intermolecular attack on **33** or a solvent-separated ion pair. A mechanism similar to this has been used to explain similar behavior observed during the hydrolysis of various *N*-(sulfonatooxy)acetanilides.[8,10]

The intramolecular nucleophilic attack of the acyl oxygen on the ortho carbons of **33**, which generates **12** and **13**, must not be a highly efficient process because **5** and **6** compete very effectively with the intramolecular process at low concentration. The 4-methoxy product **16** cannot be generated by a direct nucleophilic attack, but is probably generated by the sequence of eq 3. There is precedent for such a sequence in reactions of monocyclic analogues of **1**.[8,15]



Of course, **17** cannot be generated by any of the processes shown in Scheme IV. Less reactive analogues of **1**, such as **29**, decompose predominately or exclusively by S–O bond cleavage in MeOH or EtOH to generate the corresponding *N*-hydroxy compound.[10,11] This same process apparently accounts for **17**, although this is a minor reaction of **1** (ca. 3% yield) in MeOH due to the more rapid N–O bond cleavage reactions of **1** compared to its monocyclic analogues.[5,10] Although the S–O bond cleavage process is apparently suppressed by **5** or **6** (see Results) this has no observable effect on rate constants (Table I) because of the minor contribution of this process to the overall reaction rate.

The reduction of **1** by **6** to generate **25** is not entirely unprecedented. Several reducing agents such as I^- and Fe^{2+} have been shown in the past to reduce **1** and its analogues either by reduction of the nitrenium ion or direct reduction of the substrate.[6,10,14] In this case the kinetics indicate that reduction occurs via the nitrenium ion because 0.1 M **6** generates about 30% of the reduction product **25** with no observable effect on reaction rates (Tables I and IV). The cyclic voltammetry on **5** and **6** provide an explanation for the lack of reduction of **1** by **5**. According to these experiments, **5** is ca. 2.5 kcal/mol more difficult to oxidize than **6** in MeOH. This energy difference would be sufficient to lower the yield of the reduction product from ca. 30% to 0% if the reduction competes with efficient nucleophilic trapping, since a $\Delta\Delta G^{\ddagger}$ of 2.5 kcal/mol would decrease the rate constant for reduction by about 60-fold at 35 °C. The oxidation product **28** has previously been shown to be generated along with equimolar quantities of *N*-methylaniline during certain reactions of the radical cation of **6**.[9b] No attempt to detect *N*-methylaniline was made in this study.

There are reasonable alternatives to the mechanism of Scheme IV. The available data do not require that the *O*-sulfates **18** and **19** are formed by internal return from an ion pair, but do require that the pathway leading to these materials is distinct from the pathway leading to the other solvolysis products and the products of trapping by **5** and **6**. The *O*-sulfates could be formed by a concerted process[16] that does not involve any intermediate, or they may be formed through the intermediacy of a π-complex.[17] The apparent high efficiency of trapping of **33** by **5** and **6**, but their complete inability to trap the process leading to **18** and **19**, does suggest that cationic intermediates may not be involved in the formation of these rearrangement products. Experiments designed to provide definitive evidence concerning this matter are in progress. The rate and product effects of **5** and **6** do provide evidence for a two-step mechanism in the formation of the other solvolysis products and indicate that formation of the intermediate ion is rate limiting.

The results of this study are considerably different from our previous examination of the reactions of **5** and **6** with the *N*-aryl-*O*-pivaloylhydroxylamines (**4**).[3] In that case the adducts **7–11** were generated by a reaction which exhibited second order kinetics. The kinetics and other data indicated that an S_N2 process was responsible for the formation of the adducts. Some of the adducts obtained in this study (**20**, **26**) exhibit structures similar to those observed in the earlier study, but the lack of an overall rate effect (Table I) and the independence of product yields with the concentration of **5** in the range 0.1–0.4 M and **6** in the range 0.1–1.0 M (Tables III and IV) show that none of these materials are generated by an S_N2 process. The results indicate that steric hindrance at the nitrogen of **1** is at least partly responsible for this change in mechanism. The lack of any significant products due to attack of the ortho

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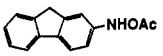
carbons of **5** or **6** (see structures **8** and **10** of eq 2) also suggests that the environment at the reactive sites of **33** is sterically congested. This study provides the first evidence that products such as **20** and **26**, in which bond formation occurs directly at the nitrogen of an ester derivative of an *N*-arylhydroxamic acid, can be formed by an S_N1 process. This is somewhat surprising because all available calculations show that the charge on *N*-acyl-*N*-arylnitrenium ions is predominately delocalized on the ortho and para carbons of the aromatic ring.[18] Studies are now underway on less reactive analogues of **1**, such as **1a** and **29**, to determine if these compounds react with **5** and **6** and, if so, by what mechanism.[11]

The adducts **2** and **3** isolated from the reaction of **1** or **1a** with deoxyguanosine residues of DNA or deoxyguanosine[2] are structurally similar to those obtained in this study. Homogeneous conditions under which guanosine reacts with **1** in yields which are sufficiently high for mechanistic studies have not yet been discovered, but it now appears, based on these results, that this reaction is likely to be an S_N1 process. This is in contrast to the conclusion reached by ourselves[3] and others[19] that deacylated analogues of **1** such as **34** and **35**, which have also



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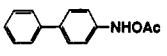

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been implicated as carcinogens,[20] are likely to react with guanosine via an S_N2 mechanism. Obviously much work remains to be done before a full understanding of the nucleophilic substitution reactions of these species is obtained.

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Registry No. **1**, 138235-69-5; **5**, 62-53-3; **6**, 121-69-7; **12**, 138235-70-8; **13**, 138235-71-9; **14**, 1838-56-8; **15**, 2784-86-3; **16**, 138235-72-0; **17**, 53-95-2; **18**, 138235-73-1; **19**, 138235-74-2; **20**, 138235-75-3; **21**, 138235-76-4; **22**, 138235-77-5; **23**, 138235-78-6; **24**, 138235-79-7; **25**, 53-96-3; **26**, 138235-80-0; **27**, 138235-81-1; **28**, 101-61-1.

Supplementary Material Available: Tabulation of IR and ^1H NMR data for **12**, **13**, **16**, **20–24**, **26**, and **27** and ^{13}C NMR and selected COSY-90 NMR spectra for the same compounds (14 pages). Ordering information is given on any current masthead page.

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Biocatalytic Resolutions of Sulfinylalkanoates: A Facile Route to Optically Active Sulfoxides

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Two methods are presented for kinetic resolutions of compounds containing ester and sulfoxide functionalities (sulfinylalkanoates). In the first a crude lipase preparation from *Pseudomonas* sp. (K10) mediates enantioselective hydrolysis of these esters in an aqueous environment. The second method uses the same lipase preparation to promote enantioselective transesterifications with alcohols in hexane. Both procedures are suitable for preparation of sulfinylalkanoates where the ester and sulfoxide groups are separated by one or two methylene units (sulfinylacetates and sulfinylpropanoates) but compounds with three methylene "spacer groups" (sulfinylbutanoates) are not substrates for the lipase under either set of conditions.

Compounds containing both ester and sulfoxide functionalities are useful reagents for organic synthesis.[1,2] Sulfinylacetate **I**, for instance, can be used in asymmetric aldol reactions providing, after reduction, chiral unsubstituted enolate equivalents (eq 1).[3–5] Knoevenagel condensations of sulfinylacetate **I** with nonenolizable aldehydes afford α,β-unsaturated sulfoxides[6–8] which can be elaborated via conjugate additions, directed by the sulfoxide functionality (eq 2).[9,10] Moreover, sulfinylacetates **I** are reagents for the SPAC reaction with enolizable aldehydes (eq 3),[11] a powerful transformation which creates

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